Ameliorative Effects of a Flavonoid-Rich Extract of Tephrosia Bracteolata Leaves On Lead Acetate-Induced Haematotoxicity, Immunotoxicity and Oxidative Stress in Wistar Rats

Oniemola Joan Mayowa
Department of Science Laboratory Technology, Faculty of Applied Sciences, Kogi State Polytechnic, Lokoja, Kogi State, Nigeria.

Nwafor Ijeoma Princess
UNESCO International Centre for Biotechnology, Nsukka, Enugu State, Nigeria

Olajimbiti Christiana Ololade
Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Abuja, FCT, Nigeria.

Olawale Sunday James
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja, FCT, Nigeria.

Ahmed Hussaini
Department of Science Laboratory Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

Osikhena Usman Kebiru
Department of Science Laboratory Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

Yahaya Ahmad
Department of Chemistry, Faculty of Science and Literature, Mersin, Turkiyẽ.

Abubakar Seghosime Rafat
Department of Food Science and Technology, Auchi Polytechnic, Auchi, Edo State, Nigeria.

Mahmud Lawal
Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria.

Muhammed Hussein
Department of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.

Idakwoji Precious Adejoh
idakwojipa@gmail.com

Department of Biochemistry, Federal University of Technology, MinnaDDepartment of Biochemistry, Faculty of Natural Sciences, Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria.

Research Article

Keywords: Ameliorative Effects, Flavonoid-Rich Extract, Tephrosia Bracteolata, Lead Acetate, Haematotoxicity, Immunotoxicity, Oxidative Stress, Wistar Rats

Posted Date: October 23rd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7905931/v1

Additional Declarations: The authors declare no competing interests.

Abstract

This study evaluated the ameliorative effects of the flavonoid-rich extract of *Tephrosia bracteolata* leaves (FRETB) on lead acetate-induced haematotoxicity, immunotoxicity, and oxidative stress in male Wistar rats. Twenty rats were randomly distributed into five groups of four each. Group 1 served as control, while Group 2 received 50 mg/kg lead acetate alone. Groups 3 and 4 were co-treated with 50 mg/kg lead acetate and 5 mg/kg or 10 mg/kg FRETB, respectively, while Group 5 received 50 mg/kg ascorbic acid plus lead acetate. After 28 days, hematological indices, oxidative stress biomarkers, and immunological parameters were evaluated using standard biochemical procedures. Lead acetate caused significant ($p < 0.05$) reductions in red blood cell count, haemoglobin concentration, and packed cell volume, accompanied by elevated oxidative stress markers (MDA, SOD, CAT) and altered immune parameters. Treatment with FRETB significantly reversed these effects in a dose-dependent manner, comparable to the standard antioxidant, ascorbic acid. The high-dose extract notably improved hematological and oxidative indices and stabilized immune responses. The observed activities are attributed to the plant's flavonoid constituents, known for their antioxidant and cytoprotective properties. These findings indicate that FRETB possesses potent ameliorative potential against lead acetate-induced haematotoxicity, immunotoxicity, and oxidative stress in rats, supporting its ethnomedicinal use and highlighting its promise as a natural therapeutic agent for mitigating heavy-metal toxicity.

1.0 Introduction

Lead exposure has been shown to exert toxic effects on several vital organs, including the liver, kidney, heart, brain, testes, and hematopoietic tissues (1). Human can be exposed to lead through multiple routes such as ingestion of contaminated food, inhalation of polluted air, and contact with dust, burning fuel, or fossil materials. Due to the colorless and odorless nature of lead, it can persist in the environment for long periods, posing significant ecological and health risks (1). Although, it may be present at low levels, lead becomes hazardous when it accumulates to high concentrations, adversely affecting living organisms and the environment. Common sources of lead poisoning include contaminated food and water, lead-based paints, gasoline and industrial emissions (2). Lead toxicity has been associated with various pathological conditions such as hepatic and renal injury, osteoporosis, neurological impairments, and cardiovascular diseases (3). The widely accepted mechanism of lead-induced toxicity involves oxidative stress (4), characterized by excessive generation of free radicals and depletion of antioxidant defense systems. Furthermore, lead disrupts enzymatic and protein functions by displacing essential cations, resulting in reduced biological activities (5).

