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Restorative effects of methanol leaf extract of *Uvaria chamae* on some biochemical and pharmacological parameters of paracetamol-induced liver damage in Wistar albino rats

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Abstract

Objectives To evaluate the therapeutic potential of *Uvaria chamae* extracts in mitigating paracetamol-induced hepatic injury in rats.

Methods Rats were administered paracetamol to induce hepatic toxicity and subsequently treated with *Uvaria chamae* extracts. Biochemical markers of liver and kidney function, oxidative stress, and lipid profile were measured. Antioxidant enzyme activities and electrolyte levels were also assessed. Liver tissues were examined histologically to evaluate structural integrity.

Results Treatment with *Uvaria chamae* extracts produced significant reductions ($p < 0.05$) in markers of hepatic and renal injury, including AST, ALT, ALP, GGT, MDA, total and direct bilirubin, total cholesterol, LDL, TAG, urea, and creatinine. Treated groups also showed a significant decrease in final body weight compared with the induced but untreated group. Antioxidant parameters—including SOD, catalase, and GPx—were significantly elevated ($p < 0.05$). Total protein, albumin, HDL, and potassium levels also increased following treatment. Histological examination revealed normal liver architecture in treated groups, with no observed lesions.

Conclusions *Uvaria chamae* extracts ameliorated biochemical and structural indicators of paracetamol-induced hepatic injury in rats. These findings support its potential as a therapeutic candidate for liver damage associated with paracetamol toxicity.

Keywords *Uvaria chamae*, Paracetamol-induced hepatotoxicity, Antioxidant activity, Biochemical biomarkers, Histopathology

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Background

Paracetamol is an analgesic that is also used as an anti-pyretic globally [1]. It is one of the most accessible over-the-counter medications, commonly used across all age groups, from infants to the elderly [2]. However, its affordability and availability have led to frequent misuse and overconsumption. Excessive self-medication remains a significant factor contributing to numerous cases of paracetamol toxicity [3]. In the liver, paracetamol is primarily broken down by relevant enzymes, chiefly the microsomal cytochrome P450 (CYP450) system, resulting in non-toxic metabolites. Specifically, this process leads to the production of large amounts of reactive species. Under normal therapeutic conditions, approximately 90% of paracetamol undergoes sulfation and glucuronidation, leading to renal excretion in its non-toxic form [4]. However, excessive doses saturate these pathways, causing an increased proportion of paracetamol to be metabolised for detoxification, eventually overwhelming the system and leading to toxicity. NAPQI is normally detoxified by conjugation with glutathione, rendering it harmless before excretion [5]. However, in overdose scenarios, excessive NAPQI production depletes glutathione reserves, resulting in its accumulation and subsequent liver cell damage [6]. Mitochondrial and cytosolic glutathione depletion hinders the excretion of toxic byproducts, thereby worsening hepatotoxicity [7] and replenishing glutathione levels. Glutathione stores are restored by hydrolysing N-acetylcysteine (NAC) to cysteine, mitigating liver damage and reducing the necessity for liver transplantation [8]. The administration of NAC depends on the patient's plasma paracetamol concentration and the time elapsed before hospital admission. *Uvaria chamae* is a medicinal shrub indigenous to West Africa [9]. Traditionally, the roots and bark of *Uvaria chamae* have been employed in benefits [10]. Research has shown that the root and leaf extracts of *Uvaria chamae* possess antidiabetic and hypolipidemic [11] as well as antimalarial properties [12]. Additionally, *Uvaria chamae* is abundant in natural antioxidants [13]. Since paracetamol-induced hepatotoxicity is primarily linked to oxidative stress and free radicals, the antioxidant-rich nature of *Uvaria chamae* suggests its potential hepatoprotective effects. Although multiple studies have explored the phytochemical composition and antioxidative benefits of *Uvaria chamae* leaves, limited research has investigated the in vivo impact of methanol extracts and their fractions on biochemical and pharmacological parameters in rat livers affected by paracetamol toxicity. Furthermore, studies examining the histological effects of these extracts and identifying the active phytochemicals in the most potent fraction remain relatively unexplored. Therefore, this study aims to assess the

possible hepatoprotective properties of *Uvaria chamae* leaf extracts in paracetamol-induced liver damage.

Methods

Plant materials

Fresh leaves of *Uvaria chamae* were gathered from Umuakashi, in Nsukka, Enugu State, Nigeria. The plant was validated by a taxonomist, Mr. Alfred Ozioko, at the Biodiversity and Conservation Programme (BDCP), Aku Road, Nsukka, Enugu State. A voucher specimen was assigned the identification number (InterCEDD/A11/003 *Uvaria chamae*) and subsequently stored in the BDCP herbarium.

Preparation of plant extracts

Fresh *Uvaria chamae* leaves weighing 6 kg were collected, carefully cleaned with clean water, and allowed to air-dry in a shaded area. Following drying, they were ground into a fine powder. Subsequently, extraction with 80% methanol took place over a 72-h period via cold maceration. Whatman filter paper No. 1 was used to filter the mixture, and a magnetic stirrer was used to concentrate the filtrates at 40°C. For additional examination, the resultant extracts were placed in a cool, airtight container.

Fractionation of the methanol extract

The crude methanol extract was adsorbed onto silica gel mesh (60–120) and loaded onto a silica gel-packed column for chromatographic separation. Elution was carried out using a stepwise solvent gradient of increasing polarity. The column was first eluted with 100% dichloromethane (DCM) (500 mL), followed by DCM:ethyl acetate mixtures at 95:5 (400 mL), 90:10 (400 mL), 80:20 (400 mL), and 70:30 (400 mL). This was continued with 100% ethyl acetate (500 mL), then ethyl acetate:methanol mixtures at 90:10 (300 mL) and 80:20 (300 mL). Final elution was performed with 100% methanol (500 mL) to obtain the most polar fractions. Fractions were collected in 50 mL aliquots, monitored by thin-layer chromatography (TLC), and pooled based on similar chromatographic profiles for further analysis.

