

## The Antioxidant Effects of Capparis Ovata and Deferasirox in Patients with Thalassaemia Major

Handan Duman<sup>1</sup>, Duran Canatan<sup>1\*</sup>, Güçhan Alanoglu<sup>2</sup>, Recep Sutçu<sup>3</sup> and Tufan Nayır<sup>4</sup>

<sup>1</sup>Department of Pediatric Hematology, Suleyman Demirel University, Turkey

<sup>2</sup>Department of Adult Hematology, Suleyman Demirel University, Turkey

<sup>3</sup>Department of Biochemistry, Suleyman Demirel University, Turkey

<sup>4</sup>Department of Public Health, Suleyman Demirel University, Turkey

### Abstract

Iron overload and auto-oxidation of unpaired globin chains is the main cause of oxidative stress in thalassaemia. We aimed to show the additive antioxidant effect of capparidaceae and deferasirox in thalassaemic patients. A total number of 40 thalassaemia major patient aged between 7-30 years, who have been taken regular red cell 15 cc/kg/month to maintain Hb >10 gr/dl and chelation (30 mg/kg/day ICL-670) for one year are involved. They were divided into two groups as control and study group randomly. Both study and control groups were followed by regular transfusion and chelation therapy. In addition study group has been taken capparid marmalade at the breakfast with a dose of a dessert-spoon (12.5 gr) younger than 10 years and a soup-spoon (25 gr) older than 10 years for 6 months. Hematological and biochemical parameters, ferritin at every month and oxidative-antioxidant status (MDA, CAT, Gpx, SOD) were measured at the beginning and at the end of the study. Serum ferritin and MDA levels declined significantly in both groups (for ferritin; control group p=0.00; study group p=0.00) during the study but a much more decrease occurred at MDA levels in the capparid given group (p=0.02). There was no statistically significant difference between the groups at the initial and last SOD, CAT, GPX, SOD levels. Further more in the study group a significant decrease in liver function tests has been occurred (AST p= 0.05, ALT p= 0.01). The high levels of MDA in iron overloaded thalassaemic patients is the best marker of oxidative stress. Generally decreased iron burden was associated with decreased oxidant damage. In vitro it was shown that iron chelators such as deferoxamine and deferiprone neutralize intracellular free iron and inhibit oxidation. Our findings suggest that combination of capparid with deferasirox may have additive effect on decreasing the oxidative damage and hepatotoxicity.

**Keywords:** Thalassaemia; Antioxidants; Capparis ovata

### Introduction

Iron overload is one of the contributing causes of oxidative stress in thalassaemic patients. As iron exceeds transferrin capacity a low molecular weight iron called non-transferrin bound iron (NTBI) and its portion called labile plasma iron (LPI) occurs and causes production of oxygen-free radicals resulting in depletion of protective antioxidants. Also auto-oxidation of globin chains and premature hemolysis of red cells enhances the oxidative damage at thalassaemic patients [1-3]. Oxidative stress induces apoptosis, leakage of protons from mitochondria and increases oxygen depletion causing damage to cell and organelle membranes. The long term clinical consequences of this process are heart failure, liver fibrosis or cirrhosis and endocrinopathies. The balance between the prooxidant and antioxidant levels becomes impaired while a decrease occurs in levels of antioxidant enzymes, an increase occurs in levels of MDA which is a good marker of lipid peroxidation [4].

Treatment strategies of thalassaemic patients are regular transfusion and chelation therapy to main aim is to reduce iron overload and NTBI. There are so many studies about thalassaemia and the effects of antioxidants on thalassaemia patients. In recent years use of specially plant origin antioxidants, such as fermented papaya preparation has been shown to reduce the oxidative stress and damage on several organs [3]. Silymarin, a flavonoid, acts as iron chelator and also increases intracellular glutathione content, suggesting to restore cellular antioxidant defences [5]. Oral vitamin E supplies an amelioration at rate of antioxidant/oxidant levels and inhibition at lipid peroxidation in patients with thalassaemia [6].

Capparis, a member of Capparidaceae family, is used in phytomedicine as anti-oxidative, hypolipidemic, anti-inflammatory and anti-hepatotoxic agent. Its buds contain lipids, alkaloids, flavonoid and polyphenols. It is known that the antioxidant effect of capparid is formed by the composition of flavonoids and polyphenols in its compound [7-10]. In a study done by Yadav et al it has been shown that capparid decidua extracts possess antihepatotoxic activity against alloxan induced hepatotoxicity and furthermore a decrease in oxidative stress has been observed [11]. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid in normal and streptozotocin-induced diabetic Wistar rats studied. A decrease in fasting blood glucose level and an increase in insulin level observed. Additionally a decrease in lipid peroxidation products and an increase in both enzymatic and non enzymatic antioxidant levels observed [12].

