Hepatoprotective and Nephroprotective Effect of Curcumin Against Copper Toxicity in Rats

Emad A. Hashish¹ · Shimaa A. Elgaml¹

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Abstract Curcumin is a natural anti-inflammatory and antioxidant with several potential health benefits. Although it has been examined in several metals toxicity studies, but its role in the protection against copper toxicity has not been investigated. In this study; the detoxification and antioxidant effect of curcumin were examined to determine its prophylactic/therapeutic role experimentally in rats. Forty albino rats were divided into five groups; control, CuSO₄ (4 mg/kg body weight), curcumin (80 mg/kg body weight), curcumin post-treatment (CuSO₄ for 15 days followed by curcumin for the next 15 days) and curcumin co-treatment (CuSO₄ plus curcumin for 30 days). All rats were treated orally by stomach tube for 30 days/once a day. Changes were observed in hepatic marker enzymes such as: aspartate aminotransferase (AST), alanine transaminase-(ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT), besides the serum total protein, urea and creatinine. Concentration of liver and kidney antioxidants such as: catalase (CAT), superoxide dismutase (SOD), reduced glutathione-(GSH) and malondialdehyde (MDA) were measured. An increased in the activities of liver marker enzymes, urea, creatinine and the MDA contents were detected after exposure to CuSO₄. Meanwhile, the activities of serum total protein, hepatic and renal antioxidants were decreased. Changes in all biochemical parameters were alleviated by the post-treatment and co-treatment of curcumin. Our finding suggests that the

curcumin showed protective effects on CuSO₄-induced hepatotoxicity and nephrotoxicity.

Keywords Antioxidants · Copper · Curcumin · Kidney · Liver · Rats

Introduction

Copper (Cu) is an essential element, widely distributed in both animal and human tissues. It is a component of a number of metalloenzymes such as catalase, peroxides and cytochrome oxidase [1]. High level of Cu in drinking water causes a potential environmental hazard. The source of Cu could be in building, sheets, strips and many alloy products [2]. Cu is a catalyst which leads to oxidative stress through the formation of reactive oxygen species (ROS) and destructive damage for lipid peroxidation [3]. It can be absorbed into the systemic circulation from the gastrointestinal tract, lungs, and skin. It binds to plasma albumin and amino acids in the portal blood, then transported to the liver where it is incorporated into ceruloplasmin and released into the plasma. The main target organs of CuSO₄ are liver and kidney leading to hepatotoxicity and renal failure [4, 5]. Therefore, studies on the copper toxicity are of especial interest.

Curcumin is considered as a safe nutritional dietary supplement. It is the principal bioactive phenolic component in turmeric spice (*Curcuma longa*). It has several broad spectrum biological functions such as, anti-inflammatory, anti-mutagenic and antioxidant activities, besides being widely used as a spice and coloring agent [6]. Curcumin is used in traditional medicine due to its potential health beneficial effects with high efficacy as a cleanser of the body. It is used to treat many disorders such

✉ Shimaa A. Elgaml
drshimaa_68@yahoo.com

¹ Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

as cough, anorexia, sinusitis, hepatic and renal diseases [7]. Since it has been reported in several health aspects and disease conditions, the interest in the use of curcumin as a protective and therapeutic agent against copper toxicity has been investigated.

The current study describes the potential role of curcumin as a hepatoprotective and nephro protective effect against copper sulphate (CuSO_4) toxicity in rats. Several biochemical, antioxidant and histopathological assays were utilized to assess the role of curcumin in ameliorating the copper-induced hepatic and renal toxicity.

Materials and Methods

Experimental Animals

Forty female albino rats (Laboratory Animal Colony, Helwan, Egypt) aged from 12 to 16 weeks and weighing 100–120 g was used in this study. Eight rats were randomly selected for each group in stainless steel cages. Rats were housed in the center for the laboratory animal at the Faculty of Veterinary Medicine, Zagazig University, Egypt. Rats were maintained on 12 h light-darkness cycle with controlled temperature (21–24 °C) and relative humidity (50–60 %) and were given standard diet and water ad libitum throughout the study. All ethical considerations for the use of animals were considered carefully. The study is complied with the Animal Welfare Act and the whole experimental work was approved by Veterinary Committee.

