Send Orders for Reprints to reprints@benthamscience.ae

Infectious Disorders – Drug Targets, **2015,** *15,* **000-000**

Therapeutic Value of Combined Therapy with Deferiprone and Silymarin as Iron Chelators in Egyptian Children with Beta Thalassemia Major

Adel A. Hagag[1,*], Mohamed S. Elfaragy[1], Shaymaa M. Elrifaey[1] and Amal E. Abd El-Lateef[2]

[1]*Pediatrics and* [2]*Clinical Pathology Departments, Faculty of Medicine, Tanta University, Egypt*

A.A. Hagag

Abstract: *Background*: Beta Thalassemia is inherited anemia characterized by absent or reduced synthesis of β-globin chains of hemoglobin, caused by β-globin gene mutations resulting in chronic hemolytic anemia that requires 'repeated blood transfusion with resulting iron overload'. Silymarin has iron chelating activity in thalassemic patients with iron overload. *Aim of the work*: was to study the therapeutic value of combined therapy of Deferiprone and silymarin as iron chelators in Egyptian children with beta thalassemia with iron overload'. *Patients and Methods*: 'This study was conducted on 80 beta thalassemic children with their serum ferritin more than 1000 ng/ml who were divided into two groups'. Group I included 40 patients who were treated with oral Deferiprone and silymarin for 9 months. Group II included 40 patients who were treated with oral Deferiprone and placebo for 9 months. *Results*: 'There were no significant differences in serum ferritin, iron and TIBC between group I and group II before the study but after regular chelation therapy, serum ferritin and iron were significantly lower in group I than group II. No statistically significant differences in serum creatinine, blood urea, ALT, AST and bilirubin levels between Group I and Group II before and after chelation therapy were observed'. *Conclusion*: Deferiprone in combination with silymarin are better iron chelators than Deferiprone and placebo. *Recommendations*: 'Extensive multicenter studies in large number of patients with longer follow up period 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'.

Keywords: Deferiprone, iron overload, silymarin, Thalassemia.

INTRODUCTION

'Thalassemias are heterogeneous group of inherited anemias' [1]. β-thalassemias are 'characterized by absent or reduced synthesis of β-globin chains of hemoglobin', 'caused by mutations of β-globin gene' cluster with reduction of hemoglobin, decreased RBCs production and anemia [2, 3]. In Egypt, β-thalassemia 'is the commonest cause of chronic hemolytic anemia' representing a major public health problem. It is particularly common in populations of Upper Egypt and peoples of Delta and Red Sea Hill Region [4, 5].

The standard treatment for 'thalassemia is repeated blood transfusion which provides the patients with healthy red blood cells and can lead to iron overload' [6]. 'Iron loading in thalassemia depends on the volume of transfused blood and the amount accumulated from increased gastrointestinal iron absorption mediated by Hepcidin down-regulation and Ferroportin up- regulation [7, 8].

Hepcidin regulates 'iron transport across gut mucosa, thereby preventing excess iron absorption and maintaining normal iron levels within the body'. Ferroportin is a transmembrane protein that transports iron from inside to outside the cell [9]. As each unit of packed red cells contains about 200 mg iron, iron overload can occur easily in patients with repeated blood transfusion. For this reason, patients must undergo chelation therapy [10, 11] as 'excess iron is deposited in body organs' causing organ damage [12].

*Address correspondence to this author at the 16 Elemam Moslem street branched from Elhelw Street, Tanta, Gharbia, Egypt; Tel: 01005020768; E-mail: adelhagag20@yahoo.com

1871-5265/15 $58.00+.00

Deferiprone is bidentate oral 'iron chelator that began clinical trials in UK in 1980. 'It was first licensed for use in thalassaemia in India, followed by European Union and other countries outside US and Canada, in 1990s' [13]. Deferiprone (Ferriprox) gained FDA approval in October 2011. It is approved in 65 countries [14]. Some studies show no differences between Deferiprone and Desferrioxamine [15]. Deferiprone daily dose that had been evaluated most thoroughly is 75 mg/kg/day, given in three divided doses [16].

