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Protective Effect of Curcumin on Antioxidant Defense System and Oxidative Stress in Liver Tissue of Iron Overloading Rats

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ABSTRACT

Iron overload (IOL) is one of the most common metal related toxicity. Under this circumstance, excessive iron deposition in liver will lead to further injury such as hepatocellular necrosis, inflammation, fibrosis and even to carcinoma. In this study, the effect of a nature flavonoid, curcumin, on IOL-induced rats liver oxidative damage was investigated. Sixty male albino rats were randomly divided into three equal groups. Group I: Control group, Group II: Iron-overload group, received six doses (three doses per week) of ferric hydroxide poly maltose (100 mg kg^{-1} b.wt.) by intraperitoneal injections (IP) and Group III: Iron overload+curcumin group, received six doses of 100 mg kg^{-1} b.wt. ferric hydroxide poly maltose (IP) and curcumin (100 mg kg^{-1} b.wt.) as one daily oral dose until the sacrificed day. Blood samples for serum separation and liver tissue specimens were collected three times, after three, four and five weeks from the onset of the experimental. Serum iron profile (Iron, Total iron Binding Capacity (TIBC), Unsaturated Iron Binding Capacity (UIBC), transferrin (Tf) and transferrin saturation% (TS%)}, ferritin, albumin, total protein, total cholesterol and triacylglycerols levels and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined. Moreover, iron, L-malondialdehyde (L-MDA), reduced glutathione (GSH), nitric oxide (NO) and total nucleic acid (TNA) levels and glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities in the liver were also determined. The obtained results revealed that, IOL resulted in significant increase in serum iron, TIBC, Tf, TS% and ferritin levels and AST and ALT activities and also increased liver iron, L-MDA and NO levels. Meanwhile, it decreased serum UIBC, albumin and total protein and liver GSH, TNA levels and GPx, CAT and SOD activities, compared those of the control group. Administration of curcumin to IOL rats significantly decreased serum iron, TIBC, Tf, TS%, ferritin levels, AST and ALT activities and liver iron, L-MDA and NO levels, with significant increases in serum UIBC, albumin, total protein and total cholesterol levels and in liver GSH, CAT and SOD activities compared with the IOL group. The results of this study may demonstrate the antioxidant potential of curcumin against iron overload induced hepatic oxidative stress in rats.

Key words: Curcumin, antioxidant enzymes, iron overload, oxidative stress, iron profile, rats

INTRODUCTION

Iron is an essential micronutrient for all living organisms and mainly presents in protein-bound forms such as heme and non-heme proteins (Boldt, 1999). It is essential for many metabolic functions (oxygen transport and utilization, DNA synthesis, electron transport), but it becomes toxic

when accumulated (Crisponi and Remelli, 2008). It promotes oxidative stress in the livers of overloaded animals and human (Cornejo *et al.*, 2005), as it participates in the fenton reaction, the most basic reaction of oxidative stress and Reactive Oxygen Species (ROS) (Gitto *et al.*, 2001). In hepatocytes, iron excess induces cellular injury and functional abnormalities by lipid peroxidation (LPO) process (Piperno *et al.*, 2002), as excessive iron deposition leads to fibrosis (Arezzini *et al.*, 2003) and subsequently development of liver cirrhosis (Stal *et al.*, 1990), or hepatocellular carcinoma (Siah *et al.*, 2005).

Iron-removal therapy is an effective life-saving strategy that may be achieved by antioxidants, iron chelators and/or free radical scavenging compounds as flavonoids (polyphenols) (Zhang *et al.*, 2006). Flavonoids are phenolic compounds widely distributed in plants which exert multiple biological effects, including antioxidant and free radical scavenging abilities Negre-Salvayre and Salvayre, 1992), in addition to their iron chelating activity (Blache *et al.*, 2002) as they were suggested to present a strong affinity to iron ions (Borsari *et al.*, 2001).

Curcumin is a polyphenol extracted from the rhizomes of the Indian spice turmeric (Dattani *et al.*, 2010) and possess multiple pharmacological properties (Aggarwal and Sung, 2009a). It is 10 times more active as an antioxidant or free radical scavenger than vitamin E (Jyoti *et al.*, 2009), show hepatoprotective property (Shapiro *et al.*, 2006), as well as protection against iron-induced LPO by forming a complex with ferric ions (Reddy and Lokesh, 1994b). Accordingly, the present study investigates the protective effect of curcumin as natural antioxidant against iron overload induced hepatic oxidative stress in rats.

MATERIALS AND METHODS

Animals: Sixty white male albino rats of 8-10 weeks old and weighing 180- 220 g were used in the experimental investigation of this study. Rats were obtained from the Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University, housed in separated metal cages and kept at constant environmental and nutritional conditions during the experimental period. The animals provided with a constant supply of standard pellet diet and fresh, clean drinking water ad libitum.

