



Green tea consumption improves the therapeutic efficacy of deferoxamine on iron overload in patients with α -thalassemia major: a randomized clinical study

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(Received 29 May, 2015, Accepted 25 July, 2015)

(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Green tea (*Camellia sinensis*) is an important medicinal plants due to its potent antioxidant effects and metal chelating properties. This study was designed to explore the effects of green tea (GT) against iron overload in α -thalassemia major (TM) patients who were under regular chelation therapy with deferoxamine (DFO). Based on the standard method, 14 TM patients were, randomly, selected and their blood samples were collected immediately before and one month after GT administration (12 gr/day). Then, the blood levels of iron, ferritin, total iron-binding capacity (TIBC), lipid peroxidation (LPO), and hematological profile were determined.

A significant decrease in the levels of iron ($p<0.001$), ferritin ($p<0.01$), LPO ($p<0.001$) and white blood cells ($p<0.05$) and a remarkable increase in TIBC levels ($p<0.001$) were noticed in the blood samples of TM patients thirty days after GT therapy while no significant changes were observed in the other hematological parameters. GT could be used as a supplement to DFO therapy in TM patients.

Keywords: Green tea, Iron, α -thalassemia major, Oxidative stress

INTRODUCTION

α -thalassemia major (TM) is a widespread genetic disorder (Weeraphan *et al.*, 2013). TM patients suffer a partial or complete lack of ability for synthesizing hemoglobin α -chains which leads to insufficient hemoglobin content, less red blood cells (RBCs) production, and the subsequent anemia (Gharagozloo *et al.*, 2014; Kukreja *et al.*, 2013). Iran is located on the world thalassemia belt (Miri *et al.*, 2013) such that according to the report of Iran's Ministry of Health, the average prevalence of TM has been estimated to be 3.6% in this country (Zeinalian *et al.*, 2013).

One of the major consequences of this syndrome is iron overload due to ineffective erythropoiesis and overall hemolysis in main organs such as blood, liver, heart, and endocrine glands (Kukreja *et al.*, 2013; Hoffbrand, 2001). Although, iron is critical trace element of biological systems such as enzymes and metalloproteins (Kasperczyk *et al.*, 2015), its toxicity may predispose TM patients to oxidative stress (Kassab-Chekir *et al.*, 2003). Kalpravidh *et al.* (2010) showed that iron

overload is associated with elevated levels of Malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) in RBC samples of TM patients.

Next to water, tea is the most popular drink consumed in the world (Gupta *et al.*, 2002). For instance, average consumption of black tea for any Iranian individual is 3.56 g/day (Falahi and Hedayati, 2013). This aromatic drink is prepared from the *Camellia sinensis* (CS) as different types including green, black, or oolong tea (Jain *et al.*, 2013). Among these, green tea (GT) has attracted much attention due to its antimicrobial (Reygaert, 2014), anti-inflammatory (Crisponi and Remelli, 2008), antioxidant (Forester and Lambert, 2011), and anticarcinogenic properties (Moezizadeh, 2013). Moreover, tea was traditionally used to eliminate alcohol and improve blood flow (Dufresne and Farnworth, 2000). The health-promoting effects of GT are mainly attributed to its polyphenolic compounds, particularly flavonoids such as catechins, which form 30%-40% of the dry leaf weight (Jain *et al.*, 2013).

Catechins include epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and (-)-epigallocatechin-3-gallate (EGCG). Among these, EGCG is the most abundant subtype, and is thought to be responsible for the majority of the biological activities of GT (Jain *et al.*, 2013; Bhardwaj and Khanna, 2013). Extraction of these active agents into drinking tea is both temperature and time dependent. Thus, preparation of tea is important, as using hot water let it to be better at scavenging free radicals than using cold water (Forester and Lambert, 2011). As reviewed by Nelson and Poulter (2004), the effects of drinking tea with meals on non-haem iron absorption has been confirmed. Moreover, a previous study showed the efficacy of GT in reduction of oxidative stress parameters in iron-loaded rats (Ounjaijean *et al.*, 2008). According to importance of iron toxicity in TM patients, in this research the effects of the co-administration of GT were studied in the patients that were under regular chelation therapy with deferoxamine (DFO).

