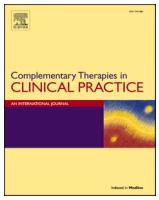
Complementary Therapies in Clinical Practice 53 (2023) 101804

Contents lists available at ScienceDirect

Complementary Therapies in Clinical Practice

journal homepage: www.elsevier.com/locate/ctcp



Grape seed extract in combination with deferasirox ameliorates iron overload, oxidative stress, inflammation, and liver dysfunction in beta thalassemia children

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ARTICLE INFO

Keywords:
Beta thalassemia
Grape seed extract
Inflammation
Iron overload
Liver dysfunction
Oxidative stress

ABSTRACT

Background and purpose: Iron overload in the body is associated with serious and irreversible tissue damage. This study aimed to investigate the iron-chelating, antioxidant, anti-inflammatory, and hepatoprotective activities of grape seed extract (GSE) supplement as well as its safety in β-thalassemia major (β-TM) pediatric patients receiving deferasirox as a standard iron-chelation therapy.

Materials and methods: The children were randomly allocated to either GSE group (n = 30) or control group (n = 30) to receive GSE (100 mg/day) or placebo capsules, respectively, for 4 weeks. The serum levels of iron, ferritin, total iron-binding capacity (TIBC), alanine transaminase (ALT), aspartate aminotransferase (AST), tumor necrosis factor alpha (TNF-α), high-sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), and glutathione (GSH) as well as superoxide dismutase (SOD) activity and hemoglobin (Hb) concentration were measured pre-and post-intervention.

Results: GSE supplement significantly attenuated the serum levels of iron ($p = 0.030$), ferritin ($p = 0.017$), ALT ($p = 0.000$), AST ($p = 0.000$), *TNF-α ($p = 0.000$)*, and *hs-CRP ($p = 0.001$)*. The TIBC level ($p = 0.020$) significantly enhanced in the GSE group compared with the placebo group. Moreover, GSE supplement remarkably improved the oxidative stress markers, MDA ($p = 0.000$) and GSH ($p = 0.001$). The changes in the SOD activity ($p = 0.590$) and Hb concentration ($p = 0.670$) were not statistically different between the groups.

Conclusion: GSE supplement possesses several health beneficial influences on children with β-TM by alleviating iron burden, oxidative stress, inflammation, and liver dysfunction.

1. Introduction

β-Thalassemia major (β-TM) is a common hereditary hemolytic anemia, which is characterized by a defect in the synthesis of beta-globin chains of hemoglobin [1]. A high prevalence of β-thalassemia has been reported in certain areas of the world such as the Mediterranean, Middle-East, and Southeast Asia [2]. It is well known that the regular and frequent blood transfusion, as the main treatment for β-TM, results in the excess levels of iron in the body [3]. In non-transfusion-dependent thalassemia, iron overload can develop owing to ineffective erythropoiesis, which leads to the enhanced intestinal iron absorption by decreasing the serum levels of iron regulatory hormone, hepcidin [4]. Excess iron can be deposited in tissues and organs, leading to the oxidative damage and inflammation [5]. Moreover, iron overload can induce ferroptosis, a type of iron regulated cell death, which plays an important role in the pathogenesis of a variety of diseases [6,7]. Iron accumulation in the body is associated with serious and irreversible damage to various organs, including liver, pancreas, heart, and gonads, which can cause cirrhosis, diabetes, cardiac impairment, and hypogonadism as well as increased mortality [8].

Iron chelation therapy, as a standard clinical strategy, is used to attenuate the excessive levels of iron and prevent the toxic effects related to the iron deposition in tissues [9]. The standard iron chelators such as deferoxamine, deferasirox, and deferiprone have *serious adverse reactions*, which limit their long-term use in clinical practice [10,11]. Therefore, it is necessary to explore and develop safer and more effective iron chelating agents as alternative or adjuvant for treatment of β-TM. In recent years, the discovery and development of novel iron chelating

