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Review Article

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Synergic Treatment of Plant-Based Antioxidants with Iron Chelators for Iron Overload in Transfusion-Dependent-Thalassemia Patients: A Systematic Review

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ABSTRACT

The combined use of plant-based antioxidants and iron chelators presents a synergistic treatment approach that effectively tackles both iron overload and the accompanying oxidative stress in individuals with transfusion-dependent Thalassemia (TDT). Plant-based antioxidants counteract reactive oxygen species (ROS) and oxidative damage, whereas iron chelators effectively bind excess iron, reducing the body's iron concentration. This combined therapy can be beneficial in improving TDT patients with iron overload. We systematically reviewed the literature exploring the plant-based antioxidants with iron chelators for iron overload in transfusion-dependent Thalassemia Patients. All fourteen included studies were randomized clinical trials, employing various randomization methods including simple randomization, double-blinded, triple-blinded, and crossover designs. The included studies enrolled participants across different age groups, including both young and adult patients. Despite the variability in plant-based antioxidants with iron-chelating properties, the key findings were as follows: Nine studies reported a significant reduction in iron overload, eight studies observed a marked decrease in oxidative stress markers, and five studies demonstrated reduced liver enzyme levels, suggesting potential hepatoprotective effects. All included studies reported significant effects of various supplements on key biomarkers, including total iron (Fe), ferritin, total iron-binding capacity (TIBC), total antioxidant capacity (TAC), malondialdehyde (MDA), and liver enzymes (AST, ALT). Silymarin, green tea, and grape seed extract (GSE) supplementation demonstrated notable reductions in total Fe, Ferritin, ASL, and ALT levels. Additionally, these supplements increased TIBC levels, suggesting improved iron metabolism. In contrast, quercetin and curcumin supplementation did not show a statistically significant difference compared to control groups in these outcomes.

Keywords: Plant-based antioxidant; Transfusion-dependent thalassemia; Supplement

INTRODUCTION

In recent years, there has been growing interest in the therapeutic potential of plant-based antioxidants in conjunction with iron chelators for managing iron overload and oxidative stress in Transfusion-dependent thalassemia (TDT) patients. Fruits, vegetables, and herbs are rich sources of plant-based antioxidants, which have the potential to eliminate free radicals and protect against damage caused by oxidative stress.

In several studies, these antioxidants, which also include polyphenols, flavonoids, carotenoids, and

vitamins C and E, have shown potent antioxidant properties [1,2].

The combination of plant-based antioxidants with iron chelators offers a synergistic treatment approach that addresses both iron overload and the associated oxidative stress in TDT patients. Plant-based antioxidants combat reactive oxygen species (ROS) and oxidative damage, while iron chelators bind excess iron to lower the body's concentration of iron. This combined therapy may improve the overall efficiency of managing iron overload in TDT patients. Preclinical studies have demonstrated the beneficial interactions between iron chelators and plant-based antioxidants. In contrast to DFP or DFO treatment alone, co-administration of curcumin and iron chelator increased antioxidant activity by enhancing the speed of non-transferrin-bound iron (NTBI) removal by DFP [3]. A well-known antioxidant, green tea, also showed synergistic effects in the thalassaemic mouse model by delaying the deposition of hepatic iron in regular iron-loaded thalassemic mice [4].

These findings highlight the therapeutic value of combining plant-based antioxidants with iron chelators to manage TDT patients' iron overload. This synergistic strategy intends to treat both iron-induced toxicity and oxidative stress by utilising plant-based compounds' antioxidant characteristics and chelators' iron-binding capacities.

Moreover, combined administration of iron chelators, Dimercaptosuccinic acid (DMSA) and monoisoamyl dimercaptosuccinic acid MiADMSA), with vitamins C and E reduced lead-induced oxidative stress and improved antioxidant status compared to monotherapy with iron chelators alone in rats[5]. Therefore, plant-based antioxidants became a promising supplement to enhance iron chelating in thalassemia patients.

This study aims to conduct a comprehensive literature review on the therapeutic benefits of combining plant-based antioxidants and iron chelators as a synergistic treatment approach for managing transfusion-dependent thalassemia (TDT) patients.

MATERIALS AND METHODS

Literature Search

The comprehensive literature search was done systematically to evaluate plant-based antioxidants' clinical efficacy and safety in transfusion-dependent thalassemia patients. The included studies were searched by three researchers independently from PubMed, Ovid Medline, and Scopus databases and backreferences of the articles till February 2024. Following the Cochrane guidelines, Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA), the search included the keywords: “beta thalassemia” AND “antioxidant” or “antioxidant therapy” OR “antioxidant treatment.” The randomised controlled studies published in English were included in this review.

Study selection

All obtained articles were imported into Zotero software, and duplicate articles were removed. The titles and abstracts were screened by three researchers independently, and those articles that did not appear to be relevant were excluded. Two authors independently reviewed the full-text articles and the disagreement on study selections was solved by consensus with the third author. Inclusion criteria include (1) transfusion-dependent thalassemia patients receiving any chelating therapy of any age, (2) treatment which used plant-based antioxidants therapy, (3) investigation parameters for antioxidants or iron levels, and (3) randomised controlled trials (RCT). Exclusion criteria are (1) non-transfusion-dependent thalassemia patients, (2) any other haemoglobinopathies rather than transfusion-dependent β-thalassemia major (3) studies without antioxidant parameters or iron study (4) any study designs rather than RCT and (5) any languages rather than English. If a study comparing two or more antioxidant therapy, only plant-based study results were selected.

Data abstraction and quality assessment

Two reviewers (P.S.O and M.T.K) extracted the data independently. The characteristics of the studies included author, year of publication, country, study design, sample size, targeted participant and age of participants, blood transfusion history, type and

dose of iron chelating agents, type, dose and duration of plant-based antioxidant therapy, investigation parameters, response and type of adverse reaction of plant-based antioxidant agents. The quality of the studies was assessed by two reviewers (C.S.R and A.N.U) using Joanna Briggs Institute (JBI)'s critical appraisal tools for RCT (supplement 1). The tool comprises multiple questions assessing the potential risk for selection bias, administration bias, assessment bias and participant bias.

Data analysis

A meta-analysis was conducted using STATA version 16.0 (STATA Corporation, Texas, USA). Owing to the expected heterogeneity within and between studies, we used the random-effects REML model, which takes into consideration between- and within-study variations and provides a more conservative analysis of the studies than the fixed-effects model.

The effect of different types of plant-based supplements were estimated changes in total Fe, serum ferritin, TIBC, ALT and AST in both between groups (placebo and intervention) and within groups (before and after studies). Subgroup analysis based on the types of supplements was also conducted.

Due to the limited number of papers, publication bias was not assessed.

RESULT

Study characteristics

This systematic review included a total of 14 studies that met the inclusion criteria. These studies were conducted over the past decade (2009-2024) in two countries, specifically Iran (12 studies) and Egypt (2 studies). The studies examined the effects of silymarin[6,7,8,9,10,11], curcumin[12,13], green tea [14], quercetin [15,16], alpha lipoic acid (ALA) [17], Iranian oak (Quercus brantii) [18] and Grape seed extract (GSE) [19] as dietary supplements. All 14 studies were randomized clinical trials, with various types of randomization methods employed, including simple randomized [7,8,14], double-blinded [6,11,12,13,15,16,18,19], and triple-blinded, cross-over[9,10,17]designs. These three randomized, triple-blind studies [9,10,17] used stratification method as participants were grouped as AB/BA cross over with 2-3week washout period between treatments.

All of the included studies were published in English and were available as full-text papers. All adult patients included in the studies were individuals with beta thalassemia major (BTM) who were dependent on transfusions, receiving them at least four times per year. These patients had already been undergoing chelation therapy using either Desferioxamine or Deferasirox, with varying dosages as indicated in **Table 1**.

Description of Included Patients

The detailed characteristics of all patients are shown in Table 1. The Prisma flow chart reveals our initial literature search which yielded 471 published studies, of which 31 were excluded based on titles and abstract (Figure 1). After a subsequent review, we included a total of 14 articles. Table 1 summarizes the characteristics of all patients included in this review. Among the 821 patients diagnosed with BTM, the mean age of participants is different. Three studies [7,8,19] was done on children (n=171) with the age range from 5-16 years. The mean serum ferritin levels were >1000 ng/ml. The range of treatment duration of plant-based antioxidants with iron chelators was from 4 weeks to 36 weeks.