MATERIALS AND METHODS

A. Chemicals

All chemicals were obtained from Sigma-Aldrich unless otherwise stated. Also, GT were purchased from local manufacturers, Torbat-Heydarieh, Iran.

B. Study design

The study protocol was approved by institute review board of Islamic Azad University, Shahreza Branch, Shahreza, Iran with code number of (19710603922002) and it was carried out in accordance with the ethical standards laid down in the World Medical Association Declaration of Helsinki.

The patients including 14 beta-thalassemia children (9 female and 5 male) with ages 9-15 years who were under regular chelation therapy with DFO (34±4 mg/kg/day; intravenous), were, voluntarily, recruited from 9-Day Hospital in Torbat-Heydarieh, Iran. At the beginning of the study, 5 mL of peripheral blood was collected from each patient. Then, they received a 12 g/day of GT infusion for one month and at the end of treatment, 5 mL of peripheral blood was collected again. Hematological parameters, lipid peroxidation (LPO) level, serum iron, ferritin, and total iron-binding capacity (TIBC) were measured in blood samples before and after administration of GT. It should be noted that preparation of GT infusion was educated to patients based on current protocols in Iranian traditional medicine. Briefly, 200 mL of hot water (60-70 °C) was added to 6 g of GT and left to cool at room temperature for 5 min.

C. Iron assay

The iron serum level was measured using iron photometric assay kit (Pars Azmun, Tehran, Iran) according to the reaction of Fe (2+) with ferrozine and formation of ferrous ferene (blue complex). The color

change was measured at 600 nm with a spectrophotometer.

D. Ferritin assay

The amounts of ferritin in serum samples were determined using a ferritin ELISA kit (Ideal Tashkhis, Tehran, Iran) according to the manufacturer instructions.

E. TIBC assay

To quantify TIBC in serum samples, TIBC assay kit (Darman Kave, Tehran, Iran) was used according to the instructions of the manufacturer. Briefly, an extra amount of iron was added to the serum samples (in an alkaline pH) so that transferrin was saturated with iron. Then, the unbound iron was determined using the ferrozine reagent and finally, unbound iron binding capacity (UIBC) and TIBC were calculated from the following formula:

UIBC = iron added - iron remaining

TIBC = UIBC + serum iron

F. LPO assay

LPO was measured in serum samples based on thiobarbituric acid reactive substances (TBARS) method (Nili-Ahmadabadi *et al.*, 2011). Briefly, serum samples were mixed with 20% trichloroacetic acid (TCA) and the precipitate was dispersed into 0.05 M H₂SO₄. Then, thiobarbituric acid (TBA) (0.2% in 2 M sodium sulfate) was added and heated for 60 min in boiling water bath. Lipid peroxides were extracted by n-butanol and absorbance was measured at 532 nm.

G. Determination of hematological parameters

Complete blood counts including white blood cell (WBC), hemoglobin (HG), hematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), platelet count (PC), and RBC were measured using an automated cell counter (Celltac Alpha, Nihon kohden, Germany).

H. Statistical analysis

The results were analyzed using IBM SPSS Statistics 21.0 software (New York: IBM Corp). Data were expressed as the mean ± standard deviation (SD). The paired-samples t-test was used to compare treatment alone (DFO) and co-administration of the supplement and treatment (DFO+GT) values. The significance level was set at 0.05.

RESULTS

A. Effects of GT on iron

Serum iron concentration is shown in Fig. 1; a statistically significant decrease (23.9 ± 9.5%) was noticed in the serum iron by co-administration of DFO+GT in comparison with DFO (p<0.001).

B. Effects of GT on ferritin

As presented in Fig. 2, the serum ferritin level of the samples considerably decreased (p<0.01) by DFO+GT to values that are 20 ± 16% less than that of DFO.

C. Effects of GT on TIBC

According to Fig. 3, TIBC significantly increased ($22.4 \pm 12.8\%$) by DFO+GT compared to that of with DFO ($p < 0.001$).

D. Effects of GT on LPO

As shown in Fig. 4, the LPO levels in serum samples considerably decreased by DFO+GT to $25.3 \pm 2.8\%$ less than that of DFO ($p < 0.001$).

