Efficacy of Bacopa monnieri in Mitigating Lead-Induced Blood Toxicity in Mice Compared to Synthetic Antioxidants

Flora Shah
Gujarat University
Venmathi Muthu
Saveetha Institute of Medical and Technical Sciences (SIMATS)
Nayan Jain
Gujarat University
Sakthivel Muthu
Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS)
Jamith Basha Abdul Wahid
Northern Border University
Mumtaj Shah
King khalid University
Karuppiah Nagaraj
surfactantnagaraj@gmail.com

Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS)

Research Article

Keywords: Toxicity, Ethnopharmacology, bioactive compounds, Chromatography, Clinical toxicology, Chemico-biological interaction, Lead Toxicity, Bacopa monnieri, Hematological parameters, Antioxidant

Posted Date: July 31st, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7161974/v1

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Biological Trace Element Research on November 6th, 2025. See the published version at https://doi.org/10.1007/s12011-025-04849-x.

Abstract

The current study investigates the protective and therapeutic potential of Bacopa monnieri extract and synthetic antioxidants against lead-induced hematological toxicity in mice. HPLC analysis of Bacopa monnieri extract revealed a rich profile of bioactive compounds, including apigenin, luteolin flavonoids, bacosides, and bacopasaponins. In vivo experiments demonstrated significant hematological alterations in lead-exposed groups, with dose-dependent reductions in hemoglobin, red blood cell (RBC) count, platelet count, hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), alongside increases in white blood cell (WBC) count. Lead exposure resulted in hemoglobin declines of up to 51.82% and RBC reductions of up to 59.32% in high-dose groups, indicating severe hematotoxic effects. Co-administration of Bacopa monnieri extract or synthetic antioxidants mitigated these alterations, with Bacopa monnieri showing superior protection by maintaining hemoglobin, RBC, and platelet levels closer to control values. Curative treatments further restored hematological parameters near baseline, highlighting the efficacy of Bacopa monnieri in reversing lead-induced toxicity. The findings of the study revealed dose-dependent statistically significant ($p < 0.001$) alterations in hematological parameters of lead intoxicated mice groups as compared to control groups, while co-treatment with synthetic antioxidants or Bacopa monnieri extract conferred protection and reduce these toxic effects. Notably, Bacopa monnieri showed higher efficacy in mitigating lead-induced toxicity to hematological system. These data imply that Bacopa monnieri has better potential to be an effective ameliorative therapeutic agent against lead toxicity as compared to synthetic alternatives.

1. Introduction

The increasing prevalence of environmental toxins, particularly heavy metals like lead, has led to growing concerns regarding their impact on human health. Lead toxicity is considered as a serious environmental hazard of public health concern at global level due to its persistent occupational and industrial exposure [1]. Strong neurotoxic lead is known to cause a variety of harmful effects, including abnormalities in the hematological system. Exposure to lead upsets the delicate equilibrium between pro-oxidant and antioxidant processes, mostly by producing too many reactive oxygen species [2]. Oxidative stress brought on by this imbalance oxidizes structural proteins and modifies the permeability of blood cell membranes, ultimately resulting in hematological toxicity. The prevalence of lead poisoning worldwide, especially in areas where it is endemic, highlights the critical need for efficient treatment approaches to lessen the negative consequences of the metal.

Bluish-white lustrous lead is extensively preferred metal in the world because of its unique physico-chemical properties of softness, low tensile strength, corrosion resistance, heaviness, malleability, ductility and poor conductibility. Lead is still used vehemently for numerous industrial and domestic activities in the developing countries for the production of explosives, fusible alloys, paint, crystal glass, kitchen utensils, crayons, ceramic glaze, pencil, pesticides, radiation shields, cosmetics, ayurvedic medicines, pipes, ship breaking instruments, building construction material and ammunition [3]. Major sources of lead exposure involved lead battery factories engaged in manufacturing and recycling, mining

activities, smelting, refining, and cottage industries. Environmental contamination associated with non-biodegradable lead metal is a serious danger as its accumulation leads to bio-magnification and pose continuous threat to living organisms via its entry into food chain through myriads of pathways including food, water, soil or air [4].

Lead poisoning, sometimes referred to as plumbism or saturnism, happens when the body accumulates high concentrations of the heavy element lead [5]. Lead poisoning has serious, systemic effects on many different organ systems. According to Luo *et al.* (2007), clinical symptoms of lead poisoning can include anemia, encephalopathy, mental retardation, cognitive deficits, lethargy, loss of appetite, weight loss, dizziness, abdominal pain, constipation, vomiting, irritability, fatigue, anxiety, and the appearance of blue lines on the gums (known as lead lines). In extreme cases, the condition can result in coma and death [6].

It is believed that in living things, the hematological system is extremely susceptible to lead toxicity [7]. Blood is an important marker of physiological changes since it is a vital connective tissue that is constantly undergoing metabolic activities. Changes in hematological parameters might therefore function as early and trustworthy markers of the toxicity of lead on different tissues. The hemopoietic system has been demonstrated to be particularly negatively impacted by lead exposure [8]. As erythrocytes have a significant affinity for lead, they usually contain the majority of the lead metal in the blood. Lead causes elevated levels of aminolevulinic acid (ALA) and excessive formation of reactive oxygen species (ROS) by interfering with the activity of δ-aminolevulinic acid dehydratase (ALAD), which in turn impairs heme synthesis [9]. Hemoglobin oxidation results from ROS-induced membrane peroxidation, which in turn causes RBC hemolysis and, eventually, anemia.

Chelation therapy is the mainstay of current therapeutic techniques to mitigate lead toxicity. However, chelation therapy has severe limits and side effects, even though it is effective in lowering lead levels [10]. In this regard, there is increasing interest in investigating substitute treatments that may provide a safer and more efficient way to treat lead-induced poisoning. Antioxidant treatment is one of the best alternatives that has shown promise. Synergism of synthetic antioxidants can prove to be beneficial in counteracting the free radical mediated oxidative stress in lead toxicity. Herbal treatments have garnered interest due to their possible therapeutic benefits, in addition to synthetic antioxidants [11]. Being well-known for its therapeutic properties and long use in traditional medicine, *Bacopa monnieri* has showed great potential as an antioxidant and neuroprotective agent [12, 13]. *Bacopa monnieri* is a rich source of pharmacologically active phytochemicals, such as luteolin and apigenin, flavonoids, bacosides, and bacopasaponins. It has also demonstrated strong free radical scavenging action and antioxidant properties [14]. Because of this, it can be a great alternative therapeutic agent for treatment and prevention of oxidative stress and hematological toxicity induced by heavy metal lead.

This study was undertaken to thoroughly assess the ameliorative effects of synthetic antioxidants (N-Acetyl Cysteine, Ascorbic acid, Tocopheryl acetate and Thiamine Mixture) as a combinational therapy approach and *Bacopa monnieri* as herbal antioxidant therapy approach in a model of lead-induced

hematological toxicity in male Swiss albino mice. In comparative analysis of effectiveness of lead toxicity treatment with synthetic and herbal antioxidants, the research hopes to shed light on the mechanism *via* which these ameliorative agents lessen the harmful effects of lead and pinpoint the best therapeutic approaches for treatment of lead poisoning. The results of this study should have a major impact on public health, especially in areas where lead exposure is still a major concern. Administration of *Bacopa monnieri* could also prove to be new, safe, and very effective treatment for lead intoxication.