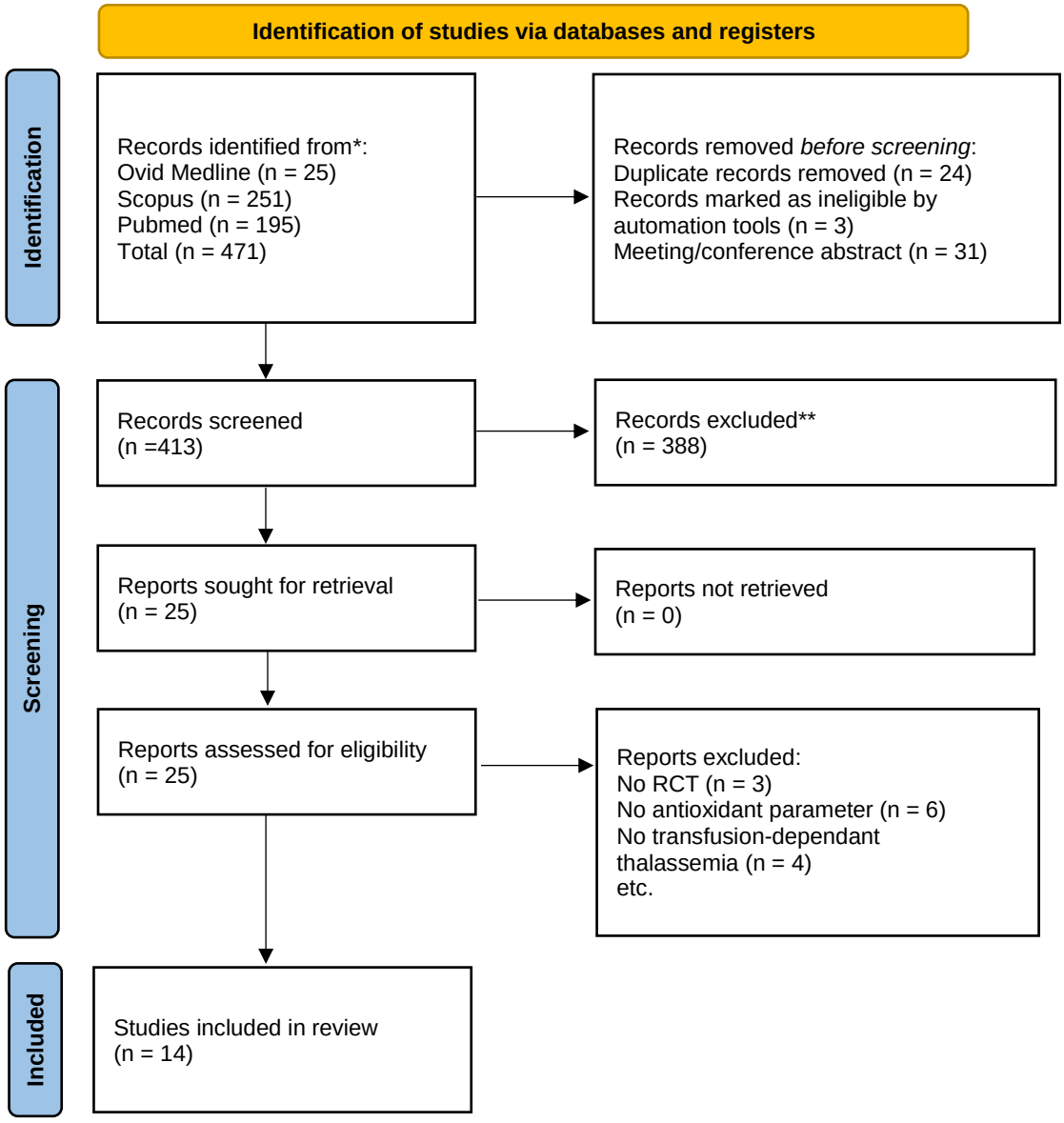

Figure 1. PRISMA flow diagram of articles included in current review

Meta-analysis results

1. Total Fe

This study aimed to investigate the effect of different supplementations on total Fe levels of BTM patients. From the pool of 14 studies, eight of them measured total Fe levels [7,8,9,11,12,14,15,19]. Among the eight studies, four utilized silymarin as a supplementation [7,8,9,11], while the remaining studies used curcumin [12], GSE[19], green tea [14] and quercetin [15] as supplements.

a. Silymarin and green tea: The analysis of the eight studies that measured total Fe levels revealed that there was a significant reduction in total Fe levels in the intervention groups administered with silymarin and green tea as supplementation when compared with control. Silymarin can reduce 45.40 µg/dL (95%CI: -73.54 to -17.25) and green tea can reduce 115.00 µg/dL (95%CI: -143.32 to -86.16). This suggests that these supplements may have an inhibitory effect on Fe absorption or utilization in the body.

b. Grape seed extract: The treatment with grape seed extract (GSE) affected total iron (Fe) levels. The GSE treatment group exhibited a reduction in serum Fe levels of 19.00 µg/dL (95% CI: -39.44 to 1.44) compared to the control group.

c. Quercetin and Curcumin: On the other hand, there was no significant difference between the intervention and control groups that used quercetin and curcumin as supplementation. This implies that these supplements did not have a significant effect on total Fe levels in the respective groups.

d. Before and after comparison: Most products can reduce Fe levels after supplementation; however, curcumin and quercetin do not show significant reductions, both clinically and statistically. Grape seed extract (GSE) reduces Fe by 38.00 µg/dL (95% CI: -61.01 to -14.99), green tea reduces Fe by 49.00 µg/dL (95% CI: -76.23 to -21.54), and overall silymarin studies reduce Fe by 69.46 µg/dL (95% CI: -102.80 to -36.12), respectively.

2. Ferritin

Among the pool of 14 studies, 9 studies 6,7,8,9,11,12,14,15,19 measured serum ferritin level out of which 5 studies 6,7,8,9,11 used silymarin as supplementation. The remaining 4 studies were done using curcumin 12 , GSE19 , quercetin 15 and green tea 12 as supplementation.

a. Silymarin: All five studies that used silymarin as supplementation demonstrated a reduction in ferritin levels. When considering all five studies collectively, the overall results indicated a statistically significant reduction in ferritin levels, with a mean reduction of 393.10 ng/mL (95% CI: -691.97 to -94.22).

b. Grape seed extract and green tea: Ferritin levels are found to have effect by GSE and green tea. GSE treatment group showed a reduction of serum ferritin level 240.30 (95%CI: -467.98 to -12.62) and green tea treatment group showed reduction of serum ferritin level 831.69 (95%CI: -1143.79 to -519.59) compared to control group.

c. Curcumin and quercetin: Ferritin levels are found to be not affected by curcumin and quercetin supplementation.

d. Before and after comparison: From the baseline, some supplements could reduce serum ferritin levels [GSE -648.00 (-909.55 to -386.45), Green tea -589.00 (-869.13 to -308.87), Quercetin -258.68 (-525.35 to 8.09)] after supplementation. On the other hand, curcumin has not statistically changed of reducing the ferritin level -74.45 ng/mL (95%CI: -309.49 to 160.59).

Silymarin could reduce ferritin levels in all 5 studies and overall amount of reduction is 1219.54 ng/mL from baseline (95%CI; -1802.94 to -636.14) which is statistically significant. The effect of silymarin on reducing ferritin levels from baseline becomes apparent at 1.5 months, though it is not statistically significant at this stage. However, with prolonged treatment, a statistically significant reduction in ferritin levels is observed, showing continuous decreases at 3 months 645.09 ng/mL (95%CI: -1243.10 to -51.08), 6 months 822.40 ng/mL (95%CI: -1527.37 to -117.43), and 9 months 1056.60 ng/mL (95% CI: -1724.52 to -388.68).

3. TIBC

a. A total of six studies—specifically, Quercetin 15, GSE 19, and four silymarin studies 7, 8, 9, 11 examined the effects on changes in TIBC levels. Overall, TIBC was found to be increased by 20.31

µg/dL (95% CI: -7.21 to 47.75), although this was not statistically significant. Three silymarin studies 7, 8, 9, GSE 19 and Quercetin11 study reported improvements in TIBC. However, it is important to note that the study by Moayedi B et al.11 presented contrasting results, indicating a decrease in TIBC following the use of silymarin.

b. Before and after comparison: Similar results were observed before and after comparison for different supplements.

4. ALT

A total of seven studies 6,7,8,11,13,16,19 measured ALT levels. An overall reduction of ALT levels 7.74 IU/L (95%CI: -14.67 to -0.81) was observed in intervention groups compared to control and the finding is statistically significant. This reduction appears to be primarily due to the effects of GSE supplementation [-27.61 IU/L (95%CI: -39.24 to -15.98)] and quercetin supplementation [-14.00 IU/L (95% CI: -28.67 to 0.67)], rather than silymarin. In the before and after comparison, a significant reduction in ALT levels was observed only with GSE supplementation [-22.83 IU/L (95% CI: -34.67 to -10.99)].

5. AST

Among the seven studies examining AST levels with different supplementations (GSE19, quercetin 16, curcumin13 and silymarin 6, 7, 8, 11), a reduction was observed in nearly all studies. Compared to control groups, the overall reduction in AST levels in supplementation group was -5.83 IU/L (95% CI: -10.21 to -1.49), which is statistically significant. GSE supplementation led to a reduction in AST levels in both between-group [-11.70 IU/L (95% CI: -17.00 to -6.40)] and within-group [-8.11 IU/L (95% CI: -14.31 to -1.91)] comparisons. In contrast, quercetin reduced AST levels only in the between-group comparison but not within-group. No effect on AST level is observed any silymarin supplementation either comparison with control or from baseline.

Non-metanalysis findings

Transferrin

Serum transferrin levels were measured in two studies involving silymarin [6] and quercetin [14] supplementations. Quercetin significantly increased transferrin levels compared to the control group (p=0.045).

