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NEPHRO PROTECTIVE EFFECT OF *VITELLARIA PARADOXA* LEAVES ON BENZENE-INDUCED WISTAR RATS

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ABSTRACT
The protective effect of *Vitellaria paradoxa* leaf extract against benzene-induced toxicity was investigated in Wistar rats. Thirty male rats weighing between 150 and 200 g were randomly distributed into five groups (n = 6). Benzene (0.2 ml/kg) was administered to induce toxicity, while treatment groups received 200 mg/kg and 400 mg/kg of the n-hexane extract. Vitamin E (200 mg/kg) served as the reference treatment for 21 days. Body weight changes, renal function indices and oxidative stress markers were evaluated at the end of the experimental period. Exposure to benzene reduced weight gain and increased serum urea, creatinine and bilirubin levels when compared with the normal control group. Antioxidant enzyme activities, including superoxide dismutase, catalase and glutathione, were also reduced, while malondialdehyde levels increased significantly. Administration of *Vitellaria paradoxa* extract improved body weight gain, reduced renal biomarkers, restored antioxidant enzyme activity and lowered lipid peroxidation in a dose-related manner. The effect observed at 400 mg/kg was comparable to that of vitamin E. These findings indicate that *Vitellaria paradoxa* leaf extract may protect against benzene-induced nephrotoxicity, possibly through antioxidant mechanisms.

KEYWORDS: Nephrotic damage, benzene, *Vitellaria paradoxa*, Creatinine, Urea.

INTRODUCTION

Benzene is a volatile aromatic hydrocarbon commonly used in industrial processes and chemical production (Miller et al., 2022). Due to its widespread use, exposure may occur through inhalation, contaminated water, or occupational activities. Benzene has been associated with toxic effects on several organs, including the liver, lungs, brain and kidneys (Irato & Santovito, 2021). Continuous exposure may disrupt normal cellular processes and contribute to tissue injury through oxidative stress mechanisms (Wang et al., 2024).

The kidneys are highly susceptible to toxic injury because they continuously filter blood and concentrate metabolic waste products (Imenez-Silva & Mohebbi, 2022). Damage to renal tissues caused by toxic substances can impair kidney function and may progress to severe renal complications if left unmanaged (Yadav et al., 2024). Oxidative stress has been identified as one of the major mechanisms involved in chemically induced nephrotoxicity (Piko et al., 2023).

Medicinal plants remain an important source of therapeutic agents due to the presence of biologically active compounds (Dar et al., 2023). Many plants contain flavonoids, alkaloids, tannins and terpenoids, which possess antioxidant and anti-inflammatory activities (Parvin et al., 2023). These compounds may help reduce

free radical generation and protect tissues from oxidative damage (Bittner et al., 2021).

Vitellaria paradoxa, commonly known as shea tree, is a medicinal plant widely used in traditional medicine (Bairy et al., 2023). Different parts of the plant have been reported to possess antioxidant, antimicrobial, anti-inflammatory and protective properties (Baky et al., 2022). The plant contains phytochemicals such as triterpenes, catechins and flavonoids, which may contribute to its biological activity (Ojo et al., 2021).

Based on these observations, this study investigated the effect of *Vitellaria paradoxa* leaf extract on benzene-induced nephrotoxicity in Wistar rats by assessing renal function and oxidative stress markers.

MATERIALS AND METHODS

Animals

Thirty male Wistar rats weighing between 150 and 200 g were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology, University of Port Harcourt, Rivers State, Nigeria. They were housed in standard laboratory cages at the Faculty of Basic Medical Sciences, Niger Delta University, Bayelsa State, Nigeria.

The animals were maintained under standard laboratory conditions with a 12-hour light/dark cycle and adequate ventilation. Standard pellet feed and clean drinking water were provided ad libitum throughout the experimental period. Prior to the commencement of the study, the rats were allowed to acclimatize for 14 days.

Ethical Approval

Ethical approval for this study was obtained from the Ethical Committee of Niger Delta University, Bayelsa State, Nigeria. All experimental procedures involving animals were carried out in accordance with the guidelines for the care and use of laboratory animals.

