

Original Research Article

***Rauwolfia vomitoria* leaves as a natural candidate for drug-induced liver injury management: A biochemical and toxicological analysis**

Ijeoma Princess Nwafor^{1,2*}, Israel Ogwuche Ogra^{2,3}, John Wassagwa², Emeka John Dingwoke², Nnamdi Okereke⁴

¹Department of Biochemistry, University of Nigeria, Nsukka, Enugu State, Nigeria ²UNESCO International Centre for Biotechnology, Nsukka 410001, Enugu State, Nigeria, ³Biological Institute, Tomsk State University, 36 Lenin Avenue, Tomsk, 634050, Russian Federation, ⁴School of Veterinary Medicine, University of Lancashire, United Kingdom

*For correspondence: **Email:** princess.ijeoma05@gmail.com

Sent for review: 21 July 2025

Revised accepted: 19 November 2025

Abstract

Purpose: To evaluate the hepatoprotective and nephroprotective effects of *Rauwolfia vomitoria* ethanol leaf extract in a rat model of paracetamol-induced toxicity.

Methods: Acute oral toxicity of paracetamol was determined using 18 rats divided into 6 groups and administered graded doses up to 5,000 mg/kg and the lethal dose (LD₅₀) was determined. Thereafter, 24 adult male Wistar albino rats were randomized into four groups (n = 6/group): Group 1 (distilled water, control; 2 mL/kg); Group 2 (hepatotoxic control, single oral paracetamol 2000 mg/kg on day 15); Group 3 (extract control, *R. vomitoria* 250 mg/kg, oral gavage, once daily for 14 days); Group 4 (extract pre-treatment, *R. vomitoria* 250 mg/kg, oral gavage, once daily for 14 days, followed by paracetamol 2000 mg/kg on day 15). Animals were sacrificed 24 h after paracetamol administration. Serum was analyzed for liver enzymes, renal markers, oxidative stress markers, inflammatory cytokines and serum proteins.

Results: Paracetamol induced marked hepatotoxicity and nephrotoxicity ($p < 0.001$). Extract pretreatment (250 mg/kg) significantly attenuated paracetamol-induced elevations in serum alanine aminotransferase (66.9 %), aspartate aminotransferase (65.1 %) and alkaline phosphatase (51.5 %; $p < 0.001$), and lowered creatinine and urea (52.4 and 45.7 %, respectively; $p < 0.001$). Oxidative damage and pro-inflammatory cytokines were reduced by 42 – 46 % ($p < 0.001$). The extract alone did not produce any adverse changes when compared with control ($p > 0.05$).

Conclusion: Prophylactic oral administration of *Rauwolfia vomitoria* ethanol leaf extract markedly mitigates single-dose paracetamol-induced liver and kidney injury. The effects are associated with antioxidant and anti-inflammatory actions. Further studies are needed to isolate and characterize its bioactive compounds for clinical applications.

Keywords: *Rauwolfia vomitoria*, Hepatotoxicity, Oxidative stress, Inflammatory cytokines, Liver enzymes, Nephroprotection

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Acute liver failure is characterized by severe impairment of liver function, classically

presenting with coagulopathy, jaundice, and hepatic encephalopathy in the absence of preexisting chronic liver disease [1]. In developed nations, the condition is rare: reported incidence

estimates vary by study but are generally on the order of ≤ 10 cases per 1,000,000 population per year. For example, population-based surveillance in metropolitan Atlanta, USA, reported an annualized incidence of 5.5 cases per million, and studies from the United Kingdom/Scotland and other European centers report incidence estimates of fewer than 10 cases per million per year [2-4]. Viral hepatitis remains a leading cause of acute liver failure globally, while drug-induced liver injury (DILI), particularly acetaminophen/paracetamol overdose in many high-income settings, is a major and preventable contributor to ALF in those countries [4].

The liver plays a central role in drug metabolism and detoxification of xenobiotics, making it highly susceptible to damage from these substances [4]. Drug metabolizing enzymes (DMEs) within the liver are crucial for maintaining homeostasis by processing drugs and toxins, facilitating their excretion through the kidneys or bile. Cytochrome P-450 enzymes in the liver are particularly important for the biotransformation of drugs into either active or inactive metabolites, which underscores the liver's vulnerability to toxic compounds [5]. In recent years, there has been an increased interest in the use of herbal supplements, natural products, and traditional medicines, given their role in various biological activities [6]. Medicinal plants are a significant source of modern pharmaceuticals, with nearly 25 % of current medicines derived from them [6]. *Rauwolfia vomitoria* (Apocynaceae), commonly known as poison devil's pepper, is a small-to-medium-sized evergreen tree featuring glossy, oblong to elliptic leaves, clusters of small white flowers, and red globular fruits. It is native not only to Nigeria but also occurs in other West and Central African countries such as Ghana and Cameroon [7]. The bark and roots, rich in monoterpene indole alkaloids such as reserpine, ajmaline, ajmalicine, serpentine, and yohimbine, are the primary parts used medicinally [8,9]. These phytochemicals are known for their antihypertensive, sedative, aphrodisiac, and antioxidant properties [8,9]. Traditionally, preparations from the roots and bark have been used to treat conditions including diarrhea, jaundice, fever, mental disorders, hypertension, and male reproductive issues [7].