Animals

Male albino Wistar rats, with weights ranging from 120 to 140 g, were obtained from Emmanuel Farms in Nsukka, Enugu State. The rats were maintained in metallic cages in an ambient environment in the laboratory animal facility. Rats were allotted 1 week for acclimatisation prior to the initiation of the experiment. Throughout this period, they were fed Vital Feeds pellets containing 16% crude protein, 5.25% crude fibre, 5.50% crude ash, 1.10% calcium, and 4.00% crude oil and fats, sourced from a distributor in Nsukka, Enugu State, with

unlimited availability of food and water. Upon completion of the acclimatisation period, the animals were weighed, organised into groups of five rats, and labelled appropriately. This study was conducted and reported in accordance with Animal Research Reporting in vivo Experiments (ARRIVE) guidelines. Animals were handled carefully in line with institutional ethical guidelines. The study was considered and approved by the Faculty of Biological Science Research Ethics Committee with the approval number UNN/FBS/EC/1092. We confirm that all methods were carried out in accordance with relevant guidelines and regulations.

Acute toxicity studies

The acute toxicity study (LD50) was performed in accordance with the methodology outlined by Lorke [14]. The experimental subjects were categorized into two distinct phases: phase I and phase II. Each phase was divided into four groups, with each subgroup comprising three rats.

Experimental design

Group 1: Standard control

Group 2: Induction excluding treatment

Group 3: Induction using 2000 mg/kg of paracetamol, followed by treatment with 100 mg/kg body weight of crude extract

Group 4: Induction using 2000 mg/kg of paracetamol, followed by treatment with 200 mg/kg body weight of crude extract

Group 5: Induction using 2000 mg/kg of paracetamol, followed by treatment with 100 mg/kg body weight of the ethyl acetate fraction

Group 6: Induction using 2000 mg/kg of paracetamol, followed by treatment with 200 mg/kg body weight of the ethyl acetate fraction

Group 7: Induction using 2000 mg/kg of paracetamol, followed by treatment with 100 mg/kg body weight of the methanol fraction

Group 8: Induction using 2000 mg/kg of paracetamol, followed by treatment with 200 mg/kg body weight of the methanol fraction

Group 9: Induction using 2000 mg/kg of paracetamol, followed by treatment with 100 mg/kg body weight of silymarin

In the curative study, hepatotoxicity was induced in groups 2 to 9 using paracetamol administered on days 1, 2, and 3. Thereafter, treatment was administered to groups 3 to 9 from days 4 to 13. Paracetamol-induced liver injury was achieved using a dose of 2000 mg/kg [15]. The experiment lasted for 14 days, after which all animals were sacrificed. Blood samples were collected, and

the sera were stored in labelled plain specimen bottles for serological analysis. Liver tissues were preserved in 10% formalin, labelled accordingly, and processed for histological examination.

Biochemical assays and analytical methods

Measurement of aspartate aminotransferase (AST) activity

The activity of AST was assessed via the Reitman-Frankel colorimetric technique [16] with a Quimica Clinica Applicada (QCA) test kit.

Measurement of alkaline phosphatase (ALP) activity

The method of Klein et al. [17] was employed to determine ALP activity in serum via a QCA test kit.

Measurement of alanine aminotransferase (ALT) activity

ALT activity was quantified following the Reitman-Frankel colorimetric method [16] via a QCA test kit.

Measurement of gamma-glutamyl transferase (GGT) activity

The enzymatic activity of GGT was determined utilizing the method outlined by Tietz [18].

Measurement of total serum protein concentration

The Biuret method was used to estimate total protein levels, employing a QCA test kit following the procedure reported by Lubran [19].

Measurement of serum albumin concentration

Serum albumin concentration was assessed according to Doumas et al. [20] using a QCA test kit.

Estimation of total and direct bilirubin levels in serum

Total bilirubin (TBIL) and direct bilirubin (DBIL) concentrations were estimated via the method of Jendrassik and Grof [21].

Measurement of oxidative stress parameters

The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) and the concentration of malondialdehyde (MDA) were evaluated according to the method of Fridovich [22], Aebi [23], Paglia and Valentine [24], and Varshney and Kale [25] respectively.

Determination of lipid profile

Total cholesterol, low-density lipoprotein, high-density lipoprotein, and triglycerides levels were measured following the method of Allain et al. [26], Assman et al. [27], and Albers et al. [28] respectively utilising QCA kits.

Measurement of serum electrolyte concentrations

Sodium ion (Na^+), potassium ion (K^+), chloride ion (Cl^-), and bicarbonate ion (HCO_3^-) concentrations were measured following Tietz's method [18] using a Teco diagnostic kit.

Measurement of urea and creatinine

Serum urea and creatinine concentrations were measured via Tietz [29] with a Randox diagnostic kit.

Estimation of random blood sugar (RBS) concentration

Random blood glucose levels were measured using an Accu-Chek Active glucometer by Roche Diagnostics.

Assessment of body weight

The body weight of both control and test animals was recorded before and after the study. The difference in body weight was noted and analysed accordingly.

Histological examination

Histological analysis of liver tissue samples was conducted via Suvarna et al. [30].

Statistical analysis

Data were expressed as mean $\pm$ SD. Statistical significance was analyzed using one-way and two-way analysis of variance (ANOVA). Mean comparisons were performed using Duncan's multiple range test. The SPSS software, version 20, was employed for data analysis.

Results**Acute toxicity test on the extract of *Uvaria chamae* leaves**

As in Table 1, the result of the acute toxicity test of the extract of *Uvaria chamae* leaves in Stage 1 and Stage 2 showed no mortality after 24 h in the tested mice up to 5000 mg/kg.

Table 1 The result of acute toxicity test on the extract of *Uvaria chamae* leaves

Groups	No of mice	Dosage (mg/kg)	Mortality
Stage one			
1	3	10	0/3
2	3	100	0/3
3	3	1000	0/3
Stage two			
1	1	1600	0/3
2	1	2900	0/3
3	1	5000	0/3

Curative effect of *Uvaria chamae* leaf extracts on liver enzyme markers' activity in rats with paracetamol-induced hepatic injury

Table 2 presents the results of a curative assessment, examining the impact of extracts and fractions of *Uvaria chamae* leaves on the activity of liver enzyme markers associated with paracetamol-induced liver damage.

There was a significant ($p < 0.05$) reduction in the activities of AST, ALT, ALP, and GGT in the treated groups compared to group 2 (induced but untreated). Furthermore, there were no significant ($p > 0.05$) alterations in the activities of AST, ALT, ALP, and GGT in the treated groups compared to Group 1 and 9 (induced and treated with silymarin).

