

**Beneficial effects of *Cinnamomum burmannii* in the
treatment of diabetes mellitus**

by

Maria Leonor Tavares da Silva

A thesis submitted in partial fulfilment for the requirements for the degree of
PhD at the University of Central Lancashire

May 2015

STUDENT DECLARATION FORM

Concurrent registration for two or more academic awards

Either *I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution

Material submitted for another award

Either *I declare that no material contained in the thesis has been used in any other submission for an academic award and is solely my own work

Collaboration

Where a candidate's research programme is part of a collaborative project, the thesis must indicate in addition clearly the candidate's individual contribution and the extent of the collaboration. Please state below:

Signature of Candidate Maia Leonor Tavares da Silva

Type of Award Doctoral

School School of Forensic and Applied Sciences

Abstract

Diabetes mellitus (DM) is a major global health disorder currently affecting over 380 million people worldwide. Its prevalence is on constant increase rising to about 592 million by 2035. In addition, there are about 1 billion people who are pre-diabetic and they will eventually join the diabetic group. Moreover, it costs the Governments of the world around 31 trillion to diagnose, treat and care for diabetic patients. If left untreated, DM will lead to end organ failures in the body. Both exercise and diet have important roles for glycaemia control in DM. Plant food has been employed as potential complementary therapeutics in the treatment of DM. Cinnamon is a spice which has beneficial properties on both animal and human models. These properties have been related with its bioactive compounds via an anti-oxidant effect. The study investigated the effects of cinnamon in the treatment of DM employing both animal and human studies.

Chemical characterization of antioxidant capacity of aqueous cinnamon extract (ACE) was employed in this study, including the total phenols quantification, HPLC identification and quantification of the major phenolic compounds and the anti-oxidant capacity (Ferric Reducing Antioxidant Power (FRAP) method and superoxide anion radicals scavenging activity). In animal studies, young male Wistar rats were divided into, 6 groups namely, 3 normal groups: normal untreated (n=6), normal treated 75 mg/Kg cinnamon (n=6) and normal treated 150 mg/Kg (n=5); and 3 diabetic groups: diabetic untreated (n=5), diabetic treated 75 mg/Kg cinnamon (n=6) and diabetic treated 150 mg/Kg (n=5). DM was induced with a single intraperitoneal injection of streptozotocin (STZ; 60 mg/kg in citrate buffer) to the animals. Normal rats received an equivalent volume (0.3 ml) of citrate buffer alone. Two weeks after confirming DM, two groups of normal and diabetic rats (STZ-treated) received either 75 mg/Kg or 150 mg/kg cinnamon daily over a period of 11 weeks. The untreated normal and diabetic groups only received distilled water daily. Body weights, food consumption, blood glucose levels (BGL), blood biochemical parameters, anti-oxidant status were measured *in vivo*. In *in vitro* studies, cation levels in the pancreas, liver, kidneys, heart and serum, insulin secretion from the pancreas, distribution of pancreatic alpha and beta cells in the pancreas and the fibrosis in the heart were measured using established techniques. In human study, a total of 30 non-diabetic subjects were selected and allocated in 2 groups namely oral glucose tolerance test (n=15) and OGTT followed by cinnamon tea administration (n=15) (6 g cinnamon/100 ml). SPSS software was used for statistical analysis. A p -value ≤ 0.05 was considered significant.

Data from antioxidant characterization of ACE show a high concentration of total phenols and a strong antioxidant capacity of cinnamon. The major phenolic compounds identified were cinnamaldehyde, cinnamic acid, coumarin and cinnamyl alcohol. The results from animal study show that cinnamon treatment significantly ($p < 0.05$) decreased food consumption in diabetic rats, but significantly increased the body weight with higher dose in diabetic rats. Cinnamon treatment also showed a significant ($p < 0.05$) reduction in blood glucose levels at week 3 with higher dose and no effect on blood biochemical parameters. Furthermore, cinnamon seems to improve the anti-oxidant status in serum of normal and diabetic rats, especially in diabetic rats treat with 150 mg/Kg doses. The results for the serum cations content revealed that cinnamon treatment significantly decreased Na^+ , Ca^{2+} and Se^{2+} in normal rats and decreased Na^+ , Ca^{2+} and Mg^{2+} in diabetic rats ($p < 0.05$). In the heart, cinnamon with both doses had significant effect ($p < 0.05$) in normal and diabetic rats, namely, increases in Mg^{2+} , K^+ , Ca^{2+} and Fe^{2+} levels. In the liver, kidney and pancreas, cinnamon treatment seemed to have no significant effect on most of cations analysed. The results also show that cinnamon administration can decrease insulin secretion in normal rats and increased the hormone in diabetic rats in a marked dose-dependent manner. The distribution of left ventricle heart fibrosis slightly decreased with cinnamon treatment, especially with high dose in diabetic rats. Concerning to the distribution of pancreatic beta and alpha cells, the results show that cinnamon seems to improve the number of insulin and glucagon positive cells in diabetic rats. The human study data in non-diabetic subjects revealed that postprandial BGL were lower with cinnamon tea administration. Moreover, cinnamon administration showed an improvement of BGL area under the curve following OGTT. It also leads to both a significant ($p < 0.05$) decrease of the maximum concentration and a variation of maximum concentration of BGL in the blood.

In conclusion, the data from animal studies revealed some beneficial effects of cinnamon treatment in diabetic rats through improvement in body weight, food consumption and BGL. The results also suggest a possible positive influence of cinnamon on heart fibrosis prevention and on insulin secretion in diabetic animal. Moreover, cinnamon tea ingestion seemed to exert a beneficial hypoglycaemic effect in non-diabetic subjects during postprandial period. The high phenolic compounds content and anti-oxidant capacity may contribute to these health benefits. The cellular mechanism(s) of action of cinnamon administration is not fully understood regarding its antioxidant effect or its ability to regulate blood glucose.

Table of contents

1	Introduction	2
1.1	Epidemiology of DM	3
1.2	Classification and characterization of T1DM and T2DM	4
1.3	Aetiology of T1DM	5
1.4	Complications of DM	7
1.5	Treatment of DM	8
1.6	Use of plants in DM: cinnamon as a therapeutic approach	10
1.6.1	Chemical constitutions of cinnamon	12
1.6.2	Animal and human studies using cinnamon	15
1.6.3	Cinnamon as a potential antioxidant	28
1.6.4	Anti-inflammatory properties of cinnamon	29
1.6.5	Bioavailability and toxicity of cinnamon bioactive compounds	30
1.7	Working hypothesis	33
1.7.1	Mains aims	33
2	Materials and Methods	36
2.1	Materials	36
2.1.1	<i>Cinnamomum burmannii</i> sticks	36
2.1.2	Chemical analysis	36
2.1.3	Animal studies	37
2.1.4	Human studies	38
2.2	Methods	39
2.2.1	Preparation of aqueous cinnamon extract (ACE)	39
2.2.2	Quantification of total phenols content	40
2.2.3	Simultaneous identification and quantification of phenolic compounds	40
2.2.4	Characterization of antioxidant capacity of ACE	41
2.2.5	Experimental design	43
2.2.6	Induction of type 1 diabetes mellitus	44
2.2.7	Measurement of food consumption	44
2.2.8	Measurement of body weight	44
2.2.9	Measurement of blood glucose levels	44
2.2.10	Oral glucose tolerance test (OGTT)	45
2.2.11	Blood collection and biochemical analysis	45
2.2.12	Serum total antioxidant status measurement	45
2.2.13	Tissues collection and processing	46
2.2.14	Cation measurement in tissues and serum	46
2.2.15	Determination of <i>in vitro</i> pancreatic insulin release	47
2.2.16	Measurement of fibrosis in left heart ventricle	48
2.2.17	Distribution of pancreatic beta and alpha cell by Immunofluorescence	49
2.2.18	Ethical considerations of human study	50
2.2.19	Subjects	50
2.2.20	Experimental design of human study	50

2.2.21	Preparation of Oral Glucose Tolerance Test employed in human.....	51
2.2.22	Data collection	52
2.3	Statistical analysis.....	53
3	Results	55
3.1	Quantification of total phenols	55
3.2	Identification and quantification of phenolic compounds	57
3.3	Characterization of antioxidant capacity	60
3.3.1	FRAP (Ferric Reducing Antioxidant Power) method.....	60
3.3.2	Superoxide anion scavenging activity	60
3.4	Effect of ACE on body weight of normal and diabetic rats.....	61
3.5	Effect of ACE on food consumption of normal and diabetic rats	63
3.6	Effect of ACE on blood glucose level of normal and diabetic rats.....	65
3.7	Oral glucose tolerance test of normal and diabetic rats.....	67
3.8	Effect of ACE on biochemical parameters of normal and diabetic rats.....	69
3.8.1	Effect of ACE on triglycerides (TG)	69
3.8.2	Effect of ACE on total cholesterol (TC).....	70
3.8.3	Effect of ACE on high-density lipoprotein (HDL)	71
3.8.4	Effect of ACE on low-density lipoprotein (LDL).....	72
3.8.5	Effect of ACE on albumin	73
3.8.6	Effect of ACE on hemoglobin A1c (HbA1c)	74
3.8.7	Effect of ACE on creatinine.....	75
3.9	Effect of ACE on organs weight of normal and diabetic rats.....	76
3.9.1	Effect of ACE on pancreas weight.....	76
3.9.2	Effect of ACE on heart weight.....	77
3.9.3	Effect of ACE on liver weight	78
3.9.4	Effect of ACE on kidney weight.....	79
3.9.5	Effect of ACE on soleus muscle weight	80
3.9.6	Organs weight ratio.....	81
3.10	Effect of ACE on total antioxidant status of normal and diabetic rats	85
3.11	Effect of ACE on tissue and serum cations content of normal and diabetic rats	86
3.11.1	Effect of ACE on cation levels in <u>serum</u> of diabetic and normal rats.....	87
3.11.2	Effect of ACE on cation levels in <u>heart</u> of diabetic and normal rats	94
3.11.3	Effect of ACE on cation levels in <u>liver</u> of diabetic and normal rats	101
3.11.4	Effect of ACE on cation levels in <u>kidney</u> of diabetic and normal rats	107
3.11.5	Effect of ACE on cation levels in <u>pancreas</u> of diabetic and normal rats	114
3.11.6	Effect of ACE on different cation contents of normal and diabetic rats per tissue for comparison.....	121
3.12	<i>In vitro</i> pancreatic insulin release in normal and diabetic rats	131
3.13	Effect of ACE on heart fibrosis of normal and diabetic rats.....	134
3.13.1	Distribution of left heart ventricle fibrosis of untreated and treated normal rats	134

3.13.2	Distribution of left heart ventricle fibrosis in untreated and treated diabetic rats	138
3.13.3	Quantification of interstitial fibrosis of left heart ventricle in untreated and treated normal and diabetic rats.....	142
3.14	Effect of ACE on distribution of insulin and glucagon containing cells in pancreatic islet of normal and diabetic rats.....	143
3.14.1	Distribution of insulin in the pancreatic islet of normal and diabetic rats ...	143
3.14.2	Distribution of glucagon in the pancreatic islet of normal and diabetic rats	147
3.15	Sample characterization of human study	150
3.16	Effect of ACE on blood glucose level after Oral Glucose Tolerance Test of human	156
4	Discussion.....	161
4.1	Anti-oxidant capacity and major phenolic compounds of ACE	162
4.2	Effect of ACE on body weight of normal and diabetic rats.....	163
4.3	Effect of ACE on food consumption of normal and diabetic rats.....	164
4.4	Effect of ACE on blood glucose and OGTT of normal and diabetic rats	165
4.5	Effect of ACE on blood biochemical parameters of normal and diabetic rats	167
4.6	Effect of ACE on organs of normal and diabetic rats.....	169
4.7	Effect of ACE on antioxidant profile of normal and diabetic rats	171
4.8	Effect of ACE on cation levels of normal and diabetic rats.....	172
4.9	Effect of ACE on insulin secretion from pancreatic islet of normal and diabetic rats	177
4.10	Effect of ACE on heart fibrosis of normal and diabetic rats	178
4.11	Effect of ACE on distribution of alpha and beta pancreatic cell of normal and diabetic rats	180
4.12	Effect of cinnamon tea on blood glucose level after OGTT of non-diabetic humans.....	181
4.13	Proposed mechanism(s) of action of ACE.....	184
4.14	Conclusion.....	194
4.15	Scope for future studies	195
5	References	197
	APPENDIX	223
	PUBLICATIONS	230
	MEETING AND COLLABORATION WORK	233

Acknowledgments

I would like to thank to my Director of Studies, Professor Jaipaul Singh for guidance, supervision, leadership and support during this PhD programme. I would also to extend my thanks to Professor Jaipaul Singh for friendship, his great encouragement during the program and his unlimited help.

I would also like to express my sincere thanks to my 1st supervisor Professor Fernanda Mesquita for the opportunity to make this PhD programme come true for me. It was my desire to do a PhD and Professor Mesquita was so kind to facilitate this. I would also like to thanks her for supervision, friendship, advice and excellent leadership during the course of PhD program. I am sincerely grateful for the financial support Professor Fernanda Mesquita has given me to complete my PhD and to attend national and international meetings.

I would thanks to my 2nd supervisor Professor Alexandra Bernardo for supervision, guidance, friendship, magnificent support and the excellent unlimited help during the PhD programme. I am also especially grateful for her help and advice during the chemical analysis of some aspects of my experimental work.

I would thanks to Professor Margarida Moncada for her indispensable and wonderful support in chemical analysis in the PhD thesis. I would thanks to Professor Paula Pereira for her help in animal experiments and excellent encouragement during the programme. I would thanks to Dr. Belmira Carrapiço, Professor Berta São Bráz, Dr. Adriana and the technician Inês for their indispensable support in animal experiments. I would thanks to my colleague Dr. Carolina Santos for help in animal experiments. My gratitude also extended to her friendship and personal support.

I would thanks to Professor José Martins dos Santos for his kind help in microscopy study. My gratitude also extended to Dr. Gonçalo Borrecho for his indispensable support and help in microscopy of the heart study.

I would like thanks to Cooperativa de Ensino Superior Egas Moniz for financial support during PhD programme.

I would like to thank to Professor Ernest Adeghate for indispensable and excellent support in microscopy of the pancreas study. My gratitude also extended to Dr. Rasheed Hameed who helped me in this microscopy study. I would thanks to Dr. Tamar Sorribes and Dr. Jim Donnelly who helped me in cation analysis of this work.

Finally, my grateful go to all my family for their constant personal support and encouragement.

Abbreviation

7OHC	7-hydroxycoumarin
AAgs	Islet autoantigens
ABCG	Adenosine triphosphate-binding cassette subfamily G
ACAT	Acetyl-CoA acetyltransferase
ACE	Aqueous cinnamon extract
Ach	Acetylcholine
AGEs	Advanced glycation endproducts
AMPK	AMP-activated protein kinase
APC	Antigen-presenting cells
AUC	Area under the curve
BGL	Blood glucose level
BMI	Body mass index
bw	Body weight
CAB	Chromotropic aniline blue
CD	Carbohydrate
CE	Cinnamon extract
C _{max}	Maximum concentration
COX-2	Cyclooxygenase -2
CyP2A6	Cytochrome P450
DAG	Diacylglyceride
DC	Diabetic cardiomyopathy
DM	Diabetes Mellitus
DT150	Diabetic treated with 150 mg/kg body weight
DT75	Diabetic treated with 75 mg/kg body weight
EFSA	European Food Safety Authority
ER	Endoplasmic reticulum
ERK	Extracellular-signal regulated kinase1/2
FBG	Fasting blood glucose level
FC	Free cholesterol
FCR	Folin Ciocalteu reagent
FFA	Free fatty acid
FGT	Fasting glucose test
FM	Fat mass
FPG	Fasting plasma glucose
FRAP	Ferric reducing antioxidant power
GAD65	Glutamic acid decarboxylase 65
GC-MS	Gas-chromatography mass spectrometry
GIR	Glucose infusion rate
GLP	Glucagon-like peptide
GLUT	Glucose transporter protein
GS1	Glycogen synthase
GSK 3K	Glycogen synthase 3 kinase
H ₂ O ₂	Hydrogen peroxide
HDL	High-density lipoprotein
HG	Hyperglycaemia
HbA1c	Haemoglobin A1c
HLA	Leukocyte antigen
HPLC	High-performance liquid chromatography
HSPs	Heat shock proteins
IDDM	Insulin Dependent Diabetes Mellitus
IFN- γ	Interferon- γ
IGF	Insulin-like growth factor
IL	Interleukin

IP	Intra-peritoneally
IP	Intraperitoneally injection
IR β	Insulin receptor β
IRS	Insulin receptor substrate
JNK	C-jun-N terminal kinase
L	Lipid
LDL	Low-density lipoprotein
LG-MS	Liquid-chromatography mass spectrometry
LPO	Lipid peroxidation level
MAG	Mono-acylglyceride
MAPK	Mitogen-activated protein kinase
MM	Muscular mass
MTP	Microsomal transfer protein
MTTP	Microsomal triacylglycerol transfer protein
NADH	Nicotinamide adenine dinucleotide hydride
NF-kB	Nuclear factor kB
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NO	Nitric oxide
NOS	Nitric oxide synthase
NOX	NADPH oxidase
NPC L1	Niemann-Pick C1-like 1
Npc111	Niemann-Pick c1-like 1
O ₂ ⁻	Superoxide anion
OGTT	Oral glucose tolerance test
OGTT	Oral glucose tolerance test
P	Protein
PB2	Postprandial 2h blood glucose level
PBS	Phosphate buffer solution
PK1	Phosphoinositide-dependent protein kinase 1
PEPCK	Phosphoenolpyruvate carboxykinase
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
Pten	Phosphatase and tensin homologue
ROS	Reactive oxygen species
SAPK	Stress-activated protein kinase
SEM	Standard errors of the mean
SREBP	Sterol regulatory element binding protein
Srebp 1c	Sterol regulatory element-binding protein 1c
STZ	Streptozotocin
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TAS	Total antioxidant status
TAS	Total Antioxidant Status
TBARS	2-Thiobarbituric acid reactive substrate
TC	Total cholesterol
TDI	Tolerable daily intake
TEV	Total energy value
TG	Triglycerides
TNF- α	Tumor necrosis factor-alfa
TTP	Tristetraprolin
TTP	Tristetraprolin
UCPs	Uncoupling Proteins
ZnT8	Zinc transporter-8
ΔC_{max}	Variation of maximum concentration

Chapter One

INTRODUCTION

1 Introduction

Diabetes Mellitus (DM) is a major global health metabolic disorder which is characterized by hyperglycaemia (HG) resulting from dysregulation of insulin secretion and/or insulin action or both (Banting et al., 1991; Opie, 1901). DM can cause long-term complications in different organs in the body including the heart, kidney, liver, eye, parts of the gastrointestinal tract and the nerves (Fowler, 2008). The prevalence of this disease was estimated to increase from 360 million people in 2011 to 552 million in 2030 (Whiting et al., 2011) and to 592 million in 2035 (Guariguata et al., 2014). Most of these cases will be type 2 diabetes mellitus (T2DM), which is strongly associated with a sedentary lifestyle, hereditary and high calorie diet and obesity (Murea et al., 2012).

Type 1 diabetes mellitus (T1DM), although fewer in cases, the incidence indicates that it has also been increasing by 2-5% (Maahs et al., 2010). On the basis of the aetiology, T1DM may be due to immunological destruction of pancreatic β cells resulting in insulin deficiency. Its pathogenesis involves environmental triggers that may activate autoimmune mechanisms in genetically susceptible individuals, leading to progressive loss of pancreatic islet β cells. Most of the acute effects of this disease can be controlled by insulin replacement therapy, but there are long-term adverse effects on blood vessels, nerves and other organ systems (Belle et al., 2011).

The treatment of this disorder has been mainly attribute to pharmacological therapies (Bailey, 2013). Nevertheless, the nutritional approach can be a powerful tool in diabetes management. Plants, namely, cinnamon have demonstrated beneficial properties on animal have employing clinical studies, where the therapeutic properties have been related with its chemical compounds via an anti-oxidant effect (Dugoua et al., 2007; Many et al., 2012). However, a systematic review revealed that high quality human trial supporting the efficacy of this spice are lacking. Furthermore, more investigation is needed to understand its cellular and molecular mechanisms of actions of cinnamon is this disease (Ulbricht et al., 2011).

1.1 Epidemiology of DM

In 2013, over 380 million people were confirmed diabetics and this number will increase to 552 million in 2030 and to 592 million in 2035, with significantly more cases from the developing countries compared to the developed countries (Guariguata et al., 2014; Whiting et al., 2011). A study representing 130 countries worldwide projected rapid increase in the number of diabetic people compared with the projected number for growth in the population alone, estimating it to be by 55% in 2035 (Guariguata et al., 2014). This represented mainly the 40-59 age-group people. Currently, China has the highest number of diabetic people (over 98.4 million), followed by India (70.1 million). Europe is the second region with higher increase of diabetic people, an increase in 22.4% from 2013 to 2035. In Portugal, the percentage of diabetic aged between 20-79 years was estimated to be 13% of the national population in 2013. This number is estimated to increase by 15,8% in 2035 (Guariguata et al., 2014). Furthermore, 174.8 million people were estimated to have undiagnosed diabetes in 2013 (Beagley et al., 2013). Moreover, the prevalence rate of pre diabetic patients increased from 11.6% to 35.3% from 2003 to 2011 in England (Mainous et al., 2014).

These projections of diabetes prevalence lead to estimate its effect on serious long term complications of this disorder and also in health care cost. Bagust and co-authors revealed that with this increase of T2DM, the diabetes-related complications would be also risen rapidly by 2045. This can also lead an increase of health care cost for patient with T2DM by to 25% from 2035 to 2045 in UK (Bagust et al., 2002).

Regarding T1DM, data from epidemiologic study revealed that the incidence has been also increased worldwide by 2-5%. The prevalence for T1DM was estimated to 1 in 300 patients, in US (Maahs et al., 2010). Currently, diabetes diagnosis, its treatment and health care for patients in the United States cost the Government \$245 billion USAD in 2012, including direct medical costs and reduced productivity (ADA, 2013a).

1.2 Classification and characterization of T1DM and T2DM

DM is classified mainly in two types, namely, type 1 Diabetes Mellitus (T1DM) and type 2 Diabetes Mellitus (T2DM), which are associated with different aetiologies.

T1DM represents approximately 5-10% of all cases of diabetes and results from progressive loss of pancreatic islet mass through a process involving autoimmunity mechanisms targeted of molecules that are expressed in the pancreatic beta cells (S. Han et al., 2013). T2DM, on the other hand, is characterized by insulin resistance and it is due to multifactorial aetiologies including both genetic and non-genetic factors, in which both physical inactivity, obesity and diet have been demonstrated to be determinants in T2DM (Poulsen et al., 1999; Tuomilehto et al., 2001). It represents more than 90% of all cases of diabetes.

Diabetes symptoms are similar in both types of DM, but they develop most rapidly and more severely in T1DM than T2DM. Some common symptoms of DM due to elevated HG include polyuria, polydipsia, polyphagia, weight loss and blurred vision (Shin et al., 2012; Weinger et al., 1995). In long-term, DM can lead to the development of complications in different organs of the body, such as, the eye (retinopathy) with potential vision loss (Kern et al., 2010), the kidney (nephropathy) that can lead to renal failure (Min et al., 2012), peripheral neuropathy or an inability to feel or touch (Jolivald et al., 2013), and the heart (cardiomyopathy or heart failure) (Brom et al., 2010). In addition, DM can also carry a high risk of large vessel damage as atherosclerosis commonly associated with metabolic abnormalities. These are caused by diabetes, which induced vascular dysfunction, such as, hypertension, hyperlipidaemia and hyperglycemia. Diabetes can also increase the risk of stroke, myocardial infarction and death (Beckman et al., 2002). These long-term complications of DM will be detailed in section 1.4.

Currently, this disorder is diagnosed by either undertaking a fasting glucose test (FGT) or oral glucose tolerance test (OGTT) and confirmed by haemoglobin A1c (HbA1c) measurement. A person is diagnosed diabetic when fasting plasma glucose (FPG) for at least 8 h fasting is more than or equal to 126 mg/dl (7.0 mmol/l) or 2-h plasma glucose is more than or equal to 200 mg/dl (11.1 mmol/l) during an OGTT (using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water) or HbA1c is more than or equal to 6,5% or 48 mmol/l (X. Zhang et al., 2010).

1.3 Aetiology of T1DM

As mentioned above, T1DM results from dysregulation of immune responses allowing auto-reactive T cells against pancreatic β -cell. According to Rabinovitch and co-authors (1998), T cells initiated a cascade of immune/inflammatory process in the islet and consequently to β cell destruction and death. After, certain β cell protein can act as autoantigens and processed by antigen-presenting cells (APC), a immunogenic signal which can activate T cells ($CD4^+$), namely Th1 subset (Rabinovitch & Suarez-pinzon, 1998). Figure 1.1 shows a schematic model of the process, which is involved in the development of T1DM. It is now possible to create a T1DM model of DM using a drug called streptozotocin (STZ). In addition, other chemicals can also be used to induce T1DM including alloxan. In STZ-induced diabetes mellitus model, the pancreatic β cell in islets of Langerhans are destroyed leading to impaired insulin production and consequently to hyperglycaemic state (Akbarzadeh et al., 2007).

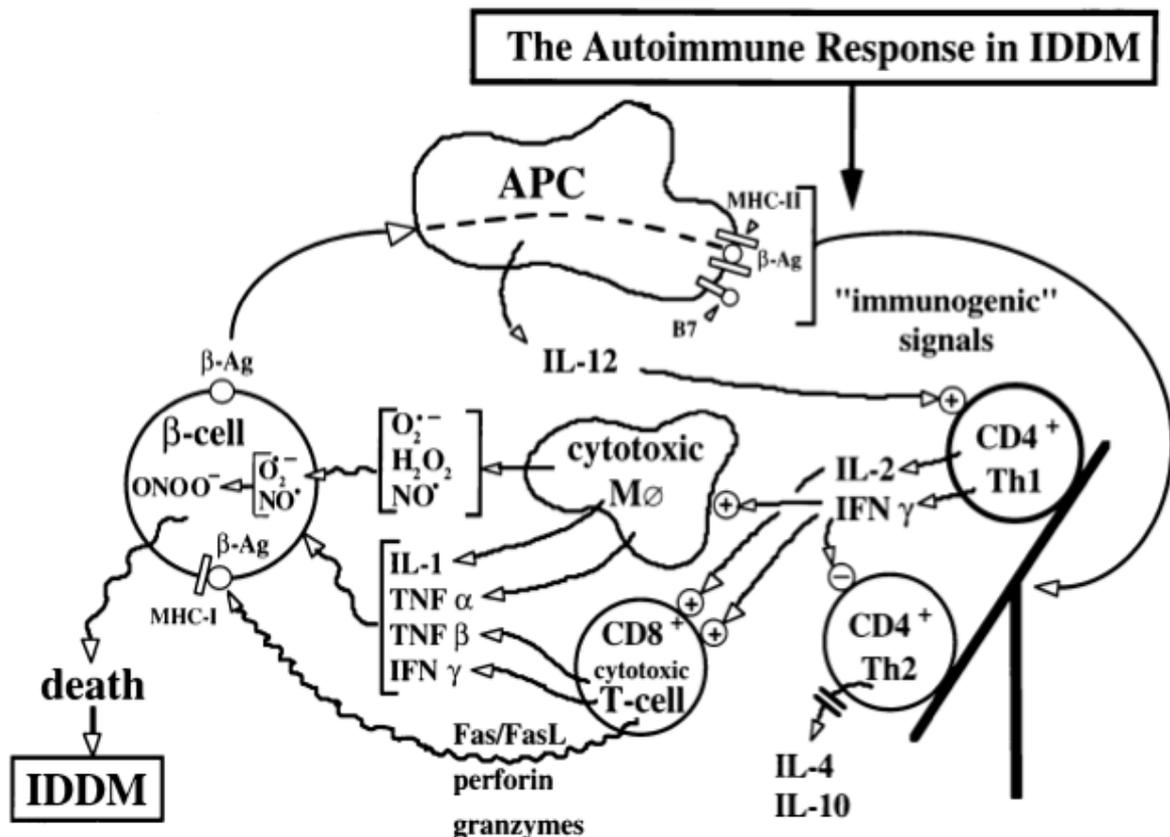


Figure 1.1: Schematic diagram showing the proposed biochemical mechanism(s) of immune system cells, cytokines and oxidative stress involved in destruction of pancreatic β cell T1DM. Taken from Rabinovitch and co-authors study (Rabinovitch & Suarez-pinzon, 1998).

The subset of auto-reactive T cells, Th1 induces the production of pro-inflammatory cytokines IL-2 and IFN γ which inhibit the Th2 production. In later Th2 is responsible for the production of IL-4 and IL-10 (Serreze et al., 2001).

Different biochemical mechanisms have been proposed for islet β cell damage during the genesis of T1DM. These include through pro-inflammatory cytokines activation, free radicals action and destruction of β cell by interaction between cytotoxic T cell and β cell via Fas receptor, via macrophages and CD8⁺ T cells activation (Jun et al., 1999; Maedler et al., 2001; Rabinovitch & Suarez-pinzon, 1998).

Macrophages can produce pro inflammatory cytokines IL-1 and TNF α , which can bind to specific receptors on β cell and in turn individual cytokines, can inhibit insulin release in rat islet. *In vivo* and *in vitro* studies have demonstrated that the high glucose levels can increase pro inflammatory cytokines production. These include interleukin (IL)- 1 β (Maedler et al., 2002), tumor necrosis factor-alfa (TNF- α) (L.-F. Lee et al., 2005) and interferon- γ (IFN- γ) (X. Huang et al., 1995).

IL-1 β is a pro-inflammatory cytokine that contributes to β cell glucotoxicity (Maedler et al., 2002; Moran et al., 2013). This cytokine is released in hyperglycaemic state and it seems to act on pancreatic β cell affecting the synthesis and secretion of insulin (Ling et al., 1993) and in turn it promotes β cell apoptosis (Maedler et al., 2002). This mechanism of action may be through nuclear factor kB (NF-kB) activation (Maedler et al., 2002). Persaud and co-authors, in 2004, also suggested that the increased of hyperglycaemia-induced IL-1 β could induce cyclo-oxygenase (COX)-2 expression contributing to β cell dysfunction (Persaud et al., 2004).

TNF- α and IFN γ are another cytokines that play an important role in T1DM. TNF- α has been associated with the initiation T1DM acting by activation of islet-specific pancreatic lymph node cells (L.-F. Lee et al., 2005) and IFN γ has been associated with development of this disease (X. Huang et al., 1995).

On the other hand, the macrophages can also activated the production of oxygen free radicals superoxide (O₂^{•-}), hydrogen peroxide (H₂O₂) and nitric oxide (NO[•]), which may damage the β cell membrane (Thayer et al., 2011). In STZ-induced diabetic rats, nitric oxide (NO[•]) seems to play an important role in dysfunction of pancreatic β cells induced by inflammatory stimulation (Kaneto et al., 1995).

These free radicals inactivate mitochondrial and cytosolic proteins leading to decreased oxidative phosphorylation, decreased glycolysis, decreased ATP levels and consequently

impaired insulin secretion and production of advanced glycation end-products (AGEs) and their receptors AGE activation (Niedowicz & Daleke, 2005; Rabinovitch & Suarez-pinzon, 1998). Oxidative phosphorylation is believed to be the inductor of reactive oxygen species (ROS) in the mitochondrion, which produces superoxide radical, a highly reactive free radical (Kwong & Sohal, 1998). The uncoupling proteins (UCPs) appear also to contribute to an increase in the superoxide production in diabetic rats. These proteins facilitate dissipation of high membrane protonic potential and thus a limited respiration rate, which is responsible for ROS production in mitochondria (Korshunov et al., 1997).

Glucose autoxidation is another possible mechanism to ROS production. High plasma glucose level can react with hydrogen peroxide in the presence of transition metals (iron and copper) forming hydroxyl radical (Robertson et al., 2003).

Other important source of free radicals production in DM is advanced glycation end-product (AGE). The interaction of glucose with proteins leads to the formation of these products that promote free radicals formation and subsequently quench and block anti proliferative effects of nitric oxide (NO) The AGEs activate the transcription factor NF- κ B and thus, enhanced the production of NO, which is believed to be a mediator of islet beta cell damage (Cellek et al., 2004; Mariappan et al., 2010; Wautier et al., 1994).

Some enzymes can also to be considered as potential sources of ROS. Nitric oxide synthase (NOS) is an enzyme that induces nitric oxide production, which has been shown to produce free radicals during catalytic cycle. In STZ-induced diabetic rats, NOS expression is increased (Koo & Vaziri, 2003). *In vitro* studies also suggested an integrated pathway in pancreatic β cell linking the pro inflammatory cytokines with lipoyxygenase and NADPH oxidase (NOX)-1 activation (Weaver et al., 2012).

1.4 Complications of DM

DM has several long term complications that can take many years to develop depending on when the disease was diagnosed and treatment started. These complications are related to blood vessel diseases and are generally classified into small vessel diseases (micro-vascular diseases), which include the eyes, kidneys and nerves and large vessel diseases (macro-vascular diseases) that involve the heart and blood vessels (Fowler, 2008).

Regarding the micro-vascular disease, diabetic patients can develop retinopathy, which has been classified as the most common micro-vascular complication of diabetes, and this is

closely related to hyperglycaemic (HG) and hypertension. There is much evidence that patients with T1DM develop retinopathy within 20 years of diagnosis (Fowler, 2008). Another micro-vascular complication is nephropathy leading to renal failure in both T1DM and T2DM. According to UKPDS 64 Study (The United Kingdom Prospective Diabetes Study), approximately one quarter of patients will develop micro albuminuria or nephropathy within 10 years of the disease. It was also estimated in this work that the time spent in each stage of nephropathy was about 19 years for patients with no nephropathy at diagnosis of diabetes, 11 years for those with micro albuminuria, 10 years for those with macro albuminuria and 2.5 years for those with elevated plasma creatinine (Adler et al., 2003). On the other hand, neuropathy appears also as a long-term complication of DM and the risk of development depends on the magnitude and duration of HG. This complication can manifest as sensory, focal/plurifocal and autonomic neuropathies, such as, sensitivity to touch, muscle weakness, chronic pain and numbness (Adler et al., 2003).

Another long-term complication due to DM is related to macro-vascular disease. Diabetic cardiomyopathy (DC) is one of those complications that can occur leading to structural, functional and metabolic changes in the myocardium. Data have demonstrated that DM can increase the risk for cardiac dysfunction and heart failure independently of other risk factors including hypertension and obesity. DM can lead to the development of fibrosis in the heart, apoptosis and subsequently, remodelling of the myocardium. In addition, more than 78% of all diabetics will eventually die from heart disease (Asbun & Villarreal, 2006; Brom et al., 2010; Khavandi et al., 2009).

1.5 Treatment of DM

Many type 2 diabetic patients begin anti-hyperglycaemic therapy with lifestyle changes, namely with nutrition therapy and physical activity. The goals of this therapy are:

- 1) to obtain optimal metabolic outcomes, including blood glucose levels, lipid profile and blood pressure level,
- 2) to either delay or prevent and to treat the long-term complications of DM, including dyslipidaemia, cardiovascular diseases, nephropathy, neuropathy, retinopathy and others and

3) to improve their health encouraging healthy food choices and physical activity practices (Franz et al., 2010). All these practices will help in maintaining a better quality of life for diabetic patients.

For T1DM management, daily insulin administration is the major therapy, but also nutrition therapy plays an important role as well, although it may be often one of the most difficult aspects of treatment (Wise et al., 1992). The recommendations to type 1 diabetic people is to focus on achieving blood glucose goals without excessive hypoglycaemia and moreover, to ensure an adequate energy to normal growth and development (Wise et al., 1992). In T2DM, nutritional intervention should allow for the modification in lifestyle in order to reduce insulin resistance and to improve metabolic status. This can be achieved by reducing total and saturated fat, increase dietary fibres, including whole grains and to decrease sodium intake which is also strongly emphasized (Franz et al., 2010). In addition, for T1DM and T2DM patients should choose food sources of carbohydrates with low glycaemic index in order to optimize glycaemic control (Brand-Miller et al., 2006). Although non-pharmacologic therapy which includes diet, exercise and weight loss have important roles in diabetes treatment, pharmacological intervention is also required if glycaemic targets are not achieved within 2 to 3 months of lifestyle management. The pharmacological therapy can include oral drug therapy or insulin therapy. A number of oral anti-diabetic drugs have been used successfully in the treatment of T2DM. These include the five different classes namely, sulfonylurea, biguanides, alfa-glucosidase inhibitor, thiazolinediones and intestinal lipase inhibitor (A. Y. Y. Cheng & Fantus, 2005). According to the guidelines of American Dietetic Association and European Association of the Study of Endocrinology, metformin (a biguanide) is a first-line pharmacological treatment. This drug allows for high efficacy by reducing HbA1c, lowers the risk of HG, helps in weight loss and moreover, it is cost effective. When HbA1c target is not achieved in 3 months, a combination therapy is necessary to treat T2DM with other anti- diabetic drugs or insulin (Bailey, 2013). More recently new anti-diabetic agents such as incretins have been introduced in diabetic therapies, especially when metformin cannot be used for a subsequent pharmacological treatment (Bailey, 2013). Incretins are intestinal protein hormones, which help in increasing beta cell mass and also in the release of endogenous insulin from the pancreas (Bailey, 2013).

1.6 Use of plants in DM: cinnamon as a therapeutic approach

The use of the Complementary and Alternative Medicine (CAM) has been employed as potential new therapeutic agents in the management of DM for hundreds of years. Currently, there are several plant-based medicines to treat DM (Bnouham et al., 2006). However, since this research project is related to cinnamon, then emphasis will be placed only on this particular plant. The use of the plants has revealed an important role in the management of DM, in which cinnamon is one of the most frequently used plants to treat this disorder (Dugoua et al., 2007; Many et al., 2012). From 1990, a large number of medicinal plants have been demonstrated to be of importance in the treatment of this disorder, showing beneficial hypoglycaemic effects. A number of bioactive compounds have been isolated from plants displaying equal or more potent than oral hypoglycaemic agents currently in use in controlling blood glucose level (Bnouham et al., 2006). Phytotherapy is used, not only for glycaemic control, but also for the prevention and treatment of diabetic complications (R. Singh et al., 2013). Although many plants have been used in the management of different diseases including DM as complement of the diet, their mechanisms of actions still unclear.

Cinnamon is a spice that was introduced to Europe from Sri Lanka in the 16th century by the Portuguese. This spice is used in the culinary for its aromatic properties and is used as flavouring and tasting of food, beverages, chewing gums, chocolates, liquors and others (Ravindran et al., 2004). Moreover, cinnamon has been used extensively over the years for its medicinal values as well. Different studies have reported that cinnamon can act beneficially in glycaemic (Shen et al., 2010) in lipid profile control (Ping et al., 2010), in anti-inflammatory activity (Koteswara et al., 2007) and with antioxidant potential (Ranjbar et al., 2006). The therapeutic properties of this plant have been widely related with its chemical compounds, which depend on different factors including the species, the parts of the tree and more importantly, the concentrations employed in the study.

Cinnamon comes from the family *Lauraceae* and there are several species of *Cinnamomum* (Barceloux, 2009; Ravindran et al., 2004) including:

- *Cinnamomum verum* (sym. *C. zeylanicum*), commonly called “true” cinnamon or Ceylon cinnamon which is a native from Sri Lanka;
- *Cinnamomun cassia*, originated from different sources;
 - o Chinese cinnamon (syn. *C. aromaticum*), native of China-Vietnam;

- Indonesian cassia (syn. *C. burmannii*), originated from Sumatera-Java region;
- Indian cassia (syn. *C. tamala*), originated from North Eastern India;
- Saigon cassia (syn. *C. loureiroi*).

Figure 1.2 shows a typical cinnamon bark (A) and the powder (B). Generally, the bark is golden brown and this can be pulverised into powder.

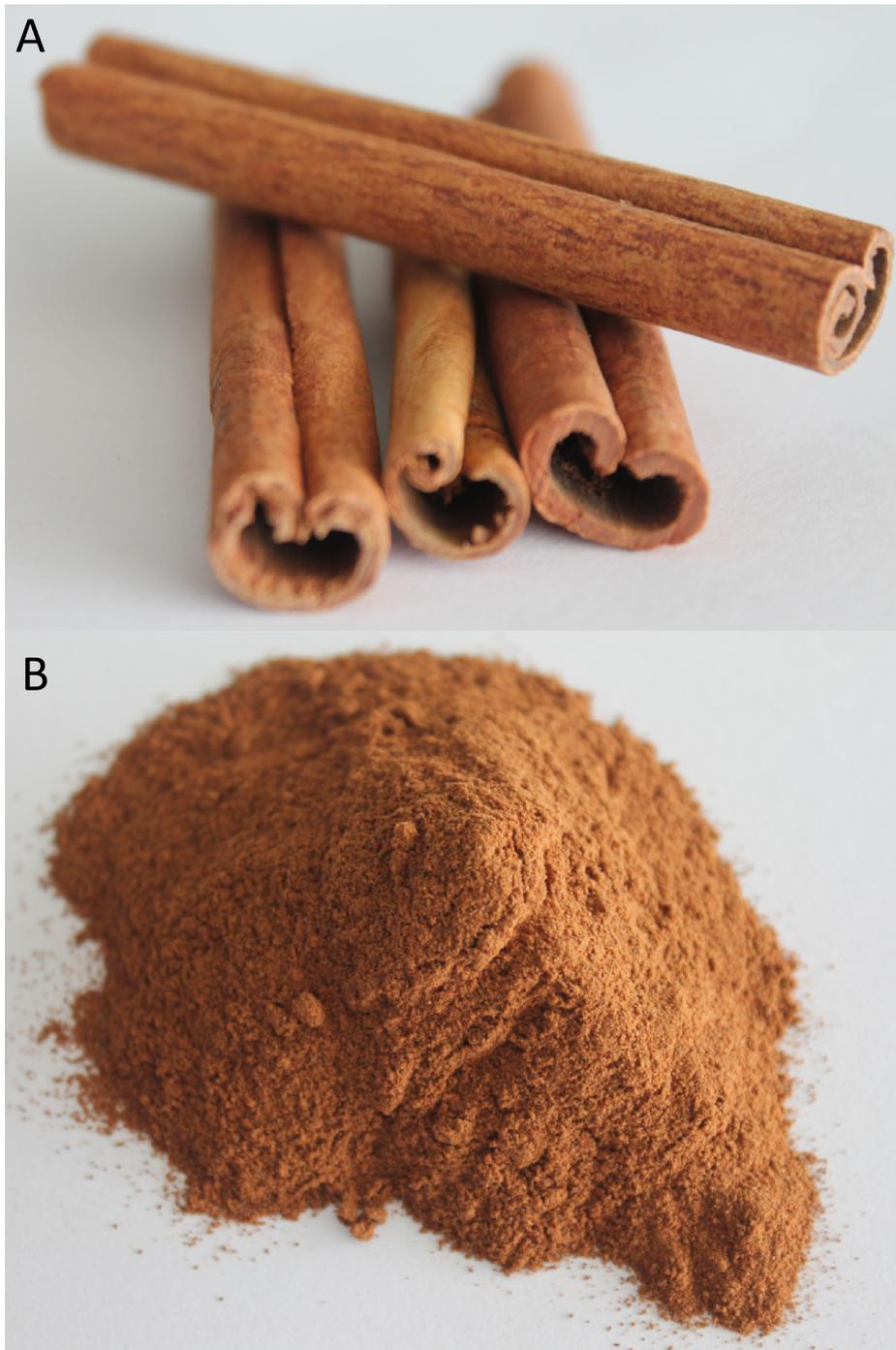


Figure 1.2: Images showing (A) bark and (B) powder of cinnamon.

1.6.1 Chemical constitutions of cinnamon

The composition of the cinnamon depends upon the species as well as the tree section (top, centre and lower segments) and on the different growth stages (Geng et al., 2011). Geng et al. demonstrated that the top and centre segments of the cinnamon bark would be more efficient for oil extraction than the whole bark (Geng et al., 2011). Moreover, at 12-years-old, the bark oil of the tree has the highest yield. One of the main compounds of oil cinnamon extract is trans-cinnamaldehyde (G. Singh et al., 2007). The bark products have a minimum of this compound in one years' of growth (33.95%) and a maximum in six years' growth (76.4%) (Geng et al., 2011). Pro-anthocyanidins are another main classes of compounds present in aqueous cinnamon extract and they have been demonstrated recently to have beneficial hypoglycaemic effect on DM (Jiao et al., 2013).

Figure 1.3 shows the chemical structures of the main cinnamon compounds including procyanidin type-A polymers, cinnamic acid, cinnamaldehyde and coumarin.

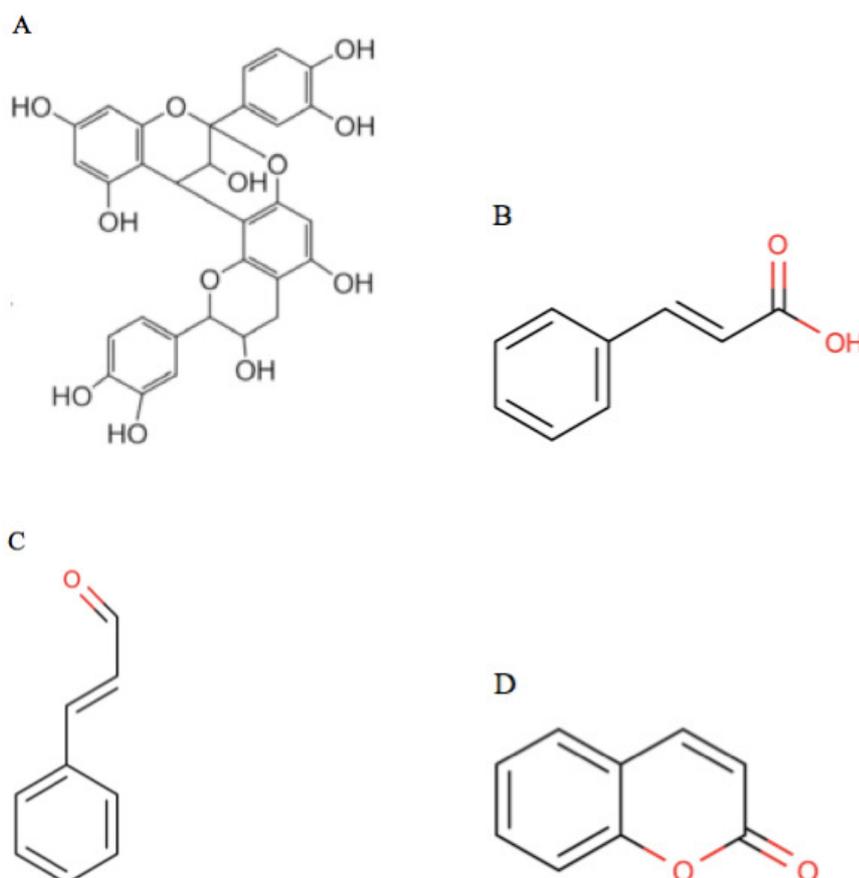


Figure 1.3: Diagram showing chemical structures of the main cinnamon compounds: (A) procyanidin type-A polymers (Jiao et al., 2013), (B) cinnamic acid*, (C) cinnamaldehyde* and (D) coumarin*. *Molecular structures made by Marvin –beans Sketch Software.

Regarding their chemical properties, these compounds vary in quantities depending on the different solvents used in the extraction process. Table 1.1 shows the major compounds of different species of cinnamon according with the different analytical chemical methods, namely high-performance liquid chromatography (HPLC), liquid-chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS) employed in each study.

Cinnamon contains also trace elements such as potassium (134.7 mg/g), magnesium (85.5 mg/g), calcium (83.8 mg/g), phosphorus (42.4 mg/g), manganese (20.1 mg/g), iron (7.0 mg/g), zinc (2.6 mg/g), chromium (0.4 mg/g), sodium (0.0 mg/g) (Gul & Safdar, 2009).

Table 1.1: Table showing the major compounds found in different cinnamon species according to extract and analytical methods.

References	Species	Cinnamon extracts	Major compounds identified	Analytical methods
(J. Yang et al., 2007)	<i>C. cassia</i>	Aqueous-methanolic	Protocatechuic acid, (-)-epicatechin, cinnamic acid, cinnamaldehyde, eugenol	HPLC
(He et al., 2005)		Methanolic	Coumarin, Cinnamic acid, Cinnamaldehyde, Eugenol	HPLC
(Geng et al., 2011)		Oil	Cis-cinnamaldehyde (1.43%), trans-cinnamaldehyde (60%), copaene (7.37%), cinnamyl alcohol acetate (1.18%), gamma-murolene (1.56%), 2-methoxycinnamaldehyde (1.85%), alpha-murolene (1.92%), beta-bisabolene (1.25%), (+)-delta-cadinene (2.92%), trans-alpha-bisabolene (2.54%), tetradecanal (0.83%)	GC-MS
(Shen et al., 2010)	<i>C. zeylanicum</i>	Aqueous	Cinnamyl alcohol and cinnamaldehyde	HPLC
(G. Singh et al., 2007)		Oil	Cinnamaldehyde (97.7%), α -Copaene (0.8%), α -Amorphene (0.5%), Cadinene (0.9%)	GC-MS
(He et al., 2005)	<i>C. wilsoni</i>	Methanolic	Cinnamaldehyde and eugenol	HPLC
	<i>C. mairei</i>		Cinnamaldehyde and eugenol	HPLC
	<i>C. loureirii</i>		Coumarin, Cinnamic acid and cinnamaldehyde	HPLC
(Anderson et al., 2004)	<i>C. burmannii</i>	Aqueous	Procyanidin Type-A oligomers; chlorogenic acid, ferulic acid, t-cinnamic acid, guaiacol, cinnamic acid methyl ester, homovanillic acid, cinnamide, isovanillic acid cinnam, 2-methoxy-cinnamaldehyde, cinnamyl alcohol, 3-methoxy-l-tyrosine, clove oil, 4-oxo-4h-1-benzopyran-carboxylic acid, p-coumaric acid, resveratrol, o-coumaric acid, vanillic acid, curcumin, vanillin azine, eugenol	HPLC
(Shan et al., 2007)		Methanolic	Procyanidin B1 (0.7%), procyanidin B2 (5.12%), procyanidin trimer (13.76%), (+)-catechin (2.61%), procyanidin dimer (1.27%), procyanidin tetramer (2.35%), (-)-epicatechin (1.02%), (E)-cinnamic acid (2.69%), (E)-cinnamaldehyde (62.18%) and (S)-cinnamaldehyde (1.95%)	GC-MS and LC-MS
(Thantsin et al., 2008)		Oil	Camphor (1.79%), 4-terpineol (0.50%), cinnamaldehyde (2.70%), δ -elemene (2.32%), α -cubebene (1.56%), α -ylangene (0.52%), caryophyllene (1.23%), epi-bicyclosesquiphellandrene (0.44%), calarene (0.94%), β -guaiene (2.14%), aromadendrene (1.47%), α -humulene (0.43%), santalene (0.77%), α -amorphene (5.39%), valencene (0.60%), α -murolene (2.40%), γ -cadinene (0.50%), δ -cadinene (5.98%), patchoulene (0.25%), α -calacorene (1.71%), cyclopentadecane (1.29%), cariphyllyl alcohol (0.49%), γ -eudesmol (1.28%), T-cadinol (1.15%), α -eudesmol (1.63%), pentadecanol (0.96%), isocurcumenol (0.55%), palmitic acid (2.48%) e elaidinsaeure (7.71%).	GC-MS

1.6.2 Animal and human studies using cinnamon

This literature search identified ten animal studies and twenty-four human studies, which have employed cinnamon. One systematic review with meta-analysis described the effect of cinnamon on T2DM (Allen et al., 2013). One systematic review (Priyanga Ranasinghe et al., 2013) described hypoglycaemic effect and a meta-analysis (W. Baker et al., 2008) explaining the effect of cinnamon on glucose control and lipid profiles. Finally, two systematic reviews described the medicinal properties of cinnamon especially in the treatment of DM. The available evidence suggests that cinnamon has hypoglycaemic, cholesterol-lowering, anti-inflammatory, antioxidant properties which may act beneficially in DM treatment (Gruenwald et al., 2010).

Effects of cinnamon on glycaemic control

Several studies have shown possible beneficial effects of cinnamon as hypoglycaemic properties. For this reason, the main research of this spice has been focused on prevention and treatment of DM (Mang et al. 2006; Allen et al. 2013). A systematic review revealed that the effect of cinnamon on glycaemia in T1DM and T2DM is yet unclear or there is conflicting scientific evidence (Ulbricht et al., 2011). Most of the *in vitro* and in animal models studies have reported a beneficial metabolic effect of cinnamon on T1DM and T2DM (see tables 1.2 and 1.3). However, numerous clinical trials in humans revealed some discrepancies regarding to the effect of cinnamon on glucose control (see tables 1.4 and 1.5). Recent literature reveals that it is still premature to recommend cinnamon supplementation to DM treatment based on the scientific evidence (Rafehi et al., 2012).

In vitro and animal study

In vitro and *in vivo* animals studies (see table 1.2 and table 1.3 for T2DM and T1DM, respectively) have reported marked beneficial effects of cinnamon and its bioactive compounds on glycaemic control (S. H. Kim & Choung, 2010; Priyanga Ranasinghe et al., 2012; Rekha et al., 2010). *In vivo* studies, it has been demonstrated that cinnamon extract (S. H. Kim et al., 2006a; Shen et al., 2010) and cinnamaldehyde

(Babu et al., 2007) administration improved fasting blood glucose levels (FBG) and improved hyper-insulinemia compared to controls. These effects were also observed with the administration of cinnamon polyphenolic extract in T1DM (Jia et al., 2009). Cinnamon oil, another administration form of this spice, also decreased FBG in T2DM rats (Ping et al., 2010). In STZ-induced diabetic rats treated with polyphenol-rich de-coumarinated extract of *C. cassia* significantly improved blood sugar and serum insulin levels compared to standard aqueous cinnamon extract containing 18% polyphenols content and 0.8% coumarin (Kumar et al., 2014). This de-coumarinated extract from cinnamon could be benefit since coumarin at high doses have hepatotoxic and carcinogenic properties (Abraham et al., 2010).

Table 1.2: A summary of studies evaluating the effects of either cinnamon or its isolated bioactive compounds on body weights, food intake and blood parameters in T2DM animal models with insulin resistance.

References	Population/duration	Interventions	Results
(S. H. Kim et al., 2006a)	n=10 rats with T2DM/ 6 weeks	Aqueous <i>Cinnamomum cassia</i> extract (50, 100, 150 and 200 mg/kg bw) / Once a day	<u>Treated group vs control:</u> ↓FBG 511.8 to 253.4 mg/dL (50 mg/kg, p<0.01), to 243 mg/dL (100 mg/kg, p<0.01), to 208.7 mg/dL (150 mg/kg, p<0.01) and to 159.7 mg/dL (200 mg/kg, p<0.001) ↓FB2 524.7 to 343.3 mg/dl (50 mg/kg, p<0.05), to 252.7 mg/dL (100 mg/kg, p<0.01), to 251.6 mg/dl (150 mg/kg, p<0.01) and to 247.3 mg/dl (200 mg/kg, p<0.01) ↑Insulin (200 mg/kg p<0.05) No significant effect on BW and food intake. <u>With 200 mg/kg dose:</u> ↓TG (221 to 121.6 mg/dl), p<0.01; ↑HDL (32.8 to 51 mg/dl), p<0.05; No significant effect on TC.
(Ping et al., 2010)	n=10 mice with T2DM/ 35 days	Cinnamon oil (25, 50 and 100 mg/kg bw) / On morning	<u>Treated group vs control:</u> ↓BFL with 50 and 100 mg/kg doses (p<0.01) ↓BGL in OGTT at 30, 60 and 120 min with 100 mg/kg dose (p<0.01) ↓TC 4.98 to 4.28 mmol/l (50 mg/kg, p<0.05) and to 3.80 mmol/l (100 mg/kg, p<0.001) ↓TG 1.03 to 0.9 mmol/L (25 mg/kg, p<0.05) to 0.87 mmol/l (50 mg/kg, p<0.01) and to 0.84 mmol/L (100 mg/kg, p<0.01)
(K Couturier et al., 2010)	n=8-10 rats with insulin resistance/ 12 weeks	<i>Cinnamomum cassia</i> powder (20 g/Kg of diet) added to food	<u>Treated group vs control:</u> ↓BW (401.15 to 392.68), p<0.05 ↓White adipose tissue , p<0.05
(Z. Lu et al., 2011)	n=9 rats with insulin resistance/ 14 days	Cinnamon bark extract (200 and 300 mg/kg bw)	<u>Treated group vs control:</u> ↓BGL 28.42 to 25.95 mmol/l with 300mg/kg doses (p<0.05)
(B Qin, 2003)	n=6 rats with Euglycemic clamp/ 3 weeks	Aqueous cinnamon extract (30 and 300 mg/kg bw)	<u>Treated group vs control:</u> ↑GIR to 118% (30 mg/kg, p<0.05) and to 146% (300 mg/kg, p<0.001); No significant effect on FBG, FFA, BW and insulin secretion after cinnamon treatment
(Chen et al., 2012)	n=8 mice <i>db-db</i> / 4 weeks	<i>Cinnamomum cassia</i> extract (CC-E) and <i>Cinnamomum tamala</i> extract (CT-E) / Gavage; once daily	<u>Treated group vs control:</u> ↓AUCs for OGTT (p<0.05) ↑Insulin level with CC-E (p<0.05) and CT-E (p<0.01) ↓TG with CC-E (p<0.001); No significant differences in TC

FBG – fasting blood glucose level; BGL – blood glucose level; PB2 – postprandial 2h blood glucose level; TG – triglycerides level; HDL – high density lipoprotein; TC – total cholesterol; HbA1c – glycosylated hemoglobin; OGTT – oral glucose tolerance test; bw – body weight; FFA – free fatty acids; AUC – area under the curve; GIR – glucose infusion rate; ↑ – increase; ↓ – decrease.

Table 1.3: A summary of studies evaluating the effects of either cinnamon or its isolated bioactive compounds on body weights, food intake and blood parameters in T1DM animal models.

References	Population/duration	Interventions	Results
(Shen et al., 2010)	n=10 rats with T1DM/ 22 days	Aqueous <i>Cinnamomum zeylanicum</i> extract (3, 30 and 100 mg/kg bw) / given by water bottles	<u>Treated group vs control:</u> ↑BW 134.8 to 153.4g (30 mg/kg) and 134.8 to 166.3 g (100 mg/kg) (p<0.05) ↓FBG 580.5 to 351.8 mg/dL (30 mg/kg) and 580.5 to 378 mg/dL (p<0.05) No effect with 3 mg/kg dose; No effect on food consumption with both doses
(Jia et al., 2009)	n=10 rats with T1DM/ 14 days	Polyphenolic extract of <i>Cinnamomum parthenoxylon</i> bark (100, 200 and 300 mg/kg bw)	<u>Treated group vs control:</u> ↓FBG by 11.1% (100 mg/kg bw), 22.5% (200 mg/kg bw) and 38.7% (300 mg/kg bw) (p<0.01) ↓BGL at 90 minute on OGTT (200 mg/kg bw) ↑Insulin level 4.85 to 13.7 μIU/mL (100 mg/kg bw), to 15.2 μIU/mL (200 mg/kg bw) and to 16.8 μIU/mL (300 mg/kg bw) (p<0.01)
(Anand et al., 2010)	n=6 rats with T1DM/ 2 months	Cinnamaldehyde (20 mg/kg bw)	<u>Treated group (initial vs final value):</u> ↓FBG 289 to 152 mg/dl (p<0.001) <u>Treated group vs control:</u> ↓FBG 323 to 248 mg/dl p<0.001); ↓HbA1c 12.7 to 6.6 % (p<0.001) ↑BW 139 to 209g (p<0.01) ↓Fluid intake 175 to 74 ml/day (p<0.001) ↑Insulin level 5.5 to 12.5 μU/mL (0.001)
(Babu et al., 2007)	n=6 rats with T1DM/ 45	Cinnamaldehyde (5, 10 and 20 mg/kg bw)	<u>Treated group vs control:</u> ↓FGL 431 to 256.9 mg/dl (5 mg/kg) to 189.4 mg/dl (10 mg/kg) and to 127.4 mg/dl (20 mg/kg) (p<0.05) <u>With 20 mg/kg dose:</u> ↑BW (165.7 vs 190.6 g) ↓HbA1c (0.97 vs 0.58 %) ↓TC (246.7 vs 113.5 mg/dl) ↓TG (38 vs 17.5 mg/dl) ↑HDL (38.5 vs 54.3 mg/dl) ↑Insulin level (8.2 vs 13.4 μU/ml) (p<0.05) No effect on food intake.

FBG – fasting blood glucose level; BW – body weight; BGL – blood glucose level; PB2 – postprandial 2h blood glucose level; TG – triglycerides level; HDL – high density lipoprotein; TC – total cholesterol; HbA1c – Glycosylated hemoglobin; OGTT – oral glucose tolerance test; bw – body weight; FFA – free fatty acids; AUC – area under the curve; GIR – glucose infusion rate; ↑ – increase; ↓ – decrease.

Taking in account the literature search, potential cellular mechanisms of cinnamon action on metabolic pathways of glycaemic control is proposed and summarized in figure 1.4. The main mechanism of action of cinnamon emphasizes on the hypothesis that cinnamon can elicit an insulin-mimetic effect through the regulation of insulin signalling (Cao et al., 2007; Bolin Qin et al., 2012). Thus, cinnamon is believed to exert its benefit effect on glucose homeostasis by the following pathways i) increasing glucose uptake in muscle and adipose tissue by glucose transporter (GLUT) 4 production and GLUT 4 translocation (Anand et al., 2010; Shen et al., 2010), ii) promoting glycogen synthesis in the liver, thereby inhibiting glycogen synthase kinase 3 β (Cao et al., 2010); and iii) decreasing gene expression of two regulators of gluconeogenesis in liver, the phosphoenolpyruvate carboxykinase (PEPCK) and the glucose-6-phosphatase (D. M. Cheng et al., 2012).

The mechanism by which cinnamon and its bioactive components regulate insulin signalling includes the activation of intracellular cascade events. Thus, the extract of this plant and its isolated compounds (hydroxychalcone) seem to stimulate the insulin receptor (IR) tyrosine auto-phosphorylation (Jarvill-Taylor et al., 2001) and then the insulin receptor substrate molecules (IRS) (Karine Couturier et al., 2011). The IRS-2 phosphorylation results in activation of phosphatidylinositol 3-kinase (PI3K), which is responsible for the activation of phosphoinositide-dependent protein kinase (PDK1). This kinase in turn activates different signalling molecules, such as protein kinase B (Akt1/PKB) that has been reported to have an important role in the regulation of protein translocation, enzymes activity and gene transcription of different enzymes (Bolin Qin et al., 2012). The Akt1/PKB can enhance protein kinase C (PKC), which in turn stimulates glucose uptake by the cell. Similarly, Akt1/PKB inhibits GSK-3 leading to an activity of glycogen synthase. In addition, cinnamon also seems to inhibit the phosphatase and tensin homolog deleted on chromosome 10 (PTEN) that is responsible to inhibit PI3K. Cinnamon extract has also demonstrated an ameliorated GLUT 4 translocation effect via another signalling pathways in 3T3-L1 adipocytes (not shown in figure) which includes enhanced phosphorylation of AMP-activated protein kinase (AMPK) (Shen et al., 2014). Finally, the effect of cinnamon on the IRS-1 insulin receptor substrate molecule can stimulate the P38-MAPK, ERK and JNK/SAPK signalling pathways via GRB-2 leading to apoptosis and cell growth (Bolin Qin et al., 2012). All these multiple effects of cinnamon and its isolated compounds help in regulating the HG induced by DM.

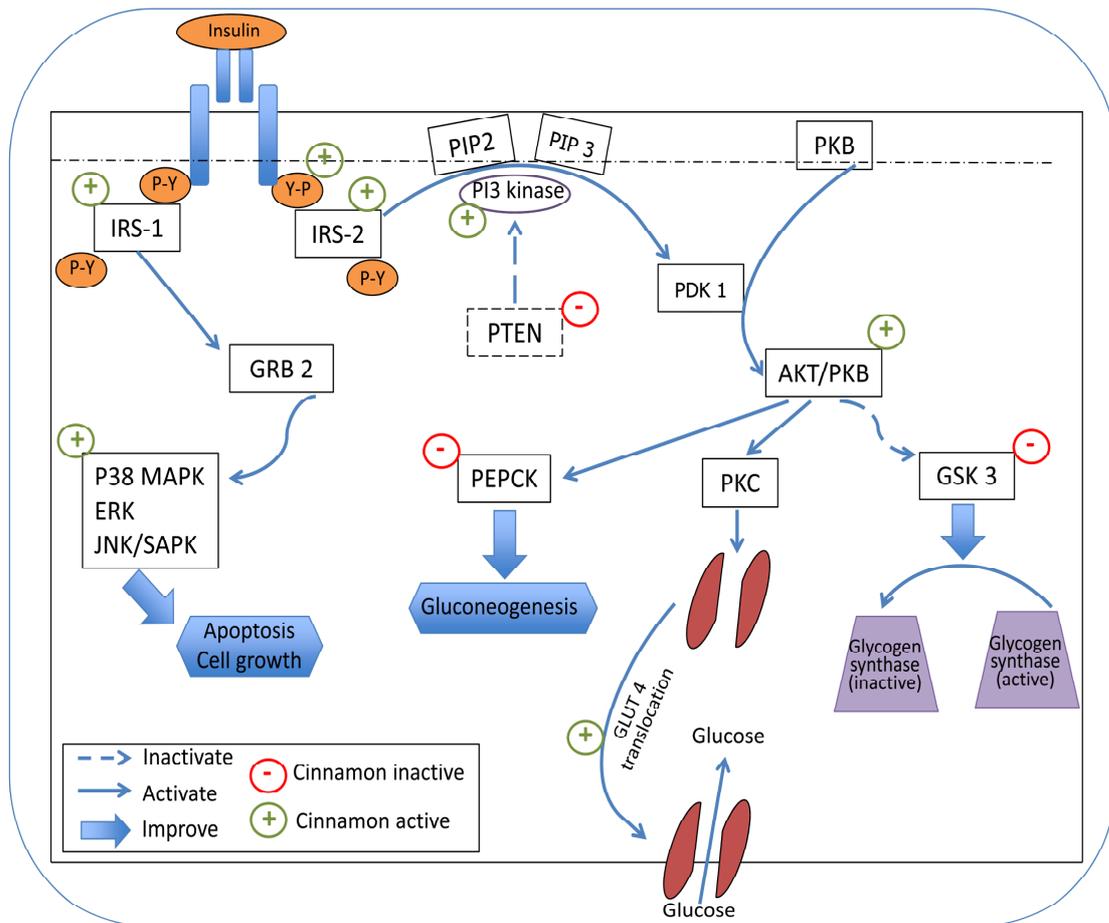


Figure 1.4: Schematic models describing tentative mechanism(s) of action of cinnamon and its isolated bioactive compounds on glycaemic control in insulin-dependent tissue. IRS, insulin receptor substrate molecule; PIP2, phosphatidylinositol 4,5-biphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate; PI3 kinase, phosphatidylinositol 3-kinase, PTEN, phosphatase and tensin homolog; GRB2, growth factor receptor-bond protein 2; p38 MAPK, p38 mitogen-activated protein kinase; ERK, extracellular-signal-regulated kinases; JNK/SAPK, c-Jun N-terminal kinase/stress-activated protein kinase; PEPCK, phosphoenolpyruvate carboxy-kinase; PDK1, 3-Phosphoinositide-dependent kinase 1; PKB/AKT, protein kinase B; PKC, protein kinase C; GSK 3, glycogen synthase kinase 3; GLUT 4, glucose transporter protein.

In addition to its insulin-mimetic effect, cinnamon and cinnamaldehyde have been demonstrated to exert a hypoglycaemic effect by regulating non-insulin sensitive glucose transporter gene expression. Cinnamon extract can increase GLUT1 gene expression (Cao et al., 2010), but this occurs in a dose-dependent manner. In L929 fibroblast cells, cinnamaldehyde promotes a maximum stimulation at a concentration of 2.0 mM (Plaisier et al., 2011), which is markedly higher than would be expected during the ingestion of cinnamon. Cinnamon powder seems to act on gene expression of another glucose transporter, namely non-insulin dependent - GLUT2 - by increasing its RNA expression in liver (Karine Couturier et al., 2011).

Another potential mechanism of action of cinnamon for glycaemic control involves its ability to reduce intestinal α -glycosidase and pancreatic α -amylase activity (Adisakwattana & Lerdsuwankij, 2011). The activity of sucrase, maltase and lactase enzymes were significantly decreased following cinnamon extract treatment in the small intestine of mice (S. H. Kim et al., 2006a). Cinnamon exerts this effect by decreasing the breakdown of disaccharides into glucose, allowing a slow absorption of glucose, and thereby, reduced postprandial blood glucose level (Hamada et al., 2013; S. H. Kim et al., 2006a).

Clinical trials in human

A total of 22 clinical trials were reviewed analysed in this study regarding the effect of cinnamon in glycaemia control on human (see tables 1.4 and 1.5). Of those trials, there are 13 studies that demonstrated an improved of FGB, PBG or area under the curve for PBG. The other 9 trials revealed that cinnamon administration had no metabolic effect on either FBG or PBG. It is important to note that only 5 studies employed a well-controlled clinical trial with the control group. This literature search, included clinical studies of healthy, T1DM, T2DM or impaired glycaemia subjects.

In healthy subjects, cinnamon powder (6 g) intake added to a high sugar meal (rice pudding) or a meal test significantly decreased PBG (Hlebowicz et al., 2007; Magistrelli & Chezem, 2012). However, lower doses (1 or 3 g) of cinnamon employed in same study did not demonstrate any significant effect in glycaemia (Hlebowicz et al., 2009). The administration of cinnamon by capsule form leads to an improvement of plasma glucose response by 13% on OGTT plus cinnamon in a dose of 5 g (T P J Solomon & Blannin, 2007). Although cinnamon ingestion by capsule form containing 3 g had no significant effect after OGTT, its ingestion for 14 days decreased glucose

response to OGTT by 5.5% at the end of experimental protocol (Thomas P J Solomon & Blannin, 2009). The area under the curve for glycaemia also significantly decreased with 6 g of cinnamon between 0 and 60 min after OGTT in healthy subjects (Beejmohun et al., 2014).

The clinical trials have shown controversial results in human model. The possible reason may be due to use of different doses, extracts and species of cinnamon as well as different forms of administration (food or capsule) or depending whether it is healthy, T2DM, T1DM or impaired glycaemia tolerance.

In either T2DM or impaired fasting glycaemia subjects, the ingestion of 1, 2 or 6 g of *C. cassia* after meals for 40 days by capsule administration decreased FBG by 18-29% at the end of experimental protocol. However, the sulfonylurea drugs taking by the participants may also help in the improvement of FBG levels observed in these participants (Khan et al., 2003). A similar study reported that the ingestion of 6 g cinnamon in capsule during 12 weeks did not significantly alter FBG compared with control group (Wickenberg et al., 2014).

The administration of 2 g of *C. cassia* for 40 days also decreased PBG (Soni & Bhatnagar, 2009). Furthermore, *C. zeylanicum* (3 g/day) and *C. cassia* (1 or 2 g/day) administration significantly decreased HbA1c (Akilen et al., 2010; Crawford, 2009; Vafa et al., 2012). Aqueous cinnamon extract intake in different doses (250 mg, 336 mg, 360 mg and 500 mg) by capsule form for 3 or 4 months also resulted in a significant decrease in FBG (T. Lu et al., 2012; Mang et al., 2006; A.-M. Roussel et al., 2009). These results provide evidence for the possible beneficial effects on the treatment of DM. However, in studies by Vanshoonbee et al. and Blavins et al., they showed that cinnamon administration (1.5 g and 1 g, respectively) have no beneficial hypoglycaemic effect (Blevins et al., 2007; Vanschoonbeek et al., 2006). The possible explanation may be due to the short intervention time (6 weeks) or due to oral anti-diabetic drugs that subjects already took. In the study with impaired glucose tolerance subjects, the administration of 6 g cinnamon had also demonstrated no significant effect on insulin response (Wickenberg et al., 2012).

A study employing T1DM subjects was found, and the results show that cinnamon capsule administration has no benefit effect during a period of 3 months (Altschuler et al., 2007).

Table 1.4: A summary of studies evaluating the effects of either cinnamon or its isolated bioactive compounds on blood parameters of healthy human subjects.

References	Population/duration	Interventions	Results
(Markey et al., 2011)	n=9 healthy subjects /1 time	<i>Cinnamomum zeylanicum</i> powder (3 g) capsule /with a test meal	No significant effect on gastric emptying, postprandial blood glucose levels, TC, LDL, HDL and appetite (p>0.05)
(Ranjbar et al., 2006)	n=54 healthy subjects /2 weeks	<i>Cinnamomum zeylanicum</i> tea (100 mg/30 mL per day)	<u>Treated group vs control:</u> ↓Plasma TBARS (38%, p<0.05); ↑Total antioxidant power (21%, p=0.01); ↑Plasma thiols (22%, p=0.03)
(Cao et al., 2008b)	n=18 healthy subjects /10 days	<i>Cinnamomum zeylanicum</i> powder tea (100 mg/300 mL/day)/ once daily	<u>Before vs final treatment:</u> ↓LPO (5.03 vs 1.28 nmol/mL, p=0.016)
(Hlebowicz et al., 2009)	n=15 healthy subjects /1 time	<i>Cinnamomum cassia</i> (1 or 3 g)/ with 300 g of rice pudding	<u>Treated group vs control:</u> ↓AUC for Postprandial insulin at minute 120 (2888.7 mU min/L vs 2381.6 mU min/L) (3g of cinnamon) ↓Fasting insulin response at 60 min (p<0.05); No effect on FGL, GER, satiety and GLP-1
(T P J Solomon & Blannin, 2007)	n=7 healthy subjects /1 time	<i>Cinnamomum cassia</i> (5 g) capsule/ after evening meal (OGTT/12h prior to OGTT)	<u>Treated group vs control:</u> ↓Plasma glucose response by 13% on OGTT+cinnamon (p<0.05) ↓Plasma glucose response by 10% when cinnamon supplementation 12h before (p<0.05) No significant difference on serum insulin concentration
(Tang et al., 2008)	n=11 healthy subjects /4 weeks	Cinnamon capsule (3 g per day) /3 time with meals	No effect on FGL, TC and TG
(Magistrelli & Chezem, 2012)	n=30 healthy and obese subjects /1 time	<i>Cinnamomum cassia</i> (6 g per day)/ with meal test	<u>Treated group vs control:</u> ↓Pos-prandial BGL at 15 (109 to 99 mg/dL, p=0.001), 30 (141 to 122 mg/dL, p<0.001), 45 (148 to 122 mg/dL, p<0.001) and 60 min (139 to 120 mg/dL, p=0.001)
(Thomas P J Solomon & Blannin, 2009)	n=8 healthy subjects /14 days	<i>Cinnamomum cassia</i> powder (3 g per day) /capsule after evening meal	<u>Before vs final treatment:</u> ↓Glucose response to OGTT by 13.1% (p<0.05) on day 1 and by 5.5% (p=0.05) on day 14; Improve insulin sensitivity (p<0.05) on day 14
(Hlebowicz et al., 2007)	n=14 healthy subjects /1 time	Cinnamon (6 g)/ with 300 g of rice pudding	<u>Treated group vs control:</u> ↓AUC for Postprandial BGL at minute 30 (13.7 vs 30.7 mmol/l), 45 (32.4 vs 68.1 mmol/l), 60 (47.3 vs 97.2 mmol/l), 90 (63.3 vs 125 mmol/l) and 120 (75 vs 139.1 mmol/l) Delayed gastric emptying (p<0.05) ↑Insulin sensitivity during OGTT (p<0.05) No significant effect on satiety

GER – gastric emptying rate; GLP-1 – glucagon-like peptide-1; LPO – lipid peroxidation level; TBARS – 2-thiobarbituric acid reactive substance; FRAP – ferric reducing antioxidant power; AUC – are under the curve; FBG – fasting blood glucose; HbA1c – haemoglobin A1C; TC – total cholesterol; LDL – low density lipoprotein, HDL – high density lipoprotein; TG – triglycerides; ↑ – increase; ↓ – decrease.

