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Nephroproctective Effect of Cocoa Leaf (*Theobroma Cacao L*) in Toluene Induced Wistar Rats

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ABSTRACT

This research examined whether *Theobroma cacao* leaf extract could protect Wistar rats from toluene-induced toxicity. Thirty male rats (150–200 g) were split into five groups of six. Toxicity was induced with toluene (50 mg/kg), while treatment groups received the extract at 200 mg/kg or 400 mg/kg. A standard group was given vitamin E (200 mg/kg). After 21 days, liver enzymes (ALT, AST, ALP), total protein, and albumin were measured. Oxidative markers (SOD, catalase, GSH, MDA) were also assessed. Toluene caused significant rises ($p < 0.05$) in ALT, AST, ALP, and MDA, and lowered total protein, albumin, SOD, catalase, and GSH. Treatment with the extract reversed these changes, with stronger effects at the higher dose. The results suggest the extract can reduce toluene toxicity, likely through its antioxidant action.

KEYWORDS: *Nephroproctective effect, Toluene, Wistar rats, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST).*

INTRODUCTION

The kidneys are essential organs responsible for maintaining internal balance in the body through the removal of metabolic waste, regulation of electrolyte levels, and control of fluid homeostasis (Hix & Nayman, 2024). They also play a key role in blood pressure regulation through hormonal mechanisms. Because of their continuous exposure to circulating substances, the kidneys are particularly susceptible to damage from toxic compounds (Lee & Park, 2026; Boima et al., 2025).

Nephrotoxicity refers to the impairment of kidney function caused by exposure to harmful substances, including drugs, environmental pollutants, and industrial chemicals (Yadav et al., 2024). This condition remains a significant public health concern, as it can progress to acute kidney injury or chronic kidney disease if not properly managed (Francis et al., 2024). The mechanisms underlying nephrotoxicity often involve oxidative stress, inflammation, and cellular damage within renal tissues (Piko et al., 2023).

Toluene is a commonly used organic solvent found in products such as paints, adhesives, and cleaning agents. Human exposure may occur through occupational activities or environmental contamination (Rajput et al., 2025; Ghobakhloo et al., 2022). Previous studies have shown that toluene can adversely affect multiple organ systems, including the kidneys, by disrupting normal cellular function and promoting oxidative damage (Ben-Attia et al., 2025; Abubakar et al., 2025). Prolonged exposure has been associated with electrolyte imbalance, structural damage, and reduced renal efficiency (Deabes & Essa, 2024).

In recent years, there has been growing interest in the use of plant-derived compounds as protective agents against chemically induced organ damage. Many medicinal plants contain bioactive constituents such as flavonoids, polyphenols, and alkaloids, which are known to possess antioxidant and anti-inflammatory properties (Dar et al., 2023; Roy et al., 2022). These compounds can help reduce oxidative stress and improve cellular defence mechanisms.

Theobroma cacao, commonly known as cocoa, is widely recognized for its nutritional and medicinal value (Rojas et al., 2022). While the seeds have been extensively studied, the leaves also contain significant amounts of phytochemicals that may contribute to protective biological effects (Patience & Richard, 2025). These phytochemicals may play a role in reducing oxidative damage and supporting tissue recovery under toxic conditions.

Therefore, this study was designed to evaluate the protective effect of *Theobroma cacao* leaf extract against toluene-induced toxicity in Wistar rats by assessing biochemical markers and oxidative stress parameters.

MATERIALS AND METHODS

Experimental Animals

This study used thirty male Wistar rats, each weighing 150–200 g. The animals were sourced from the University of Port Harcourt's animal house and later housed in the Biochemistry Department at Niger Delta University, Bayelsa State. They were maintained under standard laboratory conditions, including regulated temperature and a 12-hour light/dark cycle. Clean water and standard pellet diet were provided without restriction throughout the experiment. Before any procedures began, the rats were given two weeks to acclimate to their new environment.

Experimental Design

The animals were randomly assigned into five groups (n = 6 per group):

Group I (Normal Control): Received distilled water only for 21 days.

