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Effect of Ethanol Extract of *Xylopia aethiopica* Fruit on Oxidative Stress Indices of Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The use of *Xylopia aethiopica* fruit in folklore medicine is on the increase without caution of its toxicity.
Aim: This present study tends to assess its effect on the oxidative stress biomarkers of Wistar rats.
Methodology: The fruits of *Xylopia aethiopica* were air-dried and extracted by Soxhlet extractor using ethanol as solvent. The median lethal dose (LD_{50}) of the extract was assessed using standard method. Thirty adult Wistar rats were divided into five groups of six rats each. Animals in groups 1, 2, 3, and 4 were treated with 130, 259, 389 and 518 mg/kg body weight of *X. aethiopica* fruit extract respectively, while those in group 5 received normal animal feeds and water only. The administration was done once daily for 28 days via oral route. Oxidative stress indices were measured using standard methods.
Results: A significant decline was observed in the antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) activities in experimental animals compared with those in the control group (*P<0.05)*. In the same vein, a significant reduction was observed in the concentration of reduced glutathione in experimental animals compared with those in the control

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group (*P<0.05)*. Lipid peroxidation was however observed to increase when experimental animals were compared with those in the control group. The elevation in lipid peroxidation was significantly different when animals treated with higher doses of 259, 389 and 518 mg/kg body weight of extract were compared with those in the control group (*P<0.05)*.

Conclusion: The adverse perturbation of antioxidant indices by *Xylopia aethiopica* fruit is suggestive that it could induce oxidative stress and thus unhinged the immune system. Oxidative stress has also been implicated in several diseases thus the consumption of *Xylopia aethiopica* fruit as well as its use in folklore medicine should be discouraged especially in high doses due to its toxic nature.

Keywords: Immune system; oxidative stress; toxic nature; Xylopia aethiopica fruit.

1. INTRODUCTION

Induction of oxidative stress elicits free radicals such as nitric oxide (NO), superoxides anions (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), organic hydroperoxide (ROOH) via the release of reactive oxygen species (ROS) [1]. These ROS are highly fickle atoms because they are known to contain a lone pair of electrons in their outermost shells. They are known to be involved in processes like aging, carcinogenesis, mutagenesis, and so on, which is caused by their in-built ability to induce cellular obstruction on DNA [2]. Some operations of human elicit ROS both endogenously and exogenously, which result in enervating some disease conditions. These diseases could arise from the adverse correlation in the induction of oxidative stress and the tendency of the living system to alleviate the free radicals generated sequel to the induction of stress [3]. Worthy of note is the point that the living system is built to alleviate the destructive nature of the radicals elicited *via* the natural antioxidants that are enzymatic in nature [4]. However, synthetic antioxidants also exist which are absorbed by the body to elevate the activities of the natural ones but have been observed to have health-related risk [2]. This has resulted in the renewal of vigor in the search for antioxidants from plant sources which will work well with the living system and also increase the ability of natural antioxidants with the purpose of preventing the health issues ascribed to the synthetics antioxidants [5].

Xylopia aethiopica has a great patronage in both nutrition and ethnomedicine. The plant which also known as African Negro pepper, is popular among traditional medicine practitioners and traditional birth attendants (TBA) who utilize the fruit preparations to cause the discharge of placental after a woman has giving birth [6]. A preparation of the stem bark or fruit is helpful in the management of bronchitis, stomach aches, asthma, and dysenteric conditions [6]. The seed extract is helpful as a vermifuge for roundworms [7]. Several postnatal women eat the aqueous preparation of the fruit for its perceived antiseptic properties. Some of the women have been reported to sometimes come to the hospitals with characteristics which suggest complications in organ [8]. Medicinal plant extracts with a therapeutic property has the tendency of wrong prescription and sometimes, overdosed. The fact that *Xylopia aethiopica* is a natural product does not automatically confers on it safety and might be risky to its consumers. Chemical ingredients of the plant are perceived to be useful in preventing and managing cancerous tumors [9]. *Xylopia aethiopica* fruit is known to have alkaloids, terpenoids, flavonoids, and organic oils [10,11].

