In Vitro Study on Hepatoprotective Effect of Phyllanthus fraternus Against Lead Induced Toxicity

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Abstract

Lead is one of the most abundant and hazardous environmental contaminants of the global concern. Environmental and occupational exposure to lead has continued to pose a serious threat to the health of living organisms due to its propensity to induce toxicity in mammalian systems. The major aim of the present in vitro study was to investigate ameliorative potential of Phyllanthus fraternus against lead induced hepatotoxicity. Current synergistic study involves exposure of goat liver homogenate to different concentrations of lead acetate and specific concentration of Phyllanthus fraternus aqueous extract for particular duration. During the study, biochemical indices like lipid peroxidation (LPO), Protein levels and enzymatic activities of alkaline phosphatase (ALPase), acid phosphatase (ACPase) and succinate dehydrogenase (SDH) were measured. Results of the present study revealed that protein levels and enzymatic parameters of alkaline phosphatase as well as succinate dehydrogenase declined significantly while levels of lipid peroxidation and acid phosphatase increased significantly in lead acetate exposed goat liver homogenates as compared to control groups. The results also emphasized that effect of lead acetate was dose dependent. As the dosage of lead was increased, the intensity of damage to liver also found to be increased. Maximum alterations in all biochemical parameters were found at the dosage of 5 ppm. The results of the current study strongly suggested that lead had affected antioxidant system, protein content and activities of specific metabolic enzymes of liver homogenate. Co-administration of herbal aqueous extract of Phyllanthus fraternus (5 mg/ml) along with 5 ppm lead acetate to liver homogenate exerted an ameliorative effect and maintained the studied parameters closed to control group, which confers its protective role against lead induced hepatotoxicity.

Keywords: Lead Toxicity, Hepatotoxicity, Oxidative Stress, Phyllanthus fraternus, Hepatoprotective Activity

1 Introduction

Lead toxicity has remained as one of the most dreaded heavy metal toxicities worldwide due to its continuous prevalence in the environment. Lead is at the number 2 position on the Agency for Toxic Substances and Disease Registry’s (ATSDR) “Top 20 List” of hazardous substances. No “safe” level of lead exposure has been established yet and is the subject of much remediation research.

Ubiquitous metal lead is being used recklessly by humans for various industrial purposes especially in the manufacturing of protective paints for iron and steel, explosives, rodenticides, lead-acid batteries, cosmetics, fusible alloys, pencils, toys and ammunition. Worldwide use of lead compounds and its natural exposure due to air, water and food facilitates its entry into the food chain, causes the bioaccumulation and thus increases the possibility of having its toxic effects on humans and animals.

Lead poisoning also referred to as Plumbism, Colica pictonium, Saturnism, Devon colic or Painter's colic is the medical condition resulted due to increased levels of the heavy metal lead in the body and has remained a serious health threat since ancient times. Lead is considered as one of the most cumulative and persistent environmental pollutants with no beneficial biological role. Heavy
metal lead has been well recognized as a potent toxicant even at the very low level of exposure⁴. Lead toxicity can be encountered clinically in multiple organs especially, in liver being the major target organ for this metallic toxicant. Hepatic toxicity of lead is of major concern because liver plays an important role in carbohydrate metabolism, protein metabolism, lipid metabolism, energy production and detoxification. In terms of pathogenesis, lead exposure is known to interrupt synthesis of sulfhydryl group containing proteins, inhibit enzymes activation, alter calcium homeostasis, inhibit the formation of heme containing protein cytochrome P-450 and lower the level of available sulfhydryl antioxidant reserves in the body leading to oxidative stress⁵. Therefore, it is extremely important to investigate protective measures against adverse exposure of this hazardous element.

