Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2015; 7(4); 185-193

ISSN: 0975-5160

Research Article

Amelioration Effect of Red Cabbage Extract on Copper-Induced Hepatotoxicity and Neurotoxicity in Experimental Animals

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Available Online:14th June, 2015

ABSTRACT
Copper is an essential transitional metal, which acts as cofactors in enzyme-catalyzed reactions. It can be toxic to biological systems when it exceeds the levels of cellular needs. Red cabbage has a positive impact on human health where it is rich in minerals, vitamins, oligosaccharides, and a number of bioactive substances. The present study aimed to investigate the protective effect of red cabbage extract on copper induced hepato- and neuro-toxicity in experimental animals. Forty-eight male Sprague–Dawley rats divided into 6 groups (n=8 rats). Intoxicated group was injected intraperitoneal (i.p.) with copper sulphate ($CuSO_4$) (3 mg/kg b.w.) 5 days a week for 2 months. The other groups were orally administered with red cabbage extract (200 mg/Kg b. w.) daily for 2 months alone or with $CuSo_4$ (as pre, post and simultaneous treatment). The activity of serum aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and copper level were demonstrated. Furthermore, hepato- and neuro-antioxidant status such as reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT), lipid peroxidation (LPO), deoxiribonucleic acid (DNA), protein contents and the activity of brain acetylcholinestrase (AChE) were also estimated. The results revealed that copper administration resulted in significant injury in the liver tissue as manifested by increase in serum ALT, AST and ALP activities with increase in copper level and significant injury in the brain tissues as indicated by decrease in AChE activity associated with histopathological changes in both tissues. Moreover, administration of copper resulted in increased the level of LPO, depletion of GSH, as well as decrease in antioxidant enzyme activity of SOD, CAT in both liver and brain. The administration of red cabbage alone did not produce the previous biochemical or histological alteration. Administration of red cabbage to the animals given copper counteracted the development of full-blown liver and brain toxicities. Treatment with the extract reduced substantially both liver and brain injuries and restored the histological and biochemical parameters of hepatotoxicity and neurotoxicity towards normal. The ameliorative effect of pre-treated group is more pronounced than that of both post and simultaneous treated groups. These results suggest that red cabbage extract has ameliorative effect on copper induced hepato- and neuro-toxicity in rats via suppression of oxidative stress, and this extract may have a chelating effect on copper.

Keywords: Red cabbage, Copper, Hepatotoxicity, Neurotoxicity, Oxidative stress, Antioxidants.

INTRODUCTION

Presence of heavy metals, like nickel, cobalt, cadmium, copper, lead, chromium and mercury in air, soil and water can cause bioaccumulation affecting the entire ecosystem and pose harmful health consequences in all life forms[1,2]. Heavy metals have been used in a wide variety of human activities that have significantly increased both professional and environmental exposure. Unfortunately, disasters have highlighted the toxic effects of metals on different organs and systems[3]. Copper is an important biological trace element which is necessary for different metabolic functions and enzyme activities such as cytochrome C oxidase, lysyl oxidase, dopamine ß-hydroxylase, superoxide dismutase, tyrosinase, ascorbic acid oxidase, and ceruloplasmin, and is essential for the utilization of iron[4,5]. Copper at low concentrations is an essential element for organisms but becomes toxic at high concentrations both for plants and animals where, it can induce free radical production such as superoxide, hydrogen peroxide and the hydroxyl radicals resulting in cellular damages as DNA damage, lipid peroxidation, protein modification and other effects, all symptomatic for numerous diseases, involving cancer, cardiovascular disease, diabetes, atherosclerosis, neurological disorders. In particular, adequate copper nutrition is critical during pregnancy and lactation for normal infant development[6]. Thus, tight regulation of copper homeostasis, maintained by mechanisms involving uptake, transport, storage, and excretion is required[7].

Copper regulation is controlled mainly by the liver, where

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Table 1: The effect of red cabbage extract on serum liver function and copper content in all tested groups.

Group	AST (U/L)	ALT (U/L)	ALP (U/L)	Copper (µg/ dL)
Control	40.13±0.67	38.50±0.42	44.88±1.52	75.33±2.94
Red cabbage treated	41.88±0.67	38.25±0.59	46.25±1.26	89.25±2.82
$CuSO_4$ intoxicated	98.38±1.51[a]	78.13±1.41[a]	143.13±5.02[a]	250.25±15.36[a]
Red cabbage pre-treated	55.88±1.32[a,b]	42.38±0.73[a,b]	56.38±1.42[a,b]	121.23±4.55[a,b]
Red cabbage post-treated	73.75±1.22[a,b]	52.38±0.82[a,b]	75.40±1.58[a.b]	193.91±5.10[a,b]
Simultaneously treated	73.13±1.90[a,b]	52.50±0.82[a,b]	71.75±1.25[a.b]	163.15±4.71[a,b]

Values are expressed as mean ± SE (n=8), a: Significantly different from normal control group at $p < 0.05$. b: Significantly different from copper-intoxicated group at $p < 0.05$.