Xylopia aethiopica is characterized with numerous chemical components with various medicinal potentials [13]. The chemical components of this plant have been investigated to include saponins, sterols, carbohydrates, glycosides, mucilage, acidic compounds, tannins, balsams, cardiac glycosides, volatile aromatic oils, phenols [8,14,15], alkaloids, rutin and fixed oils [16,17]. The plant has also be known to contain vitamins such as vitamin A, vitamin B, vitamin C, vitamin D, and vitamin E, and proteins as well as several minerals such as copper, manganese and zinc [15,17]. The impact of the fruit on body weight and glucose concentration of animals has been reported [18]. The fruit has also been reported to induce dyslipidemia [19], hepatotoxicity [20] as well as renal toxicity [21]. This present study focused on examining its impact on the oxidative stress biomarkers of Wistar rats.

Fig. 1. *Xylopia aethiopica* Fruit [12]

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

The fruits of *Xylopia aethiopica* were sourced from a market in Aba, Abia State. They were identified and authenticated by Prof. Margaret Bassey of Botany and Ecological Studies Department, University of Uyo. It was assigned a voucher number of UU/PH/4e and deposited in the Herbarium of the Department of Pharmacognosy and Natural Medicine, University of Uyo, Akwa-Ibom State, Nigeria.

2.2 Extraction of Plant Materials

Extraction of the plant was carried out in the Post-graduate Laboratory of Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria. It was extracted based on the outlined method in Ogbuagu et al. [13]. The fruits were rinsed under flowing tap water to eliminate contaminants and air-dried. The plant material was milled by laboratory blender. The pulverized plant material was macerated in 250 mL of 99.8% ethanol (Sigma Aldrich) contained in a flask attached to a Soxhlet extractor coupled with condenser and heating mantle (Isomantle). It was then poured into the sample holder (thimble) and inserted in the apparatus. The side arm is lagged with glass wool. The mixture was heated using the heating mantle (Isomantle) at 60 °C and as the temperature rises it starts to evaporate, going via the extractor to the condenser. The condensate dripped into the reservoir housing the thimble. As soon as the solvent gets to the siphon it emptied itself into the flask and the process repeats itself. The process goes on until it is exhaustively extracted. The process runs for a total of 13 hours. As soon as it was set up, it was allowed to run without interruption as long as water and power supply were not interrupted. The apparatus was switched on and off and overnight running was not allowed, and the time for the complete process split over some days. The extract was poured into 1000 mL beaker and concentrated to dryness in water bath (A3672-Graffin Student Water Bath) at 35 °C. The total weight of the marc (residue) and the concentrated extract were noted. Several days was spent on the entire process. The evaporated extract was kept in the refrigerator until when the need for it arise.

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

The median lethal dose (LD_{50}) of the extract was determined using albino mice according to the method described by Airaodion et al. [22]. This method involves two phases:

In Phase one, five groups containing five mice each weighing between 20 g and 27g were fasted for 18 hours. They were respectively treated with 1000 mg/kg, 2000 mg/kg, 3000 mg/kg, 4000 mg/kg and 5000 mg/kg body weight via intraperitoneal (i.p) route and were monitored for visible signs of toxicity and mortality for 24 hours. A dosage of 1000 mg/kg recorded 0% mortality while 2000 mg/kg, 3000 mg/kg 4000 mg/kg and 5000 mg/kg recorded 100% mortality within 24 hours. Based on the value of phase one, phase two was conducted.

In Phase two, twenty-five albino mice weighing between 20 and 27g were grouped into 5 of 5 mice per group and were fasted for 18 hours. Each group was administered 1200 mg/kg, 1400 mg/kg 1600 mg/kg, 1800 mg/kg and 2000 mg/kg body weight intraperitoneally (i.p) and was observed for physical signs of toxicity and mortality within 24 hours. 1200 mg/kg recorded 0% mortality while 1400 mg/kg, 1600 mg/kg, 1800 mg/kg and 2000 mg/kg recorded 100% mortality within 24 hours. The LD_{50} was computed as geometrical means of the maximum dose yielding 0% mortality (a) and the minimum dose yielding 100% death (b).