Curative effect of extracts of *Uvaria chamae* leaves on the concentrations of liver proteins in paracetamol-induced hepatotoxicity in rats

Table 3 presents the curative study, illustrating the impact of extracts and fractions of *Uvaria chamae* leaves on liver protein concentrations in paracetamol-induced liver damage. It was noted that the total protein concentration and serum albumin concentration in the treated groups

Table 2 Curative effect of extracts of *Uvaria chamae* leaves on the activities of liver enzyme markers in paracetamol-induced hepatic damage in rats

Group	AST iu/L	ALT iu/L	ALP iu/L	GGT U/L
1	21.00 $\pm$ 5.00 ^a	5.67 $\pm$ 2.89 ^a	17.80 $\pm$ 1.00 ^a	1.01 $\pm$ 0.20 ^a
2	35.00 $\pm$ 5.00 ^b	15.33 $\pm$ 4.04 ^b	49.00 $\pm$ 1.73 ^b	3.40 $\pm$ 0.41 ^b
3	21.67 $\pm$ 2.08 ^a	5.00 $\pm$ 2.00 ^a	17.33 $\pm$ 1.15 ^a	1.40 $\pm$ 0.41 ^a
4	22.00 $\pm$ 3.20 ^a	5.67 $\pm$ 3.06 ^a	17.67 $\pm$ 1.15 ^a	1.63 $\pm$ 0.62 ^a
5	21.33 $\pm$ 3.21 ^a	5.33 $\pm$ 3.21 ^a	18.33 $\pm$ 1.53 ^a	1.62 $\pm$ 0.54 ^a
6	21.00 $\pm$ 4.58 ^a	6.67 $\pm$ 0.58 ^a	18.67 $\pm$ 0.58 ^a	1.56 $\pm$ 0.65 ^a
7	21.00 $\pm$ 6.00 ^a	5.33 $\pm$ 2.52 ^a	17.67 $\pm$ 0.58 ^a	1.48 $\pm$ 0.24 ^a
8	22.67 $\pm$ 3.06 ^a	6.00 $\pm$ 2.00 ^a	18.33 $\pm$ 0.58 ^a	1.14 $\pm$ 0.11 ^a
9	21.67 $\pm$ 6.51 ^a	5.33 $\pm$ 2.08 ^a	18.00 $\pm$ 0.00 ^a	0.88 $\pm$ 0.31 ^a

Values are expressed as mean $\pm$ SD ($n = 5$)

Superscript letters (a, b) within the same column indicate statistically significant differences between groups at $p < 0.05$. Means that share the same letter are not significantly different, while means with different letters differ significantly

Group 1: normal control

Group 2: induction, no treatment

Group 3: 100 mg/kg bw crude extract + induction with 2000 mg/kg paracetamol

Group 4: 200 mg/kg bw crude extract + induction with 2000 mg/kg paracetamol

Group 5: 100 mg/kg bw of ethyl acetate fraction + induction with 2000 mg/kg paracetamol

Group 6: 200 mg/kg bw of ethyl acetate fraction + induction with 2000 mg/kg paracetamol

Group 7: 100 mg/kg bw of methanol fraction + induction with 2000 mg/kg paracetamol

Group 8: 200 mg/kg bw of methanol fraction + induction with 2000 mg/kg paracetamol

Group 9: 100 mg/kg bw of silymarin + induction with 2000 mg/kg paracetamol

Table 3 Curative effect of extracts of *Uvaria chamae* leaves on the concentrations of liver proteins in paracetamol-induced hepatotoxicity in rats

Groups	Albumin g/dL	Total protein g/dL	Total bilirubin mg/dL	Dir. bilirubin mg/dL
1	3.27 ± 0.31 ^b	6.77 ± 0.21 ^b	0.88 ± 0.11 ^a	0.25 ± 0.04 ^a
2	4.23 ± 0.28 ^a	5.80 ± 0.32 ^a	1.15 ± 0.15 ^b	0.53 ± 0.12 ^b
3	3.05 ± 0.22 ^b	6.43 ± 0.21 ^b	0.88 ± 0.08 ^a	0.35 ± 0.13 ^a
4	3.39 ± 0.72 ^b	6.49 ± 0.38 ^b	0.91 ± 0.54 ^a	0.22 ± 0.08 ^a
5	3.36 ± 0.70 ^b	6.04 ± 0.49 ^b	0.89 ± 0.41 ^a	0.23 ± 0.09 ^a
6	3.22 ± 0.67 ^b	6.91 ± 0.20 ^b	0.90 ± 0.27 ^a	0.38 ± 0.10 ^a
7	3.39 ± 0.15 ^a	5.39 ± 0.79 ^b	0.84 ± 0.03 ^a	0.24 ± 0.06 ^a
8	3.24 ± 0.28 ^a	5.29 ± 0.13 ^b	0.81 ± 0.06 ^a	0.29 ± 0.07 ^a
9	3.07 ± 0.37 ^b	5.62 ± 0.38 ^b	0.88 ± 0.12 ^a	0.12 ± 0.07 ^a

Values are expressed as mean ± SD (n = 5)

Superscript letters (a, b) within the same column indicate statistically significant differences between groups at $p < 0.05$. Means that share the same letter are not significantly different, while means with different letters differ significantly

exhibited a significant increase ($p < 0.05$) compared to group 2 (induced but untreated). The total bilirubin concentration and direct bilirubin concentration in the treated groups exhibited a significant decrease ($p < 0.05$) compared to group 2 (induced but untreated). Nonetheless, there were no significant ($p > 0.05$) alterations in total protein concentration, serum albumin concentration, total bilirubin concentration, and direct bilirubin concentration in the treated groups as compared to group 1 (normal control) and group 9 (induced and treated with silymarin).

