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Methanol Extract of *Xylopia aethiopica* Fruits Modifies Haematological and Biochemical Status in Rats

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ABSTRACT

Herbal medicines have received greater attention as an alternative to clinical therapy leading to increasing demand; however, the prolonged use of these medicinal plants requires proper safety evaluation to avoid detrimental health conditions. This study evaluated the haematological and biochemical effects of 30-day administration of methanol extract of Xylopia aethiopica fruits (MEXAF), a plant widely used as herb and spice in many parts of the world. Twenty-four albino rats used for the study were divided into four groups of six rats each. Rats in group 1 served as the control and were treated with vehicle only while the rats in groups 2, 3 and 4 were administered 200, 400 and 600 mg/kg body weight of MEXAF. Haematological indices, lipid peroxidation, antioxidant, cardiac, liver and kidney status, lipid profile, blood glucose level, and body and organ weights changes in MEXAF-treated and control rats were evaluated using standard protocols. The presence of flavonoids, alkaloids, steroids, terpenoids, anthocyanins, carotenoids, phenols, saponins, tannins and glycosides were detected in MEXAF. Improved haematological indices and antioxidant status were observed in the MEXAF-treated rats when compared with the control. Cardioprotective, hypolipidemic, hypoglycemic, body and organ weights lowering and decreased lipid peroxidation effects were also seen in the MEXAF-treated rats when compared with control. On the other hand, nephrotoxicity and hepatotoxicity were also observed in the MEXAF-treated rats when compared with control. These show that long-term administration of high dose of methanol extract of the extract is not safe to the kidney and liver of the consumer.

Keywords: *Xylopia aethiopica*; Nephrotoxicity; Hepatotoxicity; Hypolipidemia; Hypoglycemia

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INTRODUCTION

In most parts of the world, especially in the developing countries, the use of medicinal plants to treat and manage ailments had served from the onset as the most important therapeutic measure available to man [1]. One of such widely consumed medicinal plants is *Xylopia aethiopica*, a native of the lowland, rainforest and moist fringe forest

in the Savanna zone of Africa. It is largely located in the west, central and southern parts of Africa and also seen in some parts of Asia and North America [2]. In Nigeria, it is known as 'Uda' by the Igbos of the South-East Nigeria, 'Eeruerunje' in Yoruba of the Western Nigeria and 'Kimba' in Hausa of the Northern Nigeria [3]. In Ghana, it is called 'Hwenteaa' in the Akan dialect, 'Etso' and 'So' in Ewe and Ga dialects respectively, and it is known by the Waala people from the Upper West Region of Ghana as 'Samaamdabile' [4]. Traditionally, *X. aethiopica* fruits (Figure 1) are used as spice to substitute for pepper and as medicine for the management of pain disorders such as rheumatism, headache, and colic pain neuralgia. It is steeped in alcohol and used for treating Asthma. The fruits are used in the induction of placental discharge postpartum due to its abortifacient effect [5].

Figure 1. Dried fruits of *Xylopia aethiopica*

The powdered fruit is used for the treatment of ulcers and toothache due to its antimicrobial effects which have been linked to the presence of xylopic acid [6]. It was reported in the work of Sofidiya et al. [7] that *X. aethiopica* by virtue of its flavonoid contents, exhibited antioxidant properties by inhibiting lipid peroxidation and glutathione oxidation, and this could also slow down the ageing process and improve immune response. The fruits have been shown to contain saponins [8], which have anti-inflammatory and immunostimulatory activities [9]. This may validate the traditional use of *X. aethiopica* seed as an an immune booster. *Xylopia aethiopica* seed extract has also been shown to contain bioactive compounds that could enhance the production of red blood. This supports the claims of its potentials to boost immunity as well as to increase the haematopoiesis in postpartum women [10].