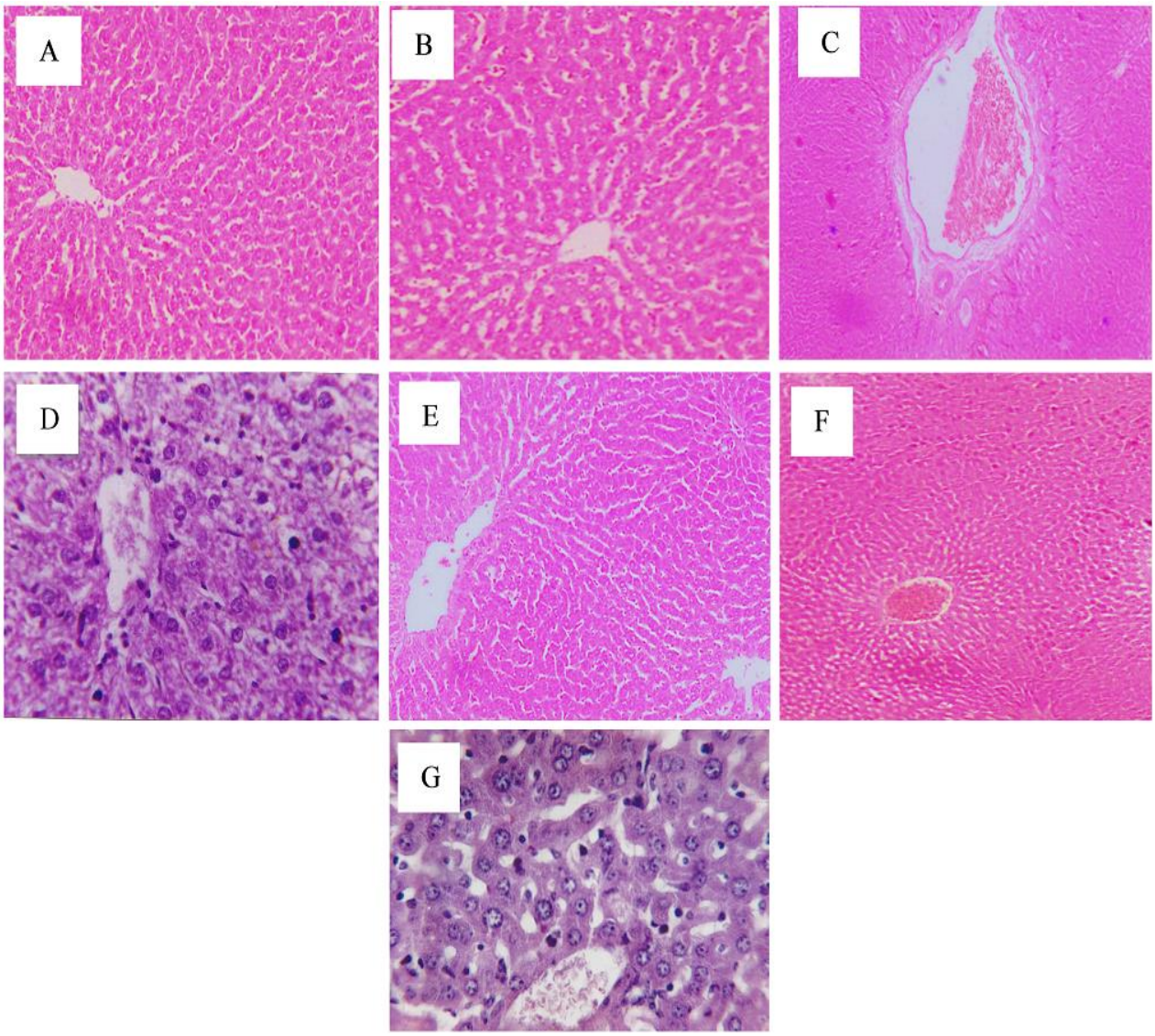

Figure 1: (A): Photomicrograph of control liver rats showing normal cellular architecture, (B): Photomicrograph of rats treated with the extract alone showing normal hepatic lobules with spherical nuclei, well defined cell membrane, normal central vein and mitotic activity, (C, D): Sections of liver rat intoxicated with copper alone showing damage of the hepatic cells. The central vein appears congested with blood cells, the sinusoidal lumens surrounding by Kupffer cells, inflammatory cells appears fibrosis around portal tract and hemorrhage in hepatic artery, (E): Section of rat liver pre-treated with the extract showing normal architectural hepatic cords, dilated blood vessels with minimal infiltration, (F): Section of rat liver simultaneously treated with the extract showing cellular infiltration and congested portal vein, (G): Section of rat liver post-treated with the extract showing congested and dilated blood vessels, pyknosis in the hepatocytes nuclei, vacuolated hepatocytes (H&E, 400x).

