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ORIGINAL ARTICLE

The Potency of Red Seaweed (*Eucheuma cottonii*) Extracts as Hepatoprotector on Lead Acetate-induced Hepatotoxicity in Mice

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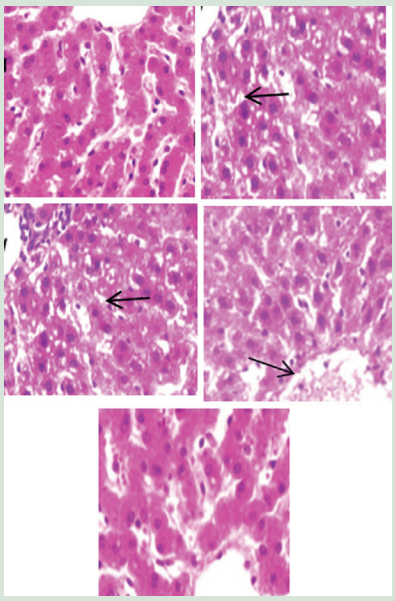
ABSTRACT

Background: Lead is one of the most toxic metals, producing severe organ damage in animals and humans. Oxidative stress is reported to play an important role in lead acetate-induced liver injury. **Aim:** This study was carried out to investigate the role of ethanol extract of *Eucheuma cottonii* in protecting against lead acetate-induced hepatotoxicity in male mice. **Materials and Methods:** The sample used fifty male mice which were divided into five groups: negative control (mice were given daily with Aquadest); positive control (mice were given daily with lead acetate 20 mg/kg body weight (BW) orally once in a day for 21 days); and the treatment group (mice were given *E. cottonii* extracts 200 mg, 400 mg, and 800 mg/kg BW orally once in a day for 25 days, and on the 4th day, were given lead acetate 20 mg/kg BW 1 h after *E. cottonii* extract administration for 21 days). On day 25, the levels of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALP), malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were measured. The data of SGOT, SGPT, ALP, MDA, SOD, and GPx were analyzed with one-way ANOVA, followed by least significant difference test. **Results:** The results showed that oral administration of lead acetate 20 mg/kg BW for 21 days resulted in a significant increase in SGOT, SGPT, ALP, and MDA levels. Moreover, there was a significant decrease in SOD and GPx levels. Treatment with *E. cottonii* extracts of 800 mg/kg BW but not with 200 mg/kg BW and 400 mg/kg BW significantly ($P < 0.05$) decreased the elevated SGPT, SGOT, ALP, and MDA levels as compared to positive control group. Treatment with *E. cottonii* extracts of 800 mg/kg BW also showed a significant increase in SOD and GPx levels as compared to positive control group. Treating mice with lead acetate showed different histopathological changes such as loss of the normal structure of hepatic cells, blood congestion, and fatty degeneration whereas animals treated with lead acetate and *E. cottonii* extracts showed an improvement in these changes and the tissue appeared with normal structures. **Conclusion:** It can be concluded that *E. cottonii* extracts could be a potent natural product and can provide a promising hepatoprotective effect against lead acetate-induced hepatotoxicity in mice.

Key words: Biochemical assays, *Eucheuma cottonii* extracts, hepatoprotector, lead acetate

SUMMARY

- In summary, Oxidative stress reported to play an important role in lead acetate induced liver injury. The lead acetate treatment significantly increased the SGOT, SGPT, ALP, MDA, and decreased the antioxidant enzymes (SOD and GPx) in liver. The inhibition of antioxidant enzymes will increase free radicals in liver tissues and might induce liver injury in mice. The presence of ethanol extract of Eucheuma cottonii with lead acetate showed protective effects as attenuating lead acetate against its liver toxicity, and this may be due to the activity of ethanol extract of Eucheuma cottonii as antioxidant. The antioxidant enzymes (SOD and GPx) were increased, and MDA, SGOT, SGPT, ALP were decreased after ethanol extract of Eucheuma cottonii administration.The enzymatic activities (SOD and GPx) and MDA in mice can be used as biomarkers of heavy metal toxicity such as lead acetate. Histopathological view of liver sections in the lead acetate treated group showed the liver damage, as compared to the negative control group. However, administration of ethanol extract of Eucheuma cottonii significantly improved the histopathological in liver of lead acetate-treated mice. From the results of this study we concluded that the ethanol extract of Eucheuma cottonii could be a potent natural product provide a promising protective effect against lead acetate induced liver toxicity in mice.



