Original Article *Iran J Ped Hematol Oncol. 2021, Vol 11, No 4, 255-262*

A Randomized, Controlled Study Evaluating the Effects of Silymarin Addition to Deferasirox on the Liver Function of Children with Transfusion-Dependent Thalassemia

Aziz Eghbali MD[1], Roghayeh Rahimi Afzal MD[2], Mojtaba Hashemi MD[2], Aygin Eghbali MD[3], Bahar Taherkhanchi MD[4], Bahador Bagheri PhD[5,6*]

1. Department of Pediatrics, Aliasghar Clinical Research Development Center, Iran University of Medical Sciences, Tehran, Iran
2. Department of Pediatrics, Arak University of Medical Sciences, Arak, Iran
3. School of Medicine, Iran University of Medical Sciences, Tehran, Iran
4. Department of Pediatrics, Erfan Niyayesh Hospital, Tehran, Iran
5. Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran
6. Center for Molecular Cardiology, University of Zurich, Schlieren, Switzerland

*Corresponding author: Dr Bahador Bagheri, Department of Pharmacology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran. Email: bagherib@semums.ac.ir,bahadordvm@yahoo.com. ORCID ID: 0000-0003-2574-8034

Received: 12 November 2020 **Accepted:** 16 May 2021

Abstract

Background: Frequent blood transfusion can lead to iron overload which is potentially dangerous for the heart and liver. Silymarin has well-documented protective effects on hepatocytes. The purpose of this study was to evaluate the hepatoprotective effects of silymarin addition to iron chelators in children with thalassemia.

Materials and Methods: This randomized, double-blinded, and placebo-controlled trial was performed on 40 subjects with thalassemia major and intermedia in Amir Kabir Hospital, Arak, Iran. Subjects were randomized 1:1 oral to 30 mg/kg deferasirox plus placebo, or deferasirox plus oral 70-140 mg silymarin (twice daily) for 6 months. Cardiac and hepatic iron levels and levels of Gamma-glutamyltransferase (GGT), Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP), total bilirubin, albumin, total protein, and total cholesterol were measured at baseline and after 6 months of treatment.

Results: The mean age of patients was 16 years and 60% of patients were female. After 6 months, there were significant increases in the levels of ALT, AST, GGT, and TG in the placebo group as compared to the silymarin group ($P < 0.05$). In contrast, ALT, AST, and GGT had significant reductions compared to the silymarin group ($P =0.05$). Patients in the placebo group had a rise in total bilirubin ($P = 0.07$), but total protein and albumin did not have significant changes in the silymarin group ($P > 0.05$). Finally, a significant improvement was noted in cardiac iron values in patients using silymarin; 22.2 ± 6.6 ms at baseline vs 26.9 ± 7.1 ms at 6 months ($P < 0.05$).

Conclusion: This study suggests that twice-daily addition of silymarin to deferasirox could improve liver function in children with thalassemia major and intermedia. Silymarin seems safe in pediatrics.

Keywords: Deferasirox, Liver Function, Silymarin, Thalassemia

Introduction

Thalassemia syndromes are considered the most common hereditary blood disorders worldwide (1). These syndromes are frequently seen in African, Mediterranean, the Middle East, and Southeast Asian populations with considerable rates of morbidity and mortality (2). Beta-thalassemia is caused by a decrease in the production of β-globin chains. More than 200-point mutations are involved in the pathogenesis of thalassemia. Homozygous or compound-heterozygous inheritance of mutations can lead to β-thalassemia major and intermedia (2-4). Transfusion is a mainstay in the treatment of thalassemia. According to the clinical status of patients, transfusion is prescribed at specific intervals. Of note, frequent transfusion causes an increase in iron levels. Due to the disability of the body to excrete excess iron, iron enters plasma and gradually exceeds the capacity of transferrin and the iron overload may be resulted. Hepatocytes, cardiomyocytes, and endocrine cells are the most vulnerable sites for damages due to iron overload (5,6). Excessive oxygen-