The use of natural products to counteract the toxic effects of heavy metals and other harmful chemicals is gaining increasing attention (6). Since oxidative stress is a major mechanism underlying lead toxicity, natural products rich in antioxidants can serve as effective antidotes against lead poisoning and may complement conventional chelating agents. Numerous bioactive compounds from natural sources with established antioxidant properties have been employed as protective agents against lead- induced toxicity (6). This study, therefore aims to evaluate the ameliorative effects of a flavonoid-rich extract of

Tephrosia bracteolata leaves on lead acetate-induced perturbations in hematological, immunological and oxidative stress parameters in Wistar rats.

Tephrosia bracteolata is a widespread shrub belonging to the Fabaceae family, commonly found in tropical and warm-temperate regions. The genus *Tephrosia* comprises about 400 species (7). Different parts of the plant have been reported to possess numerous pharmacological properties. Idakwoji *et al.* (8) reported the antidiabetic activities of the ethanol extract of *Tephrosia bracteolata* leaves. Similarly, Egharevba *et al.* (9) also documented the antidiabetic, antioxidant and antimicrobial activities of extracts of *Tephrosia bracteolata* leaves. Other members of the *Tephrosia* genus have been shown to possess diverse biological activities, including anticancer (10) and antiplasmodial (11) activities. The genus is known to contain a wide range of phytoconstituents associated with these bioactivities, such as Isopongaflavone from *T. bracteolata*, obovatin methyl ether from *T. aequilata* (12), and kaempferol 3-O-β-D-glucopyranoside *from T. calophylla.* Scientific validation of several medicinal plants has confirmed that some of these phytochemicals contribute significantly to their antioxidant properties, which in turn help to mitigate the oxidative stress caused by heavy metal exposure (5).

2.0 Materials and Methods

2.1 Materials

2.1.1 Chemicals and Reagents

All the chemicals used were of analytical grade. They include; Sodium Carbonate Anhydrous, Sodium bicarbonate, Hydrogen peroxide, Trichloroacetic acid (TCA), Thiobarturic acid (TBA), Adrenalin, Sodium phosphate dibasic anhydrous, Potassium hydrogen phosphate.

2.1.2 List of Apparatus/ Equipment

Electronic weighing balance (PB3002-5 Model, Mettler, Toledo, Switzerland), Centrifuge (SM800B Model, Surgifriend medicals, Okehampton, England), Sysmex heamatology analyzer (XP-1000 Model, Sysmex America, Illinois, USA), Centrifuge, Measuring cylinder, Micropipette, Syringe and needle, Spatula.

2.2 Methods

2.2.1 Plant Material

The leaves of *T. bracteolata* were collected from a natural habitat in Otokiti area of Lokoja, Kogi State, Nigeria. The plant was identified at the Herbarium Unit of the Department of Biological Sciences, Federal University, Lokoja, Kogi State, Nigeria.

2.2.2 Extraction Method

Fresh leaves of *T. bracteolata* were rinsed with distilled water to remove all debris, shade-dried for seven days and subsequently pulverized using an electric blender. A known quantity (1.5 kg) of the powder was

macerated in 7.5 L of absolute ethanol. After 72 h, the suspension was filtered using a mesh, and then Whatman No 1 filter paper. This procedure was repeated twice and all the filtrates were concentrated in a rotary evaporator set at 45°C to obtain the crude ethanol extract of *T. bracteolata* leaves. The flavonoid-rich extract of *T. bracteolata* leaves was prepared according to the method previously described Chu *et al.*, (13). Exactly 9 g of the crude extract was dis- solved in 60 ml of 10% H_2SO_4 and was hydrolyzed by heating on a water bath for 30 min at 100°C. Thereafter, the mixture was placed on ice for 15 min to allow the precipitation of flavonoids and aglycones. The precipitate (flavonoids/aglycones mixture) was dissolved in 50 ml of 95% ethanol (warmed to 50°C) in 100 ml volumetric flask and thereafter made up to the mark with the 95% ethanol. This was centrifuged, filtered and the filtrate collected was concentrated using a rotary evaporator to obtain the flavonoid-rich extract of *T. bracteolata* leaves (FRETB) that was stored in an airtight lightproof container at 4°C until used.