2. Materials and Methods

2.1 Experimental Animals

Cadila Pharmaceuticals in Dholka, India, supplied healthy male Swiss Albino Mice (*Mus musculus*) weighing between 30–35 gram and aged 4–5 weeks. The animals were cared for in accordance with Animal Maintenance and Registration No. 167/1999/CPCSEA, which was given by the Ministry of Social Justice and Empowerment of the Indian government.

2.2 Animal Care

The animals were housed in the animal facility of Department of Zoology, Gujarat University following the criteria established by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Individually housed in plastic enclosures, they were kept under a controlled 12-hour light-dark cycle at a temperature of 26 ± 2°C, following authorized experimental methods approved by the institutional animal ethics committee. The animals were fed normal commercial laboratory chow (Amrut laboratory animal feed from Pranav Agro Industries, which included 3625 kcal/kg energy, 22.10% crude protein, 4.10% crude oil, 4.45% crude fiber, 9.10% ash, and 0.75% sand silica) and tap water *ad libitum*. All animals were given a one-week acclimatization period before beginning treatment. The treatment was given orally once a day using a cannula attached to a hypodermic syringe, before their feeding routine.

2.3 Chemicals

Analytical grade reagents with 99% purity were used in this study. The required chemicals, such as Tocopheryl acetate (Vitamin E), Ascorbic acid (Vitamin C), N-acetyl Cysteine (NAC) and Thiamine (Vitamin B_1) were obtained from reliable standard companies like Hi-Media, Merck Laboratory Pvt. Ltd., India and Sigma-Aldrich St. Louis, MO, USA.

2.4 Preparation of Reagents

Lead acetate trihydrate, Ascorbic acid, N-Acetyl Cysteine, Thiamine, etc. reagents utilized for present *in vivo* study were prepared by dissolving in double distilled water in order to obtain the solutions of required concentrations. Tocopheryl acetate was given directly to the subjects in this study as Evion-emulsion syrup, which was obtained from Merck Laboratory.

2.5 Plant Collection and Preparation of Plant Extract

In December, whole plant of *Bacopa monnieri* was harvested from the botanical garden of Gujarat University, Ahmedabad, India. The plant was identified and authenticated by the University's botany department, and a voucher specimen was preserved in the herbarium. Following collection, the plant was washed with double-distilled water, cut into small pieces, and air-dried in a shaded, dust-free area for one week at room temperature. After drying, the plant material was ground into a coarse powder. Soxhlet extraction was then conducted with 10 grams of this powder using 100 ml of 90% ethanol at 80°C for 15 hours. The crude extract was concentrated, air-dried, and stored at -10°C in a dark container for future analysis.

2.7 Characterization of Bacopa monnieri Extract by HPLC Analysis

The primary bioactive components of the *Bacopa monnieri* ethanolic extract were identified and characterized using High Performance Liquid Chromatography (HPLC) with a Shimadzu Model LC 2010CHT and LC Solution software. A C-18 Phenomenex column (150 mm x 4.6 mm, 5 µm) was included in the HPLC system. 500 mg of the ethanolic extract was dissolved in 50 ml of HPLC-grade methanol in a 100 ml volumetric flask to prepare the sample. The extract was subjected to sonication for 10–15 minutes, cooled and made up to 100 ml with methanol. A 0.45 µm membrane filter was then used to filter the mixture. The plant extract was injected into the HPLC column in a 20 µl aliquot, and eluted using a mobile phase that contained 0.25% orthophosphoric acid in water and acetonitrile at a flow rate of 1.5 ml/min. The column oven was maintained at 25°C. The separation was carried out using gradient chromatographic technique for 45 minutes of run time. The separated components were detected using PDA detector at the wavelength of 205nm. The compounds in the chromatogram have been identified by comparing their retention time with the standard curve [15].

2.8 Experimental Design

Experimental design comprised of a total of 100 healthy adult Swiss strain male albino mice equally divided into ten experimental groups. Group-I to VIII were orally treated for 4-weeks. Group I animals were maintained without any treatment and represented untreated control group. They were given free access to feed and water *ad libitum*. Group II animals were orally administered mixture of synthetic antioxidants (N-Acetyl Cysteine-5.5mM/kg/day, Ascorbic acid-200mg/kg/day, Tocopheryl acetate-160mg/kg/day and Thiamine-30mg/kg/day) which served as synthetic antidote control group. Group III animals were orally treated with ethanolic extract of *Bacopa monnieri* (10mg/kg/day) which served as herbal antidote control group. Group IV, V and VI orally received low dose (160mg/kg/day), mid dose (266mg/kg/day) and high dose (320mg/kg/day) of lead acetate, respectively. Group VII animals were orally administered high dose (320mg/kg/day) of lead acetate along with synthetic antioxidants mixture in prescribed dosage for synergistic study. Group VIII animals were orally treated with high dose (320mg/kg/day) of lead acetate along with *Bacopa monnieri* ethanolic extract (10mg/kg/day) for synergistic study. Group IX animals were administered high dose (320mg/kg/day) of lead acetate along with prescribed dosage of synthetic antioxidants mixture for 4-weeks and supplementation of synthetic antioxidants was continued for next 2-weeks in order to analyze curative effect of synthetic antidote.

Group X animals were treated with high dose (320mg/kg/day) of lead acetate along with *Bacopa monnieri* extract (10mg/kg/day) for 4-weeks and supplementation of plant extract was continued for next 2-weeks in order to analyze the curative potential of herbal antidote. With the use of pilot studies and the body of available literature, the experimental dosages for lead acetate and antidotes were chosen based on LD_{50} values and laboratory standardization [16–18]. Table 1 provides a full description of the *in vivo* study's experimental design.

Table 1
Experimental Design for *In Vivo* Study

Type of Study Groups	Experimental Group No.	Experimental Groups	No. of Animals	Treatment Duration	Day of Autopsy
Control Study	Group - I	Untreated Control [UC]	10	4 - Weeks	29th
Groups	Group - II	Synthetic Antidote Control [A-I] (NAC-5.5mM/kg/day + Ascorbic acid-200mg/kg/day + Tocopheryl acetate-160mg/kg/day + Thiamine-30mg/kg/day)	10	4 - Weeks	29th
	Group - III	Herbal Antidote Control [A-II] (*Bacopa monnieri* ethanolic Extract) (10mg/kg/day)	10	4 - Weeks	29th
Toxin Exposed	Group - IV	Lead Acetate Low Dose [LD] (160mg/kg/day)	10	4 - Weeks	29th
Groups	Group - V	Lead Acetate Mid Dose [MD] (266mg/kg/day)	10	4 - Weeks	29th
	Group - VI	Lead Acetate High Dose [HD] (320mg/kg/day)	10	4 - Weeks	29th
Synergistic Study	Group - VII	Lead Acetate High Dose + Synthetic Antidote [HD + A-I]	10	4 - Weeks	29th
Groups	Group -VIII	Lead Acetate High Dose + Herbal Antidote [HD + A-II]	10	4 - Weeks	29th
Curative Study	Group - IX	Lead Acetate High Dose and Synthetic Antidote (4-Weeks) + Only Synthetic Antidote (2-Weeks) [HD + A-I] ©	10	6 - Weeks	43rd
Groups	Group - X	Lead Acetate High Dose and Herbal Antidote (4-Weeks) + Only Herbal Antidote (2-Weeks) [HD + A-II] ©	10	6 - Weeks	43rd

2.9 Sample Collection

After an experimental period of four weeks for synergistic investigation and six weeks for curative study animals were fasted overnight and next day sacrificed by subjecting them to an excessive dosage of diethyl ether. For the purpose of estimating hematological parameters, blood samples from each experimental group were obtained by cardiac puncture and preserved in EDTA (Ethylene Diamine Tetra Acetic Acid) vials. Collected blood samples were stored at -20°C for further analysis.

