Saudi Journal of Biological Sciences 26 (2019) 531–540

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Green tea activity and iron overload induced molecular fibrogenesis of rat liver

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ARTICLE INFO

Article history:
Received 11 April 2017
Revised 24 May 2017
Accepted 17 August 2017
Available online 18 August 2017

Keywords:
Liver fibrosis
Hepcidin
Oxidative stress
Apoptosis
Green tea
Iron overload

ABSTRACT

Iron overload toxicity was shown to associate with chronic liver diseases which lead to hepatic fibrosis and subsequently the progression to cancer through oxidative stress and apoptotic pathways. Green tea potential activity as chelating, anti-oxidative, or anti-apoptotic mechanisms against metal toxicity was poorly clarified. Here, we are trying to evaluate the anti-oxidant and anti-apoptotic properties of green tea in the regulation of serum hepcidin levels, reduction in iron overloads, and improve of liver fibrosis in iron overloaded experimental rats. Three groups of male adult rats were randomly classified into three groups and treated as follows: control rats, iron treated rats for two months in drinking water followed by either vehicle or green tea extract (AGTE; 100 mg/kg) treatment for 2 more months. Thereafter, we studied the effects of AGTE on iron overload-induced lipid peroxidation, anti-oxidant depletion, liver cell injury and apoptosis. Treatment of iron-overloaded rats with AGTE resulted in marked decreases in iron accumulation within liver, depletion in serum ferritin, and hepcidin levels. Iron-overloaded rats had significant increase in malonyldialdehyde (MDA), a marker of lipid peroxidation and nitric oxide (NO) in liver when compared to control group. Also, significant change in cytochrome c and DNA content as apoptotic markers were reported in iron treated rats. The effects of iron overload on lipid peroxidation, NO levels, cytochrome c and DNA content were significantly reduced by the intervention treatment with AGTE ($P < 0.001$). Furthermore, the endogenous anti-oxidant capacities/levels (TAC) in liver were also significantly decreased in chronic iron overload and administration of AGTE restored the decrease in the hepatic antioxidant activities/levels. Also, hepatic hepcidin was shown to be significantly correlated with oxidative and apoptotic relating biomarkers as well as an improvement in liver fibrosis of iron treated rats following AGTE treatment. In-vitro analysis showed that, the improvement in iron toxicity of the liver depend mainly on antioxidant and protective ability of green tea polyphenolic compounds especiallyepigallocatechin-3-gallate (EGCG). Our study showed that green tea extract (GTE) ameliorates iron overload induced hepatotoxicity, apoptosis and oxidative stress in rat liver via inhibition of hepatic iron accumulation; improve of liver antioxidant capacity, and down regulation of serum hepcidin as well as reduction in the release of apoptotic relating proteins.

1. Introduction

Nutritional deficiency is the main target for iron deficiency in human beings with or without anemia. Thus, for all living cells, both essential roles and toxic actions of iron were reported (Wosten et al., 2000). Also, prolonged and uncontrolled iron administration should be avoided for the potentially cellular damaging effects especially in liver cells (Mollet et al., 2016). Higher epidemic levels were reported as a result of excess iron in living cells.

In human and animal research models, iron overload was shown to be related with hereditary hemochromatosis, thalassemia, and hepatic diseases such as chronic viral hepatitis, alcoholic hepatitis (Deugnier et al., 2008). Iron (Fe) is stored within liver cells in various forms including, Fe containing enzymes, ferritin, hemosiderin, and heme (Jomova and Valko, 2011).

As a result of metal binding capacity of iron to some cellular low molecular weight proteins which act as chelators, excess iron was sequestrated, deposited within liver cells, and inducing liver tissue

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.sjbs.2017.08.007
1319-562X/

damage. The deposition of iron in hepatocytes increasing the risk of developing significant fibrosis, cirrhosis, and subsequently increase the rate of morbidity and mortality (Olynyk et al., 2005). Several research studies reported a significant association between the hepatic iron concentration (HIC), hepatotoxicity, and the prognosis of liver fibrosis (Olynyk et al., 2005).

In iron overload, excess iron promotes the generation of reactive oxygen species which stimulates severe oxidative damage to cellular organelles such as lipids, proteins, and nucleic acids (Siah et al., 2006). Also, the liberated oxidative free radicals trigger hepatic inflammation via production of some proinflammatory cytokines (TNF-α, nuclear factor κB) which participates in the pathogenesis of both acute, chronic liver damage, and even cirrhosis (Uchiyama et al., 2008).

Also, excess iron may have additional toxic effect on mitochondrial membranes. It may initiates an opening of the mitochondrial pores which conducts solutes into the mitochondria and consequently produce more damaging process such as mitochondrial depolarization, uncoupling of oxidative phosphorylation, mitochondrial swelling, and depletion in adenosine triphosphate. This finally leads to the release of proapoptotic proteins, like cytochrome c which resulting in hepatic cell necrosis or apoptosis (Uchiyama et al., 2008; Moon et al., 2010). This significant hepatocyte apoptosis plays a significant role in the progression of hepatic fibrogenesis and carcinogenesis (Kowdley, 2004). Although, many mechanisms were present to discuss hepatocellular injury, the estimate pathways involved in liver cell dysfunction and fibrogenesis remain to be sufficiently elucidated (Hubscher, 2003).

Hepcidin is a small 25-amino acid peptide produced within liver cell and can be estimated easily in tissue, serum or urine samples (Frazer et al., 2002). Most studies reported hepcidin as an iron hormone regulator whereas, deficiency in hepciden level was observed in cases with iron overload, and that overexpression of hepcidin levels were shown in subjects with severe iron deficiency and anemia (Frazer et al., 2002).

The up and down regulation of hepcidin was shown to be linked with iron disorders and in turn estimates its importance in systemic iron homeostasis. The hyposideremic activity of hepcidin regulates iron levels by inhibiting the intestinal absorption, the release of iron by macrophages, and control of the surface expression of the iron exporter ferroportin. It was shown that hepcidin was be able to bind to ferroportin, leading to the internalization and degrading of the iron exporter (Nemeth et al., 2004a,b), thereby decreasing iron availability in the circulation. So, hepcidin may has a pivotal role in hepatic fibrogenesis and severity of liver diseases (Frazer et al., 2002), and could be used as a diagnostic parameter for staging of liver fibrosis (Frazer et al., 2002).

The changes in the expression of hepcidin have been reported in many liver diseases. Lower levels of serum hepcidin/ferritin ratio were reported in iron overloaded chronic hepatitis C patients compared to HBV patients (Tan et al., 2012; Fujita et al., 2007). Also, hepatocyte apoptosis induced by iron overload was shown to be linked with the regulation of hepcidin (Ganz, 2011).

Recently, it was reported that over expression of p53 and Fas antigens as apoptosis inducing proteins in hepatoma cells participates in the regulation of hepcidin. Whereas, up regulation of these proteins has been shown to induce hepcidin gene transcription and conversely down regulation of p53 and Fas antigens resulted in down regulation of hepcidin expression (Li et al., 2013).

Chemically synthesized iron chelators have been proposed for the treatment of many diseases associated with iron overload (Pangjitet al., 2015; Kulprachakarn et al., 2014; Chansiw et al., 2014). However, more adverse effects were represented during the treatment schedules that made many pharmacologists to devote their efforts to study new treatment strategies based on naturally occurred iron chelators of plant origin.

Green tea was reported as one of the most naturally present iron chelators which showed both antioxidant and iron chelation activities in vivo and in vitro experimental models (Patel et al., 2012; Saewong et al., 2010). In iron overloaded experimental models, hepcidin levels were regulated with both drug therapy and naturally occurred iron chelators (Porter et al., 2014; Gu et al., 2013;). Although, little is known about the exact regulation mechanisms proposed under these conditions, only green tea alone or in combination with other drugs chelating toxic iron in plasma and tissues, and increasing the levels of hepcidin expression (Kautz et al., 2014; Yun and Vincelette, 2015; Upanan et al., 2015). From the previous data, our hypothesis that green tea as naturally occurred iron chelator of plant origin could ameliorates iron overload toxicity and prevent most iron related diseases especially liver diseases. Thus, in this study, we are trying to evaluate the anti-oxidant and anti-apoptotic properties of green tea in the regulation of serum hepcidin levels, reduction in iron overloads, and improve of liver fibrosis in iron overloaded experimental rats.

2. Materials and methods

2.1. Animals and experimental design

A total of 30 young albinos male Sprague Dawley rats (*Rattus norvegicus*) weighing 120–150 g have been randomly included in this study. The animals have been housed in healthy atmospheric conditions, normal feeding, drinking, and medical care based on the guidelines of the experimental animal care, college of science, King Saud University, Riyadh, Saudi Arabia. The experimental procedures were approved by the Ethics Committee of the Experimental Animal Care Society at King Saud University (Permit Number: PT 1204).

