Kanem J Med Sci 2022; 16(2): 92 - 100

ISSN 2006-4772
https://doi.org/10.36020/kjms.2022.1602.003

Original Article

Acute toxicity study of aqueous fruit extract of xylopia aethiopica (dunal) a. Rich. In albino Rats

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Abstract

Background: *Xylopia aethiopica* (Dunal) A. Rich, is a medicinal plants distributed in lowland rainforest of Guinea Savannah zones of Africa. It is widely consumed in African cuisine as spice as well used in traditional African medicine especially by pregnant women to either; ease child birth, induce placental discharge, facilitate lactation or to prevent nausea. **Objectives**: This study aimed at determining the toxicity level of the aqueous fruit extract of *Xylopia aethiopica* (Dunal) A. in albino rats. **Methodology**: Lorke's method was adopted, using twelve female albino rats of Wistar strain weighing 120 to 160g. The study was divided into two phases of three groups each. First phase were administered at the dose rate of 10, 100 and 1000 mg/kg body weight respectively (N=3). Second phase were administered at the dose rate of 1600, 2900 and 5000 mg/kg body weight (N=1). **Result**: Clinical signs of toxicity like loss of appetite, piloerection, stretching of the abdomen, one-sided movement, diarrhea and lethargy were observed in rats groups treated with 1000 and 1600 mg/kg of *Xylopia aethiopica* (Dunal) A. While mortality were observed in the rats groups treated with 2900 and 5000 mg/kg of the extract. Tissue samples collected for Histology, liver, kidney and spleen showed vascular congestion, degeneration architecture of the glomerulus accompanied with distortion of renal tubules and severe hemosiderin in the respective organs. **Conclusion**: LD_{50} of the extract was estimated to be 2154 mg/kg. The study showed that though the extract is relatively safe but indiscriminate consumption of the extract at a higher dose can be deleterious to health.

Keywords: Acute toxicity, albino rats, histology, *xylopia aethiopica* extract.

Introduction

Xylopia aethiopica (Dunal) A. Rich. family *Annonaceae*is a greenish plant with a unique aroma.[1] It is widely found in lowland rainforest, coastal brackish swamps and littoral formations; deciduous and fringing forest of Guinea Savannah Zones of Africa. It is mostly cultivated in Democratic Republic of Congo, Ethiopia, Ghana, Kenya, Mozambique, Nigeria, Senegal and Uganda.[2]The dried fruit is commonly called African pepper or Ethiopian pepper (English), Údà (Igbo), Kimbáá (Hausa) and Èerù (Yoruba), it is a seasonal plant that is mostly abundant in the Savannah regions between June to September and December to March.[3]*Xylopia aethiopica* is used in African cuisine and traditional medicines because of its pharmacological properties such as; analgesic, anti-inflammatory, anti-allergic, anti-allodynic, anti-hyperalgesic, central nervous system depressant, cytotoxic, antioxidant and hypoglycemic effects.[4-9]An infusion of the plant's bark or fruit is effective in the treatment of bronchitis, dysentery and toothaches.[10]*Xylopia aethiopica* is reported to reduce serum lipid to a

Cite this article as: Anagor O. Keren, Garba H. Sani, Zirahei J. Vandi. Acute toxicity study of aqueous fruit extract of xylopia aethiopica (dunal) a. Rich. In albino Rats. Kanem J Med Sci 2022; 16(2): 92-100

Submitted: 12th June 2022 **Accepted: 25th September 2022** **Published: 21st February, 2023**

favorable level and Post Mitochondrial Fractions (PMF) of visceral organs in experimental hyper-cholesterolemia[11] It is more effective in management of sickle cell disease when it is combined with legumes, fish and fruits.[12]