Studies have demonstrated effectiveness in lowering blood pressure using bark-derived extracts and improving male fertility with root bark preparations [8]. Given the oxidative and inflammatory nature of paracetamol-induced hepatotoxicity, and in light of evidence that *R. vomitoria* contains antioxidant and hepatoprotective compounds (e.g., squalene,

phytol, n-hexadecanoic acid) in its leaves and roots [10,11], this study investigates the hepatoprotective potential of an ethanol extract of *R. vomitoria* leaves that are both traditionally underused for liver-related ailments and rich in relevant bioactive compounds in a rat model of paracetamol-induced liver injury.

Rauwolfia vomitoria (*R. vomitoria*; Figure 1), known as "poison devil's-pepper," is a medicinal plant native to Nigeria, recognized for its diverse therapeutic phytochemicals. Traditionally, it has been used to treat conditions such as diarrhea, malaria, hypertension, mental disorders, and male infertility [14]. Studies have shown its effectiveness in lowering blood pressure [15] and enhancing male fertility [14]. Given the therapeutic potential of phytochemicals as antioxidants, this study aims to explore the hepatoprotective effects of an ethanol extract derived from *R. vomitoria* leaves in a rat model of paracetamol-induced liver injury, focusing on its ability to mitigate hepatic damage.

EXPERIMENTAL

Materials

All reagents and analytical grade chemicals used in this study were of analytical grade. They were purchased from Sigma-Aldrich (St. Louis, MO, USA) or Thermo Fisher Scientific (Waltham, MA, USA). Commercial diagnostic assay kits (ALT, AST, ALP, creatinine, urea) and ELISA kits for IL-6 and TNF- α were used according to the manufacturers' instructions. All solutions were prepared using distilled water and handled under standard laboratory conditions.

Plant material and ethical statement

This study was approved by the University of Nigeria, Nsukka (UNN), Enugu State, Nigeria, Ethics and Biosafety Committee (approval no. UNN/FBS/EC/1005). All protocols in this study were conducted in accordance with the revised ARRIVE 2.0 guidelines. *Rauwolfia vomitoria* leaves were collected on 2nd September 2019 during the rainy season, from Obukpa village, Nsukka Local Government Area (GPS: 6°54'01.2" N, 7°23'36.7" E), Enugu State, Nigeria, with the informed consent of the district head. The plant material was identified and authenticated by a taxonomist from the Bioresources Development and Conservation Program Department (BDGP), UNN, Enugu State and assigned a voucher specimen number, INTERCEDD/0035.



Figure 1: Pictorial description of *Rauwolfia vomitoria*. **Source:** [West African Plants - A Photo Guide - Rauwolfia vomitoria Afzel \(senckenberg.de\)](#)

Preparation of plant material and extraction

Prior to extraction, the fresh *Rauwolfia vomitoria* leaves were washed, air-dried, and ground into a fine powder using a mortar and pestle. The powdered plant material (150 g) was subjected to cold maceration using 1200 mL of 80 % ethanol in a flat-bottom flask for 72 h at room temperature, with occasional agitation. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The solvent was evaporated under reduced pressure at 40 °C using a rotary evaporator to obtain the dry ethanol extract, which was subsequently stored in an airtight container and refrigerated at 4 °C until use.

Qualitative and quantitative phytochemical analysis

Qualitative phytochemical screening of the crude ethanol extract was carried out to detect the presence of alkaloids, flavonoids, tannins, saponins, glycosides, and phenolic compounds, using standard procedures [12]. Quantitative determinations of total phenolic, flavonoid and alkaloid contents were also performed following the same reference protocols, with results expressed as equivalents of appropriate

standards. The phytochemical profile of the crude ethanol leaf extract of *Rauwolfia vomitoria* was assessed through standard analytical techniques, encompassing both qualitative and quantitative analyses.

Animals

A total of forty-two (42) adult male Wistar albino rats were used in the study: eighteen (18) rats for the acute oral toxicity study and twenty-four (24) rats for the hepatoprotective efficacy study. For both studies, the rats were male, 8 – 10 weeks old, and weighed 105 – 115 g. Animals were obtained from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, and acclimatized for seven days under standard laboratory conditions (25 ± 2 °C; relative humidity 45 – 60 %; 12-h light/dark cycle) with free access to commercial rodent chow and water *ad libitum*. All procedures were approved by the UNN Ethics and Biosafety Committee (approval no. UNN/FBS/EC/1005) and complied with ARRIVE 2.0 guidelines.

Acute oral toxicity (AOT) test

The acute oral toxicity of the crude ethanol leaf extract of *Rauwolfia vomitoria* was assessed in

male Wistar albino rats following a two-stage dose-escalation protocol adapted from the Lorke's method [13]. Eighteen (18) rats (8 – 10 weeks; 180 – 220 g) were randomly assigned to six groups (n = 3 per group). In Stage 1, three groups received single oral doses of 10, 100, and 1000 mg/kg, respectively, and were observed for 24 – 48 h for signs of toxicity and mortality. In Stage 2, the remaining three groups received single oral doses of 1600, 2900, and 5000 mg/kg, respectively, and were monitored for 14 days. No mortality was observed at any of the administered doses up to 5000 mg/kg, and the median lethal dose (LD₅₀) was therefore considered to be greater than 5000 mg/kg. Based on this safety profile and published precedents, a sub-lethal therapeutic dose of 250 mg/kg ($\approx 1/20$ th of the highest dose) was selected for the efficacy study [14].

