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The Effect of Ethanol Extract of *Xylopia aethiopica* Fruits on the Histomorphology of the Kidney of Albino Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author IUU designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AUE managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The study investigated the effect of ethanol extract of *Xylopia aethiopica* fruits on the histomorphology of the kidney of albino Wistar rats.
Study Design: Twenty (20) albino Wistar rats weighing between 130 – 180 g were assigned into four (4) groups of five rats each. Group 1 served as the control group. Groups 2, 3 and 4 received orally, 250 mg, 500 mg and 750 mg of *Xylopia aethiopica* ethanol extract per kilogram body weight respectively for twenty-eight (28) days.
Place and Duration of Study: Department of Anatomy, Faculty of Basic Medical Sciences, University of Uyo, Akwa Ibom State, Nigeria.
Methodology: The body and kidney weights were observed and kidney was excised for histological studies using haematoxylin and eosin staining techniques.
Results: The final body and kidney weight showed significant weight increases in test groups compared with the control. The photomicrographs revealed gradual epithelial lining degeneration,

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vascular degeneration, inflammation and renal tubular degeneration and necrosis in the test groups when compared to the control group.
Conclusion: It can, therefore, be concluded that the administered doses of the extract of *Xylopia aethiopica* have nephrotoxic effects on the kidney of albino Wistar rats.

Keywords: Xylopia aethiopica; histomorphology; nephrotoxicity; vascular degeneration; inflammation.

1. INTRODUCTION

Xylopia aethiopica is a slim, tall, evergreen, aromatic tree of the Annonaceae family whose height could reach over 20 m with a stem girth up to about 60 - 70 cm in diameter [1]. It is native to tropical African rainforests and moist fringe forests in the Savanna Zones of Africa [2]. The fruit, commonly called African pepper, is the most important part of the tree. *Xylopia aethiopica* produces complex chemical compounds, making it a useful medicinal plant. The fresh and dry fruits, leaf, stem bark and root bark essential oils showed various degrees of activity against the Gram-positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus*, the Gram-negative bacteria *Pseudomonas aureuginosa* and the yeast-like fungus *Candidia albicans* [3,4,5,6]. The compositions of these essential oils are reported to have antioxidant properties, their principal constituents being mono and sesqui-terpene hydrocarbons [7].

Xylopia aethiopica possesses a wide variety of secondary metabolites which gives it a wide range of biological activity including insecticidal, anti-tumour, anti-asthmatic, anti-inflammatory, anti-microbial, hypotensive and vasodilatory effects [8]. The conditions treated with *Xylopia aethiopica* in traditional medicine include a cough (fruits and roots), bronchitis, dysentery and biliousness (fruits and stem bark) and boils and sores, (leaves and bark) [9,1,10,11]. *Escherichia Coli* has been reported to be insensitive to the essential oils of *Xylopia aethiopica* [3] suggesting that it will not be useful in the treatment of diseases caused by it. The bark, when steeped in palm-wine, is used to treat asthma, stomach-aches, and rheumatism [1].

Xylopic acid and kaurenoic acid, the major constituents of *Xylopia aethiopica*, are reported to exhibit a number of biological activities including antimicrobial [3,12], cytotoxic [13,5], anti-HIV [14], hypotensive and diuretic [15], anti-inflammatory and anti-pyretic [16]. Other biological activities of the seeds and fruits include: intraocular pressure lowering effect [17], hypolipidaemic and anti-oxidant activities [7,18].

Ethanol extracts of the fruits of *Xylopia aethiopica* have been reported to possess analgesic properties [19]. A recent study to examine the possible mechanisms of analgesic action of ethanolic extracts of the fruits of *Xylopia aethiopica* and its kaurene diterpene constituent, xylopic acid, showed results suggesting that the anti-nociceptive effects of *Xylopia aethiopica* and xylopic acid are mediated through the opioidergic, adenosinergic, muscarinic cholinergic, NO/cGMP, and serotoninergic pathways. Additionally, xylopic acid acted on the α2-adrenergic system [19]. Another study to evaluate the effects of ethanolic extracts of *Xylopia aethiopica* on reproductive functions of adult male rats showed an increase in body weight as well as the weight of testes and epididymis, and a significant increase in caudal

Fig. 1. Fresh and Dried fruits of *Xylopia aethiopica*

sperm count [20]. Transverse sections of testis exhibited spermatogenesis. The extract treatment also showed a significant increase in serum testosterone and luteinizing hormones levels. The study clearly revealed the androgenic activity of the extract and its effects on hypothalamic-pituitary-gonadal axis [20]. Also, a study to characterize the effects of ethanolic extracts of *Xylopia aethiopica* on cancer cells showed that the extract has anti-proliferative activity against a panel of cancer cells, the main cytotoxic and DNA-damaging compound in ethanolic extracts of *Xylopia aethiopica* being ent-15-oxokaur-16-en-19-oic acid [21].