Table 1.5: A summary of studies evaluating the effects of either cinnamon or its isolated bioactive compounds on blood parameters of healthy and diabetic subjects.

References	Population/Duration	Interventions	Results
(Beejmohun et al., 2014)	n= healthy subjects/12 weeks	Ceylon cinnamon (1 g per day) capsule /after meal	<u>Treat group vs control:</u> ↓AUC for glycaemia, between 0 and 60 min by 21.2% (p<0.05)
(Altschuler et al., 2007)	n=72 T1DM subjects /90 days	Cinnamon (1 g per day) capsule/ Insulin therapy	No significant effect in number of hypoglycaemic episodes, HbA _{1c} , insulin total daily doses and weight changes
(Vanschoonbeeck et al., 2006)	n=25 T2DM subjects /6 weeks	<i>Cinnamomum cassia</i> (1,5 g per day) capsule/antidiabetic oral / 3 times with meal	No significant effect in FBG, insulin levels, glucose level on OGTT, HbA _{1c} and lipid profile (TC, HDL, LDL, TG)
(Mang et al., 2006)	n=55 T2DM subjects /4 months	Aqueous cinnamon extract (3 g/day) capsule / Oral anti-diabetics/3 times with meal	<u>Before vs final treatment:</u> ↓FBG: 9.26 vs 8.15 mmol/l No effect in TC, LDL, HDL, TG and HbA _{1c} levels
(Blevins et al., 2007)	n=43 T2DM subjects /3 months	<i>Cinnamomum cassia</i> (1 g per day) capsule/ Oral anti - diabetic/2 times with meal	No significant effect on FBG, lipid profile and HbA _{1c} and insulin levels
(Akilen et al., 2010)	n=58 T2DM subjects /12 weeks	<i>Cinnamomum cassia</i> powder capsules (2 g per day)/oral hypoglycaemic agents/ 4 times with meals	<u>Treated group vs control:</u> ↓HbA _{1c} (8.22% to 7.86%) compared with control (8.55% to 8.68%), p<0.005 No effect on lipid profile (Total cholesterol, LDL, HDL and triglycerides) and FGL compared control with treated groups
(T. Lu et al., 2012)	n=66 T2DM subjects /3 months	<i>Cinnamomum aromaticum</i> extract (120mg and 360mg per day) capsule/ oral hypoglycaemic agents	<u>Before vs final treatment:</u> ↓HbA _{1c} with low (0.67%) and high (0.93%) doses (p<0.01) ↓FBG with low (11.61 to 0.42 mmol/l) and high (2.32 to 0.93 mmol/l) doses (p<0.01) ↓TG with low (1.32 to 0.23 mmol/l) dose (p<0.01) No effect on TC, HDL and LDL
(Vafa, et al. 2012)	n=44 T2DM subjects /8 weeks	<i>Cinnamomum zeylanicum</i> (3 g per day) capsules/oral hypoglycaemic agents/ 3 time with meal	<u>Before vs final treatment:</u> ↓HbA _{1c} (7.4 vs 6.9%) ↓FBG (139.3 vs 126.5 mg/dL) and TG (163.3 vs 138.2 mg/dL) <u>Treated group vs control:</u> No significant effect in TC, HDL and LDL and anthropometric

GER – gastric emptying rate; GLP-1 – glucagon-like peptide-1; LPO – lipid peroxidation level; TBARS – 2-thiobarbituric acid reactive substance; FRAP –ferric reducing antioxidant power; AUC – are under the curve; FBG – fasting blood glucose; HbA_{1c} – haemoglobin A1C; TC – total cholesterol; LDL – low density lipoprotein, HDL – high density lipoprotein; TG – triglycerides. ↑ – increase; ↓ – decrease.

Table 1.5 (cont.): Summary of studies evaluating the effects of either cinnamon or its bioactive compounds on blood parameters of diabetic and impaired glycaemia subjects.

References	Population/Duration	Interventions	Results
(Khan et al., 2003)	n=57 T2DM subjects /40 days	<i>Cinnamomum cassia</i> (1, 3 or 6 g per day) capsule; Sulfonylurea / After meals	<u>Before vs final treatment:</u> ↓FBL (18-29%, p<0.05) ↓TG (23-30%, <0.05), LDL (7-27%, <0.05) ↓TC (12-26%, <0.05) No effect on HDL cholesterol
(Soni & Bhatnagar, 2009)	n=30 T2DM subjects /40 days	<i>Cinnamomum cassia</i> powder capsule (2 g per day) /4 times after meal	<u>Before vs final treatment:</u> ↓FBG (148.7 to 120 mg/dL), p<0.01 ↓Postprandial BGL by 12.79% (p<0.01)
(Crawford, 2009)	n=89 T2DM subjects /90 days	<i>Cinnamomum cassia</i> capsule (1 g)/diabetic medication/ 2 times with meal	<u>Before vs final treatment:</u> ↓HbA1C (8.47 to 7.64), p<0.001
(Ziegenfuss et al., 2006)	n=22 Pre-diabetes and metabolic syndrome subjects /12 weeks	Aqueous cinnamon extract (500 mg per day) capsule/ 2 times with meal	<u>Before vs final treatment:</u> ↓FBG in cinnamon-treat group by 8.4% (p<0.01) ↑Lean mass by 1.1% (p<0.002)
(Wickenberg et al., 2014)	n=17 Impaired glucose tolerance subjects /12 weeks	<i>Cinnamomum cassia</i> (6 g per day) 2 times with meal	<u>Treat group vs control:</u> No significant effect on insulin sensitivity, HbA1c, FBG
(Wickenberg et al., 2012)	n=10 Impaired glycaemia tolerance subjects / 1 time	<i>C. zeylanicum</i> (400 mg per day) capsule / 1 time (OGTT)	<u>Treat group vs control:</u> No significant effect in glucose level and insulin response
(A. Roussel et al., 2009)	n=22 Obesity with impaired fasting glycemia subjects /12 weeks	Aqueous <i>Cinnamomum cassia</i> extract (250 mg per day) capsule/2 times	<u>Before vs final treatment:</u> ↓FBG (114 to 102 mg/dL, p<0.05) ↑Plasma thiol groups (4.89 to 5.56 μMol/g prot, p<0.05) ↑FRAP (812 to 918 μMol/L, p<0.05) □o significant effect in fasting insulin level

GER – gastric emptying rate; GLP-1 – glucagon-like peptide-1; LPO – lipid peroxidation level; TBARS – 2-thiobarbituric acid reactive substance; FRAP –ferric reducing antioxidant power; AUC – are under the curve; FBG – fasting blood glucose; HbA1c – Haemoglobin A1C; TC – total cholesterol; LDL – low density lipoprotein, HDL – high density lipoprotein; TG – triglycerides. ↑ – increase ; ↓ – decrease.

Effects of cinnamon on lipid profile

From the available *in vitro* and *in vivo* animal models, evidence has shown that cinnamon and its bioactive compounds have cholesterol-lowering properties. However, the evidence on clinical trials is controversial regarding the effect of cinnamon on lipid profile. A systematic-review with meta-analysis revealed that cinnamon can improve lipid profile in T2DM (Allen et al., 2013), while another meta-analysis shows that cinnamon does not appear to improve lipid parameters in T1DM and T2DM (W. Baker et al., 2008).

In vitro and in animal study

Data from *in vitro* and *in vivo* animal studies demonstrated that cinnamon has possible benefit properties in lipid profile (see tables 1.2 and 1.3 for T2DM and T1DM, respectively). In T2DM rats, aqueous cinnamon extract (200 mg/Kg) administration for 6 weeks significantly decreased triglycerides (TG) and increased high-density lipoprotein (HDL) (Chen et al., 2012; S. H. Kim et al., 2006a). In addition, Ping et al. showed that cinnamon oil decreased total cholesterol (TC) after 35 days of a experimental protocol, compared to control group (Ping et al. 2010). One of the bioactive compounds of cinnamon, namely cinnamaldehyde, seems also to exert a beneficial effect in T2DM. In a dose of 20 mg/Kg this compound decreased TC level, TG level, and increased HDL level (Babu et al., 2007).

The mechanism(s) of action by which cinnamon and its compounds can regulate lipid profile metabolism is not clearly understood in the literature (P Ranasinghe et al., 2012). Figure 1.5 proposes and summarizes the possible mechanism(s) of action of cinnamon and its isolated compounds on lipid metabolism in the body. However, Qin and co-workers have identified some pathways involved in lipid metabolism employing small intestine enterocytes to study the effect of cinnamon and its isolated compounds (Bolin Qin et al., 2012). They showed that cinnamon can inactivate the Niemann-Pick c1-like 1 and Cd36 mRNA receptors on the enterocytes leading to a decrease in the absorption of free cholesterol (FC) and free fatty acid (FA), respectively from the gut to the cell. Furthermore, cinnamon leads to a down-regulation of chylomicron synthesis by decreasing MTTP levels and Apo B48 secretion from the enterocytes, which are **responsibly** for intestinal lipoprotein assembly. Cinnamon and its isolated compounds can also regulate cholesterol homeostasis by inducing ABCA1 expression, which in turn is responsible for

promoting cholesterol efflux from enterocytes. Cinnamon and its compounds can also decrease ABCG5 expression, which promotes cholesterol efflux from enterocytes into the gut lumen. Finally, cinnamon can down-regulate lipogenesis by decreasing Srebp 1c expression (Bolin Qin et al., 2012). Together, all these multiple effects of cinnamon and its isolated compounds help in reducing the free fatty acids and cholesterol (lipid profile) induced by DM (see figure 1.5).

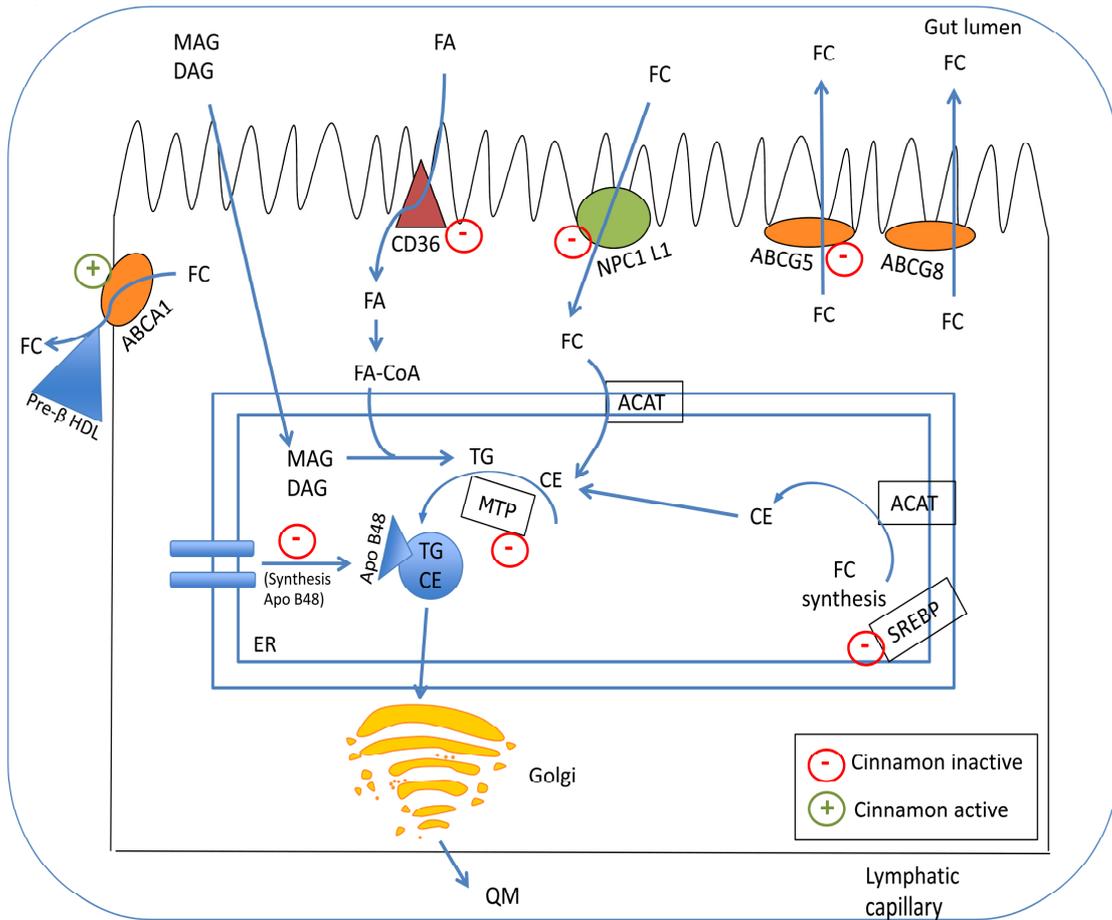


Figure 1.5: Schematic model describing tentative mechanism(s) of action of cinnamon and its isolated bioactive compounds on lipid metabolism in enterocyte cell. MAG–monoacylglyceride; DAG, diacylglyceride; FA, fatty acid; FC, free cholesterol; NPC1 L1, Niemann-Pick C1-Like 1; ABCG, ATP-binding cassette sub-family G; ACAT, acetyl-CoA acetyltransferase; TG – triglycerides; CE, cholesterol ester; MTP, microsomal transfer protein; SREBP, sterol regulatory element binding protein; ER – endoplasmatic reticulum.

Clinical trials in human

Regarding to the effect of cinnamon on lipid profile in human model, a total of 9 clinical trials were analysed (see tables 1.4 and 1.5). Most of these studies (6 in all) revealed that cinnamon had no beneficial effect on healthy and T2DM subjects.

In studies employing particularly T2DM subjects, it was demonstrated that the administration of either 1, 3 or 6 g of *C. cassia* for 40 days can lower TG, and TC levels (Khan et al., 2003). Another study also suggests that the extract of *C. aromaticum* (120 mg) by capsule can significantly decrease TG levels at the end of 3 months compared to the beginning of the experimental protocol. Thus, published data on human studies have shown some discrepancies, since the data from animal studies demonstrated marked beneficial effects on lipid profile. More studies should be performed, especially, using a well-controlled clinical trial with a control group for comparison.

1.6.3 Cinnamon as a potential antioxidant

As described in section 1.3 above, glucose in excess can cause toxic effects on pancreatic islet. Different biochemical pathways and molecular mechanisms of action for glucotoxicity have been proposed (Niedowicz & Daleke, 2005). However, all different pathways share the formation of the reactive oxygen species (ROS), which in high levels can induce defects on insulin production and/or insulin secretion leading to the apoptosis of pancreatic cells (Robertson, 2004). The oxidative stress resulted from generation of ROS has a major role in the development of DM complications. On the other hand, endogenous antioxidant mechanisms can help to defence our body, such as enzymatic system (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) and antioxidant compounds (Niedowicz & Daleke, 2005). Cinnamon extract and its different isolated bioactive compounds have been demonstrated to possess a potential source of natural antioxidants (Dragland et al., 2003) exhibiting strong free radicals scavenger activity in *in vitro* models (Mathew & Abraham, 2006; Su et al., 2007). The high phenolic content of ACE has been associated to its antioxidant properties (Dudonné et al., 2009). According to Peng, the major anti-diabetic compounds of ACE are proanthocyanidins which demonstrate to be effective in the prevention of advanced glycation-end products formation (AGEs) (Peng et al., 2008). Furthermore, cinnamon extract has been shown to have an important role on hepatic lipid peroxidation prevention by increasing the antioxidant enzyme levels of superoxide dismutase and catalase (Moselhy & Ali, 2009).

In hyper-cholesterolemic rats, cinnamon can decrease homocysteine and can also increase antioxidant enzyme levels, including, catalase, peroxidase and superoxide dismutase (Amin & El-twab, 2009). Similarly, cinnamon has been shown to possess potential antioxidants properties, not only in *in vitro* and *in vivo* animal studies, but also in human models (see tables 1.4 and 1.5). In healthy subjects, the consumption of 100 mg of cinnamon (in 300 ml tea) for 10 days seems to decrease oxidative stress by inhibiting lipid peroxidation level (Ranjbar et al., 2006). The cinnamon powder also seems to improve total antioxidant power (Ranjbar et al., 2007). ACE administration (500 mg) by capsule form for 12 weeks decreases oxidative stress in obese human with impaired fasting glycaemia (A.-M. Roussel et al., 2009).

1.6.4 Anti-inflammatory properties of cinnamon

As mentioned earlier (see section 1.3), inflammatory cytokines seem to have a role in the autoimmune pathogenesis of pancreatic beta cells dysfunction. The T1DM occurs from an immune-regulatory alteration where Th1 cells and its cytokines IFN γ , IL-2 and TNF β dominate over immune-regulatory process for Th2 and its cytokine products IL-4 and IL-10 (Rabinovitch & Suarez-pinzon, 1998). The inflammatory process is also implicated at the beginning of diabetic long term complications (King, 2008).

Apart from beneficial effect of cinnamon described previously, this spice and its isolated bioactive compounds seem also to exert a potent anti-inflammatory properties (Koteswara et al., 2007). *In vitro* studies showed that polyphenol of aqueous cinnamon extract (ACE) from *C. burmannii* can increase the anti-inflammatory marker Tristetraprolin (TTP) mRNA and protein levels (Cao et al., 2008a; Bolin Qin et al., 2012), which can interfere with TNF- α production by destabilizing its mRNA (Cao et al., 2006; Ray et al., 2013). Cinnamaldehyde, another bioactive component of cinnamon, has been demonstrated to induce a potential anti-inflammatory activity by suppressing transcription factor, NF-kB activation via three signal transduction pathways, p38 MAPK, JNK and ERK (Hong et al., 2012; D. H. Kim et al., 2007). This has a key role in the expression of pro-inflammatory cell adhesion molecules (Liao et al., 2008). Moreover, cinnamaldehyde seems to inhibit toll-like receptors 4 activation, which plays an important role in the induction of inflammatory responses by NF-kB activation (Youn et al., 2008).

The aqueous cinnamon extract administration to mice demonstrated a significant and positive effect in reducing of TNF- α and IL-6 serum levels. However, this occurs in a

dose-dependent manner especially in the lower dosage (20 and 100 mg/kg bw) (Hong et al., 2012). The higher concentrations showed IL-6 levels more elevated than control group, which suggested that the unidentified compounds of ACE might be interfere with anti-inflammatory process (Hong et al., 2012). A previous *in vitro* study has demonstrated that ACE can also decrease IL-1 mRNA levels (Bolin Qin et al., 2012). In humans trials, the addition of 3 g of cinnamon powder in the diet did not change interleukin-6 serum level (Mashhadi et al., 2013). Together, these initial studies have clearly linked a beneficial effect to both cinnamon extract and its isolated compounds in supressing inflammation in the body.

1.6.5 Bioavailability and toxicity of cinnamon bioactive compounds

The culinary spices, especially cinnamon, and its bioactive agents have been the focus of interest due to their antioxidant and anti-inflammatory properties, which are attributed to high polyphenol contents in the cinnamon (I. Baker et al., 2013). In diabetes mellitus, this plant has been of particular interest demonstrating a potential effect on the prevention of the development of this disorder exhibiting hypoglycaemic capacity (Bahadoran et al., 2013).

However, the bioavailability of cinnamon compounds could depend on many factors, namely, the food preparations process, metabolism and administration forms (C. Han & Cui, 2012; Scalbert & Williamson, 2000).

Regarding food preparation processes, recent investigations showed that cinnamon cooked (simmer for 1 hour) contained more total phenolic contents compared to cinnamon uncooked. However, although there was a slightly increase in phenolic compounds, the thermal process did not seem to affect its antioxidant and anti-inflammatory capacity based on percentage of COX-2 inhibition (I. Baker et al., 2013).

Furthermore, these authors revealed that the digested process can also influence cinnamon properties. In *in vitro* model, the cooking following digestion decreased significantly its antioxidant capacity, but increased significantly its anti-inflammatory capacity. Despite these effects, the total phenolic content was not significantly altered by digestion process (I. Baker et al., 2013).

It is interesting to note that a significantly strong positive correlation between the total phenolic content and the antioxidant capacity for uncooked, cooked and digested cinnamon

has been reported. The authors also suggested that the antioxidant capacity of cinnamon may contribute to its anti-inflammatory properties (I. Baker et al., 2013).

Cinnamon can be administrated through different forms and routes, which can also influence its bioavailability. According to Han and co-authors, the oral bioavailability of cinnamon oil can be improved by liquid loadable table administration due its poor water soluble, making more efficiency on DM treatment. The results from animal studies revealed that blood glucose level and HbA1c are significantly decreased with cinnamon oil by liquid tablets, which do not verify with only cinnamon oil alone (C. Han & Cui, 2012). However, the forms of administration not only affect the cinnamon extract bioavailability, but also the bioavailability of its bioactive isolated compounds. Coumarin is a bioactive compound of cinnamon that has been reported to possess pharmacological activity, such as anti-inflammatory, antioxidant, anti-hyperglycaemic, anti-adipogenic, anti-bacterial and anti-cancer properties (Venugopala et al., 2013). This compound belongs to a class of phenolic compounds and it is naturally presented in many plants of a variety of families, including Lauraceae i.e., cinnamon. In addition, coumarin is used from many years, as sweetener, as flavouring, food additive in combination with vanillin and stabilizer substances (Egan et al., 1990). However, human data have indicated that when exposed to high doses of coumarin, this can result in a considerable hepatotoxicity (Abraham et al., 2010). In this context, the European Food Safety Authority (EFSA) established a tolerable daily intake (TDI) value of 0.1 mg/Kg body weigh per day to health assessment of coumarin (EFSA, 2004).

After oral intake, coumarin is rapidly metabolized in human liver and it has been a very low percentage in systemic circulation (Pelkonen et al., 2000). This compound of cinnamon is catalysed by cytochrome P450 2A6 (CYP2A6) to 7-hydroxycoumarin (7OHC), which is also rapidly excreted by urine (Khayyat et al., 2013).

Coumarin is utilized as medicinal products and can be administrated by different forms. In turn, this can affect its bioavailability. The bioavailability of coumarin depends on different factors, such as forms of administration (capsule or contained in cinnamon), dietary applications and different kinetics (Abraham et al., 2011).

Human data reported that oral intake of coumarin from cinnamon tea produced the fastest uptake of 7-hydroxycoumarin (7OHC) into the plasma, approximately 30 min after administration. This is followed by oral intake of coumarin from cinnamon powder containing foods (rice pudding) and coumarin intake from capsule (coumarin-isolated or

cinnamon powder) (Abraham et al., 2011). According to these authors, this result suggests that coumarin dissolved in water is rapidly transported to the small intestine for absorption following oral ingestion.

The relative extent of coumarin absorption (measured as 7OHC excretion within 8h) also demonstrated to have the highest values (66.1%) by cinnamon tea administration. The coumarin showed lower absorption when administrated in capsule as coumarin-isolated (62.8%) or cinnamon (56%) and when administrated cinnamon in rice pudding (54.7%). The coumarin administration with capsule or foods may need more time for the capsule to break down to form molecules, which can be absorbed by the gut. Furthermore, the cinnamon powder might have a component which interferes with coumarin absorption since they do not transfer to aqueous solution (tea) (Abraham et al., 2011).

In addition, 105 min after administration, 7OHC plasma levels demonstrated the lowest plasma values for cinnamon tea and cinnamon powder in rice pudding, which showed that the 7OHC plasma levels from non-capsule administration is more rapidly metabolized, compared to capsule administration. The urinary excretion within the observation period of 8 h of 7OHC metabolite demonstrated to be 80.3% on cinnamon tea, 73.4% on cinnamon in rice pudding, 70.5% on cinnamon capsules and 58.8% on coumarin capsules, showing to have a more excretion of coumarin when administrated by non-capsule administration (Abraham et al., 2011).

It is also important to note that coumarin content also depends on the species of cinnamon. *Cinnamomum cassia* demonstrated a high content of coumarin (2650 to 7017 mg/Kg) in contrast with *Cinnamomum verum* (cinnamon true) samples, which showed very low (trace) coumarin content. However, in the last five years *C. cassia* has replaced cinnamon true (Blahová & Svobodová, 2012). This may be due to the fact that *C. cassia* is less expensive compared to *C. verum*. An analysis of cinnamon containing bakery food products on the European market demonstrated that several cases of coumarin content seemed to exceed the European Union limits (Ballin & Sørensen, 2014).

In Europe, United States and Canada *Cinnamomum cassia* has been used more widely than *Cinnamomum verum* (cinnamon true or Ceylon cinnamon). In United States, cinnamon from *C. burmannii* corresponded at 90% of imported cinnamon (Y.-H. Wang et al., 2013). Because this species has reported to be the mostly used cinnamon by the population, *C. burmannii* was the species employed in this study.

1.7 Working hypothesis

Aqueous cinnamon extract from the species *Cinnamomum burmannii* can exert beneficial and protective effects in animals with type 1 diabetes mellitus and in healthy subjects.

1.7.1 Mains aims

The mains aims of this study are:

1. To identify the major compounds from the aqueous cinnamon extract employing *Cinnamomum burmannii*;
2. To investigate the effects of aqueous cinnamon extract from *Cinnamomum burmannii* administration in streptozotocin (STZ)-induced type 1 diabetic rats compared to normal age matched controls measuring a number of parameters including its hypoglycaemic effects and its effect on the general characteristics of the animals;
3. To investigate the effects of aqueous cinnamon extract from *Cinnamomum burmannii* in post-prandial glycaemia of healthy human.

The specific aims of this study are:

1. To undertake a literature search in the subject area;
2. To determine the major phenolic compounds and antioxidant capacity of aqueous cinnamon extract from *Cinnamomum burmannii*;
3. To render the rats diabetic using streptozotocin (STZ) and to feed age-matched control and diabetic rats with different oral doses of cinnamon daily for 11-12 weeks;
4. To weight the STZ-induced T1DM and healthy rats, test the blood glucose and food consumption in a weekly basis;
5. To analysis the blood for different biochemical parameters including cations content in STZ-induced in T1DM and healthy rats;
6. To measure fibrosis of left heart ventricle STZ-induced T1DM and healthy rats;
7. To determine insulin distribution and insulin secretion in the pancreas in STZ-induced T1DM and normal rats;
8. To measure mean glycaemia values on fasting and after oral glucose tolerance test (OGTT) at 30, 60, 90 and 120 minutes (OGTT) in healthy subjects and compare with the measured glycaemia values after OGTT with aqueous cinnamon extract (OGTT cinnamon);

9. To calculate and compare the area under the curve (AUC), the maximum concentration (C_{\max}) and the variation of maximum concentration (ΔC_{\max}) after oral glucose tolerance test at 30, 60, 90 and 120 minutes in the 2 groups studied;
10. To analyse the data and write up the PhD thesis.

Novelty of this study:

This is the first study of its kind to use *C. burmannii* in animal and human studies. Moreover, this study employed the different groups of animals to compare different doses of cinnamon and moreover, the same tissues from the same animals were isolated and analysed for parameters of fibrosis in the heart, glucose in the blood, cations in the different tissues, distribution of insulin and glucagon in the pancreas and release of these hormones from pancreatic segments following stimulation.

Chapter Two

MATERIALS AND METHODS

2 Materials and Methods

2.1 Materials

2.1.1 *Cinnamomum burmannii* sticks

The *Cinnamomum burmannii* bark was purchased from Sucrame Company (Portugal) with Indonesia origin. It was provided in stick forms in individual packing and stored in a dried environmental locally until needed. The product has a batch number of L113003 (expiration date 10/2013) and provided an analysis certificated (number 003/11).

2.1.2 Chemical analysis

Reagents and solutions:

Ferric Chloride (III) hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$; $\geq 99\%$), folin-ciocalteu (2,2-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid); PA), Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; 97%), TPTZ 2,4,6-tri(2-piridil)-s-triazine, methanol (CH_3OH ; 98.9%) and nicotinamide adenine dinucleotide (NADH; $\geq 97\%$), nitroblue tetrazolium (NBT) 2-Amino-2-hydroxymethyl-propane-1,3-diol (tris) and Phenazine methosulfate (PMS; $\geq 90\%$) were the reagents used in the chemical analysis. All the reagents were P.A. grade and purchased in Sigma-Aldrich (Portugal), gallic acid-1-hydrate ($\text{C}_6\text{H}_2(\text{OH})_3\text{COOH} \cdot \text{H}_2\text{O}$; 99.5%) was purchased in Acros Organics (Portugal) and sodium carbonate (Na_2CO_3 ; 99.9%) was purchased in ICS Science group (France).

The following solutions were prepared: chloridric acid 40 mM (HCl 37%), phosphate buffer pH=7 (NaH_2PO_4 , 99%; Na_2HPO_4) purchased to Scharlau (Spain) acetate buffer 300 mM pH=3,6 ($\text{NaCH}_3\text{COO} \cdot 3\text{H}_2\text{O}$ and CH_3COOH ; 100%) purchased to AnalaR Normapur (Portugal).

For identification and quantification of each phenolic compound (HPLC method), the following reagents and materials were used: acetonitrile acid (HCOOH ; 99.9%) (Acetonitrile: For HPLC-Gradient, Carlo Erba Reagents Group, Portugal; HCOOH : Ameisensaure 98-100% zur Analyse, Merck, Portugal); acid water (HCOOH) (H_2O For HPLC, CARLO ERBA REAGENTS, Portugal); Cinnamaldehyde natural ($\geq 93\%$, Kosher

Sigma-Aldrich, Portugal); Trans-cinnamic acid ($\geq 99\%$, Aldrich, Portugal); Cinnamyl alcohol ($\geq 98\%$, Aldrich, Portugal); Coumarin ($\geq 99\%$, HPLC, Sigma, Portugal).

Equipment: High-performance liquid chromatography (HPLC) (Finnigan Survey with DAD), reverse phase C₁₈ column (250/4.6 Nucleosil 300-5 C18 of Macherey-Nagel), spectrophotometer (Perkin Elmer, Lambda 25) and analytical balance (Sartorius, $\pm 0,0001\text{g}$).

Animals: Healthy Wistar Rats

2.1.3 Animal studies

Reagents and solutions:

The following solution was prepared for diabetes induction: Concentrations of 0.1 M sodium citrate (220 ml) ($\geq 99\%$, Merck, Portugal) and 0.1 M citric acid (280 ml) (99.5%, Merck, Portugal) were prepared in distilled water (500 ml). The pH was adjusted to 4.5.

Phosphate buffer saline solution (pH 7.4) was prepared with 800 ml of distilled water, 8 g of sodium chloride (99.5%, José Manuel Gomes Santos, Portugal), 0.2 g of potassium chloride (99.6%, Merck, Portugal), 1.44 g of sodium dihydrogen phosphate (99%, Scharlau, Spain) and 0.24 g of potassium dihydrogen phosphate (99.9%, Merck, Portugal).

A solution was also prepared for oral glucose tolerance test (OGTT). An amount of 75 g of glucose (Dextrose) monohydrated (Cmd Chemicals, Portugal) was dissolved in 200 ml of distilled water.

Nitric acid and deionised water were utilized to dissolved different tissues in order to determine the measurements of cations.

The following reagents namely: 10% buffered paraformaldehyde (VWR Prolabo Chemicals, Portugal), chromotrope aniline blue (CAB), ethanol (70°, 96° and 100°) (AGA, Portugal), xylene (98.5%), (Carlo Erba Reagents Group, Portugal), paraffin (ThermoScientific, UK), Weigert's hematoxylin (Leica, UK), phosphomolybdic acid (2%) (Merck, Germany) and Entellan (Klinipath, Belgium) were used for morphological study.

Drugs: Streptozotocin (STZ) (reference S0130, Sigma-Aldrich, Spain) was utilized for diabetes induction. Similarly, Domitor injected solution and Imalgène 1000 injected solution were utilized for analgesia and anaesthesia, respectively.

Animals: Male Wistar rats (Harlan Laboratories, Nederland) were purchased for this study. A certificate of animal health was providing from Laboratory in accordance with EEC

Council Directive 92/65 (94/AM.1). Microbiological analyses certificated were provided by Harlan Laboratories (Nº de Albarán 1041190 SO).

Equipment: Glucometer (Precision Xceed, Abbott Diabetes Care, UK) and lancet (FreeStyle Precision Xceed, Abbott Diabetes Care, UK) were used to measure blood glucose levels and a technical balance was utilized to measure food intake and body weight. A nasogastric probe was used to administrate aqueous cinnamon extract to the animals.

For biochemical analysis, the study used RANDOX *RX Daytona* analyser and different commercial assay kit appropriated to each parameter measured. These included triglycerides (No. TR 3823), total cholesterol (No. CH 3810), high-density lipoprotein (No. CH 3811), low-density lipoprotein (No. CH 3841), albumin (No. AB 3800), hemoglobin A1c (No. HA 3830), creatinine (No. CH 3814) and total antioxidant status (No. NX 2332). Cation levels in serum and in organs tissue were measured by inductively coupled mass spectrometry (ICP-MS) (Thermo Electronic) equipment. Other equipment used for morphological study included microtome, optical Leica microscopy, electronic microscopy and incubator.

2.1.4 Human studies

Reagents and solutions:

A solution was prepared for OGTT and it included 75 g of glucose (Dextrose) monohydrated (Cmd Chemicals, Portugal) dissolved in 200 ml of water.

Equipments: Glucometer and lancet (FreeStyle Precision Xceed, Abbott Diabetes Care, UK) were used for the measurement of blood glucose levels. A bioimpedance balance (*Tanita*, BC-601) was utilized to measure body composition and stadiometer (*Jofre*®) was employed to measure the height of each subject.

2.2 Methods

2.2.1 Preparation of aqueous cinnamon extract (ACE)

Three different aqueous cinnamon extracts were prepared for use in this study. Two were used for (i) the quantification of phenol content and antioxidant capacity and (ii) identification of phenolic compounds. The third was used in animal studies.

a) Chemical analysis:

Quantification of total phenols content and for antioxidant capacity characterization:

Aqueous cinnamon extract (ACE) was obtained from 60 g of sticks cinnamon dissolved in 1000 ml of Millipore (18,5 Ω) distilled water. Briefly, cinnamon sticks were weighted using an analytical balance and soaked in distilled water. After 24h in room temperature, the content (ACE) was boiled for 30 minutes at 100°C and allowed to cool to room temperature. Thereafter, the aqueous cinnamon extract was filtered using *Whatman* filter paper. This method was adapted by Shen and co-authors (Shen et al., 2010). A hydro-methanolic extract (50:50) was performed with ACE obtained previously.

Identification of each phenolic compound using HPLC method:

ACE was obtained by adapting the method from Shen et al. (Shen et al., 2010). Briefly, 100 g of cinnamon sticks was soaked in 1000 ml of distilled water Millipore (18,5 Ω) for 24 hours. The solution was stirred using a magnetic stirrer at room temperature. The aqueous cinnamon extract obtained was then heated for 30 minutes at 100°C. The sample was cooled, filtered using *Whatman* filter paper and stored at -80°C for 150 minutes. After 2.5 hours, the samples were lyophilized and placed again in the freezer at -80°C until it was ready to analyse for phenolic compounds.

b) Animal studies:

For animal studies, an amount of 875 g of the sticks cinnamon was soaked into 2,500 ml of Millipore (18,5 Ω) distilled water at room temperature. After 24 h the solution was heated for 30 min at 100°C. The aqueous cinnamon extract was filtrated using *Whatman* filter paper and then stored into separated vials at -20°C until needed. This method was adapted

from Shen and co-authors (Shen et al., 2010). The aqueous cinnamon extract (ACE) was daily removed from freezer and defrosted at room temperature before administration to normal and diabetic rats.

c) Human studies:

Regarding human studies, aqueous cinnamon extract (tea) was obtained by the same process of chemical characterization. An amount 60 g of sticks cinnamon was dissolved in 1000 ml of water. Briefly, cinnamon sticks were weighted using an analytical balance (*Sartorius*, $\pm 0,0001\text{g}$) and soaked into Millipore (18,5 Ω) distilled water. After 24 h in room temperature, the cinnamon tea was boiled at 100°C and rest until achieve the room temperature. The cinnamon tea was filtered and distributed as individual doses (100 ml) to each participant.

A. ANTIOXIDANT CAPACITY OF AQUEOUS C. BURMANNII EXTRACT

The characterization of antioxidant capacity, including quantification of total phenols and antioxidant assays (FRAP - Ferric Reducing/Antioxidant Power and superoxide anion inhibition test), was done in this study in Biochemistry Laboratory at Egas Moniz University (Portugal).

2.2.2 Quantification of total phenols content

The total phenolic concentration of aqueous cinnamon extract was determined using the Folin Ciocalteu method (Prabha & Vasantha, 2011) employing gallic acid as standard. The results were expressed as mg for gallic acid equivalent (GAE)/g of extract. For this test, a volume of 375 μl of aqueous sample solution and 4 ml of sodium carbonate were added to 5 ml of Folin Ciocalteu reagent. After 15 min, the absorbance was measured at 765 nm.

2.2.3 Simultaneous identification and quantification of phenolic compounds

Sample preparation: The ACE previously obtained (section 2.2.1) was lyophilized for high-performance liquid chromatography (HPLC) method. An amount of 0.34 g of

lyophilized aqueous cinnamon extract was dissolved in 25 ml of methanol. A volume of 25 μ L of the sample was injected to the HPLC (1:50) in methanol.

For the simultaneous identification and quantification of each major phenolic compound of aqueous cinnamon extract, a HPLC technique was employed using an established method described previously by He et al (He et al., 2005). The sample was separated using a reverse phase C₁₈ column. The elution order obtained for standard compounds was obtained carrying out an isocratic method at a flow rate of 1.7 ml/min, using as eluent a solution of 25% of acetonitrile with 1% of formic acid and 75% of water with 1% of formic acid. The temperature employed for column corresponded to room temperature. The identification of cinnamaldehyde, cinnamic acid and coumarin was done using 280 nm wavelengths and for cinnamyl alcohol a wavelengths of 252 nm was employed.

Calibration curve preparation: An amount of either cinnamaldehyde, coumarin, trans-cinnamic acid or cinnamyl alcohol was weighted and dissolved in methanol. Thereafter, appropriate dilutions were made up in methanol to obtain standards for the calibration curve for each compound (see below). The number of moles of standard solutions for calibration curve was the following:

Trans-cinnamic acid (3.47×10^{-9} mol; 2.31×10^{-9} mol; 1.16×10^{-9} mol)

Cinnamyl alcohol (1.54×10^{-9} mol; 1.02×10^{-9} mol; 5.12×10^{-10} mol)

Coumarin (1.67×10^{-9} mol; 1.25×10^{-9} mol; 4.17×10^{-10} mol)

Cinnamaldehyde (1.99×10^{-9} mol; 9.93×10^{-10} mol; 4.97×10^{-10} mol; 2.48×10^{-10} mol)

The identification of each compound was obtained by comparison of retention time and absorption spectra of standard compounds with sample. The quantification of the compounds was determined by calibration curve and by peak area values of each compounds identified.

2.2.4 Characterization of antioxidant capacity of ACE

Characterization of the antioxidant capacity was done using FRAP (Ferric Reducing/Antioxidant Power) and superoxide anion ($O_2^{\cdot-}$) assays.

FRAP assay:

The method for determination of ferric reducing effect was based on the reduction, at low pH, employing a colourless ferric complex (Fe^{3+}) to a blue-coloured ferrous complex (Fe^{2+}) by electron-donating antioxidants action in 2,4,6-tri (2-piridil)-s-triazina (TPTZ) presence (Thaipong et al., 2006). The fresh working solution was prepared by mixing 25 ml of acetate buffer (300 mM, pH=3.6), into 2.5 ml of TPTZ solution (10 mM) to HCl (40 mM) and into 2.5 ml of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (20 mM). The solution was heated at 37°C. Samples (150 μl) were introduced in tubes with 2850 μl of the FRAP solution. This solution was maintained in the dark condition for 30 min. The absorbance was measured at 593 nm. *Trolox* (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as standard and the results expressed in $\mu\text{mol Trolox/L}$.

$\text{O}_2^{\cdot-}$ inhibition test:

The $\text{O}_2^{\cdot-}$ anion was generated by reacting metassulfato phenazine (PMS) and nicotinamide adenine dinucleotide hydride (NADH) and oxygen causing reduced of NBT in Formazan. The test applied was based on previously method describes (Alam et al., 2013; Morais et al., 2009). Into a glass tube with cap, a volume of 0.5 ml of sample was added to 2 ml of a solution containing NADH (189 μM) and NBT (120 μM) with Tris-HCl (40 mM, pH = 8). The reaction started after the addition of 0.5 ml of PMS (60 mM). Initially, a control sample was measured using only distilled water. After 5 min of incubation the absorbance of the control was measured at 560 nm at room temperature. Thereafter, the ACE sample absorbance was measured.

The percentage inhibition of anion $\text{O}_2^{\cdot-}$ was calculated using the following equation:

$$\%I = \frac{Abs (control) - Abs (corrected sample)}{Abs (control)} \times 100$$

B. ANIMAL STUDIES

Forty young male Wistar rats, 8-weeks old and weighing between 125-149 g were purchased from Harlan. The rats were kept for 2 weeks and maintained with 12 h:12 h

light/dark cycle at temperature (22-24°C) in humidity controlled rooms (50%). Rats were housed 3 per cage and given *ad libitum* access to food (Mucedole, 4RF21) and water.

This work had the relevant Ethical Clearance for all the procedures employed in accordance with European legislation on the use and care of laboratory animals. It was also carried out according to guidelines for Animal Experiments of Veterinary Medicine Faculty, Directorate-General of Veterinary of Portugal and the University of Central Lancashire in United Kingdom.

2.2.5 Experimental design

Forty male Wistar rats were purchased, however only 33 rats were used in this experimental protocol. One rat was dead during the protocol (unknown cause) and 6 rats were not diabetic, following injection of streptozotocin. For this reason, the results of these rats rendered were excluded for the study. Thus, animals were divided into 6 different groups, according with the following diagram as shown in figure 2.1.

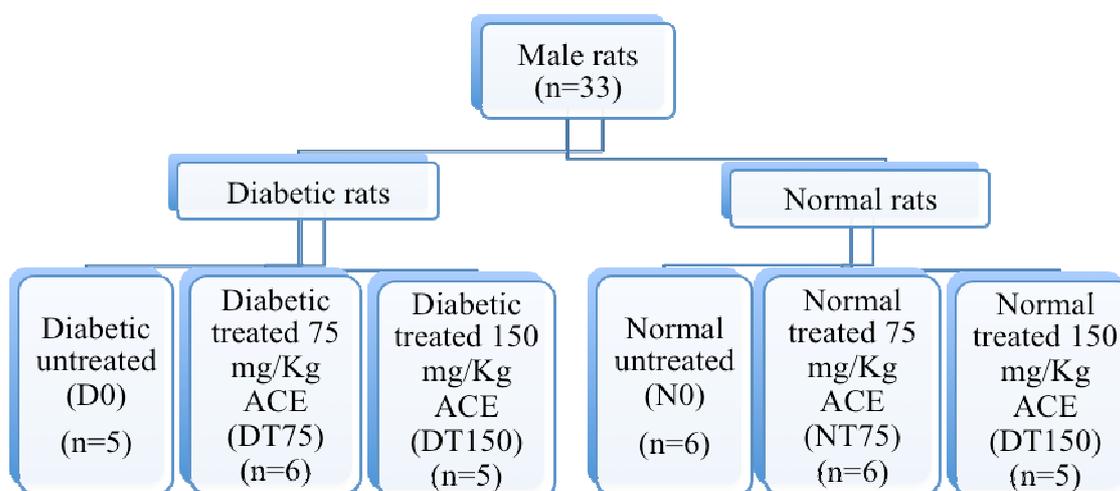


Figure 2.1: Diagram showing the experimental design for animal study.

The rats were administrated with ACE by oral gavage at 4.00 pm daily (1-1.8 ml) for 11 weeks. The rats received 75 mg/kg of ACE (DT75 and NT75 groups), 150 mg/kg of ACE (DT150 and NT150 groups) or vehicle (distilled water) (D0 and N0 groups). Either ACE or distilled water was orally administrated after 7 hours fasted, immediately before food intake, for all the animals. These animal experiments were employed in animal house of Faculty of Veterinary Medicine (Portugal).

2.2.6 Induction of type 1 diabetes mellitus

Following 2 weeks of adaptation in the animal house, diabetes was induced following overnight fasting of the rats (n=22) using streptozotocin (STZ). This process was done by a single intra-peritoneally (IP) injection (0.3 ml) containing 60 mg/Kg of body weight (E Adeghate, 1999). The STZ was weighted and freshly dissolved in 0.1 M citrate buffer solution (pH 4.5) (Bolkent et al., 2000). The normal control rats (n=18) were injected with same volume (0.3 ml) of citrate buffer solution. After 4, 7 and 14 days following STZ injection, a drop of blood from the tail end of each rat was taken to determine fasting blood glucose level using a glucometer. Diagnosis of diabetes was confirmed either 3, 7 or after 14 days of STZ injection. Rats with blood glucose level with more or equal to 220 mg/dl were diagnosed as diabetic (Shen et al., 2010). Blood glucose level was measured weekly for the remaining 11 weeks.

2.2.7 Measurement of food consumption

The food consumption was determined for all normal and diabetic animals weighting of the food weight at the beginning and the end of day. Then, the food consumption (g) was estimated for each rat according to number of animal per cage. For each experimental group, food consumption was calculated as mean \pm standard error of the mean (SEM) every week.

2.2.8 Measurement of body weight

The weights (g) of all normal and diabetic (treated and untreated) animals were measured weekly using a technical balance. For each experimental group, the mean of body weight \pm SEM was calculated every week.

2.2.9 Measurement of blood glucose levels

After fasting for 7 hours, blood glucose was weekly monitored using tail vein blood with a Glucometer, for each rat. For each experimental group blood glucose level (mg/dl) was calculated as mean \pm SEM. This was done every Wednesday. Fasting started at around 9.00 am and blood samples were taken at around 4.00 pm. Thereafter, the animals were given the cinnamon extract or vehicle followed.

2.2.10 Oral glucose tolerance test (OGTT)

Ten weeks (STZ-injection) after the experimental procedures, all untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals were subjected to an oral glucose tolerance test. Each rat was given 2 g/Kg of body weight of oral glucose solution (Jia et al., 2009) by gavage method. Blood was collected from the tail vein after overnight fasting for 12 h. The blood glucose was measurement at 0 (before glucose solution administration), 30, 60, 120 and 180 min after administration of the oral glucose solution (Jia et al., 2009) and data expressed as mean (mg/dl) \pm SEM.

2.2.11 Blood collection and biochemical analysis

After 11 weeks following STZ-injection, untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals were killed humanely under anaesthesia (Imalgène 1000) and analgesia (Dormitor) using 0.75 ml/Kg and 0.5 ml/Kg doses, respectively. Immediately, during anaesthesia blood samples were taken from the heart with a syringe. Blood was centrifuged at 5000 RPM for 5 min and serum was collected for biochemical analysis. Four days after collection, the whole blood was haemolysed for haemoglobin A1c measurement. All blood samples were stored at -80°C until analysed. Serum total cholesterol, serum high-density lipoprotein (HDL), serum low-density lipoprotein (LDL), serum triglycerides (TG), serum creatinine, serum albumin and haemoglobin A1c values were obtained using RANDOX RX Daytona analyser. All biochemical analysis of rats serum were performed in Biochemical Laboratory at Egas Moniz University (Portugal).

2.2.12 Serum total antioxidant status measurement

Serum total antioxidant status (TAS) of untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals was measured by RANDOX RX Daytona analyser with the commercially available Randox Total Antioxidant Status test kit (RANDOX-NX 2332). This method measured ABTS+ (2,2'-

Azino-di-[3-ethylbenzthiazoline sulphonate]) radical formation by spectrophotometry method (600 nm). All values were expressed as mM of Trolox/L. This analysis was performed in Biochemical Laboratory at Egas Moniz University (Portugal).

2.2.13 Tissues collection and processing

After killing the rats humanly, a mid-line abdominal incision was made to all the rats in each group and heart, pancreas, liver, kidney and soleus muscle were rapidly removed from each animal. Each organ or tissue of untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals was either used immediately, stored at -80°C or stored in appropriate solution for further use.

Fragments/sections of different parts of pancreas were collected for the measurement of tissue cation levels, microscopic study and estimation of *in vitro* pancreatic insulin release. Three transversal fragments were taken from heart. These included an apex fragment of the ventricle, a fragment in the middle of the ventricle heart and the auricles fragments, for histochemical study and cation measurements. A coronal fragment of kidney and a transversal fragment of liver were obtained to measure the cations. The tissue samples were stored at -80°C for cation analysis and in paraformaldehyde for optical microscope study of the heart.

2.2.14 Cation measurement in tissues and serum

Different cations were measured in this study of each organ tissue and in serum of untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals:

Tissues: The stored tissue samples at -80°C were thawed out at room temperature and a piece (100-200 mg) of each organ tissue (pancreas, heart, liver and kidney) was weight. Each tissue was placed in a glass vial and 1 ml of concentrated nitric acid was added. The samples were left overnight to dissolve and thereafter the tissue and sample were subsequently vortex mixed. A volume of 0.2 ml of the dissolved tissue acidic solution was placed in tubes containing 9.8 ml of deionised water and vortex mixed (Changrani et al.,

2006). This solution was used to measure the levels of different cations, including sodium (Na^+), magnesium (Mg^{2+}), potassium (K^+), calcium (Ca^{2+}), copper (Cu^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}), using Inductively Coupled Mass Spectrometry (ICP-MS) (Thermo Electronic Corporation). The results were expressed as $\mu\text{M}/100$ mg tissues.

Serum: As mentioned earlier, the serum stored at -80°C was thawed out at room temperature and centrifuge at 5000 RPM for 5 minutes. A volume of 0.2 ml of serum was taken of each sample and placed in tubes containing 9.8 ml of deionised water. The solutions obtained were vortex mixed and used to measure the levels of different cations namely, Na^+ , Mg^{2+} , K^+ , Ca^{2+} , Cu^{2+} , Fe^{2+} , Zn^{2+} , Se^{2+} and Mn^{2+} , using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Thermo Electronic Corporation). Results were calculated to express as mg/ml (100g tissue) value.

The cation measurements in tissues and serum was analysed in Chemistry Laboratory at University of Central Lancashire (Preston, UK).

2.2.15 Determination of *in vitro* pancreatic insulin release

For each rat of untreated normal ($n=6$) and normal treated with either 75 mg/Kg ($n=6$) or 150 mg/Kg ($n=5$) animals and also untreated diabetic ($n=5$) and diabetic treated either with 75 mg/Kg ($n=6$) or 150 mg/Kg ($n=5$) different sections of pancreas was removed and placed in phosphate buffer solution (PBS). The connective tissues and adherent fat were removed from the pancreas and the pancreatic segments cut into small fragments (1-2 mm^2). The pancreas fragments of each rat were placed into tubes containing 1 ml of oxygenated at 37°C PBS. All tubes were incubated uncap in a shaking water bath at 37° for 30 min in order to wash away any enzymes and hormones due to cutting of the tissues. Thereafter, the fragments from each rat pancreas (treated with either 75 mg/Kg or 150 mg/Kg bw cinnamon or untreated) were placed in oxygenated PBS solution containing the PBS solution alone (control) or PBS solution containing either glucose (16 mM) or different concentrations (10^{-5} or 10^{-6} M) of acetylcholine. The tissue was incubated in the oxygenated PBS solution in a shaking water bath at 37°C for 1 h. After incubation, the tissue was removed from the solution and was blotted dry with filter paper and weight. The tubes with PBS solution were stored for further analysis of insulin release (E. Adeghate & Ponery, 2002).

At the end of the experiment protocol, insulin secretion was estimated for each tissue by ELISA assay (Mercodia AB, Sylveniusgatan 8A, Sweden). All values for insulin secretion

were expressed as $\mu\text{g/L}/100 \text{ mg}$ tissue. These experiments were performed in *Joaquim Chaves* Laboratory (Portugal).

2.2.16 Measurement of fibrosis in left heart ventricle

The left ventricle of the heart was transversely sectioned and fixed in 10% buffered paraformaldehyde for histological observation. The fibrosis analysed in left ventricle heart was made by chromotrope aniline blue (CAB) staining for collagen (Gürtl et al., 2009). The tissue was dehydrated in a progressive graded ethanol series (96°, 70° and 100°) with 1 hour in each one. The sections of the heart were cleared with xylene for 1 hour, 2 times and after embedded in paraffin. In the next day, the embedded fragments in paraffin blocks were cut on a microtome into sections with 3.5 μm . These sections were put in glass slides in incubator at 66.4°C for 3 hours to remove the paraffin excess. This method was adapted from Antunes et al. (Antunes, Oliveira, et al., 2013). After 1 week at room temperature, tissues were stained for collagen with chromotrope aniline blue (CAB) technic according to standard methods (Zollinger, 1983). The slides were deparaffinized with xylene and rehydrated in a progressive decreasing graded ethanol series (100°, 95° and 70°) and in distilled water. Then, slides were stained with Weigert's Hematoxylin for 5 minutes and 1% phosphomolybdic acid. After washed with water, chromotrop-aniline blue (CAB) was applied for 6 minutes. Sections were dehydrated in ascending concentrations of ethanol (95° and 100°) and cleared with xylene. Sections were covered with Entellan, according with Hadi et al. (Hadi et al., 2011).

The histological images were obtained with an optical Leica microscope using 10x magnification for untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals. A total of 90 optical fields were selects from all anatomical components. A total of three observations were made under blinded assessment and a semi-quantitative analysis by *Image J Software* programme.

The fibrosis measurement in heart left ventricle was performed in Morphology Laboratory at Egas Moniz University (Portugal).

2.2.17 Distribution of pancreatic beta and alpha cell by Immunofluorescence

Pancreas tissues from the 33 rats (treated and untreated) were dehydrated in a progressive graded ethanol series (96°, 70° and 100°) with 1 hour in each one. Sections from each pancreas were cleared with xylene for 1 hour, 2 times and after embedded in paraffin. On the next day, the embedded fragments in paraffin blocks were cut on a microtome into sections with 3,5 µm. These sections were placed in electrostatic slides in incubator at 66,4°C for 3 hours to remove the paraffin excess. This method was adapted from Antunes et al. (Antunes, Borrecho, et al., 2013). Isolated pancreatic tissues were retrieved, fixed and embedded in paraffin. Sections of about 3.5 µm thickness were deparaffinised in xylene, hydrated in descending concentration of ethanol for 3 min each and washed 3 times in PBS solutions for 5 minutes each. The tissue was marked with a Dako pen to prevent solutions draining away from the tissue section. Tissues were staining, incubating the sections with blocking reagent for 30 min. The blocking reagent was then drained off and appropriate dilution of primary antibodies were applied and incubated at 4°C for 24 h. Specific antibodies for insulin (Guinea pig, 1:1000 dilution, from DakoCytomation, CA) and glucagon (Rabbit, 1:1000 dilution, from DakoCytomation, CA) were used. The slides were then washed 3 times in PBS for 5 min each and incubated with secondary antibodies conjugated with FITC or TRITC (Jackson Laboratory, USA) for 1 h and washed in PBS 3 times for 5 min each. Sections were then mounted in CITI-Floure mounting media and viewed and photographed under Ziess Axiophot Fluorescence Microscope, Germany. The insulin (n=33) and glucagon (n=33) positive cells were estimated semi-quantitatively in pancreas tissues of untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals using *Image J Software* programme. The method for this experimental protocol was adapted from the procedure described earlier by Adeghate et al. and Lotfy et al. (Ernest Adeghate et al., 2010; Lotfy et al., 2014). All the slides with tissues were posted to the Department of Human Anatomy the College of Medicine and Health Sciences, United Arabs Emirates. University in Al-Ain, United Arabs Emirates where each slide was analysed by electron microscopy and photographic images taken by Professor Ernest Adeghate and Professor Jaipaul Singh, my Director of Studies (note that I was unable to go to the UAE because of funding constraint).

C. HUMAN STUDIES

2.2.18 Ethical considerations of human study

The clinical trial was approved by Instituto Superior de Ciências da Saúde Egas Moniz Ethical Clearance and University of Central Lancashire (UCLan). All the data were collected after informed consent and protected confidentiality was guaranteed on the data processing.

2.2.19 Subjects

The human subjects for the present study were recruited from a Solidary Association in Guarda, Portugal. Recruited subjects were non-diabetic adults (fasting blood glucose level < 100 mg/dL), more than 18 years old and both genders (8 males, 22 females). Subjects on medication for glycaemia control or for any other gastrointestinal symptoms or diseases were excluded from the study. It was also necessary to exclude all participants who were prescribed with medication during the course of the study or altered medications during the interventions or pregnant mothers and those who breast-fed their child. Furthermore, it was relevant to ask the participants not ingested any cinnamon at the day before the intervention.

2.2.20 Experimental design of human study

After the subjects recruited for this study (n=30), informed consent was applied and the history taking of each subject was made by the investigator. The data collection starting with anthropometric data collection, namely, weight, high, body mass index (BMI), fat mass percentage and muscular mass percentage. Two groups were randomly constituted and they included control group (both males and females) and experimental group (both males and females) (see also figure 2.2 for experimental design).

A **control group**: After 12 h fasting, the experimental protocol consisted of the ingestion of the OGTT solution and measured of capillary blood glucose level after 30 (t_{30}), 60 (t_{60}), 90 (t_{90}) and 120 (t_{120}) min. The **experimental group**: ingested OGTT solution following the 100 ml of cinnamon tea solution.

All the subjects answered to the 24-hour dietary recall. Figure 2.2 showed a study flow chart of the experimental design protocol.

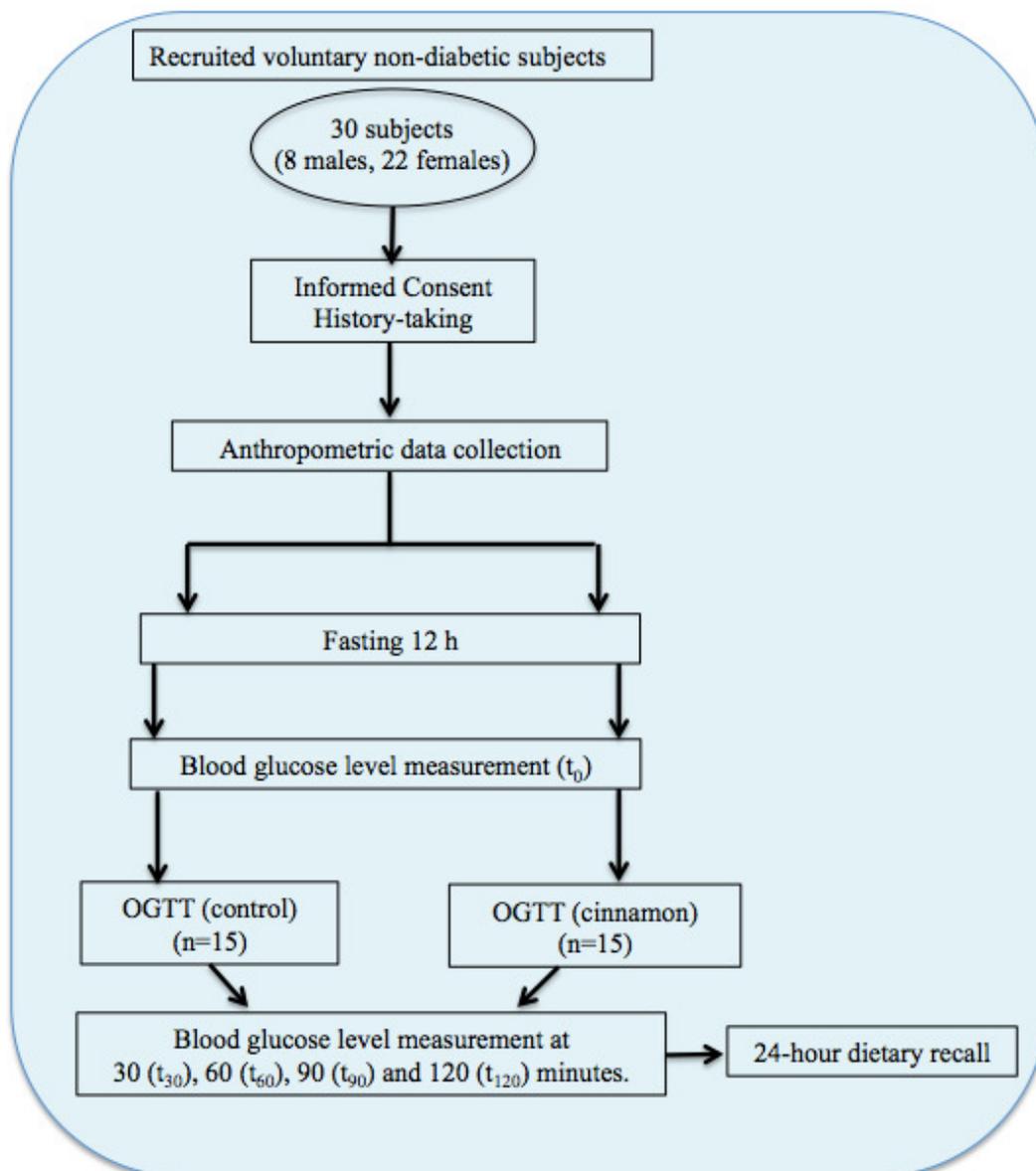


Figure 2.2: A study flow chart showing the experimental design protocol for human studies during oral administration of *C. burmannii*.

2.2.21 Preparation of Oral Glucose Tolerance Test employed in human

The glucose (dextrose) was weighted (75 g) using an analytical balance and dissolved in 200 ml of water. This solution was prepared according with American Dietetic Association (ADA, 2010a). Before the experimental protocol, the procedure was tested and standardized in order to realize identical protocol to each participant.

2.2.22 Data collection

History-taking:

The history taking was made for each participant for sample characterization. Thus, personal codification (age, gender, code), anthropometric parameters (weight, height, body mass index, fat mass, muscular mass) and clinical data regarding pathologies and medications were registered.

Assessment of dietary intake:

The daily intake was usually based on recording the dietary intake in a day by recalling the food intake in the previous 24 hours (Y. Yu & Song, 2015). These data provided a register of all foods ingested during 24 hours before the day of the intervention. The quantification of each food was determined by home measures using a spoon. The *Food Processor SQL* (version 10.5.0) programme was used to analyse the nutritional composition of the meal ingested during the day, namely total caloric value (TCV), carbohydrates (g), protein (g) and lipid (g).

Anthropometric data:

Regarding the anthropometrics data, all participants were weighted and height, body mass index (BMI), fat mass percentage and muscular mass percentage calculated. The weight was measured in fasting condition, without shoes, socks and coat using a bioimpedance balance *Tanita* (BC-601). The height was measured using a stadiometer *Jofre*, in anthropometric position – together feet, arms pending near the body, knees straight, palm of the hand against the body and head respecting the *Frankfurt* plane.

For fat mass (%) and muscular mass (%) a bioimpedance balance *Tanita* (BC-601) was also utilized. Body mass index (Kg/m^2) was measured by the following equation:

$$\text{Weight (Kg)} / \text{Height}^2 (\text{m}^2)$$

The BMI classification was obtained according with World Health Organization (WHO): Regular weight: $18.5 \text{ Kg}/\text{m}^2 \leq \text{BMI} < 24.9 \text{ Kg}/\text{m}^2$; Overweight: $25 \text{ Kg}/\text{m}^2 \leq \text{BMI} < 30 \text{ Kg}/\text{m}^2$; Obesity: $30 \geq \text{Kg}/\text{m}^2$ (WHO, 1997).

Glycaemia was measured by fasting and post-prandial period. For this, a capillary drop of blood was collected from the finger of each participant. Sterilized lancet, glucometer equipment and strips for glucometer were used for blood glucose level measurement.

The area under the curve (AUC) for blood glucose of each subject was determined as incremental area under the curve of blood glucose level during the time. AUC calculation was determinate using *Graph Pad Prim Software* (version 5.01) and this was represented as a mean \pm SEM. Maximum concentration (C_{max}) of post-prandial blood glucose level was also calculated for each participant and the variation of the maximum concentration (ΔC_{max}) as well.

2.3 Statistical analysis

The statistical analysis of the results was obtained using Statistical Package for Social Sciences (SPSS) programme, version 20.0. The significance established in this work was for all results 5% ($p \leq 0.05$). For animal studies, all data were analysed and presented as mean \pm standard error of the mean (SEM) for untreated and treated groups of both normal and diabetic rat. The *Shapiro-Wilk* ($n < 200$) was used to verify the normality of the sample distribution. Moreover, the data from the different groups (treated and untreated) as well as normal and diabetic rats were compared using *Student's t-test* and ANOVA test.

Regarding to human study, independent sample t-test was utilized for comparing total caloric value, carbohydrates, protein and lipid at day before of intervention with OGTT and OGTT + cinnamon tea administration. The mean of blood glucose levels at t_0 , t_{30} , t_{60} , t_{90} and t_{120} time between OGTT and OGTT + cinnamon tea administration were statistically analysed with repeated measures ANOVA of mixed type. A value of $p \leq 0.05$ was taken as significant. The independent samples t-test was used to assess the difference between the 2 groups for total caloric value, carbohydrates, protein and lipid, C_{max} , ΔC_{max} and $AUC_{Incremental}$ values.

Chapter Three

RESULTS

3 Results

A. ANTIOXIDANT CAPACITY OF AQUEOUS *C. BURMANNII* EXTRACT

3.1 Quantification of total phenols

The follow results presented the total phenols quantification that constituted aqueous cinnamon extract of *C. burmannii* specie. The study took into consideration that different concentrations were employed in this study (0.06 g/ml and 0.35 g/ml). Moreover, the chemical characterization regarding to total phenols content was also analysed in different concentrations of aqueous extract. The results are shown in table 3.1. The data clearly show that each concentration of cinnamon has different amounts of total phenolic compounds demonstrating to be dose-dependent, but not proportionally. It is apparent that the high dose (0.35 g/ml) contained less phenolic agent. Proportionally, 0.35 g/ml should have produced a phenolic content of around 13,335 ml/l gallic acid and not 9,247.25 ml/l gallic acid.

Table 3.1: Total phenolic content (mg/L) of aqueous extract from *C. burmannii* used in animal studies (0.35 g/ml) and human study (0.06 g/ml). Data are mean (\pm SEM), n=8.

Concentration of aqueous cinnamon extract (g/ml)	Total phenolic (mg/L gallic acid) Mean (\pm SEM)
0.35	9247.25 (\pm 15.41) ⁽¹⁾
0.06	2286.3 (\pm 48.0) ⁽²⁾

⁽¹⁾Regression equation: $y = 5.73143E-3x + 1.65000E-2$ ($r^2=0.9989$)

⁽²⁾Regression equation: $y = 1.8558E-3x + 1.6500E-3$ ($r^2=0.9982$)

In order to verify the stability of total phenolic content in ACE, it was necessary to investigate the effect of temperature on the phenolic content of ACE using storage temperature of -20°C and refrigeration temperature of 4°C for 24 hours. The effect of temperature on phenolic content of ACE is shown in table 3.2. The results show that temperature had only a slight effect on the phenolic content after 24 hours storage. There

was a small decrease in the content, typically from 2.96% at -20°C to 5.15% for refrigeration at 4°C. For this reason, it was decided that during the experimental protocol on animal study, the samples of ACE should be stored at -20°C prior to use during oral administration.

Table 3.2: Table showing total phenolic content (mg/L) of aqueous *C. burmannii* according with different temperature storage methods at 0.1 g/ml concentration. Data are mean \pm SEM, n=3.

Storage method	Total phenolic (mg/l)		Lost total phenolic compounds (%)
	Mean (\pm SEM)		
	Before storage (0 days)	After storage (1 days)	
Frozen at -20°C (*)	2767.55 (\pm 17.4)	2685.5 (\pm 10.7)	2.96
Refrigerated at 4°C (*)	2812.38 (\pm 22.5)	2667.62 (\pm 3.9)	5.15

(*)Regression equation: $y = 0.0052x - 0.0149$ ($r^2 = 0.9995$)

In another series of experiments the phenolic content of ACE employed in the animal studies was measured before and after 5 weeks of storage at -20°C. The data are shown in table 3.3. The results clearly revealed that storage at -20°C can decrease the phenolic content of cinnamon (19.48%) during 5 weeks.

Table 3.3: Table showing total phenolic content (mg/L) of aqueous *C. burmannii* utilized in animal study (0.35 g/ml), before and after 5 weeks of storage at -20°C. Data are mean (\pm SEM), n=2.

Storage method	Total phenolic (mg/l)		Lost total phenolic compounds (%)
	Mean (\pm SEM)		
	Storage time (0 days)	Storage time (5 weeks)	
Aqueous cinnamon extract	9247.25 ⁽¹⁾ (\pm 15.41)	7445.73 ⁽²⁾ (\pm 131.70)	19.48%

⁽¹⁾ Regression equation: $y = 5.73143E-3x + 1.65000E-2$ ($r^2 = 0.9989$)

⁽²⁾ Regression equation: $y = 5.50300E-3x + 8.27500E-3$ ($r^2 = 0.999$)

Together, the results have demonstrated that the method of storage of the ACE might exert a declining effect on phenolic content of the solution. The solution was freshly made and then divided up, placed in vials and stored at -20°C . Daily, each vial was thawed out and thereafter the cinnamon solution was administered to the animals. During the time course of this study, fresh solutions were made up at week 0 and on week 6.

3.2 Identification and quantification of phenolic compounds

Cinnamon is a spice that is composed of several bioactive compounds (Ding et al., 2011). In this study, it was decided to identify and quantify the major active compounds of cinnamon found in the prepared aqueous solution administered to the animals and humans using the method of HPLC. This method has the ability to both identify and quantify the bioactive compound in cinnamon. Figure 3.1 shows a typical chromatogram profile of the 4 major phenolic compounds found in ACE of *C. burmannii*, identifying 4 peaks at 5.2, 5.8, 6.6 and 8.2 min as coumarin, cinnamyl alcohol, cinnamic acid and cinnamaldehyde, respectively.

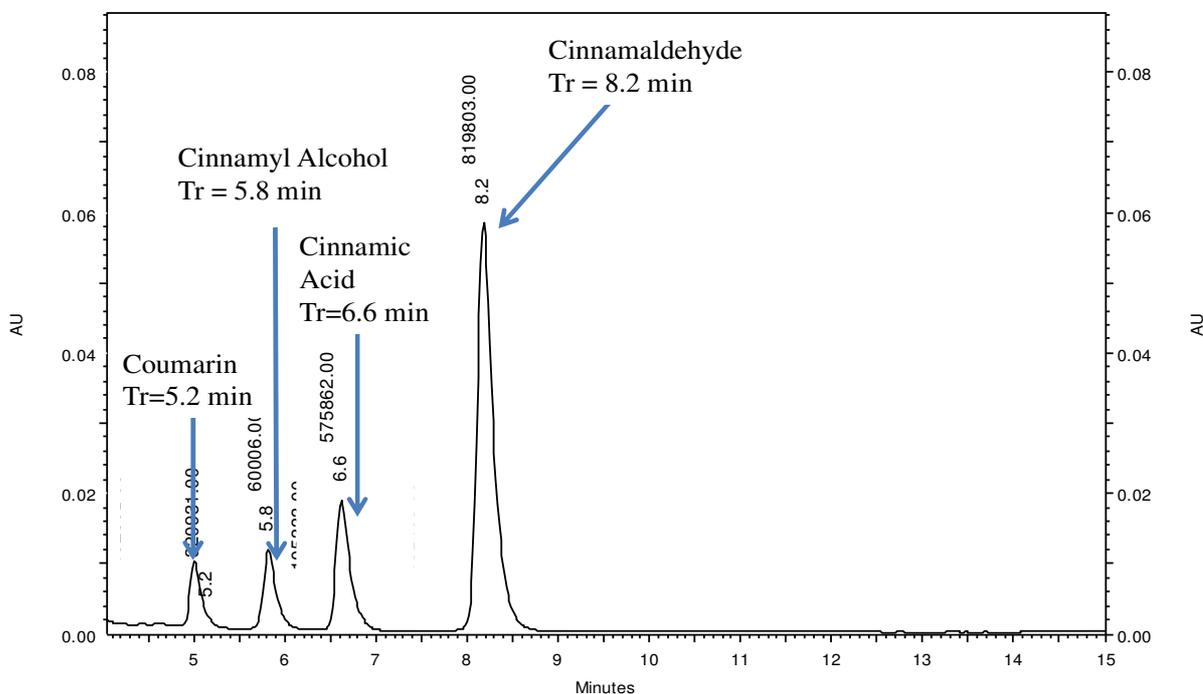


Figure 3.1: Original chromatogram showing HPLC identification of major phenolic compounds of aqueous cinnamon extract from *C. burmannii* (252 nm), at 0.1 g/ml concentration. This chromatogram is typical of 3 such different experiments.

The results also demonstrate that the major phenolic compound in this plant extract from *C. burmannii* is cinnamaldehyde (23.99%). Cinnamyl alcohol, coumarin and cinnamic acid revealed to exist in low percentage in this sample (0.21%, 1.56%, and 3.73%, respectively) (see table 3.4).

Table 3.4: Table showing percentage of major phenolic compounds of aqueous cinnamon extract (ACE) from *C. burmannii*. Data are mean, n=3. ACE – aqueous cinnamon extract.

Phenolic compounds of ACE	Peak Area from chromatogram	Amount of each phenolic compound mol/l (% in the sample)	Regression equation	r ²
Cinnamyl alcohol	60006	8.53 x 10 ⁻⁶ (0.21%)	y=7X10 ¹⁵ x - 146250	0.997
Coumarin	53410	2.29 x 10 ⁻⁵ (1.56%)	y=7X10 ¹⁵ x + 146564	0.992
Trans-cinnamic acid	529268	1.36 x 10 ⁻⁴ (3.73%)	y=4X10 ¹⁵ x + 150199	0.997
Cinnamaldehyde	4882890	9.8 x 10 ⁻⁴ (23.99%)	y=4X10 ¹⁵ x + 158094	0.995

Figure 3.2 shows chromatograms in (1) standard and in (2) the equivalent compound found in cinnamon, namely (A) coumarin, (B) cinnamic acid, (C) cinnamic aldehyde and (D) cinnamyl alcohol.