It has been shown that iron chelator agents have antioxidant effect on thalassaemic patients. After incubation of thalassaemic red cells with deferiprone it has been observed that MDA levels decreased [13]. Walter et al. [14] have compared the antioxidant effectiveness of

\*Corresponding author: Duran Canatan, Department of Pediatric Hematology, Suleyman Demirel University, Turkey, E-mail: [dcanatan@superonline.com](mailto:dcanatan@superonline.com)

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deferasirox and desferrioxamine and found a decrease in MDA levels in both groups and a positive correlation with serum ferritin levels and a reverse correlation with vitamin and alpha-tocopherol levels.

Under the highlights of these findings we hypothesize that an improvement in oxidative status of patients with thalassaemia would be possible with the combination of antioxidant plant and new oral chelator deferasirox. In the present study, we aimed to investigate the antioxidant effects of capparidaceae and deferasirox in thalassaemic patients.

## Material and Methods

### Capparis

Capparidaceae contains the following (g/mg/mcg per 100 gr) Dried: Protein = 21 g. Fat = 1.6 g. Calcium = 123 mg. Fe = 6.8 mg. Beta carotene = 165 mcg Vitamin B1 = .02 mg. Vitamin B2 = .03 mg. Niacin = 8.8 mg. Vitamin C = 5 mg. Kcal = 341; Cooked: Protein = 5.4 g. Fat = 0.2 g. Calcium = 33 mg. Fe = 2.8 mg. Beta carotene = 25 mcg. Vitamin B1 = .01 mg. Kcal = 92. Un-debittered: Protein (crude) = 29.3%. Oil = 0.7%. Ash = 3.5%. Fibre (crude) = 2.7%. Carbohydrate (soluble) (starch) = 39.5%; (sugars): Sucrose = 4.3%. D-glucose = 0.2%. D-fructose = 0.7%. Amino acids (g [16g N]<sup>-1</sup>): Aspartic acid = 7.7 g. Threonine = 1.7 g. Serine = 2.3 g. Glutamic acid = 9.0 g. Proline = 6.5 g. Glycine = 3.5 g. Alanine = 3.2 g. Valine = 4.5 g. Cysteine (performic acid oxidation) = 1.3 g. Methionine (performic acid oxidation) = 1.8 g. Isoleucine = 2.9 g. Leucine = 7.0 g. Tyrosine = 2.3 g. Histidine = 1.3 g. Lysine = 1.5 g. Arginine = 15.1 g. Minerals: Sulphur = 2.20 mg/kg<sup>-1</sup> (dry). Potassium = 0.15%<sup>-1</sup> (dry). Magnesium = 0.10% (dry). Calcium = 0.14% (dry). Na = 0.01% (dry). K = 1.03 mg/kg% (dry). Zinc = 42 mg/kg<sup>-1</sup> (dry). Iron = 10.5 mg/kg<sup>-1</sup> (dry). Manganese = 17 mg/kg<sup>-1</sup> (dry). Copper = 8 mg/kg<sup>-1</sup> (dry) [15]. Capparis marmelade was prepared from dried capparidaceae and presented to patients by company.

### Patients

Randomized, double blind and prospective study was done at Suleyman Demirel University, Department of Pediatric Hematology. The study was reviewed and approved by the Ethical Committee of Suleyman Demirel University Faculty of Medicine. The study was carried out in accordance with the ethical standards laid down in the World Medical Association Declaration of Helsinki. A total number of 40 patients with thalassaemia major aged between 7-30 years, who are on regular red cell transfusion protocol (Red cell: 15 cc/kg/month to maintain Hb >9-10 gr/dl) and chelation therapy since one year (Deferasirox: 20 mg/kg/day) were included in this study. They were divided in two groups as control and study group randomly. Study group had capparidaceae marmelade before the breakfast with a dose of a dessert-spoon (12.5 gr) who is younger than 10 years and a soup-spoon (25 gr) who are older than ten years for 6 months in addition to deferasirox therapy. Control group was only taken regular deferasirox therapy. In study group there were 13 girls and 7 boys. The mean age was 18.08 ± 5.2. In control group there were 12 girls and 8 boys and the mean age was 19.08 ± 6.2. Patients with hepatic or cardiac failure and diabetes mellitus were excluded from study.