Curative effect of extracts of *Uvaria chamae* leaves on the activities of antioxidant enzymes in paracetamol-induced hepatotoxicity in rats

In Table 4, the curative study, the effects of leaf extract and fractions of *Uvaria chamae* on concentrations/activities of in vivo antioxidant enzymes of

Table 4 Curative effect of extracts of *Uvaria chamae* leaves on the activities of antioxidant enzymes in paracetamol-induced hepatic damage in rats

Groups	MDA mg/mL	GPx U/mg	SOD U/mg	CAT U/mg
1	2.63 ± 0.49 ^a	45.33 ± 2.07 ^b	11.12 ± 0.06 ^b	7.63 ± 0.219 ^b
2	15.82 ± 1.0 ^b	16.01 ± 1.65 ^a	3.47 ± 0.01 ^a	2.51 ± 1.36 ^a
3	4.69 ± 0.26 ^a	48.99 ± 2.11 ^b	12.48 ± 0.01 ^b	7.39 ± 1.13 ^b
4	4.59 ± 0.40 ^a	47.14 ± 2.65 ^b	12.49 ± 0.00 ^b	7.45 ± 1.21 ^b
5	4.61 ± 0.26 ^a	48.82 ± 3.06 ^b	12.49 ± 0.01 ^b	7.57 ± 1.34 ^b
6	4.58 ± 0.34 ^a	48.31 ± 4.28 ^b	12.48 ± 0.002 ^b	7.82 ± 0.60 ^b
7	4.48 ± 0.27 ^a	45.49 ± 3.34 ^b	11.48 ± 0.01 ^b	8.57 ± 1.67 ^b
8	4.48 ± 0.14 ^a	45.64 ± 3.64 ^b	11.49 ± 0.01 ^b	7.14 ± 1.47 ^b
9	4.62 ± 0.11 ^a	45.01 ± 2.72 ^b	11.48 ± 0.001 ^b	7.40 ± 1.88 ^b

Values are expressed as mean ± SD (n = 5)

Superscript letters (a, b) within the same column indicate statistically significant differences between groups at $p < 0.05$. Means that share the same letter are not significantly different, while means with different letters differ significantly

paracetamol-induced liver damage were shown. The MDA levels in the treated groups exhibited a substantial decrease ($p < 0.05$) compared to group 2 (induced but untreated). The treated groups exhibited a significant increase ($p < 0.05$) in glutathione peroxidase activity, superoxide dismutase activity, and catalase activity compared to group 2 (induced but untreated). Furthermore, there were no statistically significant ($p > 0.05$) alterations in MDA concentration, glutathione peroxidase activity, superoxide dismutase activity, and catalase activity in the treated groups compared to group 1 (normal control) and group 9 (induced and treated with silymarin).

Curative effect of extracts of *Uvaria chamae* leaves on the lipid profile in paracetamol-induced hepatic damage in rats

Table 5 presents the curative study, illustrating the impact of *Uvaria chamae* leaf extracts and fractions on the lipid profile concentrations in paracetamol-induced liver injury. The treated groups exhibited a significant ($p < 0.05$) reduction in total cholesterol, LDL, and TAG concentrations compared to group 2 (induced and untreated). The HDL concentration in the treated groups exhibited a substantial increase ($p < 0.05$) compared to group 2 (induced but untreated). Furthermore, there were no significant ($p > 0.05$) alterations in total cholesterol, LDL, HDL, and TAG concentrations in the treated groups compared to group 1 (normal control) and group 9 (induced and treated with silymarin).

Curative effect of extracts of *Uvaria chamae* leaves on the electrolyte concentrations in paracetamol-induced hepatic damage in rats

Table 6 presents the curative study, illustrating the impact of extracts and fractions of *Uvaria chamae* leaves on

Table 5 Curative effect of extracts of *Uvaria chamae* leaves on the lipid profile in paracetamol-induced hepatic damage in rats

Groups	T. Chol mmol/L	LDL mmol/L	HDL mmol/L	Tag mmol/L
1	4.18 ± 0.53 ^a	1.27 ± 0.38 ^a	6.75 ± 0.22 ^b	0.77 ± 0.29 ^a
2	8.89 ± 0.49 ^b	9.42 ± 0.12 ^b	1.75 ± 0.22 ^a	1.22 ± 0.173 ^b
3	4.19 ± 0.17 ^a	1.77 ± 0.18 ^a	5.77 ± 0.15 ^b	0.75 ± 0.24 ^a
4	3.71 ± 0.55 ^a	1.75 ± 0.11 ^a	6.73 ± 0.15 ^b	0.76 ± 0.27 ^a
5	3.85 ± 0.50 ^a	1.77 ± 0.27 ^a	6.77 ± 0.35 ^b	0.72 ± 0.02 ^a
6	3.09 ± 0.12 ^a	1.77 ± 0.30 ^a	5.83 ± 0.25 ^b	0.76 ± 0.25 ^a
7	4.03 ± 0.33 ^a	2.21 ± 0.08 ^a	6.30 ± 0.10 ^b	0.81 ± 0.23 ^{ab}
8	3.75 ± 0.44 ^a	2.92 ± 0.20 ^a	7.40 ± 0.26 ^b	0.95 ± 0.287 ^{ab}
9	3.70 ± 0.61 ^a	1.06 ± 0.05 ^a	6.77 ± 0.15 ^b	0.64 ± 0.151 ^a

Values are expressed as mean ± SD (n = 5)

Superscript letters (a, b) within the same column indicate statistically significant differences between groups at $p < 0.05$. Means that share the same letter are not significantly different, while means with different letters differ significantly

Table 6 Curative effect of extracts of *Uvaria chamae* leaves on the electrolyte concentrations in paracetamol-induced hepatic damage in rats

Groups	Na ⁺ mmol/L	K ⁺ mmol/L	CL ⁻ mmol/L	HCO ₃ ⁻ mmol/L
1	134.67 ± 18.58 ^a	2.60 ± 0.400 ^b	106.67 ± 4.16 ^a	26.00 ± 1.00 ^{abc}
2	171.50 ± 6.00 ^b	3.19 ± 0.66 ^a	138.00 ± 1.00 ^b	28.00 ± 1.73 ^{cd}
3	136.77 ± 3.30 ^a	4.31 ± 0.79 ^b	106.00 ± 5.20 ^a	27.33 ± 1.15 ^{bcd}
4	133.43 ± 18.76 ^a	4.27 ± 0.50 ^b	106.33 ± 0.58 ^a	24.67 ± 0.58 ^a
5	133.30 ± 30.98 ^a	4.61 ± 0.18 ^b	107.00 ± 1.00 ^a	27.00 ± 1.00 ^{bcd}
6	135.00 ± 13.97 ^a	4.54 ± 0.18 ^b	106.33 ± 0.58 ^a	27.33 ± 1.53 ^{bcd}
7	139.03 ± 5.00 ^a	3.08 ± 0.17 ^b	107.33 ± 0.58 ^a	27.33 ± 1.15 ^{bcd}
8	132.47 ± 14.13 ^a	3.03 ± 0.54 ^b	108.00 ± 1.00 ^a	27.33 ± 1.16 ^{bcd}
9	130.87 ± 2.797 ^a	4.87 ± 0.34 ^b	105.67 ± 0.58 ^a	25.67 ± 0.58 ^{ab}

Values are expressed as mean ± SD (n = 5)

Indices within the same column with different superscript are significantly different

blood electrolyte concentrations in paracetamol-induced liver injury.