The animals were divided randomly into three groups (n = 10); Control group (rats feed on normal diets without iron), Iron overloaded group (rats feed with iron in a drinking water for two month and then left without treatment for another two month), and AGTE treated group (rats feed with iron in a drinking water for two month then treated with 100 mg/kg/day AGTE suspended in drinking water for another two month).

Iron was added to drinking water in a quantity exceeds the maximum permissible concentration (MPC; Fe^{2+} is 0.3 mg per liter) for this chemical in Ministry of Health. Thus, rats of control group supplemented only tap water, whereas iron overloaded and AGTE groups provided with drinking water containing 3 mg/L of Fe^{2+} (using 8.3 mg/L of $FeSO_4$). The dose of AGTE was selected based on previous studies (Upanan et al., 2015; Kim et al., 2009). In most animal studies, a dose range of 50–200 mg/kg body weight AGTE exhibited a good anti-inflammatory and anti-fibrotic activity and seemed to have no adverse effects on human (Saewong et al., 2010; Kim et al., 2009; Ibrahim et al., 2015). After four months, rats were sacrificed under ether anesthesia. Blood and liver tissue samples were collected and subjected for subsequent histological and biochemical analysis. For biochemical analysis in liver tissues, part of the samples was immediately frozen at −80 °C until reused.

2.2. Green tea extracts (GTE)

A microwave cabinet was used for drying freshly harvested green tea leaves (Camellia sinensis) (Upanan et al., 2015). To prepare green tea extract, hot water is used. Epigallocatechin 3-gallate (EGCG) as active constituent was estimated in green tea extract by using HPLC method. The GTE product containing 28% (w/w) EGCG was kept in the dark at −20 °C until studied (Kim et al., 2009; Saewong et al., 2010).

2.3. Estimation of total phenolic (TPC) and flavonoid contents (TFC) of aqueous green tea extract

Spectrophotometric analysis were reported to estimate total flavonoid (TFC) and phenolic contents (TPC) in green tea water extract by using 2% aluminum chloride and diluted Folin-Ciocalteu as reagents (Djeridane et al., 2006). The absorbance of the reaction mixtures produced was measured at 430 nm and 725 nm respectively. Standard calibrated curves of rutin and Gallic Acid were used to estimate flavonoids and poly phenolic compounds in green tea samples. The data obtained were expressed in mg rutin equivalent (RE) per g for flavonoids and Gallic Acid Equivalents/100 mg for poly phenolic compounds respectively (Djeridane et al., 2006).

2.4. Antioxidant analysis of aqueous green tea extracts (AGTE)

Radical scavenging and antioxidant activities of the AGTE were measured by using spectrophotometric analysis as previously (Brand et al., 1995; Mothana, 2011). Various concentrations from the extracted green tea were prepared in hot water and both radical scavenging and antioxidant activities of green tea against DPPH and β-Carotene-linoleic acid were estimated as absorbance at λ = 517 nm according to the following equations:

$$\text{Radical scavenging activity } (\%) = [(\text{Ac} - \text{As})/\text{Ac}] \times 100 \quad (1)$$

where (Ac) is the absorbance of the control and (As) is the absorbance of the sample.

$$\text{Antioxidant activity } (\%) = (\text{Abs}0 - \text{Abst})/(\text{Abs} * 0 - \text{Abs}^{\circ}\text{t}) \times 100 \quad (2)$$

where Abs0 and Abs∗ 0 are the absorbance values measured at 0 time of incubation for sample extract and control, respectively. Abst and Abs∗ t are the absorbance values for sample extract and control, respectively, at t = 120 min.

2.5. Estimation of liver function and hepcidin levels

Commercially available colorimetric assays were performed to estimate liver function tests (ALT, AST, and Bilirubin), and serum ferritin as previously reported in the literature (Thapa and Anuj, 2007; Bardou-Jacquet et al., 2014). Additionally, Serum hepcidin was measured using a validated ELISA kits, as already previously described (Koliaraki et al., 2009; Bardou-Jacquet et al., 2014).

2.6. Histopathological evaluation

Liver tissues of both iron overloaded and AGTE treated rats were investigated histologically and liver cell fibrosis scored as no fibrosis (0–1) and fibrosis (2–3) as previously reported in literature (Scheuer, 1991).

2.7. Estimation of iron in liver tissues

Flame atomic absorption spectroscopy (Perkin-Elmer 2380, Norwalk, CT 06859-0012, USA) was used to analyze iron in liver homogenates after digestion and dissolution in 0.1 mol/L HNO_3.

2.8. Estimation of malonyldialdehyde (MDA) as marker of oxidative stress

A total of 200 µl of liver tissue homogenates were used to estimate MDA by using colorimetric assay previously reported. A mixture of TBA (1.5 mL), 8.1% SDS (200 µl), 20% acetic acid (1.5 mL), and distilled water (600 µl) were added to the homogenates and the resultant pink color was measured colorimetrically at 534 nm using a spectrophotometer. MDA concentration per tissue samples was calculated against standard calibration curve (Lefevre et al., 1998; Erdincler et al., 1997).

2.9. Estimation of NO concentration in liver tissues

Indicative levels of nitric oxide (NO) in liver tissues were estimated as nitrite (NO_2^-) concentrations which considered the most stable metabolic product and can be formed by conversion of nitrate (NO_3^-) molecules by the aids of elementary zinc. To estimate nitrite concentration in liver tissues, a classic colorimetric Griess reaction was performed by adding equal volumes of liver tissue homogenates along with Griess reagent at room temperature. The absorbance of the developed color was measured colorimetrically at 570 nm using a spectrophotometer. The concentration of nitrite was calculated using standard calibrated curve of sodium nitrite standard (Hortelano et al., 1995).

2.10. Estimation of total antioxidant capacity (TAC)

Serum samples were used to determine total antioxidant capacity (TAC) by using colorimetric assay Kit (BioVision Incorporated, CA, USA). The concentrations of antioxidant capacity were measured at 570 nm using a spectrophotometer. Based on manufacturer's instructions equivalents, the results were calculated as a function of Trolox concentration as follows.

$$\text{Sa}/\text{Sv} = \text{nmol}/\mu\text{l or mM Trolox equivalent},$$

Sa = Sample amount (in nmol) which has been read from the standard curve.
Sv = The undiluted sample volume added to the wells.

2.11. Estimation of DNA content and cytochrome c as markers of apoptosis

Colorimetric analysis was used to estimate DNA content in liver tissue samples. DNA was extracted by using diphenylamine as organic solvent and the concentration of DNA was measured at 600 nm using a spectrophotometer (Gendimenico et al., 1988). Also, serum cytochrome c concentrations were determined using an immune assay ELISA kit (Zymed^R cytochrome c ELISA Kit Cat. No. 99-0040).

2.12. Statistical analysis

The data of this study have been analyzed using SPSS version 17. All data have been tabulated as mean ± SD. The statistical differences were performed by using one-way analysis of variance (ANOVA) and Student's *t*-test. $P < 0.05$ considered to be statistically significant.

3. Results

3.1. Total phenolic and flavonoid contents of aqueous green tea extract

Green extraction yields of phenolic and flavonoid compounds were listed in Table 1. Both Total phenolic compounds and flavonoid contents recorded values of 29.8 ± 5.8 mg GAE/100 g, and 16.9 ± 2.4 mg rutin/100 g of dry green tea extract respectively (Table 1).

Table 1
Biological activities, total phenolic and flavonoids constituents of aqueous green tea extract (AGTE; mg/100 g).

Contents/biological activity	AGTE (100 mg)
Phenolic content (mg/100 g)	29.8 ± 5.8
Flavonids content (mg/100 g)	16.9 ± 2.4
Radical scavenging activity (BCLA; %)	
At cons. of 500 µg/mL	82.3
At cons. 1000 µg/mL	94.2
Total antioxidant activity (DPPH; %)	89.7

3.2. In-vitro antioxidant activity of green tea

In the present study, *in vitro* antioxidant and radical scavenging activities of phenolic and flavonoid rich green tea extract were measured according to the inhibition rates of linoleic acid oxidation and DPPH radicals. AGET recorded free radical scavenging activity of 82.3% and 94.2% at concentrations of 500 and 1000 µg/mL respectively, while the same extract reported antioxidant activity with mean of 89.7% according to the β-carotene bleaching rate of green tea extract (Table 1).