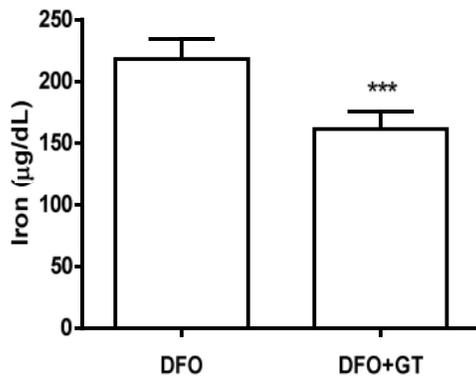


Fig. 1: Serum iron status in patients before (DFO) and after (DFO+GT) GT therapy. Results are expressed as the mean \pm SD; $n=14$ for each group. The paired-samples t-test was used to compare data. *** The difference between DFO and DFO+GT groups is significant at $p < 0.001$.

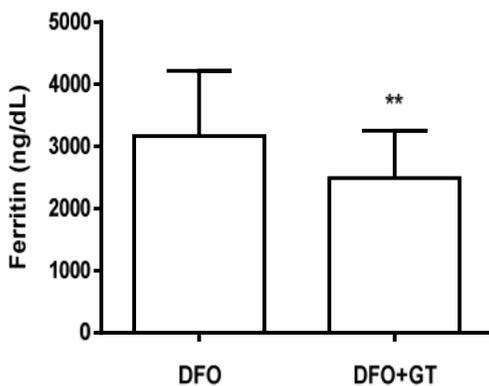


Fig. 2: Serum ferritin status in patients before (DFO) and after (DFO+GT) GT therapy. Results are expressed as the mean \pm SD; $n=14$ for each group. The paired-samples t-test was used to compare data. ** The difference between DFO and DFO+GT groups is significant at $p < 0.01$.

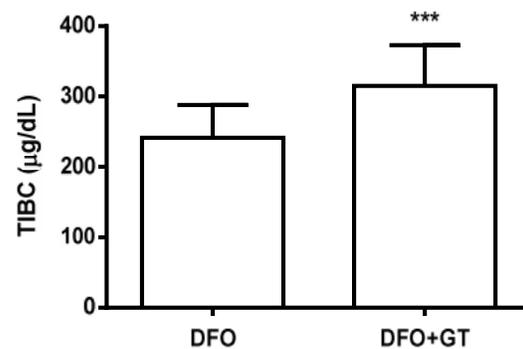


Fig. 3: Serum TIBC status in patients before (DFO) and after (DFO+GT) GT therapy. Results are expressed as the mean \pm SD; $n=14$ for each group. The paired-samples t-test was used to compare data. *** The difference between DFO and DFO+GT groups is significant at $p < 0.001$.

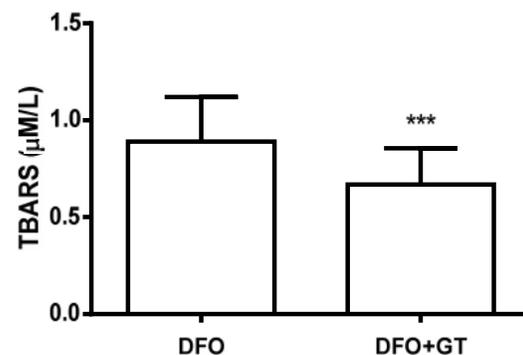


Fig. 4: Serum LPO status in patients before (DFO) and after (DFO+GT) GT therapy. Results are expressed as the mean \pm SD; $n=14$ for each group. The paired-samples t-test was used to compare data. *** The difference between DFO and DFO+GT groups is significant at $p < 0.001$.

E. Effects of GT on hematological parameters

According to Table 1, the WBC level in blood samples was significantly decreased by DFO+GT compared with DFO. No changes were observed in other hematological parameters such as HG, HCT, MCH, MCV, and RBC.

Table 1: Hematological profile of TM patients before (DFO) and after (DFO+GT) GT therapy. Results are expressed as the mean \pm SD; n=14 for each group. The paired-samples t-test was used to compare data. * The difference between DFO and DFO + GT groups is significant at $p < 0.05$. Abbreviations: White blood cell (WBC), Red blood cells (RBC), Hemoglobin (HG), Hematocrit (HCT), Mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV), and Platelet count (PC).

