

Research Article



Hepatoprotective and Antioxidant Activity of Aqueous Extract of *Cyperus rotundus* Rhizome Against Mercuric Chloride Induced Oxidative Stress in Rats

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ABSTRACT

The present study was undertaken, to evaluate the protective effect of Aqueous extract of *Cyperus rotundus* against mercuric chloride induced oxidative stress in experimental rats. Female *Albinos Wistar* rats randomly divided into four groups, were the first was served as a control, whereas the remaining groups respectively treated with: *Cyperus rotundus* (200mg/ kg b.w; by gavage), mercuric chloride (1 mg/kg body weight i.p) and combination of *Cyperus rotundus* and HgCl₂. Change in liver enzyme activities, thiobarbituric acid reactive substances (TBARS) level, antioxidants and reduced glutathione (GSH) contents were determined after 10 days experimental period. Exposure of rats to mercuric chloride caused a significant increase the lipid peroxidation level along with corresponding decrease in the reduced glutathione and various antioxidant enzymes in liver. And increase in serum: glucose and bilirubin levels, APL and transaminases activities. Furthermore, treatment with mercuric chloride caused a marked elevation of liver weight and decreased body weight. Supplementation of Aqueous extract of *Cyperus rotundus* resulted in decreased of lipid peroxidation level and in the serum: glucose and bilirubin levels and AST, ALT and APL activities were decreased along with increase in liver GSH level. The activities of antioxidants enzymes: glutathione peroxidase (GSH-Px) and glutathione -S-transferase (GST) were also concomitantly restored to near normal level by Aqueous extract of *Cyperus rotundus* supplementation to mercuric chloride intoxicated rats. Liver histological studies have confirmed the changes observed in biochemical parameters and proved the beneficial role of Aqueous extract of *Cyperus rotundus*. The results clearly demonstrate that Aqueous extract of *Cyperus rotundus* treatment augments the antioxidants defense mechanism in mercuric chloride induced toxicity and provides evidence that it may have a therapeutic role in free radical mediated diseases.

Keywords: Antioxidant enzymes, *Cyperus rotundus*, Mercury, Oxidative stress, female Rat.

INTRODUCTION

Mercury a highly toxic metal, results in a variety of adverse health effects including neurological, renal, respiratory, immune, dermatologic, reproductive and developmental sequelle¹. Due to wide use of mercury in agriculture, industrial, medical and other fields, its exposure is cannot be avoided. The toxicity the mercury compounds (elemental, inorganic and organic). Inorganic mercury present in the environment is a well- established toxicant to human health².

Exposure to mercury promotes the reactive oxygen species (ROS) formation such as hydrogen peroxides, these ROS enhances the subsequent iron and copper induced production of lipid peroxides and highly reactive hydroxyl radicals^{3,4}. Detrimental effects caused by free radicals occur when there is an imbalance between free radical production and radical scavenging capacity of antioxidant system in favour of former⁵. Mercury induced oxidative stress; make an important contribution to molecular mechanism for liver injury^{6,7}.

Recent evidences also show that mercury causes severe oxidative damage⁸, thus mercury is proved to be a potential oxidant in the category of environmental factors. Therefore, there is a need to provide protection against mercury induced toxicity. It is important to develop an effective drug for mercury to prevent the

mercury induced cellular damages. Historically, plants have been used as folk medicine against various type of disease.

Previous studies have been showed that the herbal origin antioxidants can reduce the oxidative stress induced by mercuric chloride⁹. Therefore, treatments with antioxidant and radical scavengers such as vitamin E, vitamin C and herbal antioxidants were found to decrease the oxidative stress induced mercuric chloride¹⁰. *Cyperus rotundus* extract is a folk medicinal herb containing many polyphenolic compounds, flavonoids and phenolic acids, which may act as antioxidative potential. The phytochemical studies have been showed that the major chemical component of *Cyperus rotundus* is essential oil, terpenoids and esquiterpenes^{11,12}.

Cyperus rotundus is widely used as a traditional folk medicine for treating various diseases such as gastrointestinal disorders, inflammatory and infectious diseases in Middle Eastern countries¹³. So, recently studies were subjected to its pharmacological investigations revealed their antioxidant properties^{14,15}. However, protective effects of *Cyperus rotundus* extract against oxidative stress induced by mercuric chloride in rat was not well defined.

