



Comparative Effects of Methanol Extracts of *Garcinia kola* and *Cola nitida* on Acetaminophen-Induced Liver and Kidney Damage in Wistar Rats

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Abstract

Background: Acetaminophen (paracetamol) is a widely used analgesic and antipyretic that is generally safe at therapeutic doses but can cause severe liver and kidney damage following overdose through the formation of the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). Medicinal plants with antioxidant properties have attracted attention as potential protective agents against drug-induced organ toxicity. *Garcinia kola* and *Cola nitida* are widely used in traditional medicine and possess reported antioxidant and cytoprotective activities.

Objective: To evaluate the comparative effects of methanol extracts of *Garcinia kola* and *Cola nitida* on acetaminophen-induced liver and kidney damage in Wistar rats.

Materials and Methods: Wistar rats were divided into six groups: normal control, acetaminophen-treated control, *Cola nitida* + acetaminophen, *Garcinia kola* + acetaminophen, combined *Garcinia kola* and *Cola nitida* + acetaminophen, and Vitamin E + acetaminophen. Liver function parameters (ALP, ALT, AST, and total bilirubin) and kidney function indices (urea, creatinine, potassium, sodium, and bicarbonate) were determined. Data were expressed as mean $\pm$ SD and analyzed using one-way ANOVA with Tukey's post hoc test at $p < 0.05$.

Results: Acetaminophen significantly elevated liver enzymes and renal biomarkers compared with the normal control group, indicating hepatic and renal injury. Treatment with methanol extracts of *Garcinia kola* and *Cola nitida* significantly ameliorated these alterations. The combined extract treatment produced the greatest protective effect, yielding liver and kidney function values comparable to those observed in the Vitamin E-treated group.

Conclusion: Methanol extracts of *Garcinia kola* and *Cola nitida* exhibited significant hepatoprotective and nephroprotective effects against acetaminophen-induced toxicity. Combined administration produced superior protection, suggesting greater interactions among their bioactive constituents and supporting their potential use in preventing drug-induced liver and kidney injury.

Keywords: *Garcinia kola*, *Cola nitida*, Acetaminophen, Hepatotoxicity, Nephrotoxicity, Liver function, Kidney function.

Article History

Received: 25 Apr 2026

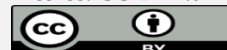
Accepted: 30 May 2026

Published: 17 Jun 2026



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How to cite this paper: Okpu, U. P., Lawson, D. S., Ehie, G. C., & Ezekwe, A. S. (2026). Comparative Effects of Methanol Extracts of *Garcinia kola* and *Cola nitida* on Acetaminophen-Induced Liver and Kidney Damage in Wistar Rats. *Journal of Phytochemistry, Ethnobotany and Traditional Medicine Systems*, 2(1), 96–100. <https://doi.org/10.54117/jpetms.v2i1.73>

Introduction

The liver and kidneys are among the most important organs involved in the metabolism, detoxification, and elimination of xenobiotics and endogenous waste products. Due to their central role in maintaining physiological homeostasis, these organs are particularly susceptible to damage caused by toxic chemicals, environmental pollutants, and therapeutic drugs. Drug-induced liver and kidney injuries remain major public health concerns and account for a significant proportion of adverse drug reactions worldwide (Ramachandran & Jaeschke, 2018).

Acetaminophen (paracetamol) is one of the most commonly used analgesic and antipyretic agents globally. Although considered safe at recommended therapeutic doses, excessive intake can result in severe hepatic and renal injury. Acetaminophen overdose is recognized as one of the leading causes of acute liver failure and is frequently associated with acute kidney injury (Jaeschke *et al.*, 2012). The toxicity of acetaminophen is largely mediated through the formation of N-acetyl-p-benzoquinone imine (NAPQI), a highly reactive metabolite generated by cytochrome P450 enzymes. Under normal physiological conditions, NAPQI is detoxified through conjugation with glutathione (Adeosun *et al.*, 2017). However, during overdose, excessive NAPQI formation depletes

intracellular glutathione stores, leading to oxidative stress, lipid peroxidation, mitochondrial dysfunction, inflammation, and cellular necrosis (Ramachandran & Jaeschke, 2018).

