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Original Research Article

Ameliorative and Synergetic Effects of Co-Administration of Honey and *Vernonia amygdalina* (Bitter leaf) Extract on Lead-Induced Wistar Rats

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ABSTRACT

Lead is a toxic environmental pollutant that disrupts oxidant-antioxidant balance, causes inflammation, and can lead to organ dysfunction. This study investigates the ameliorative and synergistic effects of honey and *Vernonia amygdalina* (VA) on lead-induced wistar rats. The study involved 35 Wistar rats, divided into five groups. Group 1 served as the control and were given feed and water only; group 2 was administered lead acetate (2.2 mg/kg) only; group 3 was treated with lead acetate (2.2 mg/kg) + honey (5g/kg); group 4 was treated with lead acetate (2.2 mg/kg) + bitter leaf extract (300mg/kg); and group 5 received lead acetate (2.2 mg/kg) + honey (5g/kg) + bitter leaf extract (300mg/kg). The animals were dosed daily orally for 6 weeks via oral cannula. After the treatment period, the rats were euthanized with sodium pentobarbital, and their blood and kidney samples were collected for further biochemical and histological analyses, respectively. The lead-exposed rats exhibited kidney damage and had high creatinine, urea, and malondialdehyde levels, while honey, bitter leaf, or both reduced these levels and increased superoxide dismutase and catalase levels. The histological data revealed that honey and bitter leaf may protect against lead-induced kidney damage when compared to the lead-induced group alone. The study revealed that honey and bitter leaf treatments effectively mitigate lead-induced kidney damage, with combining both methods being the most effective method for enhancing kidney function and structure.

***Keywords*:** Honey, *Vernonia amygdalina*, Antioxidants, Lead, nephrotoxicity, Synergistic effects

Introduction

Heavy metals are naturally occurring elements that accumulate in the environment largely due to human activities, posing a global concern given their non-biodegradable nature.[1] Lead, a harmful environmental pollutant, can cause various organ dysfunctions and disrupt the oxidant-antioxidant balance, leading to inflammatory responses in both humans and animals.[2] Prolonged or excessive lead exposure can lead to acute or chronic nephrotoxic effects, characterised by proximal tubular nephropathy, glomerular sclerosis, and interstitial fibrosis.[3]

Antioxidants have traditionally been used to alleviate common ailments such as digestive issues, colds, fevers, and morning sickness.[4] Honey, a well-known natural dietary antioxidant, has been proven to improve total plasma antioxidant capacity when humans ingest it, adding to overall health protection.[5] Although honey has been utilised for medical and household purposes for a long time, its antioxidant properties have gained recent recognition.[6]

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Honey is gaining popularity in the food industry due to its potential to combat various health issues. Its antioxidants offer preventive benefits against various conditions such as cancer, cardiovascular diseases, inflammation, neurological disorders, wound healing, infections, and ageing, driving the search for antioxidant-rich foods.[7]

Medicinal plants are renowned for their potential therapeutic properties and the ability to serve as sources for drug development.[8] Bitter leaf, an indigenous African plant with grey or brown bark, is a rich source of vitamins and mineral salts, making it a valuable component in the human diet.[9] *Vernonia amygdalina*, commonly known as bitter leaf due to its characteristic bitter taste and flavour, exhibits active properties as an anticancer, antibacterial, antimalarial, and antiparasitic agent.[10] It contains complex active components with significant pharmacological applications.[11] Given the harmful impact of lead on the environment and human health and the potential for humans to absorb and accumulate it, this study was conducted to investigate the ameliorative and synergetic effects of co-administration of honey and bitter leaf (*V. amygdalina*) on lead-induced wistar rats.

Materials and Methods

The study's methodology followed standard procedures for the collection, preparation, and analysis of samples, as well as the statistical analysis of the data.

Reagents

The study utilised analytical-grade reagents, including lead acetate from Sigma Incorporated U.S.A. and polyfloral wild honey from Ola-Osun Farm in Ilesha, Osun State.

