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Cardio-Protective Effect of *Theobroma Cacao* Leaf on Toluene-Induced Wistar Rats

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ABSTRACT

This research examined whether the n-hexane leaf extract of *Theobroma cacao* could protect against toluene-induced heart damage in Wistar rats. Thirty male rats (157–214 g) were divided into five groups of six. Cardiac toxicity was induced with toluene and treatment groups received the extract at 200 mg/kg or 400 mg/kg. Vitamin E (200 mg/kg) was used as a standard control for 21 days. Serum lipid profile (total cholesterol, HDL, triglycerides, LDL) and cardiac biomarkers (LDH, CK) were measured. Oxidative stress markers (catalase, GSH, MDA, SOD) were also assessed. Toluene significantly reduced antioxidant enzyme activity and HDL levels, while raising triglycerides, total cholesterol, LDL, LDH, CK and MDA compared to normal controls. Treatment with the extract reversed these changes in a dose-dependent manner, with the 400 mg/kg dose showing better protection, comparable to vitamin E. The results indicate that *T. cacao* leaf extract has cardioprotective and antioxidant properties that may mitigate toluene-induced heart injury.

ARTICLE DETAILS

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INTRODUCTION

Cardiovascular diseases continue to be a major cause of illness and death across the globe (Naveed et al., 2024). Disorders including coronary heart disease, hypertension, heart failure and atherosclerosis still play a significant role in worldwide health problems (Adhikary et al., 2022). As a result, considerable attention has been directed toward identifying agents capable of protecting the heart against structural and functional damage.

Cardio-protection involves mechanisms that help preserve the integrity and function of cardiac tissues by reducing oxidative stress, inflammation and cellular injury (Dar et al., 2025). Although conventional drugs are available for the management of cardiovascular disorders, their long-term use may be associated with adverse effects and toxicity (German et al., 2022). This has encouraged increased interest in medicinal plants as alternative therapeutic agents.

Medicinal plants are a source of bioactive compounds like flavonoids, alkaloids, tannins, saponins and polyphenols. Many of these substances have antioxidant and anti-inflammatory effects (Sharifi-Rad et al., 2021; Liu et al., 2021).

Theobroma cacao, widely called cocoa, is a medicinal plant well known for its nutritional and therapeutic value (Rajangam et al., 2025). Beyond its role in food manufacturing, various parts of the plant have been shown to have antioxidant and protective biological effects, which are attributed to its polyphenolic compounds and flavonoids (Jean-Marie et al., 2022).

Toluene is a volatile organic solvent found in paints, adhesives, gasoline and industrial products (Ghobakhloo et al., 2023). Exposure to toluene has been linked to harmful effects on multiple organs, such as the liver, kidneys, nervous system and heart (Lima, 2023). Oxidative stress, caused by excessive production of reactive oxygen species, is recognized as a key mechanism underlying toluene-induced tissue damage (El-Hagrasy et al., 2025).

Based on these findings, this study examined whether *Theobroma cacao* leaf extract could protect the heart from toluene-induced damage in Wistar rats. This was done

by measuring cardiac biomarkers, lipid profile and oxidative stress parameters.

METHODOLOGY

Collection and Preparation of Plant Material

Fresh *Theobroma cacao* leaves were gathered from Amarata community in Yenagoa Local Government Area, Bayelsa State, Nigeria. Their identity was confirmed at the Department of Pharmacognosy, Niger Delta University. The leaves were washed, then air-dried in the shade at room temperature for two weeks. After drying, they were ground into a coarse powder using a mechanical grinder. A 500 g portion of the powder was soaked in 2 liters of n-hexane for 48 hours with periodic stirring to ensure thorough extraction. The mixture was filtered through muslin cloth followed by Whatman filter paper. The filtrate was concentrated using a rotary evaporator at 40°C to obtain the crude extract, which was then stored in an airtight container until needed for the study.

Experimental Animals

The study used thirty healthy male Wistar rats, each weighing between 157 and 214 g. The animals were sourced from the Pharmacology Department's animal facility at the University of Port Harcourt, Rivers State, Nigeria. They were then housed in clean plastic cages under standard laboratory conditions at the Animal House of the Faculty of Basic Medical Sciences, Niger Delta University, Bayelsa State. Environmental conditions included a 12-hour light/dark cycle, sufficient ventilation and regulated temperature. Standard pelleted food and clean drinking water were available at all times. A 14-day acclimatization period was allowed before the study began.

