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Research Article

Safety assessment of *Uvaria chamae* root extract: acute and subchronic toxicity studies

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Aqueous root extract; Uvaria chamae; Kidney; Liver; Pancreas

ABSTRACT

Background: *Uvaria chamae* is a medicinal plant that is used in many parts of the world in the treatment of diabetes, and other diseases. However, research is needed to ascertain the beneficial and adverse effects of the consumption of the extract. In this study, we evaluated the acute and subchronic oral toxicity and safety of the aqueous root extract of *Uvaria chamae* in Sprague-Dawley rats. **Methods:** In the phase one of the acute toxicity study, the aqueous extract of the root was administered in single doses of 10, 100 and 1000 mg/kg body weight. In the phase two, the extract was administered in single doses of 600, 2900 and 5000 mg/kg body weight. In the sub-chronic toxicity study, the extract was administered at doses of 200, 500, 1000, 2000, 3000 and 5000 mg/ kg body weight/day for 28 days. **Results:** The acute toxicity study showed the LD_{50} of the extract to be above 5000 mg/kg body weight. In the subchronic toxicity study, oral administration of the extract induced a significant ($p < 0.05$) increase in monocytes count and platelets. There was a significant increase ($p<0.05$) in AST at 5000 mg/kg compared to the other concentrations administered. At 2000 mg/kg of extract the ALT level was significantly ($p<0.05$) elevated compared to control and the lower concentrations, while the ALP was significantly ($p<0.05$) reduced at the doses of 200, 500, 1000, and 2000 mg/kg when compared to control and the other concentrations. There were no significant changes in total protein and albumin at the different doses of the extract. Moreover, the *Uvaria chamae* extract did not significantly alter kidney function parameters such as creatinine, BUN, Na^+, K^+, HCO_3^- and Cl^- in comparison to control. Histopathological evaluation showed mild vascular congestion and infiltrates of lymphocytes in the liver and kidney. We also noted well-formed islet cells of Langerhans in rats administered doses ≤ 1000 mg/kg body weight of the extract. **Conclusion:** Data from the study showed that the consumption of the extract at any level in the management of diseases may have potential health benefits for the management of anemia.

INTRODUCTION

Uvaria chamae (P. Beauv), commonly called *Awuloko* or *Ayiloko* by the I+gala people, or *Oko aja* or *Eruju* by the Yorubas or *Kas Kaifi* by the Hausas in Nigeria, grows naturally in the savannah and rain forest regions of Africa and tropical areas of the world. It is also commonly known as finger root or bush banana and belongs to the family *Annonaceae.* It is an evergreen plant which grows to a height of 3.6 – 4.5 m. The fruits are yellow when ripe and have a sweet pulp which is widely eaten. All parts of the plant are fragrant with wide spread medicinal use among traditional West African medicine practitioners. The leaves are eaten as vegetables. *Uvaria chamae* stem ashes are used as salt substitute in food. The leaf juice is applied to wounds, sores, ulcers and cuts while the leaf infusion is used to treat injuries, swellings, ophthalmia irritis and conjunctivitis. The root-decoction is used as a purgative and lotion, and for the treatment of piles, menorrhagia, epistaxis, hematuria and hemolysis (Omajali et al., 2011). Sap from the root and stem is applied to wounds and sores, and the root is made into a drink and body-wash for edematous condition (Burkill, 1985). The root

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bark yields an oleo-resin that is taken internally for catarrhal inflammation of mucous membranes, respiratory catarrh and gonorrhea. The root infusion is used to cure abdominal pains (Omajali et al., 2011). In folk medicine, extracts of the root, bark and leaves are used to treat gastroenteritis, malaria fever, vomiting, diarrhea, wounds, sore throat and inflamed gums (Okwu, 2003). *Uvaria chamae* users commonly add one tablespoonful of pulverized root sample in a cup of hot or warm tea or pap (*akamu* amongst the Igbo and *Ogi* or *Akamu* amongst the Yorubas in Nigeria) - a traditional porridge made from ground maize twice daily. One tablespoonful of the pulverized sample is equivalent to about 333 mg of the aqueous extract. It has been reported that *Uvaria chamae* has hypoglycaemic, antifungal, bacteriostatic and antimalaria properties (Onoagbe et al.,1999; Okokon et al., 2006; Ogbulie et al., 2007; Okwuosa et al., 2012). The root of *Uvaria chamae* has also been reported to contain C-benzylated monoterpenes, aromatic oils (Lasswell and Hufford, 1977), flavanones, C-benzylated flavanones (Hufford and Lasswell 1976), and C-benzylated dihydrochalcones (Lasswell and Hufford, 1977). Despite the wide spread use of *Uvaria chamae*, in traditional medicine, toxicological data on the plant are scarce. This study was carried out to assess the acute and subchronic toxicity studies of the aqueous root extract of *Uvaria chamae* in Sprague Dawley rats, with the purpose of providing information on the safety of this widely use medicinal plant.

