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Research Article

Hepatoprotective Effect on *Theobroma Cacao L.* In Toluene-Induced Wistar Rats

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Abstract

This research investigated whether an n-hexane leaf extract of Theobroma cacao could protect against toluene-induced liver damage in male Wistar rats. Liver injury was evaluated by measuring serum enzymes (ALP, ALT, AST), total protein, albumin and liver tissue oxidative stress markers (MDA, GSH, SOD, catalase). Thirty-five healthy male rats were divided into five groups (n = 6). Group 1 (normal control) and Group 2 (positive control) received distilled water. Groups 3 and 4 were given 200 mg/kg and 400 mg/kg of the extract, respectively. Group 5 received 200 mg/kg vitamin E daily for 21 days. Groups 2–5 all received a single dose of toluene (50 mg/kg) before starting treatment. After 21 days, the rats were sacrificed under chloroform anesthesia; blood was collected for liver function tests and liver tissues were homogenized for antioxidant assays. Toluene significantly ($p < 0.05$) increased serum MDA, AST, ALP and ALT, while decreasing total protein, albumin, SOD, catalase and GSH compared to normal controls. Treatment with the extract (especially the 400 mg/kg dose) reversed these abnormalities: MDA, AST, ALP and ALT fell, while total protein, albumin and antioxidant enzyme levels rose relative to the positive control. These results indicate that T. cacao leaf extract contains antioxidant phytochemicals that may provide hepatoprotective benefits.

Keywords: *N-Hexane, Theobroma cacao, Serum Alanine Aminotransferase, Aspartate Aminotransferase, Alkaline Phosphatase.*

INTRODUCTION

The liver plays a crucial role in metabolic processes, detoxification and maintaining overall homeostasis (Georgiou-Siafis & Tsiftsoglou, 2023). Exposure to toxic substances like toluene, a widely used industrial solvent, can lead to significant liver damage through mechanisms involving oxidative stress and inflammation (Umicevic et al., 2022). Toluene-induced hepatotoxicity is characterized by elevated levels of liver enzymes and histopathological alterations, underscoring the need for effective hepatoprotective agents (Arkoub et al., 2022).

Natural products rich in polyphenols have garnered attention for their potential in mitigating liver damage. Cocoa (*Theobroma cacao*) is renowned for its high flavonoid content, which exhibits strong antioxidant and anti-inflammatory properties (Sitarek et al., 2024). Studies have demonstrated that cocoa (*Theobroma cacao*) extracts can alleviate oxidative stress and improve liver function in animal models subjected to high-fat diets. For instance (Igbayilola et al., 2024) reported that flavonoid-rich cocoa (*Theobroma cacao*) extract significantly reduced markers of oxidative stress and inflammation in Wistar rats fed a high-fat diet. Similarly, (Chang et al., 2022) found that cocoa (*Theobroma cacao*) extract protected against ethanol-induced liver injury in Sprague-Dawley rats by enhancing antioxidant defenses and reducing liver enzyme levels.

While these findings highlight the hepatoprotective potential of cocoa (*Theobroma cacao*) extracts, research specifically focusing on cocoa leaf (*Theobroma cacao*) extracts remains limited. Given that different parts of the cocoa (*Theobroma cacao*) plant may possess varying phytochemical profiles, it is imperative to investigate whether cocoa leaf (*Theobroma*

cacao) extract offers similar protective effects against liver damage. This study aims to evaluate the hepatoprotective efficacy of cocoa leaf (*Theobroma cacao*) extract in Wistar rats subjected to toluene-induced hepatotoxicity, focusing on its impact on liver enzyme activities, oxidative stress markers and histopathological changes.

MATERIALS AND METHODS

Collection and Identification of Plant Sample

Fresh *Theobroma cacao* leaves were gathered from Amarata, Yenagoa, Bayelsa State, Nigeria.

Preparation of Plant Extract

A large batch of plant leaves was collected and dried under shade at room temperature for two weeks. After drying, the leaves were ground into a coarse powder. Five hundred grams (500 g) of this powder was soaked in 2 liters of n-hexane and left to stand for 48 hours with occasional stirring. The mixture was filtered through cheesecloth and the filtrate was evaporated using a rotary evaporator at 40°C. The resulting dry residue was then reconstituted with 10% Tween 80 as needed.

Experimental Animals

Thirty healthy male albino Wistar rats, each weighing 147–212 g, were obtained, the animals underwent a 14-day acclimatization period under standard laboratory conditions.