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https://doi.org/10.1016/j.ctcp.2023.101804
Received 13 August 2022; Received in revised form 28 September 2023; Accepted 1 October 2023
Available online 11 October 2023

agents with high efficacy and low toxicity have been the focus of much research [11,12]. In this sense, polyphenols as natural phytochemicals [13], have drawn much scientific attention due to their significant iron chelating activities with minimal toxicity against healthy tissues [14]. Polyphenols can be categorized into several major classes such as flavonoids, phenolic acids, stilbenes, and lignans [15,16]. Flavonoids are one of the most important polyphenolic compounds [17], which have specific chemical structure with iron chelating affinity [18]. These natural compounds contain chelation sites that can bind iron to form a stable iron-flavonoid complex [18]. Several mechanisms are linked to the role of flavonoids in the treatment of iron overload [19]. Flavonoids can ameliorate iron burden in a direct or indirect way. These natural polyphenols decrease the iron accumulation by iron binding effect, which is a direct way for the attenuation of iron overload. *Indirectly*, flavonoids reduce the iron burden by modulating a variety of proteins and signaling pathways [19]. Flavonoids also have significant antioxidant and anti-inflammatory effects, which can diminish the iron overload-induced oxidative damage and inflammation [20,21].

In the last few years, one of the phytochemicals, which has been extensively evaluated for its pharmacological effects, is grape seed extract (GSE) [22]. GSE, as a flavonoid-rich supplement, contains several important polyphenolic compounds such as proanthocyanidins, catechin, epicatechin, and gallic acid [23,24]. Numerous experimental and clinical studies demonstrate that GSE has various biological and pharmacological effects, including antioxidant [25,26], anti-inflammatory [27,28], antibacterial [29], cardioprotective [22], hepatoprotective [30], anticancer [31,32], anticarcinogenic [33], antiviral [34], and neuroprotective effects [35]. Nowadays, GSE is used in the pharmaceutical, cosmetic, and food industries owing to its health beneficial effects [22]. 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 [36]. Studies have revealed that proanthocyanidins possess marked iron chelating activities in vitro [37]. Proanthocyanidins also have protective influences against iron overload mediated-oxidative damage in vivo [38–40].

To the best of our knowledge, so far, no clinical studies have been conducted to investigate the influence of GSE supplement on iron overload in children with β-TM. Therefore, the present study aimed to evaluate *the* iron-chelating effect and safety of GSE supplement as well as its influences on oxidative stress, inflammation, and liver function in β-TM pediatric patients receiving deferasirox as the only standard iron chelator.

2. Materials and methods

2.1. Study population

This randomized clinical trial was conducted from July 2021 to May 2022. In brief, a total of 60 children with β-TM were recruited from the thalassemia clinic of Shahid Baghaei 2 Hospital, affiliated with Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The recruited patients were transfusion-dependent thalassemia children receiving regular blood transfusions at Shahid Baghaei 2 Hospital. They had been previously identified through anemia workup and confirmed by genetic test.

Male and female children aged 12–18 years with β-TM were eligible for inclusion in the study if they had ferritin level ≥1000 ng/ml and a history of taking deferasirox as the only iron chelation therapy with a constant dose for at least one month before the intervention period. The exclusion criteria were as follows: taking any antioxidants, any nutritional supplements or other iron chelating drugs, including deferoxamine and deferiprone during one month before the study, a history of hepatitis B or C infection, autoimmune diseases, HIV infection, chronic inflammatory diseases, diabetes mellitus or infectious diseases. The withdrawal criteria were changes in the therapeutic regimen during the study, taking any antioxidants, any nutritional supplements or other iron chelating drugs such as deferoxamine and deferiprone within the intervention period.

2.2. Study design and intervention

At the beginning of the study, a general questionnaire, including demographic, anthropometric, and clinical characteristics was completed for each participant. The patients who met the mentioned inclusion criteria were randomly assigned into two groups, including GSE and placebo groups by permuted-block randomization technique. The present trial was double-blinded and neither the patients nor the investigators were aware of type of treatment assignment until the end of the study. The GSE group (n = 30) received deferasirox at daily dose of 28 mg/kg and GSE capsules (100 mg/day) for 4 weeks. The control group (n = 30) received identical capsules containing 100 mg starch and deferasirox (28 mg/kg/day) during the study. The dosage of GSE was chosen based on the data derived from previous clinical trials indicating the beneficial effects of GSE on the inflammatory biomarkers and lipid profiles [28,41]. The patients were advised to take deferasirox on empty stomach and capsules with or after meal with one glass of water and not to alter their habitual diet during the study. The compliance of the patients was evaluated by making phone call every week and asking them about the consumption of drugs and any possible side effects. The patients were also asked to give back the remaining capsules and the percentage of patients' compliance was determined based on the number of returned capsules by each patient. The data of participants with the compliance over 90 % were analyzed at the end of the trial.