In combination with the roots of *Blighia sapida* and leaves of *Newbouldia laevis*, the alcoholic extract of fruits of *X. aethiopica* is used for increasing menstrual blood flow. The combination is also administered orally to terminate an unwanted pregnancy [11]. The work of Nwangwa [12] reported a significant dose-dependent decrease in the semen parameters (sperm count and motility) as well as degenerative changes in the testicular morphology. Similarly, Nnodim et al. [13] reported a significant decrease in the serum levels of follicle stimulating hormone, luteinizing hormone, testosterone, prolactin and oestradiol in rats treated with the aqueous extract of *X. aethiopica* fruit. Meanwhile, Chris-Ozoko et al. [14] reported that aqueous extract of *X. aethiopica* fruits significantly decreased total body weight, liver weight and kidney weight as well as mild nephritis and hepatitis. The methanol extract of *X. aethiopica* fruits is rich in 58 bioactive compounds, some of which have positive health effects while some might not or even may have negative health potentials [15]. *Xylopia aethiopica* fruits have been shown to be useful in herbal medicine as well as in local delicacies as spice; however, the prolonged use without proper safety evaluation

could be detrimental to health [16]. This study was therefore designed to investigate the haematological and biochemical effects of methanol extract of *X. aethiopica* fruits as an index of safety to human consumers.

EXPERIMENTAL SECTION

Plant Materials

Plant materials used in this study were dried fruits of *Xylopia aethiopica* collected from a habitat in Orba, Udenu Local Government Area, Enugu State, Nigeria. It was authenticated at the Herbarium of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State. The fruits were ground into coarse powder of which 1 Kg was macerated in 4 litres of methanol (JHD, China), stirred and allowed to stand for 48 hours in an air-tight container. The suspension was filtered using a mesh and then Whatman No. 42 filter paper. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C. The brownish concentrate (hereafter referred to as methanol extract of *Xylopia aethiopica* fruits- (MEXAF)) was stored in well-labelled sterile beaker covered with foil at 4°C till used.

Management of Experimental Animals

Animals used in the study were male Wistar albino mice of body weight 28-32 g and male Wistar albino rats of body weight 165-183 g. The animals were obtained from Animal Farm of Faculty of Veterinary Medicine, University of Nigeria Nsukka, Enugu State. Before the experiment, the animals were acclimatized to standard laboratory conditions of standard humidity, room temperature and 12 hours light/12 hours darkness cycle in the Animal Farm of the Department of Biochemistry, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria. The animals had free access to water and were fed pelletized growers mash (Vital Feeds Nig. Ltd). They received humane care throughout the experimental period in accordance with the Ethical Rules and Recommendations of the University of Nigeria Committee on the Care and Use of Laboratory Animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985).

Acute Toxicity Study on the Extract

Investigation on the acute toxicity profile of the extract was carried out using the method of Aebi [17]. Eighteen mice were used for this test which was done in two phases. In phase I, nine mice were divided into 3 groups of 3 mice each; rats in group 1 were administered 10 mg/kg body weight while rats in groups 2 and 3 were administered 100 and 1000 mg/kg body weight of the extract, respectively. The extract was administered orally as a single dose to each mouse. The experimental mice were observed for 24 hours for abnormal behaviour, general body conditions, morbidity and mortality. The body weight of each mouse was measured before extract administration and 24 hours post administration to access if there was any significant body weight change. Based on the result of phase I, phase II was conducted. The remaining 9 mice divided into 3 groups of 3 mice each were administered increased dose of 1600, 2000 and 2900 mg/kg body weight of extract, respectively. The mice were observed for another 24 hours.

Experimental Design for Rat Study, and Haematological and Biochemical Analyses

Twenty-four male Wistar albino rats were divided into 4 groups of 6 rats each and were orally treated with MEXAF once a day for 30 days as shown in Table 1. The doses used by Nnodim et al. [13] were adopted in this study with little modification.

Table 1. Experimental design for toxicological studies of methanol extract of *Xylopia aethiopica* fruits (Number of rats per group, n=6)

Groups	Description	Treatment
Group 1	Normal control	Administered with 5 ml/kg body weight (b.w.) of distilled water
Group 2	Treatment group	Administered with 200 mg/kg b.w. of methanol extract of *Xylopia aethiopica* fruits (MEXAF)
Group 3	Treatment group	Administered with 400 mg/kg body weight of MEXAF
Group 4	Treatment group	Administered with 600 mg/kg body weight of MEXAF