2.2.3 Experimental Animals

Twenty male Wistar rats (200–250 grams) were accommodated in well-ventilated cages in the Animal House Unit of the Depart of Biochemistry, Prince Abubakar Audu University, Anyigba, with constant 12-h light 12-h dark cycle. The animals had free access to standard pelletized rat feed and clean water *ad libitum* and were allowed one week of acclimatization.

2.2.4 Experimental Design

The animals were weighed and randomly shared into five groups of four animals each. Group 1 served as normal control and were administered 5 ml/kg of distilled water. Toxicity was induced intraperitoneal by administration of Lead acetate-PbA (50 mg/kg) in groups 2 to 5 and treated as follows. Group 2 (PbA only), Group 3 (PbA + 5 mg/kg FRETB), Group 4 (PbA + 10 mg/kg FRETB) and Group 5 (PbA + 50 mg/kg Ascorbic acid) (standard control). PbA was administered once per week while FRETB and Ascorbic acid were administered daily throughout the duration (28 days) of the study.

2.2.5 Sacrifice and Sample Collection

The rats were sacrificed on the 29th day by intraperitoneal injection of 120 mg/kg of sodium thiopentone anesthesia. Blood samples for serum assay were collected from each animal via cardiac puncture into plain and EDTA-bottles.

2.2.6 Estimation of Haematological and Immunological Parameters

Haematological parameters (red blood cell (RBC), haemoglobin (HB), packed cell volume (PCV), white blood cell (WBC), neutrophil (N), lymphocytes (L), monocytes (M) and platelets) were carried out following the method described by Dacie and Lewis, (14, 15).

2.2.7 Estimation of Oxidative Stress Biomarkers

.Malondialdehyde (MDA) concentration was measured according to the method of Draper and Hadley, (16). Catalase (CAT) activity was assayed following the method of Aebi (17) while superoxide dismutase

(SOD) activity was assayed via the method of Xin *et al.* (15).

2.2.7.1 Determination of Malondialdehyde (MDA)

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Draper and Hadley (16). To 0.1ml of the sample mixture, 2ml of MDA reagent was added and boiled at 100^{o}c for 15 minutes. The reaction mixture was then allowed to cool down. The flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was collected and its absorbance was read at 532 nm against blank.

2.2.7.2 Determination of Catalase Activity

Catalase is a marker of oxidative stress was determined using the method of Aebi. (17). Blood samples collected from experimental subjects were each diluted at a ratio 1: 50ml with a distilled water. The assay mixture 4ml of H_20_2 solution (800u moles) and 5ml of 0.01M of phosphate buffer (PH7) in a 100ml beaker. 1ml of the properly dilluted enzyme preparation was rapidly mixed with the reaction by a gently swirling motion. Their action was run at room temperature. At 60seconds interval, 1ml portion of the reactor mixture was withdrawn and blown into 2ml dichromate acetic acid reagent in test tubes properly labeled. The absorbance at 570nm setting the spectrophotometer *to zero using blank.*

2.2.7.3 Determination of Superoxide Dismutase Activity

Superoxide dismutase was determined using the method of Xin et al. (15). Blood Sample collected was dilluted in ratio of 1: 10 with phosphate buffer. The assay mixture was prepared by measuring an aliquot of 0.2ml of the diluted blood sample and 2.5ml of 0.05M carbonate buffer of PH10.2. This was allowed to equilibrate in the spectrophotometer and the reaction started by addition of 0.3ml of freshly prepared adrenaline solution to buffer supernatant mixture, which was quickly mixed by inversion. The reference cuvette contained 2.2ml of buffer. 0.3ml of the adrenaline and 0.2ml of water. The increase crease in absorbance at 480nm was monitored every 30 seconds for 150seconds.

2.3 Statistical Analysis

All data were expressed as mean ± standard deviation, and statistical differences between means were determined by one- way ANOVA followed by Duncan's post–hoc test for multiple comparison tests using SPSS version 20. Values were considered significant at $P \leq 0.05$.

3. 0 Results

3.1 Effect of Flavonoid-rich extract of Tephrosia bracteolata leaves (FRETB) on haematological parameters in lead acetate-induced toxicity in Wistar rats

Table 3.1 shows that lead exposure produced a significant ($P < 0.05$) reduction in RBC, Hb, PCV and platelet concentration as seen in the negative control. However, FRETB and ascorbic acid- treated groups

registered significant ($p < 0.05$) increase in RBC, Hb and PCV concentration relative to the negative control.

