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Acute Iron Overload and Potential Chemotherapeutic Effect of Turmeric in Rats

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ABSTRACT

Iron is an essential element in the body, being found in functional form in hemoglobin, myoglobin, cytochromes, enzymes with iron sulphur complexes and other iron-dependent enzymes. At the same time, excess iron in the body is associated with toxic effects and posses health problems. The present study was undertaken to evaluate the protective effect of turmeric against acute iron overload in rats. In this study, forty male rats were divided into four experimental groups; normal control rats received single i.p injection of normal saline; excess iron treated rats received single i.p injection of iron dextran 500 mg/kg b.wt; turmeric treated rats received 1% turmeric powder containing diet 7 day before induction of acute iron overload and iron plus turmeric treated rats received both turmeric and iron at the same doses mentioned before. At the end of the experiment, the animals were sacrificed, blood samples were collected, and the liver, testes, kidneys, spleen and heart were separated and saved frozen for subsequent biochemical analysis. Laboratory investigations consisted of liver function tests (ALT, AST, total bilirubin, total protein, albumin and globulin) and kidney function tests (urea, uric acid and creatinine), measurement of lipid peroxidation indices and the antioxidative GST and GPX enzymatic activities and GSH level. In addition, serum and tissue iron content was determined to test the magnitude of iron toxicity and the antioxidant potential of turmeric supplementation. Acute iron overload markedly increased serum and tissue iron concentration and iron profile (TIBC, transferrin and transferrin saturation), while decreased UIBC level. Iron deposition in liver, kidneys, testes, spleen and heart tissues associated with oxidative stress and increased malondialdehyde level with significant alterations in antioxidant status (GST, GPX enzymatic activities and GSH content) indicating severe toxic effect of excess iron on different organs. Moreover, iron overloaded rats exhibited significant changes in liver and kidney functions. Treatment with turmeric significantly reduced serum and tissue iron levels, ameliorated the oxidative stress and antioxidant status, and had protective effect on liver and kidney function. We concluded that turmeric had a potential chemoprotective effect against acute iron overload which is attributed to its chelating effect on iron and improvement of antioxidant status.

***Key words**: Turmeric, Iron overload, Malondialdehyde, Antioxidant*

INTRODUCTION

Iron is an essential micronutrient for 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[1]. 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[2]. Under these conditions, promotion of free radical generation by free iron is considered a major action of the

transition metal, leading to oxidative damage to biomolecules, functional alterations of subcellular organelles, and loss of cell viability. 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[3].

Administration of ferric nitrilotriacetate had been reported to result in hepatic iron loading associated with extensive peroxidation of membrane lipids in vivo[4]. In addition, it had been demonstrated that ferric nitrilotriacetate induced oxidative stress could lead to hepatocytes apoptosis[1], DNA damage and liver necrosis in rats[5]. From these data, iron control could be a universal approach to disease prevention, two chelating agents; desferroxamine and deferriprone were used to prevent iron overload. Although both drugs were very effective, they exert several side effects and disadvantages[6]. Medicinal plants and their active principles had received great attention as a potentially antioxidant agents for prevention and treatment of several diseases.

Galaris and Pantopoulos (2008) reported that turmeric (Curcuma longa rhizomes), commonly used as a spice, was well documented for its medicinal properties in Indian and Chinese systems of medicine. It had been widely used for the treatment of several types of diseases[7]. Turmeric consumption may reduce the risk of some form of cancers and render other protective biological effects[8]. These biological effects of turmeric had been attributed to its constituent curcumin that had been widely studied for its antioxidant, anti-inflammatory, anti-angiogenic, wound-healing and anti-cancer effects[9]. Also, it had potential therapeutic effects against neurodegenerative, cardiovascular, pulmonary, metabolic and autoimmune diseases[10]. In addition, curcumin exerted hepatoprotective effects in various animal models of liver injury such as carbon tetrachloride[11], endotoxin[12] and thioacetamide[13].

The present study was designed to determine the chemoprotective effect of turmeric on ameliorating iron overload induced oxidative stress and perturbation of antioxidant status in rats.

MATERIALS AND METHODS

Chemicals and medicinal plants:

Iron dextran (Feracyl; 50 mg iron/ml) was obtained from STEROP laboratories, Avenue de Scheut, Brussel, Belgium. Atomic spectrophotometry standard solutions for iron (Fe in 3% nitric acid) were purchased from Ricca Chemical Company (Fenton Mo). Reduced glutathione (GSH) and thiobarbituric acid (TBA) were purchased from Fluka (Buchs, Switzerland). Nitric acid, 5,5- dithiobis-(2-nitrobenzoic acid) (DTNB, Ellman's reagent), and 1-chloro-2,4-dinitrobenzene (CDNB) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Biodiagnostic kits for measuring ALT, AST, total bilirubin, total protein, albumin, urea, uric acid, creatinine, TIBC and transferrin. All the other reagents were of analytical, high-performance liquid chromatography (HPLC), or the best available pharmaceutical grade. Turmeric powder was purchased from local markets in Alexandria, Egypt. It was identified by botanists at Faculty of Agriculture, Alexandria University, Egypt.

