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Methanolic Extract of *Xylopia aethiopica* Ameliorates Acetaminophen-Induced Liver Damage in Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author OSF designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author BFO managed the analyses of the study and literature searches. 'Ikanone CEOI managed the tissue analysis and the references. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Aims: To examine the ameliorative effect of the methanolic extract of *Xylopia aethiopica* in acetaminophen-induced hepatic damage male Wistar rats.
Study Design: Twelve male rats were randomly grouped into four. "A" = Control, "B" = Paracetamol-induced hepatotoxic (PCM-IHT), "C" = 200 mg/kg b.w treated and "D" = 400 mg/kg b.w treated.
Place and Duration of Study: Department of Biochemistry, Lagos State University, Ojo Lagos, Nigeria between November 2011 and March 2012.
Methodology: "A" received 3.0 ml of distilled water, "B" received 350 mg/kg b.w/day of Paracetamol, "C" after been induced (B) was treated with 200 mg/kg b.w/day and "D" after been induced (B) was treated with 400 mg/kg b.w/day of the plant extract. Two days after the last treatment, the serum was used to assay for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and acid phosphatase

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(ACP) using Randox commercial enzyme kits.
Results: The activities of ALT and AST reduced from 85.83±3.4 U/L and 148.2±9.75 U/L in the induced groups to 39.33±1.67 U/L and 68.84±5.72 U/L when treated with 200mg/kg and 35.67±2.46 U/L and 38.26±3.0 U/L when treated with 400 mg/kg respectively. There was no significant difference when 200 and 400 mg/kg b.w treatments were compared with the control (ALT = 38.0±0.40 U/L and AST = 53.71±2.70 U/L) *(P>.05)*. Similarly, the activities of ALP and ACP reduced from 240.8±3.98 U/L and 152.2±7.91 U/L in the induced groups to 126.0±7.62 U/L and 56.17±1.55 U/L when treated with 200 mg/kg and 188.5±4.71 U/L and 93.55±2.18 U/L when treated with 400mg/kg respectively. The 200 and 400 mg/kg b.w treatments appeared to be significantly different from each other but with former being positively correlated with the control (ALP = 135.8±6.74 U/L and ACP = 61.96±4.12 U/L).
Conclusion: The methanolic extract of *Xylopia aethiopica* possessed hepatoreparative property especially in acetaminophen-induced hepatotoxicity; however, its toxicity needs to be examined.

Keywords: Xylopia eathiopica; acetaminophen; hepatoreparative; hepatotoxicity; alanine aminotransferase; aspartate aminotransferase; alkaline phosphatase; acid phosphatase.

ABBREVIATIONS

PCM-IHT: *Paracetamol-Induced Hepatotoxic;* ***b.w:*** *body weight;* ***ALT:*** *Alanine aminotransferase;* ***AST:*** *Aspartate aminotransferase;* ***ALP:*** *Alkaline phosphatase;* ***ACP:*** *Acid phosphatase;* ***SGOT:*** *Serum glutamate oxaloacetate transaminase;* ***SGPT:*** *Serum glutamate pyruvate transaminase;* ***GGTP:*** *Gamma glutamyltranspeptidase/transferase;* ***5NT:*** *5´-nucleotidase;* ***H and E:*** *Hematoxylin and Eosin*

1. INTRODUCTION

Natural remedies from medicinal plants are considered to be generally effective and safe alternative treatments for diseases, though they could pose threat in the visceral tissues if incessantly taken because of the lack of rationalization and quantification. Well above average of the world populations rely heavily on the use of traditional medicine which is predominantly based on plant materials due to its cost effective, availability and potency [1]. *Xylopia aethiopica is* one of the most pungent spices plants that are native to the low land rain forests and moist fringe forests in the savanna zones and coastal regions of Africa [2,3,4]. It has a savory aroma and has been used in Ayurvedic medicine in the treatment of headache, neuralgia, cough, carminative, purgative, rheumatism, bronchitis, dysentery, biliousness, female sterilization, abortifacient and skin infections [5,3,4,6,7]. Monoterpenes and sesquiterpenes have been found as components of the volatile oil of this plant [8,9]. Phytoanalysis of the organic and aqueous extracts of this plant had revealed the presence of carbohydrates, glycoside, flavonoids, saponins, tannins, phytosterols and alkaloids [10,11]. Although some extracts of this plant have antioxidant properties, others have cytotoxic effects on a wide range of cancer cell lines [12,13] and one of its main components responsible for this cytotoxic effect, as isolated by Choumessi [14] was ent-15-oxokaur-16-en-19-oic acid.