[DOI: 10.18502/ijpho.v11i4.7169]

activated products are usually produced in response to the iron overload and hurt the hepatocytes which may eventually lead to cirrhosis (7,8). Iron chelators like deferoxamine, deferasirox, and deferiprone are widely used to manage iron overload. However, current therapies are not always beneficial, and the addition of any useful medicines can be considered (9,10). Silymarin as an antioxidant agent is composed of flavonoids, polyphenols, and silibinin (also known as silybin) as a major constituent (11-14). In recent years, several studies have been done to test the iron-chelating and hepatoprotective effects of silymarin in thalassemia. Of note, current findings are far from conclusion, particularly in young children. And this was the aim of the trial. The present investigation was constructed to evaluate the effects of silymarin addition to iron chelators on liver function in children aged > 5 years with thalassemia major and intermedia.

Materials and Methods

Study design

This randomized, placebo-controlled, and double-blinded study was implemented on 40 thalassemia major (TM) and thalassemia intermedia (TI) patients in Amir-Kabir Hospital, Arak, Iran. Figure1 shows consort diagram detailing study subjects. A definite diagnosis of thalassemia was done by hemoglobin electrophoresis. The presence of target cells in blood smear and isolated microcytic anemia were suggestive for thalassemia major. Children with TM aged > 5 years with increased levels of alanine transaminase (ALT) and aspartate transaminase (AST) were included in the study. All participants and caregivers were masked to treatment allocation. Sealed envelopes with an enclosed assignment were used for allocation concealment. A computer-generated sequence was used for randomization. Subjects were randomized 1:1 oral to 30 mg/kg deferasirox (exjade®, NovartisPharma AG, Switzerland) plus placebo (identical to silymarin tablet) or deferasirox (exjade®) plus oral 70-140 mg silymarin twice daily (Livergol®, GolDaru, Iran) for 6 months. Patients aged > 12 received 140 mg silymarin twice daily and younger children were given 70 mg silymarin twice daily. Participants were excluded if they had hepatitis B, C, human immune deficiency (HIV), change in chelation regimen within the last 6 months, and renal failure. Patients were discontinued from the study for these reasons: safety, loss to follow-up, and voluntary discontinuation.

Ethical consideration

The local Ethics Committee approved the study (IR.ARAKMU.REC.1397.109) and written informed consent was taken from patients, parent(s), or the legal representative prior to trial participation. The clinical trial registry number was IRCT20150119020715N9.

Efficacy assessment

Levels of ALT, AST, total bilirubin, albumin, total protein, gamma-glutamyl transferase (GGT), ferritin, total cholesterol, cardiac and hepatic T2* values were measured at baseline and 6 months after the start of the study. Cardiac and hepatic T2* were measured by MR scan (1.5T Siemens, Germany). Levels of GGT and ferritin were measured by ELISA (Abcam, UK) and serum levels of other parameters were assessed using commercially available kits (Pars Azmoon, Iran). The primary endpoint of the study was a change in the level of total bilirubin and the secondary endpoints were changes in ALT and AST levels.

Safety assessment

Physical examination was done at baseline and every month by physicians. For assessment of side effects of the drugs, patients were monitored weekly for any systemic upset and hypersensitivity. Patients or their parents were asked to immediately call the clinic in case of any medical problem.

Data analysis

The total number of 50 patients was calculated for randomization according to

[DOI: 10.18502/ijpho.v11i4.7169]

the assumption of 15 % dropout in the number of the study patients with an 80 % power at a significance level of 0.05 for changes in GGT and transaminases with a sample size of 43 patients. For data analysis, the Student *t-test*, x2 test, and Fisher's exact were used. A two-way analysis of variance was used for subgroup analysis. The level of significance was $P < 0.05$. The analysis was done using SPSS software version 21.0, Chicago, USA.