Assessment of acute toxicity of crude ethanol leaf extract of *Rauwolfia vomitoria*

Acute oral toxicity (AOT) of the ethanol leaf extract of *Rauwolfia vomitoria* was assessed in albino rats following a modified protocol described by Lorke [13]. Prior to the experiment, the animals were fasted overnight and then divided into four (4) groups, each consisting of six (6) rats. The extract was administered orally to each of groups 3 and 4 at doses of 250 mg/kg body weight, respectively. The animals were monitored for acute toxicity, indicated by mortality, over two days, and for sub-chronic toxicity over an additional fourteen (14) days. The median lethal dose (LD₅₀) was calculated using Eq 1.

$$LD_{50} = \sqrt{XY} \dots\dots\dots (1)$$

Where X represents the highest dose with no mortality, and Y represents the lowest dose with mortality. The LD₅₀ was estimated to be 5000 mg/kg. Based on previous studies on the hepatoprotective effects of medicinal plant extracts in the models [17], a sub-lethal dose of 250 mg/kg was selected as the therapeutic dose for subsequent experiments to ensure efficacy while minimizing toxicity.

Induction of hepatotoxicity

The twenty-four (24) male Wistar albino rats were randomized into four groups (n = 6 per group) after acclimatization. Hepatotoxicity was induced by a single high oral dose of paracetamol (2000 mg/kg body weight) on day 15. Treatments were administered once daily by oral gavage for 14 consecutive days prior to paracetamol challenge, according to the

following design: Group I (normal control) received distilled water (2 mL/kg) without paracetamol; Group II (hepatotoxic control) received distilled water for 14 days followed by paracetamol on day 15; Group III (extract control) received *R. vomitoria* ethanol leaf extract (250 mg/kg) for 14 days without paracetamol; and Group IV (extract pre-treated + paracetamol) received the extract (250 mg/kg) for 14 days followed by paracetamol on day 15. After the final treatment, all animals were fasted overnight with free access to water and sacrificed 24 h after paracetamol administration for blood collection and serum preparation for biochemical and cytokine analyses. This prophylactic design is consistent with established models where *R. vomitoria* extract has demonstrated hepatoprotective efficacy against paracetamol-induced liver injury in rats [14].

In vivo design

The experiment involved twenty-four (24) albino rats that were acclimatized for seven (7) days under optimal conditions with free access to food and water. Following acclimatization, the rats were randomly assigned to four groups, each comprising six (6) rats [18]. In Group 3, the rats received an oral dose of 250 mg/kg body weight of ethanol extract from *Rauwolfia vomitoria* leaf once daily for fourteen consecutive days prior to the induction of liver damage on the 15th day. All treatments across the groups were administered via oral intubation.

Group 1 (control) received 2 mL/kg body weight (bw) of distilled water without liver damage induction; Group 2 was administered an oral single dose of 2000 mg/kg bw of paracetamol to induce liver damage but received no treatment; Group 3 was orally treated with 250 mg/kg bw of the extract without liver damage induction; Group 4 was administered an oral single dose of 2000 mg/kg bw of paracetamol to induce liver damage, followed by oral treatment with 250 mg/kg bw of the extract.

Before the experiment, the rats were fasted for 24 h, with access to water. Blood samples were then collected via the retro-orbital plexus into non-heparinized tubes to obtain serum for the determination of serum albumin and total protein levels.

Sample collection and processing

At the end of the experiment, all rats were fasted overnight with free access to water and anaesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) administered

intraperitoneally. Blood was collected via the retro-orbital plexus into plain (non-heparinized) tubes and allowed to clot at room temperature ($25 \pm 2^\circ\text{C}$) for 30 min. The clotted samples were centrifuged at $3000 \times g$ for 10 minutes at room temperature to obtain serum. The separated serum was transferred into clean microtubes and stored at -20°C until biochemical, oxidative stress, and cytokine analyses were performed.

Determination of serum albumin level

Serum albumin levels were determined using the Bromocresol Green (BCG) method, a spectrophotometric technique that quantifies the binding of albumin to bromocresol green. The absorbance was measured at 578 nm, with the absorbance value being directly proportional to the albumin concentration in the sample.

Biochemical assays

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by monitoring the rate of oxaloacetate and pyruvate formation from aspartate and alanine, respectively, and the subsequent reaction with 2,4-dinitrophenylhydrazine to form hydrazones, measured spectrophotometrically at 546 nm (Reitman and Frankel method, as adopted in Randox kits). Serum alkaline phosphatase (ALP) activity was determined by the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol at 405 nm. Serum urea concentration was measured using the urease–Berthelot method, where indophenol blue formed is read at 600 nm. Serum creatinine was determined by the Jaffe reaction, based on the formation of a creatinine–picrate complex measured at 520 nm. Serum albumin was estimated using the bromocresol green (BCG) dye-binding method, in which albumin–BCG complex was measured at 628 nm. Total protein was measured and the protein–Coomassie dye complex was quantified at 595 nm, using bovine serum albumin (BSA) as standard. All analyses were performed with commercially available diagnostic kits (Randox Laboratories Ltd., Crumlin, UK) following the manufacturer's protocols. Serum samples were assayed in duplicate, and results were expressed as U/L for enzymes, mg/dL for urea and creatinine, and g/dL for albumin and total protein.