Abbreviations Used: SGOT: Serum Glutamic Oxaloacetic Transaminase, SGPT: Serum Glutamic Pyruvate Transaminase, ALP: Alkaline Phosphatase, MDA: Malondialdehyde, SOD: Superoxide Dismutase, GPx: Glutathione Peroxidase.

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INTRODUCTION

The environmental contamination by heavy metals has increased drastically along with the rapid development of modern industry. Among these metals is lead, of which its levels have increased substantially during the past few years. Lead exposure occurs through the respiratory and gastrointestinal systems, and lead which is ingested and absorbed is stored mainly in the liver, kidney, and bone. Elevated lead levels in the

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body have been associated with nephrotoxic, hepatotoxic, neurotoxic, and cardiovascular diseases.[1,2]

In living systems, liver is considered to be highly sensitive to toxic agents. The study of lead acetate in enzymatic activities such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) has been found to be of great value in experimental liver damage.[3] The mechanism behind lead hepatotoxicity is the oxidative stress and it develops when there is an imbalance between the generation of reactive oxygen species (ROS) and the scavenging capacity of antioxidants in the liver.[4,5] A previous study confirmed the possible involvement of ROS or free radicals such as superoxide ion (O_2^-), nitrogen oxide (NO), and hydroxyl radical (OH^-) in lead-induced toxicity.[6,7] Malondialdehyde (MDA), one of the well-known secondary products of lipid peroxidation after exposure to ROS and free radicals, may be used as an indicator of cell membrane injury. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals.[8] The most widely used assay for lipid peroxidation is the MDA formation. The level of MDA, the end product of lipid peroxidation, is measured by the thiobarbituric acid (TBA)-reactive substance method. The concentration of MDA is the direct evidence of toxic processes caused by free radicals.[9]

Antioxidant activity or inhibition of generation of free radicals plays a crucial role in providing protection against such hepatic damage. Vitamins are ideal antioxidants to increase tissue protection from oxidative stress due to their easy, effective, and safe dietary administration in a large range of concentrations.[10] Antioxidants such as Vitamin E and Vitamin C were found to improve hepatic conditions significantly when treated in animals with lead acetate-induced damage. The antioxidant activity of Vitamin E is targeted primarily toward the lipid component of cells. Antioxidants such as Vitamin E and Vitamin C have been shown to inhibit free radical formation and are effective in minimizing lipid peroxidation in several different biological systems. Vitamin E and Vitamin C are natural antioxidants which prevent the increased production of free radicals induced by oxidative damage to lipids and lipoproteins in various cellular compartments and tissues.[11]

Recently, there has been an increased interest in the therapeutic potential of marine products or medicinal plants having antioxidant properties in reducing free radical-induced tissue injury. Marine plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects.[12,13]

In particular, seaweeds are a very important and commercially valuable resource for the food industry and are used in traditional medicine.[14] The abundantly cultivated edible red seaweed, *Eucheuma cottonii*, grows very rapidly in pristine water in Southeast Asia and can be harvested every 45 days for human use. It contains high amounts of dietary fibers, minerals, vitamins, antioxidants, polyphenols, phytochemicals, proteins, and polyunsaturated fatty acids and has medicinal uses.[15] *E. cottonii* is one of the main seaweeds species cultivated in Tamiang Gulf of South Kalimantan. Previous studies showed that *E. cottonii* has the best antihyperlipidemic and *in vivo* antioxidant activities, which significantly reduce body weight (BW) gain, elevated erythrocyte GSH-Px, and reduced plasma lipid peroxidation of high-fat diet rats toward the values of normal rats.[16] The polyphenol-rich *E. cottonii* has tumor-suppressive activity through apoptosis induction, downregulating the endogenous estrogen biosynthesis, and improving antioxidative status in the rats.[17]

The present study is intended to investigate hepatoprotective activity of ethanolic extract of *E. cottonii* against lead acetate-induced liver damage in mice.

MATERIALS AND METHODS

Chemicals

Lead acetate was purchased from Sigma-Aldrich Chemicals (USA). All the other chemicals and solvents used in this study were of highest purity and analytical grade and were purchased from Sigma-Aldrich Chemicals (USA). Reagent kits for assay of SGPT, SGOT, and ALP were obtained from Kristalindo (Indonesia). Reagent kits for the determination of MDA, glutathione peroxidase (GPx), and superoxide dismutase (SOD) were purchased from Kristalindo (Indonesia).