Antioxidants

MDA

Seven studies [10,12,14,16,17,18,19] measured MDA levels using various supplements, including Silymarin, curcumin, green tea, quercetin, alpha-lipoic acid (ALA), Iranian oak (*Quercus brantii*), and GSE. Due to inconsistencies in the unit presentation of MDA, a meta-analysis was not performed. Mean serum MDA levels significantly decreased in all patients with β-thalassemia major (β-TM) after treatment with silymarin, curcumin, green tea, ALA, *Quercus brantii*, and GSE. However, daily supplementation with quercetin for three months, in conjunction with DFO treatment, resulted in an insignificant decrease in MDA levels.

TAC (Total antioxidant capacity)

Among the five studies[10,14,16,17,18] that measured total antioxidant capacity (TAC), supplementation with curcumin, green tea, and *Quercus brantii* significantly increased TAC levels. In contrast, quercetin and alpha-lipoic acid (ALA) did not have significant effects on TAC levels.

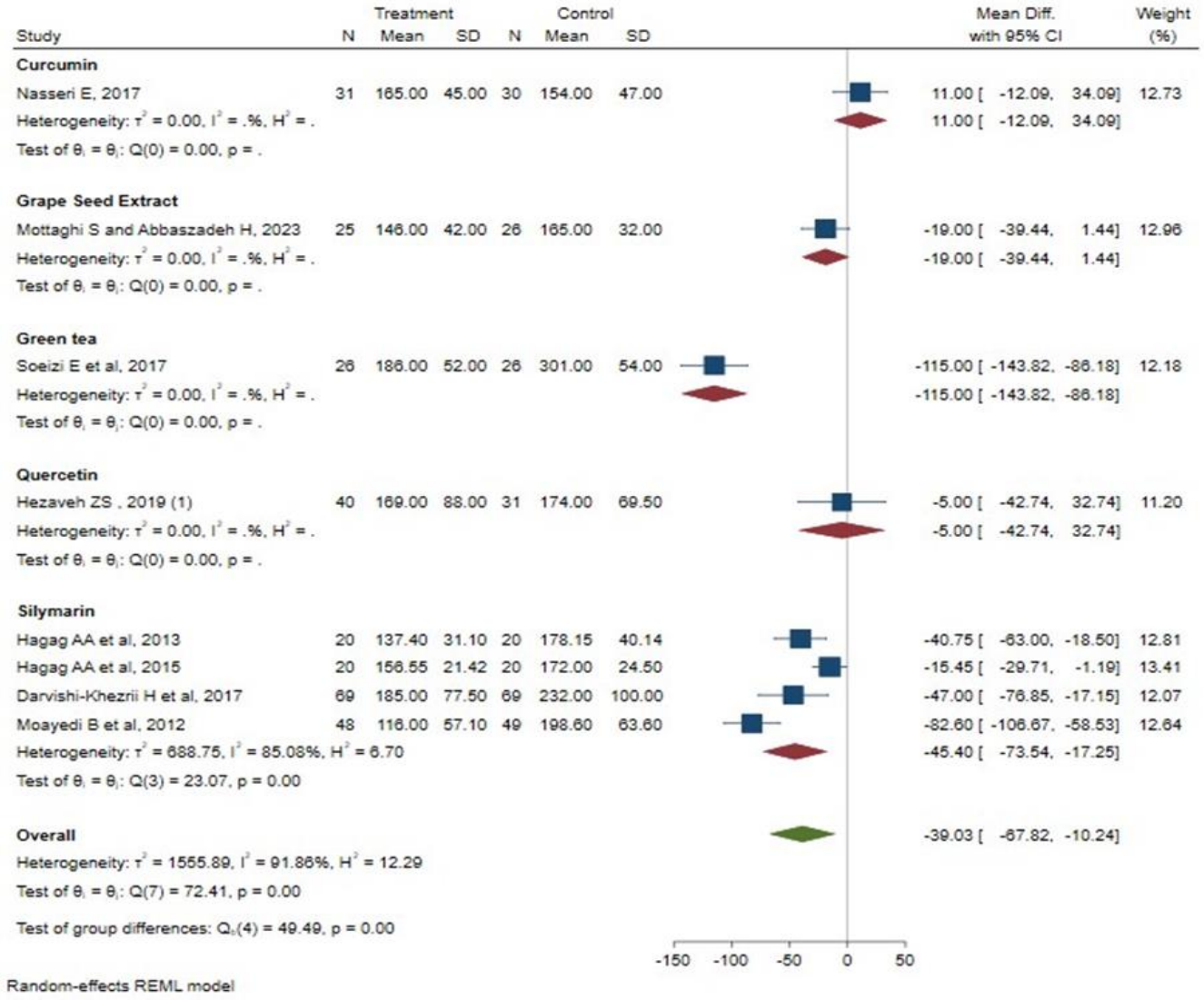

Figure 2. Forest plot on difference in serum Fe level between plant-based supplementations and placebo among thalassemic patients

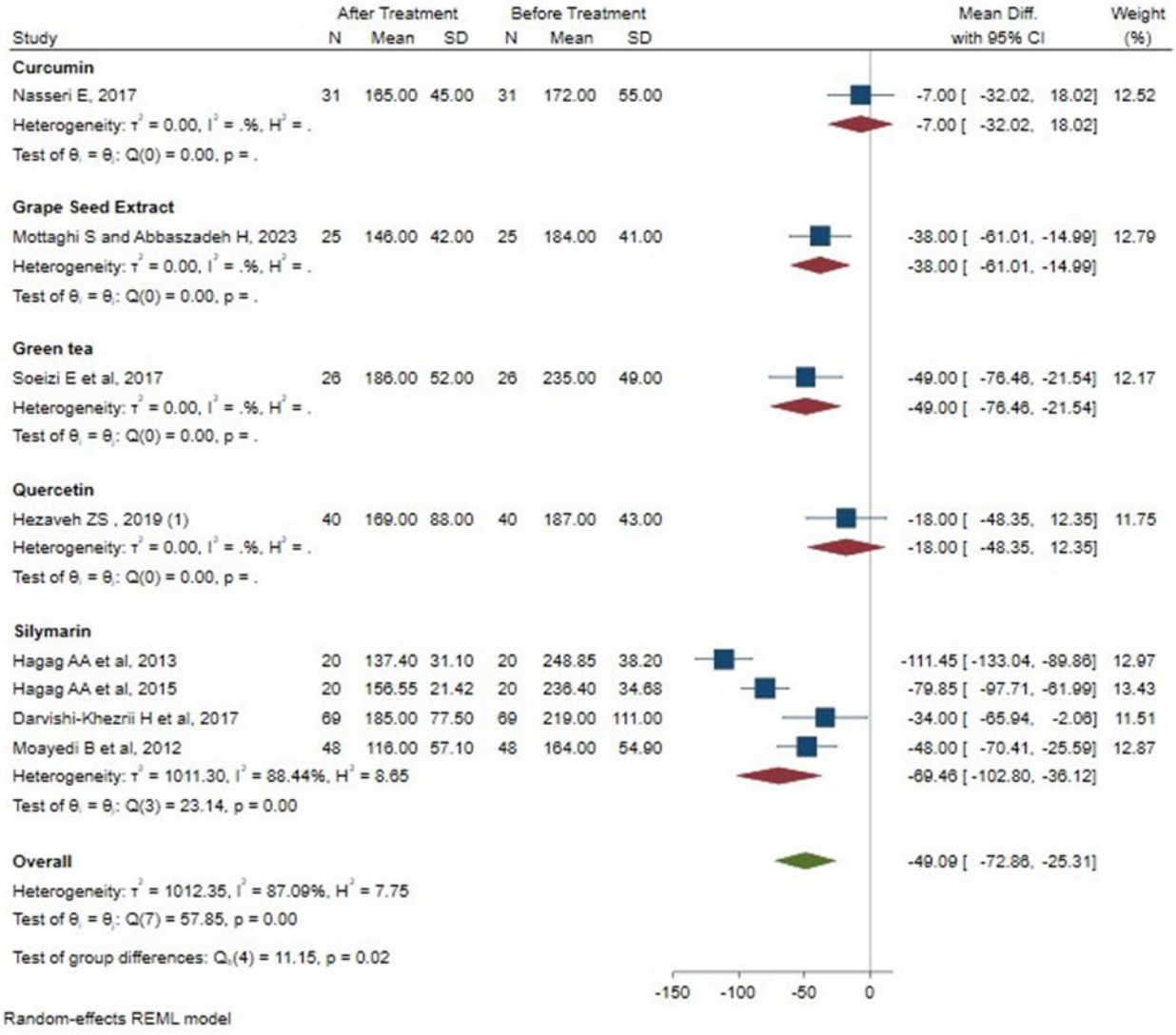

Figure 3. Forest plot on changes in serum Fe level by different plant-based supplementations from their baseline