Group II (Toluene Control): Received distilled water and a single dose of toluene (50 mg/kg body weight).

Group III (Low Dose Extract): Received 200 mg/kg body weight of *Theobroma cacao* leaf extract following toluene exposure.

Group IV (High Dose Extract): Received 400 mg/kg body weight of *Theobroma cacao* leaf extract following toluene exposure.

Group V (Standard Control): Received vitamin E (200 mg/kg body weight) following toluene exposure.

Toluene was administered as a single dose prior to the commencement of treatment, and all treatments were given daily for 21 days.

Plant Collection and Extraction

Fresh *Theobroma cacao* leaves were gathered from Amarata, Yenagoa, Bayelsa State, Nigeria. Their identity was confirmed by Professor Kola Ajibesin of the Department of Pharmacognosy, Niger Delta University. The leaves were dried in the open air at room temperature and then crushed into a coarse powder. A 500 g portion of this powder was mixed with 2 liters of n-hexane and left to stand for 48 hours with occasional stirring. The mixture was then filtered, and the liquid portion was concentrated to yield the crude extract used in this study.

Sample Collection

After the treatment period ended, the animals were anesthetized with chloroform and euthanized. Blood was drawn via cardiac puncture and left to clot before being centrifuged at 2000 rpm for 10 minutes to separate the serum. The liver tissues were removed, washed with normal saline, and then homogenized to measure oxidative stress markers.

Biochemical Analysis

Serum samples were employed to measure biochemical parameters, including ALT, AST, ALP, total protein, albumin, urea, creatinine, and bilirubin. Oxidative stress markers (SOD, catalase, GSH, and MDA) were assessed in liver homogenates using established laboratory protocols.

Statistical Analysis

Results from the study are presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used for statistical evaluation, followed by an appropriate post hoc test for multiple group comparisons. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Table 1: Effect of *Theobroma cacao* Extract on Body Weight

Group	Day 0 (g)	Day 21 (g)	Weight Gain (g)
Normal Control	153.16 ± 3.86[a]	206.50 ± 12.11[a]	53.34[a]
Toluene Control	159.00 ± 2.83[b]	191.60 ± 11.62[b]	32.60[b]
Toluene + 200 mg/kg	159.00 ± 3.35[b]	197.00 ± 6.32[c]	38.00[c]
Toluene + 400 mg/kg	158.30 ± 4.23[b]	199.17 ± 8.28[c]	40.87[c]
Toluene + Vitamin E	158.50 ± 2.74[b]	202.17 ± 8.35[a]	43.67[c]

Values are expressed as mean ± SD (n = 6). Different superscripts indicate significant difference ($p < 0.05$).

Table 2: Effect on Renal Function Parameters

Group	**Urea (mg/dL)**	**Creatinine (mg/dL)**	**Bilirubin (mg/dL)**
Normal Control	67.83 ± 2.65[a]	0.67 ± 0.02[a]	0.39 ± 0.01[a]
Toluene Control	108.16 ± 7.77[b]	2.90 ± 0.07[b]	1.05 ± 0.06[b]
Toluene + 200 mg/kg	91.16 ± 4.85[c]	1.75 ± 0.20[c]	0.67 ± 0.04[c]
Toluene + 400 mg/kg	83.09 ± 3.58[d]	1.27 ± 0.03[d]	0.58 ± 0.02[d]
Toluene + Vitamin E	76.15 ± 3.63[a]	0.60 ± 0.07[a]	0.49 ± 0.02[e]

Values are expressed as mean ± SD (n = 6). Different superscripts indicate significant difference ($p < 0.05$).