Experimental animals

Male Swiss albino mice (Mus musculus L), weighing approximately 25–30 g (2.5–3 months), were obtained from Veteriner Farma, Surabaya, Indonesia, for experimental purpose. They were housed in plastic cages in an air-conditioned room with temperature maintained at 25°C ± 3°C, relative humidity of 50% ± 5%, and 12 h alternating light and dark cycles. The mice were provided *ad libitum* with tap water and fed with standard commercial mice chow. The Animal Ethics Committee of Airlangga University, Surabaya, Indonesia, has approved experimental protocol.

Preparation of ethanol extract of *Eucheuma cottonii*

Plant material and extract preparation of *E. cottonii* were collected from Surabaya, Indonesia. *E. cottonii* plant materials were cleaned with running tap water and chopped into pieces. They were dried under shade at ambient temperature for 5 days, and the air-dried *E. cottonii* were then ground to powder for extraction. The powdered *E. cottonii* (1 kg) was macerated with ethanol (5 L) for a week at 37°C. The supernatant was then collected and filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The filtrate was concentrated by evaporation with a vacuum rotary evaporator at 45°C. The extract was dried at reduced pressure, stored at 0–4°C, and used for the experimentation.

Experimental design

The fifty male mice (Mus musculus L) were divided randomly into five groups as follows: negative control group (mice were given daily with Aquadest); positive control group (mice were given daily with Aquadest and lead 20 mg/kg BW orally once in a day for 21 days); and the treatment group (mice were given *E. cottonii* extracts with ethanol 200 mg, 400 mg, and 800 mg/kg BW orally once in a day for 25 days and lead acetate 20 mg/kg BW was given on 4th day, 1 h after the *E. cottonii* extract administration for 21 days). On day 25, blood samples were obtained by cardiac puncture into chilled tubes and centrifuged at 3000 rpm for 20 min, and then sera were stored at −85°C until assay.

Biochemical assays

Serum biochemical marker activities of SGPT, SGOT, and ALP were assayed spectrophotometrically according to the standard procedures using commercially available diagnostic kits.

The level of serum MDA was determined spectrophotometrically with TBA solution. In brief, to 150 µl of serum sample, the following were added: 1 ml trichloroacetic acid (TCA) 17.5%, 1 ml of 0.66% TBA, mixed well by vortex, incubated it in boiling water for 15 min, and then allowed to cool. Then, 1 ml of 70% TCA was added, and the mixture was let to stand at room temperature for 20 min, centrifuged at 2000 rpm for 15 min, and the supernatant for taken out for scanning spectrophotometrically.

Portions of liver were immediately washed in ice-cold physiological saline and homogenized in 50 mM potassium phosphate (pH 7.4)

to render 10% homogenate. The homogenate was centrifuged at 4000 rpm for 15 min at 4°C. The supernatant was used for SOD and GPx analyses.

Histopathological study

On day 25, all animals from every group were sacrificed and the liver was separated by dissection procedure. Pieces of liver obtained from each group were immediately fixed in 10% formalin solution. The formalin-fixed kidneys were embedded in paraffin, and serial sections were made and stained with hematoxylin and eosin. The stained sections were examined under a light microscope.

Statistical analysis

Data were presented as means ± standard errors. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with least significant difference test using a statistical package program (SPSS version 17.0).

RESULTS

Effects of *Eucheuma cottonii* extract on lead acetate-induced changes in the serum hepatic enzymes

An increase in the serum hepatic marker enzymes (SGOT, SGPT, and ALP) indicates liver damage. Analysis of these hepatic marker enzymes has been done to evaluate the hepatoprotective effect of ethanol extract of *E. cottonii* in lead acetate-treated mice. Positive control (lead acetate-treated mice) showed a statistically significant ($P < 0.05$) increase in serum hepatic enzymes (SGOT, SGPT, and ALP) compared with the negative control. In contrast, the groups pretreated with ethanol extract of *E. cottonii* (800 mg/kg BW but not 200 mg/kg BW and 400 mg/kg BW) showed statistically significantly ($P < 0.05$) decreased enzyme (SGOT, SGPT, and ALP) levels in a dose-dependent manner with respect to the positive control toward normalization and close to the negative control group [Table 1].