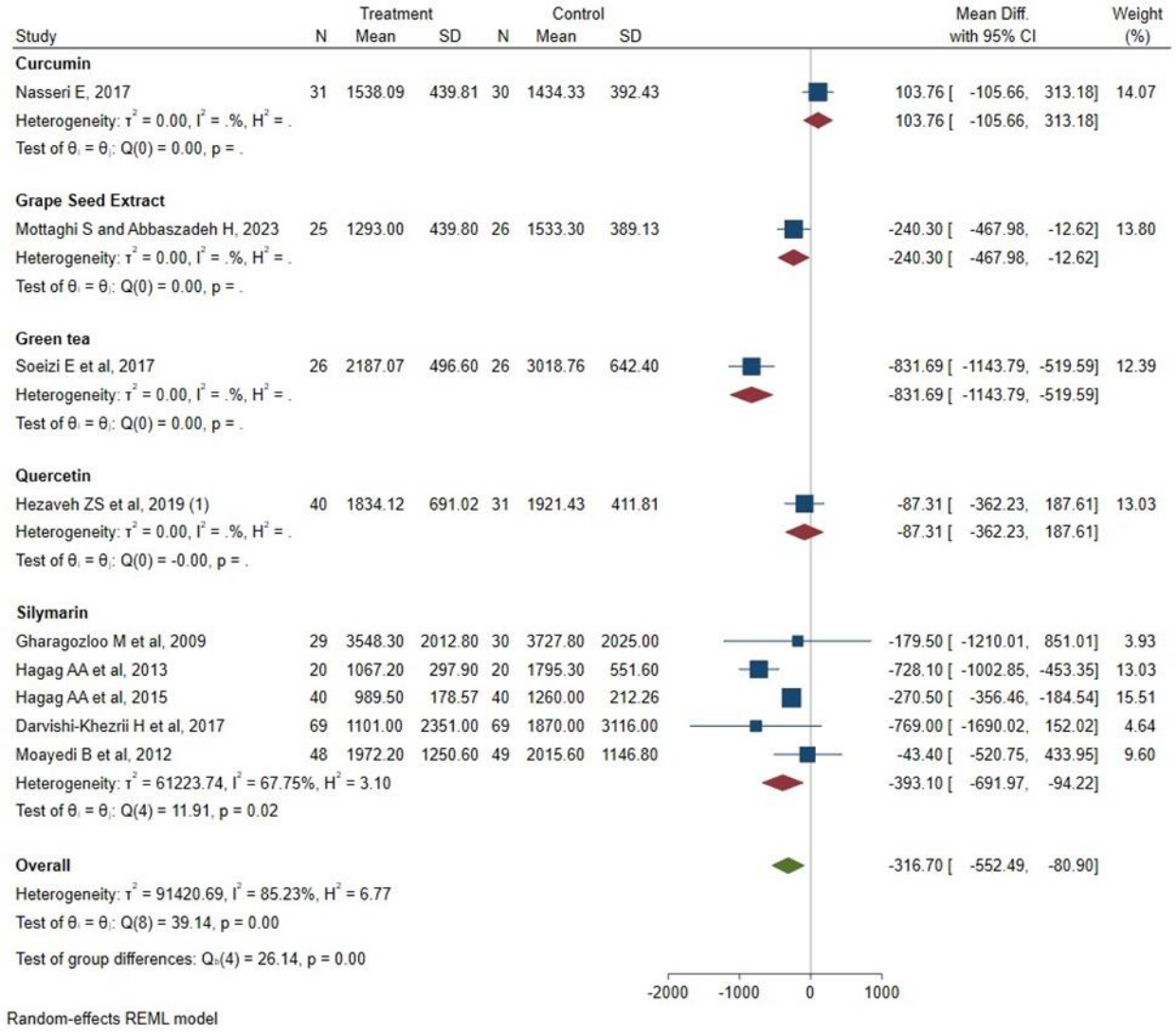

Figure 4. Forest plot on difference in serum ferritin level between plant-based supplementations and placebo among thalassemic patients

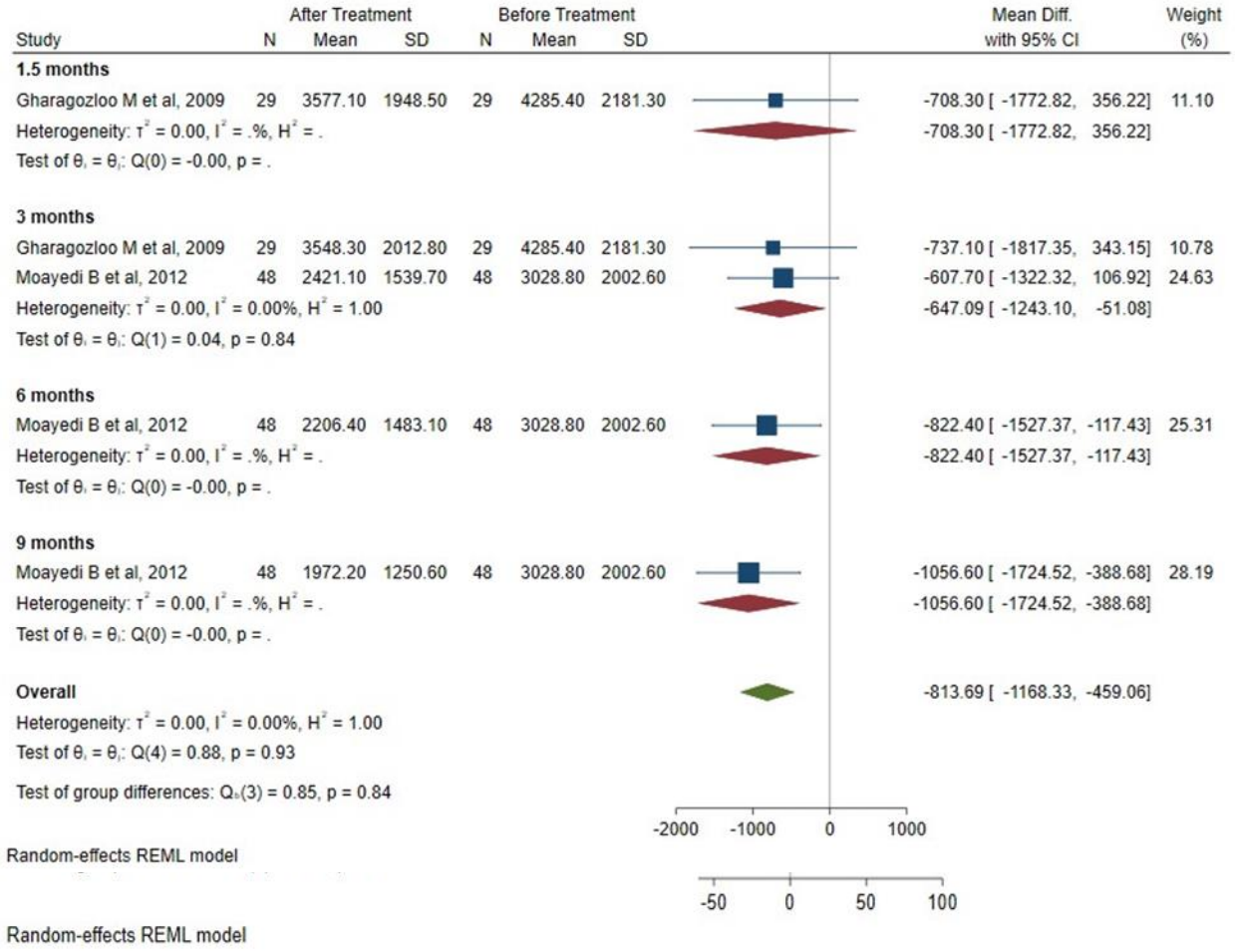

Figure 5. Forest plot on changes in serum ferritin level by Silymarin supplementations by different duration