On the 31st day, after an overnight fasting, body weights of all the experimental rats were measured and the rats were sacrificed under mild chloroform anaesthesia. Blood samples were collected from each rat into plain and anticoagulated tubes. The blood samples collected in plain tubes were allowed to clot for 15 minutes and centrifuged at 3000 rpm for 10 minutes after which the sera were separated into well-labelled clean tubes and used for biochemical analyses. The blood samples in the EDTA-anticoagulated tubes were used for haematological analyses using Abacus-380 Haematology auto-analyzer. The weights of the kidney, heart and liver were measured and the organ/total body weight percent of each organ on day 31 were calculated. Blood glucose concentration, and body and organ weights were determined. The body weight gain of each rat was calculated as present body weight minus initial body weight for days 7, 14, 21 and 28. The biochemical parameters analyzed and methods used include: activities of catalase (CAT) [18], glutathione peroxidase (GPx) [19], glutathione reductase (G-Red.) [20], aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [21], and alkaline phosphatase (ALP) [22], creatine kinase (CK) [23] and the concentrations of glutathione [24], malondialdehyde (MDA) [25], total bilirubin [26], urea [27], creatinine [28], total cholesterol (TC) [29], triacylglycerol (TAG) [30], high density lipoprotein (LDL) [31] and low density lipoprotein (LDL) [32]. Serum sodium concentration was determined using colorimetric method based on modified Maruna and Trinders method as described by Enemchukwu et al. [33]. Serum potassium concentration was determined using the turbidometric method as described by Allain et al. [28]. The body weight gains were calculated as:

Day 7 body weight gain=day 7 body weight – day 0 body weight;

Day 14 body weight gain=day 14 body weight – day 0 body weight;

Day 21 body weight gain=day 21 body weight – day 0 body weight;

Day 28 body weight gain=day 28 body weight – day 0 body weight

Statistical Analysis

Raw data obtained from the laboratory were analyzed using one-way analysis of variance (ANOVA) for all parameters except for body weight gains that were analyzed using two-way ANOVA in Statistical Product and Service Solutions (SPSS), version 18. The results were expressed as mean ± standard deviation (SD) and presented in tables. Significant differences of the result were established at $p<0.05$ for all results.

RESULTS AND DISCUSSION

Acute Toxicity Profile of Methanol Extract of *Xylopia aethiopica* Fruits

There were no mortality and significant behavioural and body weight changes in the mice after 24 hours of acute administration of MEXAF. This observation suggests that MEXAF is relatively safe at the doses administered within 24 hours (Table 2).

Table 2. Acute toxicity profile of methanol extract of *Xylopia aethiopica* fruits (n=3)

Group	Doses (mg/kg. body weight)	Mortality	Significant body weight change	Behavioural change
Group 1	10	0/3	Nil	Nil
Group 2	100	0/3	Nil	Nil
Group 3	1000	0/3	Nil	Nil
Group 4	1600	0/3	Nil	Nil
Group 5	2000	0/3	Nil	Nil
Group 6	2900	0/3	Nil	Nil

Effect of Methanol Extract of *Xylopia aethiopica* Fruit on Haematological Indices

There was a dose-dependent significant ($p<0.05$) increase in packed cell volume (PCV), haemoglobin (Hb) concentration, and red blood cell (RBC) and white blood cell (WBC) counts in the MEXAF-treated rats when compared to control. Similar trends were observed for platelet (PLT) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) values of MEXAF-treated rats when compared with control (Table 3). These results corroborate with the reports of Abaidoo et al. [10] and [34] that *X. aethiopica* fruits extract increases Hb and PCV levels. The increased Hb and PCV levels as well as RBC count on administration of the extract suggests that the extract may have stimulatory effect on RBC production. Levels of Hb, RBC and PCV are associated with the total population of red blood cells while MCH, MCHC and MCV relates to individual red blood cells. It is widely known that erythropoiesis is restricted to the bone marrow; however, Corzo-Martinez et al. [35] reported that garlic can enhance erythropoiesis in the spleen which is termed as garlic-induced extramedullary haemopoiesis. Since garlic which is rich in similar phytochemicals as *X. aethiopica* has been shown to enhance erythropoiesis [36]; it is then assumed that the increased Hb concentration, PCV and RBC count in this study may have resulted from stimulation of splenic erythropoiesis by *X. aethiopica* phytoconstituents. The increase might have also resulted from stimulation of production of erythropoietin, a hormone that stimulates erythropoiesis [37]. The significant rise in PLT count may be attributed to enhanced stimulation of thromboprotein, a glycoprotein hormone produced in the liver and kidneys that stimulates the production and differentiation of megakaryocytes, which regulates the production of PLTs [38]. This may support the use of *X. aethiopica* in wound healing [39]. The crucial role of WBC in defending the body against infection and tissue damage is well known. The significant ($p<0.05$) increase in WBCs would invariably lead to an increase in the granulocytes such as neutrophils, which responsibly fight infectious agents by phagocytosis. Hence, this study lends

credence to the use of *X. aethiopica* as an immunostimulant in the treatment of immune-related ailments as proposed by Sarah et al. [40].