Silymarin, an extract from the Silybum marianum (milk thistle) plant containing various flavonolignans (with silybin being the major one), has received major attention over the last decade as a herbal remedy for acute and chronic liver and gall bladder disorders. 'It is well known to possess a strong antioxidant and hepatoprotective effects' [17]. 'There are some studies that were designed to investigate the therapeutic activity of silymarin as adjuvant iron chelator in patients with thalassemia under conventional iron chelation therapies' [18, 19].

AIM OF THE WORK

'The aim of this work was to study the therapeutic value of combined therapy with Deferiprone and silymarin as iron chelators in Egyptian children with beta thalassemia major with iron overload'.

PATIENT AND METHODS

This study was performed after research ethical committee approval and informed written parental consent from all participants and was conducted on 80 beta thalassemic children who were attendants to 'Hematology Unit, Pediatric Department, Tanta University Hospital in the period between' October 2012 and October 2014. Patients included in this study have serum ferritin levels more than 1000 ng/ml and were divided into two groups by simple random allocation, Group I received combination of daily oral Deferiprone 75 mg/kg/day divided into three doses [16] and 'oral silymarin in the form of Legalon tablets 140 mg, one hour before each meal (3 times daily) for 9 months' [20] while group II received daily oral Deferiprone 75 mg/kg/day divided into three doses and placebo for 9 months.

Inclusion Criteria

Children with β-thalassemia major with serum ferritin of more than '1000 ng/ml who did not receive any iron chelation therapy before the start of this study'.

Exclusion Criteria

β-thalassemic children with 'serum ferritin < 1000 ng/ml or who received any iron chelation therapy before the start of this study' or who used the drugs in irregular manner during this study or who suffered from hepatitis (A or B or C) before or during this study.

'All the children in both groups were subjected to the following':

1) 'Complete history taking with special attention to onset of thalassemia, chelation therapy, frequency of blood transfusion'.
2) 'Thorough clinical examination with special attention to pallor, jaundice, mongoloid facies, hepatomegaly and splenomegaly or splenectomy'.
3) 'Laboratory investigations including':
   - 'Complete blood count'.
   - 'Hemoglobin electrophoresis'.
   - 'Liver functions including serum bilirubin level, alanine transferase (ALT) and aspartate transferase (AST)'.
   - 'Renal functions including blood urea and serum creatinine'.
   - 'Serum iron status including serum ferritin, iron and iron binding capacity'.
   - 'Liver and renal functions and serum iron status were done twice in studied patients; once before the start of chelation therapy and once after 9 months of chelation therapy'.

Specimen Collection and Handling

Four ml of venous blood were collected 'using sterile needles through gentle venipuncture after sterilization of site of puncture by alcohol, and collected samples were divided into'; 2 ml in a plain glass tube which was used for estimation of serum iron, ferritin and TIBC [21-24], one ml was delivered on 20 uL EDTA solution for complete blood count including reticulocytic count and differential WBCs which was done on leishman stained peripheral blood smear with evaluation using ERMA

PCE-210 N cell counter [25] and one ml was added to 2 ml hemolysate for Hb electrophoresis [26].

Statistics

'Statistical analysis of the present study was conducted, using the mean, standard deviation and chi-square test by SPSS Version 16' [27].

RESULTS

'There were no significant differences between group I and group II regarding age, sex, age of onset of thalassemia, frequency of blood transfusion, clinical presentations' and pre-transfusion hemoglobin levels, WBCS and platelets counts (Table **1**).

Table 1. Clinical and laboratory data of the studied Patients at the start of the study.