Herbal plants can be used for therapeutic purposes, as they possess the potential to provide protective effects by counteracting the toxicity. Traditional knowledge does not provide systematic and mechanism wise ameliorative effects of various herbal plant extracts. *Phyllanthus fraternus* Webster, commonly known as *Bhumiyamalki*, family Euphorbiaceae, is a traditional herbal drug used in Unani and Ayurvedic system of medicine. *Phyllanthus fraternus* is considered as a ‘Natural liver protector’. *Phyllanthus* species are well known for their preventive and curative role in various hepatic disorders and are used in several ethno-medicines in indigenous health care systems in India⁶. Recently, it has attracted the attention of researchers because of its wide range of pharmacological properties, including hepatoprotective, antioxidative, lipid lowering, antidiabetic, antiviral and antifungal actions⁷. Phytochemical screening of the *Phyllanthus fraternus* whole plant extract revealed the presence of biologically active constituents such as alkaloids especially phyllanthin as well as hypophyllanthin, flavonoids, tannins, anthraquinones, saponins, glycosides, resins, terpenes, sterols, quercetin and phenols⁸,⁹. The plant is widely employed for the treatment of liver diseases such as hepatitis, jaundice and liver cancer. It is extensively used as digestive stimulant, pain reliever, diuretic, antispasmodic agent and fever reducer¹⁰,¹¹. It is also utilized to certain extent for treating disorders including anemia, diabetes, hypertension, anorexia, cold, tuberculosis, urinary stone, viral and bacterial infections.

Keeping this perspective in mind, investigation of the possible therapeutic role of aqueous extract of *Phyllanthus fraternus* as a protective agent against lead induced hepatic toxicity is necessary. The objective of study was to understand the mechanism of lead toxicity on various biochemical changes such as alterations in lipid peroxidation, protein content and enzyme activities in goat liver homogenate in order to avoid the ethical concern of involving experimental animals. Another objective of the present study was to search for an agent, which could help in the amelioration of lead toxicity with maximum cost effectiveness and minimum side effects.

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Therefore, to bridge this gap, an antioxidant, *Phyllanthus fraternus* extract was used as a therapeutic agent against lead intoxication in this study.

2 Materials and Methods

2.1 Experimental design

In the present study, experimental protocol was designed into two separate phases. During the first phase, goat liver homogenate cultures were exposed for the specific period of time to different concentrations of lead acetate selected on the basis of LD₅₀ value and reported literature⁶ in order to evaluate the lead induced alterations in selected biochemical parameters like lipid peroxidation, protein content and enzymatic activities of alkaline phosphatase, acid phosphatase as well as succinate dehydrogenase *in vitro*. The second phase involves co-administration of plant aqueous extract of *Phyllanthus fraternus* to lead acetate exposed homogenate for specific time duration to investigate an ameliorative effect of the antioxidant against lead toxicity.

2.1 Chemicals

The analytical reagent (AR) grade lead acetate trihydrate having 99% purity was obtained from HIMEDIA Laboratory Pvt. Ltd., Mumbai, India. All the other chemicals of AR grade used in the present study were procured from Sigma and Merck Laboratory Pvt. Ltd., India. The herbal plant extract was prepared from the *Phyllanthus fraternus* whole plant powder obtained from L.V.G. (Recognized Ayurvedic Herbal Store), Ahmedabad, India.

2.2 Preparation of plant (*Phyllanthus fraternus*) extract¹³

12.5 gm of coarsely grinded whole plant powder of *Phyllanthus fraternus* was mixed in 250 ml double distilled water and kept in orbital shaker incubator for 48 hrs at the speed of 100 rpm at 37°C. The plant extract content thus obtained was first filtered through ordinary filter paper and then through Whatman filter paper no. 1. The filtrate was collected and evaporated till dryness. 100 mg of crude extract was dissolved in 10 ml of double distilled water and used for the present investigation.

2.3 Preparation of lead acetate solutions

0.010 gm of lead acetate was dissolved in 100 ml of double distilled water to prepare the stock solution of 100 ppm. A definite volume of this stock solution was used in a final volume of the reaction mixture, so as to get the required concentrations of lead acetate. 1 ppm, 3ppm and 5 ppm concentrations of lead acetate were used in the present study.