Preparation of Plant Extract

Fresh leaves of *Vitellaria paradoxa* were collected from Bayelsa State, Nigeria and identified by Professor Kola Ajibesin of the Department of Pharmacognosy, Niger Delta University. The leaves were washed thoroughly and air-dried under shade at room temperature for two weeks. The dried leaves were then ground into a coarse powder using a mechanical grinder.

A total of 500 g of the powdered sample was soaked in 2 L of n-hexane for 48 hours with intermittent stirring to ensure proper extraction. The mixture was filtered using muslin cloth followed by Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 40°C to obtain the crude extract. The extract was stored in a clean airtight container until required for the experiment.

Experimental Design

The animals were randomly divided into five groups containing six rats each:

Group I (Normal Control): Received distilled water throughout the study period.

Group II (Benzene Control): Received benzene (0.2 ml/kg body weight) every 48 hours for 21 days.

Group III (Low Dose Treatment): Received benzene (0.2 ml/kg body weight) every 48 hours and 200 mg/kg body weight of *Vitellaria paradoxa* extract daily.

Group IV (High Dose Treatment): Received benzene (0.2 ml/kg body weight) every 48 hours and 400 mg/kg body weight of *Vitellaria paradoxa* extract daily.

Group V (Standard Control): Received benzene (0.2 ml/kg body weight) every 48 hours and vitamin E (200 mg/kg body weight) daily.

The treatment period lasted for 21 consecutive days.

Sample Collection

At the end of the experimental period, the animals were anesthetized using chloroform and sacrificed. Blood samples were collected through cardiac puncture into plain sample bottles and allowed to clot at room temperature. The samples were centrifuged at 2000 rpm for 10 minutes to obtain serum for biochemical analysis.

The kidneys were excised immediately, rinsed in cold normal saline and homogenized for the assessment of oxidative stress markers.

Preparation of Tissue Homogenate

Kidney tissues were weighed and homogenized in ice-cold phosphate buffer (0.1 M, pH 7.4) to obtain a 10% tissue homogenate. The homogenate was centrifuged at 1000 × g for 10 minutes at 4°C. The resulting supernatant was collected and used for the determination of antioxidant enzyme activities and lipid peroxidation levels.

Determination of Renal Function Parameters

Serum urea concentration was determined using the Berthelot enzymatic method, while serum creatinine was estimated using the Jaffe colorimetric kinetic method with commercial diagnostic kits (Agappe Diagnostics, Switzerland). Serum bilirubin concentration was determined using the method described by Jendrassik and Grof (1938).

Determination of Oxidative Stress Markers

Oxidative stress parameters including superoxide dismutase (SOD), catalase, reduced glutathione (GSH) and malondialdehyde (MDA) were determined using standard laboratory procedures. SOD activity was determined according to the method of Marklund and Marklund (1974), catalase activity by the method of Aebi (1984) and reduced glutathione according to Beutler (1967). Lipid peroxidation was estimated by measuring malondialdehyde levels.

Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by an appropriate post hoc test for multiple comparisons. Differences between groups were considered statistically significant at $p < 0.05$.

RESULTS

Effect of *Vitellaria paradoxa* Extract on Body Weight

Body weight increased in all experimental groups during the study period; however, the increase observed in benzene-treated rats was markedly lower when compared with the normal control group. Rats exposed to benzene alone showed a significant reduction ($p < 0.05$) in percentage weight gain, indicating the adverse effect of benzene on normal growth and metabolism.

Treatment with *Vitellaria paradoxa* extract improved body weight gain in a dose-dependent manner. Animals treated with 400 mg/kg of the extract showed greater improvement when compared with those treated with 200 mg/kg. The group treated with vitamin E also demonstrated improved weight gain comparable to the high-dose extract group.

Table 1: Effect of *Vitellaria paradoxa* Extract on Body Weight.

Groups	Mean Weight Before Treatment (g)	Mean Weight After Treatment (g)	Percentage Weight Change (%)
Normal Control	158.83 ± 2.79[a]	195.50 ± 3.45[a]	35.67 ± 0.00[a]
Benzene Control	161.17 ± 2.48[a]	170.17 ± 2.86[b]	9.00 ± 0.00[b]
Benzene + 200 mg/kg Extract	160.67 ± 3.08[a]	182.17 ± 5.60[c]	21.50 ± 0.00[c]
Benzene + 400 mg/kg Extract	162.00 ± 3.00[a]	192.17 ± 4.67[a]	30.17 ± 0.00[a]
Benzene + Vitamin E	162.83 ± 1.83[a]	194.83 ± 2.14[a]	32.00 ± 0.00[a]

Values are expressed as mean ± SD (n = 6). Values with different superscripts differ significantly at $p < 0.05$.