Drug and antioxidants: The drug and antioxidant compounds used in the present study were:

Haemojet(R): Haemojet ampoules were produced by Amriya Pharma India for European Egyptian Pharma India, Alexandria, Egypt. Haemojet was obtained as pack of three ampoules of two ml solution. Each ampoule contains elemental iron (100 mg) as ferric hydroxide polymaltose complex

Curcumin: Pale orange's yellow crystalline powder (purity~95%), of Indian production, purchased from El-Goumhoria Co., Cairo-Egypt. Curcumin was dissolved in dimethyl sulphoxide (DMSO) and administered to animals daily by a dose of 100 mg kg^{-1} b.wt. (Naik *et al.*, 2011).

Induction of iron overload: Iron overload was induced by intraperitoneal injections of sex doses (three doses per week) of (100 mg kg^{-1} b.wt.) ferric hydroxide polymaltose complex (Zhao *et al.*, 2005).

Experimental design: Rats were randomly divided into three groups; each group containing twenty rats as follows:

Group I (control group): Received no drugs or chemicals and served as control for all other groups.

Group II (iron-overload): Received six doses (three doses per week) of 100 mg kg^{-1} b.wt. (ferric hydroxide polymaltose) administered as intraperitoneal (IP).

Group III (iron-overload+Curcumin): Administered intraperitoneal (IP) with six doses (three doses per week) of 100 mg kg^{-1} b.wt. (ferric hydroxide polymaltose) followed by oral administration of curcumin at the dose level of 50 mg Kg^{-1} b.wt. until the end of the experiment.

Sampling: Random blood samples and liver tissue specimens were collected from all animals groups (control and experimental groups) at the end of each experimental periods after 3, 4 and 5 weeks from the onset of curcumin treatment:

- **Blood samples:** Blood samples for serum separation were collected by ocular vein puncture in dry, clean and screw capped tubes and serum were separated by centrifugation at 2500 rpm for 15 min. The clean, clear serum was separated by Automatic pipette and received in dry sterile samples tube, then kept in a deep freeze at -20°C until used for subsequent biochemical analysis
- **Liver tissues:** At the end of the each experimental period, rats were sacrificed by cervical decapitation. The liver specimens were quickly removed and weighted, then perfused with cold saline to exclude the blood cells and then blotted on filter papers and stored at -20°C. Briefly, half of liver tissues were cut, weighed and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH7.4) to make 10% homogenates. The homogenates were centrifuged at 5,000 r.p.m for 15 min at 4°C then the supernatants were used for the subsequent biochemical analysis

The other half of livers were weighed and putted into glass flask, then 5 volumes of mixed acid (nitric acid: perchloric acid, 4:1) were added, heated until large amount of white vapors could be seen. The volumes of the digested samples were adjusted to 10 mL with double distilled water and the obtained solutions were used to analyze iron contents.

Biochemical analysis: Serum iron and Total Iron Binding Capacity (TIBC), ferritin, albumin, total Protein, total cholesterol, triacylglycerols levels and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, were determined according to the methods described by Makino *et al.* (1988), Dawson *et al.* (1992), Young (2001), Gendler (1984), Goodman *et al.* (1988), Stein (1987) and Young (2001), respectively.

Moreover, total iron in the liver, L-malondialdehyde (L-MDA), reduced glutathione (GSH), nitric oxide (NO) and total nucleic acid (TNA) levels and glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities were determined according to the methods described by Parker *et al.* (1967), Esterbauer *et al.* (1982), Beutler (1957), Montgomery and Dymock (1961), Spirin (1958), Gross *et al.* (1967), Sinha (1972) and Packer and Glazer (1990), respectively.

Statistical analysis: The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple test. All analyses were performed using the statistical package for social science 13.0 software, (SPSS, 2009). Values of $p<0.05$ were considered to be significant.

RESULTS

The results presented in (Table 1 and 2) revealed that, iron-overload caused a significant increase in serum total iron, TIBC, Tf, TS% and ferritin levels and AST and ALT activities and also increased liver iron, L-MDA and NO levels. Meanwhile, it decreased serum UIBC, total cholesterol,triacylglycerols, total protein, albumin and liver GSH, TNA levels and Gpx, CAT and SOD activities when compared with the control group. Administration of curcumin in iron-overloaded rats significantly decreased serum total iron, TIBC, Tf, TS%, ferritin levels, AST and ALT activities and liver total iron, L-MDA and NO levels with significant increases in serum UIBC, total protein, albumin and total cholesterol levels and in liver GSH, CAT and SOD activities when compared with the iron-overload group.

DISCUSSION

When iron accumulates in the body and exceeds the antioxidant defense, the cells undergo oxidative stress (Crisponi and Remelli, 2008), that causes cell damage (Papanikolaou and Pantopoulos, 2005). Generally the results support a beneficial effect of antioxidants, includng flavonoids that are substances with both chelating and free radical scavenging properties (Fraga a nd Oteiza, 2002), from which curcumin possibly be a very useful medicine for improvement of the antioxidant defence system against IOL-induced oxidative stress in rats.