Natural products and medicinal plants have attracted considerable attention as alternative therapeutic agents for preventing and managing drug-induced organ toxicity. Their beneficial effects are often attributed to the presence of bioactive phytochemicals possessing antioxidant, anti-inflammatory, and cytoprotective activities (Ayepola *et al.*, 2013).

Garcinia kola, commonly known as bitter kola, is widely distributed throughout West and Central Africa and has been used traditionally in the management of numerous ailments. The plant contains bioactive compounds such as kolaviron, biflavonoids, and phenolic constituents that exhibit potent antioxidant, anti-inflammatory, and membrane-stabilizing activities (Adaramoye *et al.*, 2008; Ayepola *et al.*, 2013). Studies have demonstrated that kolaviron protects tissues against oxidative stress by scavenging free radicals, inhibiting lipid peroxidation, and enhancing endogenous antioxidant defenses (Adaramoye *et al.*, 2008). Similarly, *Cola nitida* is an economically and medicinally important plant rich in polyphenols, alkaloids, caffeine, and theobromine. These phytochemicals have been associated with antioxidant, anti-inflammatory, and organ-protective properties (Erukainure *et al.*, 2021). Previous studies have suggested that extracts of *Cola nitida* may mitigate oxidative damage and preserve cellular integrity under conditions of toxic stress (Erukainure *et al.*, 2021).

Although several investigations have independently reported the beneficial effects of *Garcinia kola* and *Cola nitida*, limited information exists regarding their comparative protective effects against acetaminophen-induced liver and kidney damage. Furthermore, the possibility of greater interactions between both plants remains poorly understood. This study was therefore designed to evaluate and compare the effects of methanol extracts of *Garcinia kola* and *Cola nitida* on acetaminophen-induced hepatic and renal damage in Wistar rats using selected biochemical markers of liver and kidney function.

Methodology

Study Area and Ethical Approval

The study was conducted at the animal house of the Department of Physiology, College of Health Sciences, University of Port Harcourt. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Basic Medical Sciences, Rivers State University with reference number RSU/FBMS/REC/25/227 on the 2nd of April 2025. Experiments were conducted according to Faculty of Basic Medical Sciences Ethics Committee Guidelines for the Care and Use of Laboratory Animals.

Plant Material

Garcinia kola and *Cola nitida* seeds were obtained from Mile 1 Market, Port Harcourt, Rivers State, Nigeria. The plant materials were identified and authenticated by Dr. M. G. Ajuru at the Herbarium of the Department of Plant Science and Biotechnology, Rivers State University, Nigeria. Voucher

specimens with accession numbers RSUPbH0152 and RSUPbH0151 were deposited in the departmental herbarium for future reference.

The seeds of *Garcinia kola* and *Cola nitida* were dried and pulverized. The powdered seeds (500 g each) were extracted with methanol using a Soxhlet apparatus. Approximately 2.5 L of methanol was used, and extraction was carried out at 78–80°C for 6–8 h until the siphon solvent became clear, indicating complete extraction. The extracts were concentrated under reduced pressure using a rotary evaporator at 40°C and 40 rpm and further dried on a water bath to remove residual solvent. The dried extracts yielded approximately 80 g each, corresponding to a percentage yield of 16%, and were stored in airtight amber containers at 4°C until further analysis. Twenty grams of the dried extract was dissolved in 100 ml of distilled water to produce a stock solution of 200 mg/ml.

Drug Materials

Liver and kidney damage were induced using acetaminophen (paracetamol), which at toxic doses is metabolized to the reactive intermediate N-acetyl-p-benzoquinone imine (NAPQI), leading to glutathione depletion, oxidative stress, hepatocellular necrosis, and renal tubular injury. Following a 14-day pretreatment period with the plant extracts or vitamin E, a single oral dose of 1000 mg/kg body weight of acetaminophen was administered on day 15 to induce toxicity. This dose was selected based on its established effectiveness in producing organ damage in rats without excessive mortality (Ilavenil *et al.*, 2016). After administration, the animals were closely monitored for clinical signs of toxicity, including lethargy, reduced activity, loss of appetite, and piloerection, to confirm successful induction of hepatotoxicity and nephrotoxicity.

