Review Article

Therapeutic Value of Silymarin as Iron Chelator in Children with Beta Thalassemia with Iron Overload

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Abstract

Beta thalassemia is an inherited hemoglobin disorder resulting in chronic hemolytic anemia. The most common treatment for thalassemia is blood transfusion which is necessary to provide the patients with healthy red blood cells containing normal hemoglobin. Repeated blood transfusion leads to iron overload. Excess iron is deposited in body organs as liver, heart and endocrine glands causing organ damage. Iron chelation therapy is the main way to treat iron overload in beta thalassemia major. Silymarin and its biologically active component Silybin are strong antioxidant and have documented iron chelating activities in patients with beta-thalassemia major. The aim of this review was to spotlight on the therapeutic value of silymarin as iron chelator in children with beta thalassemia major with iron overload.

Keywords: Thalassemia; Silymarin; Iron overload

Introduction

Thalassemias are heterogeneous group of inherited anemias that collectively represents the most common monogenic disorders**.** β-thalassemia are characterized by absent or reduced synthesis of β-globin chains of hemoglobin, caused by mutations of β-globin gene cluster resulting in reduced hemoglobin in RBCs, decreased RBCs production and anemia [1]. In Egypt, β-thalassemia is the commonest cause of chronic hemolytic anemia and represents a major public health problem. It was estimated that 1000/1.5 million per year live birth suffer from thalassemia disease in Egypt with carrier rate of 9-10% [2,3].

The most common treatment for thalassemia is blood transfusion which is necessary to provide the patients with healthy red blood cells containing normal hemoglobin. Repeated blood transfusion leads to iron overload [4]**.** Excess iron is deposited in body organs as liver, heart and endocrine glands causing organ damage [5].

Iron loading in thalassemia depends on volume of transfused blood and amount accumulated from gut absorption. In β-thalassemia increased gastrointestinal iron absorption is mediated by down-regulation of hepcidin and up-regulation of ferroportin [6].

Role of Hepcidin Ferroportin System in Iron Overload

Hepcidin is a key regulatory hormone of iron homeostasis produced by hepatocytes, in response to iron loading [7]. Increased hepcidin release in thalassemia major generates a negative feedback loop [8] that inhibits intestinal iron absorption and iron release from hepatic stores and from macrophages [9]. Increased hepcidin in thalassemia major could be explained by transfusion therapies in thalassemia major, which suppress the erythropoietic drive and increase body iron load, both of which increase hepcidin level [10] or it might reflect the effects of concomitant minor infections or other inflammatory stimuli that increase hepcidin level [11].

Hepcidin deficiency in thalassemia intermedia may be the key factor allowing excessive iron absorption and development of iron overload in this milder form of anemia that remain mostly transfusion independent, but develop iron overload [12-16]. Hepcidin production is suppressed by pathological signal from an expanded population of erythrocyte precursors that fail to mature to fully differentiated erythrocytes or hypoxia which suppresses hepcidin directly in hepatocyte as patients with thalassemia intermedia had lower oxygen delivery to tissues due to lower hemoglobin concentration and high proportion of fetal hemoglobin [14-16] (Figure 3).

The sole known molecular target of hepcidin is the protein ferroportin which acts as transmembrane conduit for the transfer of cellular iron to plasma. The binding of hepcidin to ferroportin on the membranes of iron-exporting cells induces the endocytosis and proteolysis of ferroportin and thereby decreases the delivery of iron to plasma. In iron-overload disorders, ferroportin is hyperactive, stimulating intestinal iron absorption and the release of iron from macrophages, and causing an increase in plasma iron concentrations, transferrin saturation, and iron deposition in the liver and other organs [17,18] (Figure 1).

Mechanism of Iron Toxicity

Iron is highly reactive and easily alternating between ferrous (Fe^{2+}) and ferric (Fe^{3+}) states in a process which results in the gain and loss of electrons generating harmful free radicals (atoms or molecules with unpaired electrons). These can damage lipid membranes, organelles and DNA causing cell death and the generation of fibrosis [5].

In healthy human, iron is absorbed by duodenal enterocytes and circulates in the plasma bound to transferrin. Then iron is stored in monocyte-macrophage system and parenchymal cells of liver [5]. So iron is 'kept safe' by binding to molecules as transferrin in blood and storage in the form of ferritin inside cells [19].