$$LD_{50} = \sqrt{ab}$$

2.4 Experimental Design

Thirty adult Wistar rats used in this study were purchased from the University of Uyo, Nigeria. They were allowed to acclimatize for seven days prior to the start of the treatment. The weights were determined and were separated into five groups of six rats each. Groups A, B, C, D served as the experimental groups, while group E served as the control. Animals in group A were exposed to 130 mg/kg body weight (10% of LD_{50}) of *X. aethiopica* fruit extract, those in group B were treated with 259 mg/kg body weight (20% of LD_{50}) of *X. aethiopica* fruit extract, those in group C were exposed to 389 mg/kg body weight (30% of LD_{50}) of *X. aethiopica* fruit extract, those in group D were treated with 518 mg/kg body weight (40% of LD_{50}) of *X. aethiopica* fruit extract, while those in group E (control) received normal animals feeds and water only. The treatment was done once daily for 28 days via oral route. After 28 days treatment, the animals were sacrificed under ether anaesthesia in a desiccator after an overnight fast. Blood was taken from the rats through cardiac puncture.

2.5 Determination of Oxidative Stress Indices

2.5.1 Determination of superoxide dismutase (SOD) activity

Principle: This procedure involves production of superoxide radical of riboflavin and its detection by hydroxylamine hydrochloride. The nitrite reacts with sulphanilic acid to yield diazonium compound which then reacts with naphthylamine to yield red azo compound whose absorbance is measured at 543 nm.

This assay was done according to the method of Mohammad et al. [23] in which 1.4 mL aliquot of the reaction mixture involved 1.1 mL of 50 mM phosphate buffer (pH 7.4), 0.075 mL of 20 mML^{-1} methionine, 0.4 mL of 1% (v/v) Triton X-100, 0.075 mL of 10 mM hydroxylamine and 0.1 mL of 50 mM EDTA. The aliquot (1.75 mL) was added to 0.1 mL of the sample and incubated at 30^{o}C for 15 min. This was followed by addition of 80 µL of 50 µM riboflavin and then the tubes were exposed for 9 minutes to 200 watts Philip lamp. After exposure time, 1 mL of Greiss reagent was added and the absorbance of the colour formed was measured at 543 nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50 % of nitrate formation under the assay condition.

2.5.2 Determination of catalase (CAT) activity

Principle: The method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2 with the formation of perchloric acid as unstable intermediate. The acetate produced is measured colorimetrically at 610 nm.

This was assayed by the method described by Sinha [24]. The reaction mixture (1.5 mL) contained 1.0 mL of 0.01 M phosphate buffer, 0.1 mL of the sample and 0.4 mL of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 mL dichromate acetic acid reagent (5% potassium dichromate and glacial acetic acid mixed in 1:3 ratio). Then, the absorbance was measured at 610 nm. CAT activity was expressed as µmol of H_2O_2 consumed/min/mg protein.

2.5.3 Determination of glutathione peroxidase (GPx) activity

Principle: The activity of GPx was determined by measuring the decrease in GSH concentration after incubating the sample in the presence of hydrogen peroxide and sodium azide.

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

The activity of glutathione peroxidase was assayed by the method described by Rotruck et al*.* [25]. The reaction mixture contained 0.2 mL of 0.4 M Tris- buffer, pH 7.0, 0.2 mL of EDTA, and 0.1 mL of 10 mM sodium azide, 0.2 mL of 10 mM glutathione and 0.1 mL of 0.2 mM. H_2O_2. The content was incubated at 37 ^{o}C for 10 minutes. The reaction was terminated by the addition of 0.4 ml 10% (v/v) TCA and centrifuged at 5000

rpm for 5 minutes. The supernatant was assayed for glutathione by Ellman's method. Exactly 3.0 mL disodium hydrogen phosphate solution and 1.0 mL of DNTB reagent were added to 2.0 mL of the supernatant. The standard was taken and treated in similar manner. The absorbance was read at 412 nm and expressed in terms of glutathione consumed /min/mg protein.

2.5.4 Determination of lipid peroxidation

Principles: Lipid peroxidation in the supernatant fractions was determined spectrophotometrically by assessing the concentration of thiobarbituric acid reactive substances (TBARS) as described by Varshney and Kale [26]. The results were expressed in malondialdehyde (MDA) formed relative to an extinction coefficient of 1.56 x 10^6 mol/cm.