Experimental Animals

The 30 healthy male Wistar rats weighing between 120–140 g used for this study were acclimatized for two weeks under standard laboratory conditions (12-hour light/dark cycle, temperature 25 ± 2 °C, relative humidity 50–60%) with free access to standard rat feed and water ad libitum.

Experimental Design

The sample size was determined using the resource equation method for animal experiments. After acclimatization, a total of 30 Wistar rats were used, the rats were weighed and randomly assigned to experimental groups using a computer-generated randomization method, ensuring that each animal had an equal chance of allocation to any group. Group sizes were kept equal throughout the study; they were allocated into six groups of five animals each. The calculated resource equation value ($E = N - G$) was 24, this indicated an adequate sample size for detecting treatment-related differences among groups. The animals were all given free access to pelleted rat feed and water throughout the period of experimentation.

Group A - Normal Control - given free access to pelleted rat feed and water.

Group B - Acetaminophen (PCM) only - Acetaminophen only (1000 mg/kg b.w.)

Group C - *Cola nitida* + PCM - *Cola nitida* extract (200 mg/kg b.w.) + Acetaminophen (1000 mg/kg b.w.)

Group D - *Garcinia kola* + *Cola nitida* + PCM - *Garcinia kola* extract (200 mg/kg b.w.) + *Cola nitida* (200 mg/kg b.w.) + Acetaminophen (1000 mg/kg b.w.)

Group E - *Garcinia kola* + PCM - *Garcinia kola* (200 mg/kg b.w.) + Acetaminophen (1000 mg/kg b.w.)

Group F - Vitamin E + PCM - Vitamin E (50 mg/kg b.w.) + Acetaminophen (1000 mg/kg b.w.)

At the end of the 14-day treatment period, all animals were fasted overnight and sacrificed under light anaesthesia. Blood was collected via cardiac puncture, with the aid of a 5 ml capacity syringe and transferred to a plain blood bottle. Blood samples were allowed to clot and subsequently centrifuged at 3000 rpm for 10 min to obtain serum for biochemical analysis.

Biochemical Analysis

Serum liver and kidney function parameters were determined using commercially available diagnostic reagent kits according to the manufacturers' instructions. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, urea, creatinine, sodium, potassium, and bicarbonate concentrations were assayed using standard colorimetric and enzymatic spectrophotometric methods. Absorbance readings were measured using a UV-visible spectrophotometer at the appropriate wavelengths specified by the assay protocols, and

concentrations were calculated relative to corresponding standards supplied with the kits.

Statistical Analysis

Data from biochemical analyses were expressed as mean $\pm$ SD. One-way ANOVA was used to assess differences among groups, followed by Tukey's post-hoc test where applicable. Significance was set at $p < 0.05$. Analyses were performed using Microsoft Excel 2013 with the Real Statistics Resource Pack add-in.

Results

Alkaline Phosphatase (ALP), Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and total bilirubin were significantly elevated ($p < 0.05$) in the acetaminophen-treated group compared with the normal control (Table 1), indicating hepatic injury. Administration of *Cola nitida* or *Garcinia kola* extracts alone produced significant reductions ($p < 0.05$) in these parameters, showing notable hepatoprotective effects. The combined extract and vitamin E groups exhibited the greatest decreases, with enzyme and bilirubin levels closely comparable to those of the normal control, suggesting improved protective synergy.

Table 1. Effects on Liver Function Parameters

Groups	ALP (U/L)	ALT (U/L)	AST (U/L)	T. Bilirubin (mg/dL)
Normal Control	57.37 $\pm$ 0.64 ^{ac}	21.20 $\pm$ 1.40 ^a	45.13 $\pm$ 2.34 ^a	0.42 $\pm$ 0.01 ^{ab}
Acetaminophen (PCM) only	140.20 $\pm$ 2.60 ^d	37.50 $\pm$ 1.28 ^d	96.30 $\pm$ 1.32 ^e	0.82 $\pm$ 0.08 ^c
Test group 1 (<i>Cola nitida</i> + PCM)	68.93 $\pm$ 0.25 ^b	22.47 $\pm$ 1.22 ^{ac}	63.03 $\pm$ 1.47 ^c	0.46 $\pm$ 0.02 ^b
Test group 2 (<i>Garcinia kola</i> + <i>Cola nitida</i> + PCM)	53.53 $\pm$ 0.91 ^a	18.73 $\pm$ 0.42 ^{ab}	43.50 $\pm$ 0.98 ^{ab}	0.31 $\pm$ 0.03 ^a
Test group 3 (<i>Garcinia kola</i> + PCM)	67.53 $\pm$ 1.52 ^{ab}	25.27 $\pm$ 3.31 ^c	50.83 $\pm$ 3.27 ^{cd}	0.30 $\pm$ 0.06 ^a
(VIT E + PCM)	49.80 $\pm$ 1.87 ^c	18.17 $\pm$ 0.35 ^{abc}	44.93 $\pm$ 0.15 ^{acd}	0.29 $\pm$ 0.08 ^a