Animals and experimental design:

Forty healthy adult male Sprague-Dawley rats, weighing 200 ± 10 g and aged 7–8 weeks, were provided from the Faculty of Veterinary Medicine, Alexandria University, Egypt. All animals were housed in gang cages maintained in a room with controlled environment conditions and a 12-h light–dark cycle. The animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals, and the study protocol was approved by the local authorities. All efforts were made to minimize the number of animals used and their suffering. After 2 weeks of acclimatization, all animals were randomly divided into 4 experimental groups of 10 rats each. Rats in control group received single i.p injection of normal saline, those in iron overload group received single dose of iron dextran 500 mg/kg b.wt by i.p injection, while those in turmeric group received 1% turmeric powder mixed with basal diet given for 8 days and those in iron overload + turmeric group received 1% turmeric powder mixed with basal diet 7 days before iron treatment (same dose as in iron

overload group). Twenty-four hours after the injection of saline or iron, the rats were anesthetized with ketamine/xylazine (7.5:10 mg/kg, 1 mg/kg i.p). Then, blood samples were collected from the inner canthus of the eye by heparinized capillary tube into 2 ml clean test tube. Following standing in room temperature for at least 30 min, the blood was centrifuged at 3400 xg for 10 min at that point the serum was separated, transferred to eppendorf tubes, and stored at -20 ˚C prior to measure the concentrations of iron, and determine other biochemical parameters. Immediately after the collection of blood samples, the animals were euthanized, and their livers, heart, testes, spleen and kidneys were quickly excised, rinsed in ice-cold saline and used immediately or stored frozen at -80 ˚C until analysis.

Serum and tissue iron concentration:

200 mg tissues were weighed in a 10 ml microwave digestion vessels. An aliquot of 2 ml nitric acid was added into vessels. The tightly capped vessels were placed in hot air oven and digested with 15 min stepwise rise to 200 ˚C which was maintained for 10 min. After cooling to room temperature, the resulting solution was mixed with another 2 ml of distilled water. The solution was then transferred to 10 ml metal-free polypylene conical tube and store at 4 ˚C until analysis. To determine iron concentration in serum, an aliquot of 0.5 ml of serum was mixed with 1.5 ml nitric acid in microwave vessels and digested under same condition as described above. A VARIAN spectroAA-20 plus GTA-96 flameless graphite furnace AAS was used to quantify Fe concentration in tissues and serum.

Biochemical blood serum analysis:

Using commercially available diagnostic kits (Vitro Scient Co., Germany), the activities of the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), as well as the serum total bilirubin, total protein, albumin, urea, uric acid, creatinine, TIBC and transferrin contents were spectrophotometrically determined following the manufacturer's instructions.

Oxidative stress and antioxidant status:

Tissue homogenates were separately prepared from frozen liver, heart, testes, spleen and kidneys samples in 10 volumes of 0.1 M Tris–EDTA buffer (pH 7.4) and centrifuged at 8000 xg for 30 min at 4 ˚C. Aliquots of the supernatant were utilized for the spectrophotometrical assessment of the levels of the following: lipid peroxidation (LPO), assessed as the production of the thiobarbituric acid reactive substances (TBARS) in the presence of BHT[14] reduced glutathione, by using Ellman's reagent[15] glutathione S-transferase (GST, EC 2.5.1.18) activity, as the rate of GSH conjugation of CDNB[16] glutathione peroxidase (GPX, EC 1.11.1.9) activity was measured using reduced glutathione and cummene hydroperxide as substrate by the modified method of Paglia and Valentine[17].

Statistical analysis:

The obtained data were statistically analyzed for intergroup differences by using the GLM procedure of the SAS statistical package[18]. All data are expressed as means ± standard error (SE) of the means.

RESULTS

Serum iron concentration and iron profile:

The serum iron concentration in iron challenged rats was significantly increased than that in control group. Simultaneously; TIBC, transferrin and transferrin saturation were increased while UIBC was significantly lower in iron overloaded rats than that in control ones ($P<0.05$). Treatment with turmeric significantly decreased serum iron, TIBC, transferrin and transferrin saturation while increased serum UIBC than those in iron overload rats (Table 1).