MATERIALS AND METHODS

Plant material and extraction

Fresh root pieces of wild *Uvaria chamae* grown in Igwo, Oyo state of Nigeria and harvested in 2012 was purchased from the herbal market, at Oyingbo, in Lagos, Nigeria. The root pieces were identified and authenticated by a taxonomist at the department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria as root of *Uvaria chamae*. A voucher specimen of the plant with reference number UBH 266 has been deposited in the University's Plant Biology and Biotechnology Department Herbarium. The plant roots were thoroughly washed, cut into small pieces and sun dried. A mechanical grinder was used to grind the roots into a uniform powder (pulverization). The pulverized sample was kept in a bowl and soaked in distilled water. The water level was about 2 cm above the sample layer; there was intermittent stirring to allow for percolation and maceration. This process continued for 72 hours to allow for effective extraction (Onoagbe and Esekheigbe 1999). After 72 hours, the sample was filtered using a cheese cloth, and the filtrate was further filtered with Buchner's funnel, freeze dried (3720 g of plant material + 26.4 liters of distilled water yielded 167 g after extraction and freeze drying i.e. 167 g aqueous extract = 3720 g dried *Uvaria Chamae* root), and stored in the freezer.

Animals

Sprague Dawley rats were purchased from the Department of Anatomy, University of Benin, Benin City, Nigeria. The animals were housed in clean cages under standard laboratory conditions of temperature, humidity and light. They had free access to standard laboratory diet and distilled water for a period of two weeks for acclimatization. Approval for the study was obtained after presentation of the protocol to the Board of the Department of Biochemistry, University of Benin, Benin City, Nigeria.

Acute oral toxicity

The method as described by Lorke (1983) was used for the acute toxicity study. In this method there are two phases: phase I requires 3 animals per group, while phase II requires one animal per group. In this study, twelve (12) Sprague Dawley (average body weight 156.46 ± 3.1 g) rats were used. In the first phase (Phase I) of the extract administration, nine rats were divided into 3 groups of 3 rats each. The set of animals in the 3 groups were administered 10 mg, 100 mg, and 1000 mg/kg body weight of extract respectively. After an overnight fast, animals were observed before the administration of a single dose of the extract using an oral gastric gavage. After dosing, food was withheld for about 2 hours to prevent asphyxia or regurgitation, and the animals were observed individually during the first 30 minutes, and then 2, 4, 6, 24 and 48 hours after treatment. The observation focused on mortality, changes in behavior, skin, eyes, fur and somatomotor activity. In the second phase (Phase II), three specific doses were chosen based on the results of the first phase. They were divided into 3 groups of one rat per group: group 1A (1600 mg/kg body weight of the extract); group 2A (2900 mg/kg body weight of the extract); and group 3A (5000 mg/kg body weight of the extract). After an overnight fast, animals were observed before the administration of a single dose of the extract. They were observed for 24 hours for signs of toxicity and mortality.

Subchronic oral toxicity

Forty-two (42) Sprague Dawley rats were used for this study and grouped according to sex and weight (average body weight 195.66 ± 4.70 g). Animals were divided into 7 groups (6 rats in each group) as follows: group 1 received distilled water; group 2 received 200 mg/kg body weight of the extract; group 3 received 500 mg/kg body weight of the extract; group 4 received 1000 mg/kg body weight of the extract; group 5 received 2000 mg/kg body weight of the extract; group

Table 1. Effect of the extract on liver enzymes in Sprague Dawley rats

	Control (0 mg)	200 mg/ kg body wt.	500 mg/ kg body wt.	1000 mg/ kg body wt.	2000 mg/ kg body wt.	3000 mg/ kg body wt.	5000 mg/ kg body wt.
LDH (U/L)	25.7 ± 1.9[a]	20.3±0.6[a]	18.2±0.3[b]	18.8±0.8[b]	18.0±0.7[b]	19.3±0.8[a]	17.0±5.0[b]
AST (U/L)	247.1±11.9[a]	290.5±14.4[a]	292.0±9.7[a]	285.3±9.9[a]	275.0±15.7[a]	296.0±4.0[a]	341.0±8.0[b]
ALT (U/L)	44.9±1.2[a]	30.0±2.0[a]	37.5±3.5[a]	42.0±6.0[a]	44.0±4.7[b]	38.7±2.4[a]	26.0± 3.0[a]
ALP (U/L)	32.0 ± 1.4[a]	8.0±0.01[b]	8.1± 3.0[b]	23.0±2.3[b]	5.0±0.5[b]	9.0±1.7[b]	37.0±15.0[a]