2.3. Preparation of drugs

The GSE supplement capsules were supplied by Shari Company (Tehran, Iran) as 100 mg capsules. In the current study, starch powder was used to make placebo capsules. Each placebo capsule was filled with 100 mg starch powder. The GSE and placebo capsules were completely similar in size, shape, and color.

2.4. Blood samples and biochemical measurements

A 10-ml venous blood sample was taken from each patient at baseline and after 4 weeks of intervention. The specimens were immediately centrifuged at 4000 rpm for 10 min to separate serum and finally, the supernatant was stored at −80 °C until bioanalysis. The serum iron and total iron binding capacity (TIBC) levels were measured by colorimetric assays using Pars Azmoon kits (Karaj, Iran). The serum ferritin level was quantified by enzyme-linked immunosorbent assay (ELISA) using Monobind kit (Lake Forest, CA, USA). The automated blood cell counter (Sysmex, Japan) was used to estimate the hemoglobin (Hb) concentration. A commercial human tumor necrosis factor alpha (TNF-α) kit (Invitrogen, USA) was used to measure the serum TNF-α level. The serum high-sensitivity C-reactive protein (hs-CRP) level was quantified by ELISA kit (DBC, Canada) according to the manufacturer's protocol. The serum levels of alanine transaminase (ALT) and aspartate aminotransferase (AST) were determined by spectrophotometric technique using Pars Azmoon kits (Karaj, Iran). The serum levels of malondialdehyde (MDA) and glutathione (GSH) as well as superoxide dismutase (SOD) activity were measured by colorimetric assay using ZellBio kits (Germany) according to the manufacturer's instruction.

2.5. Sample size

The sample size was estimated on the basis of change in serum ferritin concentration as a primary outcome. We expected a difference of 500 ng/ml in serum ferritin level following the intervention, by taking

into account a confidence level of 95 % and 80 % power [42]. The required sample size was 24 patients in each group. Considering a dropout rate of 20 %, the sample size was increased to 60 patients (30 in each group).

2.6. Ethical considerations

The present study protocol was approved by the medical ethics committee of Ahvaz Jundishapur University of Medical Sciences (IR.AJUMS.REC.1399.307). The authors declare that the investigation has been performed in adherence with the Declaration of Helsinki. In this clinical trial, the written informed consent was provided by the participants' parents before enrolment in the study. All the patients could withdraw from the study at any time. *The patients' personal information* was kept confidential. This project was registered in the Iranian Registry of Clinical Trials (IRCT) website (No. IRCT20180603039959N2).

2.7. Statistical analysis

Statistical analysis of data was performed using the SPSS software (IBM SPSS Statistics for Windows, version 22). The Kolmogorov–Smirnov was used to evaluate the normal distribution of values. The independent-samples *t*-test was applied to compare differences between the two groups at baseline. *Comparisons* of any differences in biochemical variables between the GSE and placebo groups after 4 weeks of intervention were carried out using the analysis of covariance (ANCOVA) test, adjusting for baseline values. The changes in outcomes were compared within the groups at baseline and after intervention by the paired samples *t*-test. The qualitative variables were compared between the two groups by Chi-square test. *P* value < 0.05 was considered statistically significant.

3. Results

The study flow diagram indicating the patients' enrollment, allocation, follow-up, and analyses is represented in Fig. 1. In the present clinical trial, among 60 β-TM pediatric patients initially enrolled, 5 patients from the GSE group (2 discontinued the consumption of GSE supplement, 2 changed the iron chelation regimen, 1 refused to cooperate with laboratory tests) and 4 patients from the placebo group (3 were unwilling to continue, 1 changed the iron chelation regimen) withdrew from the study and finally, the trial was completed with 51 patients (25 in the GSE group and 26 in the placebo group).