it can be mobilized into the circulation or excreted via the bile[8]. In chronic copper poisoning, copper is gradually deposited in the liver without producing any significant sign. When the hepatic copper storage capacity is exceeded, it may result in hepatocellular necrosis and consequently the liberation of copper from the liver into the blood stream produces hemolysis, jaundice, and renal insufficiency[9]. Despite the highest concentration of copper being in liver, it is also found in the nervous system reaching concentrations around 70 μM in the cerebrospinal fluid and 200 μM in the synaptic cleft[10]. Wilson's disease is characterized by copper toxicity that typically affects the hepatic and nervous systems severely. This autosomal recessive disorder is caused by mutations in the *ATP7B* gene found on chromosome 13[11]. Chelating agents and zinc are effective treatments for copper toxicity, via formation of complexes, particularly with glutathione and other small molecules to be excreted, but are inefficient in most patients with fulminant hepatic failure[12].

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. Several developing countries as well as developed countries are using natural herbal medicines. Due to economic conditions and availability, plants are the main source of phyto-constituents which treat infectious diseases in some developing countries[13]. Red cabbage (*Brassica oleracea* L.; family Brassicaseae) is a commonly used dietary supplement that is rich in anthocyanins (ATHs) such as cyanidin-3- diglucoside-5-glucoside derivatives with various acetylated groups connected to the diglucoside, mostly sinapoyl esters[14]. In addition, the presence of natural antioxidants such as ascorbic acid, α-tocopherol, *β*-carotene, lutein etc. has been reported in the red cabbage extract[15,16]. Anthocyanins are glycosylated polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium (flavylium) salts accounting for the colors in several fruits, vegetables and flowers[17]. They are water soluble, which facilitates their incorporation into aqueous food systems. ATHs have been reported to be beneficial to health as potent antioxidants and to improve visual acuity[18]. Their activities ranged from inhibition of DNA damage in cancer cells *in vitro*[19], induction of insulin production in isolated pancreatic cells[20], reduction in inflammatory responses[21] to protection against age-related decline in brain function[22]. The red cabbage is excellent sources of fibers. Insoluble fiber helps to prevent constipation and reduce colorectal cancer risk. Soluble fiber helps to reduce blood cholesterol and blood sugar, thereby reducing the risk of heart disease and diabetes[23,24].

The present study aimed to investigate the antioxidant effect of red cabbage extract on hepatotoxicity and neurotoxicity induced by copper in experimental animals with investigation the probable role of this extract as chelating agent to get ride the excess copper from the body.

MATERIALS AND METHODS

Chemicals

$CuSO_4$ was purchased from ICN pharmaceutical company (USA). All other chemicals and solvents used in this study were of the highest purity and analytical grade, and

