

Full Length Research Paper

Effect of crocin and curcumin on biochemical alterations associated with iron overload-induced liver injury in rats

Shohda A. EL-Maraghy, Sherine M. Rizk and Maha M. El-Sawalhi*

Biochemistry Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt.

Accepted 21 October, 2020

The present study was undertaken to evaluate the possible ameliorating effect of crocin and curcumin on certain biochemical alterations associated with iron overload-induced liver injury in rats. 5 groups of rats were used, a normal control group received daily i.p. injections of saline and 4 groups received daily i.p. injections of ferric nitrilotriacetate (FeNTA) for 8 successive days, the dose of iron was increased during the experimental period (from 6 to 15 mg Fe/kg). The first iron overloaded group kept without further treatment and served as a positive control group. The second iron overloaded group received daily i.p. injections of crocin (200 mg/kg) in saline. The 3rd and the 4th iron overloaded groups received orally either 0.5% carboxy methyl cellulose (CMC) or curcumin (100 mg/kg) in CMC respectively. Treatment started 3 days before and concurrently with iron administration for 8 days. Results revealed that iron-induced liver injury was reflected by significant changes in the liver function indices, hyperammonemia and reduced serum urea level. A significant deposition of iron in liver was associated with enhanced oxidative and nitrosative stress status. Moreover, iron overloaded rats exhibited significant alterations in liver energy metabolism together with diminished ureogenesis and a decline in dimethylarginine dimethylaminohydrolase activity. Supplementation with either crocin or curcumin ameliorated most of the biochemical changes induced by iron overload in rat liver. A function that may be beneficial for populations at risk for iron overload.

Key words: Iron overload, liver, rat, oxidative stress, energy metabolism, ureogenesis, crocin, curcumin.

INTRODUCTION

Iron is an essential micronutrient for the growth, development and long-term survival of most organisms. However, because human beings have no active mechanism to control iron excretion, excess iron, regardless of the route of entry, accumulates in parenchymal organs and threatens cell viability (Yajun et al., 2005). Iron overload syndromes are classified as genetic (hereditary hemochromatosis) or secondary (most commonly in patients who require long-term blood transfusions, as in severe anemias and thalassemia). In addition, there are many diseases that show mild iron deposition or dysregulation of body iron distribution. Such conditions include chronic hepatitis C, alcoholic liver disease and non-alcoholic steatohepatitis (Britton et al., 1994; Kohgo et al., 2008).

Indeed, when iron-buffering capability is overwhelmed, oxidative stress-induced cell damage may arise, mainly in the liver, the main storage site for iron in the body (Yajun et al., 2005; Kohgo et al., 2008).

As a redox-active transition metal, iron generates reactive oxygen species (ROS) via the Fenton and Haber-Weiss reactions. ROS react directly with proteins, lipids and nucleic acids and induce oxidative stress by depleting cellular stores of antioxidants. ROS also influence multiple cell signaling pathways important to cell survival, proliferation and death (Aust et al., 1985; Valko et al., 2005). Administration of ferric nitrilotriacetate (FeNTA) has been reported to result in hepatic iron loading (Matsuura, 1983) associated with extensive peroxidation of membrane lipids in vivo (Matsuura, 1983; Suzumura et al., 2000; Deiana et al., 2004). In addition, it has been demonstrated that FeNTA-induced oxidative stress could lead to hepatocyte apoptosis (Yajun et al., 2005), DNA damage and liver necrosis in rats (Matos et al., 2001).

*Corresponding author. E-mail: mahaelsawalhi@yahoo.com.
Tel.: +202 33450283. Fax: +202 23635140

Trends on applying nutritional antioxidants in diseases related to oxidative stress have gained immense interest in recent years.

Crocin (crocetin digentiobiose ester), a unique water soluble carotenoid contained in the stigmas of *Crocus sativus* Linne (saffron) and in the fruits of *Gardenia jasminoides* Ellis (Pfister et al., 1996) has attracted research attention for its extensive pharmacological effects. One of the important mechanisms by which crocin and crocetin exert their beneficial biological effects is their ability to modulate redox status of organisms via the antioxidant activity of these water -soluble pigments (Chen et al., 2008). Crocin has been reported to possess several medicinal properties such as neuroprotection (Ochiai et al., 2004), anti-atherogenic (He et al., 2005), anti-carcinogenic and anti- tumor (Abdullaev, 2002) activities. Furthermore, crocin has been shown to attenuate the acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats (Lin and Wang, 1986). However, little is known about the effect of this carotinoide on iron-induced liver injury.

On the other hand, curcumin is a hydrophobic polyphenol derived from the rhizome (turmeric) of the herb *Curcuma longa*. Curcumin exhibits potent antioxidant, anti-inflammatory, anti-microbial and anti-carcinogenic activities (Anand et al., 2008). It also has potential therapeutic effects against neurodegenerative, cardiovascular, pulmonary, metabolic and autoimmune diseases (Aggarwal and Harikumar, 2009). In addition, curcumin exerted hepatoprotective effects in various animal models of liver injury such as carbon tetrachloride (Park et al., 2000; Fu et al., 2008), endotoxin (Kaur et al., 2006) and thioacetamide (Shapiro et al., 2006). Moreover, curcumin has properties of an iron chelator (Jiao et al., 2006) and has been reported to reduce the toxic effects of iron loading in rat liver epithelial cell line (Messner et al., 2009).