Table 3. Effect of methanol extract of *Xylopia aethiopica* fruit on the haematological indices

Groups	PCV (%)	Hb (g/l)	RBC (×1012/L)	WBC (×109/l)	PLT (×109/l)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1	40.25 ± 1.73[a]	13.43 ± 0.61[a]	7.17 ± 0.76[a]	7.32 ± 1.79[a]	366.25 ± 12.18[a]	60.00 ± 0.82[a]	16.28 ± 0.29[a]	26.05 ± 0.76[a]
Group 2	45.05 ± 2.03[b]	15.36 ± 0.55[b]	8.43 ± 0.41[b]	10.39 ± 0.89[b]	419.25 ± 15.52[b]	65.25 ± 2.06[b]	16.45 ± 0.24[a]	28.70 ± 0.81[b]
Group 3	50.59 ± 0.52[c]	16.64 ± 0.50[c]	9.77 ± 0.20[c]	13.49 ± 1.00[c]	486.50 ± 9.47[c]	75.75 ± 2.50[c]	17.70 ± 0.29[b]	29.05 ± 0.70[b]
Group 4	58.03 ± 1.09[d]	19.24 ± 0.44[d]	10.62 ± 0.81[c]	15.58 ± 0.67[d]	546.75 ± 16.32[d]	86.00 ± 3.92[d]	19.68 ± 0.79[c]	29.16 ± 0.91[b]

Data are mean ± SD (n=4). Values with different superscripts in a column are significantly different at $p<0.05$. haemoglobin (Hb), red blood cell count (RBC), white blood cell count (WBC), path cell volume (PCV), Platelet count (PLT), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

Effects of Methanol Extract of *Xylopia aethiopica* Fruits on Lipid Profile

Treatment of rats with MEXAF significantly ($p<0.05$) reduced in a dose-dependent manner, the TC, LDL and TAG concentrations of sera of rats in groups 2-4 when compared with control (group 1). Conversely, the HDL concentration was significantly ($p<0.05$) increased, in a dose-dependent manner, in MEXAF-treated rats when compared with control (Table 4). This result is similar to the reports of Omeh et al. [8] and Abaidoo et al. [10] which showed that extracts of *X. aethiopica* produced dose-dependent reduction in serum TC, TAG and LDL levels and elevation in levels of HDL. This could be associated with the presence of hypolipidemic components in the extract. The observation showed that MEXAF could play beneficial roles in dyslipidemia and reduction in the risks associated with hypercholesterolemia such as cardiovascular diseases [41]. These findings validate the reported medicinal uses of *X. aethiopica* fruits in ethnomedicine as lipid-lowering agent in many parts of the world [42,43]. Several mechanisms may be involved in the reduction in cholesterol concentrations such as a decrease in food intake (appetite) and absorption, an increase in breakdown of the available cholesterol in the plasma and a decrease in the *in vivo* biosynthesis of cholesterol. The reduction in plasma lipids observed in this study is believed to be mediated via the documented *X. aethiopica*-induced inhibition of dietary lipid absorption in the gastrointestinal tract, which is thought to be achieved via a reduction in the bile salts which are required for cholesterol absorption in small intestine [44]. A study by Adewale et al. [45] showed that aqueous extract of *X. aethiopica* has anti-lipase activity, which may suggest one mechanism of lipid lowering effect observed in this study. Further studies are needed to identify other specific mechanism(s) of hypolipidemic effect demonstrated in this study.

Table 4. Effects of methanol extract of *Xylopia aethiopica* fruits on the lipid profile

Group	TC (mmol/l)	HDL (mmol/l)	LDL (mmol/l)	TAG (mmol/l)
Group 1	4.25 ± 0.30^{c}	1.33 ± 0.22^{a}	2.59 ± 0.45^{c}	1.68 ± 0.10^{d}
Group 2	3.68 ± 0.13^{b}	2.00 ± 0.16^{b}	1.43 ± 0.27^{b}	1.25 ± 0.13^{c}
Group 3	3.23 ± 0.17^{a}	2.28 ± 0.13^{bc}	0.78 ± 0.18^{a}	0.88 ± 0.10^{b}
Group 4	3.10 ± 0.27^{a}	2.55 ± 0.26^{c}	0.41 ± 0.43^{a}	0.60 ± 0.08^{a}

Data are mean ± standard deviation (SD) (n=4). Values with different superscript in a column are statistically significant at $p<0.05$.