Copper Sulphate and Curcumin

Copper sulphate (CuSO_4), was purchased from Sigma Chemicals Company for Pharmaceutical Industries, St Louis, Missouri, USA and used for the induction of toxicity at a dose of 4 mg/kg b.w. orally by stomach tube [8]. Curcumin was purchased from Sigma Chemicals Company for Pharmaceutical Industries, St Louis, Missouri, USA (Cat No. C7727). It was dissolved in 1 % gum acacia solution just before usage [9], and used at a dose of 80 mg/kg b.w. orally by stomach tube.

Experimental Design

Rats were divided into five groups and acclimatized for 14 days before starting the experiment. Group I ($n = 8$): negative control rats were fed on clean healthy food and water throughout the experimental period and left under normal condition without any treatment. Group II ($n = 8$): positive control, rats were administered CuSO_4 orally

(4 mg/kg b.w.) daily for 30 days. Groups III ($n = 8$): curcumin treated group; rats were orally administered 80 mg/kg b.w. curcumin daily for 30 days. Group IV ($n = 8$): curcumin post treated group, rats were orally administered CuSO_4 (4 mg/kg b.w.) for 15 days followed by curcumin 80 mg/kg b.w. for the next 15 days. Group V ($n = 8$): curcumin co-treated group, rats were daily administered CuSO_4 (4 mg/kg b.w.) and 80 mg/kg b.w. curcumin simultaneously for 30 days. All rats were sacrificed at day 31st. Samples of blood, liver and kidney tissues were collected for further analysis. All the experimental procedures were carried out at the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Blood Sampling

Two–three ml blood was collected from the retro-orbital venous plexus of rats under sterile septic condition at the 31st day. Blood was allowed to flow smoothly into the tubes without anticoagulant, left to clot for 2 h at room temperature then centrifuged at 3000 rpm for 10 min. The clear supernatant serum was collected using sterile disposable Pasteur pipettes. The collected serum was transferred to 1.5 ml dry, sterile micro tubes (Eppendorf, Germany) for further biochemical analysis.

Estimation of Biochemical Parameters

Parameters were measured colorimetrically using spectrophotometer 5010 v5⁺ (Berlin, Germany) for biochemical serum analysis using commercial kits provided by Biomerieux, Egypt. Serum hepatic marker enzymes, including the activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) [10], serum alkaline phosphatase (ALP) [11], and serum gamma glutamyl transferase (GGT) [12] were measured. Serum total protein [13], urea [14] and serum creatinine [15] were colorimetrically determined.

Antioxidants and (MDA) Lipid Peroxidation Assay

Liver and kidney from all groups were collected at day 31 of the experiment. One gram of each liver and kidney sample was added to 9 ml of normal saline 0.9 % and homogenized using electrical tissue homogenizer and centrifuged at 3000 rpm for 15 min. The supernatant was collected and used for the estimation of antioxidants (CAT, SOD and GSH) and malondialdehyde (MDA), the marker of lipid peroxidation [16]. The catalase activity (CAT) [17], superoxide dismutase (SOD) [18], reduced glutathione (GSH) [19] and malondialdehyde (MDA) [20] were determined.

Histopathological Assays

Rats were anesthetized with diethyl ether and sacrificed. A portion of liver and kidney tissue from all groups was collected and immediately prepared for hisopathological examination [21]. Simply, the specimen labeled and fixed in 10 % neutral buffered formalin solution overnight. Fixed tissues were processed after washing and preserved in 70 % ethanol, dehydrated in a series of graded concentrations of ethyl alcohol and cleared in xylene. Tissue were embedded in paraffin waxes (melted paraffin at 55–60 °C), casting and cutting at 4–5 µm thickness. These sections were placed on top of glass slides, routinely stained with Hematoxylin and Eosin (H&E) to be examined and visualized under the microscope.

