

Original Research Article

Subchronic toxicity study of ethanolic extract of *Uvaria chamae* root in rats

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Abstract

Purpose: To assess the safety of ethanol extract of *Uvaria chamae* root consumption for the treatment of diseases in traditional medicine.

Methods: *Uvaria chamae* was extracted with ethanol, and administered orally to rats at doses ranging from 200 to 5000 mg/kg/day for 28 days while distilled water was administered to the control group, the survived animals (43 %), were then euthanized, and blood collected for biochemical and haematological markers. Histopathological examination of the pancreas, liver and kidney sections were also done.

Results: There was a significant ($p < 0.05$) elevation in serum AST and a significant ($p < 0.05$) reduction in LDH at 500 mg and 200 mg respectively. Serum BUN was significantly ($p < 0.05$) reduced, while chloride and potassium ions were significantly ($p < 0.05$) elevated at 200 and 500 mg respectively. However, there was a significant ($p < 0.05$) decrease in mean corpuscular haemoglobin (MCHC) at 200 mg, and a significant ($p < 0.05$) increase in platelets at 500 mg/kg of extract. Examination of haematoxylin and eosin stained sections of pancreas showed well-formed islets; mild portal vascular congestion in liver sections; and periportal and interstitial infiltrates of lymphocytes in the liver and kidney sections of the surviving animals respectively.

Conclusion: The consumption of extract at low doses may not be harmful.

Keywords: *Uvaria chamae*, Safety, Kidney, Liver, Pancreas, Toxicity

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INTRODUCTION

Uvaria chamae (*P. Beauv*) belongs to the family of *Annonaceae*. It is a climbing large shrub or small tree native to the tropical rain forest of West and Central Africa where it grows in wet and coastal shrub lands [1]. It is extensively branched with sweet, aromatic and alternate leaves commonly used to cure diseases like

piles, epistaxis, menorrhagia, hematuria and heal injuries [2]. It is a common ingredient for the preparation of *Agbo* in Lagos, Nigeria chiefly for febrile conditions in children. In folk medicine it is used to treat diabetes, gonorrhoea and respiratory infections. It is also used to make pomade in Ghana and for severe abdominal pain and dysentery. In Sierra Leone, it is used to treat yellow fever, jaundice and cough, while in

Senegal, it is used for treating renal and infantile rickets [2].

Some *U. chamae* users commonly cut the root into small pieces and soak in a bottle containing local gin (alcohol) and drink a small glass full (about 30 ml) daily. Previous studies have reported antifungal, bacteriostatic and antimalaria properties of *Uvaria chamae* preparations [4-6]. The chemical composition of *U. chamae* root include C-benzylated monoterpenes, dihydrochalcones and aromatic oils [7], flavanones and C-benzylated flavanones [8].

Although, the acute and subchronic toxicity studies of aqueous extract of *Uvaria chamae* had earlier been reported [9], herbal practitioners also use ethanol extract to treat various diseases, hence this study was designed to ascertain the safety of ethanol extract of this medicinal plant.

EXPERIMENTAL

Plant material and extraction

Fresh root pieces of wild *Uvaria chamae* grown in Igwo, Oyo state of Nigeria were purchased from the herbal market, at Oyingbo, in Lagos, Nigeria. Root pieces of the plant were identified and authenticated by Dr. Henry Akinnibosun, a taxonomist, at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria with a voucher number UBH 266, and deposited at the Herbarium [9]. The root sample was pulverized and then soaked in absolute ethanol for 72 hours with intermittent stirring to allow for percolation and maceration [10]. It was then rotary evaporated, and freeze dried to obtain 139 g from 3312 g of the plant root and stored in the freezer at 4 °C until used.

Animals

Sprague Dawley rats were purchased from the Department of Anatomy, University of Benin, Benin City, Nigeria. The rats [average body weight 190.49 ± 2.86 g] were housed in clean cages under standard laboratory conditions of temperature, humidity and light at the animal house, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City and had free access to standard laboratory diet and distilled water for a period of two weeks for acclimatization. Ethical approval for the study was obtained from the Ethical Board of the Department of Biochemistry, University of Benin, Benin City.

Subchronic oral toxicity

Forty-two (42) Sprague Dawley rats were used for this study and grouped per sex and weight (average body weight 190.49 ± 2.86 g) into seven groups as follows: group one (Normal rats + distilled water), group two (Normal rats + 200 mg/kg of extract), group three (Normal rats + 500 mg/kg of extract), group four (Normal rats + 1000 mg/kg of extract), group 5 (Normal rats + 2000 mg/kg body weight of extract), group six (Normal rats + 3000 mg/kg body weight of extract) and group seven (Normal rats + 5000 mg/kg of extract). Both control and test groups had six (6) rats per group.