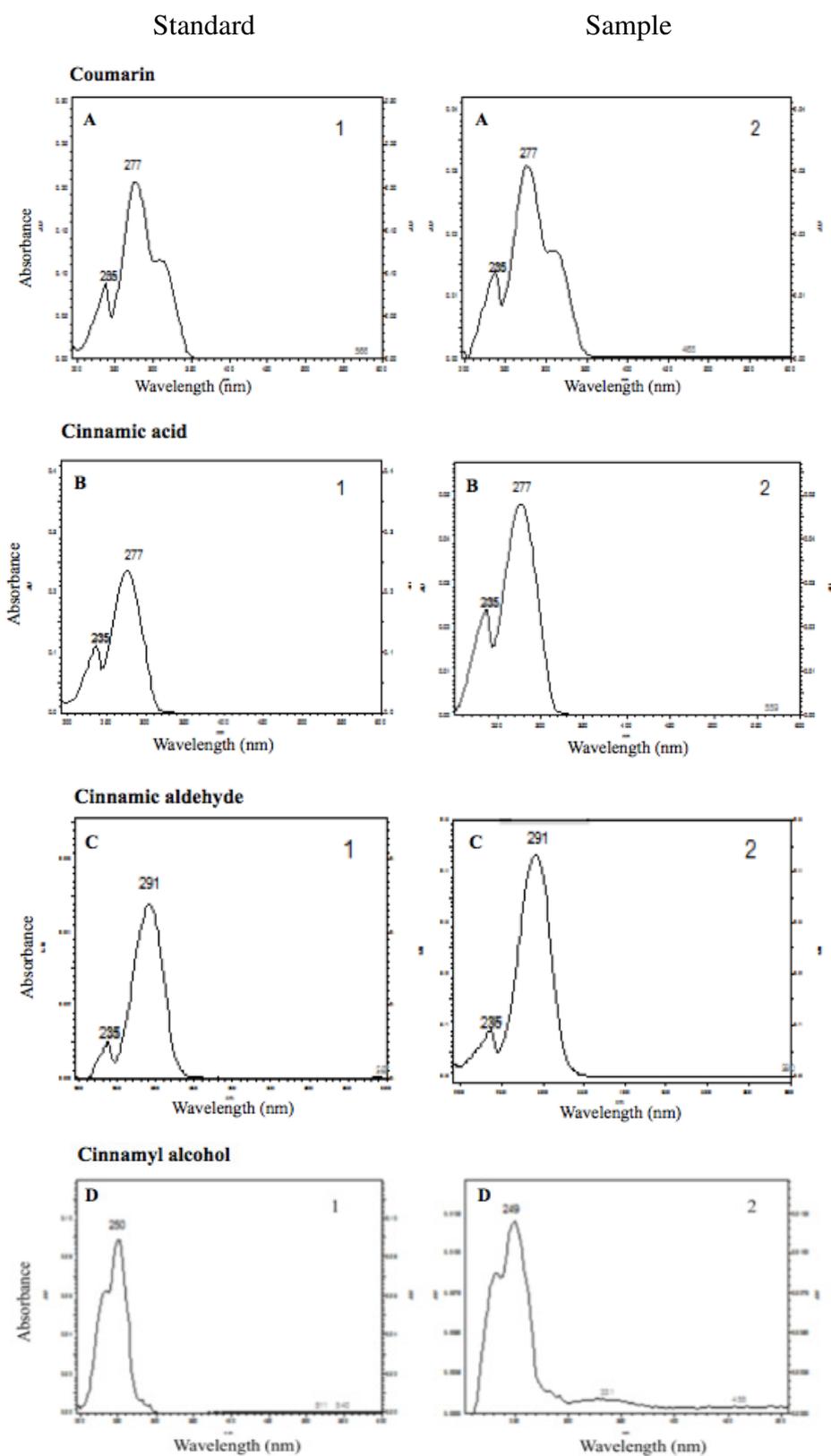


Figure 3.2: Absorption spectra of (A) coumarin, (B) cinnamic acid, (C) cinnamic aldehyde and (D) cinnamyl alcohol identified in (1) standard compound and in (2) ACE, using HPLC method.

3.3 Characterization of antioxidant capacity

3.3.1 FRAP (Ferric Reducing Antioxidant Power) method

Table 3.5 shows the antioxidant capacity using FRAP method in cinnamon tea (0.06 g/ml). The data revealed a strong anti-oxidant capacity as determined by FRAP test (11,779.0 ±294.7 μmol Trolox/l).

Table 3.5: Anti-oxidant capacity of aqueous cinnamon extract (ACE) from *C. burmannii*. The results are expressed as mean (±SEM), n=6.

	Antioxidant capacity (FRAP) (μmol Trolox/l ^(*)) Mean (±SEM)
Aqueous cinnamon extract	11779.0 (±294.7)

^(*) Regression equation: $y = 1.8558E-3x + 1.6500E-3$ ($r^2=0.9982$)

3.3.2 Superoxide anion scavenging activity

In another series of experiments, the superoxide anion scavenging activity of aqueous cinnamon extract was determined and the data presented in the figure 3.3. The results show that the aqueous cinnamon extract can induce an inhibitory capacity of superoxide scavenging activity in a dose-dependent manner reaching 96% at 1143 mg/l gallic acid (half of the total phenols concentrations).

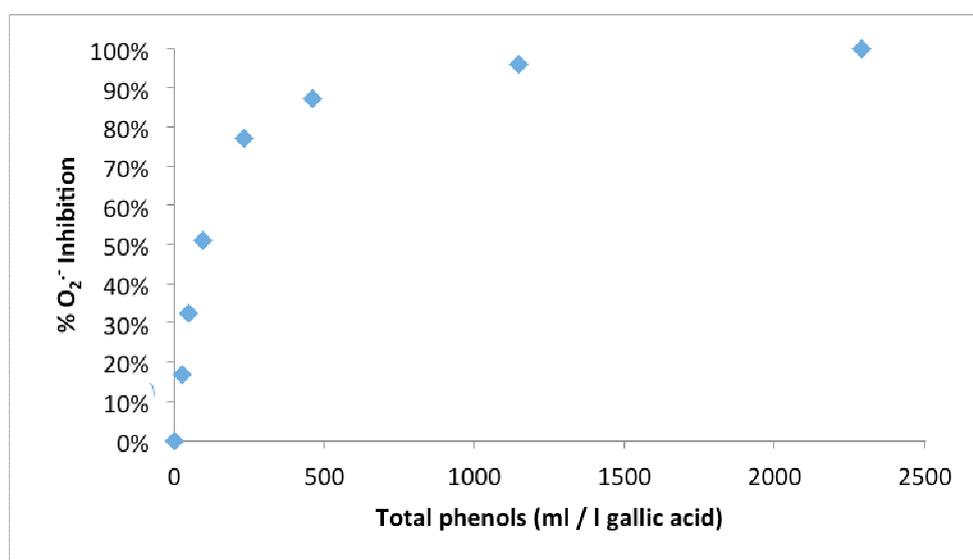


Figure 3.3: Dose-dependent curve showing the percentage inhibition of superoxide anion by aqueous cinnamon extract from *C. burmannii*.

B. ANIMAL STUDIES

3.4 Effect of ACE on body weight of normal and diabetic rats

The mean weight (\pm SEM) of all the rats when they arrived in the animal house was 228.9 ± 8.46 g ($n=40$). Following 2 weeks of adaption in the laboratory, the mean (\pm SEM) weight of all the rats increased to 287 ± 11.2 g.

The rats were divided into 2 groups. One group was rendered diabetic with STZ and the other group only received citrate buffer and they acted as control. Both control and diabetic animals were divided up into three further sub-groups (3 diabetic and 3 controls). Two diabetic groups received either 75 mg/Kg bw ACE or 150 mg/Kg bw ACE. The other diabetic group only obtained distilled water daily. Similarly, two control groups received either either 75 mg/Kg bw ACE or 150 mg/Kg bw ACE. The third control group only received distilled water daily. All the animals were treated over a period of 11 weeks.

The mean (\pm SEM) time course weights of normal and diabetic treated and untreated rats are shown in Figure 3.4 (A and B, respectively). The results show that the weight of the normal (both treated and untreated) rats (Figure 3.4A) increased gradually over the experimental period, from the start of the experimental protocol at week zero to week 11. The data also show that the normal rats (untreated and treated) weight between 271.5 and 311.1 g at week 0 and then the weights increased to around 358 and 424 g after 11 weeks of the experiments. However, these results shown no significant ($p>0.05$) differences in the weights in the three groups of normal rats over the experimental period (compared weeks 0 to week 11). It is noteworthy that the rats fed with 75 mg/kg of cinnamon daily gained slightly more weight than the other 2 groups, but these values were not significant.

For diabetic rats, the results in Figure 3.4B also show the weights of all these groups of diabetic rats. The weights increased gradually over the experimental period but the weights of the rats treated with 150 mg/kg of cinnamon extract were significantly ($p<0.05$) higher compared to the untreated diabetic animals at weeks 1, 3, 4, 5 and 11. Treatment with 150 mg/Kg bw of cinnamon was more effective than treatment with 75 mg/Kg bw in weight gain over the experimental period. These results clearly show that a high dose of cinnamon had a beneficial effect on the body weight gain of diabetic rats. For the sake of clarity the weights of normal and diabetic treated and untreated rats using 150 mg/Kg of cinnamon were compared and the data presented in Figure 3.4C. The data clearly show that the

normal rats whether treated or untreated gained significantly ($p < 0.05$) more weight than diabetic animals (compared the groups in Figure 3.4C). Cinnamon treatment at 150 mg/kg bw improved weight gain for diabetic rats. These results clearly show that cinnamon treatment at 150 mg/kg bw had a significant beneficial effect on the diabetic rats.

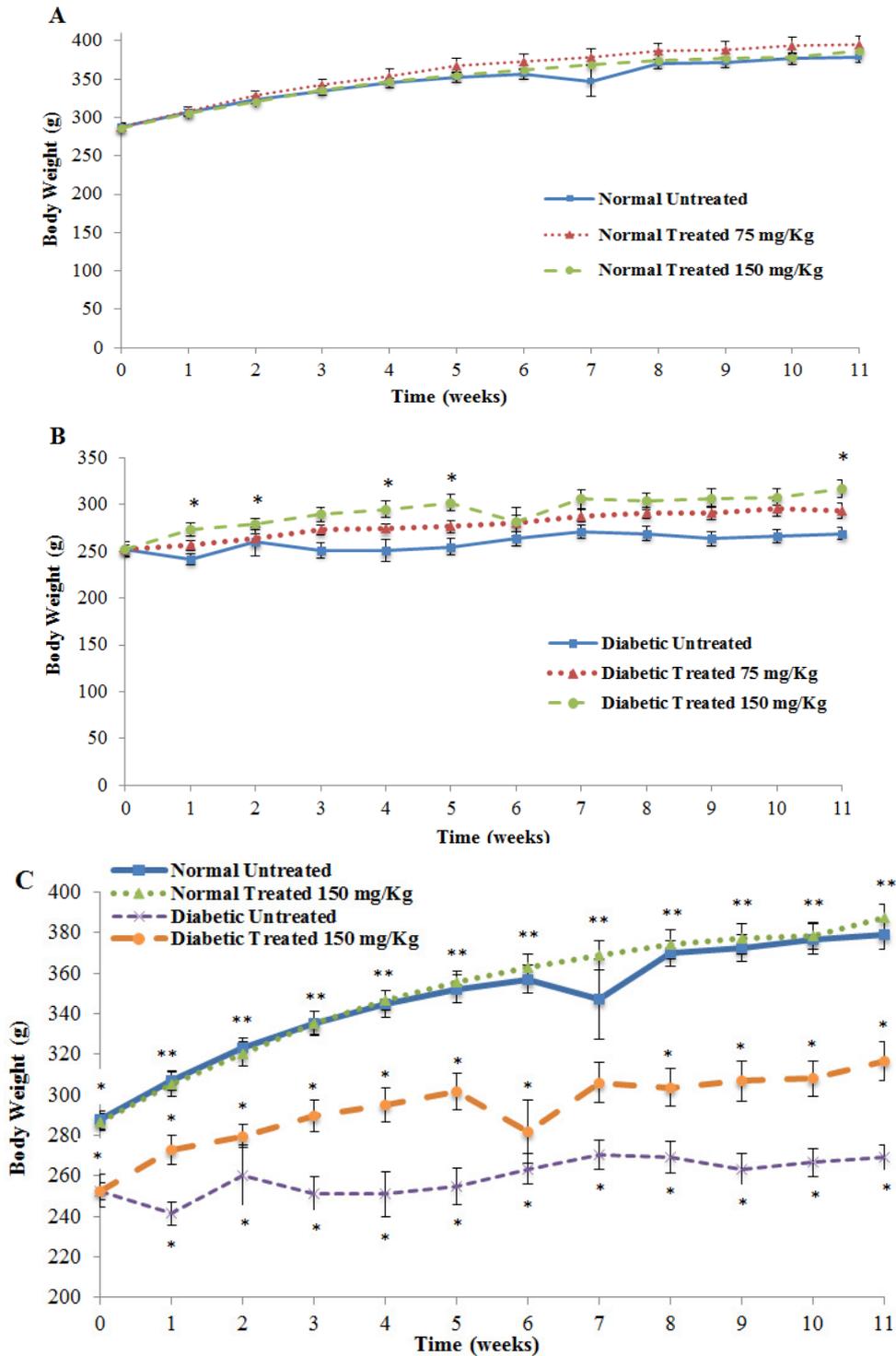


Figure 3.4: Time course changes in body weights (g) of normal (A), diabetic (B) and both normal and diabetic rats treated with 150 mg/Kg (C) over the experimental period of 11

weeks. Data are mean (\pm SEM); n=5-6 rats. ANOVA test was used to compare untreated with treated rats ($*p<0.05$) in both normal and diabetic rats (A, and B). Figure C shows the data for normal and diabetic treated and untreated rats with 150 mg/Kg of cinnamon for comparison, $**p<0.05$ for normal rats compared to diabetic $*p<0.05$ (C).

3.5 Effect of ACE on food consumption of normal and diabetic rats

The effect of ACE on food consumption was also investigated in this study (see Figure 3.5).

The results show no significant differences in the data comparing untreated normal rats with treated animals using the high dose of cinnamon (150 mg/Kg) over the whole experimental period of 11 weeks (Figure 3.5, A). The results also show that food consumption in all three groups (treated and untreated) decreased over the experimental period compared to week 0 (before treatment) and week 11 (end of treatment). These are unexpected results since these rats seem to gain weight constantly over the experimental period of 11 weeks (see Figure 3.5).

In diabetic rats (Figure 3.5, B) there is a slightly decrease in food consumption almost constantly over the experimental several of 11 weeks. The results revealed that all three groups of diabetic rats (either treated and untreated) consumed significantly ($p<0.05$) more food than age-matched normal animals. Regarding the diabetic rats (either treated and untreated), the data show similar pattern of food consumption over the time course of the experiments. During weeks 5, 6, and 11, the animals treated with 75 mg/Kg cinnamon ate significantly less ($p<0.05$) food than diabetic untreated rats. The treatment with 150 mg/kg bw in diabetic rats seems to decrease food consumption compared with untreated diabetic rats, during the experimental protocol. However, for higher dose of cinnamon extract the results were not significant ($p>0.05$).

From the experiments, it is noteworthy that diabetic untreated and treated rats consumed more food than control untreated and treated rats. The untreated diabetic rats consumed more or less the same amount of food over the 11 weeks. In contrast, the untreated control rats ate more food at week 1, but the consumption declined gradually over the experimental period. The same observation is noticed for treated control and diabetic rats.

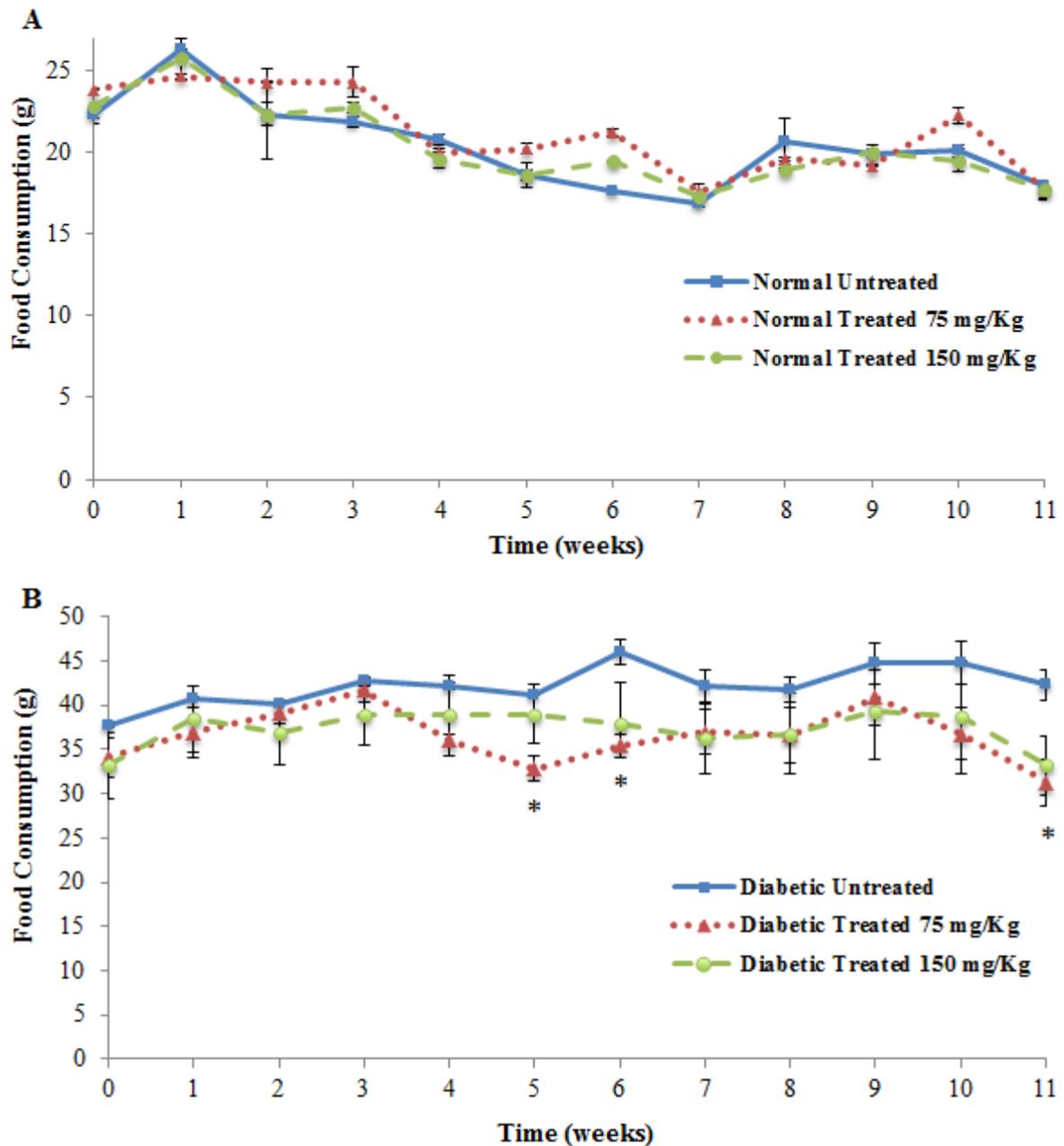


Figure 3.5: Time course changes in food consumption (g) over the experimental period in age-matched normal (A) and diabetic (B) untreated and cinnamon treated (75 mg/Kg or 150 mg/Kg body weight daily) rats. Data are mean \pm SEM; n=6 rats in normal and normal-treated 75mg/Kg; n=5 in normal-treated 150 mg/Kg. ANOVA test was used to compared diabetic untreated with diabetic treated with cinnamon rats (*p<0.05).

3.6 Effect of ACE on blood glucose level of normal and diabetic rats

Figure 3.6 shows the blood serum glucose levels in normal and diabetic treated and untreated rats. The results show that cinnamon treatment had very little or no effect on fasting blood glucose in normal rats (Figure 3.6A). At only week 6 there was a small, but no significant, reduction ($p>0.05$) in blood glucose following treatment with 150 mg/Kg of cinnamon compared to untreated normal. At weeks 7 and 8 fasting blood glucose seemed to increase slightly in normal rats treated with 75 mg/kg compared untreated-normal and treated-normal with 150 mg/kg body weight of cinnamon.

The results show that all 3 groups of diabetic rats had elevated fasting blood glucose at the start of the experimental period confirming diabetes (compared Figure 3.6A and Figure 3.6B). The data also show that the diabetic untreated group had more, but not significantly ($p>0.05$) increase in blood glucose level compared to treated groups at week 0 at the start of the experiments. However, daily administration of cinnamon with either 75 mg/Kg or 150 mg/Kg only reduced fasting blood glucose slightly compared to the untreated diabetic group. A concentration of 150 mg/Kg of cinnamon extract was somewhat more effective in reducing blood glucose compared with 75 mg/Kg. A significant difference ($p<0.05$) occurred only at weeks 3 compared treated 150 mg/kg with untreated group.

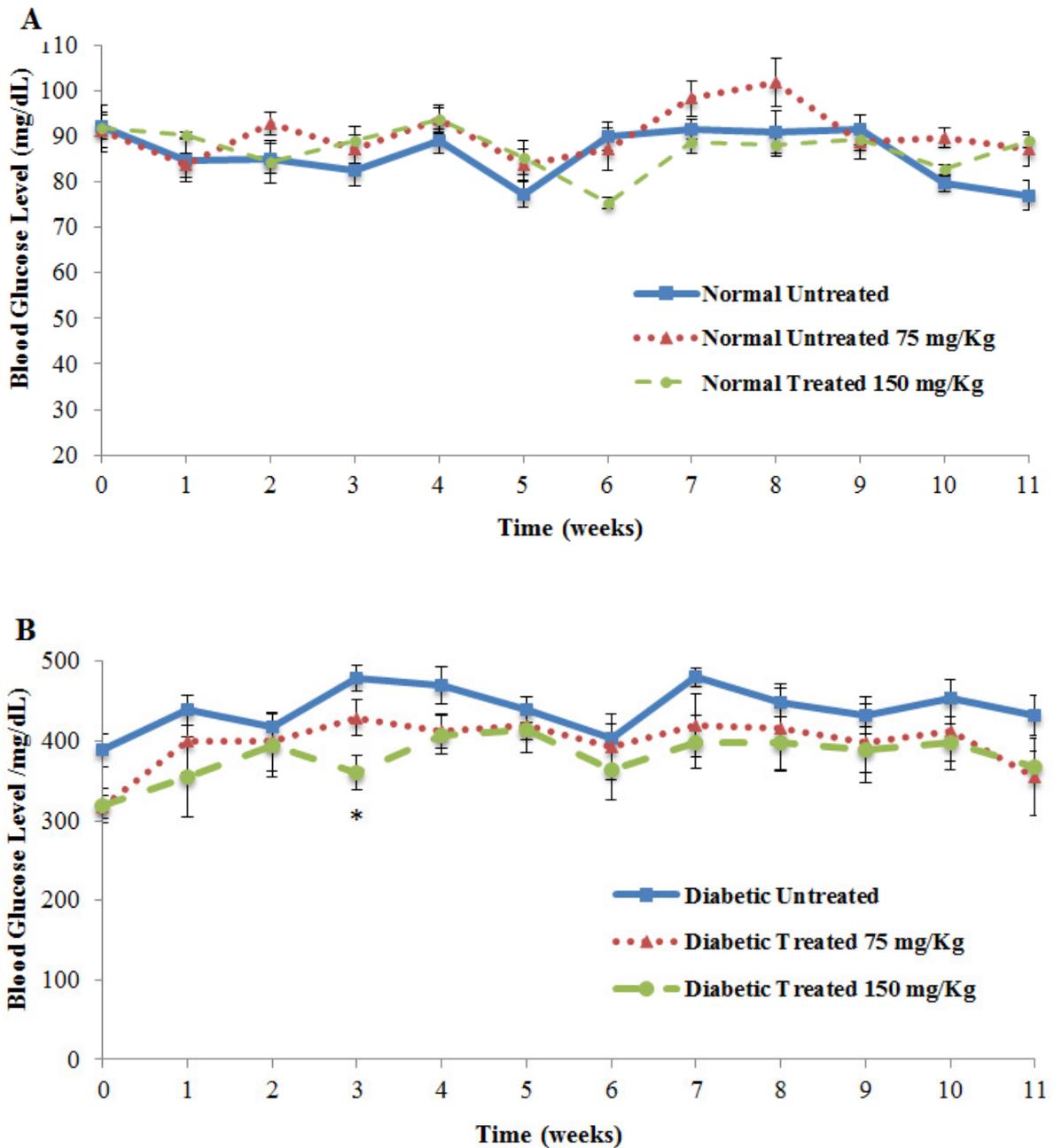


Figure 3.6: Time course changes in fasting blood glucose level in age-matched normal A) and diabetic (B) both untreated and treated rats. Animals were given either 75 mg/Kg or 150 mg/Kg of cinnamon on a daily basis. Data are mean \pm SEM; n=6 rats in normal and normal-treated 75 mg/Kg; n=5 in normal-treated 150 mg/Kg. ANOVA test was used to compared diabetic untreated with diabetic treated with cinnamon rats (*p<0.05).

3.7 Oral glucose tolerance test of normal and diabetic rats

Figure 3.7 shows the time course of the oral glucose tolerance test for all 6 sub-groups of rats. The data show that the rats treated with 150 mg/Kg of cinnamon had slightly elevated blood glucose either before the administration or 30 minutes after the OGTT compared to normal untreated or normal-treated with 75 mg/Kg of cinnamon but these values were not significant ($p>0.05$). Furthermore, the results show that the untreated normal rats seem to metabolize glucose better than the normal-treated rats with either 75 mg/Kg or 150 mg/Kg of cinnamon. However, these differences were not significant ($p>0.05$) in the overall data (Figure 3.7).

The results confirm hyperglycaemia in diabetic rats compared to normal. The results also show that both treated and untreated diabetic rats had elevated blood glucose compared to normal. Furthermore, the results revealed that there was no significant differences between treated and untreated rats diabetic ($p>0.05$).

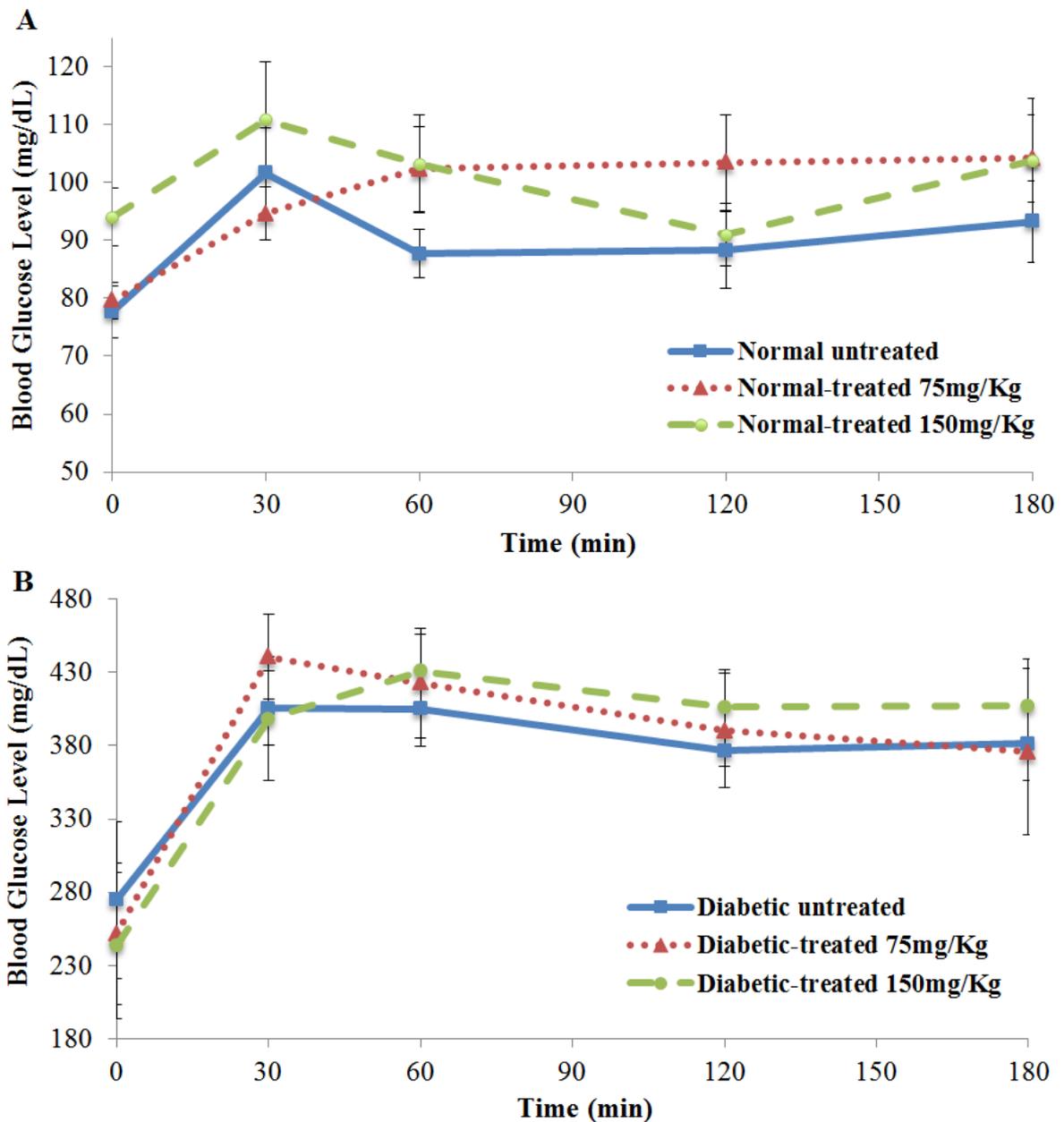


Figure 3.7: Time course changes on fasting blood glucose level on week 10 of the experimental period following on oral glucose tolerance test (OGTT) in age-matched normal (A) and diabetic (B) both untreated and treated (75 mg/Kg or 150 mg/Kg of cinnamon) rats either before (0 min) and after (30-180 min) glucose administration. Data are mean \pm SEM; n=5-6 rats. ANOVA test was used to compared untreated with treated with cinnamon rats ($p>0.05$).

3.8 Effect of ACE on biochemical parameters of normal and diabetic rats

3.8.1 Effect of ACE on triglycerides (TG)

Figure 3.8 shows the data for blood triglycerides in all 6 sub-groups. The results revealed that serum TG increased in diabetic rats compared to normal rats in both treated and untreated animals. However, these increases on TG in diabetic rats was close to significant level ($p=0.08$) compared to normal. The levels of TG was slightly higher in treated normal rats compared with untreated normal rats. In diabetic rats, the levels of TG in treated diabetic rats was slightly lower than untreated diabetic rats. However, these results had no significant differences ($p>0.05$), showing that cinnamon treatment had no effect on serum TG levels.

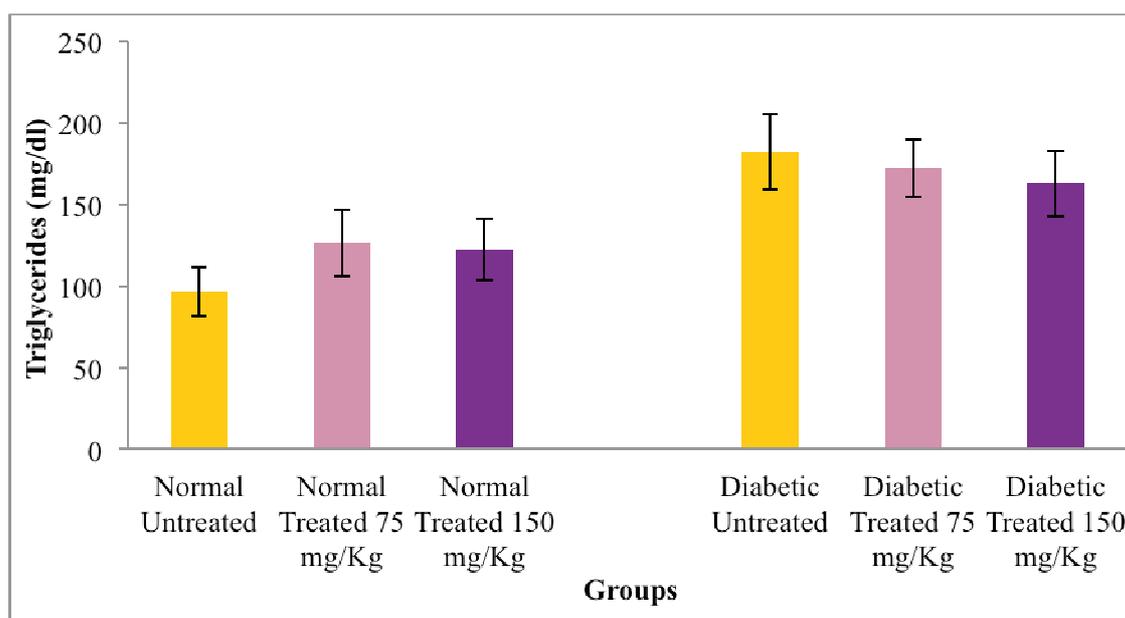


Figure 3.8: Bar charts showing the serum levels of tryglicerides (TG) in age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats 11 weeks after the start of the treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150 mg/Kg, diabetic and diabetic-treated 150 mg/Kg. ANOVA test was used mean values ($p>0.05$) for diabetic compared to normal.

3.8.2 Effect of ACE on total cholesterol (TC)

Figure 3.9 shows the data for total cholesterol in serum of the 6 sub-groups of rats. The results also show that there are no significant changes in TC levels in either normal or diabetic rats. Moreover, the data also reveal that cinnamon (75 mg/Kg or 150 mg/Kg) treatment over 11 weeks had no significant effect of TC in both normal and diabetic rats. However, TC levels in the diabetic animals were slightly elevated compared to normal.

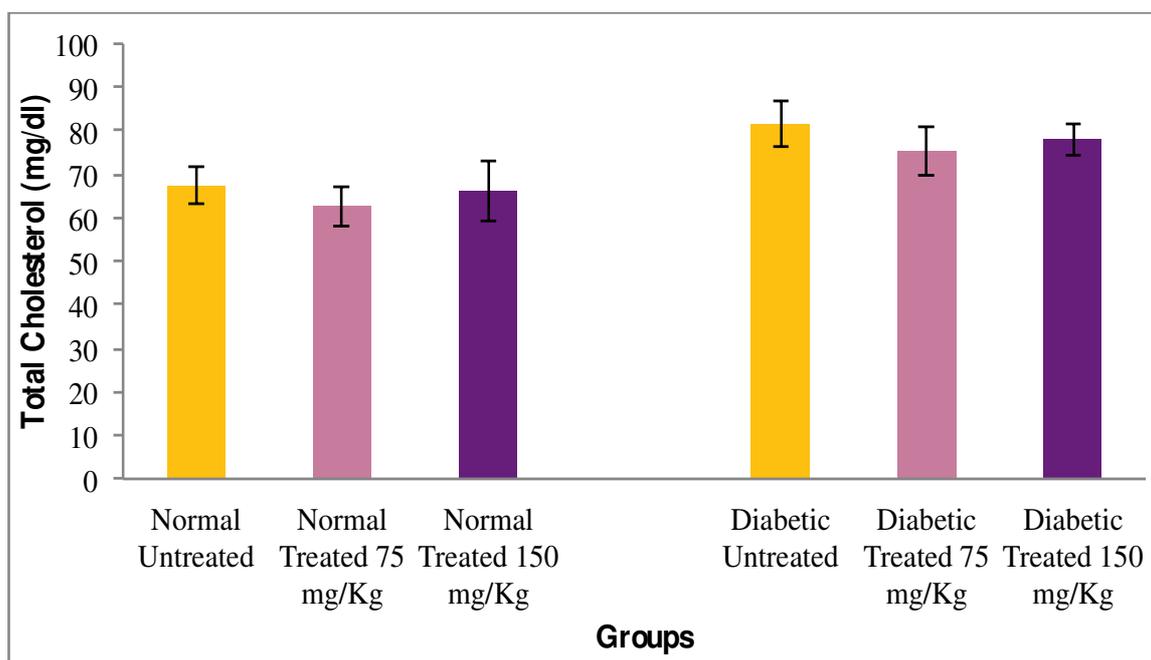


Figure 3.9: Bar charts showing the serum levels of total cholesterol (TC) in age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats 11 weeks after the start of the treatment. Data are mean \pm SEM; n=6 rats in normal, normal-treated 75 and diabetic-treated 75 groups; n=5 rats in normal-treated 150, diabetic and diabetic-treated 150. ANOVA test was used mean values ($p>0.05$).

3.8.3 Effect of ACE on high-density lipoprotein (HDL)

Figure 3.10 shows the data for levels of HDL in the serum of all 6 sub-groups of rats. The results show that cinnamon treatment had no significant ($p>0.05$) effect on HDL level in either normal and diabetic rats, comparing untreated with treated animals. In addition, the results demonstrated that untreated and treated (150 mg/kg) diabetic rats had significantly higher ($p<0.05$) levels of serum HDL compared to treated (75 mg/kg and 150 mg/kg) normal rats.

The results also show that diabetic treated rats (150 mg/Kg) revealed significantly high HDL levels than normal treated rats (75 or 150 mg/Kg) ($p<0.05$). Normal treated rats (75 mg/Kg) revealed significantly ($p<0.05$) low HDL levels compared with untreated diabetic rats.

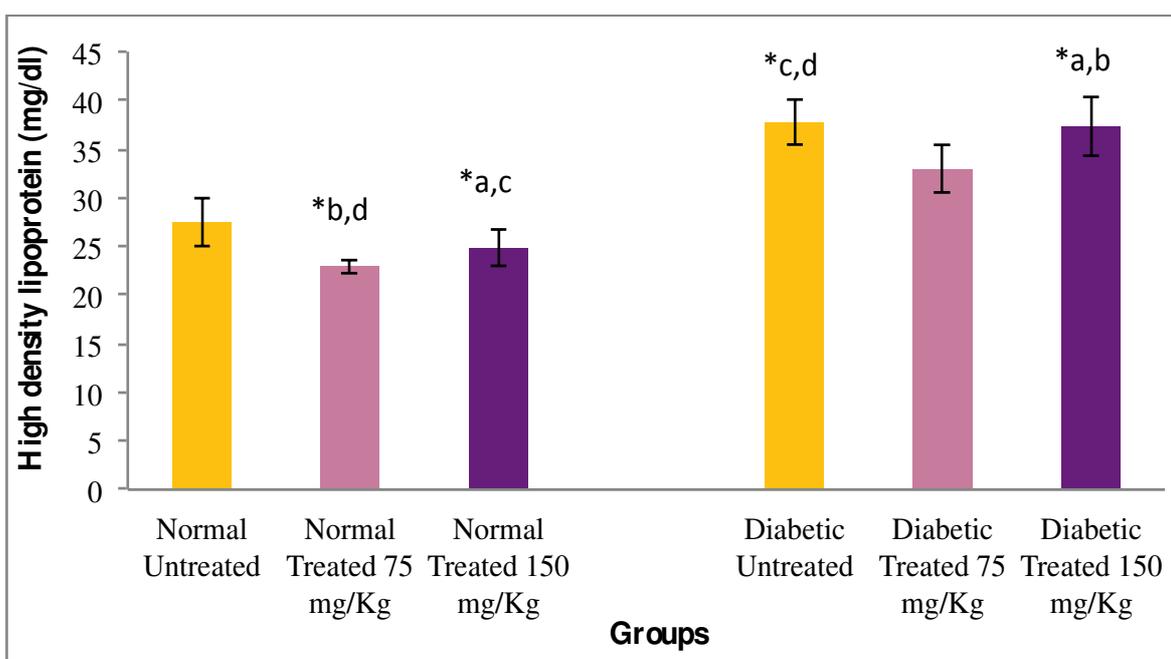


Figure 3.10: Bar charts showing serum levels of high density lipoprotein (HDL) in age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats 11 weeks after the start of the treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p<0.05$). ANOVA test was used to compared normal and diabetic rats (* $p<0.05$).

3.8.4 Effect of ACE on low-density lipoprotein (LDL)

Figure 3.11 shows the data for levels of LDL in the serum of all 6 sub-groups of rats. The data show no significant changes ($p>0.05$) comparing normal with STZ-induced diabetic rats, either treated and untreated animals. In addition, the data show that LDL levels increase slightly in the untreated diabetic rats compared to normal. Cinnamon treatment seem to reduce the LDL levels in the diabetic treated rats, but the values were not significant different ($p>0.05$).

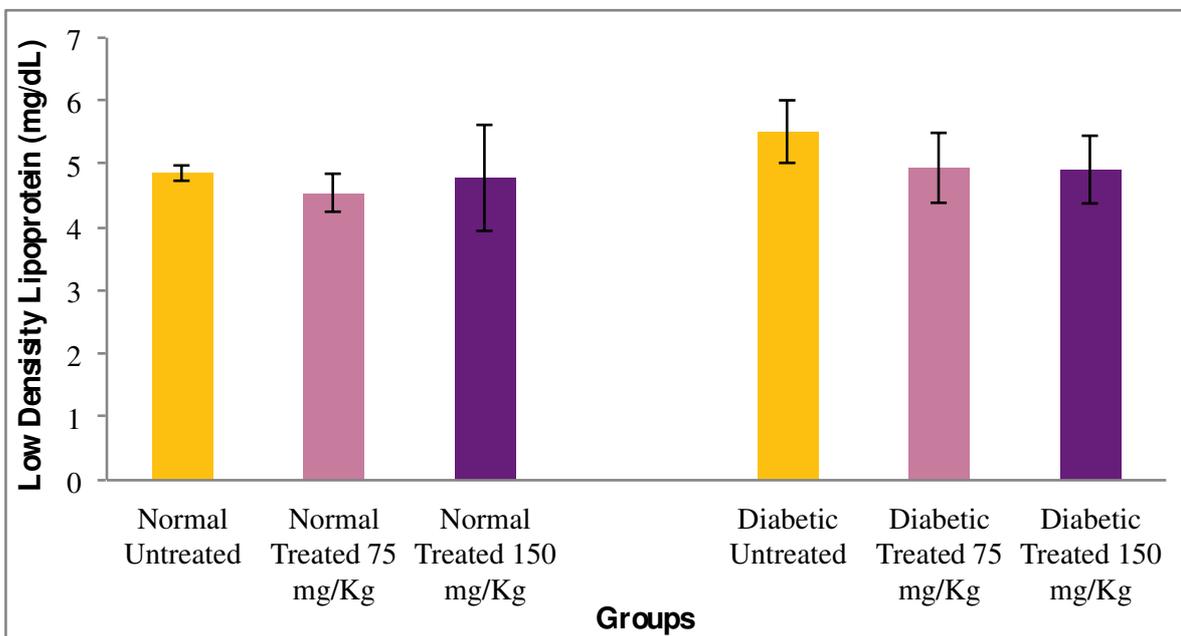


Figure 3.11: Bar charts showing the serum level of low density lipoprotein (LDL) from age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats 11 weeks after the start of the treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150. ANOVA test was used to compared normal and diabetic rats ($p>0.05$).

3.8.5 Effect of ACE on albumin

Figure 3.12 shows the data for levels of albumin in the serum of all 6 sub-groups of rats. The results show that serum albumin levels remained approximately the same in normal treated and untreated rats. The same is also true for untreated and treated diabetic rats. However, the levels of albumin in the diabetic rats decreased significantly ($p < 0.05$) compared to normal. Moreover, treated (150 mg/kg) diabetic rats had a significantly decreased of albumin levels compared to normal and treated (75 mg/kg) normal rats ($p < 0.05$).

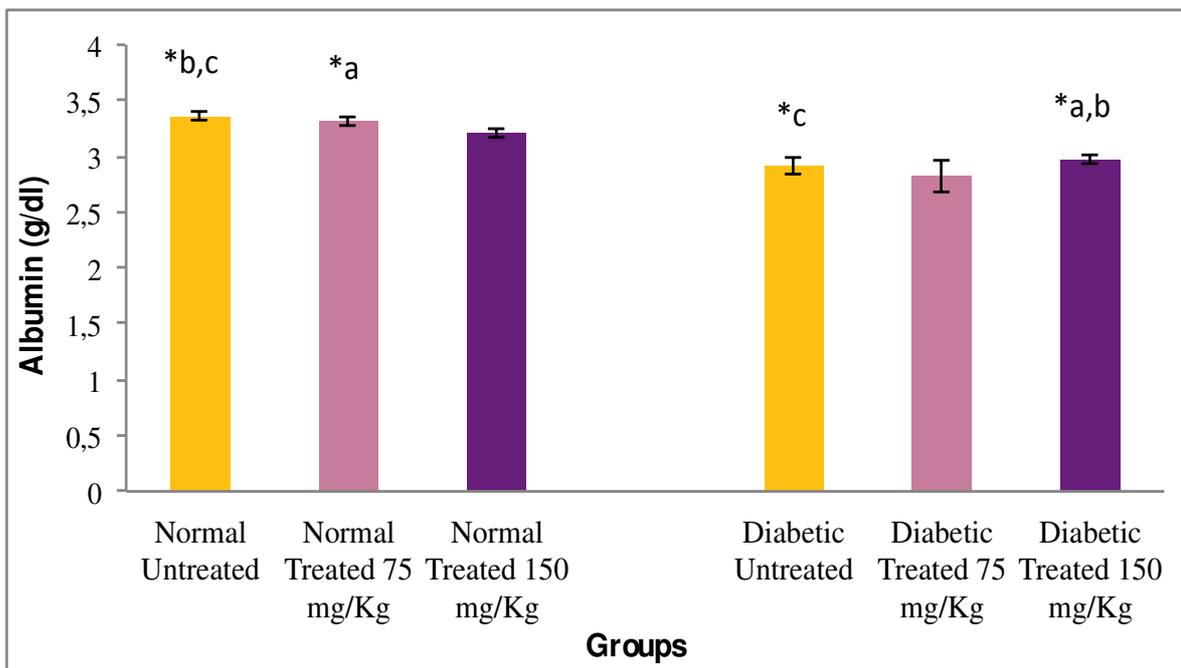


Figure 3.12: Bar charts showing the serum levels of serum albumin in age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats, 11 weeks after the start of the treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p < 0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats ($*p < 0.05$).

3.8.6 Effect of ACE on hemoglobin A1c (HbA1c)

Figure 3.13 shows the data for levels of HbA1c in the serum of all 6 sub-groups of rats. The results show that diabetic rats (untreated and treated) had significantly ($p < 0.05$) elevated levels of HbA1c compared with age-matched normal treated and untreated rats. The results also show that cinnamon (75 mg/Kg or 150 mg/Kg) treatment had no significant effect on HbA1c in either normal or diabetic rats ($p > 0.05$). Together, the data clearly demonstrated that the diabetic rats were confirmed in having diabetes.

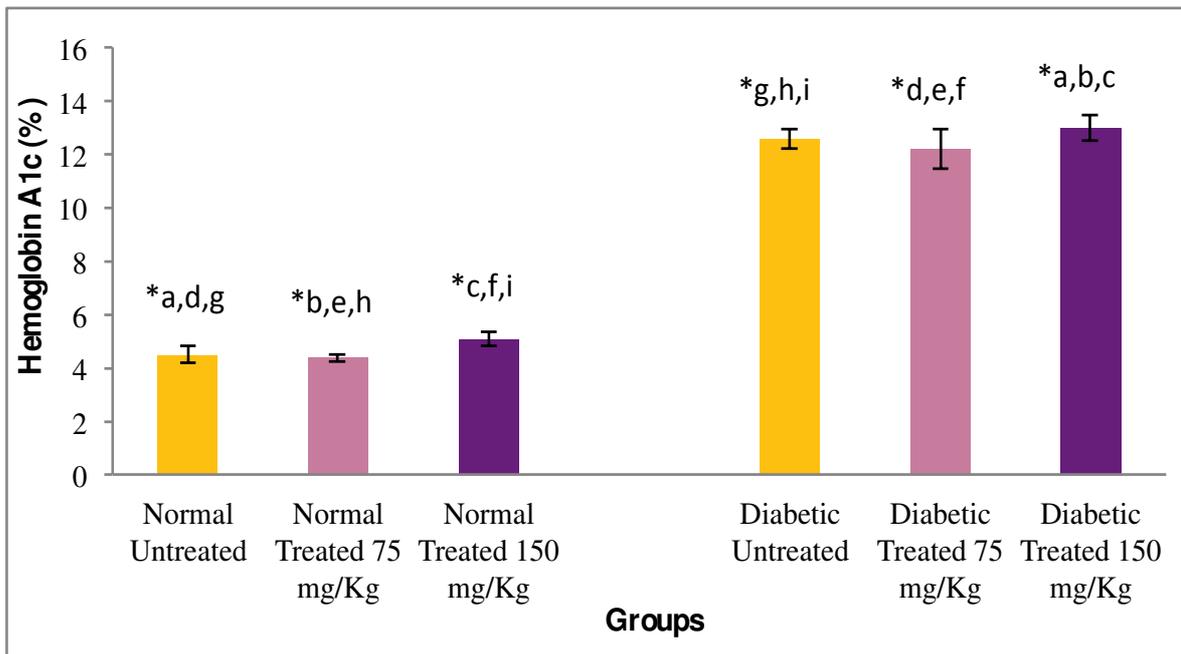


Figure 3.13: Bar charts showing hemoglobin A1c (%) in serum of age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats at 11 week of the experimental period. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p < 0.05$). ANOVA test was used to compared normal and diabetic rats ($*p < 0.05$).

3.8.7 Effect of ACE on creatinine

Figure 3.14 shows the data for levels of creatinine in the serum of all 6 sub-groups of rats. The results show that there are no significant ($p>0.05$) changes in the levels of creatinine in the serum of either normal or diabetic rats. Moreover, cinnamon (75 mg/Kg or 150 mg/Kg) had no significantly effect on blood serum creatinine levels in either diabetic or age-matched normal rats ($p>0.05$).

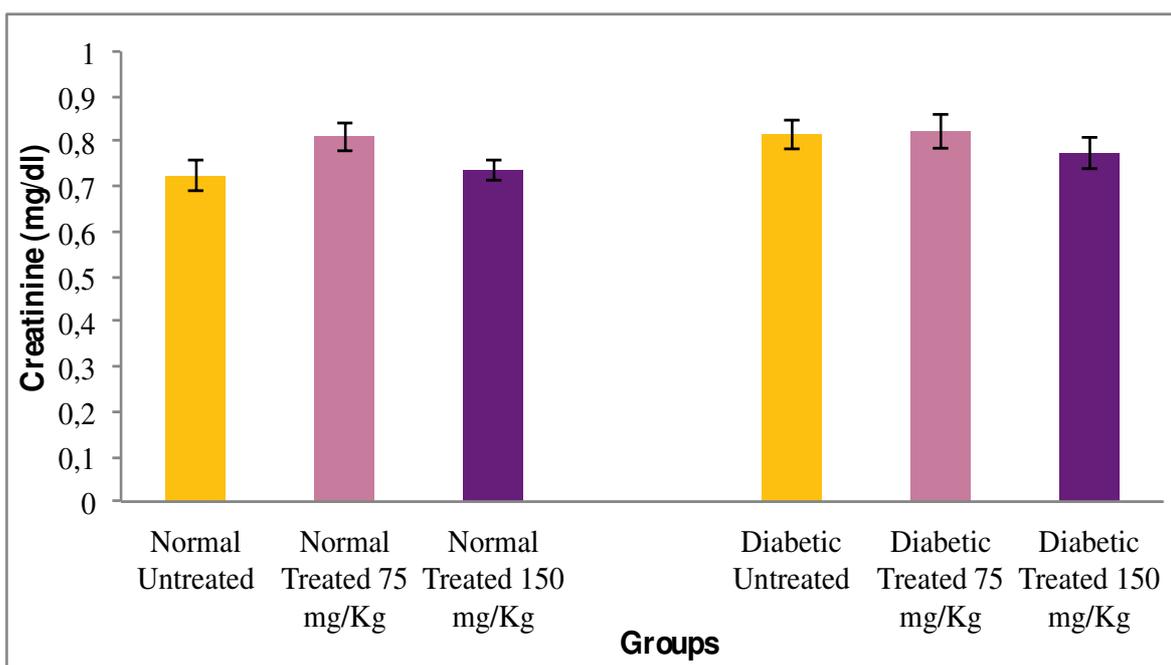


Figure 3.14: Bar charts showing serum levels of creatinine of age-matched normal and STZ-induced diabetic untreated and cinnamon (75 mg/Kg or 150 mg/Kg) treated rats at 11 week of the experimental period. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150. ANOVA test was used to compared normal and diabetic rats ($p>0.05$).

3.9 Effect of ACE on organs weight of normal and diabetic rats

3.9.1 Effect of ACE on pancreas weight

Figure 3.15 shows the data for pancreas weight of all 6 sub-groups of rats. The data show no significant differences in the weights of the pancreas in either normal or STZ-induced diabetic rats. Moreover, cinnamon had no detectable effect in the weights of the pancreas comparing treated (75 mg/Kg or 150 mg/Kg) with untreated animals (Figure 3.15).

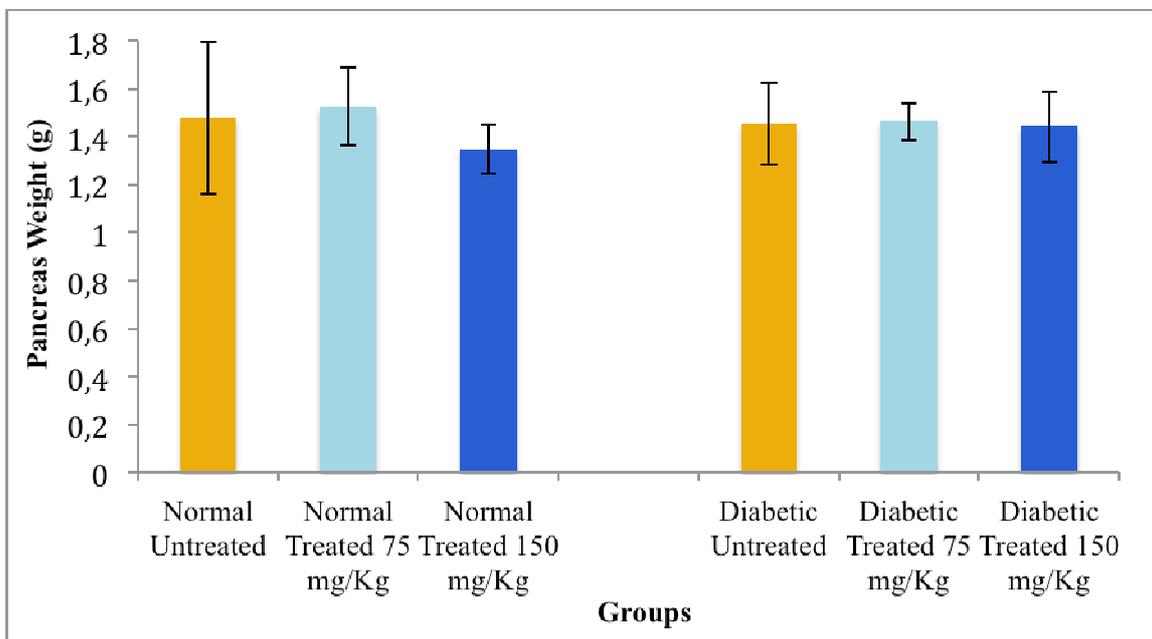


Figure 3.15: Bar charts showing the mean (\pm SEM) weights (g) of the pancreas in age-matched normal and STZ-induced diabetic untreated and treated with cinnamon (75 mg/Kg or 150 mg/Kg) rats after 11 weeks following cinnamon treatment. Data are mean \pm SEM, n=6 rats in normal, normal-treated 75 and diabetic-treated 75 groups; n=5 rats in normal-treated 150, diabetic and diabetic-treated 150. ANOVA test was used to compared normal and diabetic rats ($p>0.05$).

3.9.2 Effect of ACE on heart weight

Figure 3.16 shows the data for heart weight of all 6 sub-groups of rats. The results show no significant differences in the weights of normal and diabetic treated and untreated rats. Moreover, cinnamon treatment (75 mg/Kg or 150 mg/Kg) rats had no effect on the weights of the hearts for each group comparing cinnamon treated with untreated rats. It is noted that the untreated diabetic heart weight was less compared to normal untreated hearts. Cinnamon treatment on diabetic hearts seemed to improve the weight, but not significantly ($p>0.05$) (Figure 3.16).

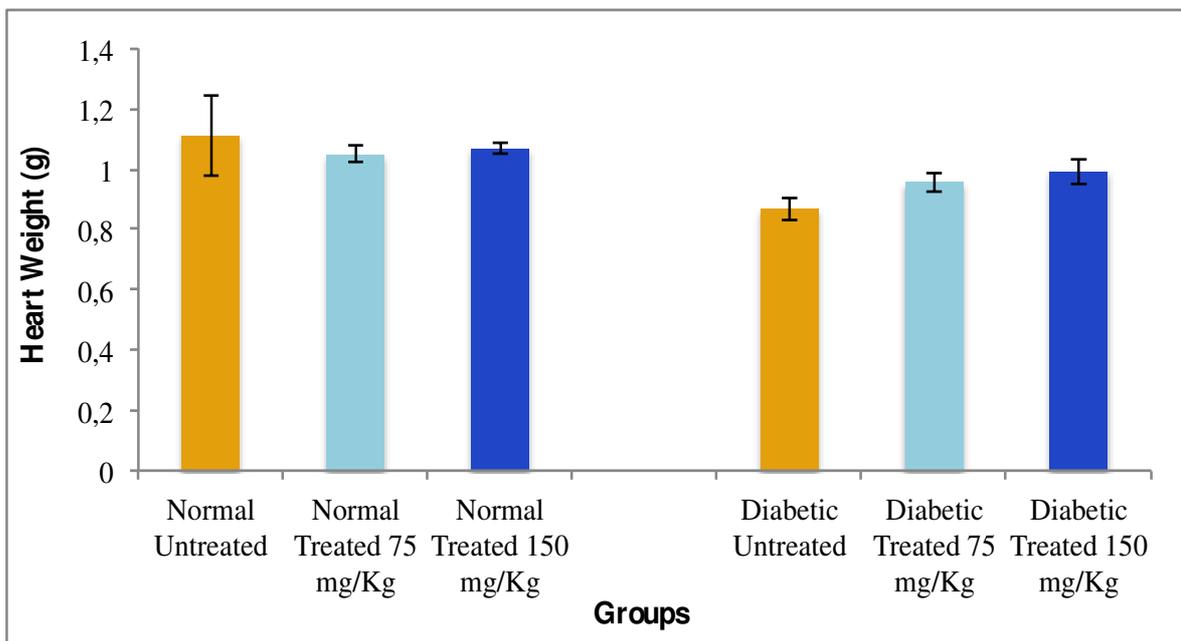


Figure 3.16: Bar charts showing the mean (\pm SEM) weights (g) of the hearts taken in age-matched normal and STZ-induced untreated and treated with cinnamon (75 mg/Kg or 150 mg/Kg) rats after 11 weeks following cinnamon treatment. Data are mean \pm SEM, $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150. ANOVA test was used to compared normal and diabetic rats ($p>0.05$).

3.9.3 Effect of ACE on liver weight

Figure 3.17 shows the data for liver weight of all 6 sub-groups of rats. The results show that the weights of the liver (mean \pm SEM) of treated (75 mg/kg or 150 mg/Kg) normal and diabetic treated rats were not significantly ($p>0.05$) different from the liver weight of untreated normal and diabetic rats. However, cinnamon treatment with higher doses (150 mg/Kg) in diabetic rats had significantly high liver weights compared to untreated and treated (150 mg/kg) normal rats ($p<0.05$). Cinnamon treatment with low doses (75 mg/Kg) diabetic rats also revealed a significantly higher liver weight compared with treated (150 mg/kg) normal rats ($p<0.05$) (Figure 3.17).

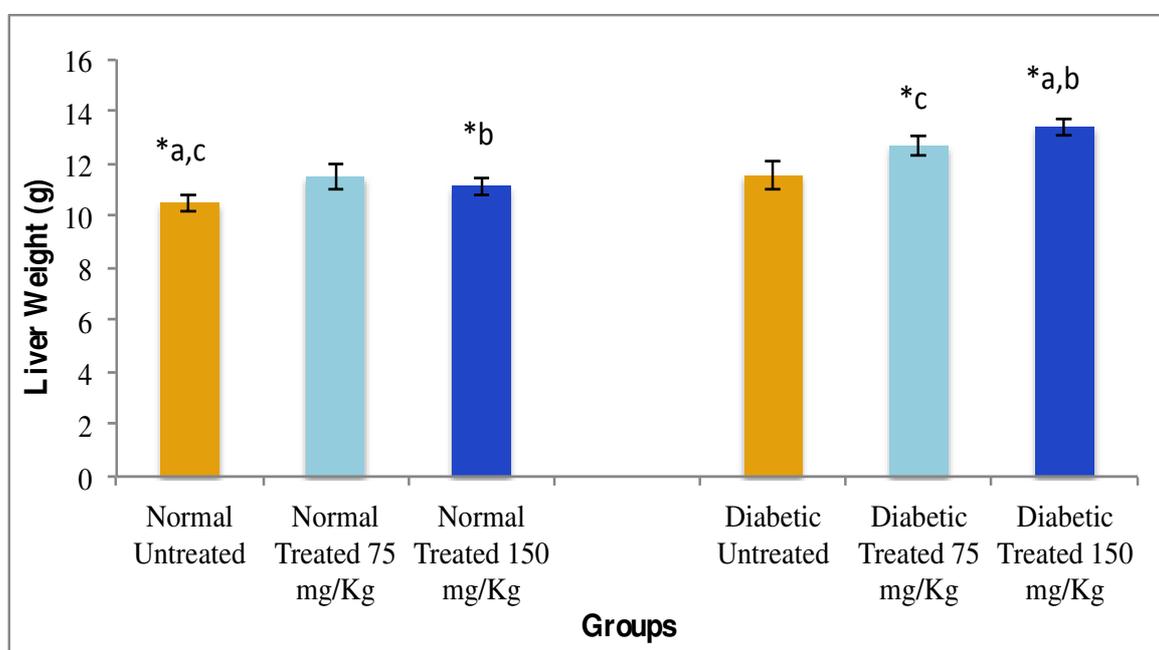


Figure 3.17: Bar charts showing the mean (\pm SEM) weights (g) of the liver taken from age-matched normal and STZ-induced diabetic untreated and treated rats with cinnamon (75 mg/Kg or 150 mg/Kg) after 11 weeks following cinnamon treatment. Data are mean \pm SEM; n=6 rats in normal, normal-treated 75 and diabetic-treated 75 groups; n=5 rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p<0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats (* $p<0.05$).

3.9.4 Effect of ACE on kidney weight

Figure 3.18 shows the data for the weights of the kidney for all 6 sub-groups of rats. The data show that mean (\pm SEM) weights of the kidney of treated (75 mg/kg and 150 mg/kg) diabetic rats were significant ($p < 0.05$) higher compared to untreated and treated age-matched normal rats. The results also revealed that treated (75 mg/kg and 150 mg/kg) diabetic rats had more kidney weights compared to untreated diabetic rats. However, there was no differences in this data ($p > 0.05$) (Figure 3.18).

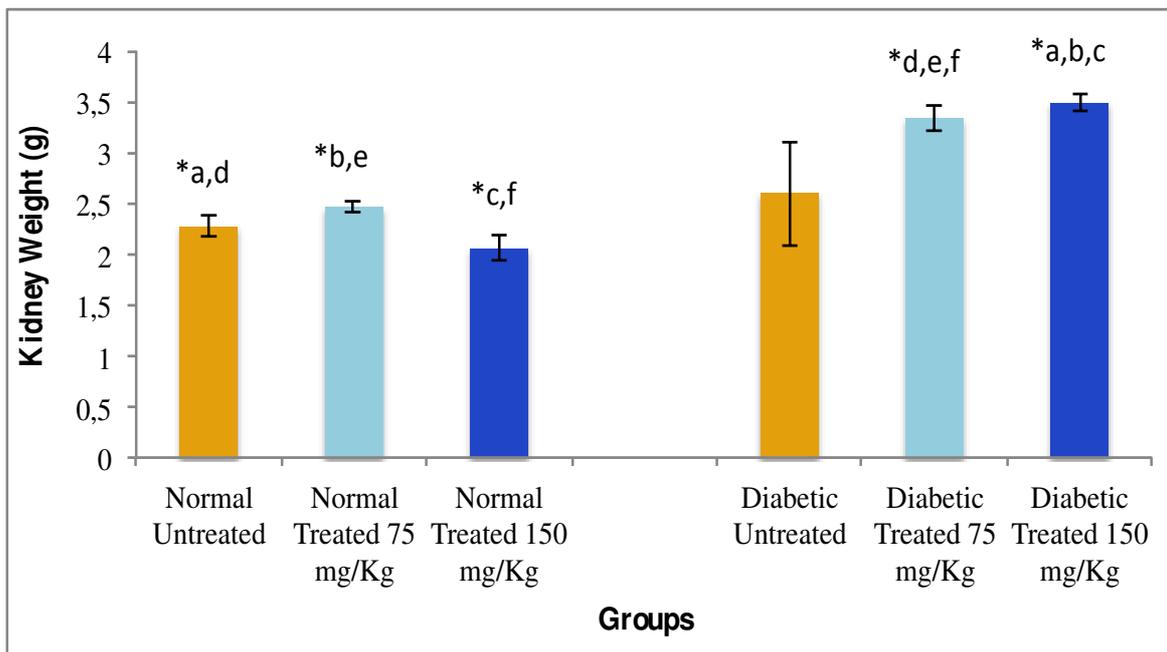


Figure 3.18: Bar charts showing the mean (\pm SEM) weights (g) of the kidney taken from age-matched normal and STZ-induced diabetic untreated and treated with cinnamon (75 mg/Kg or 150 mg/Kg) rats after 11 weeks following cinnamon treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p < 0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats ($*p < 0.05$).

3.9.5 Effect of ACE on soleus muscle weight

Figure 3.19 shows the data for soleus muscle weight of all 6 sub-groups of rats. The data show that the skeletal soleus muscle isolated from treated (75 mg/Kg or 150 mg/Kg cinnamon) diabetic and normal rats weighted more than untreated diabetic and normal rats, respectively, but the values were not significant ($p > 0.05$). However, the results for the untreated diabetic rats show a significant ($p < 0.05$) decrease in the soleus muscle compared to treated normal rats (Figure 3.19).

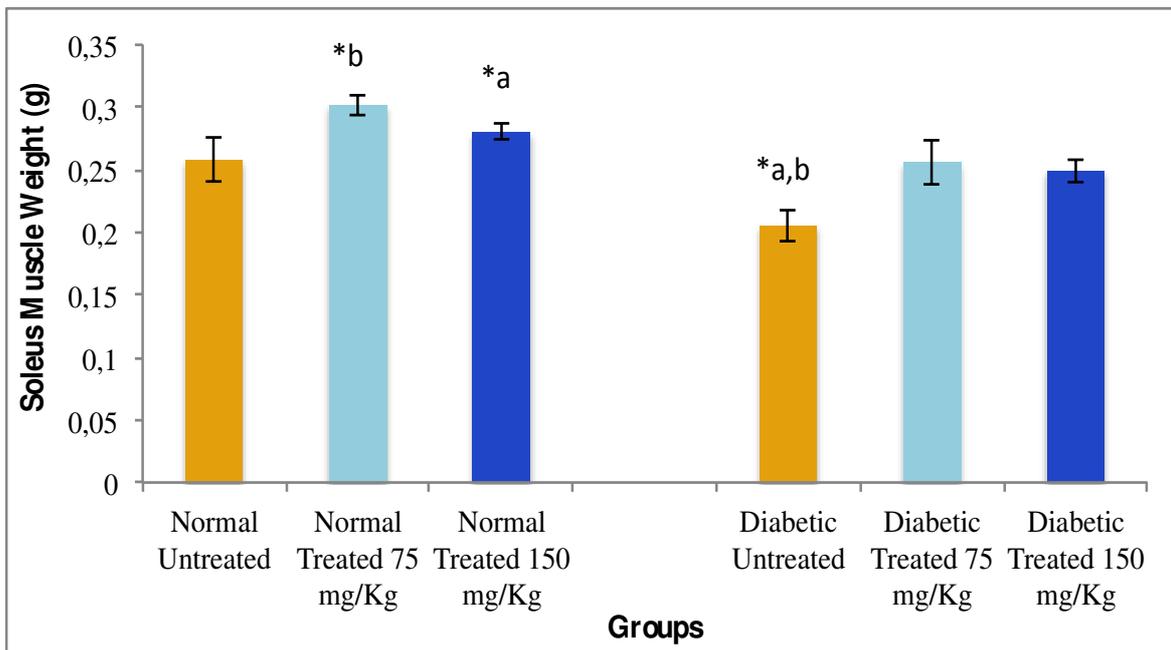


Figure 3.19: Bar charts showing the mean (\pm SEM) weights (g) of the skeletal soleus muscle taken from age-matched normal and STZ-induced diabetic untreated and treated rats with cinnamon (75 mg/Kg or 150 mg/Kg) after 11 weeks following cinnamon treatment. Data are mean \pm SEM; $n=6$ rats in normal, normal-treated 75 and diabetic-treated 75 groups; $n=5$ rats in normal-treated 150, diabetic and diabetic-treated 150; Values sharing common superscript letters are significantly different ($p < 0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats ($*p < 0.05$).

3.9.6 Organs weight ratio

Table 3.6 and Table 3.7 show the tissue or organ weight to body weight ratios for heart, pancreas (table 3.6) and for liver, kidney and soleus muscle (table 3.7). The results revealed a slightly decrease in heart weight to body weight ratio in treated normal (either 75 mg/kg and 150 mg/kg) and diabetic rats (150 mg/kg) compared to untreated normal and diabetic rats. The pancreas weight to body weight ratio was lower in treated normal (150 mg/kg) and diabetic (either 75 mg/kg and 150 mg/kg) rats compared to untreated normal rats. On other hand, liver weight to body weight ratio in treated normal (either 75 mg/kg and 150 mg/kg) and diabetic rats (75 mg/kg) slightly increased compared to untreated normal and diabetic animals. The kidney weight to body weight ratio, similarly to liver weight ratio, increased in treated (75 mg/kg) normal and diabetic (either 75 mg/kg and 150 mg/kg) compared to untreated normal and diabetic animals. Finally, the soleus muscle weight to body weight ratio had the same or a slight increase in treated normal and diabetic compared to untreated animals. Finally, it is noteworthy that all of untreated diabetic organs of body weight ratio was higher than untreated normal organs to body weight ratio (table 3.6 and table 3.7).

After statistical analysis, the results revealed that heart weight to body weight ratio in untreated and treated (both doses) diabetic animals were significant ($p < 0.05$) higher than normal treated with 75 mg/Kg of cinnamon. In pancreas, no significant effect was found between different groups from normal and diabetic animals (table 3.6). Regarding the liver, the results from table 3.7 showed that in normal rats, both untreated and treated had significant lower ($p < 0.05$) liver weight to body weight ratio compared with diabetic untreated and treated animals. However, cinnamon treatment demonstrated no significant effect on normal and diabetic animals compared untreated and treated rats.

In the kidney, normal untreated and treated rats also differ significantly ($p < 0.05$) from diabetic treated rats with both doses of cinnamon. Diabetic treated rats had significantly ($p < 0.05$) more kidney weight to body weight ratio with cinnamon administration (both doses), compared with normal rats. Furthermore, no significant effect was found between untreated and treated diabetic rats (table 3.7).

Finally, in soleus muscle weight to body weight ratio in diabetic animals treated with 75 mg/Kg of ACE demonstrated a significant ($p < 0.05$) increase compared with untreated normal rats (Table 3.7).

Table 3.6: Data showing mean (\pm SEM) the weights of the heart and pancreas and the mean (\pm SEM) heart to body weight ratio and pancreas to body weight ratio, at week 11 following experimental protocol. Data are mean \pm SEM; n= 5 to 6 per group. Values sharing common superscript letters in the same column are significantly different ($p<0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats (* $p<0.05$).

Groups	Body weight (g)	Heart weight (g)	Heart to body <u>ratio</u>	Pancreas weight (g)	Pancreas to body <u>ratio</u>
Normal untreated	378.8 \pm 7.21	1.11 \pm 0.13	0.0029 \pm 0.00029	1.48 \pm 0.32	0.0039 \pm 0.00088
Normal treated 75 mg/kg	395.8 \pm 9.89	1.05 \pm 0.03	0.0026 \pm 0.00004 ^{a,b,c}	1.53 \pm 0.16	0.0038 \pm 0.00035
Normal treated 150 mg/kg	387.5 \pm 6.38	1.07 \pm 0.02	0.0028 \pm 0.00009	1.35 \pm 0.1	0.0035 \pm 0.00024
Diabetic untreated	269.1 \pm 6.22	0.87 \pm 0.04	0.0032 \pm 0.00007 ^a	1.45 \pm 0.17	0.0053 \pm 0.00068
Diabetic treated 75 mg/kg	293.3 \pm 8.49	0.96 \pm 0.03	0.0033 \pm 0.00008 ^b	1.46 \pm 0.08	0.0050 \pm 0.00037
Diabetic treated 150 mg/kg	316.5 \pm 9.57	0.99 \pm 0.04	0.0031 \pm 0.00008 ^c	1.44 \pm 0.15	0.0046 \pm 0.00046

Table 3.7: Data showing the liver to body weight ratio, kidney to body weight ratio and soleus to body weight ratio at week 11 following experimental protocol. Data are mean \pm SEM; n= 5 to 6 per group. Values sharing common superscript letters in the same column are significantly different ($p < 0.05$). ANOVA test was used to compared untreated and treated diabetic and normal rats ($*p < 0.05$).

Groups	Body weight (g)	Liver weight (g)	Liver to body <u>ratio</u>	Kidney weight (g)	Kidney to body <u>ratio</u>	Soleus weight (g)	Soleus to body <u>ratio</u>
Normal untreated	378.8 \pm 7.21	10.50 \pm 0.32	0.0278 \pm 0.00092 ^{a,b,c}	2.27 \pm 0.1	0.0060 \pm 0.00030 ^{a,b}	0.26 \pm 0.02	0.0007 \pm 0.00004 ^a
Normal treated 75 mg/kg	395.8 \pm 9.89	11.48 \pm 0.52	0.0290 \pm 0.00087 ^{d,e,f}	2.47 \pm 0.06	0.0062 \pm 0.00017 ^{c,d}	0.30 \pm 0.01	0.0008 \pm 0.00001
Normal treated 150 mg/kg	387.5 \pm 6.38	11.13 \pm 0.35	0.0288 \pm 0.00097 ^{g,h,i}	2.06 \pm 0.13	0.0053 \pm 0.00037 ^{e,f}	0.28 \pm 0.01	0.0007 \pm 0.00002
Diabetic untreated	269.1 \pm 6.22	11.54 \pm 0.54	0.0430 \pm 0.00204 ^{a,d,g}	2.60 \pm 0.52	0.0097 \pm 0.00198	0.21 \pm 0.01	0.0008 \pm 0.00005
Diabetic treated 75 mg/kg	293.3 \pm 8.49	12.66 \pm 0.35	0.0432 \pm 0.00058 ^{b,e,h}	3.34 \pm 0.13	0.0114 \pm 0.00043 ^{a,c,e}	0.26 \pm 0.02	0.0009 \pm 0.00035 ^a
Diabetic treated 150 mg/kg	316.5 \pm 9.57	13.40 \pm 0.32	0.0424 \pm 0.00043 ^{c,f,i}	3.49 \pm 0.08	0.0110 \pm 0.00043 ^{b,d,f}	0.25 \pm 0.01	0.0008 \pm 0.00002

3.10 Effect of ACE on total antioxidant status of normal and diabetic rats

Figure 3.20 shows the effect of ACE on antioxidant status of in normal and diabetic rats. Oral administration of aqueous *C. burmannii* extract shows that cinnamon can improve the antioxidant status in both normal and diabetic rats. However, these results are not statistically significant ($p>0.05$). In diabetic animals, a dose of 150 mg/Kg seems to be more effective in both normal and diabetic animals compared to untreated normal and diabetic rats rats (figure 3.20).