The purpose of this study was to evaluate the protective role of aqueous extract of *Cyperus rotundus* on mercury chloride induced oxidative stress in rats.



MATERIALS AND METHODS

All chemicals used in this work were purchased from sigma chemical company. Laboratory animals, *Albinos Wistar* female rats, were brought from the Algiers Pasteur institute at the age of 4 weeks, with an average live weight of 200g. They were located in a room with an ambient temperature of $21\pm 1^{\circ}\text{C}$ and up to 12h of light daily. The rats were divided into four experimental groups; each consists of six rats. The first group was served as the control. The second group was given aqueous extract of *Cyperus rotundus* at a dose of 200mg/kg body weight, while the third group (HgCl_2) was intraperitoneally given mercuric chloride at a dose of 1 mg/kg body weight. Finally, the fourth group: aqueous extract of *Cyperus rotundus* was given (200mg/kg body weight) 5min before HgCl_2 (1 mg/kg body weight) and continued up to 10 days after mercuric chloride treatment. The treatment of all groups was lasted for 10 consecutive days.

Twenty four hours after the last administration the blood was collected by retro- orbital sinus puncture from each anesthetized rat. After centrifugation at 3000 rpm for 10min, the serum was separated immediately and stored at -20°C until determination of: glucose and bilirubin levels and enzymes (AST, ALT and ALP) activities. Subsequently, rats were decapitated and liver were removed.

Cyperus rotundus rhizome aqueous extract preparation

Rhizome plant was washed well with water, dried at room temperature in the dark and then ground in an electric grinder to obtain a coarse powder. Then 50 g of the plant powder was suspended in 500 mL distilled water and heated under reflux for 30 min. The decoction obtained was centrifuged, filtered, frozen at -20°C and then lyophilized. The yield of the dry product was about 25% w/w, which was stored at -20°C until used.

Tissue preparation

About 500mg of liver was homogenized in 4ml of buffer solution of phosphate buffered saline (w/v: 500mg tissue with 4ml PBS, PH 7.4) homogenates were centrifuged at 10.000xg for 15min at 4°C . The resultant supernatant was used for determination of: reduced GSH, Thiobarbituric acid- reactive substance (TBARS) levels, and the activities of: GSH-PX and GST.

Determination of glucose and bilirubin levels and enzymes

Serum glucose, total bilirubin and direct bilirubin levels and AST, ALT and ALP activities were determined using automatic analyses.

Determination of lipid peroxidation (LPO)

Lipid peroxidation level in the liver was measured by the method of Buege and Aust (1978)¹⁶. 125 μl of supernatant were homogenized by sonication with 50 μl of PBS, 125 μl of 20% TCA + BHT 1% (TCA-BHT) in order to precipitate

proteins, and centrifuged (1000xg, 10min, 4°C), afterwards, 200 μl of supernatant were mixed with 40 μl of HCl (0,6M), and 160 μl of TBA dissolved in tris (120 mM). And the mixture was heated at 80°C for 10min; the absorbance was measured at 530nm. The amount of TBARS was calculated by using a molar extinction coefficient of $1.56\times 10^5 \text{ M/Cm}$.

Determination of reduced glutathione (GSH)

GSH content in liver was measured spectrophotometrically by using Ellman's reagent (DTNB) as a colouring reagent, following the method described by Weick bekeretory (1988)¹⁷.

Determination of glutathione-S-transferase (GST) (EC.5.1.18)

The cytosolic glutathione-S-transferase activity was determined spectrophotometrically at 37°C by method of Habig et al. (1974)¹⁸. The reaction mixture (1ml) contained 0.334ml of 100mM phosphate buffer (PH 6.5), 0.033ml of 30mM CDNB and 0.33ml of reduced Glutathione. After pre-incubating for 2min, the reaction was started by adding 0.01ml of diluted cytosol and the absorbance was followed for 3min at 340 nm. The specific activity of GST is expressed as μmole of GSH-CDNB conjugate formed/ min /mg protein using extinction coefficient of $9.6 \text{ Mm}^{-1} \text{ cm}^{-1}$

Determination of GSH-Px (E.C.1.11.1.9)

Glutathione peroxidase (EC 1.11.1.9) activity was modified from the method of Flohe and Gunzler (1984)¹⁹. for the enzyme reaction, 0.2ml of the supernatant was placed into a tube and mixed with 0.4ml GSH (reduced glutathione, sigma product, analytical grade), and the mixture was put into an ice bath for 30min. Then the mixture was centrifuged for 10min at 3000rpm, 0.48ml of the supernatant was placed into a cuvette, and 2.2ml of 0.32M Na_2HPO_4 and 0.32ml of 1m mol/l 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB, sigma) were added for color development. The absorbance at wavelength 412nm was measured with a UV spectrophotometer after 5min. The enzyme activity was calculated as a decrease in GSH within the reaction time as compared with the non-enzyme reaction.

