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Effects of acute oral administration of aqueous extract of *Syzygium guineense* root on the kidney of albino Wistar rats

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Abstract

The first step in evaluating toxicity of a substance is to test for acute toxicity of that substance. The focus of acute oral toxicity is on possible short-term adverse effects from one or more oral exposures given within a 24-hour period. The purpose of this research was to investigate the effects of acute oral administration of *Syzygium guineense's* root extract on the kidney of albino Wistar rats. *S. guineense* belongs to the family *Myrtaceae*. It is used against cancer and stomach ache, and as antihelmintic and purgative. A total of fifteen (15) young healthy male Wistar rats (8-10 weeks old) weighing between 140 g and 165 g were used for this study. The study was designed and conducted in two (2) phases: phase I and phase II using established protocols. In phase I, twelve (12) rats were divided into four (I-IV) groups of three rats each. Group I was designated as the control group, while groups II, III and IV were administered with 10 $mgkg^{-1}$, 100 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ respectively. In phase II, three rats were used and shared into three (I-III) groups of one rat each. Groups I, II and III were administered with 1600 $mgkg^{-1}$, 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$ respectively. Data were analyzed using GraphPad Prism version 9.2.0. Results from this research were presented as mean±SEM. Relative kidney weights showed significant decrease. Plasma sodium and urea concentrations showed significant increase. Kidney sections from the treated groups showed no remarkable changes. It is recommended that the root of *S. guineense* should be used with caution.

Keywords: acute oral toxicity, *Syzygium guineense*, renal function, histology, albino Wistar rat

INTRODUCTION

Acute oral toxicity is crucial in assessing the safety of chemicals and pharmaceuticals, focusing on potential short-term negative effects from single oral exposure, a prevalent mode of exposure in real-world situations[1]. Studies on acute toxicity offer insights on the possibility of acute toxicity in humans, the target organs of toxicity, the course of drug-induced clinical findings over time, the right dosage for multiple-dose toxicity studies, and the toxicity differences between species[2].

Syzygium guineense belongs to the family *Myrtaceae*. It is a tree that grows 10 to 15 meters high and has a thick, twisted bole, relatively low branching, a moderately dense crown, and drooping branches[3, 4]. The whole plant has anticancer activity[5,6]. *S. guineense* root infusion is used as an anthelmintic and purgative, and for the treatment of stomachache[6]. The leaf of *S. guineense* is used as anti-inflammatory, analgesic, antibacterial, antimalarial, antidiarrheal, antidiabetic (*in vitro*), antioxidant and antihypertensive[7, 8, 9]. The stem bark of *S. guineense* has antispasmodic activities and is used in the management of tuberculosis[6, 10].

Kidneys are two bean-shaped, smooth, reddish-brown organs, located retroperitoneally and are attached ventrally to the posterior abdominal wall[11]. The kidneys are responsible for regulation of fluid, electrolyte and acid-base balance; detoxification; elimination of metabolic waste products and excretion of nitrogenous wastes such as creatinine and urea from the body. Estimates of the plasma or serum levels of certain compounds and electrolytes are typically used as biomarkers for kidney function. In addition, the kidneys serve endocrine functions[12, 13, 14]. The functional unit of the kidney is called the nephron, which consists of renal corpuscle and uriniferous tubules[15]. The present research is aimed at investigating the acute oral effects of aqueous root extract of *S. guineense* on the kidney of albino Wistar rats.

MATERIALS AND METHODS

Collection and identification of *S. guineense*'s roots

Fresh roots of *S. guineense* were obtained from a herb seller at Maiduguri Monday market in Borno State, Nigeria. The plant was identified and authenticated at the Department of Biological Sciences, University of Maiduguri, Nigeria, where a Voucher number UMDB001 was deposited at the herbarium. The plant material was dried under shade, pulverized and subjected to exhaustive soxhlet aqueous extraction. Continuous hot extraction is a procedure that uses a Soxhlet extractor, a glass-based device. The powdered root of *S. guineense* was placed in a porous bag, filled with water, and heated. The water evaporated, flowed through the condenser, and then into the extraction chamber, where it contacted the root of *S. guineense* to be extracted. The water level in the extraction chamber reached the top of the siphon, and the process repeated until all the drug is extracted.

Animal care

The rats were procured from the animal house of the Department of Biochemistry, Faculty of Science, University of Maiduguri, Nigeria. The research was also conducted in the same place. The rats had access to feed (Vital Feed Growers, Grand Cereals Ltd., Jos-Nigeria) and water *ad libitum*. Standard guidelines as required by the ethical committee of the University of Maiduguri for the care and use of laboratory animals was strictly followed throughout the research period.