The results also show that 150 mg/kg bw of cinnamon was more effective in both normal and diabetic rats as an antioxidant compared to 75 mg /kg bw and untreated normal and diabetic animals. These results suggest that cinnamon increased the antioxidant level of treated diabetic rats compared to untreated diabetic rats. In addition, the results also show that antioxidant level decreased in diabetic untreated rats compared to normal untreated animals, suggesting that diabetes is responsible for oxidation.

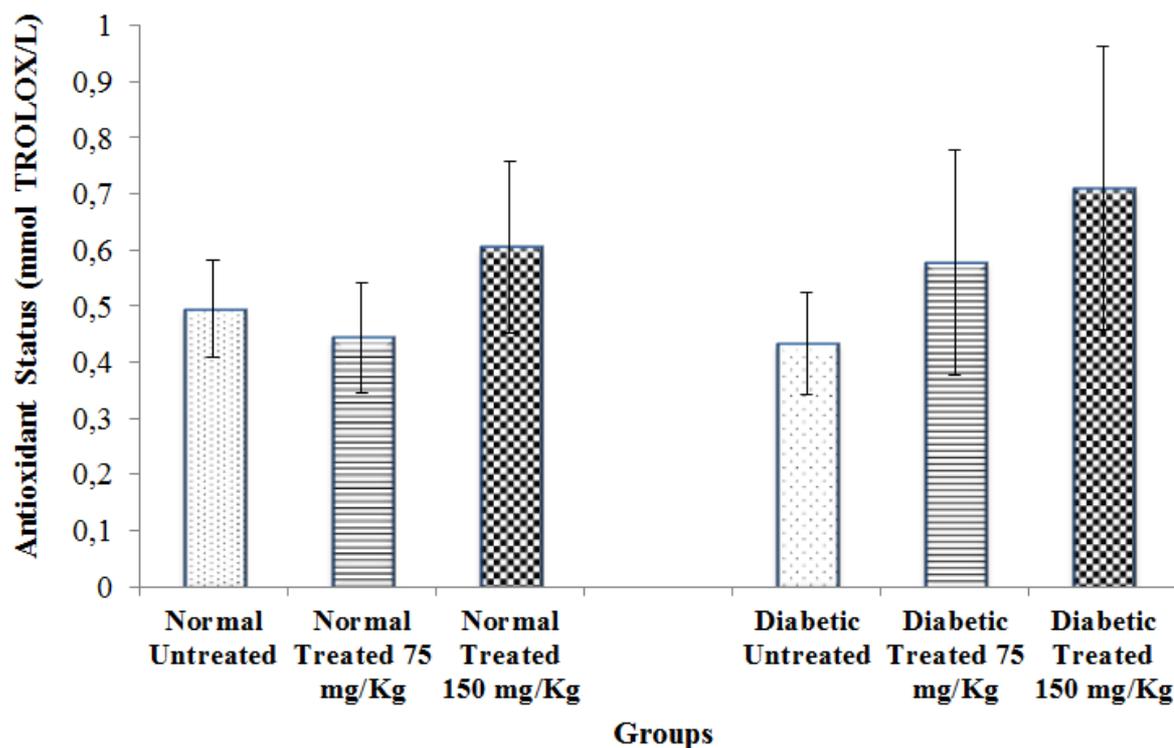


Figure 3.20: Bar charts showing the antioxidant effect of cinnamon (either 75 mg/Kg or 150 mg/Kg) in normal and diabetic rats compared to untreated and treated animals at 11 week of the experimental period. Data are mean \pm SEM; n= 4 rats in normal, normal-treated 150, diabetic-treated 75 and diabetic-treated 150 groups; n= 3 in normal-treated 75 and diabetic groups. ANOVA test was used to compared untreated and treated diabetic and normal rats ($p>0.05$).

3.11 Effect of ACE on tissue and serum cations content of normal and diabetic rats

Cations play major physiological and pathophysiological roles in the body depending on their concentrations in both serum and other organs and tissues of the body. In this series of experiments, levels of sodium (Na^+), magnesium (Mg^{2+}), potassium (k^+), calcium (Ca^{2+}), copper (Cu^{2+}), iron (Fe) and zinc (Zn^{2+}) were measured in blood serum and in heart, pancreas, liver and kidney using ICP-MS method. The results are presented from figure 3.21 to figure 3.59 and are all expressed as mg/ml for serum and mg/mL/100g for organs tissues. The mean value (\pm SEM) of cations levels for each group is represented.

3.11.1 Effect of ACE on cation levels in serum of diabetic and normal rats

Serum sodium level

Figure 3.21 shows the effect of ACE on sodium levels in serum of normal and diabetic rats. The results show a significant decrease ($p < 0.05$) in serum sodium in normal treated rats (both doses) compared to normal untreated rats. There was also a significant decrease ($p < 0.05$) of serum sodium in diabetic treated with 75 mg/kg of aqueous cinnamon extract compared to diabetic untreated (Figure 3.21).

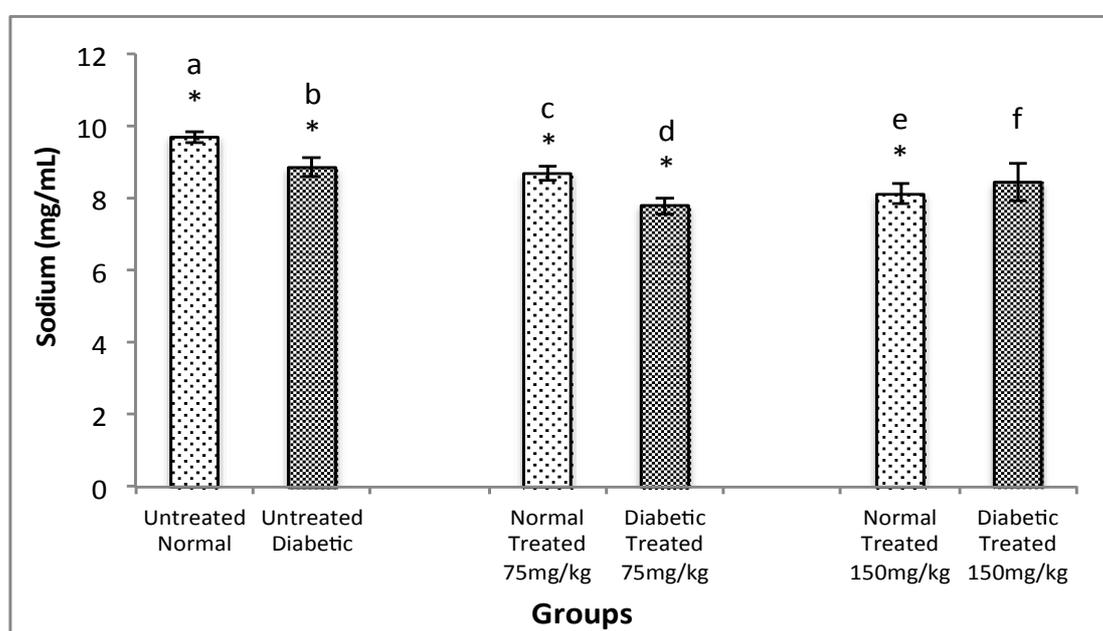


Figure 3.21: Bar charts showing the levels of sodium (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared to b, c and e; $p < 0.05$ for b compared to d.

Serum magnesium level

Figure 3.22 shows the effect of ACE on magnesium levels in serum of normal and diabetic rats. The results show a significant decrease in magnesium levels in diabetic rats treated with 75 mg/kg of aqueous cinnamon extract compared with untreated diabetic rats. However, no significant effect was observed with a high dose of cinnamon in diabetic rats or in normal untreated and treated rats (Figure 3.22).

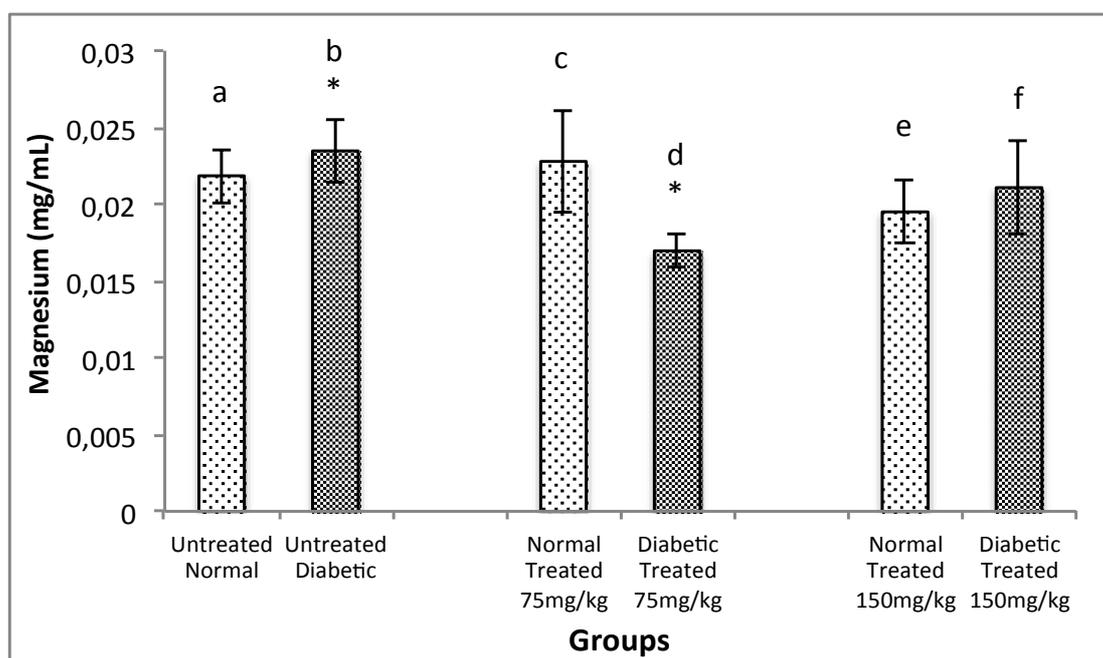


Figure 3.22: Bar charts showing the levels of magnesium (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6; * p<0.05 for b compared to d.

Serum potassium level

Figure 3.23 shows the effect of ACE on potassium levels in serum of normal and diabetic rats. The results show that there was no significant effect in untreated and treated diabetic rats and untreated and treated normal rats (Figure 3.23).

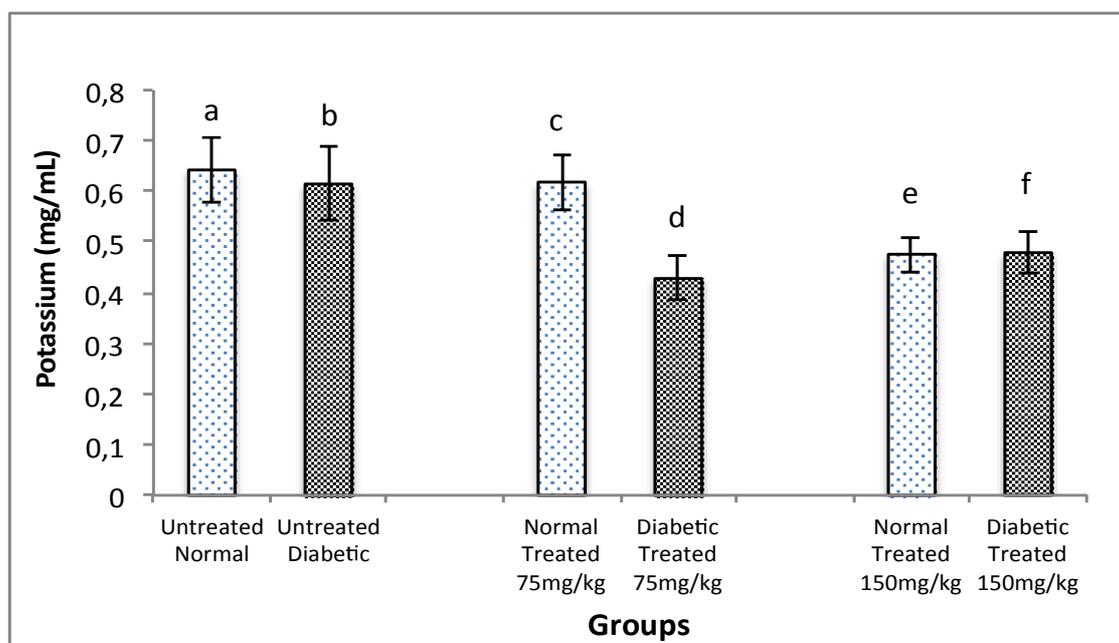


Figure 3.23: Bar charts showing the levels of potassium (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Serum calcium level

Figure 3.24 shows the effect of ACE on calcium levels in serum of normal and diabetic rats. The results show a significant decrease ($p < 0.05$) in serum calcium in normal and diabetic treated rats with 75 mg/kg aqueous cinnamon extract compared to normal and diabetic untreated rats, respectively. The results clearly demonstrate that cinnamon treatment seems to reduce the levels of Ca^{2+} in the serum (Figure 3.24).

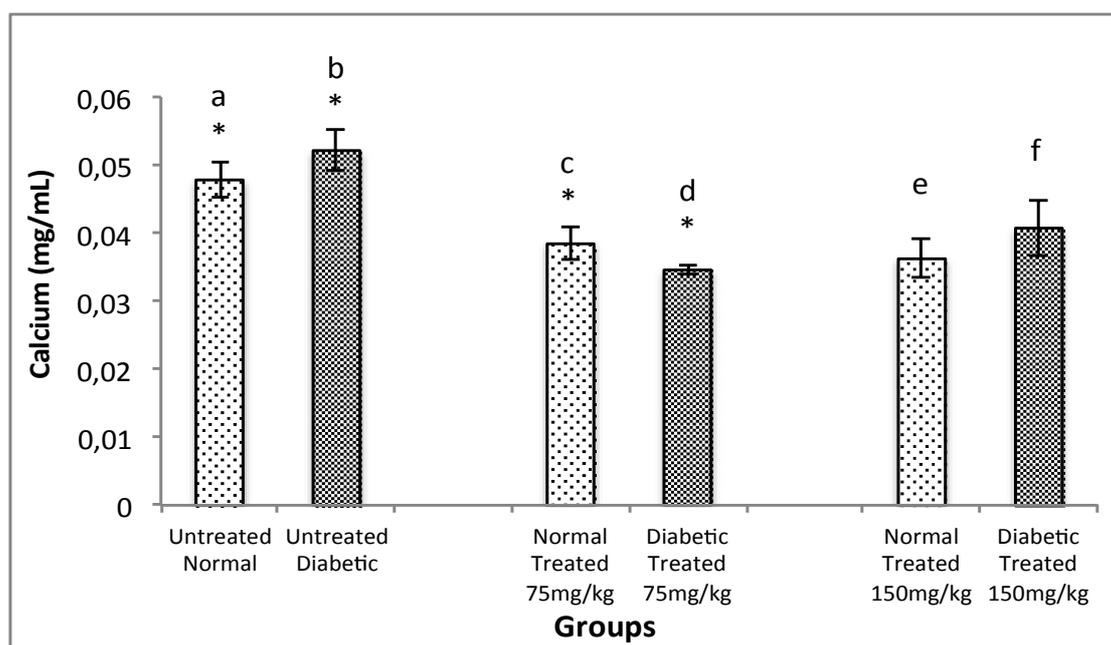


Figure 3.24: Bar charts showing the levels of calcium (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared c; $p < 0.05$ for b compared to d.

Serum copper level

Figure 3.25 shows the effect of ACE on copper levels in serum of normal and diabetic rats. The results show that there was no significant effect on serum copper in untreated and treated diabetic rats and untreated and treated normal rats (Figure 3.25).

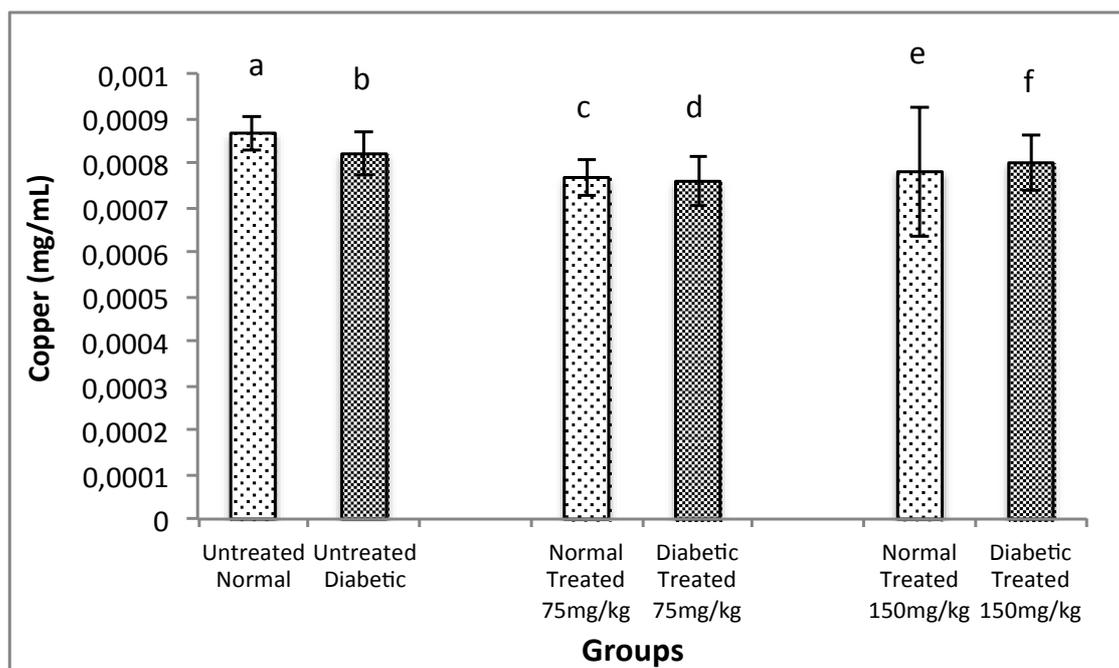


Figure 3.25: Bar charts showing the levels of copper (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Serum iron level

Figure 3.26 shows the effect of ACE on iron levels in serum of normal and diabetic rats. The results show that there was no significant effect on serum iron level in untreated and treated diabetic rats compared with untreated and treated normal rats. However, the serum levels of iron seems to reduce slightly in all the treated group, except for control treated with 75 mg/Kg cinnamon extract (Figure 3.26).

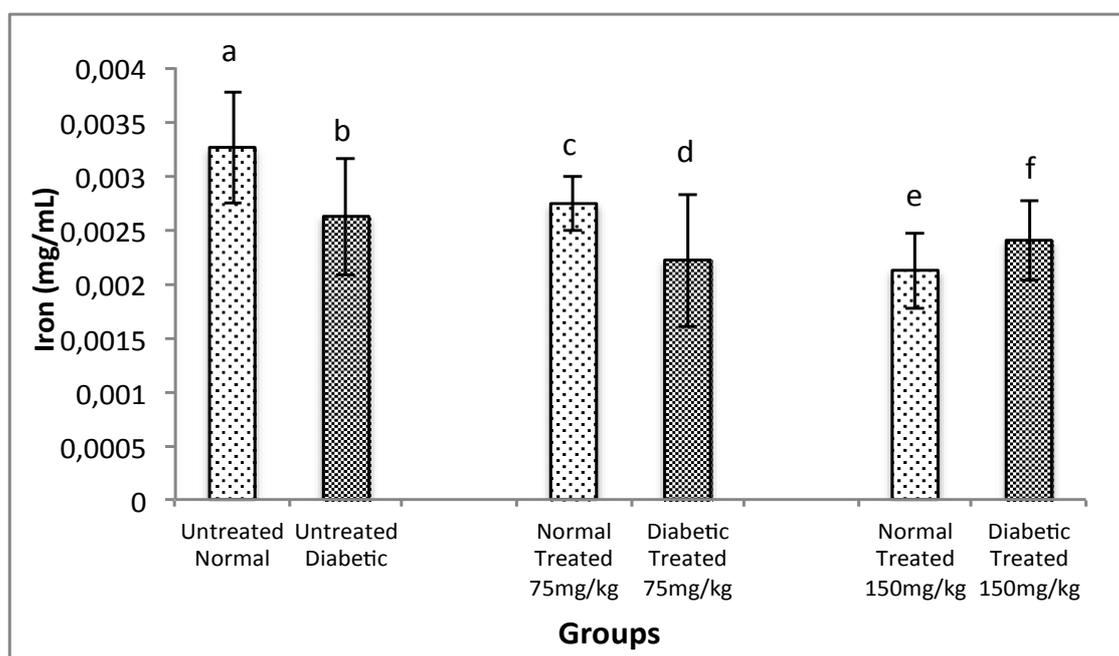


Figure 3.26: Bar charts showing the levels of iron (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Serum zinc level

Figure 3.27 shows the effect of ACE on zinc levels in serum of normal and diabetic rats. The results show that there was no significant effect on serum zinc in untreated and treated diabetic rats and untreated compared to treated normal rats (Figure 3.27).

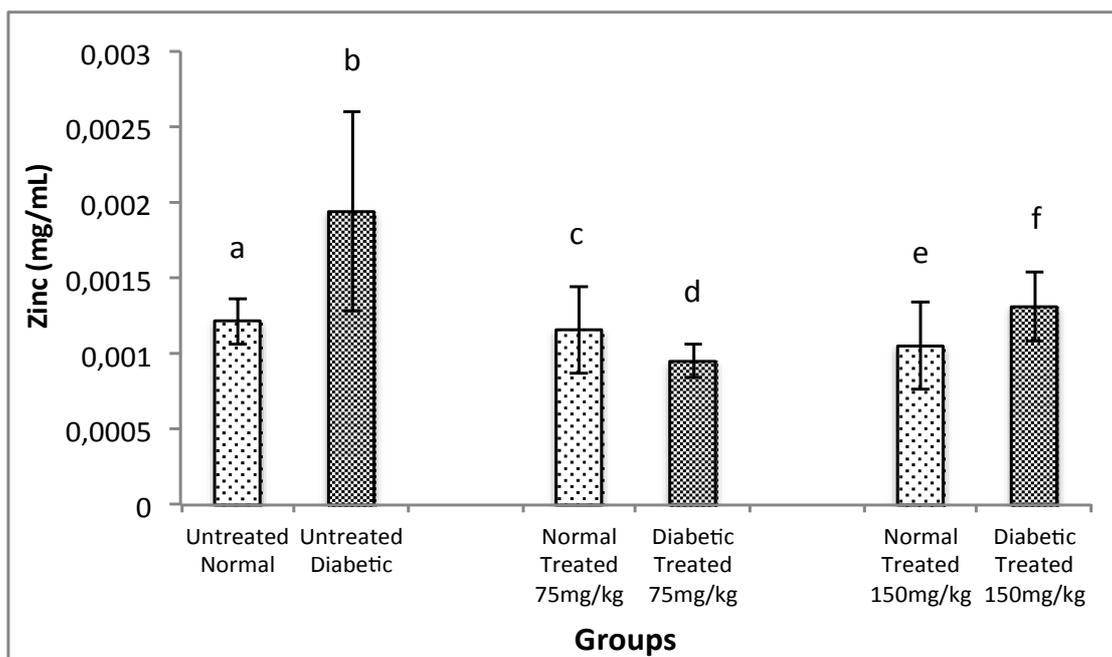


Figure 3.27: Bar charts showing the levels of zinc (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

3.11.2 Effect of ACE on cation levels in heart of diabetic and normal rats

Heart sodium level

Figure 3.28 shows the effect of ACE on sodium levels in hearts of normal and diabetic rats. The heart sodium results show a significant decrease ($p < 0.05$) in diabetic treated rats with 75 mg/kg and 150 mg/kg of aqueous cinnamon extract compared to untreated normal rats (Figure 3.28).

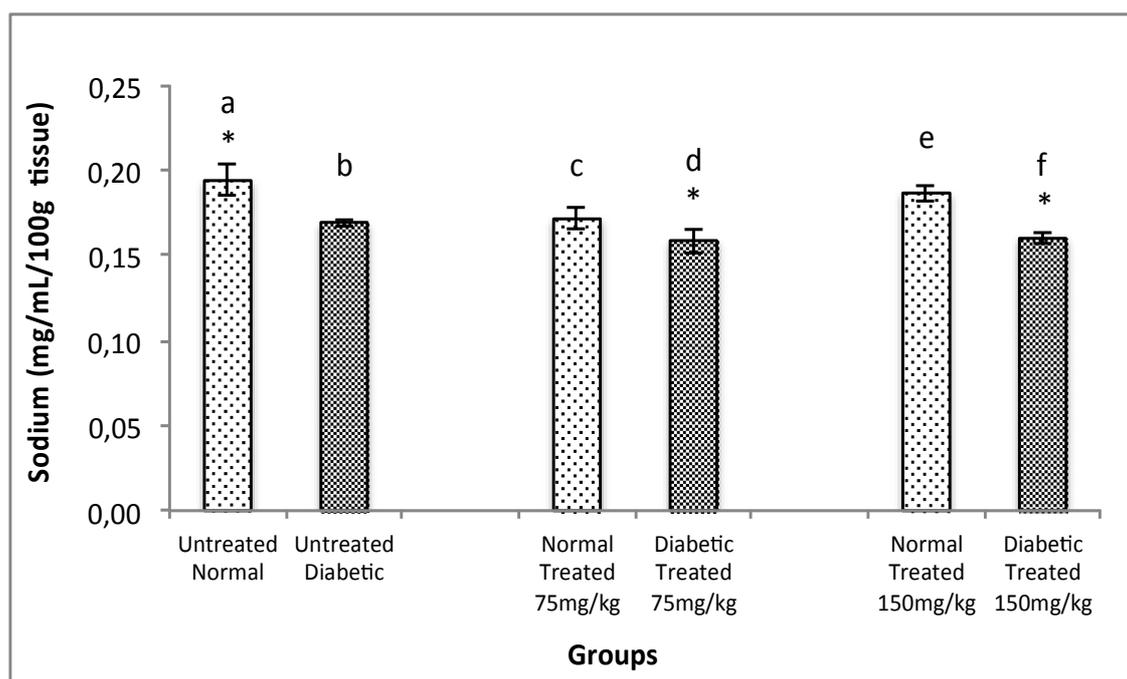


Figure 3.28: Bar charts showing the levels of sodium (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared to d and f.

Heart magnesium level

Figure 3.29 shows the effect of ACE on magnesium levels in hearts of normal and diabetic rats. The results show that untreated diabetic rats had significant ($p < 0.05$) less magnesium in the heart than untreated normal rats. However, the treatment with both doses (75 and 150 mg/kg) of cinnamon increase significantly ($p < 0.05$) the magnesium levels in heart. The treated diabetic rats with high dose (150 mg/kg) had significantly ($p < 0.05$) more magnesium than treated 75 mg/kg and 150 mg/kg normal rats (Figure 3.29).

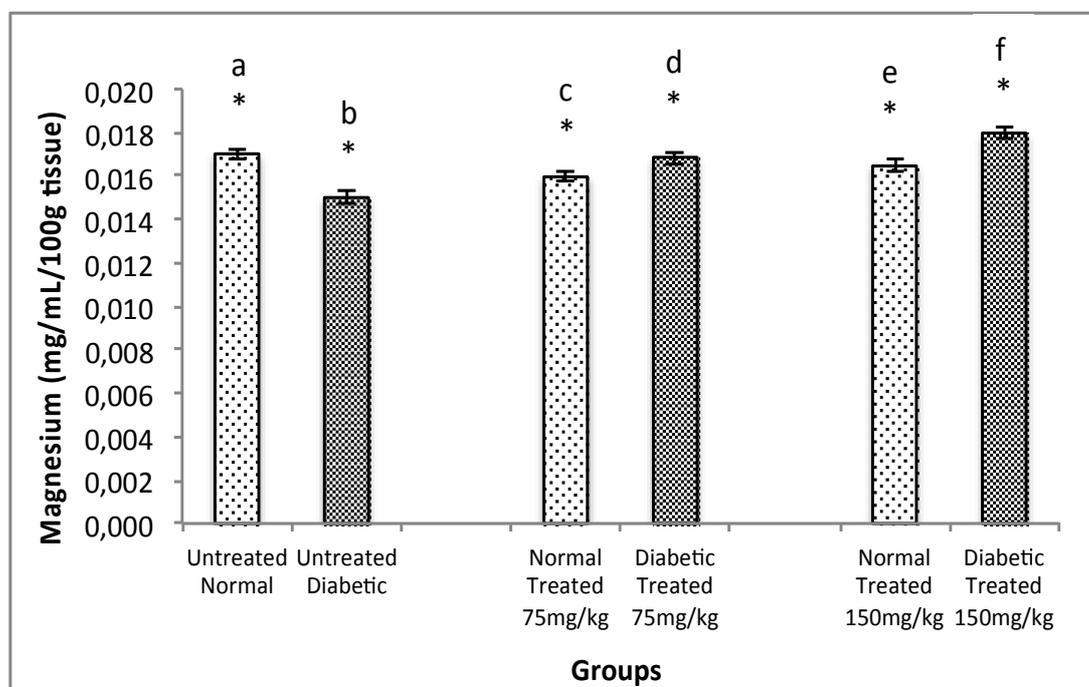


Figure 3.29: Bar charts showing the levels of magnesium (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared b; $p < 0.05$ for a compared c; $p < 0.05$ for b compared to d and f; $p < 0.05$ for f compared to c and e.

Heart potassium level

Figure 3.30 shows the effect of ACE on potassium levels in hearts of normal and diabetic rats. The results show that treatment with 75 and 150 mg/kg of cinnamon in diabetic rats (d and f) increased significantly ($p < 0.05$) potassium levels in heart compared to untreated diabetic rats (b). Furthermore, the heart of treated (150 mg/kg) diabetic rats (f) contains significantly ($p < 0.05$) more potassium levels compared to treated (150 mg/kg) normal rats (e) (Figure 3.30).

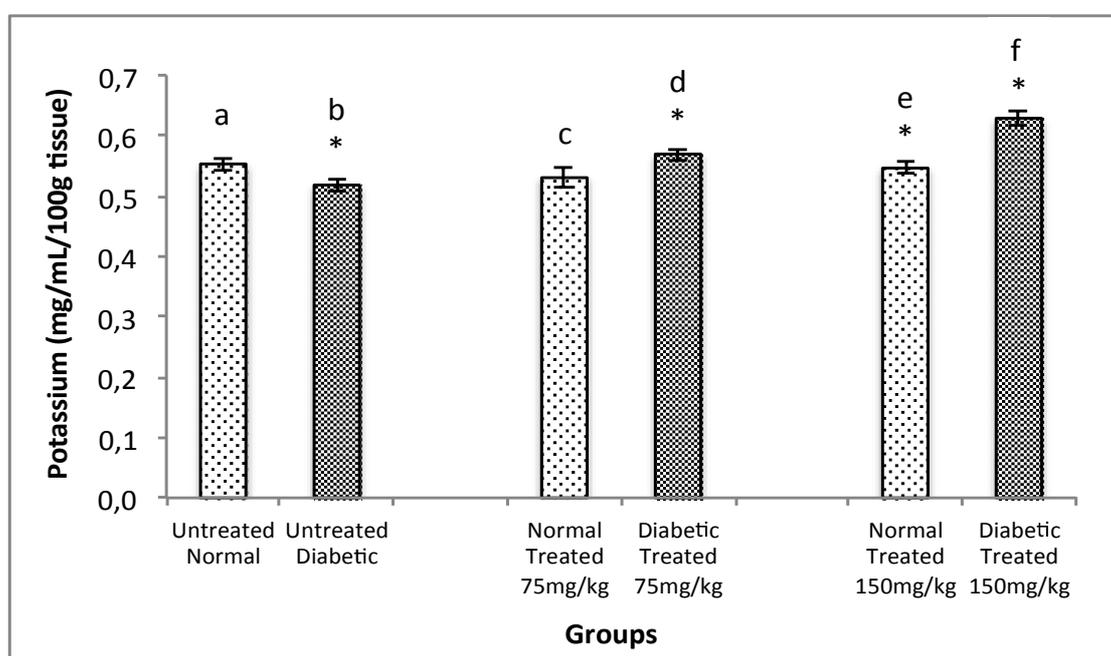


Figure 3.30: Bar charts showing the levels of potassium (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for b compared to d and f; $p < 0.05$ for e compared to p.

Heart calcium level

Figure 3.31 shows the effect of ACE on calcium levels in hearts of normal and diabetic rats. The data show that untreated diabetic rat hearts contain more Ca^{2+} compared to untreated rats. Furthermore, the results also show that treatment with 150 mg/kg of cinnamon in diabetic rats increased significantly ($p < 0.05$) calcium levels in heart compared to untreated diabetic rats. No significant difference was found in treated normal rats compared to untreated normal rats (Figure 3.31).

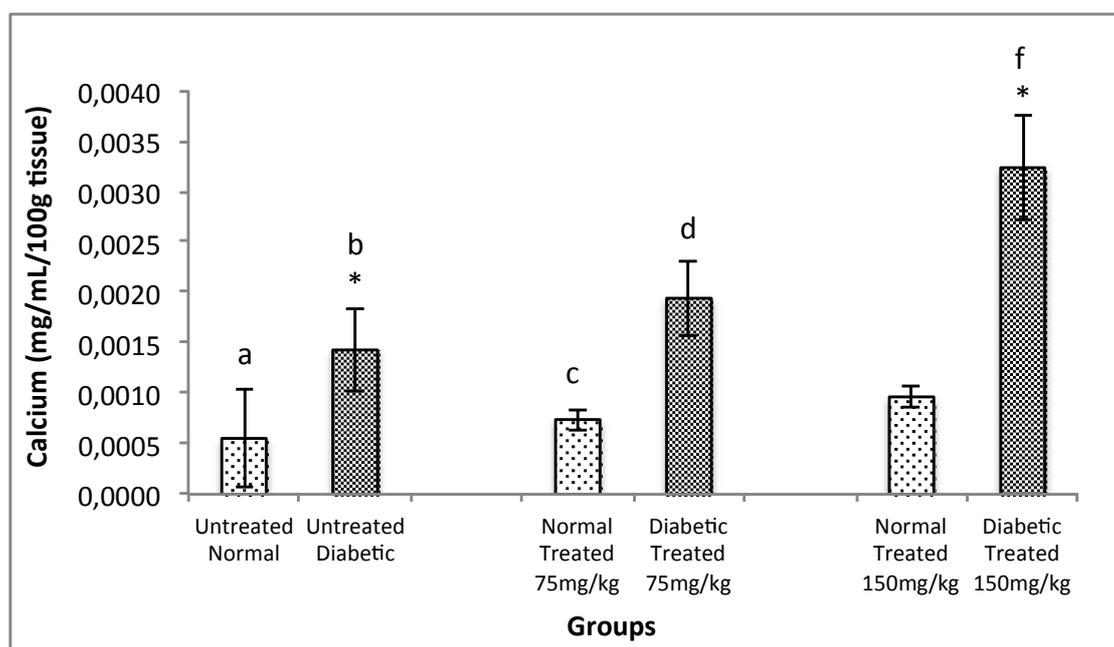


Figure 3.31: Bar charts showing the levels of calcium (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for b compared to f.

Heart copper level

Figure 3.32 shows the effect of ACE on copper levels in hearts of normal and diabetic rats. The results show that the heart of untreated diabetic rats contained significantly less copper ($p < 0.05$) than untreated normal rats. Furthermore, treatment with cinnamon (75 mg/Kg) demonstrated the opposite effect, with a significantly decrease in copper level in normal rats compared with untreated normal rats. The copper level also increased significantly in diabetic rats compared to untreated diabetic rats (Figure 3.32).

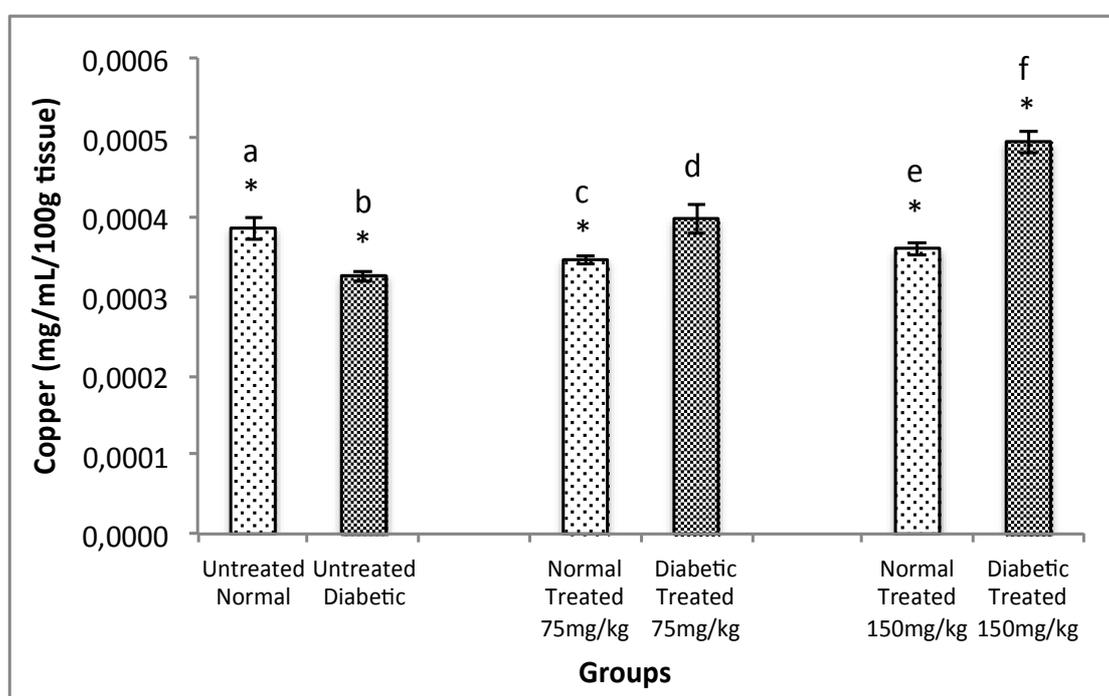


Figure 3.32: Bar charts showing the levels of copper (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. * $p < 0.05$ for f compared with a, b, c, and e.

Heart iron level

Figure 3.33 shows the effect of ACE on iron levels in hearts of normal and diabetic rats. The results show that hearts from diabetic untreated rats contain less iron compared to untreated normal. The results also show that treatment with high dose (150 mg/kg) of cinnamon increased significantly ($p < 0.05$) iron levels in normal and diabetic rats compared to untreated normal and diabetic rats, respectively. No significant difference was found between treatment with 75 mg/Kg of cinnamon comparing normal with diabetic animals (Figure 3.33).

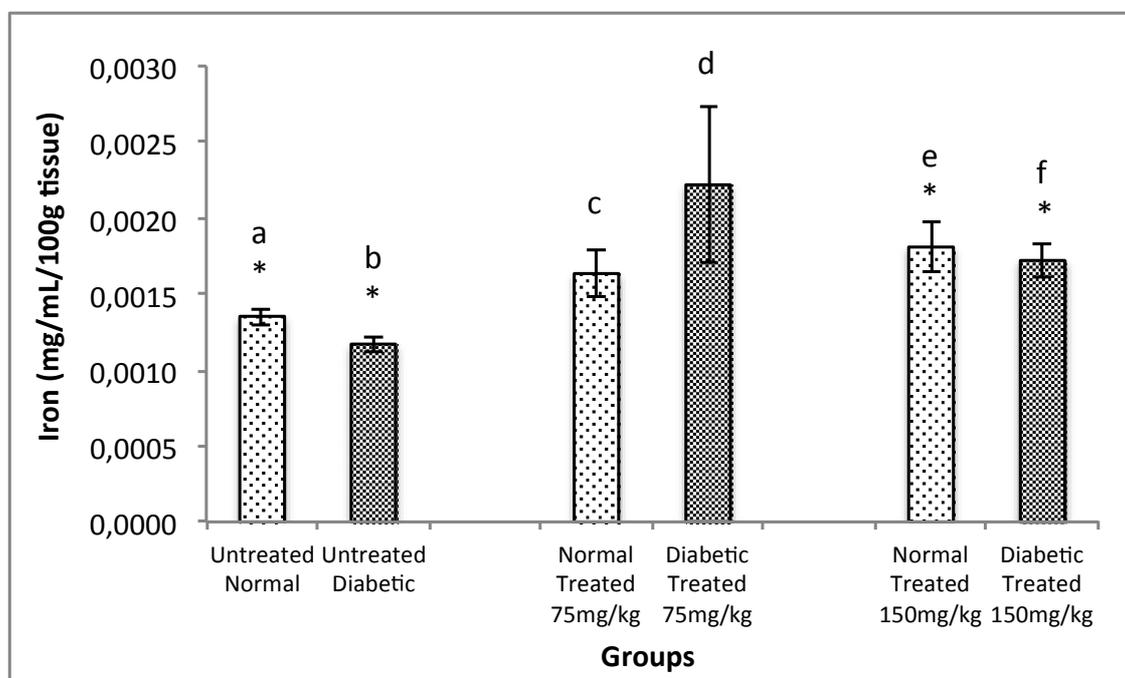


Figure 3.33: Bar charts showing the levels of iron (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared to e; $p < 0.05$ for b compared to f.

Heart zinc level

Figure 3.34 shows the effect of ACE on zinc levels in hearts of normal and diabetic rats. The results show that 75 mg/kg of cinnamon treatment decreased significantly ($p < 0.05$) zinc levels in the hearts of normal rats (c) compared to untreated normal rats (a). However, no significant difference was found in hearts of treated diabetic rats compared to untreated diabetic rats. The data further show that hearts from diabetic rats (f) treated with 150 mg/kg cinnamon contain more zinc compared to control treated rats (e) with same dose of cinnamon. However, this results was not significantly ($p > 0.05$) (Figure 3.34).

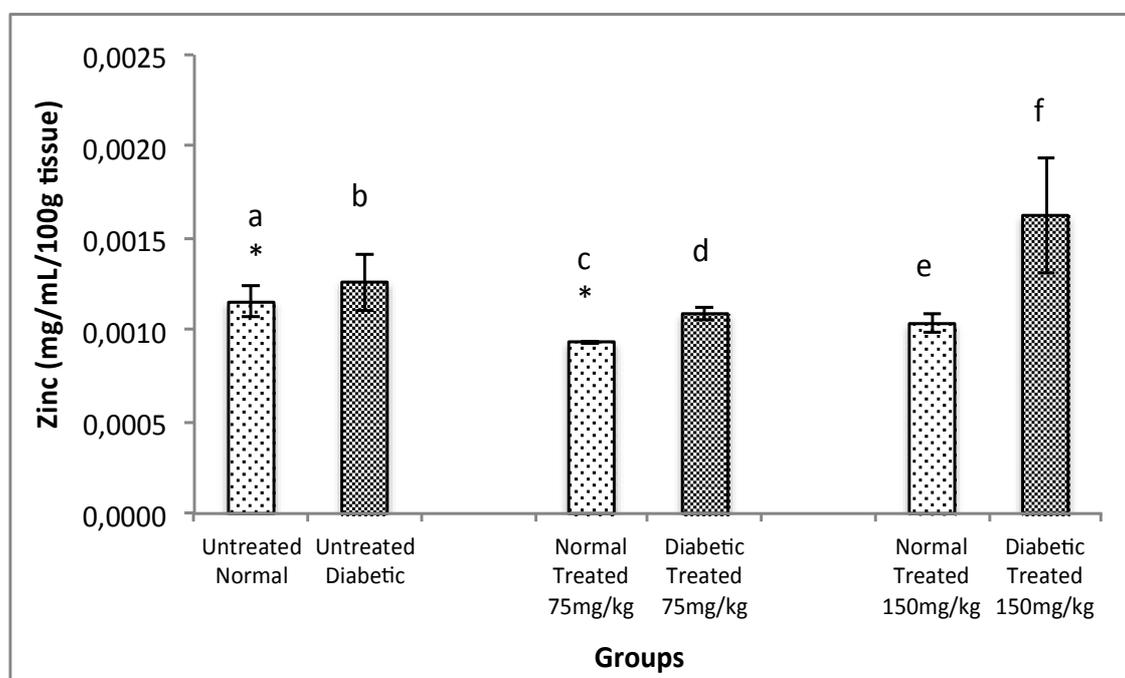


Figure 3.34: Bar charts showing the levels of zinc (mg/ml/100g tissue) in hearts of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared c.

3.11.3 Effect of ACE on cation levels in liver of diabetic and normal rats

Liver sodium level

Figure 3.35 shows the effect of ACE on sodium levels in livers of normal and diabetic rats. The results show that no significant difference was found in treated diabetic and treated normal rats compared to untreated diabetic and untreated normal rats (Figure 3.35).

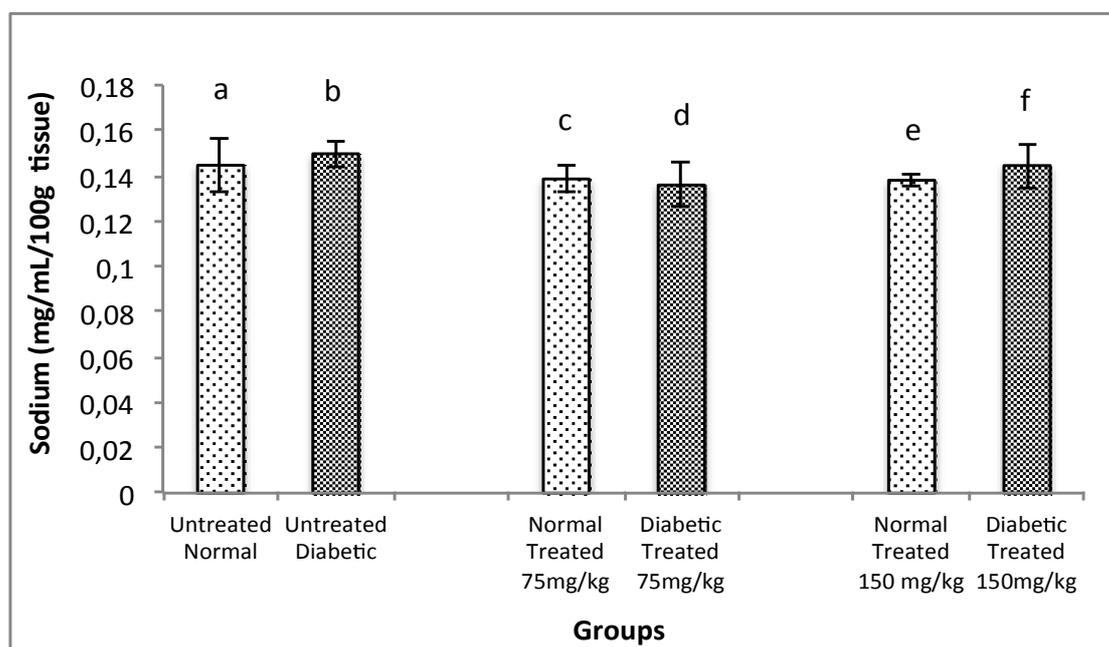


Figure 3.35: Bar charts showing the levels of sodium (mg/ml/100g tissue) in the livers of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Liver magnesium level

Figure 3.36 shows the effect of ACE on magnesium levels in livers of normal and diabetic rats. The results show that both doses of cinnamon can reduce liver magnesium level in the diabetic rats compared to untreated diabetic animals with both 75mg/Kg and 150 mg/Kg cinnamon, however these was no significant decrease in liver magnesium levels. In addition, the liver of diabetic rats (treated and untreated) contains less magnesium level compared to the respective controls (treated and untreated) (Figure 3.36).

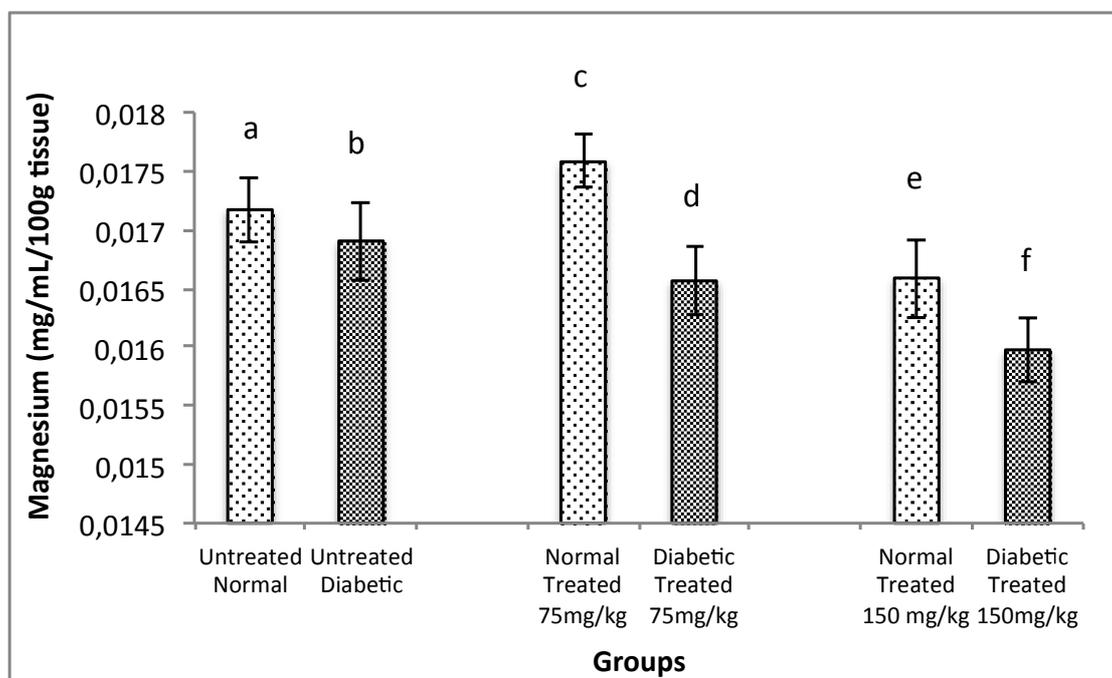


Figure 3.36: Bar charts showing the levels of magnesium (mg/ml/100g tissue) in the livers of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Liver potassium level

Figure 3.37 shows the effect of ACE on potassium levels in livers of normal and diabetic rats. The data show that a high dose (150 mg/Kg) of cinnamon can reduce liver potassium level in the both normal (e) and diabetic (f) rats compared to normal and diabetic untreated rats, however these results were not significantly different. The results also show that cinnamon extract had no effect on liver potassium level in normal and diabetic untreated and treated with 75 mg/Kg cinnamon (Figure 3.37).

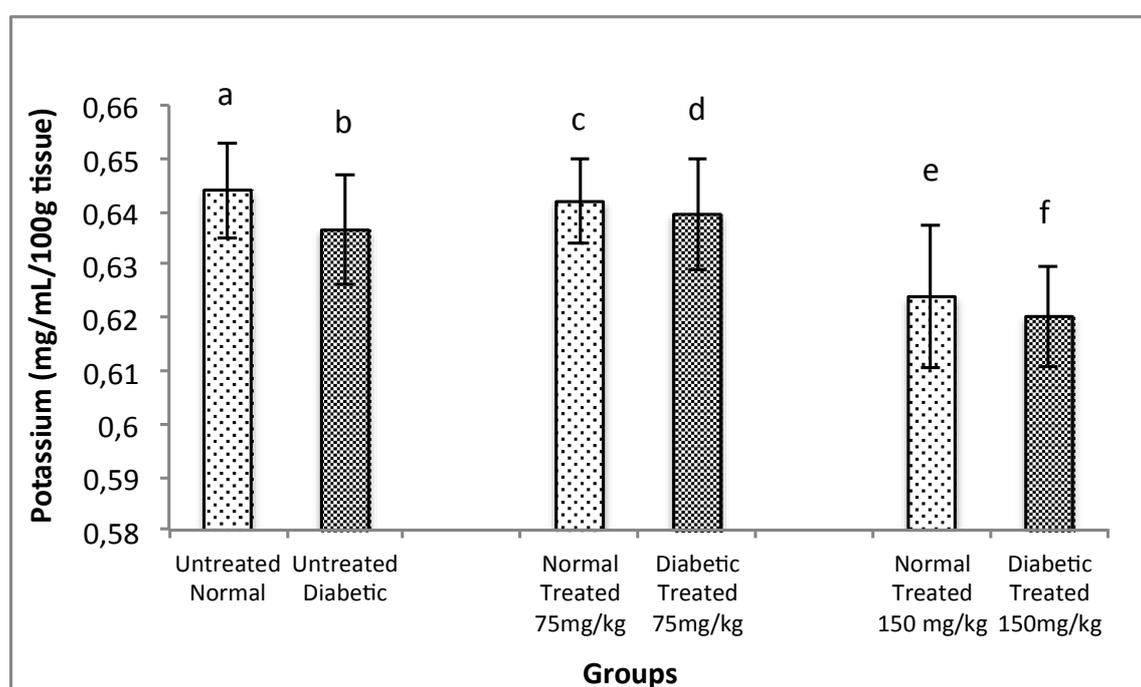


Figure 3.37: Bar charts showing the levels of potassium (mg/ml/100g tissue) in the livers of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Liver iron level

Figure 3.38 shows the effect of ACE on iron levels in livers of normal and diabetic rats. The results show that the liver of untreated diabetic rats (b) had significant ($p < 0.05$) less iron than untreated normal rats (a). Treated (75 mg/kg) diabetic rats (d) also had significantly ($p < 0.05$) less iron than treated (75 mg/kg) normal rats (c). The results show that cinnamon seems to decrease liver iron levels in treated normal rats compared to untreated normal rats but only with 75 mg/Kg dose. Furthermore, cinnamon administration seems had no effect in liver iron level in diabetic rats compared to untreated diabetic rats (Figure 3.38).

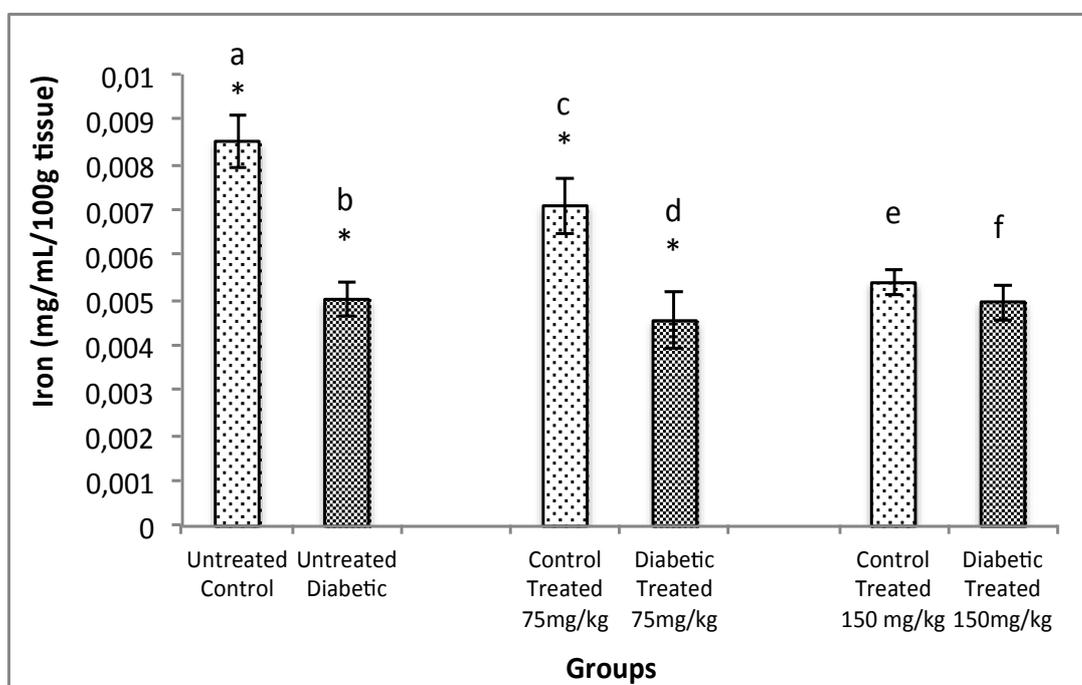


Figure 3.38: Bar charts showing the concentration of iron (mg/ml/100g tissue) untreated rats in the livers in normal and diabetic and treated normal and diabetic treated following daily administration of with 75 mg/kg or 150 mg/kg cinnamon of the rats. Data are mean \pm SEM, $n=5-6$; * $p < 0.05$ for a compared to b; $p < 0.05$ for c compared to b and to d.

Liver copper level

Figure 3.39 shows the effect of ACE on copper levels in liver of normal and diabetic rats. The results show no significant differences in liver copper levels in treated diabetic and treated normal rats compared to untreated diabetic and untreated normal rats in copper levels (Figure 3.39).

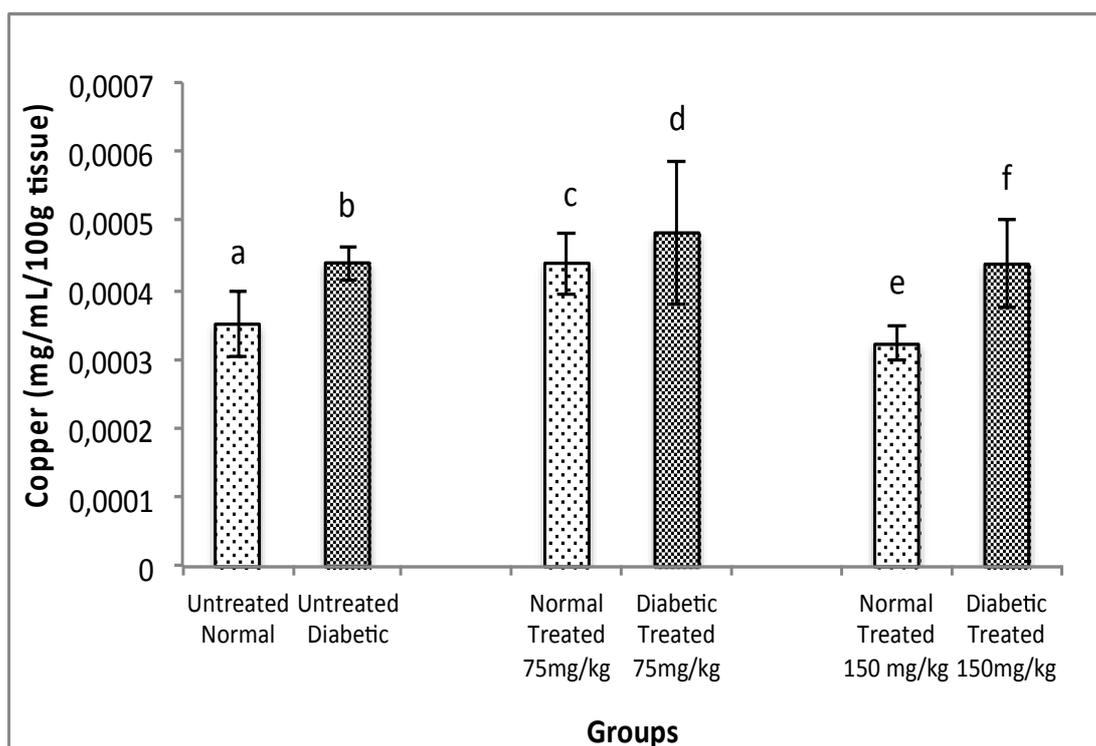


Figure 3.39: Bar charts showing the levels of copper (mg/ml/100g tissue) in the livers of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Liver zinc level

Figure 3.40 shows the effect of ACE on zinc levels in liver of normal and diabetic rats. The results show that no significant differences ($p>0.05$) in liver zinc level in treated diabetic and treated normal rats compared to untreated diabetic and untreated normal rats in zinc levels (Figure 3.40).

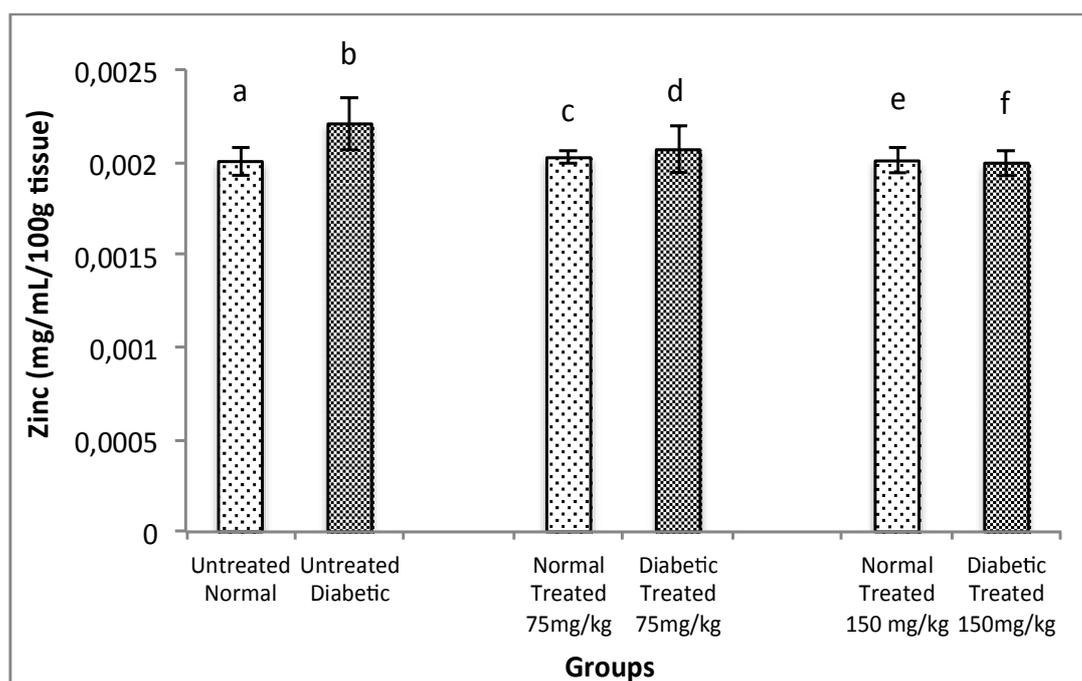


Figure 3.40: Bar charts showing the levels of zinc (mg/ml/100g tissue) in the livers of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

3.11.4 Effect of ACE on cation levels in kidney of diabetic and normal rats

Kidney sodium level

Figure 3.41 shows the effect of ACE on sodium levels in kidney of normal and diabetic rats. The results show that there were no significant changes in kidney sodium levels in treated diabetic and treated normal rats compared to untreated diabetic and treated normal rats in zinc levels (Figure 3.41).

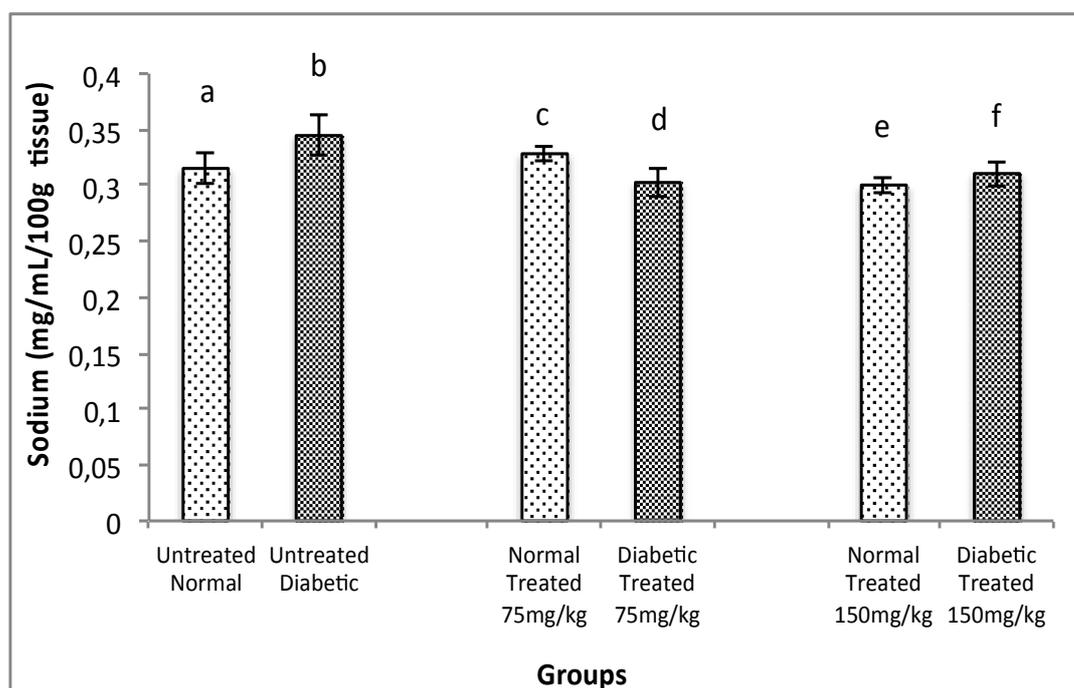


Figure 3.41: Bar charts showing the levels of sodium (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Kidney magnesium level

Figure 3.42 shows the effect of ACE on magnesium levels in kidney of normal and diabetic rats. The results show that treated (150 mg/kg) diabetic rats (f) had significantly ($p < 0.05$) less magnesium than treated (150 mg/kg) normal rats (e). However, there were no significant changes in kidney sodium levels in treated (75 mg/kg) diabetic and normal rats compared to untreated diabetic and normal rats (Figure 3.42).

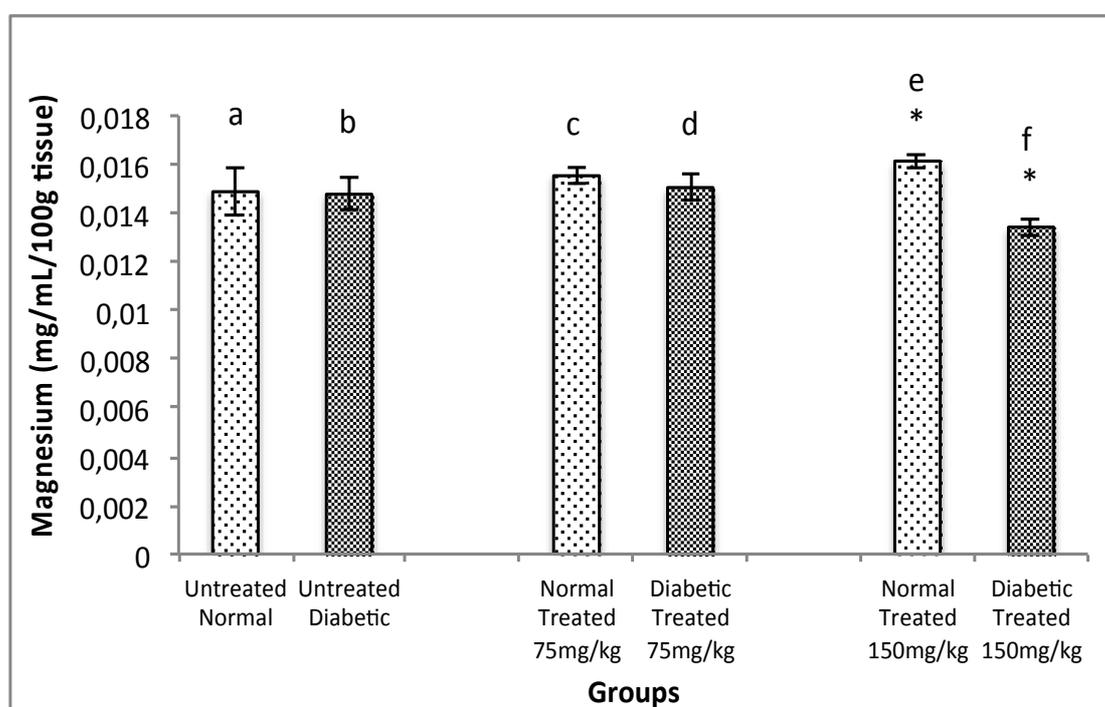


Figure 3.42: Bar charts showing the levels of magnesium (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. * $p < 0.05$ for e compared to f.

Kidney potassium level

Figure 3.43 shows the effect of ACE on potassium levels in kidney of normal and diabetic rats. The results show that there were no significant changes in kidney potassium levels in treated diabetic and treated normal rats compared to untreated diabetic and untreated normal rats. However, cinnamon seems to slightly decrease kidney potassium level in treated 150 mg/Kg diabetic rats compared to untreated diabetic rats (Figure 3.43).

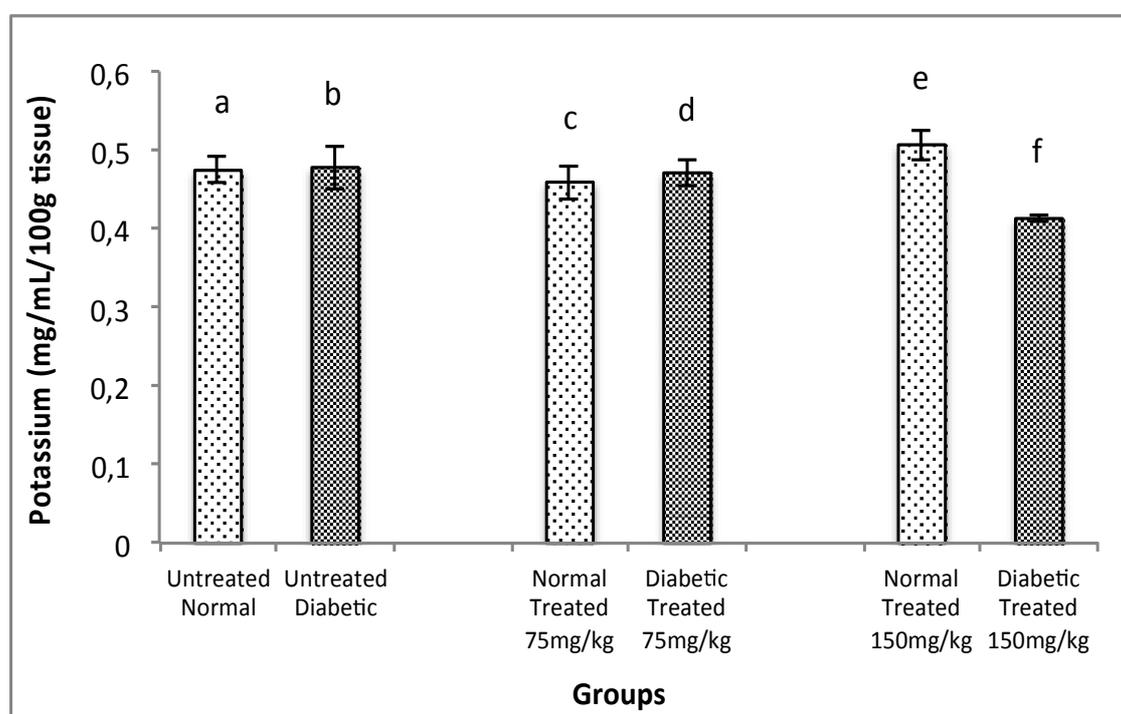


Figure 3.43: Bar charts showing the levels of potassium (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Kidney calcium level

Figure 3.44 shows the effect of ACE on calcium levels in kidney of normal and diabetic rats. The results show that the diabetic untreated kidneys contain more or less the calcium compared to untreated normal kidneys. However, cinnamon treatment seem to cause large and significant ($p < 0.05$) decreases kidney calcium level in both normal and diabetic treated rats employing 150 mg/kg cinnamon compared to untreated normal and diabetic rats (Figure 3.44, compare a and b with e and f).

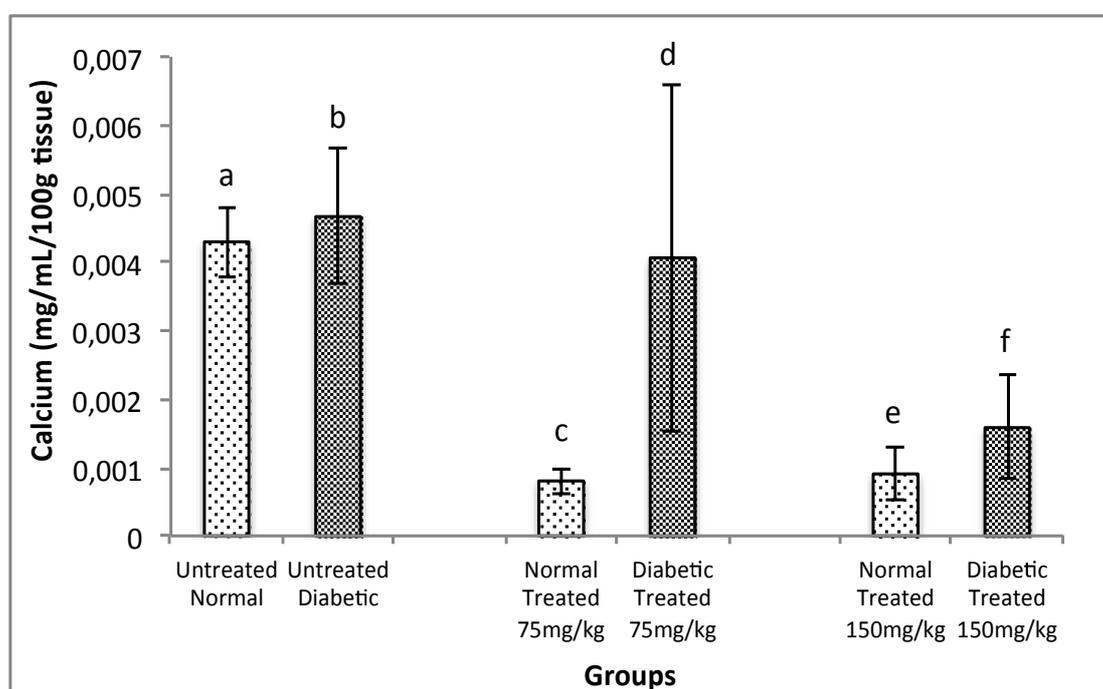


Figure 3.44: Bar charts showing the levels of calcium (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. Note that $p < 0.05$ for e and f compared to a and b, respectively. No significant differences was observed between different groups (a, b, c, d, e and f) ($p > 0.05$).

Kidney iron level

Figure 3.45 shows the effect of ACE on iron levels in kidney of normal and diabetic rats. The results show that untreated diabetic rats (b) had a slightly decrease iron level compared to untreated normal rats (a). Cinnamon treatment (either 75 and 150 mg/kg, c and e) significantly decrease ($p < 0.05$) the iron levels in normal rats (c and e) compared to untreated normal rats (a). Treated diabetic rats (d) with 75 mg/Kg cinnamon seem to decrease kidney iron levels compared to untreated diabetic animals (b). In contrast, kidney iron levels seem to increase in diabetic rats (f) treated with 150 mg/Kg cinnamon compared to treated controls rats (e). However, there were no significant changes in kidney iron levels in treated diabetic rats (c and e) compared to untreated diabetic rats (b) (Figure 3.45).