Table (1): Effect of acute iron overload and turmeric on serum iron profile of rats

Groups	Iron (µg/dl)	TIBC (µg/dl)	UIBC (µg/dl)	Transferrin (µg/dl)	Transferrin saturation %
I	187.34 ± 2.33 [c]	494.92 ± 7.27 [b]	307.58 ± 4.08 [c]	4.11 ± 0.89 [b]	37.85 ± 1.41 [c]
II	746.86 ± 4.76 [a]	1015.91 ± 21.14 [a]	269.05 ± 3.95 [d]	9.23 ± 0.27 [a]	73.51 ± 1.00 [a]
III	177.29 ± 3.44 [c]	507.27 ± 11.08 [b]	329.98 ± 3.05 [b]	4.79 ± 0.32 [b]	34.94 ± 1.30 [c]
IV	638.46 ± 6.71 [b]	1036.38 ± 18.97 [a]	397.92 ± 5.94 [a]	10.67 ± 0.45 [a]	61.61 ± 0.89 [b]

The values are the means ± S.E
Means in the same columns with different superscript letters are significantly different at ($p<0.05$). Group I: control, Group II: Iron, Group III: Turmeric, Group IV: Iron + Turmeric.

Tissue iron concentration:

The iron concentration significantly tend to accumulated in the examined tissues following iron overload than those in control group ($P<0.05$). Administration of turmeric not statistically decreased iron concentration in all examined tissues as compared to iron overload rats (Table 2).

Table (2): Effect of acute iron overload and turmeric on iron concentration (µg/g dry tissue) of liver, spleen, testes, heart and kidneys of rats

Groups	Liver	Spleen	Testes	Heart	Kidney
I	139.56 ± 1.89 [b]	264.48 ± 2.54 [b]	112.27 ± 2.51 [b]	97.12 ± 1.12 [b]	93.86 ± 1.41 [b]
II	423.15 ± 3.12 [a]	534.92 ± 4.76 [a]	296.68 ± 2.48 [a]	167.92 ± 2.04 [a]	179.73 ± 1.72 [a]
III	131.63 ± 2.34 [b]	249.39 ± 4.71 [b]	109.75 ± 1.99 [b]	89.82 ± 1.45 [b]	91.52 ± 1.72 [b]
IV	411.87 ± 3.67 [a]	522.59 ± 3.48 [a]	287.72 ± 2.01 [a]	161.52 ± 1.87 [a]	169.71 ± 1.89 [a]

The values are the means ± S.E
Means in the same columns with different superscript letters are significantly different at ($p<0.05$). Group I: control, Group II: Iron, Group III: Turmeric, Group IV: Iron + Turmeric.

Oxidative stress and antioxidant status:

In relation to control group, iron overloaded rats had significant greater level of MDA in liver, heart, testes, spleen and kidneys. Accumulation of iron in all tissues caused induction in GST enzymatic activity and GSH content with reduction in the activity of GPX in all examined tissues ($P<0.05$). Treatment with turmeric significantly lowered the level of MDA and induced the increase in GST and GPX enzymatic activities and GSH level in all examined tissues (Table 3).

Table (3): Effect of acute iron overload and turmeric on MDA level (nmole/g wet tissue), GPX activity (IU/g wet tissue), GST activity (mole CDNB/min/g wet tissue) and content of reduced glutathione (µmole/g wet tissue) of liver, spleen, testes, heart and kidneys of rats