6 received 3000 mg/kg body weight of the extract; and group 7 received 5000 mg/kg body weight of the extract. The animals in all the groups were fasted overnight and observed before the administration of the extract. The extract was administered using an oral gastric gavage once a day (between 8.00 a.m. – 9.00 a.m.) for 28 days. Groups 2 - 7 received 200, 500, 1000, 2000, 3000, and 5000 mg/kg body weight of the extract dissolved in distilled water. The animals were observed for signs of toxicity and mortality daily throughout the experimental period. Observations included visual and auditory responses, movements, respiration, skin and hair coat, salivation, lacrimation, diarrhea, and response to proprioceptive stimulus. Neurological observations included tactile response and response to tail pinch, locomotor and equilibrium. On the 28th day, the animals were fasted overnight, euthanized by decapitation and blood collected for hematological assessment (blood collected in EDTA tubes) and biochemical assays (blood collected in tubes without anti-coagulants). Laparotomy was performed on the animals and organs were immediately collected for histopathological evaluation.

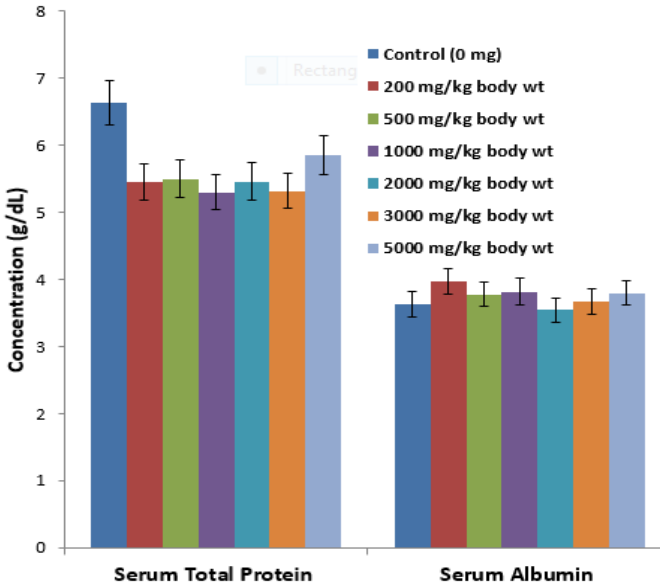

Fig. 1: Effect of oral administration of different doses of the extract on serum proteins in Sprague Dawley rats. Serum concentrations of total protein and albumin in the liver were expressed as mean ± SEM. Values were not significantly different among the groups ($P > 0.05$).

Blood Analysis

Serum lactate dehydrogenase (LDH), total protein, albumin, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), amylase, blood urea nitrogen (BUN), electrolyte and creatinine were determined using VITROS® DT 60 11 chemistry system**.** The system uses VITROS DT slides to perform a number of discreet clinical tests on serum or plasma specimen. All reactions needed for a single quantitative measurement take place within the multilayered analytical element of the slide. The unique properties of these slides permit reliable analyses with a very small volume of sample. All processes performed by the analyzer are controlled by a self-contained microcomputer. The communication with the microcomputer is through the keyboard, which communicates with the user through messages that appear on the display screen and print outs. Manufacturer: KNAUER. GMI SKUN#: 8195-10-0020 model**.** For hematological analysis, white blood cell count, lymphocyte, monocytes, granulocytes, red blood cell (RBC), packed cell volume, hemoglobin (Hgb), mean corpuscular volume (MVC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets were determined using PCE-210N (full automatic Blood cell counter).

Histopathological examination

Isolated organ tissues were fixed in 10% solution of buffered formalin (pH 7.4) followed by dehydration and then embedded in paraffin wax. The organs were sectioned and stained with hematoxylin and eosin (H&E) prior to microscopic examination.

Statistical analysis

Data are expressed as mean ± standard error of the mean (S.E.M.) one-way ANOVA with Duncan's multiple range test performed to assess differences between groups (SPSS 21.0 for Windows). Values of $p < 0.05$ were considered statistically significant.