2.10 Experimental Parameters

In this study, hematological parameters of Hemoglobin (Hb), Total RBC, Total WBC, Platelets, Hematocrit (HCT), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) were analyzed from blood samples of all experimental animals using automated hematology analyzer [Model: CELL-DYN 3700 SYSTEM] working on the principle of measurement of blood parameters by the Electrical Impedance method and equipped with an automated sample loader module.

2.11 Statistical Analysis

The *in vivo* study data were statistically analysed using GraphPad Prism software, version 5.03. The results were represented as mean ± standard error of mean (SEM) for each parameter (n = 10). Comparison among different groups was made by One-way Analysis of Variance (ANOVA) followed by Tukey's *post hoc* test. At the significance level of $p < 0.05$, a statistically significant difference was considered.

3. Results

3.1 Characterization of the Ethanolic Extract of Bacopa monnieri

HPLC analysis of an ethanolic extract of *Bacopa monnieri* confirmed the presence of considerable number of bioactive phytochemicals in the forms of apigenin and luteolin flavonoids, as well as derivatives of *jujubogenin* in the forms of bacosides and bacopasaponins (Fig. 1).

3.2 Hematological Parameters

Results of present *in vivo* study revealed statistically significant ($p < 0.001$) alteration in hematological parameters of lead administered groups of animals as compared to control groups. Results emphasized statistically significant decline in Hemoglobin level (Table-2), Total RBC count, Platelet count (Table-3), Hematocrit value, Mean corpuscular volume and Means corpuscular hemoglobin level (Table-4) as well as increment in Total WBC count (Table-3) in lead acetate exposed Groups IV, V and VI of mice as compared to control groups. The toxic effect observed was dose-dependent in manner (Table-5).

Table 2 presents the effect of lead and antioxidants on blood hemoglobin levels in control and treated mice *in vivo*. The untreated control group (Group I) had an average hemoglobin level of 13.70 ± 0.060 g/dL. Mice administered with a synthetic antioxidant mixture (Group II) showed a hemoglobin level of 13.40 ± 0.075 g/dL, while those treated with *Bacopa monnieri* extract (Group III) had a level of 13.50 ± 0.080 g/dL. In contrast, mice exposed to low, mid, and high doses of lead acetate (Groups IV, V, and VI) exhibited significantly lower hemoglobin levels, with values of 11.40 ± 0.063 g/dL, 8.29 ± 0.009 g/dL, and 6.60 ± 0.058 g/dL, respectively ($p < 0.001$). Co-administration of high-dose lead with synthetic antioxidants (Group VII) or *Bacopa monnieri* extract (Group VIII) protected against reduction and maintained hemoglobin levels to 11.90 ± 0.092 g/dL and 12.50 ± 0.071 g/dL, respectively. Additionally, curative study groups with high-dose lead-exposure and co-treatment with synthetic antioxidants (Group IX) or *Bacopa monnieri* extract (Group X) for 6-weeks duration resulted in restoration of hemoglobin levels to 13.10 ± 0.042 g/dL and 13.30 ± 0.080 g/dL, respectively.

Table 2
Effect of Lead and Antioxidants on Blood Hemoglobin Level of Control and Treated Mice *In Vivo*

Group No.	Experimental Groups	Experimental Parameter
		Hemoglobin (Hb) g/dL
Group-I	Untreated Control (UC)	13.70 ± 0.060
Group-II	Synthetic Antioxidants Mixture (A-I)	13.40 ± 0.075
Group-III	*Bacopa monnieri* Extract (A-II)	13.50 ± 0.080
Group-IV	Lead Acetate Low Dose (LD)	11.40 ± 0.063[a*]
Group-V	Lead Acetate Mid Dose (MD)	8.29 ± 0.009[a*]
Group-VI	Lead Acetate High Dose (HD)	6.60 ± 0.058[a*]
Group-VII	HD + A-I	11.90 ± 0.092[b*]
Group-VIII	HD + A-II	12.50 ± 0.071[b*]
Group-IX	HD + A-I ©	13.10 ± 0.042[b*]
Group-X	HD + A-II ©	13.30 ± 0.080[b*]
p – Values: [*]p < 0.001		
Values are expressed as mean ± SEM; (n = 10)		
[a] as compared to control group		
[b] as compared to toxin (high dose lead acetate) exposed group (Group VI)		

No significant difference was noted between untreated control and antidotes control groups

Table 3 illustrates the impact of lead exposure and antioxidant treatments on various hematological parameters in mice. In the untreated control group (Group I), baseline values were Total RBC at 7.67 ± 0.010 M/µl, Total WBC at 0.82 ± 0.002 K/µl, and Platelets at 1093.0 ± 1.578 K/µl. The synthetic antioxidants mixture (Group II) and *Bacopa monnieri* extract (Group III) had similar values to the control group, indicating no significant changes. However, lead acetate exposure significantly altered these parameters. Mice exposed to low dose lead acetate (Group IV) exhibited reduced Total RBC (5.03 ± 0.002 M/µl), increased Total WBC (1.54 ± 0.009 K/µl), and decreased Platelets (962.00 ± 0.955 K/µl). Mid dose (Group V) and high dose lead acetate (Group VI) resulted in even more pronounced reductions in Total RBC, with values of 4.84 ± 0.004 M/µl and 3.12 ± 0.002 M/µl, respectively, and Platelets dropping to

610.00 ± 0.558 K/µl and 466.50 ± 0.004 K/µl. Total WBC counts increased to 2.01 ± 0.002 K/µl and 2.36 ± 0.001 K/µl in these groups. Co-administration of antioxidants improved these hematological parameters, with high dose lead plus synthetic antioxidants (Group VII) showing Total RBC at 7.05 ± 0.001 M/µl, Total WBC at 0.98 ± 0.001 K/µl, and Platelets at 966.00 ± 1.000 K/µl, and high dose lead with *Bacopa monnieri* (Group VIII) showing even better results with Total RBC at 7.12 ± 0.001 M/µl, Total WBC at 0.97 ± 0.001 K/µl, and Platelets at 980.90 ± 1.149 K/µl. Continued treatment with antioxidants for curative study (Groups IX and X) showed further improvements, with Total RBC returning to 7.63 ± 0.001 M/µl and 7.64 ± 0.001 M/µl, Total WBC decreasing to 0.92 ± 0.002 K/µl and 0.91 ± 0.001 K/µl, and Platelets increasing to 1058.0 ± 1.174 K/µl and 1075.0 ± 0.919 K/µl.