2.4 Sample collection

The goat liver was used as a vital organ for the study. Liver samples weighing approximately 250–350 gm of healthy adult goat (*Capra hircus*) were obtained from the approved local slaughter house. After...
sacrificing the animal, fresh liver tissue was brought to the laboratory under frozen condition and used immediately. The appearance of fresh tissue was dark reddish-brown color. Liver tissue was washed in normal saline, blotted dry by pressing between 2-3 folds of filter paper and divided into different experimental groups.

2.5 Experimental groups

The experimental protocol includes six experimental groups: (I) Control Group, (II) Herbal Antioxidant (Phyllanthus fraternus extract-5 mg/ml) Exposed Group, (III) Low Dosage Lead Acetate (1 ppm) Exposed Group, (IV) Mid Dosage Lead Acetate (3 ppm) Exposed Group (V) High Dosage Lead Acetate (5 ppm) Exposed Group and (VI) Lead Acetate (High Dosage-5 ppm) and Herbal Antioxidant (Phyllanthus fraternus extract-5 mg/ml) (co-administration) Exposed Group.

2.6 In vitro study

The tissues of different experimental groups were subjected to the process of homogenization with constant pace and speed under suitable condition of 4°C in chilled glass mortar pestles in order to maintain the viability. The fresh liver homogenates obtained were whitish red in appearance due to haemolysis occurred during the homogenization process. The liver homogenate samples in all the tubes were exposed to aqueous solutions of various concentrations of lead acetate (1 ppm, 3 ppm and 5 ppm) and plant extract of Phyllanthus fraternus (5 mg/ml) for 30 minutes time duration. The unexposed control and exposed liver homogenates were maintained at same conditions in 5% CO$_2$ containing BOD incubator at 37°C and subjected to study of various biochemical indices for investigating ameliorative effect of the antidote against lead induced hepatotoxicity.

2.7 Biochemical analysis

To analyze free radical induced cell injury by lead acetate, the levels of lipid peroxides were determined in liver homogenates. The measurement of lipid peroxidation (LPO) in the liver homogenates of control and exposed groups were done by the method of Ohkawa et al. A 10% of tissue homogenate of liver was prepared in ice cold 0.1M phosphate buffer solution (pH=7.4) for the estimation of lipid peroxidation levels. For investigation of toxic effect of lead acetate on the protein metabolism, levels of soluble proteins were estimated in goat liver homogenates by the method of Lowery et al. At the end of the lead acetate treatment as well as Phyllanthus fraternus exposure, certain specific parameters of goat liver, including enzyme activities of alkaline phosphatase, acid phosphatase and succinate dehydrogenase were also investigated. The alkaline phosphatase and acid phosphatase activities were analyzed by the method of Bessey et al while the activity of succinate dehydrogenase was determined by method of Beatty et al. 0.1 gm of liver tissue was homogenized in the known amount of double distilled water for the estimation of soluble proteins, alkaline phosphatase, acid phosphatase and succinate dehydrogenase activities.

2.8 Statistical analysis

Student’s ‘t – test’ was used for the statistical analysis of the data. For each parameter (n=5), the data were expressed as mean ± SEM after subjecting to Student’s ‘t – test’ using GraphPad software for the interpretation of results. The significance difference was statistically considered at the level of p < 0.05.

3 Results

3.1 Lipid peroxidation

Results of the LPO in the goat liver homogenate exposed to different concentrations of lead acetate, Phyllanthus fraternus extract and related control in vitro are given in table 1. Their percentage of difference, due to the lead acetate and herbal extract exposure with respect to their control, is given in table 2. The lead acetate exposure was found to increase production of thiobarbituric acid reactive substances (TBARS) significantly, which is marked by increased LPO levels in goat liver homogenates. The increase in lipid peroxidation was dose dependent. Lipid peroxidation at low dosage (1 ppm) and medium dosage (3 ppm) exposure was represented as 6.8% (p < 0.05) and 19.64% (p < 0.01), respectively. Addition of 5 ppm lead acetate in liver homogenate caused highly significant increase in lipid peroxidation compared to control group as represented by 35.24% (p < 0.001). Addition of aqueous extract of Phyllanthus fraternus (5 mg/ml) to homogenate did not cause any significant effect on LPO level. However, simultaneous addition of lead acetate (5 ppm) and aqueous extract (5 mg/ml) in goat liver homogenate significantly reduced (22.09%) lead induced lipid peroxidation as compared to lead (5 ppm) exposed group (Table 2).