The leaves of *Xylopia aethiopica* plant are grinded to powdered form, by people of Congo.[2] The people in eastern part of Nigeria use *Xylopia aethiopica* fruit as spices in their local dishes and to serve as blood tonic after child birth; which is attributed to its antiseptic potentials – to inhibit blood loss.[6] It is also used to assist nursing mothers in breast feeding (to increase milk yield),[1]and also in the treatment of digestive system hyper-motility (diarrhea)[3] and as an anti-diabetic agent.[14]The leaves and roots are boiled as tonic called 'Agbo' in Nigeria and used to treat fever (antipyretic). In Ivory Coast, women who newly put to bed are asked to take *Xylopia aethiopica* blood tonic, it is also taken to encourage fertility and to ease child birth.[15,6].*Xylopia aethiopica* is used in the treatment of amenorrhea and as such is seen to be capable of inducing abortion.[2]
Traditional medicine has been proven to account for more than 90% of rural populace health's needs.[16]In as much as these plants may be administered in most disease conditions over a long period of time, without proper dosage monitoring and knowledge of its toxic effect it can cause advance effects (resulting from prolong usage). The use of herbs requires better understanding of the toxicity dosage, purity, suitable extraction solvent as well as adverse effect.[17]Every substance consumed by humans has either a therapeutic, nutritional or toxic effect on the body. The body is capable of adjusting itself to cope with toxic effect of substances if the threshold of intoxication is not exceeded, hence there will be no excessive organ damage.[18]Therefore, information on incidence of renal and hepatic toxicity involved in consuming medicinal herbs without caution is needed.[19] The aim of this study is to determine the acute toxicity and LD_{50} (lethal dose that will lead to mortality in 50%) of the aqueous fruit extract of *Xylopia aethiopica* in albino rats.

Materials and method

Collection, Identification and Storage of Plant Material

Dried fruits of *Xylopia aethiopica* were purchased from Monday market, Maiduguri, Borno State. It was identified and authenticated by a plant Taxonomist in Department of Biological Science Faculty of Sciences, University of Maiduguri, Borno state. The dried fruits were pulverized into course powdered form using mortar and pestle and was then stored for later use.

Extraction of Plant Material

The dried fruit coarse powder weighing 330g was soaked in 3 liters of distilled water and refluxed for two hours in a continuous extraction (soxhlet) apparatus as described by Adienbo, et al.[7] The solution was filtered with a filter paper and the filtrate was evaporated in an oven at 40^{o}C to produce the extract. The extract was weighed (44.9 g extract) then stored in an air-tight container and refrigerated. The yield of the extract was calculated using the formula; Yeild = weight of extract (g)/weight of plant material (g) x 100%

Preparation of Extract Stock Solution

The stock solution of 250 mg/ml of the extract was prepared by dissolving 2 g of the extract in 8 ml of distilled water which served as the vehicle. The volume of solution corresponding to the dose administered to each animal was obtained using the formula: Volume (ml) = (Dose ($mg.kg^{-1}$) x Body weight (kg)) / (Concentration ($mg.ml^{-1}$)).

Animal Treatment

A total of twelve (12) female albino rats of Wistar strain were purchased from the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. They were kept in the Animal house of the Department of Biochemistry, University of Maiduguri, Borno State for 2 weeks to acclimatize before the commencement of the experiment. They were fed with grower mash (Vital Feed, Grand Cereal, Jos, Nigeria) and water *ad libitum.* The research was conducted in accordance with the ARRIVE Guidelines: Reporting of *in Vivo* Experiment.[20]

Acute Oral Toxicity Study

The study was carried out according to the method described by Lorke.[21]The study was divided into two phases. In the first phase, nine female rats were randomly divided into three groups of three rats per group and were given the aqueous fruit extract of *Xylopia aethiopica* at 10 $mgkg^{-1}$, 100 $mgkg^{-1}$ and 1000 $mgkg^{-1}$ body weight orally (using oro-gastric cannula), respectively (Table 1). The rats were

observed for signs of toxicity and/or mortality for 24 hours and then 14 days. In the second phase of the study, three female rats were randomly divided into three groups of one rat each, and were given 1600 $mgkg^{-1}$, 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$ body weight of aqueous fruit extract of *Xylopia aethiopica* respectively (Table 1). The rats were also observed for signs of toxicity and/or mortality for 24 hours and then 14 days. Autopsy was performed on any rat that died and the kidney, liver and spleen were removed, and fixed in 10% formalin and processed for light microscopy. The lethal dose (LD_{50}) was calculated using the formula;

Ld50 $\sqrt{D0 \times D100,}$= Where; D0 = Highest dose that gave no mortality and D100 = lowest dose that produce mortality.