In iron overload, excess iron is sequestered in the cells of the monocyte-macrophage system and then the liver [20]. But when these organs get filled up and have no ability to store more iron, it is bound to transferrin and when the capacity of transferrin is saturated, iron
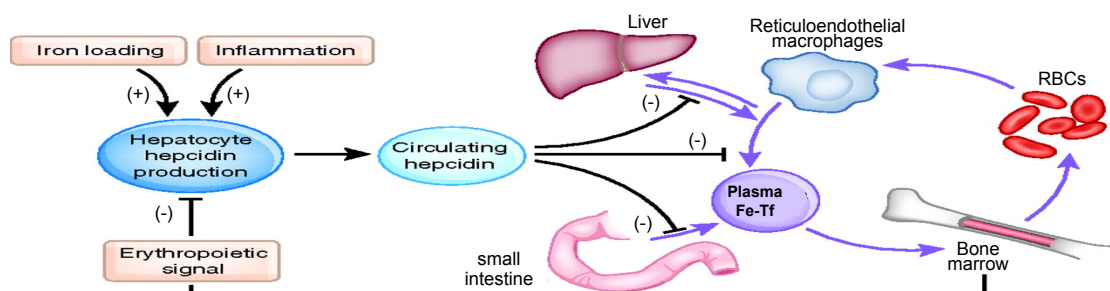
Figure 1: Role of hepcidin in iron overload [18].

Figure 2: Mechanism of iron toxicity in iron overload [23].
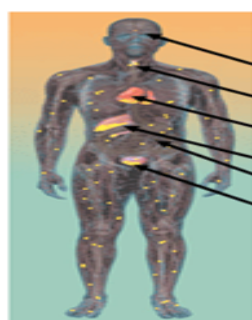
Figure 3: Clinical Sequelae of iron overload [29].

circulates in the bloodstream as extracellular non-transferrin-bound iron (NTBI) which is very toxic [21]. There is labile plasma iron (LPI) or 'free iron' which is a directly chelatable component of NTBI. It is highly toxic as it can catalyze and form harmful free hydroxyl radicals [22].

LPI is detected in patients with transfusional iron overload. It isn't detected in healthy person [10]. LPI is thought to be the iron that loads cells via a mechanism other than the transferrin receptor. Voltage-dependent calcium channels have been hypothesized as the route of entry of LPI. LPI is taken up excessively by cells leading to iron-overload pathologies. This raises the cell labile iron pool (LIP) [23]. With sustained iron loading, the iron deposits can exceed the storage and detoxification capacity of ferritin and might eventually also transform into hemosiderin. When LIP levels exceed the cell antioxidant capacity they evoke the formation of reactive oxygen species (ROS) that lead to cell damage by affecting lipids, proteins and nucleic acids. The resulting

'free iron' damages many tissues in the body and is fatal unless treated by iron chelation therapy [24] (Figure 2).

Clinical Feature of Iron Overload

Excess iron is deposited in major organs especially liver, heart, pancreas, thyroid, pituitary gland and other endocrine organs resulting in organ damage [25]. Liver is the principal site for iron storage and has the largest capacity for excess iron storage with resulting collagen deposition and portal fibrosis within 2 years from the start of transfusion regimen [26]. When liver capacity is exceeded, iron is deposited in other organs. In the heart, iron collects in the cardiac muscle fiber particularly in the ventricular wall, septum, papillary muscles and the epicardium. Signs of myocardial damage due to iron overload present as arrhythmia, cardiomegaly, heart failure and pericarditis [27]. Iron loading of the anterior pituitary can disrupt sexual maturation. Many patients suffer growth failure due to growth hormone deficiency or defective synthesis of insulin-like growth factor. Excess iron can also

damage thyroid, parathyroid and adrenal glands. It can cause skin hyperpigmentation and diabetes [28].

Investigations for Assessment of Iron Overload

Serum markers

I-Serum ferritin: Ferritin is the iron-storage protein [5]. Serum ferritin assay is easy to perform, well established, generally correlating with body iron stores. Inflammation, hepatitis, liver damage, hemolysis and ineffective erythropoiesis may falsely increase ferritin while vitamin C deficiency may depress it. A sudden and unexpected rise in ferritin should prompt a search for hepatitis, other infections or inflammatory conditions [29,30].