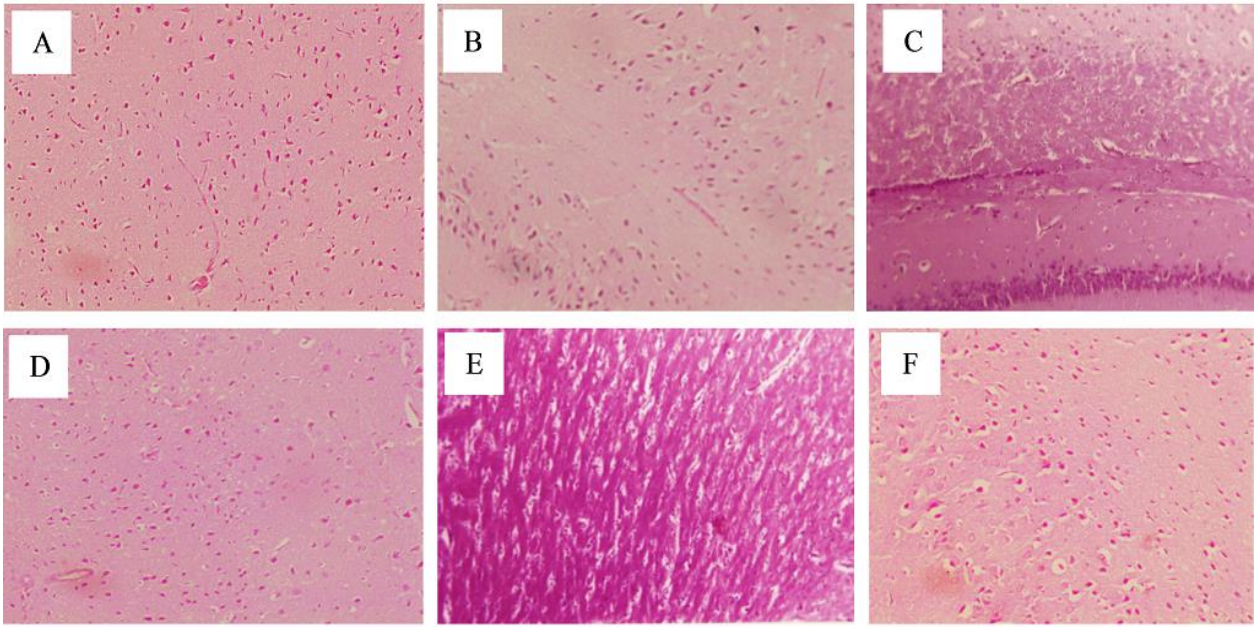

Figure 2: (A): Control rat brain showing normal histological architecture and normal nerve cells, (B): Section of brain treated with the extract alone showing no significant histological alteration in the architectural structure compared with normal control, (C): Section of brain rat treated with toxic alone showing ruptured nerve cell membranes, vacuolated cytoplasm, necrotic and pyknotic nuclei and dilated blood capillary, (D): Section of brain pre-treated with the extract showed preserving near normal architecture of the brain tissue, (E): Section of brain rat simultaneously treated with the extract showing regenerated nerve cell and blood capillary, (F) Section of rat brain post treated with the extract showing the neuroprotective activity as a significant recovery of neuronal damage, and decreased necrosis comparing to copper treated group (H&E, 200x).

Table 2: The effect of red cabbage extract on antioxidant status and lipid peroxidation in liver tissues.

Group	GSH (µg/g tissue)	SOD (µg/g tissue)	CAT (µmol/min/g tissue)	TBARS (µmol/g tissue)
Control	126.88±2.70	196.75±8.75	118.75±2.60	54.4±1.80
Red cabbage treated	127.5±4.51	286.13±10.40	117.13±4.90	64.92±2.59
$CuSO_4$ intoxicated	39.63±2.30 [a]	145.38±6.19 [a]	35.81±2.85 [a]	190.13±1.14[a]
Red cabbage pre-treated	98.63±2.42[a,b]	261.38±12.20[a,b]	94.29±1.66[a]	72.25±1.16[a,b]
Red cabbage post-treated	72.04±2.13[a,b]	184.5±2.49[a,b]	64.49±1.67[a,b]	115.38±4.35[a,b]
Simultaneously treated	81.93±2.13[a,b]	180.5±1.57[a,b]	57.95±2.23[a,b]	105.63±4.18[a,b]

Values are expressed as mean ± SE (n=8), a: Significantly different from normal control group at $p < 0.05$. b: Significantly different from copper-intoxicated group at $p < 0.05$.

purchased from Sigma-Aldrich chemic (Deisenhofer, Germany).

Preparation of red cabbage extract

Red cabbage was obtained from local market, the outer leaves were removed and the inner ones were cut into small pieces and dried in an oven at 50 ° C. For each 100 g of dried leaves, 200 ml of acidified ethanol (50% v/v ethanol, 1% acetic acid) was added, with constant stirring by a magnetic stirrer for 16 hrs. The mixture was filtered followed by removal of the solvent on the rotator evaporator and concentrated using freeze dryer (floor model LY-5-FM Snijders, Holland) to give a dark-brown extract[25].

Determination of total anthocyanins in red cabbage extract

The total content of anthocyanins in the red cabbage was measured spectrophotometrically using molar extinction coefficient of cyaniding-3,5-diglucoside (26900 L/cm mol) according to Chandrasekhar *et al.*,[25]. Anthocyanin content was calculated to be 177 mg/100g extract.

Animals

Forty-eight male Sprague Dawley rats weighing 80-100 gm, obtained from animal house of National Research Centre, Giza, Egypt. Rats were housed in stainless steel cages at a constant temperature 25±2 ˚C with alternating 12 hours light and dark cycles and allowed water and food (laboratory chow) *ad libitum*. The rats were acclimatized to laboratory condition for 10 days before commencement of the experiment. Animal procedures were performed in accordance with Guidelines for Ethical Conduct in the Care and Use of Animals at the National Research Centre, Egypt.