Experimental Design

The animals were randomly assigned to five groups, with six rats per group, housed in standard plastic cages. The treatment protocol was as follows:

Group I (Normal control): Received distilled water only for 21 days.
Group II (Positive control): Received daily distilled water plus a single dose of toluene (500 mg/kg body weight).
Group III (Treatment group 1): Given a single dose of toluene (500 mg/kg) followed by 21 days of daily extract at 200 mg/kg body weight.
Group IV (Treatment group 2): Given a single dose of toluene (500 mg/kg) followed by 21 days of daily extract at 400 mg/kg body weight.
Group V (Standard control): Given a single dose of toluene (500 mg/kg) followed by 21 days of daily vitamin E (200 mg/kg body weight).

Collection of samples

On the 21st day, all rats were anesthetized with chloroform and euthanized. Blood was collected by cardiac puncture into plain bottles and left to stand for 30 minutes to allow clotting. The samples were then centrifuged at 2000 RPM for 10 minutes and the resulting supernatant (serum) was collected for biochemical analysis. Portions of the liver were also taken and used to prepare homogenates for antioxidant assays.

RESULTS

Table 1 displays the mean body weights of toluene-treated Wistar rats before and after 21 days of treatment with *Theobroma cacao* extract. Table 2 shows the mean serum levels of AST, ALP, ALT, total protein and albumin. Table 3 presents the mean liver concentrations of SOD, catalase, GSH and MDA.

Table 1: Effects of Toluene and *Theobroma cacao* on the mean body weight of the Wistar rats

GROUPS	DAY 0 (g)	DAY 21 (g)	MEAN WEIGHT CHANGE (g)
Normal control treated with distilled water	153.17 ± 3.87[a]	206.50 ± 12.11 [a]	52.80 [a]
positive control -treated with toluene 500 mg/kg b.w)	159.00 ± 2.83 [b]	191.67 ± 11.62 [b]	32.67 [b]
Test group treated with *theobroma cacoa* (200 mg/kg b.w) + toluene 500 mg/kg b.wt)	159.00 ± 3.35 [b]	197.00± 6.32 [c]	38.00 [c]
Test group 2 treated with *theobroma cacoa* (400 mg/kg b.w) + toluene 500 mg/kg b.wt)	158.00.5± 4.23 [b]	199.17 ± 8.28 [c]	41.17 [c]
Standard control treated with vitamine(200 mg/kgb.w + toluene 500 mg/kg b.wt)	158.5 ± 2.74 [b]	202.17 ± 8.33 [a]	43.67 [c]

Data are expressed as the mean ± SD (n = 6). Means within the same column carrying the same superscripts are not significantly ($p < 0.05$) different.

As shown in Table 1, administration of toluene (500 mg) significantly ($p < 0.05$) reduced the mean weight gain (32.68) compared to the normal control (52.80). In contrast, treatment with *Theobroma cacao* extract at 200 mg/kg and 400 mg/kg resulted in a significant ($p < 0.05$) increase in mean weight gain (38.00 and 41.17, respectively) relative to the positive control.

Table 2: The protective role of *Theobroma cacao* on the liver of Toluene induced albino wistar Rats

GROUPS	ALT (U/L)	AST (U/L)	ALP (U/L)	TP (g/dl)	ALB (mg/dl)
Normal control treated with distilled water	74.32±2.78 [a]	80.52±3.21 [a]	82.8±1.52 [a]	8.11±0.54 [a]	4.73±0.11 [a]
Positive control -treated with toluene 500mg/kg b.w)	128.18±6.48 [b]	158.72±3.24 [b]	158.72±3.24[b]	2.06±0.08 [b]	1.10±0.15 [b]
Test group treated with *theobroma cacoa* (200 mg/kg b.w) + toluene)	109.91±7.19 [c]	130.75±2.41 [c]	129.17±3.53[c]	3.92±0.21 [c]	2.64±0.19 [c]
Test group 2 treated with *theobroma cacoa* (400 mg/kg b.w) (+toluene b.w)	93.84±5.06 [d]	118.86±2.18 [d]	115.49±4.60[d]	4.65±0.22 [d]	3.03±0.19 [c]
Standard control treated with vitamin e (200 mg/kg b.w)	88.15±4.16 [e]	101.16±8.99 [e]	105.94±5.14[e]	6.78±0.29 [e]	4.06±0.05 [a]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

As shown in Table 2, toluene administration significantly ($p < 0.05$) elevated serum ALT (128.18±6.48), AST (158.72±3.24) and ALP (158.72±3.24) activities in the positive control group (Group 2), while significantly ($p < 0.05$) reducing total protein (2.06±0.08) and serum albumin (1.10±0.15) compared to normal control rats. However, treatment with *Theobroma cacao* at 200 mg/kg and 400 mg/kg significantly ($p < 0.05$) lowered ALT (109.91±7.19 and 93.84±5.06), AST (130.75±2.41 and 118.86±2.18) and ALP (129.17±3.53 and 115.49±4.60) in a dose-dependent manner. The extract also significantly increased total protein (3.92±0.21 and 4.65±0.22) and albumin (2.64±0.19 and 3.03±0.19) relative to the toluene-only group.

