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Original Article

Effects of Acute Oral Administration of *Syzygium guineense* Root Extract on the Liver of Albino Wistar Rats

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ABSTRACT

Acute oral toxicity is the general side effects that a substance causes after one or more doses are administered over a period of 24 hours. *Syzygium guineense* is used in the treatment of diabetes, diarrhea and epilepsy. The purpose of this research was to investigate the effects of acute oral administration of *Syzygium guineense's* root extract on the liver of albino Wistar rats. A total of fifteen young healthy male albino Wistar rats (8-10 weeks old) weighing between 140g-and 165g were used for this study, which was designed and conducted in two (2) phases: phase I and phase II. In phase I, twelve 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. Liver function parameters evaluated include albumin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase and total protein. Data were analyzed using GraphPad Prism version 9.2.0. Results from this research were presented as mean±SEM. There was no mortality in relation to the treatment with the extract. Alanine aminotransferase and Aspartate aminotransferase increased significantly. Groups administered with 1600 $mgkg^{-1}$ (E) showed enlarged central vein. Therefore, there is need to be cautious when administering the root of *Syzygium guineense* orally.

Keywords: Acute effect, liver, *Syzygium guineense.* albino Wistar rat

INTRODUCTION

Acute oral toxicity is defined as the general side effects that a substance causes after one or more doses are administered over a brief period of time (24 hours). In order for a substance to be considered acutely toxic, side effects must appear soon after administration within at least 14 days (Knych *et al*., 2022). The primary regulatory motivation behind the use of acute oral toxicity testing is for labeling and classification (Sewell *et al*., 2023). The products of medicinal plants that are ingested orally need to be evaluated for oral toxicity.

About 80% of the population of the world uses medicinal plants (WHO, 2022). The oral route is the most common means of medicinal plant ingestion (Kim and De Jesus, 2023). The consumption of medicinal plants is considered safe by many people who use them (Mensah *et al*., 2019). However, some medicinal plants may be toxic when introduced into the biological system. Such toxicity, if present, may or may not be noticed because dose and duration of time play important roles in the manifestation of toxicity.

Syzygium guineense is a medicinal plant that grows in moist soil, open woodland, and lower montane forests (0–2100 m) (Ruffo *et al*., 2002). It is called water berry in English, 'Malmoo' in Hausa, and 'Igi-aro' or 'Adere' in Yoruba (Oladosu *et al*., 2017). *Syzygium guineense* is used for various medicinal purposes such as in the treatment of diabetes, diarrhea, dysentery, and sore throats (Odugbemi and Akinsulire, 2008). The root is soaked in water and consumed for the treatment of epilepsy and stomach aches, as well as a purgative and anthelmintic (Maroyi, 2008). However, despite being widely used traditionally, there has not yet been a thorough scientific investigation of its safety, especially its aqueous root extract.

The liver is the primary target organ of toxicity in the body (Messelmani *et al*., 2022). It is the focal point for preserving metabolic balance. Its main functions include breaking down food from the intestines,

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generating hormones and proteins, biotransformation, excreting bile, and managing energy metabolism. Additionally, it has endocrine and immunological functions (Leiskau and Baumann, 2017).

The structure of the liver is ideal for defining patterns of liver toxicity, but its classification is based on biochemical tests. The present research is aimed at determining the effect of acute oral administration of root extract of *Syzygium guineense* on the histology and functions of the liver.

MATERIALS AND METHODS

Collection and identification of plant material

Fresh roots of *Syzygium guineense* were obtained from a herb seller at Maiduguri Monday market in Borno State, Nigeria. The plant was identified and authenticated by an experienced plant taxonomist from the Department of Biological Sciences, University of Maiduguri, Nigeria. The plant material was dried under shade, pulverized and subjected to exhaustive soxhlet aqueous extraction.

Animal husbandry

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*.

Experimental Design

With little modifications, the acute oral toxicity study was conducted according to Lorke's method (Lorke, 1983). A total of fifteen (15) young healthy male Wistar rats (8-10 weeks old) weighing between 140-165g 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 (**Table 1**).

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 (**Table 1**).

Table 1: **Experimental Design**

PHASE I			PHASE II		
Doses (mg/kg)	Number of rats	Mortality	Doses (mg/kg)	Number of rats	Mortality
0	3	0	1600	1	0
10	3	0	2900	1	0
100	3	0	5000	1	0
1000	3	0			

All experimental animals were dosed once. Phase I rats were observed for fourteen days. Starting from the first hour of dosing, they were observed on hourly bases for the first 24 hours, and then at approximately the same time on daily bases thereafter. Phase II rats were observed for a duration of 24 hours only after dosing, 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 75mg/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 obtained were used for assessment of liver function. Livers from all the rats were cleared of any adherent tissues, weighed and fixed 10% formalin.