Serum and liver iron and serum TIBC, Tf, TS% and ferritin levels were significantly elevated in the iron loaded rats while serum UIBC was significantly decreased. When rats were administerd with curcumin, serum and liver iron and serum TIBC, Tf, TS% and ferritin concentrations were significantly decreased and serum UIBC was significantly increased than that of IOL group (Tables 1, 2).

These results came in accordance with the recorded data of Papanikolaou and Pantopoulos (2005) who reported that when plasma Fe content exceeds the Iron Binding Capability (IBC) of transferrin, iron accumulates in the body and causes cell damage. Also, Crisponi and Remelli (2008) reported that, serum iron was 75% higher in rats receiving cholesterol-free diet together with iron dextran treatment compared with those of the untreated control group and also presented higher TS.

Additionaly, Zhang *et al.* (2006) recorded that, hepatic iron in mice were significantly increased after administration of 500 mg kg^{-1} iron-dextran for 45 days. Moreover, Silva *et al.* (2008) observed that, liver iron was significantly influenced by iron-dextran administration, as treated rats showed 6-fold levels higher than that of the control group. Furthermore, Torti and Torti (2002) recorded that, treatment with iron increased basal levels of ferritin H. Also Zhang *et al.* (2006) reported that, when mouse under IOL, serum ferritin level was significantly increased. Cornejo *et al.* (2007) observed that, chronic iron overload leads to a substantial increase in liver NOS activity in rats, a feature that is accompanied by ferritin induction.

Excess iron induced increase in hepcidin mRNA level that was not sufficient to prevent increased intestinal iron absorption and onset of IOL, compatible with the observation that serum iron was very high in that condition and TS was more than 100%, that most certainly results in the presence of NTBI, although they were not able to assess this directly (Nahdi *et al.*, 2010). When the iron load increases, the iron binding capacity (IBC) of serum Tf is exceeded and a NTBI fraction of plasma iron appears which generates free hydroxyl radicals and induces dangerous tissue damage (Crisponi and Remelli, 2008), suggesting a novel mechanistic link between dopaminergic GSH depletion and increased Fe levels based on increased translational regulation of transferrin receptor 1(TfR1) (Kaur *et al.*, 2009).

Thus, the increase in hepatic iron pool due to GSH depletion mechanism indicate a strong relation between alterations of cellular redox condtion/increase in ROS generation due to GSH depletion with altered iron homeostasis in hepatic cell that led to iron deposition, but in conditions when GSH depletion sustainsed for long, iron-storage capacity of ferritin might be a limiting factor to sequester continuously generating free iron (Tapryal *et al.*, 2010). However, iron in excess, regulates iron storage protein ferritin synthesis by translational mechanism due to coordinated release of bound iron regulatory proteins (IRPs) from iron responsive elements present in the 5'UTR of ferritin (Andrews and Schmidt, 2007).

Curumin administration to iron overloaded rats protect against iron-induced LPO by forming a complex with Fe^{3+} (Reddy and Lokesh, 1994b). Similar results were reported by Ak and Gulcin (2008) who recorded that, the marked capacity of CUR for iron binding, suggested that its main action as a peroxidation inhibitor may be related to its IBC, as it might chelate the Fe^{2+} with its binding sites, hydroxyl and methoxyl groups. Additionally, Kalpravidh *et al.* (2010) observed that, during the treatment of **β**-thalassemia/HbE patients with curcuminoids for 12 month, serum NTBI levels were significantly reduced during the first 6 months and slightly increased afterward

Table 1: Effect of treatment with curcumin on some biochemical blood parameters in iron overloaded male rats