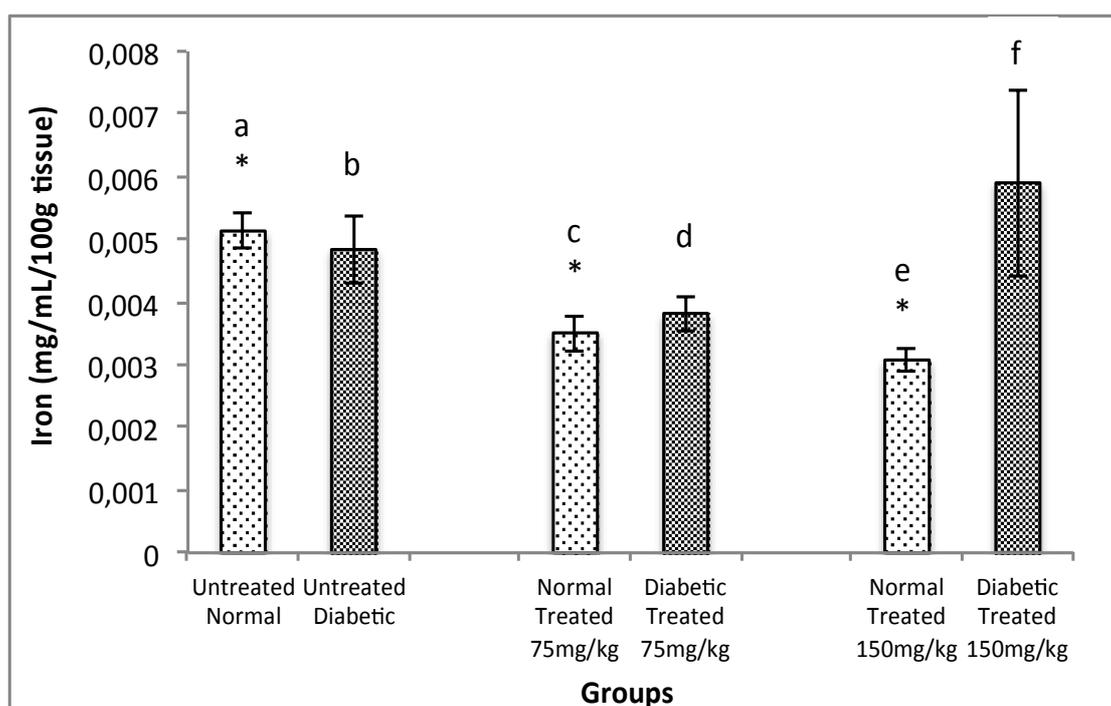


Figure 3.45: Bar charts showing the levels of iron (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. * $p < 0.05$ for a compared to c and to e.

Kidney copper level

Figure 3.46 shows the effect of ACE on copper levels in kidney of normal and diabetic rats. The results show that kidneys of untreated diabetic rats (b) had significant ($p < 0.05$) more copper levels than kidneys from untreated normal rats (a). The same occurs in treatment group, with either 75 or 150 mg/kg cinnamon in which diabetic rats (d and f) had significant ($p < 0.05$) more kidney copper levels than treated (75 and 150 mg/kg) normal rats (c and e), respectively. In general, the data clearly demonstrated that diabetes seem to cause an accumulation in copper in the kidneys of the animals compared to normal rats. However, cinnamon treatment did not alter the kidney copper level in diabetic rats compared to untreated diabetic rats (Figure 3.46).

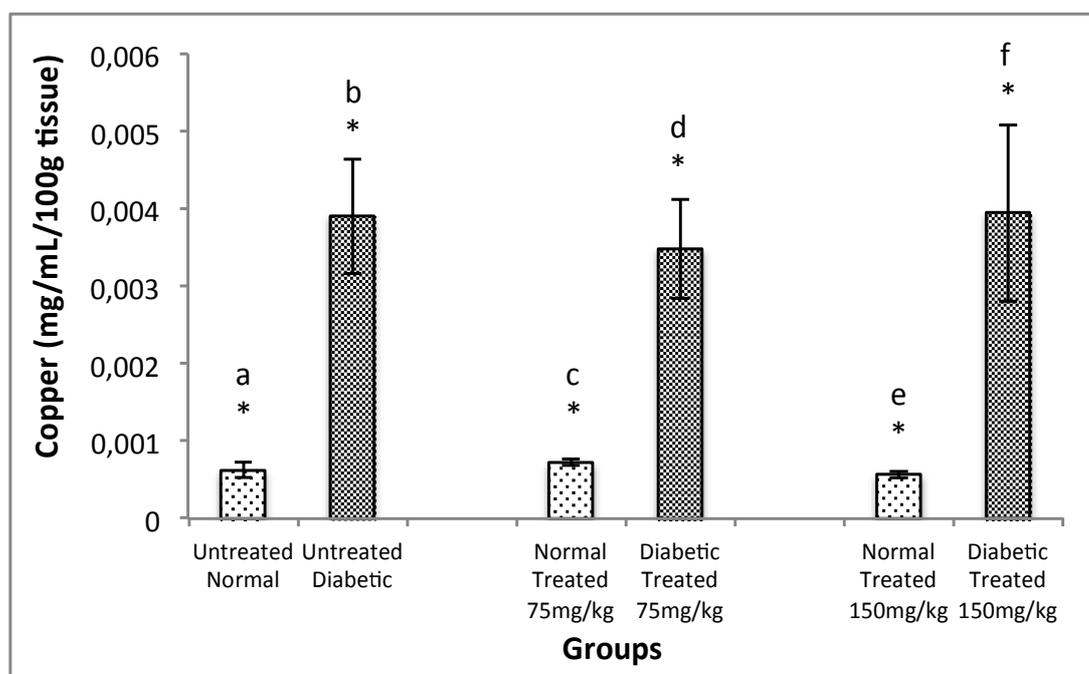


Figure 3.46: Bar charts showing the levels of copper (mg/ml/100g tissue) in the kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. * $p < 0.05$ for a compared to b; $p < 0.05$ for c compared to d; $p < 0.05$ for e compared to f.

Kidney zinc level

Figure 3.47 shows the effect of ACE on zinc levels in kidney of normal and diabetic rats. The results show that untreated diabetic rats (b) had a slight increase in kidney zinc level compared to untreated normal rats (a). Cinnamon treatment seemed did not to alter kidney zinc levels in normal rats (c and e) compared to untreated normal rats (a). The results also show that there were no significant changes in kidney zinc levels in treated diabetic and treated normal rats (d and f) compared to untreated diabetic (b) (Figure 3.47).

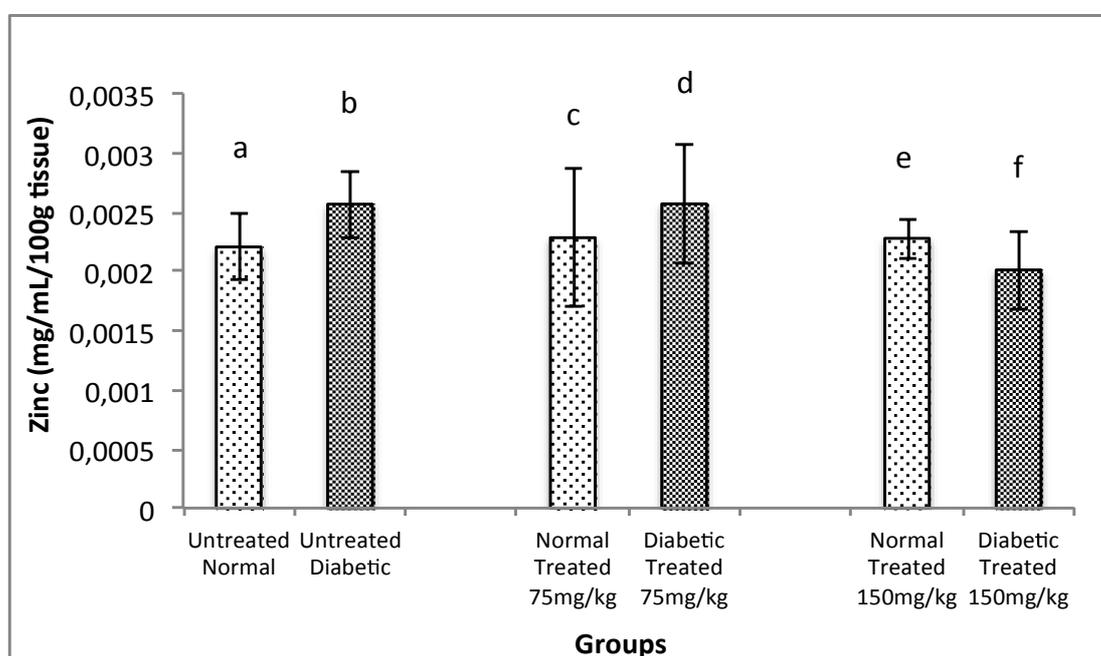


Figure 3.47: Bar charts showing the levels of zinc (mg/ml/100g tissue) in kidneys of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

3.11.5 Effect of ACE on cation levels in pancreas of diabetic and normal rats

Pancreas sodium level

Figure 3.48 shows the effect of ACE on sodium levels in pancreas of normal and diabetic rats. The data show that pancreatic sodium level was not different in untreated diabetic rats (b) compared to untreated normal rats (a). Cinnamon treatment seemed to decrease pancreas sodium level in treated (75 mg/Kg) normal rats (c) compared to untreated normal rats (a), but the data were not significant. The results also show that there were no significant changes in pancreas sodium levels in treated diabetic rats (d and f) compared to untreated diabetic (b) (Figure 3.48).

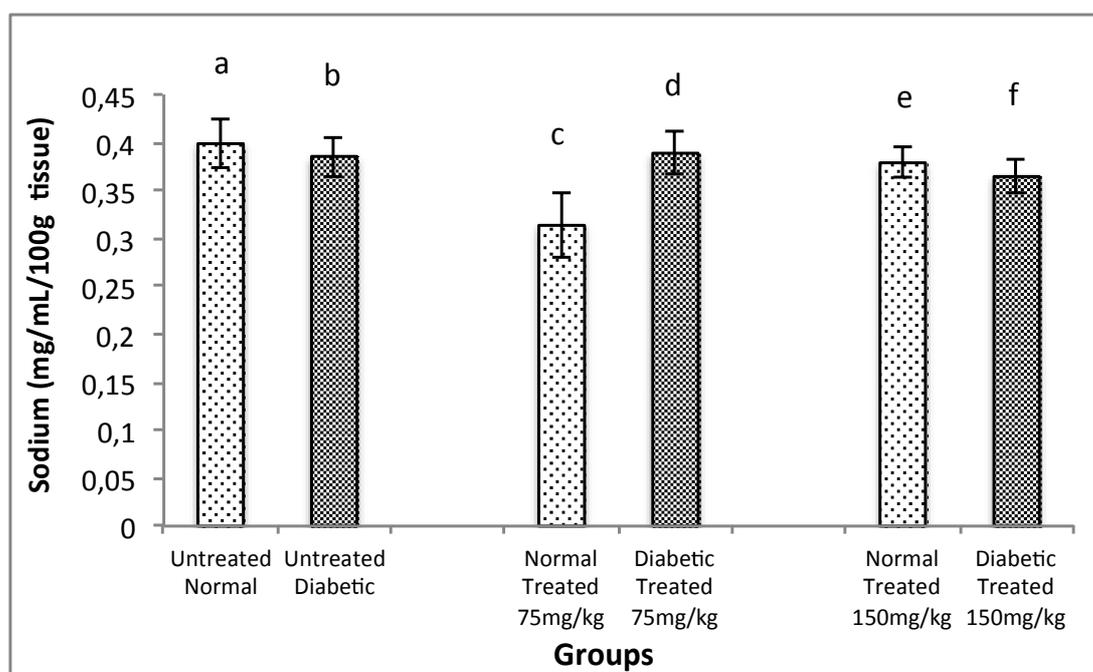


Figure 3.48: Bar charts showing the levels of sodium (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Pancreas magnesium level

Figure 3.49 shows the effect of ACE on magnesium levels in pancreas of normal and diabetic rats. The results show that there were no significant changes in pancreas magnesium levels in treated diabetic and treated normal rats compared to untreated diabetic and untreated normal rats (Figure 3.49).

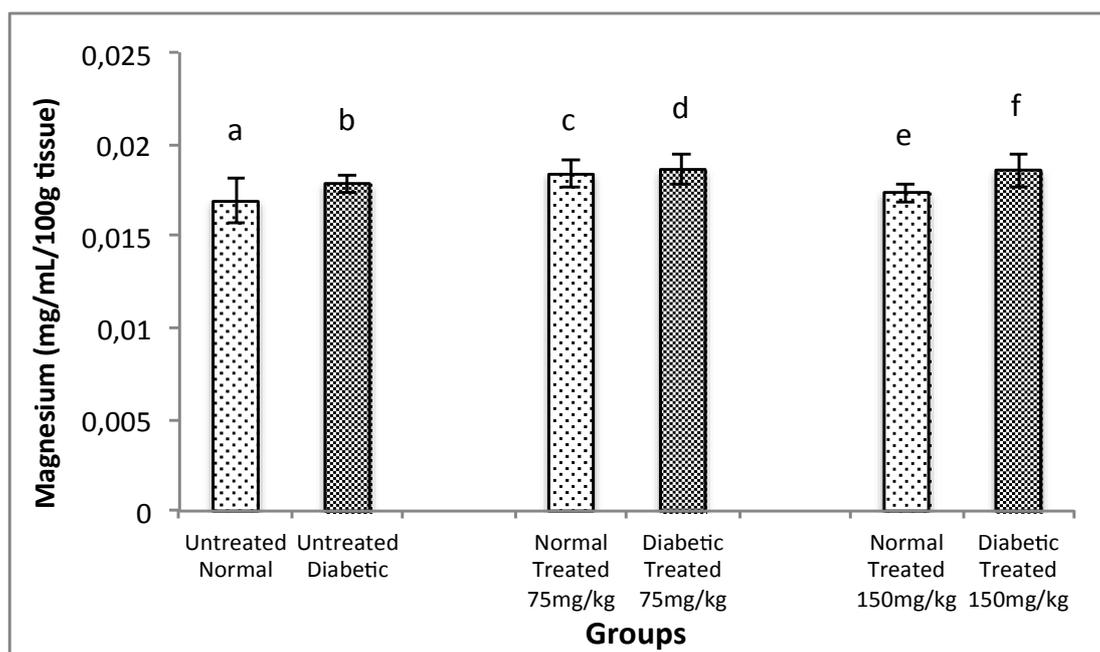


Figure 3.49: Bar charts showing the levels of magnesium (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Pancreas potassium level

Figure 3.50 shows the effect of ACE on potassium levels in pancreas of normal and diabetic rats. The results show that there were no significant changes in pancreas potassium levels in treated diabetic and normal rats compared to untreated diabetic and normal rats (Figure 3.50).

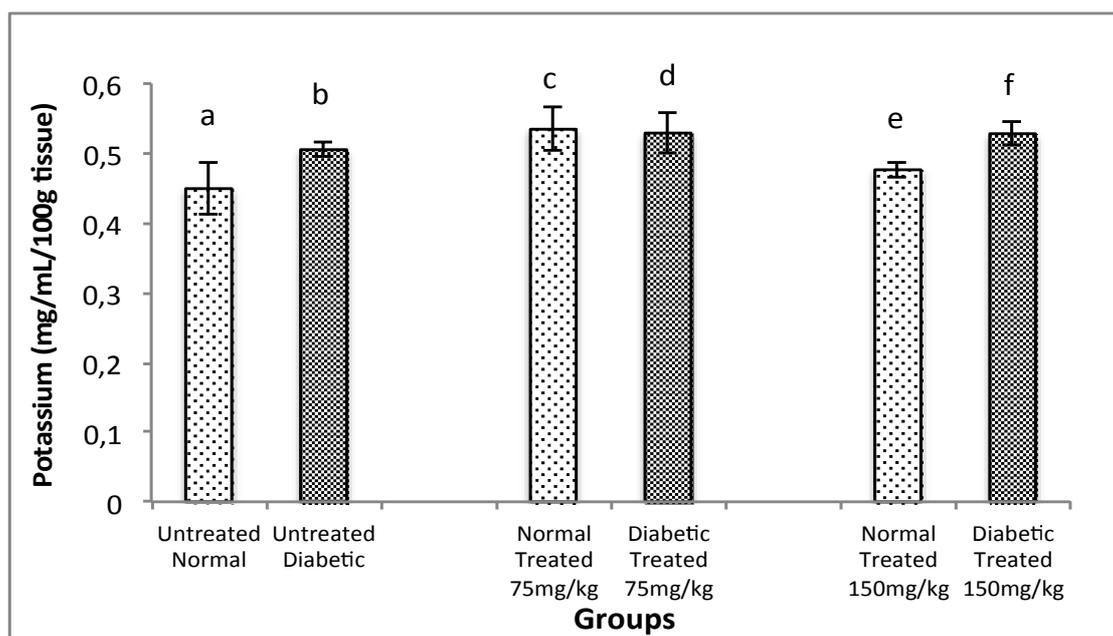


Figure 3.50: Bar charts showing the levels of potassium (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p > 0.05$).

Pancreas calcium level

Figure 3.51 shows the effect of ACE on calcium levels in pancreas of normal and diabetic rats. The results show that pancreas calcium levels were not different compared untreated diabetic rats (b) compared to untreated normal rats (a). The data also show that cinnamon treatment (75 mg/Kg) increase significantly the pancreas calcium levels in normal rats (c) compared to untreated normal rats (b) ($p < 0.05$). Cinnamon treatment (150 mg/Kg) was also more in diabetic rats (d) compared to untreated rats (b), but was not significant. The results also show that there were no significant changes in pancreas calcium levels in treated 150 mg/Kg normal and diabetic rats (e and f) compared to untreated normal (a) and untreated diabetic rats (b) (Figure 3.51).

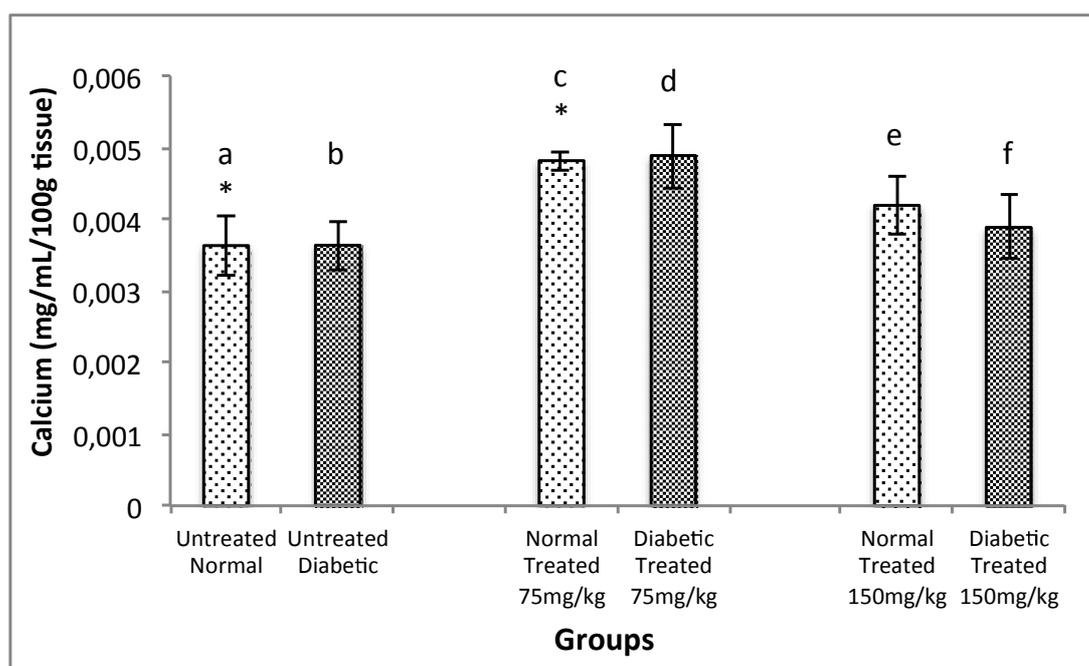


Figure 3.51: Bar charts showing the levels of calcium (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. * $p < 0.05$ for a compared to c.

Pancreas iron level

Figure 3.52 shows the effect of ACE on iron levels in pancreas of normal and diabetic rats. The results show that untreated diabetic rats (b) slightly decrease pancreas iron levels compared to untreated normal rats (a). However, cinnamon treatment (75 mg/Kg) seem to increase pancreas iron levels in normal and diabetic rats (c and d) compared to untreated normal and diabetic rats (a and b). However, this result was not significant. In contrast, cinnamon treatment with 150 mg/Kg demonstrate a slightly less pancreas iron level in normal and diabetic rats (e and f) compared to untreated normal and diabetic rats (a and b). However, these results show that there were no significant changes in pancreas iron levels in treated diabetic and normal rats compared to untreated diabetic and normal rats (Figure 3.52).

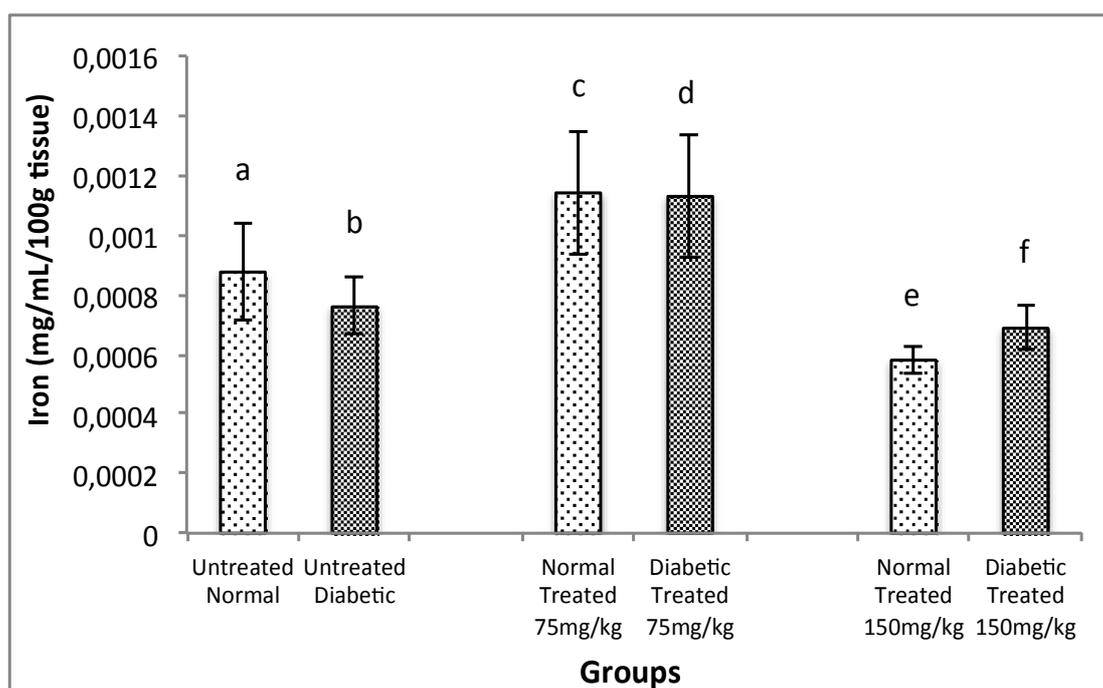


Figure 3.52: Bar charts showing the levels of iron (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Pancreas copper level

Figure 3.53 shows the effect of ACE on copper levels in pancreas of normal and diabetic rats. The results show that there were no significant changes in pancreas copper levels in treated diabetic and normal rats compared to untreated diabetic and normal rats (Figure 3.53).

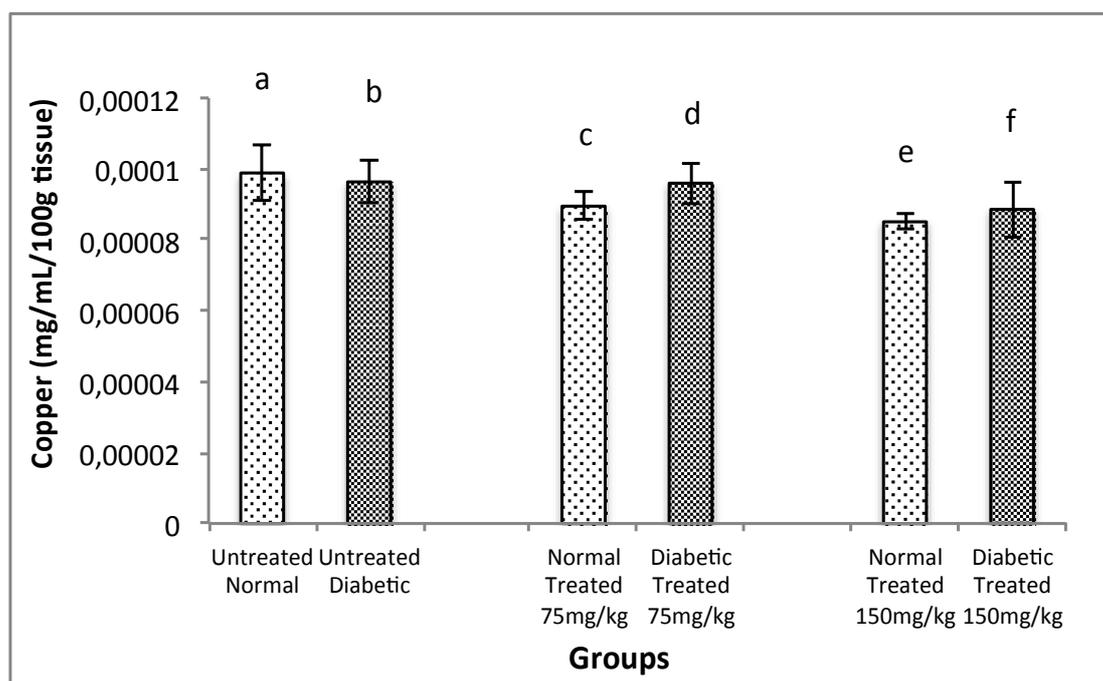


Figure 3.53: Bar charts showing the levels of copper (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

Pancreas zinc level

Figure 3.54 shows the effect of ACE on zinc levels in pancreas of normal and diabetic rats. The results revealed a slightly but not significantly decrease of pancreas zinc levels in untreated diabetic rats (b) compared to untreated normal rats (a). The data also show that there were no significant changes in pancreas zinc levels in treated diabetic and normal rats compared to untreated diabetic and normal rats, respectively. However, the data further show that a low dose of cinnamon seems to increase pancreatic zinc levels (c and d) where as high dose of cinnamon lead the opposite effect (e and f) (Figure 3.54).

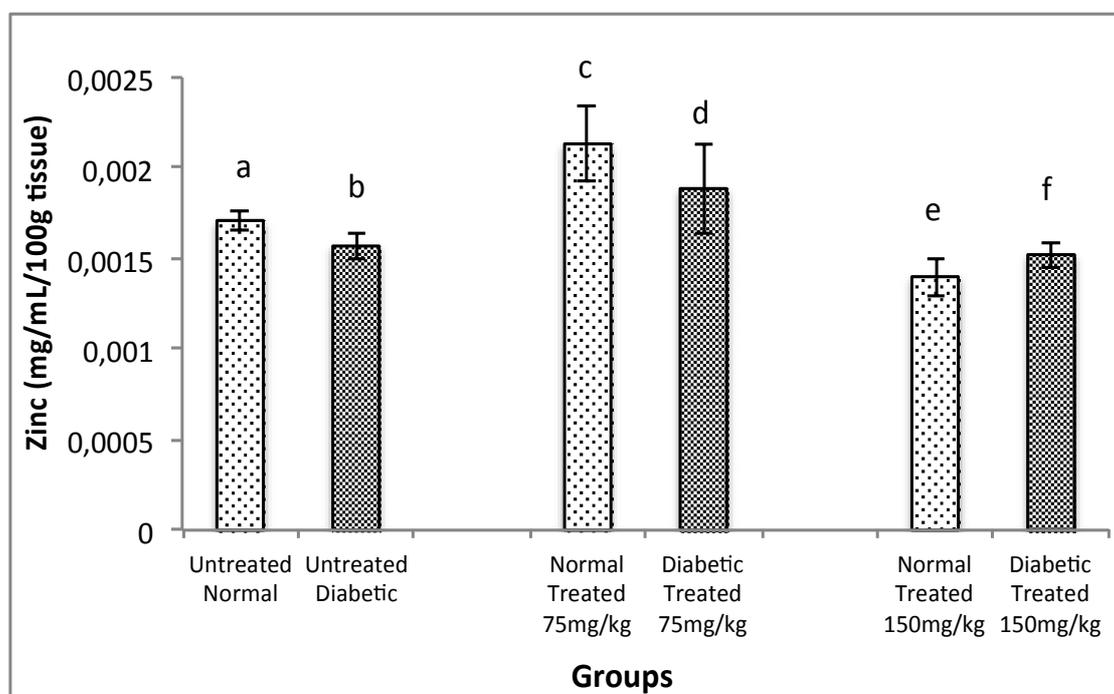


Figure 3.54: Bar charts showing the levels of zinc (mg/ml/100g tissue) in the pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, n=5-6. No significant differences was observed between different groups (a, b, c, d, e and f) ($p>0.05$).

3.11.6 Effect of ACE on different cation contents of normal and diabetic rats per tissue for comparison

Figure 3.55 shows the levels of sodium, magnesium, potassium, calcium, copper, iron and zinc (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg for comparison. The results show that ACE administration had no significant effect on potassium, copper, iron and zinc serum levels in treated with either 75 or 150 mg/Kg doses normal and diabetic rats compared to untreated normal and diabetic rats, respectively. However, data suggest that cinnamon administration with 75 mg/Kg in normal rats decrease significantly sodium and calcium levels ($p < 0.05$) compared to untreated normal rats. In addition, data also show that in diabetic rats, this dose of cinnamon decrease significantly ($p < 0.05$) sodium, magnesium and calcium levels compared to untreated diabetic rats.

Cinnamon at 150 mg/Kg administration revealed a significant decrease of sodium levels in normal rats ($p < 0.05$) compared to untreated normal rats. However, no significantly effect was observed in diabetic animals serum after cinnamon treatment with 150 mg/Kg.

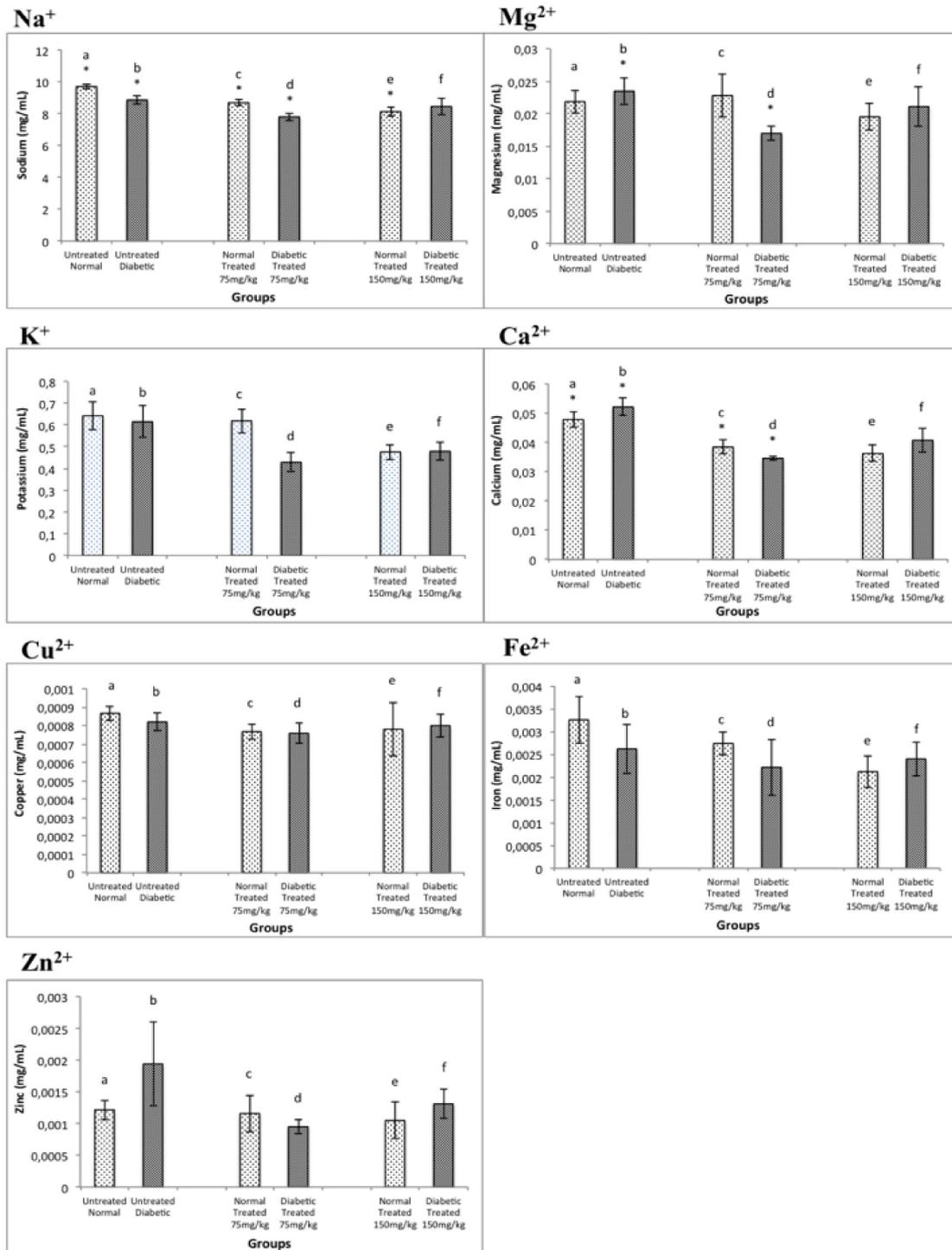


Figure 3.55: Bar charts showing the levels of sodium, magnesium, potassium, calcium, copper, iron and zinc (mg/ml) in serum of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks for comparison. Data are mean \pm SEM, n=5-6; * p<0.05.

Figure 3.56 shows the levels of sodium, magnesium, potassium, calcium, copper, iron and zinc (mg/ml/100g tissue) in heart of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg for comparison. The results show that ACE administration had no significant effect only on copper heart levels in treated with either 75 or 150 mg/Kg doses normal and diabetic rats compared to untreated normal and diabetic rats, respectively.

The data show that cinnamon administration with 75 mg/Kg in normal rats decreases significantly magnesium and zinc levels ($p < 0.05$) and increases iron levels, compared to untreated normal rats. However, in diabetic rats, cinnamon administration with 75 mg/Kg increases significantly ($p < 0.05$) magnesium and potassium levels compared with untreated diabetic rats. Regarding the administration of cinnamon at 150 mg/Kg, in normal-treated rats, there was no significant effect compared to untreated normal rats. In addition, in diabetic rats, high dose of cinnamon seem to have a significant effect, namely increased magnesium, potassium, calcium and iron levels ($p < 0.05$) compared to untreated diabetic rats.

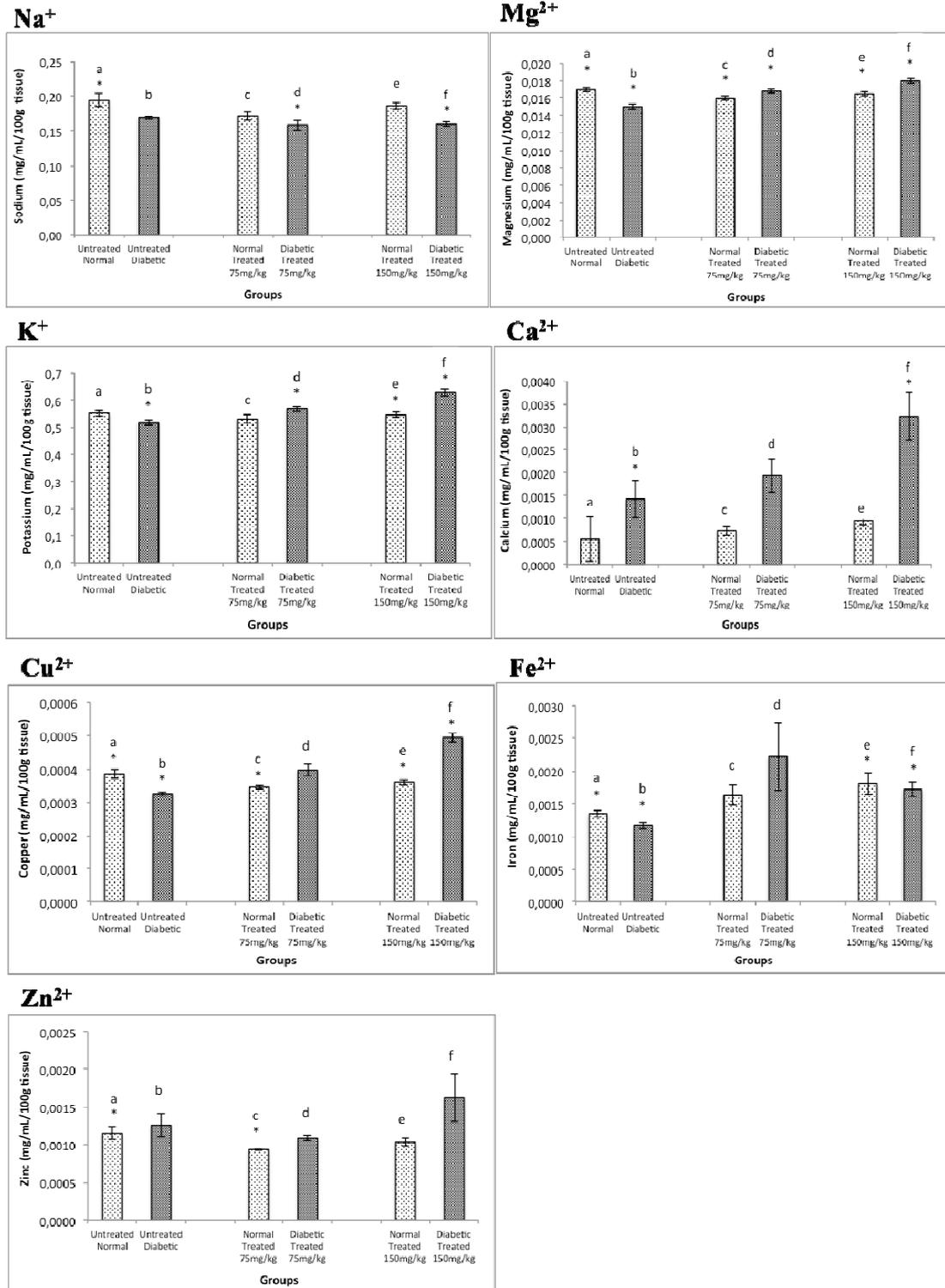


Figure 3.56: Bar charts showing the levels of sodium, magnesium, potassium, calcium, copper, iron and zinc (mg/ml/100g tissue) in heart of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks for comparison. Data are mean \pm SEM, n=5-6; * p<0.05.

Figure 3.57 shows the levels of sodium, magnesium, potassium, copper, iron and zinc (mg/ml/100g tissue) in liver of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg. The results show that ACE administration had no significant effect on sodium, magnesium, potassium, copper and zinc liver levels in treated with either 75 or 150 mg/Kg normal and diabetic rats compared to untreated normal and diabetic rats, respectively.

The data show that cinnamon administration with 75 mg/Kg had no significant effect in normal and diabetic rats compared to untreated normal and diabetic rats, respectively. In addition, cinnamon 150 mg/Kg dose demonstrated had no significantly effect on normal rats compared to untreated normal rats.

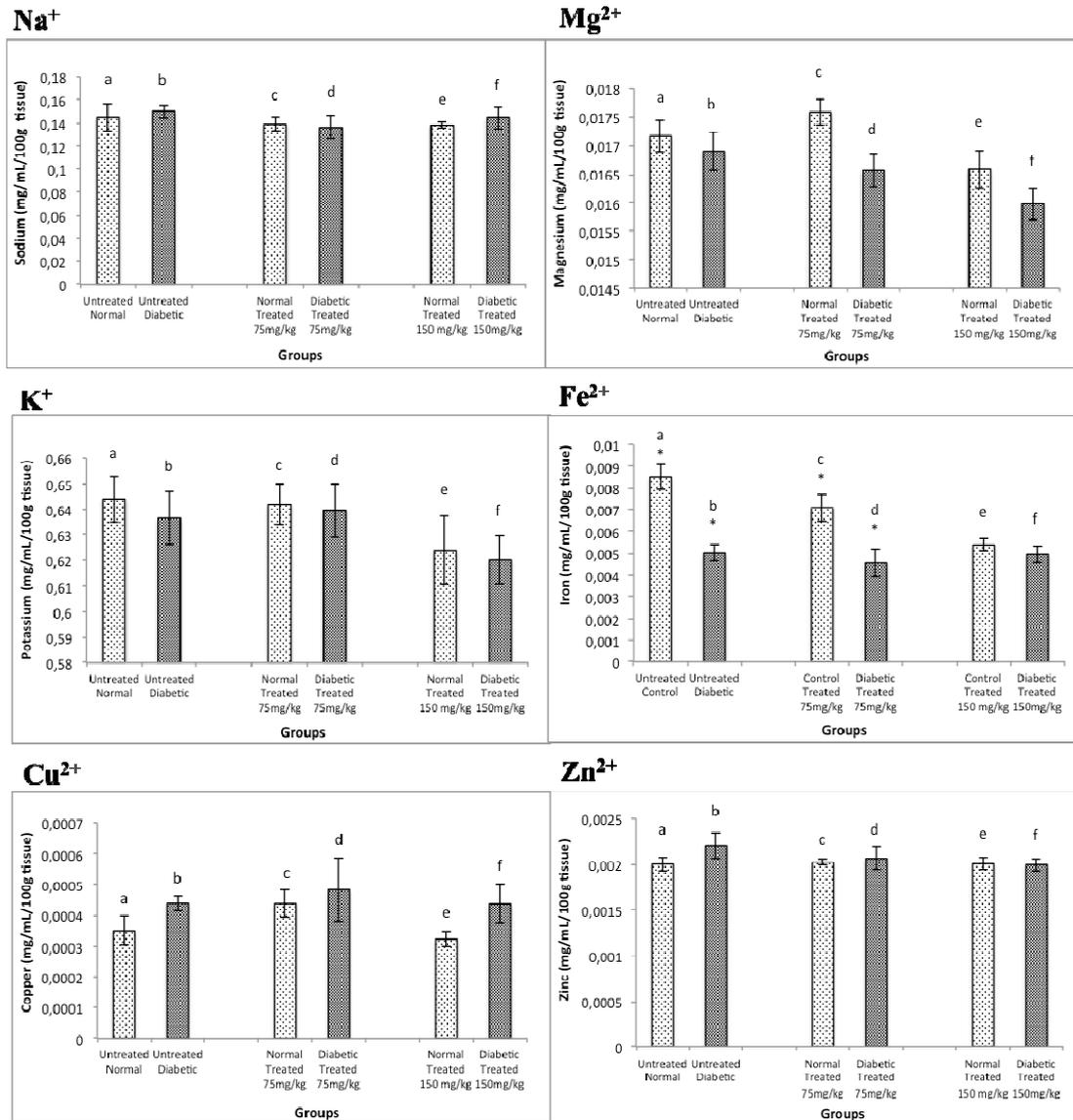


Figure 3.57: Bar charts showing the levels of sodium, magnesium, potassium, copper, iron and zinc (mg/ml/100g tissue) in liver of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks for comparison. Data are mean \pm SEM, n=5-6; * p<0.05.

Figure 3.58 shows the levels of sodium, magnesium, potassium, copper, iron, copper and zinc (mg/ml/100g tissue) in kidney of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg. The results show that ACE administration had no significant effect on sodium, magnesium, potassium, calcium, copper and zinc kidney levels in treated with either 75 or 150 mg/Kg normal and diabetic rats compared to untreated normal and diabetic rats, respectively. However, the data show that cinnamon administration with 75 mg/Kg and 150 mg/Kg significantly decrease iron levels in normal treated rats compared to untreated normal rats ($p < 0.05$).

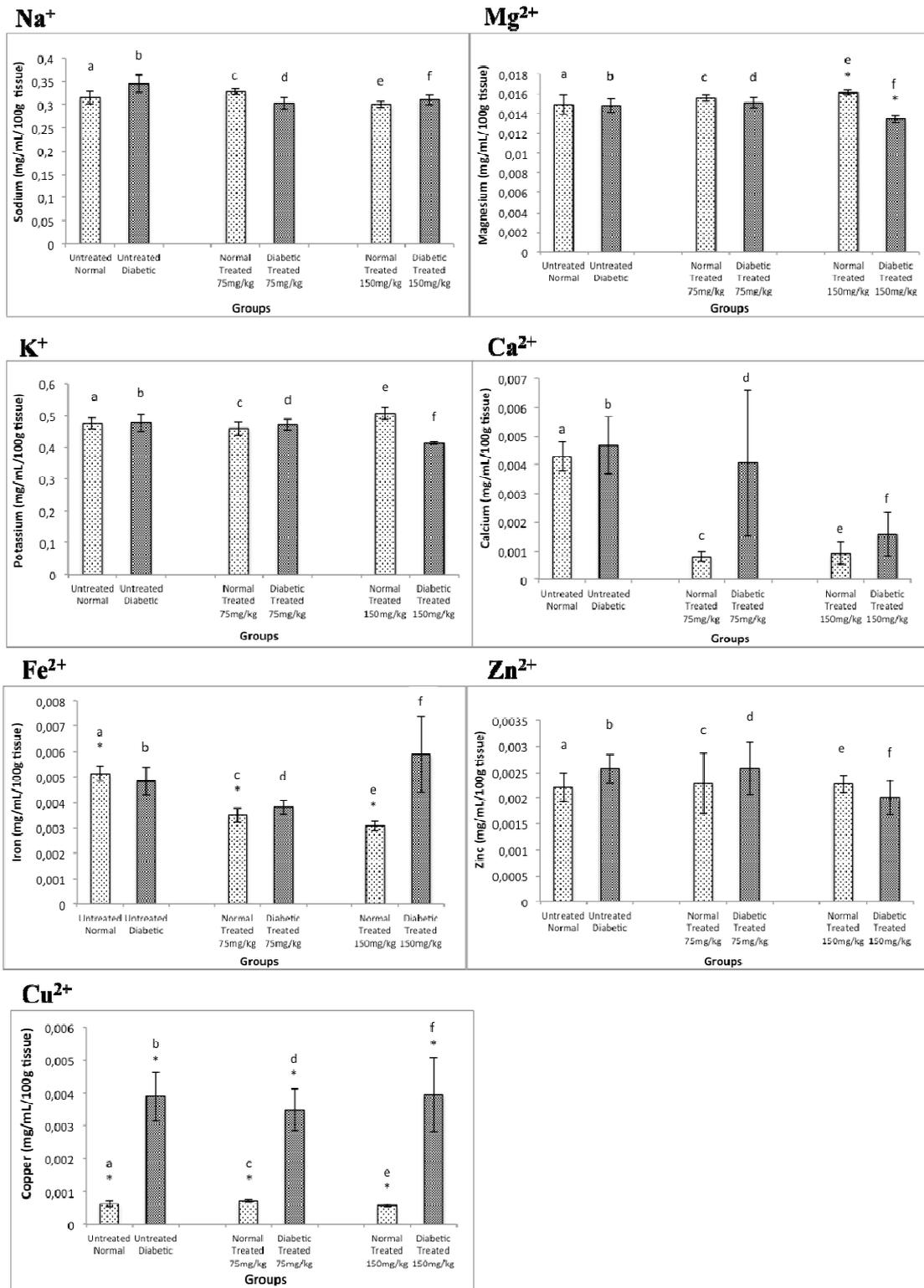


Figure 3.58: Bar charts showing the levels of sodium, magnesium, potassium, calcium, iron, zinc and copper (mg/ml/100g tissue) in kidney of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks for comparison. Data are mean \pm SEM, n=5-6; * p<0.05.

Figure 3.59 shows the levels of sodium, magnesium, potassium, calcium, copper, iron, copper and zinc (mg/ml/100g tissue) in pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg. The results show that ACE administration had no significant effect on sodium, magnesium, potassium, iron, copper and zinc pancreas levels in treated with either 75 or 150 mg/Kg normal and diabetic rats compared to untreated normal and diabetic rats, respectively. The data show that cinnamon administration with 75 mg/Kg significantly can increase calcium levels ($p < 0.05$) in normal treated rats compared to untreated normal rats.

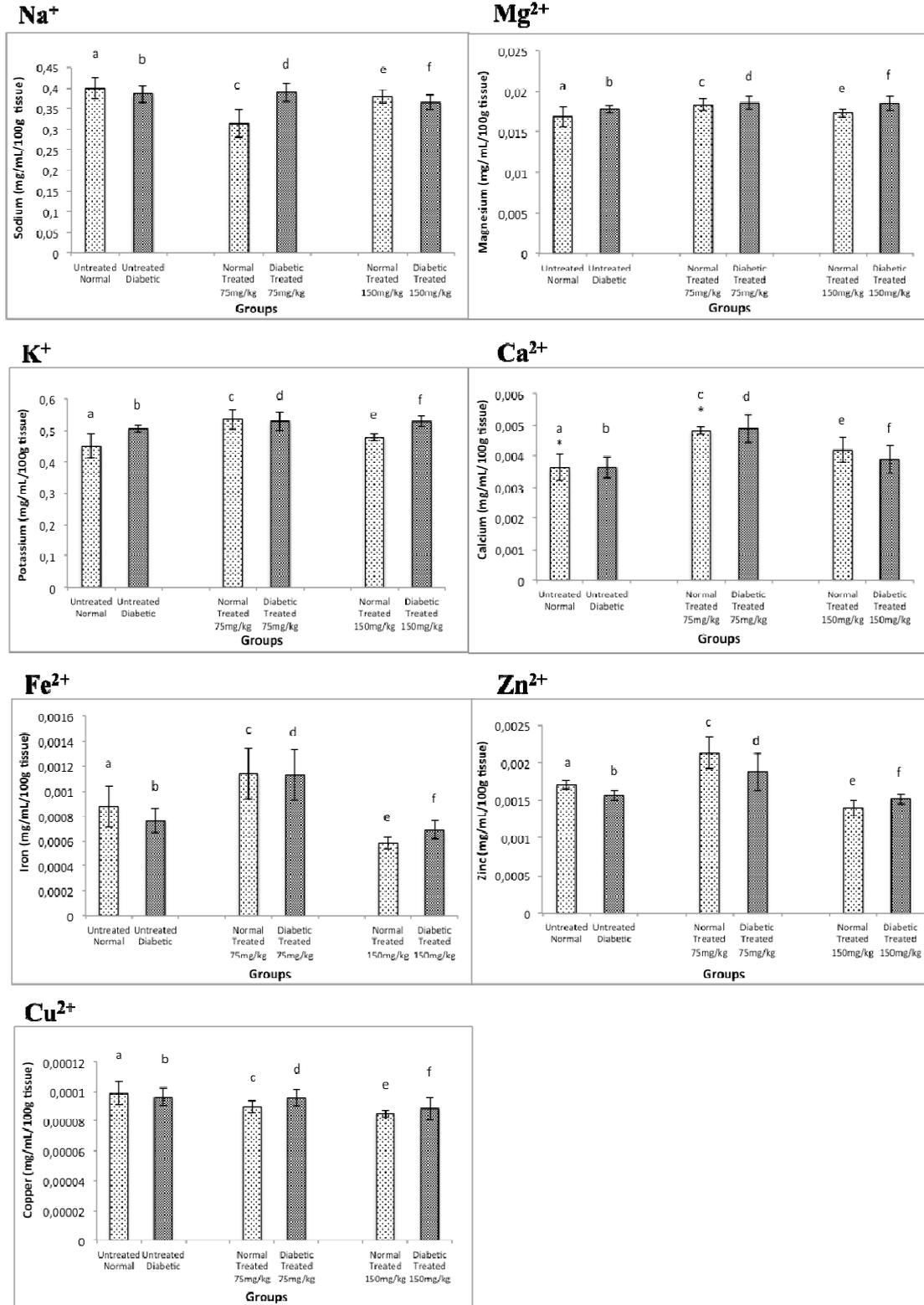


Figure 3.59: Bar charts showing the levels of sodium, magnesium, potassium, calcium, copper, iron, zinc and copper (mg/ml/100g tissue) in pancreas of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks for comparison. Data are mean \pm SEM, n=5-6; * p<0.05.

3.12 *In vitro* pancreatic insulin release in normal and diabetic rats

In normal rats, the pancreatic fragments stimulated with agonists of insulin secretion (glucose or acetylcholine) without cinnamon treatment demonstrated increase insulin secretion with glucose and 10^{-6} M acetylcholine. However, cinnamon treatment with either 75 or 150 mg/Kg seem to decrease insulin secretion after glucose or acetylcholine stimulator. Note that cinnamon treatment with higher doses seemed to inhibit the insulin secretion in normal rats either with basal conditions or stimulated with agonist.

Figure 3.60 shows the effect of either glucose (16 mM) or Ach (10^{-5} and 10^{-6} M) on insulin secretion in control untreated and treated rats with 75 mg/kg and 150 mg/kg cinnamon. The results show that basal insulin secretion was significantly higher in untreated rat pancreatic segments compared to treated pancreatic segments ($p < 0.05$). The pancreatic fragments stimulated with agonists of insulin secretion (either glucose or acetylcholine) without cinnamon treatment demonstrated increase insulin secretion with glucose and 10^{-6} M acetylcholine. However, cinnamon treatment with either 75 or 150 mg/Kg seem to decrease insulin secretion after glucose or acetylcholine stimulator. Note that cinnamon treatment with higher doses seemed to inhibit the insulin secretion in normal rats either with basal conditions or stimulated with agonist. A significant result ($p < 0.05$) was observed in normal treated (150 mg/Kg) rat compared with untreated normal rats after acetylcholine stimulations.

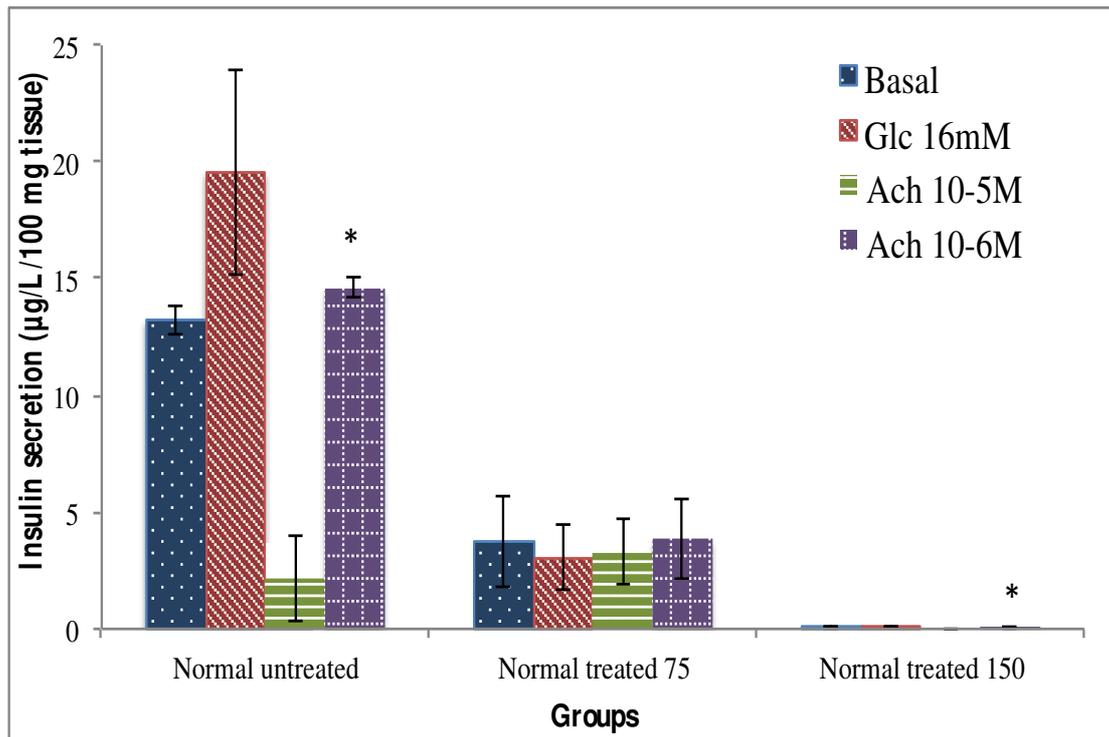


Figure 3.60: Bar charts showing *in vitro* pancreatic insulin secretion from untreated and treated (75 and 150 mg/Kg of cinnamon) normal rat under basal condition and incubated with either glucose (16 mM) or acetylcholine (10^{-5} M or 10^{-6} M). ANOVA test was used for compared normal untreated rats with treated with of cinnamon both incubated with acetylcholine (10^{-6} M) (* $p < 0.05$). Data are mean \pm SEM, n = 4-6 rats.

Figure 3.61 shows the effect of either glucose or ACh on insulin secretion in diabetic untreated and treated rats with 75 mg/kg and 150 mg/kg cinnamon. The results in figure 3.61 showed that in basal conditions the untreated diabetic pancreatic segments produced low mean levels of insulin output. However, cinnamon treatment with 150 mg/Kg of cinnamon seemed to increase insulin secretion from pancreatic segments of diabetic rats under both under basal conditions and during stimulation with either glucose or 10^{-5} M acetylcholine.

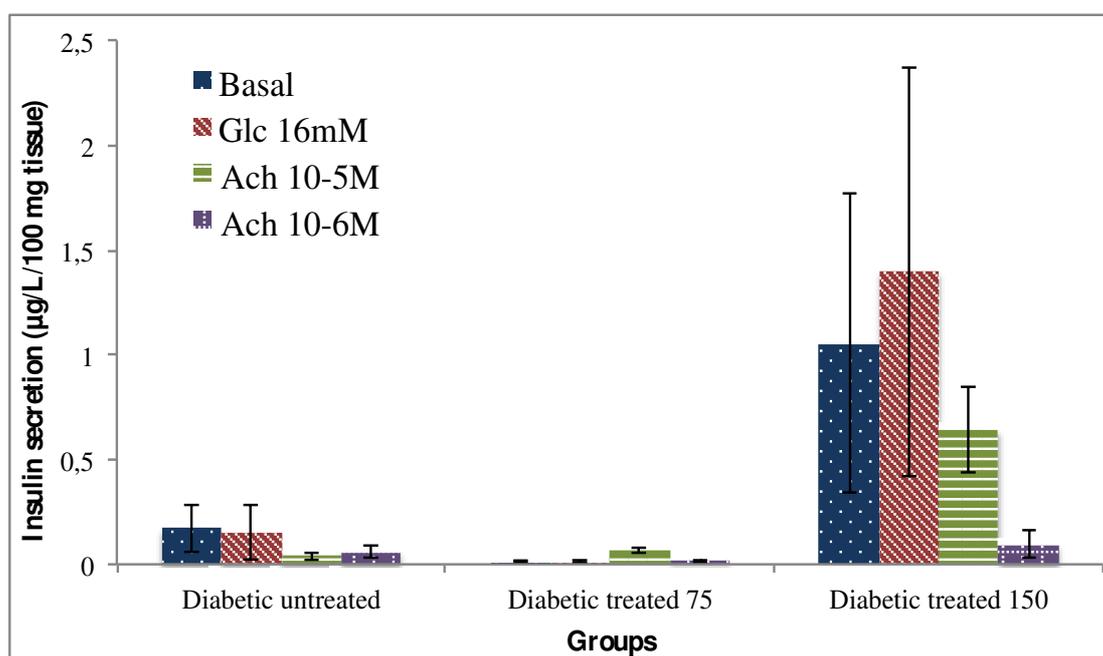


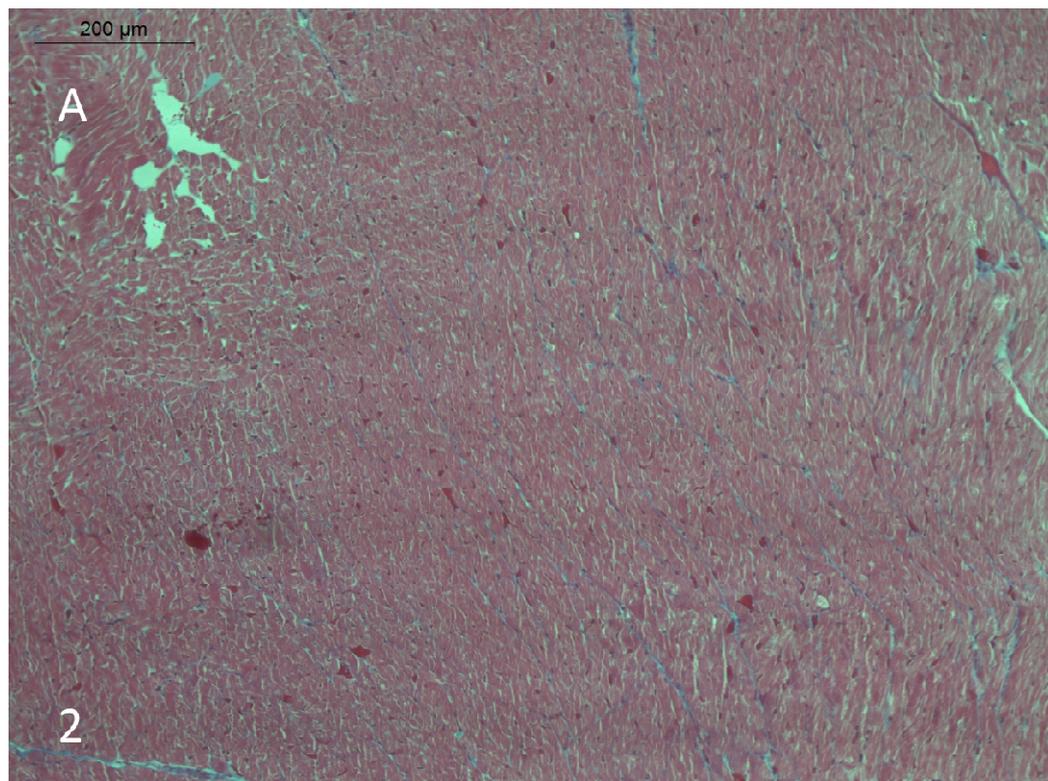
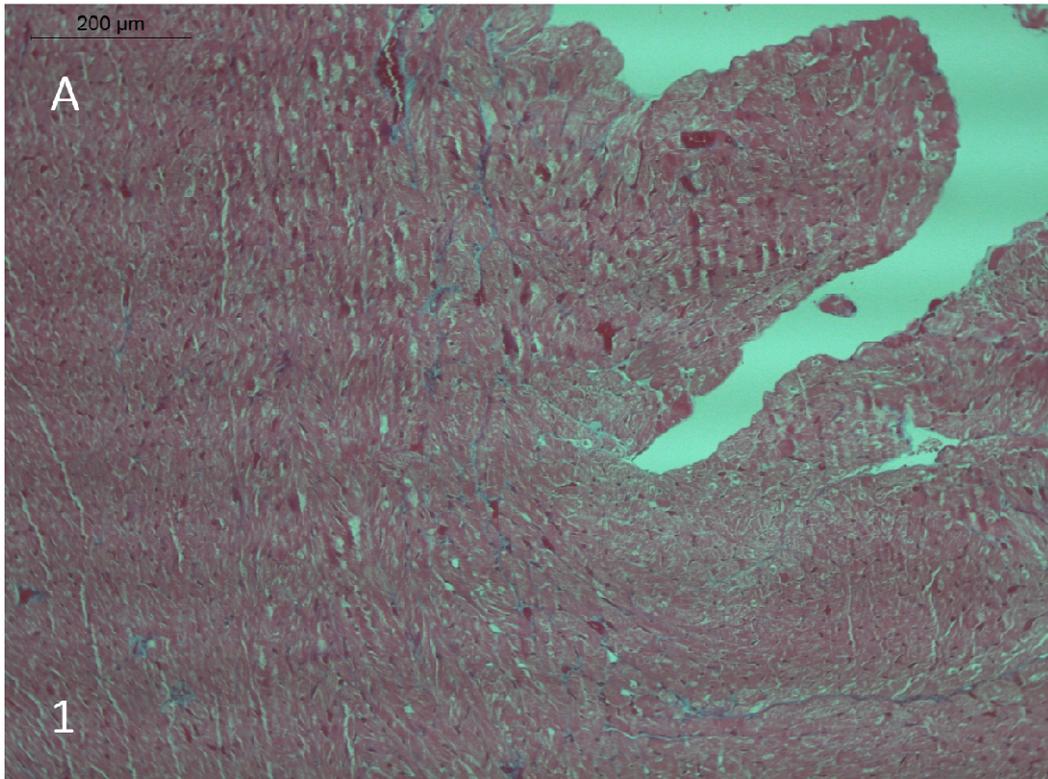
Figure 3.61: Bar charts showing *in vitro* pancreatic insulin secretion from untreated and treated (75 and 150 mg/Kg of cinnamon) diabetic rat under basal condition and incubated with either glucose (Glc) (16 mM) or acetylcholine (ACh) (10^{-5} M or 10^{-6} M). Note that no significant ($p > 0.05$) effect was observed between untreated rats compared with treated both 75 or 150 mg/Kg of cinnamon, in basal or incubated with glucose (16 mM) or acetylcholine (10^{-5} or 10^{-6} M). Data are mean \pm SEM, $n = 4-6$ rats.

3.13 Effect of ACE on heart fibrosis of normal and diabetic rats

3.13.1 Distribution of left heart ventricle fibrosis of untreated and treated normal rats

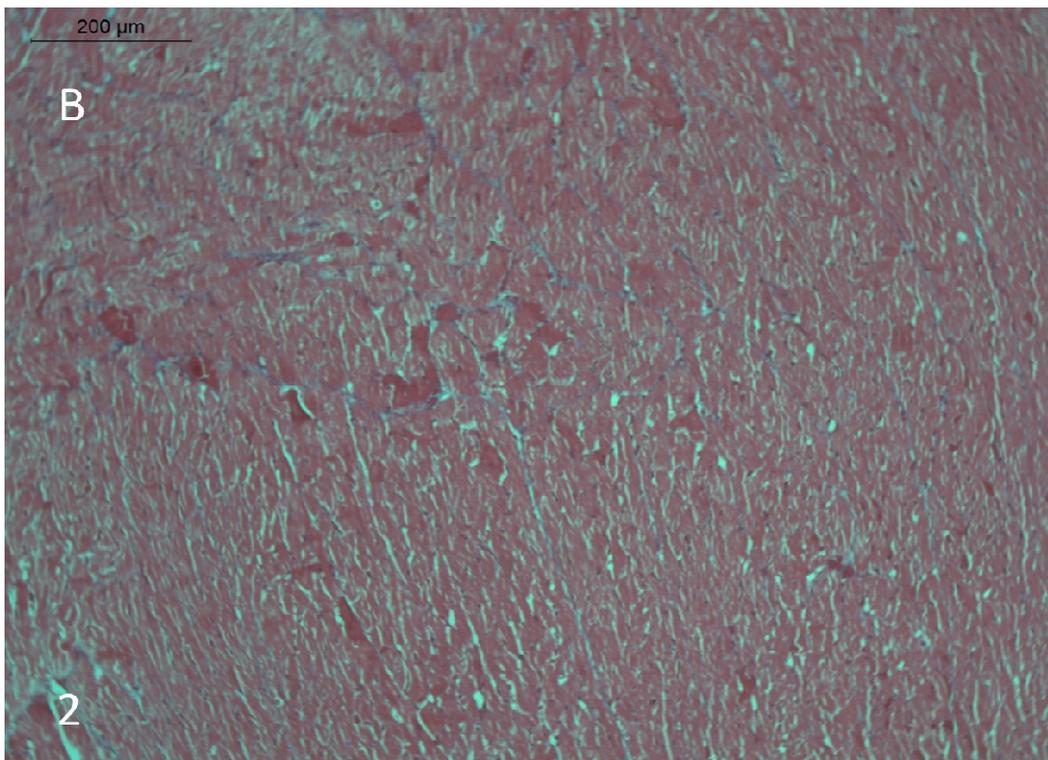
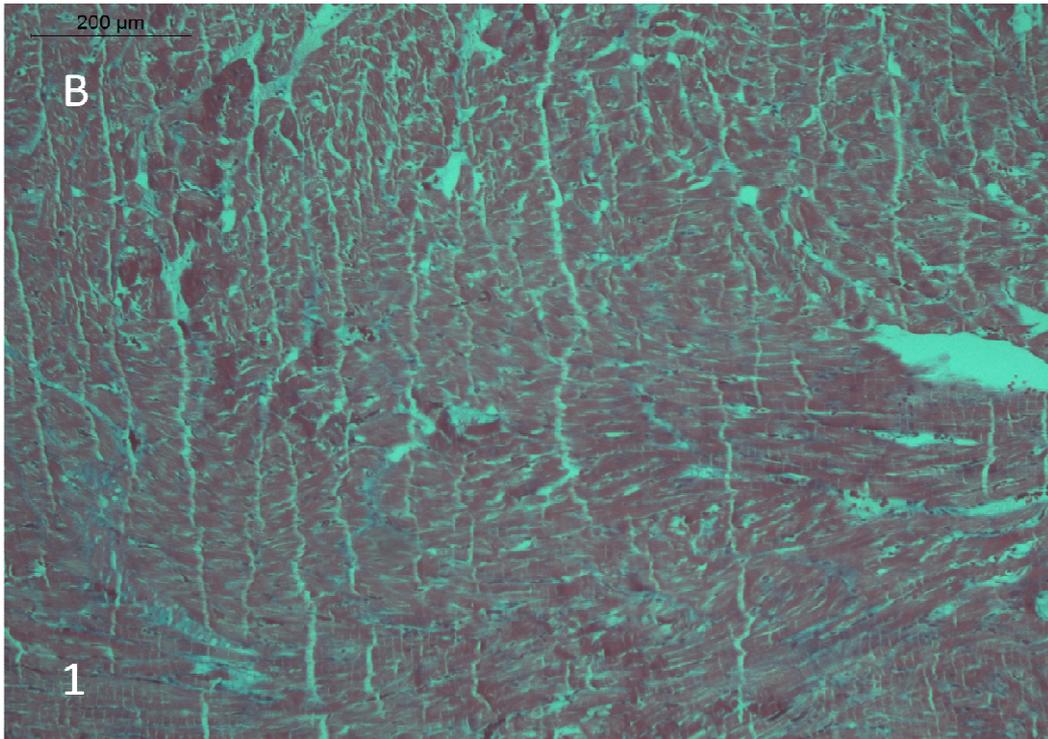
This study examined any beneficial effect of cinnamon treatment on the morphology and fibrosis development in the left ventricle of the diabetic and normal hearts compared to untreated cardiac tissues.

Figures 3.62 - 3.64 show the optical microscopy of normal ventricle of the heart of untreated (A) and tissue treated with either 75 mg/kg (B) and 150 mg/kg (C) normal rats, respectively. The data presented in each figure represents 2 different hearts for each group (either untreated or treated) of rats. The microscopy pictures show the large light blue zone that represents the vessels of the heart and the red zone, representing muscle of heart. Additionally, the pictures revealed that there was fibrotic tissue, represented by dark blue zone in normal rats either with or without cinnamon treatment. The results also revealed that the hearts of normal rats treated with aqueous cinnamon extract (with both doses) seem to have lower fibrotic tissue (represented by dark blue zone) when compared untreated normal rats.



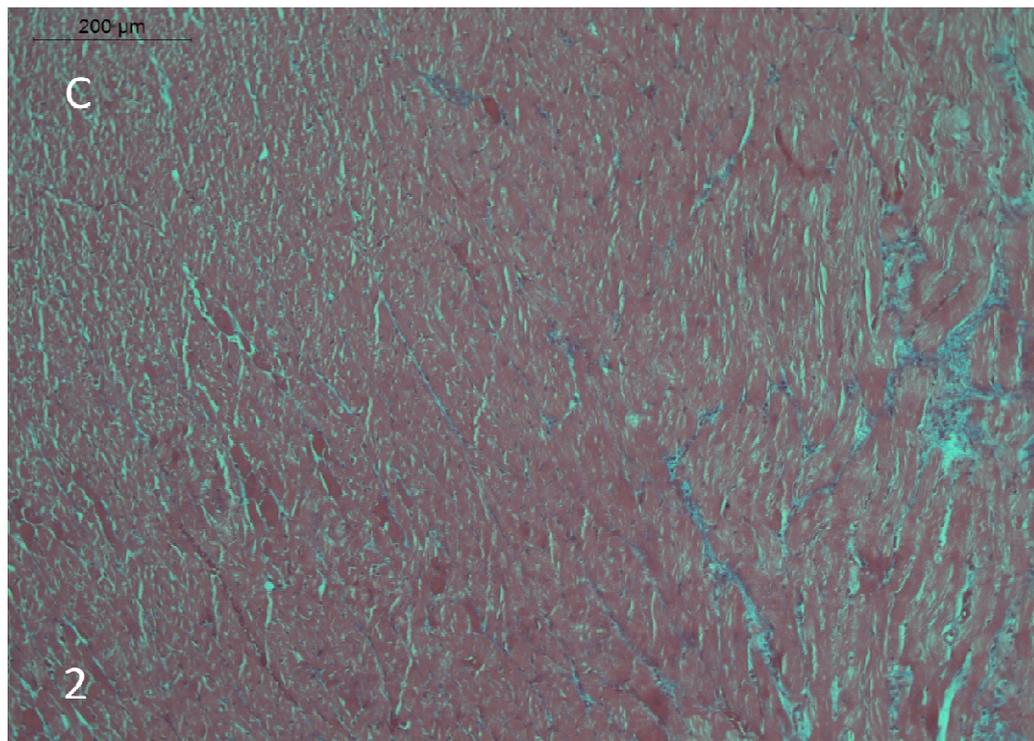
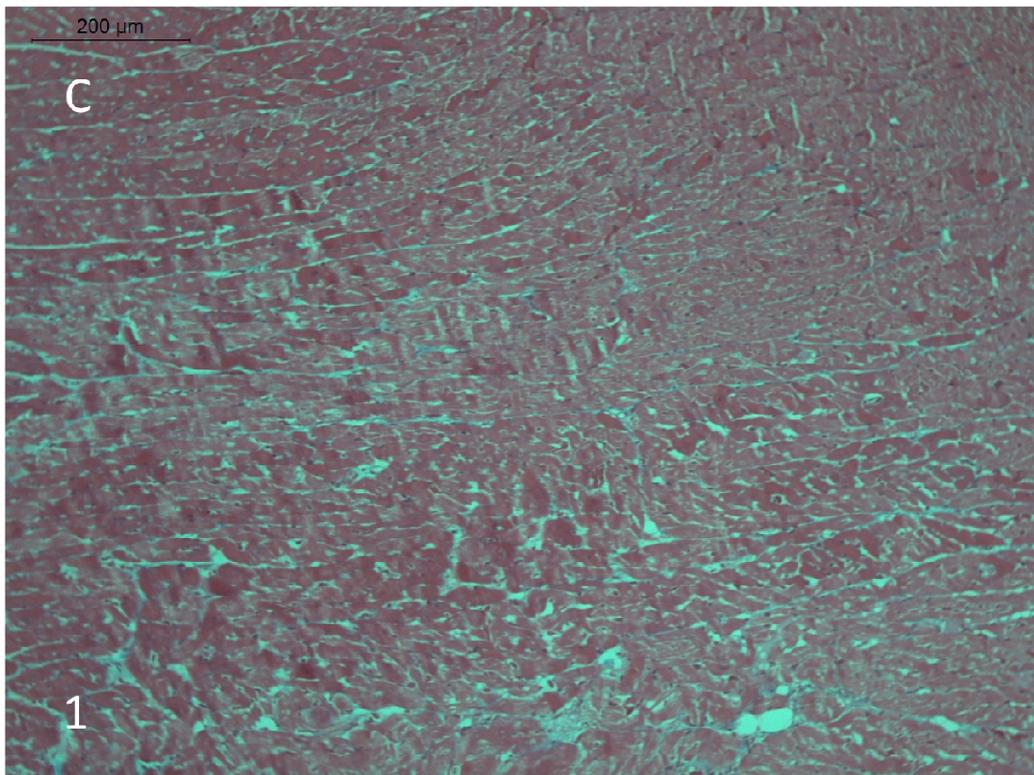
Untreated normal (A)

Figure 3.62: Original micrographs (A) showing interstitial fibrosis observed in the left ventricle of the heart of normal untreated rats. Histological observations represented in this figure were taken from the hearts of 2 different rats of each normal group, for comparison. (CAB staining, magnification 10X)



Normal treated with 75 mg/Kg bw of cinnamon (B)

Figure 3.63: Original micrographs (B) showing interstitial fibrosis observed in left ventricle of the heart of normal rats treated with 75 mg/kg of cinnamon extract over a period of 11 weeks. Histological observations represented in this figure were taken from the hearts of 2 different rats of each normal group, for comparison. (CAB staining, magnification 10X).