The aim of the current study was to evaluate the possible ameliorating effect of crocin and curcumin on certain biochemical alterations associated with iron overload-induced liver injury in rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (170 - 200 g) were obtained from laboratory animals' farm of the Egyptian organization for biological products and vaccines – Cairo – Egypt. They were housed in the animal house of Faculty of Pharmacy, Cairo University, Cairo, Egypt. Rats were kept under controlled conditions, fed standard chow diet, provided with water *ad libitum* and left for an initial adaptation period of 1 week before any experimental manipulation.

Chemicals

Ferric nitrate, nitrilotriacetic acid disodium salt, crocin, curcumin, enzymes and coenzymes were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemicals were from Analar grade or from the purest grade available.

Preparation of ferric nitrilotriacetate (FeNTA)

Ferric nitrate was dissolved in 1 N HCl. To produce FeNTA solution, 162 ml of a 0.1 M ferric nitrate solution was added to 100 ml of 0.08 M disodium nitrilotriacetate solution and the pH was adjusted to 7.4 with sodium bicarbonate powder under magnetic stirring. The mixture was prepared immediately before use (Awai et al., 1979).

Experimental design

Animals were randomly allocated into 5 groups (8 rats each). The first group received daily i.p. injections of appropriate volumes of saline for 8 days and served as a normal control group. The animals in the other 4 groups received daily i.p. injections of FeNTA for 8 successive days in the following sequence: 6 mg Fe/kg b.w. for 2 days, 9 mg Fe for the next 2 days, 12 mg Fe for the following 2 days and 15 mg Fe for the last 2 days. The first iron overloaded group kept without further treatment and served as a positive control group. The second iron overloaded group received daily i.p. injections of crocin (200 mg/kg b.w.) (Hosseinzadeh et al., 2005) dissolved in saline. The third and the fourth iron overloaded groups received orally either 0.5% carboxy methyl cellulose (CMC) or curcumin (100 mg/kg b.w) (Park et al., 2000) dissolved in 0.5% CMC respectively. Treatment started 3 days before and concurrently with iron administration for 8 days. At the end of the experimental period, animals were sacrificed by decapitation. Blood was collected into plain centrifuge tubes and EDTA-containing centrifuge tubes and the liver of each animal was rapidly isolated, washed with ice cold saline and blotted dry.

Biochemical analysis

The first set of blood samples was allowed to clot, centrifuged at 1000 x g at 4°C for 15 min and the separated sera were used for the estimation of the following biochemical parameters: alanine amino transferase (ALT) and aspartate amino transferase (AST) activities as well as serum total protein, albumin and urea levels using standard diagnostic kits (Biocon, Germany). Serum ornithine transcarbamylase (OTC) activity was determined according to the method of Ceriotti and Gazzaniga (1967). Other blood samples, collected on EDTA, were centrifuged at 1000 x g at 4°C for 15 min and the separated plasma samples were used for determination of ammonia level using kits provided by Randox, UK.

6 pieces of liver were weighed and appropriately treated for the separation and estimation of the parameter being measured. The first piece of liver was stored at -80°C for the assay of liver iron content, using Zeeman atomic absorption spectrophotometer 4100 ZL (Perkin Elmer) after sample preparation as described by Parker et al. (1967). The second and the third portions of the liver were used to prepare 10% homogenate in 1.15% KCl and 5% homogenate in 5% meta-phosphoric acid, using Potter-Elvehjem glass homogenizer, centrifuged at 1000 x g and 3000 x g at 4°C for 15 min respectively. The supernatant of the first homogenate was used for the assay of hepatic malondialdehyde (MDA) level (Yoshioka et al., 1979). The supernatant of the second homogenate was used for the assay of hepatic glutathione (GSH), pyruvate (Pyr) and lactate (Lac) contents according to the methods of Beutler et al. (1963), Hohorst (1965) and Bucher et al. (1965) respectively. The fourth portion of the liver tissue was homogenized in ice-cold 3 M perchloric acid to form 10% homogenate and centrifuged at 1000 x g, at 4°C for 20 min. Hepatic ATP content was determined in the supernatant by the spectrofluorometric method of Lowry et al. (1964). using shimadzu spectrofluorimeter RF 1501. A fifth piece of liver tissue was used to prepare 5% homogenate in Tris-sucrose buffer pH 7.4 and divided into 2 aliquots. The first one was centrifuged at 2000 x g, at 4°C for 10 min and the resultant supernatant was used

Table 1. Effect of crocin (200mg/kg i.p.) and curcumin (100 mg/kg p.o.) on liver function indices, serum urea and plasma ammonia levels of iron overloaded rats.