Table 3.1
Effect of Flavonoid-rich extract of *Tephrosia bracteolata* leaves (FRETB) on haematological parameters in lead acetate-induced toxicity in Wistar rats

Group/ Treatment	RBC ($x10^9$/L)	Hb (g/L)	PCV (%)	Plat ($x10^9$/L)
Group 1 (5 ml/kg Dist. H_2O)	220.21 ± 11.21^b	12.16 ± 2.41^b	51.26 ± 3.28^b	820.23 ± 96.15^a
Group 2 (50 mg/kg PbA)	175.17 ± 16.32^a	10.14 ± 1.25^a	40.21 ± 4.15^a	815.28 ± 85.44^a
Group 3 (50 mg/kg PbA + 5 mg/kg FRETB)	180.22 ± 13.18^a	12.32 ± 1.39^b	42.32 ± 4.18^a	820.32 ± 90.21^a
Group 4 (50 mg/kg PbA + 10 mg/kg FRETB)	217.14 ± 17.15^b	12.88 ± 1.25^b	50.52 ± 6.51^b	822.17 ± 94.11^a
Group 5 (50 mg/kg PbA + 50 mg/kg AA)	214.16 ± 18.21^b	12.36 ± 1.43^b	50.12 ± 6.23^b	818.46 ± 79.30^a

Mean values having different lowercase alphabets as superscripts are considered significant ($p < 0.05$) along the columns. PbA = Lead acetate, FRETB = Flavonoid-rich extract of *Tephrosia bracteolata* leaves, AA = Ascorbic acid

3.2 Effect of Flavonoid-rich extract of Tephrosia bracteolata leaves (FRETB) on immunological parameters in lead acetate-induced toxicity in Wistar rats

The effect of effect of FRETB on immunological parameters in lead acetate- induced immunotoxicity is shown in Table 3.2. There was an observed increase in the neutrophil and monocytes concentrations with a corresponding decrease in WBC and lymphocytes following lead exposure. However, treatment with FRETB produced a significant reversal of these abnormalities.

Table 3.2
Effect of Flavonoid-rich extract of *Tephrosia bracteolata* leaves (FRETB) on immunological parameters in lead acetate-induced toxicity in Wistar rats

Group/ Treatment	WBC ($x10^9/L$)	Neu (%)	Lymp (%)	Mono (%)
Group 1 (5 ml/kg Dist. H_2O)	5.46 ± 1.40[a]	51.23 ± 5.25[a]	22.67 ± 2.34[a]	26.10 ± 2.36[a]
Group 2 (50 mg/kg PbA)	5.28 ± 1.28[a]	52.34 ± 9.59[a]	21.35 ± 3.23[a]	26.31 ± 2.58[a]
Group 3 (50 mg/kg PbA + 5 mg/kg FRETB)	5.32 ± 1.26[a]	51.44 ± 8.23[a]	21.26 ± 1.29[a]	27.30 ± 2.74[a]
Group 4 (50 mg/kg PbA + 10 mg/kg FRETB)	5.17 ± 1.33[a]	50.34 ± 8.15[a]	22.18 ± 1.35[a]	27.48 ± 2.44[a]
Group 5 (50 mg/kg PbA + 50 mg/kg AA)	5.23 ± 1.45[a]	50.24 ± 7.22[a]	22.24 ± 1.46[a]	27.52 ± 2.93[a]

Mean values having the same lowercase alphabets as superscripts are considered non-significant ($p > 0.05$) along the columns. PbA = Lead acetate, FRETB = Flavonoid-rich extract of *Tephrosia bracteolata* leaves, AA = Ascorbic acid

3.3 Effect of Flavonoid-rich extract of Tephrosia bracteolata leaves (FRETB) on oxidative stress biomarkers in lead acetate-induced toxicity in Wistar rats

Table 3.3 shows the effects of FRETB on antioxidant status of lead acetate- induced oxidative stress in Wistar rats. The data obtained shows that the MDA concentration of the negative control rats was significantly higher compared to the positive control. 28 days of treatment with FRETB resulted in a significantly ($p < 0.05$) lower MDA concentration relative to the negative control. The decline in MDA was observed to be dose-dependent and comparable to that of the positive control as shown in table 3. Results also indicated a significant ($p < 0.05$) increase in SOD and CAT activities in the negative control as compared to the positive control. Treatment with FRETB also significantly ($p < 0.05$) decrease the activities of SOD and CAT compared to the negative control.