	Animal groups								
	Control (weeks)			Iron overload (weeks)			Curumin+iron overload (weeks)		
Parameters	3	4	5	3	4	5	3	4	5
Total iron (μg dL^{-1})	138.50±7.03^{b}	144.50±3.66^{c}	161.00±7.82^{b}	230.75±3.52^{a}	232.80±8.22^{a}	260.75±11.84^{a}	147.25±4.50^{b}	165.40±4.40^{b}	185.25±3.09^{b}
TIBC (μg dL^{-1})	211.50±5.30^{c}	221.25±4.46^{d}	243.50±10.99^{b}	289.25±2.75^{a}	300.00±7.34^{a}	336.50±14.86^{a}	216.00±10.78^{c}	247.25±8.86^{c}	269.75±7.77^{b}
UIBC (μg dL^{-1})	75.00±2.27^{a}	76.75±0.95^{b}	82.00±3.44^{ab}	54.75±0.85^{b}	58.75±2.81^{c}	73.75±2.25^{b}	83.25±2.98^{a}	81.75±4.15^{b}	58.25±1.49^{b}
Transferrin (g L^{-1})	1.50±0.04^{c}	1.55±0.03^{a}	1.70±0.08^{b}	2.04±0.04^{a}	2.10±0.04^{a}	2.37±0.10^{a}	1.85±0.04^{b}	1.71±0.07^{a}	1.50±0.07^{c}
TS (%)	65.25±1.70^{bc}	65.25±0.25^{b}	66.50±0.65^{b}	79.75±1.32^{a}	79.25±1.25^{a}	77.50±0.29^{a}	73.75±1.80^{ab}	68.00±1.78^{b}	69.00±1.08^{b}
Ferritin (ng mL^{-1})	2.07±0.08^{b}	2.25±0.03^{b}	3.33±0.04^{b}	2.47±0.05^{a}	2.60±0.04^{a}	3.75±0.07^{a}	2.38±0.10^{a}	2.54±0.04^{a}	2.74±0.01^{c}
AST (U L^{-1})	78.60±6.07^{d}	94.00±1.58^{c}	72.40±6.17^{c}	152.60±5.56^{a}	149.80±4.93^{a}	148.00±6.63^{a}	132.60±2.66^{b}	114.60±4.68^{b}	125.60±2.94^{b}
ALT (U L^{-1})	14.80±1.28^{c}	15.60±0.75^{c}	16.60±1.21^{b}	25.60±1.08^{a}	34.40±2.11^{a}	31.80±2.35^{a}	20.80±1.53^{b}	18.40±2.14^{bc}	20.20±1.07^{b}
Total Cholesterol (mg dL^{-1})	110.25±5.65^{a}	90.76±7.44^{ab}	89.25±7.98^{b}	68.75±9.43^{b}	78.75±4.23^{b}	77.00±2.38^{b}	121.25±1.89^{a}	103.50±5.33^{a}	110.50±3.97^{a}
Triacylglycerols (mg dL^{-1})	94.00±5.80^{a}	78.00±3.87^{a}	82.00±5.67^{a}	74.50±4.17^{b}	66.75±4.77^{a}	71.75±1.32^{a}	90.25±4.66^{a}	77.00±4.66^{a}	79.25±6.01^{a}
Albumin (g dL^{-1})	3.02±0.17^{b}	3.44±0.18^{a}	3.84±0.39^{a}	2.04±0.13^{c}	2.72±0.08^{b}	1.98±0.10^{b}	4.48±0.21^{a}	3.96±0.19^{a}	4.14±0.16^{a}
Total protein (g dL^{-1})	6.36±0.31^{b}	6.90±0.44^{ab}	7.00±0.64^{a}	4.58±0.25^{c}	5.92±0.25^{b}	3.44±0.22^{b}	8.58±0.34^{a}	7.49±0.40^{a}	6.68±0.25^{a}

Data are presented as (Mean±S.E.). S.E: Standard error, Mean values with different superscript letters in the same row are significantly different at ($p<0.05$)

Table 2: Effect of treatment with curcumin on some biochemical liver parameters in iron overloaded male rats

	Animal groups								
	Control (weeks)			Iron overload (weeks)			Curumin+iron overload (weeks)		
Parameters	3	4	5	3	4	5	3	4	5
Total iron (μg dL^{-1})	290.00±17.32^{c}	323.33±14.53^{c}	353.33±26.03^{c}	1266.67±46.67^{a}	1313.33±17.64^{a}	1373.33±12.02^{a}	1046.67±49.10^{b}	1106.67±44.85^{b}	1156.67±62.27^{b}
MDA (nmol g^{-1})	131.60±5.07^{b}	139.80±6.99^{b}	136.00±8.33^{c}	189.60±18.38^{a}	197.80±9.34^{a}	182.00±5.72^{a}	157.20±13.28^{ab}	154.40±5.05^{b}	160.60±7.00^{ab}
GSH (nmol g^{-1})	87.60±3.75^{a}	82.60±1.50^{a}	80.00±0.89^{a}	76.00±1.23^{b}	75.40±0.93^{b}	73.60±0.93^{b}	78.60±1.21^{b}	77.80±0.86^{b}	78.20±0.86^{a}
GPX(U g^{-1})	0.49±0.02^{b}	0.54±0.01^{a}	0.17±0.02^{a}	0.58±0.01^{a}	0.44±0.02^{bc}	0.23±0.02^{a}	0.55±0.06^{a}	0.37±0.04^{c}	0.25±0.04^{a}
CAT (U g^{-1})	14.78±0.33^{a}	14.94±0.23^{a}	14.84±0.29^{a}	12.16±0.37^{b}	12.46±0.39^{b}	12.72±0.29^{b}	15.18±0.19^{a}	14.42±0.20^{a}	14.50±0.91^{a}
SOD (U g^{-1})	304.00±19.65^{a}	270.00±7.07^{a}	278.00±18.55^{a}	174.40±10.27^{c}	174.00±16.31^{b}	145.00±9.22^{c}	236.00±10.77^{b}	238.00±3.74^{a}	248.00±15.30^{b}
NO (μmol g^{-1})	0.159±0.002^{b}	0.164±0.002^{b}	0.153±0.003^{b}	0.193±0.004^{a}	0.225±0.02^{a}	0.176±0.004^{a}	0.153±0.01^{b}	0.153±0.001^{b}	0.142±0.002^{c}
TNA (μg g^{-1})	0.43±0.04^{a}	0.37±0.03^{a}	0.45±0.04^{a}	0.32±0.04^{a}	0.25±0.03^{b}	0.33±0.03^{a}	0.40±0.05^{a}	0.36±0.05^{ab}	0.40±0.15^{a}