Table 3 the antioxidant effect of *Theobroma cacao* on the liver of Toluene induced wistar albino rats

GROUPS	SOD (U/mg)	CATALASE (U/mg)	GSH (U/mg)	MDA (U/mg)
Normal control	8.98±0.28 [a]	8.73±0.53 [a]	8.56±0.53 [a]	2.37±0.23 [a]
Positive control with Toluene (500 mg/kg)	1.87±0.17 [b]	1.81±0.18 [b]	1.79±0.10 [b]	18.33±1.28 [b]
Test group 1 with Toluene (500mg/kg) and *Theobroma cacao* (200 mg/kg)	4.44±0.40 [c]	3.55±0.41 [c]	4.04±0.16 [c]	13.55±1.57 [c]
Test group 2 with Toluene (500mg/kg) and *Theobroma cacao* (400 mg/kg)	5.71±0.37 [d]	4.91±0.56 [d]	5.28±0.26 [d]	7.81±0.85 [d]
Standard control with Vitamin E (200 mg/kg)	5.64+0.47 [d]	5.97±0.09 [e]	5.82±1.99 [d]	7.34±0.52 [d]

Data are expressed as the mean ± SD (n = 5). Means within the same column carrying same superscripts are not significantly ($p < 0.05$) different.

Table 3 reveals that toluene administration in the positive control group significantly ($p < 0.05$) decreased SOD (1.87±0.17), catalase (1.81±0.18) and GSH (1.79±0.10) levels, while markedly increasing MDA (18.33±1.28). In contrast, treatment with Theobroma cacao at 200 mg/kg and 400 mg/kg significantly ($p < 0.05$) elevated SOD (4.44 ± 0.40 and 5.71 ± 0.37), catalase (3.55 ± 0.41 and 4.91 ± 0.56) and GSH (4.04 ± 0.16 and 5.28±0.26) and reduced MDA (13.55 ± 1.57 and 7.81 ± 0.85) in a dose-dependent manner compared to the toluene-only group.

DISCUSSION

Theobroma cacao (cacao) is known for its medicinal potential, largely due to its high content of polyphenols, flavonoids and alkaloids. Recent studies have reported its antioxidant, anti-inflammatory and anticancer properties (Sitarek et al., 2024). Cacao exhibits strong antioxidant activity by scavenging free radicals and inhibiting lipid peroxidation, mainly because of its flavonoid content (Cañas et al., 2023). It also shows anti-inflammatory effects by modulating inflammatory mediators and suppressing NF-κB signaling (Vaillancourt et al., 2022).

This study investigated whether the n-hexane leaf extract of *T. cacao* could protect against liver injury caused by a high dose of toluene in male Wistar rats. Toluene significantly reduced mean weight gain compared to normal controls. However, treatment with the extract at 200 mg/kg and 400 mg/kg significantly ($p < 0.05$) improved weight gain relative to the positive control, suggesting that the extract may promote weight gain.

Toluene also caused a significant ($p < 0.05$) increase in serum ALT, AST and ALP and a decrease in albumin and total protein compared to normal controls (Table 2), indicating hepatocyte damage and leakage of enzymes into the bloodstream (Metra et al., 2022). Treatment with the extract (both doses) significantly reversed these changes, as did vitamin E (200 mg/kg).

Toluene significantly increased serum MDA (lipid peroxidation marker) and decreased SOD, catalase and GSH in the positive control group (Table 3). This suggests oxidative damage and failure of antioxidant defenses (Dehghan-Haghighi et al., 2022). Treatment with the extract at 200 mg/kg and 400 mg/kg significantly and dose-dependently reduced MDA and raised antioxidant enzyme activities. Similar effects were seen with vitamin E. These findings align with earlier reports (Sitarek et al., 2024; Wiryanthini et al., 2020) showing that *T. cacao* can reduce toluene-induced liver and nerve damage and boost antioxidants.

The liver-protective effect of the extract is likely due to its rich phytochemical content, with the higher dose (400 mg/kg) being more effective. Further research is needed to understand the exact mechanisms and to explore potential applications of *T. cacao* for liver health.

CONCLUSION

The results of this study suggest that the n-hexane extract of *Theobroma cacao* (cocoa) contains antioxidant compounds that may help shield the liver from toluene-induced injury in Wistar rats. The protective effect was dose-dependent, meaning it varied with the amount of extract administered. Although these outcomes are encouraging, further research is necessary to clarify the precise mechanism by which this extract exerts its protective action against toluene-related liver damage.

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