History	Group I (No=40)	Group II (No=40)	ANOVA	
			F	P
Age (months) Range Mean ± SD	 60-84 6.10 ± 1.61	 62-90 6.68 ± 1.64	2.04	0.11
Sex Males Females	 20 20	 18 22	0.100	0.752
Age of onset of thalassemia (months) Range Mean ± SD	 6-36 11.5 ± 4.3	 6-36 10.45 ± 4.13	0.63	0.97
Age of 1st transfusion (months) Range Mean ± SD	 6-36 10.93 ±10.96	 6-36 10.60 ± 6.67	0.52	0.66
Inter-transfusion interval (days) Range Mean ± SD	 21-42 25.67 ± 6.51	 21-49 31.67 ± 8.99	1.25	0.29
Frequency of blood transfusion Every 2 week Every 3 week Every 4 week Every 6 week	 8 cases 20 cases 10 cases 2 case	 10 cases 10 cases 18 cases 2 case	2.921	0.404
Clinical data	**Number (%)**	**Number (%)**	**Chi-square**	
			X^2	P
Pallor	40 (100%)	40 (100%)	0.00	1.00
Jaundice	23 (57.5%)	25(62.5%)	5.53	0.13
Hepatomegaly	40(100%)	40(100%)	0.00	1.00
Splenomegaly	31 (77.5%)	32 (80%)	0.74	0.86
Splenectomy	9 (22.5%)	8 (20%)	1.27	0.73
Laboratory data				
Hemoglobin (gm /dl) Range Mean ± SD	 7-8 7.3 ± 0.32	 7-8.8 7.72 ± 0.60	0.93	0.63
WBCS (thousands/ mm^3) Range Mean ± SD	 5.9-15.2 7.43 ±2.22	 5.5-22 7.73 ±2.31	0.97	0.09
Platelets (thousands/ mm^3) Range Mean ± SD	 364.4-619 425.5 ± 35.39	 369-699 421.20 ± 25.71	0.24	0.85

Table 2. Renal and hepatic functions and iron status in studied patients 'before and after chelation therapy'.

	Group I (No=20)	Group II (No=20)	t value	p value
Serum creatinine (mg/dl)				
Before (mean ± SD)	0.63±0.11	0.62±0.10	t1. 0.07	p1. 0.94
After (mean ± SD)	0.59±0.07	0.61±0.07	t2. 2.0	p2. 0. 48
t3 value	1.24	1.55		
p3 value	0.22	0.13		
Blood urea (mg/dl)				
Before (mean ± SD)	35.30±6.25	33.75±6.4		
After (mean ± SD)	36.95±5.44	35.45±5.47	t1. 1.06	p1. 0.29
t3 value	2.31	1.93	t2. 0.98	p2. 0.33
p3 value	0.32	0.68		
ALT(U/L)				
Before (mean ± SD)	26.25±3.64	26.30±2.71	t1. 0.84	p1. 0.93
After (mean ± SD)	27.50±3.16	28.20±3.63	t2. 1.42	p2. 0.17
t3 value	0.90	2.83		
p3 value	0.73	0.11		
AST(U/L)				
Before (mean ± SD)	22.65 ±4.51	23.75±5.85		p1. 0.40
After (mean ± SD)	23.7±3.77	24±9.4.05	t1. 0.84	p2. 0.26
t3 value	0.50	0.47	t2. 1.14	
p3 value	0.62	0.64		
Total bilirubin (mg/dl)				
Before (mean ± SD)	3.19±0.40	3.15±0.43	t1. 0.82	p1. 0.41
After (mean ± SD)	3.23±0.51	3.24±0.47	t2. 0.09	p2. 0.92
t3 value	0.54	0.53		
p3 value	0.44	0.35		
Ferritin (ng/ml)				
Before (mean ± SD)	1901±563.38	1885.2±510.54	t1 0.41	p1. 0.68
After (mean ± SD)	989.5±178.57	1260± 212.26	t2. 3.99	p2 0.000*
t3 value	6.53	5.40		
p3 value	0.000*	0.000*		
Iron (ug/dl)				
Before (mean ± SD)	236.40±34.68	227.7±35.49	t1. 2.20	p1. 0.40
After (mean ± SD)	156.55±21.42	172±40.24.51	t2. 6.33	p2. 0.04*
t3 value	0.7.91	5.72		
p3 value	0. 000*	0.000*		
TIBC (ug/dl)				
Before (mean ± SD)	192.45±10.53	191.85±9.81	t1. 0.29	p1. 0.77
After (mean ± SD)	275.20±10.08	265.9±9.19	t2. 2.33	p2. 0.03*
t3 value	24.18	21.72		
p3 value	0.000*	0.000*		