RESULTS

Acute toxicity; Clinical observations

Apart from monitoring toxicity by mortality, other means of doing so include lacrimation, refusal of feed,

diarrhoea, paralysis, bleeding from the nose, mouth, or genitals, changes in the skin, fur, eyes mucous membrane, salivation, and tremors. However, in phases I and II in this study none of the above mentioned indices of toxicity were observed**.** At the end of the 48 hours' observation in the phase I and phase II of the acute toxicity study, there was no mortality in any of the groups or treatment-related signs of toxicity, and no death was recorded at 5000 mg/kg body weight of the extract administration. So, the LD_{50} value of the extract by oral administration was greater than 5000 mg/kg body weight. The method described by Lorke (1983) was used to determine the acute toxicity of the extract.

Sub-chronic oral toxicity; Clinical observations

Following a single treatment with the extract in the acute toxicity study, no exterior signs of toxicity were observed. Daily oral administration of the extract at doses of 200, 500, 1000, 2000, 3000 and 5000 mg/kg /body weight for 28 days did not induce any obvious symptoms of toxicity and mortality in the rats.

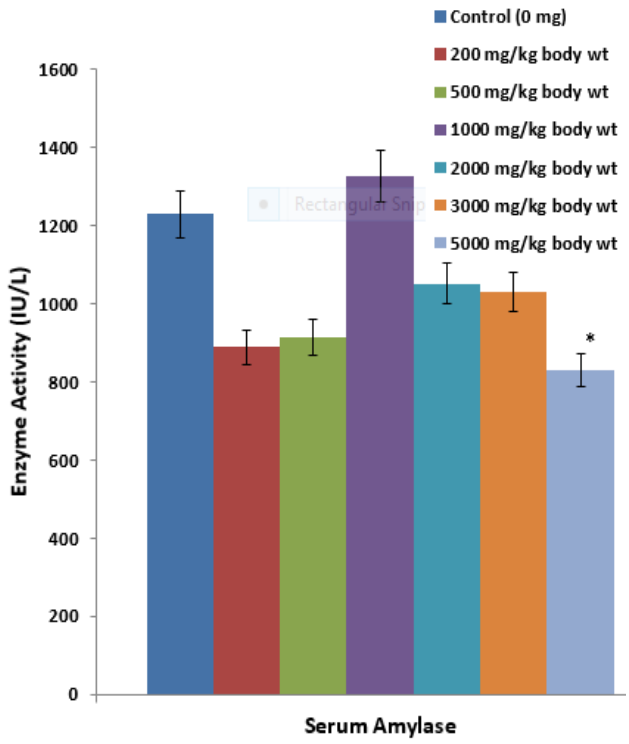

Fig. 2. Effect of oral administration of different doses of the extract on serum amylase in Sprague Dawley rats. Serum concentrations of amylase were expressed as mean ± SEM. *P < 0.05 is significant compared to control.

Biochemical assessment

We noted significant decrease ($p < 0.05$) in serum LDH and ALP activities in animals administered 200, 500, 1000, and 2000 mg of the extract compared to the control group (Table 1).

