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 providing a promising protective effect against lead acetate induced liver toxicity in mice.

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...
body have been associated with nephrotoxic, hepatotoxic, neurotoxic, and cardiovascular diseases.\[12\]

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.\[13\] 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 polysaturated fatty acids and has medicinal uses.\[15\] *E. cottonii* is one of the main seaweed 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 Veterinar 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].

**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. Lead produces
oxidative damage in the liver by enhancing lipid peroxidation, causes
liver dysfunction, and increases free radical damage. 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. 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. *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. *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 SGT, SGPT, and ALP are recommended
for the assessment of hepatocellular injury in preclinical studies as it is

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

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

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

| Groups                        | MDA (nmol/mL) | SOD (U/mg) | GPx (U/mg) |
|-------------------------------|---------------|------------|------------|
| Negative control              | 5.79±0.86     | 9.33±1.17  | 45.38±6.57 |
| Positive control              | 9.24±1.57     | 4.81±0.92  | 33.43±3.42 |
| *E. cottonii* (mg/kg BW)      |               |            |           |
| 200                           | 8.38±0.52     | 4.27±0.84  | 31.27±5.63 |
| 400                           | 7.93±0.89     | 5.92±1.01  | 34.52±4.86 |
| 800                           | 7.13±0.62     | 7.02±0.83  | 39.27±3.41 |
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 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.[23,24] 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) 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$_2$O$_2$, GPx is a selenoenzyme which plays a major role in the reduction of H$_2$O$_2$ 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.[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.[23,26] 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 countering 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.

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**Conflicts of interest**

There are no conflicts of interest.

**REFERENCES**

1. Mudipalli A. Lead hepatotoxicity and potential health effects. Indian J Med Res 2007;126:518-27.
2. Adikwu E, Deo O, Geoffrey OB, Enimeya DA. Lead organ and tissue toxicity: Roles of mitigating agents (Part 1). Br J Pharm Toxicol 2013;4:232-40.
3. Akilavalli N, Radhika J, Brindha J. Hepatoprotective activity of *Ocimum sanctum* against lead induced toxicity in albino rats. Asian J Pharm Clin Res 2011;4:84-7.
4. Hsu PC, Guo YL. Antioxidant nutrients and lead toxicity. Toxicology 2002;180:33-44.
5. Patrick L. Lead toxicity part II: The role of free radical damage and the use of antioxidants in the pathology and treatment of lead toxicity. Altern Med Rev 2006;11:114-27.
6. Xu J, Lian LJ, Wu C, Wang XF, Fu WY, Xu LH. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. Food Chem Toxicol 2008;46:1488-94.
7. Mehana EE, Abdel Raheim MA, Meki KM. Ameliorated effects of green tea extract on lead induced liver toxicity in rats. Exp Toxicol Pathol 2010;13:173-80.
8. Ibrahim NM, Eweis EA, El-Beltagi HS, Abdel-Mobdy YE. Effect of lead acetate toxicity on experimental male albino rat. Asian Pac J Trop Biomed 2012;2:41-6.
9. Atta AM, Ibrahim FA, Nabil GM, Aziz SW. Antioxidant effects of ginger (*Zingiber officinale Roscoe*) against lead acetate-induced hepatotoxicity in rats. Afr J Pharm Pharmacol 2013;7:1213-9.
10. Upadhyay AK, Mathur R, Bhaduria M, Nirala SK. Therapeutic influence of zinc and ascorbic acid against lead induced biochemical alterations. Therapie 2009;64:383-8.
11. Aziz FM, Maulood IM, Chawsheen MA. Effects of melatonin, Vitamin C and E alone or in combination on lead-induced injury in liver and kidney organs of rats. Pak J Zool 2014;46:1425-31.
12. Vishnu Priya V, Niveda S, Pratiksha G, Gavathi R. A review of hepatoprotective natural products. Rec Res Sci Tech 2010;2:49-52.
13. Jackie T, Haleagrahara N, Chakravarthi S. Antioxidant effects of *Etlingera elatior* flower extract against lead acetate – Induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. BMC Res Notes 2011;4:67.
14. Yang G, Fei X, Wang G, Chung IK. Growth of *Gracilaria lemaneiformis* under different cultivation conditions and its effects on nutrient removal in Chinese coastal waters. Aquaculture 2006;254:249-55.
15. Matanjun P, Mohamed S, Mustapha NM, Muhammad K. Nutrient content of tropical edible seaweeds, *Eucheuma cottonii*, *Caulerpa lentillifera* and *Sargassum polycystum*. J Appl Phvcol 2009;21:75-80.
16. Matanjun P, Mohamed S, Muhammad K, Mustapha NM. Comparison of cardiovascular protective effects of tropical seaweeds, *Kappaphycus alvarezi*, *Caulerpa lentillifera*, and *Sargassum polycystum*, on high-cholesterol/high-fat diet in rats. J Med Food 2010;13:792-800.
17. Namvar F, Mohamed S, Fard SG. Polyphenol-rich seaweed (*Eucheuma cottonii*) extract suppresses breast tumour via hormone modulation and apoptosis induction. Food Chem 2012;130:736-82.
18. Haleagrahara N, Jackie T, Chakravarthi S, Rao M, Kulur A. Protective effect of *Etlingera elatior* (torch ginger) extract on lead acetate – Induced hepatotoxicity in rats. J Toxicol Sci 2010;35:683-71.
19. Wang J, Yang Z, Lin L, Zhao Z, Liu Z, Liu X. Protective effect of naringenin against lead-induced oxidative stress in rats. Biol Trace Elem Res 2012;146:354-9.
20. Newairy AS, Abdou HM. Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem Toxicol 2009;47:813-8.
21. Moussa SA, Bashandy SA. Biophysical and biochemical changes in the blood of rats exposed to lead toxicity. Rom J Biophys 2008;18:123-33.
22. Grattagliano I, Bonfrete L, Diogo CV, Wang HH, Wang DQ, Portincasa P. Biochemical mechanisms in drug-induced liver injury: Certainties and doubts. World J Gastroenterol 2009;15:4865-76.
23. Sharma V, Pandey D. Protective role of *Tinospora cordifolia* against lead-induced hepatotoxicity. Toxicol Int 2010;17:12-7.
24. Abd El Kader MA, El-Sammad NM, Taha H. The protective role of rosemary (*Rosmarinus officinalis*) in lead acetate induced toxicity in rats. J Appl Sci Res 2012;8:3071-82.
25. Baxla SL, Gora RH, Kerketta P, Kumar N, Roy BK, Patra PH. Hepatoprotective effect of *Curcuma longa* against lead induced toxicity in Wistar rats. Vet World 2013;6:664-7.
26. Mannem P. Protective effects of ginger extract against lead induced hepatotoxicity in male albino rats. IOSR J Environ Sci Toxicol Food Technol 2014;8:53-9.