Treatment schedule

Rats were divided into 6 groups with 8 rats in each group. *Group1*: served as control group, rats fed a plain chow diet for 2 months. *Group2:* served as red cabbage treated group, rats were orally received the extract by gavage (200 mg/Kg b.w., daily) for 2 months. *Group3*: served as toxic group, rats were injected intraperitoneal (i.p.) with $CuSO_4$ (3 mg/Kg b.w.) 5 days a week for 2 months. *Group 4:* served as pre-treated group, rats were orally received red cabbage extract (200 mg/Kg b.w. daily) start one week before (i.p.) injection with $CuSO_4$. (3 mg/Kg b.w.) for 2 months. *Group 5:* served as post treated group, rats were injected with $CuSO_4$ (3 mg/Kg b.w.) start one week before oral treatment with the extract (200 mg/Kg b.w., for 2 month.). *Group 6*: served as simultaneously treated group, rats received both $CuSO_4$ (3 mg/Kg b.w.) and the extract (200 mg/Kg b.w.) at the same time for 2 months.

At the end of the treatment protocol, food was withheld for 16-18 hr., animals were anesthetized with ether and blood samples were drawn from the retro-orbital plexus of the individuals of all groups. Samples were left to clot then centrifuged at 3000 rpm for 15 minutes to separate the sera. The serum was used for the assay of aspartate and alanin-aminotransferase (AST and ALT), alkaline phosphatase (ALP) activities spectrophotometrically according to the manufacturer's instructions, using reagent kits obtained from Biomerieux (France). The concentration of copper was assayed in serum according to the manufacturer's instructions, using reagent kit obtained from Randox, CU 2340 (Randox laboratories Ltd., Ardmore, Antrim, UK, BT29, 4QY).

After blood collection, all animals were sacrificed by decapitation and the liver and brain of each animal was rapidly excised, weighed, washed with saline and portion of each liver and brain was preserved in 10% formalin in saline and subjected to histopathological examination. The remaining part of each liver and brain divided into 2 parts. The first part was immediately homogenized in ice-cold 10% sucrose buffer. The homogenate was centrifuged at 1700 rpm at 4 ºC and the resulting supernatant was stored at -70 ºC for biochemical analysis. The second part was immediately homogenized in ice-cold 0.9 % saline for DNA assay.

Histopathological investigation

Immediately after sacrifice, Small pieces of liver and brain tissues were fixed in 10% formalin solution, dehydrated with 90% ethanol, embedded in paraffin, cut into thin sliced section (5 µm thickness), and stained with haematoxylin-eosin dye and then observed under microscope[26].

Biochemical analysis

Liver and brain tissue homogenates were used for the estimation of the content of reduced glutathione GSH according to the method of Beutler *et al.*[27], superoxide dismutase (SOD) and catalase (CAT) activities were determined according to Marklund and Marklund[28], and Sinha[29] respectively. The extent of lipid peroxidation was assayed by the measurement of thiobarbituric acid reactive substances (TBARS) according to Conrad *et al.*[30]. DNA was extracted from liver and brain tissues and assayed

Table 3: The effect of red cabbage extract on antioxidant status and lipid peroxidation in brain tissues.

Group	GSH (µg/g tissue)	SOD (µg/g tissue)	CAT (µmol/min/g tissue)	TBARS (µmol/g tissue)
Control	180.63±7.40	202±2.90	78.16±1.04	50.38±0.08
Red cabbage treated	192.38±2.44	193.25±5.75	75.7±0.60	49.48±0.92
$CuSO_4$ intoxicated	50.68±0.90 [a]	49.79±0.91 [a]	24.1±0.85 [a]	117.75±3.06 [a]
Red cabbage pre-treated	142.88±6.9[a,b]	143.7±7.70[a,b]	60±1.31[a,b]	60.46±1.34[a,b]
Red cabbage post-treated	93.25±2.28[a,b]	89.1±2.82[a,b]	39.9±1.17[a,b]	89.1±2.65[a,b]
Simultaneously treated	96.75±3.23[a,b]	86.93±2.68[a,b]	38±0.80[a,b]	81.68±2.23[a,b]

Values are expressed as mean ± SE (n=8), a: Significantly different from normal control group at $p < 0.05$. b: Significantly different from copper-intoxicated group at $p < 0.05$.

Table 4: The effect of red cabbage extract on DNA and protein in liver tissues.

Group	Protein (mg/g tissue)	DNA (µg/g tissue)
Control	9.73±0.19	183.60±18.10
Red cabbage treated	9.29±0.14	157.61±11.74
Cu SO_4 intoxicated	4.41±0.17 [a]	58.80±2.7[a]
Red cabbage pre-treated	8.04±0.22[a,b]	115.60±2.9[a,b]
Red cabbage post treated	5.64±0.13[a,b]	84.92±3.21[a,b]
Simultaneously treated	5.30±0.15[a,b]	85.10±2.25[a,b]

Values are expressed as mean ± SE (n=8), a: Significantly different from normal control group at $p < 0.05$. b: Significantly different from copper-intoxicated group at $p < 0.05$.