Table 3
Effect of Lead and Antioxidants on Hematological Parameters of Control and Treated Mice *In Vivo*

Group No.	Experimental Groups	Experimental Parameters		
		Total RBC M/µl	Total WBC K/ µl	Platelets K/µl
Group-I	Untreated Control (UC)	7.67 ± 0.010	0.82 ± 0.002	1093.0 ± 1.578
Group-II	Synthetic Antioxidants Mixture (A-I)	7.64 ± 0.010	0.83 ± 0.001	1086.0 ± 0.856
Group-III	*Bacopa monnieri* Extract (A-II)	7.66 ± 0.008	0.82 ± 0.001	1089.0 ± 0.895
Group-IV	Lead Acetate Low Dose (LD)	5.03 ± 0.002[a*]	1.54 ± 0.009[a*]	962.00 ± 0.955[a*]
Group-V	Lead Acetate Mid Dose (MD)	4.84 ± 0.004[a*]	2.01 ± 0.002[a*]	610.00 ± 0.558[a*]
Group-VI	Lead Acetate High Dose (HD)	3.12 ± 0.002[a*]	2.36 ± 0.001[a*]	466.50 ± 0.004[a*]
Group-VII	HD + A-I	7.05 ± 0.001[b*]	0.98 ± 0.001[b*]	966.00 ± 1.000[b*]
Group-VIII	HD + A-II	7.12 ± 0.001[b*]	0.97 ± 0.001[b*]	980.90 ± 1.149[b*]
Group-IX	HD + A-I ©	7.63 ± 0.001[b*]	0.92 ± 0.002[b*]	1058.0 ± 1.174[b*]
Group-X	HD + A-II ©	7.64 ± 0.001[b*]	0.91 ± 0.001[b*]	1075.0 ± 0.919[b*]
p – Values: [*]p < 0.001				
Values are expressed as mean ± SEM; (n = 10)				
[a] as compared to control group				
[b] as compared to toxin (high dose lead acetate) exposed group (Group VI)				

No significant difference was noted between untreated control and antidotes control groups

Table 4 presents the impact of lead exposure and antioxidant treatments on various hematological parameters in mice. In the untreated control group (Group I), the hematocrit (HCT) was 35.80 ± 0.004%, mean corpuscular volume (MCV) was 50.60 ± 0.086 fL, and mean corpuscular hemoglobin (MCH) was 17.40 ± 0.041 pg. The synthetic antioxidants mixture (Group II) and *Bacopa monnieri* extract (Group III) showed similar values to the control group. Lead acetate exposure led to significant reductions in these parameters: low dose (Group IV) resulted in HCT at 24.00 ± 0.387%, MCV at 47.00 ± 0.258 fL, and MCH at

16.90 ± 0.027 pg; mid dose (Group V) showed HCT at 21.60 ± 0.074%, MCV at 45.50 ± 0.073 fL, and MCH at 16.70 ± 0.056 pg; and high dose (Group VI) resulted in HCT at 18.10 ± 0.107%, MCV at 44.00 ± 0.152 fL, and MCH at 15.50 ± 0.073 pg. Co-administration of high dose lead with antioxidants demonstrated improved parameters: high dose lead with synthetic antioxidants (Group VII) had HCT at 34.50 ± 0.081%, MCV at 49.00 ± 0.106 fL, and MCH at 16.20 ± 0.056 pg; high dose lead and *Bacopa monnieri* (Group VIII) showed HCT at 34.80 ± 0.104%, MCV at 49.10 ± 0.087 fL, and MCH at 16.50 ± 0.073 pg. Continued treatment with antioxidants for curative study of 6-weeks (Groups IX and X) further improved values, with HCT at 35.10 ± 0.032% and 35.30 ± 0.101%, MCV at 50.00 ± 0.191 fL and 50.00 ± 0.134 fL, and MCH at 16.90 ± 0.095 pg and 17.10 ± 0.101 pg, respectively.

Table 4

Effect of Lead and Antioxidants on Hematological Parameters of Control and Treated Mice *In Vivo*

Group No.	Experimental Groups	Experimental Parameters		
		HCT (%)	MCV (fL)	MCH (pg)
Group-I	Untreated Control (UC)	35.80 ± 0.004	50.60 ± 0.086	17.40 ± 0.041
Group-II	Synthetic Antioxidants Mixture (A-I)	35.90 ± 0.001	50.10 ± 0.111	17.00 ± 0.094
Group-III	*Bacopa monnieri* Extract (A-II)	35.90 ± 0.002	50.40 ± 0.067	17.30 ± 0.047
Group-IV	Lead Acetate Low Dose (LD)	24.00 ± 0.387[a*]	47.00 ± 0.258[a*]	16.90 ± 0.027[a*]
Group-V	Lead Acetate Mid Dose (MD)	21.60 ± 0.074[a*]	45.50 ± 0.073[a*]	16.70 ± 0.056[a*]
Group-VI	Lead Acetate High Dose (HD)	18.10 ± 0.107[a*]	44.00 ± 0.152[a*]	15.50 ± 0.073[a*]
Group-VII	HD + A-I	34.50 ± 0.081[b*]	49.00 ± 0.106[b*]	16.20 ± 0.056[b*]
Group-VIII	HD + A-II	34.80 ± 0.104[b*]	49.10 ± 0.087[b*]	16.50 ± 0.073[b*]
Group-IX	HD + A-I ©	35.10 ± 0.032[b*]	50.00 ± 0.191[b*]	16.90 ± 0.095[b*]
Group-X	HD + A-II ©	35.30 ± 0.101[b*]	50.00 ± 0.134[b*]	17.10 ± 0.101[b*]
p – Values: [*]p < 0.001				
Values are expressed as mean ± SEM; (n = 10)				
[a] as compared to control group				
[b] as compared to toxin (high dose lead acetate) exposed group (Group VI)				

No significant difference was noted between untreated control and antidotes control groups

Table – 5 Gross Effect of Lead and Antioxidants on Mice Blood *In Vivo.* (% of difference with respect to their control groups)

Sr. No.	Biochemical Parameters	Group IV (LD)	Group V (MD)	Group VI (HD)	Group VII (HD + A-I)	Group VIII (HD + A-II)	Group IX (HD + A-I) ©	Group X (HD + A-II) ©
1.	Hemoglobin	16.79	39.49	51.82	13.14	8.76	4.38	2.92
2.	Total RBC	34.42	36.90	59.32	8.08	7.17	0.52	0.39
3.	Total WBC	87.80*	145.12*	187.80*	19.51*	18.29*	12.20*	10.98*
4.	Platelets	11.99	44.19	57.32	11.62	10.26	18.13	1.65
5.	Hematocrit	32.96	39.66	49.44	3.63	2.79	1.96	1.40
6.	Mean Corpuscular Volume	7.11	10.08	13.04	3.16	2.96	1.19	1.19
7.	Mean Corpuscular Hemoglobin	2.87	4.02	10.92	6.90	5.17	2.87	1.72