*Significant. t1 comparison between group I and group II before chelation therapy, t2 comparison 'between group I and group II after chelation therapy and t3 comparison between the same group (as group I or II before and after chelation therapy)'.

'There were no statistically significant differences in serum creatinine, blood urea, serum bilirubin, ALT and AST between Group I and Group II before and after chelation therapy' (Table **1**).

ʻThere were no statistically significant differences in serum ferritin, iron and TIBC between group I and group II before chelation therapy' while there were significantly lower serum ferritin and iron and higher TIBC in group I than group II after chelation therapy (Table **2**).

DISCUSSION

ʻThalassemias are the most common chronic hemolytic anemia in Middle East [28]. Silymarin is an herbal drug with antioxidant and free radical scavenging abilities. It also acts as an iron chelator by binding Fe (III) [20]. This study was carried out on 80 β-thalassemic children who were divided into two groups'; group I included 40 children with beta thalassemia who were treated with combination of oral Deferiprone and silymarin and group II included 40 children with beta thalassemia who were treated with oral Deferiprone and placebo for 9 months.

In the current study, there were no significant differences in serum levels of ferritin, iron and TIBC before the start of chelation between group I and group II but after chelation therapy, there were significantly lower serum ferritin and iron and significantly higher TIBC in group I than group II.

ʻThis is in agreement with Gharagozloo *et al*. 2009 [18] who assessed the efficacy of silymarin and Desferroxamine compared with Desferroxamine alone in removing excess iron in 48 patients with beta thalassemia and found significantly lower serum ferritin and iron and significantly higher TIBC in patients who received silymarin and Desferroxamine than patients who received Desferrioxamine alone', Moayedi *et al*. 2013 [29] who studied ʻtherapeutic effects of silymarin' in patients with β-thalassemia. Patients in their study were divided into 2 groups; group I included 49 patients treated with Desferrioxamine and silymarin' and group II included 48 patients treated with Desferrioxamine and placebo for 9 months and they found significant reduction of serum ferritin in patients receiving silymarin therapy than patients receiving placebo', and Hagag *et al.*, 2013 [19] who compared combination therapy of silymarin and Exjade with Exjade and placebo in 40 patients with thalassemia major and found significant reduction in serum ferritin and iron in patients who received silymarin and Exjade than patients who received Exjade alone.

On the other hand; Adibi, *et al.*, 2012 [30] found no significant changes in liver iron concentration after silymarin use and they recommended to evaluate this drug by longer course of treatment to clarify its effects on reduction of liver iron concentration.

Variation between our study and Adibi, *et al*. study could be explained by different mode of evaluation of serum iron status (serum ferritin in our study versus liver iron concentration in Adibi, *et al*. study) and variation in severity of iron load and duration of silymarin therapy between both studies.

ʻIn this study, there were no significant differences in renal and liver functions in group I before and after chelation therapy. This is in agreement with Gharagozloo, *et al.*, 2009' [18], Moayedi *et al.*, 2013 [29] and Hagag *et al.*, 2013 [19] who concluded that; ʻthalassemic patients with iron overload can be safely treated with combination of silymarin and Desferrioxamine or silymarin and Exjade with no detectable abnormalities in complete blood count, liver and renal functions due to silymarin use'.