Table 5: The effect of red cabbage extract on DNA, protein and AChE in brain tissues

Group	Protein (mg/g tissue)	DNA (µg/g tissue)	AChE ng/g tissue
Control	6.07±0.11	186.88±13	27.23±2.7
Red cabbage treated	5.91±0.16	167.53±7.44	26.00±1.5
$CuSO_4$ treated	1.07±0.12 [a]	58.00±2.84[a]	19.60±2.13[a]
Red cabbage pre- treated	3.98±0.13[a,b]	113.6±3.68[a,b]	25.00±1.5[b]
Red cabbage post treated	3.10±0.19[a,b]	84.10±3.13[a,b]	22.00±1.9[a,b]
Simultaneously treated	2.43±0.22[a,b]	89.51±5.16[a,b]	25.55±1.6[a,b]

Values are expressed as mean ± SE (n=8), a: Significantly different from normal control group at $p < 0.05$. b: Significantly different from copper-intoxicated group at $p < 0.05$.

according to the method described by Zhou *et al.*[31]. The total protein content in liver and brain was assayed according to Lowry *et al.*[32]. Acetylcholinesterase (AChE) was determined in brain tissue using reagent kit obtained from WKEA Med Supplies Corp (China) according to the manufacturer's instructions.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). Results of biochemical studies were statistically analyzed using one-way analysis of variance (ANOVA). In case of the significant F-ratio, *Posthoc Bonferroni's* test for multiple comparisons was done. All statistics were done using SPSS (Version 17) for Windows (Chicago, IL, USA). Differences were considered significant at a *P* value less than 0.05.

RESULTS

Biochemical analysis

Liver enzymes represent the activities of AST, ALT and ALP in the serum of control and all experimental groups were shown in Table (1). The results showed that the intoxicated $CuSO_4$ group induced a significant increase in the activities of these enzymes compared to those values of control group. While there was a significant decrease in the activity of liver enzymes in all treated groups compared to copper intoxicated group. The ameliorative effect of pre-treatment is more effective than that of both post and simultaneous treatment. There was no significant change in the activity of these enzymes in red cabbage treated group compared to control group. Serum copper content (Table 1) was significantly increased in $CuSO_4$ intoxicated group as compared to control group. While the content of serum copper was significantly decreased in all treated groups as compared to $CuSO_4$ intoxicated group. The ameliorative effect of pre-treated group is more effective than that of both post and simultaneous treated group. There was no significant change in the level of copper in in red cabbage treated group compared to control group.

Hepatic histopathological features of control and experimental groups of rats were illustrated in (Fig. 1A-G). In this study, liver sections of the control rat showed the normal hepatocytes architecture and the central vein. Damage to hepatic structure integrity induced by $CuSO_4$ (3 mg/Kg b.w.) is further supported by our histopathological examination, where the central vein appears congested with blood cells, the sinusoidal lumens surrounding by

Kupffer cells, inflammatory cells appears, fibrosis around portal tract and hemorrhage in hepatic artery. Treatment with red cabbage extract alone (200 mg/Kg b.w.) showed normal hepatocytes architecture. The pre-treatment with red cabbage extract showed normal architecture and dilated blood vessels with minimal infiltration, the simultaneous treatment showed cellular infiltration and congested portal vein, while the post treatment showed congested and dilated blood vessels, pyknosis in the hepatocytes nuclei and vacuolated hepatocytes.

The histopathological features of control and experimental groups of brain tissues were illustrated in (Fig. 2A-F). Brain of a control rat showed normal histological architecture and normal nerve cells. The intoxicated $CuSO_4$ group showed ruptured nerve cell membranes, vacuolated cytoplasm, necrotic and pyknotic nuclei and dilated blood capillary. The treatment with red cabbage extract alone showed no significant histological alteration in the architectural structure compared with normal control. The pre-treatment showed normal architecture, the simultaneous treatment showed regenerated nerve cell and blood capillary, while the post treatment confirmed the neuroprotective activity as a significant recovery of neuronal damage, and decreased necrosis was evident against $CuSO_4$-induced oxidative damage in the brain of the rats, which is similar to their control.

The level of GSH and TBARS as well as the activities of both SOD and CAT were measured as an index of antioxidant status of liver and brain tissues. Table (2) and (3) showed that the injection of $CuSO_4$ decreased the level of GSH and the activities of SOD and CAT and increased the level of TBARS in liver and brain tissues as compared to control group. While the treatment with red cabbage extract showed a significant increase in level of GSH and the antioxidant enzyme activities with a significant decrease in the level of TBARS in liver and brain tissues as compared to $CuSO_4$ intoxicated group. The ameliorative effect of pre-treated group is more pronounced than that of both post and simultaneous treated groups. There was no significant change in antioxidant enzyme activities or lipid peroxidation of red cabbage treated group compared to control group.