Data are presented as (Mean±SE), SE: Standard error. Mean values with different superscript letters in the same row are significantly different at ($p<0.05$)

and mRNA levels of ferritin were increased. Moreover, Bernabe-Pineda *et al.* (2004) reported that, CUR induced ferritin mRNA and reduced ferritin protein in cultured liver cell. Also, Jiao *et al.* (2006) reported that, CUR increased mRNA levels of ferritin in cultured liver cells in mice. Suggesting that iron chelation may be an additional mode of action of CUR, to redox metal-binding activity (Yang *et al.*, 2005). Also, CUR ablility to bind Fe^{+3} via its **β**-diketonate group (binding sites for metal chelation) impling that iron chelation may be a novel mechanism (Bernabe-Pineda *et al.*, 2004). Also the ability of CUR to activate transferrin-receptor 1 and iron regulatory proteins (IRPs) (indicators of iron depletion), as it enhances mRNA levels of the TfR1 in cultured liver cells of mice (Jiao *et al.*, 2006), in which con?ict between increased mRNA and decreased protein levels of ferritin, indicating another mode of action of CUR and may explain the slight decrease of serum ferritin levels (Kalpravidh *et al.*, 2010)).

Serum AST and ALT activities were significantly increased in iron loaded rats, compared with the control. Similar results were obtained by Lykkesfeldt *et al.* (2007) who recorded that, increasing of iron conc. in the liver resulted in increase in the biochemical markers of oxidative stress and damage. Also, Pardo-Andreu *et al.* (2008) reported that, hepatotoxicity was evidenced by 3-fold increases of AST and ALT. This siginicant increase of AST and ALT can be attributed to the generation of ROS and oxidative damage by excess hepatic iron (Jungst *et al.*, 2004), as numerous enzymes normally located in the cytosol are released into the blood stream (Kumar *et al.*, 2006). Also, hepatotoxicity was evidenced (Pardo-Andreu *et al.*, 2008)) by the significant correlation between the levels of liver lipid peroxide and AST and ALT in serum in iron-injected animals. Curumin administration to iron overloaded rats induced significant decreases in AST and ALT. Similarly, Reddy and Lokesh (1996) observed that, administration of CUR to rats injected with Fe^{3+} significantly lowered serum ALT and AST activities. In addition to Naik *et al.* (2011) reported that, CUR at different doses signi?cantly restored the elevated serum marker enzymes in different experimental animal models, during CCl_4 and isoproterenol (ISO)-induced liver and cardiac toxicity, resp., indicating that this spice principle reduces the severity of iron toxicity by lowering the LPO, due to its antioxidative (Naik *et al.*, 2011) and radical scavenging properties (Masuda *et al.*, 2001), as the significant decreasing of ALT and AST had a high degree of correlation with liver lipid peroxides, following iron induced liver toxicity (Reddy and Lokesh, 1996). Moreover, this observed anti-LPO effect of CUR may be attributed to CUR redox metal-binding activity (Yang *et al.*, 2005).

Iron overload resulted in significant increase in serum albumin and total protein concentrations compared with the control rats. Similar results were recorded by Benhar *et al.* (2002) who reported that, the cytotoxic and reactive aldehydic by-products of LPO induced by iron, as MDA, thiobarbituric reactive substances (TBARS) and 4-hydroxy-2-non-enal (4-HNE) impair cellular function and protein synthesis. Additionally, Arnal *et al.* (2010) recorded that, the elevated levels of ROS are related to the pathogenesis of various diseases as a result of damage to important biomolecules such as lipids, proteins and DNA (Heim *et al.*, 2002). These results are consistent with releasing cytotoxic products of LPO that may impair cellular functions, thus stimulating O-dependent processes such as LPO (Galleano and Puntarulo, 1992) and protein oxidation (Boisier *et al.*, 1999), in which the formation of MDA-protein adducts was confirmed in iron loaded liver rats (Khan *et al.*, 2002) and the greater formation of these MDA-macromolecules adducts could be a potential mechanism in iron-induced hepatic injury (Zhang *et al.*, 2006).