Supplementation of aqueous extract of Phyllanthus fraternus as an ameliorative agent resulted in significant reduction in elevated MDA (malondialdehyde) levels in lead exposed group.

3.2 Protein levels

Lead acetate exposure caused the significant decline in the protein levels in goat liver homogenates compared to control (Table 1). The decrease in the protein content at 1 ppm and 3 ppm exposure was represented as 13.06% (p < 0.02) and 22.40% (p < 0.001), respectively. Maximum reduction of protein content (30.90%) in the liver homogenate was observed at 5 ppm concentration of lead acetate (p < 0.001). Administration of aqueous extract of Phyllanthus fraternus (5 mg/ml) to homogenate did not cause any significant effect. However, simultaneous supplementation of lead acetate (5 ppm) and Phyllanthus fraternus aqueous extract (5 mg/ml) in liver homogenate significantly increased (41.76 %) protein level compared to lead acetate (5 ppm) exposed group (Table 2).

Supplementation of aqueous extract of Phyllanthus fraternus as a
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A therapeutic agent resulted in significant maintenance of protein levels against lead intoxication.

Table 1: Biochemical parameters of control and exposed liver homogenates in vitro (n=5, mean ± SEM)

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group I Control</th>
<th>Group II PF (5 mg/ml)</th>
<th>Group III LA (LD)(1 ppm)</th>
<th>Group IV LA (MD) (3 ppm)</th>
<th>Group V LA (HD) (5 ppm)</th>
<th>Group VI LA (HD) (5 ppm)+PF (5 mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO(^a)</td>
<td>5019.23±81.46</td>
<td>4981.14±18.65(^{NS})</td>
<td>5365.39±92.12(^{*})</td>
<td>6706.92±247.15(^{**})</td>
<td>6787.86±155.81(^{***})</td>
<td>5288.46±0.71(^{*})</td>
</tr>
<tr>
<td>Protein(^b)</td>
<td>20.90±0.470</td>
<td>21.84±0.785(^{NS})</td>
<td>18.17±0.702(^{*})</td>
<td>16.219±0.569(^{*})</td>
<td>14.44±0.157(^{*})</td>
<td>20.47±0.213(^{NS})</td>
</tr>
<tr>
<td>ALPase(^c)</td>
<td>3.15±0.300</td>
<td>3.02±0.238(^{NS})</td>
<td>1.42±0.309(^{**})</td>
<td>1.29±0.274(^{**})</td>
<td>1.16±0.236(^{**})</td>
<td>3.00±0.003(^{NS})</td>
</tr>
<tr>
<td>ACPase(^d)</td>
<td>5.52±0.006</td>
<td>5.54±0.011(^{NS})</td>
<td>5.78±0.013(^{***})</td>
<td>6.06±0.006(^{***})</td>
<td>7.02±0.006(^{***})</td>
<td>5.58±0.013(^{***})</td>
</tr>
<tr>
<td>SDH(^e)</td>
<td>411.05±4.616</td>
<td>410.41±3.912(^{NS})</td>
<td>357.78±8.824(^{***})</td>
<td>357.78±8.824(^{***})</td>
<td>320.68±8.532(^{***})</td>
<td>399.82±0.447(^{*})</td>
</tr>
</tbody>
</table>

\(p\) – Values: \(*p < 0.05\), \(**p < 0.01\), \(**p < 0.001\); PF - Phyllanthus fraternus extract, LA - Lead Acetate, LD - Low Dose, MD - Mid Dose, HD - High Dose