Table 3: Effect on Oxidative Stress Markers

Group	**SOD (U/mg)**	**Catalase (U/mg)**	**GSH (U/mg)**	**MDA (U/mg)**
Normal Control	9.05 ± 0.23[a]	9.76 ± 0.62[a]	8.24 ± 0.32[a]	2.03 ± 0.12[a]
Toluene Control	1.50 ± 0.32[b]	1.72 ± 0.20[b]	1.97 ± 0.10[b]	10.36 ± 1.29[b]
Toluene + 200 mg/kg	4.01 ± 0.19[c]	3.64 ± 0.45[c]	3.91 ± 0.45[c]	6.94 ± 0.36[c]
Toluene + 400 mg/kg	6.07 ± 0.55[d]	5.35 ± 0.90[d]	6.19 ± 0.60[d]	4.68 ± 0.56[d]
Toluene + Vitamin E	6.34 ± 0.29[d]	5.99 ± 0.39[d]	6.57 ± 0.66[d]	4.24 ± 0.75[d]

Values are expressed as mean ± SD (n = 6). Different superscripts indicate significant difference ($p < 0.05$).

DISCUSSION

The present study evaluated the protective effect of *Theobroma cacao* leaf extract against toluene-induced toxicity in Wistar rats. The findings clearly show that exposure to toluene disrupted normal physiological and biochemical processes, while treatment with the plant extract improved these alterations.

A reduction in body weight gain was observed in animals exposed to toluene, suggesting that the toxicant may have interfered with normal metabolic activity. Reduced weight gain following exposure to toxic substances has been associated with impaired nutrient utilization and increased physiological stress. However, administration of *Theobroma cacao* extracts improved weight gain, indicating a possible recovery of metabolic function.

The significant increase in serum urea and creatinine levels observed in the toluene-treated group indicates impaired kidney function. These biomarkers are widely used indicators of renal integrity, and their elevation suggests reduced glomerular filtration and accumulation of metabolic waste products. This finding is consistent with previous reports that organic solvents can induce nephrotoxicity through direct cellular damage and oxidative stress mechanisms (Choi et al., 2018; Cullen et al., 2019).

Treatment with *Theobroma cacao* extract significantly reduced the levels of urea and creatinine, suggesting an improvement in renal function. The effect was more pronounced at the higher dose, indicating a dose-dependent response. This improvement may be attributed to the presence of bioactive compounds in cocoa leaves, particularly flavonoids and polyphenols, which are known to enhance antioxidant defence and protect cellular structures from damage (Adeyemi et al., 2021).

Oxidative stress plays a central role in chemically induced tissue injury. In this study, toluene exposure led to a marked reduction in antioxidant enzymes, including SOD, catalase, and GSH, alongside an increase in MDA, a marker of lipid peroxidation. This pattern indicates that toluene promoted the generation of reactive oxygen species, which overwhelmed the endogenous antioxidant system and resulted in cellular damage.

Administration of *Theobroma cacao* extract reversed these changes by increasing antioxidant enzyme activity and reducing MDA levels. This suggests that the extract helped restore the balance between oxidants and antioxidants. The observed antioxidant effect may be linked to the ability of phytochemicals present in cocoa leaves to scavenge free radicals and stabilize cellular membranes. Similar findings have been reported in studies where plant extracts rich in polyphenols improved oxidative stress parameters in toxic models (Bottino et al., 2019).

The protective effect observed in this study was comparable to that of vitamin E, a well-known antioxidant, further supporting the role of oxidative stress modulation in the mechanism of action of *Theobroma cacao*. The dose-dependent improvement seen with the extract suggests that higher concentrations may provide greater protective benefits.

The findings indicate that the protective effect of *Theobroma cacao* leaf extract is likely mediated through its antioxidant properties, which help reduce oxidative damage, preserve cellular integrity, and support functional recovery of affected tissues.

CONCLUSION

These results support the potential application of *Theobroma cacao* leaves as a low-cost and accessible therapeutic option in the management of toxin-induced organ damag

REFERENCES

Abubakar, M. G., Hamza, A. B., Ibrahim, A. G., & Rabiu, S. (2025). Toxicological Impact of Petroleum Vapours on Liver and Kidney Function: A Comprehensive Review. *Direct Research Journal of Public Health and Environmental Technology*, *10*(3), 31-57.

Adeyemi, T. R., Adewumi, B. L., &Olawoye, B. T. (2021). The antioxidant and anti-inflammatory potential of cocoa leaves (*Theobroma cacao*) in experimental models. *Journal of Medicinal Plants Research, 15*(5), 210–219.