Effect of *Vitellaria paradoxa* Extract on Renal Function Parameters

Administration of benzene caused a significant increase ($p < 0.05$) in serum urea, creatinine and bilirubin levels when compared with the normal control group, indicating renal impairment.

Treatment with *Vitellaria paradoxa* extract reduced these elevated biochemical markers in both treatment groups. The reduction was more pronounced in animals treated with 400 mg/kg of the extract, suggesting a dose-related protective effect. Similar improvements were observed in the vitamin E-treated group.

Table 2: Effect of *Vitellaria paradoxa* Extract on Renal Function Parameters.

Groups	Urea (mg/dL)	Creatinine (mg/dL)	Bilirubin (mg/dL)
Normal Control	66.69 ± 2.13[a]	0.69 ± 0.03[a]	0.37 ± 0.02[a]
Benzene Control	120.80 ± 2.84[b]	3.19 ± 0.19[b]	1.11 ± 0.05[b]
Benzene + 200 mg/kg Extract	106.01 ± 8.10[c]	1.86 ± 0.16[c]	0.71 ± 0.06[c]
Benzene + 400 mg/kg Extract	87.82 ± 3.88[d]	1.28 ± 0.03[d]	0.61 ± 0.02[d]
Benzene + Vitamin E	78.29 ± 2.64[e]	0.92 ± 0.10[e]	0.55 ± 0.03[e]

Values are expressed as mean ± SD (n = 6). Values with different superscripts differ significantly at $p < 0.05$.

Effect of *Vitellaria paradoxa* Extract on Oxidative Stress Markers

Benzene exposure significantly reduced ($p < 0.05$) the activities of antioxidant enzymes including superoxide dismutase (SOD), catalase and reduced glutathione (GSH) when compared with the normal control group. In contrast, malondialdehyde (MDA) concentration increased significantly following benzene administration, indicating enhanced lipid peroxidation and oxidative stress.

Treatment with *Vitellaria paradoxa* extract improved antioxidant enzyme activities and reduced MDA concentration in a dose-dependent manner. The high-dose extract group showed greater restoration of antioxidant status and reduction in oxidative damage. Comparable effects were observed in the vitamin E-treated group.

Table 3: Effect of *Vitellaria paradoxa* Extract on Oxidative Stress Markers.

Groups	SOD (U/mg)	Catalase (U/mg)	GSH (U/mg)	MDA (U/mg)
Normal Control	10.30 ± 0.49[a]	8.68 ± 0.50[a]	8.69 ± 0.50[a]	1.96 ± 0.13[a]
Benzene Control	1.92 ± 0.16[b]	1.96 ± 0.09[b]	2.00 ± 0.09[b]	13.46 ± 0.97[b]
Benzene + 200 mg/kg Extract	4.32 ± 0.22[c]	3.97 ± 0.39[c]	4.58 ± 0.18[c]	7.07 ± 0.17[c]
Benzene + 400 mg/kg Extract	6.24 ± 0.15[d]	5.03 ± 0.23[d]	6.25 ± 0.25[d]	4.82 ± 0.17[d]
Benzene + Vitamin E	7.25 ± 0.44[e]	6.35 ± 0.23[e]	5.50 ± 0.23[e]	4.87 ± 0.70[d]

Values are expressed as mean ± SD (n = 6). Values with different superscripts differ significantly at $p < 0.05$.

DISCUSSION

The findings of this study demonstrate that exposure to benzene caused marked alterations in body weight, renal function indices and oxidative stress parameters in Wistar rats. These changes indicate that benzene exposure adversely affected normal physiological and biochemical processes, particularly those associated with kidney function and antioxidant defence mechanisms.

A significant reduction in body weight gain was observed in the benzene-treated group when compared with the normal control animals. Reduced weight gain following benzene exposure may be associated with metabolic disturbances, impaired nutrient utilization and increased oxidative stress caused by the toxicant (Li et al., 2024). Similar reductions in body weight have been reported in experimental animals exposed to benzene and related environmental toxicants, where prolonged exposure interfered with normal metabolic activities and growth performance (Cui et al., 2022).