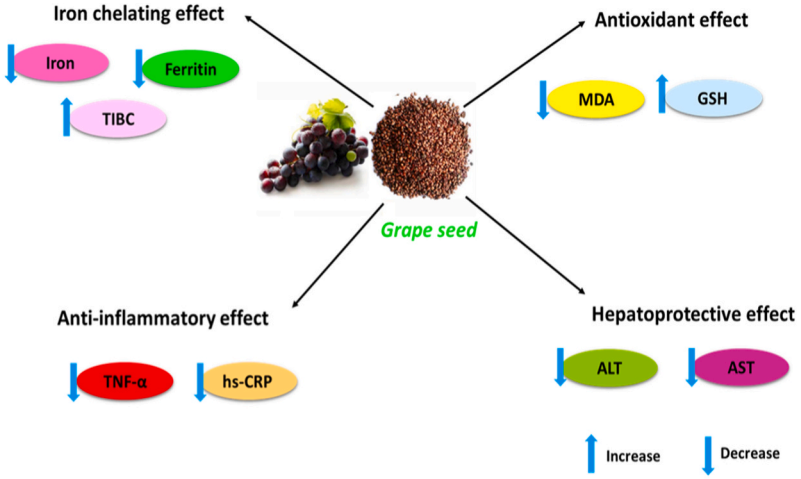
GSE supplement was well tolerated and no serious adverse reactions related to the consumption of GSE supplement were observed during the trial. Only 5 patients reported transient abdominal pain at the beginning of the study. Anthropometric, demographic, and clinical characteristics of the participants are shown in Table 1. There were no significant differences in baseline characteristics between the GSE and placebo groups at the onset of the study. The patients in the GSE and placebo groups had a mean age of 16.1 (±8.80) and 16.3 (±5.40) years, respectively, with no significant difference in age between the two groups ($p = 0.90$). Among the male patients, 18 patients were in the GSE group and 15 patients in the placebo group. Among the female patients, 7 patients were in the GSE group and 11 patients in the placebo group.

The changes in three parameters of iron status, including serum iron, ferritin, and TIBC in GSE and placebo groups in children with β-TM are summarized in Table 2. After 4 weeks of intervention, the serum levels of iron ($p = 0.030$) and ferritin ($p = 0.017$) significantly decreased in GSE group compared with placebo group. There was a significant increase in the TIBC level ($p = 0.020$) in the GSE group compared with the placebo group. The changes in oxidative markers, including MDA, GSH, and SOD in GSE and placebo groups are shown in Table 3. GSE supplement significantly reduced the serum level of MDA ($p = 0.000$) at the end of the study. The serum level of GSH ($p = 0.001$) was significantly higher in the GSE group compared with the placebo group. No significant changes were observed in SOD activity ($p = 0.590$) in the GSE group.

Table 1
Baseline characteristics of β-TM pediatric patients in GSE and placebo groups.

Variables	GSE group (n = 25)	Placebo group (n = 26)	*p* value
Age (year)	16.1 ± 8.80	16.3 ± 5.40	0.90
Sex n (male/female)	18/7	15/11	0.39
Weight (kg)	25.1 ± 8.10	26.2 ± 8.12	0.56
Height (cm)	125 ± 23.00	126 ± 21.00	0.84
BMI (kg/m^2)	15.81 ± 2.60	16.4 ± 3.10	0.38
Splenectomy n (%)	1 (2.80)	2 (5.55)	0.56
Deferasirox dosage (mg/kg)	27.01 ± 4.18	28.16 ± 3.02	0.18
Deferasirox treatment duration (month)	5.01 ± 2.12	4.41 ± 2.78	0.31

Data are represented as mean ± standard deviation, or numbers (%).
p values resulted from independent samples *t*-test for quantitative variables and from the Chi-square test for qualitative variables between the two groups.
β-TM: β-thalassemia major; GSE: grape seed extract; n: number; BMI: body mass index.



TIBC: total iron-binding capacity; MDA: malondialdehyde; GSH: glutathione; TNF-α: tumor necrosis factor alpha; hs-CRP: high-sensitivity C-reactive protein; ALT: alanine transaminase; AST: aspartate aminotransferase.

Fig. 1. CONSORT flow diagram of the study.

Table 2
Parameters of iron status in β-TM pediatric patients in GSE and placebo groups.

Variables	Measurement period	GSE group (n = 25)	Placebo group (n = 26)	*p* value
Iron (μg/dl)	**Baseline**	184 ± 41.00	179 ± 25.71	0.540[b]
	After intervention	146 ± 41.70	165 ± 31.50	0.030[c]
	***p* value[a]**	.0000	0.040	
Ferritin (ng/ml)	**Baseline**	1941 ± 501.78	1987 ± 414.91	0.670[b]
	After intervention	1293 ± 439.80	1533.3 ± 389.13	0.017[c]
	***p* value[a]**	.0000	.0000	
TIBC (μg/dl)	**Baseline**	251.63 ± 40.60	241.30 ± 31.12	0.230[b]
	After intervention	279.41 ± 41.21	257.49 ± 36.88	0.020[c]
	***p* value[a]**	0.005	.0400	

Data are represented as mean ± standard deviation.
p value[a] is reported based on the analysis of paired samples *t*-test.
p value[b] is reported based on the analysis of independent samples *t*-test.
p value[c] is reported based on the analysis of covariance.
β-TM: β-thalassemia major; GSE: grape seed extract; n: number; TIBC:total iron binding capacity.