\(NS\) - Non significant v/s control group; a = nano moles of MDA / 100 mg tissue weight / 60 minutes; b = mg Protein / 100 mg fresh tissue weight; c, d = \(\mu\) moles of p-nitrophenol released / 30 minutes / 100 mg tissue weight; e = \(\mu\)g formazan formed / 15 minutes / 100 mg tissue weight

Table 2: Gross effect of lead and Phyllanthus fraternus on biochemical parameters of goat liver homogenates in vitro (% of difference with respect to their control as well as lead exposed homogenate cultures)

<table>
<thead>
<tr>
<th>Biochemical Parameters</th>
<th>Group III Lead Acetate (1 ppm)</th>
<th>Group IV Lead Acetate (3 ppm)</th>
<th>Group V Lead Acetate (5 ppm)</th>
<th>Group VI Lead Acetate (5 ppm)(^a)</th>
<th>Group VI Lead Acetate (5 ppm)+P. fraternus(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipid Peroxidation</td>
<td>6.8*</td>
<td>19.64*</td>
<td>35.24*</td>
<td>5.36*</td>
<td>22.09</td>
</tr>
<tr>
<td>Protein Levels</td>
<td>13.06</td>
<td>22.4</td>
<td>30.9</td>
<td>2.06</td>
<td>41.76*</td>
</tr>
<tr>
<td>Alkaline Phosphatase</td>
<td>54.78</td>
<td>59.10</td>
<td>63.07</td>
<td>4.76</td>
<td>158.62*</td>
</tr>
<tr>
<td>Acid Phosphatase</td>
<td>4.71*</td>
<td>9.78*</td>
<td>27.17*</td>
<td>1.09*</td>
<td>20.51</td>
</tr>
<tr>
<td>Succinate Dehydrogenase</td>
<td>12.96</td>
<td>28.47</td>
<td>43.88</td>
<td>2.73</td>
<td>73.32*</td>
</tr>
</tbody>
</table>

\(^a\) All values are expressed in % of decrease or \(^*\)increase; a – compared to control group; b – compared to Group V

3.3 Alkaline phosphatase (E.C.3.1.3.1) [E.C. - Enzyme Commission Number]

Lead acetate exposure to goat liver homogenate for 30 minutes brought about a significant reduction in the alkaline phosphatase activity. Results revealed that alkaline phosphatase activity markedly decreased as the dose of lead acetate was increased in goat liver homogenate, and it remained less than control always (Table 1). The decline in the enzyme activity at 1 ppm and 3 ppm exposure was represented as 54.78% (p < 0.01) and 59.10% (p < 0.01), respectively. Maximum retardation of enzyme activity (63.07%) in liver homogenate was noted at 5 ppm exposure of lead acetate (p < 0.001).
Addition of 5 mg/ml aqueous extract of *Phyllanthus fraternus* to homogenate did not cause any significant effect. However, simultaneous addition of the lead acetate (5 ppm) and aqueous extract (5 mg/ml) in goat liver homogenate significantly maintained (4.76%) reduction in enzyme activity as compared to control (Table 2). Supplementation of aqueous extract of *Phyllanthus fraternus* significantly maintained alkaline phosphatase activity closest to control group and exerted protective effect against lead toxicity.

### 3.4 Acid phosphatase (E.C.3.1.3.2)

Results revealed that acid phosphatase activity significantly increased as the dose of lead acetate was increased in goat liver homogenate (Table 1). The increase in the enzyme activity at 1 ppm and 3 ppm lead exposure in homogenate cultures was represented as 4.71% (p < 0.0001) and 9.78% (p < 0.0001), respectively. Statistically extremely significant elevation of p < 0.0001 (27.17%) of enzyme activity in liver homogenate was observed at 5 ppm concentration of lead acetate. Addition of 5 mg/ml aqueous extract of *Phyllanthus fraternus* to homogenate did not cause any significant effect. However, simultaneous addition of lead acetate (5 ppm) and aqueous extract (5 mg/ml) in goat liver homogenate significantly ameliorated (20.51%) the enzyme activity (Table 2). Addition of aqueous extract of *Phyllanthus fraternus* as the therapeutic agent significantly maintained acid phosphatase activity in lead exposed liver homogenate nearest to control group.