Little is known about the biochemical mechanisms of action of silymarin [17]. But it may be due to reduction of iron absorption as concluded by Hutchinson *et al*. 2010 [31] who studied the effects of silybin in reduction of iron absorption in 10 homozygous patients for hemochromatosis. ʻPatients in their study consumed a vegetarian meal containing 13.9 mg iron with: 200 ml water; 200 ml water and 140 mg silybin; or 200 ml tea. Blood was drawn once before, then 0.5, 1, 2, 3 and 4 h after meal. Consumption of Silybin reduces the postprandial increase in serum iron compared with water ($P<0.05$) and tea ($P<0.05$) and they concluded that silybin has the potential to reduce iron absorption, and could be used as adjuvant treatment in patients with hemochromatosis' [31].

Improvement in patients group receiving silymarin in our study may be due to the antioxidant property of silymarin that lead to direct scavenging of free radicals and chelating free Fe and Cu which is mainly effective in the gut and may decrease iron absorption [32] or may be explained by the changes of serum hepcidin after silymarin use in patients with thalassemia as found in Moayedi *et al.*, 2013 study [29].

Hepcidin is a key regulatory hormone of iron homeostasis produced by hepatocytes, in response to iron loading [33]. Increased hepcidin release in thalassemia major generates a negative feedback loop [34] that inhibits intestinal iron absorption and iron release from hepatic stores and from macrophages [35]. Increased hepcidin in thalassemia major could be explained by regular transfusion therapies which suppress the erythropoietic drive and increase body iron load, both of which increase hepcidin level [36].

CONCLUSION

'From this study we concluded that, Deferiprone and silymarin are better iron chelators in thalassemic children with iron overload than Deferiprone and placebo'.

RECOMMENDATIONS

'Extensive multicenter studies in large number of patients with longer follow up period and more advanced methods of assessment of iron status are recommended to clarify the exact role of silymarin in reduction of iron over load in children with beta thalassemia'.

STUDY LIMITATION

There are some limitations to give recommendations for routine use of silymarin as iron chelator in children with beta thalassemia from this study including:

- Small sample of the patients included in this study that needs to be expanded to include large number of patients in multicenters studies.
- Duration of follow up must be longer to assess the side effects of silymarin in prolonged use and to give valid conclusions and recommendations as the use of iron chelation in thalassemic patients is lifelong.
- Method of assessment of iron overload in this study is only serum ferritin and serum iron which may be not enough for assessment of iron load in thalassemic patients and therefore it is better to use other methods of assessment of iron overload as liver iron concentration and hepatic MRI, which till now are not widely used in Egypt, to detect the actual improvement of iron overload with silymarin use.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGMENTS

Many thanks for patients of thalassemia who participated in this work, hoping for them good health and happy life.

REFERENCES

[1] Miccio, A.; Cesari, R.; Lotti, F. The cure of thalassemia by bone marrow transplantation. *Blood Rev.*, **2007**, *16*, 81-85.

[2] Surapon, T. Thalassemia syndrome. In: Advances *in the study of genetic disorders*; Ikehara, K., Ed.; 1st edition. In Tech. Croatia, **2011**; Ch.6, pp.102-137.

[3] Galanello, R.; Origa, R. Beta-thalassemia. *Orphanet J Rare Dis*., **2010**, *5*, 11-15.

[4] El Dakhakhny, A.M.; Hesham, M.A.; Mohamed, S.E.; Mohammad, F.N. Quality of life of school age thalassemic children at Zagazig City. *Journal of American Science*, **2011**, *7* (1), 186-197.

[5] Shawky, R.M.; Kamal, T.M. Thalassemia intermedia: An overview. *Egyptian Journal of Medical Human Genetics*, **2012**, *13*(3), 245-255.