Table 3
Oxidative stress markers in β-TM pediatric patients in GSE and placebo groups.

Variables	Measurement period	GSE group (n = 25)	Placebo group (n = 26)	*p* value
MDA (μmol/L)	**Baseline**	6.87 ± 0.98	6.91 ± 0.63	0.830[b]
	After intervention	3.78 ± 0.71	6.85 ± 5.20	0.000[c]
	***p* value[a]**	0.000	0.340	
GSH (μmol/L)	**Baseline**	9.87 ± 4.21	10.12 ± 3.68	0.790[b]
	After intervention	14.61 ± 5.91	10.94 ± 2.57	0.001[c]
	***p* value[a]**	0.000	0.270	
SOD (U/ml)	**Baseline**	29.34 ± 8.65	27.98 ± 9.41	0.520[b]
	After intervention	29.87 ± 9.68	28.67 ± 9.41	0.590[c]
	***p* value[a]**	0.800	0.750	

Data are represented as mean ± standard deviation.
p value[a] is reported based on the analysis of paired samples *t*-test.
p value[b] is reported based on the analysis of independent samples *t*-test.
p value[c] is reported based on the analysis of covariance.
β-TM: β-thalassemia major; GSE: grape seed extract; n: number; MDA: malondialdehyde; GSH: glutathione; SOD: superoxide dismutase.

Table 4 represents changes in the inflammatory biomarkers, *TNF-α* and hs-CRP in GSE and placebo groups. *The serum* levels *of TNF-α* (p = 0.000) and hs-CRP (p = 0.001) significantly decreased in the GSE group compared with the placebo group after 4-week treatment. Changes in the liver enzymes and Hb concentration in GSE and placebo groups are shown in Table 5. Treatment with GSE supplement resulted in a significant reduction in ALT (p = 0.000) and AST (p = 0.000) levels in children with β-TM. At the end of the trial, changes in the Hb concentration (p = 0.670) were not statistically different between the two groups.

Table 4
Inflammatory biomarkers in β-TM pediatric patients in GSE and placebo groups.

Variables	Measurement period	GSE group (n = 25)	Placebo group (n = 26)	*p* value[1]
TNF-α (pg/ml)	**Baseline**	7.86 ± 0.86	8.01 ± 0.71	0.420[b]
	After intervention	5.36 ± 0.63	7.91 ± 0.62	0.000[c]
	***p* value[a]**	0.000	0.520	
hs-CRP (mg/L)	**Baseline**	13.43 ± 6.21	13.56 ± 5.67	0.920[b]
	After intervention	9.91 ± 4.81	14.61 ± 6.71	0.001[c]
	***p* value[a]**	0.010	0.470	

Data are represented as mean ± standard deviation.
p value[a] is reported based on the analysis of paired samples *t*-test.
p value[b] is reported based on the analysis of independent samples *t*-test.
p value[c] is reported based on the analysis of covariance.
β-TM: β-thalassemia major; GSE: grape seed extract; n: number; TNF-α: tumor necrosis factor alpha; hs-CRP: high-sensitivity C-reactive protein.

4. Discussion

GSE, as a natural supplement, is used in traditional medicine for treatment of many diseases [43]. Although previous preclinical and clinical studies have indicated the biological and therapeutic influences of GSE, there is no evidence for its influence on excess iron in β-TM patients. In this study, the effects of GSE supplement on iron overload, oxidative stress, inflammation, liver function, and Hb concentration as well as its safety have been evaluated for the first time in children with β-TM through a randomized, double-blind, placebo-controlled clinical trial. Our results showed the beneficial effects of GSE supplement on iron status, oxidative stress, inflammatory markers, and liver function.