### 3.5 Succinate dehydrogenase (E.C.1.3.99.1)

A significant decline was observed in the activity of succinate dehydrogenase in lead exposed homogenate cultures compared to control (Table 1). Reduction in enzyme activity at low dosage (1 ppm), medium dosage (3 ppm), and high dosage (5 ppm) exposure was represented as 12.96 % (p < 0.001), 28.47 % (p < 0.001) and 43.88 % (p < 0.001), respectively. Administration of aqueous extract of *Phyllanthus fraternus* to homogenate did not cause any significant effect. However, co-administration of lead (5 ppm) and herbal extract (5 mg/ml) in liver homogenate significantly ameliorated (73.32%) succinate dehydrogenase activity as compared to lead (5 ppm) exposed group (Table-2) and exerted protection against lead induced hepatotoxicity.

### 4 Discussions

Data of the present study showed statistically significant elevated levels of malondialdehyde (MDA) in lead exposed groups compared to control group, which might be due to formation of highly reactive species having unpaired electrons known as free radicals. Oxidative stress results when the balance between antioxidant system and reactive oxygen species (ROS) is lost. Results of several studies suggested increased amount of ROS in lead-exposed animals, which corroborate with our data18,19. The possible suggested mechanism for enhanced production of reactive oxygen species such as superoxide ions, hydroxyl radicals and hydrogen peroxide can be depletion of intracellular free radical scavenger glutathione. Most important consequence includes the peroxidation of membrane lipids, with an increase in the permeability of cell membrane rendering the tissue susceptible to free radical injury20.

The results emphasized that co-administration of herbal extract of *Phyllanthus fraternus* to lead exposed liver homogenates cultures significantly reduced the increased levels of lipid peroxides. The various antioxidants present in the herbal extract exerted ameliorating effect by lowering free radical levels. The plant extract of *Phyllanthus fraternus* inhibits the chain reaction and exerts an antioxidant effect.

Reduction in protein content of lead exposed homogenates might be due to binding of lead with the sulphydryl group containing proteins and interference with number of enzyme systems essential to cellular metabolism. In the present study, decline in protein levels could be attributed to their damage by singlet oxygen mainly due to oxidation of essential amino acids. Further, MDA formed during lipid peroxidation could react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities21. The other factors responsible for alteration in protein metabolism might be inhibition of protein biosynthesis due to the impairment of peptide chain initiation. The decrease in proteins might be as a result of increased proteolysis and the reduced incorporation of amino acids into proteins22 or increased deamination of amino acids in the liver and kidney. Thus, one of the reasons for liver toxicity in the current study might be the decreased availability of proteins necessary for growth and differentiation of tissues and enzyme systems.

The plant extract of *Phyllanthus fraternus* is able to prevent cell injury by maintaining sulphydryl groups of membrane binding proteins. The hepatoprotective, anti-inflammatory, antioxidant and membrane stabilizing properties of the plant can be attributed to its anticholesteric action, reduction in free radicals and reduction in cell protein necrosis as well as glutathione depletion reduction potential.

Many products of LPO such as hydro peroxides can inhibit protein synthesis and alter enzyme activity23. Results of current study elucidated that lead acetate exposure caused significant depletion in activity of alkaline phosphatase in liver. Alkaline phosphatases are a group of enzymes, which hydrolyze phosphate esters at alkaline pH. Reduced alkaline phosphatase activity might be attributed to the alteration in cell membrane permeability in addition to lead induced imbalance between synthesis and degradation of enzyme.