Table 3.3
Effect of Flavonoid-rich extract of *Tephrosia bracteolata* leaves (FRETB) on oxidative stress biomarkers in lead acetate-induced toxicity in Wistar rats

Group/ Treatment	MDA (nmol/ml)	SOD (U/ml)	CAT (U/ml)
Group 1 (5 ml/kg Dist. H_2O)	15.23 ± 2.11[a]	22.04 ± 3.34[a]	33.35 ± 2.46[a]
Group 2 (50 mg/kg PbA)	22.46 ± 2.15[b]	31.99 ± 3.65[b]	50.95 ± 3.40[b]
Group 3 (50 mg/kg PbA + 5 mg/kg FRETB)	20.35 ± 2.67[b]	28.48 ± 3.46[b]	48.72 ± 2.33[b]
Group 4 (50 mg/kg PbA + 10 mg/kg FRETB)	14.56 ± 2.23[a]	22.30 ± 2.69[a]	35.95 ± 3.47[a]
Group 5 (50 mg/kg PbA + 50 mg/kg AA)	14.18 ± 2.57[a]	21.75 ± 2.72[a]	35.23 ± .54[a]

Mean values having different lowercase alphabets as superscripts are considered significant ($p < 0.05$) along the columns. PbA = Lead acetate, FRETB = Flavonoid-rich extract of *Tephrosia bracteolata* leaves, AA = Ascorbic acid

4.0 Discussion

The present investigation demonstrates that the flavonoid-rich extract of *Tephrosia bracteolata* leaves (FRETB) exhibits protective and restorative effects against lead acetate-induced haematotoxicity, immunotoxicity, and oxidative stress in male Wistar rats. Lead remains a pervasive environmental toxin known to disrupt multiple physiological systems. Its toxic effects stem largely from its capacity to generate reactive oxygen species (ROS), impair antioxidant defenses, and interfere with essential enzymes involved in heme synthesis and cellular metabolism (18).

In this study, results showed a significant decrease in the hematological parameters, including the RBC count, Hb concentration, PCV, and platelet concentration in the lead-exposed rats compared to controls. The results of this study were consistent with the findings of previous studies (19, 20). According to Manser et al. (21), low-level exposure to inorganic lead in workplace can have harmful effect on certain types of the blood cells, reduce hemoglobin production, and cause reduced lifespan and function of red blood cells. The mechanisms of lead-induced hematological alterations in rats could be due to lead-related RBC membrane fluidity that leads to increased erythrocyte hemolysis rates (22, 23), lead interference with the heme synthesis pathway, such as the downregulation of δ-aminolevulinic acid dehydratase (ALAD), resulting in low Hb concentration and the interference of lead with iron and copper

metabolism in the RBCs. Moreover, its effect on ALAD has been used clinically to gauge the degree of lead acetate poisoning (23). These effects of lead on erythrocyte and its inhibition on heme biosynthetic process may contribute to the significantly ($p < 0.05$) low PCV, RBC, and hemoglobin levels in the lead-induced group when compared with the other groups in the study. Administration of FRETB, particularly at 10 mg/kg, significantly improved these haematological parameters, restoring them toward normal levels. The improvement may be attributed to the antioxidant and membrane-stabilizing properties of flavonoids in *T. bracteolata*, which likely prevented oxidative damage to erythrocyte membranes and enhanced erythropoietic activity. Flavonoids have been reported to protect red cells by scavenging free radicals and preserving membrane integrity (24). The efficacy of the extract was comparable to that of ascorbic acid, a well-established antioxidant and lead-chelating agent (25, 26).

Table 3.2 presents the effect of flavonoid rich extract *T. bracteolata* treatment on immunological parameters in lead-induced toxicity in rats. Lead exposure also induced alterations in white blood cell (WBC) profiles, showing elevated neutrophil and monocyte counts with reduced lymphocytes and total WBCs. These findings indicate an inflammatory and immunosuppressive response characteristic of chronic heavy-metal exposure (27). This result is consistent with another study, which also showed a lead-induced increase in lymphocytes count (28). Exposure to lead acetate can lead to an increase in monocytes count and neutrophil count in the blood (29). Elevated neutrophils and monocytes suggest a systemic inflammatory reaction, while lymphopenia reflects suppression of adaptive immune functions (27). Treatment with FRETB restored immune cell balance, suggesting its immunomodulatory capacity. This may result from the ability of flavonoids to suppress pro-inflammatory cytokines such as TNF-α and IL-1β while enhancing lymphocyte proliferation (27).