The co-treatment with GSE and deferasirox was more effective in reducing the serum levels of *iron* and ferritin and enhancing the TIBC level compared with deferasirox alone in children with β-TM. These results highlight that GSE supplement has iron-binding ability in clinical setting. A high content of flavonoids such as proanthocyanidins has been found in GSE [44]. In vitro studies have shown the iron chelating potential of proanthocyanidins [37]. In vivo and clinical evidence exhibits that flavonoids, as natural polyphenols, possess marked iron chelating effects [19,42]. For instance, iron chelating activities of flavonoids such as curcumin and silymarin have been reported in patients with β-TM [42,45]. Curcumin, a polyphenolic compound present in turmeric, ameliorated iron overload in β-TM patients by diminishing the level of non-transferrin bound iron (NTBI) [42]. Silymarin, as a natural compound consisting of several flavonolignans, attenuated iron burden in patients with β-TM by reducing the serum level of iron [45].

A growing body of evidence demonstrates that iron overload can encourage the generation of free radicals, leading to the oxidative stress and tissue damage [5]. Indeed, iron overload triggers cytotoxicity via oxidation of cell components such as membrane lipids, proteins, and nucleic acids, which can lead to the tissue injury and disease initiation and progression [5]. Iron accumulation is implicated in a specific form of cell death named ferroptosis [6]. Iron overload induces lipid peroxidation, which can result in the ferroptosis [7]. Oxidative stress plays an important role in the pathophysiology of β-thalassemia [46]. Several

Table 5
Liver enzymes and Hb concentration in β-TM pediatric patients in GSE and placebo groups.

Variables	Measurement period	GSE group (n = 25)	Placebo group (n = 26)	*P* value[1]
ALT (U/L)	**Baseline**	41.83 ± 11.11	43.71 ± 11.71	0.489[b]
	After intervention	19 ± 28.10	46.61 ± 10.71	0.000[c]
	***p* value[a]**	0.000	0.280	
AST (U/L)	**Baseline**	50.41 ± 12.12	51.13 ± 11.14	0.790[b]
	After intervention	42.3 ± 10.16	53.98 ± 9.13	0.000[c]
	***p* value[a]**	0.003	0.240	
Hb (g/dl)	**Baseline**	8.31 ± 0.63	8.42 ± 0.61	0.457[b]
	After intervention	8.51 ± 0.56	8.57 ± 0.62	0.670[c]
	***p* value[a]**	0.160	0.310	

Data are represented as mean ± standard deviation.
p value[a] is reported based on the analysis of paired samples *t*-test.
p value[b] is reported based on the analysis of independent samples *t*-test.
p value[c] is reported based on the analysis of covariance.
β-TM: β-thalassemia major; GSE: grape seed extract; n: number; ALT: alanine transaminase; AST: aspartate aminotransferase; Hb: hemoglobin.

studies have demonstrated the reduced levels of antioxidant enzymes and elevated levels of MDA in β-TM patients [47]. Preclinical and clinical evidence indicates that GSE has a potent protective effect against oxidative stress [26,48]. GSE supplement can remarkably improve the body's redox system [48,49]. Our results showed that GSE supplement had a significant modulating effect on the oxidative markers, MDA and GSH in children with β-TM, which is associated with its potent antioxidant activity. Glutathione is found to act as a crucial element of the erythrocytes antioxidant defense mechanism against free radicals [50]. The upregulation of glutathione levels of erythrocytes reduces the oxidative stress, thereby enhancing resistance of erythrocytes to hemolysis and improving erythropoiesis in β-TM patients [45]. Elimination of free radicals and other reactive oxygen species (ROS) from the body is the function of antioxidant enzymes such as SOD [51]. In this clinical trial, GSE supplement could not remarkably enhance the SOD activity in children with β-TM. Some clinical studies display that grape products containing polyphenols have no significant effect on SOD activity [52]. Therefore, it appears that GSE supplement confers its antioxidant effects by increasing the glutathione levels in β-TM pediatric patients.

Scientific evidence indicates that β-TM disease is related to a chronic inflammatory state [53–55]. The high levels of inflammatory biomarkers such as TNF-α and hs-CRP have been reported in patients with β-TM [53,54,56,57]. The increased iron store in the body is the major cause of chronic inflammation in β-TM patients [58]. Iron plays a fundamental role in the inflammation process. Iron-induced oxidative stress can cause the overexpression of pro-inflammatory cytokines [12]. Several experimental and clinical studies have reported the anti-inflammatory potential of GSE and its constituents [28,59,60]. GSE supplement can significantly reduce the levels of TNF-α and hs-CRP in clinical setting [28]. The findings of our study indicated that the serum levels of TNF-α and hs-CRP remarkably decreased after 4 weeks of treatment with GSE supplement. Therefore, the underlying mechanism by which GSE supplement attenuates the serum levels of TNF-α and hs-CRP in β-TM children can be attributed to its anti-inflammatory properties.