The data of current investigation also revealed the increase in acid phosphatase activity in lead acetate exposed liver homogenates. Acid phosphatase, a lysosomal enzyme is involved in a number of activities such as phagocytosis24, autolysis, dissolution of tissue.
components, fat absorption in intestine, cellular differentiation and keratinization. Alteration in activity might be due to direct inhibitory effect of accumulated lead and fat as well as disturbed balance between synthesis and degradation of enzyme in liver.

Results accumulated in the present biochemical analysis revealed a considerable loss of SDH activity in the liver homogenate exposed to lead acetate. Changes in the SDH activity of liver revealed alterations in its oxidative energy metabolism indicating lesions in TCA cycle, which may affect conversion of succinate to fumarate leading to blockage in the Kreb's cycle which would result into reduction in ATP synthesis. Decreased SDH activity also indicates a possible alteration in mitochondrial structure and functions of mitochondrial enzymes due to accumulation of lead in mitochondria. Lead may uncouple oxidative phosphorylation, which may reflect on the slow rate of TCA cycle. Thus, depression of SDH activity reflects upon the altered state of oxidation and energy metabolism of a damaged liver.

The *Phyllanthus fraternus* extract also manifested maintenance of alkaline phosphatase, acid phosphatase and succinate dehydrogenase enzyme activities in goat liver homogenate nearest to control. The mechanism of action of herbal extract seemed to be mainly by virtue of detoxification, because it is very powerful reducing agent which participates in oxidation – reduction reactions and acts as an antioxidant. It has been suggested that the plant extract must be having the important role either in production of enzymes that scavenge the free radicals or / and regulation of antioxidant levels of the body.

*Phyllanthus fraternus* aqueous extract acts not only as a powerful free radical scavenger but also an effective hepatoprotective agent due to its antioxidant property. The primary active components of *Phyllanthus fraternus* responsible for hepatoprotective activity are phyllanthin, hypophyllanthin and triacontanal. Thus, antioxidative and hepatoprotective properties of medicinal plant *Phyllanthus fraternus* significantly ameliorates lipid peroxidation by reducing oxidative stress which subsequently provides protection against lead induced alterations in protein content as well as enzyme activities in goat liver homogenate.

5 Conclusions

In conclusion, the findings of present study suggest the role of oxidative mechanisms in lead-acetate induced liver damage. From the current *in vitro* study, it can be clearly elucidated that heavy metal lead affects the anti-oxidative as well as other biochemical indices of goat liver possibly by inducing oxidative stress. Present research study also suggested that lead metal appears to cause an imbalance in the antioxidant defense system by inhibiting some related enzymes, thereby enhancing the free radical mediated peroxidation of lipids. The study findings also confirm that lead adversely affects the protein content as well as energy and oxidative metabolism of goat liver. Thus, from the present *in vitro* study, it can be clearly concluded that lead exposure has a definitely destructive effect on the structural, metabolic and functional status of liver.

The current investigation also emphasized that co–administration of aqueous extract of *Phyllanthus fraternus* in liver homogenate significantly exerts protective effects against lead intoxication due to its well-known antioxidant and hepatoprotective properties. Thus, it is clearly revealed that *Phyllanthus fraternus* has a significant role in alleviating the lead toxicity. It acts as a therapeutic eliminator of heavy metals. Hence, this antidote could be a very effective and beneficial ameliorating agent against lead induced hepatic toxicity in the world over.

The present investigation has elucidated the mechanism of action of lead induced hepatotoxicity and also suggested its amelioration, which can be considered as a significant contribution in the field of mitigation of plumbism in endemic regions.

6 Competing interests

There are no conflicts of interests.

7 Author’s contributions

The present research work was carried out by equal contribution of both the authors. NKJ and FCS designed the experimental protocol, carried out literature review and draft the manuscript. FCS participated in collection of data through experimental work and performed statistical analysis. The final draft of the manuscript was reviewed and edited under the guidance of NKJ. Both the authors read and approved the final manuscript.

7 References