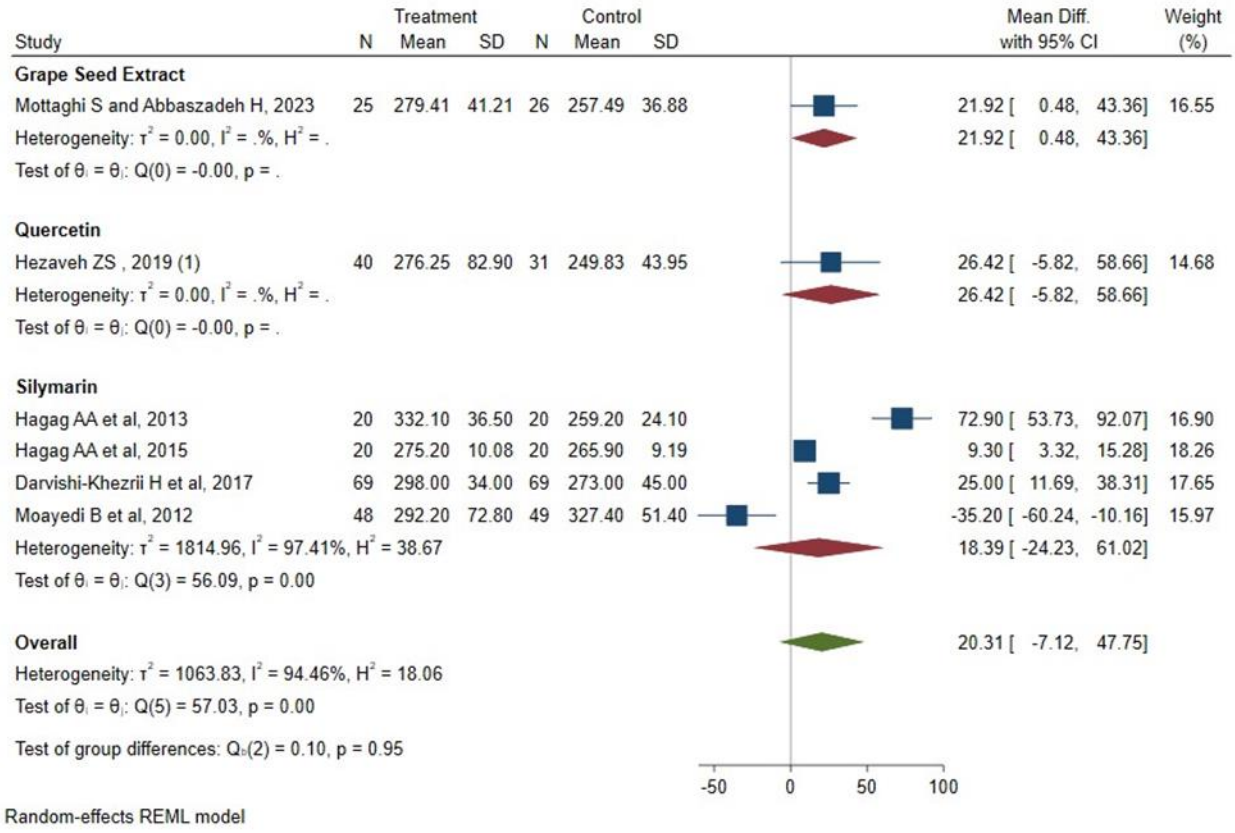

Figure 6. Forest plot on difference in TIBC level between different plant-based supplementations and placebo among thalassemic patients

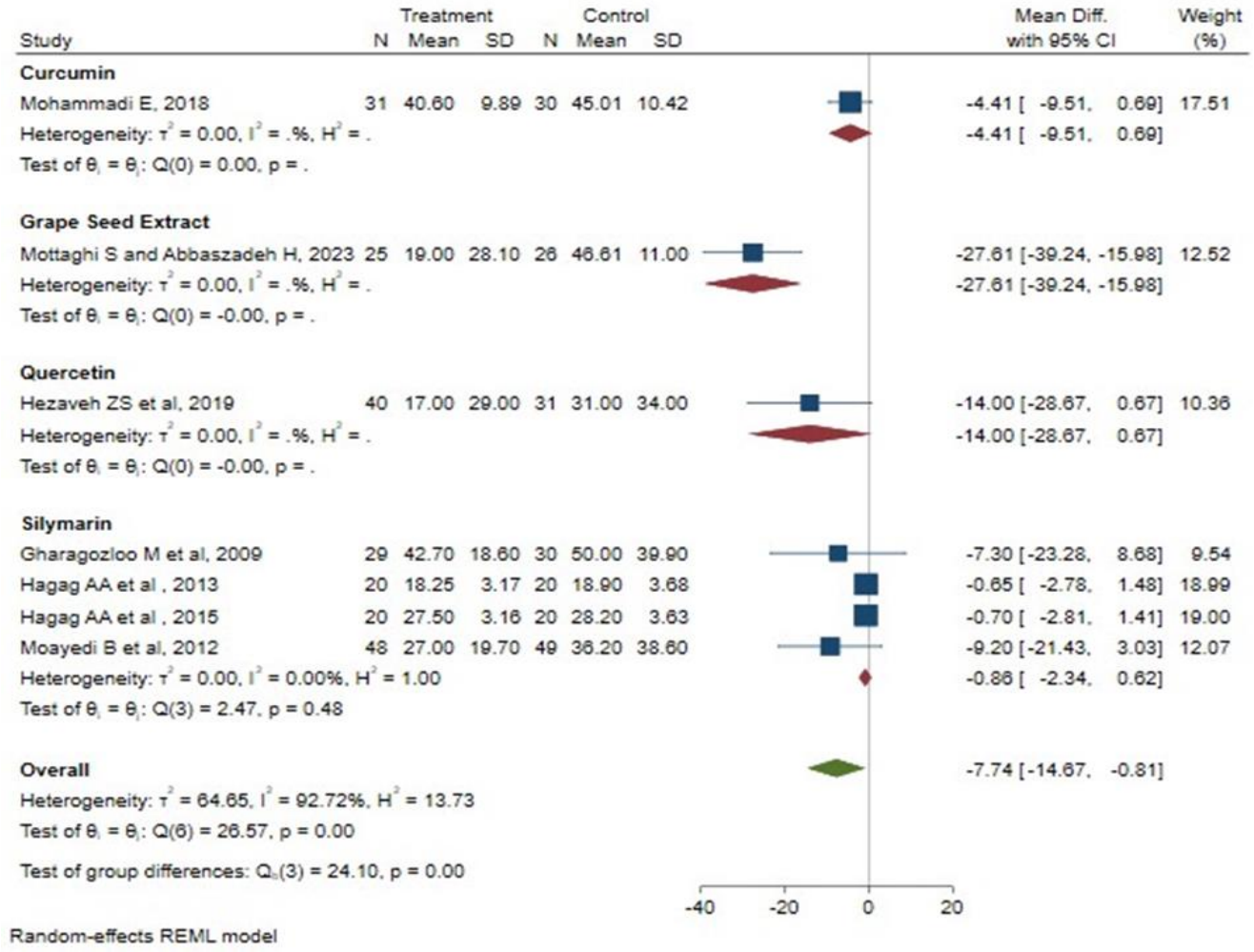

Figure 7. Forest plot on difference in ALT enzyme level between plant-based supplementations and placebo among thalassemic patients

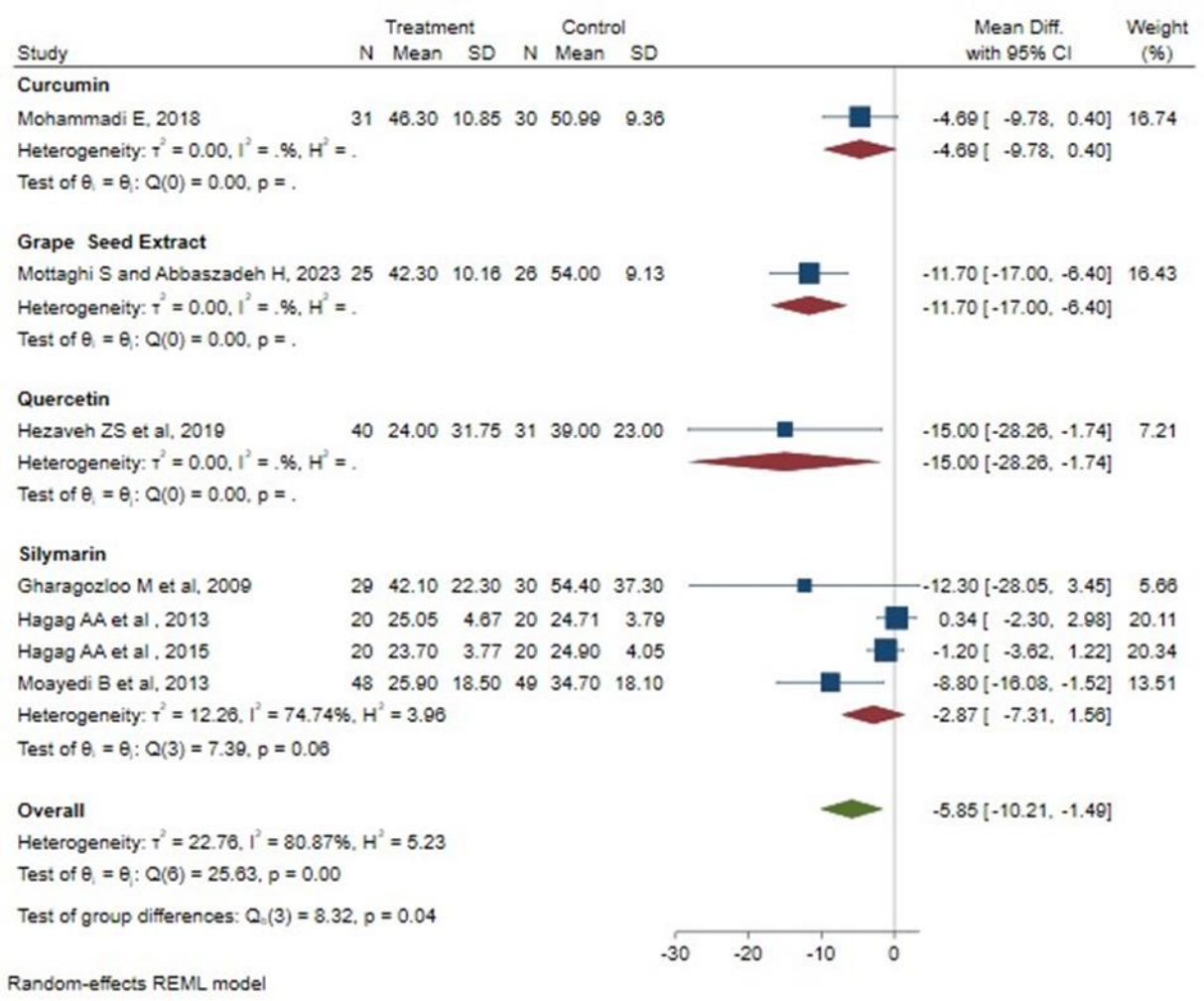

Figure 8. Forest plot on difference in AST enzyme level between plant-based supplementations and placebo among thalassemic patients

DISCUSSION

To the best of our knowledge, this study represents the first comprehensive meta-analysis to evaluate the clinical efficacy and safety of plant-based antioxidant supplements in managing transfusion-dependent thalassemia. The findings indicate that supplements such as Silymarin, Curcumin, Green Tea, Quercetin, Alpha lipoic acid (ALA), Iranian Oak (*Quercus brantii*) and Grape seed extract (GSE) significantly enhance antioxidant levels and serum iron profiles.