	Normal control	Iron overload	Crocin + Iron overload	CMC + Iron overload	Curcumin in CMC + Iron overload
ALT (U/l)	41.82 ± 1.77	73.92 ± 4.16 ^a	53.77 ± 4.77 ^b	75.55 ± 6.41 ^a	48.67 ± 2.57 ^c
AST (U/l)	78.57 ± 4.18	119.27 ± 6.54 ^a	88.99 ± 6.60 ^b	122.32 ± 8.98 ^a	85.52 ± 5.08 ^c
OTC (U/l)	5.06 ± 0.35	7.57 ± 0.45 ^a	5.43 ± 0.28 ^b	7.70 ± 0.55 ^a	5.48 ± 0.45 ^c
Total protein (g/dl)	5.70 ± 0.45	4.35 ± 0.21 ^a	6.18 ± 0.31 ^b	4.03 ± 0.40 ^a	6.02 ± 0.33 ^c
Albumin(g/dl)	3.6 ± 0.21	2.76 ± 0.09 ^a	3.5 ± 0.23	2.47 ± 0.16 ^a	3.37 ± 0.29
Urea (mg/dl)	45.13 ± 1.2	28.07 ± 2.31 ^a	38.30 ± 1.92 ^b	31.24 ± 2.99 ^a	50.56 ± 2.64 ^c
Ammonia(μmol/l)	100.71 ± 11.87	287.4 ± 18.75 ^a	120 ± 14.37 ^b	307.0 ± 28.30 ^a	103.4 ± 11.58 ^c

Values are expressed as means ± S.E. of 7 observations.

a: significant difference from normal control group at p < 0.05. b: significant difference from iron overloaded group at p < 0.05.

c: significant difference from CMC (vehicle for curcumin) + iron overloaded group at p < 0.05.

for estimating hepatic (nitrite/nitrate) as an index of nitric oxide (NO) contents (Miranda et al., 2001). Whereas, the second aliquot was centrifuged at 105,000 x g at 4°C for 30 min using Dupont Sorvall Combiplus ultracentrifuge, the separated cytosolic fraction was used for the assay of hepatic OTC (Ceriotti and Gazzaniga, 1967), arginase (Mia and Koger, 1978) and dimethylarginine dimethylaminohydrolase (DDAH) (Knipp and Vašák, 2000) activities, as well as hepatic citrulline (Boyde and Rahmatullah, 1980) contents. The last portion of liver was homogenized (1:4) in 0.5% sodium dodecyl sulfate solution, incubated for 15 min at room temperature then, 4% sulphosalicylic acid was added (1: 0.6) v/v, mixed with vortex and centrifuged at 12,000 x g at 4°C for 20 min. The supernatant was used for estimation of hepatic amino acids; arginine, ornithine and glutamine (Romano et al., 1990) contents using amino acid analyzer Eppendorf Biotronik LC 3000. The protein content of the cytosolic fraction was determined by the method of Lowry et al. (1951).

Statistical analysis

The data were expressed as means ± S.E. and compared using one way analysis of variance (ANOVA). Comparisons among groups were made according to Tukey-Kramer's multiple comparisons test. The significance level was tested at p < 0.05.

RESULTS

Effect of crocin and curcumin on liver function indices, serum urea and plasma ammonia levels of iron overloaded rats. As shown in Table 1, iron-induced liver injury resulted in a significant increase in serum ALT, AST and OTC activities, associated with a significant reduction in serum total protein and albumin levels compared with the normal control group. Furthermore, iron overloaded rats exhibited hyperammonemia together with reduced serum urea level. Administration of either crocin or curcumin significantly reduced the elevated activities of the enzymes markers of liver injury and restored the altered total protein, urea and ammonia levels of iron overloaded rats to approach the normal control values.

Effect of crocin and curcumin on iron deposition, oxidative and nitrosative stress markers as well as energy metabolism in liver of iron overloaded rats

Data summarized in Table 2 indicated that intraperitoneal

injections of FeNTA caused a significant deposition of iron in rat liver. Both crocin and curcumin significantly reduced hepatic iron deposition. However, the values of both crocin and curcumin treated groups were still significantly higher than the normal control ones.

The results also demonstrated that administration of excess iron induced oxidative and nitrosative stress as evidenced by the remarkable increase in liver MDA and NO levels together with depletion of hepatic GSH content as compared to the normal control group. Treatment with either crocin or curcumin succeeded to normalize the elevated hepatic MDA and NO levels and replenish GSH content.

In addition, iron overload resulted in a significant decrease in liver ATP and pyruvate contents accompanied with a significant increase in lactate level as well as hepatic lactate/pyruvate ratio. Both crocin and curcumin were able to replenish hepatic ATP content and to restore the altered lactate, pyruvate and their ratio to the normal control values.

Effect of crocin and curcumin on ammonia metabolism and dimethylarginine dimethylaminohydrolase (DDAH) activity in liver of iron overloaded rats

Results presented in Table 3 revealed that iron overloaded rats showed reduced activities of two of the urea synthesizing enzymes, OTC and arginase accompanied with reduction of DDAH enzyme activity as compared to their activities in liver of normal control rats. Moreover, a significant decrease in citrulline and arginine contents, insignificant decrease in ornithine contents together with a significant increase in glutamine contents were also observed in the liver of iron loaded rats. Administration of either crocin or curcumin, enhanced hepatic OTC, arginase and DDAH activities as compared to that of iron overloaded rats. In addition, both crocin and curcumin normalized hepatic citrulline and arginine contents and reduced the elevated glutamine contents to near the normal control values.

Table 2. Effect of crocin (200 mg/kg i.p.) and curcumin (100 mg/kg p.o.) on iron deposition, oxidative and nitrosative stress markers as well as energy metabolism in liver of iron overloaded rats.