Results

Baseline characteristics of study subjects

Of the 51 patients who were included 8 patients did not receive the treatments (3 did not use the medications, and 5 met the exclusion criteria). Three patients were lost to follow up and 40 participants had complete observation over 6 months. Table I shows the baseline characteristics of subjects. The mean age of patients was 16 years and 60% of patients were female. Of a total of 40 patients, 6 patients had TI. At baseline, no significant differences were noted in patients' characteristics. The volume of transfusion was about 10 ml/kg in both groups and there were no significant differences in total transfusion requirements. Transfusion intervals were from 19 to 61 days with a mean of 18 ± 7 days. That is why our range is a bit wide. Patients had a different history of chelators prior to study during the study they were given deferasirox 30 mg/kg per day for 6 months and there was no dose reduction or dose elevation. Besides, no other chelator was added for patients during the study. The mean level of 6-month serum ferritin was 2802 ± 1348 ng/dL in the placebo group and it was 2998 ± 1488 ng/mL in the silymarin group. Transfusion was indicated for patients with pre-transfusion hemoglobin levels of 9-9.5 mg/mL.

Myocardial iron

As shown in Table II, myocardial T2* increased from 22.1 ± 7.7 ms at baseline to 23.9 ± 4.9 ms at 6 months ($P = 0.5$). In the silymarin group, it elevated from 22.2 ± 6.6 ms at baseline to 26.9 ± 7.1 ms at 6 months ($P < 0.05$). The between-group difference was not statistically significant at 6 months ($P = 0.07$).

Hepatic iron

As presented in Table II, no change was seen in hepatic T2* from a baseline of 6.6 ± 5.6 ms to 6.7 ± 5.4 ms in the placebo group ($P = 0.7$). In the silymarin group, hepatic T2* slightly increased from 9.9 ± 8.9 ms at baseline to 10.0 ± 7.2 ms at 6 months ($P = 0.7$). At 6 months, there were no significant differences between the 2 study groups ($P = 0.09$).

Liver function

As shown in Figure 2A, there was a rise in total bilirubin in the placebo group. Also, there were significant decreases in total protein and albumin in the mentioned group ($P = 0.01$). As presented in Figure 2B, after 6-month treatment there was a significant reduction in total bilirubin ($P = 0.04$). Compared to baseline, there were no significant changes in total protein and albumin in the silymarin group. Differences between the two groups were statistically significant ($P < 0.05$). Furthermore, as shown in Figure 3A, except for TG and cholesterol, significant increases were noted in ALT, AST, GGT in the placebo group over the 6 months ($P = 0.05$). As shown in Figure 3B, in the silymarin group and except in TG and cholesterol, significant reductions were observed in ALT, AST, and GGT as compared to baseline ($P < 0.05$). The between-group differences were statistically significant at 6 months ($P < 0.01$).

Adverse effects

The most common reported adverse effects were mild gastrointestinal symptoms seen in 4 subjects (10 %) in both groups. One patient (2.5 %) had transient skin rashes in the silymarin group. No patient died and no patient was withdrawn due to severe adverse effects of treatments.

[DOI: 10.18502/ijpho.v11i4.7169]

Table I: Baseline characteristics of participants

Characteristics	Placebo (n=20)	Silymarin (n=20)	P
Age, y	16.1 ± 5.3	16.3 ± 8.1	0.2
Age (range)	5 -22	5- 21	0.3
Female	14 (70)	10 (50)	0.1
Haemoglobin (mg/dL)	9.1 ± 1.7	9.9 ± 1.9	0.8
Serum ferritin (ng/dL)	2802 ± 1348	2988 ± 1488	0.6
ALT (U/L)	119.5 ± 2.81	122 ± 4.7	0.3
AST (U/L)	128 ± 4.74	133.2 ± 4.92	0.3
T. Bili (mg/dL)	3.12 ± 0.2	3.16 ± 0.16	0.7
TG (mg/dL)	129.5 ± 5.41	117 ± 4.93	0.9
Cholesterol (mg/dL)	161.9 ± 3.88	171.2 ± 3.52	0.9
Total protein (g/dL)	7.53 ± 0.10	7.07 ± 0.23	0.2
Albumin (g/dL)	4.16 ± 0.03	4.01 ± 0.04	0.4
GGT (U/L)	41.70 ± 1.11	46.90 ± 1.37	0.8

Data are shown as mean ± SD and number (%); alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin (T.Bili), triglyceride (TG), gamma-glutamyl transferase (GGT)