The pathophysiology of thalassemia is characterized by high oxidative stress and increased reactive oxygen species (ROS) production, with reduced glutathione levels observed in the RBCs in transfusion-dependent thalassemia (TDT) patients [20]. Consequently, the exploration of various molecules with antioxidant properties has emerged as a potential therapeutic strategy in β-thalassemia.

Silymarin, an herbal antioxidant, has been found to increase antioxidant levels and decrease oxidative stress [20]. It is effective in preventing cardiovascular diseases (CVDs) with fewer side effects compared to other antioxidants[21]. Silymarin can scavenge reactive oxygen species (ROS) and enhance antioxidant enzymes, making it more potent than vitamin E, with at least ten times greater effectiveness. Furthermore, silymarin improves membrane stability and aids in tissue regeneration[21].

Silymarin supplementation

In this study, we explored the protective properties of silymarin and its constituents in β-thalassemia major (β-TM) patients, assessing its potential for clinical application as an antioxidant therapy. Across all studies, the administered dose of silymarin was 140 mg, taken three times daily.

In Egypt, two clinical studies[7,8] were conducted on children with BTM who had not undergone any iron chelation therapy prior to the start of the study. The initial investigations revealed no noteworthy disparities in serum ferritin, iron, TIBC, serum creatinine, blood urea, ALT, AST, and bilirubin levels between the groups. However, after administering chelation therapy, there was a significant decrease in serum ferritin and iron levels, while TIBC levels increased in the group receiving silymarin as an addition. These findings indicate that the combined treatment of the iron chelator (Deferoxamine) with silymarin offers improved iron chelation and poses no known toxicity, suggesting its safe usage in children.

Among other three studies [6,9,10] which were done on adult patients and used silymarin as supplementation, all studies demonstrated a significant reduction in ferritin levels, which was statistically significant. Serum ferritin level reflects the efficacy of silymarin in removing excess iron. Most of the included studies showed that the combined therapy (silymarin plus desferrioxamine) was more effective than desferrioxamine alone in reducing serum iron burden.

These findings may support the hypothesis that silymarin is an iron chelator that can penetrate cells and take part in formation of a complex of lipophilic low-molecular-weight flavonoids that might penetrate cells and consequently chelate iron from cells[9]. This iron-mobilising shuttle effect and increase in accessible and chelatable iron pool increases the efficacy of standard iron chelators, leading to a decrease in the iron stores of vital organs including the liver, which was demonstrated by the reduction liver enzymes [10].

Clinical studies investigating the iron-chelating effect of silymarin in patients with β-thalassemia major (β-TM) have shown that its efficacy may depend on the duration of treatment[10,22]. From the metanalysis data, ferritin levels among the treated groups showed significant variation based on the duration of treatment. Longer duration of treatment resulted in the difference being statistically significant with the continuous reduction of ferritin levels at 3month, 6month and 9month. These findings imply that the duration of treatment with silymarin supplementation significantly influences the serum ferritin levels in the respective groups.

Iron acts as a catalyst for redox reactions, leading to the generation of ROS and oxidative stress (OS). Silymarin, used as an adjuvant therapy with iron chelators, shows promising effects on antioxidants and OS in β-thalassemia patients, interacting directly with ROS to eliminate free radicals[9]. Silymarin reduces serum oxidative stress and enhances antioxidant capacity in individuals with β-

thalassemia, providing potential additional benefits when used alongside iron chelators[9].

Silymarin administration reduces MDA and protein carbonyl levels in thalassemia patients, indicating its beneficial impact in mitigating oxidative stress[9]. Silymarin's cellular protection may restore GSH levels and serum oxidant status, despite its low bioavailability, a daily dose of 420 mg can still demonstrate antioxidant effects in these patients.

Silymarin promotes antioxidant defenses through various mechanisms, including free radical scavenging, inhibition of ROS-mediated lipid and protein peroxidation, and activation of protective molecules[23]. In vitro studies demonstrate silymarin's protective effect against oxidative stress caused by thiacetamide in rats[24]. Additionally, silymarin shows potential in protecting against doxorubicin-induced damage and increasing redox state and total GSH content in rats' liver and intestine. While data on gene expression effects is limited, some studies suggest silymarin may upregulate GSH-related genes and enhance endogenous antioxidant enzymes[25].

Curcumin

Curcumin (1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is a polyphenolic compound isolated from the Curcuma longa plant. Curcumin is the primary bioactive compound found in turmeric. Chemically, it is a bis-α, β-unsaturated, β-diketone that exhibits keto-enol tautomerism[26]. It is the most abundant curcuminoid found in turmeric[27]. Curcumin is reported to possess a wide range of biological activities such as anti-inflammatory[28]. Over the past two decades, extensive research has documented curcumin's ability to scavenge reactive oxygen species (ROS) such as O_2^-, OH^-, NO^- and $ONOO^-$ radicals [29] and its anti-inflammatory effects through inhibition of pro-inflammatory cytokine and transcription factor production [29].

Effect of Curcumin on total iron and ferritin levels in the body

Curcumin is a promising natural bioactive compound with the ability to bind to iron[30], exhibiting affinity for iron similar to that of clinically used iron chelators[31]. Although not all iron in the body is extractable by curcumin, studies suggest that a safe, well-tolerated dose can chelate a significant fraction of body iron. In support of this idea, mice fed a diet high in curcuminoids showed decreased levels of liver ferritin, indicating a reduced iron burden[32]. In another study, liver ferritin was decreased in mice after a 12-week dietary supplementation with 1.5% curcumin but not in the group fed 0.4% curcumin[33]. However, evidence on the effects of curcumin in patients with β-thalassemia major is limited, with very few numbers of human randomized clinical trials have been conducted in this topic.

Results from the study by Nasseri et al.[12] revealed that 12 weeks of curcumin treatment did not significantly reduce Hb, serum iron, and ferritin levels. These findings contrast with previous animal research by[35]. However, they align with the results from[36] who reported that daily intake of 500 mg curcuminoids for 12 months did not alter Hb and serum ferritin levels in 10 β-thalassemia patients. The discrepancies among these studies may be due to differences in the subjects' characteristics, including normal healthy mice[35], subclinical iron-deficient subjects[34], and β-thalassemia major patients[36]. Additionally, variations in curcumin dose, intervention duration, and study methodology could influence the outcomes. In Nasseri's study, the mean baseline ferritin level was not very high, indicating proper chelation before starting curcumin treatment, which could explain the notable but non-significant decrease in ferritin levels at the trial's end. More research is needed to fully elucidate curcumin's effects on total body iron stores as measured by serum ferritin in beta-thalassemia patients. Available evidence suggests that curcumin may help reduce free iron and liver damage, but its impact on overall iron overload remains unclear [36].

Effects of Curcumin supplementation on serum MDA

Curcumin is well-known for its potent non-enzymatic antioxidant, anti-inflammatory and anticancer properties. MDA is a frequently used biomarker for oxidative stress, with higher level serum levels indicating increased oxidative stress in the body [12]. In Nasseri's study, curcumin supplementation reduced MDA levels in patients with β-thalassemia major. A double-blind randomized controlled trial found that curcumin in combination with deferoxamine,

improved antioxidant status by lowering MDA levels in these patients. Both Curcumin[12,13] and curcuminoid[37] supplementation have been shown to be effective in reducing MDA levels and improving antioxidant status in patients with various forms of β-thalassemia, potentially through their iron-chelating and antioxidant properties.

Green Tea

Effects of Green Tea on serum iron and ferritin levels

The health benefits of green tea are primarily due to its polyphenol, mainly catechins, which exhibit antioxidant effects by scavenging reactive oxygen species (ROS) and chelating metal ions[38]. In a study involving transfusion-dependent thalassemia (TDT) patients, green tea was used alongside deferoxamine as an antioxidant supplement. After eight weeks, serum iron and ferritin levels in the intervention group significantly decreased by 21.15% and 20.36%, respectively[14]. Similarly, Al-Momen et al., showed that liver iron concentration and serum ferritin level were significantly reduced in green tea intervention group after 12 months, although there was no significant difference in packed blood cell transfusion between control and intervention groups [39]. These findings suggested that the potential effects of green tea on iron chelating effects in transfusion-dependent thalassemia patients.

Effects of Green Tea on antioxidant parameters (MDA, TAC)

Green tea is a popular nutraceutical antioxidant, with its catechins enhancing antioxidant activity by increasing serum superoxide dismutase and catalase expression[40]. Malondialdehyde (MDA), a marker of oxidative stress, is generated from the ROS-catalyzed degradation of membrane phospholipids[40]. Soeizi et al. demonstrated that green tea consumption reduced serum MDA levels by 35.26%, compared to a 2.33% decrease in the control group, though no significant changes were found in serum TAC levels[14]. Koonyosying et al. reported that green tea, combined with the iron chelator DFP, reduced tissue MDA levels in iron-loaded BKO mice and decreased ROS levels in a dose-dependent manner in RINm5F insulinoma cells[41]. These findings suggest that green tea supplementation can benefit iron-overload patients by improving antioxidant parameters.