Test animals were administered doses of medicinal plant extract ranging from 200 mg/kg body weight to 5000 mg/kg. Control rats were given distilled water. Animals in all the groups were fasted overnight and observed for 10-20 min before the administration of extract. *Uvaria chamae* extract was administered using an oral gastric gavage once a day (between 8.00 a.m. – 9.00 a.m.) for 28 days. The animals were observed for signs of toxicity and mortality daily throughout the experimental period. The animals administered ethanol root extract at 1000, 2000, 3000 and 5000 mg/kg did not survive up to the 28th day of study. However, daily oral administration of ethanolic *Uvaria chamae* root extract at 200 or 500 mg/kg body weight for 28 days did not induce any obvious symptoms of toxicity and mortality. On the 28th day, animals in groups 1 – 3 were fasted overnight, euthanized by decapitation and blood collected in appropriate tubes for biochemical and haematological assessments. Organs (pancreas, liver and kidney) were collected for histopathological evaluation.

Blood analysis

Serum lactate dehydrogenase (LDH), total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), amylase, blood urea nitrogen (BUN), electrolyte and creatinine were determined using Vitros 350 System Chemistry Ortho-Clinical Diagnostics [New Jersey, USA.IHVN/LAB/1001924]. White blood cell count, lymphocyte, monocytes, granulocytes, red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelets were determined using PCE-210 Haematological Analyzer [Full Automatic Blood Cell Counter-Model PCE-210 N Erma Inc., Tokyo, Japan].

Histopathological examination

The organs (pancreas, liver and kidney) were harvested and immediately submerged in 10 % buffered formalin for 72 hours. The tissues were subsequently processed, embedded in paraffin, sectioned (4 μm) and stained with haematoxylin and eosin (H & E) [11] prior to microscopic examination.

Statistical analysis

The biochemical and haematological data were expressed as mean ± standard error of the mean (SEM). The difference between the groups was tested using ANOVA. Duncan's multiple range test was used to test for significant difference among the means ($p < 0.05$).

RESULTS

Biochemical, haematological and histopathological profiles

Total protein and albumin levels were not significantly ($p > 0.05$) altered in the groups treated with ethanolic extract when compared to control (Figure 1). There was a significant ($p < 0.05$) decrease in serum LDH activity in the animals administered 200 mg ethanolic extract (Figure 2). Serum AST activity was significantly ($p < 0.05$) increased in the rats administered 500 mg of ethanolic extract when compared to the control group (Figure 2). The administration of ethanolic extract to the animals did not significantly ($p > 0.05$) alter serum amylase activity among the groups (Figure 3).

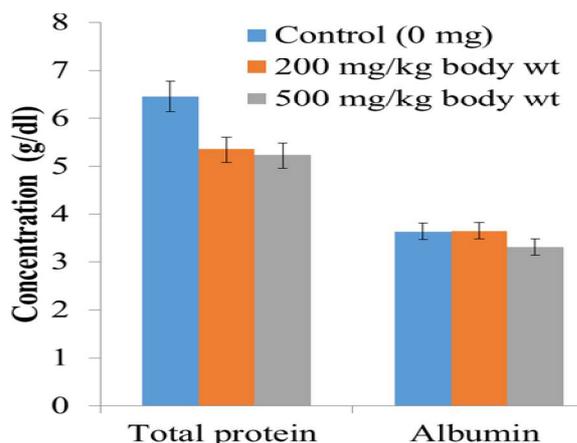


Figure 1: Effect of oral administration of 200 and 500 mg/kg body weight of ethanol extract of *U. chamae* on serum proteins in Sprague Dawley rats. Serum concentrations of total protein and albumin in the liver were expressed as mean ± SEM. Values were not significantly different among the groups ($p > 0.05$).