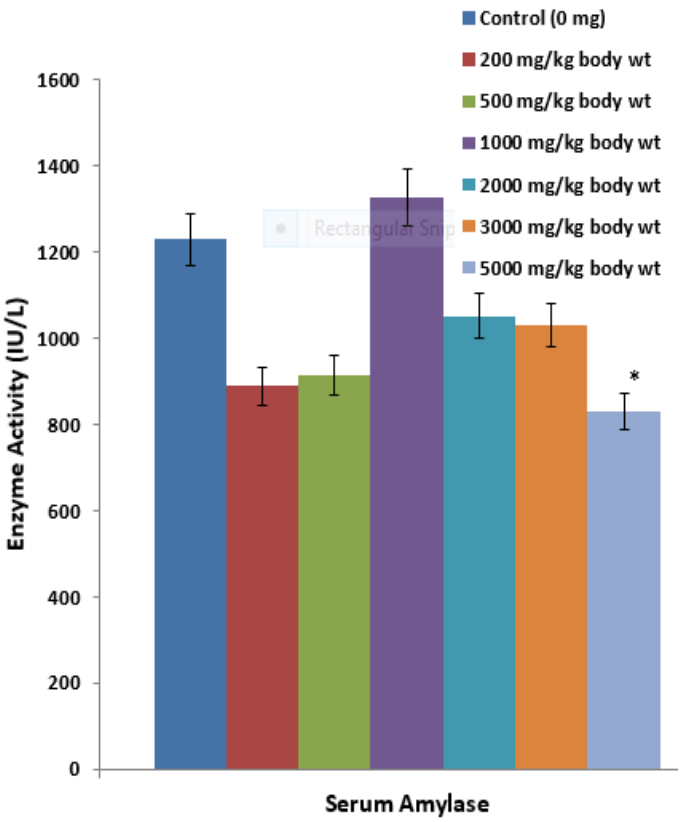

Fig. 3. Effect of oral administration of different doses of the extract on kidney function test in Sprague Dawley rats. Serum concentrations of BUN and creatinine were expressed as mean ± SEM. Values are not significantly different among the groups ($P > 0.05$).

Total protein and albumin levels were not significantly ($p > 0.05$) altered in the groups treated with the extract compared to the control group (Figure 1). There was a significant decrease ($p < 0.05$) in serum amylase activity in the group treated with 5000 mg of the extract compared to the control group (Figure 2). The levels of serum electrolytes assayed were not significantly ($p > 0.05$) altered in the groups treated with the extract compared to the control group (Table 2). We did not observe any significant changes in the levels of serum BUN and creatinine in animals treated with the extract compared to the control group (Figure 3).

Repeated daily oral administrations of the extract at 200, 500, 1000, 2000, 3000 and 5000 mg/kg body weight for 28 days. Serum concentrations of LDH, AST, ALT, ALP were expressed as mean ± SEM. Values in the same row with different superscripts are significantly different at $p < 0.05$. LDH, lactate dehydrogenase; AST, aspartate amino transferase; ALT, alanine amino transferase; ALP, alkaline phosphatase.

Table 2. Effect of oral administration of different doses of the extract on serum electrolytes in Sprague Dawley rats

	Control (0 mg)	200 mg/Kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.	3000 mg/kg body wt.	5000 mg/kg body wt.
Na^+ (mmol/L)	139.5±1.4[a]	141.8±2.8[a]	137.8±1.6[a]	138.0±3.5[a]	141.4±7.1[a]	135.8±2.7[a]	153.0±11.0[a]
K^+ (mmol/L)	5.7±0.2[a]	4.8±0.8[a]	6.1±0.5[a]	5.1±0.6[a]	7.7±0.9[a]	7.2±0.5[a]	7.1±0.2[a]
Cl^- (mEq/L)	109.8±1.3[a]	115.0±2.6[a]	116.8±3.9[a]	123.8±7.5[a]	124.8±2.1[a]	118.5±3.5[a]	125.5±2.5[a]
HCO_3-(mEq/L)	18.8±0.6[a]	21.5±2.5[a]	15.5±3.2[a]	18.0±3.5[a]	16.3±2.9[a]	14.5± 1.2[a]	20.5±9.5[a]

Serum concentrations of Na^+, K^+, Cl^-, and HCO_3^- were expressed as mean ± SEM. Values were not significantly different among the groups ($P > 0.05$).

Table 3. Effect of oral administration of different doses of the extract on white blood cells, platelets and differentials in Sprague Dawley rats

	Control (0 mg)	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000 mg/kg body wt.	3000 mg/kg body wt.	5000 mg/kg body wt.
WBC (10^3µL)	5.9±0.7[a]	4.2±1.1[a]	6.7±0.5[a]	5.6±1.1[a]	3.6±1.6[a]	6.3±1.7[a]	7.7±0.6[a]
LY (%)	88.3±1.8[a]	77.2±9.9[a]	91.3±1.7[a]	90.1±3.6[a]	75.4±3.2[a]	78.4±10.6[a]	92.6±1.2[a]
MO (%)	3.3±0.4[a]	2.4±0.7[a]	4.2±0.8[a]	2.5±0.2[a]	14.5±0.4[b]	15.0±1.3[b]	3.6±0.6[a]
GR (%)	8.5±1.7[a]	3.4±0.5[a]	4.5±0.8[a]	3.7±0.2[a]	13.4±0.7[a]	6.6±3.6[a]	3.8±0.6[a]
Platelets (10^3µL)	217.3 ±58.0[a]	310.0±57.8[a]	408.0±8.3[a]	376.3±69.1[a]	369.7±54.8[a]	404.2 ± 53.9[a]	438.0±72.1[a]

Concentrations of WBC, LY, MO, GR, and Platelets were expressed as means ± SEM. Values in the same row with different superscripts are significantly different at $P < 0.05$. Abbreviations: WBC, white blood cell; LY, lymphocyte; MO, monocyte; GR, granulocyte.