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Plant Collection and Preparation of Crude Methanolic Extract:
Fresh twigs with leaves of mature *Vernonia amygdalina* were collected from the Kere, Idi Oro Area, Ogbomoso, Oyo State, Nigeria, on January 27, 2018. The voucher number for the collected *Vernonia amygdalina* leaves is LHO 820.
The leaves were air-dried and processed into a powder, from which a crude methanolic extract was obtained. 2 kg of this powder was soaked in 10 litres of methanol for 3 days in order to obtain the extract. The extract mixture was filtered after 3 days with filter paper, and the filtrate was concentrated using a rotary evaporator to obtain a paste-like extract that was later dried in an oven at 45 degrees Celsius.

Ethical Approval
All experimental procedures and materials were approved by the Faculty of Basic Medical Sciences' Ethical Research Committee of Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria. The ERC Approval Number is ERCFBMSLAUTECH:020/05/2024.

Animals' management and procedure
Thirty-five male Wistar rats (weighing 175-250 g) were acclimatised for two weeks and divided into five groups of seven animals per group. The animals in group 1 served as controls, and they were given feed and water only; group 2 was administered with lead acetate (2.2 mg/kg) only; group 3 was treated with lead acetate (2.2 mg/kg) + honey (5g/kg); group 4 was treated with lead acetate (2.2 mg/kg) + bitter leaf extract (300 mg/kg); and group 5 received lead acetate (2.2 mg/kg) + honey (5g/kg) + bitter leaf extract (300 mg/kg). The animals were dosed daily orally for 6 weeks via oral cannula. After the treatment period, the rats were euthanized with sodium pentobarbital, and their blood and kidney samples were collected for further analysis.

Biochemical assays
The serum and kidney tissue samples were used to analyse various biochemical parameters, including creatinine, urea, superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT).
The serum creatinine and urea levels were determined by the method specified in the RANDOX kit. The tissue's SOD activity was evaluated using Misra and Fridovich's technique.[12] The capacity of superoxide dismutase to block the auto-oxidation of epinephrine at pH 10.2 provides a simple foundation for this assay. The MDA concentration was determined by the method of Varshney and Kale.[13] The estimation of lipid peroxidation is based on the interaction of malondialdehyde (MDA) with thiobarbituric acid (TBA), which forms an MDA-TBAR adduct that absorbs strongly at 532 nm. The CAT activities were measured using the Aebi method of Aebi.[14] CAT activity was determined by measuring the decrease in absorbance at 240 nm.

Histological Analysis
The kidney tissues were fixed in 10% formosaline and processed for histological analysis using hematoxylin and eosin (H and E) staining. The tissue sections underwent a thorough preparation process, including dewaxing and alcohol baths. The sections were stained with Harris hematoxylin, washed, counterstained with 1% eosin, dehydrated, cleared in xylene, and mounted in dibutyl phthalate xylene (DPX) for further analysis.

Statistical analysis
The data obtained for each group were analysed and expressed as the mean ± SEM. A one-way ANOVA was used to determine the level of significance ($P < 0.05$) across the groups, and the Duncan Multiple Range Post Hoc test was performed to indicate specifically where the significance exists. The Statistical Package for Social Sciences (version 20) was used for the analysis.