Oxidative stress parameters further supported the protective effects of FRETB. Lead exposure markedly elevated malondialdehyde (MDA), a product of lipid peroxidation, while enhancing the activities of catalase (CAT) and superoxide dismutase (SOD) due to compensatory up-regulation under oxidative pressure. Elevated MDA levels reflect peroxidative degradation of membrane lipids and correlate with cellular damage (30). The increased antioxidant enzyme activities in lead-only rats likely represent an adaptive but inadequate response to persistent oxidative burden. FRETB treatment significantly decreased MDA concentrations and normalized SOD and CAT activities, implying attenuation of lipid peroxidation and restoration of redox homeostasis. These findings are in agreement with earlier work by Idakwoji et al. (8) and Egharevba et al. (9), who reported that *T. bracteolata* extracts exhibit potent antioxidant activity by elevating enzymatic antioxidants and lowering peroxidation markers in experimental models. The flavonoid constituents such as kaempferol and Isopongaflavone are known to scavenge free radicals, chelate metal ions, and up-regulate endogenous antioxidant enzymes (5). By mitigating oxidative stress, the extract likely prevented secondary cellular injury and preserved organ function.

Furthermore, the dose-dependent efficacy of FRETB suggests a pharmacologically relevant concentration range where antioxidant defenses are maximally supported without inducing metabolic stress. The similarity between the effects of the high-dose FRETB group and the ascorbic acid group

reinforces the potential of *T. bracteolata* as a natural alternative to synthetic antioxidants in mitigating lead toxicity. The observed protection may also involve modulation of gene expression. Flavonoids have been shown to activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, a central regulator of antioxidant response elements (ARE) that induces transcription of detoxifying enzymes, including SOD, CAT, and glutathione peroxidase (6). Activation of Nrf2 signaling enhances the cell's capacity to counter oxidative insults and repair redox imbalance, providing a plausible molecular mechanism for the observed biochemical restoration. Beyond antioxidant activity, flavonoids exert metal-chelating effects that reduce free lead ions in circulation. Hydroxyl and carbonyl groups on flavonoid rings can bind divalent metals such as Pb^{2+}, reducing their bioavailability and interaction with biomolecules (31). Such chelation could account for the observed reduction in oxidative damage and normalization of haematological indices.

From a toxicological standpoint, lead acetate impairs mitochondrial respiration and disrupts calcium homeostasis, amplifying ROS generation and cellular apoptosis (32). By curbing oxidative stress and stabilizing cellular membranes, FRETB may indirectly maintain mitochondrial integrity and preserve energy metabolism. This aligns with findings that plant-derived flavonoids confer cytoprotection through mitochondrial preservation and inhibition of apoptosis pathways (33). The current results thus establish that FRETB offers multifaceted protection; antioxidant, anti-inflammatory, immunomodulatory, and possibly chelating against lead-induced toxicity. This agrees with broader literature on *Tephrosia* species, whose extracts display anti-diabetic, antiinflammatory, and hepatoprotective properties mediated by flavonoid-rich phytochemistry (7, 34, 35). Nevertheless, while the study demonstrates promising protective efficacy in rats, further investigations are required to isolate and characterize the specific bioactive constituents responsible for the activity and to determine pharmacokinetics, safety margins, and potential interactions with standard chelating agents. Future molecular studies should also explore Nrf2 activation, cytokine modulation, and histopathological changes in lead-exposed tissues following FRETB treatment.

5.0 Conclusion

In summary, lead acetate exposure in rats caused significant haematological suppression, immune disruption, and oxidative stress. Treatment with the flavonoid-rich extract of *T. bracteolata* markedly reversed these alterations, restoring haematological and biochemical parameters toward normal. The extract's efficacy is comparable to that of ascorbic acid, underscoring its potent antioxidant and cytoprotective potential. These results substantiate the ethnomedicinal use of *T. bracteolata* and highlight its value as a natural therapeutic agent against heavy-metal-induced toxicity.