Effects of Methanol Extract of *Xylopia aethiopica* Fruits on Antioxidant and Lipid Peroxidation Status

The mean serum catalase and glutathione peroxidase activities of MEXAF-treated rats were significantly ($p<0.05$) higher when compared with control. On the other hand, the glutathione reductase activities of MEXAF-treated rats were significantly ($p<0.05$) lower when compared with control. The concentrations of reduced glutathione were higher in MEXAF-treated rats than in control. On the other hand, MEXAF-treated rats had lower MDA activity when compared with control (Table 5). It was reported that the administration of aqueous extracts of *X. aethiopica* fruits to rats intoxicated with carbon tetrachloride (CCl_4) elevated the serum antioxidant enzyme activities [46]. Glutathione peroxidase catalyzes the reduction of hydrogen peroxide (H_2O_2) to water (H_2O); oxidized glutathione (GSSG) is produced in the process and it is recycled to its reduced form by glutathione reductase (G-Red) using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a coenzyme [47]. The reduced glutathione, in turn, reduces oxidized enzyme systems and other metabolites such as ascorbate. Due to its high concentration and role in maintaining redox state in the cells, glutathione is considered one of the most essential cellular antioxidants. Glutathione regenerates most important antioxidants; it reduces tocopherol radical of vitamin E [48]. The antioxidant activity of *X. aethiopica* is associated with its polyphenolic contents such as β-pinene, *p*-cymene, α-cadinol, trans-pinacaveol, α-pinene and 1,8-cinaole [49]. These phytoconstituents might be responsible for the boosting of antioxidant status of rats in this study. A study by Dada et al. [50] earlier reported that catalase found in the blood of most living cells decomposes H_2O_2 to water (H_2O) and molecular oxygen (O_2), thereby making it unreactive and harmless. Increased activities of these antioxidant enzymes may be as a result of the stimulatory effect by phytoconstituents of the extract on the enzymes as well as the combined activities of the antioxidant phytoconstituents and the inherent antioxidant enzymes, sparing some antioxidant enzymes, and preventing the burden of oxidative stress in the system [51]. The inverse relationship exists between the antioxidants and lipid peroxidation status has been recognized [52]. Malondialdehyde is the major product of lipid peroxidation; it reacts

with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts which may give rise to diseases [53]. In this study, the concentration of malondialdehyde was significantly ($p<0.05$) lower in the MEXAF-treated rats when compared to control. This finding agrees with the report of Sofidiya et al. [7] that methanol extract of *X. aethiopica* exhibited antioxidant properties by inhibiting lipid peroxidation and glutathione oxidation which could slow down the ageing process and improve immune response. The improvement of antioxidant status of experimental rats by MEXAF could be attributed to the bioactive components of the plant such as alkaloids, flavonoids, saponins and tannins [54].

Table 5. Effects of methanol extract of *Xylopia aethiopica* fruits on the antioxidant and lipid peroxidation status

Study group	Antioxidant enzyme activities (IU/L)			Malondialdehyde (MDA) (mg/dl)	Reduced glutathione (GSH) (mg/dl)
	Catalase	GPx	G-Red		
Group 1	4.44 ± 0.25^{a}	22.35 ± 1.96^{a}	24.45 ± 2.61^{a}	2.91 ± 0.32^{a}	2.45 ± 0.29^{a}
Group 2	6.44 ± 0.42^{b}	28.81 ± 2.32^{a}	24.85 ± 1.75^{a}	2.81 ± 0.35^{b}	3.00 ± 0.29^{a}
Group 3	7.52 ± 0.39^{c}	34.89 ± 2.44^{b}	23.71 ± 1.49^{b}	2.19 ± 0.07^{c}	3.60 ± 0.22^{a}
Group 4	8.83 ± 0.30^{d}	42.74 ± 2.12^{c}	21.69 ± 1.82^{c}	1.07 ± 0.11^{d}	4.50 ± 0.22^{b}

Data are mean ± standard deviation (n=4). Values with different superscripts in a column are significantly different at $p<0.05$. GPx=Glutathione peroxidase while G-Red=glutathione reductase.