Liver is the fundamental organ in the metabolism and detoxification of xenobiotics (drugs and toxins). Thus, drugs affect the liver more frequently than any other organ and this places liver at greater risk for damages induced by toxic substances [15]. Xenobiotics in the liver via the portal system undergo complex metabolic processes through phases of drug metabolisms to be converted to hydrophilic substances, readily soluble in the blood stream and easily eliminated thereafter through conjugation among other processes [16]. Certain drugs are converted to such reactive molecules capable of causing peroxidation of the unsaturated fatty acyl components of the cell membrane, reacting with the extracellular membrane proteins, binding to the cell receptors and developing affinity for the membrane sterols [17,18,19]. Paracetamol (acetaminophen) is an analgesic drug capable of inhibiting prostaglandin at the central nervous system thereby reducing the pains and fever [20]. It is usually a liver-metabolized product of prodrugs, acetanilide/phenacetin [21,22]. These prodrugs can undergo hydrolysis in the liver to form aniline-derivatives such as acetaminophen-N-hydroxamide and N-acetylimidoquinone, which are hepatotoxic and nephrotoxic substances due to their affinity for thiol group of cysteine amino acid in globular proteins found in these organs [21]. From the work of Van den Heuvel et al. [23], a baseline combined average of 1.959 mg/kg body weight of acetaminophen was estimated as LD_{50}. By this information, the acute oral toxicity of acetaminophen is likely to be low according to the harmonized integrated hazard classification system. Higgins et al, [24] and Van den Heuvel et al. [23] by oral administration of acetaminophen, have observed ptosis, lethargy, abnormal gait, lacrimation, sedation, narcosis and paralysis in laboratory animals. Acetaminophen, among other drugs, when administered in excess may induce oxidative damage in the liver cells and this will lead to cellular necrosis [25]. Consequently, certain enzyme markers have been used to evaluate the extent of damages to the liver. Among the marker enzymes are serum glutamate oxaloacetate transaminase (SGOT)/aspartate transaminase (AST), serum glutamate pyruvate transaminase (SGPT)/alanine transaminase (ALT), γ-glutamyltranspeptidase/transferase (GGTP/GGT), 5´-nucleotidase (5NT), alkaline phosphatase (ALP) and acid phosphatase (ACP) [26]. These enzymes generally determined the protein turnover rate in the disease stage in tandem with shuttling of ketoacidic molecules. Hepatonecrosis is majorly characterized by increase in the activities of the aforementioned enzymes especially ALT and ALP both in the organ and serum and subsequent release of bilirubin.

Hepatoreparative agents are indeed needed to complement the defence mechanism of this organ. To this effect, extracts from medicinal plants had gained much impact as antioxidants, antimicrobial, antinfarction, antisclerosis and antinecrosis. This work is therefore aimed at repairing the liver-damage caused by acetaminophen administration in Wistar rats using methanolic extract of *Xylopia aethiopica*. Furthermore, the interplay of AST, ALT, ALP and ACP are used as an index to assess the nature of liver damage and repair offered by the methanolic extract of this medicinal plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Xylopia aethiopica* were obtained as green foliage from a local herb seller at Iyana Iba market, Ojo Local Government Area, Lagos State Nigeria. The plant was authenticated at the Department of Botany, Faculty of Science, Lagos State University, Ojo Lagos State Nigeria. The leaf sample was oven-dried at 40^{o}C for seven days.

2.2 Preparation of Extract

As described by Adebayo et al. [27] the leaves of *Xylopia aethiopica* were collected and dried for seven days after which they were blended into fine powder and 400g of this blended leaves were extracted with 95% methanol (BDH) by soaking for 72 hours in an air-tight jar. The methanolic content was evaporated in an oven at 25^{o}C and gave a yield of 7.5g

2.3 Experimental Animals

Twelve male Wistar strain rats obtained from the University of Agriculture, Abeokuta, Ogun State, Nigeria weighing between 87-100g were used for this research. Animals were maintained in 12 hour light: 12 hour dark at a controlled temperature (25 ± 2^{o}C), humidity (60 ± 5%) and kept in the animal house of the Department of Biochemistry, Lagos State University, Lagos State. The animals were allowed to acclimatize for one week in a plastic cage. Feed and water were given *ad libitum*. All animals were treated in accordance with the recommendations of Institute for Laboratory Animal Research Guides for the Care and Use of Laboratory Animals [28,29].