Treatment with *Vitellaria paradoxa* extract improved body weight gain in a dose-dependent manner, with the 400 mg/kg group showing values close to the normal control and vitamin E-treated groups. This observation suggests that the extract may possess protective properties capable of reducing the metabolic stress associated with benzene toxicity. The improvement observed may be linked to the presence of bioactive phytochemicals such as flavonoids, triterpenes, catechins and phenolic compounds known for their antioxidant activities (Nwozo et al., 2023). Comparable findings have been reported in studies involving medicinal plants rich in polyphenols, where restoration of body weight was associated with reduced oxidative damage and improved physiological function (Ribeiro et al., 2026).

The significant elevation in serum urea, creatinine and bilirubin levels observed in benzene-exposed rats indicates impairment of renal function. Urea and creatinine are important biomarkers used in assessing glomerular filtration efficiency and their increased concentrations suggest renal tissue damage and reduced excretory capacity (Gowda et al., 2010). Elevated bilirubin levels may also indicate impaired clearance and increased oxidative injury associated with toxic exposure. Similar increases in renal biomarkers following benzene administration have been documented in previous studies involving chemically induced nephrotoxicity (Qin et al., 2025).

Administration of *Vitellaria paradoxa* extract significantly reduced serum urea, creatinine and bilirubin levels when compared with the benzene control group. The improvement was more pronounced at the higher dose, indicating a dose-related protective effect. These findings are consistent with earlier reports demonstrating the nephroprotective and antioxidant properties of *Vitellaria paradoxa* and other medicinal plants containing flavonoids and triterpenoids (Mihailović et al., 2021). The reduction in renal biomarkers observed in this study may be associated with stabilization of renal cell membranes, improvement in filtration efficiency and reduction of oxidative damage within kidney tissues (Dennis & Witting, 2017).

Oxidative stress is considered one of the major mechanisms involved in benzene-induced organ toxicity. In the present study, exposure to benzene significantly reduced the activities of antioxidant enzymes including SOD, catalase and GSH, while MDA concentration increased markedly. Reduced antioxidant enzyme activity suggests depletion of the endogenous antioxidant defence system following excessive production of reactive oxygen species (Rao et al., 2025). Increased MDA levels further indicate enhanced lipid peroxidation and oxidative damage to cellular membranes (Mohideen et al., 2023). Similar findings have been reported in experimental studies where benzene exposure induced oxidative stress through excessive free radical generation and depletion of antioxidant reserves (Mohammed, 2026).

Treatment with *Vitellaria paradoxa* extract restored antioxidant enzyme activities and reduced MDA concentration in a dose-dependent manner. The higher dose of the extract demonstrated stronger antioxidant activity and produced effects comparable to vitamin E, which served as the standard antioxidant treatment. These findings suggest that the extract may help maintain oxidant-antioxidant balance and reduce lipid peroxidation in renal tissues. Similar antioxidant effects have been reported in studies involving plant extracts rich in phenolic compounds and flavonoids (Tungmunnithum et al., 2018).

The protective effects observed in this study may therefore be attributed to the antioxidant phytochemicals present in *Vitellaria paradoxa*, which may act by scavenging reactive oxygen species, stabilizing cellular membranes and improving endogenous antioxidant defence systems (Idih et al., 2020). The dose-dependent response observed further supports the therapeutic potential of the plant extract in reducing benzene-induced nephrotoxicity.

The results obtained from this study are consistent with previous reports on the nephroprotective and antioxidant effects of medicinal plants against chemically induced toxicity (Daoud et al., 2026). The findings further support the potential use of *Vitellaria paradoxa* as a natural therapeutic agent for managing oxidative stress-related renal damage.

CONCLUSION

The findings from this study indicate that *Vitellaria paradoxa* leaf extract reduced benzene-induced alterations in renal function and oxidative stress parameters in Wistar rats. The extract improved antioxidant enzyme activity and decreased lipid

peroxidation in a dose-dependent manner. These results suggest that *Vitellaria paradoxa* possesses protective properties against benzene-induced nephrotoxicity, possibly through antioxidant mechanisms. Further studies are recommended to investigate the active compounds and molecular pathways involved.

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