All values are expressed in % of decrease or *increase

Table 5 summarizes the gross effects of lead exposure and antioxidant treatments on various blood parameters in mice, expressed as the percentage difference compared to control groups. Lead acetate administration resulted in significant alterations: low dose (Group IV) led to a decrease in hemoglobin by 16.79%, decrease in total RBC by 34.42%, increase in total WBC by 87.80%, decrease in platelets by 11.99%, decrease in hematocrit by 32.96%, decrease in mean corpuscular volume (MCV) by 7.11%, and decrease in mean corpuscular hemoglobin (MCH) by 2.87% as compared to control group of animals. The mid dose (Group V) showed a decrease in hemoglobin by 39.49%, decrease in total RBC by 36.90%, increase in total WBC by 145.12%, decrease in platelets by 44.19%, decrease in hematocrit by 39.66%, decrease in MCV by 10.08%, and decrease in MCH by 4.02% as compared to control group. The high dose (Group VI) resulted in a decrease in hemoglobin by 51.82%, decrease in total RBC by 59.32%, increase in total WBC by 187.80%, decrease in platelets by 57.32%, decrease in hematocrit by 49.44%, decrease in MCV by 13.04%, and decrease in MCH by 10.92% as compared to control group of mice. In contrast, co-administration of high dose lead with synthetic antioxidants (Group VII) and high dose lead with *Bacopa monnieri* (Group VIII) mitigated these effects and protected against reductions in hemoglobin, total RBC, and other parameters being less pronounced. For instance, Group VII (HD + A-I) showed a decrease in hemoglobin by 13.14%, decrease in total RBC by 8.08%, increase in total WBC by 19.51%, decrease in platelets by 11.62%, decrease in hematocrit by 3.63%, decrease in MCV by 3.16%, and decrease in MCH by 6.90%. Group VIII (HD + A-II) displayed a decrease in hemoglobin by 8.76%, decrease in total RBC by 7.17%, increase in total WBC by 18.29%, decrease in platelets by 10.26%, decrease in hematocrit by 2.79%, decrease in MCV by 2.96%, and decrease in MCH by 5.17% as

compared to control group. Curative treatment with antioxidants (Groups IX and X) showed even smaller deviations, with Group IX (HD + A-I ©) and Group X (HD + A-II ©) exhibiting minimal differences compared to controls, indicating significant ameliorative effects.

Table 6 presents the gross effects of lead exposure and antioxidant treatments on mice blood parameters, expressed as percentage differences with respect to high dose lead acetate exposed Group VI. In Group VI mice, which were exposed to high dose lead acetate, significant changes were observed in various hematological parameters. In Group VI animals, the hemoglobin level was reduced by 51.82%, while total RBC decreased by 59.32%, and total WBC increased by 187.80% as compared to control group. Platelets decreased by 57.32%, hematocrit dropped by 49.44%, mean corpuscular volume (MCV) fell by 13.04%, and mean corpuscular hemoglobin (MCH) was reduced by 10.92% as compared to control group. In contrast, the addition of antioxidants showed considerable protection against alteration in hematological parameters. For instance, Group VII (HD + A-I) demonstrated an increase in hemoglobin by 80.30%, an increase in total RBC by 125.96%, and a decrease in total WBC by 58.47% compared to the high dose lead group. Platelets and hematocrit also improved by 107.07% and 90.61%, respectively in Group VII animals as compared to Group VI. MCV and MCH showed decrease by 11.36% and 4.52%, respectively in Group VII animals as compared to Group VI. Group VIII (HD + A-II) displayed an even more pronounced effect, with an increase in hemoglobin by 89.39%, an increase in total RBC by 128.21%, and a decrease in total WBC by 58.90% as compared to Group VI. Platelets and hematocrit increased by 110.27% and 92.27%, respectively, while MCV and MCH decreased by 11.59% and 6.45%, respectively in Group VIII animals as compared to Group VI. Groups IX and X, which received continuous antioxidant treatment after withdrawal of lead acetate showed near-normalization of these parameters to control group. Group IX (HD + A-I ©) achieved an increase in hemoglobin by 98.48%, an increase in total RBC by 144.55%, and a decrease in total WBC by 61.02% as compared to Group-VI. Platelets and hematocrit increased by 126.80% and 93.92%, respectively, with MCV and MCH decrease by 13.64% and 9.03%, respectively in Group IX animals as compared to Group VI. Similarly, Group X (HD + A-II ©) exhibited an increase in hemoglobin by 101.52%, an increase in total RBC by 144.87%, and a decrease in total WBC by 61.44% in Group X animals as compared to Group VI. Platelets and hematocrit rose by 130.44% and 95.03%, respectively, while MCV and MCH showed decrease by 13.64% and 10.32%, respectively in Group X animals as compared to Group VI. These results highlight the efficacy of antioxidants in mitigating the adverse effects of high dose lead exposure on blood parameters.

Table – 6 Gross Effect of Lead and Antioxidants on Mice Blood *In Vivo.* (% of difference with respect to high dose lead acetate exposed groups)

Sr. No.	Biochemical Parameters	Group VI (HD) (Relative to Control)	Group VII (HD + A-I)	Group VIII (HD + A-II)	Group IX (HD + A-I) ©	Group X (HD + A-II) ©
1.	Hemoglobin	51.82	80.30*	89.39*	98.48*	101.52*
2.	Total RBC	59.32	125.96*	128.21*	144.55*	144.87*
3.	Total WBC	187.80*	58.47	58.90	61.02	61.44
4.	Platelets	57.32	107.07*	110.27*	126.80*	130.44*
5.	Hematocrit	49.44	90.61*	92.27*	93.92*	95.03*
6.	Mean Corpuscular Volume	13.04	11.36*	11.59*	13.64*	13.64*
7.	Mean Corpuscular Hemoglobin	10.92	4.52*	6.45*	9.03*	10.32*

All values are expressed in % of decrease or *increase

Results of present study has emphasized significant role of co-administration of synthetic antioxidant mixture or *Bacopa monnieri* extract in high dose lead-exposed mice groups of synergistic study. Group VII and Group VIII revealed statistically significant protective effect against alteration in hematological parameters as depicted by increase in hemoglobin (80.30% and 89.39% respectively), Total RBC (125.96% and 128.21% respectively), Platelets (107.07% and 110.27% respectively), Hematocrit (90.61% and 92.27% respectively), MCV (11.36% and 11.59% respectively), MCH (4.52% and 6.45% respectively) and reduction in Total WBC (58.47% and 58.90% respectively) as compared to high dose lead exposed mice Group VI (Table-6).

Comparing the mice in the curative study groups IX and X to the mice in the high dose lead administered group VI, the curative study groups animals showed statistically significant amelioration against reductions in hemoglobin, total RBC, platelets, hematocrit, MCV, and MCH as well as elevation in total WBC. Groups VII and VIII, which received high doses of lead with synthetic and herbal antidotes respectively, did not exhibit the same improvements as compared to Groups IX and X in the curative study. Curative study Groups IX and X depicted significant increase in Hemoglobin content (98.48% and 101.52% respectively), Total RBC (144.55% and 144.87% respectively), Platelet count (126.80% and 130.44% respectively), Hematocrit value (93.92% and 95.03% respectively), MCV (13.64% and 13.64% respectively), MCH (9.03% and 10.32% respectively) and decrease in Total WBC (61.02% and 61.44% respectively) as compared to their respective high dose lead exposed Group VI (Table-6). These findings demonstrated that synthetic antioxidants mixture and *Bacopa monnieri* extract administration for 2-weeks aftermath withdrawal of lead acetate in respective group IX and Group X of animals resulted into

normalization of all hematological parameters nearest to control group with more pronounced and remarkable curative effect of herbal antidote as compared to synthetic antioxidants mixture against lead induced hematological toxicity.