3.3. Liver function tests

Iron overload produces significant increase in the levels of ALT and AST activity and TB concentration and decrease in the levels of albumin in iron treated rats compared to control group as shown in Fig. 1. In AGET treated rats, significant improvement was reported in the levels of ALT and AST activity and TB concentration and increase in the levels of albumin to words normal values compared to iron overloaded rats (Fig. 2). Non-significant changes were detected for all parameters between the control and group IV (Fig. 2).

3.4. Effects of aqueous green tea extract (AGTE) on iron accumulation, serum ferritin and hepcidin in iron overloaded rats

The data obtained showed no change in water, food consumption or behavior of rats during iron overload. In iron treated rats, iron administration produce about 2.8 fold increase in total hepatic iron content when compared to related values of control group as shown in figure (2A). Treatment with green tea resulted in significant reduction in the levels of iron content in the livers of iron overloaded rats by fold change of 1.6 ($P < 0.01$). Also, serum ferritin was studied as marker of iron overload. Chronic iron loads resulted in a significant increase by about 350-fold change in ferritin when compared to healthy control rats. In AGTE group, green tea administration produces significant reduction by about 125-fold change in the levels of ferritin ($P < 0.001$) (Fig. 2B).

3.5. Effects of AGTE on hepatic lipid peroxidation and antioxidant profile

Also, in this study, we trying to study the anti-oxidant activity of green tea against oxidative stress induced by iron overloads. Chronic iron overloads showed significant increase in the levels of hepatic malonyldialdehyde (MDA), a corresponding biological compound commonly used as an index of oxidative stress. Whereas, significant improvement in the levels of MDA towards normal levels was reported in iron overloaded rats following treatment with green tea extract ($P < 0.01$, $P < 0.001$ Fig. 3A). Additionally, the increase in oxidative stress in iron overloaded rats was associated with a marked increase in liver NO levels ($P < 0.001$), and decrease in total antioxidant capacity (TAC) ($P < 0.001$). Green tea supplementation significantly reduced the elevation in hepatic NO levels and increase or improve TAC in iron overloaded rats as shown in Figs. 3B and 2C.

3.6. Effects of AGTE on hepatic DNA content and serum cytochrome C

Hepatic DNA content and serum cytochrome C were estimated as markers of apoptosis induced by iron overloads. Chronic iron overloads resulted in significant increase in serum cytochrome C and reduction in DNA content compared to normal control group ($P < 0.01$). A significant decrease in serum levels of cytochrome c and increase in DNA content were reported in iron overloaded rats following treatment with AGTE

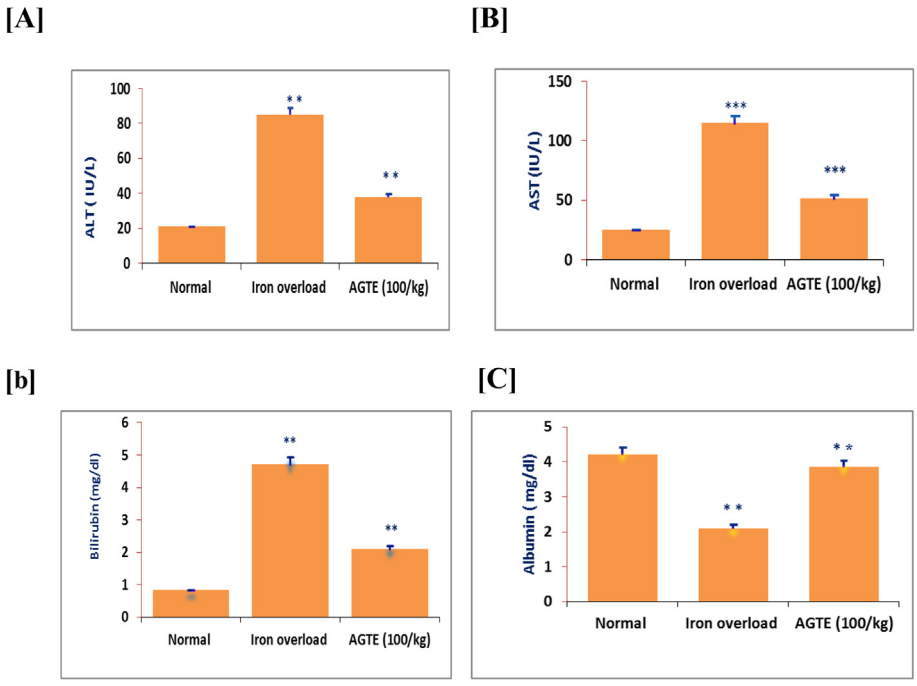

Fig. 1. Effect of aqueous green tea extract (AGTE) on the levels of liver function biomarkers in overload and green tea treated experimental rats. All values represent mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$ compared to control; Student's *t*-test.

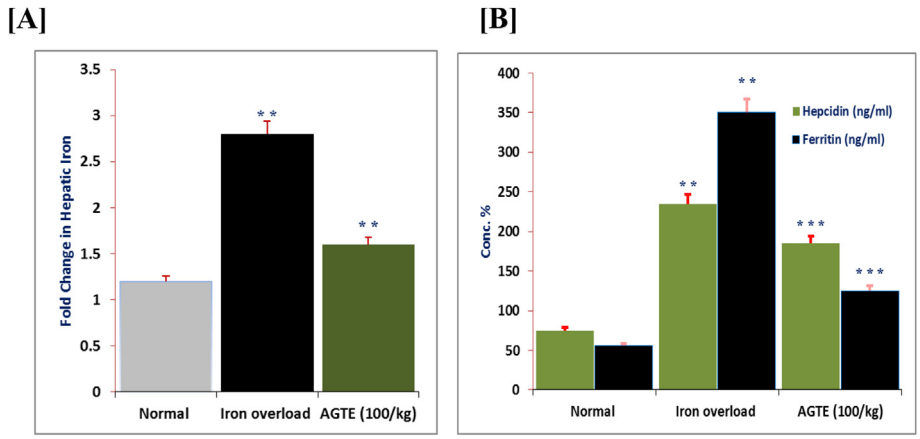

Fig. 2. Potential effects of aqueous green tea extract (AGTE) on iron accumulation, serum ferritin, and hepcidin in experimental rats. [A] Fold change in the level of hepatic iron (μmole/g tissue) in iron overloaded and green tea treated rat livers in relation to control group. [B] Fold change in hepatic hepcidin and ferritin levels of iron overloaded and green tea treated rat livers in relation to control group. All values represent mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$ compared to control; Student's *t*-test.

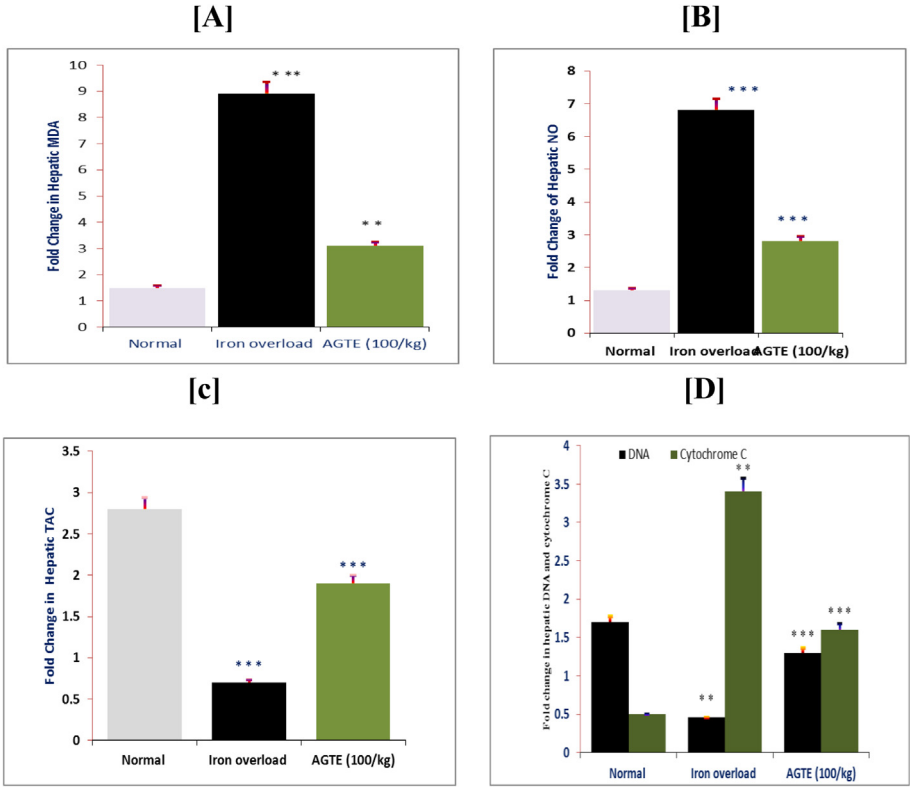

Fig. 3. Effect of iron deposition on oxidative stress, NO levels, and apoptotic related biomarkers (DNA, cytochrome C) in livers, and potential effects of aqueous green tea extract (AGTE). [A] Fold change of hepatic malonyldialdehyde (MDA) as a marker of oxidative stress in liver tissues (nmole/g wet tissue). MDA levels significantly increased in livers of iron overloaded rats compared to both control and AGTE treated groups. [B] Fold change in hepatic nitric oxide (NO) (nmole/g wet tissue) in liver. Levels of NO showed significant increase in iron overloaded rats than in control rats, whereas significant reduction in NO levels were reported in iron overloaded rats following green tea treatments (AGTE; 100/kg). [C] Fold change in total antioxidant capacity (TAC; nmol/mM) in hepatic cells of iron overloaded and green tea treated rats in relation to control rats. Significant decrease in TAC activity was reported in iron overloaded rats compared to control and green tea treated rats. [D] Fold change in hepatic DNA content and cytochrome C as markers of apoptosis. Significant decrease in DNA content and increase in cytochrome C in iron overloaded rats compared to control rats, and significantly improved in iron overloaded rats following AGTE treatments. All values represent mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ compared to control; Student's *t*-test.