Table 4. Effect of oral administration of different doses of the extract on Red Cell Indices in Sprague Dawley rats

	Control (0 mg)	200 mg/kg body wt.	500 mg/kg body wt.	1000 mg/kg body wt.	2000mg/kg body wt.	3000 mg/kg body wt.	5000 mg/kg body wt.
RBC (10^6µL)	5.1±0.8[a]	6.5±1.0[a]	6.3±0.5[a]	5.2±0.2[a]	5.7±1.3[a]	6.2±0.3[a]	7.6±1.0[a]
Hgb (g/dL)	9.6±1.4[a]	13.6±3.2[a]	11.9±1.1[a]	9.5±0.5[a]	13.6±2.5[a]	11.5±0.6[a]	13.3±2.3[a]
PCV (%)	29.3±4.4[a]	36.9±6.9[a]	35.4±3.1[a]	29.2±1.5[a]	41.5±6.7[a]	35.5±1.5[a]	40.1±6.2[a]
MCV (fl)	56.7±2.1[a]	56.5±2.1[a]	56.3±0.5[a]	55.9±0.9[a]	61.5±3.1[a]	57.5±1.0[a]	52.2±1.1[a]
MCH (pg)	18.3±1.1[a]	19.1±0.8[a]	18.9±0.2[a]	18.1±0.4[a]	19.7±1.1[a]	18.7±0.1[a]	17.7±1.1[a]
MCHC (g/dL)	32.3±0.7[a]	33.8±0.3[a]	33.6±0.4[a]	32.4±0.3[a]	32.1±0.8[a]	32.4±0.7[a]	32.9±0.8[a]

Concentrations of RBC, Hgb, PCV, MCV, MCH, and MCHC were expressed as mean ± SEM. Values are not significantly different among the groups ($P > 0.05$). Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Hgb, hemoglobin; PCV, pack cell volume; RBC, red blood cell.

Hematological parameters

The white blood cells and platelets were not significantly ($p > 0.05$) altered in rats treated with the extract. There was a significant ($p < 0.05$) increase in the monocytes levels in rats administered 2000 and 3000 mg/kg of the extract compared to the control group (Table 3). The red cell indices were not significantly ($p > 0.05$) altered in the treatment groups versus the control group (Table 4).

Histopathological analysis

The liver of rats treated with the extract showed mild portal vascular congestion, mild periportal infiltrates of lymphocytes when compared to the control group (Plates 1, 2, 3, 4, 5 and 6). Similarly, we also noted mild interstitial vascular congestion and dilatation, and mild infiltrates of lymphocytes in the kidney of rats treated with the extract compared to the control group (Plates 7, 8, 9, 10, 11 and 12). However, in the pancreas, treatment with the extract showed well-formed islets and normal exocrine glands at doses ≤ 1000 mg/kg body weight compared to the control group (Plates 13, 14, 15, 16, 17 and 18).