Histological Study

Harvested tissues (kidney, liver and spleen), were fixed in neutral buffered formalin (NBF) for 24 hours. They were dehydrated by immersing in a series of alcohol gradient, cleared in xylene, impregnated with molten paraffin wax in hot oven, and embedded in paraffin blocks. Then the tissues (paraffin blocks) were sectioned at a thickness of 5 µm with a rotary microtome. Ribbons containing every 8th to 10th sections were collected and gently floated on a tissue floatation bath at 40 ^{0}C and picked up on glass microscopic slides. Before staining, the tissue sections were deparaffinized by xylene and hydrated with alcohol. After rinsing the slide in distilled water, sections were stained regressively with Harris' hematoxylin for 10 min. The sections were washed in tap water and dipped into 1% acid alcohol for differentiation and to remove excess stain. The sections were rinsed briefly in running tap water to remove excess acid. The slides were then placed in saturated sodium bicarbonate solution for 3 minutes and counter stained in 1% alcoholic eosin for 1 min. the H & E stained sections were dehydrated by increasing concentration of alcohol and cleared in xylene and were mounted using DPX mountant and glass cover slips for general histology.

Results

Acute Oral Toxicity of *Xylopia aethiopica* Extract

The yield of the extract was calculated using the formula; Yield = weight of extract/weight of plant material x 100%. Weight of extract = 44.9g, Weight of plant material = 330g. Yield = 44.9/330 x100%= 0.136 x100% = 13.6%.

The rats that receive *Xylopia aethiopica* fruit extract at 1000 mg/kg, 1600 mg/kg, 2900 mg/kg and 5000 mg/kg showed toxicity signs such as loss of appetite, lethargy, piloerection, stretching of the abdomen and one-sided movement and diarrhea, within 24 hours post administration. The rats that receive the extract at 2900 mg/kg and 5000 mg/kg died within 24 hours while the other rats survived to the 14th day post administration. The signs of toxicity that were observed in rats treated with the extract at 1000 mg/kg and 1600 mg/kg started reducing on the 4th day and were not noticeable by day 7, the rats appeared normal.

LD50 =$\sqrt{D0 \times D100,}$ D_o =1600 mg/kg and

D_{100} =2900 mg/kg. LD_{50}= $\sqrt{1600 \times 2900}$ =

$$\sqrt{4,640,000} = 2154 mg/kg.$$

Histological Observations

The kidney of rats treated with aqueous fruit extract of *Xylopia aethiopica* at 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$showed degeneration of glomerulus (Figure 1A and 1B), degeneration of the epithelium of Bowman's capsule and distortion of convoluted tubules (Figure 2A and 2B). Collecting duct of the rats that received 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$ of the extract revealed degenerated collecting duct as shown in Figure 3A and 3B. The rats treated with aqueous fruit extract of *Xylopia aethiopica* at 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$ showed congestion of central veins in the liver tissue (Figure 4A and 4B). The spleen of the rats treated with aqueous fruit extract of *Xylopia aethiopica* at 2900 $mgkg^{-1}$ and 5000 $mgkg^{-1}$showed severe hemosiderins at red pulp with a clear white pulp (Figure 5A and 5B).