Statistical Analysis

Statistical Analysis System software package were used to analyze the data by one-way analysis of variance, ANOVA [22]. All data showed a normal distribution and passed equal variance testing. Group II were compared to group I, where groups III–V were compared to group II. Significant differences between means were determined at a level of ($P < 0.05$ or $P < 0.01$) by Duncan's multiple range tests.

Results

Serum hepatic marker enzymes (AST, ALT, ALP and GGT) of all treated rats (Fig. 1). The results showed that CuSO₄ induced significant ($P < 0.01$) increase in the activities of hepatic enzymes when compared to the negative control. Oral administration of curcumin alone did not change the activities of those enzymes indicating the safe use of curcumin as a natural product. The activities of AST, ALT,

ALP and GGT enzymes were significantly ($P < 0.05$ or $P < 0.01$) decreased in both curcumin post- and co-treated groups when compared to the CuSO₄ treated group. However, the ameliorative effect of the curcumin post-treatment was more pronounced than the curcumin co-treatment with CuSO₄.

Serum total protein, urea and creatinine activities showed significant ($P < 0.01$) decrease in the serum total protein with significant ($P < 0.01$) increase in serum urea and creatinine activities in the CuSO₄ treated group when compared with the negative control (Fig. 2). The administration of curcumin alone did not affect the activities of these parameters indicating the safe use of curcumin. However, significant ($P < 0.05$ or $P < 0.01$) increases in the serum total protein with significant ($P < 0.01$) decreased in serum urea and creatinine activities were showed in the curcumin post-treated and curcumin co-treated groups when compared with the CuSO₄ treated group. The ameliorative effect of post-treatment of curcumin on the concentration of serum total protein, urea and creatinine was more prominent than that of the curcumin co-treatment with CuSO₄.

Hepatic and renal antioxidants and MDA showed a significant ($P < 0.05$) decrease in CAT, SOD and GSH activities with a significant ($P < 0.05$) increase in MDA activity in CuSO₄ treated group when compared with the negative control (Figs. 3, 4). Curcumin is a strong antioxidant; it acts as an oxygen free radicals scavenger. The administration of curcumin alone to rats did not affect the activities of all these enzymes and MDA. Meanwhile activates of these enzymes were significantly ($P < 0.05$ or $P < 0.01$) increased in curcumin-post treated and co-treated groups. Meanwhile MDA was significantly ($P < 0.01$) less than CuSO₄ treated group. The curcumin post treated group showed an improvement in these enzymes activities and the MDA more than curcumin co-treated group.

Fig. 1 Hepatic serum enzymes showing significant increase in the levels of serum AST, ALT, ALP and GGT activities in the positive control comparing with the negative control group. Curcumin, curcumin post treated and co-treated groups (III–V) exhibited a decrease in the activity of these enzymes comparing with the positive

control. Values are expressed as mean $\pm$ SE. Significant difference was detected [#] $P < 0.01$ compared the positive control (CuSO₄ treated) with the negative control. ^{*} $P < 0.05$ or ^{**} $P < 0.01$ for the comparison between groups III–V with the positive control.

Fig. 2 Levels of serum total protein, urea and creatinine. Values are expressed as mean $\pm$ SE. Significant difference was detected $^{\#}P < 0.01$ compared the CuSO_4 treated (positive control) group with

the negative control, while $*P < 0.05$ or $**P < 0.01$ for the comparison between groups III–V with positive control

Fig. 3 Concentration of antioxidants (CAT, SOD and GSH) and lipid peroxidation (MDA) in the liver. Values are expressed as mean $\pm$ SE. Significant difference was detected $^{\#}P < 0.01$ compared the positive

control with the negative control. $*P < 0.05$ or $**P < 0.01$ for the comparison between groups III–V with the positive control