Oxidative stress and antioxidant assays

Serum lipid peroxidation was determined by estimating malondialdehyde (MDA) using the thiobarbituric acid-reactive substances (TBARS) method. In this assay, MDA reacts with thiobarbituric acid under acidic conditions and

heating to form a pink chromogen, which was measured spectrophotometrically at 532 nm [15]. Results were expressed as $\mu\text{mol/L}$ of serum. Serum reduced glutathione (GSH) reacts with 5,5'-dithiobis (2-nitrobenzoic acid; DTNB) to form a yellow-coloured 5-thio-2-nitrobenzoic acid (TNB) product, quantified at 412 nm. Results were expressed as $\mu\text{mol/L}$ of serum. Superoxide dismutase (SOD) activity was assayed following a standard method that measures the inhibition of epinephrine auto-oxidation at alkaline pH [16]. The rate of inhibition was recorded spectrophotometrically at 480 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit the auto-oxidation of epinephrine by 50 %. Results were expressed as U/mL of serum. All assays were performed in duplicate with appropriate blanks, and values were calculated against standard calibration curves where applicable.

Inflammatory cytokine measurement

Serum interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) concentrations were quantified using rat-specific sandwich ELISA kits (Elabscience Biotechnology Inc., Houston, TX, USA; Catalog nos: E-EL-R0015 for IL-6 and E-EL-R0019 for TNF- α). The assays are based on the capture antibody–antigen–biotinylated detection antibody system, with horseradish peroxidase (HRP)–streptavidin conjugate used for signal amplification. After adding tetramethylbenzidine (TMB) substrate, a blue color developed and was converted to yellow upon addition of stop solution. Absorbance was read at 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA). Standard curves were generated using recombinant rat cytokine standards provided with the kits, and concentrations of IL-6 and TNF- α in the serum samples were calculated by four-parameter logistic (4-PL) regression. The assay sensitivity was $< 2 \text{ pg/mL}$ for IL-6 and $< 5 \text{ pg/mL}$ for TNF- α , with a detection range of 7.8 – 500 pg/mL. All samples and standards were assayed in duplicate, and intra- and inter-assay coefficients of variation (CVs) were $< 10 \%$. Results are expressed in pg/mL of serum.

Determination of total protein

Protein concentration was determined using the Bradford assay, with bovine serum albumin (BSA) as the standard. Sample aliquots were incubated for 20 min at 26°C with 250 μL of Bradford reagent (Sigma-Aldrich, USA). The absorbance was subsequently measured at 595 nm using a Cary 300 UV-visible

spectrophotometer (Agilent Technologies, Santa Clara, USA) to quantify the protein content.

Statistical analysis

Analyses were conducted with GraphPad Prism version 9.0 (GraphPad Software Inc., San Diego, CA, USA). Data are expressed as mean $\pm$ standard deviation (SD) for each group ($n = 6$). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test to determine significant differences among groups. Statistical significance was accepted at $p < 0.05$. All assumptions for ANOVA, including normality of data distribution and homogeneity of variances, were checked before analysis.

RESULTS

The percentage yield of the crude ethanol extract from *R. vomitoria* leaves was 15 % (w/w). The extract exhibited a sticky texture and a dark brown color. Phytochemical analysis (Table 1) of the crude extract identified the presence of several bioactive compounds.

Qualitative analysis revealed that the extract contains a variety of bioactive compounds, including alkaloids, flavonoids, glycosides, saponins, and tannins, which are recognized for their potential therapeutic properties and may contribute to the extract's pharmacological effects. The results of the acute toxicity (LD_{50}) test for the crude ethanol leaf extract of *R. vomitoria*, conducted in two stages on albino rats, are detailed in Table 2. No mortality was observed at any of the administered doses during both stages of the test.

The results indicate that even at the highest administered dose of 5000 mg/kg body weight, no mortalities were observed in either stage of the acute toxicity test. This suggests that the crude ethanol leaf extract of *Rauwolfia vomitoria* has a relatively low toxicity profile in albino rats under the conditions of this study.

Table 2: Acute toxicity (LD_{50}) test of crude ethanol leaf extract of *Rauwolfia vomitoria* in albino rats

Stage 1		
Group	Dosage (mg/kg bw)	Mortality
1	10	0/3
2	100	0/3
3	1000	0/3

Stage 2		
Group	Dosage (mg/kg bw)	Mortality
1	1600	0/3
2	2900	0/3
3	5000	0/3

Figure 1 illustrates the effect of the ethanol leaf extract of *Rauwolfia vomitoria* on serum albumin levels in rats. The data show a significant ($p \leq 0.05$) reduction in total serum albumin concentrations in the group with induced hepatotoxicity that did not receive treatment, compared to the control group. However, there was no statistically significant ($p \leq 0.05$) difference in total serum albumin levels between the group with induced hepatotoxicity that was treated with the extract and the control group.

Figure 2 shows the results of the investigation on the effect of the ethanol leaf extract of *Rauwolfia vomitoria* on serum total protein levels in albino rats. A significant ($p \leq 0.05$) decrease in total serum protein concentrations was observed in the group with induced hepatotoxicity that received no treatment, compared to the control group. However, there was no significant difference in total serum protein levels between the group with induced hepatotoxicity (which were treated with the extract) and control group ($p > 0.05$).

These findings suggest that the ethanol leaf extract of *Rauwolfia vomitoria* has a protective effect on serum total protein levels, effectively mitigating the decrease induced by hepatotoxicity and maintaining levels similar to those in the control group.