Tables

Table 1: Summary of administration of aqueous fruit extract of *Xylopia aethiopica*

Phases	Groups	Number of Rats	Treatment
1	I	3	10 mg/kg
	II	3	100 mg/kg
	III	3	1000 mg/kg
2	IV	1	1600 mg/kg
	V	1	2900 mg/kg
	VI	1	5000 mg/kg

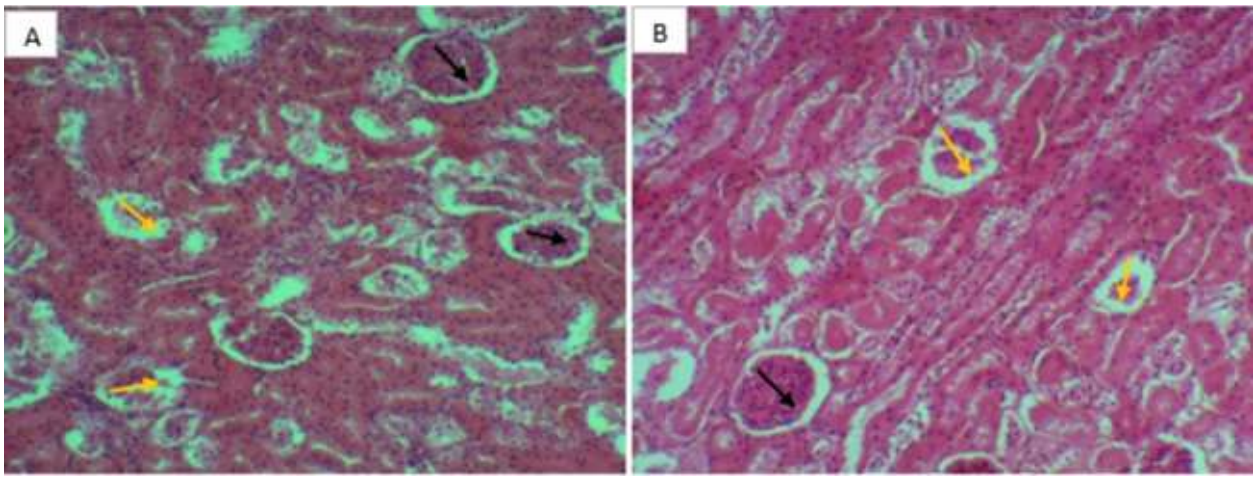

Figure 1: Photomicrograph of rat kidney showing glomerular degeneration (yellow arrows) and intact glomerulus (black arrow). A received 2900 $mgkg^{-1}$ while B received 5000 $mgkg^{-1}$ of aqueous fruit extract of *Xylopia aethiopica* respectively. H & E x100

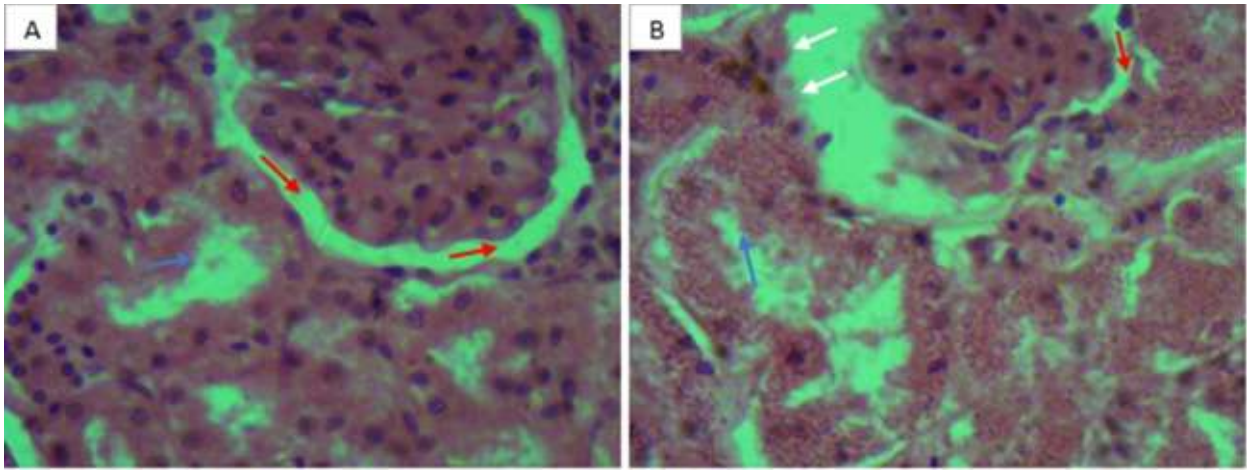

Figure 2: Photomicrograph of rat kidney showing Bowman's space (red arrow), degenerated epithelial lining of Bowman's capsule (white arrows), and distortion of convoluted tubule (blue arrow). A received 2900$mgkg^{-1}$ while B received 5000$mgkg^{-1}$ of aqueous fruit extract of *Xylopia aethiopica* respectively. H & E x400