Determination of liver function parameters

Parameters for determination of liver function were determined using commercially available kit as described by the manufacturer. Parameters evaluated include albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total protein (TP).

Histological analysis

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

Effects of the Extract on Body and Liver Weights

There was no mortality in relation to the treatment with the extract (**Table 1**). **Table 2** shows the mean body weights (initial and final), body weight differences and liver weights (absolute and relative). The body weights in all the groups showed no significant difference (**Table 2**). Absolute liver weights showed no significant difference among all the groups. However, the relative liver weight in the groups administered with 100 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ of the extract decreased significantly ($P<0.05$) when compared with the control group.

Effects of the Extract on Liver Function

Results of liver function test showed no significant difference in albumin (ALB), alkaline phosphatase (ALP) and total protein (TP) among all the groups (**Table 3**). However, alanine aminotransferase (ALT) increased significantly (P<0.02) in the group administered with 1000mg/kg when compared with the control group. Aspartate aminotransferase (AST) also increased significantly (P<0.01) when the groups treated with 10 $mgkg^{-1}$ and 100 $mgkg^{-1}$ were compared with the control group. Also, the group administered with 1000 $mgkg^{-1}$ showed significant increase (*P*<0.001) in AST.

Table 2: Showing the Body and Liver Weights

	Body Weight (g)			Liver Weight	
Doses ($mgkg^{-1}$)	FBW	IBW	BWD	Relative (%)	Absolute (g)
0	198.90± 8.14	150.30± 0.52	44.87± 1.30	4.49 ±0.38	6.23± 0.37
10	192.27± 15.44	147.40± 14.15	53.60± 4.33	3.49± 0.00	6.71± 0.54
100	214.13± 1.16	160.80± 5.72	40.00± 2.31	3.15± 0.24*	6.74± 0.47
1000	202.67± 3.93	162.57± 1.70	46.60± 8.66	3.14± 0.09*	6.40± 0.14

IBM= Initial Body Weight; FBW=Final Body Weight; BWD= Body Weight Difference. *=p<0.05

Table 3: Biomarkers of Liver Functions

Doses ($mgkg^{-1}$)	ALB (g/dL)	ALP (g/dL)	ALT (IU/L)	AST (IU/L)	TP (g/dL)
0	4.00± 0.40	27.70± 4.50	2.67± 0.33	2.83± 0.17	5.87± 0.09
10	3.10± 0.06	15.20± 1.91	8.00± 2.31	4.00± 0.58**	5.30± 0.23
100	3.60± 0.06	22.30± 1.91	3.50± 0.29	3.17± 0.17**	6.77± 1.36
1000	3.47± 0.32	30.50± 5.72	8.50± 1.44*	7.00± 0.58***	5.90± 0.35

SEM: Standard Error of Mean; ALB: Albumin; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; TP: Total Protein. *P<0.05; **P<0.01; ***P<0.001

Effects of the Extract on the Histology of the Liver

Sections of the liver from the groups administered with 0 $mgkg^{-1}$ to 5000 $mgkg^{-1}$ are shown in **Figure 2.** Plate **A** is a liver section from the group administered with 0 $mgkg^{-1}$ (control). The figure shows normal cytoarchitecture of the liver with central vein (CV) at the center and sinusoids (blue arrow) radiating from it. Hepatocytes are indicated with black arrow. Groups administered with 10 $mgkg^{-1}$ (B), 100 $mgkg^{-1}$ (C) and 1000 (D) $mgkg^{-1}$ showed no remarkable difference from the control group. Groups administered with 1600 $mgkg^{-1}$ (E) showed enlarged central vein, a feature that is more pronounced in groups administered with 2900 $mgkg^{-1}$ (F) and 5000 $mgkg^{-1}$ (G). In these groups, lymphocytes (indicated by green arrows) are also observed.