Table 1: Qualitative phytochemical analysis of the crude ethanol leaf extract of *Rauwolfia vomitoria*

Phytochemical	Test used	Result
Alkaloids	Dragendorff's test	Present (Precipitation)
Flavonoids	Hinoda's test	Present (Pink-red coloration)
Glycosides	Kellar-Kiliani's test	Present (Green-blue coloration)
Saponins	Frothing test	Present (Stable froth formation)
Tannins	Ferric chloride test	Present (Blue-black coloration)

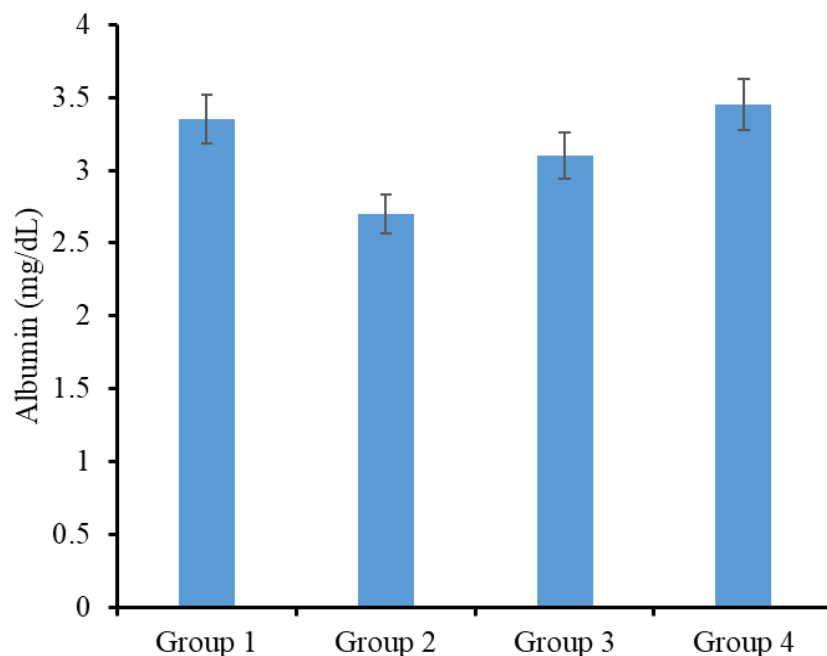


Figure 1: Effect of crude ethanol extract of *Rauwolfia vomitoria* leaves on serum albumin levels in albino rats. Group 1: Control (2 mL/kg distilled water, no liver damage); Group 2: Paracetamol-induced hepatotoxicity (2000 mg/kg bw, untreated); Group 3: Extract only (250 mg/kg bw, no liver damage); Group 4: Extract + paracetamol (250 mg/kg bw + 2000 mg/kg bw paracetamol). Values are expressed as mean $\pm$ SD (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.001 vs control group (G1). # p < 0.05, ## p < 0.01, ### p < 0.001 vs hepatotoxic group (G2)

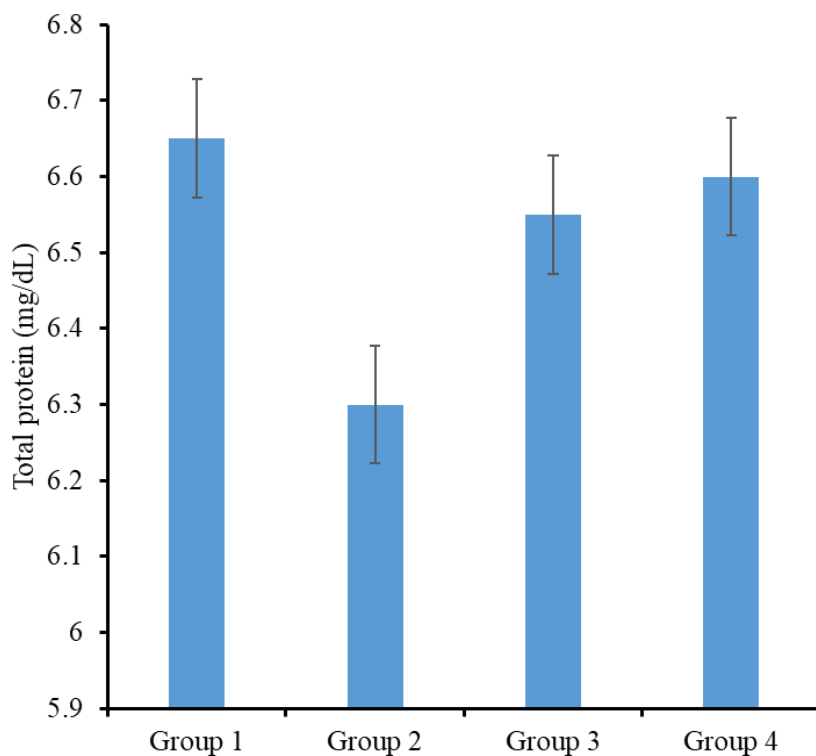


Figure 2: Effect of crude ethanol extract of *Rauwolfia vomitoria* leaves on serum total protein levels in albino rats. Group 1: Control (2 mL/kg distilled water, no liver damage); Group 2: Paracetamol-induced hepatotoxicity (2000 mg/kg bw, untreated); Group 3: Extract only (250 mg/kg bw, no liver damage); Group 4: Extract + paracetamol (250 mg/kg bw + 2000 mg/kg bw paracetamol). Values are expressed as mean $\pm$ SD (n = 6). * p < 0.05, ** p < 0.01, *** p < 0.001 vs control group (G1). # p < 0.05, ## p < 0.01, ### p < 0.001 vs hepatotoxic group (G2)

The biochemical and physiological effects of *Rauwolfia vomitoria* extract are summarized in Table 3. Group 3 (extract only) did not differ significantly from the control group, confirming that the extract alone at 250 mg/kg had no adverse effects. Paracetamol administration (G2) caused significant elevations in alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) compared with the control ($p < 0.001$), indicating severe hepatocellular damage. Pretreatment with *R. vomitoria* (G4) significantly reduced these enzyme levels compared with the hepatotoxic group ($p < 0.01$), although values did not fully return to normal.