Quercetin

Quercetin, a flavone family member found in apples, onions, tea, red wines, and berries[42], has a catechol moiety that likely serves as its iron chelation site. Its high binding energy values indicate that quercetin is a potent chelating agent, capable of sequestering iron and preventing its involvement in oxidation reactions[43]. Animal studies have shown that quercetin can reduce hepatic iron overload, decrease serum ferritin levels, increase fecal iron excretion, limit intestinal iron absorption, and reduce basolateral iron efflux into the bloodstream[44,45].

Hezaveh et al.[15] demonstrated that quercetin is a valuable supplement for transfusion-dependent thalassemia major patients. A 12-week combined treatment of DFO and 500 mg quercetin significantly improved hs-CRP, serum iron, ferritin, TS, and transferrin levels compared to placebo, though it did not significantly affect TIBC and TNF-α. Another study by Hezaweh et al[16] investigated quercetin's effects on oxidative stress and hepatic function in beta-thalassemia major patients receiving DFO, finding no reduction in oxidative stress. These findings should be validated with larger sample sizes, extended follow-up periods, and varying quercetin doses.

Alpha lipoic acid (ALA)

Alpha-lipoic acid (ALA), also known as thioctic acid, is a water- and fat-soluble antioxidant that serves as a cofactor for several specific mitochondrial enzymes[46]. ALA is synthesized endogenously in both animals and plants. Once absorbed into cells and tissues, ALA is reduced to dihydrolipoic acid (DHLA)[46]. Both ALA and DHLA possess metal-chelating properties, leading to a reduction in the production of reactive oxygen species (ROS)[47].

Previous animal model study[48] has reported that alpha-lipoic acid (ALA) supplementation reduces malondialdehyde (MDA) production and serum iron levels. In addition to its role as a reactive oxygen species (ROS) scavenger, ALA regulates inflammatory signaling pathways. ALA is also effective in

maintaining cellular antioxidant status by stimulating the uptake or increasing the synthesis of endogenous antioxidants and enzymes with antioxidant activity[49,50].

In a crossover randomized controlled clinical trial conducted by Sharifi-Z et al.,[17] the effects of 600 mg/day alpha-lipoic acid (ALA) supplementation on iron levels, lipid profile, and markers of oxidative stress were evaluated in β-thalassemia major (β-TM) patients. The study found that ALA consumption significantly reduced serum ferritin, malondialdehyde (MDA), and the MDA/LDL-C ratio, while increasing HDL-C levels. However, when compared with placebo, only the reduction in serum ferritin was statistically significant. Meta-analysis could not include the effect of ALA on serum ferritin due to differences in the units of measurement (ng/L).

Irania Oak (Quercus brantii)

In recent years, significant research attention has focused on exploring the pharmaceutical potential of herbal flavonoids for their protective properties[51]. These secondary metabolites of plants typically feature a 15-carbon skeleton comprising two phenyl rings. Among the notable genera, Quercus species stand out for their rich polyphenolic content and widespread traditional uses globally. These species have been employed traditionally as antifungal, antidiarrheal, and astringent agents, addressing conditions such as hemorrhoids, tonsillitis, and inflammation of mucosal surfaces[52].

The diverse traditional uses and complex phytochemical compositions of Quercus species make them compelling subjects for biological and toxicological investigations[51]. Antioxidant activity is a pivotal biological attribute of these natural compounds, crucial for enhancing food quality, stability, and serving as nutraceuticals to counteract free radical chain reactions in biological systems[51,52]. Many of these antioxidant's function as scavengers[53]of free radicals and active oxygen species, thereby underscoring the biological significance of plants and their extracts rich in antioxidants[54,55,56].

Flavonoids, the largest subgroup of herbal polyphenols, include tannins and play a critical role in these antioxidant properties. The genus Quercus, belonging to the Fagaceae family and comprising around 500 species, includes prominent varieties like *Quercus brantii* found predominantly in central and northern Iran[57]. The fruit of the oak tree, known as an acorn, is enclosed in a cup-like structure called a gland and contains substantial amounts of vitamins, nutrients, and carbohydrates, making it a notable nutritional component.

In clinical studies[18], patients received a combined regimen of desferrioxamine and 140 mg of Quercus extract orally, three times daily before meals, with good tolerance and no reported side effects. Quercus extracts have shown significant efficacy in inhibiting free radicals, enhancing the body's antioxidant reserves, and reducing markers of tissue oxidative damage associated with iron overload in thalassemia patients. When used adjunctively with standard iron chelators, Quercus extracts may improve oxidative stress measurements in these patients, offering additional therapeutic benefits.

Furthermore, aqueous oak extracts have demonstrated potential as liver protectors and antidiabetic agents due to their beneficial effects on liver parameters and glucose levels[54]. However, further well-designed studies are needed to confirm the antioxidant effects of Quercus extracts, particularly through advanced biochemical analyses of oxidative stress markers such as reactive oxygen species (ROS) in fresh samples using techniques like flow cytometry.

Grape seed extract (GSE)

In the last few years, one of the phytochemicals, which has been extensively evaluated for its pharmacological effects, is grape seed extract (GSE)[58]. GSE, as a flavonoid-rich supplement, contains several important polyphenolic compounds such as proanthocyanidins, catechin, epicatechin, and gallic acid[59,60].

Nowadays, GSE is used in the pharmaceutical, cosmetic, and food industries owing to its health beneficial effects[58]. Growing evidence indicates that proanthocyanidins, as the major components of GSE,

can remarkably attenuate free radicals concentration and inflammatory mediators by modulating several molecular targets and signaling cascades. These natural compounds have the potential to chelate metals with their o-diphenol groups[61]. Studies have revealed that proanthocyanidins possess marked iron chelating activities in vitro[62]. Proanthocyanidins also have protective influences against iron overload mediated-oxidative damage in vivo[63,64].

In Mottaghi S and Abbaszadeh H's study[19], the effects of GSE supplement on iron overload, oxidative stress, inflammation, liver function, and Hb concentration as well as its safety had been evaluated for the first time in children with β-TM through a randomized, double-blind, placebo-controlled clinical trial. The results showed the beneficial effects of GSE supplement on iron status, oxidative stress, inflammatory markers, and liver function.

Co-administration of GSE with deferasirox was found to be more effective in lowering serum iron and ferritin levels and increasing total iron-binding capacity (TIBC) compared to deferasirox alone in β-TM children[65]. These findings underscore the iron-binding potential of GSE in clinical settings, supported by its high flavonoid content, especially proanthocyanidins[65], which exhibit strong iron chelating properties as demonstrated in vitro[62] and in clinical studies[13,19].

Overall, Mottaghi S and Abbaszadeh H's study[19] demonstrates that GSE supplementation offers multiple health benefits for pediatric patients with β-TM, attributed to its iron chelating, antioxidant, anti-inflammatory, and hepatoprotective properties. The findings suggested that GSE can serve as a promising adjunctive therapy with deferasirox for alleviating iron overload, oxidative stress, inflammatory conditions, and liver dysfunction in this patient population. Further research is warranted to explore the full therapeutic potential and optimal dosage of GSE in managing β-TM and related conditions.

CONCLUSION

In summary, the examination of 14 studies revealed significant findings regarding the impact of various supplements on total iron (Fe) and ferritin levels. Silymarin, green tea and quercetin effectively reduced total iron and ferritin levels, suggesting potential inhibitory effects on iron absorption or utilization. Moreover, silymarin consistently decreased ferritin levels across all studies using it as a supplementation, with greater reductions observed over longer treatment durations (3, 6, and 9 months). Grape seed extract (GSE) also demonstrated significant reductions in both serum iron and ferritin levels compared to controls. Conversely, curcumin supplementation did not result in significant changes in iron and ferritin levels between the intervention and control groups, indicating limited impact in βTM patients. These results emphasize the necessity for additional research to explore the underlying mechanisms and potential consequences of these supplementation effects on iron metabolism.