Table II: Myocardial and hepatic iron values at baseline and 6 months later

Value	Placebo group (n=20)	Silymarin group (n=20)
Myocardial iron (msec)		
Baseline	22.1 ± 7.7	22.2 ± 6.6
6-month	23.9 ± 4.9	26.9 ± 7.1*
Hepatic iron (msec)		
Baseline	6.6 ± 5.6	9.9 ± 8.9
6-month	6.7 ± 5.4	10.0 ± 7.2

Data are shown in mean ± SD. * P < 0.05 vs. baseline

[DOI: 10.18502/ijpho.v11i4.7169]

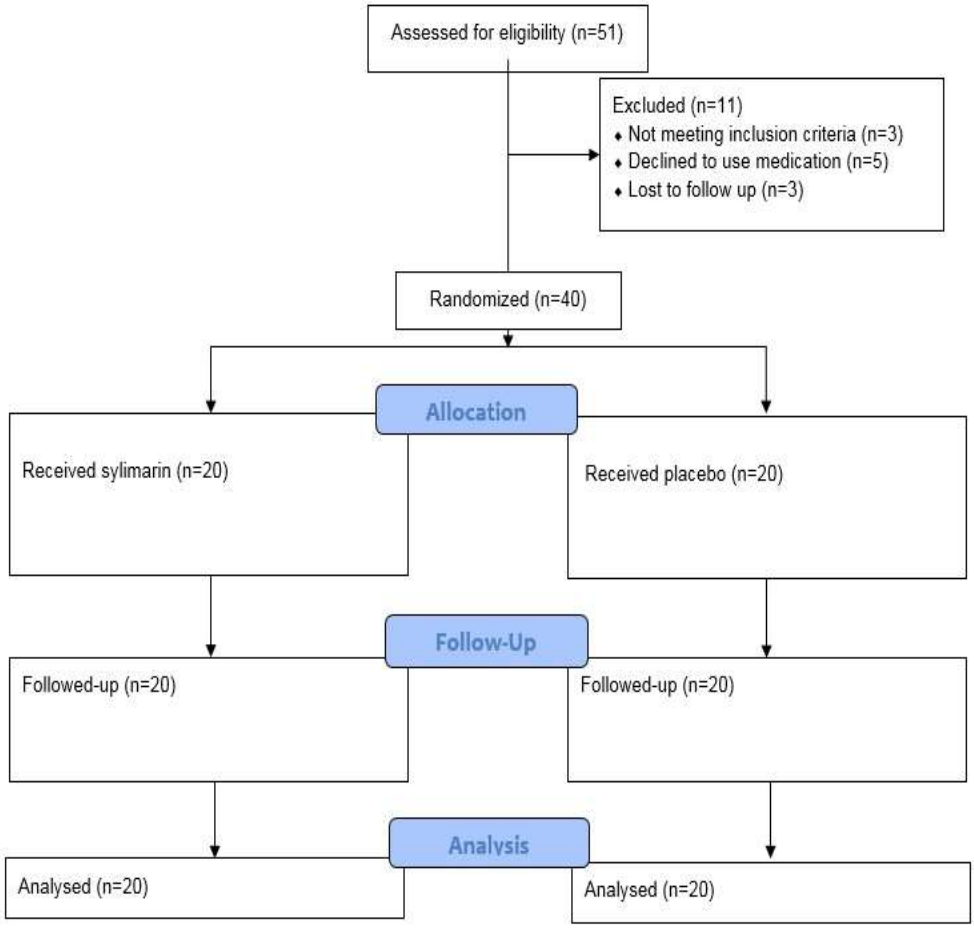

Figure1. Consort diagram detailing study subjects

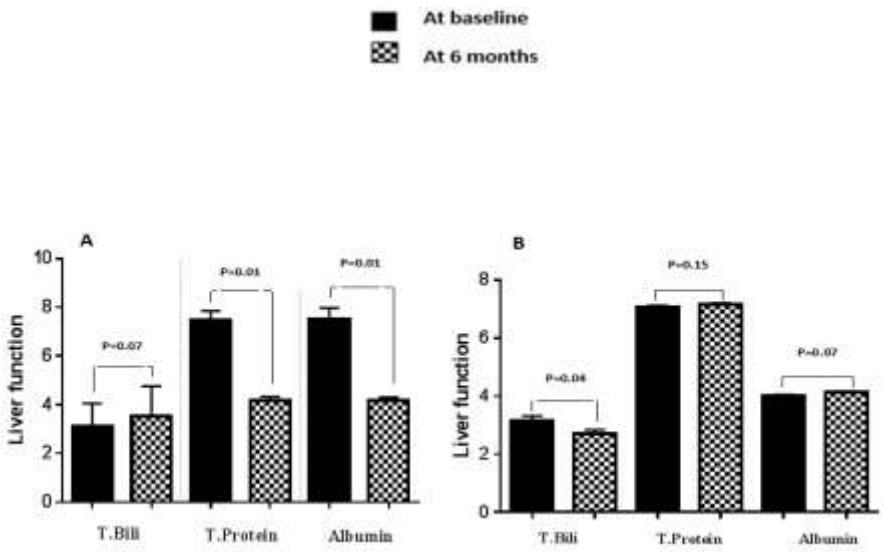

Figure 2. Effects of placebo (A) and silymarin (B) on total bilirubin, total albumin, and albumin at baseline and after 6 months of treatment

[DOI: 10.18502/ijpho.v11i4.7169]

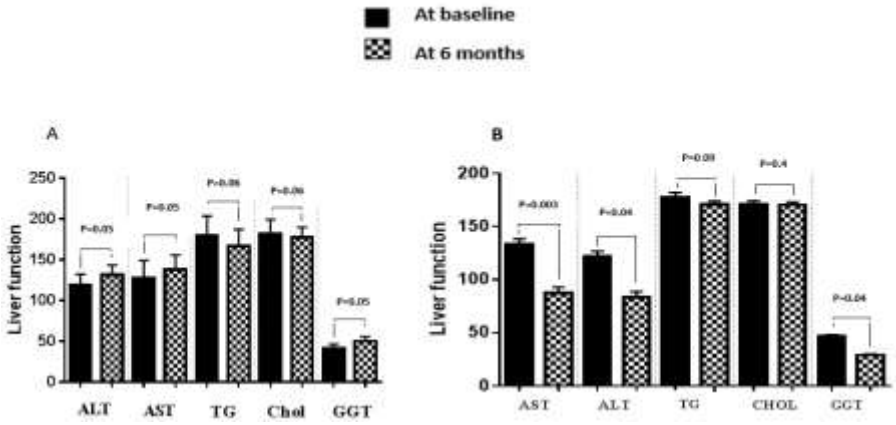

Figure 3. Effects of placebo (A) and silymarin (B) on liver enzymes, triglyceride, and cholesterol at baseline and after 6 months of treatment

Discussion

This randomized and placebo-controlled trial showed that a 6-month treatment with the combination regime of deferasirox and silymarin is more effective than deferasirox alone to improve hepatic function in transfused patients. However, no change was seen in ALP values. Maintaining the normal hepatic function is of clinical importance if the treatment can be continued over a long time. Iron overload due to frequent blood transfusion is the main complication of thalassemia major. Transfusion intervals in thalassemia major are usually every 2-5 weeks. But due to the presence of thalassemia intermedia patients (6 patients), the transfusion range was from 19 to 61 days in our study. Thalassemia intermedia patients usually require a blood transfusion in a larger time frame. Also, patients with thalassemia major and intermedia have usually an increased rate of iron gastrointestinal absorption (15). Excessive