Renal function markers (creatinine and urea) were significantly increased in G2 compared with G1 ($p < 0.001$), but extract pretreatment (G4) markedly lowered both parameters relative to G2 ($p < 0.01$). Similarly, oxidative stress was evident in G2, with increased malondialdehyde (MDA) and decreased reduced glutathione (GSH) and superoxide dismutase (SOD) levels ($p < 0.001$ vs G1). These changes were significantly reversed in G4 ($p < 0.01$ vs G2), though not completely normalized. Inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) were elevated in G2 ($p < 0.001$ vs G1) and were significantly reduced in G4 ($p < 0.01$ vs G2). Serum albumin and total protein were also significantly reduced in G2 ($p < 0.001$ vs G1) but were restored by the extract in G4, with no significant difference from control ($p > 0.05$). Collectively, these results demonstrate that *R. vomitoria* extract alone is non-toxic and that it confers significant hepatoprotective, antioxidant,

anti-inflammatory, and nephroprotective effects against paracetamol-induced toxicity.

The biochemical and physiological effects of *Rauwolfia vomitoria* extract on liver enzymes, kidney function, oxidative stress markers, inflammatory cytokines, and serum proteins are summarized in Table 3. The hepatotoxic group (G2) demonstrated significantly elevated levels of ALT, AST, and ALP, indicating severe liver damage in comparison with the control (G1). Treatment with *R. vomitoria* (G4) significantly reduced these enzyme levels when compared with G1, although they did not fully return to normal, reflecting the extract's hepatoprotective effect. Similarly, elevated creatinine and urea levels in G2 suggested renal impairment secondary to hepatotoxicity, which was ameliorated by the extract treatment (G4), highlighting its nephroprotective potential.

Oxidative stress markers revealed increased MDA and decreased antioxidant markers (GSH and SOD) in G2, consistent with oxidative damage in comparison with controls (G1). Treatment with *R. vomitoria* (G4) significantly reduced MDA levels and restored GSH and SOD activity, suggesting potent antioxidant effects. Elevated pro-inflammatory cytokines (IL-6 and TNF- α) in G2 indicated an inflammatory response, which was significantly mitigated in G4, demonstrating the extract's anti-inflammatory properties. Additionally, serum albumin and total protein levels were markedly lower in G2, reflecting impaired liver synthetic function in comparison with G1.

Table 3: Effects of *Rauwolfia vomitoria* extract on biochemical parameters, oxidative stress markers, and inflammatory cytokines

Parameter	Control group (G1)	Hepatotoxic group (G2)	Extract-treated without hepatotoxicity (G3)	Extract-treated with hepatotoxicity (G4)
Liver enzymes (U/L)				
ALT	32.4 $\pm$ 3.2	145.8 $\pm$ 10.3***	34.6 $\pm$ 2.7	48.2 $\pm$ 4.5####
AST	28.7 $\pm$ 3.0	162.4 $\pm$ 12.7***	29.3 $\pm$ 2.5	56.7 $\pm$ 6.1####
ALP	75.3 $\pm$ 4.5	212.8 $\pm$ 18.5***	78.1 $\pm$ 3.8	103.2 $\pm$ 9.2####
Kidney function (mg/dL)				
Creatinine	0.7 $\pm$ 0.1	2.1 $\pm$ 0.3***	0.8 $\pm$ 0.1	1.0 $\pm$ 0.2####
Urea	25.2 $\pm$ 3.1	62.8 $\pm$ 5.4***	26.7 $\pm$ 3.4	34.1 $\pm$ 4.1####
Oxidative stress markers				
MDA (μ mol/L)	3.2 $\pm$ 0.4	7.8 $\pm$ 0.6***	3.4 $\pm$ 0.3	4.5 $\pm$ 0.5####
GSH (μ mol/L)	8.7 $\pm$ 0.5	3.1 $\pm$ 0.4***	8.5 $\pm$ 0.4	7.2 $\pm$ 0.6####
SOD (U/mg protein)	128.2 $\pm$ 10.5	57.3 $\pm$ 6.2***	130.4 $\pm$ 12.1	102.8 $\pm$ 9.7####
Inflammatory cytokines (pg/mL)				
IL-6	8.3 $\pm$ 0.6	24.7 $\pm$ 2.1***	7.9 $\pm$ 0.5	13.5 $\pm$ 1.7####
TNF- α	5.4 $\pm$ 0.4	20.1 $\pm$ 1.8***	5.6 $\pm$ 0.3	10.8 $\pm$ 1.3####

Values are expressed as mean $\pm$ standard deviation (SD), n = 6. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control group (G1). * $p < 0.05$, ## $p < 0.01$, #### $p < 0.001$ vs hepatotoxic group (G2). ALT = Alanine transaminase; AST = Aspartate transaminase; ALP = Alkaline phosphatase; MDA = Malondialdehyde; GSH = Reduced glutathione; SOD = Superoxide dismutase; IL-6 = Interleukin-6; TNF- α = Tumor necrosis factor-alpha

Treatment with *R. vomitoria* restored these protein levels, underscoring its role in maintaining liver function and overall protein synthesis. These findings collectively highlight the therapeutic potential of *R. vomitoria* extract in managing paracetamol-induced hepatotoxicity and associated complications.