(100 mg/kg; $P < 0.001$) as compared with iron treated rats (Fig. 3D). The improvement of DNA content and reduction in the expression in cytochrome c clearly confirms the preventive and anti-apoptotic role of green tea against iron overload toxicity.

3.7. Effects of AGTE on expression of hepatic hepcidin in the serum of iron overloaded rats

Serum hepcidin levels were studied as marker of molecular fibrogenesis induced by iron overload. Chronic iron loads resulted

in a significant increase by about 235-fold change in hepatic hepcidin compared to healthy control rats. In AGTE group, green tea administration produces significant reduction by about 185-fold change in the levels of hepcidin as compared with iron loaded rats ($P < 0.001$) (Fig. 2B).

The correlative effect of the expression of hepatic hepcidin in liver tissues was shown to be significantly correlated with liver cell damage, lipid peroxidation, oxidative stress, and apoptosis resulted in relation to iron overloads. The expression of hepcidin in both iron overloaded and green tea treated rats correlated negatively with hepatic iron content, serum ferritin, oxidative stress markers (MDA, NO), apoptosis markers (DNA, cytochrome C), and positively with liver function, TAC, and fibrosis score as shown in Table 2. Also, liver cell fibrosis was shown to be closely related with iron toxicity as measured by hepatic iron content, ferritin, and hepcidin levels in iron overloaded and AGRTE treated rats as shown in Table 3.

4. Discussion

In the present study, the effects of excess iron administration on hepatic tissues and cellular functions were evaluated in experimental rats. In rats treated with chronic iron the data obtained showed significant increase in hepatic iron contents. This increment was associated with significant changes in liver cell function, lipid peroxidation, and subsequent deficiency in both antioxidant defense mechanisms as well as progression of liver cell apoptosis, and fibrosis.

Excess iron deposition demonstrates evidence of tissue damage and alterations in cellular function of most biological organs especially liver tissues (Bancroft and Gamble, 2002). Previous research studies reported that excessive hepatic iron loads lead to the progression of liver fibrosis, significant fibrosis, cirrhosis, and subsequently increased the rates of morbidity and mortality (Britton, 2000; Olynyk et al., 2005). There are many sources relating to excess iron in human organs such as increased absorption of dietary iron, chronic liver and diseases associated with hemolytic anemia as well as drinking water with higher amounts of iron which effects on human health (Abou-Seif et al., 2004; Badria et al., 2007).

Liver is one of the most important organs for vital biological activities such as metabolism, metal storage and detoxification (Tao and Gitlin, 2003). According to our results, a significant elevation in ALT and AST enzyme activity, TB level, and decrease in albumin were reported in iron overloaded rats. Also, significant changes in liver tissue cells was reported such as hepatocellular necrosis, mononuclear cells infiltration, activation of Kupffer cells, and sporadic cell necrosis as well as apoptosis. The increment in the levels of liver function may be related to disturbance in the transport function of hepatocytes, leakage, and subsequent release of hepatocellular enzymes out of hepatic cells (Mohammadyari et al., 2014; Aneja et al., 2013). Also, iron loads produce significant damage in hepatic parenchyma which results in the failure of normal uptake, conjugation, excretion, and subsequent increase in TB concentration (Dubey and Mehta, 2014).

In the current study significant increase in hepatic iron content and serum ferritin were reported in rats following iron treatment. A greatest iron deposition and density were reported in the hepatic cells of iron treated rats which is closely related to the severity of liver fibrosis scores (0–1 vs 2–3). The deposition of iron was significantly linked with cellular hepatic damage, fibrosis score and elevation in liver function. Our data were in accordance with previous research reports which suggested an association between excess iron deposition and hepatic diseases such as chronic viral hepatitis and alcoholic hepatitis (Deugnier et al., 2008). Also, the increase in serum ferritin was significantly related to excess iron which stored within liver cells in various forms especially ferritin and hemosiderin (Jomova and Valko, 2011), releasing liver tissue damage and initiates fibrosis (Olynyk et al., 2005). Similarly, several research studies reported a significant association between the hepatic iron concentration (HIC), hepatotoxicity, and the prognosis of liver fibrosis (Olynyk et al., 2005).

In the current study, oxidative stress markers; MDA, NO and TAC were estimated in iron overloaded rats. The data showed significant increase in the serum levels MDA, NO, and decrease in the levels TAC in iron treated compared with healthy control rats. The data obtained significantly correlated with hepatic iron content, liver function, and staging of liver fibrosis.

In normal hepatic cells, there was a homeostatic balance between the levels of ROS produced and antioxidant enzymes whereas mitochondria, peroxisomes, and microsomes produce a significant levels of ROS which maintained in a constant levels by action of enzymes like superoxide dismutase, catalase, and glutathione peroxidases, which degrade ROS to nontoxic by-products (Britton, 2000; Pietrangelo, 2003). In excess iron conditions, severe oxidant damage of cellular macromolecules was reported such as lipid peroxidation of cellular membranes and organelles, amino acid oxidation, DNA strand breaks, and protein fragmentation (Pietrangelo, 2003). Also, increased ROS, and oxidative stress were reported in AML-12 mouse hepatocytes following treatment with excess iron (Messner et al., 2013). The increase in hepatic iron was associated with excess ferritin levels which suggested behaving like inflammatory cytokine which may activate hepatic stellate cells to initiate the process of fibrogenesis (Ruddell et al., 2009). Recently, excess iron supplementation

Table 2
Correlation between hepatic hepcidin levels and different variables among iron overload and green tea treated rats.

Variables	Hepatic hepcidin levels			
	Iron overload		Green tea treated (AGTE; mg/100 g)	
	r	P	r	P
ALT (IU/L)	0.21	0.001	0.45	0.01
AST (IU/L)	0.38	0.001	0.158	0.01
Albumin (mg/dl)	0.89	0.001	0.174	0.01
Bilirubin (mg/dl)	0.58	0.001	0.85	0.01
Hepatic iron	−0.75	0.001	−0.89	0.05
Ferritin	−0.28	0.001	−0.39	0.01
MDA	−0.128	0.01	−0.265	0.001
NO	−0.358	0.001	−0.450	0.01
TAC	0.871	0.01	0.125	0.01
DNA content	−0.25	0.001	−0.37	0.01
Cytochrome c	−0.510	0.01	−0.650	0.01
Fibrosis score (0–1; 2–3)	0.248	0.001	0.312	0.001

Table 3
Correlation between scores of liver cell fibrosis and iron toxicity measured by hepatic hepcidin, iron and ferritin levels in iron overload and green tea treated rats.

Variables	Liver cell fibrosis			
	Iron overload		Green tea treated (AGTE; mg/100 g)	
	0–1	2–3	0–1	2–3
Hepatic iron	0.754**	0.981***	−0.120**	−0.168***
Ferritin	0.167**	0.298***	−0.399**	−0.541***
Hepatic hepcidin levels	0.235**	0.147***	−0.378**	−0.156***

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$.

initiates hepatic oxidative stress, induction of both inflammatory and immune mediators, and hepatocellular ballooning injury which resulting in the prognosis of NASH (Handa et al., 2016).

Cell death was recognized as promoters of liver damage and prognosis of hepatic fibrosis in all liver diseases such as acute and chronic viral hepatitis (Jaeschke et al., 2012 Canbay et al., 2004). The studying of biochemical markers of cell death in liver pathophysiology and fibrosis enables in the management of new therapeutic targets of liver diseases (Friedman et al., 2013; Ni et al., 2012).