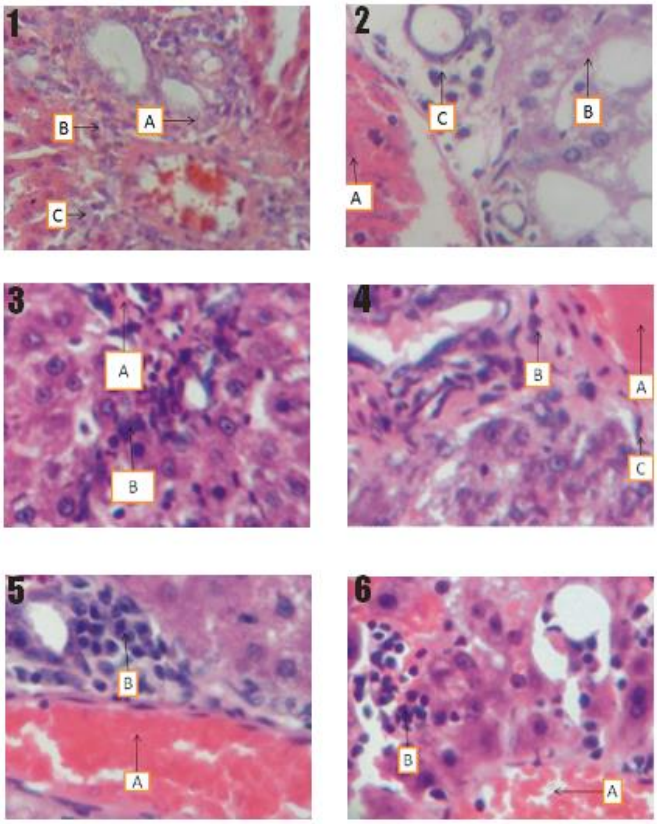

Fig. 6. Photomicrograph of section of rat liver after 28 days of treatment with the extract at doses of 200, 500, 1000, 2000 and 5000 mg/kg body weight/day (plates 2-6 respectively) as compared to the control (plate1). There is vascular congestion [A] and periportal infiltrates of lymphocytes [B] in plates (2-6). Sections were stained with H&E X 400 (plates 1-6).

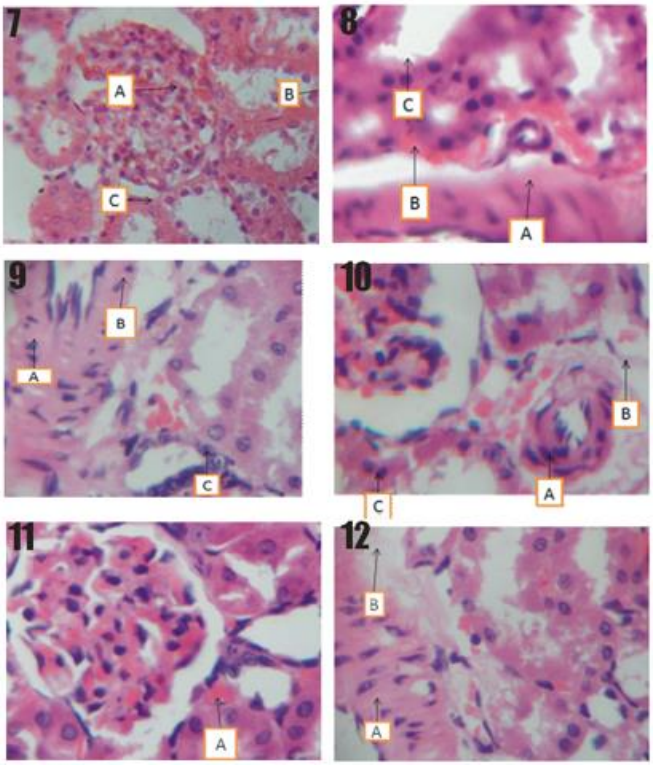

Fig. 7. Photomicrograph of section of rat kidney after 28 days of treatment with the extract at doses of 200, 500, 1000, 2000 and 5000 mg/kg body weight/day (plates 8-12 respectively) compared to the control (plate 7). There is mild interstitial vascular congestion [A] and edema [B] and mild infiltrates of lymphocytes [C] in plates (8-12). Sections were stained with H&E X 400 (plates 7-12).

DISCUSSION

In this study, we presented a comprehensive safety evaluation of the aqueous root extract of *Uvaria chamae* by performing acute and subchronic toxicity studies in Sprague Dawley rats. In the acute toxicity study, no animal died within 24 hours of the administration of the extract. Lorke (1983) suggested that LD_{50} > 5000 mg/kg body weight is thought to be safe. Similarly, according to the acute toxicity grading standards (Duan and Liang, 2011; Aniagu, 2005), if the LD_{50} is greater than 5000 mg/kg, the drug is considered as practically non-toxic. The LD_{50} of *Uvaria chamae* aqueous extract was found to be higher than 5000 mg/kg. The absence of death among the rats in all the doses administered seems to support this claim. Hence, the oral administration of the aqueous extract of *Uvaria chamae* can be said to be practically non-toxic.

Subchronic toxicity studies have been reported to be valuable in evaluating the safety of xenobiotics (Hodge, 1949). In the 28 days subchronic oral toxicity study, animals of both sexes were used. We did not observe any death in the groups administered the extract. Daily oral administration of the extract for 28 days