Protein quantification

Protein was measured by the method of Bradford (1976)²⁰, using bovine serum albumin as the standard.

Histopathological examination

Liver from autopsied animals were excised out and fixed in formalin (10%). five micron thick section were prepared by using microtome and these section were stained with hematoxyline and eosin. For histological alterations these slides were observed under light microscope.



Statistical analysis

The data were subjected to student *t* test for comparison between groups. The values are expressed as mean \pm SEM. Significance level was set at $P < 0.05$, $P < 0.01$, $P < 0.001$.

RESULTS

Effects of treatments on body, absolute and relative liver weights

The variations in body and relative liver weights of animals subjected to different treatments were shown in Table 1. During the course of present investigations, it was observed that the control body weight and aqueous extract of *Cyperus rotundus* treated group have increased progressively, contrary in $HgCl_2$ treated rats, results revealed significant decrease in body weight gain as compared to the control. Besides, a significant increase of $HgCl_2$ treated group in absolute and relative liver weights and in *Cyperus rotundus* treated group as compared to the control.

Effects of treatments on serum biochemical Parameters

Treatment with $HgCl_2$ caused a significant increase in the activities of AST, ALT and ALP as compared to the control. Only *Cyperus rotundus* treatment did not show any significant alteration. However, the combined treatment of *Cyperus rotundus* with mercuric chloride results in gradual recovery in AST, ALT and ALP activities as compared to the control (table 2). The content of serum glucose of the $HgCl_2$ treated group tended to be higher compared to the control. Total bilirubin and direct bilirubin levels in $HgCl_2$ treated animals were increased,

but the co-administration of *Cyperus rotundus* with $HgCl_2$ has produced a recovery in the above mentioned biochemical variables.

Effects of treatments on hepatic oxidative stress parameters

Mercuric chloride exposure a highly significant depleted in reduced glutathione (GSH) level, GPx and GST activities. And a significant increase in liver lipid peroxidation level in mercury intoxicated rats was noticed. *Cyperus rotundus* alone treatment did not show any significant decline. In combined treatment of *Cyperus rotundus* with mercuric chloride a highly significant increase in reduced glutathione (GSH) level, GPx and GST activities. And a significant depletion in lipid peroxidation level was recorded with respect to mercury intoxicated rats (Figs. 1 and 2).

Histological studies

Mercuric chloride induces various pathological alterations in liver of rats. These alterations were characterized by centrilobular necrosis, degranulation, destruction of membrane cells, cytoplasmic vacuolization (Fig. 3C). In combination group where *Cyperus rotundus* was administered with mercuric chloride showed reparative changes. Liver showed prominent recovery in the form of normal hepatocytes and very less centrilobular necrosis. Pronounced sinusoid with granular hepatocyttoplasm was also evident (Fig.3D). Liver of the control group had a regular histological structure with a characteristic pattern of hexagonal lobules (Fig. 3A). Furthermore, no histological alterations were observed in the liver of *Cyperus rotundus* treated group (Fig. 3B).

Table 1: Changes in body and absolute and relative kidney weights of control and rats treated with *Cyperus rotundus* (CR), mercuric chloride, and combined treatment of mercuric chloride with *Cyperus rotundus* after 10 days of treatment.

| Parameters | Treatment groups | | | |
|------------------------------------|------------------|------------------|------------------|------------------|
| | Control | CR | $HgCl_2$ | CR+ $HgCl_2$ |
| Initial body weight (g) | 160 \pm 12 | 164.2 \pm 16 | 174.15 \pm 25 | 168.5 \pm 17 |
| Final body weight (g) | 180.25 \pm 12 | 167 \pm 10 | 154 \pm 6.2 | 175.75 \pm 7.5 |
| Absolute Liver weight (g) | 5.1 \pm 0.3 | 5.4 \pm 0.3 | 7.27 \pm 0.9** | 7.0 \pm 0.4** |
| Relative Liver weight (g/100g b.w) | 0.028 \pm 0.02 | 0.032 \pm 0.03 | 0.047 \pm 0.1 | 0.037 \pm 0.09 |