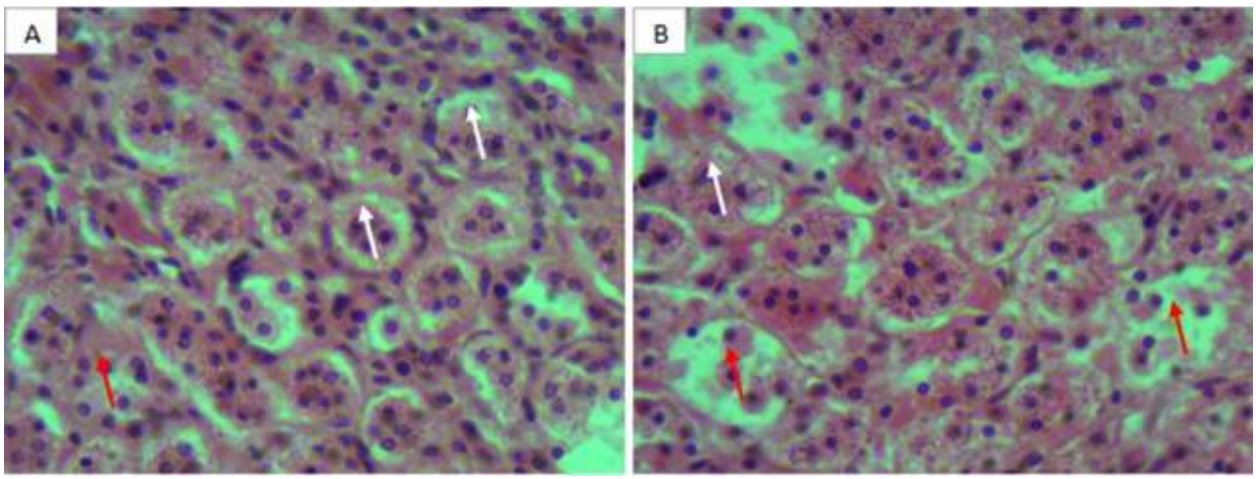

Figure 3: Photomicrograph of rat kidney showing degenerated collecting duct (red arrows) and intact collecting duct (white arrows) lined by simple squamous epithelium. A received 2900 $mgkg^{-1}$ while B received 5000 $mgkg^{-1}$ of aqueous fruit extract of *Xylopia aethiopica* respectively. H & E x400.