Experimental Design

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

Group I: Given distilled water every day for 21 days.

Group II: Received a single dose of toluene (500 mg/kg body weight) only.

Group III: Given a single dose of toluene (500 mg/kg), then treated daily with 200 mg/kg of *Theobroma cacao* extract for 21 days.

Group IV: Given a single dose of toluene (500 mg/kg), then treated daily with 400 mg/kg of *Theobroma cacao* extract for 21 days.

Group V: Given a single dose of toluene (500 mg/kg), then treated daily with vitamin E (200 mg/kg) for 21 days

Sample Collection

After the treatment period ended, the rats were anesthetized with chloroform and euthanized. Blood was collected via cardiac puncture into plain bottles and left at room temperature to clot. The samples were then centrifuged at 2000 rpm for 10 minutes to isolate serum for biochemical assays. Heart tissues were rapidly removed, washed with cold normal saline and homogenized to measure antioxidant parameters.

Determination of Cardiac Biomarkers and Lipid Profile

Cardiac biomarkers, specifically creatine kinase (CK) and lactate dehydrogenase (LDH) were measured using serum samples. Lipid profile parameters; total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were also determined according to the procedures outlined in the Randox biochemical kit manual.

Determination of Oxidative Stress Parameters

Oxidative stress markers in heart tissue homogenates namely catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA) and reduced glutathione (GSH) were measured using standard laboratory techniques. CAT activity was determined by the method described by Aebi (1984). SOD activity was assayed following the protocol of Misra & Fridovich (1972). GSH levels were estimated according to Ellman's (1959) procedure. Lipid peroxidation was assessed by measuring MDA concentration using the thiobarbituric acid reactive substances (TBARS) method of Ohkawa et al. (1979).

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 conducted in accordance with the guidelines for the care and use of laboratory animals.

Statistical Analysis

Statistical evaluation of the results was performed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test. Data are presented as mean ± standard deviation and differences were deemed statistically significant when $p < 0.05$.

RESULTS

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

Groups	Day 0 (g)	Day 21 (g)	Mean Weight Change (g)
Normal Control	153.17 ± 3.87^{a}	206.50 ± 12.11^{a}	53.33 ± 0.00^{a}
Toluene Control	159.00 ± 2.83^{a}	191.67 ± 11.62^{b}	32.67 ± 0.00^{b}

Toluene + 200 mg/kg Extract	158.50 ± 4.32[a]	199.17 ± 8.28[c]	40.67 ± 0.00[c]
Toluene + 400 mg/kg Extract	158.50 ± 4.32[a]	199.17 ± 8.28[c]	40.67 ± 0.00[c]
Toluene + Vitamin E	—	—	—

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

Table 2: Effect of *Theobroma cacao* Extract on Cardiac Biomarkers and Lipid Profile

Groups	LDL (mg/dL)	Total Cholesterol (mg/dL)	TG (mg/dL)	CK (U/L)	HDL (mg/dL)	LDH (U/L)
Normal Control	44.15 ± 3.00[a]	78.31 ± 3.89[a]	5.58 ± 0.39[a]	9.92 ± 0.76[a]	57.25 ± 1.82[a]	12.14 ± 0.67[a]
Toluene Control	106.77 ± 9.54[b]	136.72 ± 5.37[b]	18.75 ± 1.49[b]	28.65 ± 2.30[b]	30.48 ± 3.32[b]	37.89 ± 4.10[b]
Toluene + 200 mg/kg Extract	77.78 ± 3.16[c]	125.24 ± 4.44[c]	9.09 ± 0.68[c]	20.96 ± 1.38[c]	46.75 ± 2.27[c]	21.09 ± 1.87[c]
Toluene + 400 mg/kg Extract	64.60 ± 4.31[d]	104.72 ± 11.38[d]	5.96 ± 0.60[d]	13.36 ± 1.80[d]	50.38 ± 3.01[d]	17.33 ± 1.32[d]
Toluene + Vitamin E	35.86 ± 4.04[e]	98.07 ± 4.31[d]	5.95 ± 0.55[d]	11.47 ± 0.83[d]	53.73 ± 3.40[d]	16.63 ± 0.58[d]