Fig. 4 Concentration of antioxidants (CAT, SOD and GSH) and lipid peroxidation (MDA) in the kidney. Values are expressed as mean $\pm$ SE. Significant difference was detected $^{\#}P < 0.01$ compared

the positive control with the negative control. $**P < 0.01$ for the comparison between groups III–V with the positive control

Histopathological Observations Liver of CuSO_4 treated group showed periportal acute cell swelling of hepatocytes which appeared swollen with normal nuclei and granular

cytoplasm (arrow head). The adjacent hepatocytes were atrophied and appeared condensed with more eosinophilic cytoplasm. In addition, individual hepatocytes revealed

coagulative necrosis represented by karyolysis of their nuclei (zigzag arrow). The portal area showed mild hyperplasia of the lining epithelium of bile ducts (arrow) with presence of newly formed bile ductules (white arrow) (Fig. 5a). Liver of curcumin treated group showed normal architecture of the hepatic lobules where the central vein (arrow head) was normal and surrounded with intact hepatocytes (arrows) (Fig. 5b). Curcumin post-treated and curcumin co-treated groups showed nearly normal hepatic architecture when compared with CuSO_4 treated group. In the curcumin post treated group, the hepatic lobules showed normal centrilobular hepatocytes (arrow) with slightly dilated hepatic sinusoids (zigzag arrow) and mild vacuolar degeneration of the adjacent hepatocytes (Fig. 5c). Curcumin co-treated group showed mild vacuolar and hydropic degenerations of hepatocytes (zigzag arrow) adjacent to the central vein (arrow), besides mild hypertrophy of Kupffer cells (Fig. 5d). No significant lesions were detected in the portal area. Renal tissues of CuSO_4 treated group showed signs of degeneration represented by cloudy swelling, vacuolar and hydropic degenerations in the lining epithelium of some renal tubules (arrow head) with cystic dilatation (hydronephrosis) (zigzag arrow) of numerous medullary renal tubules in addition to, contracted glomerular tufts (arrow), (Fig. 6a).

Regarding rats treated with curcumin alone, the renal tissue appeared mostly normal. The majority of the glomeruli were intact (arrow) and a few renal tubules appeared dilated and cystic (zigzag arrow) (Fig. 6b). Curcumin post-treated and curcumin co-treated group showed a better improvement of the renal tissue when compared with CuSO_4 treated group. Curcumin post treated group showed mild cloudy swelling of the lining epithelium of convoluted tubules. Moreover, a few renal tubules appeared dilated and lined by flattened epithelium (arrow) (Fig. 6c). Curcumin co-treated group showed mild shrinkage of the glomerular tufts (arrow) with slight dilation of the Bowman's capsule, besides mild vacuolation of the lining epithelium of renal tubules (Fig. 6d).

Discussion

Curcumin is a potent polyphenolic antioxidant. The protective effect of curcumin against copper sulphate-induced hepatotoxicity and nephrotoxicity was evaluated in the present study. Curcumin demonstrates the ability to diminish the alterations associated with copper toxicity. This is probably due to the incorporation with some stem cells to regenerate such lost cell masses [8]. Curcumin is

Fig. 5 Liver histopathology (H&E). **a** Positive control, (CuSO_4 treated) group II, showing acute cell swelling of hepatocytes adjacent to the portal area (arrow head). A few individual hepatocytes showed coagulative necrosis with presence of faintly stained nuclei (zigzag arrows). The wall of the bile duct appeared thick (arrow) with newly formed bile ductules (white arrow) $\times 1200$. **b** Group III showing

normal hepatocytes (arrows) around the central vein (arrow head) $\times 300$. **c** Group VI showing normal hepatocytes (arrows) and dilated hepatic sinusoids (zigzag arrow), $\times 1200$. **d** Group V showing mild vacuolation of hepatocytes (zigzag arrows) adjacent to the central vein (arrow) $\times 300$