The salt and chloride concentrations in the treated groups exhibited a substantial decrease ($p < 0.05$) compared to group 2 (induced but untreated). The potassium concentration in the treated groups exhibited a substantial rise ($p < 0.05$) compared to group 2 (induced but untreated). Furthermore, there were no significant ($p > 0.05$) alterations in sodium, potassium, and chloride concentrations in the treated groups compared to group 1 (normal control) and group 9 (induced and treated with silymarin). Furthermore, there was no significant ($p > 0.05$) alteration in bicarbonate concentration as compared to all the controls.

Curative effect of extracts of *Uvaria chamae* leaves on the concentrations of kidney proteins in paracetamol-induced hepatic damage in rat

Table 7 presents the impact of extracts and fractions of *Uvaria chamae* leaves on blood renal function indicators in the context of paracetamol-induced liver damage. The urea and creatinine concentrations in the treated groups exhibited a substantial decrease ($p < 0.05$) compared to group 2 (induced but untreated). Furthermore, there were no significant ($p > 0.05$) alterations in urea and creatinine concentrations in the treated groups compared to group 1 (normal control) and group 9 (induced and treated with silymarin).

Curative effects on random blood glucose levels in paracetamol-induced liver damage following treatment with crude extract, ethyl acetate fraction, methanol fraction of *Uvaria chamae* leaves, and silymarin

Table 8 presents the effect on concentrations of random blood sugar and changes in weight by crude extract, ethyl acetate fraction, methanol fraction of *Uvaria chamae* leaves, and silymarin on paracetamol-induced liver damage.

There was no significant ($p > 0.05$) change in RBS in the preventive and curative studies when compared with all the controls. A significant reduction ($p < 0.05$) in final weight was observed in all treated groups compared to group 2 (induced, untreated). No significant change ($p > 0.05$) in final weight was observed in all treated groups compared to group 1 (normal control) and group 9 (induced and treated with silymarin).

Table 7 Curative effect of extracts of *Uvaria chamae* leaves on the concentrations of kidney proteins in paracetamol-induced hepatic damage in rat

Groups	Urea mg/dL	Creatinine mg/dL
1	56.00 ± 7.21 ^a	1.70 ± 0.44 ^a
2	98.90 ± 6.26 ^b	3.49 ± 0.22 ^b
3	65.23 ± 6.48 ^a	1.38 ± 0.26 ^a
4	66.40 ± 5.77 ^a	1.38 ± 0.21 ^a
5	64.23 ± 5.22 ^a	1.32 ± 0.19 ^a
6	64.20 ± 5.650 ^a	1.37 ± 0.21 ^a
7	64.57 ± 6.21 ^a	1.40 ± 0.35 ^a
8	67.63 ± 7.66 ^a	1.44 ± 0.36 ^a
9	60.47 ± 8.84 ^a	1.44 ± 0.31 ^a

Values are expressed as mean ± SD (n = 5)

Superscript letters (a, b) within the same column indicate statistically significant differences between groups at $p < 0.05$. Means that share the same letter are not significantly different, while means with different letters differ significantly

Table 8 Effect on random blood sugar and weight changes by crude extract, ethyl acetate fraction, methanol fraction of *Uvaria chamae* leaves, and silymarin on paracetamol-induced liver damage

Groups	Final RBS (mg/dL)	Initial weight (g)	Final weight (g)
1	67.40 ± 5.13 ^a	86.00 ± 9.31 ^a	78.00 ± 6.48 ^a
2	66.40 ± 9.24 ^a	90.4 ± 25.65 ^a	119.2 ± 21.67 ^b
3	68.25 ± 5.12 ^a	87.2 ± 16.47 ^a	86.8 ± 18.99 ^a
4	69.20 ± 6.87 ^a	86.8 ± 24.45 ^a	79.4 ± 25.45 ^a
5	67.40 ± 3.85 ^a	86.2 ± 15.88 ^a	81.8 ± 17.54 ^a
6	67.25 ± 18.08 ^a	87.2 ± 12.22 ^a	82.3 ± 7.23 ^a
7	61.00 ± 9.89 ^a	89.2 ± 51.84 ^a	85.75 ± 29.55 ^a
8	67.20 ± 9.76 ^a	84.2 ± 20.07 ^a	80 ± 14.74 ^a
9	64.80 ± 12.39 ^a	85.00 ± 7.58 ^a	85.8 ± 37.51 ^a

Values are expressed as mean ± SD (n = 5)
Superscript letters (a, b) within the same column indicate statistically significant differences between groups at *p* < 0.05. Means that share the same letter are not significantly different, while means with different letters differ significantly

Photomicrograph of liver sections showing the curative effect of methanol extracts of *Uvaria chamae* leaves on paracetamol-induced hepatic injury in rats (groups 1 to 9)

As in Plate 1, the photomicrograph of liver sections showed the following results.
Plate 2 shows mild hepatic damage with cellular vacuolation. All other liver photomicrographs across groups 1 and 3 to 9 demonstrated normal histological architecture. The hepatocyte plates appeared intact, and no lesions or pathological alterations were observed in any group. Overall, the liver tissues maintained normal cellular morphology, indicating preserved structural integrity throughout all experimental groups.