Table 2: Changes in biochemical parameters of control and rats treated with *Cyperus rotundus* (CR), mercuric chloride, and combined treatment of mercuric chloride with *Cyperus rotundus* after 10 days of treatment.

| Parameters | Experimental groups | | | |
|--------------------------|---------------------|------------------|------------------|-----------------|
| | Control | CR | $HgCl_2$ | CR+ $HgCl_2$ |
| Glucose (mg/dl) | 1.68 \pm 0.28 | 1.39 \pm 0.24 | 2.0 \pm 0.47 | 1.72 \pm 0.17 |
| SGOT(U/L) | 40.8 \pm 0.73 | 41.61 \pm 0.6 | 50.13 \pm 12 | 43.1 \pm 3.2 |
| SGPT(U/L) | 45 \pm 3.4 | 50.12 \pm 0.7 | 73.72 \pm 18* | 47.39 \pm 4.2 |
| ALP(U/L) | 259.34 \pm 42 | 260.6 \pm 27 | 326.19 \pm 51 | 274.8 \pm 17 |
| Total Bilirubin(mg/dl) | 9.42 \pm 0.45 | 11.75 \pm 0.84 | 16.97 \pm 5.2* | 10.12 \pm 1.9 |
| Direct Bilirubin (mg/dl) | 2.7 \pm 0.65 | 0.2 \pm 0.49 | 3.4 \pm 0.99 | 3.07 \pm 0.38 |

Values are given as mean \pm SEM for group of 6 animals each. * $P \leq 0.05$, compared to controls.

** $P \leq 0.01$, compared to controls. *** $P \leq 0.001$, compared to controls.



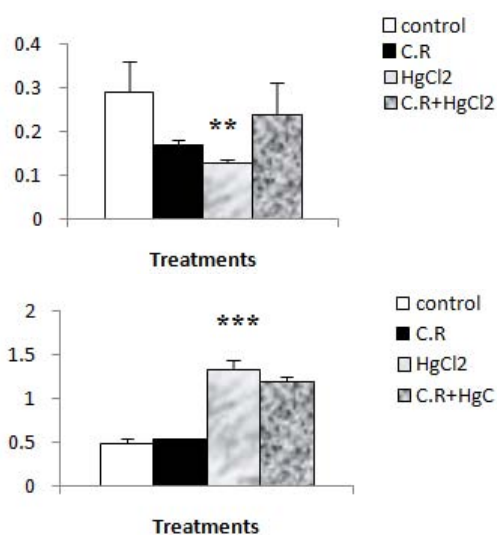


Figure 1: Reduced glutathione (nmol/ mg protein) and TBARS (nmol MDA/mg protein) levels in liver of control and rats treated with *Cyperus rotundus*, mercuric chloride, and combined treatment of *Cyperus rotundus* with mercuric chloride after 10 days of treatment. Values are given as mean \pm SEM for group of 6 animals each significant difference: * compared to controls (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

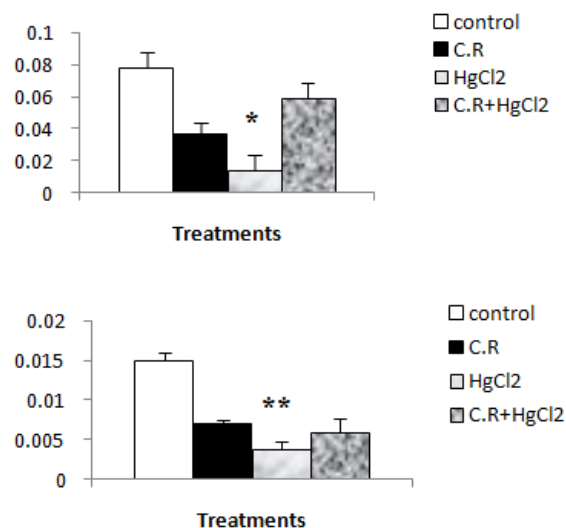


Figure 2: Enzyme activities of GPx ($\mu\text{mol GSH/ mg protein}$) and GST ($\text{nmol /min/mg protein}$) in liver of control and rats treated with *Cyperus rotundus*, mercuric chloride, and combined treatment of *Cyperus rotundus* with mercuric chloride after 10 days of treatment. Values are given as mean \pm SEM for group of 6 animals each significant difference: * compared to controls (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