Experimental design

With little modifications, the acute oral toxicity study was conducted according to Lorke's method[16]. A total of fifteen (15) young healthy male Wistar rats (8-10 weeks old) weighing between 140-165 g were used for this study. The study was designed and conducted in two (2) phases: phase I and phase II. In phase I, twelve (12) rats were divided into four (I-IV) groups of three rats each. Group I was designated as the control group, while groups II, III and IV were administered with 10 $mgkg^{-1}$, 100 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ respectively. In phase II, three rats were used and shared into three (I-III) groups of one rat each. Groups I, II and III were administered with 1600 $mgkg^{-1}$, 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$ respectively.

All experimental animals were dosed once. Phase I rats were observed for fourteen days. Starting from the first hour after treatment, they were observed on hourly basis for the first 24 hours, and then at approximately the same time on daily basis thereafter. Phase II rats were observed for a duration of 24 hours only after treatment, during that period, they were observed on hourly bases. At the end of the experiment, the rats were euthanized by intraperitoneal injection of ketamine chloride 75 mg/kg (Hospira Inc. Lake Forest, USA). Blood samples were collected in plain bottles by cardiac puncture and centrifuged at 3500 rpm (using 896 MSE minor centrifuge, England) for 10 minutes. Sera were used for assessment of kidney function. Kidneys from all the rats were cleared of any adherent tissues, weighed and fixed 10% formalin.

Determination of kidney function parameters

Assessment of kidney function was conducted using commercially available kit as described by the manufacturer. Parameters evaluated included bicarbonate, chloride, cholesterol, creatinine, potassium, sodium and urea.

Histological preparation

The tissues were dehydrated in ascending grades of alcohol (50% - 100%), impregnated and embedded in paraffin, sectioned at 5µm, mounted on glass slide, cleared in xylene, rehydrated in descending grades of alcohol (100% - 50%), and finally stained using hematoxylin and eosin. Tissue sections were viewed using Olympus biological microscope (CX23, Japan). All photomicrographs were produced at magnification of one hundred (×100).

Data analysis

Data were analyzed using GraphPad Prism version 9.2.0. Results from this research were presented as mean±SEM. *P*-values less than 0.05 were considered statistically significant.

RESULTS

Acute oral toxicity of the aqueous root extract of *S. guineense*

Administration of various doses of the extract in phase I of the study did not cause death or any observable signs of toxicity in the treated groups. Likewise, administration of the various doses in phase II did not result in mortality. However, at 5000 $mgkg^{-1}$ the rat stayed motionless and thereafter remained sluggish for about an hour.

Effects of the extract on body and kidney weights

Table 1 showed the body weight, body weight differences and kidney (absolute and relative) weight. Absolute kidney weight was obtained by weighing the kidneys directly using (AIPI-SS2) digital scale, GSS Scale (Suzhou) Co., Ltd. China. Relative kidney weight was obtained by dividing the absolute kidney

weight with the body multiplied by 100. The body weights in all the groups showed no significant difference. Absolute kidney weights showed no significant difference among all the treated groups. However, relative kidney weights showed significant decrease. The relative kidney weight in the group administered with 10 mg/kg of the extract showed significant decrease (p<0.05) when compared with the control group. Likewise, the groups administered with 100 mg/kg and 1000 mg/kg of the extract also showed significant decrease (p<0.01) in relative kidney weight when compared with the control group.

Effects of the *S. guineense* aqueous root extract on renal functions

Results of renal functions were presented in Table 2. Cholesterol, creatinine, bicarbonate, chloride and potassium showed no significant changes in all the treated groups. However, urea in the group administered with 1000 mgkg^{-1} increased significantly (p<0.05) when compared with the control group. Likewise, plasma sodium concentration also showed significant (p<0.05) increase in the groups administered with 10 mgkg^{-1}, 100 mgkg^{-1} and 1000 mgkg^{-1} when compared with the control group.

Effects of the *S. guineense* aqueous root extract on the histology of the kidney

Sections of kidneys from the control group and the groups administered with 10, 100 and 1000 mgkg^{-1}, as well as those administered with 1600, 2900 and 5000 mgkg^{-1} were shown in Figure 1. Sections from the control group and the group administered with 10 mgkg^{-1} showed intact normal renal corpuscle (RC), Bowman's capsule (BC) and renal tubule (☆). Lymphocytes are indicated by letter (L). At doses of 1600, 2900 and 5000 mgkg^{-1} renal tubules did not differ remarkably from the control group.