In the current study, cytochrome c and hepatic DNA content as apoptotic markers were estimated in iron overloaded rats. The data showed significant increase in the level of cytochrome c and reduction the hepatic DNA content in rats treated with excess iron compared to healthy control rats.

Previous research studies showed that excess iron induce damage of the mitochondrial inner membrane and initiate opening of the mitochondrial pores via oxidative stress mechanism. This leads to depletion in adenosine triphosphate and the release of cytochrome c, a proapoptotic protein of hepatic cell apoptosis (Uchiyama et al., 2008). Thus, excess iron either alone or by exerting oxidative stress synergizes in promoting hepatic cell necrosis or apoptosis (Ni et al., 2012, Moon et al., 2010). These data also clearly show that iron overload can cause apoptotic cell death in isolated hepatocytes via cell DNA fragmentation produced by ROS via oxidative stress mechanism whereas the production of ROS was the first event probably occurs in iron overloaded hepatocytes (Allameha et al., 2008). Also, it was reported that oxidative damage of DNA resulting from excess iron may lead to the development of hepatocellular carcinoma (HCC). The increased DNA damage was associated with the increased frequency of mutations in p53, inducing apoptosis and progression of liver cell cancer (Hussain et al., 2000).

Iron hemostasis was significantly regulated via synthesis of hepcidin hormone which released into the blood circulation to regulate metabolism of systemic iron (Fung and Nemeth, 2013; Kuhn, 2015). In iron overload conditions, hepcidin was considered recently as one of the novel reliable markers for iron metabolism in tissue, urine, and serum samples (Porter et al., 2014; Chauhan et al., 2014; Ikuta, 2015).

In the current study, serum hepcidin was estimated in iron overloaded rats. The data showed a significant increase in the serum levels of hepcidin in iron treated compared with healthy control rats.

Liver cells were shown to be the main precursor for the production of hepcidin as pro-hepcidin (inactive form) which subsequently activated via cleavage of a furin site through prohormone convertase furin process (Gu et al., 2013; Kautz et al., 2014). Under stimulation processes, the role of hepcidin is to control of local iron fluxes within liver cells (Yun and Vincelette, 2015), whereas the increase in its content was shown to be linked with serum ferritin levels in iron overloaded and inflammatory diseases (Nemeth et al., 2004a,b). The expression of hepcidin in iron overload diseases was significantly influenced by many biological factors especially the growth differentiation factor 15 and the twisted gastrulation factor 1(Kautz et al., 2014; Kartikasari et al., 2008). However, the mechanisms by which oxidative stress and apoptosis induced by iron overload effects on hepcidin regulation are still unclear.

In the current study, in rats treated with excess iron the expression of hepcidin correlated negatively with hepatic iron content, serum ferritin, oxidative stress markers (MDA, NO), apoptosis markers (DNA, cytochrome C), and positively with liver function, TAC, and staging of liver fibrosis. The expression of hepcidin was shown to be associated with iron content and oxidative stress parameters in iron overloaded patients with low-risk myelodysplastic syndrome (Ghoti et al., 2011). Regarding to apoptosis, many studies reported that induction of apoptosis has been suggested in the regulation of hepcidin in most liver diseases linked with iron overload (Schattenberg et al., 2006; Ganz, 2011).

Also, it was reported that the expression of over-expression of p53, and Fas antigens in liver cells participate in the down regulation of hepcidin expression (Li et al., 2013). In contrary, our results were the first to discuss up regulation of hepcidin expression in iron overloaded models via overexpression of cytochrome C and lower DNA content as markers of apoptosis. This may be due to oxidative stress and inflammatory cytokines produced as result of iron overload (Villarroel et al., 2012; Nemeth et al., 2004a,b). Many therapeutic treatments were reported as iron chelators which characterized by some limitations such as lower specificity, limited capacity to enter cells and higher side effects especially hepatic failure (Gagliardo et al., 2008; Cappellini et al., 2006). Thus, herbal medicine may be evaluated as alternative therapeutic strategies against iron overload.

Further we are trying to highlight on the hepatoprotective effects of GTE against iron overloaded-inducing apoptotic effects triggered over the production of ROS. The mechanistic roles underlying the protective effect of AGTE against excess iron hepatotoxicity need for more clarifications. Based upon evidences of previous work and our current results, several protective pathways could be drawn. It was recommended that food products of plant origin, especially tea contain high amounts of phenolic and polyphenolic constituents that were reported to reduce the susceptibility to certain human diseases including cancer (He et al., 2001). In the present study, high levels of polyphenolic (29.8 mg) and flavonoid (16.9 mg) were found in 100 g of AGTE, whereas epigallocatechin-3-gallate (EGCG) constitutes up to 28%. The data obtained were different from previously reported studies which showed higher EGCG contents of the extract (Roomi et al., 2016). This may be related to brewing time, type of tea, commercial brand and producing country (Nash and Ward, 2016).

In our study, hepatic cell injury and elevated circulating markers of hepatocyte injury such as serum ALT, AST, bilirubin, and reduction in albumin level caused by iron overload were all

restored to normal ranges following treatment with green tea extracts. This may be related to the presence of flavonoids and polyphenols constituents in green tea, whereas its ability to stabilize and preserve the integrity of the hepatocyte membrane, stimulating hepatocyte regeneration, and hepatocellular protein synthesis, to repair damaged hepatic tissues were estimated previously (Safer et al., 2015; Pezeshki et al., 2016). Also, in the same manner increased hepatic iron content, and both serum ferritin and hepcidin levels in iron treated rats were shown to be normalized into normal levels following green tea administration. This may be owing to the chelating activity of green tea against iron and minimizing its content in plasma and liver tissues (Saewong et al., 2010).

The hepatoprotective effects of green tea extract were shown to be associated with its considerable antioxidant capacity and antifree radical activities against excess iron inducing oxidative stress (Tomaszewska et al., 2015; Gramza et al., 2005).So, we further estimated the free radical scavenging activity of AGTE by both DPPH (89.2%) and β-carotene (82.3% and 94.2% at 500 and 1000 μg/mL respectively) tests. The data obtained proposed that the phenolic constituents of AGTE have the ability to suppress the chain reactions of lipid peroxidation via free radical scavenging mechanism. Our results are in line with other research work which reported that green tea extracts (AGTE) have considerable free radicals scavenging activity (Panat et al., 2016).

Consistent with the *in vitro* observations, AGTE was able to reverse *in vivo* lipid peroxidation induced by deposition of excess iron in liver tissue. Thus, this showed that green tea could provide essential antioxidant effects during chronic iron overload alongside to its role as an iron-chelating agent. The antioxidant property of green tea may be related to the presence of more phenolic and polyphenolic constituents especially epigallocatechin-3-gallate (EGCG) (He et al., 2001; Roomi et al., 2016; Nash and Ward, 2016). The increase in MDA as a potential marker for lipid peroxidation during iron overload was associated with elevation in hepatic NO levels which could be an alternative pathway to minimize oxidative stress. This proposed pathway was supported by the fact that green tea activity induced significant decrease in MDA, depletion in the levels of hepatic NO along with significant improve in total antioxidant capacity (TAC) of iron overloaded rats.

Most studies reported that systemic toxicities of hepatic cells are associated with the release of many chemical mediators such as NO and proinflammatory cytokines which in turn induce liver cell damage. The presence of these mediators in higher concentrations plays an integral part in hepatic fibrogenesis (Ojiako et al., 2015; Poli, 2000; Shuto et al., 2004). The improvement in the levels of lipid peroxides, NO, TAC as well as damaged hepatic cells may be related to the promising antioxidant and antiradical scavenging activity of green tea constituents against harmful oxidative free radicals (Safer et al., 2015). Similarly, other research studies reported that some polyphenol-rich plant extracts have the capability to ameliorate hepatic cell injury induced by LPS (Amat et al., 2010) or iron overload (Badria et al., 2015; Ounjaijean et al., 2008). Thus, green tea extracts have been shown to have a positive effect on hepatocytes via antiradical activity against oxidative stress free radicals initiated from excess iron toxicity.