The findings of the study demonstrated that the co-administration of a high dose of lead acetate with either a synthetic antioxidant mixture or *Bacopa monnieri* extract produced notable protective effects against alteration in the hematological damage caused by lead. In particular, the body's capacity to combat oxidative stress was improved when lead acetate and synthetic antioxidants were combined, leading to improvements in important blood parameters like hemoglobin and red blood cell counts due to synergistic effect. In a similar vein, significant protection against lead toxicity was obtained by combining lead acetate with *Bacopa monnieri* extract, which is well known for its strong antioxidant qualities. The abundant range of flavonoids and bioactive substances included in the plant extract facilitated the neutralization of reactive oxygen species leading to the restoration of normal hematological function. These results show that both therapeutic approaches greatly reduce the negative consequences of exposure to lead, indicating that they may be useful as therapeutic agents to prevent or ameliorate lead-induced oxidative damage and enhance blood health in general.

4. Discussion

The findings of current *in vivo* study showed that, as compared to control groups, mice administered with lead acetate had significant alterations in hematological parameters of hemoglobin, total RBC count, total WBC count, platelets, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin. Exposure to lead has a substantial effect on biosynthesis of heme, peroxidase, cytochrome and catalase [19]. Lead obstructs a number of enzymatic stages in heme biosynthesis pathway; most notably delta-aminolevulinic acid dehydratase (ALAD), which is extremely vulnerable to the harmful effects of lead. Due to the fact that 70% of blood lead binds to ALAD, lead interferes with the enzyme's ability to function by directly attaching to the -SH groups that are necessary for the catalytic activity of the enzyme [20]. ALAD catalyzes formation of porphobilinogen from delta-aminolevulinic acid (ALA) and ferrochelatase, which incorporates iron into protoporphyrin. Failure to condense two molecules of delta-aminolevulinic acid (ALA) for formation of porphobilinogen by ALAD and insertion of iron into protoporphyrin by ferrochelatase results in depressed heme formation [21]. Heme synthesis impairment prevents the improvement in red blood cell population. Lead is also known to readily inhibit porphobilinogen synthase in erythrocytes responsible for regulating H_b synthesis *in vivo* and *in vitro* [22]. The decline in Hb aftermath lead administration was also noted in other scientific studies in accordance of our results [23, 24]. Because of their strong affinity for lead, red blood cells usually contain most of the circulating lead in the blood stream [25]. Due to a number of key factors, including the inhibition of heme and hemoglobin biosynthesis, induction of hemoglobin auto-oxidation, alteration of erythrocyte morphology by direct interaction of lead metal with RBC membranes, induction of lipid peroxidation [26] and limited ability of RBCs to repair their damaged components, a severe oxidative stress is generated in lead-exposed RBCs, which ultimately results in their decreased survival (Caylak *et al.* 2008).

Lead exposure may account for increasing fragility of erythrocytes [27] and inducing hemolysis as well as origin of defective cells that can be eliminated by spleen [28]. Lead's inhibitory effect on the essential erythrocyte enzyme glucose-6-phosphate dehydrogenase and the erythrocytes' shortened life span [29] could be the cause of the decline in hemoglobin and total red blood cell count. Results of lead toxicity mediated reduction in RBC observed in our study corroborates with the findings of other scientific researchers [30, 31]. Reduced heme synthesis, which results in anemia, is one of the most significant impacts of lead exposure on the hematological system. Decrease in red blood cell survival, increase in the rate of RBC destruction, decrease in the rate of RBC synthesis, decrease in the production of hemoglobin in the bone marrow, or combination of all these mechanisms may be responsible for the development of anemia in lead-induced toxicity [32].

Given that the red blood cell count, hemoglobin concentration, and packed cell volume all affect the validity of these indexes, lead acetate's toxic effects on hemoglobin concentration and red blood cell count may have contributed to the changes in mean corpuscular volume and mean corpuscular hemoglobin concentration observed in this study. Following exposure to lead, the resulted alteration in Platelet count, hematocrit, MCV, and MCH were consistent with findings of prior studies [33]. Lead-induced tissue inflammation could be the cause of the elevated Total WBC levels. Increase in Total WBC count obtained in our study also corroborates with the findings of other researchers [34, 35].

Lead induced alterations in hematological parameters were ameliorated by co-supplementation of mixture of synthetic antioxidants comprising of Ascorbic acid, Tocopheryl acetate, N-acetyl cysteine and Thiamine (Group-VII and IX) or ethanolic extract of *Bacopa monnieri* (Group-VIII and X). Vitamin-C has been reported as a good antioxidant to overcome lead induced hematotoxicity (Sharma and Panwar, 2013). Animals are more vulnerable to the hemolytic effects of lead poisoning when they are deficient in Vitamin-E. Our results corroborate with the reports of other researchers which highlight that, in lead-treated animals, Vitamin-C and Vitamin-E significantly increased packed cell volume, hemoglobin concentration, red blood cell count, and neutrophil percentage while significantly decreasing white blood cell count and lymphocyte percentage. Kamruzmman (2006) and Haque (2005) also reported that lead content in blood, liver and kidney was significantly reduced following administration of Vitamin-C and Vitamin-E. N-Acetyl Cysteine exhibited antioxidant capacity against lead toxicity via promoting maintenance of intracellular reduced glutathione levels and scavenging of free radicals [33, 36]. Thiamine has been shown to have antioxidant potential because of its ability to scavenge free radicals and ability to form complex with lead metal resulting into its excretion.

It has been demonstrated that *Bacopa monnieri*, a multi-purpose traditional herb with a wide range of medicinal uses, exhibits antioxidant effects by chelating metal ions, breaking oxidative chain reactions, scavenging reactive oxygen species such as peroxides, superoxides, and hydroxyl radicals, and enhancing the activities of antioxidative defense enzymes [37]. It also functions as a potent blood purifier. Phytochemical screening of *Bacopa monnieri* extract has revealed presence of variety of primary bioactive constituents. These included flavonoids such as glucoronyl-7-apigenin and glucortonyl-7-luteolin, alkaloids, D-mannitol, potassium salts, and steroids, as well as saponins such as *hersaponin,*

jujubogenin, and *pseudojujubogenin* [32]. Numerous studies revealed that the presence of distinctive active constituent, a dammarane type tri-terpenoid saponin known as "Bacoside-A" as chemical derivative of *jujubogenin* in *Bacopa monnieri* was primarily responsible for the pharmacological actions of plant, including its antioxidant activity. HPLC analysis data also supported the presence of these phytochemicals in *Bacopa monnieri* extract in our study.