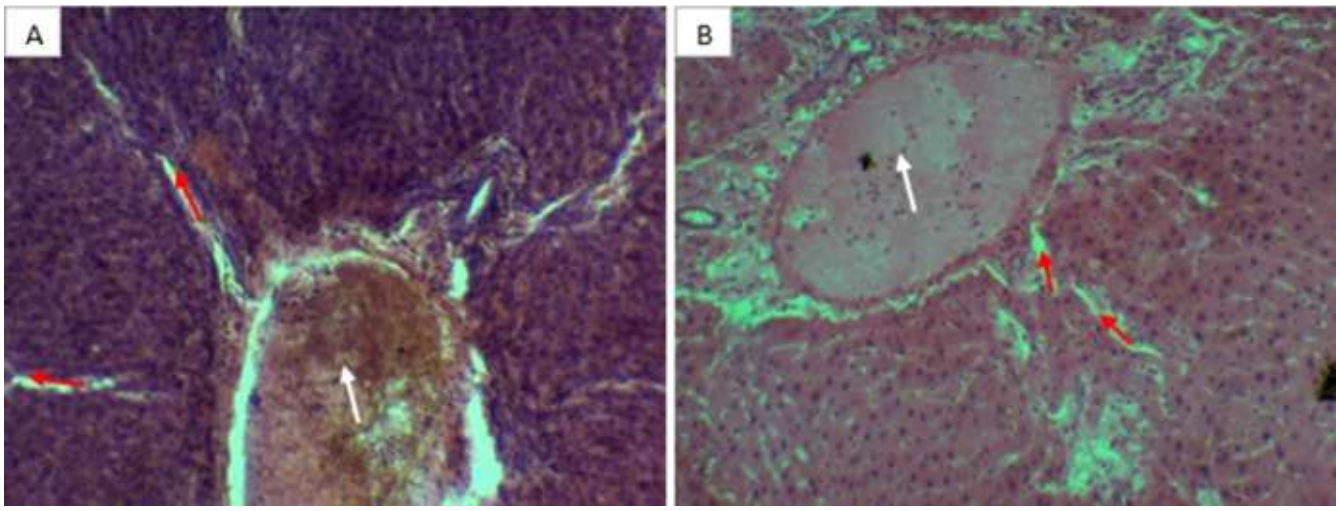

Figure 4: Photomicrograph of rat liver showing congestion of central vein (white arrow), sinusoid (red arrows). A received 2900 mgkg^{-1} while B received 5000 mgkg^{-1} of aqueous fruit extract of *Xylopia aethiopica* respectively. H & E x100

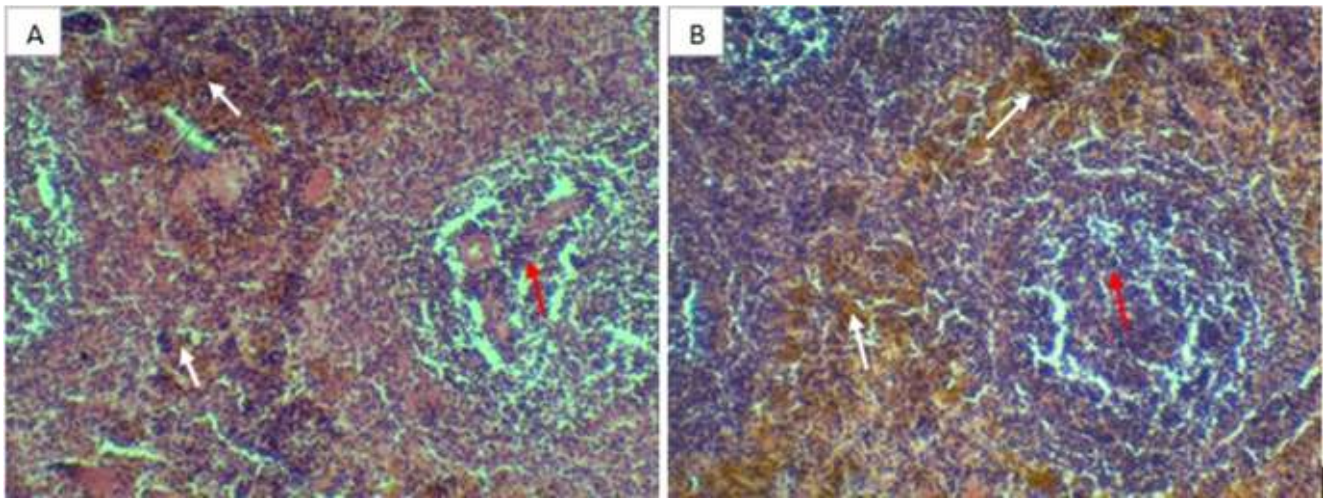

Figure 5: Photomicrograph of rat spleen showing severe hemosiderins (white arrows) and white pulp (red arrows). A received 2900 mgkg^{-1} while B received 5000 mgkg^{-1} of aqueous fruit extract of *Xylopia aethiopica* respectively. H & E x100.