		Group I	Group II	Group III	Group IV
MDA	Liver	156.72±1.89 ^{c}	271.38±2.54 ^{a}	108.37±1.78 ^{d}	211.25±1.85 ^{b}
	Spleen	77.94±1.45 ^{c}	129.39±2.17 ^{a}	55.35±1.39 ^{d}	102.83±1.72 ^{b}
	Testes	98.82±1.09 ^{c}	261.27±1.87 ^{a}	67.91±1.73 ^{d}	215.73±2.11 ^{b}
	Heart	112.86±2.03 ^{c}	265.62±2.05 ^{a}	77.19±2.01 ^{d}	203.48±1.79 ^{b}
	Kidney	54.68±1.48 ^{c}	111.92±1.35 ^{a}	36.75±1.82 ^{d}	93.75±1.14 ^{b}
GPX	Liver	17.23±0.97 ^{c}	14.86±1.01 ^{d}	23.74±2.41 ^{a}	19.35±1.03 ^{b}
	Spleen	10.15±1.01 ^{c}	6.46±0.78 ^{d}	15.84±1.13 ^{a}	12.87±1.34 ^{b}
	Testes	12.37±0.87 ^{c}	9.87±0.72 ^{d}	15.39±1.01 ^{a}	10.96±0.89 ^{b}
	Heart	14.17±0.74 ^{c}	9.38 ±1.03 ^{d}	19.27±1.11 ^{a}	12.48±1.13 ^{b}
	Kidney	8.65±0.72 ^{c}	5.31±0.65 ^{d}	12.01±0.89 ^{a}	7.14±0.87 ^{b}
GST	Liver	417.23±6.73 ^{d}	485.23±7.45 ^{c}	522.75±6.53 ^{b}	543.27±8.46 ^{a}
	Spleen	247.98±5.23 ^{c}	322.34±6.78 ^{b}	409.29±6.12 ^{a}	413.76±7.64 ^{a}
	Testes	409.55±4.87 ^{b}	489.11±7.54 ^{a}	511.65±6.98 ^{a}	523.65±5.87 ^{a}
	Heart	128.45±3.65 ^{c}	210.85±5.34 ^{b}	289.73±4.67 ^{a}	302.98±4.62 ^{a}
	Kidney	161.87±4.87 ^{c}	236.63±4.67 ^{b}	312.65±4.98 ^{a}	325.71±5.67 ^{a}
GSH	Liver	17.73±2.87 ^{c}	27.62±2.65 ^{b}	36.98±3.11 ^{a}	38.65±3.98 ^{a}
	Spleen	14.68±1.98 ^{d}	21.98±2.01 ^{c}	26.19±2.11 ^{b}	31.87±2.23 ^{a}
	Testes	22.65±2.03 ^{b}	29.43±1.98 ^{a}	31.98±1.76 ^{a}	32.76±2.03 ^{a}
	Heart	9.76±1.01 ^{b}	12.98±1.11 ^{a}	11.65±0.98 ^{a}	12.45±1.04 ^{a}
	Kidney	13.78±1.67 ^{b}	17.67±1.12 ^{a}	18.87±1.65 ^{a}	18.73±1.03 ^{a}

The values are the means ± S.E

Means in the same rows with different superscript letters are significantly different at ($p<0.05$). Group I: control, Group II: Iron, Group III: Turmeric, Group IV: Iron + Turmeric.

Liver and kidney functions:

Iron overload resulted in liver and kidney damage as assessed by significant increase in ALT, AST enzymatic activities, and total bilirubin, total protein, albumin, urea, uric acid and creatinine levels than those in control group ($P<0.05$). Administration of turmeric showed a protective effect on liver and kidney as the activities of ALT and AST, levels of total bilirubin, urea, uric acid and creatinine were significantly lowered than those in iron overload (Table 4).

Table (4): Effect of acute iron overload and turmeric on liver and kidney functions of rats

ALT (IU/l)	**12.87±0.9 [c]**	**37.23±1.03 [a]**	**8.76±0.8 [d]**	**28.87±1.02 [b]**
AST (IU/l)	57.45±3.61 [c]	129.75±3.01 [a]	41.56±3.12 [d]	97.65±2.87 [b]
Bilirubin (mg/dl)	1.23±0.05 [c]	1.46±0.08 [a]	1.12 ±0.06 [d]	1.38±0.05 [b]
T. protein (g/dl)	6.7±0.2 [b]	7.8±0.3 [a]	7.9±0.3 [a]	7.6±0.1 [a]
Albumin (g/dl)	4.22±0.05 [b]	5.01±0.6 [a]	4.92±0.05 [a]	5.03±0.03 [a]
Globulin (g/dl)	2.48±0.02 [d]	2.79±0.03 [b]	2.98±0.03 [a]	2.57±0.02 [c]
Uric acid (mg/dl)	3.54±0.02 [c]	5.25±0.03 [a]	2.76±0.02 [d]	4.34±0.03 [b]
Creatinine (mg/dl)	1.2±0.01 [c]	1.7±0.01 [a]	0.92±0.00 [d]	1.5±0.01 [b]
Urea (mg/dl)	37.56±0.74 [c]	49.62±1.01 [a]	28.98±0.99 [d]	43.76±1.02 [b]

The values are the means ± S.E

Means in the same rows with different superscript letters are significantly different at ($p<0.05$). Group I: control, Group II: Iron, Group III: Turmeric, Group IV: Iron + Turmeric.