Fig. 6 kidney histopathology (H&E). **a** Positive control, (CuSO_4 treated) group II, showing shrunken glomerular tufts with dilated Bowman's capsule (*arrow*) and cystic dilation of renal tubules (*zigzag arrow*). Some renal tubules showed cloudy swelling of their lining epithelium with obliterated lumina (*arrow head*) $\times 300$. **b** Group III showing normal glomerular tufts (*arrow*) and mild cystic dilation of

the adjacent renal tubules (*zigzag arrow*) $\times 300$. **c** Group VI showing cystic dilation of the renal tubules which were lined by flattened epithelium (*arrows*) with pressure atrophy of the adjacent renal tissue $\times 1200$. **d** Group V showing slightly dilated Bowman's capsule (*arrow*) with contracted glomerular tufts $\times 1200$

shown to be hepatoprotective against copper sulphate-induced (CuSO_4) hepatotoxicity. The hepatoprotective effect may attribute to the low absorption of copper in the intestine with an increased intestinal metabolism. The curcumin pro-oxidant can induce the GSH-antioxidant response and thus provide hepatic protection [23].

Hepatic marker enzymes (AST, ALT, ALP and GGT) are usually elevated in hepatic damage due to the membrane damage. Alterations in the hepatic enzymes were observed in our study, an increase in the activities of the hepatic enzymes in serum of the CuSO_4 treated group was detected. It was shown that CuSO_4 can induce hepatic toxicity [8]. No changes in the activities of these enzymes was detected after oral administration of curcumin; this suggests that curcumin could be safe on liver [9]. The disruption in the hepatic enzymes activities in CuSO_4 treated rats was ameliorated by both curcumin post- and co-treated groups. Significant decrease in the activities of these enzymes in sera was detected when compared with CuSO_4 treated group. However, this effect was more profound and more effective in curcumin post-treated group. The concentration of these enzymes approximately came back close to the normal concentration. The decreased activities of the hepatic enzymes in the curcumin post treated group support the protective and therapeutic effects

of curcumin. Curcumin is shown to prevent the leakage of hepatic enzymes into the serum and thus supports the hepatocytes from CuSO_4 -induced hepatotoxicity. The significant decrease in the activities of these enzymes in the curcumin co-treated group suggests a chelating effect of curcumin. These results were supported by the histopathological findings of the liver as it showed a better improvement of the hepatic tissue.

In the present study, a significant decrease in serum total protein concentration was detected in the CuSO_4 treated group when compared with the negative control. Hypoproteinemia may be induced due to hepatocellular injury or inflammation induced by CuSO_4 toxicity which leads to the disturbance of protein biosynthesis [8]. Significant increase in the serum total protein concentration was observed in the curcumin post- and co-treated groups. This effect was more pronounced in the curcumin post-treated group, which may attribute to the therapeutic ability of curcumin to stimulate the regeneration of hepatic tissue. This illustrates that increase in the protein synthesis in the damaged hepatic cells was more effective than its chelating effect which was represented by the previous studies [24].

Curcumin is shown to be nephroprotective against copper sulphate-induced (CuSO_4) nephrotoxicity. Significant increase in the concentrations of urea and creatinine in

CuSO₄ treated group. This suggests that the kidney is unable to excrete these products, which indicates an impairment of the kidney functions [4]. These effects may be due to the changes in the tubular re-absorption threshold, renal blood flow and the rate of glomerular infiltration [25]. This effect of CuSO₄ on kidneys has been described previously [26]. The oral administration of curcumin did not change the activities of these parameters and suggested a safe use of curcumin on kidneys. Curcumin had a therapeutic and chelating effect against CuSO₄ induced nephrotoxicity. The curcumin post- and co-treated groups showed a significant decrease in these parameters when they compared with CuSO₄ treated group. This suggests a therapeutic and chelating effect of curcumin against CuSO₄-induced nephrotoxicity. This effect of curcumin in ameliorating the activities of urea and creatinine in the sera were previously described [27]. These results were supported by the histopathological findings of the kidney as it showed a better improvement of the renal tissue.