2.4 Experimental Design

The model described by Chakraborti and Handa [30] was employed with some modifications. The rats were divided into four groups of three rats each. The animals in group A served as control and were given 3.0ml of distilled water once per day for 2 days by oral gavage. The animals in group B (untreated) were administered Paracetamol once per day at 350 mg/kg body weight (b.w) by oral gavage for 2 days and were tagged Paracetamol-induced hepatotoxic (PCM-IHT) group. The animals in groups C were administered with paracetamol once per day at 350 mg/kg body weight for 2 days and 200 mg/kg body weight of methanolic extract of *Xylopia aethiopica* were orally administered once per day for a week and were tagged 200 mg/kg b.w treated. The animals in group D were also administered once per day with 350 mg/kg body weight of Paracetamol for 2 days and 400 mg/kg body weight of methanolic extract of *Xylopia eathiopica* were orally administered once per day for a week.

2.5 Blood Collection and Preparation of Serum

Two days after the last treatment, the rats were anaesthetized with chloroform (SIGMA) prior to dissection. The blood was obtained by cardiac puncture and allowed to clot for 10 minutes. The blood was centrifuged in a sample bottle at 3000 rpm for 15 minutes at room temperature. The serum obtained was pipetted and used to analyze for the presence of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and acidic phosphatase using Randox commercial kits.

2.6 Histoarchitectural Study

Immediately after the collection of blood, the liver tissue was carefully excised, chilled and perfused with ice-cold saline (0.8% w/v) and patted dry. The liver tissue was weighed and the percentage proportion by mass was estimated. The liver tissue was fixed in 10% (w/v) buffered formalin and embedded in paraffin. A thin sectional cut of 4-5µm across the inner linking flap of the two lobes was made. The microtome was stained with hematoxylin and eosin *(H and E)* stains and examined under a compound microscope *(magnification = x100)*

for histoarchitectural changes [31]. Severity of lesions was graded according to the criteria described by Shackelford et al, [32]. Degree of lesions was graded from zero to five depending on severity: 0 = no lesion; 1 = minimal lesion (< 1%); 2 = slight lesion (1-25%); 3 = moderate lesion (26-50%); 4 = moderately severe/high lesion (51-75%); 5 = severe/high lesion (76-100%). In order to compare the degree of histopathological lesions in different groups, mean histopathological scores were used for further analysis and calculated by dividing the sum of the score per grade of affected rats by the total number of examined rats.

2.7 Statistical Analysis

Data obtained were analyzed using Tukey HSD one way ANOVA with GraphPad Prism (Version 5.0). Similarly, ALT/AST and ACP/ALP were compared using two ways ANOVA analysis. All data were presented as mean±SEM. The means were considered significant at *P<.05*.

3. RESULTS

Antihepatotoxic activity of the methanolic extract of *Xylopia aethiopica* was examined in this work following hepatic damage induced by oral administration of acetaminophen at 350 mg/kg b.w. Hepatotoxic was confirmed as a result of colour change observed in the liver of paracetamol-induced hepatotoxic (PCM-IHT) group as compared to the control. Similarly, there was a higher percentage proportion by mass of the liver of PCM-IHT (5.33) when compared to other groups, Table 1. Furthermore, all enzymes studied in this work were essentially and significantly higher in PCM-IHT group as compared to the control *(P<.05)*.

Table 1. Acetaminophen increased the liver-body weight percentage proportion in hepatotoxic male Wistar rats *(weight range = 87 – 100g, Data were presented as mean±SEM, n = 3)*

Group/Treatment	Body weight (g)	Liver weight (g)	% proportion of liver by mass
A (Control)	92.40±2.42	4.32±0.23	4.68
B (PCM-IHT)	94.24±1.49	5.02±0.91	5.33[†]
C (200 mg/kg b.w treated)	93.72±1.64	4.82±1.01	5.14
D (400 mg/kg b.w treated)	92.69±1.66	4.49±0.29	4.84

[†]*An increased in the percentage proportion of the liver by mass was an indication of the presence of fibrous tissue characterized by steatosis and necrosis*

The activity of ALT (alanine transferase) in all the groups is shown in Fig. 1. The activity of this enzyme in the PCM-IHT (85.83±3.4 U/L) was significantly higher when compared with the control (38.0±0.40 U/L), 200 mg/kg b.w (39.33±1.67 U/L) and 400 mg/kg b.w (35.67±1.67 U/L) groups *(P<.0001)*. There was a significant reduction in the activity of this enzyme when treated with both 200 and 400 mg/kg b.w and these effects were positively correlated with the control with no significant difference among them *(P>.05)*.