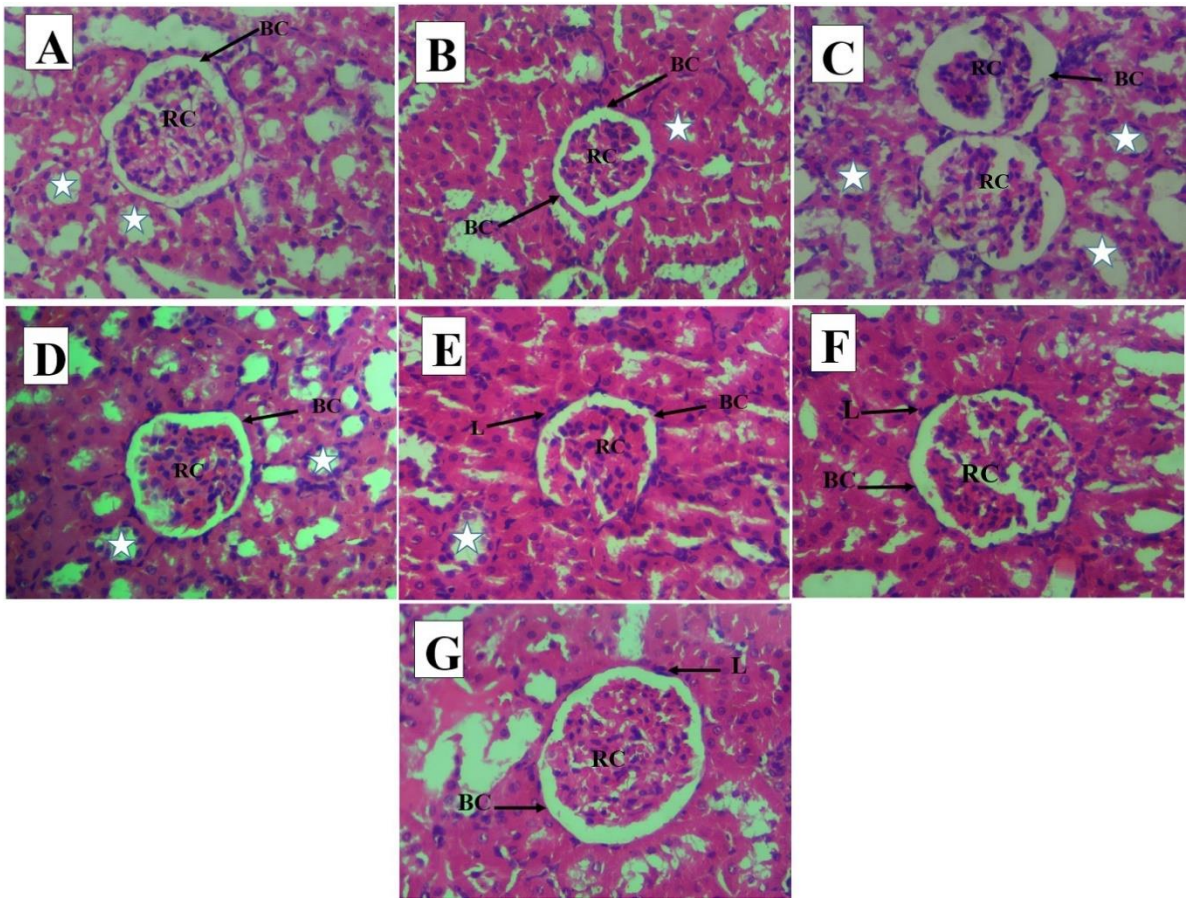

Figure 1: Sections from kidneys, plate (A) control received 0 mgkg^{-1}, plate (B) received 10 mgkg^{-1}, plate (C) received 100 mgkg^{-1}, plate (D) received 1000 mgkg^{-1}, plate (E) received 1600 mgkg^{-1}, plate (F) received 2900 mgkg^{-1} and plate **(**G) received 5000 mgkg^{-1}. Sections from the treated groups showed no remarkable differences from the control group, except for the lymphocytes observed at doses of 1600 mgkg^{-1}, 2900 mgkg^{-1} and 5000 mgkg^{-1} (Haematoxylin and Eosin).