Effects of *Eucheuma cottonii* extract on lead acetate-induced changes in antioxidant and malondialdehyde

Lead acetate enhances the intracellular formation of ROS causing hepatic damage. In the present study, we analyzed the hepatic levels of several antioxidants (SOD and GPx) and MDA. Positive control (lead acetate-treated mice) showed statistically significant ($P < 0.05$) decrease in the level of SOD and GPx compared with negative control group; meanwhile, a statistically significant ($P < 0.05$) increase in MDA level was detected. Groups pretreated with ethanol extract of *E. cottonii* (800 mg/kg BW) showed a statistically significant ($P < 0.05$) increase in the level of SOD and GPx with statistically significant ($P < 0.05$) decrease in MDA level compared with lead acetate-treated mice toward the normal level and close to the negative control [Table 2].

Effects of *Eucheuma cottonii* extract on lead acetate-induced liver damage

Histological observations in the negative control show that liver tissues were normal. In the positive control, mice administered only with lead acetate showed loss of the normal structure of hepatic cells, blood congestion, and fatty degeneration. Lead acetate thus had deleterious effect on liver tissues. The mice treated with *E. cottonii* extract 800 mg/kg BW but not with 200 mg/kg BW and 400 mg/kg BW showed an improvement in these changes and the tissue appeared with normal structures. Observations indicate that the testicular toxic effects of lead acetate were reduced by *E. cottonii* extract [Figure 1].

Table 1: Effects of *Eucheuma cottonii* extract on lead acetate-induced changes in the serum hepatic marker enzymes

Groups	Means±SD		
	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
Negative control	61.20^{a}±4.23	25.68^{a}±2.83	161.28^{a}±8.62
Positive control	87.20^{b}±6.01	41.36^{b}±3.24	221.52^{b}±9.32
E. cottonii (mg/kg BW)			
200	85.15^{b}±5.63	42.52^{b}±3.21	212.73^{b}±8.67
400	80.31^{b}±4.81	39.20^{b}±2.95	207.54^{b}±9.48
800	73.43^{c}±4.62	33.70^{c}±3.19	185.82^{c}±8.51

Superscripts within each column indicate significant difference between the means ($P<0.05$). *E. cottonii*: *Eucheuma cottonii*; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvate transaminase; ALP: Alkaline phosphatase; SD: Standard deviation; BW: Body weight

Table 2: Effects of *Eucheuma cottonii* extract on lead acetate-induced changes in antioxidants, malondialdehyde, superoxide dismutase, and glutathione peroxidase

Groups	Means±SD		
	MDA (nmol/mL)	SOD (U/mg)	GPx (U/mg)
Negative control	5.79^{a}±0.86	9.35^{a}±1.17	45.38^{a}±4.67
Positive control	9.24^{b}±1.57	4.81^{b}±0.92	33.43^{b}±3.42
E. cottonii (mg/kg BW)			
200	8.38^{b}±0.92	4.27^{b}±0.84	31.27^{b}±5.63
400	7.93^{b}±0.89	5.92^{b}±1.01	34.52^{b}±4.86
800	7.13^{c}±0.62	7.02^{c}±0.83	39.27^{c}±3.41

Superscripts within each column indicate significant difference between the means ($P<0.05$). GPx: Glutathione peroxidase; MDA: Malondialdehyde; SOD: Superoxide dismutase; SD: Standard deviation; *E. cottonii*: *Eucheuma cottonii*; BW: Body weight

DISCUSSION

Lead is one of the most toxic metals, producing severe organ damage in animals and humans. Studies have shown that the liver is one of the primary targets in lead-associated toxicity.[2] Lead produces oxidative damage in the liver by enhancing lipid peroxidation, causes liver dysfunction, and increases free radical damage.[18,19] Antioxidant enzymatic levels are applied as markers of oxidative stress. Based on the present study, it can be revealed that lead-induced toxicity might result in decreased tissue activities of enzymatic antioxidants such as SOD and GPx. The decrease of SOD and GPx activities might predispose the examined tissue of mice to oxidative stress, because these enzymes catalyze the decomposition of ROS.[6,20] The levels of these antioxidants might provide a clear indication on the extent of cytotoxic damage that occurs in hepatic tissue. Therefore, some authors have postulated that antioxidants should be one of the important components of an effective treatment of lead poisoning.[4,5] *E. cottonii* is an edible species of Pacific red seaweeds obtained from water resources of Kalimantan, Indonesia, which is a potential source of a variety of compounds such as dietary fibers, Vitamin C, α-tocopherol, minerals, fatty acids, and protein.[15] *E. cottonii* is a rich source of antioxidants (Matanjun, 2010), which can significantly prevent tissue damage by stimulating the wound healing process and also possess anti-inflammatory activity.