iron can enter cells and propagates oxidative and inflammatory damages which may finally lead to liver dysfunction and heart failure (5). To blunt the damages, iron chelators are frequently administered either sequentially or asynchronously. Deferoxamine, deferasirox, and deferiprone are current iron chelators (8). Different combination modalities of iron chelators have been investigated during recent years (16). Of note, in some patients, even combination therapies are not sufficient to achieve a considerable response, and the mentioned complications would be a source of concern. To resolve it, many studies have dealt with the beneficial effects of other drugs added to iron chelators. Silymarin has well-established antioxidant effects and can reduce inflammation in the hepatocytes. It may also delay hepatic fibrogenesis and accelerate cell regeneration (10). Silymarin is obtained from milk thistle (Silybum marianum) which grows up in various world regions including Europe, Middle East, South America, and Mediterranean countries (9,17). It has a long history of treating liver diseases, particularly cirrhosis and hepatitis. The remarkable antioxidant and scavenging properties of silymarin confer fortified protection against the hepatotoxicity of drugs. Some studies have also proposed immune-modulatory and anti-cancer effects for silymarin (18). During recent years several studies have been implemented to assess the effects of silymarin in thalassemia and results are consistent with them. A work by Gharagozloo et al showed that 3 months of treatment with 420 mg daily silymarin added to deferoxamine was able to significantly reduce ALP and glutathione levels in patients older than 12 yet reported no significant improvement on ferritin (19). Compared with this study, children with