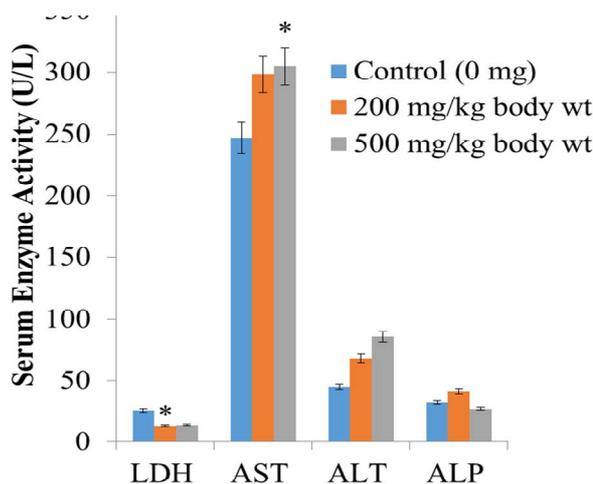


Figure 2: Effect of oral administration of 200 and 500 mg/kg body weight of ethanolic extract of medicinal plant on serum liver enzymes in Sprague Dawley rats. Serum concentrations of LDH, AST, ALT and ALP in the liver were expressed as mean ± SEM; * $p < 0.05$ is significant compared to control

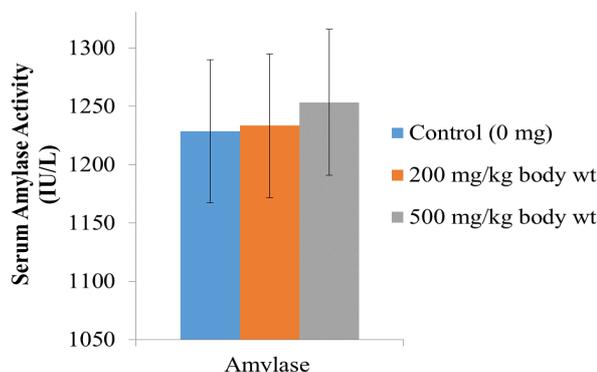


Figure 3: Effect of oral administration of 200 and 500 mg/kg body weight of ethanolic extract of *Uvaria chamae* on serum amylase in Sprague Dawley rats; serum concentration of amylase was expressed as mean ± SEM. Values were not significantly different among the groups ($p > 0.05$)

However, K^+ and Cl^- ions were significantly ($p < 0.05$) increased by the administration of 200 or 500 mg/kg of ethanolic extract compared to the control group (Figure 4). Serum BUN level was significantly ($p < 0.05$) reduced by the administration of 200 or 500 mg/kg of ethanolic extract compared to control (Figure 5). There was a significant ($p < 0.05$) increase in the platelets of rats treated with 500 mg of the extract compared to control (Figure 6). The red cell indices were not significantly ($p > 0.05$) altered in the treatment groups versus the control group (Figure 7). There was a significant ($p < 0.05$) decrease in MCHC levels when compared to control group at 200 mg of the extract administration (Figure 7).

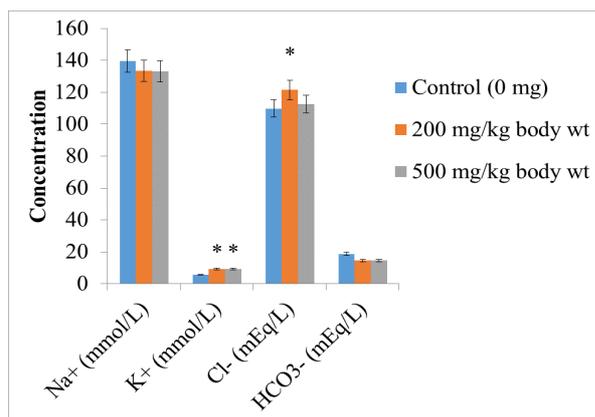


Figure 4: Effect of oral administration of 200 and 500 mg/kg body weight of ethanolic extract of *U. chamae* on serum electrolytes in Sprague Dawley rats. Serum concentrations of Na⁺, K⁺, Cl⁻ and HCO₃⁻ were expressed as mean ± SEM; *p < 0.05 is significant compared to control

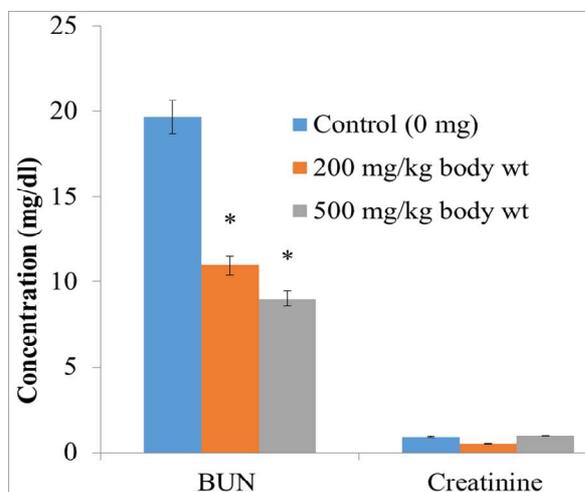


Figure 5: Effect of oral administration of 200 and 500 mg/kg body weight of ethanolic extract of medicinal plant on serum BUN and creatinine in Sprague Dawley rats. Serum concentrations of BUN and creatinine were expressed as mean ± SEM. * P < 0.05 is significant compared to control