Effect of Methanol Extract of *Xylopia aethiopica* Fruit on the Liver and Cardiac Status

The liver is an organ of paramount importance that is involved in the maintenance of metabolic function and detoxification from the exogenous and endogenous challenges such as xenobiotics, drugs, viral infections and chronic alcoholism. These roles make the liver susceptible to the toxicity from these agents. Hence, if the protective mechanisms of the liver are overpowered or compromised by these toxic agents, the result is hepatic injury [55]. Dose-dependent significant ($p<0.05$) elevation in the serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphate (ALP) were observed in MEXAF-treated rats when compared with control. Similarly, treatment of rats with MEXAF significantly ($p<0.05$) elevated, in a dose-dependent manner, the total bilirubin concentration when compared with control (Table 6). Liver enzymes such as ALT, AST and ALP are markers of liver status; function and integrity of the liver [56]. These enzymes are produced and housed mainly in the hepatocytes and cardiac muscles. During liver injury, these liver enzymes leak into circulation; hence, their activities in the serum becomes elevated [56]. The significant elevation in ALP, AST and ALP activities on administration of MEXAF may be attributed to the damage of structural integrity of the liver [57]. This finding is in contrast with the report of Abaidoo et al. [10] and Orji et al. [58] in which the methanol extract of *X. aethiopica* fruit had no significant effect on ALP, ALT and AST activities. However, it agrees with the report that the methanol extract of *X. aethiopica* fruits elevates ALT activity [59]. Variations among these results could be attributed to differences in doses administered and treatment durations.

Serum bilirubin concentration is generally considered a good test of liver function since it reflects the liver's ability to take up, process and secret bilirubin into bile [60]. The total serum bilirubin concentrations of MEXAF-treated

rats were significantly ($p<0.05$) elevated, in a dose-dependent manner, when compared to control (Table 6). As a marker of liver damage, elevation in serum total bilirubin concentration implies that MEXAF possesses hepatotoxic effects. This finding corroborates the result of the serum activities of the liver maker enzymes. This result agrees with the study by Chris-Ozoko et al. [14] which demonstrated that administration of large dose of *X. aethiopica* elicited histopathological effects on kidney and liver of experimental animals. The dose-dependent decrease in mean creatine kinase activity in the MEXAF-treated rats when compared with the control as observed in this study indicates that the extract may possess cardioprotective effects. The presence of flavonoids, antioxidant phytochemicals with lipid lowering effects in *X. aethiopica* has was detected in this study as well as reported by Holy et al. [61]. Lowering of lipids reduces the formation of atherosclerotic plaques and arterial stiffness. This can be achieved by scavenging free radicals such as superoxide anions and lipid peroxy radicals, thereby preventing the oxidation of LDL that is atherogenic [61].

Table 6. Effects of methanol extract of *Xylopia aethiopica* fruits on the liver and cardiac status

Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	T. Bil. (mg/dl)	CK (IU/L)
Group 1	15.25 ± 2.06^{a}	23.00 ± 1.83^{a}	3.93 ± 0.25^{a}	2.43 ± 0.21^{a}	84.9 ± 2.5^{d}
Group 2	22.50 ± 2.08^{b}	33.00 ± 0.82^{b}	$4.65 \pm 0.13b^{c}$	3.50 ± 0.08^{b}	74.7 ± 2.6^{c}
Group 3	31.00 ± 1.63^{c}	39.75 ± 2.50^{c}	4.45 ± 0.24^{c}	3.95 ± 0.17^{c}	67.2 ± 1.5^{b}
Group 4	39.50 ± 2.08^{d}	49.50 ± 3.11^{d}	4.85 ± 0.13^{d}	4.90 ± 0.26^{d}	53.4 ± 2.8^{a}

Data are mean ± standard deviation (n=4). Values with different superscripts in a column are statistically significant at $p<0.05$. ALT=alanine aminotransferase; AST=aspartate aminotransferase; ALP=alkaline phosphatase; CK=creatine kinase, T. Bil.=total Bilirubin.