In the current study, green tea also showed significant antiapoptotic activity against the progression of liver cell apoptosis in rats following treatment with excess iron. In green tea treated rats, there was a significant decrease in the levels of cytochrome c and increase in hepatic DNA content as apoptotic relating biomarkers. Pervious research studies showed that the imbalance between oxidative burden and defense mechanism induces severs damage in cell organelles especially, DNA damage and a progression of cell death or apoptosis (Li et al., 2003). It was reported that normal apoptosis plays an important part in development process of multicellular organisms; however its abnormal induction could attributed with motivation of various diseases (Neeta, 2007). Previous research studies showed that excess iron induce damage of the mitochondrial inner membrane and initiate opening of the mitochondrial pores via oxidative stress mechanism. This leads to depletion in adenosine triphosphate and the release of cytochrome c, a proapoptotic protein of hepatic cell apoptosis (Ni et al., 2012; Uchiyama et al., 2008). Thus, excess iron either alone or by exerting oxidative stress synergizes in promoting hepatic cell necrosis or apoptosis (Ni et al., 2012). The improvement in the levels of cytochrome c and hepatic DNA content following treatment with green tea similarly matched with recently published data which showed that GTE offered partial hepatic protection through minimization of DNA fragmentation, apoptotic genes as caspases enzymes alongside with the healing view of hepatic parenchyma, and regeneration of hepatocytes or reduction in liver fibrosis (Ibrahim et al., 2015).

The anti-apoptotic activity of green tea extract against excess iron may be related to gallate groups of catechins especially EGCG. These phenolic compounds have a radical scavenging ability via chelating divalent transition metal ions via their ortho-hydroxyphenolic groups, preventing the generation of LPO (Somdet et al., 2012; Leopoldini et al., 2011; Lamberta and Eliasa, 2010). Also, green tea rich catechins minimize damage of DNA molecules by nullifying the effects of ROS through ultra-rapid electron transfer from catechins to ROS free radicals or activation of intracellular signaling cascades which acts as potent anti-apoptotic signaling (Lamberta and Eliasa, 2010; Anderson et al., 2001; Pan et al., 2007). Also, many research studies go in the same line and supported that green tea catechins especially, EGCG compounds and its methylated metabolite protect liver cells against necrosis and apoptosis by suppressing the expression proapoptotic genes (caspase-3) via preventing the cytochrome c release from mitochondria to cytosol (Kagaya et al., 2002; Al-Refai et al., 2014; Hisamura et al., 2006).

More studies concluded that whole GTE was more stable and liable than pure ECGC due to the presence of other antioxidant constituents, (Kaszkin et al., 2004) that act synergistically and exert their full beneficial effect as total extracts (Loew and Kaszkin, 2002). This is why we select to work on the natural extracts of green tea rather than its ingredients.

Finally, based on our data, the antioxidant, anti-apoptotic, and chelating properties of whole AGTE active constituents may contribute in its efficacy as anti-fibrotic when treating iron overload toxicity.

5. Conclusion

Our study showed that green tea extract (GTE) ameliorates iron overload induced hepatotoxicity, apoptosis and oxidative stress in rat liver via inhibition of hepatic iron accumulation; improve of liver antioxidant capacity, and down regulation of serum hepcidin as well as reduction in the release of apoptotic relating proteins.

References

Abou-Seif, M., Badria, F., Houssein, W., 2004. Amelioration of iron overload-induced oxidative stress in rats using natural antioxidant and iron chelating agent. Arab. J. Lab. Med. 30, 193–206.

Allameha, A., Amini-Harandia, A., Osati-Ashtianib, F., O'Brienc, P.J., 2008. Iron overload induced apoptotic cell death in isolated rat hepatocytes mediated by reactive oxygen species. Iran. J. Pharm. Res. 7 (2), 115–121.

Al-Refai, A.S., Ali, A.B.H., Kamal, K.A., 2014. Immunohistochemical study of the effect of green tea extract on methotrexate-induced oral mucositis in albino rats. J. Cytol. Histol. 5, 227.

Amat, N., Upur, H., Blazekovic, B., 2010. In vivo hepatoprotective activity of the aqueous extract of *Artemisia absinthium* against chemically and