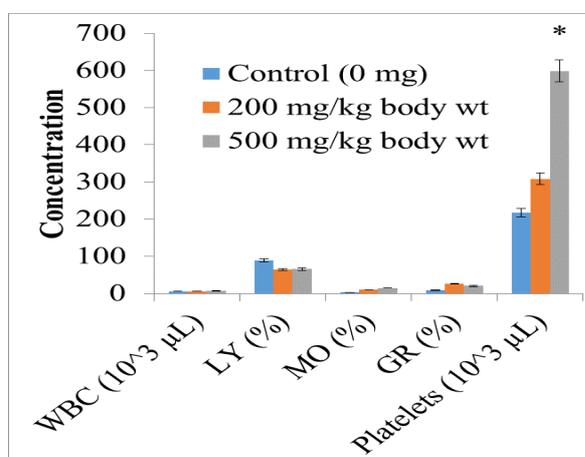


Figure 6: Effect of oral administration of 200 and 500 mg/kg body weight of ethanolic extract of *Uvaria chamae* on white blood cells, platelets, total and differentials in Sprague Dawley rats. The

concentrations of WBC, LY, MO, GR and platelets were expressed as mean ± SEM. * P < 0.05 is significant compared to control.

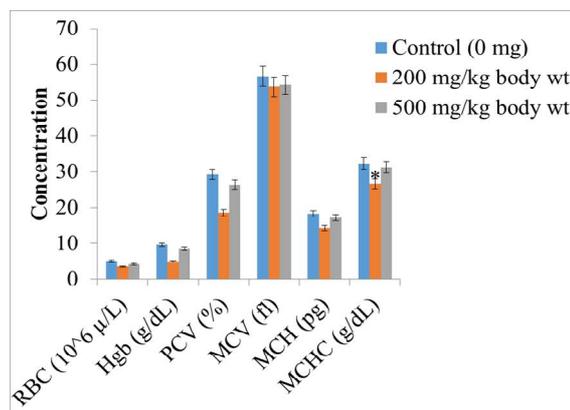


Figure 7: Effect of oral administration of 200 mg and 500 mg/kg body weight of ethanolic extract of *Uvaria chamae* on red cell indices in Sprague Dawley rats. The concentrations of RBC, Hgb, PCV, MCV, MCH and MCHC were expressed as mean ± SEM. * P < 0.05 is significant compared to control.

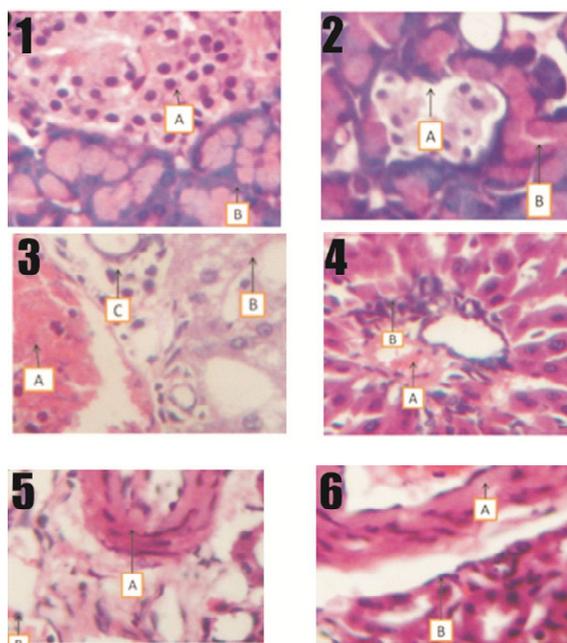


Figure 8: Photomicrographs of rat pancreas, liver and kidney sections stained with H&E after 28 days of treatment with ethanolic extract of *U. chamae*. **Plates 1 and 2** (200 and 500 mg respectively) of rat pancreas showed well-formed normal islets [A], and normal exocrine glands [B]. **Plates 3** (200 mg) of rat liver showed mild vascular congestion [A], normal hepatocyte architecture [B], mild periportal infiltrates of lymphocytes [C], and **Plate 4** (500 mg) of rat liver showed mild portal congestion [A] and periportal infiltrates of lymphocytes [B]. **Plates 5 and 6** (200 mg and 500 mg respectively) of rat kidney showed mild interstitial vascular congestion [A] and mild infiltrates of lymphocytes [B]. Magnification x 400.