When rats were administerated with curcumin, serum albumin and total protein concentrations were significantly increased than that of iron loaded group. These results agree well with those

obtained by Yousef *et al.* (2010) who reported that, CUR restored plasma total protein, albumin, globulin and bilirubin as a consequence of liver protection, similar to the results obtained of rats challenged with sodium arsenite and treated concomitantly with CUR for 30 days (Yousef *et al.*, 2008). Such protective effect of CUR can be attributed to its antioxidant (Wei *et al.*, 2006) and free radical scavenging activity (Priyadarsini *et al.*, 2003), in addition to its anti-LPO and anti-protein oxidation effects (Farooqui and Horrocks, 1998), in which binding to Fe^{2+} could be another mechanism underlying its ability to prevent protein oxidation in epileptic rats (Jyoti *et al.*, 2009).

The significant decrease in serum total cholesterol and triacyglycerols concentrations in iron-overload after three weeks only followed by non siginificant decrease until the end of the experiment, compared with the control rats, may be explained by Silva *et al.* (2008) who reported that, hepatic injury triggered by iron excess may increase the concentrations of secondary serum metabolites, such as cholesterol, triacylglycerols and glucose and also recorded that, treatment with Fe-dextran in male rats increased serum triacylglycerols level, but had no effect on the cholesterol level. However, Araujo *et al.* (1995) and Turbino-Ribeiro *et al.* (2003) observed the absence of alteration in serum cholesterol in rabbits receiving Fe-dextran injections. Curcumin administeration significantly increased serum concentrations of triacyglycerols after three weeks only and total cholesterol allover the experimental period than that of IOL group (Table 1). These results may agree with the data of Olszanecki *et al.* (2005) who reported that, CUR inhibited atherogenesis and did not in?uence the conc. of cholesterol or triglycerides in blood, in mice model. However, contradictory results were recorded by Aggarwal and Harikumar (2009) who recorded the protective effects of curcumin in rabbits with experimental atherosclerosis, as indicated by inhibition of lipoperoxidation of subcellular membranes and low-density lipoprotein oxidation, brought a significant decrease in blood triglyceride in diabetic rats and has hypocholesterolemic effects (Ramirez-Tortosa *et al.*, 1999). That can be explained by the Baum *et al.* (2007) who reported that, any decrease in cholesterol levels by CUR may be due to inhibition of dietary cholesterol absorption and not because of its effects on cholesterol synthesis.

The obtained results revealed that iron overload resulted in significant increase in liver L-MDA concentrations. The results agree well with those recorded by Asare *et al.* (2006) who observed that, all of the indices of LPO including MDA were increased to a signi?cantly greater extent (~5fold) in the iron-supplemented rats compared with the control rats. Additionally, Jagetia and Reddy (2011) reported that, treatment of isolated mouse liver mitochondrial fraction with ferric ion resulted in a time dependent elevation in the LPO up to 30 min post-treatment, where a maximum LPO was observed, with increased TBARS by ~60%, suggesting thereby production of oxidative stress in which MDA, a measure of free radical generation, is an end product of LPO (Golechha *et al.*, 2011) that is usually used as an indirect indicator of ROS (Turner *et al.*, 1997), in which iron-associated LPO induced by dietary IOL in animals results in aldehyde conjugation of liver microsomal proteins (aldehyde-protein adducts), suggesting that covalent modification of microsomal proteins may alter their function and might lead to cellular injury and iron-associated hepatotoxicity (Valerio and Petersen, 1998). When rats were administerated with curcumin hepatic L-MDA concentrations were significantly decreased than that of iron loaded group (Table 2). These obtained results agree with the data of Reddy and Lokesh (1996) who reported that, MDA level measured as TBARS and lipid peroxides in which liver lipid peroxides measured as TBARS were lowered, by 24% due to oral administration of CUR and by 39 and 34%, respectively, due to oral administration of CUR and eugenol in Fe-injected rats. Also, Naik *et al.* (2011) observed that, CUR significantly reduced the elevated MDA levels in different target tissues in CCl_4-induced liver injury and ISO-induced cardiac

necrosis and signi?cantly inhibits LPO process by preventing the formation of MDA from fatty acids, due to the antioxidant property of CUR in vivo which ultimately protects membrane damage leading to hepato- and cardio-protection. Furthermore, CUR regulates the LPO by altering the antioxidant enzyme activities (Reddy and Lokesh, 1994a) and reduces Fe^{3+}/Fe^{2+} which may result in the inhibition of LPO (Minotti and Aust, 1987) and decrease of MDA formation in various target tissues.