Values are presented as Mean $\pm$ Standard Deviation (SD); values with different superscript letters differ significantly at $p < 0.05$.

Urea, creatinine, potassium, sodium, and bicarbonate levels were significantly elevated ($p < 0.05$) in the acetaminophen-treated group compared with the normal control, indicating renal impairment. Administration of *Cola nitida* or *Garcinia kola* extracts alone led to significant reductions ($p < 0.05$) in all these parameters, demonstrating their nephroprotective

effects. The groups treated with the combined extracts and Vitamin E showed the most pronounced decreases, with values not significantly different from the control. This suggests that the combined extracts exerted a greater protective effect, comparable to vitamin E, in restoring renal function and mitigating acetaminophen-induced kidney damage.

Table 2. Effects on Kidney Function Parameters

Groups	Urea (mg/dL)	Creatinine (mg/dL)	K ⁺ (mmol/L)	Na ⁺ (mmol/L)	HCO ₃ ⁻ (mmol/L)
Normal Control	17.03 $\pm$ 0.22 ^{ab}	0.31 $\pm$ 0.03 ^a	3.85 $\pm$ 0.09 ^a	136.47 $\pm$ 1.85 ^{abc}	16.04 $\pm$ 0.12 ^a
Acetaminophen (PCM) only	33.15 $\pm$ 1.28 ^f	1.14 $\pm$ 0.03 ^d	6.85 $\pm$ 0.34 ^d	156.91 $\pm$ 6.85 ^d	34.36 $\pm$ 2.54 ^d
Test group 1 (<i>Cola nitida</i> + PCM)	18.55 $\pm$ 0.88 ^d	0.44 $\pm$ 0.08 ^b	5.26 $\pm$ 0.31 ^b	146.97 $\pm$ 2.39 ^a	22.56 $\pm$ 2.63 ^b
Test group 2 (<i>Garcinia kola</i> + <i>Cola nitida</i> + PCM)	18.31 $\pm$ 0.77 ^{ae}	0.27 $\pm$ 0.02 ^c	3.06 $\pm$ 0.16 ^c	133.19 $\pm$ 2.89 ^b	13.58 $\pm$ 0.82 ^c
Test group 3 (<i>Garcinia kola</i> + PCM)	15.42 $\pm$ 0.06 ^{bcd}	0.28 $\pm$ 0.06 ^c	3.41 $\pm$ 0.47 ^c	132.25 $\pm$ 5.25 ^b	16.41 $\pm$ 1.09 ^{ac}
VIT E + PCM	12.74 $\pm$ 0.18 ^e	0.31 $\pm$ 0.01 ^a	3.12 $\pm$ 0.23 ^c	136.01 $\pm$ 4.02 ^{bc}	12.02 $\pm$ 1.44 ^c

Values are presented as Mean $\pm$ Standard Deviation (SD); values with different superscript letters differ significantly at $p < 0.05$.