Procedure: Acetic acid 1.5 mL (20%; pH 3.5), 1.5 mL of 0.8% thiobarbituric acid and 0.2 mL of 8.1% sodium dodecylsulphate was added to 0.1 mL of the sample and heated at 100 ^{o}C for 60 min. After centrifugation at 1200×*g* for 10 min, the organic layer was separated and absorbance measured at 532nm using a spectrophotometer. Malondialdehyde (MDA) is an end product of lipid peroxidation, which reacts with thiobarbituric acid to form pink chromogen–thiobarbituric acid reactive substance. It was calculated using a molar extinction coefficient of 1.56×10^6 mol/cm and expressed as nanomoles of MDA/tissue. The concentration of MDA (nmol/ml) was calculated by using the formula:

$$\text{Concentration of the test} = \frac{\mathbf{Abs\ (test) - Abs\ (blank)}}{\mathbf{1.56 \times 10^6}}$$

2.5.5 Determination of glutathione concentration

Principles: Glutathione (reduced) was measured according to the method of Jollow et al. [27]. Reduced glutathione (GSH) forms the bulk of non-protein sulfhydryl groups. This method is based on the formation of relatively stable yellow colour when Ellman's reagent is added to a sulfhydryl compound, 2-nitro-5-thiobenzoic acid, the chromophoric product resulting from the reaction of Ellman's reagent with reduced glutathione.

Procedure: Equal quantities of the sample and 10% trichloroacetic acid were mixed and centrifuged at 4000 x g for 15 minutes to separate the proteins. To 0.5 mL of the supernatant, 4.5 mL of Ellman's reagent was added. The mixture was vortexed and the absorbance read at 412 nm within 15 min.

2.6 Statistical Analysis

Data were subjected to analysis of variance using Graph Pad Prism. Results were presented as Mean ± Standard Error of the Mean (SEM). One-way analysis of variance (ANOVA) was used to compare the mean, followed by Tukey's post hoc test. Differences between means were considered to be significant at $p<0.05$.

3. RESULTS

3.1 Median Lethal Dose (LD_{50}) Result

The visible signs of toxicity of *X. aethiopica* fruit extract observed in this study are excitation, decreased motor activity, paw licking, increased respiratory rate, gasping and coma which could be followed up by death. In the first phase of the median lethal dose determination, no death was observed in the group administered 1000 mg/kg body weight of *X. aethiopica* fruit extract. However, all the animals died in the groups exposed to 2000, 3000, 4000, and 5000 mg/kg body weight of *X. aethiopica* fruit extract respectively (Table 1). In the same vein, in the second phase of medial lethal dose determination, no death was recorded in the group treated with 1200 mg/kg body weight of *X. aethiopica* fruit extract while 100% mortality was recorded in the groups treated with 1400, 1600, and 1800 mg/kg body weight of *X. aethiopica* fruit extract respectively as presented in Table 1.

The median lethal dose (LD_{50}) was computed as geometrical average of the maximum dose yielding 0% death (a) and the minimum dose yielding 100% death (b).

$$LD_{50} = \sqrt{ab}$$

Where a = 1200 mg/kg

b = 1400 mg/kg

LD_{50}= 1296.15 mg/kg

3.2 Effect of Extracts of *Xylopia aethiopica* Fruit on Oxidative Stress Indices of Animals after 28 Days of Treatment

The impact of extract of *Xylopia aethiopica* fruit extract on oxidative stress parameters of animals

after 28 days of treatment is presented in Figs. 2-6. A significant reduction was seen in the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) in experimental animals compared with those in the control group (*P<0.05)*. In the same vein, a noticeable reduction was seen in the concentration of reduced glutathione in experimental animals compared with those in the control group (*P<0.05)*. Lipid peroxidation was however observed to increase when experimental animals were compared with those in the control group. The elevation in lipid peroxidation was significantly different when animals treated with higher doses of 259, 389 and 518 mg/kg body weight of extract were compared with those in the control group (*P<0.05)*.