Liver GSH concentrations were significantly decreased in the iron loaded rats as compared with control group. The obtained results are nearly similar to those of Pardo-Andreu *et al.* (2008) who reported that, Fe-dextran administration in liver tissue evidenced by 34% decrease of its GSH content, in Fe loaded rats. Also, Kalpravidh *et al.* (2010) recorded that, the reduced GSH levels of RBC in **β**-thalassemia/HbE patients were also significantly decreased concomitant with the increase in GPx activities. GSH, that is an important non-protein thiol and work in conjunction with GPx, is involved in protection against free radicals, peroxides and xenobiotics (Rahman and MacNee, 2000) and maintain the overall cellular redox state upon oxidation (Formigari *et al.*, 2007), indicating a strong relation between alterations of cellular redox condtion/increase in ROS due to GSH depletion (Gong *et al.*, 2010). Curcumin administration to iron loaded rats resulted in significant increase in liver GSH level compared with the IOL-group. Similarly, Mohanty *et al.* (2004) reported that, Curcuma longa exhibited significant antioxidant activity as it restored GSH level activity and reduced LPO compared to control in I/R-induced myocardial injuries rats. Moreover, Kalpravidh *et al.* (2010) explored that the antioxidant activity of curcuminoids resulted in a signi?cant decrease in the SOD and GPx concomitant with an increase in GSH levels in RBC throughout treatment **β**-thalassemia/Hb E patients and this increasing in cellular GSH content can be attributed to the antioxidant activity of curcuminoids. CUR is able to donate H atom from the phenolic groups directly to $O_2^{\bullet-}$ and $^{\bullet}OH$ (Biswas *et al.*, 2005) and thus lowering ROS level (Venkatesan *et al.*, 2000). The significant restore of depleted GSH levels in target organs with CUR could be either due to its enhanced synthesis or due to improved glutathione reductase activity (Naik *et al.*, 2011).

The obtained results revealed that, IOL resulted in significant decrease in liver CAT and SOD activities when compared with the control group. Similar results was reported by Galleano and Puntarulo (1997) who observed that, under IOL there was a significant decrease of CAT activity in rat liver. Also, Jagetia and Reddy (2011) observed that, IOL drastically reduced GPx by ~2 fold, in the mouse mitochondrial fraction and caused inhibition in the CAT activity which was reduced to less than half of the control value and induced significant decline in the SOD activity by 3.25 folds, when compared to the control. Chronic iron administration induces adaptive responses involving stimulation of the antioxidant defenses (Jagetia and Reddy, 2011), including a derangement of some antioxidant mechanisms of the liver cell occurs (GSH depletion, decreased SOD and CAT activities) concomitantly with a substantial LPO response (Galleano and Puntarulo, 1992), that may be not efficiently scavenge ROS in IOL, in which excessive ROS accumulation, causes an antioxidant status imbalance, leading to LPO and GSH depletion (Kim *et al.*, 2006). Generally SOD and GP_X constitute a mutually supportive enzyme system of the first line cellular defense against oxidative injury (Kalpravidh *et al.*, 2010)), in which SOD enzymes converts superoxide into less toxic H_2O_2 (Geisser, 1997) and CAT detoxifies H_2O_2 into H_2O (Khan *et al.*, 2009) as one molecule of CAT can convert ~6 million molecules of H_2O_2 to H_2O and O_2 each minute (Valko *et al.*, 2006), thus Fe-induced oxidative injury, as the SOD/GPx ratio might be indicative of increased ROS when enzymes are not efficiently scavenging ROS, could be considered a pathway leading to cell death (Formigari *et al.*, 2007).

Curcumin administration to iron-loaded rats resulted in significant increases in liver CAT and SOD activities compared with the IOL group (Table 2). These obtained results are of the same harmony with the data of Iqbal *et al.* (2003) recorded that, curcumin enhances the activity of CAT, GR, GPx and GST in liver and kidney of mice. Likewise, Farombi and Ekor (2006) reported that, CUR decreased the reduction in activities of CAT and GPx by 31 and 55%, resp., in gentamicin-induced renal oxidative damage. Additionally, Naik *et al.* (2011) observed that, CUR treatment (100 and 200 mg kg^{-1} b.w.) restored significantly the decline in GPx and SOD levels in liver and cardiac tissues due to CCl_4- and ISO-treatment, resp. Thus, CUR (1500 and 500 ppm) inhibited LPO induced by Fe (Valerio and Petersen, 1998) as the supplementation of CUR regulates the LPO by altering the antioxidant enzyme activities (Reddy and Lokesh, 1994a). This marked capacity of CUR as a peroxidation inhibitor may be related to its iron chelating activity with its binding sites hydroxyl and methoxyl groups (Ak and Gulcin, 2008), in addition to its free radical scavenging (Masuda *et al.*, 2001) and antioxidative properties (Kelloff *et al.*, 1996).