Results and Discussion

Biochemical Studies
Lead toxicity is a significant environmental and health concern due to its severe biological impacts, including renal system damage, oxidative stress, and enzymatic activities in humans and animals. This study investigates the effects of lead exposure on rats' biochemical parameters and investigates the potential benefits of *Vernonia amygdalina* and honey, both individually and in combination. In the current investigation, rats exposed to lead had significantly higher urea levels than the control group, showing the nephrotoxic effects of lead (Table 1). Lead exposure is linked to decreased renal function, as evidenced by elevated urea and creatinine levels, which are crucial indicators of kidney health and glomerular filtration rate.[15]
Creatinine levels were observed to be higher in rats treated with lead compared to controls. The increases were normalised in all animals when fed bitter leaf, honey, or a combination of honey and bitter leaf (Table 1). Sharma *et al.*[16] reported similar findings, observing elevated urea and creatinine levels in lead-exposed rats, indicating renal damage.
Lead exposure has also been shown to affect antioxidant defence pathways, generating oxidative stress. Catalase (CAT) and superoxide dismutase (SOD) are key antioxidant enzymes that protect cells from oxidative stress by neutralising reactive oxygen species (ROS). In this study, lead-treated rats had considerably lower catalase activity than the control group, indicating impaired antioxidant defences (Table 1). This is similar with prior studies by Flora *et al.*[17] who found that lead exposure lowers catalase activity, adding to oxidative stress. Interestingly, the study found no significant difference in SOD activity across the groups ($P = 0.313$), showing that SOD is less susceptible to lead-induced oxidative stress than catalase. This contrasts with some studies that have found reduced SOD activity in lead-exposed animals.[18] The discrepancy could be due to differences in exposure duration, lead concentration, or the specific animal model used.
Malondialdehyde (MDA) is a by-product of lipid peroxidation and serves as a marker for oxidative stress and cellular damage. It was observed that bitter leaf and honey administration (both alone and combined) restored MDA levels closer to control values is intriguing (Table 1). This potentially suggests that these natural substances enhanced antioxidant defences and promoted repair mechanisms.These natural compounds might have aided in repairing cell membrane damage caused by lipid peroxidation, potentially influencing MDA levels. Previous research has demonstrated that natural antioxidants can reduce lipid peroxidation and oxidative stress caused by heavy metals.[19]
The study looked at the possible therapeutic effects of bitter leaf and honey on lead intoxication. Bitter leaf (*Vernonia amygdalina*) is reported to have antioxidant and hepatoprotective effects.[20] Honey, a naturally occurring antioxidant, has been found to reduce oxidative stress and improve immunological function.[21] The combined treatment of bitter leaf and honey restored urea and creatinine levels, indicating kidney protection. Furthermore, the co-administration restored catalase activity to comparable levels with the control group, showing the synergistic antioxidant benefits of these natural compounds (Table 1).
Histological Studies
Photomicrographs of kidney sections in the control group reveal normal architecture, with normal glomeruli, mesengial cells, capsular spaces, renal tubules, interstitial spaces, and no pathological lesion observed in higher magnification (Plate 1). Lead poses a significant threat to the renal system in animals, resulting in detrimental histopathological changes.[22] Renal injury is a known consequence of lead exposure.[23]
Photomicrographs of kidney sections of lead-only group show poor architecture, glomeruli with atrophic mesengial cells, degenerated tubules, tubular necrosis, moderate fibrosis, and severe infiltration of inflammatory cells in interstitial spaces (Plate 2). Lead exposure has been shown to induce significant histological changes in the kidneys of experimental animals. A study by Sharma *et al.*[16] reported that lead acetate administration in rats led to glomerular atrophy, tubular necrosis, and interstitial inflammation in the kidneys. Similarly, Hsu

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and Guo observed that chronic lead exposure caused glomerular sclerosis, tubular degeneration, and interstitial fibrosis in rat kidneys.[24] This study found enlargement of epithelial cells in renal tubules, particularly proximal tubules, and tubular necrosis, a severe injury causing kidney disease. This result corroborated with the findings of Mukherjee *et al*.[25] The enlargement may be due to increased metabolic activity in response to injury, as proximal tubules re absorb nutrients and other substances.[26] Kidney metabolism undergoes profound modifications to adapt to oxygen and nutrient shortage during injury, resulting in kidney function loss.[27]