Normal treated with 150 mg/Kg bw of cinnamon (C)

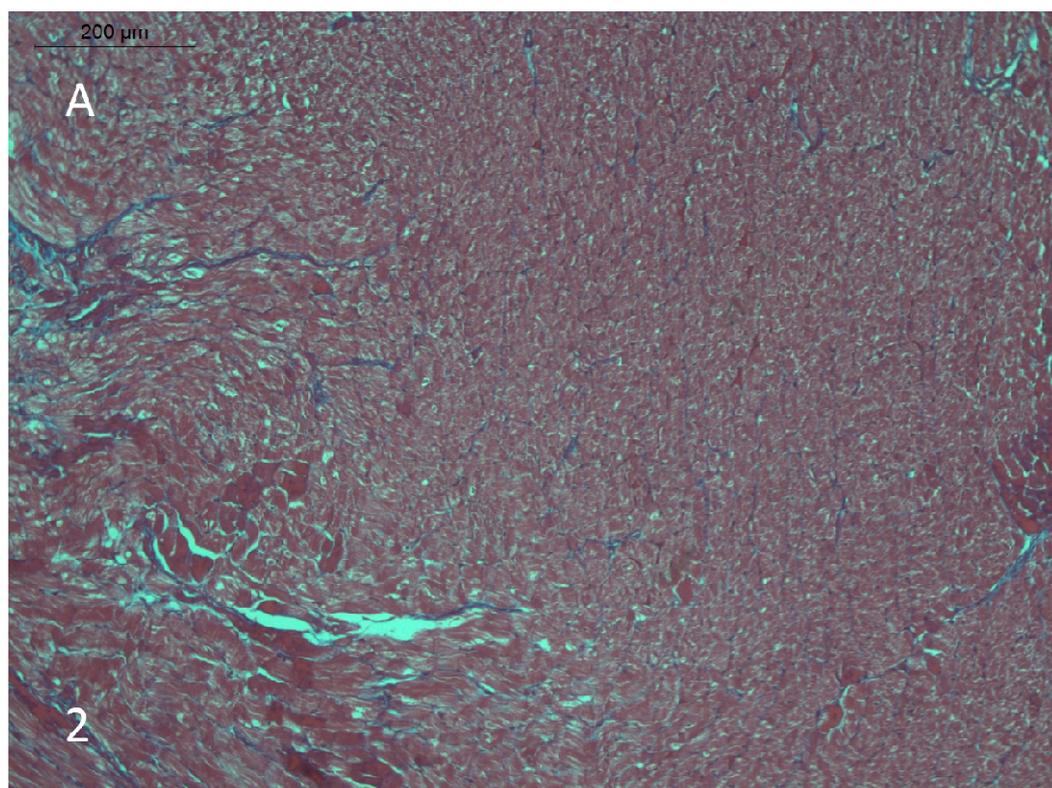
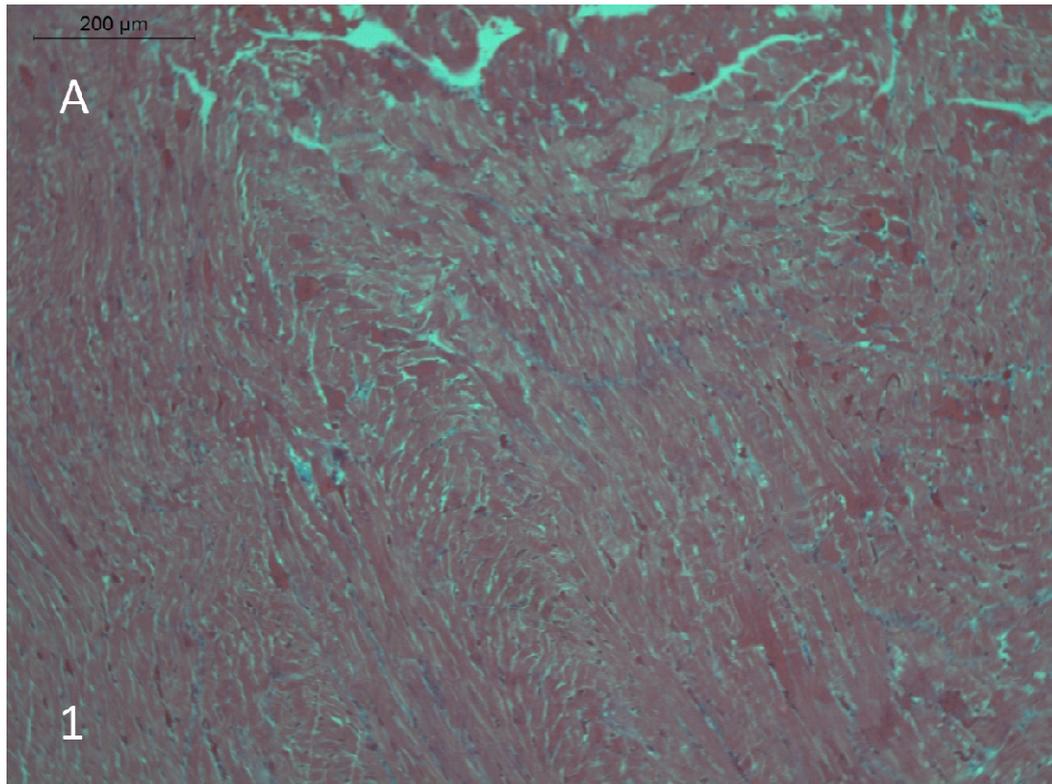
Figure 3.64: Original micrographs showing (C) interstitial fibrosis observed in left ventricle of the heart of normal rats treated with 150 mg/kg of cinnamon extract over a period of 11 weeks. Histological observations represented in this figure were taken from the hearts of 2 different rats of each normal group, for comparison. (CAB staining, magnification 10X)

3.13.2 Distribution of left heart ventricle fibrosis in untreated and treated diabetic rats

Figures 3.65 - 3.67 show the optical microscopy of the left ventricle of hearts taken from diabetic rats during untreated (A) and treated with either 75 mg/kg (B) or 150 mg/kg (C) aqueous cinnamon extract over a period of 11 weeks. The data presented in each figure represents 2 different hearts for each group (either untreated or treated) of rats.

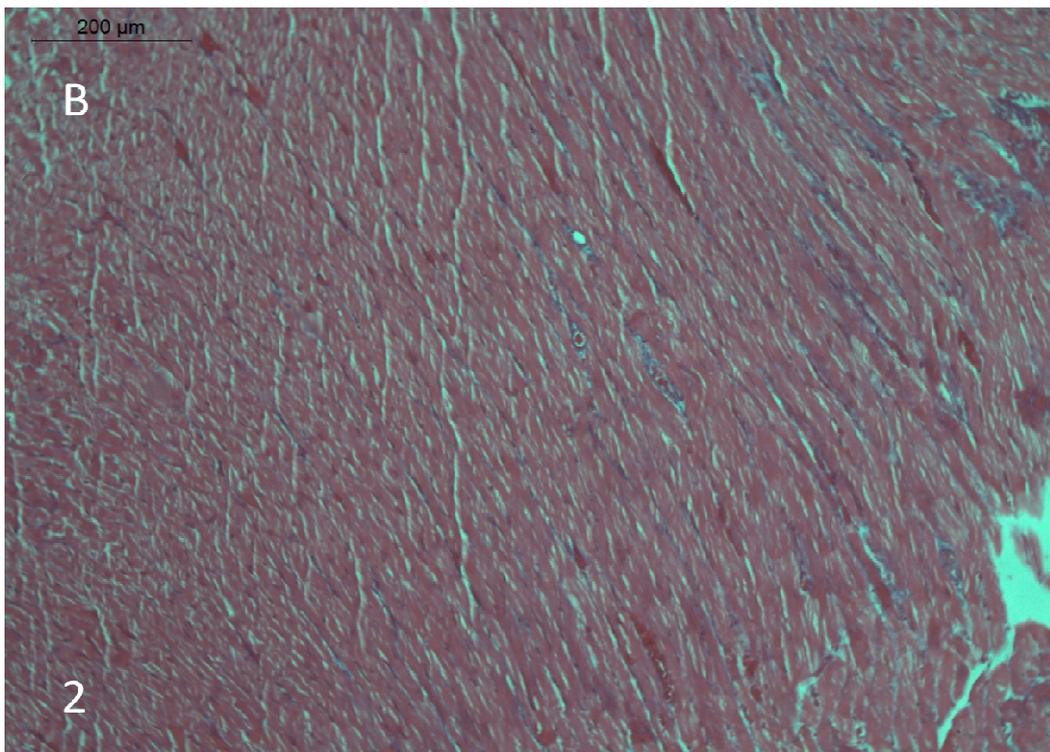
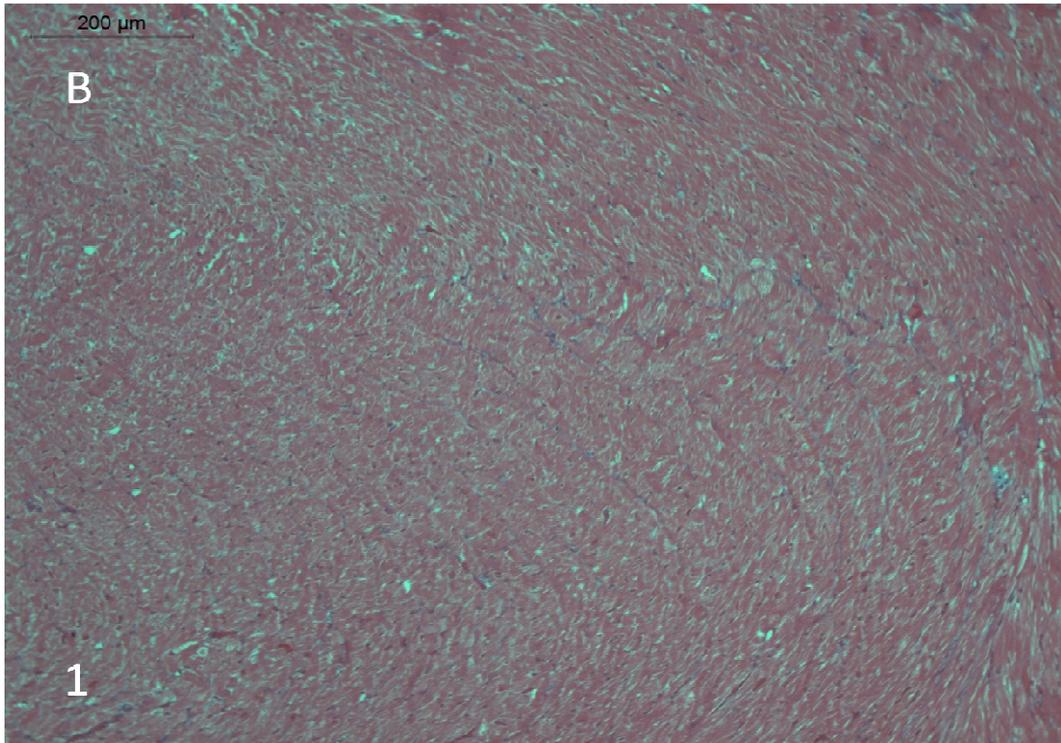
The micrographs show the large light blue zone that represents the vessels of the heart and the red zone, representing muscle of heart. Additionally, the micrographs further reveal that there is fibrotic tissue, represented by dark blue zone in diabetic rats with or without cinnamon treatment. However, the fibrosis was much more prominent in the untreated diabetic hearts.

The results revealed that STZ-induced diabetic rats treated with aqueous cinnamon extract seems to have lower fibrotic tissue (represented by dark blue zone) when compared untreated diabetic rats. However, this result was much more prominent with the high dose (150 mg/Kg) of cinnamon. The fibrosis seems to be slightly higher in hearts obtained from animals treated with 75 mg/Kg cinnamon compared with untreated diabetic rats. In this case, cinnamon treatment seems to have more beneficial effect when a higher dose was administered to the diabetic animals.



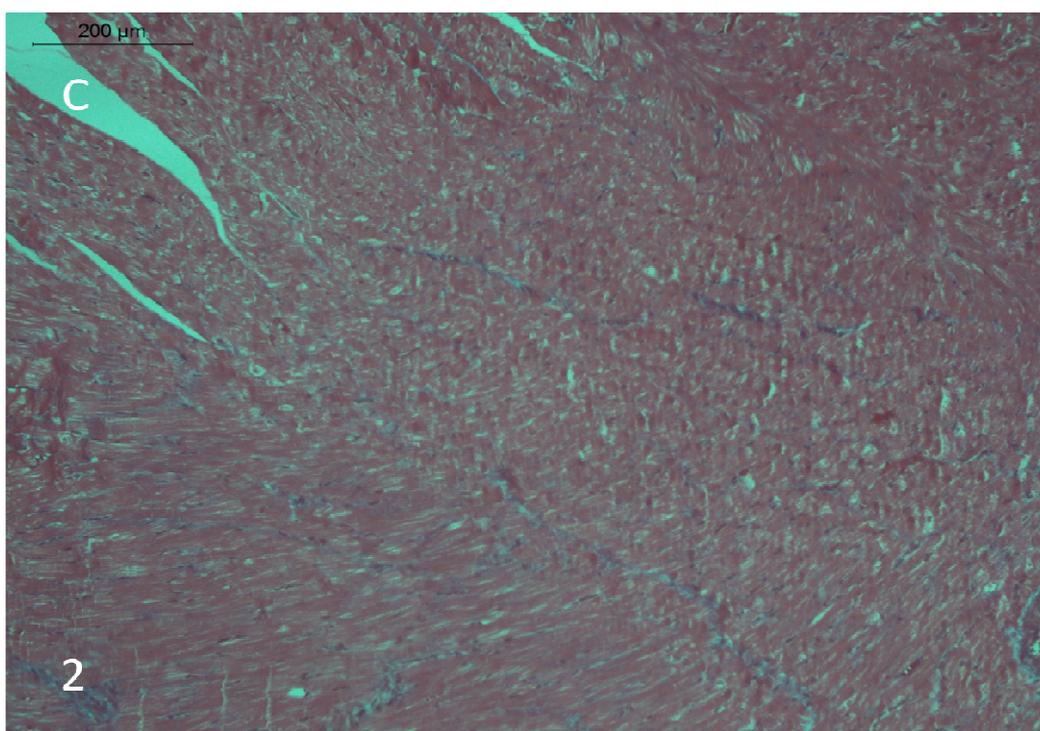
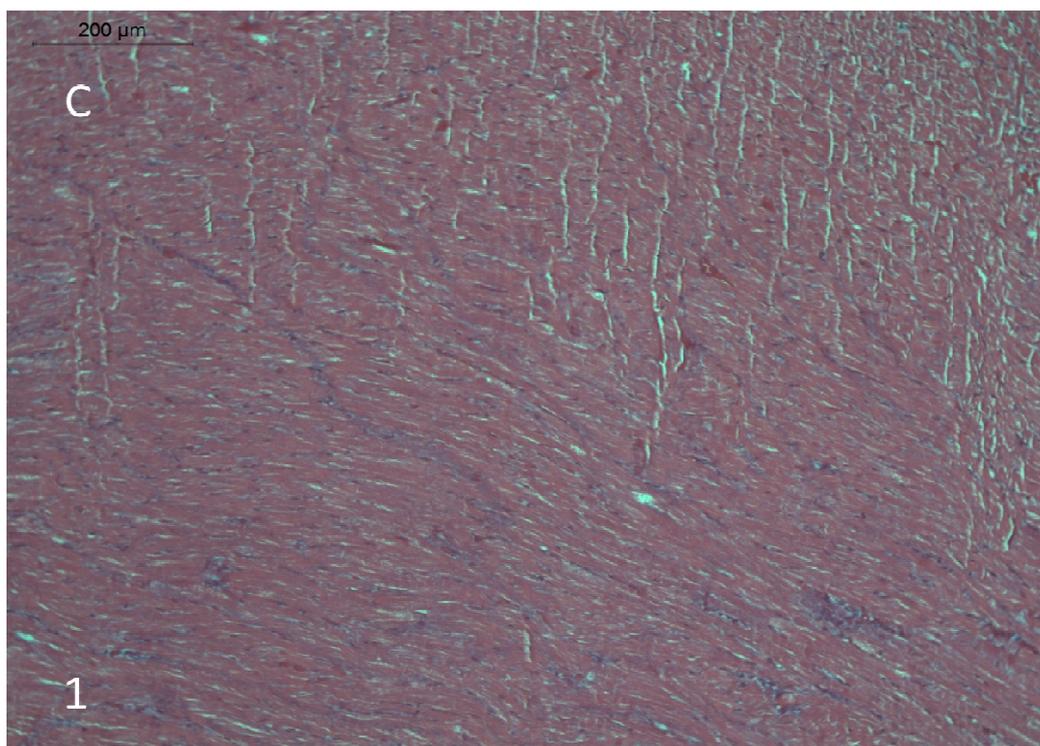
Untreated diabetic (A)

Figure 3.65: Original micrographs (A) showing interstitial fibrosis observed in the left ventricle of hearts of diabetic untreated rats. Histological observations represented in this figure were taken from left ventricles of 2 different diabetic hearts from the untreated group, for comparison. (CAB staining, magnification 10X)



Diabetic treated with 75 mg/Kg bw of cinnamon (B)

Figure 3.66: Original micrographs (B) showing interstitial fibrosis observed in the left ventricle of the heart of diabetic rats treated with 75 mg/kg of cinnamon extract over a period of 11 weeks. Histological observations represented in this figure were taken from the left ventricle of 2 different hearts from diabetic treated group, for comparison. (CAB staining, magnification 10X)



Diabetic treated with 150 mg/Kg bw of cinnamon (C)

Figure 3.67: Original micrographs (C) interstitial fibrosis observed in the left ventricle of the heart of diabetic rats treated with 150 mg/kg of cinnamon extract over a period of 11 weeks. Histological observations represented in this figure were taken from left ventricles of 2 different hearts in the treated group, for comparison. (CAB staining, magnification 10X)

3.13.3 Quantification of interstitial fibrosis of left heart ventricle in untreated and treated normal and diabetic rats

The following results are represented as the mean (\pm SEM) values of left ventricle fibrosis of hearts in normal and diabetic both untreated and treated rats with 75 mg/Kg and 150 mg/kg body weight of aqueous cinnamon extract daily over a period of 11 weeks (Figure 3.68).

The results have shown that the hearts of diabetic untreated rats developed significantly ($p < 0.05$) more interstitial fibrosis than untreated normal rats during the experimental protocol. In addition, cinnamon treatment seems to decrease the mean fibrosis in hearts of diabetic rats compared to untreated diabetic rats. This effect was more marked and significant ($p < 0.05$) with the high dose (150 mg/Kg bw) of cinnamon compared to untreated diabetic hearts. In normal rats, the results show that cinnamon treatment had no effect ($p > 0.05$) in interstitial fibrosis values of treated (75 mg/kg) rats compared with untreated normal rats.

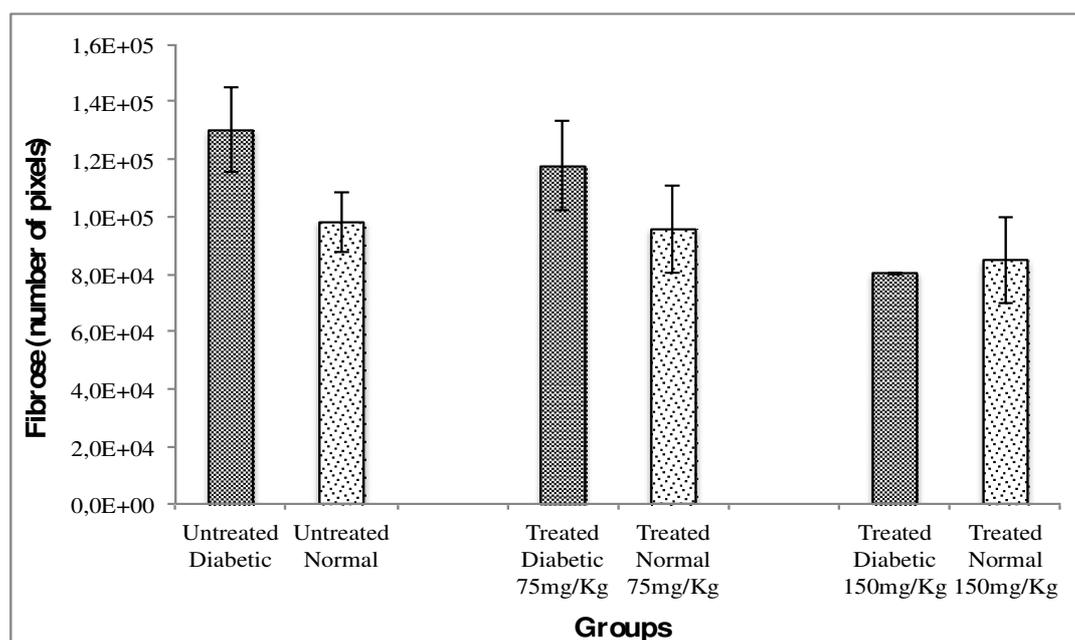


Figure 3.68: Bar charts showing the interstitial fibrosis (number of pixels) in heart of normal and diabetic untreated rats compared to normal and diabetic treated rats with either 75 mg/kg or 150 mg/kg of aqueous cinnamon extract orally over a period of 11 weeks. Data are mean \pm SEM, $n=5-6$. Note that 150 mg/kg cinnamon seems to decrease significantly ($p < 0.05$) fibrosis in the diabetic and control hearts compared to untreated diabetic and normal hearts. ANOVA was used to compare normal and diabetic rats ($p > 0.05$).

3.14 Effect of ACE on distribution of insulin and glucagon containing cells in pancreatic islet of normal and diabetic rats

In another series of experiments it was investigate how cinnamon may exert its hypoglycaemic effect in the endocrine pancreas of diabetic rats. Cinnamon could either act like insulin or as a growth factor repairing damaged Islet of Langerhans destroyed by STZ treatment. Alternatively, it could act as an antioxidant. As such, it was relevant to find out how cinnamon treatment over a period of 11 weeks could improve pancreatic islet status and morphology following STZ-treatment. In this study, STZ was employed to destroy the Islets of Langerhans in rats. Thereafter, the animals were treated with cinnamon for 11 weeks to find out if the plant extract can exert a beneficial effect on the animals by repairing the partially damage islets due of the STZ treatment. Both morphological and immune-histochemical studies were performed by measuring the distribution of insulin and glucagon containing cells in the pancreas of untreated normal (n=6) and normal treated with either 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals and also untreated diabetic (n=5) and diabetic treated either with 75 mg/Kg (n=6) or 150 mg/Kg (n=5) animals. Figures 3.69 and 3.71 show the results of immunofluorescence original micrographs highlighting the distribution of insulin and glucagon positive islets in the pancreatic segments of normal and diabetic untreated and treated (75 mg/Kg or 150 mg/Kg) rats.

3.14.1 Distribution of insulin in the pancreatic islet of normal and diabetic rats

The results presented in figure 3.69 show that the pancreas of normal untreated (1A) rats contains more insulin positive cells per islets compared to pancreas of diabetic untreated rats (2A). In addition, the results revealed that treatment with cinnamon seemed to increase the number of insulin positive cells per islet in diabetic rats (2B and 2C) and seemed to decrease the number of insulin positive cells per islet in normal rats (1B and 1C). A dose of 150 mg/Kg of cinnamon in diabetic treated rats was slightly more effective in improving the number of insulin islet positive cells. On the other hand, cinnamon, when administrated at 75 mg/Kg seemed to exert only a

small beneficial effect in persevering the integrity of the beta cells in the islets comparing to 150 mg/Kg aqueous cinnamon extract. These results demonstrated that cinnamon treatment did not significantly change the number of insulin positive cells per islet in normal rats, but it was more pronounced in the diabetic pancreas (compare 2A with 2B and 2 C).

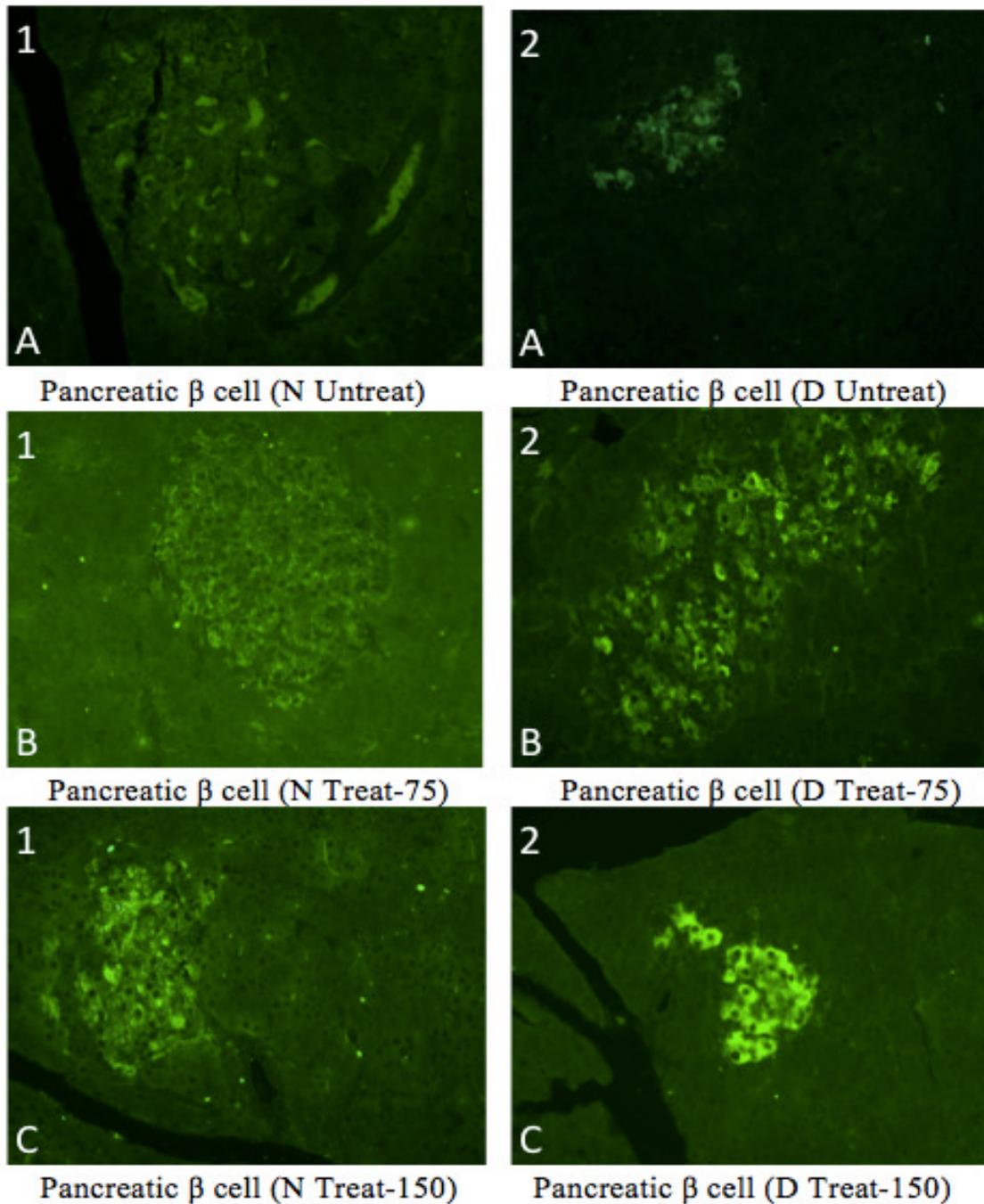


Figure 3.69: Original micrographs of immunofluorescence showing the distribution of insulin positive cells in normal (N) untreated (1A) and diabetic (D) (2A) untreated rat pancreas and in normal and diabetic rats treated with both 75 mg/Kg (1B normal treated, 2B diabetic treated) and 150 mg/Kg (1C normal treated, 2C diabetic treated) aqueous cinnamon extract for 11 weeks. Magnification: X 400. These micrographs are typical of 5 different animals for each.

In another series of experiments the number of insulin positive cells per islet was semi-quantitatively counted from the micrographs obtained from untreated and treated (75 mg/Kg and 150 mg/Kg) normal and diabetic rats.

Figure 3.70 shows the percentage of islet positive cells in the pancreas of normal and diabetic untreated and treated rats with either 75 mg/Kg or 150 mg/Kg of aqueous cinnamon extract. The data show that STZ-induced diabetic rat pancreas contains less insulin positive cells compared to untreated normal rat pancreas. Treatment with aqueous cinnamon extract for 11 weeks seemed to improve the number of insulin positive cells in diabetic rats, but not in normal rats. A dose of 150 mg/Kg of cinnamon was more effective in preventing the islets compared to 75 mg/Kg treatment over 11 weeks. However, this result was not significant ($p > 0.05$).

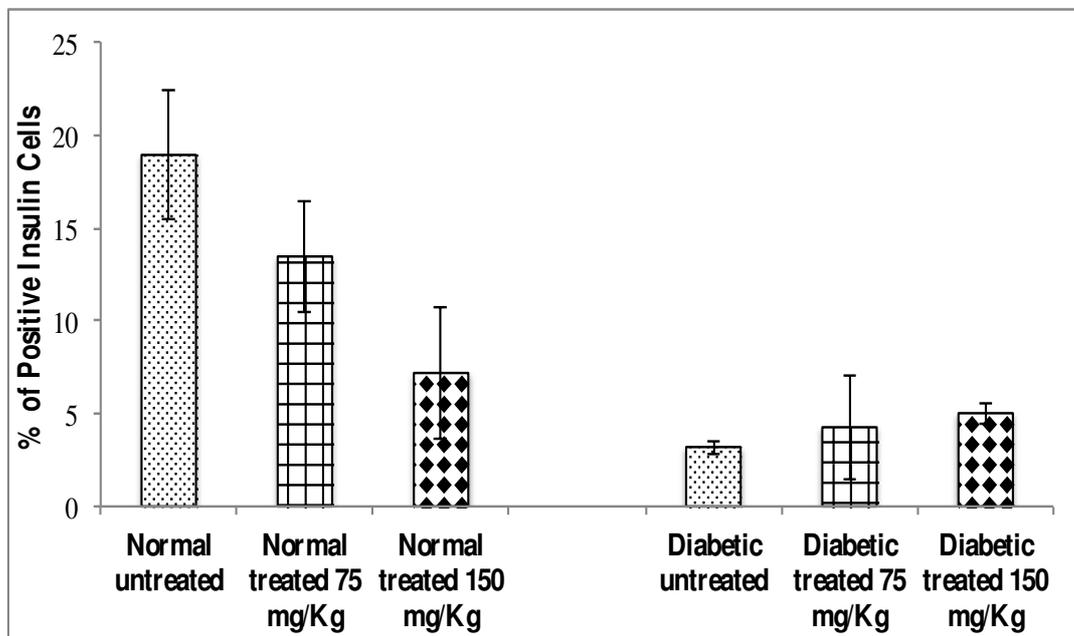


Figure 3.70: Bar chart showing the percentage (%) of islet positive insulin cells in untreated normal and diabetic rats and treated with either 75 mg/Kg or 150 mg/Kg of aqueous cinnamon extract for 11 weeks. Data are mean \pm SEM, $n=5-6$ per group. No significant effect was found between treated and untreated diabetic rats ($p > 0.05$), ANOVA Test. Data obtained from original micrographs as shown if figure 3.69. Note that the diabetic untreated and treated pancreas contained significantly ($p < 0.05$) less insulin positive cells compared to normal untreated and treated pancreas. Cinnamon seems to have a small beneficial effect on the diabetic pancreas by improving insulin positive cells.

3.14.2 Distribution of glucagon in the pancreatic islet of normal and diabetic rats

The results presented in Figure 3.71 show that the pancreas of untreated normal rats contains less glucagon positive cells per islets compared to pancreas of diabetic untreated rats. In addition, the results revealed that treatment of both normal and STZ-induced diabetic rats with either 75 mg/Kg or 150 mg/Kg aqueous cinnamon extract for 11 weeks seems to increase the number of glucagon positive cells per islet in both normal and diabetic treated rats compared to untreated, respectively. Cinnamon, when administrated at 75 mg/Kg seems to have a more pronounced beneficial effect in persevering the integrity of the alpha cells in the islets in normal rats. However, in diabetic rats cinnamon 150 mg/Kg had more beneficial effect in presenting the islets.

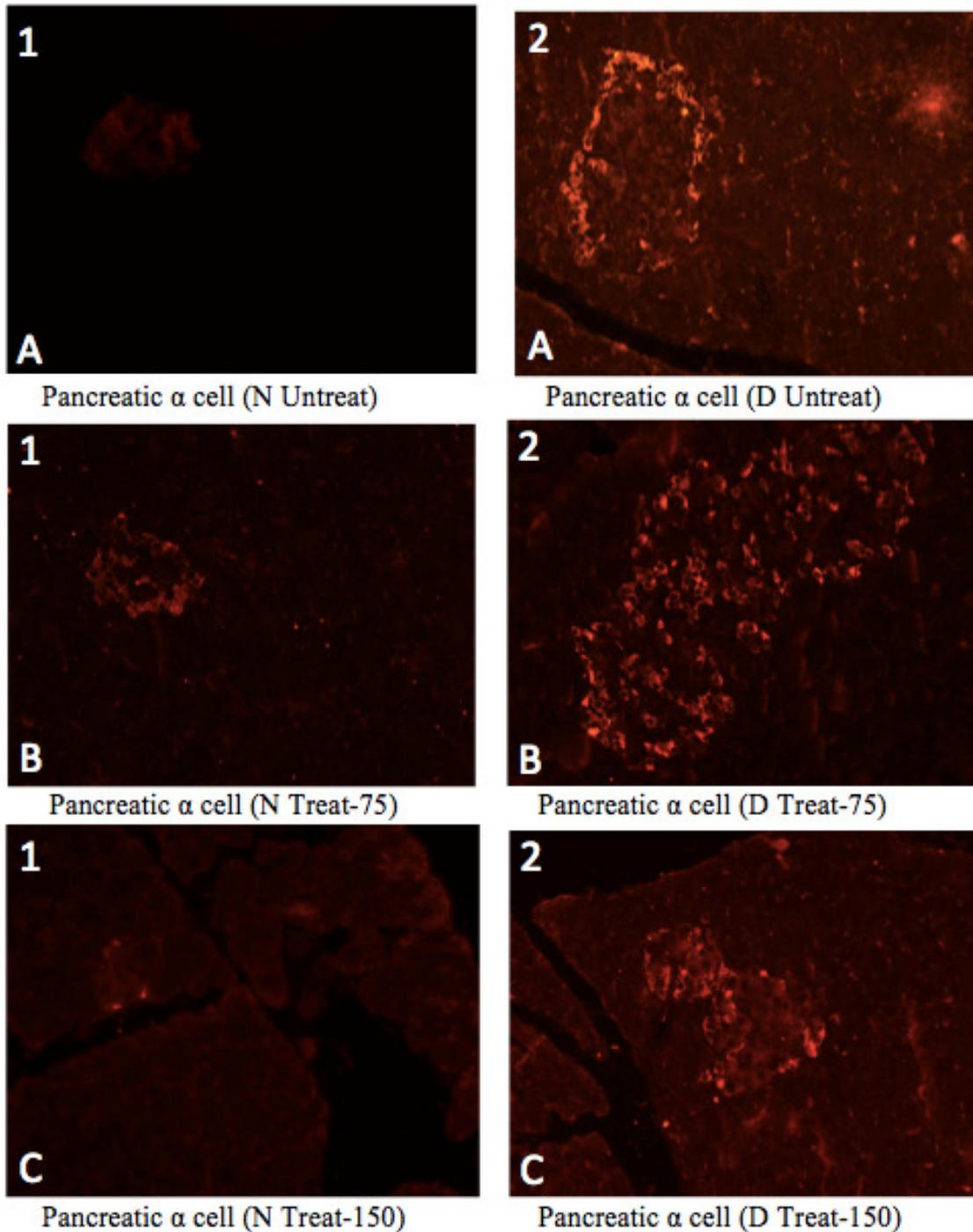


Figure 3.71: Original micrographs of immunofluorescence showing the distribution of glucagon positive cells in normal (N) untreated (1A) and diabetic (D) untreated (2A) rats pancreas and in normal and diabetic rats treated with both 75 mg/Kg (1B normal treated, 2B diabetic treated) and 150 mg/Kg (1C normal treated, 2C diabetic treated) aqueous cinnamon extract for 11 weeks. Magnification: X 400. Micrographs are typical of different pancreas taken for 5-6 different rats.

In another series of experiments the number of glucagon positive cells per islet were semi-quantitatively counted from the micrographs obtained from untreated and treated (75 mg/Kg and 150 mg/Kg) normal and diabetic rats. The mean (\pm SEM) values are presented in figure 3.72.

The results in figure 3.72 shows the percentage of glucagon positive cells in the pancreas of untreated normal and diabetic and in pancreas of normal and diabetic treated with either 75 mg/Kg or 150 mg/Kg of aqueous cinnamon extract. The results further show that pancreas of untreated diabetic rats contain significantly ($p < 0.05$) more glucagon positive cells compared to untreated normal rats. Treatment of cinnamon seemed to improve significantly ($p < 0.05$) the number of glucagon positive cells in both normal and diabetic rats pancreas. A dose of 150 mg/Kg of cinnamon was more effective in improving islet number in the diabetic rats.

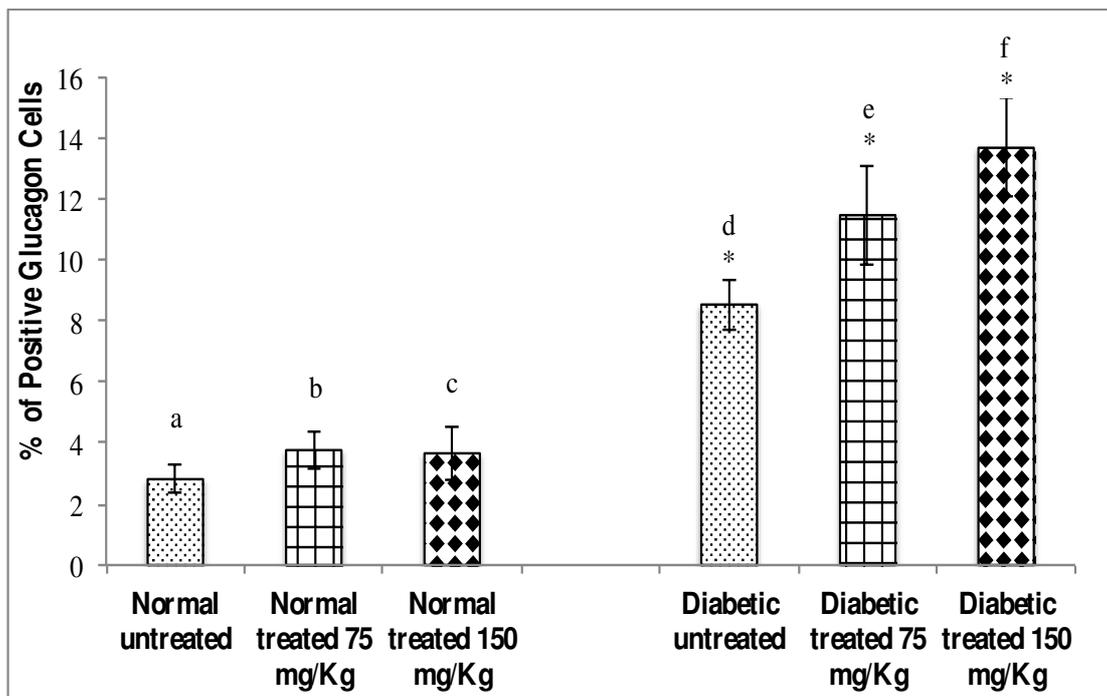


Figure 3.72: Bar chart showing the percentage (%) of islet positive glucagon cells in untreated normal and diabetic rats and treated with either 75 mg/Kg or 150 mg/Kg of aqueous cinnamon extract for 11 weeks. Data are mean \pm SEM, $n=5-6$ per group. Note that significant effect was found between treated and untreated diabetic rats ($*p < 0.05$, for diabetic compared to control) compared to untreated and treated normal rats. ANOVA Test. Data obtained from original micrographs as shown if figure 3.71.

C. HUMAN STUDIES

Cinnamon has been ingested by human for thousands of years as food, flavouring, beverages and as plant-based medicinal drug (Jakheta et al., 2010). Cinnamon is ingested as a tea, powder and tablets in the different doses. This part of the study investigated the effect of cinnamon on healthy human subjects measuring its effect on postprandial glycaemia. All subjects were asked to drink 100 ml of cinnamon tea (0.06 g/mL) in the morning on fasting followed by OGTT (oral glucose tolerance test).

3.15 Sample characterization of human study

The characterizations of the subjects of this study are represented in the following figures and tables regarding the distribution by gender, age, clinical record and anthropometric parameters.

Thirty non-diabetic subjects constitute the sample of this work. They included 22 (73.3%) females and 8 (26.7%) males.

Figure 3.73 represents the age distribution of all 30 healthy subjects employed in this study. The results show that most of the subjects were between 20-30 (n=12; 40%) and 41-50 (n=11; 36.7%) years old. The mean of age sample was 35.3 (\pm 1.9) years old. The minimum age was 20 years old and maximum age was 53 years old.

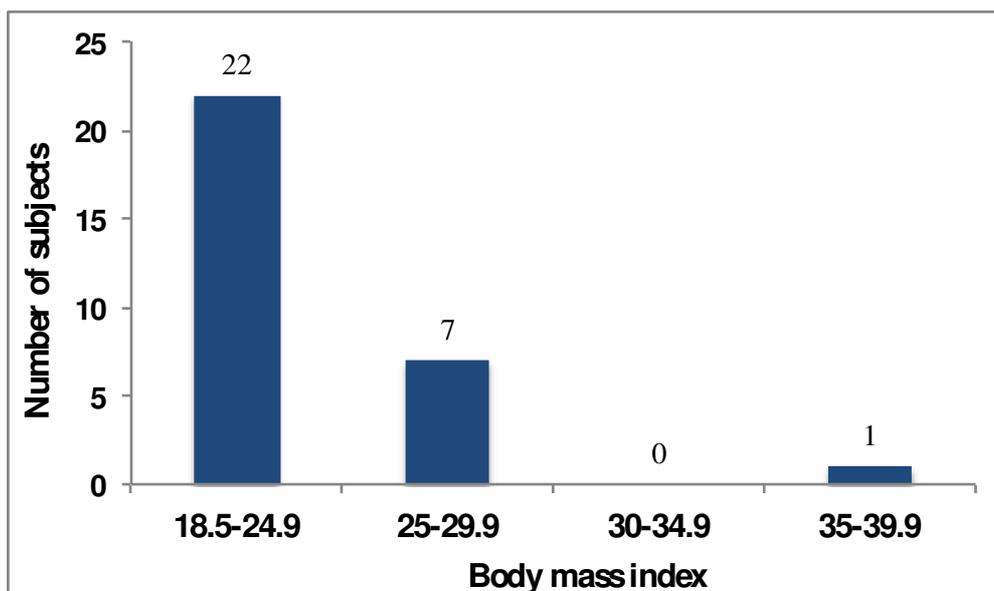


Figure 3.73: Bar chart showing the age distribution of the subjects. Values are expressed as number. Note that most of them fall in the age groups 20-30 and 41-50 years.

Table 3.8 shows the clinical records of the subjects regarding any pathologies and pharmacological therapies. The results of the table 3.8 show the clinical records of the subjects employed in this study, demonstrating that the participants had no diabetes mellitus, gastrointestinal or other diseases. Three participants had allergy and 2 participants took anti-inflammatory drug.

Table 3.8: Characterization of clinical record of participants employed in this study.

		n		Percentage (%)	
		Yes	No	Yes	No
Pathologies	Diabetes <i>Mellitus</i> 2	0	30	0	100
	Gastrointestinal disease	0	30	0	100
	Allergy	2	28	9.7	90.3
	Others	0	30	0	100
Pharmacologic therapy	<u>Anti-lipidemic</u>	0	30	0	100
	Anti-inflammatory	2	29	6.4	93.6
	<u>Antirritmic</u>	0	30	0	100

Table 3.9 shows the anthropometric parameters, namely weight and height of the subjects. The data show that the weight mean of the participants of this study was 66.7 (± 1.8) Kg (n=30) and the mean height was 164.9 (± 1.7) cm (n=30). The weights of the subject varied for 52.3 Kg to 90 Kg and the heights varied for 153 cm to 184 cm.

Table 3.9: Weight and height mean ($\pm SEM$) values of subjects.

	n	Minimum	Maximum	Mean ($\pm SEM$)
Weight (Kg)	30	52.3	90.0	66.7 (± 1.8)
Height (cm)	30	153.0	184.0	164.9 (± 1.7)

Table 3.10 shows the mean, minimum and maximum values regarding to anthropometric parameters, namely, body mass index (BMI), fat mass and muscular mass among the 30 subjects employed in this study. The results show that regarding to anthropometric parameters, the mean body mass index (BMI) in both gender corresponded to a regular weight of 24.4 (± 0.6) and 24.5 (± 0.7) Kg/m² for men and women, respectively. The fat mass mean ($\pm SEM$) percentage of the participants were 16.4 (± 0.9) and 27.8 (± 1.2), for man and women, respectively. The muscular mass mean ($\pm SEM$) of the participants were 61.7 (± 2.9) Kg and 42.0 (± 1.1) Kg for man and women, respectively.

Table 3.10: Body mass index (BMI), fat mass and muscular mass mean ($\pm SEM$) values of subjects. BMI – Body mass index; M – Men; W – women.

Anthropometric parameters	Gender	n	Minimum	Maximum	Mean ($\pm SEM$)
BMI (Kg/m²)	M	8	21.3	26.9	24.4 (± 0.6)
	W	22	19.7	35.1	24.5 (± 0.7)
Fat mass (%)	M	8	11.0	19.3	16.4 (± 0.9)
	W	22	17.0	36.2	27.8 (± 1.2)
Muscular mass (Kg)	M	8	48.7	70.8	61.7 (± 2.9)
	W	22	26.5	49.1	42.0 (± 1.1)

The data for the BMI classes are presented as bar charts in figure 3.74. The results show that most of the participants from both genders have regular weight (73.3%,

n=22). Only one subject was obese (3.3%) and 7 subjects had borderline overweight (23.3%).

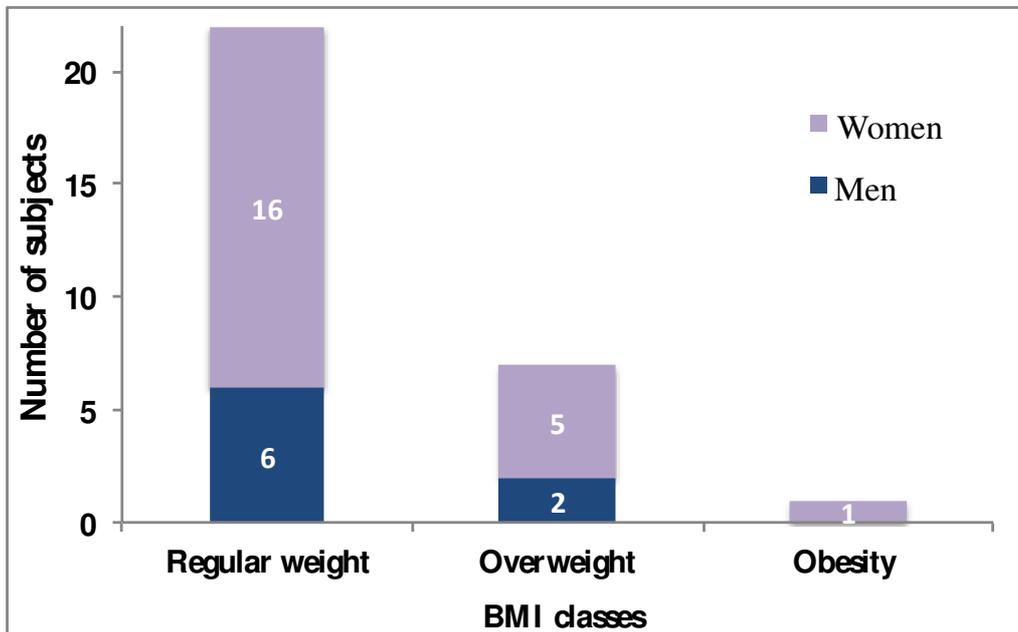


Figure 3.74: Distribution of gender by BMI classes. (BMI – body mass index. Regular weight: $18.5 \text{ Kg/m}^2 \leq \text{BMI} < 24.9 \text{ Kg/m}^2$; Overweight: $25 \text{ Kg/m}^2 \leq \text{BMI} < 30 \text{ Kg/m}^2$; Obesity: $30 \geq \text{Kg/m}^2$) (WHO, 1997).

Table 3.11 and figures 3.77 to 3.80 represent the results obtained regarding to nutritional parameters of the subjects on the day before either OGTT_(control) and OGTT_(cinnamon). These including, total energy value (TEV), carbohydrates (CD), protein (P) and lipid (L) ingested by participants. The results in table 3.15 revealed that there was no significant difference between dietetic ingestion on the day before of the intervention either with or without cinnamon tea ($p > 0.05$) in relation to total energy value, carbohydrates, protein and lipid. These results allowed to confirm the homogeneity between groups.

Table 3.11: Data showing total energy value (TEV), carbohydrates (CD), protein (P) and lipid (L) ingested at the day before of intervention by participants. Data are mean \pm SEM, n=30; *Independent sample Student's t-test* was used to statistical analysis.

Dietary Parameters	Day before at	Day before at	<i>p</i>
	OGTT	OGTT+cinnamon tea	
	Mean (\pm SEM)	Mean (\pm SEM)	
TEV (Kcal)	1708.01 (\pm 97.04)	1736.51(\pm 113.74)	0.850
CD (g)	216.04 (\pm 19.32)	225.40 (\pm 15.33)	0.707
P (g)	75.66 (\pm 6.15)	77.67 (\pm 6.49)	0.823
L (g)	58.54 (\pm 4.6)	58.47 (\pm 6.61)	0.993

TEV: total energy value; CD: carbohydrates; P: protein; L: lipid

Figures 3.75 to 3.78 show bar chart with the protein (g), lipid (g), carbohydrate (g) and total energy value (Kcal) mean (\pm SEM) ingested by the participants on the day before of OGTT_(control) or before OGTT_(cinnamon).

The results show that mean dietary intake of protein (figure 3.75), lipid (figure 3.76), carbohydrate (figure 3.77) and total energy (figure 3.78) before OGTT and before OGTT with cinnamon ingestion were lower in women that in men.

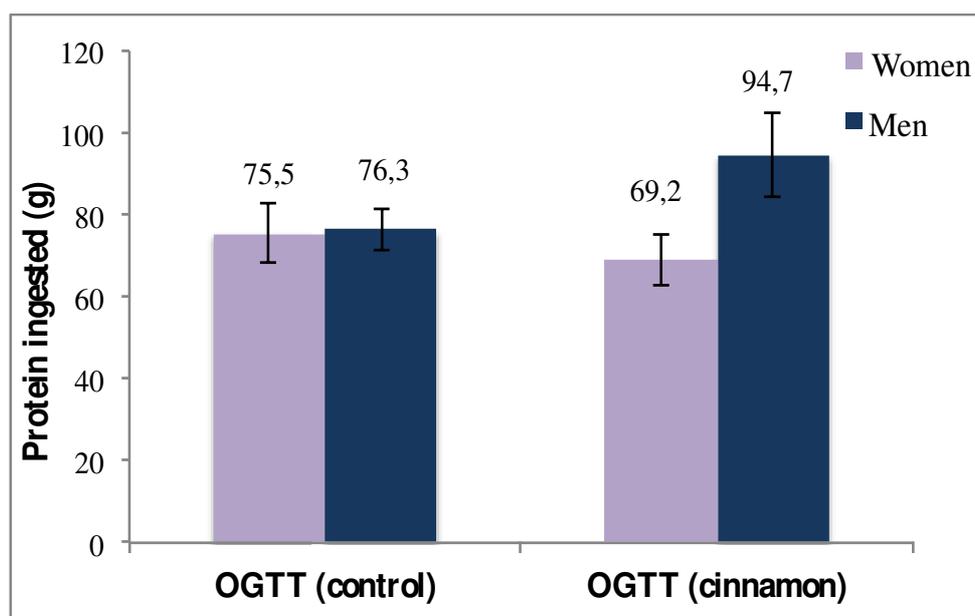


Figure 3.75: Bar charts showing protein (g) ingested by both women and man on the day before the OGTT_(control) and OGTT_(cinnamon). Data are mean \pm SEM (n=30).

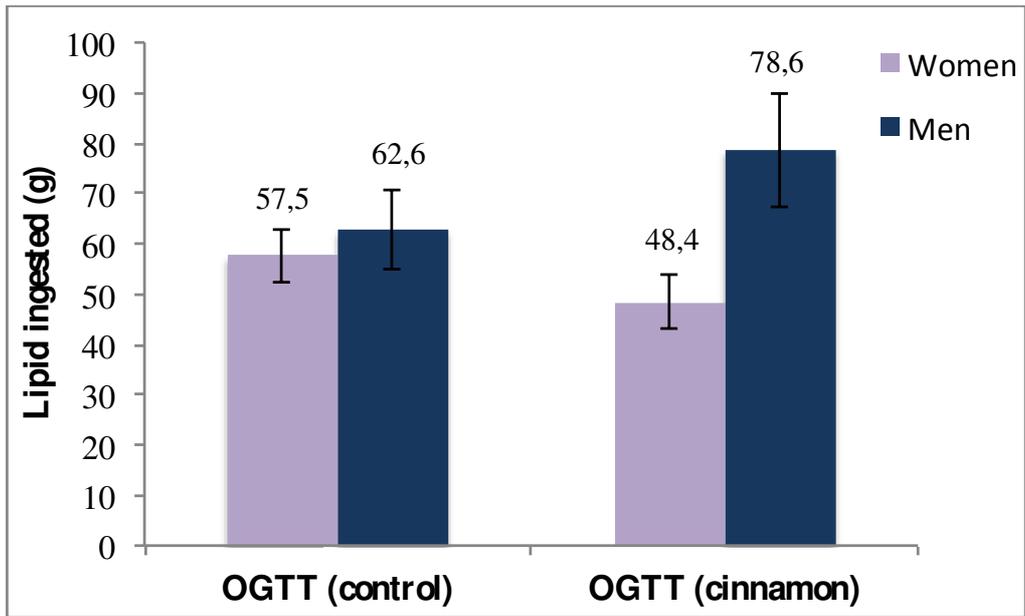


Figure 3.76: Bar charts showing lipid (g) ingested by both women and man on the day before the OGTT_(control) and OGTT_(cinnamon). Data are mean \pm SEM (n=30).

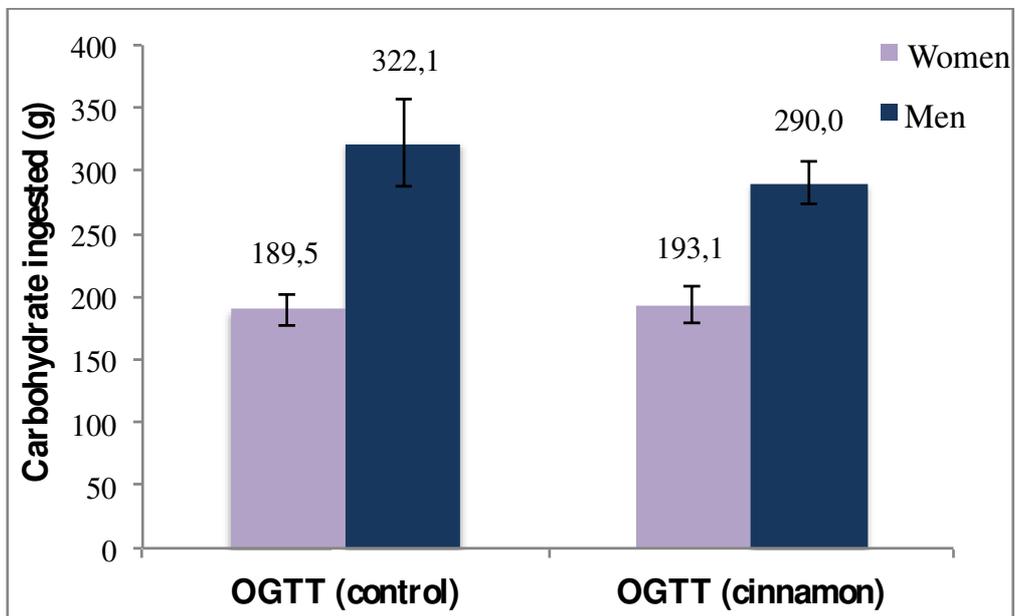


Figure 3.77: Bar charts showing carbohydrate (g) ingested by both women and man on the day before the OGTT_(control) and OGTT_(cinnamon). Data are mean \pm SEM (n=30).

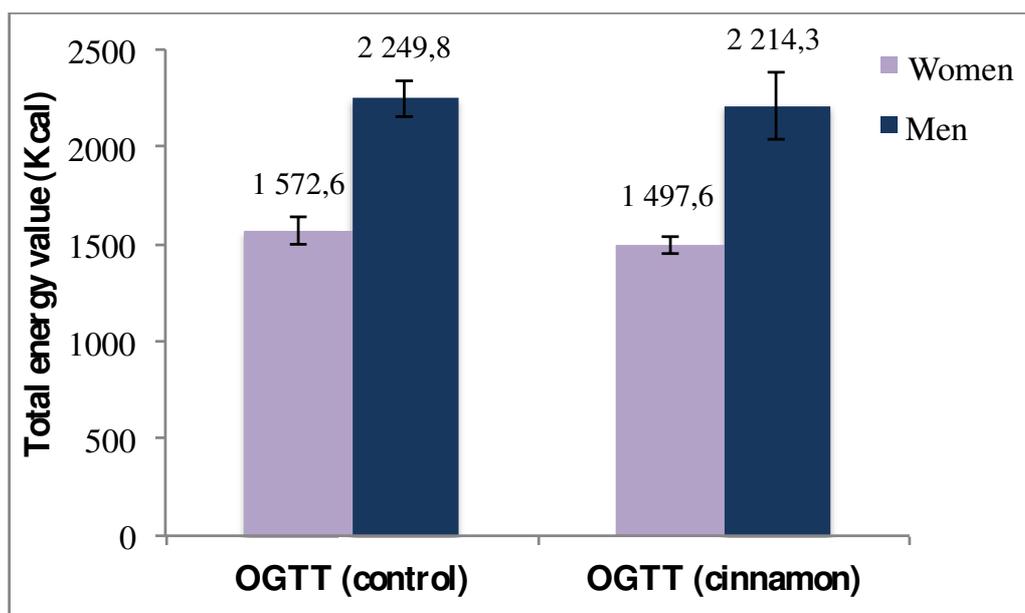


Figure 3.78: Bar charts showing total energy values (g) ingested by both women and man on the day before the OGTT_(control) and OGTT_(cinnamon). Data are mean \pm SEM (n=30).

3.16 Effect of ACE on blood glucose level after Oral Glucose Tolerance Test of human

In order to analysed the effect of aqueous cinnamon extract from *C. burmannii* on blood glucose levels of normal subjects, a glucose tolerance test (OGTT) either with or without cinnamon tea administration was undertaken.

Table 3.12 shows capillary glycaemia values obtained after OGTT with and without cinnamon tea administration. Values were expressed on fasting (t_0), and 30 (t_{30}), 60 (t_{60}), 90 (t_{90}) and 120 (t_{120}) minutes after OGTT administration. The individual results from the OGTT are represented in of 30 different tests.

The results from the Table 3.12 show that blood glucose levels obtained after OGTT + cinnamon tea administration were lower compared with blood glucose levels obtained after OGTT alone at 30, 60, 90 and 120 min. Statistical analysis revealed that there was no interaction between the independent and repeated measures factors ($p=0.209$), which means that it is not possible to infer about differences in BGL in different moments. However, the data shown that the administration of cinnamon tea

after OGTT slightly decreased BGL mean values compared to OGTT in the absence of cinnamon ingestion (figure 3.79).

Table 3.12: Data showing capillary glycaemia obtained by oral glucose tolerance test (OGTT) Data are mean \pm SEM, n=30.

Time	OGTT (n=15)		OGTT + cinnamon tea (n=15)	
	Min/Max	Mean (\pm SEM)	Min/Max	Mean (\pm SEM)
t_0	4.22 – 5.49	4.97 (\pm 0.1)	4.11 – 5.49	4.99 (\pm 0.1)
t_{30}	7.38 – 15.65	10.14 (\pm 0.4)	5.72 – 14.49	8.87 (\pm 0.4)
t_{60}	4.16 – 14.32	8.75 (\pm 0.5)	4.61 – 13.82	8.24 (\pm 0.4)
t_{90}	4.77 – 16.43	7.66 (\pm 0.5)	4.44 – 10.71	7.29 (\pm 0.3)
t_{120}	4.00 – 7.71	6.40 (\pm 0.2)	3.89 – 7.22	5.86 (\pm 0.2)

* p < 0.05, compared glycaemia mean values between OGTT and OGTT + cinnamon tea.

Figure 3.79 represents the time course blood mean glucose level for the participants (n=30) during OGTT and OGTT + cinnamon tea.

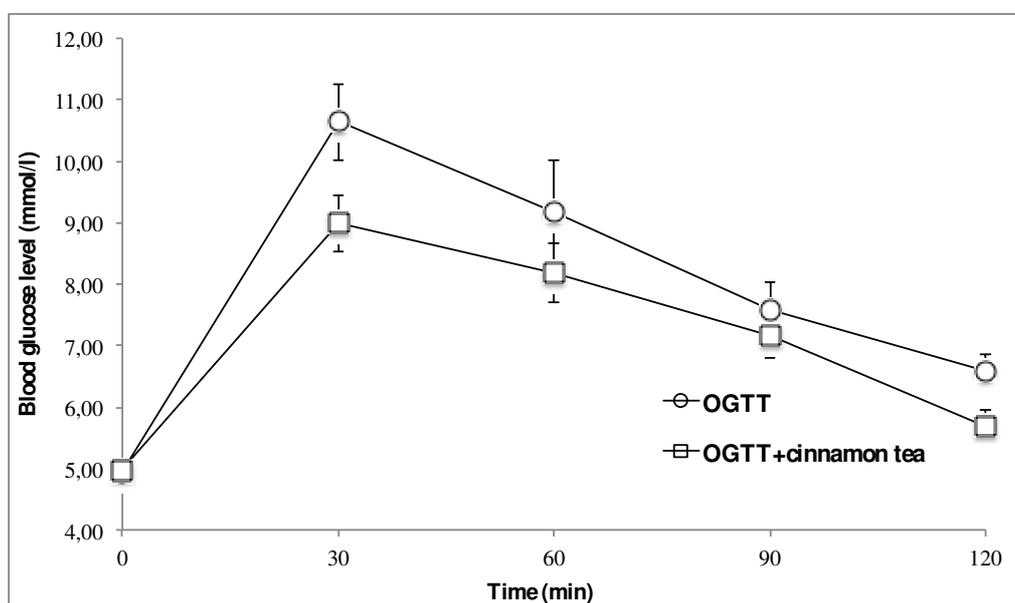


Figure 3.79: Mean (\pm SEM) time course of blood glucose concentration in 30 normal subjects after OGTT (\ominus) and OGTT + cinnamon tea (\boxminus).

The same was observed regarding the maximum and minimum blood glucose levels values in which, blood glucose levels after OGTT + cinnamon tea administration were

lower than OGTT alone. Minimum values of blood glucose levels at t_{60} were the only results that demonstrated to be lower in OGTT compared to OGTT + cinnamon tea administration. Furthermore, the results obtained at t_{30} time representing the maximum values confirmed that glycaemic values to be normal when compared with references values (ADA, 2013b).

Figure 3.80 shows the (A) maximum concentration (C_{\max}), (B) variation of maximum concentration (ΔC_{\max}) and (C) area under the curve (AUC) with regarding blood glucose level after OGTT and OGTT + cinnamon tea, for comparison. The results reveal that the ingestion of cinnamon tea to OGTT result improve the area under the curve for OGTT with cinnamon compared with OGTT without cinnamon ingestion ($p>0.05$). The blood glucose level at maximum concentration and the variation of maximum concentration show a significant decrease in OGTT + cinnamon tea ingestion ($p<0.05$). These results suggest a beneficial effect of cinnamon in glucose homeostasis after high sugar ingestion, regulating the maximum glucose concentration in blood during a period of 120 minutes.

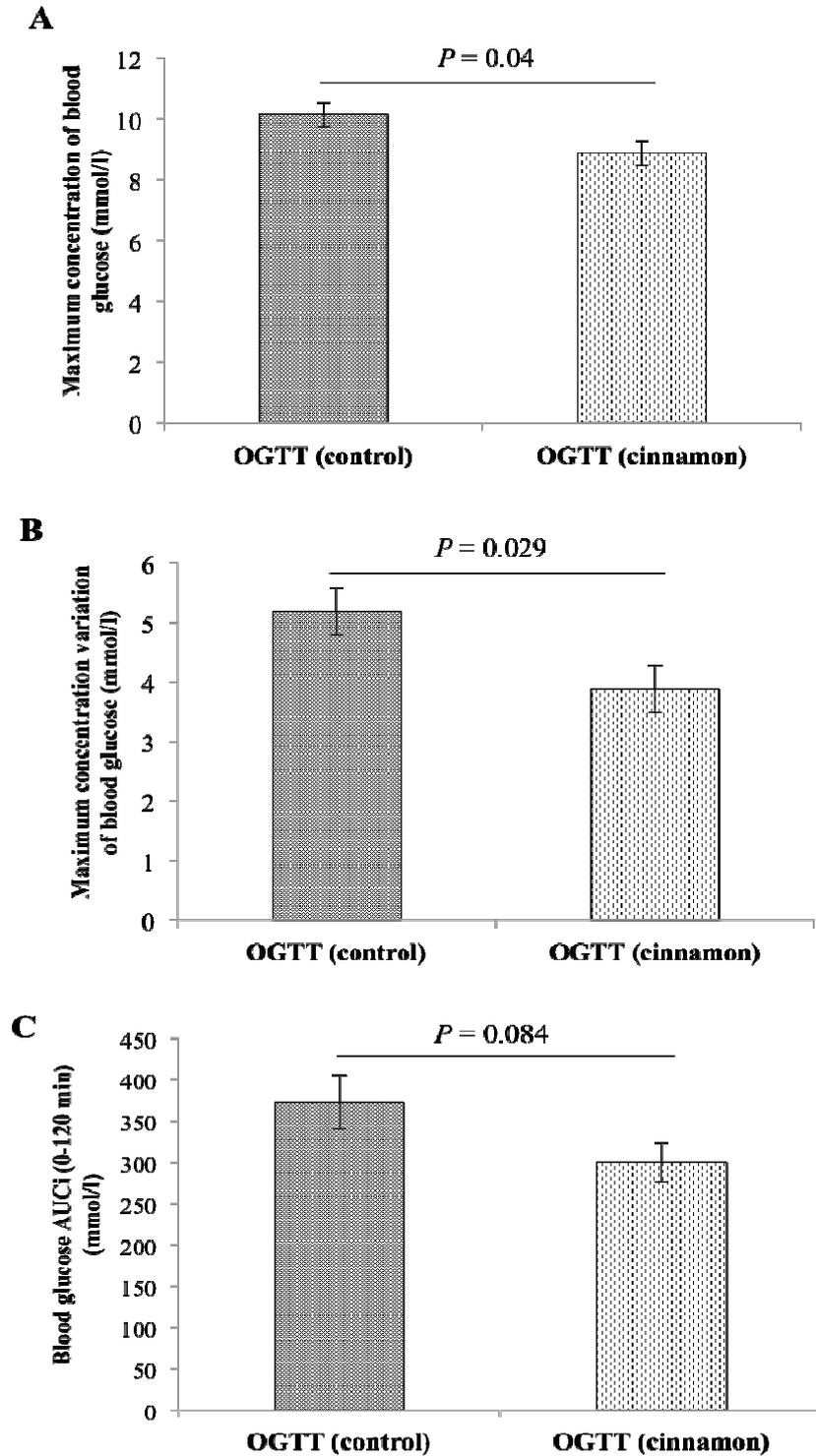


Figure 3.80: Data showing the (A) blood glucose maximum concentration (C_{max}), (B) blood glucose of maximum concentration variation (ΔC_{max}) and (C) blood glucose area under the curve (AUC) with in healthy subjects after OGTT and OGTT + cinnamon tea administration. Data are mean \pm SEM, $n=30$; $p<0.05$; *T-student test* was used to statistical analysis between OGTT and OGTT + cinnamon tea administration.

Chapter Four

DISCUSSION

4 Discussion

This study investigated the beneficial effect of aqueous cinnamon extract in *in vivo* healthy rat and human models employing streptozotocin (STZ)-induced type 1 diabetic rats and compared with age-matched healthy control animals and healthy human subjects.

Diabetes mellitus (DM) is a major global health metabolic disorder estimated to increase markedly worldwide. It is also an economic burden to the Governments of the world estimated to cost in excess of £1 trillion annually to diagnose, treat and care for diabetic patients so that they can have a better quality of life. In Portugal, in particular, DM is highly prevalent (Guariguata et al., 2014). This disease can lead to numerous long-term complications including retinopathy, cardiomyopathy, neuropathy, nephropathy, foot ulcers and many others, all resulting in end-organ failure over time if left untreated (Fowler, 2008). As such it is very important to diagnose the diabetes earlier and either to prevent or to treat the disorder at a very early stage. In developing countries DM is a very prevalent disorder and it is too costly for the people to get medical help (ADA, 2010b; Whiting et al., 2011). As such plant-based medicine has been used to treat many disorders including cancers, mental disorders, DM and several others. One such plant is cinnamon. This plant has many species and a particular one is *Cinnamomum burmannii*. Previous studies employing different species of cinnamon have shown that this plant contains numerous compounds, which can exert different effects including anti-hyperglycaemic, anti-inflammatory, anti-oxidant and anti hyperlipidaemic benefits (Ulbricht et al., 2011).

This discussion will be focused on the following:

- 1) The chemical characterization of anti-oxidant capacity of aqueous cinnamon extract employed in animal and human studies;
- 2) Cinnamon spices from *C. burmannii* species to treat DM both normal and diabetic animal model assessing its effect on biochemical parameters, structure and function of the pancreas, the heart and cation levels in different organs;
- 3) Cinnamon tea to improve postprandial blood glucose level on healthy subjects and;

- 4) Possible mechanism(s) of action of the active anti-diabetic compounds of cinnamon.

4.1 Anti-oxidant capacity and major phenolic compounds of ACE

The results from the present study demonstrated that total phenolic contents of aqueous cinnamon extract from *C. burmannii* specie increased, but not proportionally with increased concentrations of the cinnamon extract. The total phenolic content analysed in aqueous cinnamon extract employed in the present study revealed a higher mean values (2286.3 mg/l gallic acid), compared with a previous study (Prasad et al., 2009). This difference could be due to different part of the tree used for analyses since that in Prasad and co-authors work it was analysed the extract of cinnamon leaf (943.7 µg/g gallic acid) (Prasad et al., 2009). The storage method (-20°C) used for aqueous cinnamon extract employed in the present study demonstrated a loss of total phenolic contents by 20% after 5 weeks of storage at -20°C. This result obtained in the present study is not in full agreement with a previous study, which reported that total phenolic contents of the cinnamon fruit juice decreased by 29% after 15 days of storage at -25°C. Such different results may be attributed to a lower temperature used in juice fruit, -25°C instead of -20°C or the way the fruit juice or extracted is prepared for the study (Mirsaeedghazi et al., 2014). Nevertheless, the results clearly showed that storage at very low temperature over time might reduce the phenolic contents of cinnamon and it would be advisable to use the freshly prepared extract for experimentation. The major phenolic compounds identified in cinnamon extract form *C. burmannii* were cinnamaldehyde > cinnamic acid > coumarin > cinnamyl alcohol with 23.99%, 3.73%, 1.56% and 0.21%, respectively, which are consistent with another published data (Y.-H. Wang et al., 2013).

The anti-oxidant potential of aqueous cinnamon extract employed in this study was analysed *in vitro* using the reducing power FRAP assay and the superoxide anion scavenging activity. The results have shown that cinnamon can exert a strong anti-oxidant capacity determined though FRAP assay (11779.0 ±294.7 µmol Trolox/l). According to the previous studies the cinnamon extract from the bark revealed less reducing power than cinnamon extract from the powder (Hossain et al., 2008; M. Lu

et al., 2011). However, the uniformity absence of the extraction methods could contribute to the difference observed in different previous studies (Hossain et al., 2008; Khristi et al., 2014; M. Lu et al., 2011). Moreover, the total phenolic content could also depend of intrinsic characteristics of cinnamon, which depend from species, growth stages, parts of plant or plant origin (Geng et al., 2011; He et al., 2005). Cinnamon extract also revealed a high capacity to inhibit superoxide scavenging in a dose-dependent manner (96%). These result is in agreement with previous studies, which demonstrated a high anti-oxidant capacity and an excellent superoxide anion scavenging activity in a dose-dependent manner by 74.5% (Prasad et al., 2009).

The phenol compounds identified in the sample employed in the present work could be responsible for its anti-oxidant properties observed in this study both in *in vitro* and *in vivo* animal model. Data from *in vitro* study demonstrated a significant relationship between anti-oxidant properties and total phenolic content in plants, suggesting that phenols are the bioactive compounds which contributed to their antioxidants capacity (Dudonné et al., 2009). In addition, the high total phenolic contents in *C. zeylanicum* were also suggested to be associated with the high reducing power of this spice (Prasad et al., 2009).