[6] Pathare, A.; Taher, A.; Daar, S. Practical chelation protocol based on stratification of thalassemia patients. *Ann Hematol.*, **2010**, *89*(4), 405-409.

[7] Crisponi, G.; Remelli, M. Review: Iron chelating agents for the treatment of iron overload. *Coordin Chem Rev*., **2008**, *252* (11), 1225-1240.

[8] Zhao, N.; Zhang, A.S.; Enns, C.A. Iron regulation by hepcidin. *J Clin Invest.*, **2013**, *123*(6), 2337-43.

[9] Gardenghi, S.; Marongiu, M.F.; Ramos, P.; Guy, E.; Breda, L.; Chadburn, A.; Liu, Y.; Amariglio, N.; Rechavi, G.; Rachmilewitz, E.A.; Breuer, W.; Cabantchik, Z.I.; Wrighting, D.M., Andrews, N.C.; de Sousa, M.; Giardina, P.J.; Grady, R.W.; Rivella, S. Ineffective erythropoiesis in β-thalassemia is characterized by increased iron absorption mediated by down-regulation of Hepcidin and up-regulation of Ferroportin. *Blood*, **2007**, *109*(11), 5027-5035.

[10] Prabhu, R.; Prabhu, V.; Prabhu, R.S. Iron overload in beta thalassemia-a review. *J Biosci Tech*., **2009**, *1*(1), 20-31.

[11] Kattamis, A.; Ladis, V., Berdousi, H.; Kelekis, N.L.; Alexopoulou, E.; Papasotiriou, I.; Drakaki, K.; Kaloumenou, I.; Galani, A.; Kattamis, C. Iron chelation treatment with combined therapy with deferiprone and deferroxamine: A 12-month trial. *Blood Cells Mol Dis.*, **2006**, *36*(1), 21-25.

[12] Aessopos, A.; Berdoukas, V.; Tsironi, M. The heart in transfusion dependent homozygous thalassaemia today: Prediction, prevention and management. *Eur J Haematol*., **2008**, *80* (1), 93-106.

[13] Kwiatkowski, J.L. Oral iron chelator. *Pediatr Clin N Am.*, **2008**, *55*(2), 461-482.

[14] Traynor, K. Deferiprone approved for iron overload. *Am J Health Syst Pharm.,* **2011**, *68*(22), 2106.

[15] Ha, S.Y.; Chik, K.W.; Ling, S.C.; Lee, A.C.; Luk, C.W.; Lam, C.W.; Ng, I.O.; Chan, G.C. A randomized controlled study evaluating the safety and efficacy of deferiprone treatment in thalassemia major patients from Hong Kong. *Hemoglobin*, **2006**, *30*(2), 263-274.

[16] Cappellini, M.D.; Musallam, K.M.; Taher, A.T. Overview of iron chelation therapy with desferrioxamine and deferiprone. *Hemoglobin*, **2009**, *33*(1), 58-69.

[17] Valenzuela, A.; Garrido, A. Biochemical bases of the pharmacological action of the flavonoid silymarin and of its structural isomer silibinin. *Biol Res.*, **2002**, *27*, 105-112.

[18] Gharagozloo, M.; Moayedi, B.; Zakerinia, M.; Hamidi, M.; Karimi, M.; Maracy, M.; Amirghofran Z. Combined therapy of silymarin and desferrioxamine in patients with beta-thalassemia major: A randomized double-blind clinical trial. *Fundam Clin Pharmacol*., **2009**, *23*(3), 359-65.

[19] Hagag, A.A.; Elfrargy, M.S.; Gazar, R.A.; Abd El-Lateef A.E. 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, DOI: 10.4084/ MJHID.2013.065.

[20] Gharagozloo, M.; Khoshdel, Z.; Amirghofran, Z. The effect of an iron chelator, silybin, on the proliferation and cell cycle of Jurkat cells: A comparison with desferrioxamine. *Eur J Pharmacol*., **2008**, *589*(1-3), 1-7.