immunologically induced liver injuries in mice. J. Ethnopharmacol. 131, 478–484.
Anderson, R.F., Fisher, L.J., Hara, Y., Harris, T., Mak, W.B., Melton, L.D., Packer, J.E., 2001. Green tea catechins partially protect DNA from (·)OH radical-induced strand breaks and base damage through fast chemical repair of DNA radicals. Carcinogenesis 22, 1189–1193.
Aneja, S., Vats, M., Aggarwal, S., Sardana, S., 2013. Phytochemistry and hepatoprotective activity of aqueous extract of *Amaranthus tricolor* Linn. roots. J. Ayurveda Integr. Med. 4 (4), 211–215.
Badria, F., Mandour, R., Ghanem, A., 2007. Impact of iron overload in drinking water on animal and human health in Dakahlyia governorate and role of catechins as iron chelator. J. Environ. Sci. 33, 25–45.
Badria, F.A., Ibrahim, A.S., Badria, A.F., Elmarakby, A.A., 2015. Curcumin attenuates iron accumulation and oxidative stress in the liver and spleen of chronic iron-overloaded rats. PLoS One 10 (7), e0134156. 31.
Bancroft, J.D., Gamble, M., 2002. Theory and Practice of Histological Techniques. Churchill Livingstone, London, New York and Philadelphia.
Bardou-Jacquet, E., Philip, J., Lorho, R., Ropert, M., Latournerie, M., Houssel-Debry, P., Guyader, D., Loréal, O., Boudjema, K., Brissot, P., 2014. Liver transplantation normalizes serum hepcidin level and cures iron metabolism alterations in HFE hemochromatosis. Hepatology 59 (3), 839–847 (Wiley-Blackwell).
Brand, W.W., Cuvelier, H.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol. 82, 25.
Britton, R.S., 2000. Mechanisms of iron toxicity. In: Barton, J.C., Edwards, C.Q. (Eds.), Hemochromatosis: Genetics, Pathophysiology, Diagnosis and Treatment. Cambridge University Press, Cambridge, UK, pp. 229–238.
Canbay, A., Friedman, S., Gores, G.J., 2004. Apoptosis: the nexus of liver injury and fibrosis. Hepatology 39, 273–278.
Cappellini, M.D., Cohen, A., Piga, A., Bejaoui, M., Perrotta, S., Agaoglu, L., Aydinok, Y., Kattamis, A., et al., 2006. A phase 3 study of deferasirox (ICL670), a once-daily oral iron chelator, in patients with beta-thalassemia. Blood 107, 3455–3462.
Chansiw, N., Pangjit, K., Phisalaphong, C., Porter, J.B., Evans, P., Fucharoen, S., Srichairatanakool, S., 2014. Effect of a novel oral active iron chelator: 1-(Nacetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) iniron-overloaded and non-overloaded mice. Asian Pac. J. Trop. Med. 7 (Suppl. 1), S155–S161.
Chauhan, R., Sharma, S., Chandra, J., 2014. What regulates hepcidin in polytransfused b-thalassemia major: erythroid drive or store drive? Indian J. Pathol. Microbiol. 57 (1), 39–42.
Deugnier, Y., Brissot, P., Loreal, O., 2008. Iron and the liver: update 2008. J. Hepatol. 48, S113–S123.
Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., Vidal, N., 2006. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chem. 97, 654–660.
Dubey, S., Mehta, S.C., 2014. Hepatoprotective activity of Euphorbia Hirta Linn. Plant against carbon tetrachloride-induced hepatic injury in rats. Int. Conf. Food Bio Med. Sci. Bangkok (Thailand) 1, 28–29.
Erdincler, D.S., Seven, A., Inci, F., Beger, T., Candan, G., 1997. Lipid peroxidation and antioxidant status in experimental animals: effects of aging and hypercholesterolemia diet. Clin. Chim. Acta 265, 77–84.
Frazer, D.M., Wilkins, S.J., Becker, E.M., Vulpe, C.D., McKie, A.T., Trinder, D., Anderson, G.J., 2002. Hepcidin expression inversely correlates with the expression of duodenal iron transporters and iron absorption in rats. Gastroenterology 123, 835–844.
Friedman, S.L., Sheppard, D., Duffield, J.S., Violette, S., 2013. Therapy for fibrotic diseases: nearing the starting line. Sci. Transl. Med. 5, 167sr1.
Fujita, N., Sugimoto, R., Takeo, M., Urawa, N., Mifuji, R., Tanaka, H., Kobayashi, Y., Iwasa, M., Watanabe, S., Adachi, Y., Kaito, M., 2007. Hepcidin expression in the liver: relatively low level in patients with chronic hepatitis C. Mol. Med. 13 (1–2), 97–104.
Fung, E., Nemeth, E., 2013. Manipulation of the hepcidin pathway for therapeutic purposes. Haematologica 98 (11), 1667–1676.
Gagliardo, B., Faye, A., Jaouen, M., Deschemin, J.C., Canonne-Hergaux, F., Vaulont, S., Sari, M.A., 2008. Production of biologically active forms of recombinant hepcidin, the iron-regulatory hormone. FEBS J. 275, 3793–3803.
Ganz, T., 2011. Hepcidin and iron regulation, 10 years later. Blood 117, 4425–4433.
Gendimenico, G.J., Bouquin, P.L., Tramposch, K.M., 1988. Diphenylamine-colorimetric method for DNA assay: a shortened procedure by incubating samples at 50 degrees C. Anal. Biochem. 173 (1), 45–48. 15.
Ghoti, H., Fibach, E., Westerman, M., Gordana, O., Ganz, T., Rachmilewitz, E.A., 2011. Increased serum hepcidin levels during treatment with deferasirox in iron-overloaded patients with myelodysplastic syndrome. Br. J. Haematol. 153 (1), 118–120.
Gramza, A., Korczak, J., Amarowicz, R., 2005. Tea polyphenols – their antioxidant properties and biological activity – a review. Pol. J. Food Nutr. Sci. 14 (55), 219–235.
Gu, S., Song, X., Zhao, Y., Guo, J., Fei, C., Xu, F., Gu, S., Song, X., Zhao, Y., Chang, C., Li, X., 2013. The evaluation of iron overload through hepcidin level and its related factors in myelodysplastic syndromes. Hematology 18 (5), 286–294.
Handa, P., MorganStevenson, V., Maliken, B.D., Nelson, J.E., Washington, S., Westerman, M., Yeh, M.M., Kowdley, K.V., 2016. Iron overload results in hepatic oxidative stress, immune cell activation, and hepatocellular ballooning injury, leading to nonalcoholic steatohepatitis in genetically obese mice. Am. J. Physiol. Gastrointest. Liver Physiol. 310 (2), G11727. 15.
He, P., Noda, Y., Sugiyama, K., 2001. Green tea suppresses lipopolysaccharide-induced liver injury in d-galactosamine-sensitized rats. J. Nutr. 131 (5), 1560–1567.
Hisamura, F., Kojima-Yuasa, A., Kennedy, D.O., Matsui-Yuasa, I., 2006. Protective effect of green tea extract and tea polyphenols against FK506-induced cytotoxicity in renal cells. Basic Clin. Pharmacol. Toxicol. 98, 192–196.
Hortelano, S., Dewez, B., Genaro, A.M., Diaz-Guerra, M.J., Bosca, L., 1995. Nitric oxide is released in regenerating liver after partial hepatectomy. Hepatology 21, 776–786.
Hubscher, S.G., 2003. Iron overload, inflammation and fibrosis in genetic haemochromatosis. J. Hepatol. 38, 521–525.
Hussain, S.P., Raja, K., Amstad, P.A., Sawyer, M., Trudel, L.J., Wogan, G.N., Hofseth, L. J., Shields, P.G., Billiar, T.R., Trautwein, C., Hohler, T., Galle, P.R., Phillips, D.H., Markin, R., Marrogi, A.J., Harris, C.C., 2000. Increased p53 mutation load in nontumorous human liver of Wilson disease and hemochromatosis: oxyradical overload diseases. Proc. Natl. Acad. Sci. USA 97, 12770–12775.
Ibrahim, M.A., Khalaf, A.A., Galal, M.K., Ogaly, H.A., Hassan, H.M.A., 2015. Ameliorative influence of green tea extract on copper nanoparticle-induced hepatotoxicity in rats. Nanoscale Res. Lett. 10 (1), 363.
Ikuta, K., 2015. Two novel potential markers for iron metabolism: hepcidin and non-transferrin-bound iron (NTBI). Rinsho Ketsueki 56 (2), 194–203.
Jaeschke, H., McGill, M.R., Ramachandran, A., 2012. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: lessons learned from acetaminophen hepatotoxicity. Drug Metab. Rev. 44, 88–106.
Jomova, K., Valko, M., 2011. Advances in metal-induced oxidative stress and human disease. Toxicology 283, 65–87.
Kagaya, N., Tagawa, Y., Nagashima, H., Saijo, R., Kawase, M., Yagi, K., 2002. Suppression of cytotoxin-induced cell death in isolated hepatocytes by tea catechins. Eur. J. Pharmacol. 450, 231–236.
Kartikasari, A.E., Roelofs, R., Schaeps, R.M., Kemna, E.H., Peters, W.H., Swinkels, D.W., Tjalsma, H., 2008. Biochim. Biophys. Acta 1784, 2029–2037.
Kaszkin, M., Beck, K.F., Eberhardt, W., Pfeilschifter, J., 2004. Unravelling green tea's mechanisms of action: more than meets the eye. Mol. Pharmacol. 65 (1), 15–17.
Kautz, L., Jung, G., Valore, E.V., Rivella, S., Nemeth, E., Ganz, T., 2014. Identification of erythroferrone as an erythroid regulator of iron metabolism. Nat. Genet. 46 (7), 678–684.
Kim, H.K., Yang, T.H., Cho, H.Y., 2009. Antifibrotic effects of green tea on in vitro and in vivo models of liver fibrosis. World J. Gastroenterol. 7 (15), 5200–5205.
Koliaraki, V., Marinou, M., Vassilakopoulos, T.P., Vavourakis, E., Tsochatzis, E., Pangalis, G.A., Papatheodoridis, G., Stamoulakatou, A., Swinkels, D.W., Papanikolaou, G., Mamalaki, A., 2009. A novel immunological assay for hepcidin quantification in human serum. PLoS One. 4 (2), e4581.
Kowdley, K.V., 2004. Iron, hemochromatosis and hepatocellular carcinoma. Gastroenterology 127, S79–S86.
Kuhn, L.C., 2015. Iron regulatory proteins and their role in controlling ironmetabolism. Metallomics 7 (2), 232–243.
Kulprachakarn, K., Chansiw, N., Pangjit, K., Phisalaphong, C., Fucharoen, S., Hider, R. C., Santitherakul, S., Srichairatanakool, S., 2014. Iron-chelating and anti-lipid peroxidation properties of 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one (CM1) in long-term iron loading b-thalassemic mice. Asian Pac. J. Trop. Biomed. 4 (8), 663–668.
Lamberta, J.D., Eliasa, R.J., 2010. The antioxidant and pro-oxidant activities of green tea polyphenols: a role in cancer prevention. Arch. Biochem. Biophys. 501 (1), 65–72.