Liver injury following lead exposure is well characterized by elevated levels of plasma hepatic marker enzymes which indicate cellular leakage and loss of functional integrity of hepatic membrane architecture. The serum enzyme markers such as SGOT, SGPT, and ALP are recommended for the assessment of hepatocellular injury in preclinical studies as it is

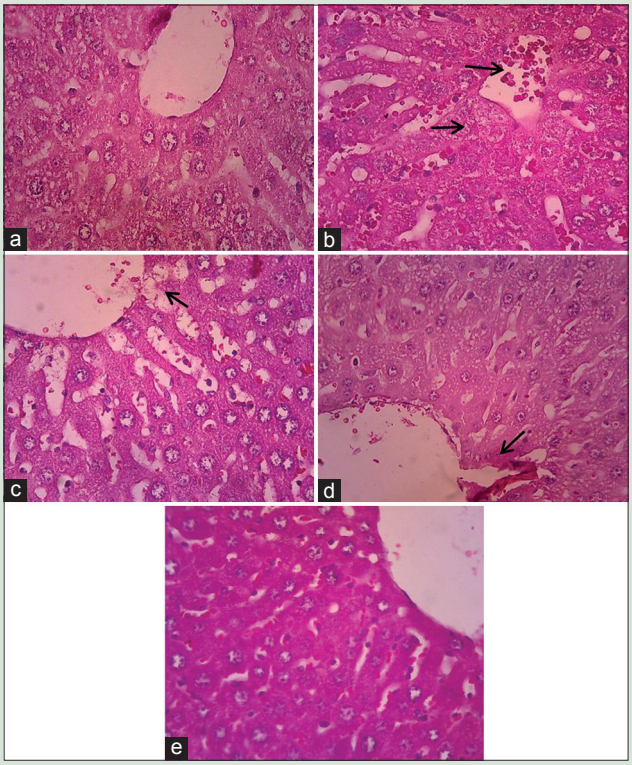

Figure 1: Histological study of liver tissue in control and experimental groups of rats. Normal morphological view of liver sections in negative control (a). Histopathological view of liver sections in lead acetate-treated group (b), treated with *Eucheuma cottonii* extract of 200 mg/kg body weight (c) and 400 mg/kg body weight (d) shows loss of the normal structure of hepatic cells, blood congestion, and fatty degeneration (indicated by arrows) as compared to negative control. Mice treated with *Eucheuma cottonii* extract 800 mg/kg body weight show an improvement in these changes and the tissue appears with normal structures (e)

considered a more specific and sensitive indicator of liver damage. Low levels of SGOT, SGPT, and ALP are normally found in the blood, but when the liver is damaged or diseased, it releases SGOT, SGPT, and ALP into the bloodstream, which makes a rise in SGOT, SGPT, and ALP levels. Most increases in SGOT, SGPT, and ALP levels are caused by liver damage.[21,22]

The current work revealed an increase in the levels of SGOT, SGPT, and ALP in lead acetate-treated mice in comparison to the negative control and this may be due to the degeneration of hepatocytes by necrosis which causes leakage of these enzymes into blood circulation. Similar observation was reported by Moussa and Bashandy (2008). Mehana *et al.* (2010) and Attia *et al.* (2013) have reported that lead acetate treatment induced significant elevation of serum SGOT, SGPT, and ALP activities. Furthermore, Ibrahim *et al.* (2012) reported that the high SGOT, SGPT, and ALP activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue. Our results indicated that ethanol extract of mangosteen pericarp has hepatoprotective activity against lead acetate-induced hepatotoxicity, where the pretreated groups with ethanol extract of *E. cottonii* 800 mg/kg BW showed an improvement in the SGOT, SGPT, and ALP levels. This might be through its direct action on free radicals of lead acetate to prevent the liver cellular damage by maintaining its membrane integrity. Reduction of serum transaminases near-normal levels suggested regeneration of hepatocytes with healing of hepatic parenchyma.