[21] Dawson, D.W. The accuracy and clinical interpretation of serum ferritin assays. *Clin Lab Haematol*., **1992**, *14*(1),47-52.

[22] Bishop, N.L.; Fudy, E.P.; Schoeff, L. Priniciples and correlations In: *Clinical chemistry*; Harris, N., Winter, W., Eds.; 5th edition, Lippinocott Williams and Wilkins: Philadelphia, **2000**, pp.180-220.

[23] Peters, T.; Giovanniello, T.J.; Apt, L.; Ross J.F. A simple improved method for the determination of serum iron. *J Lab Clin Med*., **1956**, *48*(2), 280-8.

[24] Beard, J.L. Iron Biology in immune function, muscle metabolism and neuronal functioning. *J Nutr*., **2001**, *131*, 568-580.

[25] Monica, C. Strategy of blood safety in Africa region. In: *District laboratory practice in tropical countries*; Tito, A., Lewis, S.M., Eds.; 2nd edition. Cambridge low price Egyptian edition **2005**; chapter 4, pp. 268-378.

[26] Schedlauer, L.M.; Pass, K.A. Cellulose acetate/ citrate agar electrophoresis of filter paper hemolysates from heel stick. *Pediatrics*, **1989**, *83*(5 Pt 2), 839-42.

[27] Levesque, R. SPSS programming and data management: *A guide for SPSS and SAS Users*, 4th edition, SPSS Inc, Chicago, **2007**.

[28] Khalifa, A.S.; Baffico, M.; Heshmat, N.M. Relationship between the hematological phenotype and type of beta thalassemic mutation in Egyptian Children. *Egy J Hematol*., **1995**, *20*(2), 103-137.

[29] Moayedi, B.; Gharagozloo, M.; Esmaeil, N.; Maracy, M.R.; Hoorfar, H. A randomized double-blind, placebo-controlled study of therapeutic effects of silymarin in β-thalassemia major patients receiving desferrioxamine. *European Journal of Hematology*, **2013**, *90*(3), 202-209.

[30] Adibi, A.; Azin, S.H.; Behjat, S.M. Therapeutic effects of deferoxamine and silymarin versus deferoxamine alone in β-thalassemia major based on findings of liver MRI. *J Res Med Sci*., **2012**, (1), 73-78.

[31] Hutchinson, C.; Bomford, A.; Geissler, C.A. The iron-chelating potential of silybin in patients with hereditary haemochromatosis. *Eur J Clin Nutr*,, **2010**, *64*(10), 1239-41.

[32] Peter, F. Surai, Silymarin as a Natural Antioxidant: An Overview of the Current Evidence and Perspectives. *Antioxidants*, **2015**, *4*(1), 204-247.

[33] Park, C.H.; Valore E.V.; Waring A.J.; Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. *J Biol Chem*., **2001**, *276*(11), 7806-10.

[34] Ramos, E.; Kautz, L.; Rodriguez, R.; Hansen, M.; Gabayan, V.; Ginzburg Y.; Roth, M.P.; Nemeth, E.; Ganz T. Evidence for distinct pathways of hepcidin regulation by acute and chronic iron loading in mice. *Hepatology*, **2011**, *53*(4), 1333-41.

[35] Ganz, T. Hepcidin in iron metabolism. *Curr Opin Hematol*., **2004**, *11*(4), 251-4.

[36] Papanikolaou, G.; Tzilianos, M.; Christakis, J.I.; Bogdanos, D.; Tsimirika, K.; MacFarlane, J.; Goldberg, Y.P.; Sakellaropoulos, N.; Ganz, T.; Nemeth, E. Hepcidin in iron overload disorders. *Blood*, **2005**, *105*(10), 4103-4105.

Received: January 17, 2015 **Revised: July 30, 2015** **Accepted: July 30, 2015**