Ameliorative potential of the synthetic antioxidant's mixture and *Bacopa monnieri* exhibited protective role against lead induced hematotoxicity in synergistic study groups. A significant amelioration observed in all studied hematological parameters of curative study groups receiving 4-weeks exposure of lead acetate and 6-weeks antidote treatment suggested that lead toxicity mediated oxidative stress *in vivo* could be reversible by exogenous supplementation of pharmacological manipulations rich in antioxidant potential. The antioxidant qualities of both synthetic and herbal antidotes may be responsible for lowering of lipid peroxidation, preserving the activity of delta-aminolevulinic acid dehydratase (ALAD), stabilizing the plasma membrane of red blood cells, and lowering hemolysis in these cells, which in turn preserve blood hemoglobin, total WBC count, platelets, MCV, and MHC nearest to the control group. Therefore, by reducing oxidative stress and ensuring the preservation of antioxidant equilibrium in Swiss albino mice cells, synthetic antioxidants and *Bacopa monnieri* functioned as mitigating agents against lead-induced hematological toxicity. This work clearly shows that lead exposure severely exaggerated the hematological system in Swiss albino mice by inducing oxidative stress, which changes blood cell membrane permeability and oxidizes structural proteins. Lead co-administration with synthetic antioxidants or *Bacopa monnieri* extract provided significant protection against this toxicity, with the latter proving to be more effective. This therapeutic efficacy is most likely owing to the wide range of active phytochemicals present in *Bacopa monnieri*, which function as powerful free radical scavengers. The findings indicate that lead-induced hematological damage can be both transient and reversible through use of *Bacopa monnieri* and synthetic antioxidants, emphasizing their ameliorative potential as safer antidotes against lead toxicity. This study provides important information for establishing novel treatment strategies in occupational toxicology and pharmacology to combat lead poisoning.

Declarations

Acknowledgement

Dr. FS is thankful to the Department of Life-Science, University School of Sciences, Gujarat University, Gujarat, India for providing lab and research facility. The authors extend their appreciation to the Deanship of Scientific Research at Northern Border University, Arar, KSA for funding this research work through the project number "NBU-FFR-2024-1329-16". The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the General Research Project under the grant number (RGP 2/252/45).

Competing Interests: Authors have declared that there are no conflicts of interests.

Ethical approval: N/A

Informed consent: N/A

Author Contributions: Conceptualization, Writing- Original Draft and Supervision: FS and NJ.; Writing - Review & Editing: KN, JBAW, MS, Data curation, Validation: SM.; Resources, Project Administration: VM.

Data Availability Statement: N/A

Funding statement: Deanship of Scientific Research at Northern Border University, Arar, KSA (NBU-FFR-2024-1329-16). Deanship of Scientific Research at King Khalid University (RGP 2/252/45).

References

1. Augustyniak, A., Bartosz, G., Cipak, A., Duburs, G., Horákova, L.U., Luczaj, W., Majekova, M., Odysseos, A.D., Rackova, L., Skrzydlewska, E. and Stefek, M. 2010. Natural and synthetic antioxidants: an updated overview. *Free Radical Research*, 44(10), pp.1216-1262.
2. ATSDR (Agency for Toxic Substances and Disease Registry). 2001. The nature and extent of lead poisoning in children in the United States: A report to Congress. *Atlanta: US Department of Health and Human Services; DHHS Report*, no. 99-2966.
3. Bhattacharya, S.K., Kumar, A., and Ghosal, S., 2000. Effect of *Bacopa monniera* on animal models of Alzheimer disease and perturbed central cholinergic markers of congnition in rats. In: Siva Sankar, D.V., (Editor). *Molecular Aspects of Asian Medicines*, PJD publications, New York.
4. Ercal, N., Treeratphan, P., Lutz, P., Hammond, T.C. and Matthews, R.H. 1996. N-acetyl cysteine protects CHO cells from lead-induced oxidative stress, *Toxicology*, 108: 57-64.
5. Flora, Gagan, Deepesh Gupta, and Archana Tiwari. 2012. Toxicity of lead: a review with recent updates. *Interdisciplinary Toxicology*, 5.2: 47-58.
6. Flora, Swaran JS, Govinder Flora, and Geetu Saxena. 2006. Environmental occurrence, health effects and management of lead poisoning. In Lead, *Elsevier Science BV* pp. 158-228.
7. Garza, A., Vega, R., and Soto, E., 2006. Cellular mechanisms of lead neurotoxicity. *Medical Science Monitor: International Medical Journal of Experimental and Clinical Research,* 12 (3), RA57-65.
8. Grant, L.D., 2009. Lead and compounds. In: Lippmann, M. *Environmental Toxicants: Human Exposures and Their Health Effects*, 3rd edition. Wiley-Inter Science.
9. Haque, M.M., 2005. Effects of calcium carbonate, potassium iodide and zinc sulfate in lead induced toxicities in mice. *MS Thesis, Department of Pharmacology*, BAU, Mymensingh.
10. Harishekar, M.B., 2003. Studies on alcohol-lead interactive hepatotoxicity. *Ph.D. Thesis, Rajiv Gandhi University of Health Sciences*, Bangalore, Karnataka. pp. 77-97.
11. Iavicoli, I., *et al.*, 2003. Effects of low doses of dietary lead on red blood cell production in male and female mice. *Toxicology Letters,* 137, 193-199.
12. Jacob, B., *et al.*, 2000. The effects of low level of blood lead on hematological parameters in children. *Environmental Research*, 82, 150-159.

13. Jyoti, A. and Sharma, D. 2006. Neuroprotective role of *Bacopa monniera* extract against aluminium-induced oxidative stress in the hippocampus of rat brain, *Neurotoxicology*, 27, 451-457.
14. Kamruzzaman, 2006. Effects of ascorbic acid (Vitamin C) and α-tocopherol (Vitamin E) in lead induced toxicities in rats. *MS thesis, Department of Pharmacology*, BAU, Mymensingh.
15. Kosmachevskaya, Olga V., Natalia N. Novikova, and Alexey F. Topunov. 2021. Carbonyl stress in red blood cells and hemoglobin. *Antioxidants*, 10, 2:253.
16. Kumar, A., Kumar, A., MMS, C.P., Chaturvedi, A.K., Shabnam, A.A., Subrahmanyam, G., Mondal, R., Gupta, D.K., Malyan, S.K., Kumar, S.S. and A. Khan, S., 2020. Lead toxicity: health hazards, influence on food chain, and sustainable remediation approaches. *International journal of environmental research and public health*, 17(7), p.2179.
17. Lanphear, B.P., *et al.*, 2000. Cognitive deficits associated with blood lead concentrations <10 µg/100 ml in US children and adolescents. *Public Health Reports,* 115 (6): 521-529.
18. Lodia, S., and Kansal, L., 2012. Antioxidant activity of *Rubia cordifolia* against lead toxicity. *International Journal of Pharmaceutical Sciences and Research*, 3 (7): 2224-2232.
19. Luo, T., *et al.*, 2018. Chronic exposure to low doses of Pb induces hepatotoxicity at the physiological, biochemical and transcriptomic levels of mice. *Environmental Toxicology*, 1-9. https://doi.org/10.1002/tox.22706
20. Madiha, M.A., Abeer, A.A., and Amany, H.M., 2011. Roles of N-acetyl cysteine, methionine, vitamin C and vitamin E as antioxidants against lead toxicity in rats. *Australian Journal of Basic and Applied Sciences*, 5 (5): 1178-1183.
21. Merrill, J.C., Morton, J.J.P. and Soileau, S.D., 2007. Metals. In: A.W. Hayes. *Principles and Methods of Toxicology*, 5th edition. CRC Press. ISBN 084933778X.
22. Phillips, John D. 2019. Heme biosynthesis and the porphyrias. *Molecular Genetics and Metabolism*, 128, 3: 164-177.
23. Qureshi, N., Sharma, R., Mogra, S. and Panwar, K. 2010. Amelioration of lead induced alterations in ovary of Swiss mice by antioxidant vitamins, *Journal of Herbal Medicine and Toxicolgy*, 4(1): 89-95.
24. Rameshwari, R., Abirami, H., and Catharin, S., 2013. Pharmacological actions of *Bacopa monnieri*: A review. *International Journal of Pharmacy and Industrial Research,* 3 (2), 158-167.
25. Russo, A., *et al.*, 2003. Free radical scavenging capacity and protective effect on DNA damage of *Bacopa monnieri*. *Phytotherapy Research*, 17, 870-875.
26. Sakai, Tadashi. 2000. Biomarkers of lead exposure. *Industrial health*, 38, 2: 127-142.
27. Sharma, R., and Panwar, K., 2013. Treatment strategies against female reproductive lead toxicity: A review. *Journal of Chemical, Biological and Physical Sciences,* 3 (4), 2793-2802.
28. Srivastava, S.S., Kappor, K.R., and Kakkar, P., 2009. *Bacopa monnieri* modulates antioxidant responses in brain and kidney of diabetic rats. *Environmental Toxicology and Pharmacology,* 27 (1), 62-69. doi: 10.1016/j.etap.2008.08.007.