However, co-administration of honey or bitter leaf appears to have reduced the lead-induced histological changes in the kidneys. Photomicrographs of lead and honey kidney slices showed a rather normal architecture with minor vascular congestion (Plate 3). This shows that honey may have contributed to the kidneys' structural integrity by lowering lead-induced oxidative stress and inflammation. Omotoso *et al*. discovered that honey supplementation reduced lead-induced oxidative stress and histological alterations in the kidneys of rats.[28] These advantages apply to the antioxidant components found in honey, such as flavonoids and phenolic acids. Honey is reported to have antioxidant and nephroprotective effects and has been used to treat wounds since ancient times till nowadays.[29]

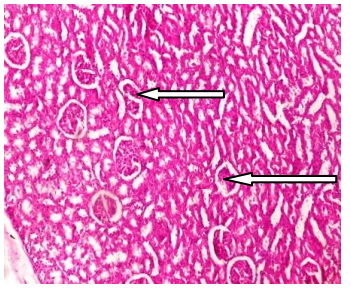
Similarly, lead and bitter leaf kidney sections displayed a reasonably typical architecture, with hyperplasia in mesenchymal cells but no significant renal cortex lesions (Plate 4). Adesanoye and Farombi discovered that bitter leaf extract reduced lead-induced oxidative damage and histological abnormalities in the liver of rats.[30] These protective effects were achieved through the antioxidant and metal-chelating properties of the plant's phytochemicals, which included flavonoids and saponins. Bitter leaf (*Vernonia amygdalina*) is known for its anti-inflammatory, antioxidant, and liver-protective qualities and its medicinal properties are well established.[31]

Interestingly, photomicrographs of lead, honey, and bitter leaf group kidney sections exhibited dilated renal tubules containing eosinophilic components (Plate 5). This observation shows that the combination of lead exposure and therapies (honey and bitter leaf) may have caused some tubular damage or obstruction. Further research is required to determine the underlying processes and potential synergistic or antagonistic effects of honey and bitter leaf in the presence of lead toxicity.

Table 1: Effects of Lead and Antioxidant Treatments on Biochemical Parameters in Wistar Rats

Test	Urea (µmol/l)	Creatinine (mgl/l)	Catalase(µmol/mg) protein	SOD (µmol/mg) protein	MDA (µmol/mg) protein
Control	7.93 ± 0.43^{a}	1.45 ± 0.14^{a}	2.82 ± 0.12^{b}	0.57 ± 0.15^{a}	0.75 ± 0.19^{a}
Lead Only	11.69 ± 0.57^{c}	2.48 ± 0.24^{b}	1.51 ± 0.09^{a}	0.27 ± 0.17^{a}	3.27 ± 0.07^{d}
Lead + Bitter leaf	10.03 ± 0.66^{b}	1.65 ± 0.09^{a}	2.25 ± 0.12^{a,b}	0.25 ± 0.05^{a}	1.61 ± 0.08^{b}
Lead + Honey	8.97 ± 0.39^{a,b}	1.90 ± 0.06^{a}	2.19 ± 0.19^{a,b}	0.33 ± 0.08^{a}	2.02 ± 0.09^{c}
Lead + Bitter leaf + Honey	9.64 ± 0.28^{b}	1.60 ± 0.25^{a}	2.50 ± 0.51^{b}	0.40 ± 0.08^{a}	1.46 ± 0.14^{b}

All values were expressed as mean ± SEM of 5 determinations. Values in the same column with different superscript indicate significant difference at P<0.05.



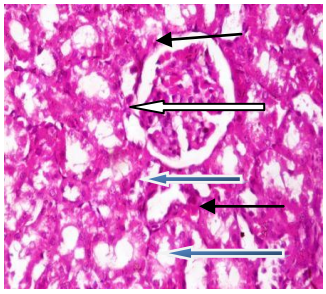
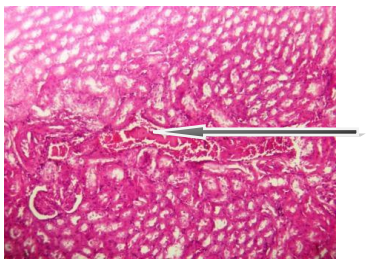


Plate 1: Control Group
Photomicrographs of kidney sections of the control group reveal normal architecture with no pathological lesions.