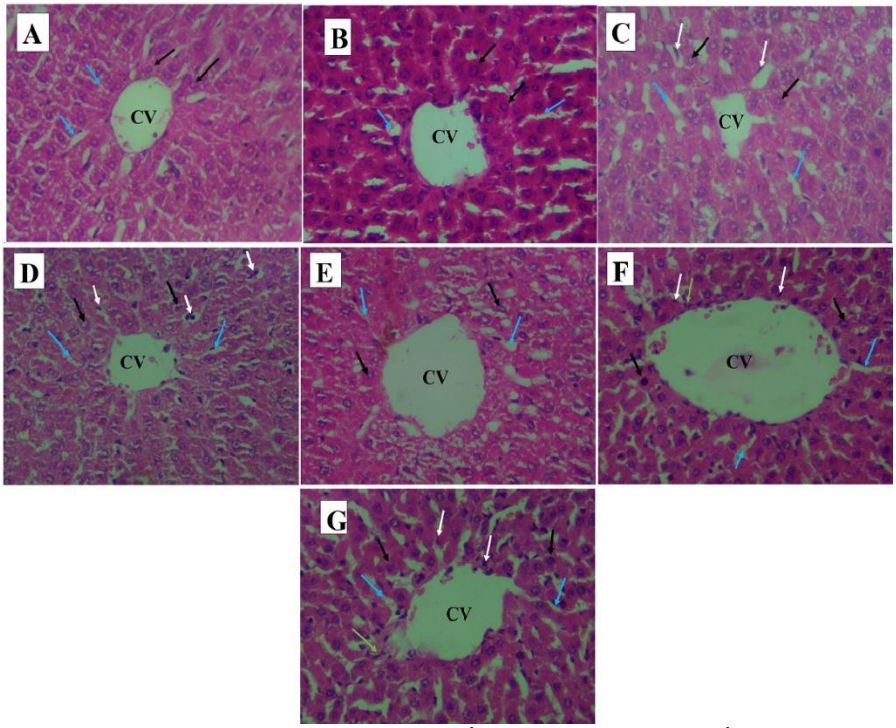

Figure 2 Sections from livers, (A) control received 0 $mgkg^{-1}$, (B) received 10 $mgkg^{-1}$, (C) received 100 $mgkg^{-1}$, (D) received 1000 $mgkg^{-1}$, (E) received 1600 $mgkg^{-1}$, (F) received 2900 $mgkg^{-1}$ and **(**G) received 5000 $mgkg^{-1}$. (H & E, ×100)

DISCUSSION

The median lethal dose (LD_{50}) of the *Syzygium guineense* root extract was found to be more than 5000 mg/kg body in the acute oral toxicity study. The extract of *Syzygium guineense* is classified under category 5 of the United Nations' categorization criteria for chemicals; substances in this category have relatively low acute toxicity but may, in certain situations, pose a hazard to vulnerable people (Globally Harmonized System of Classification and Labelling of Chemicals, 2023). Significant decrease in the percentage liver-body weight ratio in the present study suggests that the aqueous root extract of *Syzygium guineense* has some degree of toxicity. Sellers et al (2007) reported that decrease in organ weight is treatment related. Such decrease of organ weight in toxicity study is one of the most important indicators of toxicity (Piao *et al*., 2013). Significant decrease in organ weight in this study can be supported by the work of Abebe et al (2023) where they reported decrease in relative organ weight of uterus and ovaries in female rats exposed to methanolic leave extract of *Syzygium guineense*. Other medicinal plant extracts that caused significant decrease in liver weight include methanolic stem bark extract of *Entada abyssinica* (Obakiro *et al*., 2021) and ethanolic leaf extract of *Ziziphus spina-christi* (Sabah *et al*., 2021).

The presence of inflammatory cells in the sections of the liver might be attributed to the effect of the plant extract, which might have interfered with the biochemical processes taking place in the liver, resulting in the generation and accumulation of free radicals, which in turn initiated inflammatory processes.

Damaged hepatocyte membranes, overt hepatocyte necrosis, toxicity, inflammation, trauma, hypoxia, induction, or cytoplasmic blebbing are only a few of the insults that can result in ALT leakage (Anadón *et al*., 2019). Significant increase in ALT in this research could be due to its possible leakage from the hepatocyte membrane resulting from *Syzygium guineense*-induced toxicity. The main factor contributing to elevated blood aminotransaminase activity is damage to hepatocytes (Panteghini and Bais, 2019). However, damage to hepatocytes was not clearly observed at light microscopic level in this research but it is possible that the integrity of hepatocyte membrane must have been affected by the extract. The significant increase in the serum ALT activity indicated liver damage (Anadón *et al*., 2019).

Dose-dependent significant increase in AST across all the treated groups seemed to be caused by the extract and it suggests liver toxicity. Although, aspartate aminotransferase is also present in cardiac and skeletal muscles, kidney, brain, pancreas and spleen as well as lung and erythrocytes (Ndrepepa, 2021), but its significant increase in the present research can be supported by the presence of significant increase in ALT which suggests that they were released from hepatocytes. Other root extract that caused significant increase in AST include *Carex baccans*, *Potentilla fulgens* (Roy *et al*., 2012) and *Telfaria occidentalis* (Ogunmoyole *et al*., 2019).

CONCLUSION

Acute oral administration of aqueous root extract of *Syzygium guineense* did not cause mortality. However, significant increase in ALT and AST suggests some level of liver toxicity. Therefore, there is need to be cautious while administering the root of *Syzygium guineense* orally.

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