[DOI: 10.18502/ijpho.v11i4.7169]

lower mean age and tested smaller doses (420 mg vs 140-280) were included in the present study. Also, patients with TI were included. In the investigation by Hagag, it was shown that silymarin addition (420 mg daily) to deferoxamine had no effects on levels of ALT, AST, and bilirubin but it was effective to reduce ferritin levels. In this study, young children were included (2.5 to 6 years) (20). A Work from Moayedi showed that 9-month therapy with 420 mg silymarin added to deferoxamine significantly reduced ALP, AST, and ALT in subjects with TM (21). In the present investigation, patients who were above 5 years old (16 years vs 20.5 years) were studied. Besides, smaller doses (140-280 mg) and more markers of the hepatic function could be evaluated for 6 months (GGT, total bilirubin, albumin, and total protein). A crossover investigation by Darvishi-Khezri demonstrated that 420 mg silymarin can reduce the iron content of the heart and liver shown by T2* MRI (22). In this work, silymarin was added to different iron chelators and individuals were not matched for them. In addition, silymarin effects on liver function were not assessed. In the present trial, mean age was low and liver function was tested in patients with TM and TI and a fixed dose of one iron chelator was used throughout the investigation. Taken together, it seems that silymarin addition to iron chelators has fruitful effects to ameliorate the liver damages of iron overload, however, some weak points should be taken into account; current data are not derived from large clinical trials, therefore more trials are still required for better understating about silymarin in thalassemia. Besides, all studied patients were not heavily iron overloaded, and it can be investigated by future works. Finally, none of the antioxidant compounds including silymarin has been shown to resolve the anemia in patients with thalassemia. Quite recently it was shown that 420 mg silymarin combined with iron chelators are effective to reduce serum levels of CRP and interleukins. This cross-over trial indicates that such therapy is associated with an improvement in inflammatory responses (23). Compared to the present study, it was a cross-over trial continued for 12 weeks. The duration of treatment was shorter and a larger amount of silymarin was used. The present study has some limitations that should be addressed. The study was single-center and findings should be confirmed in double-blinded and multicenter trials with larger sample sizes.

Conclusion

The current study indicates that deferasirox and silymarin combination can improve liver function in children with thalassemia major. This therapy seems safe.

Acknowledgments

This study was supported with a grant from the vice-chancellor of research of Arak University of Medical Sciences (grant no.3143). The authors are thankful to the staff of Amir Kabir Hospital for their assistance with this project.

Conflict of interest

The authors declare no conflict of interest.

References

1.Muncie H, Campbell J. Alpha and beta thalassemia. Am Fam Physician 2009;80(4):339-344.

2.Higgs D, Weatherall D. The alpha thalassaemias. Cell Mol Life Sci 2009;66(7):1154-1162.

3.Singer S. Variable clinical phenotypes of alpha-thalassemia syndromes. Sci World J 2009;9:615-625.

4.Pignatti CB, Gamberini MR. Complications of thalassemia major and their treatment, Expert Rev Hematol 2011; 353-366.

5.Piel FB, Weatherall DJ. The α-thalassemias. N Engl J Med 2014;371:1908-1916.