DISCUSSION

This study highlights the hepatoprotective effects of *Rauwolfia vomitoria* ethanol extract in a paracetamol-induced hepatotoxicity model, focusing on biochemical parameters, oxidative stress markers, inflammatory cytokines, and serum proteins. The findings suggest that the extract exhibits significant potential in mitigating hepatic and systemic damage caused by excessive paracetamol intake.

Liver enzymes such as ALT, AST, and ALP are well-established biomarkers of hepatocellular damage [17,18]. The significant elevation of these enzymes in the hepatotoxic group (G2) confirms liver injury caused by the toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). This metabolite depletes glutathione stores and induces oxidative stress, leading to hepatocyte damage [17,18]. Treatment with *R. vomitoria* (G4) significantly reduced ALT, AST, and ALP levels, reflecting a protective effect on liver integrity. Although enzyme levels did not fully normalize, the reduction highlights the extract's potential to partially restore liver function through its antioxidant and membrane-stabilizing properties. Importantly, Group 3 (extract only) was included to evaluate the safety of *R. vomitoria* in the absence of liver injury. The lack of significant changes in ALT, AST, and ALP in G3 compared with the control group indicates that the extract alone did not exert hepatotoxic effects. This finding supports the safety of the extract at the tested dose and reinforces that the improvements observed in G4 were attributable to its protective role against paracetamol-induced hepatotoxicity rather than any confounding intrinsic effect.

Paracetamol-induced hepatotoxicity is often associated with secondary effects on renal function, as indicated by elevated creatinine and urea levels in G2 [18]. These changes signify impaired renal clearance and oxidative stress affecting the kidneys. Treatment with *R. vomitoria* significantly reduced these markers in G4, suggesting its nephroprotective effects. This observation aligns with the antioxidant and anti-inflammatory properties of the extract, which mitigate systemic toxicity and reduce the burden on renal tissues. Oxidative stress is a key driver

of paracetamol-induced liver damage, evidenced by elevated malondialdehyde (MDA) levels and depleted antioxidant markers such as glutathione (GSH) and superoxide dismutase (SOD) in G2. MDA, a product of lipid peroxidation, indicates extensive oxidative damage to hepatocytes [8]. The extract's ability to significantly reduce MDA levels while restoring GSH and SOD activities in G4 suggests a potent antioxidant mechanism [6]. This may be attributed to the presence of bioactive phytochemicals, such as alkaloids, flavonoids, and tannins, which are known for their free radical-scavenging properties [6].

Inflammatory cytokines, particularly IL-6 and TNF- α , are pivotal in the pathophysiology of paracetamol-induced hepatotoxicity [4]. Elevated levels of these cytokines in G2 reflect an inflammatory response driven by oxidative damage and immune activation. Treatment with *R. vomitoria* significantly reduced IL-6 and TNF- α levels in G4, indicating anti-inflammatory effects. This reduction may result from the extract's ability to modulate cytokine production and suppress pro-inflammatory signaling pathways.

Paracetamol-induced hepatotoxicity impaired liver synthetic function, as demonstrated by reduced serum albumin and total protein levels in G2 [17,18]. These decreases signify compromised protein synthesis, which is crucial for maintaining metabolic and structural integrity. Treatment with *R. vomitoria* restored these parameters in G4, highlighting its role in supporting liver recovery and protein metabolism. The hepatoprotective effects of *R. vomitoria* may involve multiple mechanisms. The extract scavenges free radicals and enhances endogenous antioxidant defenses, thereby reducing oxidative damage [17,18]. Additionally, it suppresses pro-inflammatory cytokines, alleviating inflammation and tissue damage. The reduction in liver enzyme levels suggests that the extract stabilizes hepatocyte membranes, preventing enzyme leakage into circulation. Furthermore, the improvement in GSH levels indicates that the extract replenishes glutathione stores, facilitating the detoxification of NAPQI.

These findings align with previous studies, which reported the therapeutic potential of *R. vomitoria* in reducing oxidative stress, inflammation, and hepatocellular damage in various experimental models [8,10]. Similar protective effects have been documented for other medicinal plants used in traditional medicine, such as *Silybum marianum* (milk thistle), *Curcuma longa* (turmeric), and *Glycyrrhiza glabra* (licorice), all of which contain bioactive phytochemicals with established hepatoprotective properties [6]. This

emphasizes the importance of phytochemicals in mitigating hepatotoxicity and supporting liver function.

CONCLUSION

The study demonstrates that *Rauwolfia vomitoria* ethanol extract possesses significant hepatoprotective and nephroprotective properties. By attenuating liver enzyme levels, restoring antioxidant defenses, reducing inflammation, and improving serum protein levels, the extract shows promise as a therapeutic agent for managing paracetamol-induced hepatotoxicity and associated complications. Future studies should focus on isolating and characterizing the specific bioactive compounds responsible for these effects and evaluating their potential for clinical applications.

DECLARATIONS

Acknowledgement/Funding

The authors wish to express their gratitude to the management of the Department of Biochemistry, University of Nigeria, Nsukka, for generously providing bench space for this research. We also extend our thanks to the laboratory technicians in the same department for their technical assistance and to Patrick A. Chuka Egbuna for his supervision and insightful comments, which greatly enhanced the manuscript.