Effect of Methanol Extract of *Xylopia aethiopica* Fruit on Kidney Status

Treatment of rats with MEXAF significantly ($p<0.05$) elevated the serum creatinine concentrations when compared with control (Table 7). The kidney is responsible for clearance of creatinine in circulation and when the kidney is damaged, serum creatinine concentration is increased; hence, creatinine concentration is a common marker of kidney function. The increase in serum creatinine concentrations in the MEXAF-treated rats indicates nephrotoxicity. This result is in line with the findings of Obhakhan et al. [62], who also reported an increase in mean serum creatinine level in rats administered cold water extract of *X. aethiopica* fruits. Also, MEXAF-treated rats had elevated serum urea concentrations when compared with control. Urea is major nitrogenous end product of protein and amino acid catabolism, produced in the liver and distributed throughout intracellular and extracellular fluid. It is also excreted out of circulation by the kidney and the serum concentration is elevated during kidney disorders such as kidney failure, blockage of the urinary tract by a kidney stone, congestive heart failure, dehydration, fever, shock and bleeding in the digestive tract. A dose and duration-dependent kidney erosion in rats treated with ground *X. aethiopica* leaves was reported by Chandrasoma et al. [63]. These reports suggest that high doses of the extract at long duration might elicit nephrotoxicity. It was observed that MEXAF-treated rats had elevated serum sodium concentrations when compared with controls. This result is also in line with the report of Obhakhan et al. [62], who reported an increase in mean serum sodium concentration of rats treated with aqueous extracts of *X. aethiopica* fruits. Sodium is a vital electrolyte that helps with electrical signals and regulation of total amount of water in the

body. Renal failure as a result of abnormal excretion of water with inadequate intake leads to an increase in sodium ion concentration in the blood. This suggests that high doses of MEXAF over a long period of time may elicit a diuretic effect, causing the excretion of more body fluids. It was also observed that MEXAF-treated rats had significant ($p<0.05$) decrease in serum potassium concentration when compared with control. Potassium is needed for the proper function of all cells, tissues, and organs in the human body. It is also crucial to proper cardiac function and plays a key role in skeletal and smooth muscle contraction, making it necessary for normal digestive and muscular function. It has been demonstrated that during acute renal failure, osmotic diuresis, and renal tubular acidosis, renal potassium loss is increased and hence, serum potassium level will decrease [64]. In general, findings of this study showed that MEXAF induces nephrotoxicity at the doses administered for 30 days.

Table 7. Effect of methanol extract of *Xylopia aethiopica* fruit on the kidney status

Group	Creatinine Conc. (mg/dl)	Urea Conc. (mg/dl)	Potassium Conc. (mg/dl)	Sodium Conc. (mg/dl)
Group 1	1.70 ± 0.03^{a}	24.50 ± 2.65^{a}	5.45 ± 0.24^{d}	4.85 ± 0.71^{a}
Group 2	1.77 ± 0.05^{a}	29.25 ± 2.22^{b}	4.55 ± 0.21^{c}	4.65 ± 0.17^{a}
Group 3	1.97 ± 0.10^{b}	35.00 ± 1.63^{c}	3.75 ± 0.24^{b}	5.55 ± 0.33^{b}
Group 4	2.15 ± 0.17^{c}	40.75 ± 2.22^{d}	3.08 ± 0.17^{a}	6.63 ± 0.17^{c}

Results expressed in means ± standard deviation (n=5); mean values with letters as superscript in a column are considered significant ($p < 0.05$).

Effects of Methanol Extract of *Xylopia aethiopica* Fruits on Glucose Concentration

Administration of MEXAF to rats reduced the glucose level significantly ($p<0.05$) in a dose-dependent manner when compared with control (Table 8). This study agrees with results of Ogbonnia et al. [65] that *X. aethiopica* fruits possess glucose-lowering effects. A polyherbal formulation of *Alstonia congensis* bark and *X. aethiopica* fruits also showed hypoglycemic effects on alloxan-induced diabetic rats [66]. The mechanisms of this anti-hyperglycemic effect could be inhibition of α-amylase activity and other key enzymes linked with hyperglycemia [45,67]. Other possible mechanisms include reduced absorption of dietary glucose [68] and increased breakdown of plasma glucose by glycolysis via activation of glycolytic enzymes.

Table 8. Effects of methanol extract of *Xylopia aethiopica* fruits on the glucose concentrations

Groups	Glucose concentration (mg/dl)
Group 1	181.75 ± 8.90^{d}
Group 2	142.25 ± 10.14^{c}
Group 3	121.75 ± 5.38^{b}
Group 4	95.75 ± 10.21^{a}

Data are mean ± standard deviation (n=6). Values with different superscripts in a column are significantly different at $p<0.05$.