Discussion

The utilisation of botanical substances for disease treatment is a prevalent practice not only in impoverished African nations such as Nigeria but across the globe. In

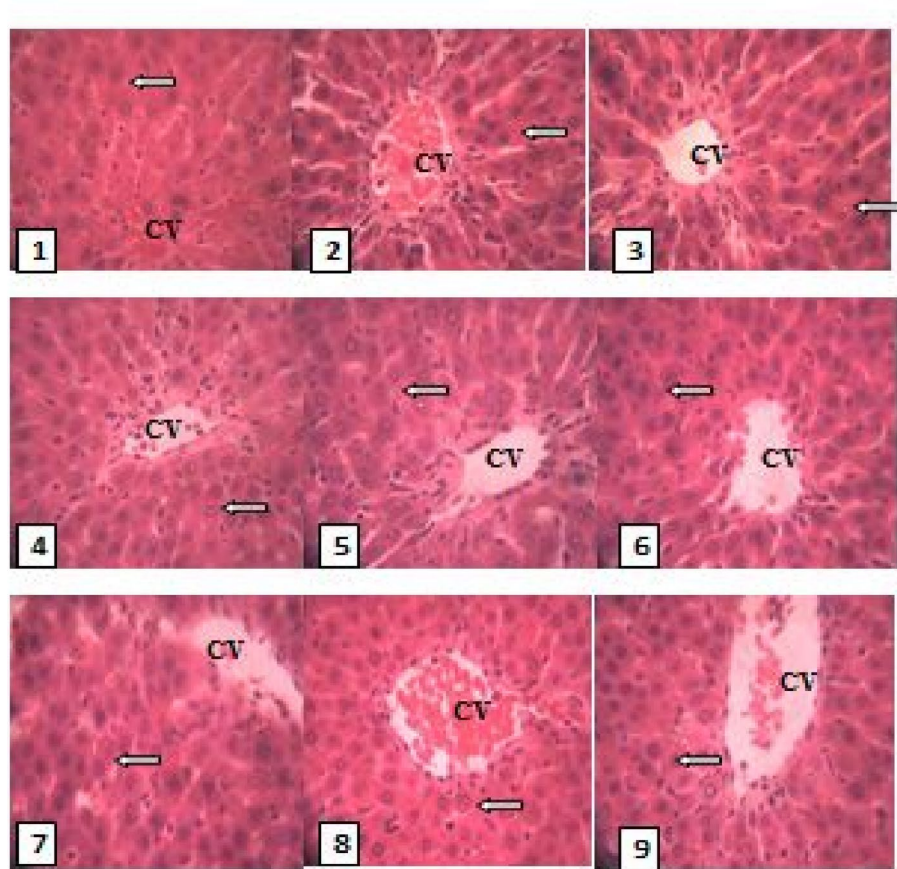


Plate 1 Photomicrograph of liver sections showing the ameliorative effect of methanol extracts of *Uvaria chamae* leaves on paracetamol-induced hepatic injury in rats (groups 1 to 9)

this study, the acute toxicity profile of *Uvaria chamae* leaf extract demonstrated no evidence of morbidity or mortality in mice up to 5000 mg/kg body weight (Table 1). This finding corroborates the earlier reports by Olumese et al. [31], who established that the LD₅₀ of the methanol extract exceeded 2000 mg/kg body weight, and aligns with the ameliorative observations of Enabulele and Ifeka [32], who employed OECD guidelines. The absence of acute toxicity at such high concentrations significantly enhances the therapeutic relevance and safety considerations of *U. chamae* as a potential hepatoprotective agent.

Table 2 illustrates the extract's modulatory effects on liver enzyme markers following paracetamol-induced hepatic injury. The significant reductions ($p < 0.05$) in AST, ALT, ALP, and GGT activities in all treated groups relative to group 2 (induced, untreated) suggest substantial amelioration of hepatocellular damage. These biochemical improvements are consistent with previously reported hepatoprotective effects of other medicinal plants, such as *Agave americana* [33, 34], indicating that phytochemical constituents can effectively mitigate xenobiotic-induced hepatic injury. The mechanisms by which plant extracts protect the liver are multifaceted and may include antioxidant defence, enhancement of detoxification pathways, suppression of inflammation, promotion of hepatocyte repair, antifibrotic activity, and modulation of the gastrointestinal–hepatic axis. Accordingly, several bioactive constituents within *U. chamae* likely contributed to the restoration of hepatic metabolic and detoxification functions.

Perturbations in protein metabolism, particularly reductions in serum albumin and total protein levels, are hallmark features of hepatic insufficiency. Table 5 shows that treatment with *U. chamae* extract and its fractions significantly increased ($p < 0.05$) serum albumin and total protein concentrations relative to the induced, untreated group, underscoring improved synthetic liver function. Similarly, significant reductions in total and direct bilirubin levels reflect enhanced hepatic clearance capacity and reduced hepatocellular compromise. These changes further consolidate the extract's therapeutic potential in restoring normal physiological processes disrupted by paracetamol-induced toxicity.

Lipid peroxidation, indicated by elevated malondialdehyde (MDA), represents a central mechanism of oxidative injury in hepatotoxic states. In contrast, antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, and catalase constitute essential defences against reactive oxygen species (ROS) [35]. Table 6 shows that *U. chamae* extract and its fractions significantly reduced MDA levels while simultaneously enhancing antioxidant enzyme activities ($p < 0.05$), thereby demonstrating potent antioxidative efficacy. These findings not

only validate the extract's capacity to counteract oxidative hepatic injury but also point toward deeper molecular mechanisms that may underpin its hepatoprotective properties.

A growing body of evidence indicates that *Uvaria chamae* exerts its antioxidative action, at least in part, through activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway. Nrf2 regulates a suite of cytoprotective genes, including phase II detoxification enzymes and endogenous antioxidants. Recent in vitro studies using AREc32 luciferase reporter assays demonstrated that *U. chamae* root extract induces approximately a four-fold increase in Nrf2 activity, confirming its robust stimulation of the Nrf2–ARE axis [36]. Such activation enhances glutathione synthesis, elevates detoxification enzyme levels, and reinforces oxidative defence networks—mechanisms highly relevant in mitigating paracetamol-induced hepatotoxicity. This observation is further consistent with established literature showing that botanical polyphenols frequently activate Nrf2 to confer antioxidative and anti-inflammatory protection [8, 37].

In addition to antioxidant effects, *U. chamae* modulates inflammatory signalling. Popoola et al. reported that the extract significantly inhibited LPS-induced nitric oxide (NO) production in RAW 264.7 macrophages, highlighting notable anti-inflammatory capacity. The dual modulation of oxidative stress and inflammation is mechanistically coherent, as Nrf2 activation has been shown to suppress NF- κ B signalling and downstream inflammatory mediators. Therefore, the improvements in hepatic indices observed in this study may reflect a synergistic interaction between antioxidant and anti-inflammatory pathways.