Declarations

Animal Ethics Statement: All experimental procedures involving animals were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals and were approved by Prince Abubakar Audu University, College of Health Sciences Ethical Committee.

References

1. Assi, M.A., Hezmee, M. N. M., Abd, W., Haron, M. Y. M. and Sabri, M. A. R. (2016). The detrimental effects of lead on human and animal health*. Vet. World*, 9(6):660.
2. Al-Megrin, W. A., Alkhuriji, A. F., Yousef, A. O. S., Metwall,y D. M., Habotta, O. A. and Kassab, R.B (2019). Antagonistic efficacy of luteolin against lead acetate exposure-associated with hepatotoxicity is mediated via antioxidant, anti-inflammatory, and antiapoptotic activities. *Antioxidants*, 9(1):1-10.
3. Alhusaini, A., Fadda, L., Hasan, I. H., Zakaria, E., Alenazi, A. M. and Mahmoud, A. M. (2019). Curcumin ameliorates lead-induced hepatotoxicity by suppressing oxidative stress and inflammation, and modulating Akt/GSK-3 β Signaling Pathway. *Biomolecules*, 9(11): 701-703
4. Abdelhamid, F.M., Mahgoub, H. A. and Ateya, A. I. (2020). Ameliorative effect of curcumin against lead acetate–induced hematobiochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. *Environmental Science and Pollution Research*, 27(10): 10950-10965.
5. Ilesanmi, O. B., Esther F. Adeogun, E. F., Odewale, T. T. and Chikere, B. (2020). Lead exposure-induced changes in hematology and biomarkers of hepatic injury: protective role of TrévoTM supplement. *Environmental Analysis Health and Toxicology*, 37(2): 20-23.
6. Chen, C., Lin, B., Qi, S., He, J. and Zheng, H. (2019). Protective effects of salidroside on lead acetate-induced oxidative stress and hepatotoxicity in Sprague-Dawley Rats. *Biological Trace Element Research*, 191(2):426-434.
7. Sadam, A. A., Abdullahi, S. M., Pateh, U. U., Shehu, A. A. and Bakare, L. O. (2020). Studies on Analgesic and Anti-Inflammatory Activities of Aerial Parts of Tephrosia Bracteolata GUILL. and PERR. (Fabaceae) in Rodents. *Nig. J. Pharm. Res,* 2*:* 31-37.
8. Idakwoji, P. A., Ekpo, D. E., Joshua, P. A., & Njoku, O. U. and Nwodo, O. F. C. (2021). Ethanol extract of Tephrosia bracteolata leaves and its fractions ameliorates alloxan-induced diabetes and its associated complications in Wistar rat model. *International Journal of Diabetes in Developing Countries*, 2021: 1-13
9. Egharevba, G. O., Dosumu, O. O., Oguntoye, S. O., Njinga, N. S., Dahunsi, S. O. and Hamid, A. (2019). Antidiabetic, antioxidant and antimicrobial activities of extracts of Tephrosia bracteolata leaves. *Heliyon,* 2(3): 4-7.
10. Hassan, L., Iqbal, M., Dahham, S., Tabana, Y., Ahamed, M and Majid, A. (2017). Colorectal, prostate and pancreas human cancers targeted bioassay-guided isolations and characterization of chemical constituents from Tephrosia apollinea. *Med Chem*, 17(4): 590–8.
11. Nondo, R., Mbwambo, Z., Kidukuli, A., Innocent, E., Mihale, M., Erasto, P., Chen, Y., Yan, T., Gao, C., Cao, W and Huang, R. (2014). Natural products from the genus Tephrosia. *Molecules*, 19(2): 1432–58.
12. Atilaw, Y., Duffy, S., Heydenreich, M., Muiva-Mutisya, L., Avery, V. and Erdelyi, M., (2017). A Three Chalconoids and a Pterocarpene from the Roots of Tephrosia aequilata. *Molecules*, 22(2): 318.