Discussion

The low yield of aqueous fruit extract of *Xylopia aethiopica* (13.6%) is an indication that only a limited part of the fruit i.e less than 20% is utilized in aqueous solution. According to Holy[22] the yield of 251g *Xylopia aethiopica* powder in 1255ml cold water and hot water was 14.9g and 30.3g respectively.

The value of the LD50 of aqueous fruit extract of *Xylopia aethiopica* (2154 mg/kg) showed that the *Xylopia aethiopica* fruit is relatively safe for consumption in rats. Ecobichon[23] recorded that plants with LD_{50} within the range of >500 to <5000 mg/kg is slightly hazardous using rats as model. Where as compared to mice in which the LD50 was estimated to be 1258.9 mg/kg as reported by Omodamiro[24]. This shows that the LD50 of *Xylopia aethiopica* fruit is specie dependent, therefore care should be taken to prevent excessive and prolong use as the LD50 might be even lesser in humans. This might lead to serious side effects that can cause tissue injuries. It is advised that humans should minimize the dosage and the period of consumption because even though the LD50 was higher in rats, there were noticeable signs of toxicity for a couple of days after a single dose of 1000 mg/kg. Since the fruits and seeds of *Xylopia aethiopica* are sometime added to meals of pregnant and nursing mothers for therapeutic effect and to help ease child birth,[14, 6] it might be of health implication to both the mother and fetus/new born if the dosage is not minimize.

The death that occurred in rats treated with aqueous fruit extract of *Xylopia aethiopica* at 2900 mg/kg

and 5000 mg/kg might be as a result of glomerular degeneration and the distortion of the kidney tubules that were seen in the kidneys coupled with the vascular congestion in the liver tissues with severe hemosiderin that was observed in the spleen. Multiple tissue/organ injuries might lead to serious health implications that can cause a lifetime disability or even death[25]. The concurrent occurrence of both liver and kidney damages in rats treated with *Xylopia aethiopica* at dose rate of 2900 mg/kg and 5000 mg/kg signifies that liver injury might lead to kidney injury and vice versa. About 20-50% of patients with acute kidney injuries were diagnosed with liver cirrhosis and patients with liver cirrhosis are more likely to develop kidney disease compared to people without any form of liver disease.[26-29]. Liver and kidney injuries are among the common cause of death in many populations as they can cause multiple organ system dysfunction that might eventually lead to dead[30,31].

The severe hemosiderin that was seen in the spleen tissue shows that consumption of *Xylopia aethiopica* fruit at a dosage of 2900 mg/kg and above may lead to destruction of erythrocytes and deposition of iron within spleen. Wang[32] reported damages of erythrocytes incisplatin treated mice, it lead to the accumulation of iron in the red pulp of spleen with defective recycling of FPN1 and ferritin protein. Accumulation of iron in some vital organs has been reported to be a major cause of morbidity and mortality in transfusion-dependent patients with β-thalassemia[33]. Therefore, the accumulation of iron within the spleen might also be a contributing factor to the death recorded in rats treated with *Xylopia aethiopica* at dose rate 2900 mg/kg and 5000 mg/kg.

Conclusion

We concluded that the estimated LD_{50} of aqueous fruit extract of *Xylopia aethiopica* is 2154 mg/kg in rats, which is slightly toxic. At 10 mg/kg and 100 mg/kg there was no toxicity signs observed, but at 1000 mg/kg and 1600 mg/kg there were noticeable signs of toxicity. Administration of high dose of 2900 mg/kg and 5000 mg/kg body weight lead to death of the rats. The study showed that though the extract is relatively safe but indiscriminate consumption of the extract at a higher dose can be deleterious to health.

Conflict of Interest: The authors decleared no conflicts of interest.

Authors' Contribution: Conception and design: All authors; Provision of study materials: Anagor KO; Collection and assembly of data: Anagor KO; Data analysis and interpretation: All authors; Initial draft of manuscript: Anagor KO; Critical review of the manuscript: Garba SH and Zirahei JV; Final approval of manuscript: All authors.

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