Ben-Attia, T., López-Maldonado, E. A., Galai, S., Bel Haj Kacem, L., & Mhamdi, A. (2025). Nephrotoxic effects of combined exposure to 85 dB (a) noise and 300 ppm toluene in Wistar rats: biochemical and histopathological analysis. *Toxicology Research*, *14*(4), tfaf116.

Boima, V., Agyekum, A. B., Ganatra, K., Agyekum, F., Kwakyi, E., Inusah, J., ... & Adu, D. (2025). Advances in kidney disease: pathogenesis and therapeutic targets. *Frontiers in Medicine*, *12*, 1526090.

Bottino, S. M., Bianchini, L. A., &Borsato, L. (2019). Oxidative stress in nephrotoxicity: Role of antioxidants and their therapeutic potential. *Journal of Toxicology, 58*(2), 102-115.

Choi, H. M., Oh, H. J., & Lee, Y. J. (2018). Mechanisms of toluene-induced nephrotoxicity: A review. *Toxicology Letters, 286*, 123–130.

Cullen, M. R., Kreiss, K., &Rosenstock, L. (2019). Nephrotoxic effects of occupational exposure to organic solvents. *American Journal of Industrial Medicine, 62*(3), 259–268.

Dar, R. A., Shahnawaz, M., Ahanger, M. A., & Majid, I. U. (2023). Exploring the diverse bioactive compounds from medicinal plants: a review. *J. Phytopharm*, *12*(3), 189-195.

Deabes, A. A., & Essa, A. (2024). Fluid and electrolyte imbalance in renal dysfunction. *Anaesthesia & Intensive Care Medicine*, *25*(5), 316-319.

Francis, A., Harhay, M. N., Ong, A. C., Tummalapalli, S. L., Ortiz, A., Fogo, A. B., ... & International Society of Nephrology. (2024). Chronic kidney disease and the global public health agenda: an international consensus. *Nature Reviews Nephrology*, *20*(7), 473-485.

Ghobakhloo, S., Khoshakhlagh, A. H., Morais, S., & Mazaheri Tehrani, A. (2023). Exposure to volatile organic compounds in paint production plants: levels and potential human health risks. *Toxics*, *11*(2), 111.

Hix, H., & Nayman, B. D. (2024). Pathophysiology of the Kidney. *Patient Transport: Medical Critical Care-E-Book: Patient Transport: Medical Critical Care-E-Book*, 18.

Lee, S. E., & Park, Y. S. (2026). Environmental pollution and its impact on kidney diseases: a comprehensive review of current evidence. *Life*, *16*(2), 291.

Patience, O., & Richard, E. A. O. (2025). Effects of Theobroma cacao aqueous seed extract on arsenic trioxide-induced kidney damage in adult wistar rats. *Dutse Journal of Pure and Applied Sciences*, *11*(3e), 117-126.

Piko, N., Bevc, S., Hojs, R., & Ekart, R. (2023). The role of oxidative stress in kidney injury. *Antioxidants*, *12*(9), 1772.

Rajput, S. K., Singh, S., & Bhardwaj, R. (2025). Toluene toxicity: Outline, management, and prognosis. In *Hazardous Chemicals* (pp. 391-404). Academic Press.

Rojas, L. M. C., Rodríguez, E. A. G., Ramirez, A. M. H., & Trujillo, A. I. U. (2022). Nutrition in cacao (Theobroma cacao L.) crops: What determining factors should be considered?. *Revista de la Facultad de Agronomía*, *121*(Especial 2), 101-101.

Roy, A., Khan, A., Ahmad, I., Alghamdi, S., Rajab, B. S., Babalghith, A. O., ... & Islam, M. R. (2022). Flavonoids a bioactive compound from medicinal plants and its therapeutic applications. *BioMed research international*, *2022*(1), 5445291.

Yadav, R., Kumar, D., Singh, J., & Jangra, A. (2024). Environmental toxicants and nephrotoxicity: implications on mechanisms and therapeutic strategies. *Toxicology*, *504*, 153784.