DISCUSSION

Acute iron overload induced significant deposition of iron in rat's organs associated with exacerbated oxidative stress status and remarkable alterations of antioxidants. Matsuura, (1983) had demonstrated iron uptake by rat's liver and induction of hepatic iron loading and iron toxicity in the liver after a single injection of ferric nitrilotriacetate. Also, Whittaker and Chanderbhan[19] reported that feeding rats diets supplemented with carbonyl iron resulted in dose related increases in liver non heme iron and lipid peroxidation. Moreover, El-Maraghy et al.,[20] demonstrated that administration of ferric nitrilotriacetate induced a significant deposition of iron in rat liver associated with oxidative stress and a remarkable increase in hepatic nitric oxide level. Also, Sarker et al.,[21] reported that mice injected with iron dextran 100 mg/kg for 5 days exhibited an increased in liver iron deposition. Administration of turmeric significantly reduced serum and tissue iron concentration, these may be attributed to iron chelator effect of curcumin; an active principle of turmeric. Several studies had demonstrated that curcumin can bind iron and it had properties of an iron chelator[22,23]. In addition, mice fed a diet high in curcuminoids showed decreased level of liver ferritin, indicative of decreased iron burden[24]. Furthermore, Thephinlap et al.,[25] found that curcuminoids were effective in chelation of plasma iron in iron loaded thalassemic mice. Our results agreed with Srichairatanakool et al.,[26] who found that curcumin act as iron chelator, mice that were fed diets supplemented with curcumin exhibited a decline in level of ferritin protein in liver. Moreover, phenolics in the turmeric meal determine the inhibitory effect of phenolic compounds on iron absorption[27].

Regarding the effect of acute iron overload on lipid peroxidation and antioxidant status, our results showed that acute iron overdose increased MDA, reduced glutathione levels, and glutathione transferase activity while decreased activity of glutathione peroxidase in different organs. Iron is a well-known inducer of reactive oxygen species. Its ability to accelerate lipid peroxidation is well established[3]. Several studies had shown that, Fe-NTA administration induced a state of a sustained oxidative stress and caused high levels of lipid peroxidation products in liver. It also induced a significant decrease in hepatic glutathione, α-tocopherol and polyunsaturated fatty acids contents[28,4]. Furthermore, Abd El-Baky[29], reported that iron overload induced oxidative stress involved the GSH depletion while GSSG and lipid peroxidation will be increased. Iron-overload can potentiate various forms of liver injury[30] with oxidative stress through the formation of hydroxyl radicals[31] and lipid peroxidation[32]. In particular, the generation of reactive oxygen species (ROS) can result in reversible and irreversible cell and tissue damage[33]. Decreased activity of GPX in different tissues after iron overload suggested the increased utilization and

subsequent depletion of this antioxidant with induction of GST activity and reduced glutathione level to counter the increased level of lipid peroxidation[34].

The antioxidant property of turmeric may be attributed to its active component, curcumin, with its conjugate structure which include two methoxylated phenols and an enol form of β-diketone which showed a typical radical trapping ability as a chain breaking antioxidant[35]. 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[7,36]. Earlier studies had shown that curcumin significantly reduced the redox activity of iron[37] and lowered the liver and serum lipid peroxide levels enhanced by iron injection[37,38]. The antioxidant effect of curcumin reduced the utilization of GST, GPX and GSH as antioxidants and subsequently preserved and their activities or content will be increased. Also, Dickinson et al.,[39] reported that curcumin caused induction of GST activity without depletion of cellular level of glutathione and in fact increased modestly, perhaps due to the ability of curcumin to induce enzymes involved in the synthesis of glutathione, such as glutamate cysteine ligase.

Liver damage by iron had been assessed by leakage of enzymes such as ALT and AST and lactate dehydrogenase into blood[28,38]. In the present study, higher activities of serum ALT, AST and total bilirubin level had 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. EL-Maraghy et al.,[20] demonstrated a reduction in the severity of iron-induced hepatotoxicity by curcumin through the correction of the altered liver function indices. Also, Fu et al.,[11] demonstrated that curcumin significantly protects the liver from injury by reducing the activities of serum AST, ALT, and ALP, and by improving the histological architecture of the liver. Moreover, Naik et al.,[40] showed that curcumin treatment reversed the elevated serum marker enzymes; AST, ALT and ALP, the increased lipid peroxidation, the decreased glutathione level, glutathione peroxidase and superoxide dismutase activities in edematous, granulomatus, liver and heart tissues during inflammation, liver injury and cardiac necrosis, respectively.

CONCLUSION

Taken together, it might be speculated that, in the current study, turmeric could improve acute iron overload induced liver dysfunction via their ability to antagonize the enhanced oxidative stress and by preserving and restoring the antioxidant status. The above mentioned findings indicate that, curcumin is a potent hepatoprotective agent against iron overload-induced biochemical alterations in rat tissues. It also implies the potential usefulness of curcumin as dietary supplements for populations at risk for iron overload to guard against its detrimental effects.

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