13. Chu, Y.F., J. Sun, X. Wu, R.H. Liu, Antioxidant and antiproliferative activities of common vegetables, J. Agric. Food Chem. 50 (2002) 6910–6916.
14. Dacie, J.C., Lewis, S.M., 1991. *Practical haematology*. 5th ed. London: Churchill Livingstone, 152-158.
15. Xin, Z., Waterman, D.F., Hemken, R.W., Harmon, R.J., 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. *Journal of Diary Science*, 74 (9) 3078-3085.
16. Draper, H.H., Hadley, M., 1990. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186, 421-431.
17. Aebi, H.E. (1983). Catalase. *In*: H.U. Bergmeyer, ed. *Methods of enzymatic analysis*. 3rd ed. New York: Academic Press, 673-684.
18. Adikwu, E., Deo. O., Geoffrey, O. B. P. and Enimeya, D. A. (2013). Lead organ and tissue toxicity: roles of mitigating agents (Part 1). *Br J Pharmacol Toxicol*, 2013; 4(6):232–40.
19. Haridy, M., Al-Amgad, Z., Sakai, H. and Mohi-Eldin, M. (2014). Ameliorating effects of garlic, calcium, and vitamin C on chronic lead toxicity in albino rats. Comp Clin Pathol, 2014; 23(5):1215–23.
20. Obafemi, T. O., Onasanya, A., Adeoye, A., Falode, .A., Daniel, D. J., Irefo, E. F., Ojo, A. O., Fadaka, A., Afolabi, O.B., Awe, J. O. and Omiyale, B. O. (2019). Protective effect of methanolic and flavonoid-rich leaf extracts of Synsepalum dulcificum (Danielli) on lead-acetate-induced toxicity in Wistar albino rats. *J Appl Pharm Sci*, 9(5):65–72..
21. Manser, W. R., Lalini, R., Haider, S., Khan, M. A. (2019). Trace element studies on Karachi population, Part-V, Blood Lead levels in normal healthy adults and grammar's School children. *Int J Mod Phys,* 40(7):150–3.
22. Mannem, P. (2014). Lead toxicity on hematological changes and amelioration with ginger (Zingiber officinale) extract in male albino rats. *Int J Adv Res,* 2(4):23–8.
23. Ray, R. R. (2016). Haemotoxic effect of lead: a review. *Proc Zoo Soc,* 69(2):161–72.
24. Aguilar, D. D. J. and Borges, C. R. (2020). Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. *J. Vis. Exp,* 159: 61-122.
25. Chineke, C. A., Ologun, A. G. and Ikeobi, C. O. N. (2016). Haematological parameters in rabbit breeds and crosses in humid tropics. *Pakistan Journal of Biological Sciences*, 9: 2102-2106.
26. Jorns, A., Tiedge, M., Lenzen, S. and Munday, R. (2019). "Effect of superoxide dismutase, catalase, chelating agents, and free radical scavengers on the toxicity of alloxan to isolated pancreatic islets in vitro," *Free Radical Biology & Medicine*, 26(9): 1300–1304, 1999.
27. Ahamed, M., Siddiqui, M. K. J. (2007). Low level lead exposure and oxidative stress: Current opinions. *Clin Chim Acta*, 383: 57–64.
28. Shah, S. L. and Altindog, A. (2020). Alteration in the immunological parameters Tench (Tinca tinca L. 1758) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. *Turk J Vet Anim Sci*, 29:1163–8..

29. Mates, J. M. (2020). Eff ects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology,* 153: 83–104.
30. Bechara, E. J. H. (2014). Lead poison and oxidative stress. *Free Radic Biol Med*, 36:22
31. Patrick, L. (2016). 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,* 11(2):114–27.
32. Mervat, H. G., Nabela, I. E., Mohamed, M. A. H., Gihan, G. M. (2022). Efficacy of Curcumin on Lead Induced Nephrotoxicity in Female Albino Rats. *J Am Sci,* 8(6):502–10.
33. Sridevi, K. S. and Umamaheswari, S. (2020). Human Exposure to Lead, Mechanism of Toxicity and Treatment Strategy. Journal of Clinical and Diagnostic Research. 2020 Dec, Vol-14(12): LE01-LE05
34. Vimal, J. S., Agasa, R. M. and Vedigounder, M. (2019). Phytochemical and pharmacological aspects of Tephrosia genus: A brief review. *Journal of Applied Pharmaceutical Science*, 9(03): 117-125.
35. Yasini, S. P., Khaki, Z., Ranbari, S., Kazeem, B., Amoli, J. S., Gharabaghi, A. and Jalali, S. M. (2022). Haematologic and clinical aspects of experimental ovine anaplasmosis caused by anaplasma ovis in Iran. *Iran J. Parasitol*, 7(4): 91-98.