Discussion

Acetaminophen administration significantly increased ALP, ALT, AST and total bilirubin levels when compared with the normal control group, indicating severe hepatic injury. ALP increased from 57.37 ± 0.64 U/L in the control group to 140.20 ± 2.60 U/L following acetaminophen administration. Similarly, AST increased from 45.13 ± 2.34 U/L to 96.30 ± 1.32 U/L, while total bilirubin increased from 0.42 ± 0.01 mg/dL to 0.82 ± 0.08 mg/dL, these findings are consistent with previous reports describing acetaminophen-induced hepatic injury (Ramachandran & Jaeschke, 2018). The observed elevation of these biochemical markers indicates hepatocellular membrane damage and leakage of intracellular enzymes into circulation. Increased bilirubin levels further suggest impairment of hepatic excretory function. Treatment with *Cola nitida* and *Garcinia kola* extracts significantly attenuated these alterations. The combined extract group produced the most pronounced hepatoprotective effect, reducing ALP, ALT, AST, and bilirubin to values comparable with those of the normal control and Vitamin E-treated groups. AST activity in the combined treatment group was 43.50 ± 0.98 U/L, which closely approximated the normal control value of 45.13 ± 2.34 U/L. Likewise, bilirubin was reduced to 0.31 ± 0.03 mg/dL. This finding is consistent with the antioxidant properties previously reported for *Garcinia kola* (Ayepola *et al.*, 2013).

The hepatoprotective activity observed may be attributed to the antioxidant and membrane-stabilizing effects of phytochemicals present in both plants. *Garcinia kola* contains kolaviron and biflavonoids known to inhibit lipid peroxidation and scavenge reactive oxygen species. *Cola nitida* also contains polyphenols, alkaloids, caffeine, and theobromine, which contribute to antioxidant protection. The superior effect observed in the combined treatment group suggests greater interactions between the bioactive constituents of both plants (Erukainure *et al.*, 2021).

Acetaminophen-induced nephrotoxicity was evident from significant increases in serum urea, creatinine, potassium, sodium, and bicarbonate levels. Urea increased from 17.03 ± 0.22 mg/dL in the control group to 33.15 ± 1.28 mg/dL, while creatinine increased from 0.31 ± 0.03 mg/dL to 1.14 ± 0.03 mg/dL. Potassium concentration also increased from 3.85 ± 0.09 mmol/L to 6.85 ± 0.34 mmol/L, and sodium concentration also increased from 136.47 ± 1.85 mmol/L to 156.91 ± 6.85 mmol/L, while bicarbonate levels increased from 16.04 ± 0.12 mmol/L to 34.36 ± 2.54 mmol/L, indicating renal dysfunction and electrolyte imbalance (Alabi & Akomolafe 2020). The increase in renal biomarkers reflects impaired glomerular filtration and tubular dysfunction resulting from acetaminophen toxicity. NAPQI-mediated oxidative stress has been implicated in renal tubular injury and acute kidney damage (Park 2020).

Administration of *Cola nitida* and *Garcinia kola* extracts significantly improved renal function. The combined extract group exhibited urea and creatinine values of 18.31 ± 0.77 mg/dL and 0.27 ± 0.02 mg/dL respectively, demonstrating marked restoration of renal function (Tauchen *et al.*, 2023). Electrolyte concentrations were also normalized, indicating improved maintenance of renal homeostasis (Tauchen *et al.*, 2023).

The nephroprotective effects observed are consistent with the antioxidant properties of both plants. Their bioactive compounds may reduce oxidative stress, preserve tubular integrity, and improve renal filtration efficiency. The combined treatment produced effects comparable to Vitamin E, suggesting strong protective activity against acetaminophen-induced nephrotoxicity (Meng *et al.*, 2025). Overall, the findings demonstrate that both *Garcinia kola* and *Cola nitida* possess significant protective effects against acetaminophen-induced liver and kidney damage, with the combined administration producing the greatest benefit.

Conclusion

The present study demonstrated that acetaminophen administration induced significant hepatic and renal injury, as evidenced by elevated liver enzymes, bilirubin, urea, creatinine, and electrolyte disturbances. Treatment with methanol extracts of *Garcinia kola* and *Cola nitida* significantly ameliorated these biochemical alterations. Among the treatment groups, the combined administration of *Garcinia kola* and *Cola nitida* produced the most pronounced hepatoprotective and nephroprotective effects, with results comparable to those obtained with Vitamin E. The findings suggest greater interactions between the phytochemical constituents of both plants and support their traditional medicinal use.

The study therefore provides scientific evidence that methanol extracts of *Garcinia kola* and *Cola nitida* may have potential as natural hepatoprotective and nephroprotective agents against acetaminophen-induced liver and kidney damage. Further studies are recommended to elucidate their molecular mechanisms of action and therapeutic potential.

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