The present investigation resulted in significant increase in MDA levels in the liver of lead acetate-treated mice in comparison to the negative control. This means that it increased the oxidative stress in the lead acetate-treated mice. It is known that lead acetate-induced oxidative stress tissue damage could be caused by two mechanisms: increased generation of ROS and by causing direct depletion of antioxidant reserves.[5,8] Intense lipid peroxidation caused by lead exposure may affect the mitochondrial and cytoplasmic membranes, causing more severe oxidative damage in the tissues and consequently releasing lipid hydroperoxides into circulation, which reflects the induction of oxidative stress.[11,23] The ethanol extract of *E. cottonii*, which behaves as a powerful antioxidant and free radical scavenger, can decrease MDA level perturbed by lead acetate in mice liver, as observed in this study. Treatment of mice with ethanol extract of *E. cottonii* at a dose of 800 mg/kg BW prevented the levels of lipid peroxidation (MDA) to rise when the mice were challenged with lead acetate. This means that ethanol extract of *E. cottonii* minimized the toxic effect of lead acetate through its antioxidant activity. The antioxidant protective mechanism decreases the oxidative stress and scavenges the free radicals which are responsible for liver damage and thus inhibit the lipid peroxidation (MDA). The findings of this study suggest that ethanol extract of *E. cottonii* could attenuate oxidative stress by decreasing the lipid peroxidation (MDA level) in lead-treated liver. A similar result had showed that Vitamin C and Vitamin E enhanced the antioxidant status and inhibited lipid peroxidation (MDA) in rats with lead acetate-induced liver injury. These findings indicate that the antioxidant activity of Vitamin C and Vitamin E is targeted primarily toward the lipid component of cells. Antioxidants such as Vitamin C and Vitamin E have been shown to inhibit free radical formation and are effective in minimizing lipid peroxidation in several different biological systems.[11]

SOD and GPx are important antioxidant enzymes. They constitute a mutually supportive defense mechanism against ROS. SOD decomposes superoxide radicals (O_2^-) to produce H_2O_2. GPx is a selenoenzyme which plays a major role in the reduction of H_2O_2 and hydroperoxide to produce nontoxic products. Therefore, the activities of these enzymes have been used to assess oxidative stress in cells. Many studies have shown that lead has a high affinity for SH groups in several enzymes such as SOD and GPx, thus it can alter antioxidant activities by inhibiting functional SH groups in these enzymes.[4,24] In the present study, the activity of SOD and GPx in mice liver dramatically decreased by lead acetate treatment. This suggested that lead acetate exposure induced oxidative stress by inhibiting the activity of this antioxidant enzyme. Interestingly, the administration of ethanol extract of *E. cottonii* increased the activities of SOD and GPx in the liver of lead-treated mice, which might be due to the ability of *E. cottonii* to reduce the accumulation of free radicals. Ethanol extract of *E. cottonii* acts as a scavenger for the oxygen-derived free radicals, thus protecting from cellular damage. This decreased SOD and GPx activity with lead acetate treatment is in agreement with previous studies.[18,19]

Histopathological results demonstrating structural changes in liver tissue of aminoglycoside metal toxic such as lead acetate were reported by some researchers.[25,26] In the present study, histopathological view of liver sections in lead acetate-treated group showed loss of the normal structure of hepatic cells, blood congestion, and fatty degeneration. Lead acetate thus had deleterious effect on liver tissues. The liver damage was considerably mild in the groups treated with *E. cottonii* extract of 800 mg/kg BW but not with 200 mg/kg BW and 400 mg/kg BW.

In summary, our data indicate that lead acetate-induced liver toxicity might be related to oxidative damage. Co-administration of *E. cottonii* extract lessened the effects of lead acetate-induced liver toxicity, possibly by inhibiting free radical-mediated process. Further investigation of these

promising protective effects of *E. cottonii* extract against lead acetate-induced liver damage may have a considerable impact on developing clinically feasible strategies to treat patients with lead acetate-induced liver failure.

CONCLUSION

It could be concluded that ethanol extract of *E. cottonii* may exert its protective actions against lead-induced hepatic injury in rats, possibly through its antioxidant mechanisms. Ethanol extract of *E. cottonii* can be a future natural product for counteracting the lead acetate intoxication. These results showed that ethanol extract of *E. cottonii* has a potential hepatoprotective effect in a dose-dependent manner that minimize or diminish the hepatotoxic effect of lead acetate.

Acknowledgments

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Conflicts of interest

There are no conflicts of interest.

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