	Normal Control	Iron overload	Crocin+ Iron overload	CMC+ Iron overload	Curcumin in CMC + Iron overload
Iron (g/g tissue)	171.3± 15.4	815.37±27.2 ^a	554.35 ± 29.5 ^{a,b}	789.89 ± 44.8 ^a	445.38 ± 33.6 ^{a,c}
GSH (mg/g tissue)	1.18± 0.1	0.38 ± 0.03 ^a	1.30 ± 0.12 ^b	0.41 ± 0.03 ^a	1.49 ± 0.16 ^c
MDA(nmol/g tissue)	61.05± 3.41	140.25±7.88 ^a	72.30 ± 11.47 ^b	125.04 ± 10.4 ^a	58.33 ± 5.15 ^c
NO (nmol/g tissue)	24.68± 1.53	49.37 ± 2.41 ^a	30.91± 2.39 ^b	53.86 ± 3.64 ^a	26.97 ± 2.61 ^c
ATP (mol/g tissue)	5.00 ± 0.31	3.70 ± 0.20 ^a	5.14 ± 0.35 ^b	3.39 ± 0.24 ^a	5.05 ± 0.54 ^c
Lac(mol/g tissue)	2.45 ± 0.20	3.64±0.23 ^a	2.23 ± 0.13 ^b	3.84±0.26 ^a	2.67 ± 0.15 ^c
Pyr (mol/g tissue)	0.135± 0.01	0.08 ± 0.007 ^a	0.115 ± 0.01 ^b	0.071±0.009 ^a	0.16 ± 0.017 ^c
Lac/Pyr ratio	20.13± 1.21	45.98 ± 3.17 ^a	23.17 ± 1.65 ^b	47.75 ± 4.45 ^a	18.42 ± 1.22 ^c

Values are expressed as means ± S.E. of 7 observations.

a: significant difference from normal control group at p < 0.05.

b: significant difference from iron overloaded group at p < 0.05.

c: significant difference from CMC (vehicle for curcumin) + iron overloaded group at p < 0.05.

Table 3. Effect of crocin (200mg/kg i.p.) and curcumin (100mg/kg p.o.) on ammonia metabolism and DDAH activity in liver of iron overloaded rats.

	Normal control	Iron overload	Crocin + Iron overload	CMC+ Iron overload	Curcumin in CMC + Iron overload
OTC (U/mg protein)	1.80 ± 0.09	1.45 ± 0.03 ^a	1.73 ± 0.04 ^b	1.29 ± 0.1 ^a	1.75 ± 0.07 ^c
Arginase (U/mg protein)	7.94 ± 0.44	5.53 ± 0.31 ^a	7.53 ± 0.49 ^b	5.30 ± 0.32 ^a	7.58 ± 0.63 ^c
Citrulline (mol/g tissue)	29.64 ± 2.09	20.08 ± 2.08 ^a	25.44 ± 2.54 ^b	18.94±2.56 ^a	29.65 ± 2.24 ^c
Ornithine (mol/g tissue)	23.79 ± 3.39	15.09 ± 1.05	19.10 ± 1.88	16.95 ± 1.79	23.97 ± 4.34 ^c
Arginine(mol/g tissue)	25.91± 2.86	14.59 ± 1.01 ^a	32.93 ± 3.34 ^b	14.99 ± 0.89 ^a	34.14 ± 3.04 ^c
Glutamine(mol/g tissue)	4.77 ± 0.54	7.58 ± 0.53 ^a	5.07 ± 0.57 ^b	7.63 ± 0.59 ^a	5.22 ± 0.61 ^c
DDAH (U/mg protein)	0.98 ± 0.08	0.74 ± 0.03 ^a	0.94 ± 0.06 ^b	0.70 ± 0.07 ^a	0.92 ± 0.04 ^c

Values are expressed as means ± S.E. of 7 observations.

a: significant difference from normal control group at p < 0.05.

b: significant difference from iron overloaded group at p < 0.05.

c: significant difference from CMC (vehicle for curcumin) + iron overloaded group at p < 0.05.

DISCUSSION

In the present study, administration of FeNTA induced a significant deposition of iron in rat liver associated with exacerbated oxidative stress status and a remarkable increase in hepatic nitric oxide level.

Study by Matsuura (1983) has demonstrated iron uptake by rat liver and induction of hepatic iron loading and iron toxicity in the liver after a single injection of FeNTA. Furthermore, FeNTA binding proteins associated with rat liver plasma membranes have been characterized (Barisani and Wessling-Resnick, 1996). Iron is a well-known inducer of reactive oxygen species. Its ability to accelerate lipid peroxidation is well established (Aust et al., 1985; Valko et al., 2005). Several studies have shown that, FeNTA administration induced a state of a sustained oxidative stress and caused high levels of lipid peroxidation products in liver (Suzumura et al., 2000; Matos et al., 2001; Deiana et al., 2004). It also induced a significant decrease in hepatic glutathione, -tocopherol and polyunsaturated fatty acids contents (Deiana et al., 2004).

On the other hand, both acute (Cornejo et al., 2001) and chronic (Cornejo et al., 2005) iron overload have been reported to increase NO production in rat liver. Such effect of iron was attributed to the enhancement of inducible nitric oxide synthase (iNOS) expression in the liver (Cornejo et al., 2005). Under inflammatory oxidative stress, NO can be withdrawn from its regular physiological course by ROS, degraded further to reactive nitrogen oxide species and can damage the cell of its origin and thereby contribute to organ dysfunction (Minin et al., 2005).