4.2 Effect of ACE on body weight of normal and diabetic rats

The present study was also designed to investigate the beneficial hypoglycaemic effects of aqueous *C. burmannii* extract on the STZ-induced diabetic rat compared to age-matched normal rats. Thus, the rats were separated into different sub-groups including diabetic and healthy age-match groups, both untreated and treated with two different doses of aqueous cinnamon extract (75 mg/Kg and 150 mg/Kg). In this study, the rats were treated for 11 weeks.

The results of this study show that all the rats gained weight for the first two weeks following adaptation in the laboratory. Following two weeks of diabetes induction by STZ, diabetic rats gained less weight compared to normal animals which is in agreement with other study (Jia et al., 2009). Over the experimental aqueous

cinnamon extract treated period of the 11 weeks, all normal rats gained weight compared to weight week zero, at just before injection of STZ. However, cinnamon treatment showed no effect on weight gain in normal rats, compared with untreated normal rats.

The diabetic rats treated with aqueous cinnamon extract showed more gained weight compared to diabetic untreated rats, but values were not significant when they were compared statistically. This result is in agreement with a previous study in which aqueous *C. zeylanicum* extract treatment improved body weight in T1DM compared to diabetic untreated rats (Shen et al., 2010). Furthermore, the bioactive compounds isolated from ACE seems also to exert an improvement of body weight, namely polyphenolic extract (Jia et al., 2009) and cinnamaldehyde (Babu et al., 2007). Other studies demonstrated no significant effect of ACE on body weight, but this occurred in T2DM animal model (S. H. Kim et al., 2006a; B Qin, 2003). The current study also suggests that the effect with high dose (150 mg/kg) of *C. burmannii* extract was more pronounced compared to low dose (75 mg/kg) in diabetic-treated rats. These results clearly show that *C. burmannii* treatment has some beneficial effects in STZ-induced diabetic rats relating to weight gain. The mechanism by which cinnamon extract seemed to improve body weights is unclear. However, considering that insulin has an anabolic effect (Murray et al., 2009), it is tempting to infer from the data of the present study that the bioactive component(s) of aqueous cinnamon extract may exert an insulinomimetic effect, namely, improving insulin receptor signalling (Qin et al., 2012) or repairing the pancreatic beta cell and its mass in diabetic rats (Ping et al., 2010). By repairing the beta cells and their mass, they in turn can produce newly synthesising insulin in the body of the rats (Lotfy et al., 2014).

4.3 Effect of ACE on food consumption of normal and diabetic rats

The present results have also shown that the normal-treated and untreated rats ate more chow (food) at the inception (0-3 weeks) than at the end of the experimental protocol, 11 weeks later. Over the experimental protocol, cinnamon treatment demonstrated no significant effect on food consumption in normal age-matched rats especially at a dose of 75 mg/Kg. However, normal rats treated with high dose of

cinnamon (150 mg/kg) seemed to consume more or less similar amount of food compared to untreated normal rats. These results are in agreement with a previous study, which showed no significant changes in food intake in healthy normal rats (Priyanga Ranasinghe et al., 2012). These are surprising results since the data for weight gain show a gradual increase in body weight with maximal weight gain at week 11. This result may not be due to cinnamon treatment since the untreated normal rats ate more or less the same amount of food in a weekly basis over the 11 weeks of treatment.

Regarding, diabetic treated rats (75 and 150 mg/Kg doses), they seemed to eat significantly ($p < 0,05$) less chow over the 11 weeks of experimental period compared to untreated diabetic rats which gained significantly ($p < 0,05$) more weight. This result is also in agreement with other studies which demonstrated that aqueous cinnamon extract (600 mg/Kg bw) decreased food intake in T1DM rats (Priyanga Ranasinghe et al., 2012). It is interesting to note that although the mean of body weight increased, the mean of food intake decreased in diabetic rats with cinnamon treatment. This probably occurred as a result of the cinnamon enhancing insulin-regulated glucose utilization, resulting in cellular glucose uptake by GLUT 4 translocation (Shen et al., 2010). The mechanism(s) involved in food intake and body weight- gained processes may be due to the stimulation of insulin receptor activity to increase tyrosine phosphorylation of IRS-1 and increase of IRS-1 mRNA expression (B Qin, 2003; Bolin Qin et al., 2012).

However, one interesting finding in this study is that diabetic treated and untreated rats ate more food than control treated and untreated rats, but the diabetic rats gained significantly less body weight compared to normal animals.

4.4 Effect of ACE on blood glucose and OGTT of normal and diabetic rats

The results of this study have also demonstrated that untreated and treated diabetic rats had significantly ($p < 0.05$) elevated blood glucose compared to normal rats over the experimental period. This was confirmed by measuring fasting blood glucose, OGTT and HbA1c in diabetic and normal rats. In normal rats no pronounced difference in blood glucose levels between treated and untreated normal rats were

observed. On the other hand, both 75 mg/Kg and 150 mg/Kg of cinnamon treatment showed a reduction in blood glucose levels over the experimental period of 11 weeks compared to untreated control rats. Only at week 3, the blood glucose level of rats treated with high dose of cinnamon extract was significantly ($p < 0.05$) lower compared to untreated diabetic rats. This result is in agreement with other study which verified that aqueous cinnamon extract of other species of cinnamon decreased blood glucose level at week 3 compared to untreated diabetic rats (Shen et al., 2010). Similarly with other works, after 6 weeks of aqueous cinnamon extract treatment no significantly effect in blood glucose levels was observed in treated-diabetic rats compared with untreated rats (B Qin, 2003). This result suggests that aqueous *C. burmannii* extract may exert some insulinotropic effect in STZ-induced diabetic rats, but this depends on the time after administration. It was more pronounced at an earlier stage compared to a later time. According to previous findings, this plant can stimulate an increase in blood insulin levels (Anand et al., 2010) and insulin activity (Anderson et al., 2004). This effect can be due to polyphenol compounds presented in cinnamon extract (Jia et al., 2009). Once elevated in the body, these polyphenols can act like insulin secretagogue or they can increase the beta cell mass in the pancreas.

Concerning the oral glucose tolerance test (OGTT), the results show that the normal untreated rats were able to metabolize glucose in the body. Following an oral glucose administration, blood glucose rises within 30 min followed by a reduction after 120 min. This was more noticeable in normal untreated and treated rats with 150 mg/Kg of cinnamon animals. However, in normal treated animals with 75 mg/Kg, blood glucose levels increased slightly over the experimental protocol.

In diabetic rats, the administration of oral glucose solution resulted in blood glucose elevation after 30 min. This level remained more or less the same in all 3 sub-groups (untreated and treated both 75 mg/Kg and 150 mg/Kg) of diabetic rats over the experimental period indicating that the diabetic rats were unable to regulate blood glucose metabolism and as such confirmed diabetes in the animals. Moreover, the treated diabetic rats show more or less the same trend suggesting that cinnamon was unable to regulate blood glucose in the diabetic rats. The results further suggest that cinnamon may exert no anti-glycaemic effect in the rats. A study analysing the effect of cinnamon bark on oral glucose tolerance test revealed that the mean percentage reduction in blood glucose from 30 to 60 min was significantly higher in cinnamon administrated group comparing to untreated diabetic group (Priyanga Ranasinghe et

al., 2012). The administration of 5 g of *C. cassia* by capsule form also significantly decreased postprandial blood glucose at 30 min after glucose solution administration (T P J Solomon & Blannin, 2007). The data from the present study and others have clearly demonstrated that cinnamon extract may not directly regulate blood glucose level upon administration in rats, but its capsule form or even its phenolic compounds administered individually may possess hypoglycaemic properties. Further experiments are required to compare the effects of aqueous cinnamon extract and its different phenolic compounds, as well as capsule forms of all the compounds and cinnamon on hyperglycaemia.

4.5 Effect of ACE on blood biochemical parameters of normal and diabetic rats

In addition to all the previous parameters, the study also measured blood biochemical parameters, such as, levels of blood triglycerides, total cholesterol, LDL, HDL, total albumin, creatinine and haemoglobin A1c (HbA1c). The results show that STZ-induced diabetes was associated with elevated levels of blood triglycerides and these enhanced values remained high following treatment with cinnamon compared to untreated diabetic rats. These data are in agreement with other studies where aqueous cinnamon extract had not significant effect on triglycerides in T1DM (Shen et al., 2010). However, the main bioactive compounds of cinnamon extract such as cinnamaldehyde revealed significantly reduction in triglycerides levels (Babu et al., 2007).

The results also show that the levels of LDL increased slightly in diabetic untreated and treated rats compared to normal rats. However, these values were not significant comparing normal with diabetic rats. Moreover, cinnamon treatment seems to improve LDL level in diabetic rats.

Regarding HDL, cholesterol increased significantly in untreated and treated (150 mg/kg) diabetic rats compared to untreated and treated normal rats. HDL is a protective cholesterol, which is beneficial to the animals. Elevated levels of HDL may be a protective mechanism to compensate for the small rise in LDL observed in this study (Dullaart et al., 2012). These elevations in LDL and HDL are reflected in the

small rise in total cholesterol in diabetic-treated and untreated rats compared to the respective normal rats.

The results of this study revealed that the effect of aqueous cinnamon extract on total cholesterol levels is in agreement with others studies. Administration of aqueous *C. zeylanicum* extract in STZ-induced type 1 diabetic rats showed no significant alteration in the total cholesterol compared to untreated group (Shen et al., 2010). In another study, the administration of cinnamon extract for 30 days reduced significantly LDL cholesterol in normal and diabetic rats at the end of experimental protocol compared to the beginning of the experimental protocol. This difference was not observed in normal and diabetic untreated rats. The HDL cholesterol and TG did not change significantly in untreated and treated normal and diabetic rats (Priyanga Ranasinghe et al., 2012). Moreover, the administration of cinnamaldehyde to T1DM for 45 days significantly improved lipid profile, suggesting that it is the isolated bioactive compounds in a higher dose which can exert beneficial effect in T1DM rats (Babu et al., 2007). In T2DM rats, aqueous cinnamon extract (S. H. Kim et al., 2006b) and cinnamon oil (Ping et al., 2010) can also exert a beneficial action on lipid parameters.

The results of this study have also shown that serum creatinine levels remain more or less the same in normal and diabetic untreated and treated rats. Aqueous cinnamon extract seems to decrease slightly creatinine levels in diabetic rats compared to untreated, which is according to others workers (Shen et al., 2010). This result suggests a positive effect of this plant extract on diabetes since serum creatinine level is an important kidney function marker that increases with development of diabetic nephropathy (Grover et al., 2012).

The serum albumin levels were significantly ($p < 0.05$) lower in untreated diabetic rats compared to normal rats. This is in agreement with a study which verified an association between low serum albumin level and diabetes mellitus (Folsom et al., 1995). However, this work suggests that aqueous cinnamon extract has no effect on serum albumin levels on STZ-induced diabetic rats.

Finally, the results have clearly demonstrated significant increases in blood levels of haemoglobin A1c (HbA1c) in both untreated and treated diabetic rats compared to respective normal rats. Medical Diabetes Expert Committees (MDEC) reported that this is an important parameter to diagnose diabetes. An animal or a person is confirmed diabetic when HbA1c is equal to or more than 6.5% (Herman et al., 2010).

This study suggests that aqueous cinnamon extract treatment did not change the levels of haemoglobin A1c in either normal or diabetic rats. This result is in agreement with previous studies which reported that cinnamon treatment had no effect in normal and diabetic rats (Priyanga Ranasinghe et al., 2012). However, cinnamaldehyde (20 mg/kg), a major component of cinnamon significantly decreased HbA1c after 45 days of the treatment in T1DM (Babu et al., 2007). The present data from the HbA1c test have further corroborated that aqueous cinnamon extract may not possess hypoglycaemic properties. Similar data were obtained with fasting glucose test presented earlier in this discussion.

Therefore, its beneficial hypoglycaemic effect is seen only in capsule form or via its individual phenolic compound. It is also possible that the body of the animals are unable to release the active hypoglycaemic content of aqueous cinnamon extract when administered orally. Together, data from HbA1c blood levels have clearly demonstrated that the rats were indeed diabetic. Moreover, cinnamon treatment seems to improve slightly fasting blood glucose levels but failed to reduce postprandial blood glucose level indicating that it is not exerting its protective and beneficial effect by regulation blood glucose in the T1DM.

4.6 Effect of ACE on organs of normal and diabetic rats

The present study has also attempted to determine if diabetes can result in either reduced or elevated organs weights compared to normal and moreover to find out if cinnamon treatment can reverse any adverse effects. Five organs and tissues were employed including the heart, the pancreas, the liver, the kidney and the soleus muscle.

The results of this study revealed that the weight of the heart of untreated diabetic rats decreased compared to age-matched normal rats, but their heart weight to body weight ratio was increased in diabetic rats indicating a sign of hypertrophy or cardiomyopathy. These results was consistent with previous study (L. Zhang et al., 2008). According to published data, in pre-diabetic mice the left ventricle remodelling presented marked hypertrophy of cardiomyocytes and increased extracellular matrix deposition, which together lead to increased heart size. The results of that study suggest that the decompensating of ventricle in advanced cardiomyopathy may have

origin in fibrotic mechanism (D'Souza et al., 2011). In the present study, treatment with cinnamon (150 mg/Kg) demonstrated a slightly decreased of heart weight to body weight ratio, suggesting that cinnamon can exert a possible beneficial effect in the diabetic rats. The same effect was observed in normal rats, but in this case with the 75 mg/Kg dose of cinnamon.

Regarding pancreas weight, untreated diabetic rats showed a slightly, but not significant decrease in pancreas weight compared to age-matched normal rats. These results are in agreement with previous studies (Shen et al., 2010). However, an unexpected result was obtained since that the pancreas weight to body weight ratio showed an increased in diabetic untreated rats compared to untreated normal rats. Another study demonstrated that untreated diabetic rats had less pancreatic weight to body weight ratio than untreated normal rats (K Couturier et al., 2010). Furthermore, cinnamon treatment did not seem to exert any beneficial effect on these parameters, which are in agreement with another study (Shen et al., 2010). In a previous study, cinnamon was shown to improve pancreatic weight to body weight ratio which may be related to the improvement of insulin sensitivity in animals (K Couturier et al., 2010). However, cinnamon may exert different actions depending on the animal condition. In a study by Couturier et al. (2010), they demonstrated a positive effect of cinnamon on the weight of the pancreas, but only in animal model of the metabolic syndrome.

The results of this study regarding the weight of the liver show an unexpected finding, in which untreated diabetic rats demonstrated a slightly increased weight compared to untreated normal rats (Anand et al., 2010; Shen et al., 2010). The same unexpected results were observed concerning liver weight to body weight ratio (Anand et al., 2010). Moreover, data from the present study revealed that cinnamon treatment did not increase significantly the weight of this organ in diabetic rats compared with untreated diabetic rats. These data are in contrast to those obtained by Shen et al. who reported a significant increase liver weight to body weight ratio (Shen et al., 2010).

The present study has shown an increase in kidney weight of untreated diabetic rats compared to untreated normal rats, but this was not significant. This finding is consistent with previous studies (Anand et al., 2010; Shen et al., 2010). A similar consistent result was observed for kidney weight to body weight ratio, showing an increase in untreated diabetic rats compared with untreated normal rats (Anand et al., 2010). Cinnamon treatment with both doses (75 and 150 mg/Kg) increased kidney

weight and kidney weight to body weight ratio in diabetic rats compared to untreated diabetic rats. This result is in agreement with another work which verified that aqueous cinnamon extract did not increase significantly kidney weight in treated animals compared with untreated T1DM rats (Shen et al., 2010). The authors from that study suggest that aqueous cinnamon extract may exert a protective effect attenuating the organ weight loss in STZ-induced diabetic rats (Shen et al., 2010), as already was observed in kidney weight from treated diabetic rats in the current study. A decreased of skeletal soleus muscle in untreated diabetic rats was observed compared to untreated normal rats ($p>0.05$). In addition, a significantly decreased was observed in untreated diabetic rats compared to treated normal rats ($p<0.05$), which are in agreement with a previous study (Shen et al. 2010). Moreover, cinnamon treatment, especially with 75 mg/Kg dose increased the skeletal soleus muscle weight to body weight ratio compared with untreated diabetic rats. This result is also in agreement with a previous finding (Shen et al., 2010). The possible mechanism can be explained through the effect of aqueous cinnamon extract in up-regulated GLUT 4 translocation, stimulating glucose uptake in muscle in diabetic rats (Shen et al., 2010).

4.7 Effect of ACE on antioxidant profile of normal and diabetic rats

The results of the present study have shown that untreated diabetic rats contained lower anti-oxidant status compared to untreated normal. This physiological mechanism can be explained since hyperglycaemia status can lead to free radical production thereby inducing an oxidative stress (Ceriello, 2000; Lipinski, 2001).

There is much evidence in the literature that cinnamon exerts its protective and beneficial effects via its anti-oxidant properties by strong scavenging activity of free radicals (Su et al., 2007). The present study measured anti-oxidant status in the blood of treated rats with both 75 and 150 mg/Kg doses of aqueous cinnamon extract following eleven weeks of cinnamon extract treatment. Treatment of both normal and diabetic rats over eleven weeks resulted in an elevation of blood antioxidant profile in both normal and diabetic rats compared to these respective untreated groups, despite not to a significant level.

Cinnamon extract has been demonstrated to have an anti-antioxidant effect *in vitro* showing an important capacity to oxidant protection (Mathew et al., 2006). However, no study has been done in *in vivo* animal model evaluating the total anti-oxidant status in serum of diabetic rats. The results of this study showed that cinnamon seems to exert a beneficial and protective effect on oxidative status. The possible bioactive compound responsible for its antioxidant properties could be the proanthocyanidins, a polyphenol compound, which was not quantified in the sample of this study. This compound is presented in aqueous cinnamon extract and can exert a beneficial effect in the prevention of advanced glycation-end product (AGE) formation (Peng et al., 2008), which is originated by reactive oxygen species during the hyperglycaemic status (Inoguchi et al., 2000; King & Loeken, 2004).

4.8 Effect of ACE on cation levels of normal and diabetic rats

Diabetes mellitus is a metabolic disorder characterized by dysregulation of carbohydrate metabolism. However, occurrence of both T1DM and T2DM can lead to a modification in intra and extracellular ionic concentration, which has been involved in pathogenesis of diabetes complication (Changrani et al., 2006; Siddiqui et al., 2014). Micronutrients and trace elements play important roles in the body participating in tissue and cellular functions including regulations of cellular mechanism and membrane potential, muscular contractions, secretions, mitochondrial activity and enzymatic reaction (Changrani et al., 2006). This study investigated the macro and trace elements status in serum and different organs and tissues such as heart, pancreas, liver, kidney and skeletal soleus muscle of normal and STZ-induced T1DM rats. The cations contents analyzed in this study were sodium (Na^+), magnesium (Mg^{2+}), potassium (K^+), calcium (Ca^{2+}), copper (Cu^{2+}), iron (Fe^{2+}) and zinc (Zn^{2+}).

The results from the present study demonstrated that no significant changes were found in **sodium** levels in untreated diabetic rats compared to normal rats in different organs tissues, such as pancreas, heart, liver and kidney. This result is not consistent with previous study especially in pancreas, in which STZ-induced T1DM rats

demonstrated a significant reduction of Na^+ content compared to untreated normal rats (Changrani et al., 2006). However, in this study the results showed a significant decrease in Na^+ levels in serum from untreated diabetic rats compared to untreated normal rats. Moreover, cinnamon does not seem to exert an effect on this cation in the different organs and tissues of diabetic and normal rats except in serum in which there was a significant decrease in the Na^+ levels.

In the present study, the results revealed that untreated STZ-induced T1DM rats had significantly less **magnesium** level in left ventricle of the heart compared with untreated normal rats. This results is in agreement with a previous study, which showed that total Mg^{2+} content decreased in cardiac tissue and in isolated cardiac ventricular myocytes from STZ-injected diabetic rats (Reed et al., 2008). The authors demonstrated that insulin treatment for 2 weeks improved Mg^{2+} homeostasis and its transport in cardiac cells, suggesting that the lack of insulin is an important factor to impair Mg^{2+} homeostasis. Accordingly, with a meta-analysis, the intake of Mg^{2+} is inversely associated with risk of type diabetes in a dose-response manner (Dong et al., 2011).

This work also revealed that the treatment with both 75 mg/Kg and 150 mg/Kg of cinnamon significantly increased the Mg^{2+} content in left ventricle of the heart of diabetic animals compared with untreated diabetic animals. Thus, these results of Mg^{2+} levels in heart suggest that aqueous cinnamon extract, especially with high a dose (150 mg/Kg), could contribute to an improvement of Mg^{2+} homeostasis in T1DM rat. Previous findings from animal studies revealed that the lower Mg^{2+} levels could lead to the increase of oxidative stress in cardiovascular tissues (Shah et al., 2011). This divalent cation is involved in several important biological processes including transport, contraction, secretion, regulation of ion channels, modulation of oxidative stress and its role in apoptotic process (Wolf et al., 2003). Mg^{2+} is also a co-factor of many enzymatic reactions in carbohydrate metabolism, binding to the enzyme and modifies its structure or act as catalytic role or binding to the substrate and mediate the interaction with the enzyme (Wolf & Trapani, 2008). However, cinnamon treatment does not seem to have any significant effect on Mg^{2+} levels in the pancreas, liver and kidney of normal and diabetic rats compared to the heart.

Another result of this study revealed that **potassium** level decreased in STZ-induced T1DM rats, but this value was not significant in the left ventricle of the heart compared to untreated normal rats. This result can be associated with lower Mg^{2+} levels in heart since a deficit of intracellular Mg^{2+} can lead to K^+ depletion. One of the mechanisms that has been described is related to Mg^{2+} depletion which in turn can impair Na^+/K^+ ATPase, which is responsible to K^+ uptake in to cells (C.-L. Huang & Kuo, 2007).

Cinnamon treatment seems to increase significantly the K^+ levels in heart of STZ-induced T1DM rats compared to untreated diabetic rats. According to a study by Tammaro et al. (2005), the increase of intracellular Mg^{2+} concentration at physiological conditions can facilitate the modulation of the voltage-dependent K^+ channel, demonstrating an important function in muscle cell (Tammaro et al., 2005). Thus, the results from the present study suggest that the improvement of Mg^{2+} homeostasis through cinnamon treatment could also be beneficial in the regulation of K^+ levels in heart. However, cinnamon treatment does not seem to have any significant effect on K^+ content in the pancreas, liver and kidney of normal and diabetic rats

The results from the present study revealed that **calcium** levels did not change significantly in heart of untreated diabetic rats compared to untreated normal rats. This results are in agreement with a previous study (J. Z. Yu et al., 1997). However, the treatment of cinnamon with high doses seems to increase the Ca^{2+} levels in hearts compared to hearts of untreated diabetic rats.

The content of Ca^{2+} in pancreas did not change significantly comparing untreated diabetic and normal rats. In addition, cinnamon treatment, especially with 75 mg/Kg dose revealed a significantly increase of Ca^{2+} levels in pancreas of normal rats compared to untreated normal rats and a slightly increased in diabetic-treated rats compared to untreated diabetic rats. However, this result was not in agreement with another results from the present study. The influx or increased level of Ca^{2+} by the pancreatic beta cell taken from normal rats treated with 75 mg/Kg should promote insulin secretion. However, this was not observed in the physiological experiments of this study involving the release of insulin following secretagogue stimulation (see discussion later). According with the literature search, Ca^{2+} is an important physiological divalent cation that can stimulate the secretion of insulin in pancreatic

beta cell. Recent data suggest that the alteration in Ca^{2+} levels in pancreatic beta cell could interfere in the insulin release (S.-N. Yang et al., 2014). Furthermore, the treatment with both 75 mg/Kg and 150 mg/Kg doses of cinnamon does not seem to have any effect on Ca^{2+} levels in the liver and kidney of normal and diabetic rats.

In the present study, the results have shown that the levels of **copper** in heart of untreated diabetic rats decreased (but not significantly) compared to untreated normal rats. This results is in accordance with previous results, which have demonstrated that in STZ-induced T1DM there was a decrease of Cu^{2+} levels in the heart (L. Zhang et al., 2013). Cardiomyopathy is one of the complications of diabetes that has been associated with copper-mediated mechanisms. Deficiency of Cu^{2+} in the heart has been reported to be responsible for the maintenance of the integrity of cardiac structure. Recent published data revealed that treatment with copper in diabetic rats markedly prevent the cardiac contractile dysfunction (L. Zhang et al., 2013).

In addition, the treatment with a high dose of (150 mg/Kg) cinnamon significantly increased the levels of Cu^{2+} in heart of diabetic rats compared to untreated diabetic rats. Thus, this result suggests that aqueous cinnamon extract treatment could also be beneficial in the prevention of cardiomyopathy in STZ-induced T1DM rats, in a dose dependent-manner. However, the levels of Cu^{2+} revealed unexpected or possibly interested results in the kidneys of untreated and cinnamon treated diabetic rats. The results demonstrated a significant increase in Cu^{2+} levels. In addition, cinnamon treatment had no significant effect in either pancreas, serum or liver of normal and diabetic rats.

The results from the present study also provided evidence that the hearts and liver of untreated diabetic rats contained less (but not significantly) **iron** levels than hearts and liver taken from untreated normal rats. These results are not in agreement with those reported by previous workers (Cristina et al., 2014). According to the literature, Fe^{2+} plays a pathogenic role in diabetes and it is responsible for such complication as heart failure. The mechanism attributed to this pathogenic role of Fe^{2+} in the heart is oxidative injury leading consequently to tissue injury (Swaminathan et al., 2007). Interesting, these are initiating events of pathological progression involving apoptosis, fibrosis and cardiac dysfunction. (Gammella et al., 2015)

The results also demonstrated that cinnamon treatment, especially with a high dose of 150 mg/Kg, seems to increase significantly the Fe^{2+} levels in the hearts of diabetic and normal rats compared to untreated diabetic and normal rats, respectively. This result suggests that cinnamon treatment with a high dose (150 mg/Kg) may not be of beneficial use in diabetes mellitus condition. However, cinnamon treatment, both 75 mg/Kg and 150 mg/Kg doses, seemed to decrease the Fe^{2+} levels in the kidney of normal rats compared to untreated normal rats. In this case, cinnamon seems to exert a protective effect in the prevention of overload of Fe^{2+} levels in the kidney. Moreover, cinnamon treatment resulted in no significant change in Fe^{2+} levels in pancreas of normal and diabetic rats.

The results from the present study have clearly shown that the levels of **zinc** in the pancreas of untreated diabetic rats do not change significantly compared the untreated normal rats. This finding is not consistent with a previous study, which revealed that the pancreas of STZ-induced T1DM rats contains significantly less Zn^{2+} levels than age-matched control rat (Changrani et al., 2006). Although there was no significant effect on tissue zinc level in untreated hearts, the treatment of cinnamon with 75 mg/Kg slightly increased the Zn^{2+} levels in pancreas of diabetic rats compared to untreated diabetic rats. This result could be a possible beneficial effect of cinnamon since that Zn^{2+} has an important role in the synthesis, storage and secretion of insulin in pancreatic beta cell (Chausmer, 1998). In addition, cinnamon could prevent the Zn^{2+} deficiency that has been associated with the increased of inflammation and oxidative damage process in tissue of diabetic animal model (C. Zhang et al., 2012). Moreover, cinnamon seems do not exert an beneficial effect in normal rats after treatment with 75 mg/Kg of cinnamon, since that its administration during a period of 11 weeks revealed a decrease in the level of Zn^{2+} in heart of normal rats compared to untreated normal rats. According to previous study, this cation can exert a beneficial role in the diabetic heart though its antioxidant action (Y. Song et al., 2005). Furthermore, cinnamon treatment had no effect on zinc levels in serum, liver and kidney of diabetic and normal rats.

4.9 Effect of ACE on insulin secretion from pancreatic islet of normal and diabetic rats

The results of the present study have demonstrated that untreated diabetic rats displayed lower basal insulin secretion levels from pancreatic islet compared to untreated normal rats. These results clearly demonstrated that untreated STZ-induced type 1 diabetic pancreas produced less insulin than untreated normal rats confirming that the animal were indeed diabetic. Others studies which induced diabetes with streptozotocin reported similar results (Lotfy et al., 2014). Furthermore, the administration of aqueous cinnamon extract with a higher dose (150 mg/Kg) during 11 weeks showed a dose-dependent increase in insulin secretion from the pancreatic beta cell at basal conditions in diabetic rats, compared with untreated diabetic rats. The maximum effect of aqueous cinnamon extract was obtained with glucose (16 mM) stimulation. Previous studies suggested that hypoglycemic effect of cinnamon may be due to stimulation of insulin secretion in STZ-induced diabetic rats (Jia et al., 2009). The increased insulin levels after cinnamon oil administration was also suggested to be due to an increase in the levels of insulin secretion (C. Han & Cui, 2012). The possible bioactive compound responsible for this effect is cinnamic acid, which is part of aqueous cinnamon extract employed in this study. Cinnamic acid can also stimulate insulin secretion in isolated islets in a concentration-dependent manner after glucose stimulation in T2DM rats (Hafizur et al., 2015).

The possible mechanism(s) of action whereby cinnamon can increase endogenous insulin secretion from the pancreas is possible through the stimulation of the gastrointestinal hormone, glucagon-like peptide (GLP-1). It is well known that GLP-1 is an incretin that can stimulate glucose-dependent insulin secretion. In human study, the ingestion of 3 g of cinnamon significantly increased the variation of maximum concentration of GLP-1 (Hlebowicz et al., 2009). The signalling transduction pathway of GLP-1 in the pancreatic cell seems to occur by its binding to G-protein coupled receptor, facilitated $G\alpha_s$ subunit activation of the complex. In turn, this activates adenylate cyclase leading to the production of cyclic AMP which induces the protein kinase A activation and secretion of insulin from the granules in pancreatic beta cell (Meloni et al., 2013). In addition, cinnamon can also act as GLP-1 mimetic action through the activation of G-protein-coupled receptor in the pancreatic cell. Recently,

trans-cinnamic acid, another bioactive compound identified in aqueous cinnamon extract employed in this study, was shown to bind to G-protein-coupled receptor and consequently the activation of phosphorylation of AMP-activated protein kinase (Kopp et al., 2014).

Another proposed possible mechanism of action of cinnamon, as demonstrated in the present study, is that cinnamon can improve the secretion of insulin from the pancreatic cells by enhancing protein levels of sirtuin. Sirt 1 can regulate insulin secretion in beta cell through the repression of UCP₂ transcription in beta cell, allowing the secretion of insulin in response to glucose (Bordone et al., 2006). In *in vitro* C6 glioma cell in rats, cinnamon polyphenols demonstrated a significant increase of sirt1 protein levels compared to cells control cultures (Bolin Qin et al., 2014).

The present study also showed an unexpected result regarding the effect of aqueous cinnamon extract on insulin secretion. In normal rats, cinnamon administration (150 mg/kg bw) significantly decreased the insulin secretion after 11 weeks of treatment with the spice compared to untreated normal rats. The possible mechanism for this decrease in insulin output is already unclear. However, this results confirmed those of a previous study in which the extract of C. Ceylon treatment potentiated the decreased of insulin response to meal in normal rats. The authors of that study showed that the effect of cinnamon extract was not due to a stimulation of insulin secretion (Beejmohun et al., 2014).

4.10 Effect of ACE on heart fibrosis of normal and diabetic rats

The results of the present study have also revealed that untreated diabetic rats developed more fibrosis than untreated normal rats. According to the literature, the possible mechanism is due to STZ-induced diabetes leading to different pathological changes allowing the development of diabetic cardiomyopathy in rats (D'Souza et al, 2011). These changes include collagen deposits in the cardiac interstitium and consequently hyperplasia of medial layer after 30 days of STZ injection (Manjarrez-gutiérrez, 2014). Left ventricular heart dysfunction has been associated with this cardiac changes of experimental animal models of diabetes (Brom et al., 2010).

The administration of aqueous cinnamon extract especially with higher doses (75 and 150 mg/Kg) decreased fibrosis in the ventricle of the left heart of diabetic rats compared to hearts from untreated diabetic rats. Although the results demonstrated in this study revealed a marked increase in fibrosis, the data, however, were not statistically significant. Treatment with aqueous cinnamon extract seems to have a beneficial effect in the prevention/treatment of fibrosis in left ventricle over 11 weeks of treatment. However, the experiments have to be prolonged for longer periods of treatment to observe significant changes. In the literature no results were found regarding the effect of aqueous cinnamon extract either in prevention or treatment of fibrosis in left ventricle of heart. For this reason, it is not possible to compare the results from this study with others.

The possible mechanism of action of the protecting effecting of cinnamon in either preventing or reducing cardiac fibrosis during DM could be attributed to the inhibition of the accumulation of advanced glycation- endproducts (AGE) in the heart through its antioxidant properties. A recent work revealed that cinnamon and its bioactive compounds, namely procyanidin-B2, could be beneficial in diabetic nephropathy in rats, another major complication of this metabolic disease. Cinnamon and procyanidin-B2 prevented AGE, thereby ameliorating the renal malfunction in diabetic rats (Muthenna et al., 2014). In *in vitro* study, cinnamon proanthocyanidins were shown to prevent the formation of AGE through reactive carbonyl scavengers (Peng et al., 2008). This mechanism could also be attributed to the extract employed in this study since its antioxidant capacity has also been demonstrated to possess a strong anion scavenging activity.

It is also tempting to suggest that the increase of Mg^{2+} levels in the left ventricle of diabetic treated rats treated with cinnamon may be involved in the development of fibrosis in the diabetic heart. According to previous histopathological study, the administration of Mg^{2+} showed a marked reduction in fibrosis in the heart of diabetic rats (Patel et al., 2014). In STZ-induced T1DM rats the lack of insulin impairs Mg^{2+} homeostasis and transport in cardiac cells (Reed et al., 2008).

4.11 Effect of ACE on distribution of alpha and beta pancreatic cell of normal and diabetic rats

The results from the present study have shown that the distribution of insulin positive beta cells in pancreas of untreated diabetic rats was different compared to untreated normal rats. The diabetic pancreas was shown to have less insulin positive beta cells compared to normal rats. This result is in accordance with a previous study (Lotfy et al., 2014). Streptozotocin (STZ) is the drug that was used to induce diabetes. This diabetogenic agent causes rapid destruction of pancreatic beta cells (Szkudelski, 2001). In the present study, it is apparent that the STZ treatment did not destroy completely all pancreatic beta cells in diabetic rat pancreas, an observation which was made by other studies (Lotfy et al., 2014). This is very important because by destroying all the beta cells there will be no endogenous insulin and as such, the animals could be demised during the course of the experiments. In addition, partially damaged beta cells tended to survive and regenerate due to the low level of circulating insulin and other endogenous growth factors in the body of the rats.

In diabetic animals, the administration of aqueous cinnamon extract seems to increase the immune-reactivity beta cells. In a previous study employing KK-A mice, it was also demonstrated that treatment with cinnamon oil for 35 days increased the area of insulin immune-reactive in beta cell of pancreas compared with untreated diabetic rats (Ping et al., 2010). In addition, the administration of both *C. tamala* and *C. cassia* (200 mg/Kg) extract also improved the insulin concentration in the pancreas of rats (Chen et al., 2012). Cinnamon and its bioactive compound may exert a protective effect by preventing beta cell death. This could be due to its anti-oxidant property. As also referred earlier in the results of this study in the antioxidant characterization experiments, aqueous cinnamon extract was revealed to possess a strong capacity to superoxide anion scavenger. This is one of the radicals originated in the diabetes induction by drugs. This free radical can in turn lead to the destruction of beta cell (Szkudelski, 2001). On the other hand, if cinnamon is acting as an incretin, as well in stimulating insulin secretion either directly or indirectly via GLP-1, the spice could probable responsible for stimulation of pancreatic beta cell replication/development (Friedrichsen et al., 2006). Thus, the results of this work suggest that cinnamon can partly repair the pancreatic beta cells and their mass in diabetic rats.

In normal rats, an unexpected result was observed in this study regarding insulin secretion. The treatment with cinnamon with both 75 and 150 mg/Kg decreased the percentage of positive beta cells in pancreas. This result is not consistent with a previous study, in which cinnamon oil treatment partly recovered the beta cells (C. Han & Cui, 2012). The possible explanation for this result is the different form of administration of cinnamon. According to the study by Han et al. (2012), the liquid loadable tables improved the bioavailability and glycaemic metabolism of cinnamon (C. Han & Cui, 2012). Another possible explanation is that cinnamon is interfering with the antibodies or with the insulin assay.

Regarding to distribution of alpha cell in pancreas, untreated diabetic rats demonstrated a higher area of glucagon distribution than untreated normal rats. Similar results were found in another study (Lotfy et al., 2014). According to previous study, in STZ-induced diabetic rats there is a marked loss of islet beta cell accompanied with an expansion of alpha cells as well as a regeneration of alpha cells (Z. Li et al., 2000). In this study, the pancreas of diabetic rats treated with both 75 and 150 mg/Kg produced dose-dependent increase in alpha cell number compared to untreated diabetic rats. No reported published data was found regarding the effect of cinnamon on the number of positive glucagon cells. For this reason, the mechanism is yet unclear. However, the results of the present study suggest that cinnamon can act as protective effect on alpha cells by exerting its antioxidant and anti-inflammatory properties. Furthermore, in normal rats, the results demonstrated that cinnamon does not seem to exert any effect on the percentage of glucagon in the alpha pancreatic cells.

4.12 Effect of cinnamon tea on blood glucose level after OGTT of non-diabetic humans

Since cinnamon has been reported previously to possess beneficial hypoglycaemic effects in both animal models and in human studies with conflicting results (see tables 1.2 to 1.5 in the introduction), it was decided to test the effect of the ingestion of cinnamon on blood glucose metabolism employing healthy human subjects

undertaking only acute OGTT experiments. The rationale was to determine if both rats and human can give more or less the same hypoglycaemic results for the spice.

The results of the present study have shown that cinnamon tea (6 g of *C. burmannii* into 100 ml water) can improve postprandial blood glucose (PBG) in healthy non-diabetic adults. The ingestion of cinnamon tea decreased blood glucose levels at 30 to 120 minutes after OGTT compared to OGTT alone (without cinnamon tea administration). However statistical analysis revealed that there is no interaction between the independent and repeated measures factors ($p=0.209$), which means that it is not possible to infer about differences in BGL in different moments. Nevertheless, these findings are not in close agreement with previous studies where cinnamon powder was demonstrated to reduce significantly PBG after 30 min of OGTT (Hlebowicz et al., 2007; Magistrelli & Chezem, 2012; T P J Solomon & Blannin, 2007). The effect of aqueous cinnamon extract at 120 min in this study seemed to exert similar results compared with other published data (Hlebowicz et al., 2007; T P J Solomon & Blannin, 2007). These differences were observed in several studies and they could be attributed to the different cinnamon preparations employed in the studies, including cinnamon tea, cinnamon powder, capsule or its individual component.

The results also show that the area under the curve for glycaemia demonstrated a large decrease after OGTT following cinnamon tea ingestion compared to OGTT alone between 0 and 120 min. The value was close to significance. In their study, Beejmohum et al. (2014) showed that administration of 6 g of cinnamon significantly decreased AUC between 0 and 60 min after OGTT in healthy subjects (Beejmohun et al., 2014). Although previous studies demonstrated that 3 g of cinnamon powder did not reduce significantly $C_{\text{máx}}$ and $\Delta C_{\text{máx}}$ BGL (Hlebowicz et al., 2009), the results from the present study have revealed that cinnamon tea, after OGTT, significantly reduced $C_{\text{máx}}$ ($p = 0.040$) and $\Delta C_{\text{máx}}$ ($p = 0.029$) compared with OGTT without cinnamon tea. This effect may be due to the concentration of cinnamon employed in this study compared with the other study. This heterogeneous results on glycaemia, also demonstrated in a meta-analysis review (Allen et al., 2013), could be attributed to variations in doses, species and formulation (cinnamon tea, powder or capsule) or study design employed in different studies.

The possible mechanisms of actions for the hypoglycaemic effect of this spice has been postulated or suggested by several authors previously. These include reducing

gastric emptying (Hlebowicz et al., 2007), insulin-mimetic action (Cao et al., 2007; Bolin Qin et al., 2012), which can lead cellular glucose uptake (Shen et al., 2010); and reducing intestinal glycosidase activity. This effect on enzyme can lead to a decrease in the breakdown of disaccharidases into glucose, allowing a slow absorption of glucose and reducing PBG level (S. H. Kim et al., 2006a).

The hypoglycaemic effect of cinnamon observed in the present study could also be attributed to the phenolic content of *C. burmannii* tea demonstrated in the present study. According to literature, the molecular mechanism(s) of action of cinnamon polyphenols include a number of processes including an increase of insulin receptor- β protein in adipocytes suggesting act beneficially in insulin signalling (Cao et al., 2007) as well as others.

In healthy animals and healthy human subjects, cinnamon has been reported to act beneficially in hyperglycaemia. The administration of *C. cassia* and *C. tamala* extracts (200 mg/Kg bw) once daily significantly reduced the blood glucose AUC for OGTT in animals (Chen et al., 2012). In healthy subjects, the ingestion of 6 g of cinnamon powder to a meal significantly decreased post-prandial BGL (Magistrelli & Chezem, 2012). However, lower doses of cinnamon powder (1 or 3 g) did not affect glycaemia (Hlebowicz et al., 2009). Moreover, a dose of 5 g of cinnamon capsule also resulted in a improvement of glucose response on OGTT (13%), which was not observed with 3 g of cinnamon capsule (Thomas P J Solomon & Blannin, 2009).

These hypoglycaemic effects demonstrated by cinnamon in animal and human healthy subjects has also been demonstrated in DM. The bioactive constituents of aqueous *C. burmannii* extract identified in the sample employed in the present study could be responsible for its benefit properties. Cinnamaldehyde, a majority compound (23.9 %) found in aqueous *C. burmannii* extract demonstrated to improved fasting blood glucose levels and improved hyper-insulinemia (Babu et al., 2007). Another component is cinnamic acid, which is also part of aqueous *C. burmannii* extract (3.7 %). Cinnamic acid can also stimulate insulin secretion in isolated islets after glucose stimulation in T2DM animals (Hafizur et al., 2015).

4.13 Proposed mechanism(s) of action of ACE

The schematic model in the figure 4.1 summarizes the main mechanism(s) of actions that may occur in DM at least in **cardiac cell** leading to apoptosis, cardiac hypertrophy, cardiomyopathy and fibrosis. The sequence of mechanisms is described in the figure. Moreover, the results obtained in this study on the heart suggest the potential beneficial effects of cinnamon in the cardiac cell. DM is characterized by the destruction of pancreatic islets leading to hyperglycaemia (Banting et al., 1991). This disorder leads to a number long-term complications in different organs (subsequently, end-organ failure) (Fowler, 2008). Cardiomyopathy is one of the complications that can cause cardiac fibrotic process, apoptosis and then demodulation of the myocardium tissue (Asbun & Villarreal, 2006; Brom et al., 2010).

The high blood glucose levels has been reported to be associated with the activation of NADPH oxidase enzyme, an enzyme presented in the membrane of the cell. In turn the NADPH oxidase activation leads to the generation of reactive oxygen species (ROS) in the cardiac cell (H. B. Lee, 2003), which contributed to the development of cardiac hypertrophy, fibrosis and cardiomyopathy (Seddon et al., 2007). These ROS can also activate signalling pathways in the cell of diabetic hearts including the activation of protein kinase C ($PKC_{\alpha/\beta}$) (Way et al., 2002). The $PKC_{\alpha/\beta}$ stimulates a sequence in the collagen promoter yielding to collagen production and consequently fibrosis and hypertrophy (X. Song et al., 2015). Furthermore, the activation of $PKC_{\alpha/\beta}$ also induces the phosphorylation of a number of proteins involved in cardiac excitation-contraction coupling and therefore disturbs cellular Ca^{2+} homeostasis in the myocardium. In STZ-induced T1DM rats, the alteration in SERCA transporters leads to dysfunctional intracellular Ca^{2+} signalling/mobilization. These events seem to interfere with the effective sequestrations of Ca^{2+} in sarcoplasmic reticulum (SR) and the overload in the cytosol (elevated diastolic calcium) and subsequently impairing diastolic dysfunction or relaxation of the heart (Trost et al., 2002).

Another possible mechanism is related with the intracellular glucose concentration increased after uptake of glucose from the blood by GLUT1 transporters. This intracellular glucose generated the activation of diacylglycerol (DAG) through the alteration of enzymes transcription. DAG can activate the $PKC_{\alpha/\beta}$, which can lead to a sequence of events including cardiac hypertrophy, cardiomyopathy and fibrosis, as

explained earlier (van Baal et al., 2005). In addition, the high glucose concentration in the cell could also promote the AGEs production (Nowotny et al., 2015), which can lead to the apoptosis in the cardiac cell (Lan et al., 2015).

Furthermore, the copper deficiency in STZ-induced T1DM rats has been reported to promote alterations of heart morphology and functions (Y. Li et al., 2005; S. Zhang et al., 2014). This mechanism could also be associated with an increase in oxidative stress due the suppression of superoxide dismutase on superoxide anion ($O_2^{\bullet-}$). This events lead to ROS increases in the cell (McCord & Fridovich, 1969). In cardiac ventricular of STZ-induced diabetic rats, the results showed a decrease of mobilization magnesium (Mg^{2+}) content to the cell (Reed et al., 2008; Tashiro et al., 2013). This dysfunction of Mg^{2+} homeostasis can lead to an impairment of the L-Type calcium channel voltage-dependent modulation, and then the influx of calcium by the cardiac cell (M. Wang & Berlin, 2007).

This study suggests that aqueous cinnamon extract (ACE) administration could be of beneficial use in STZ-induced T1DM rats by regulation of Cu^{2+} levels in heart, thus preventing the cardiomyopathy in diabetic rats. In addition, cinnamon seems to be effective as a scavenger of superoxide anion, demonstrated in *in vitro* study. In turn, this could also be of beneficial use cardiomyocytes though the prevention of ROS. Histological studies have also demonstrated that ACE can improve the fibrosis content in left ventricle of the heart of STZ-induced T1DM rats. The increases of Ca^{2+} level verified in cardiac cell of STZ-induced T1DM rats and this could be also contributed to the dysregulation of Ca^{2+} homeostasis and consequently the induction and formation of fibrosis. ACE with high doses revealed an increased of Mg^{2+} levels in ventricular tissue of diabetic rats, improving the Mg^{2+} homeostasis. This effect could contribute to the Ca^{2+} increases level in the cell by L-Type calcium channel voltage-dependent modulation.

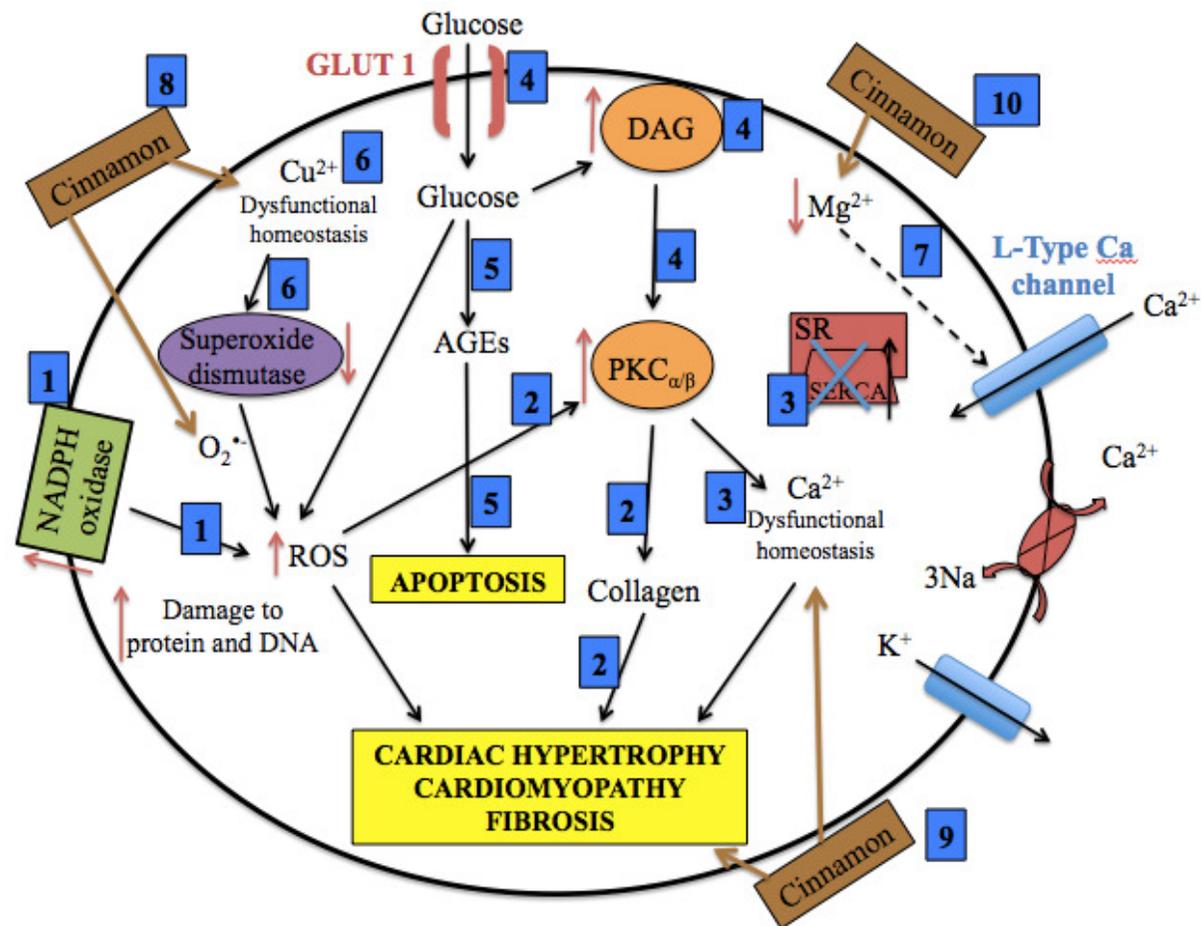


Figure 4.1: A schematic diagram showing proposed mechanisms of actions a cardiac myocyte of STZ-induced diabetic rats. The various events are triggered by diabetes and the proposed diagram also shows the potential beneficial effects of cinnamon. The explanations are given then (sequence of events 1-10).

Sequence of the events described in the figure 4.1 (1-10) representing the summarized mechanism(s) of actions in the cardiac myocyte of diabetic rats:

1. NADPH oxidase enzyme activation by the high blood glucose levels and production of reactive oxygen species (ROS) leading to the cardiac hypertrophy, fibrosis and cardiomyopathy.
2. ROS induced the activation of PKC _{α/β} , leading to collagen production and consequently fibrosis and hypertrophy.
3. PKC induced disturbance of Ca²⁺ homeostasis by alteration in SERCA transporters leading to dysfunctional intracellular Ca²⁺ signalling.
4. Glucose is taken up by GLUT1 transporters leading to the activation of diacylglycerol (DAG) through the alteration of enzymes transcription.
5. The high glucose concentration also promoted the AGEs production leading to the apoptosis in the cell.
6. Copper deficiency could promote superoxide dismutase suppression and consequently increased of superoxide anion (O₂^{•-}) leading to elevation of ROS and heart morphology and functions modification.
7. Decreased magnesium content in the cell could impair the L-Type calcium channel voltage-dependent modulation and consequently the uptake of calcium by the cell.
8. In the present study, the results suggested that cinnamon could be benefit in STZ-induced T1DM rats via the regulation of Cu²⁺ levels leading to increases in its content in heart to prevent the cardiomyopathy in diabetic rats. In addition, cinnamon seems to be effective in scavenger of superoxide anion, demonstrated in *in vitro* study, which could also beneficiate in heart cell though the prevention of ROS.

9. Histological studies demonstrated that cinnamon improved the fibrosis content in left heart ventricle of STZ-induced T1DM rats. The increases of Ca^{2+} level verified in cardiac cell of STZ-induced T1DM rats could be contributed to the dysregulation of Ca^{2+} homeostasis and consequently leading to fibrosis progression.

10. Cinnamon also could also increase cellular Mg^{2+} content in ventricular tissue of diabetic rats, thereby improving Mg^{2+} homeostasis. This effect could contribute to an increase in cytosolic Ca^{2+} level in the cell by L-Type calcium channel voltage-dependent modulation.

The schematic model in the figure 4.2 summarizes the main mechanism(s) of actions that may occur in **pancreatic islet β -cell** of STZ-induced T1DM leading to impaired insulin production (Akbarzadeh et al., 2007). The sequence of mechanisms described below is identified in figure 4.2. Moreover, figure also represented the results obtained in this study, suggesting the potential beneficial effects of cinnamon in the pancreatic β -cell. The biochemical mechanisms occurring in pancreatic β -cell damage following T1DM-induction include pro-inflammatory cytokines activation and free radicals action (Jun et al., 1999; Maedler et al., 2001).

The macrophages and cytotoxic T cell (CD8+) activate the destruction of islet β -cell by inflammatory mediators such as cytokines (IL-1, TNF $_{\alpha}$, TNF $_{\beta}$ and IFN γ), which bind to a specific receptor in β -cell activating different pathways (Rabinovitch & Suarez-pinzon, 1998). In addition, the macrophages can also stimulate the free radicals production namely, O $_2^{\cdot-}$, H $_2$ O $_2$ and OH $^{\cdot}$, which damage the cellular membrane of pancreatic β -cell and they can also cause further damaging process into the cell through H $_2$ O $_2$ (Rabinovitch & Suarez-pinzon, 1998). The free radicals, together with the actions of cytokines can lead to protein damage and DNA fragmentation and consequently mitochondrial damage (Maechler et al., 1999). The dysfunction of the mitochondrial resulted in an impairment of β -cell leading to cellular death. Moreover, these events also promote the decrease of ATP levels in the cell, leading to impaired insulin secretion (Maechler et al., 1999).

In hyperglycaemic status, the blood glucose is taken up by the pancreatic β -cell by GLUT2 transporters and in turn the glucose is phosphorylated by glucokinase (Postic et al., 1999). Thereafter, the metabolism of glucose leads to an increase in the ATP/ADP ratio promoting closure of the ATP-sensitive K $^+$ channel and consequently to the depolarization of the cell membrane (Zou et al., 2014). The membrane depolarization is associated with the promotion of calcium influx into the cell by voltage-gated Ca $^{2+}$ channel, release of intracellular calcium and consequently the exocytosis of insulin and its secretion into the blood (Zou et al., 2014). However, the excess of glucose concentration in the β -cell can develop toxic effects through the accumulation of reactive oxygen species (ROS) originated by mitochondrial dysfunction. These ROS, in excess and over time, can lead to oxidative stress, which causes impairment to the insulin gene expression and consequently a decreases in insulin synthesis and content and subsequently, reduced insulin secretion (Arcidiacono et al., 2015; Federici et al., 2001; Robertson, 2004). These event

pathways seem to occur through loss of PDX-1 gene expression and MafA protein, two essential transcription factors of insulin production (Arcidiacono et al., 2015). This study suggests that aqueous cinnamon extract (ACE) administration could be beneficial in STZ-induced T1DM rats by improving Ca^{2+} levels in pancreas thereby preventing the impairment of insulin secretion from the pancreas of Type 1 diabetic rats. The results of the present study clearly have demonstrated that although, not significantly, ACE can improve insulin secretion in STZ-induced T1DM rats. In addition, cinnamon seems to be effective as a scavenger of superoxide anion, demonstrated in *in vitro* study, which could also be of beneficial use in pancreatic β -cell through the prevention of ROS production. Histological studies demonstrated that ACE also improved the insulin content in β -cell. The high potential antioxidant properties of ACE could contribute to this beneficial effect (C. Zhang et al., 2012). Moreover, the increase of Zn^{2+} levels in pancreas of diabetic rats could also be of some benefit in the elevation of insulin content, since that Zn^{2+} has an important role in the synthesis and storage of insulin (Chausmer, 1998).

Sequence of the events described in the figure 4.2 (1-15) representing the summarized mechanism of action in the pancreatic β -cell of diabetic rats:

1. Macrophages stimulate free radicals production ($O_2^{\cdot-}$, H_2O_2 and OH^{\cdot}) leading to cellular membrane damage.
2. At the same time, the macrophages and cytotoxic T cell (CD8+) activate cytokines inflammatory, IL-1, TNF_{α} , TNF_{β} and $IFN\gamma$, which also bind to specific receptor in β -cell to cause further damage.
3. The H_2O_2 diffuse intra-cellularly to the cell and $O_2^{\cdot-}$ is activated into the cell by signalling pathways of cytokines.
4. These intracellular radicals lead to protein damage and DNA fragmentation and consequently mitochondrial dysfunction.
5. The mitochondrial dysfunction impaired β -cell leading to cellular death.
6. The mitochondrial damage also promotes the decrease of ATP in the cell, leading to impaired insulin secretion.
7. Glucose is taken up by GLUT 2 transporters and its phosphorylation by glucokinase leading to ATP elevation.
8. The ATP/ADP ratio elevation induces the ATP-sensitive K^+ channel closure and consequently to the depolarization of the membrane and opening of voltage-gated Ca^{2+} channel.
9. Exocytosis of insulin-containing granules.
10. The excess of glucose concentration in the β -cell promotes the ROS production by mitochondrial damage.

11. These ROS in excess and over time lead to impaired insulin gene expression by loss of PDX-1 gene expression and MafA protein.
12. Decreases of insulin content by impaired of insulin gene expression.
13. This study suggests that cinnamon administration could be benefit in STZ-induced T1DM rats by improving Ca^{2+} levels in pancreas leading to increase of insulin secretion in diabetic rats.
14. In addition, cinnamon seems to be effective as a scavenger of superoxide anion, demonstrated in *in vitro* study, which could also beneficiate in pancreatic β -cell though the prevention of ROS production.
15. Histological studies demonstrated that ACE improved the insulin content in β -cell, which could be attributed by potential antioxidant properties of ACE. Moreover, the increase of Zn^{2+} levels in pancreas of diabetic rats verified in this study could also beneficiate in the elevation of insulin content.

In summary, the results of this study have clearly demonstrated the potential beneficial use of cinnamon in preventing end-organ failure, at least in the heart and pancreas. It may also have the same beneficial use(s) in such other organs as the kidneys, the brain, the eyes and in the nerves in the body. However experiments have to be do it.

4.14 Conclusion

From the results of the present study, it can be concluded that the treatment of diabetic rats with aqueous cinnamon extract can result in improvements in body weight and BGL compared to untreated animals. In contrast, cinnamon did not seem to exert any beneficial effect on biochemical parameters in both normal and diabetic rats. The administration of cinnamon for 11 weeks also demonstrated a beneficial effect in its antioxidant status in both normal and diabetic animals.

The results obtained from the measurements of cations content in serum and the different organs show that in serum, cinnamon treatment significantly decreased Na^+ , Ca^{2+} and K^+ in normal rats and decrease Na^+ , Ca^{2+} and Mg^{2+} in diabetic rats ($p < 0.05$). In heart, cinnamon, with both doses, had significant effect ($p < 0.05$) in normal and diabetic rats, namely increases in Mg^{2+} , K^+ , Ca^{2+} and Fe. In liver, kidney and pancreas, cinnamon treatment seems to have no significant effect ($p > 0.05$) on the most of cations analysed from these tissues.

Treatment of cinnamon with both 75 mg/Kg and 150 mg/kg seems to stimulate the production/proliferation of pancreatic beta cell and the secretion of insulin from the pancreas in diabetic rats. In normal rats, cinnamon treatment decreased insulin secretion from the pancreatic segments incubated with secretagogues. Similarly, cinnamon improved the distribution of alpha pancreatic cells in isolated pancreatic fragments in a dose-dependent manner in diabetic rats, which was not observed in normal rats. The results also suggest a possible positive influence of cinnamon on the reduction or prevention of fibrosis in the hearts of diabetic animals compared to age-matched control rats.

Furthermore, data from the human study suggest that ingestion of cinnamon tea seems to exert a hypoglycaemic benefit effect in healthy non-diabetic subjects during postprandial period. The high phenolic compound contents and anti-oxidant capacity in aqueous cinnamon extract employed in the present study may contribute to these health benefits.

Together, the results of this study have demonstrated some beneficial effects of aqueous cinnamon in both rats and human as discussed in the proposed mechanisms of action. However, further studies are required to unravel further its precise

mechanism (s) of action(s) not only with aqueous cinnamon extract but also with its powder, its capsule and its different purified components for comparison.

4.15 Scope for future studies

This work should be continued for further studies investigation a number of other parameters to unravel how this plant-based medicine can be used effectively and successfully in the treatment of DM and other diseases. These new investigation should employ scientific techniques such as microscopy, cell culture and molecular and gene expression studies to better understood the mechanism(s) of action of this traditional spices in the treatment of DM and other diseases. Several studies can be employed in the future:

- A. Do long term aqueous cinnamon extract, cinnamon powder, cinnamon capsules and the different components of cinnamon administration over a period of 6-12 months, employing different doses and repeat the various measurements similar to those employed in this study.
- B. Investigate the effect of cinnamon on glucose uptake in L6 skeletal muscle cell line comparing the uptake with exogenous insulin in the absence and presence of a tyrosine kinase inhibitor.
- C. Investigate the effect of cinnamon and its extracted compounds individually on glucose absorption in enterocyte.
- D. Investigate the action of cinnamon and its extracted compounds individually on the stimulation of insulin gene expression in the pancreas.
- E. Investigate the anti-oxidant defense system in pancreatic tissues with cinnamon treatment.
- F. Investigate the effect of cinnamon on the stimulation of GLP-1 gene expression.

Chapter Five

REFERENCES

5 References

- Abraham, K., Pfister, M., Wöhrlin, F., & Lampen, A. (2011). Relative bioavailability of coumarin from cinnamon and cinnamon-containing foods compared to isolated coumarin: a four-way crossover study in human volunteers. *Molecular Nutrition & Food Research*, **55**(4): 644–653.
- Abraham, K., Wöhrlin, F., Lindtner, O., Heinemeyer, G., & Lampen, A. (2010). Toxicology and risk assessment of coumarin: focus on human data. *Molecular Nutrition & Food Research*, **54**(2): 228–239.
- ADA. (2010a). Diagnosis and classification of diabetes mellitus. *Diabetes Care*, **33**(1): S62–S69.
- ADA. (2010b). Standards of medical care in diabetes--2010. *Diabetes Care*, **33**(Suppl 1): S11–S61.
- ADA. (2013a). Economic costs of diabetes in the U.S. in 2012. *Diabetes Care*, **36**(4): 1033–1046.
- ADA. (2013b). Standards of medical care in diabetes-2013. *Diabetes Care*, **36**(Suppl 1): S11–S66.
- Adeghate, E. (1999). Effect of subcutaneous pancreatic tissue transplants on streptozotocin-induced diabetes in rats. III. Distribution of neuropeptides in normal and diabetic (host) pancreas. *Tissue & Cell*, **31**(1): 84–89.
- Adeghate, E., Fernandez-Cabezudo, M., Hameed, R., El-Hasasna, H., El Wasila, M., Abbas, T., & Al-Ramadi, B. (2010). Orexin-1 receptor co-localizes with pancreatic hormones in islet cells and modulates the outcome of streptozotocin-induced diabetes mellitus. *PLoS One*, **5**(1): 1–11.
- Adeghate, E., & Ponery, A. S. (2002). Ghrelin Stimulates Insulin Secretion from the Pancreas of Normal and Diabetic Rats. *Journal of Neuroendocrinology*, **14**: 555–560.
- Adisakwattana, S., & Lerdsuwankij, O. (2011). Inhibitory Activity of Cinnamon Bark Species and their Combination Effect with Acarbose against Intestinal α -glucosidase and Pancreatic α -amylase. *Plant Foods for Human Nutrition*, **66**: 143–148.

Adler, A. I., Stevens, R. J., Manley, S. E., Bilous, R. W., Cull, C. a., & Holman, R. R. (2003). Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney International*, **63**(1): 225–232.

Akbarzadeh, a., Norouziyan, D., Mehrabi, M. R., Jamshidi, S., Farhangi, a., Verdi, a. A., Mofidian, S. M. a., & Rad, B. L. (2007). Induction of diabetes by Streptozotocin in rats. *Indian Journal of Clinical Biochemistry*, **22**(2): 60–64.

Akilen, R., Tsiami, A., Devendra, D., & Robinson, N. (2010). Treatment Glycated haemoglobin and blood pressure-lowering effect of cinnamon in multi-ethnic Type 2 diabetic patients in the UK: a randomized, placebo-controlled, double-blind clinical trial. *Diabetic Medicine*, **27**: 1159–1167.

Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, **21**: 143–152.

Allen, R. W., Schwartzman, E., Baker, W. L., Coleman, C. I., & Phung, O. J. (2013). Cinnamon Use in Type 2 Diabetes: An Updated Systematic Review and Meta-Analysis. *Annals of Family Medicine*, **11**(5): 452–459.

Altschuler, J., Casella, S., MacKenzie, T., & Curtis, K. (2007). The Effect of Cinnamon on A1C Among Adolescents With Type 1 Diabetes. *Diabetes Care*, **30**(4): 6–9.

Amin, K. A., & El-twab, T. M. A. (2009). Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolemic rats: role of atorvastatine and cinnamon. *Clinical and Experimental Medicine*, **2**: 254–265.

Anand, P., Murali, K. Y., Tandon, V., Murthy, P. S., & Chandra, R. (2010). Insulinotropic effect of cinnamaldehyde on transcriptional regulation of pyruvate kinase, phosphoenolpyruvate carboxykinase, and GLUT4 translocation in experimental diabetic rats. *Chemico-Biological Interactions*, **186**(1): 72–81.

Anderson, R. A., Broadhurst, C. L., Polansky, M. M., Schmidt, W. F., Khan, A., Flanagan, V. P., Schoene, N. W., & Graves, D. J. (2004). Isolation and characterization of polyphenol type-A polymers from cinnamon with insulin-like biological activity. *Journal of Agricultural and Food Chemistry*, **52**(1): 65–70.

Antunes, E., Borrecho, G., Oliveira, P., Alves de Matos, A. P., Brito, J., Águas, A., & Martins dos Santos, J. (2013). Effects of low-frequency noise on cardiac collagen and cardiomyocyte

ultrastructure: an immunohistochemical and electron microscopy study. *International Journal of Clinical and Experimental Pathology*, **6(11)**: 2333–2341.

Antunes, E., Oliveira, P., Borrecho, G., R, M. J., Brito, J., Águas, A., Martins, J., & Santos, D. O. S. (2013). Myocardial fibrosis in rats exposed to low frequency noise. *Acta Cardiologica*, **68(3)**: 241–245.

Arcidiacono, B., Iiritano, S., Chiefari, E., Brunetti, F. S., Gu, G., Foti, D. P., & Brunetti, A. (2015). Cooperation between HMGA1, PDX-1, and MafA is Essential for Glucose-Induced Insulin Transcription in Pancreatic Beta Cells. *Frontiers in Endocrinology*, **5**: 1–10.

Asbun, J., & Villarreal, F. J. (2006). The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. *Journal of the American College of Cardiology*, **47(4)**: 693–700.

Babu, P., Prabuseenivasan, S., & Ignacimuthu, S. (2007). Cinnamaldehyde-a potential antidiabetic agent. *Phytomedicine*, **14(1)**: 15–22.

Bagust, A., Hopkinson, P. K., Maslove, L., & Currie, C. J. (2002). The projected health care burden of Type 2 diabetes in the UK from 2000 to 2060. *Diabetic Medicine: A Journal of the British Diabetic Association*, **19(Suppl 4)**: 1–5.

Bahadoran, Z., Mirmiran, P., & Azizi, F. (2013). Dietary polyphenols as potential nutraceuticals in management of diabetes: a review. *Journal of Diabetes and Metabolic Disorders*, **12(43)**: 1–9.

Bailey, T. (2013). Options for combination therapy in type 2 diabetes: comparison of the ADA/EASD position statement and AACE/ACE algorithm. *The American Journal of Medicine*, **126**: S10–S20.

Baker, I., Chohan, M., & Opara, E. I. (2013). Impact of Cooking and Digestion, In Vitro, on the Antioxidant Capacity and Anti-Inflammatory Activity of Cinnamon, Clove and Nutmeg. *Plants Foods for Human Nutrition*, **68**: 364–369.

Baker, W., Gutierrez-Williams, G., White, M., Kluger, J., & Coleman, C. (2008). Effect of Cinnamon on Glucose Control and Lipid Parameters. *Diabetes Care*, **31(1)**: 41–43.

Ballin, N. Z., & Sørensen, A. T. (2014). Coumarin content in cinnamon containing food products on the Danish market. *Food Control*, **38(1)**: 198–203.

- Banting, F. G., Best, C. H., Collip, J. B., Campbell, W. R., & Fletcher, a a. (1991). Pancreatic extracts in the treatment of diabetes mellitus: preliminary report. *Canadian Medical Association Journal*, **145**(10): 1281–1286.
- Barceloux, D. G. (2009). Cinnamon (*Cinnamomum* species). *Medical Toxicology of Natural Substances: Foods, Fungi, Medicinal Herbs, Toxic Plants, and Venomous Animals*, **55**(6): 327–335.
- Beagley, J., Guariguata, L., Weil, C., & Motala, A. A. (2013). Global estimates of undiagnosed diabetes in adults. *Diabetes Research and Clinical Practice*, **103**(2): 150–160.
- Beckman, J., Creager, M. A., & Libby, P. (2002). Diabetes and Atherosclerosis - Epidemiology, Pathophysiology, and Management. *The Journal of the American Medical Association*, **287**(19): 2570–2581.
- Beejmohun, V., Peytavy-izard, M., Mignon, C., Muscente-paque, D., Deplanque, X., Ripoll, C., & Chapal, N. (2014). Acute effect of Ceylon cinnamon extract on postprandial glycemia : alpha-amylase inhibition , starch tolerance test in rats , and randomized crossover clinical trial in healthy volunteers. *BMC Complementary and Alternative Medicine*, **14**(351): 1–11.
- Belle, T., Coppeters, K., & Herrath, M. (2011). Type 1 Diabetes: Etiology, Immunology, and Therapeutic Strategies. *Physiological Review*, **79**: 79–118.
- Blahová, J., & Svobodová, Z. (2012). Assessment of Coumarin Levels in Ground Cinnamon Available in the Czech Retail Market. *The Scientific World Journal*, **263851**: 1–4.
- Blevins, S., Leyva, M., Brown, J., Wright, J., Scofield, R., & Aston, C. (2007). Effect of Cinnamon on Glucose and Lipid Levels in Non-Insulin-Dependent Type 2 Diabetes. *Diabetes Care*, **30**(9): 2236–2237.
- Bnouham, M., Ziyat, A., Mekhfi, H., Tahri, A., & Legssyer, A. (2006). Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). *International Journal of Diabetes & Metabolism*, **14**: 1–25.
- Bolkent, S., Yanardağ, R., Tabakoğlu-Oğuz, A., & Ozsoy-Saçan, O. (2000). Effects of chard (*Beta vulgaris* L. var. *Cicla*) extract on pancreatic B cells in streptozotocin-diabetic rats: a morphological and biochemical study. *Journal of Ethnopharmacology*, **73**: 251–259.

Bordone, L., Motta, M. C., Picard, F., Robinson, A., Jhala, U. S., Apfeld, J., McDonagh, T., Lemieux, M., McBurney, M., Szilvasi, A., Easlson, E. J., Lin, S. J., & Guarente, L. (2006). Sirt1 regulates insulin secretion by repressing UCP2 in pancreatic β cells. *PLoS Biology*, **4**(2): 210–220.

Brand-Miller, J., Hayne, S., Petocz, P., & Colagiuri, S. (2006). Low – Glycemic Index Diets in the A meta-analysis of randomized controlled trials. *Diabetes Care*, **26**: 2261–2267.

Brom, C. E. Van Den, Bosmans, J. W. A. M., Vlasblom, R., Handoko, L. M., Huisman, M. C., Lubberink, M., Molthoff, C. F. M., Lammertsma, A. A., Ouwens, M. D., Diamant, M., & Boer, C. (2010). Diabetic cardiomyopathy in Zucker diabetic fatty rats: the forgotten right ventricle. *Cardiovascular Diabetology*, **9**(25): 1–7.

Cao, H., Deterding, L. J., Venable, J. D., Kennington, E. a, Yates, J. R., Tomer, K. B., & Blackshear, P. J. (2006). Identification of the anti-inflammatory protein tristetraprolin as a hyperphosphorylated protein by mass spectrometry and site-directed mutagenesis. *The Biochemical Journal*, **394**: 285–297.

Cao, H., Graves, D. J., & Anderson, R. A. (2010). Cinnamon extract regulates glucose transporter and insulin-signaling gene expression in mouse adipocytes. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology*, **17**(13): 1027–1032.

Cao, H., Polansky, M. M., & Anderson, R. A. (2007). Cinnamon extract and polyphenols affect the expression of tristetraprolin, insulin receptor, and glucose transporter 4 in mouse 3T3-L1 adipocytes. *Archives of Biochemistry and Biophysics*, **459**(2): 214–222.

Cao, H., Urban, J. F., & Anderson, R. a. (2008a). Cinnamon polyphenol extract affects immune responses by regulating anti- and proinflammatory and glucose transporter gene expression in mouse macrophages. *The Journal of Nutrition*, **138**(5): 833–840.

Cao, H., Urban, J. F., & Anderson, R. a. (2008b). Insulin increases tristetraprolin and decreases VEGF gene expression in mouse 3T3-L1 adipocytes. *Obesity*, **16**(6): 1208–2018.

Cellek, S., Qu, W., Schmidt, a M., & Moncada, S. (2004). Synergistic action of advanced glycation end products and endogenous nitric oxide leads to neuronal apoptosis in vitro: a new insight into selective nitregric neuropathy in diabetes. *Diabetologia*, **47**(2): 331–339.

Ceriello, A. (2000). Oxidative Stress and Glycemic Regulation. *Metabolism*, **49**(2): 27–29.

Changrani, N. R., Chonkar, A., Adegate, E., & Singh, J. (2006). Effects of streptozotocin-induced type 1 diabetes mellitus on total protein concentrations and cation contents in the isolated pancreas, parotid, submandibular, and lacrimal glands of rats. *Annals of the New York Academy of Sciences*, **1084**: 503–519.

Chausmer, a B. (1998). Zinc, insulin and diabetes. *Journal of the American College of Nutrition*, **17(2)**: 109–115.

Chen, L., Sun, P., Wang, T., Chen, K., Jia, Q., Wang, H., & Li, Y. (2012). Diverse mechanisms of antidiabetic effects of the different procyanidin oligomer types of two different cinnamon species on db/db mice. *Journal of Agricultural and Food Chemistry*, **60(36)**: 9144–9150.

Cheng, A. Y. Y., & Fantus, I. G. (2005). Oral antihyperglycemic therapy for type 2 diabetes mellitus. *Canadian Medical Association Journal*, **172(2)**: 213–226.

Cheng, D. M., Kuhn, P., Poulev, A., Rojo, L. E., Ann, M., & Raskin, I. (2012). In vivo and in vitro antidiabetic effects of aqueous cinnamon extract and cinnamon polyphenol-enhanced food matrix. *Food Chemistry*, **135(4)**: 2994–3002.

Couturier, K., Batandier, C., Awada, M., Hininger-Favier, I., Canini, F., Anderson, R. a, Lerverve, X., & Roussel, a M. (2010). Cinnamon improves insulin sensitivity and alters the body composition in an animal model of the metabolic syndrome. *Archives of Biochemistry and Biophysics*, **501(1)**: 158–161.