Effects of Methanol Extract of *Xylopia aethiopica* Fruits on the Body Weight of Rats

To check the effect of MEXAF-treatment on body weight of rats as well as the trend across the treatment duration, the body weight of the experimental rats were measured on days 7, 14, 21 and 28. Body weight gain was calculated

as present body weight minus initial body weight at the start of the experiment. The body weight gains of MEXAF-treated rats on day 7 were significantly ($p<0.05$) reduced in a dose-dependent manner when compared with controls. A similar trend was obtained on days 14, 21 and 28 (Table 9). This result agrees with the report of Chike et al. [69] that administration of *X. aethiopica* leaves to rats for 21 days lead to reduction in daily average feed intake as well as body weight. In addition, similar findings have been reported by Woode et al. [70]. The reduction in body weight could be due to the xylopic acid content of the extract [71]. Androgens are anabolic hormones that increase the synthesis and storage of fats and carbohydrates which result in increase in body weights. In addition, a study by reported that extract of *X. aethiopica* fruit as well as xylopic acid from the fruit possess anti-androgenic effect. This might explain the mechanism of reduction in body weight gain observed in this study.

Table 9. Effects of methanol extract of *Xylopia aethiopica* fruits on the body weight gains

Groups	Body weight gain (g)			
	Day 7	Day 14	Day 21	Day 28
Group 1	29.89 ± 2.13^{eA}	36.74 ± 1.75^{eB}	43.41 ± 3.01^{dC}	52.15 ± 2.95^{dD}
Group 2	23.02 ± 2.35^{cA}	26.38 ± 2.15^{cB}	35.34 ± 1.82^{cC}	40.15 ± 2.72^{cD}
Group 3	13.03 ± 2.29^{bA}	17.12 ± 2.03^{bB}	23.67 ± 3.57^{bC}	26.18 ± 2.68^{bC}
Group 4	6.97 ± 1.13^{aA}	10.23 ± 1.74^{aB}	13.22 ± 2.83^{aC}	17.69 ± 2.13^{aD}

Data are mean ± standard deviation (n=6). Values with different small letters of the alphabets as superscripts in a column are significantly different at $p<0.05$. Values with different capital letters of the alphabets in a row are significantly different at $p<0.05$.

Effects of Methanol Extract of *Xylopia aethiopica* Fruits on the Organ/Total Body Weight Percentage

Table 10 represents the effect of MEXAF on organ/total body weight percentage for liver, heart, and kidney of rats treated with MEXAF. There was a dose-dependent decrease in organ/total body weight percent for liver and kidney while there was no significant difference in heart/total body weight percent in the MEXAF-treated rats when compared with control. This finding agrees with the report of Chris-Ozoko et al. [14] that administration of the aqueous extract of *X. aethiopica* fruits significantly ($p<0.05$) decreased the weights of liver and kidney of rats. This finding also agrees with the results of serum levels of markers of nephro- and hepato-toxicity obtained in this study which indicate that MEXAF may be toxic to the liver and kidney at the dose administered for 30 days.

Table 10. Effect of methanol extract of *Xylopia aethiopica* fruits on the organ/body weight percentage of rats

Groups	Liver/body weight percentage (%)	Heart/body weight percentage (%)	Kidney/body weight percentage (%)
Group 1	5.54 ± 0.30^{d}	0.38 ± 0.07^{a}	0.71 ± 0.81^{bc}
Group 2	4.50 ± 0.29^{c}	0.43 ± 0.03^{b}	0.69 ± 0.11^{c}
Group 3	3.89 ± 0.33^{b}	0.50 ± 0.03^{c}	0.62 ± 0.07^{a}
Group 4	3.13 ± 0.15^{a}	0.60 ± 0.03^{d}	0.67 ± 0.02^{b}

Data are mean ± standard deviation (n=6). Values with different superscripts in a column are significantly different at $p<0.05$.

CONCLUSION

The findings from this study showed that 30-day daily oral administration of graded dose of MEXAF to rats demonstrated improved haematological and antioxidant status and reduced lipid peroxidation. Also, hypoglycemic, hypolipidemic as well as body and organ weights lowering effects were observed. On the other hand, dose-dependent toxicity to the liver and kidney was observed. Meanwhile, 24-hour acute toxicity study showed no sign of toxicity and no significant body weight change up to 2900 mg/kg body weight of MEXAF. Being an herb that is widely used as spice in delicacies in many parts of the world, it is recommended that long-term consumption of high dose of *Xylopia aethiopica* fruits should be avoided to prevent liver and kidney damage; however, low dose consumption is seen to be safe.

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