Table 1. Summary of study characteristics

Sr. No. Year Country	Type of study (Author)	Age group	Combined Tx (N)	Control N	Desferioxamine dose	Supplement Dose/day	Duration	Findings (Iron status markers)	Other Findings (Anti-oxidant markers)	Conclusion
1 2009 Iran	Randomized double-blinded clinical trial **(Gharagozloo et al)[6]**	(20.2±6.2) (17.9±3.7)	29 (D+S)	30 (D+P)	40-50m/kg over 8-12 hr 5-6 days/wk	Silymarin 140mg 3 times	5±1 wk	A significant decrease in serum ferritin levels in baseline, end of 1.5 & 3 months of silymarin therapy.	Significant improvement in glutathione levels of red blood cells and liver ALP enzyme.	No significant difference was detected between silymarin and placebo groups after 1.5- and 3-months treatment.
2 2013 Egypt	Simple randomised-clinical trial **(Hagag AA et al)[7]**	(5.17±1.8) (5.10±1.16)	20 (D+S)	20 (D+P)	20-40mg/ kg/day	Silymarin 140mg 3 times	6 mth	Serum ferritin and TIBC levels were markedly decreased in silymarin group compared with placebo (P= 0.001).	No statistically significant differences in serum creatinine, blood urea, ALT, AST and bilirubin levels between groups.	Silymarin in combination with Exjade can be safely used in the treatment of iron-loaded thalassaemic patients, with no sign of toxicity.
3 2015 Egypt	Simple randomised-clinical trial **(Hagag AA et al)[8]**	(6.10±1.61) (6.68±1.64)	40 (D+S)	40 (D+P)	75mg/kg/day	Silymarin 140mg 3 times	9 mth	Serum ferritin and iron were significantly lower in silymarin group than placebo.	No statistically significant differences in serum creatinine, blood urea, ALT, AST and bilirubin levels between silymarin and placebo groups before and after chelation therapy.	Deferiprone in combination with silymarin are better iron chelators than Deferiprone and placebo.
4 2017 Iran	Randomised, triple-blind, placebo-controlled, cross over trial**(Darvishi-Khezri H et al-a)[9]**	AB (20.4±6.82) BA (27.7±8.09)	AB sequence (37) [(D+S)-W-(D+P)] BA sequence (32) [(D+P)-W-(D+S)]		-	Silymarin 420mg	12 wk each (cross over) 2 wk (wash out)	Silymarin treatment resulted in a negative change in the serum iron and ferritin levels and a positive change in the TIBC levels.	Positive changes in cardiac and liver function in both treatment sequences of study (not statistically significant). There was a negative change in liver iron concentration in both treatment sequences.	Combined iron-chelation and silymarin therapy was effective for improving the iron-burden status in patients with β-thalassemia major.

5 2017 Iran	Randomised, triple-blind, placebo-controlled, cross over trial**(Darvishi-Khezri H et al-b)**[10]	AB (20.4±6.82) BA (27.7±8.09)	AB sequence (37) [(D+S)-W-(D+P)] BA sequence (32) [(D+P)-W-(D+S)]		-	Silymarin 420mg	12 wk each (cross over) 2 wk (wash out)	Mean serum MDA and protein CO significantly decreased in all patients with β-TM after three months of treatment with silymarin.	Serum TAC and plasma GSH were also significantly elevated after therapy with silymarin.	Silymarin was effective in decreasing serum OS and enhancing serum antioxidant capability in patients with β-thalassemia major
6 2012 Iran	Randomized double-blinded clinical trial **(Moayedi B et al)**[11]	(21.6±5.1) (19.4 ±5.8)	62 (D+S)	57 (D+P)	35-40 mg/kg/day	Silymarin 420mg	9 mth	Serum ferritin, serum iron, TIBC, hepcidin and soluble transferrin receptor levels were significantly decreased at the end of treatment in silymarin group compared with placebo.	A significant improvement in liver function test was observed in silymarin group in comparison with placebo.	Silymarin is effective at reducing iron overload in patients when used in conjunction with desferrioxamine.
7 2017 Iran	Double blinded randomized controlled clinical trial **(Nasseri E et al)**[12]	(25.97±6.92) (27.61± 6.23)	31 (D+C)	30 (D+P)	40-50 mg/kg bw at least 4day/wk	Curcumin 500mg x 2 capsules	12 wk	Serum MDA, total and direct bilirubin significantly decreased, and TAC significantly increased in the curcumin group.	Changes in Hb, serum iron, ferritin, catalase, and vitamin E were not significant in any of the two groups.	Curcumin supplementation in combination with deferoxamin improved the antioxidant status in β-thalassemia major patients.
8 2018 Iran	Double-blind randomized controlled clinical trial **(Mohammadi E. et al)** [13]	(25.97±6.92) (27.61± 6.23)	31 (D+C)	30 (D+P)	40-50 mg/kg bw at least 4day/wk	Curcumin 500mg x 2 capsules	12 wk	NTBI, ALT and AST levels were significantly reduced in curcumin group.	No significant changes in hepcidin and other variables in any of the 2 groups.	Curcumin administration alleviated iron burden and liver dysfunction by reducing NTBI, ALT, and AST levels in patients with β-thalassemia major.
9 2017 Iran	Single-blinded Randomized controlled clinical trial (Soeizi E et al)[14]	(23.15±3.33) (24.32±3.15)	26 (D+G)	26 (D+W)	20-40 mg/kg	Green tea (2.5 g /150 mL hot water) 3 cups	8 wk	Green tea significantly decreased serum levels of iron, ferritin and MDA and increased TAC compared with control group (all, $P < 0.05$).	No significant changes were seen in transferrin saturation value in both groups.	Green tea consumption had favourable effects on iron status and oxidative stress in studied subjects and may be useful in management of β–thalassemia major patients.
10 2019 Iran	Double blinded randomized clinical trial (Hezaveh ZS et al-a)[15]	(27.88±4.73) (27.71±5.49)	40 (D+Q)	31 (D+P)	Ampoules/wk (4) (10)	Quercetin 500mg	12 wk	Quercetin could reduce hs-CRP, iron, ferritin , and transferrin saturation (TS) and increase transferrin significantly when compared to the control group,	Quercetin had no significant effect on TIBC (p=0.734) and TNF-α (p=0.310).	Quercetin could ameliorate the iron status in thalassemia major, but its effect on inflammation is indistinctive.

11 2019 Iran	Double blinded randomized clinical trial **(Hezaveh ZS et al-b)[16]**	(27.88±4.73) (27.71±5.49)		42 (D+Q)	42 (D+P)	Ampoules/wk (4) (10)	Quercetin 500mg	12 wk	Quercetin could reduce ALT and TAC significantly.	There was an insignificant increase in SOD and TAC, and insignificant decrease in GPx, MDA, AST, and ALP.	Consumption of quercetin supplement daily for 3 months along with DFO treatment might be able to alter liver function, but not the oxidative stress in beta-thalassemia major patients.
12 2021 Iran	Cross-over randomized controlled clinical trial **(Sharifi-Zahabi E et al)[17]**	23.8±5.3		22 Sequence 1 [(D+A)-W-(D+P)] **OR** Sequence 2 [(D+P)-W-(D+A)]		-	Alpha lipoic Acid (ALA) 600 mg	8 wk (21 days wash out)	Serum ferritin, MDA and MDA/LDL-C ratio were decreased, and HDL-C increased significantly during ALA consumption.	ALA had no significant effects on the other biomarkers.	Supplementation with 600 mg/d ALA may decrease serum ferritin in β-TM.
13 2024 Iran	Double blinded randomized clinical trial **(Satehi MB et al)[18]**	(18.8 ± 4.9) (20.4 ± 6.8)	25 (D+QU)	19 (D+P)	-	Iranian oak (*Quercus brantii*) extract 300 mg		12 wk	Serum MDA, and protein CO, significantly decreased in the intervention group.	SOD enzyme and TAC significantly increased in comparison with the control group.	Oak given as an adjuvant therapy to standard iron chelators may provide an improvement in the OS measurements.
14 2023	Double blinded, randomized clinical trial **(Mottaghi S and Abbaszadeh H)[19]**	(16.1 ± 8.80) (16.3 ± 5.40)	25 (D+GSE)	26 (D+P)	deferasirox (28 mg/kg/day)	Grape Seed Extract (GSE) 100 mg		4 wk	Serum iron, ferritin, ALT, AST, TNF-α, and hs-CRP. TIBC level, MDA and GSH are significantly improved in the GSE group compared with the placebo group.	The changes in the SOD activity and Hb concentration were not statistically different between the groups.	GSE supplement possesses several health beneficial influences on children with β-TM by alleviating iron burden, oxidative stress, inflammation, and liver dysfunction.

Supplementary Table 1. Summary of the quality of the studies assessed by Joanna Briggs Institute (JBI)'s critical appraisal tools

Sr. No.	Authors	1. Was true randomization used for assignment of participants to treatment groups?	2. Was allocation to treatment groups concealed?	3. Were treatment groups similar at the baseline?	4. Were participants blind to treatment assignment?	5. Were those delivering the treatment blind to treatment assignment?	6. Were treatment groups treated identically other than the intervention of interest?	7. Were outcome assessors blind to treatment assignment?	8. Were outcomes measured in the same way for treatment groups?	9. Were outcomes measured in a reliable way	10. Was follow up complete and if not, were differences between groups in terms of their follow	11. Were participants analysed in the groups to which they were randomized?	12. Was appropriate statistical analysis used?	13. Was the trial design appropriate and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?
1	Gharagozloo, 2009[6]	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	Hagag,2013[7]	Yes	Unclear	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
3	Hagag, 2015[8]	Yes	Unclear	Yes	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
4	Darvishi-Khezri, 2017-a[9]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5	Darvishi-Khezri, 2017-b[10]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
6	Moayedi, 2012[11]	Yes	Unclear	No	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
7	Nasseri, 2017[12]	Yes	Yes	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	Yes
8	Mohammadi, 2018[13]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9	Soeizi, 2017[14]	Yes	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
10	Hezaveh, 2019-a[15]	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
11	Hezaveh, 2019-b[16]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
12	Sharifi-Zahabi, 2021[17]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
13	Satehi, 2024[18]	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
14	Mottaghi S and Abbaszadeh H, 2023[19]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

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