Hepatic and neuro DNA as well as protein content were significantly decreased in $CuSO_4$ intoxicated group as compared to control group (Table 4 & 5). While the treatment with red cabbage extract resulted in a significant improvement in the contents of DNA and protein. The improvement in pre-treated group is more clearly than that of both post and simultaneous treated groups. There was no significant change in DNA and protein content in red cabbage treated group compared to control.

Table (5) showed that the concentration of AChE in the brain tissues was significantly decreased in $CuSO_4$ intoxicated group as compared to control group. The treatment with red cabbage ameliorates the level of AChE when compared with $CuSO_4$ group. There was no significant change in the level of this enzyme in red cabbage treated group when compared to control group.

DISCUSSION

Copper is an integral part of many important enzymes involved in a number of vital biological processes where its over-exposure might produce wide adverse effects in different physiological systems[5,33]. In the last decade, attention has been drawn to the health promoting activity of phenolic compounds to which anthocyanins also belong[34]. Red cabbage is a rich source of anthocyanins possessing strong antioxidant activity. The anthocyanins display marked stability, and can be used as natural food colorants as well as dietary antioxidants, where they have an important role in the prevention of diseases associated with oxidative damage. Therefore, the beneficial proprieties of the red cabbage extract should be widely spread to popularize functional food and detoxifying diet reducing the risk of dangerous diseases resulting from heavy metal pollution of the environment. Moreover, it may become potentially attractive, inexpensive and readily available at a large scale, raw material for pharmaceutical, cosmetic and food industries[35]. Therefore, we tried to investigate the ameliorative effect of red cabbage extract on the hepato- and neuro-toxicity induced by $CuSO_4$ in rats.

In the present study administration of $CuSO_4$ to rats resulted in a significant elevation in AST, ALT and ALP in the serum when compared to control. Similar observations were reported in many experimental investigations on animals exposed to copper[36]. Pal *et al.*[37] explained that ALT and AST activities were significantly increased in serum of copper-intoxicated rats, indicating widespread tissue damage. Ali *et al.*[38] explained that elevated levels of serum enzymes (ALT, AST and ALP) activities are inductive of cellular leakage and loss of functional integrity of cell membrane in liver. On the other hand, treatment with red cabbage extract attenuated the activities of these enzymes indicating an improvement in liver function. The ameliorative effect in pre-treated group was more effective than post and simultaneous treated groups, which indicate the protective effect of red cabbage extract. Treatment with red cabbage extract alone did not affect the activities of these enzymes indicating the safe use of red cabbage extract.

Acetylcholine (ACh), the principal neurotransmitter of the cholinergic neurons, is one of the main neurotransmitters involved in neurodegenerative diseases[39] and is related to cognitive functions involved in the learning and memory process[40]. The synaptic cholinergic transmission depends on the AChE activity, since this enzyme promotes the hydrolysis of the neurotransmitter ACh in choline and acetic acid, resulting in the terminus of the transmission of the nervous impulse in the synapses[41]. AChE is used as a biomarker of the cholinergic function, since its activity is inhibited by different toxic agents, such as pesticides[41] and heavy metals[42]. Our results found that AChE activity was decreased in brain tissue of $CuSo_4$ intoxicated group compared to control group. While the treatment with red cabbage extract ameliorated its activity indicating its protective effect.

In agreement with our results, Pohanka[43] reported that cupric ions were able to inhibit AChE. The AChE inhibition may be due to an action of Cu^{2+} on the catalytic

site of the enzyme by an electrostatic interaction involving specific amino acidic residues in the active site[44]. Another valid explanation could be the metal ions cause conformational changes that result in loss of catalytic activity [45]. Pal *et al*.[37] explained that copper-administered animals exhibited significantly decreased serum AChE activity and impaired neuromuscular coordination and spatial memory compared to control rats. The *in vitro* studies on the inhibition of AChE by different metals and petroleum hydrocarbons indicated that lead, cadmium and copper are the most predominant inhibitor on marine snail[46]. Inhibition of AChE in fish exposed to paraqut and $CuSO_4$ (as pesticides) may serve as an indicator of hazard due to application of these chemicals in the natural environment[47]. Halatek *et al*. [48] showed that the impairment of learning and memory processes in copper-intoxicated animals might be because of the neurotoxic effects of Cu toxicity on AChE activity.