DISCUSSION

From this study the administration of ethanolic extract of *Uvaria chamae* did not produce any deleterious effects on the liver or kidney of rats, and the immunity status and pancreatic islets of the treated animals were enhanced. No deaths or clinical abnormalities were noted in the groups treated with lower doses (that is 200 and 500 mg/kg body weight) of ethanolic extract. Mortality of the animals occurred with doses of 1000 mg/kg body weight and above. This observation may be due to the high concentration of active ingredients present in the ethanolic extract of *Uvaria chamae*.

Hepatic impairment may also affect serum protein concentration [13]. Elevated serum AST and ALT levels are commonly used as sensitive markers of possible tissue damage, particularly liver damage [14]. Acute or chronic injury of the liver causes the elevation of enzyme activities in the bloodstream [15,16]. The cellular enzymes ALT and AST are present in low concentrations in serum under normal conditions. Increased synthesis of the enzymes or leakage from damaged cells may elevate enzyme concentrations in serum [13,17]. The observed non-significant changes in ALT and AST activities, and total protein and albumin levels in the rats administered 200 mg/kg of *Uvaria chamae* may be an indication of the absence of deleterious effect on liver function. The observed significant ($p < 0.05$) increase in AST and a corresponding non-significant increase in ALT when compared to control at 500 mg of *Uvaria chamae* extract indicated that the enzyme activities in the blood of animals were dose dependent.

Histopathological examination of the pancreas of rats treated with ethanolic extract, showed well-formed islets and normal exocrine glands. The healing enhancing effect on the pancreatic cells may partly be responsible for the reported hypoglycemic properties of *Uvaria chamae* extract [9].

Creatinine is a good indicator of renal function, since any elevation in the serum level is associated with marked failure of nephron function [9]. The administration of low doses of the extract (≤ 500 mg) did not significantly alter the level of creatinine in the serum, however the observed significant reduction in the level of BUN in the treatment groups is not clear, it is possible that the active ingredients in the doses of extract tested are acting differently. Some drugs have been reported to decrease BUN levels [18]. The characteristic histopathological findings of kidney

were mild interstitial vascular congestion, dilatation and mild infiltrates of lymphocytes in the kidney, which suggests that consumption of ethanolic extract of *Uvaria chamae* at the tested dose of ≤ 500 mg may not have significant adverse effects on renal function.

Hematopoietic system has been reported to be one of the most sensitive targets for toxic substances [19]. Hematological indices can be used to assess the degree of the deleterious effect of a plant extract on blood function of an animal [20]. Moreover, significant changes in the hematological indices have been reported to have higher predictive value for human toxicity when data from animal investigations are extrapolated to human settings and situations [21].

The observed significant increase in platelets at 500 mg of extract may not be indicative of adverse hematological changes, but suggestive of the extract possessing hematopoietic potentials, since the observed alterations were not reflected in the biochemical and histopathological parameters evaluated. A decrease in MCHC may be a failure in blood osmoregulation or plasma osmolarity [22], however in this study the observed significant ($p < 0.05$) decrease at 200 mg of extract was not evidenced in the other indices of red cells which are in keeping with features of anemic condition.

Histopathological examination of the pancreas of rats treated with ethanolic extract, showed well-formed islets and normal exocrine glands. The healing enhancing effect on the pancreatic cells may partly be responsible for the reported hypoglycemic properties of *Uvaria chamae* extract [9]. The stained liver sections showed that a low dose of *Uvaria chamae* root extract may have caused mild lymphocytic infiltrates in the portal region, and renal interstitium. Hence, the consumption of the extract may boost the immunity of the people who use it to treat various illnesses.

CONCLUSION

The result of this study has demonstrated that lower doses of 200-500 mg/kg body weight of extract did not produce any harmful effects in the treated Sprague Dawley rats. However, at higher doses of the medicinal plant over 50% deaths occurred in the treated animals. It may therefore not be advisable for the use of high doses of this medicinal plant for the treatment of diseases. Our data was in rats which could only provide an indirect assessment of the ethanolic root extract for human use.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors 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 read and approved the manuscript for publication.

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