II-Transferrin saturation (TS): It is usually extremely high in regularly transfused patients and its level may suggest the site of iron accumulation (reticuloendothelial iron overload alone is associated with normal TS, whereas parenchymal iron overload leads to a high TS) [31]. TS above 50% is suggestive of high iron load [32].

Tissue Iron Concentration or Distribution

I-Liver iron concentration (LIC): LIC is now regarded as the reference standard for estimating body iron load and can accurately predict total body iron stores using the formula: Total body iron stores in mg/kg = 10.6 x LIC (in mg/g dry weight). Normal LIC values are up to 1.8 mg/g dry weight [19]. LIC is particularly useful when new chelating regimes are being used [33].

Liver biopsy: Inadequate sample size (<1 mg/g dry weight or about 2.5 cm core length) or uneven distribution of iron, as in case of cirrhosis, may give misleading results [34].

Superconducting quantum interference device (SQUID): LIC can also be measured accurately using SQUID. Only four machines are currently available worldwide. SQUID is very expensive and require trained staff [33].

Hepatic MRI: LIC can also now be measured using MRI. This technique can be applied with little training at any center with a reasonably up-to-date MRI machine [34].

Cardiac iron concentration (CIC):

Heart function: Regular monitoring of left ventricular ejection fraction is essential. Mild reductions in left ventricular function were used as the basis to intensify iron chelation therapy. Left ventricular function can be assessed using MRI, MUGA or echocardiography [31].

Cardiac MRI: Estimation of myocardial iron using MRI is becoming increasingly available. T2 value in tissues shortens as iron concentration increases. Myocardial T2 <20 ms indicates increased myocardial iron and is associated with decreased LV function [30].

Iron load of other tissues and organs:

Assessment of iron load in pancreas and pituitary gland using MRI is currently available. An increase in pancreas R2 indicates iron overload and needs modification of chelation therapy even if cardiac and hepatic iron estimates are stable [35].

Iron toxicity markers

Plasma non-transferrin bound iron (NTBI) and Labile plasma iron (LPI): NTBI or LPI promotes the formation of free hydroxyl radicals and peroxidation of membrane lipids and are directly involved in iron induced tissue toxicity [36,37].

Other markers of oxidative damage: Malondialdehyde is increased, while a wide range of antioxidants are depleted [19].

Measurement of urinary and fecal iron excretion: Urinary iron excretion can assist in evaluation of desferrioxamine effects (about half of total iron excreted in urine) or deferiprone (over 80% of iron excreted in urine) while fecal iron excretion can assist in evaluation of deferasirox [19].

Treatment of iron overload

Iron chelation therapy: Iron chelation therapy is the main way to treat iron overload in beta thalassemia major. The goals of iron chelation therapy are to prevent iron stores from reaching levels at which tissue damage occurs and to remove excess iron already present, thereby reversing tissue/organ dysfunction [23]. Effective protection against iron toxicity requires detoxification of both extracellular iron stores (non-transferrin bound or labile plasma iron) and intracellular iron stores (labile iron pool). NTBI and LPI appear within minutes of clearing of the iron chelator from the body. Once low levels of iron have been achieved, it is theoretically more appropriate to reduce the dose than to interrupt or decrease the frequency of iron chelator [38].

Types of iron chelators

Conventional iron chelators: 1-Desferrioxamine (DFO) is the major iron-chelating treatment of transfusional iron overload [39]. The standard recommended dose is 20-40 mg/kg by slow subcutaneous (SC) infusion over 8-12 hours using an infusion pump for 6 nights a week [40]. SC bolus of desferrioxamine twice daily may be indicated when an infusion pump is not available or when 10-hour infusions are not tolerated [41] and may be also considered if the patient is not at high risk of heart disease [19]. Intravenous desferrioxamine in high-risk β-thalassemia can be used in either 24-hour continuous infusion or intermittent high dose for 8-10 hours per day [42].