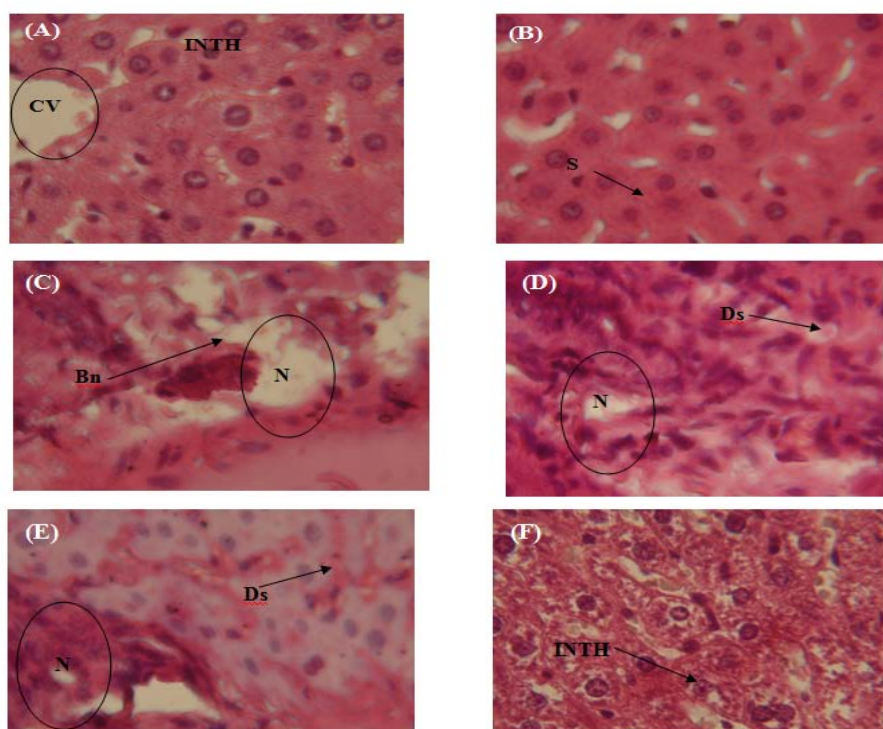


Figure 3: Microscopic evaluation of hepatic tissue from (A) control, (B) treated with *Cyperus rotundus*, (C, D, E) mercuric chloride and (F) *Cyperus rotundus* pre-and post treated with mercuric chloride after 10 days of treatment, section were stained using the hematoxylin-eosin method (400X). Bn: Bright nuclei, Ds: destruction of membrane cells, N: Necrosis, INTH: Intact hyapatocyte cells, CV: central vein, S: Sinusoid. *Cyperus rotundus* co-administrated with mercuric chloride shows granular cytoplasm and normal hepatocytes.

DISCUSSION

The present investigation revealed that mercuric chloride intoxication causes significant increase in lipid peroxidation and glucose, bilirubin levels, AST, ALT and ALP activities, and significant decrease in reduced glutathione, glutathione peroxidase and glutathione-s-transferase in liver.

The principal toxic effects of mercury involve interaction with a large number of cellular processes, including the formation of complexes with free thiols and protein thiol groups, which may lead to oxidative stress²¹. Due to its sulfhydryl group binding capability, HgCl₂ can also inhibit the activities of many enzymes, especially those involved in the cellular glucose uptake, gluconeogenesis, fatty acid;