2. MATERIALS AND METHODS

2.1 Plant Materials

Dried fruits of *Xylopia aethiopica* were obtained from Itak Ikot Akap, Ikono Local Government Area of Akwa Ibom State, Nigeria. The fruits were identified at the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. The fruits were washed, dried at room temperature and then pulverized using a manual grinder. The pulverized fruits, about 825g, were extracted with 70% volume/volume ethanol in Soxhlet apparatus for 48 hours, and the ethanol filtrate was concentrated with a rotary vacuum evaporator (Heidolph, VV2000, Germany) at 45^0C. This yielded 63g (7.64%) of crude extract which was stored in a sealed glass beaker and preserved in a refrigerator at -4°C.

2.2 Determination of Median Lethal Dose (LD_{50})

The acute toxicity of the *Xylopia aethiopica* extract was tested on mice using the modified method and calculation proposed by Lorke (1983) [22]. Different doses of the plant extract based on body weight of the animals were administered to the animals in seven groups. Each group received 300 mg/kg, 600 mg/kg, 900 mg/kg, 1,200 mg/kg, 1,500 mg/kg, 1,800 mg/kg and 2,100 mg/kg body weight. The animals were monitored and examined after 24 hours for mortality. Maximum dose with 0% mortality was recorded to be 900 mg/kg while minimum dose with 100% mortality was 1800 mg/kg. These two doses were used to calculate the $LD_{50.}$

2.3 Experimental Animals and Design

Twenty (20) albino Wistar rats weighing between 130 – 180 g were obtained from the Animal House, Faculty of Basic Medical Science, University of Uyo, Nigeria. They were fed with pelleted rodent feed and drinking water *ad libitum*. The animals were maintained in standard laboratory conditions. The animals were divided into four (4) groups with five (5) animals in each group. Group 1 served as the control and received 1 ml of 10% tween 80 which was the solvent for the crude extract. Groups 2, 3 and 4 were administered 250 mg, 500 mg and 750 mg of the extracts of *Xylopia aethiopica* per kilogram body weight of the animals.

2.4 Termination of Experiment and Tissue Processing

After the last extract administration, the animals were starved for 24 hours to empty their bowels and stabilize the levels of biochemical markers before sacrifice. Twenty-four (24) hours after the last administration, the rats were anaesthetized with chloroform-soaked in cotton wool in the desiccators. The kidney of each rat was harvested and weighed immediately and then put into 10% neutral buffered formal saline, with the container well-labelled.

Organ sections were passed through the processes of fixation, dehydration, clearing, infiltration, embedding, sectioning and staining with Haematoxylin and Eosin (H and E) for examination under a light microscope. Photomicrographs of the tissue sections were taken using a digital camera fitted to a light microscope at a magnification of X100 and X400. This was carried out as described by Umoh et al. [23].

2.5 Statistical Analysis

The data obtained were expressed as Mean ± SEM. SPSS version 20.0 was used to perform one-way analysis of variance (ANOVA) as well as Turkey post hoc multiple comparisons of the data. Test values of $p < 0.01$ were considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Median lethal dose (LD_{50})

The LD_{50} was calculated based on Lorke's formulae; $LD_{50} = \sqrt{ab}$; a = maximum dosage that result in 0% mortality and b = minimum dosage

that result in 100% mortality. a = 900 mg/kg and b = 1800 mg/kg. The LD_{50} was calculated to be 1,272.79 mg/kg.

3.1.2 Body and kidney weight

The result of the body weight and weight of kidney of the animals in the study are presented in Table 1.

3.1.3 Histology of the kidney

The photomicrographs of the histology of the kidney are presented according to the groups. The images are presented in two magnifications; x400.