2-Deferiprone was first licensed for use in thalassaemia in India, followed by European Union and other countries outside US and Canada, in 1990s [43]. According to official European licensing agency (EMEA), Deferiprone could be used as a second line drug for removing iron in patients who are unable to use Desferrioxamine or in whom DFO therapy has proven ineffective [8]. Deferiprone daily dose that had been evaluated most thoroughly is 75 mg/kg/day in three divided doses [44].

3-Deferasirox had been licensed as first-line monotherapy for thalassemia major in over 70 countries worldwide including the US and the EU [45]. The drug is taken orally, once daily, preferably before meal and is dissolved in water (or apple juice) using a non-metallic stirrer. A recommended dose is 20-30 mg/kg [46].

Silymarin as new era in iron chelation: Silymarin is a flavonolignan complex isolated from Silybin marianum which is already being used for the treatment of liver disorders characterized by degenerative necrosis and functional impairment [47]. It protects hepatocytes from injury caused by ischemia, radiation, iron overload, and viral hepatitis [48].

Besides its hepatoprotective activity, several studies have shown that silymarin is a strong antioxidant that is capable of scavenging both free radicals and reactive oxygen species. By inhibiting lipid peroxidation, silymarin protects against hepatic toxicity induced by a wide variety of agents [47].

In beta thalassemia with iron overload a wide range of antioxidants are depleted and there is an interest in the use of antioxidants

	Gharagozloo et al. [50]	Moayedi et al. [51]	Hagag et al. [52]	Adibi et al. [54]
Number and age of studied patients	59 patients aged 12 years or older divided into 2 groups	97 patients aged 12 years or older divided into 2 groups	40 patients aged 2.5-6 years divided into 2 groups	37 patients aged 12 years or older divided into 2 groups
Method used for assessment of iron overload	Serum ferritin, serum iron, TIBC, glutathione levels of RBCs and liver functions	Serum ferritin, iron, TIBC, soluble transferrin receptor, hepcidin and liver functions	Serum ferritin, serum iron, TIBC, renal and liver functions	Liver iron concentration by MRI
Duration of the study	3 months	9 months	6 months	6 months
Dose of silymarin	140 mg three times per day	140 mg three times per day	140 mg three times per day	140 mg three times per day
Drug used with silymarin	Desferroxamine	Desferroxamine	Deferasirox	Desferroxamine
Effects of silymarin in iron overload	Significantly reduced serum iron and ferritin with Significant improvement in liver alkaline phosphatase and glutathione levels of red blood cells	Significantly reduced serum iron, ferritin, hepcidin, soluble transferrin receptor with significant improvement in liver functions	Significantly reduced serum iron and serum ferritin with no detectable abnormalities in complete blood count, liver or renal functions	No significant changes in liver iron concentration after silymarin use for 6 months

Table 1: Comparison of silymarin values in iron chelation in different studies.

or naturally occurring products with antioxidant properties in thalassemia syndromes with iron overload [19]. Silymarin has also iron chelating activities however but little is known about the biochemical mechanisms of action of these substances [49]. There are some studies designed to investigate the therapeutic activity of silymarin in patients with thalassemia major under conventional iron chelation therapy [50-52].

Gharagozloo et al. [50] investigated the therapeutic activity of oral silymarin in patients with thalassemia major under conventional iron chelation therapy in 3 months randomized, double-blind, clinical trial in 59 beta-thalassemia major patients who were randomized to receive a silymarin tablet (140 mg) three times per day plus desferrioxamine (group I) or desferrioxamine and placebo (group II). Clinical and laboratory tests were assessed at the beginning and the end of the trial. This study revealed that combined therapy of silymarin and desferrioxamine were well tolerated and more effective than desferrioxamine and placebo in reducing serum ferritin level. Significant improvement in liver alkaline phosphatase and glutathione levels of red blood cells was also observed in silymarin-treated beta-thalassemia patients.