### Methods

We measured hematological and biochemical parameters, ferritin at every month and oxidative-antioxidant status (MDA, CAT, Gpx, SOD) at the beginning and at the end of the study. Blood samples were taken as late as possible, at least 3-4 weeks after the last transfusion. For hemogram and antioxidant levels 2 ml venous blood with EDTA

and for biochemical parameters and ferritin 4 ml venous blood was taken to simple polystyrene tubes. Hemogram, biochemical parameters and ferritin levels were studied at the same day and the samples for the antioxidant study stored at -80°C and analyzed by spectrophotometric method. MDA levels were evaluated with Drapper and Hadley's method [16], SOD levels with Wolliam's method [17], CAT with Aebi's method [18] and Gpx with Paglia and Valentine's method [19].

### Statistical Analysis

We used Mann-Whitney U test for comparing control and study group and Wilcoxon test for comparing the levels before and after therapy with capparidaceae. The values of p<0.05 were accepted as significant.

### Results

At the beginning of the study MDA levels were higher in the study group than the control group (p=0.023) and after the use of capparidaceae MDA levels decreased both in the study and control groups (p=0.02, 0.05, respectively, (Table 1) and significance between two groups regressed. (p=0.817). A significant decrease in SOD concentration in both of the groups was obtained but there was no statistically significant difference between the groups. (p=0.146) A minimal increase in Gpx (p=0.511) and not statistically significant decrease in catalase concentration was found (p=0.838) (Table 2).

At the beginning of the study AST levels were 97.3 ± 75.7 and after 6 month use of capparidaceae the levels were measured as 58.5 ± 23.5 and this decrease was statistically significant. (p=0.05) At the control group AST levels were 73.4 ± 48.4 and after 6 month the levels were 51.6 ± 21.1 and this decrease was not statistically significant (p=0.08).

Baseline ALT levels were 129.0 ± 123.3 and after the use of capparidaceae a significant decrease also occurred in study group (p=0.01) and there was no statistically significant decrease in control group (p=0.12) (Table 3).

As shown in Table 4, at baseline ferritin levels were high both in control and study group and after 6 month usage of level ferritin level declined significantly both in control and study group (p=0.00, p=0.00 respectively, (Table 4).

### Discussion

Prematurely denatured erythrocytes, increased iron absorption and iron accumulation due to transfusions causes inevitably enlargement of non-transferrin bound iron and labile plasma iron pool in thalassaemic patients [2,20]. NTBI was found in patients whose transferrin saturation was higher than 45 % and LPI at levels higher than 85%. This proportion of iron generates reactive oxygen radicals by Fenton reaction causing peroxidative damage to cell and organelle membranes, in long term to cell death. Previous studies have demonstrated the increased oxidative injury and insufficiency in antioxidant cellular defence mechanisms in thalassaemic patients. Also auto-oxidation of globin chains, intramedullary ineffective erythropoiesis and low levels of adult hemoglobin enhance the oxidative damage [21,22]. It is

	MDA at the beginning level	MDA at the ending level	p**
control group	118.08 ± 41.4	71.35 ± 34.3	0.05
study group	160.46 ± 62.5	84.59 ± 52.7	0.02
p*	0.023	0.817	

\* Mann-Whitney U \*\* Wilcoxon test

Table 1: MDA levels in patients with thalassaemia major.

	SOD at the beginning level	SOD at the ending level	p**	Catalase at the beginning level	Catalase At the ending level	p**	Gpx-at the beginning level	Gpx- At the ending level	p**
control	2027.7 ± 427.0	1232.1 ± 356.1	0.00	423.7 ± 318.1	276.9 ± 250.8	0.278	59.0 ± 12.4	62.3 ± 8.5	0.184
study	1858.3 ± 370.2	1053.1 ± 267.3	0.00	366.8 ± 280.9	240.2 ± 172.0	0.133	55.2 ± 11.4	60.1 ± 15.4	0.526
p*	0.177	0.146		0.569	0.838		0.298	0.511	

\* Mann-Whitney U \*\* Wilcoxon test

Table 2: SOD, Catalase and GPX levels in patients with thalassemia major.

	AST at the beginning level	AST at the ending level	p**	ALT at the beginning level	ALT at the ending level	p**
control	73.4 ± 48.4	51.6 ± 21.1	0.08	87.6 ± 69.5	56.5 ± 30.6	0.12
study	97.3 ± 75.7	58.5 ± 23.5	0.05	129.0 ± 123.3	62.3 ± 28.0	0.01
P*	0.224	0.398		0.174	0.461	

\* Mann-Whitney U \*\* Wilcoxon test

Table 3: AST and ALT levels in patients with thalassemia major.

believed that oxidative stress aggravates the symptoms of many diseases including hemolytic anemias as beta thalassemias [3].