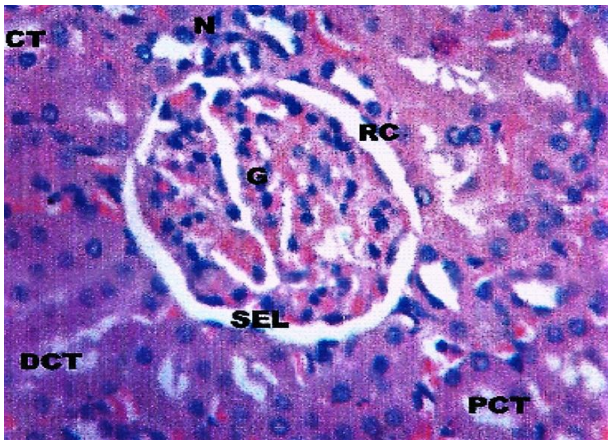
3.2 Discussion

The kidney tissue is composed of glands with highly modified secretary units and highly specialized ducts. The Kidneys excrete urine, produced by modification of blood plasma filtrates. The excretory role of the kidneys exposes them to several xenobiotics and toxic substances hence they are very susceptible to damage by these substances. The present study shows the effect of *Xylopia aethiopica*

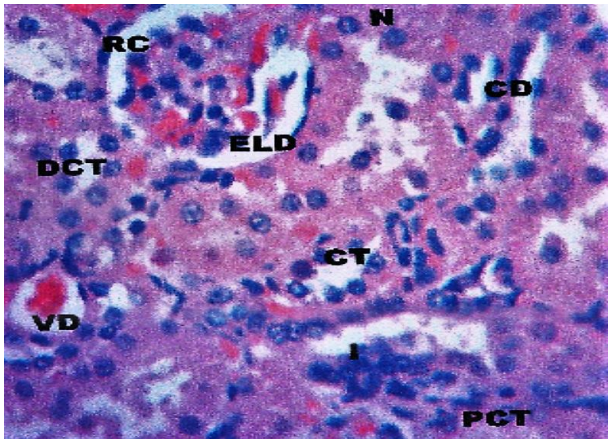
Table 1. Body and Kidney Weight of Albino Wistar Rats Administered Ethanol Extract of *Xylopia aethiopica* in Gram

Groups	Mean weight of organ	Initial body weight	Final body weight	% body weight gained
Group 1	0.58 ± 0.04	140.25 ± 4.11	161.00 ± 7.79	14.80
Group 2	0.70 ± 0.07[a]	147.75 ± 3.40	173.50 ± 11.03	17.43
Group 3	0.72 ± 0.04[a]	163.50 ± 4.51	195.50 ± 10.63	19.57
Group 4	0.80 ± 0.07[a]	179.75 ± 8.77	215.00 ± 4.16	20.03

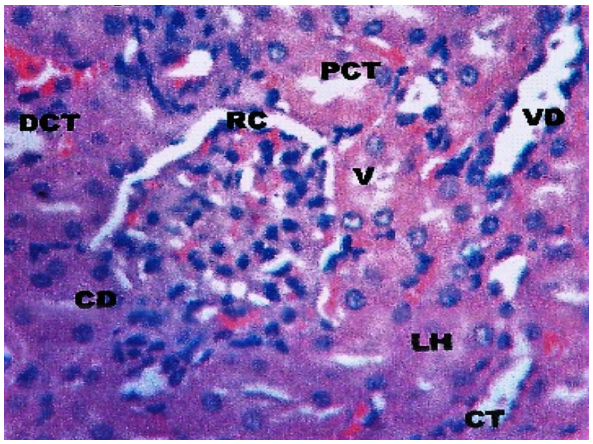
a = significantly different from group 1 (p<0.05). Values are reported as a mean ± Standard deviation.



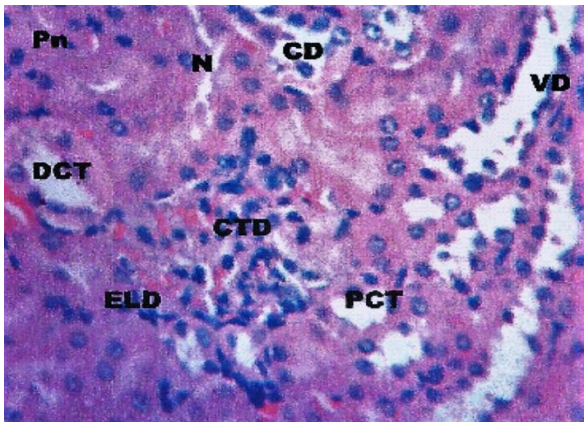
Group 1 – Kidney tissue without treatment (Control) revealed normal cellular pattern with areas of distal convoluted tubules (DCT), proximal convoluted tubules (PCT), nephrons (N), glomerulus (G), squamous epithelial lining (SEL), connecting tubule (CT) and renal corpuscle (RC). There is no evidence of cellular abnormality seen.