Table 1. The Median lethal dose (LD_{50}) of *Xylopia aethiopica* fruit extract

Study Phase/ (Animal)	Dosage of Extract (mg/kg) b.w	No of Mice per Group	No. of Death Recorded	% Mortality
PHASE ONE				
I	1000	5	0	0
II	2000	5	5	100
III	3000	5	5	100
IV	4000	5	5	100
V	5000	5	5	100
PHASE TWO				
I	1200	5	0	0
II	1400	5	5	100
III	1600	5	5	100
IV	1800	5	5	100
V	2000	5	5	100

LD_{50}= 1296.15 mg/kg

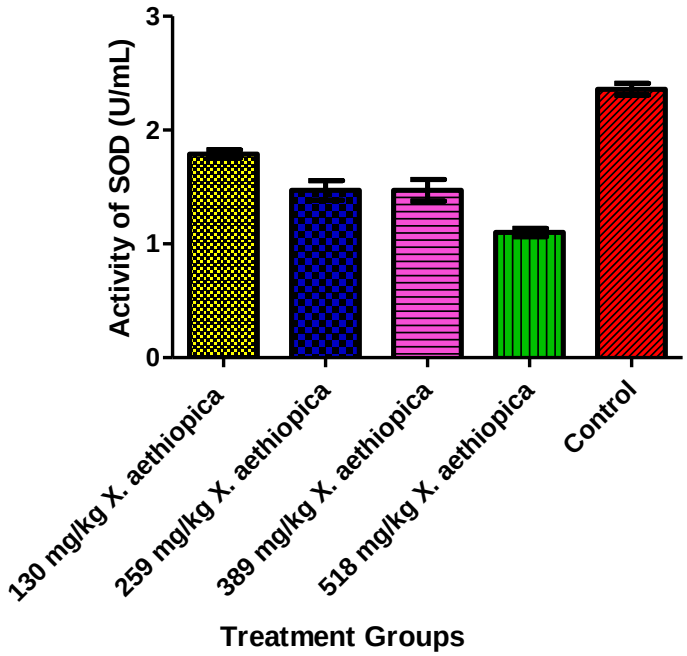

Fig. 2. Effect of *X. aethiopica* fruit extract on the Activity of Superoxide Dismutase (SOD) in Animals after 28 days of Treatment

Each bar represent mean ± SD of treatment groups with n = 6

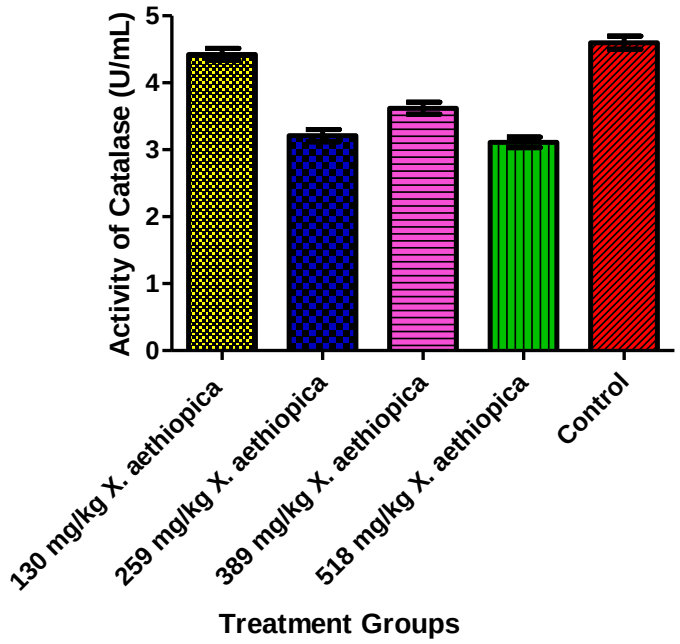

Fig. 3. Effect of *X. aethiopica* fruit extract on the Activity of Catalase in Animals after 28 days of Treatment