[DOI: 10.18502/ijpho.v11i4.7169]

6.Efremov GD. Dominantly inherited beta-thalassemia. Hemoglobin 2007;31:193-207.
7.Taher AT, Cappellini MD. How I manage medical complications of β-thalassemia in adults. Blood 2018;132:1781-1791.
8. Aydinok Y, Porter JB, Piga A, Elalfy M, Beshlawy A, Kilinç Y, et al. Prevalence and distribution of iron overload in patients with transfusion-dependent anemias differs across geographic regions: results from the CORDELIA study. Eur J Haematol 2015; 95 (3):244-253.
9.Lengyel G, Tulassay Z. Antioxidant therapy in chronic liver diseases. Orv Hetil 2006; 147:1113-1118.
10.Saller R, Melzer J, Reichling J, Brignoli R, Meier R. An updated systematic review of the pharmacology of silymarin. Forsch Komplementarmed 2007;14:70-80.
11.Bares JM, Berger J, Nelson JE, Messner DJ, Schildt S, Standish LJ, et al. Silybin treatment is associated with reduction in serum ferritin in patients with chronic hepatitis C. J Clin Gastroenterol 2008; 42(8), 937–944.
12.Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. Afr J Biotechnol 2008;7(18):3188–3192.
13.Hagag A. Therapeutic value of combined therapy with deferiprone and silymarin as iron chelators in Egyptian children with beta thalassemia major. Infect Disord Drug Targets 2015;15(3):189–195.
14.Alidoost F, Gharagozloo M, Bagherpour B, Jafarian A, Sajjadi SE, Hourfar H , et al. Effects of silymarin on the proliferation and glutathione levels of peripheral blood mononuclear cells from beta-thalassemia major patients. Int Immunopharmacol 2006; 6:1305–1310.
15.Nemeth E. Iron regulation and erythropoiesis. Curr Opin Hematol 2008; 15:169–175.
16.Eghbali A, Shokri P, Afzal R, Bagheri B. A 1-year randomized trial of deferasirox alone versus deferasirox and deferoxamine combination for the treatment of iron overload in thalassemia major. Transfus Apher Sci. 2019;58:429-433.
17.Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin from experimental pharmacology to clinical medicine. Ind J Med Res 2006; 124:491–504.
18. Ramakrishnan G, Lo Muzio L, Elinos-Báez CM, Jagan S, Augustine TA, Kamaraj S, et al. Silymarini nhibited proliferation and induced apoptosis in hepatic cancer cells. Cell Proliferation 2009; 42:229-240.
19. Gharagozloo M, Moayedi B, Zakerinia M, Hamidi M, Karimi M, Maracy M, et al. Combined therapy of silymarin and desferrioxamine in patients with beta-thalassemia major: a randomized double-blind clinical trial. Fundam Clin Pharmacol 2009; 23(3):359-365.
20. Hagag A, Elfrargy M, Gazar R, Abd El-Lateef A. Therapeutic value of combined therapy with deferasirox and silymarin on iron overload in children with beta thalassemia. Mediterr J Hematol Infect Dis 2013; 5(1): e2013065-e2013069.
21. Moayedi B, Gharagozloo M, Esmaeil N, Maracy MR, Hoorfar H, Jalaeikar M. A randomized double-blind, placebo-controlled study of therapeutic effects of silymarin in β-thalassemia major patients receiving desferrioxamine. Eur J Haematol 2013; 90(3):202-209.
22.Darvishi-Khezri H, Salehifar E, Kosaryan M, Karami H, Mahdavi M, Alipour A, et al. Iron-chelating effect of silymarin in patients with β-thalassemia major: A crossover randomised control trial. Phytother Res 2018;32(3):496-503.
23.Darvishi-Khezri H, Kosaryan M, Karami H, Salehifar E, Mahdavi M, Alipour A, et al. Can use of silymarin improve inflammatory status in patients with β-thalassemia major? a crossover, randomized controlled trial. Complement Med Res 2021;28(2):123-130.

[DOI: 10.18502/ijpho.v11i4.7169]