Group 2 – Plate C (X100) and D (X400) of Kidney tissue treated with 250 mg/kg of *Xylopia aethiopica* revealed onset of disruption of renal corpuscles (RC), vascular degeneration (VD), proximal convoluted tubules (PCT), distal convoluted tubles (DCT) enthothelial lining degeneration (ELD), cellular degeneration (CD), Inflammation (I), Nephrones (N) and proliferation of pyknotic nuclei (Pn) as compared to control group.



Group 3 – Kidney tissue with 500 mg/kg of *Xylopia aethiopica* revealed partial tubular degeneration, epithelial lining degeneration (ELD), connecting tubules (CT), vascular degeneration (VD), vacuolation (V), loops of Henle (LH) cellular degeneration, proximal convoluted tubules and distal convoluted tubules as compared to control group.



Group 4 – Kidney tissue with 750 mg/kg of *Xylopia aethiopica* revealed distal convoluted tubules (DCT). Proximate convoluted tubules (PCT), Cellular degeneration (CD), nephrons (N), complete tubular degeneration (CTD), epithelial lining degeneration (ELD), vascular degeneration (VD) and proliferation of the pyknotic nuclei (Pn) as compared to control group.

(a common spice for preparation of dishes by the locals of SouthEast and SouthSouth Nigeria) ethanol extract on the kidney of albino rats. Several medicinal potentials of the plant have been reported hence its widespread usage in preparation of local meals. Extracts of *Xylopia aethiopica* have been reported to contain hypolipidaemic agent, thus being able to decrease plasma cholesterol and triglyceride levels, suggesting that the extract can be used to reduce cardiovascular risk factors [18].

This study reveals moderate to severe damage to renal cellular structure and features such as tubular degeneration, vascular degeneration and cellular degeneration, endothelial lining degeneration and regions of inflammation on the kidney in the treated groups. The renal toxicity observed is done dependent with the greatest damage observed at the highest administered dose of 750 mg/kg. The study corroborates with other studies on the effect of the leaves of *Xylopia aethiopica* on the histology of the kidney of albino rats [24]. Obhakha et al. [24] reported that administration of the leaves extract of the plant to albino wistar rat is associated with glomerular and cellular degeneration, tubular disruption, vacuolation and haemorrhage, parenchymal obstruction and tubular wall enlargement in the kidneys of the animals in a dose-dependent manner. The nephrotoxicity resulting from continuous intake of *Xylopia aethiopica* fruit may be due to its phytochemical contents like the diterpenic acids, saponins, tannins and sterols [25]. Phytochemical constituents in the fruit and seed of *Xylopia aethiopica* include diterpenic acids (xylopic acid, kaurenoic acid, 15-oxo-kaurenoic acid, etc.), diterpenic alcohols (kauran-16-α-oil, etc.), acyclic compounds, essential oils, volatile oils, alkaloids, glycosides, saponins, tannins, sterols and criminal [25,26]. These phytochemicals, singly or in synergy may be responsible for the effects observed in this study.

Furthermore, *xylopia aethiopica* administration to albino Wistar rats has been reported to result in elevated creatinine, urea and electrolytes (chloride, sodium and potassium) levels. Increased concentration of serum creatinine and urea is an indicator of renal damage as well as reduced body mass. Hence, the extract negatively impacted the kidney [27]. The

aqueous extract of *xylopia aethiopica* has also been shown to have a toxic effect on the kidney of albino Wistar rats although body weight of the experimental animals was observed to increase compared to the control [28]. Increased body weight and kidney weight following administration of the plant extract was observed in the present study and are in alignment by the previous report by Chris-Ozoko et al. The gain in body weight may have resulted from the effect of steroids which are found in the *Xylopia aethiopica*.

Contrary to the several reports of toxicity of *Xylopia aethiopica* to the kidney of an experimental animal, Adewale and Orhue, reported a nephroprotective effect of aqueous *Xylopia aethiopica* extract on carbon tetrachloride-induced renal toxicity in albino rats [29]. The observed nephroprotective effect was attributed to the antioxidant potential of the plant. The report also exist that *Xylopia aethiopica* ameliorates radiation-induced decreases in antioxidant status of experimental animals, thus having a beneficial effect by inhibiting oxidative damage in brains of exposed rats. It also protects against gamma-radiation-induced testicular damage in wistar rats and protects against adverse effects of whole body radiation [30].

4. CONCLUSION

The study has shown that *Xylopia aethiopica* administration to experimental animals results in renal toxicity which manifests as endothelial lining degeneration, vascular and tubular degeneration and inflammation of the kidney. However, further studies are required to substantiate the exact molecular nephropathologic effect of *Xylopia aethiopica.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was carried out with the principle and guidelines of the ethical committee for conduction of animal studies in the College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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