Although CYP450 activity was not directly assessed in the present study, emerging phytochemical and pharmacological evidence suggests that *U. chamae* constituents may modulate cytochrome P450 enzyme systems. This possibility is biologically plausible for several reasons: 1. *U. chamae* contains flavonoids, phenolic acids, and alkaloids, phytochemical classes well documented to interact with CYP isoforms, including CYP2E1—the enzyme responsible for converting paracetamol into its toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). 2. Several structurally related plant extracts have demonstrated the capacity to inhibit CYP2E1 activity, thereby reducing the bioactivation of hepatotoxic compounds. For instance, a hepatoprotective fraction from *Entada africana* inhibited CYP2E1 while simultaneously enhancing Nrf2-regulated antioxidant responses, leading to substantial mitigation of oxidative liver damage [38]. 3. Given the extract's observed hepatoprotective efficacy in the present study, CYP modulation may constitute an

additional mechanistic layer, either by limiting NAPQI formation or attenuating downstream oxidative stress. Future studies are warranted to confirm whether *U. chamae* directly modulates CYP450 isoforms, using microsomal enzyme assays, qPCR, or proteomic analyses. Such investigations would be critical for establishing herb-drug interaction profiles, especially for patients taking medications metabolised via CYP pathways.

Significant reductions ($p < 0.05$) in cholesterol, triacylglycerol, and low-density lipoprotein in treated groups (relative to group 2) further underscore the extract's capacity to restore metabolic homeostasis, consistent with reports that hepatoprotective botanicals ameliorate pathological hypercholesterolaemia [39]. Table 6 shows that serum electrolyte concentrations were similarly improved, with sodium and chloride significantly decreased ($p < 0.05$) and potassium significantly elevated ($p < 0.05$), suggesting partial restoration of renal–hepatic functional integration following injury. The unchanged bicarbonate levels ($p > 0.05$) indicate that acid–base balance remained stable [40]. Table 7 further demonstrates significant reductions in serum urea and creatinine, suggesting improved renal function secondary to hepatic recovery. The extract did not significantly influence glucose metabolism, as evidenced by stable random blood sugar levels (Table 8). The observed weight reductions in treated animals (Table 8) may reflect improved metabolic equilibrium without deviation from homeostatic norms, consistent with non-toxic, physiological restoration. Histological observations (Plate 1) confirmed marked restoration of hepatocyte architecture, validating the biochemical and molecular evidence for the extract's curative efficacy.

Conclusion

Collectively, the findings from this study suggest that *Uvaria chamae* leaf extract confers hepatoprotection through a combination of antioxidative, anti-inflammatory, and potentially xenobiotic-modulating mechanisms. The integration of Nrf2 activation, reductions in oxidative stress, improved hepatic synthetic and metabolic functions, and possible modulation of CYP450-mediated toxin formation underscore the extract's multifaceted therapeutic potential. These results provide compelling evidence for the extract's potential as a natural remedy for hepatotoxicity and warrant further investigation into its bioactive compounds and mechanisms of action. Further mechanistic studies, particularly those assessing CYP enzyme activity and Nrf2-target gene expression, are essential to deepen the understanding of *U. chamae*'s hepatoprotective pathways and to evaluate its suitability for clinical translation.

Study limitations

Lack of direct mechanistic confirmation.

However, although the results indicate that antioxidant, anti-inflammatory, and perhaps Nrf2- and CYP450-mediated pathways mediate the hepatoprotective action of *Uvaria chamae*, the pathways were not directly evaluated through molecular or enzymatic methods. Therefore, mechanistic inferences are indirect.

One-time, one model, experimental design.

The researchers aimed at examining acute hepatotoxicity of paracetamol in one rodent model. This restricts the extrapolation to chronic liver damage, other hepatotoxic agents, and prolonged therapeutic application in humans.

Authors' contributions

Author CUV conceptualized the study, wrote the protocol, and wrote the draft of the manuscript. Author PEJ designed the study, wrote the protocol, carried out the formal analysis of the study and reviewed the manuscript. Author OCE managed the literature searches, carried out investigation and wrote draft of the manuscript. Author ESI designed the study, managed the laboratory analysis of the study and reviewed/edited the draft manuscript. Author AOE managed the literature searches, carried out laboratory investigation and wrote the draft of the manuscript. All authors read and approved the final manuscript for publication.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was considered and approved by the Faculty of Biological Science Research Ethics Committee with the approval number UNN/FBS/EC/1092. Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Ayoub SS (2021) Paracetamol (acetaminophen): a familiar drug with an unexplained mechanism of action. *Temperature* 8(4):351–371. <https://doi.org/10.1080/23328940.2021.1886392>