Liver damage by iron had been assessed by leakage of enzymes such as ALT and AST and lactate dehydrogenase into blood (Suzumura et al., 2000; Manjunatha and Srinivasan, 2006). In the present study, higher activities of serum ALT, AST and OTC (an indicator of hepatocytes mitochondrial damage) have been found in response to iron overload-induced oxidative stress. Such increased activities might be attributed to the leakage of these enzymes from the injured liver cells into the blood stream because of the altered liver membrane permeability.

Meanwhile, iron hepatotoxicity, also resulted in reduction of serum total protein and albumin levels in the present study. That observation could be ascribed to changes in protein and free amino acid metabolism and their synthesis in the injured liver cells and /or increased protein degradation.

In the current study, iron overloaded rats exhibited a significant decrease in liver ATP content accompanied by a significant increase in hepatic lactate/ pyruvate ratio. Depletion of hepatic ATP level has been observed in other models of experimental iron overload (Ceccarelli et al., 1995; Fujimori et al., 2004). Such depletion reflects mitochondrial dysfunction that might results from enhanced lipid peroxidation associated with excess iron (Britton et al., 1994; Ceccarelli et al., 1995). In addition, Bacon et al., (1993) have reported that chronic iron overload decreases hepatic mitochondrial cytochrome C oxidase activity and hepatic ATP levels. Moreover, the observed increase in hepatic NO contents induced by iron loading in our study might also participate in reduction of liver ATP contents, as NO and its derivatives peroxynitrite are known to inhibit mitochondrial respiration by a variety of means (Brown, 2001).

Alteration in mitochondrial oxidative mechanism with the resultant reduction in supply of ATP from substrate oxidation would enhance glycolysis as a significant alternative ATP-producing pathway. This was evidenced in the present study by the marked increase in hepatic lactate/pyruvate ratio an indicator of free cytosolic NADH /NAD⁺ ratio. A high intracellular NADH/NAD⁺ ratio inhibit several major metabolic pathways and exacerbate the intracellular formation of additional ROS (Roy et al., 1997).

Impairment of energy metabolism is expected to lead to several alterations in hepatic cell metabolism especially in energy metabolism-linked parameters such as ureogenesis. In the present study, iron overloads significantly diminished hepatic urea synthesis capacity, the main route of ammonia detoxification. This was evidenced by a remarkable increment in plasma ammonia level, accompanied by a marked decrement in serum urea level. In addition, iron overloaded rat showed reduced activities of two of the urea cycle enzymes namely OTC and arginase and decreased levels of urea cycle intermediates citrulline and arginine, together with a significant increase in hepatic glutamine contents.

Earlier studies have demonstrated decreased activity of OTC, the second enzyme of the urea cycle (Cascales et al., 1979; Thurlow et al., 1980; Riggio et al., 1992) and arginase the final enzyme in the cycle (Cascales et al., 1979; Tabuchi et al., 2000) in various experimental models of liver injury. Such reduced activities could be attributed to the leakage of these enzymes from the injured liver cells into the blood stream as reflected in the present study by the increased serum OTC activity. It might also result from liver dysfunction and disturbance in the biosynthesis of these enzymes and/or increased proteolytic degradation in response to iron overload-induced in-

jury.

In the current study, the drop in ammonia consumption by the urea cycle was largely compensated by a significant increase in hepatic glutamine contents. Such observation is on line with that reported by Cascales et al. (1979) in thioacetamide-induced liver injury in rats.

Citrulline is a nonproteogenic - amino acid that occurs as a metabolite of the urea cycle produced by the mitochondrial OTC. It is also known as a product of conversion of arginine to NO by nitric oxide synthase and as a product of conversion of side chain methylated derivatives of L- arginine by dimethylarginine dimethylaminohydrolase (DDAH) enzyme which is highly expressed in the liver (Knipp and Vašák, 2000). The finding that iron overloaded rats showed a significantly reduced hepatic citrulline contents could be related to the decreased OTC and DDAH activities observed in the liver of iron overloaded rats in the present study. The sensitivity of DDAH to oxidative stress is conferred by the presence of a reactive cysteine residue in the active site of the enzyme, which is also affected by NO via S-nitrosylation resulting in diminished DDAH activity (Leiper et al., 2002).

Arginine is a semi-essential amino acid which occurs as an intermediate of the urea cycle and serves as a common substrate for both arginase and NOS enzymes. Consequently the observed reduction of hepatic arginine contents in iron overloaded rats could be interpreted on the basis of increased consumption of arginine in the synthesis of NO that was markedly increased in the liver of those rats.

The findings of the present study indicated that administration of either crocin or curcumin ameliorated most of the biochemical alterations associated with iron overload in rat liver. This is the first report on the hepatoprotective potentials of crocin against iron overload-induced liver injury in rats. Meanwhile, previous studies have demonstrated efficacy of curcumin in reducing the severity of iron-induced hepatotoxicity and oxidative stress. However, the effect of curcumin on perturbations of energy metabolism and ammonia detoxification in iron overloaded rats has not been studied yet.