Oxidation and production of glutathione. GSH, the primary intracellular antioxidant and the conjugating agent, was shown to be depleted and to have impaired function in Hg toxicity. A single Hg ion can bind to and cause irreversible excretion of up to two GSH molecules²². In fact, GSH serves as a primary line of cellular defense against Hg compounds. Released Hg ions form complexes with GSH and cysteine results in greater activity of the free Hg ions, disturbing GSH metabolism and damaging cells²³. As a result of binding of mercury to glutathione, levels of GSH are lowered in the cell and decrease the anti-oxidant potential of the cell. Antioxidant enzymes such as glutathione peroxidase and glutathione S -transferase play a major role in the intracellular defense against oxygen radical damage to aerobic cells. Chung et al., (1982)²⁴ demonstrated that 10mg/kg of mercury caused time dependent decreases in the activities of the enzyme of the glutathione metabolism pathway in the rat kidney. Girardi and Elias (1995)²⁵ reported that mercury inhibits the activities of redox cycles enzymes. Recent finding Necib et al., (2013)²⁶ proved our point that this antioxidant enzymes show decreased level following mercury intoxication. Because of the low activity of antioxidant enzymes in the liver and decreased content of GSH, the liver is hypothesized to be highly susceptible to oxidative stress. Mercury induced oxidative stress turn to severe; the inbuilt mechanism of body fails to alleviate the damage. It has been demonstrated that mercury decreases the anti-oxidative systems and produces oxidative damages via H_2O_2 generation there by leading to lipid peroxidation²⁷. All these possible mechanisms of mercuric chloride toxicity may lead to the formation of reactive oxygen species (ROS), as found in the present investigation. Therefore, an increase in the formation of ROS by mercuric chloride may induce membrane biochemical and functional alterations and thus induced liver cell damage.

Further, mercury intoxication also induces a significant elevation in serum and: bilirubin levels and AST, ALT and ALP activities. This increase may be due to cellular necrosis of hepatocytes, which causes increase in the permeability of cell resulting release of transaminases and ALP in blood stream²⁸. This confirms our earlier reports on histopathological alterations in liver induced by mercury intoxication²⁹.

It was observed that *Cyperus rotundus* extract when given in combination with mercuric chloride significantly increases liver GSH level, GSH-Px and GST activities as antioxidant potential and thereby declines the level of lipid peroxidation, which in turn reduces the transaminases and ALP activities and glucose, total bilirubin and direct bilirubin levels in serum. In present investigation, the elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and directly detoxifies reactive species³⁰. Glutathione, as both a carrier of mercury and an antioxidant, has specific roles in protecting the body from mercury toxicity. Glutathione, specifically bind with

methylmercury, forms a complex that prevents mercury from binding to cellular proteins and causing damage to both enzymes and tissue³¹. Glutathione-mercury complexes also reduce intracellular damage by preventing mercury from entering tissue and cells, and becoming an intracellular toxin. The elevated level of GSH-Px and GST by *Cyperus rotundus* as compared to the $HgCl_2$ may have facilitated the conjugation reaction of xenobiotics metabolism and may have increased the availability of non-critical nucleophile for inactivation of electrophiles and therefore might be playing a major role in metalloprotection. The *C. Rotundus* is a traditional herbal medicine used widely as antibacterial, antimalarial, sedative, antispasmodic, anti-inflammatory and relieve diarrhea^{14,15,32}. Previous studies have been showed that *C. roduntus* and its ingredient compounds inhibit the free radical generation and act as antioxidant and free radical scavengers and its has also been demonstrated that treatment with *C. rotundus* inhibits the generation of superoxide radicals^{33,11,34} and recent evidence suggested that GSH-PX and GST play a significant role in the elimination of H_2O_2 and lipid peroxidative stress in rats³⁵. Thus, inhibition this enzymes may results in the accumulation of the H_2O_2 with subsequent oxidation of lipids. The present study has showed that MDA levels were significantly increased and the GSH-PX, GST activities were decreased with treated both dose of CR in mercuric chloride groups when compared with control groups which conformed with the histopathological evaluation of liver tissue. Flavonoids are the major component in the volatile oil of CR which are able to inhibit the oxidants and to protect the cell membrane by the restoration of lactate dehydrogenase (LDH) when compared treatment with dose of *C. Rotundus*. This study assess the effects of oxygen free radical scavengers, both mercuric chloride injury and increase of TBA-reactive substance were inhibited by the treatment of free radical scavengers. *C.R* extract is an effective free radical scavenger showing antioxidant activity against reactive oxygen production and protecting the damage caused by free radicals³⁶. This result is supported with biochemical and histopathological findings which the effect of CR on mercuric chloride induced oxidative stress in rats. The hepatoprotective effect of CR could be attributed to the improvement of antioxidant status of the animals of the presence of free radical scavenging substances such as flavonoid oil³⁷.

In conclusion, our study indicate that *Cyperus rotundus* extract have hepatoprotective effect against mercuric chloride induced oxidative stress in rats which may be related to its antioxidant effect.

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