29. Sujatha, K., *et al.*, 2011. Hematobiochemical changes of lead poisoning and amelioration with *Ocimum sanctum* in wistar albino rats. *Veterinary World*, 4 (6), 260-263.
30. Sumathi, T., and Devaraj, N.S., 2009. Effect of *Bacopa monniera* on liver and kidney toxicity in chronic use of opioids. *Phytomedicine*, 16, 897-903.
31. Tiwari, S., and Tripathi, I., 2012. Lead pollution-An overview. *International Research Journal of Environmental Science,* 1, 84-86.
32. Tong, S., Schirnding, Y.E.V. and Prapamontol, T., 2000. Environmental lead exposure: a public health problem of global dimensions. *Bulletin of World Health Organization*, 78(9), pp.1068-1077.
33. Toz, Hasan, and Yeter Deger. 2018. The effect of chitosan on the erythrocyte antioxidant potential of lead toxicity-induced rats. *Biological Trace Element Research* 184, 1: 114-118.
34. Tripathi, Y.B. *et al.*, 1996. *Bacopa monnieri* Linn. as an antioxidant: mechanism of action. *Indian Journal of Experimental Biology,* 34 (6), 523-526.
35. UNEP, 2010. Final review of scientific information on lead, Version of December. *United Nations Environment Programme, Chemicals Branch, DTIE*. UNEP, Geneva, Switzerland. pp. 20-78.
36. Wang, C., Zhang, Y., Liang, J., Shan, G., Wang, Y. *et al.*, 2006. Impacts of ascorbic acid and thiamine supplementation at different concentrations on lead toxicity in testis. *Clin Chim Acta*, 370: 82-88.

Figures

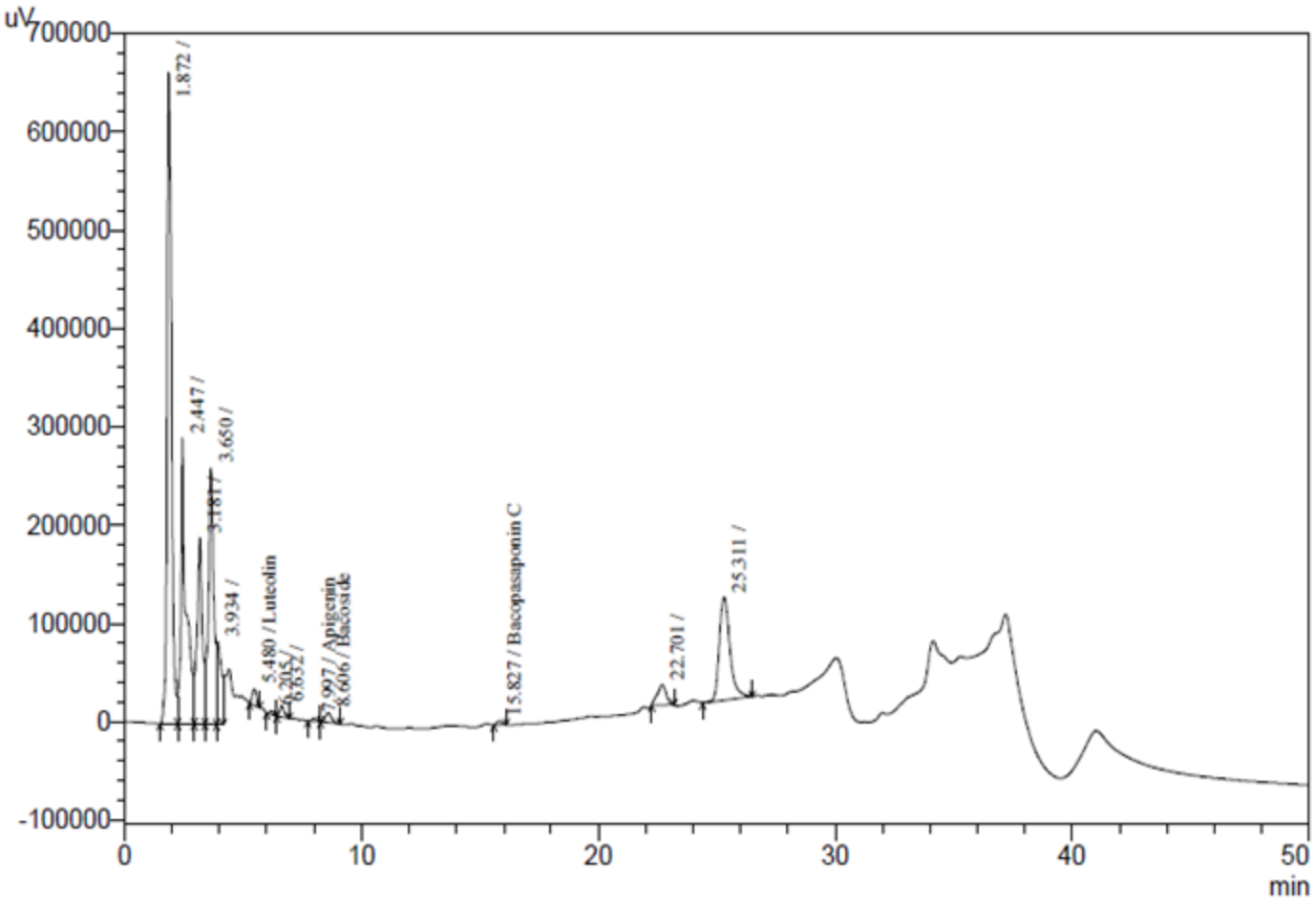

Figure 1

HPLC Analysis of Ethanolic Extract of *Bacopa monnieri*