Several studies have demonstrated that curcumin can bind iron and it has properties of an iron chelator (Baum and Ng, 2004; Jiao et al., 2006; Messner et al., 2009). In addition, mice fed a diet high in curcuminoids showed decreased levels of liver ferritin, indicative of decreased iron burden (Jiao et al., 2006). On the other hand, the observed decrease in liver iron deposition induced by crocin might also implicate a sort of binding or interaction between crocin and iron. To the best of our knowledge such effect has not been investigated yet. However, a recent study has shown that retinoid treatment significantly decreased hepatic iron content and iron-induced oxidative stress *in vitro* and *in vivo* (Tsuchiya et al., 2009). Nevertheless, further studies are needed to explore the effect of the unique carotenoid crocin on iron binding and deposition.

Among the constituents of saffron, crocin is the most abundant and with established antioxidant effects (Abdullaev, 1993; Chen et al., 2008). The present study provides further evidence for the antioxidant properties of crocin as reflected by its effectiveness in reducing the elevated hepatic MDA and NO levels and in restoring the depleted GSH levels of iron overloaded rats.

Meanwhile, curcumin is known to exhibit a strong antioxidant activity. It is a potent scavenger of a variety of ROS including superoxide anion radicals, hydroxyl radicals and nitrogen dioxide radicals (Maheshwari et al., 2006; Anand et al., 2008). Earlier studies have shown that curcumin significantly reduced the redox activity of iron (Reddy and Lokesh, 1996) and lowered the liver and serum lipid peroxide levels enhanced by iron injection (Reddy and Lokesh, 1996; Manjunatha and Srinivasan, 2006).

In the present investigation, the reduction in the severity of iron-induced hepatotoxicity by both crocin and curcumin was revealed by the correction of the altered liver function indices. The effect of crocin is on line with its reported effect against acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats (Lin and Wang, 1986). Whereas that of curcumin is in agreement with its lowering effect on the elevated serum enzymes activities induced by iron administration (Reddy and Lokesh, 1996; Manjunatha and Srinivasan, 2006).

Moreover, the efficacy of crocin and curcumin in ameliorating the toxic effect of iron overload was accompanied by a significant improvement in hepatic energy metabolism and restoration of cellular redox homeostasis as evidenced by replenishing of ATP content and normalizing the elevated lactate/pyruvate ratio in the liver of iron overloaded rats. Such effect of crocin might be attributed to the effect of its aglycone crocetin which has been shown to enhance the recovery of cellular ATP, reduce mitochondrial damage in rat liver (Dhar et al., 2005) and to stimulate oxygen consumption (Singer et al., 2000) in experimental animals following hemorrhagic shock. Meanwhile, the capacity of curcumin to preserve mitochondrial oxidative mechanism with the resultant increase in supply of ATP from substrate oxidation could be related to its reported inhibitory effect on lipid peroxidation and protein oxidation in rat liver mitochondria (Wei et al., 2006).

Taken together, it might be speculated that, in the current study, both crocin and curcumin could improve iron overload induced liver dysfunction via their ability to antagonized the enhanced oxidative and nitrosative stress and by preserving mitochondrial structure as evidenced by restoring the increased serum OTC activity of iron overloaded rats. Moreover, both of them were also able to improve the altered mitochondrial functions as reflected by the enhancement of the energy metabolism and energy requiring processes such as ureogenesis resulting in reduction of iron-induced hyperammonemia and liver glutamine contents. Finally, the observed ability of crocin and curcumin to normalize the altered hepatic OTC, arginase and

DDAH activities could be related to the restoration of citrulline and arginine amino acids content in the liver of iron overloaded rats. The above mentioned findings indicate that, crocin is a potent hepatoprotective agent as effective as curcumin against iron overload-induced biochemical alterations in rat liver. It also implies the potential usefulness of both crocin and curcumin as dietary supplements for populations at risk for iron overload to guard against its detrimental effects.

REFERENCES

- Abdullaev FI (1993). Biological effects of saffron. *Biofactors* 4: 83-86.
- Abdullaev FI (2002). Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). *Exp. Bio. Med.* 227:20-25.
- Aggarwal BB, Harikumar KB (2009). Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int. J. Biochem. Cell Biol.* 41:40-59.
- Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harik-umar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN, Aggarwal BB (2008). Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. *Biochem. Pharmacol.* 76:1590-1611.
- Aust SD, Morehouse LA, Thomas CE. (1985). Role of metals in oxygen radical reactions. *Free Radic. Biol. Med.* 1: 3-25.
- Awai M, Narasaki M, Yamanoi Y, Seno S (1979). Induction of diabetes in animals by parental administration of ferric nitriloacetate. *Am. J. Pathol.* 95: 663-674.
- Bacon BR, O'Neill R, Britton RS (1993). Hepatic mitochondrial energy production in rats with chronic iron overload. *Gastroenterol.* 105: 1134-1140.
- Barisani D, Wessling-Resnick M (1996). Fe-nitritoltri-acetic acid-binding proteins associated with rat liver plasma membranes. *Hepatology.* 24: 934-938.
- Baum L, Ng A (2004). Curcumin interaction with copper and iron suggests one possible mechanism of action in Alzheimer's disease animal models. *J. Alzheimers Dis.* 6:367-377.
- Beutler E, Duron O, Kelly BM (1963). Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 61:882-888.
- Boyde TR, Rahmatullah M (1980). Optimization of conditions for the colorimetric determination of citrulline, using diacetyl monoxime. *Anal. Biochem.* 107:424-431
- Britton RS, Ramm GA, Olynyk J, Singh R, O'Neill R, Bacon BR (1994). Pathophysiology of iron toxicity. *Adv. Exp. Med. Biol.* 356:239-253.
- Brown GC (2001). Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. *Biochim. Biophys. Acta* 1504: 46-57.
- Bucher T, Czok R, Lamprecht W, Latzko E (1965). In *Methods of Enzymatic Analysis* (Bergmeyer HU, ed.) pp: 253-259.
- Cascales M, Feijóo B, Cerdán S, Cascales C, Santos-Ruiz A (1979). The effect of thioacetamide on urea cycle enzymes of rat liver. *Clin. Chem. Clin. Biochem.* 17:129-132.
- Ceccarelli D, Gallesi D, Giovannini F, Ferrali M, Masini A (1995). Relationship between free iron level and rat liver mitochondrial dysfunction in experimental dietary iron overload. *Biochem. Biophys. Res. Commun.* 209: 53-59.
- Cerretti G, Gazzaniga A (1967). Accelerated micro and ultramicro procedure for ornithine carbamoyl-transferase (OCT) determination. *Clin. Chim. Acta* 16: 436-439.
- Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, Jia L, Yin H-X, Chen C (2008). Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. *Food Chem.* 109:484-492
- Comejo P, Tapia G, Puntarulo S, Galleano M, Videla LA, Fernández V (2001). Iron-induced changes in nitric oxide and superoxide radical generation in rat liver after lindane or thyroid hormone treatment. *Toxicol. Lett.* 119: 87-93.