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

Table 3: Effect of *Theobroma cacao* Extract on Oxidative Stress Parameters

Groups	**Catalase (U/mg)**	**SOD (U/mg)**	**GSH (U/mg)**	**MDA (U/mg)**
Normal Control	8.76 ± 0.72[a]	10.03 ± 0.72[a]	8.70 ± 0.26[a]	1.52 ± 0.26[a]
Toluene Control	1.95 ± 0.06[b]	2.06 ± 0.12[b]	1.61 ± 0.20[b]	7.79 ± 0.34[b]
Toluene + 200 mg/kg Extract	4.15 ± 0.15[c]	4.32 ± 0.14[c]	3.86 ± 0.37[c]	3.97 ± 0.16[c]
Toluene + 400 mg/kg Extract	5.96 ± 0.44[d]	5.91 ± 0.25[d]	4.27 ± 0.38[d]	4.12 ± 0.10[d]
Toluene + Vitamin E	5.95 ± 0.28[d]	6.41 ± 0.20[d]	6.45 ± 0.52[e]	3.11 ± 0.62[e]

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

DISCUSSION

Exposure to toluene produced marked alterations in cardiac biomarkers, lipid profile and antioxidant parameters, indicating the development of cardiac injury and oxidative stress in experimental animals. Reduced body weight gain observed in the toluene-treated group may reflect metabolic disturbances and impaired physiological function associated with toxic exposure (Kivimäki et al., 2023). Similar reductions in weight gain following exposure to organic solvents have been reported in earlier toxicological studies (Dekant & Dekant, 2023).

Increased serum levels of LDH and creatine kinase observed in this study suggest damage to cardiac tissues. These enzymes are commonly released into circulation following cellular injury and membrane disruption in the myocardium (Munir, 2024). Elevated triglycerides, LDL and total cholesterol levels alongside reduced HDL levels further indicate altered lipid metabolism associated with cardiovascular risk (Kosmas et al., 2023). Similar findings

have been reported in studies involving chemically induced cardiotoxicity where oxidative stress contributed to lipid abnormalities and cardiac dysfunction (Qianqian et al., 2024). Administration of *Theobroma cacao* extract significantly reversed these biochemical changes in a dose-dependent fashion. The observed decrease in triglycerides, total cholesterol, LDH, CK and LDL, along with a rise in HDL, points to a protective action on heart tissue and lipid metabolism. These findings align with earlier studies documenting the cardioprotective and cholesterol-lowering effects of cocoa-derived phytochemicals (Santos et al., 2025).
Oxidative stress is a key factor in the tissue damage caused by toluene. In this study, giving toluene significantly lowered the activity of antioxidant enzymes (catalase glutathione and superoxide dismutase) and greatly increased malondialdehyde (MDA) levels. The drop in antioxidant activity likely means that too many reactive oxygen species were produced, overwhelming the body's natural defenses (Rao et al., 2025). The rise in MDA also points to increased lipid peroxidation and oxidative harm to cell membranes (Azeez et al., 2023).
Administration of *Theobroma cacao* extract restored antioxidant enzyme activities and reduced lipid peroxidation in treated animals. The antioxidant effect observed may be attributed to phytochemicals such as flavonoids and polyphenols present in cocoa leaves, which are known to scavenge free radicals and stabilize cellular membranes (Kim et al., 2014). Similar antioxidant and cardioprotective effects of *Theobroma cacao* have been documented in previous studies involving oxidative stress-induced cardiac injury (Beshel et al., 2022).
The higher dose of the extract demonstrated greater protective activity and produced effects comparable to vitamin E treatment, suggesting a dose-related response. The findings indicate that *Theobroma cacao* leaf extract may help reduce toluene-induced cardiotoxicity through antioxidant and membrane-stabilizing mechanisms.

CONCLUSION

The findings of this study indicate that *Theobroma cacao* leaf extract reduced toluene-induced alterations in cardiac biomarkers, lipid profile and oxidative stress parameters in Wistar rats. The extract improved antioxidant enzyme activity and reduced lipid peroxidation in a dose-dependent manner. These results suggest that *Theobroma cacao* possesses cardioprotective properties that may help protect against chemically induced cardiac damage. Further studies are recommended to investigate the molecular mechanisms and active compounds responsible for these protective effects.

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