Lefevre, G., Beljean-Leymarie, M., Beyerle, F., Bonnefont-Rousselot, D., Cristol, J.P., Thérond, P., Torreilles, J., 1998. Evaluation of lipid peroxidation by measuring thiobarbituric acid reactive substances. Ann. Biol. Clin. (Paris) 56, 305–319.
Leopoldini, M., Russo, N., Toscano, M., 2011. The molecular basis of working mechanism of natural polyphenolic antioxidants. Food Chem. 125, 288–306.
Li, N., Hao, M., Phalen, R.F., Hinds, W.C., Nel, A.E., 2003. Particulate air pollutants and asthma. A paradigm for the role of oxidative stress in PM-induced adverse health effects. Clin. Immunol. 109, 250–265.
Li, X., Xu, F., Karoopongse, E., Marcondes, A.M., Lee, K., Kowdley, K.V., Miao, C.H., Trobridge, G.D., Campbell, J.S., Deeg, H.J., 2013. Allogeneic transplantation, Fas signaling, and dysregulation of hepcidin. Biol. Blood Marrow Transpl. 19, 1210–1219.
Loew, D., Kaszkin, M., 2002. Approaching the problem of bioequivalence of herbal medicinal products. Phytother. Res. 16 (8), 705–711.
Messner, D.J., Rhieu, B.H., Kowdley, K.V., 2013. Iron overload causes oxidative stress and impaired insulin signaling in AML-12 hepatocytes. Dig. Dis. Sci. 58 (7), 1899–1908.
Mollet, I.G., Patel, D., Govani, F.S., Giess, A., Paschalaki, K., Periyasamy, M., Lidington, E.C., Mason, J.C., Jones, M.D., Game, L., Ali, S., Shovlin, C.L., 2016. Low dose iron treatments induce a DNA damage response in human endothelial cells within minutes. PLoS One 11 (2), e0147990.
Mohammadyari, A., Razavipour, S.T., Mohammadbeigi, M., Negahdary, M., Ajdary, M., 2014. Explore in-vivo toxicity assessment of copper oxide nanoparticle in Wistar rats. J. Biolo. today's world 3 (6), 124–128.
Moon, M.S., Richie, J.P., Isom, H.C., 2010. Iron potentiates acetaminophen-induced oxidative stress and mitochondrial dysfunction in cultured mouse hepatocytes. Toxicol. Sci. 118, 119–127.
Mothana, R.A., 2011. Anti-inflammatory, antinociceptive and antioxidant activities of the endemic Soqotraen Boswelliaelongata Balf. f. and JatrophaunicostataBalf. f. in different experimental models. Food Chem. Toxicol. 49, 2594.
Nash, L.A., Ward, W.E., 2016. Comparison of black, green and rooibos tea on osteoblast activity. Food Funct. 7 (2), 1166–1175.
Neeta, S., 2007. Apoptosis in health and disease and modulation of apoptosis for therapy: an overview. Indian J. Clin. Biochem. 22 (2), 6–16.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., Ganz, T., 2004a. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J. Clin. Invest. 113 (9), 1271–1276.
Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., Ward, D.M., Ganz, T., Kaplan, J., 2004b. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 306, 2090–2093.
Ni, H.M., Williams, J.A., Yang, H., Shi, Y.H., Fan, J., Ding, W.X., 2012. Targeting autophagy for the treatment of liver diseases. Pharmacol. Res. 66, 463–474.
Ojiako, O.A., Chikezie, P.C., Ogbuji, A.C., 2015. Radical scavenging potentials of single and combinatorial herbal formulations in vitro. J. Tradit. Complement Med. 6 (2), 153–159.
Olynyk, J.K., St Pierre, T.G., Brunt, E.M., Bacon, B.R., 2005. Duration of hepatic iron exposure increases the risk of significant fibrosis in hereditary hemochromatosis: a new role for magnetic resonance imaging. Am. J. Gastroenterol. 100, 837–841.
Ounjaijean, S., Thephinlap, C., Khansuwan, U., Phisalapong, C., Fucharoen, S., Porter, J.B., Srichairatanakool, S., 2008. Effect of green tea on iron status and oxidative stress in iron-loaded rats. Med. Chem. 4, 365–370.
Pan, M.H., Lin, C.C., Lin, J.K., Chen, W.J., 2007. Tea polyphenol (−)-epigallocatechin 3-gallate suppresses heregulin-beta1-induced fatty acid synthase expression in human breast cancer cells by inhibiting phosphatidylinositol 3-kinase/Akt and mitogen-activated protein kinase cascade signaling. J. Agric. Food Chem. 55, 5030–5037.
Panat, N.A., Maurya, D.K., Ghaskadbi, S.S., Sandur, S.K., 2016. Troxerutin, a plant flavonoid, protects cells against oxidative stress-induced cell death through radical scavenging mechanism. Food Chem. 194, 32–45.
Pangjit, K., Banjerdpongchai, R., Phisalaphong, C., Fucharoen, S., Xie, Y.Y., Lu, Z.D., Hider, R.C., Srichairatanakool, S., 2015. Characterisation of a novel oral iron chelator: 1-(N-acetyl-6-aminohexyl)-3-hydroxy-2-methylpyridin-4-one. J. Pharm. Pharmacol. 67 (5), 703–713.
Patel, N., Masaratana, P., Diaz-Castro, J., Latunde-Dada, G.O., Qureshi, A., Lockyer, P., Jacob, M., Arno, M., Matak, P., Mitry, R.R., Hughes, R.D., Dhawan, A., Patterson, C., Simpson, R.J., McKie, A.T., 2012. BMPER protein is a negative regulator of hepcidin and is up-regulated in hypotransferrinemic mice. J. Biol. Chem. 287 (6), 4099–4106.
Pezeshki, A., Safi, S., Feizi, A., Askari, G., Karami, F., 2016. The effect of green tea extract supplementation on liver enzymes in patients with nonalcoholic fatty liver disease. Int. J. Prev. Med. 1 (7), 28.
Pietrangelo, A., 2003. Iron-induced oxidant stress in alcoholic liver fibrogenesis. Alcohol 30, 121–129.
Poli, G., 2000. Pathogenesis of liver fibrosis: role of oxidative stress. Mol. Aspects Med. 21, 49–98.
Porter, J.B., Walter, P.B., Neumayr, L.D., Evans, P., Bansal, S., Garbowski, M., Weyhmiller, M.G., Harmatz, P.R., Wood, J.C., Miller, J.L., Byrnes, C., Weiss, G., Seifert, M., Grosse, R., Grabowski, D., Schmidt, A., Fischer, R., Nielsen, P., Niemeyer, C., Vichinsky, E., 2014. Mechanisms of plasma non-transferrin bound iron generation: insights from comparing transfused diamond blackfan anaemia with sickle cell and thalassaemia patients. Br. J. Haematol. 167 (5), 692–696.
Roomi, M.W., Kalinovsky, T., Rath, M., Niedzwiecki, A., 2016. A nutrient mixture inhibits glioblastoma xenograft U-87 MG growth in male nude mice. Exp. Oncol. 38 (1), 54–56.
Ruddell, R.G., Hoang-Le, D., Barwood, J.M., Rutherford, P.S., Piva, T.J., Watters, D.J., Santambrogio, P., Arosio, P., Ramm, G.A., 2009. Ferritin functions as a pro inflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology 49 (3), 887–900.
Saewong, T., Ounjaijean, S., Mundee, Y., Pattanapanyasat, K., Fucharoen, S., Porter, J. B., Srichairatanakool, S., 2010. Effects of green tea on iron accumulation and oxidative stress in livers of iron-challenged thalassemic mice. Med. Chem. 6 (2), 57–64.
Safer, A.M., Afzal, M., Hanafy, N., Mousa, S., 2015. Green tea extract therapy diminishes hepatic fibrosis mediated by dual exposure to carbon tetrachloride and ethanol: a histopathological study. Exp. Ther. Med. 3, 794.
Schattenberg, J.M., Galle, P.R., Schuchmann, M., 2006. Apoptosis in liver disease. Liver Int. 26, 904–911.
Scheuer, P.J., 1991. Classification of chronic viral hepatitis: a need for reassessment. J. Hepatol. 13, 372–374.
Shuto, Y., Kataoka, M., Higuchi, Y., Matsuura, K., Hijiya, N.Y., Amamoto, S., 2004. Roles of CD14 in LPS-induced liver injury and lethality in mice pretreated with *Propionibacterium acnes*. Immunol. Lett. 94, 47–55.
Siah, C.W., Ombiga, J., Adams, L.A., Trinder, D., Olynyk, J.K., 2006. Normal iron metabolism and the pathophysiology of iron overload disorders. Clin. Biochem. Rev. 27, 5–16.
Somdet, S., Kulprachakarn, K., Pangjit, K., Pattanapanyasat, K., Fuchaeron, S., 2012. Green tea extract and epigallocatechin 3-gallate reduced labile iron pool and protected oxidative stress in iron-loaded cultured hepatocytes. Adv. Biosci. Biotechnol. 3, 1140–1150.
Tan, T.C., Crawford, D.H., Franklin, M.E., Jaskowski, L.A., Macdonald, G.A., Jonsson, J. R., Watson, M.J., Taylor, P.J., Fletcher, L.M., 2012. The serum hepcidin: ferritin ratio is a potential biomarker for cirrhosis. Liver Int. 32 (9), 1391–1399.
Tao, T.Y., Gitlin, J.D., 2003. Hepatic copper metabolism: insights from genetic disease. Hepatology 37, 1241–1247.
Thapa, B.R., Anuj, W., 2007. Liver function tests and their interpretation. Indian J. Pediatr. 74, 671.
Tomaszewska, E., Winiarska-Mieczan, A., Dobrowolski, P., 2015. Hematological and serum biochemical parameters of blood in adolescent rats and histomorphological changes in the jejunal epithelium and liver after chronic exposure to cadmium and lead in the case of supplementation with green tea vs black, redor white tea. Exp. Toxicol. Pathol. 67, 331–339.
Uchiyama, A., Kim, J.S., Kon, K., Jaeschke, H., Ikejima, K., Watanabe, S., Lemasters, J.J., 2008. Translocation of iron from lysosomes into mitochondria is a key event during oxidative stress-induced hepatocellular injury. Hepatology 48, 1644–1654.
Upanan, S., Pangjit, K., Uthaipibull, C., 2015. Combined treatment of 3-hydroxypyridine-4-one derivatives and green tea extract to induce hepcidin expression in iron-overloaded b-thalassemic mice. Asian Pac. J. Trop. Biomed. 5 (12), 1010–1017.
Villarroel, P., Le Blanc, S., Arredondo, M., 2012. Interleukin-6 and lipopolysaccharide modulate hepcidin mRNA expression by HepG2 cells. Biol. Trace Elem. Res. 150, 496–501.
Wosten, M.M., Kox, L.F., Chamnongpol, S., Soncini, F.C., Groisman, E.A., 2000. A signal transduction system that responds to extracellular iron. Cell 103, 113–125.
Yun, S., Vincelette, N.D., 2015. Update on iron metabolism and molecular perspective of common genetic and acquired disorder, hemochromatosis. Crit. Rev. Oncol. Hematol. 95 (1), 12–25.