2. Els JR, Taljaard L, Strover B (2020) Delayed presentation of paracetamol overdose. *Afr J Emerg Med* 10(3):170–172. <https://doi.org/10.1016/j.afjem.2020.02.003>
3. Mazzeuskaya LL, Sangkuhl K, Thorn CF, FitzGerald GA, Altman RB, Klein TE (2015) PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. *Pharmacogenet Genomics* 25(8):416–426. <https://doi.org/10.1097/FPC.0000000000000150>
4. Pacifici GM, Allegaert K (2015) Clinical pharmacology of paracetamol in neonates: a review. *Curr Ther Res* 77:24–30. <https://doi.org/10.1016/j.curtheres.2014.12.001>
5. Offor SJ, Amadi CN, Chijioke-Nwauche I, Manautou JE, Orisakwe OE (2022) Potential deleterious effects of paracetamol dose regime used in Nigeria versus that of the United States of America. *Toxicol Rep* 9:1035–1044. <https://doi.org/10.1016/j.toxrep.2022.04.025>
6. Behrends V, Giskeødegård GF, Bravo-Santano N et al (2019) Acetaminophen cytotoxicity in HepG2 cells is associated with a decoupling of glycolysis from the TCA cycle, loss of NADPH production, and suppression of anabolism. *Arch Toxicol* 93:341–353. <https://doi.org/10.1007/s00204-018-2371-0>
7. Villanueva-Paz M, Morán L, López-Alcántara N et al (2021) Oxidative stress in drug-induced liver injury (DILI): from mechanisms to biomarkers for use in clinical practice. *Antioxidants* 10(3):390. <https://doi.org/10.3390/antiox10030390>
8. Vairetti M, Di Pasqua LG, Cagna M, Richelmi P, Ferrigno A, Berardo C (2021) Changes in glutathione content in liver diseases: an update. *Antioxidants* 10(3):364. <https://doi.org/10.3390/antiox10030364>
9. Cairney DG, Beckwith HK, Al-Hourani K et al (2016) Plasma paracetamol concentration at hospital presentation has a dose-dependent relationship with liver injury despite prompt treatment with intravenous acetylcysteine. *Clin Toxicol (Phila)* 54(5):405–410. <https://doi.org/10.3109/15563650.2016.1159309>
10. Emordi JE, Agbaje EO, Oreagba IA, Iribhogbe OI (2018) Antidiabetic effects of the ethanolic root extract of *Uvaria chamae* in alloxan-induced diabetic rats. *Adv Pharmacol Sci* 2018:1314941. <https://doi.org/10.1155/2018/1314941>
11. Emordi JE, Agbaje EO, Oreagba IA, Iribhogbe OI (2016) Antidiabetic and hypolipidemic activities of hydroethanolic root extract of *Uvaria chamae* in streptozotocin-induced diabetic albino rats. *BMC Complement Altern Med* 16(1):468. <https://doi.org/10.1186/s12906-016-1450-0>
12. Adepiti AO, Iwalewa EO (2016) Evaluation of the combination of *Uvaria chamae* and amodiaquine in murine malaria. *J Ethnopharmacol* 193:30–35. <https://doi.org/10.1016/j.jep.2016.07.035>
13. Thomas PS, Essien EE (2020) Antiglycation, antioxidant, and cytotoxic activities of *Uvaria chamae* root and essential oil composition. *Nat Prod Res* 34(6):880–883. <https://doi.org/10.1080/14786419.2018.1504048>
14. Lorke D (1983) A new approach to practical acute toxicity testing. *Arch Toxicol* 54(4):275–287. <https://doi.org/10.1007/BF01234480>
15. Rotundo L, Pyrsopoulos N (2020) Liver injury induced by paracetamol and challenges associated with intentional and unintentional use. *World J Hepatol* 12(4):125–136. <https://doi.org/10.4254/wjh.v12.i4.125>
16. Reitman S, Frankel S (1957) A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 28(1):56–63. <https://doi.org/10.1093/ajcp/28.1.56>
17. Klein B, Read PA, Babson AL (1960) Rapid method for the quantitative determination of serum alkaline phosphatase. *Clin Chem* 6:269–275
18. Tietz NW (1976) Fundamentals of clinical chemistry. WB Saunders, Philadelphia, p 897
19. Lubran MM (1978) The measurement of total serum proteins by the biuret method. *Ann Clin Lab Sci* 8(2):106–110
20. Fawcett JK, Scott JE (1960) A rapid and precise method for the determination of urea. *J Clin Pathol* 13(2):156–159. <https://doi.org/10.1136/jcp.13.2.156>
21. Jendrassik L, Grof P (1938) Simplified photometric methods for the determination of bilirubin. *Biochem J* 297:81–89
22. Fridovich I (1989) Superoxide dismutases: an adaptation to a paramagnetic gas. *J Biol Chem* 264(14):7761–7764
23. Catalase AH (1983). In: Bergmeyer HU (ed) Methods of enzymatic analysis, 3rd edn. Verlag Chemie, Weinheim, pp 273–286
24. Paglia DE, Valentine WN (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 70:158–169
25. Varshney R, Kale RK (1990) Effects of calmodulin antagonists on radiation-induced lipid peroxidation in microsomes. *Int J Radiat Biol* 58:733–743
26. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of serum total cholesterol. *Clin Chem* 20:470–475
27. Assmann G, Jabs HU, Kohnert U, Nolte W, Schriewer H (1984) Determination of low-density lipoprotein cholesterol. *Clin Chim Acta* 140:77–83
28. Albers JJ, Warnick GR, Cheng MC (1978) Determination of high-density lipoprotein cholesterol. *Lipids* 13:926–932
29. Tietz NW (1994) Tietz textbook of clinical chemistry, 2nd ed. WB Saunders, Philadelphia, p 751
30. Szunyogova E, Parson SH. Histological and Histochemical Methods, Theory and Practice, 5th Ed. *J Anat.* 2016;228(5):887. <https://doi.org/10.1111/joa.12390>
31. Olumese FE, Omoagbe GI, Eze GI, Omoruyi FO (2016) Safety assessment of *Uvaria chamae* root extract: acute and subchronic toxicity studies. *J Afr Assoc Physiol Sci* 4(1):53–60
32. Enabulele OI, Ifeka LJ (2022) Immunomodulatory and antimicrobial activities of methanol extract of *Uvaria chamae* leaves in rats. *Bayero J Pure Appl Sci* 15(1):271–276
33. Ayenew KO, Wasihun Y (2023) Hepatoprotective effect of methanol extract of *Agave americana* leaves on paracetamol-induced hepatotoxicity in Wistar albino rats. *BMC Complement Med Ther* 23(1):99
34. Zerbato V, Sanson G, Deluca M et al (2022) The impact of serum albumin levels on COVID-19 mortality. *Infect Dis Rep* 14(3):278–286
35. Jomova K, Alomar SY, Alwasel SH et al (2024) Several lines of antioxidant defense against oxidative stress. *Arch Toxicol* 98(5):1323–1367. <https://doi.org/10.1007/s00204-024-03696-4>
36. Popoola TD, Guetchueng ST, Ritchie KJ et al (2021) Potent Nrf2-inducing, antioxidant, and anti-inflammatory effects of *Uvaria chamae*. *BMC Complement Med Ther* 21(1):234. <https://doi.org/10.1186/s12906-021-03404-0>
37. Niziński P, Krajewska A, Oniszczuk T et al (2025) Hepatoprotective effect of kaempferol: a review. *Molecules* 30(9):1913. <https://doi.org/10.3390/molecules30091913>
38. Kouam AF, Owona BA, Fifen R et al (2020) Inhibition of CYP2E1 and activation of Nrf2 signaling by *Entada africana*. *Heliyon* 6(8):e04602. <https://doi.org/10.1016/j.heliyon.2020.e04602>
39. Venmathi Maran BA, Iqbal M, Gangadaran P et al (2022) Hepatoprotective potential of Malaysian medicinal plants. *Molecules* 27(5):1533. <https://doi.org/10.3390/molecules27051533>
40. Jung B, Martinez M, Claessens YE et al (2019) Diagnosis and management of metabolic acidosis: guidelines from a French expert panel. *Ann Intensive Care* 9(1):92. <https://doi.org/10.1186/s13613-019-0563-2>

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