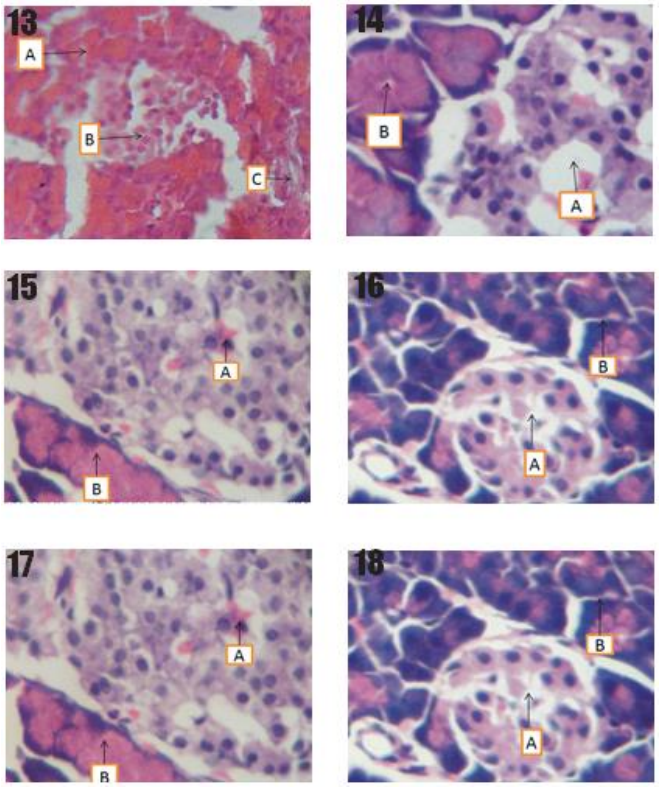

Fig. 8. Photomicrograph of section of rat pancreas after 28 days of treatment with the extract at doses of 200, 500, 1000, 2000 and 5000 mg/kg body weight/day (14-18 respectively) as compared to the control (13). There were well formed islets [A], normal exocrine glands [B] and mild vascular congestion [C]). Sections were stained with H&E X 400 (13-18)

Induced a significant ($p < 0.05$) increase in AST and a reduction in ALT at the dose of 5000 mg/kg body weight when compared to the control group. 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 (Burtis and Ashwood, 1999; Pincus and Schaffner, 1996). Hepatic impairment may also affect serum protein concentration (Burtis and Ashwood, 1999). Elevated serum AST and ALT levels are commonly used as sensitive markers of possible tissue damage, particularly liver damage (Ramaiah, 2007). Acute or chronic injury of the liver causes the elevation of the enzyme activities in the bloodstream (Han et al., 2011; Ozer et al., 2008). The observed changes in AST and ALT levels in the blood of animals administered the extract was dose dependent. The consumption of low doses of the extract (< 5000 mg/kg body weight) may not adversely affect liver function.

There were no significant changes in serum creatinine, BUN and electrolytes levels among the groups. This may be indicative of no adverse effect of the extract on renal function since the elevation of creatinine in the serum has been reported to be associated with marked failure of nephron function (Lameire et al., 2005). In the histological examination, we noted well-formed islets and normal exocrine glands. The observed enhancing effect of the extract on the pancreatic cells may partly explain the traditional use of the plant extract in the management of diabetes.

Hematopoietic system has been reported to be one of the most sensitive targets for toxic substances (Harper, 1973). The oral administration of the extract induced a significant ($p < 0.05$) increase in monocytes count at the tested doses of 2000 and 3000 mg/kg when compared to the control group. Although these changes may be indicative of adverse hematological changes at the respective doses, but it may also be suggestive of the extract possessing immune-stimulatory potentials, since the observed alterations were not reflected in other related parameters investigated at similar doses. However, our histopathological data showed that the extract may have some active ingredients that provoked the immune system to produce the lymphocytes seen in the renal interstitial space and the Kupffer cells lining the sinusoids of the liver. Further studies are needed to determine whether the infiltration of the lymphocytes into the kidney and liver will be beneficial or have adverse consequences.

The goal of the subchronic evaluation was to establish a 'no observed adverse effect level' NOAEL and to identify the specific organ or organs that are affected by the extract after repeated exposure. Overall, our data shows that the liver, kidney and pancreas are possible targets of extract consumption. While the aqueous extract consumption at ≤ 1000 mg/kg body weight may accrue health benefits through the enhancement of the pancreatic cells, the liver and kidney revealed immune boosting activity by the extract as there was 'no observed adverse effect level' for the extract.

CONCLUSION

The oral dose of the extract administered did not produce acute toxicity in Sprague Dawley rats. Hence, its single oral dose of LD_{50} for rats is in excess of 5000 mg/kg body weight. In the 28 days subchronic oral toxicity study, we established 'no observed adverse effect level' for the extract. The consumption of the extract (≤ 1000 mg/kg body weight) enhanced the pancreatic tissues, and provoked the local immune systems of the liver and kidney. Further studies are needed to determine the key ingredients responsible for the acclaimed traditional therapeutic applications of the root extract in the management of diseases.

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