Couturier, K., Qin, B., Batandier, C., Awada, M., Hininger-Favier, I., Canini, F., Lerverve, X., Roussel, A. M., & Anderson, R. A. (2011). Cinnamon increases liver glycogen in an animal model of insulin resistance. *Metabolism: Clinical and Experimental*, **60(11)**: 1590–1597.

Crawford, P. (2009). Effectiveness of cinnamon for lowering hemoglobin A1C in patients with type 2 diabetes: a randomized, controlled trial. *Journal of the American Board of Family Medicine*, **22(5)**: 507–512.

Cristina, S., López-chaves, C., Trenzado, C. E., Aranda, P., López-jurado, M., Gómez-aracena, J., Montes-bayón, M., Sanz-medel, A., & Llopis, J. (2014). Changes in Iron Metabolism and Oxidative Status in STZ-Induced Diabetic Rats Treated with Bis (maltolato) Oxovanadium (IV) as an Antidiabetic Agent. *The Scientific World Journal*, **706074**: 1–6.

D'Souza, A., Howarth, F. C., Yanni, J., Dobryznski, H., Boyett, M. R., Adeghate, E., Bidasee, K. R., & Singh, J. (2011). Left ventricle structural remodelling in the prediabetic Goto-Kakizaki rat. *Experimental Physiology*, **96**(9): 875–88.

Ding, Y., Wu, E. Q., Liang, C., Chen, J., Tran, M. N., Hong, C. H., Jang, Y., Park, K. L., Bae, K., Kim, Y. H., & Kang, J. S. (2011). Discrimination of cinnamon bark and cinnamon twig samples sourced from various countries using HPLC-based fingerprint analysis. *Food Chemistry*, **127**(2): 755–760.

Dong, J., Xun, P., He, K., & Qin, L. (2011). Magnesium Intake and Risk of Type 2 Diabetes: Meta-analysis of prospective cohort studies. *Diabetes Care*, **34**(9): 2116–2122.

Dragland, S., Senoo, H., Wake, K., Holte, K., & Blomhoff, R. (2003). Several Culinary and Medicinal Herbs Are Important Sources of Dietary Antioxidants. *Human Nutrition and Metabolism*, **133**: 1286–1290.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, **57**(5): 1768–1774.

Dugoua, J.-J., Seely, D., Perri, D., Cooley, K., Forelli, T., Mills, E., & Koren, G. (2007). From type 2 diabetes to antioxidant activity: a systematic review of the safety and efficacy of common and cassia cinnamon bark. *Canadian Journal of Physiology and Pharmacology*, **85**(9): 837–847.

Dullaart, R. P. F., Annema, W., de Boer, J. F., & Tietge, U. J. F. (2012). Pancreatic β -cell function relates positively to HDL functionality in well-controlled type 2 diabetes mellitus. *Atherosclerosis*, **222**(2): 567–573.

EFSA. (2004). Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contacts with Food (AFC) on a request from the Commission related to Coumarin. *The EFSA Journal*, **104**: 1–36.

Egan, D., O'Kennedy, R., Moran, E., Cox, D., Prosser, E., & Thornes, R. D. (1990). The pharmacology, metabolism, analysis, and applications of coumarin and coumarin-related compounds. *Drug Metabolism Reviews*, **22**(5): 503–529.

- Federici, M., Hribal, M., Perego, L., Ranalli, M., Caradonna, Z., Perego, C., Usellini, L., Nano, R., Bonini, P., Bertuzzi, F., Marlier, L. N., Davalli, A. M., Carandente, O., Pontiroli, A. E., Melino, G., Marchetti, P., Lauro, R., Sesti, G., & Folli, F. (2001). High glucose causes apoptosis in cultured human pancreatic islets of Langerhans: a potential role for regulation of specific Bcl family genes toward an apoptotic cell death program. *Diabetes*, **50**(6): 1290–1301.
- Folsom, A. R., Ma, J., Eckfeldt, J. H., Nieto, F. J., Metcalf, A., & Barnes, R. W. (1995). Association with Diabetes Mellitus and Other Cardiovascular Risk Factors But not with Prevalent Cardiovascular Disease or Carotid Artery Intima-Media Thickness. *Annals of Epidemiology*, **5**: 186–191.
- Fowler, M. J. (2008). Microvascular and Macrovascular Complications of Diabetes. *Clinical Diabetes*, **26**(2): 77–82.
- Franz, M. J., Powers, M. a, Leontos, C., Holzmeister, L. A., Kulkarni, K., Monk, A., Wedel, N., & Gradwell, E. (2010). The evidence for medical nutrition therapy for type 1 and type 2 diabetes in adults. *Journal of the American Dietetic Association*, **110**(12): 1852–1889.
- Friedrichsen, B. N., Neubauer, N., Lee, Y. C., Gram, V. K., Blume, N., Petersen, J. S., Nielsen, J. H., & Møldrup, A. (2006). Stimulation of pancreatic β -cell replication by incretins involves transcriptional induction of cyclin D1 via multiple signalling pathways. *Journal of Endocrinology*, **188**(3): 481–492.
- Gammella, E., Recalcati, S., Rybinska, I., Buratti, P., & Cairo, G. (2015). Iron-Induced Damage in Cardiomyopathy: Oxidative-Dependent and Independent Mechanisms. *Oxidative Medicine and Cellular Longevity*, **2015**: 1–10.
- Geng, S., Cui, Z., Huang, X., Chen, Y., Xu, D., & Xiong, P. (2011). Variations in essential oil yield and composition during *Cinnamomum cassia* bark growth. *Industrial Crops and Products*, **33**(1): 248–252.
- Grover, G., Gadpayle, A. K., & Sabharwal, A. (2012). Identifying patients with diabetic nephropathy based on serum creatinine in the presence of covariates in type-2 diabetes: A retrospective study. *Biomedical Research-India*, **23**(4): 615–624.
- Gruenewald, J., Freder, J., & Armbruester, N. (2010). Cinnamon and health. *Critical Reviews in Food Science and Nutrition*, **50**(9): 822–834.

- Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, **103**(2): 137–149.
- Gul, S., & Safdar, M. (2009). Proximate composition and mineral analysis of cinnamon. *Pakistan Journal of Nutrition*, **8**(9): 1456–1460.
- Gürtl, B., Kratky, D., Guelly, C., Zhang, L., Gorkiewicz, G., Das, S. K., Tamilarasan, K. P., & Hoefler, G. (2009). Apoptosis and fibrosis are early features of heart failure in an animal model of metabolic cardiomyopathy. *International Journal of Experimental Pathology*, **90**(3): 338–346.
- Hadi, A. M., Mouchaers, K. T. B., Schali, I., & Grunberg, K. (2011). Rapid quantification of myocardial fibrosis: a new macro-based automated analysis. *Cellular Oncology*, **34**: 343–354.
- Hafizur, R. M., Hameed, A., Shukrana, M., Ali, S., Chishti, S., Kabir, N., & Siddiqui, R. A. (2015). Cinnamic acid exerts anti-diabetic activity by improving glucose tolerance in vivo and by stimulating insulin secretion in vitro. *Phytomedicine*, **22**: 297–300.
- Hamada, Y., Nagasaki, H., Fuchigami, M., Furuta, S., Seino, Y., Nakamura, J., & Oiso, Y. (2013). The alpha-glucosidase inhibitor miglitol affects bile acid metabolism and ameliorates obesity and insulin resistance in diabetic mice. *Metabolism: Clinical and Experimental*, **62**(5): 734–742.
- Han, C., & Cui, B. (2012). Improvement of the bioavailability and glycaemic metabolism of cinnamon oil in rats by liquid loadable tablets. *The ScientificWorld Journal*, **681534**: 1–6.
- Han, S., Donelan, W., Wang, H., Reeves, W., & Yang, L.-J. (2013). Novel autoantigens in type 1 diabetes. *American Journal of Translational Research*, **5**(4): 379–392.
- He, Z.-D., Qiao, C.-F., Han, Q.-B., Cheng, C.-L., Xu, H.-X., Jiang, R.-W., But, P. P.-H., & Shaw, P.-C. (2005). Authentication and quantitative analysis on the chemical profile of cassia bark (cortex cinnamomi) by high-pressure liquid chromatography. *Journal of Agricultural and Food Chemistry*, **53**(7): 2424–2428.
- Herman, W. H., & Fajans, S. S. (2010). Hemoglobin A1c for the diagnosis of diabetes: practical considerations. *Polskie Archiwum Medycyny Wewnętrznej*, **120**: 37–40.

Hlebowicz, J., Darwiche, G., Björgell, O., & Almér, L.-O. (2007). Effect of cinnamon on postprandial blood glucose, gastric emptying, and satiety in healthy subjects. *The American Journal of Clinical Nutrition*, **85**(6): 1552–6.

Hlebowicz, J., Hlebowicz, A., Lindstedt, S., Bjo, O., Ho, P., Holst, J. J., & Darwiche, G. (2009). Effects of 1 and 3 g cinnamon on gastric emptying, satiety, and postprandial blood glucose, insulin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and ghrelin concentrations in. *American Journal of Clinical Nutrition*, **89**: 815–821.

Hong, J.-W., Yang, G.-E., Kim, Y. B., Eom, S. H., Lew, J.-H., & Kang, H. (2012). Anti-inflammatory activity of cinnamon water extract in vivo and in vitro LPS-induced models. *BMC Complementary and Alternative Medicine*, **12**(237): 1–8.

Hossain, M., Barry-ryan, C., & Martin-diana, A. (2008). Antioxidant activity of spice extracts and phenolics in comparison to synthetic antioxidants. *Rasayan Journal of Chemistry*, **1**(4): 751–756.

Huang, C.-L., & Kuo, E. (2007). Mechanism of hypokalemia in magnesium deficiency. *Journal of the American Society of Nephrology*, **18**(10): 2649–2652.

Huang, X., Yuang, J., Goddard, A., Foulis, A., James, R. F., Lernmark, A., Pujol-Borrell, R., Rabinovitch, A., Somoza, N., & Stewart, T. a. (1995). Interferon expression in the pancreases of patients with type I diabetes. *Diabetes*, **44**(6): 658–664.

Inoguchi, T., Li, P., Umeda, F., Yu, H. Y., Kakimoto, M., Imamura, M., Aoki, T., Etoh, T., Hashimoto, T., Naruse, M., Sano, H., Utsumi, H., & Nawata, H. (2000). High glucose level and free fatty acid stimulate reactive oxygen species production through protein kinase C-dependent activation of NAD(P)H oxidase in cultured vascular cells. *Diabetes*, **49**: 1939–1945.

Jakhetia, V., Patel, R., Khatri, P., Pahuja, N., Garg, S., Pandey, A., & Sharma, S. (2010). Cinnamon: a pharmacological Review. *Journal of Advanced Scientific Research*, **1**(2): 19–23.

Jarvill-Taylor, K. J., Anderson, R. a, & Graves, D. J. (2001). A hydroxychalcone derived from cinnamon functions as a mimetic for insulin in 3T3-L1 adipocytes. *Journal of the American College of Nutrition*, **20**(4): 327–336.

- Jia, Q., Liu, X., Wu, X., Wang, R., Hu, X., Li, Y., & Huang, C. (2009). Hypoglycemic activity of a polyphenolic oligomer-rich extract of *Cinnamomum parthenoxylon* bark in normal and streptozotocin-induced diabetic rats. *Phytomedicine*, **16**: 744–750.
- Jiao, L., Zhang, X., Huang, L., Gong, H., Cheng, B., Sun, Y., Li, Y., Liu, Q., Zheng, L., & Huang, K. (2013). Proanthocyanidins are the major anti-diabetic components of cinnamon water extract. *Food and Chemical Toxicology*, **56**: 398–405.
- Jolivalt, C., Calcutt, N., & Masliah, E. (2013). Similar pattern of peripheral neuropathy in mouse models of type 1 diabetes and Alzheimer's disease. *Neuroscience*, **27**: 405–412.
- Jun, H. S., Yoon, C. S., Zbytnuik, L., van Rooijen, N., & Yoon, J. W. (1999). The role of macrophages in T cell-mediated autoimmune diabetes in nonobese diabetic mice. *The Journal of Experimental Medicine*, **189**(2): 347–358.
- Kaneto, H., Fujii, J., Seo, H. G., Suzuki, K., Matsuoka, T., Nakamura, M., Tatsumi, H., Yamasaki, Y., Kamada, T., & Taniguchi, N. (1995). Apoptotic cell death triggered by nitric oxide in pancreatic beta-cells. *Diabetes*, **44**(7): 733–738.
- Kern, T. S., Tang, J., & Berkowitz, B. (2010). Validation of structural and functional lesions of diabetic retinopathy in mice. *Molecular Vision*, **16**: 2121–2131.
- Khan, A., Safdar, M., Ali Khan, M. M., Khattak, K. N., & Anderson, R. a. (2003). Cinnamon improves glucose and lipids of people with type 2 diabetes. *Diabetes Care*, **26**(12): 3215–3218.
- Khavandi, K., Khavandi, A., Asghar, O., Greenstein, A., Withers, S., Heagerty, A. M., & Malik, R. A. (2009). Diabetic cardiomyopathy--a distinct disease? *Best Practice & Research. Clinical Endocrinology & Metabolism*, **23**(3): 347–360.
- Khayyat, M. H., Vahdati-mashhadian, N., & Eghbal, S. (2013). Inter-individual Variability of Coumarin 7-hydroxylation (CYP2A6 activity) in an Iranian Population. *Iranian Journal Basic Medical Sciences*, **16**: 610–614.
- Khristi, V., Elias, J., Dave, N., & Patel, V. (2014). Gene Expression Analysis of Anti-oxidative Enzymes in Yeast against Oxidative Stress in Presence of *Cinnamomum zeylanicum*. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, **5**(5): 1387–1396.

- Kim, D. H., Kim, C. H., Kim, M.-S., Kim, J. Y., Jung, K. J., Chung, J. H., An, W. G., Lee, J. W., Yu, B. P., & Chung, H. Y. (2007). Suppression of age-related inflammatory NF-kappaB activation by cinnamaldehyde. *Biogerontology*, **8**(5): 545–554.
- Kim, S. H., & Choung, S. Y. (2010). Antihyperglycemic and antihyperlipidemic action of Cinnamomi Cassiae (Cinnamon bark) extract in C57BL/Ks db/db mice. *Archives of Pharmacal Research*, **33**(2): 325–333.
- Kim, S. H., Hyun, S. H., & Choung, S. Y. (2006a). Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. *Journal of Ethnopharmacology*, **104**: 119–123.
- Kim, S. H., Hyun, S. H., & Choung, S. Y. (2006b). Antioxidative effects of Cinnamomi cassiae and Rhodiola rosea extracts in liver of diabetic mice. *BioFactors*, **26**(3): 209–219.
- King, G. L. (2008). The role of inflammatory cytokines in diabetes and its complications. *Journal of Periodontology*, **79**: 1527–1534.
- King, G. L., & Loeken, M. R. (2004). Hyperglycemia-induced oxidative stress in diabetic complications. *Histochemistry and Cell Biology*, **122**(4): 333–338.
- Koo, J.-R., & Vaziri, N. D. (2003). Effects of diabetes, insulin and antioxidants on NO synthase abundance and NO interaction with reactive oxygen species. *Kidney International*, **63**(1): 195–201.
- Kopp, C., Singh, S. P., Regenhard, P., Müller, U., Sauerwein, H., & Mielenz, M. (2014). Trans-cinnamic acid increases adiponectin and the phosphorylation of AMP-activated protein kinase through g-protein-coupled receptor signaling in 3T3-L1 adipocytes. *International Journal of Molecular Sciences*, **15**(2): 2906–2915.
- Korshunov, S. S., Skulachev, V. P., & Starkov, A. A. (1997). High protonic potential actuates a mechanism of production of reactive oxygen species in mitochondria. *FEBS Letters*, **416**(1): 15–18.
- Koteswara, Y., Fang, S., & Tzeng, Y. (2007). Evaluation of the anti-inflammatory and anti-proliferation tumoral cells. *Journal of Ethnopharmacology*, **114**: 78–85.
- Kumar, K., Issac, A., Ninan, E., Kuttan, R., & Maliakel, B. (2014). Enhanced anti-diabetic activity of polyphenol-rich de-coumarinated extracts of Cinnamomum cassia. *Journal of Functional Foods*, **10**: 54–64.

- Kwong, L. K., & Sohal, R. S. (1998). Substrate and Site Specificity of Hydrogen Peroxide Generation in Mouse Mitochondria 1. *Archives of Biochemistry and Biophysics*, **350**(1): 118–126.
- Lan, K.-C., Chiu, C.-Y., Kao, C.-W., Huang, K.-H., Wang, C.-C., Huang, K.-T., Tsai, K.-S., Sheu, M.-L., & Liu, S. H. (2015). Advanced Glycation End-Products Induce Apoptosis in Pancreatic Islet Endothelial Cells via NF- κ B-Activated Cyclooxygenase-2/Prostaglandin E2 Up-Regulation. *Plos One*, **10**(4): e0124418.
- Leach, M., & Kumar, S. (2008). Cinnamon for diabetes mellitus (Protocol). *Cochrane Database of Systematic Reviews*, **CD007170**(1): 1–77.
- Lee, H. B. (2003). Reactive Oxygen Species-Regulated Signaling Pathways in Diabetic Nephropathy. *Journal of the American Society of Nephrology*, **14**(90003): 241S–245S.
- Lee, L.-F., Xu, B., Michie, S. a, Beilhack, G. F., Warganich, T., Turley, S., & McDevitt, H. O. (2005). The role of TNF-alpha in the pathogenesis of type 1 diabetes in the nonobese diabetic mouse: analysis of dendritic cell maturation. *Proceedings of the National Academy of Sciences of the United States of America*, **102**(44): 15995–6000.
- Li, Y., Wang, L., Schuschke, D. a, Zhou, Z., Saari, J. T., & Kang, Y. J. (2005). Marginal dietary copper restriction induces cardiomyopathy in rats. *The Journal of Nutrition*, **135**(9): 2130–2136.
- Li, Z., Karlsson, F. a., & Sandler, S. (2000). Islet loss and alpha cell expansion in type 1 diabetes induced by multiple low-dose streptozotocin administration in mice. *Journal of Endocrinology*, **165**(1): 93–99.
- Liao, B.-C., Hsieh, C.-W., Liu, Y.-C., Tzeng, T.-T., Sun, Y.-W., & Wung, B.-S. (2008). Cinnamaldehyde inhibits the tumor necrosis factor-alpha-induced expression of cell adhesion molecules in endothelial cells by suppressing NF-kappaB activation: effects upon IkappaB and Nrf2. *Toxicology and Applied Pharmacology*, **229**(2): 161–171.
- Ling, Z., Veld, P. A. I. N. T., & Pipeleers, D. G. (1993). Interaction of Interleukin-1 With Islet p-Cells. *Diabetes*, **42**: 56–65.
- Lipinski, B. (2001). Pathophysiology of oxidative stress in diabetes mellitus. *Journal of Diabetes and Its Complications*, **15**(4): 203–210.

- Lotfy, M., Singh, J., Rashed, H., Tariq, S., Zilahi, E., & Adeghate, E. (2014). The effect of glucagon-like peptide-1 in the management of diabetes mellitus: cellular and molecular mechanisms. *Cell and Tissue Research*, **358**(2): 343–358.
- Lu, M., Yuan, B., Zeng, M., & Chen, J. (2011). Antioxidant capacity and major phenolic compounds of spices commonly consumed in China. *Food Research International*, **44**(2): 530–536.
- Lu, T., Sheng, H., Wu, J., Cheng, Y., Zhu, J., & Chen, Y. (2012). Cinnamon extract improves fasting blood glucose and glycosylated hemoglobin level in Chinese patients with type 2 diabetes. *Nutrition Research*, **32**(6): 408–412.
- Lu, Z., Jia, Q., Wang, R., Wu, X., Wu, Y., Huang, C., & Li, Y. (2011). Hypoglycemic activities of A- and B-type procyanidin oligomer-rich extracts from different Cinnamon barks. *Phytomedicine*, **18**(4): 298–302.
- Maahs, D., West, N., Lawrence, J., & Mayer-David, E. (2010). Epidemiology of Type 1 Diabetes. *Endocrinology Metabolism Clinics of North America*, **39**(3): 481–497.
- Maechler, P., Jornot, L., & Wollheim, C. B. (1999). Hydrogen peroxide alters mitochondrial activation and insulin secretion in pancreatic beta cells. *The Journal of Biological Chemistry*, **274**(39): 27905–27913.
- Maedler, K., Sergeev, P., Ris, F., Oberholzer, J., Joller-jemelka, H. I., Spinas, G. A., Kaiser, N., Halban, P. A., & Donath, M. Y. (2002). Glucose-induced β cell production of IL-1 β contributes to glucotoxicity in human pancreatic islets. *The Journal of Clinical Investigation*, **110**(6): 851–860.
- Maedler, K., Spinas, G. A., Lehmann, R., Sergeev, P., Weber, M., Fontana, A., Kaiser, N., Donath, M. Y., & Fasli, F. F. (2001). Glucose Induces β -Cell Apoptosis Via Upregulation of the Fas Receptor in Human Islets. *Diabetes*, **50**: 1683–1690.
- Magistrelli, A., & Chezem, J. C. (2012). Effect of ground cinnamon on postprandial blood glucose concentration in normal-weight and obese adults. *Journal of the Academy of Nutrition and Dietetics*, **112**(11): 1806–1809.

- Mainous, A. G., Tanner, R. J., Baker, R., Zayas, C. E., & Harle, C. a. (2014). Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *British Medical Journal Open*, **4**: 1–8.
- Mang, B., Wolters, M., Schmitt, B., Kelb, K., Lichtinghagen, R., Stichtenoth, D. O., & Hahn, A. (2006). Effects of a cinnamon extract on plasma glucose, HbA_{1c}, and serum lipids in diabetes mellitus type 2. *European Journal of Clinical Investigation*, **36**(5): 340–344.
- Manjarrez-gutiérrez, G. (2014). Anatomopathological findings during development of diabetic cardiomyopathy in rats. *Cirugía Y Cirujanos*, **82**(1): 10–17.
- Manya, K., Champion, B., & Dunning, T. (2012). The use of complementary and alternative medicine among people living with diabetes in Sydney. *BMC Complementary and Alternative Medicine*, **12**(2): 1–5.
- Mariappan, N., Elks, C. M., Sriramula, S., Guggilam, A., Liu, Z., Borkhsenius, O., & Francis, J. (2010). NF-kappaB-induced oxidative stress contributes to mitochondrial and cardiac dysfunction in type II diabetes. *Cardiovascular Research*, **85**(3): 473–483.
- Markey, O., McClean, C. M., Medlow, P., Davison, G. W., Trinick, T. R., Duly, E., & Shafat, A. (2011). Effect of cinnamon on gastric emptying, arterial stiffness, postprandial lipemia, glycemia, glycemia, and appetite responses to high-fat breakfast. *Cardiovascular Diabetology*, **10**(78): 1–33.
- Mashhadi, N. S., Ghiasvand, R., Askari, G., Feizi, A., Hariri, M., Darvishi, L., Barani, A., Taghiyar, M., Shiranian, A., & Hajishafiee, M. (2013). Influence of ginger and cinnamon intake on inflammation and muscle soreness endured by exercise in Iranian female athletes. *International Journal of Preventive Medicine*, **4**(Suppl 1): S11–S15.
- Mathew, S., & Abraham, T. E. (2006). Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chemistry*, **94**(4): 520–528.
- McCord, J., & Fridovich, I. (1969). Superoxide Dismutase an enzymic function erythrocyte protein (Hemocuprein). *The Journal of Biological Chemistry*, **244**(22): 6049–6055.
- Meloni, a R., Deyoung, M. B., Lowe, C., & Parkes, D. G. (2013). GLP-1 receptor activated insulin secretion from pancreatic b-cells: mechanism and glucose dependence. *Diabetes, Obesity and Metabolism*, **15**: 15–27.

Min, T. Z., Stephens, M. W., Kumar, P., & Chudleigh, R. a. (2012). Renal complications of diabetes. *British Medical Bulletin*, **104**: 113–127.

Mirsaeedghazi, H., Emam-Djomeh, Z., & Ahmadkhaniha, R. (2014). Effect of frozen storage on the anthocyanins and phenolic components of pomegranate juice. *Journal of Food Science and Technology*, **51(2)**: 382–386.

Morais, Z. B., Pintão, A. M., Costa, I. M., Calejo, M. T., Bandarra, N. M., & Abreu, P. (2009). Composition and In Vitro Antioxidant Effects of Jellyfish *Catostylus tagi* from Sado Estuary (SW Portugal). *Journal of Aquatic Food Product Technology*, **18**: 90–107.

Moran, A., Bundy, B., Becker, D. J., DiMeglio, L. a, Gitelman, S. E., Goland, R., Greenbaum, C. J., Herold, K. C., Marks, J. B., Raskin, P., Sanda, S., Schatz, D., Wherrett, D. K., Wilson, D. M., Krischer, J. P., Skyler, J. S., Pickersgill, L., de Koning, E., Ziegler, A.-G., Böhm, B., Badenhop, K., Schloot, N., Bak, J. F., Pozzilli, P., Mauricio, D., Donath, M. Y., Castaño, L., Wägner, A., Lervang, H. H., Perrild, H., Mandrup-Poulsen, T., Pociot, F., & Dinarello, C. a. (2013). Interleukin-1 antagonism in type 1 diabetes of recent onset: two multicentre, randomised, double-blind, placebo-controlled trials. *Lancet*, **381(9881)**: 1905–1915.

Moselhy, S. S., & Ali, H. K. H. (2009). Hepatoprotective effect of cinnamon extracts against carbon tetrachloride induced oxidative stress and liver injury in rats. *Biological Research*, **42(1)**: 93–98.

Murea, M., Ma, L., & Freedman, B. I. (2012). Genetic and Environmental Factors Associated With Type 2 Diabetes and Diabetic Vascular Complications. *The Review of Diabetic Studies*, **9(1)**: 6–22.

Murray, R. K., Bender, D. A., Botham, K. M., Kennelly, P. J., Victor, W., & Weil, P. A. (2009). *Harper's Illustrated Biochemistry*. (Mc Graw Hill, Ed.) (28 th). Mc Graw Hill. 899-900.

Muthenna, P., Raghu, G., Kumar, P. A., Surekha, M. V., & Reddy, G. B. (2014). Effect of cinnamon and its procyanidin-B2 enriched fraction on diabetic nephropathy in rats. *Chemico-Biological Interactions*, **222**: 68–76.

Niedowicz, D. M., & Daleke, D. L. (2005). The Role of Oxidative Stress in Diabetic Complications. *Cell Biochemistry and Biophysics*, **43**: 289–330.

- Nowotny, K., Jung, T., Hohn, A., Weber, D., & Grune, T. (2015). Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes. *Biomolecules*, **5**: 194–222.
- Opie, E. (1901). The Relation of Diabetes Mellitus to Lesions of the Pancreas. Hyaline Degeneration of the Islands of Langerhans. *Journal of Experimental Medicine*, **5(5)**: 527–540.
- Patel, B. M., Raghunathan, S., & Porwal, U. (2014). Cardioprotective effects of magnesium valproate in type 2 diabetes mellitus. *European Journal of Pharmacology*, **728(1)**: 128–134.
- Pelkonen, O., Rautio, A., Raunio, H., & Pasanen, M. (2000). CYP2A6: a human coumarin 7-hydroxylase. *Toxicology*, **144**: 139–147.
- Peng, X., Cheng, K.-W., Ma, J., Chen, B., Ho, C.-T., Lo, C., Chen, F., & Wang, M. (2008). Cinnamon bark proanthocyanidins as reactive carbonyl scavengers to prevent the formation of advanced glycation endproducts. *Journal of Agricultural and Food Chemistry*, **56(6)**: 1907–1911.
- Persaud, S. J., Burns, C. J., Belin, V. D., & Jones, P. M. (2004). Glucose-induced regulation of COX-2 expression in human islets of Langerhans. *Diabetes*, **53(Suppl 1)**: S190–S192.
- Ping, H., Zhang, G., & Ren, G. (2010). Antidiabetic effects of cinnamon oil in diabetic KK-Ay mice. *Food and Chemical Toxicology*, **48**: 2344–2349.
- Plaisier, C., Cok, A., Scott, J., Opejin, A., Bushhouse, K. T., Salie, M. J., & Louters, L. L. (2011). Effects of cinnamaldehyde on the glucose transport activity of GLUT1. *Biochimie*, **93(2)**: 339–344.
- Postic, C., Shiota, M., Niswender, K. D., Jetton, T. L., Chen, Y., Moates, J. M., Shelton, K. D., Lindner, J., Cherrington, A. D., & Magnuson, M. A. (1999). Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *Journal of Biological Chemistry*, **274(1)**: 305–315.
- Poulsen, P., Kyvik, K. O., Vaag, A., & Beck-Nielsen, H. (1999). Heritability of Type II (non-insulin-dependent) diabetes mellitus and abnormal glucose tolerance - a population-based twin study. *Diabetologia*, **42**: 139–145.
- Prabha, M. R., & Vasantha, K. (2011). Antioxidant, Cytotoxicity and Polyphenolic Content of *Calotropis procera* (Ait.) R. Br. Flowers. *Journal of Applied Pharmaceutical Science*, **1(7)**: 136–140.

Prasad, K. N., Yang, B., Dong, X., Jiang, G., Zhang, H., Xie, H., & Jiang, Y. (2009). Flavonoid contents and antioxidant activities from *Cinnamomum* species. *Innovative Food Science and Emerging Technologies*, **10**(4): 627–632.

Qin, B. (2003). Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. *Diabetes Research and Clinical Practice*, **62**(3): 139–148.

Qin, B., Dawson, H. D., Schoene, N. W., Polansky, M., & Anderson, R. A. (2012). Cinnamon polyphenols regulate multiple metabolic pathways involved in insulin signaling and intestinal lipoprotein metabolism of small intestinal enterocytes. *Nutrition*, **28**: 1172–1179.

Qin, B., Panickar, K. S., & Anderson, R. a. (2014). Cinnamon polyphenols regulate S100 β , sirtuins, and neuroactive proteins in rat C6 glioma cells. *Nutrition*, **30**(2): 210–217.

Rabinovitch, A., & Suarez-pinzon, W. L. (1998). Cytokines and Their Roles in Pancreatic Islet beta-Cell Destruction and Insulin-Dependent Diabetes Mellitus. *Biochemical Pharmacology*, **55**(97): 1139–1149.

Rafehi, H., Ververis, K., & Karagiannis, T. C. (2012). Controversies surrounding the clinical potential of cinnamon for the management of diabetes. *Diabetes, Obesity and Metabolism*, **14**(1): 493–499.

Ranasinghe, P., Jayawardana, R., Galappaththy, P., Constantine, G. R., Gunawardana, N. D. V., & Katulanda, P. (2012). Review Article Efficacy and safety of “true” cinnamon (*Cinnamomum zeylanicum*) as a pharmaceutical agent in diabetes: a systematic review and meta-analysis. *Diabetic Medicine*, **29**: 1480–1492.

Ranasinghe, P., Perera, S., Gunatilake, M., Abeywardene, E., & Gunapala, N. (2012). Effects of *Cinnamomum zeylanicum* (Ceylon cinnamon) on blood glucose and lipids in a diabetic and healthy rat model. *Pharmacognosy Research*, **4**(2): 73–79.

Ranasinghe, P., Pigera, S., Premakumara, G. S., Galappaththy, P., Constantine, G. R., & Katulanda, P. (2013). Medicinal properties of “true” cinnamon (*Cinnamomum zeylanicum*): a systematic review. *BMC Complementary and Alternative Medicine*, **13**(275): 1–10.

Ranjbar, A., Ghaseminejhad, S., Takalu, H., & Baiaty, A. (2007). Anti oxidante Stress Potential of Cinnamon (*Cinnamomum zeylanicum*) in Operating Room Personnel; A

Before/After Cross Sectional Clinical Trial. *International Journal of Pharmacology*, **3(6)**: 482–486.

Ranjbar, A., Ghasmeinezhad, S., Zamani, H., Malekirad, A. A., & Baiaty, A. (2006). Antioxidative stress potential of *Cinnamomum zeylanicum* in humans: a comparative cross-sectional clinical study. *Therapy*, **3**: 113–117.

Ravindran, P. N., Babu, K. N., & Shylaja, M. (2004). Cinnamon and Cassia: The Genus *Cinnamomum*. In C. Press (Ed.), *CRC Press*.

Ray, D., Shukla, S., Allam, U. S., Helman, A., Ramanand, S. G., Tran, L., Bassetti, M., Krishnamurthy, P. M., Rumschlag, M., Paulsen, M., Sun, L., Shanley, T. P., Ljungman, M., Nyati, M. K., Zhang, M., & Lawrence, T. S. (2013). Tristetraprolin mediates radiation-induced TNF- α production in lung macrophages. *PloS One*, **8(2)**: 1–12.

Reed, G., Cefaratti, C., Berti-Mattera, L. N., & Romani, A. (2008). Lack of insulin impairs Mg²⁺ homeostasis and transport in cardiac cells of streptozotocin-injected diabetic rats. *Journal of Cellular Biochemistry*, **104(3)**: 1034–1053.

Rekha, N., Balaji, R., & Deecaraman, M. (2010). Antihyperglycemic and antihyperlipidemic effects of extracts of the pulp of *Syzygium cumini* and bark of *Cinnamomum zeylanicum* in streptozotocin-induced diabetic rats. *Journal of Applied Biosciences*, **28**: 1718–1730.

Robertson, R. P. (2004). Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *The Journal of Biological Chemistry*, **279(41)**: 42351–42354.

Robertson, R. P., Harmon, J., Tran, P. O., Tanaka, Y., & Takahashi, H. (2003). Perspectives in Diabetes. *Diabetes*, **52**: 581–587.

Roussel, A., Hininger, I., Benaraba, R., Ziegenfuss, T. N., & Anderson, R. A. (2009). Antioxidant Effects of a Cinnamon Extract in People with Impaired Fasting Glucose That Are Overweight or Obese. *Journal of the American College of Nutrition*, **28(1)**: 16–21.

Roussel, A.-M., Hininger, I., Benaraba, R., Ziegenfuss, T. N., & Anderson, R. a. (2009). Antioxidant effects of a cinnamon extract in people with impaired fasting glucose that are overweight or obese. *Journal of the American College of Nutrition*, **28(1)**: 16–21.

Scalbert, A., & Williamson, G. (2000). Dietary Intake and Bioavailability of Polyphenols. *The Journal of Nutrition*, **130**: 2073–2085.

Seddon, M., Looi, Y. H., & Shah, A. M. (2007). Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. *Heart*, **93**(8): 903–907.

Serreze, D. V., Chapman, H. D., Post, C. M., Johnson, E. a, Suarez-Pinzon, W. L., & Rabinovitch, A. (2001). Th1 to Th2 cytokine shifts in nonobese diabetic mice: sometimes an outcome, rather than the cause, of diabetes resistance elicited by immunostimulation. *Journal of Immunology*, **166**(2): 1352–1359.

Shah, N. C., Liu, J. P., Iqbal, J., Hussain, M., Jiang, X. C., Li, Z., Li, Y., Zheng, T., Li, W., Sica, A. C., Perez-Albela, J. L., Altura, B. T., & Altura, B. M. (2011). Mg deficiency results in modulation of serum lipids, glutathione, and NO synthase isozyme activation in cardiovascular tissues: Relevance to de novo synthesis of ceramide, serum Mg²⁺ and atherogenesis. *International Journal of Clinical and Experimental Medicine*, **4**(2): 103–118.

Shan, B., Cai, Y.-Z., Brooks, J. D., & Corke, H. (2007). Antibacterial properties and major bioactive components of cinnamon stick (*Cinnamomum burmannii*): activity against foodborne pathogenic bacteria. *Journal of Agricultural and Food Chemistry*, **55**(14): 5484–5490.

Shen, Y., Fukushima, M., Ito, Y., Muraki, E., Hosono, T., Seki, T., & Ariga, T. (2010). Verification of the Antidiabetic Effects of Cinnamon (*Cinnamomum zeylanicum*) Using Insulin-Uncontrolled Type 1 Diabetic Rats and Cultured Adipocytes. *Bioscience, Biotechnology, and Biochemistry*, **74**(12): 2418–2425.

Shen, Y., Honma, N., Kobayashi, K., Jia, L. N., Hosono, T., Shindo, K., Ariga, T., & Seki, T. (2014). Cinnamon Extract Enhances Glucose Uptake in 3T3-L1 Adipocytes and C2C12 Myocytes by Inducing LKB1-AMP- Activated Protein Kinase Signaling. *PloS One*, **9**(2): 1–9.

Shin, H.-J., Kim, J.-H., Han, S.-W., & Kim, H.-J. (2012). Polyuria with the Concurrent manifestation of Central Diabetes Insipidus (CDI) & Type 2 Diabetes Mellitus (DM). *Electrolyte Blood Press*, **10**: 26–30.

Siddiqui, K., Bawazeer, N., & Joy, S. S. (2014). Variation in Macro and Trace Elements in Progression of Type 2 Diabetes. *The Scientific World Journal*, **461591**: 1–9.

- Singh, G., Maurya, S., DeLampasona, M. P., & Catalan, C. a N. (2007). A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. *Food and Chemical Toxicology*, **45**(9): 1650–1661.
- Singh, R., Kaur, N., Kishore, L., & Gupta, G. K. (2013). Management of diabetic complications: a chemical constituents based approach. *Journal of Ethnopharmacology*, **150**: 51–70.
- Solomon, T. P. J., & Blannin, A. K. (2007). Effects of short-term cinnamon ingestion on in vivo glucose tolerance. *Diabetes, Obesity & Metabolism*, **9**(6): 895–901.
- Solomon, T. P. J., & Blannin, A. K. (2009). Changes in glucose tolerance and insulin sensitivity following 2 weeks of daily cinnamon ingestion in healthy humans. *European Journal of Applied Physiology*, **105**: 969–976.
- Song, X., Qian, X., Shen, M., Jiang, R., Wagner, M. B., Ding, G., Chen, G., & Shen, B. (2015). Protein kinase C promotes cardiac fibrosis and heart failure by modulating galectin-3 expression. *Biochimica et Biophysica Acta*, **1853**: 513–521.
- Song, Y., Wang, J., Li, X., & Cai, L. (2005). Zinc and the diabetic heart. *Biometals*, **18**(4): 325–332.
- Soni, R., & Bhatnagar, V. (2009). Effect of Cinnamon (*Cinnamomum Cassia*) intervention on Blood Glucose of Middle Aged Adult Male with Non Insulin Dependent Diabetes Mellitus (NIDDM). *Ethno-Medicine*, **3**(2): 141–144.
- Su, L., Yin, J.-J., Charles, D., Zhou, K., Moore, J., & Yu, L. (Lucy). (2007). Total phenolic contents, chelating capacities, and radical-scavenging properties of black peppercorn, nutmeg, rosehip, cinnamon and oregano leaf. *Food Chemistry*, **100**(3): 990–997.
- Swaminathan, S., Fonseca, V., Alam, M., & Shah, S. (2007). The role of iron in diabetes and its complications. *Diabetes Care*, **30**(7): 1926–1933.
- Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. *Physiological Research*, **50**(6): 537–546.
- Tammaro, P., Smith, A. L., Crowley, B. L., & Smirnov, S. V. (2005). Modulation of the voltage-dependent K⁺ current by intracellular Mg²⁺ in rat aortic smooth muscle cells. *Cardiovascular Research*, **65**(2): 387–396.

- Tang, M., Larson-Meyer, D. E., & Liebman, M. (2008). Effect of cinnamon and turmeric on urinary oxalate excretion, plasma lipids, and plasma glucose in healthy subjects. *The American Journal of Clinical Nutrition*, **87**(5): 1262–1267.
- Tashiro, M., Inoue, H., & Konishi, M. (2013). Magnesium Homeostasis in Cardiac Myocytes of Mg-Deficient Rats. *PLoS ONE*, **8**(9): 18–25.
- Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, **19**: 669–675.
- Thantsin, K., Zhang, Q., Yang, J., & Wang, Q. (2008). Composition of semivolatile compounds of 10 *Cinnamomum* species from China and Myanmar. *Natural Product Research*, **22**(7): 576–583.
- Thayer, T. C., Delano, M., Liu, C., Chen, J., Padgett, L. E., Tse, H. M., Annamali, M., Piganelli, J. D., Moldawer, L. L., & Mathews, C. E. (2011). Superoxide production by macrophages and T cells is critical for the induction of autoreactivity and type 1 diabetes. *Diabetes*, **60**(8): 2144–2151.
- Trost, S. U., Belke, D. D., Bluhm, W. F., Meyer, M., Swanson, E., & Dillmann, W. H. (2002). Overexpression of the sarcoplasmic reticulum CA²⁺-ATPase improves myocardial contractility in diabetic cardiomyopathy. *Diabetes*, **51**(4): 1166–1171.
- Tuomilehto, J., Lindstrom, J., Eriksson, J., Valle, T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukkaanniemi, S., Laakson, M., Louheranta, A., Rastas, M., Salminen, V., & Uusitupa, M. (2001). Prevention of type 2 Diabetes Mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *The New England Journal of Medicine*, **344**(18): 1343–1350.
- Ulbricht, C., Seamon, E., Windsor, R. C., Armbruster, N., Bryan, J. K., Costa, D., Giese, N., Gruenwald, J., Iovin, R., Isaac, R., Serrano, J. M. G., Tanguay-Colucci, S., Weissner, W., Yoon, H., & Zhang, J. (2011). An evidence-based systematic review of cinnamon (*Cinnamomum* spp.) by the Natural Standard Research Collaboration. *Journal of Dietary Supplements*, **8**(4): 378–454.
- Vafa, M., Mohammadi, F., Shidfar, F., Sormaghi, M. S., Heidari, I., Golestan, B., & Amiri, F. (2012). Effects of cinnamon consumption on glycemic status, lipid profile and body

composition in type 2 diabetic patients. *International Journal of Preventive Medicine*, **3(8)**: 531–536.

Van Baal, J., de Widt, J., Divecha, N., & van Blitterswijk, W. J. (2005). Translocation of diacylglycerol kinase theta from cytosol to plasma membrane in response to activation of G protein-coupled receptors and protein kinase C. *The Journal of Biological Chemistry*, **280(11)**: 9870–9878.

Vanschoonbeek, K., Thomassen, B. J. W., Senden, J. M., Wodzig, W. K. W. H., & Loon, L. J. C. Van. (2006). Cinnamon Supplementation Does Not Improve Glycemic Control in Postmenopausal Type 2 Diabetes Patients. *The Journal of Nutrition*, **136**: 977–980.

Venugopala, K. N., Rashmi, V., & Odhav, B. (2013). Review Article Review on Natural Coumarin Lead Compounds for Their Pharmacological Activity. *BioMed Research International*, **963248**: 1–14.

Wang, M., & Berlin, J. R. (2007). Voltage-dependent modulation of L-type calcium currents by intracellular magnesium in rat ventricular myocytes. *Archives of Biochemistry and Biophysics*, **458(1)**: 65–72.

Wang, Y.-H., Avula, B., Nanayakkara, N. P. D., Zhao, J., & Khan, I. A. (2013). Cassia cinnamon as a source of coumarin in cinnamon-flavored food and food supplements in the United States. *Journal of Agricultural and Food Chemistry*, **61(18)**: 4470–4476.

Wautier, J. L., Wautier, M. P., Schmidt, a M., Anderson, G. M., Hori, O., Zoukourian, C., Capron, L., Chappey, O., Yan, S. D., & Brett, J. (1994). Advanced glycation end products (AGEs) on the surface of diabetic erythrocytes bind to the vessel wall via a specific receptor inducing oxidant stress in the vasculature: a link between surface-associated AGEs and diabetic complications. *Proceedings of the National Academy of Sciences of the United States of America*, **91(16)**: 7742–7746.

Way, K. J., Isshiki, K., Suzuma, K., Yokota, T., Zvagelsky, D., Schoen, F. J., Sandusky, G. E., Pechous, P. a, Vlahos, C. J., Wakasaki, H., & King, G. L. (2002). Expression of connective tissue growth factor is increased in injured myocardium associated with protein kinase C beta2 activation and diabetes. *Diabetes*, **51(9)**: 2709–2718.

Weaver, J. R., Holman, T. R., Imai, Y., Jadhav, A., Kenyon, V., Maloney, D. J., Nadler, J. L., Rai, G., Simeonov, A., & Taylor-Fishwick, D. A. (2012). Integration of pro-inflammatory

cytokines, 12-lipoxygenase and NOX-1 in pancreatic islet beta cell dysfunction. *Molecular and Cellular Endocrinology*, **358**(1): 88–95.

Weinger, K., Jacobson, A. M., Draelos, M. T., Finkelstein, D. M., & Simonson, D. C. (1995). Blood Glucose Estimation and Symptoms During Hyperglycemia and Hypoglycemia in Patients With Insulin-Dependent Diabetes Mellitus. *The American Journal of Medicine*, **98**: 22–31.

Whiting, D. R., Guariguata, L., Weil, C., & Shaw, J. (2011). IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, **94**(3): 311–321.

WHO. (1997). *Obesity - Preventing and Managing the Global Epidemic*. Geneva, 1-275.

Wickenberg, J., Lindstedt, S., Berntorp, K., Nilsson, J., & Hlebowicz, J. (2012). Ceylon cinnamon does not affect postprandial plasma glucose or insulin in subjects with impaired glucose tolerance. *The British Journal of Nutrition*, **107**(12): 1845–1849.

Wickenberg, J., Lindstedt, S., Berntorp, K., Nilsson, J., & Hlebowicz, J. (2014). Cassia cinnamon does not change the insulin sensitivity or the liver enzymes in subjects with impaired glucose tolerance. *British Journal of Nutrition*, **13**(96): 1–6.

Wise, J. E., Kolb, E. L., & Sauder, S. E. (1992). Effect of glycemic control on growth velocity in children with IDDM. *Diabetes Care*, **15**(7): 826–830.

Wolf, F. I., Torsello, A., Fasanella, S., & Cittadini, A. (2003). Cell physiology of magnesium. *Molecular Aspects of Medicine*, **24**: 11–26.

Wolf, F. I., & Trapani, V. (2008). Cell (patho)physiology of magnesium. *Clinical Science*, **35**: 27–35.

Yang, J., Chen, L.-H., Zhang, Q., Lai, M.-X., & Wang, Q. (2007). Quality assessment of cortex cinnamomi by HPLC chemical fingerprint, principle component analysis and cluster analysis. *Journal of Separation Science*, **30**(9): 1276–1283.

Yang, S.-N., Shi, Y., Yang, G., Li, Y., Yu, J., & Berggren, P.-O. (2014). Ionic mechanisms in pancreatic β cell signaling. *Cellular and Molecular Life Sciences*, **71**(21): 4149–4177.

Youn, H. S., Lee, J. K., Choi, Y. J., Saitoh, S. I., Miyake, K., Hwang, D. H., & Lee, J. Y. (2008). Cinnamaldehyde suppresses toll-like receptor 4 activation mediated through the inhibition of receptor oligomerization. *Biochemical Pharmacology*, **75**(2): 494–502.

Yu, J. Z., Rodrigues, B., & McNeill, J. H. (1997). Intracellular calcium levels are unchanged in the diabetic heart. *Cardiovascular Research*, **34**(1): 91–98.

Yu, Y., & Song, Y. (2015). Three clustering patterns among metabolic syndrome risk factors and their associations with dietary factors in Korean adolescents: based on the Korea National Health and Nutrition Examination Survey of 2007-2010. *Nutrition Research and Practice*, **9**(2): 199–206.

Zhang, C., Lu, X., Tan, Y., Li, B., Miao, X., Jin, L., Shi, X., Zhang, X., Miao, L., Li, X., & Cai, L. (2012). Diabetes-Induced Hepatic Pathogenic Damage, Inflammation, Oxidative Stress, and Insulin Resistance Was Exacerbated in Zinc Deficient Mouse Model. *PLoS ONE*, **7**(12): 3–10.

Zhang, L., Cannell, M. B., Phillips, A. R. J., Cooper, G. J. S., & Ward, M.-L. (2008). Altered calcium homeostasis does not explain the contractile deficit of diabetic cardiomyopathy. *Diabetes*, **57**(8): 2158–2166.

Zhang, L., Ward, M.-L., Phillips, A. R., Zhang, S., Kennedy, J., Barry, B., Cannell, M. B., & Cooper, G. J. (2013). Protection of the heart by treatment with a divalent-copper-selective chelator reveals a novel mechanism underlying cardiomyopathy in diabetic rats. *Cardiovascular Diabetology*, **12**(123): 1–27.

Zhang, S., Liu, H., Amarsingh, G. V., Cheung, C. C. H., Hogg, S., Narayanan, U., Zhang, L., McHarg, S., Xu, J., Gong, D., Kennedy, J., Barry, B., Choong, Y. S., Phillips, A. R. J., & Cooper, G. J. S. (2014). Diabetic cardiomyopathy is associated with defective myocellular copper regulation and both defects are rectified by divalent copper chelation. *Cardiovascular Diabetology*, **13**(100): 1–18.

Zhang, X., Gregg, E. W., Williamson, D. F., Barker, L. E., Thomas, W., Bullard, K. M., Imperatore, G., Williams, D. E., & Albright, A. L. (2010). A1C level and future risk of diabetes: a systematic review. *Diabetes Care*, **33**(7): 1665–1673.

Ziegenfuss, T. N., Hofheins, J. E., Mendel, R. W., Landis, J., & Anderson, R. a. (2006). Effects of a water-soluble cinnamon extract on body composition and features of the

metabolic syndrome in pre-diabetic men and women. *Journal of the International Society of Sports Nutrition*, **3**(2): 45–53.

Zollinger, U. (1983). Rechtsmedizin Die Chromotrop-Anilinblau-Färbung zur besseren Darstellung frischer Herzmuskelfaserschädigungen*. *Z Rechtsmed*, **90**: 269–275.

Zou, C.-Y., Gong, Y., & Liang, J. (2014). Metabolic signaling of insulin secretion by pancreatic β -cell and its derangement in type 2 diabetes. *European Review for Medical and Pharmacological Sciences*, **18**: 2215–2227.

APPENDIX

The following tables represent the results (mean \pm SEM) in animal study regarding to blood glucose, body weight, food intake, OGTT-blood glucose, antioxidant status, biochemical parameters, insulin secretion from pancreatic β cell, % of positive pancreatic cells, fibrosis in the heart and the cations levels in serum, heart, liver, kidney and pancreas. In addition the results (mean \pm SEM) in human study is also represented regarding to blood glucose in human study.

Table A1.1: Table showing mean \pm SEM values for blood glucose level over the experimental period.

Blood Glucose (mg/dl) \pmSEM/Week	Normal Untreated	Normal Untreated 75 mg/Kg	Normal Treated 150 mg/Kg	Diabetic Untreated	Diabetic Treated 75 mg/Kg	Diabetic Treated 150 mg/Kg
0	92.3 \pm 3.0	91.2 \pm 3.6	91.8 \pm 5.2	388.2 \pm 21.0	316.7 \pm 14.5	318.8 \pm 21.4
1	84.8 \pm 4.9	83.7 \pm 2.7	90.2 \pm 0.8	438.6 \pm 18.5	399.2 \pm 38.6	355.2 \pm 50.5
2	85.2 \pm 3.2	92.8 \pm 2.6	84.4 \pm 4.7	418.0 \pm 18.3	399.2 \pm 36.2	394.6 \pm 39.0
3	82.5 \pm 3.4	87.2 \pm 3.1	89.0 \pm 3.0	479.0 \pm 16.8	429.2 \pm 22.1	360.2 \pm 21.7
4	89.0 \pm 2.6	93.8 \pm 3.2	93.6 \pm 2.6	470.2 \pm 23.0	412.5 \pm 21.8	407.4 \pm 24.5*
5	77.3 \pm 2.9	83.7 \pm 3.5	85.2 \pm 3.7	439.6 \pm 15.9	420.5 \pm 19.5	413.8 \pm 29.2
6	90.0 \pm 3.0	87.2 \pm 4.7	75.4 \pm 1.3	403.6 \pm 18.4	393.0 \pm 41.6	364.6 \pm 38.6
7	91.7 \pm 2.2	98.3 \pm 3.9	88.8 \pm 2.5	480.2 \pm 12.2	419.3 \pm 40.1	398.8 \pm 32.9
8	91.0 \pm 4.7	101.8 \pm 5.3	88.2 \pm 2.7	448.6 \pm 18.0	416.5 \pm 54.7	397.8 \pm 33.1
9	91.5 \pm 3.3	88.8 \pm 3.7	89.4 \pm 2.4	432.6 \pm 23.1	397.7 \pm 49.3	388.4 \pm 28.7
10	79.7 \pm 1.7	89.7 \pm 2.1	82.8 \pm 1.1	454.4 \pm 23.2	412.0 \pm 48.1	398.8 \pm 23.3
11	77.0 \pm 3.2	87.2 \pm 3.8	89.0 \pm 1.4	432.4 \pm 25.2	354.3 \pm 48.7	367.8 \pm 18.7

* p < 0.05, for diabetic treated group compared to diabetic untreated group

Table A1.2: Table showing mean \pm SEM values for body weight over the experimental period.

Body Weight (g) \pmSEM/Week	Normal Untreated	Normal Treated 75 mg/Kg	Normal Treated 150 mg/Kg	Diabetic Untreated	Diabetic Treated 75 mg/Kg	Diabetic Treated 150 mg/Kg
0	287.6 \pm 4.3	287.1 \pm 5.6	286.6 \pm 4.2	231.0 \pm 4.1	255.2 \pm 7.4	271.7 \pm 8.2
1	306.7 \pm 4.7	307.9 \pm 5.2	305.3 \pm 6.2	241.4 \pm 5.8	256.3 \pm 5.0	273.0 \pm 7.2*
2	322.9 \pm 4.9	329.3 \pm 5.6	320.2 \pm 5.9	260.5 \pm 15.0	263.7 \pm 5.1	279.5 \pm 5.7*
3	335.1 \pm 5.9	342.6 \pm 6.8	335.4 \pm 5.8	251.1 \pm 8.3	272.8 \pm 5.4	289.5 \pm 7.9
4	345.0 \pm 6.6	354.5 \pm 8.6	346.6 \pm 4.8	251.0 \pm 11.3	274.3 \pm 5.0	295.1 \pm 8.5*
5	352.1 \pm 7.0	367.6 \pm 10.1	355.4 \pm 5.6	254.8 \pm 9.2	276.5 \pm 6.8	301.7 \pm 8.9*
6	356.9 \pm 6.9	372.5 \pm 10.0	362.5 \pm 6.8	263.5 \pm 7.4	279.8 \pm 8.6	281.7 \pm 15.5
7	347.1 \pm 20.0	378.9 \pm 10.1	368.9 \pm 7.2	270.7 \pm 7.2	288.0 \pm 6.0	306.0 \pm 9.8
8	369.7 \pm 6.4	386.6 \pm 10.3	374.1 \pm 7.3	269.0 \pm 7.8	290.9 \pm 6.3	303.5 \pm 9.2
9	372.2 \pm 6.7	387.7 \pm 11.4	377.0 \pm 7.7	263.5 \pm 7.9	291.1 \pm 7.2	306.7 \pm 10.1
10	376.6 \pm 7.5	393.5 \pm 11.2	378.2 \pm 6.5	266.6 \pm 7.0	296.1 \pm 8.4	307.9 \pm 8.7
11	378.8 \pm 7.2	395.8 \pm 9.9	387.5 \pm 6.4	269.1 \pm 6.2	293.3 \pm 8.5	316.5 \pm 9.6*

* p < 0.05, for diabetic treated group compared to diabetic untreated group

Table A1.3: Table showing mean±SEM values for food intake over the experimental period.

Food intake (g) ± SEM/ Week	Normal Untreated	Normal Treated 75 mg/Kg	Normal Treated 150 mg/Kg	Diabetic Untreated	Diabetic Treated 75 mg/Kg	Diabetic Treated 150 mg/Kg
0	22.4±0.3	23.9±0.0	22.9±1.1	37.8±0.3	34.1±2.3	33.2±3.7
1	26.3±0.0	24.7±0.2	25.7±1.3	40.8±0.1	36.9±2.9	38.4±3.7
2	22.4±0.7	24.3±0.1	22.4±2.8	40.2±0.1	39.0±1.1	36.8±3.7
3	21.9±0.3	24.3±0.9	22.7±0.3	42.7±0.5	41.7±1.3	38.9±3.5
4	20.8±0.3	20.0±0.0	19.6±0.6	42.2±0.4	36.0±0.6	38.9±4.6
5	18.7±0.8	20.3±0.3	18.6±0.3	41.1±0.0	32.8±1.4*	39.0±3.4
6	17.6±0.0	21.3±0.2	19.4±0.1	46.0±1.4	35.4±1.3*	37.9±4.6
7	16.9±0.2	17.6±0.5	17.3±0.1	42.2±1.8	36.9±2.5	36.2±3.9
8	20.7±1.4	19.6±0.1	18.9±0.1	41.8±1.4	36.6±3.1	36.7±4.6
9	19.9±0.0	19.2±0.0	20.1±0.4	44.8±2.3	40.9±3.2	39.3±5.5
10	20.1±0.4	22.3±0.5	19.5±0.7	44.8±2.4	36.8±2.9	38.6±6.3
11	18.0±0.1	17.8±0.5	17.7±0.5	42.3±1.7	31.3±2.6*	33.2±3.4

*p < 0.05, for diabetic treated group compared to diabetic untreated group

Table A1.4: Table showing mean±SEM values for OGTT-blood glucose level over the experimental period.

OGTT - Blood Glucose (mg/dl) ± SEM	Normal Untreated	Normal Treated 75 mg/Kg	Normal Treated 150 mg/Kg	Diabetic Untreated	Diabetic Treated 75 mg/Kg	Diabetic Treated 150 mg/Kg
0 min	77.7±4.4	79.7±3.2	94.0±5.0	274.8±53.3	251.5±48.2	243.4±49.8
30 min	101.7±7.8	94.7±4.6	110.8±10.1	405.6±25.2	440.5±29.1	398.4±42.3
60 min	87.7±4.2	102.3±7.4	103.2±8.5	405.0±25.1	422.7±37.6	430.8±25.1
120 min	88.3±6.7	103.5±8.3	91.0±5.4	376.4±10.2	390.3±39.2	406.2±26.0
180 min	93.3±7.0	104.2±7.5	103.8±10.7	381.2±24.9	375.7±56.8	407.0±31.7

Table A1.5: Table showing mean±SEM values for antioxidant status over the experimental period.

Antioxidant Status (mmol TROLOX /l)	Mean ± SEM
Normal Untreated	0.495±0.09
Normal Treated 75 mg/Kg	0.443±0.1
Normal Treated 150 mg/Kg	0.605±0.15
Diabetic Untreated	0.43±0.09
Diabetic Treated 75 mg/Kg	0.578±0.20
Diabetic Treated 150 mg/Kg	0.71±0.25

Table A1.6: Table showing mean±SEM values for biochemical parameters over the experimental period.

Biochemical Parameters ± SEM	Normal Untreated	Normal Treated 75 mg/Kg	Normal Treated 150 mg/Kg	Diabetic Untreated	Diabetic Treated 75 mg/Kg	Diabetic Treated 150 mg/Kg
HbA1c (%)	4.5±0.3 ^{a,d,g}	4.4±0.2 ^{b,c,h}	5.1±0.2 ^{c,f,i}	12.6±0.4 ^{g,h,i}	12.2±0.7 ^{d,e,f}	13.0±0.5 ^{a,b,c}
TC (mg/dl)	67.3±4.2	62.7±4.6	66.0±6.9	81.6±5.5	75.3±5.5	78.0±3.7
HDL (mg/dl)	27.5±2.5	23.0±0.7 ^{b,d}	24.9±2.0 ^{a,c}	37.7±2.3 ^{c,d}	33.0±2.5	37.3±3.0 ^{a,b}
LDL (mg/dl)	4.9±0.1	4.5±0.3	4.8±0.9	5.5±0.5	4.9±0.6	4.9±0.5
TG (mg/dl)	96.4±14.9	126.3±20.3	122.3±19.0	181.8±23.0	172.0±17.8	162.7±20.0
Albumin (g/dl)	3.4±0.0 ^{b,c}	3.3±0.0 ^a	3.2±0.0	2.9±0.1 ^c	2.8±0.1	3.0±0.0 ^{a,b}
Creatinine (mg/dl)	0.7±0.0	0.8±0.0	0.7±0.0	0.816±0.0	0.824±0.0	0.775±0.0

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.7: Table showing mean±SEM values for insulin secretion from pancreatic β cell tissue *in vitro* over the experimental period.

Insulin Secretion from pancreatic β cell tissue <i>in vitro</i> ± SEM	Basal	Glucose (16mM)	Achetylcholine (-5M)	Achetylcholine (-6M)
Normal Untreated	13.19±0.613	19.52±4.388	2.14±1.841	14.6±0.454*
Normal Treated 75 mg/Kg	3.71±1.920	3.04±1.409	3.27±1.412	3.84±1.683
Normal Treated 150 mg/Kg	0.04±0.040	0.08±0.034	0±0.000	0.02±0.021*
Diabetic Untreated	0.17±0.112	0.15±0.128	0.04±0.017	0.06±0.036
Diabetic Treated 75 mg/Kg	0.01±0.009	0.01±0.008	0.03±0.018	0.03±0.023
Diabetic Treated 150 mg/Kg	1.06±0.715	1.4±0.975	0.64±0.203	0.09±0.066

* p < 0.05, for normal treated group compared to normal untreated group

Table A1.8: Table showing mean±SEM values for % of positive pancreatic cells over the experimental period.

% of Positive Pancreatic Cells ± SEM	% of Positive Insulin Cells	% of Positive Glucagon Cells
Normal Untreated	18.92±3.45	2.81±0.46
Normal Treated 75 mg/Kg	13.45±2.99	3.77±0.62
Normal Treated 150 mg/Kg	7.19±3.57	3.67±0.87
Diabetic Untreated	3.19±0.36	8.54±0.83
Diabetic Treated 75 mg/Kg	4.27±2.84	11.47±1.64
Diabetic Treated 150 mg/Kg	5.02±0.57	13.68±1.61

Table A1.9: Table showing mean±SEM values for fibrosis content over the experimental period.

Fibrosis content (Number of pixels)	Mean ± SEM
Normal Untreated	98038.75±10367.67
Normal Treated 75 mg/Kg	95502±15335.16
Normal Treated 150 mg/Kg	84833.63± 14973.38
Diabetic Untreated	130028.33±14537.11
Diabetic Treated 75 mg/Kg	117507.5±15446.19
Diabetic Treated 150 mg/Kg	80332.25±418.7

Table A1.10: Table showing mean±SEM values for cations levels in serum over the experimental period.

Cations Levels in Serum (mg/ml)	Normal Untreated	Normal Treated 75 mg/kg	Normal Treated 150 mg/kg	Diabetic Untreated	Diabetic Treated 75 mg/kg	Diabetic Treated 150 mg/kg
Sodium	9.7± 0.1527 ^{*a,b,c}	8.68± 0.185 ^{*b,d}	8.11± 0.29 ^{*c}	8.85± 0.245 ^{*a,d}	7.79± 0.230 ^{*a}	8.45± 0.515
Magnesium	0.022± 0.0018	0.0227± 0.0033	0.0195± 0.002	0.023± 0.00203 ^{*a}	0.017± 0.0011 ^{*a}	0.021± 0.00304
Potassium	0.642± 0.0645	0.618± 0.0549	0.476± 0.035	0.614± 0.0725	0.428± 0.0437	0.48± 0.0428
Calcium	0.048± 0.0025 ^{*a}	0.038± 0.0023 ^{*a}	0.036± 0.00287	0.05± 0.0029 ^{*b}	0.034± 0.00079 ^{*b}	0.041± 0.0040
Iron	0.0033± 0.00051	0.0027± 0.00026	0.0021± 0.00035	0.0026± 0.00054	0.0022± 0.00062	0.0024± 0.00037
Copper	0.00087± 0.000038	0.0007± 0.00004	0.00078± 0.0001	0.00082± 0.0000502	0.00076± 0.00005	0.0008± 0.00006

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.11: Table showing mean±SEM values for cations levels in heart over the experimental period.

Cations Levels in Heart (mg/ml/100g tissue)	Normal Untreated	Normal Treated 75 mg/kg	Normal Treated 150 mg/kg	Diabetic Untreated	Diabetic Treated 75 mg/kg	Diabetic Treated 150 mg/kg
Sodium	0.194± 0.0091 ^{*a}	0.171± 0.00578	0.186± 0.00465	0.17± 0.00234	0.159± 0.0073 ^{*a}	0.16± 0.003 ^{*a}
Magnesium	0.017± 0.0003 ^{*a,b}	0.016± 0.0002 ^{*b}	0.016± 0.0003	0.015± 0.0003 ^{*a,c}	0.017± 0.0003 ^{*c}	0.018± 0.0003 ^{*c}
Potassium	0.553± 0.0109	0.531± 0.0158	0.55± 0.0098	0.52± 0.0113 ^{*a}	0.569± 0.01 ^{*a}	0.629± 0.0119 ^{*a}
Calcium	0.0006± 0.0005	0.0007± 0.0001	0.0009± 0.0001	0.001± 0.0004 ^{*a}	0.0019± 0.0004	0.0032± 0.0005 ^{*a}
Iron	0.0014± 0.00006 ^{*a}	0.0016± 0.0002	0.0018± 0.0002 ^{*a}	0.0012± 0.00005 ^{*a}	0.0022± 0.0005	0.0017± 0.0001 ^{*a}
Copper	0.0004± 0.00001 ^{*d}	0.0003± 5.45E-06 ^{*c}	0.0004± 0.000008 ^{*b}	0.0003± 0.0000070 ^{*a}	0.0004± 0.00002	0.0005± 0.000014 ^{*a,b,c,d}
Zinc	0.0012± 0.00008 ^{*a}	0.0009± 9.13E-06 ^{*a}	0.001± 0.00005	0.0013± 0.0001	0.0012± 0.00004	0.0016± 0.0003

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.12: Table showing mean±SEM values for cations levels in liver over the experimental period.

Cations Levels in Liver (mg/ml/100g tissue)	Normal Untreated	Normal Treated 75 mg/kg	Normal Treated 150 mg/kg	Diabetic Untreated	Diabetic Treated 75 mg/kg	Diabetic Treated 150 mg/kg
Sodium	0.145±0.012	0.139±0.006	0.138±0.003	0.15±0.006	0.136±0.009	0.144±0.01
Magnesium	0.017±0.0002	0.018±0.0002	0.0166±0.0003	0.017±0.0003	0.0165±0.0003	0.016±0.0003
Potassium	0.644±0.009	0.642±0.0078	0.624±0.0134	0.636±0.0102	0.639±0.0104	0.620±0.0095
Iron	0.0085±0.0006 ^a	0.0071±0.00061 ^b	0.0054±0.00029	0.005±0.0004 ^a	0.0045±0.00062 ^b	0.005±0.0004
Copper	0.00035±0.000048	0.00044±0.000044	0.00032±0.000025	0.00044±0.000025	0.00048±0.000103	0.00044±0.000063
Zinc	0.002±0.000075	0.002±0.000034	0.00201±0.00006	0.0022±0.00014	0.0021±0.00012	0.002±0.000069

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.13: Table showing mean±SEM values for cations levels in kidney over the experimental period.

Cations Levels in Kidney (mg/ml/100g tissue)	Normal Untreated	Normal Treated 75 mg/kg	Normal Treated 150 mg/kg	Diabetic Untreated	Diabetic Treated 75 mg/kg	Diabetic Treated 150 mg/kg
Sodium	0.315±0.0134	0.329±0.0065	0.301±0.0071	0.345±0.0175	0.303±0.0129	0.311±0.0112
Magnesium	0.0149±0.001	0.0155±0.0003	0.0161±0.0003	0.0148±0.0007	0.0151±0.0005	0.0134±0.0004
Potassium	0.474±0.0167	0.459±0.0214	0.507±0.0189 ^a	0.478±0.0276	0.470±0.0169	0.413±0.0036 ^a
Calcium	0.0043±0.0005	0.0008±0.0002	0.0009±0.0004	0.0047±0.001	0.0041±0.0025	0.0016±0.0008
Iron	0.005±0.00027 ^{a,b}	0.0035±0.00029 ^a	0.0031±0.00019 ^b	0.0048±0.00053	0.0038±0.00028	0.0059±0.00149
Copper	0.00062±0.00009 ^a	0.00072±0.00003 ^b	0.0005±0.00004 ^c	0.0039±0.00075 ^a	0.0035±0.00064 ^b	0.00395±0.00114 ^c
Zinc	0.0022±0.00029	0.0023±0.00058	0.0023±0.00017	0.0026±0.00028	0.0026±0.0005	0.00201±0.00033

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.14: Table showing mean±SEM values for cations levels in pancreas over the experimental period.

Cations Levels in Pancreas (mg/ml/100g tissue)	Untreated Normal	Normal Treated 75 mg/kg	Normal Treated 150 mg/kg	Untreated Diabetic	Diabetic Treated 75 mg/kg	Diabetic Treated 150 mg/kg
Sodium	0.399±0.025	0.313±0.033	0.379±0.015	0.386±0.0209	0.389±0.022	0.365±0.017
Magnesium	0.017±0.0012	0.0183±0.0007	0.0174±0.0005	0.018±0.0005	0.0186±0.0008	0.0186±0.0009
Potassium	0.449±0.037	0.535±0.03	0.476±0.011	0.505±0.011	0.529±0.028	0.528±0.016
Calcium	0.0036±0.0004 ^a	0.0048±0.0001 ^a	0.00419±0.0004	0.00363±0.0003	0.0049±0.0004	0.0039±0.00043
Iron	0.00088±0.00016	0.00114±0.00021	0.00058±0.000043	0.00076±0.000095	0.00113±0.0002	0.00069±0.00007
Copper	0.000099±7.70E-06	0.000089±3.704E-06	0.000085±0.000002	0.000096±5.87E-06	0.000096±5.79E-06	0.000089±7.799E-06
Zinc	0.00171±0.000057	0.00213±0.000204	0.00140±0.00011	0.00157±0.0000734	0.0019±0.00025	0.00152±0.0000701

* p < 0.05, in the same row values sharing a common superscript letters are significantly different.

Table A1.15: Table showing mean±SEM values for incremental area under the curve (AUC_i), maximum concentration (C_{max}) and variation of maximum concentration (ΔC_{max}) blood glucose levels in serum over the experimental period.

Blood Glucose in human study	OGTT_(control) Mean (±SEM) (mmol/L)	OGTT_(cinnamon) Mean (±SEM) (mmol/L)
AUC_i (0-120 min)	403.73 (±48.5)	297.47 (±33.9)
C_{max}	10.63 (±0.6)	8.98 (±0.5)*
ΔC_{max}	5.71 (±0.6)	4.0 (±0.5)*

* p < 0.05, for cinnamon group compared to control group

PUBLICATIONS

Papers:

1. Bernardo, M.A; **Silva, M.L.**; Santos, E.; Moncada, M.; Brito, J.; Proença, L.; Singh, J.; Mesquita, M.F. (2015) Effect of cinnamon tea on postprandial glucose concentration. *Journal of Diabetes Research*, **913651**: 1-6
2. **Silva, M.L.**; Bernardo, M.A.; Singh, J.; Mesquita, M.F. Metabolic function of cinnamon and proposed mechanism for the control of dysglycemia and dyslipidemia: a review. *International Journal of Food Sciences and Nutrition*. (Submitted)

Abstracts:

1. Bernardo MA, **Silva ML**, Santos, E, Moncada, M, Brito, J, Proença, L, Singh, J, Mesquita, MF (2015). Effect of Polyphenols-rich cinnamon tea on post-prandial glycaemia on human. 9th World Congress on Polyphenols Applications, St. Julian's, Malta. (Poster presentation)
2. Moncada, M, Jorge AR, Bernardo MA, **Silva ML**, Brito, J, Singh, J, Mesquita, MF (2015). Effect of cinnamon powder addition to Portuguese cake on post-prandial glycaemia and antioxidant capacity. 9th World Congress on Polyphenols Applications, St. Julian's, Malta. (Poster presentation)
3. **Silva ML**, Pereira P, Bernardo MA, Moncada MM, Singh J, Mesquita MF (2013). Effect of *Cinnamomum Burmannii* in Streptozotocin-induced type 1 Diabetes Mellitus. 37th Congress of the International Union of Physiological Sciences, Birmingham, England. (Poster presentation)
4. **Silva ML**, Singh J, Mesquita MF (2013). Effect and mechanism of action of *Cinnamomum Burmannii* in STZ-induced diabetic rats. Internal research group organized by Director of studies. School of Forensic and Investigative Sciences, UCLan, Preston, England. (Oral presentation)

5. Antunes F, Moncada M, Bernardo MA, **Silva ML**, Auxtero MD, Pereira P, Mesquita MF (2013). Study of antioxidant capacity of aqueous cinnamon extract of *Cinnamomum Burmannii* species. 4th Journeys of Health Sciences of Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal. (Oral presentation)
6. **Silva ML**, Pereira P, Bernardo MA, Moncada MM, Singh JP, Mesquita MF (2012). Effects and Action Mechanisms of *Cinnamomum Burmannii* in Diabetes Mellitus – A Research Project. 3rd Journeys of Health Sciences, Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal. (Poster presentation)
7. Mendonça H, **Silva ML**, Vicente AF, Mesquita MF (2012). Nutritional Quality Assessment of School Lunch on Almada City Hall Schools. 3rd Journeys of Health Sciences, Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal. (Poster presentation)
8. **Silva ML**, Singh J, Mesquita MF (2012). Effect and mechanism of action of *Cinnamomum Burmannii* in diabetes Mellitus. Internal research group organized by Director of studies and supervisor in Instituto superior de Ciências da Saúde Egas Moniz, Caparica, Portugal. (Oral presentation)
9. Camolas J, Guerreiro M, Teixeira D, **Silva ML**, Carmo I. (2012) Evaluation of the therapeutic effectiveness of a nutritional intervention in obese individuals. 16th Obesity Portuguese Congress, Algarve, Portugal. (Oral presentation)
10. **Silva ML**, Mendonça H, Mesquita MF (2011). Nutritional aspects in patients infected with human immunodeficiency virus (HIV). 2nd Journeys of Health Sciences, Instituto Superior de Ciências da Saúde Egas Moniz, Caparica, Portugal. (Oral presentation)
11. Mendonça H, **Silva ML** (2011). Ways of Cardiovascular Prevention. 3rd Workshop – Highlights of the year in cardiovascular prevention in lifestyles: Nutrition, Portuguese Society of Cardiology, Lisbon Portugal (Oral presentation)

**MEETING AND COLLABORATION
WORK**

1. Jury of Scientific Committee for oral communication of 4th Journeys of Nutrition Sciences. Instituto superior de Ciências da Saúde Egas Moniz, Caparica, Portugal (March, 2015).
2. Attend a graduate research skills training: “Managing transfer from MPhil to PhD” and “Undertaking your literature review” (February, 2013).
3. Member of Scientific Committee of 2nd Journeys of Nutrition Sciences. Instituto superior de Ciências da Saúde Egas Moniz, Caparica, Portugal (March, 2013).
4. Attend on Course on Laboratory Animal Science: XI Course on Laboratory Animal Science organized by Center for Interdisciplinary Research in Animal Health (CIISA) of Veterinary Medicine Faculty in collaboration with Utrecht University (March, 2012).