Table 1: Body and Kidney Weights

	Body Weight (g)			Kidney Weight (g)	
Doses ($mgkg^{-1}$)	**FBW**	**IBW**	**BWD**	**Relative (%)**	**Absolute (g)**
0	198.90±8.14	150.30±0.52	44.87±1.30	0.47±0.04	0.67±0.02
10	192.27±15.44	147.40 ±14.15	53.60±4.33	0.36±0.02*	0.68±0.02
100	214.13±1.16	160.80±5.72	40.00±2.31	0.30±0.02**	0.65±0.04
1000	202.67±3.93	162.57±1.70	46.60±8.66	0.31±0.02**	0.62±0.02

IBM= Initial Body Weight; FBW=Final Body Weight; BWD= Body Weight Difference. *p<0.05; **p<0.01 compared with control

Table 2: Biomarkers of Renal Functions

	(mg/dL)					(mmol/L)	
Doses ($mgkg^{-1}$)	**Cholesterol**	**Creatinine**	**Urea**	**Bicarbonate**	**Chloride**	**Potassium**	**Sodium**
0	55.40±8.60	2.30±1.35	31.47±1.65	84.50±3.18	21.00±2.31	9.67±0.78	88.79±12.50
10	42.70±7.33	0.71±0.01	34.87±11.06	103.50±10.10	22.00±3.46	17.90±11.74	116.30±3.06*
100	60.47±5.51	0.60±0.06	42.87±4.34	82.00±1.73	19.00±1.16	6.75±0.49	111.70±8.20*
1000	70.70±5.77	0.97±0.09	62.87±11.45*	88.50±4.91	28.50±3.75	6.07±0.32	114.17±0.26*

*p<0.05 compared with control group

DISCUSSION

In this study, the absence of deaths at 5000 mg/kg of the extract is an indication of safety. Acute oral toxicity study of the aqueous root extract of *S. guineense* revealed that it is not lethal. This finding is in line with the work of Loha [17]. According to the United Nations[18], the root extract of *S. guineense* has relatively low toxicity and belongs to category 5. Plant leaves extracts of *Pentaclethra macrophylla* and *Psidium guajava*, and that of *Spondias purpurea* also have oral LD_{50} greater than 5000 mg/kg [19]. Substances or mixtures having oral median lethal dose greater than 5000 mg/kg are considered practically non-toxic or safe [19, 20].

Significant decrease in absolute kidney weight in all the treated groups suggests that the root extract of *S. guineense* might be toxic to the kidneys to some level. Sellers et al reported that decrease in organ weight is treatment related. Such a decrease of organ weight in toxicity study is one of the most important indicators of toxicity [21, 22]. Significant decrease in relative kidney weight in this study can be supported by the work of Abebe *et al.* where they reported decrease in relative organ weight of uterus and ovaries when methanolic leaves extract of *S. guineense* was administered to female rats [23].

Decreased water excretion will go along with decreased sodium excretion; this implies increased reabsorption of water secondary to increased re-absorption of sodium in the renal tubules. This is suggestive of probable anti-diuretic effect of the plant. Such an increase of plasma sodium could cause high blood pressure and cardiovascular diseases [24]. Conditions such as acute renal injury, advanced chronic renal disease and glomerular diseases cause the kidneys to fail to perform their normal functions, and primarily cause high levels of sodium in the blood [25].

Approximately 50% of the urea excreted by the kidneys in glomerular filtration is reabsorbed[26]. Significant increase in plasma urea might be due to toxic effects on the renal tubules, renal parenchyma, and blockages in the urine outflow tract such as calculi, crystalluria, or other obstructions, in addition to glomerular alterations[27]. Significant increase in the plasma concentration of urea in this research might be due to the toxic effects of the extract on the renal tubules or renal parenchyma. This finding is in line with the findings of Abebe[28]. Ethanolic extract of *Chromolaena odorata*[29] and methanolic root extract of *Taraxacum officinale*[30] were also reported to have caused significant increase of plasma urea. However, significant increase in plasma urea level might not be an indication of renal impairment because plasma creatinine levels in the treatment groups did not show any significant increase[30].

Histologically, no conspicuous differences were observed between the kidneys of the control and the treated groups. This finding is in line with the findings of Loha[17] and Abebe *et al* [28] who reported the absence of histologically remarkable differences between the kidneys in the control group and the treated groups.

CONCLUSION

The study revealed that oral administration of aqueous root extract of *S. guineense* caused decrease in relative kidney weight and increase in the plasma concentrations of sodium and urea in albino Wister rats. Therefore, it is recommended that the root of *S. guineense* should be consumed with caution.

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