One of the best-known consequences of excess copper is the alterations in the oxidative stress markers and peroxidative damage of membrane lipids. Oxidative stress occurs when the formation of the reactive oxygen species (ROS) exceeds the ability of the cells to defend themselves and became cytotoxic. The superoxide anion (O₂⁻), hydroxyl radicals (OH) and hydrogen peroxide (H₂O₂) are elaborating during the oxidative stress causing damage to cell constituents as membrane lipids. Lipid peroxidation is a free radical-mediated chain reaction occurs by the reaction of lipid radicals and oxygen to form peroxy radicals [5]. Catalase and GSH catalyze the conversion of hydrogen peroxide to water. SOD catalyses the destruction of O₂ and protects various organs from lipoperoxidation and the damage induced by ROS. Decreased SOD and GSH activities or increased O₂ could result in lipoperoxidation and oxidative stress with the imbalance of oxidant/antioxidant system [18].

Copper induces oxidative damage by ROS and decreases the activities of some antioxidant enzymes such as CAT and SOD which have free radical scavenging activity [28]. Copper caused lipids damage and so lipid peroxidation generation with the significant decrease in the hepatic and the renal CAT, SOD and GSH in the CuSO₄ treated group. The essential antioxidants defense systems CAT, SOD and GSH play an essential role in protection against oxidative stress [28]. The inhibitory effect of copper on CAT, SOD and GSH may contribute to oxidative modification of the enzymatic proteins and biomembrane lipids by ROS. The ability of curcumin to prevent liver dysfunction and the alterations of the antioxidant parameters may be due to the fact that curcumin possess scavenging free radicals properties and antioxidant activity [29]. Therefore, the protec-

tive effects of curcumin may be related to their ability to chelate/sequester copper via formation of complexes [30]. MDA is an end-product of lipid peroxidation. The activity of MDA of liver and kidney was increased in the CuSO₄ treated group. The data does not prove that the mechanism of copper injury is by lipid peroxidation but it is strongly suggested that it is playing an important role. These results were close to previously reported [31].

The curcumin post- and co-treated groups showed an improvement in antioxidant enzymes (CAT, SOD and GSH) with a decrease in MDA in the liver and the kidney. Curcumin has strong antioxidant potential, acts as oxygen free radicals scavenger [32, 33]. It significantly inhibit the reactive oxygen species (ROS) generation like superoxide anions and hydrogen peroxide by macrophages activation, which plays an important role in the inflammation [33]. This explains that during the necrosis the dead cells were the first one that was able to accumulate the copper. As when the cells died, copper is released into the blood stream to be excreted in the urine and the hepatocytes regenerated with formation of scar tissue. The renewal of hepatocytes perhaps because of the role of certain growth factors or interstitial stem cells with some plasticity which able to generates the hepatocytes [34]. Also bone marrow cells may regenerate new glomerular endothelial cells, showing some plasticity beside the differentiating of the wide range of specialized cells and the promoting angiogenesis in regions of progressive glomerular lesions [35]. Curcumin causes indirect reduction of ROS through a decrease of O₂ and the down regulation of the expression of some NADPH oxidase subunits such as p67phox, p22phox and NOX4, which is useful against inflammation, a common process during kidney injury [29]. This might explain the cytoprotective effects of Curcumin.

In conclusion, the present study showed that the exposure to copper sulphate induce adverse effects and profound alterations on the hepatic and renal functions associated with oxidative stress and diminished activities of antioxidant enzymes. Curcumin exhibited ameliorating effects against CuSO₄-induced hepatotoxicity and nephrotoxicity. An improvement in the biochemical, antioxidant enzymes (CAT, SOD and GSH) and MDA was detected with curcumin treated groups. The curcumin post-treatment was more potent than the curcumin co-treatment. Therefore, the use of curcumin is recommended as a prophylactic measure against CuSO₄-induced hepatotoxicity and nephrotoxicity.

Compliance with Ethical Standards

Conflict of interest The authors declare that there are no conflicts of interest in the current work.

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