Moayedi et al. [51] studied therapeutic effects of silymarin in β-thalassemia major patients in randomized double-blind, placebo-controlled study. Patients were treated with combination of desferrioxamine and silymarin (n= 49) or desferrioxamine and placebo (n=48) for 9 months. The serum levels of ferritin, iron, total iron-binding capacity, soluble transferrin receptor, hepcidin and liver function were determined at the baseline and after 9 months therapy. Serum ferritin levels decreased significantly from the beginning to the end of silymarin treatment (3028.8 ± 2002.6 vs. 1972.2 ± 1250.6 ng/mL); however, no significant changes in serum ferritin was observed in the patients receiving placebo (2249.0 ± 1304.2 vs. 2015.6 ± 1146.8), significantly reduced serum iron, serum hepcidin and soluble transferrin receptor and significant improvement in liver function tests was observed in silymarin group in comparison with placebo and they concluded that silymarin is effective at reducing iron overload in patients when used in conjunction with desferrioxamine.

Hagag et al. [52] studied the therapeutic value of combined therapy with deferasirox and silymarin on iron overload in children with beta thalassemia. Patients were treated with combination of silymarin and deferasirox (n=20) or deferasirox and placebo (n=20) for 6 months. They found that, serum ferritin and serum iron were significantly lower in patients who received silymarin and deferasirox than patients who received deferasirox and placebo with no detectable abnormalities in complete blood count, liver or renal functions due to silymarin use and

they concluded that combined therapy of silymarin and deferasirox depleted iron stores more successfully than deferasirox alone and so they recommended to use combination of silymarin and deferasirox in treatment of iron overloaded thalassemic children.

Despite the iron chelating activity of silymarin suggests its possible application in chelation therapy of iron overload [53]; little is known about the biochemical mechanisms of action of these substances [49]. The biological effects of silymarin are different from other iron chelators, probably due to antioxidant activity of silymarin, which causes pro-oxidant effect via iron-catalyzed oxidation with subsequent generation of reactive oxygen species [53].

On the other hand Adibi et al. [54] 2012 studied the therapeutic effects of deferoxamine and silymarin versus deferoxamine alone in β-thalassemia major based on findings of liver MRI in 37 cases of thalassemia major older than 12 years in 6 months treatment. Patients included in this study were divided into 2 groups; group I included 22 patients who were treated with desferroxamine and placebo and group II included 15 patients who were treated with desferroxamine and silymarin. The mean liver iron concentrations before therapy were 290 ± 62 µmol/g and 328 ± 27.8 µmol/g in group I and group II respectively while the mean liver iron concentrations after 6 months of intervention were 290.4 ± 65.4 µmol/g in group I and 334.6 ± 27.9 µmol/g in group II. No group experienced a significant inter group differences in LIC before and after the 6-months trial (p=0.43). They concluded that administration of silymarin did not cause significant changes in liver iron concentration and recommended evaluating a longer course of treatment with this drug to clarify their effects on reduction of liver iron concentration.

Table 1 shows comparison between different studies that were done to evaluate the therapeutic values of silymarin in iron chelation; three studies found that silymarin has good therapeutic effects as iron chelator through reduction of serum iron and serum ferritin while one study found that silymarin has no effect on reduction of liver iron concentration.

Toxicity of silymarin

Although silymarin has low oral absorption, oral dosages of 420 mg/day have shown some therapeutic potential, with good tolerability and largely free of adverse effects [54]. An average daily dose of silymarin (420 mg/day for 41 months) was found to be non-toxic, relative to placebo, in clinical trials [55]. Drug-drug interaction and liver toxicity by interference with co-drugs by induction or inhibition of cytochrome- P450 is a major concern for the use of silymarin [56]. Studies were performed to investigate the potential for hepatotoxicity,

cytochrome-P450 isoenzymes induction and inhibition on dry extract from *S. marianum*. The results indicated that interference or hepatotoxicity of the dry extract from *S. marianum* at the recommended maximum daily dose of 420 mg/day of silymarin which is equivalent to 210 mg silybin is unlikely and is considered safe [57].

Conclusion

From this review we concluded that, silymarin can be used as iron chelator in iron-loaded thalassemic children at the recommended safe daily dose of 420 mg/day of silymarin especially if combined with traditional iron chelating agents as desferroxamine or deferisorax.

Recommendations

From this review we can recommend extensive multicenter studies in large number of patients with longer duration of follow up and more advanced methods of assessment of iron status to clarify the exact role of silymarin in reduction of iron over load in thalassemic children and long term side effects due to lifelong use of iron chelators.

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