Each bar represent mean ± SD of treatment groups with n = 6

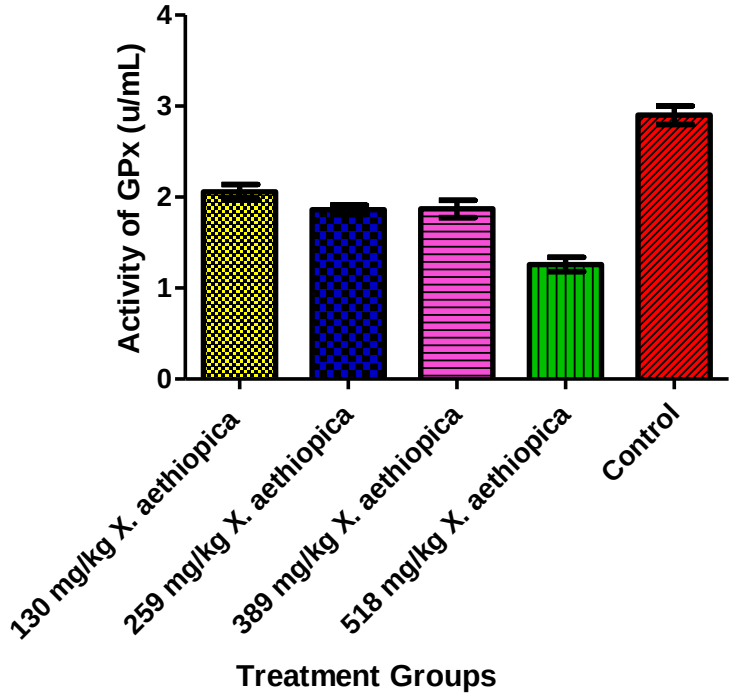

Fig. 4. Effect of *X. aethiopica* fruit extract on the Activity of Glutathione Peroxidase (GPx) in Animals after 28 days of Treatment

Each bar represent mean ± SD of treatment groups with n = 6

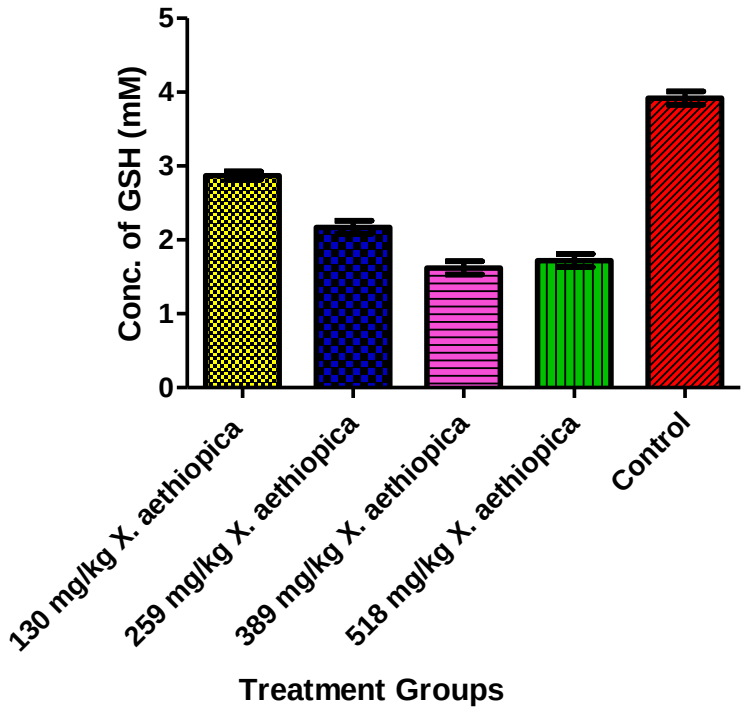

Fig. 5. Effect of *X. aethiopica* fruit extract on the Concentration of Reduced Glutathione (GSH) in Animals after 28 days of Treatment

Each bar represent mean ± SD of treatment groups with n = 6

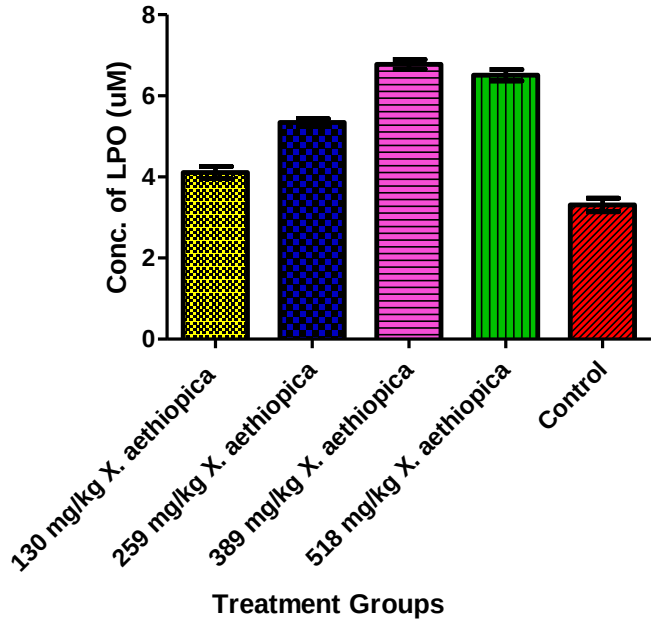

Fig. 6. Effect of *X. aethiopica* fruit extract on Lipid Peroxidation of Animals after 28 days of Treatment

Each bar represent mean ± SD of treatment groups with n = 6

4. DISCUSSION

The acute toxicity study of *X. aethiopica* fruit extracts led to 100% death at a dose of 1400 mg/kg bodyweight and above. This reveals that this fruit could be greatly toxic. The visible symptoms of toxicity seen in the animals were excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma and death.