Furthermore chelating excess iron using antioxidants to ameliorate the oxidative stress in thalassemics are the novel treatment approach for supportive therapy. Tesoriere et al. administered 600 mg/day vit E to 15 thalassemia intermedia patient resulted in decrease at MDA levels and increase in vit E levels for 3 months [6]. There are so much trials investigating antioxidant effects of different plant flavonoids such as polyphenols, rutin and curcumin. It has been known that rutin has protective effect on cytosol and curcumin protects red cell membranes and tea polyphenols have protection effect on both membranes and cytosol [23]. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) which are components of green tea have the ability of iron binding and free radical scavenging activities as they also have decretion effect on the the level of NTBI at iron overloaded erythrocytes [24]. Ounjaijean et al. [25], showed a decrease in iron levels of plasma and a decrease in formation of red cell ROS in rats challenged with iron. Another study in which fermented papaya preparation is used in thalassemic patients also showed an increase at glutathione content of red blood cells, platelets and polymorphonuclear leukocytes, and reduced ROS, membrane lipid peroxidation resulting in reduced sensitivity to hemolysis and phagocytosis by macrophages and improved PMN ability to generate oxidative burst [26].

Previous studies have shown that serum malonyldialdehyde (MDA) levels which is an end product of lipid peroxidation, was higher in thalassemic patients than healthy controls accompanied by depletion of antioxidant levels [27-29]. One study correlated serum MDA levels with ferritin values, suggesting high iron levels produces oxidative injury to cells [26]. Similarly in the present study increased levels of ferritin and MDA values was found in both of the groups at the beginning of the study showing our patients exposed to high iron overload condition and oxidative stress. After six month in at both groups serum ferritin levels decreased significantly (control p=0.00, study p=0.00) as they were under treatment of deferasirox. As ferritin levels decreased, oxidative stress decreased too but a more significant improvement occurred at MDA levels in the at study group (control p=0.05, study p=0.02) indicating cappariss restored the oxidative environment. It is known that cappariss is a rich source of antioxidant phytochemicals such as flavonoids [30,31]. These flavonoids enter in to lipid bilayer of the cell membranes acting as anti-apoptotic or cytoprotective agent and protects the cell from death caused by reactive oxidative species [32].

This is the first report showing antioxidant effect of cappariss ovata on patients with thalassemia. The results obtained from different studies on chelators also shows antioxidant capacity of them. It was found a decrease at lipid peroxidation by deferipron in thalassemic patients [13]. It has been showed a decrease in MDA levels with combination therapy of deferoxamine and Fe<sup>2+</sup> chelator DP (2,2-dipyridyl) in iron loaded human liver Hep-G2 cells [33]. A recent report compared antioxidant capacity of deferoxamine to deferasirox and resulted in equally effectiveness in decreasing MDA levels for 12 months and a positive correlation between MDA and ferritin and inverse correlation with vit C and alpha tocopherol [14]. Our findings are consistent with previous reports as we obtained a significant decrease in MDA levels in both groups who are on deferasirox (control p=0.05, study p=0.02). This shows chelating free iron by deferasirox, not only decrease the transfused iron also counteracts the redox active iron at the membranes.

There are ambiguous results for antioxidant enzyme levels such as Glutathione-peroxidase, catalase and superoxide-dismutase in the literature. Some authors suggest increased activity due to compensation mechanism of increased oxidative stress [34,35] and the others suggest decreased activity [13,36]. In our study we found not significant changes at three enzymes.

It is known that hepatic susceptibility in thalassemic patients is very common because of transfusion related HCV infections, hepatic siderosis, other infectious agents, iron induced glucose intolerance and chronic medications. There are reports about antihepatotoxic activity of cappariss. p-Methoxy benzoic acid isolated from Cappariss spinosa extract administered against carbontetrachloride and paracetamol induced hepatotoxicity *in vivo* and thioacetamide and galactosamine induced hepatotoxicity in isolated rat hepatocytes, proving antihepatotoxic activity of the plant. Consistent with literature our patients had high values of liver function tests at the beginning of the study. After 6 month a decrease occurred in both groups but a significant decrease obtained in cappariss given group (AST p=0.05, ALT p=0.01) supporting its anti-hepatotoxic activity.

As conclusion, oxidative damage is seen because of the increased iron overload in thalassemic patients. The increase in MDA levels is the best marker of this damage. In the present study we obtained a decrease in the levels of MDA and liver function tests with using cappariss. So it is possible that a combination of cappariss with deferasirox (ICL670) may be useful to improve the damage of oxidative stress and hepatotoxicity.

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	Ferritin at the beginning level	Ferritin at the ending level	p**
Control	4406.8 ± 2414.3	3597.6 ± 2163.8	0.00
Study	4654.2 ± 1845.3	3147.8 ± 1245.4	0.00
P*	0.227	0.957	

\* Mann-Whitney U \*\* Wilcoxon test

Table 4: Ferritin levels in patients with thalassaemia major.

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