Parameters	DFO	DFO + GT	P value
WBC (n/ μ L)	(15.75 \pm 14.54) $\times 10^3$	(13.98 \pm 13.12) $\times 10^3$	0.04 *
RBC (n/ μ L)	(3.32 \pm 0.74) $\times 10^6$	(3.37 \pm 0.59) $\times 10^6$	0.89
HG (g/dL)	8.52 \pm 1.84	8.57 \pm 1.55	0.95
HCT (%)	26.34 \pm 5.42	26.52 \pm 4.30	0.77
MCH (pg)	25.86 \pm 2.97	25.55 \pm 3.09	0.97
MCV (fl)	79.60 \pm 5.21	78.89 \pm 5.03	0.98
PC (n/ μ L)	(371 \pm 159) $\times 10^3$	(393 \pm 181) $\times 10^3$	0.98

DISCUSSION

In this study, we have explored the effects of GT, as a supplement, on iron overload in TM patients who were under regular chelation therapy with DFO. Our preliminary investigations showed that serum iron levels in TM patients were 23% higher than the maximum values in normal persons. Our data confirmed the iron overload in TM patients which is probably due to different in kinetic of iron in TM patients. Increased iron absorption, hemolysis and iron overproduction are mainly responsible for these extra levels in TM patients (Kukreja *et al.*, 2013). One month after administration of GT, iron and serum ferritin contents were significantly decreased in TM patients. Previously, Ounjaijean *et al.* (2008) showed a reduction of the plasma iron by GT administration in iron-loaded rats. Based on the available evidence, it seems that GT improves iron excretion and prevents absorption in small intestine just like that does each chelator. This chelating effect could be related to some active ingredients of GT such as EGCG and ECG (Mandel *et al.*, 2006). These active agents are thought to interfere with iron absorption by forming insoluble complexes in the gastrointestinal lumen leading to decrease in bioavailability of iron (Samman *et al.*, 2001).

TIBC and ferritin contents are associated to the quantity of iron stored in the human body; however, other genetic and acquired conditions could alter their serum levels (Camaschella and Poggiali, 2009). Therefore, elevation in TIBC serum level following GT therapy could be due to improvement of iron distribution following GT administration.

Iron overload is known to induce reactive oxygen species (ROS) via the Haber-Weiss reaction (Brissot *et al.*, 2012). Furthermore, free iron has been documented to catalyze conversion of hydrogen peroxide to more reactive free radical ions via the Fenton reactions (Crisponi and Remelli, 2008) and subsequent elevation in LPO. Iron induces oxidative damage in plasma membrane and in different subcellular targets including hepatocyte and cardiac mitochondria (Brissot *et al.*,

2012). In the present study, we found a valuable effect of GT against LPO, as a marker for oxidative stress. GT's catechins are characterized by the dihydroxyl or trihydroxyl groups on A or B ring of their chemical structure which lead to significant antioxidative properties. This antioxidative effect is improved by the presence of the trihydroxyl structure in the D-ring (gallate) in ECG and EGCG. The polyphenolic structure allows electron delocalization, conferring high reactivity to quench free radicals (Bogdanski *et al.*, 2012). Thus, in addition to preventive effects of GT against iron toxicity, GT's catechins decrease iron mediated oxidative damages (Forester and Lambert, 2011). On the other hand, Orino *et al.* (2001) showed that an increased serum ferritin level could be a defensive mechanism of the body against oxidative stress. Therefore, the antioxidant action of GT may also lead to the reduction of serum ferritin in treated patients.

Several studies indicated that oxidative stress could be related to inflammatory pathways in TM patients (Walter *et al.*, 2006). On the other hand, Chatterjee *et al.* (2012) showed that inflammation could decrease by GT administration. It seems that reduced WBC may be associated with antioxidative and anti-inflammatory effects of GT and improving immune function.

CONCLUSION

Taking collectively, co-administration of DFO and GT possesses the potential to decrease toxic iron levels, reduce oxidative stress, and thereby improves DFO therapy in TM patients.

ACKNOWLEDGMENTS

Authors wish to thank Mr Ahmad Reza Mohsenzadeh and Mrs Farzaneh Hosseini who assisted authors in the research. Authors also thank the assistance of 9-Day Hospital in Torbat-Heydarieh, Iran. This study was the MSc thesis of the first author that was supported by Islamic Azad University, Shahreza Branch, Shahreza, Iran.

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