One of the most important complications of iron overload is liver damage [61]. In the present clinical trial, the elevated serum levels of ALT and AST, as biomarkers of liver injury, were observed in children with β-TM. The liver is considered as the major target for iron-induced damage because it is the main iron storage site in the body, produces transferrin as the major iron-carrier protein and hepcidin as the iron-regulatory hormone [61]. Excessive iron accumulation in liver tissue can trigger the formation of ROS and inflammatory cytokines [62]. It has been reported that the iron overload-induced ferroptosis plays an important role in the initiation of hepatic inflammation process [6]. In the current study, GSE supplement remarkably decreased the serum levels of ALT and AST. Therefore, it seems that one of the most important mechanisms mediating the protective effect of GSE supplement on hepatocytes is the suppression of hepatic oxidative stress and lipid peroxidation by its antioxidant and free radicals scavenging activities. Flavonoids of GSE supplement may penetrate hepatocytes and consequently chelate iron from cells, leading to a reduction in the liver iron content, which was demonstrated through a decrease in ALT and AST levels in this clinical trial. In vivo studies show that proanthocyanidins of GSE have marked hepatoprotective impacts [63]. Several studies provide evidence supporting the beneficial effects of GSE on the liver function [30,64].

In our study, GSE supplement did not significantly affect Hb level in β-TM children. Clinical trials indicate that the dietary polyphenol supplementation has no effect on the hemoglobin concentration in patients with β-TM [14]. In the present clinical trial, the combination therapy of GSE supplement (100 mg/day) and deferasirox (28 mg/kg/day) was more effective than deferasirox alone in attenuating iron burden in pediatric patients with β-TM. Based on the iron shuttle hypothesis [42], GSE supplement may have a synergistic influence with iron chelating activity of deferasirox. This natural supplement might penetrate cells and mobilize excess intracellular iron from tissues into circulation where deferasirox binds iron and speeds up its excretion.

GSE supplement was well tolerated and the participants experienced no serious adverse reactions. Clinical studies have explored that GSE can be considered as a safe supplement in adult individuals [41]. To date, there is no data on the safety and tolerability of GSE supplement in children with β-TM. This is the first report supporting the GSE supplement as a safe natural compound at dose of 100 mg for treatment of β-TM pediatric patients. Despite the promising findings, which highlight an iron chelating role for GSE supplement in clinical setting, the current study had some limitations. To minimize the effects of potential confounders such as dietary supplements, the patients were advised not to take any nutritional supplements during the study. However, there was no accurate information about the intake of dietary supplements or antioxidants during the clinical trial. Due to budget constraint, we were unable to measure more oxidative stress and inflammatory biomarkers as well as other indicators of iron status such as hepcidin and ferroportin, which could be effective in better interpretation of the results.

5. Conclusion

In summary, our study demonstrates that GSE supplement possesses several health-promoting effects on β-TM pediatric patients owing to its iron chelating, antioxidant, anti-inflammatory, and hepatoprotective activities. The results of this clinical trial suggest that GSE supplement can serve as a promising iron chelating agent in combination *with deferasirox* for improving iron burden, oxidative stress, inflammatory state, and liver dysfunction in children with β-TM. Further studies are warranted to provide more evidence on the iron chelating efficacy and beneficial influences of GSE supplement in adult patients with β-TM.

Funding sources

This article is extracted from Sayeh Mottaghi subspecialist thesis, which was approved and financially supported by Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran (Project grant No. Th-9908).

Authors' statement

The authors confirm contribution to the manuscript as follows: Sayeh Mottaghi: Study conception and design; Data collection; Analysis and interpretation of results; Manuscript writing; Critical revision of the manuscript. Hassan Abbaszadeh: Study conception and design; Analysis and interpretation of results; Manuscript writing; Critical revision of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Declaration of competing interest

The authors declare no conflicts of interest.

Acknowledgements

We would like to express our gratitude to all the β-TM pediatric patients who participated in this study. We would also like to thank the personnel of thalassemia clinic of Shahid Baghaei 2 Hospital for providing facilities as well as the staff of Shari Company for providing GSE capsules.

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