- Cornejo P, Varela P, Videla LA, Fernández V (2005). Chronic iron overload enhances inducible nitric oxide synthase expression in rat liver. *Nitric Oxide* 13: 54-61.
- Deiana M, Rosa A, Casu V, Piga R, Assunta Dessi M, Aruoma OI (2004). L-Ergothioneine modulates oxidative damage in the kidney and liver of rats in vivo: studies upon the profile of polyunsaturated fatty acids. *Clin. Nutr.* 23:183-193.
- Dhar A, Cherian G, Dhar G, Ray G, Sharma R, Banerjee SK (2005). Molecular basis of protective effect by crocetin on survival and liver tissue damage following hemorrhagic shock. *Mol. Cell. Biochem.* 278:139-146.
- Fu Y, Zheng S, Lin J, Ryerse J, Chen A (2008). Curcumin protects the rat liver from CCl4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. *Mol. Pharmacol.* 73: 399-409.
- Fujimori H, Ozaki K, Matsuura T, Matsushima S, Narama I, Pan-Hou H (2004). Effect of iron lactate overloading on adenine nucleotide levels and adenosine 3'-monophosphate forming enzyme in rat liver and spleen. *Biol. Pharm. Bull.* 27: 1371-1375.
- He SY, Qian ZY, Tang FT, Wen N, Xu G, Sheng L (2005). Effect of crocin on experimental atherosclerosis in quails and its mechanisms. *Life Sci.* 77: 907-921.
- Hohorst HJ. (1965). In *Methods of Enzymatic Analysis* (Bergmeyer HU, ed.) pp: 260-263.
- Hosseinzadeh H, Sadeghnia HR, Ziaee T, Danaee A (2005). Protective effect of aqueous saffron extract (*Crocus sativus* L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats. *J. Pharm. Pharm. Sci.* 8: 387-393.
- Jiao Y, Wilkinson J, Christine Pietsch E, Buss JL, Wang W, Planalp R, Torti FM, Torti SV (2006). Iron chelation in the biological activity of curcumin. *Free Radic. Biol. Med.* 40: 1152-1160.
- Kaur G, Tirkey N, Bharrhan S, Chanana V, Rishi P, Chopra K (2006). Inhibition of oxidative stress and cytokine activity by curcumin in amelioration of endotoxin-induced experimental hepatotoxicity in rodents. *Clin. Exp. Immunol.* 145: 313-321.
- Knipp, M, Vašák, M (2000). A colorimetric 96-well microtiter plate assay for the determination of enzymatically formed citrulline. *Anal. Biochem.* 286: 257-264.
- Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J (2008). Body iron metabolism and pathophysiology of iron overload. *Int. J. Hematol.* 88: 7-15.
- Leiper J, Murray-Rust J, McDonald N, Vallance P (2002). S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions between nitric oxide synthase and dimethylarginine dimethylaminohydrolase. *Proc. Natl. Acad. Sci.* 9: 13527-13532.
- Lin JK, Wang CJ (1986). Protection of crocin dyes on the acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats. *Carcinogenesis* 7: 595-599.
- Lowry OH, Passonneau JV, Hasselberger FX, Schulz DW (1964). Effect of ischemia on known substrates and cofactors of the glycolytic pathway in brain. *J. Biol. Chem.* 239: 18-30.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265-275.
- Maheshwari RK, Singh AK, Gaddipati J, Srimal RC (2006). Multiple biological activities of curcumin: a short review. *Life Sci.* 78:2081-2087.
- Manjunatha H and Srinivasan K (2006). Protective effect of dietary curcumin and capsaicin on induced oxidation of low-density lipoprotein, iron-induced hepatotoxicity and carrageenan-induced inflammation in experimental rats. *FEBS J.* 273: 4528-4537.
- Matos HR, Capelozzi VL, Gomes OF, Mascio PD, Medeiros MH (2001). Lycopene inhibits DNA damage and liver necrosis in rats treated with ferric nitrilotriacetate. *Arch. Biochem. Biophys.* 396:171-177.
- Matsuura R (1983). Uptake of iron and nitrilotriacetate (NTA) in rat liver and the toxic effect of Fe-NTA. *Acta Med. Okayama.* 37:393-400.
- Messner DJ, Sivam G, Kowdley KV (2009). Curcumin reduces the toxic effects of iron loading in rat liver epithelial cells. *Liver Int.* 29: 63-72.