In this study, a noticeable decrease was recorded in superoxide dismutase (SOD) activity in animals exposed to *Xylopia aethiopica* fruit extract at all tested doses when compared with control animals at *P<0.05* (Fig. 2). This suggested that *Xylopia aethiopica* fruit extract might be toxic and could possibly play a role in the production of free radicals. This agreed with the results of Nnodim et al. [28] who observed that *Xylopia aethiopica* fruit induced oxidative stress.

Observation from this study showed that treatment with ethanol extract of *Xylopia aethiopica* fruit was observed to greatly reduce the activity of catalase in animals treated with 259, 389 and 518 mg/kg of *Xylopia aethiopica* fruit extract when compared to animals in the control group at *P<0.05* (Fig. 3). This might be an indication that *Xylopia aethiopica* fruit extract at these doses might be toxic and possibly lead to induction of oxidative stress. This agreed with the findings of Nnodim *et al*. [28], who reported that *Xylopia aethiopica* fruit generated free radicals in treated animals.

Treatment with extract of *Xylopia aethiopica* fruit significantly down-regulated glutathione peroxidase (GPx) activity at all tested doses (Fig 4). The biological role of glutathione peroxidase is to change hydroperoxides of lipid to their respective alcohols and release hydrogen peroxide to form water [29,30]. This is in consonance with the results of Nnodim et al. [28], who reported that *Xylopia aethiopica* fruit significantly reduced the activity of glutathione peroxidase of treated animals. Glutathione peroxidase is one of the enzymatic antioxidant with the capacity to defend the system and combat oxidative stress [31]. The reduction in the activity of glutathione peroxidase observed in this study might be an indication that *Xylopia aethiopica* fruit has the propensity to induce oxidative stress.

In this study, administration of ethanol extract of *Xylopia aethiopica* fruit resulted in a decline in the concentration of reduced glutathione at all doses when compared with control group (Fig. 5). The noticeable decrease observed in the reduced glutathione level in this study might be an indication that *Xylopia aethiopica* fruit enhances the conjugation of GSH with acetaldehyde [32]. This may have resulted from the direct reactive oxygen species (ROS) producing potential of *Xylopia aethiopica* fruit and/or a decrease in GSH synthesis.

In this study, administration of *Xylopia aethiopica* fruit extract was seen to have noticeably led to a surge in the malondialdehyde (MDA) levels indicating increased peroxidation and catabolism of the antioxidant defense mechanisms. Malondialdehyde is a product of lipid peroxidation of polyunsaturated fatty acids [33,34]. Furthermore, visible destruction of tissues in lipid peroxidation caused by free radicals could cause membrane injury followed by decrease in the membrane fluid content. This is in line with the study of Nnodim et al. [28], who observed that *Xylopia aethiopica* fruit significantly elevated lipid peroxidation (Fig. 6).

The depletion in antioxidant enzymes activities seen in this study agrees with the work of Somnez et al. [35]. The biochemical mechanism by which *Xylopia aethiopica* caused reduction in enzymatic antioxidants is currently unclear. It might be postulated that the consumption of this plant extract could cause oxidative stress and hence generate free radicals which could result in membrane destruction through lipid peroxidation and protein oxidation [36-38]. Thus, the reduction in the activities of superoxide dismutase (SOD), catalase (CAT), as well as glutathione peroxidase (GPx), elevated lipid peroxidation. This became visible by the increased level of MDA observed in this present investigation.

5. CONCLUSION

The adverse perturbation of antioxidant indices by *Xylopia aethiopica* fruit is suggestive that it could induce oxidative stress and thus negatively impact the immune system. Oxidative stress has also been implicated in different diseases, thus the consumption of *Xylopia aethiopica* fruit as well as its use in folklore medicine should be discouraged especially in high doses due to its toxic nature.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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