Iron overload resulted in significant increase in liver NO levels when compared with the control group. Similar results were recorded by Hida *et al.* (2003) who reported that, after lipopolysaccharide injection; 24-72 h preloaded rats with colloidal iron showed a strong inducible nitric oxide synthase (iNOS) induction in liver Kupffer cells and increased NO levels in liver and blood. Also Cornejo *et al.* (2007) confirmed that, chronic iron overload leads to a substantial increase in liver NOS activity which is related to upregulation of iNOS expression (Cornejo *et al.*, 2005). That can be attributed to the complex interrelationships between Fe and $^{\bullet}NO$ (Galleano *et al.*, 2004) which can result in changes in $^{\bullet}NO$ production in vivo in whch $^{\bullet}NO$ is considered as a fundamental intercellular and intracellular signaling molecule essential for the maintenance of homeostasis (Kagan *et al.*, 2001).

Curcumin treatment to iron overloaded rats resulted in significant decreased liver NO levels compared with the non treated IOL group. The data obtained are in harmony with Lin *et al.* (1999) that explored, several natural polyphenolic compounds such as CUR were demonstrated to inhibit lipopolysaccharide-induced NO production. Also Goel *et al.* (2008) reported that, CUR ef?ciently reduces the activation of iNOS in primary cultures of rat macrophages. Moreover, Aggarwal and Sung (2009) recorded that, CUR has the capability to scavenge reactive oxygen and nitrogen species. These radical scavenging ability of CUR and also the in vitro and in vivo ablities to reduce oxidative stress during in?ammatory conditions by down regulation of NO formation, in which CUR is lipophilic and has strong antioxidant potential (Yadav *et al.*, 2011) due to presence of hydroxyl (Kurup *et al.*, 2007), phenolic, methoxy and diketonic groups in its structure, thus CUR may be effective in decreasing IOL-induced NO level and inhibiting lipopolysaccharide-induced NO production that occur by blocking iNOS gene expression (Lin *et al.*, 1999).

Iron overload resulted in significant decrease in liver TNA levels when compared with the control group. Similarly, Zecca *et al.* (2004) reported that, iron is a major generator of ROS that lead to damage of lipids, proteins carbohydrates and nucleic acids. Also, Knobel *et al.* (2006) recorded that, iron is known to induce DNA damage in human colon tumor cell and in hepatocyte (Morel *et al.*, 1997). Moreover, Diaz-Castro *et al.* (2010) observed that, iron overload in control and anaemic rats caused DNA damage when both types of diets (with normal-Fe content (45 mg kg^{-1}) or IOL (450 mg kg^{-1})) were given. That can be explained as redox active metal ions such as Fe^{2+} or Cu^{+} that are localized or bound to the DNA react with H_2O_2 (generated by oxidative stress) to form highly reactive $^{\bullet}OH$ in immediate proximity to DNA (Perron and Brumaghim, 2009), in which $^{\bullet}OH$

then abstracts the 4' hydrogen atom from the deoxyribose sugar backbone, leaving a DNA radical adduct that rearranges, ultimately cleaving the phosphodiester backbone and resulting in strand scission (Rai *et al*., 2005).

Curcumin administration to iron overloaded rats non significantly increased liver TNA concentration compared with the IOL-group. These results are in conformity with the data reported by Perron *et al*. (2008) that assayed 12 different phenolic compounds, showing that all of the compounds with catechol or gallol groups inhibited 50% of the DNA damage from Fe^{2+} and H_2O_2 (IC50) at concentrations between 1-59 lM. Also, Perron and Brumaghim, 2009 reported that, polyphenols, generally, protect DNA from damage in systems involving Fe^{2+} at biologically relevant, low micromolar concentrations. That can be explained by Zhao *et al*. (2005) who observed that, verbascoside (polyphenol) inhibited DNA damage from the Fenton reaction in a dose-dependent manner and attributed this antioxidant activity to iron binding by the polyphenol. Also, Morel *et al*. (1993, 1994) also showed that the ability of polyphenols to chelate and remove iron from iron-loaded hepatocytes correlates with cytoprotective effects of these compounds. Moreover, CUR induce apoptosis through a variety of mechanisms including down regulation of nuclear factor-kappa B gene products, activated protein-1, cyclooxygenase-2 and ROS as well as up regulation of pro-apoptotic gene (Bax) and Bak, p53 and growth arrest and DNA damage-inducible gene 153 (Shankar and Srivastava, 2007).

The obtained results indicate that, iron overload induced oxidative stress that revealed in alteration of the blood and liver biochemical parameters in rats and curcumin treatment can increase antioxidant status, reducing iron content and lipid peroxidation in the blood and liver tissue of iron overloaded rats. It can be concluded that the ingestion of antioxidant rich foods may prevent or delay primary and secondary effects associated with iron overload-related diseases. This protective antioxidant effect may be due to radical scavenging and/or iron chelation activities. We recommended using this common dietary flavonoid regularly in food with a further study as it may be a new source of medicine for protection against iron overload diseases.

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