The activity of AST (aspartate transferase) is shown in Fig. 2. The activity of this enzyme was highly elevated in PCM-IHT group (148.2±9.75 U/L) with about three-fold increased as compared to the control (53.71±2.7 U/L). This value was significantly higher than the 200

mg/kg b.w (68.84±5.72 U/L) and 400mg/kg b.w (38.26±3.0 U/L) (P<.0001). Apparently, 200 mg/kg b.w treatment was significantly lower than the 400 mg/kg b.w treatment *(P>.01)*.

Fig. 3 shows the activity of alkaline phosphatase (ALP) in the paracetamol-induced male Wistar rats. There was a statistical difference between the activity of this enzyme in the control (135.8±6.74 U/L) and PCM-IHT (240.8±3.98 U/L) *(P<.05)*. Though, the activity of ALP 400 mg/kg b.w treated group seemed to be significantly higher than the control and 200 mg/kg treated groups at *P<.001* and *P<.0001* respectively, but 200 mg/kg b.w treated group showed no statistical difference in the activity of this enzyme when compared to the control. This indicated that the 200 mg/kg b.w (group C) was more active than the higher concentration of the same plant extract.

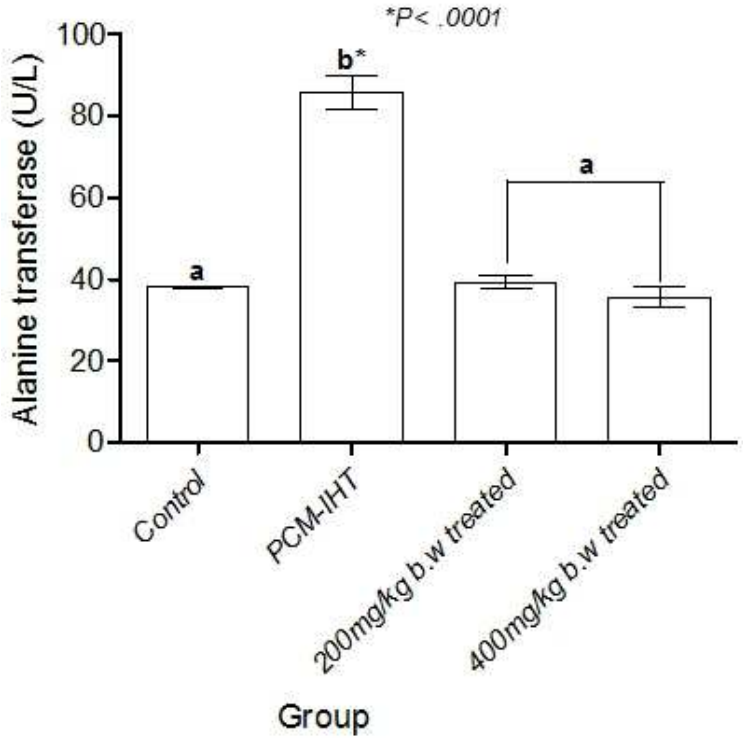


Fig. 1. Activity of alanine transferase in paracetamol-induced hepatotoxic male Wistar rats

Each bar represents enzyme activity and were presented as mean±SEM (n = 3). Bars with different alphabets were statistically different when compared with the control

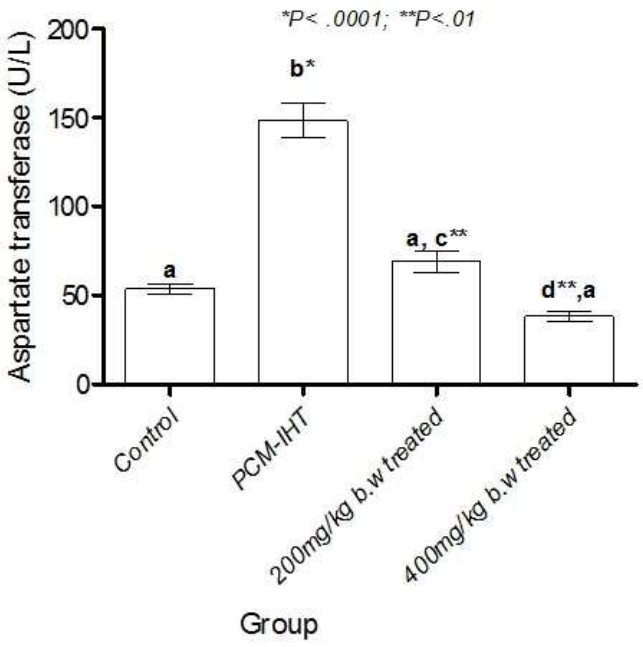


Fig. 2. Activity of aspartate transferase in paracetamol-induced hepatotoxic male Wistar rats