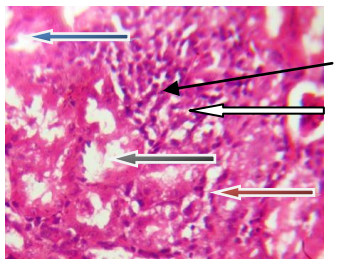
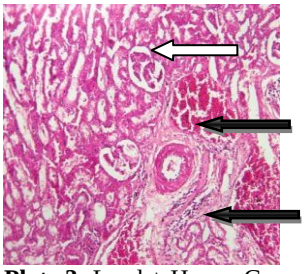


Plate 2: Lead Only Group
Photomicrographs of kidney sections exposed to lead-only reveal poor architecture and severe inflammatory cell infiltration in interstitial spaces.



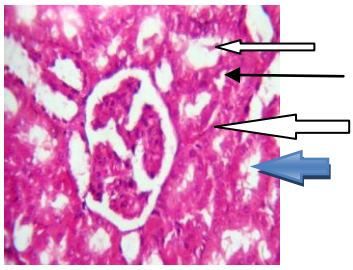
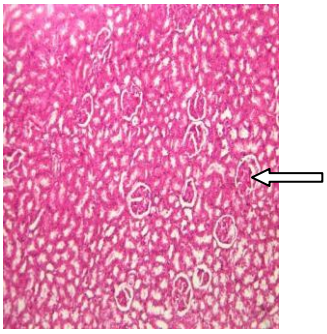


Plate 3: Lead + Honey Group
Photomicrographs of kidney sections with lead and honey reveal a normal architecture with minor vascular congestion



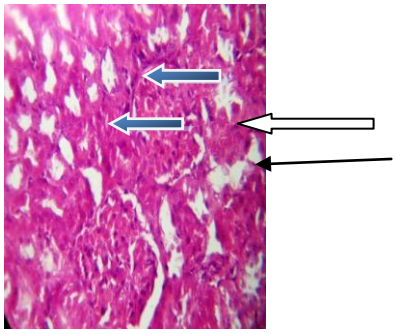


Plate 4: Lead + Bitter leaf Group
Photomicrographs of kidney sections with lead and bitter leaf reveal a typical architecture with hyperplasia in mesenchymal cells, but no significant renal cortex lesions.

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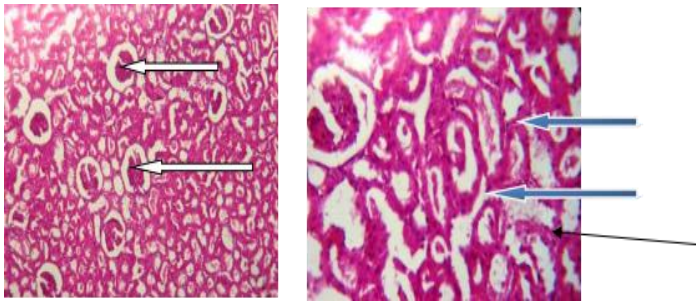

Plate 5: Lead + Honey + Bitter leaf Group
Photomicrographs of kidney sections exposed to lead, honey, and bitter leaf reveal dilated renal tubules with eosinophilic components.

Conclusion

The current study found that honey and bitter leaf extracts can protect against kidney damage caused by lead exposure. It also shows that lead exposure reduced catalase activity, which was partially recovered with honey and restored to normal levels when combined with bitter leaf. Bitter leaf treatment also reduced malondialdehyde levels, a marker of lipid peroxidation and oxidative stress. The study suggests that these natural compounds may help maintain kidney structural integrity and protect against lead-induced kidney damage in animal models.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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