Ethical approval

This study was approved by the University of Nigeria (UNN) Ethics and Biosafety Committee (approval no. UNN/FBS/EC/1005).

Availability of Data and Materials

The data set generated during this study is available upon reasonable request from the corresponding authors, Ijeoma Princess Nwafor (princess.ijeoma05@gmail.com) and Emeka John Dingwoke (dinhimself@yahoo.com, emeka.jd@unescoicb.org.ng)

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities

pertaining to claims relating to the content of this article will be borne by the authors. All Authors have read and approved the manuscript. Ijeoma Princess Nwafor conducted data collection, analysis, and interpretation. Ijeoma Princess Nwafor, Israel Ogwuche Ogra, John Wassagwa, Emeka John Dingwoke and Nnamdi Okereke contributed to manuscript writing, data analysis, manuscript editing and review.

REFERENCES

- Bernal W, Wendon J. Acute liver failure. *N Engl J Med* 2013; 369(26): 2525-2534. doi: 10.1056/NEJMra1208937
- Bower WA, Johns M, Margolis HS, Williams IT, Bell BP. Population-based surveillance for acute liver failure. *Am J Gastroenterol* 2007; 102(11): 2459-2463. doi: 10.1111/j.1572-0241.2007.01388.x.
- Bretherick AD, Craig DG, Masterton G, Bates C, Davidson J, Martin K, Iredale JP, Simpson KJ. Acute liver failure in Scotland between 1992 and 2009; incidence, aetiology and outcome. *QJM* 2011; 104(11): 945-956. doi: 10.1093/qjmed/hcr098
- Rotundo L, Pyrsopoulos N. Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol* 2020; 12(4): 125-136. doi: 10.4254/wjh.v12.i4.125.
- Corsini A, Bortolini M. Drug-induced liver injury: The role of drug metabolism and transport. *J Clin Pharmacol* 2013; 53(4): 463-474. DOI: 10.1002/jcph.22
- Vaou N, Stavropoulou E, Vaidarou CC, Tsakris Z, Rozos G, Tsigalou C, Bezirtzoglou E. Interactions between medical plant-derived bioactive compounds: Focus on antimicrobial combination effects. *Antibiotics (Basel)* 2022; 11(8): 1014. <https://doi.org/10.3390/antibiotics11081014>.
- Adegoke V, Okpakpor E, Uwaya D, Ozolua R, Salawu O. Toxicological evaluation of hydro-alcohol root extract of *Rauwolfia vomitoria* Afzel (Apocynaceae). *Trop J Pharm Res* 2024; 23(10): 1639-1646. <https://doi.org/10.4314/tjpr.v23i10.7>
- Lobay D. *Rauwolfia* in the treatment of hypertension. *Integr Med (Encinitas)* 2015; 14(3): 40-46.
- Kumar S, Singh A, Bajpai V, Srivastava M, Singh BP, Kumar B. Structural characterization of monoterpene indole alkaloids in ethanolic extracts of *Rauwolfia* species by liquid chromatography with quadrupole time-of-flight mass spectrometry. *J Pharm Anal* 2016; 6(6): 363-373. doi: 10.1016/j.jpha.2016.04.008.
- Iwu IC, Chijioke C, Iwuji SC, Obiagwu I, Onu UL, Ukaoma AA, Chijioke-Okere M, Ezekoye MO. Chemical and medicinal properties of *Rauwolfia vomitoria* (AFZEL) harvested from South-Eastern Nigeria. *Asian J Chem Sci* 2021; 10(4): 56-71. <https://doi.org/10.9734/ajocs/2021/v10i419103>.
- Asoro II, Ebuehi OAT, Igwo-Ezikpe MN. GC-MS analysis of the *Rauwolfia vomitoria* Ethanol Extracts. *Eur J Med Trop J Pharm Res*, November 2025; 24(11): 1412

- Plants 2021; 32(6): 34–45. <https://doi.org/10.9734/ejmp/2021/v32i630398>.
12. Harborne JB. *Phytochemical methods: A guide to modern techniques of plant analysis*. 3rd Edition. Springer Science & Business Media 1998.
13. Lorke D. A new approach to practical acute toxicity testing. *Archives Toxicol* 1983; 53: 275-289.
14. Eluwa MA, Ekanem TB, Udoh PB, Ekong MB, Asuquo OR, Akpantah AO, Nwakanma AO. Teratogenic effect of crude ethanolic root bark and leaf extracts of *Rauwolfia vomitoria* (Apocynaceae) on Nissl substances of albino wistar rat fetuses. *Neurosci J* 2013; 2013: 906731. doi: 10.1016/0003-2697(79)90738-3.
15. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95(2): 351–358.
16. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for SOD. *J Biol Chem* 1972; 247(10): 3170–3175.
17. Fu S, Wu D, Jiang W, Li J, Long J, Jia C, Zhou T. Molecular biomarkers in drug-induced liver injury: Challenges and future perspectives. *Front Pharmacol* 2020; 10: 1667. <https://doi.org/10.3389/fphar.2020.01667>
18. Kalas MA, Chavez L, Leon M, Taweesedt PT, Surani S. Abnormal liver enzymes: A review for clinicians. *World J Hepatol* 2021; 13(11): 1688-1698. <https://doi.org/10.4254/wjh.v13.i11.1688>.