- Mia DVM, Koger BS (1978). Direct colorimetric determination of serum arginase in various domestic animals. *Am. J. Vet. Res.* 39:1381-1383.
- Minin EA, Buchwalow IB, Wellner M, Palmes D, Spiegel HU, Neumann J, Boecker W, Herbst H (2005). L-Arginine-NO-cGMP signaling following acute liver injury in the rat. *Exp. Toxicol. Pathol.* 57:161-171
- Miranda KM, Espey MG, Wink DN (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide.* 5:62-71.
- Ochiai T, Ohno S, Soeda S, Tanaka H, Shoyama Y, Shimeno H (2004). Crocin prevents the death of rat pheochromocytoma (PC-12) cells by its antioxidant effects stronger than those of alpha-tocopherol. *Neurosci. Lett.* 362:61-64.
- Park EJ, Jeon CH, Ko G, Kim J, Sohn DH (2000). Protective effect of curcumin in rat liver injury induced by carbon tetrachloride. *J. Pharm. Pharmacol.* 52:437-440.
- Parker MM, Humoller FL, Mahler DJ (1967). Determination of copper and zinc in biological materials. *Clin. Chem.* 13:40-48.
- Pfister S, Meyer P, Steck A, Pfander, H (1996). Isolation and structure elucidation of carotenoid-glycosyl esters in *Gardenia* fruits (*Gardenia jasminoides* Ellis) and saffron (*Crocus sativus* Linne). *J. Agric. Food Chem.* 44:2612-2615.
- Reddy AC, Lokesh BR (1996). Effect of curcumin and eugenol on iron induced hepatic toxicity in rats. *Toxicol.* 107:39-45.
- Riggio O, Merli M, Capocaccia L, Caschera M, Zullo A, Pinto G, Gaudio E, Franchitto A, Spagnoli R, D'Aquillino E (1992). Zinc supplementation reduces blood ammonia and increases liver ornithine transcarbamylase activity in experimental cirrhosis. *Hepatology.* 16:785-789.
- Romano M, Diomedea L, Guiso G, Caccia S, Perego C, Salmons M (1990). Plasma and brain kinetics of large neutral amino acids and a striatum monoamines in rats given aspartame. *Food Chem. Toxicol.* 28:317-321.
- Roy S, Sen CK, Tritschler HJ, Packer L (1997). Modulation of cellular reducing equivalent homeostasis by alpha-lipoic acid. Mechanisms and implications for diabetes and ischemic injury. *Biochem. Pharmacol.* 53:393-399.
- Shapiro H, Ashkenazi M, Weizman N, Shahmurov M, Aeed H, Bruck R (2006). Curcumin ameliorates acute thioacetamide-induced hepatotoxicity. *J. Gastroenterol. Hepatol.* 21:358-366.
- Singer M, Stidwill RP, Nathan A, Gainer JL (2000). Intravenous crocinate prolongs survival in lethal Hypoxemia. *Crit. Care Med.* 28:1968-1972.
- Suzumura K, Hashimura Y, Kubota H, Ohmizu H, Suzuki T (2000). Antioxidative property of T-0970, a new ureidophenol derivative. *Free Rad. Res.* 32:255-264
- Tabuchi S, Gotoh T, Miyanaka K, Tomita K, Mori M (2000). Regulation of Genes for Inducible Nitric Oxide Synthase and Urea Cycle Enzymes in Rat Liver in Endotoxin Shock. *Biochem. Biophys. Res. Commun.* 268:221-224.
- Thurlow PM, Desai RK, Newberne PM, Brown H. (1980). Aflatoxin B₁ acute effects on three hepatic urea cycle enzymes using semiautomated methods: A model for 'Reye's syndrome' *Toxicol. Appl. Pharmacol.* 53: 293-298.
- Tsuchiya H, Akechi Y, Ikeda R, Nishio R, Sakabe T, Terabayashi K, Matsumi Y, Ashla AA, Hoshikawa Y, Kurimasa A, Suzuki T, Ishibashi N, Yanagida S, Shiota G (2009). Suppressive effects of retinoids on iron-induced oxidative stress in the liver. *Gastroenterology.* 136:341-350
- Valko M, Morris H, Cronin MT (2005). Metals, toxicity and oxidative stress. *Curr. Med. Chem.* 12:1161-1208.
- Wei QY, Chen WF, Zhou B, Yang L, Liu ZL (2006). Inhibition of lipid peroxidation and protein oxidation in rat liver mitochondria by curcumin and its analogues. *Biochim. Biophys. Acta* 1760:70-77.
- Yajun Z, Hongshan C, Baoxi S, Dengbing Y, Jianhua S, Xinshun G, Li Y, Yi C (2005). Translocation of Bax in rat hepatocytes cultured with ferric nitrilotriacetate. *Life Sci.* 76:2763-2772.
- Yoshioka T, Kawada K, Shunada T, Mori M (1979). Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. *Am. J. Obstet. Gynecol.* 135: 372-376.