In this study, damage to hepatic structure integrity induced by $CuSO_4$ is further supported by our histopathological examination, where the central vein appears congested with blood cells, the sinusoidal lumens surrounding by Kupffer cells, inflammatory cells appears, fibrosis around portal tract and hemorrhage in hepatic artery. The histopathological features of brain tissues showed that $CuSO_4$ administration induced ruptured nerve cell membranes, vacuolated cytoplasm, necrotic and pyknotic nuclei and dilated blood capillary. In addition, there was a marked decrease in the antioxidant defense system as manifested by the significant increase in lipid peroxidation; reduce the level of GSH and a significant decrease in the activity of antioxidant enzyme activity of SOD and CAT. This can be explain by the fact that, although normally, copper bound to proteins, it may be released and become free to catalyze the formation of highly reactive hydroxyl radicals, which can directly damage lipids, proteins, and nucleic acids[49]. Oxidative damage has been linked to chronic copper-overload and/or exposure to excess copper caused by accidents, occupational hazards, and environmental contamination. Additionally, copper-induced oxidative damage has been implicated in disorders associated with abnormal copper metabolism and neurodegenerative changes[33] (Gaetke and Chow, 2003). SOD, CAT and GSH are important scavengers of superoxide ion and hydrogen peroxide. These enzymes prevent generation of hydroxyl radical and protect the cellular constituents from oxidative damage[50].

On the other hand, treatment with red cabbage extract counteracted the development of full-blown liver and brain toxicities, through restored the biochemical parameters as well as oxidative status of hepatotoxicity and neurotoxicity towards normal with minimal histologic abnormalities, The ameliorative effect of pre-treatment was more effective than post and simultaneous treatment. In addition, the level of TBARS was significantly increased in copper intoxicated group, as compared to control group in both tissues. While the treatment with red cabbage extract attenuated the level of TBARS in treated groups when compared to copper group in both tissues. Posmyk *et al*.[51] mentioned that high Cu^{+2} concentration provoked oxidative stress and enhanced TBARS content in tissues. A lower level of TBARS was correlated with high ATH content, it could be resulted from the fact that PhC, which could be also substrates for different peroxidases, can protect against metal stress. Jagdish *et al.*[15] recorded that the presence of flavonoids was reported in red cabbage species and flavonoids have good therapeutic potential in inflammation and pain. Red cabbage extract has prevented oxidative stress induced in liver and brain of animals because of the presence of isothiocyanates (glucosinolate), vitamins A, B, C and anthocyanins which were found to have the strongest antioxidant power. Anthocyanins in cabbage extract significantly attenuated alterations in the cardiac and hepatic antioxidants and lipid peroxidation, and histopathological changes in cardiac and hepatic tissue[16].

The results also revealed that the contents of DNA and protein were significantly decreased in copper intoxicated group as compared to control group, while the treatment with red cabbage extract improved the levels of protein and DNA. The ameliorative effect in red cabbage pre-treated group was more pronounced than that in post and simultaneous treated groups. This can be due to that free copper causes toxicity through generating reactive oxygen species such as superoxide, hydrogen peroxide, and the hydroxyl radical resulted in damaging proteins, lipids and DNA[52]. Experimental studies confirmed that copper is also capable of inducing DNA strand breaks and oxidation of bases[53]. Otherwise, Sarma and Sharma[54] showed that DNA stabilization resulted from a direct anthocyanins interaction with phosphate backbone. Probably cyanidin and DNA associate to form a complex and such a co-pigmentation protects both DNA and ATH from the damage caused by OH. Similarly, Mas *et al*.[55] confirmed the abilities of different anthocyanins-monoglucosides to stabilize DNA triple-helical complexes. Moreover, anthocyanins being antimutagens are probably able to protect DNA by reacting with electrophilic metabolites or by masking nucleophilic centers of DNA[56].

Chelating agents are commonly used to enhance urinary copper excretion. Chelators compete with binding sites for metals and produce a water-soluble complex with copper, which is then excreted into urine or bile[12]. In the present study copper contents in copper group was significantly increased when compared to control group, while the concentration of copper was significantly decreased in treated groups as compared to copper group. This may be due to the chelation of copper by red cabbage extract-containing sulphur, our finding was in consistent with Lopes *et al*.[57] who explained that red cabbage anthocyanins have a high tendency to chelate heavy metals. In particular, the hydroxyl and carbonyl groups of phenolic compounds can strongly bind copper and iron. This chelating behavior renders phenolic compounds strong candidates in preventing metal-catalyzed free radical formation.

CONCLUSION

According to the results obtained in the present study, red cabbage extract played a protective role against copper toxicity in rat liver and brain via suppression of oxidative

stress, and this extract may have a chelating effect on copper where it contains sulphur which may form a complex with copper to be less toxic and can be excreted via urine or bile. However, further studies are needed to fully elucidate the mechanism of this protection.

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