Each bar represents enzyme activity and were presented as mean±SEM (n = 3). Bars with different alphabets were statistically different when compared with the control

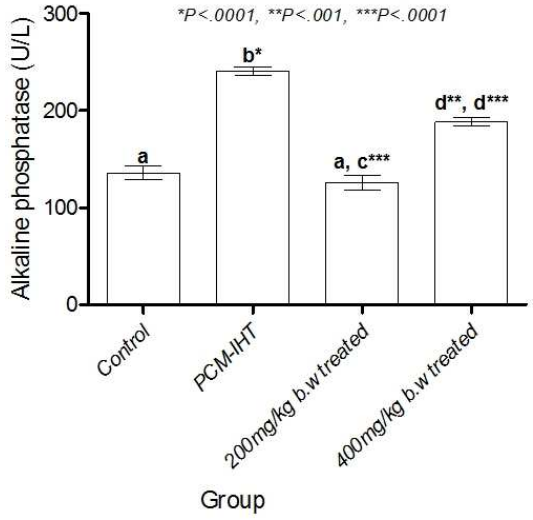


Fig. 3. Activity of alkaline phosphatase in paracetamol-induced hepatotoxic male Wistar rats

Each bar represents enzyme activity and were presented as mean±SEM (n = 3). Bars with different alphabets were statistically different when compared with the control

The activity of acid phosphatase (ACP) is shown in Fig. 4. There was little similarity as compared to alkaline phosphatase. The activity of this enzyme was significantly higher in PCM-IHT (152.2±7.91 U/L) than the control group *(P<.0001)*. Though, both groups C and D were statistically lower than PCM-IHT *(P<.0001)*, but it seemed 200 mg/kg b.w treatment (56.17±1.55 U/L) produced a more significant effect in reducing the activity of this enzyme with positive correlation with the control (61.96±4.12 U/L) *(P>0.05)* as compared to 400 mg/kg b.w treated group with relatively higher activity (93.55±2.18 U/L) than both the control and 200 mg/kg b.w treated group *(P<.001)*.

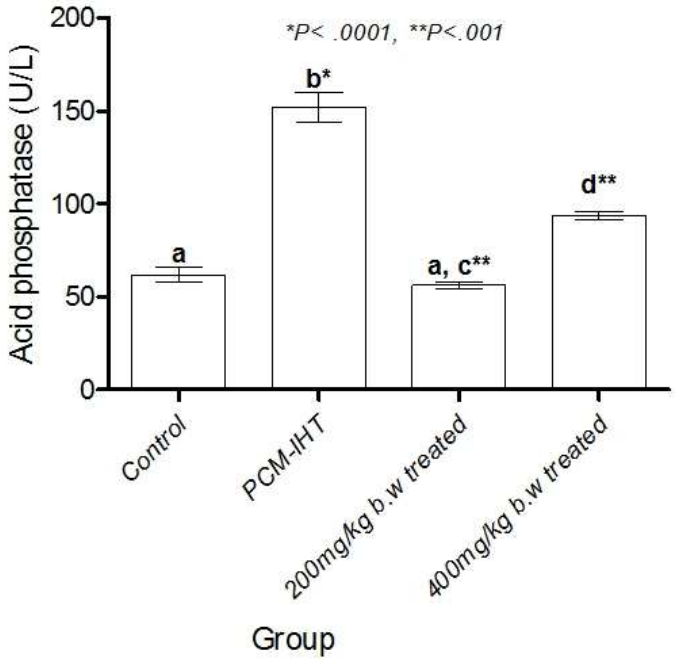


Fig. 4. Activity of acid phosphatase in paracetamol-induced hepatotoxic male Wistar rats

Each bar represents enzyme activity and were presented as mean±SEM (n = 3). Bars with different alphabets were statistically different when compared with the control

The activities of all these enzymes were paired to check the significant effect the treatment had on each of the enzyme as compared to its counterpart. Fig. 5 shows the result of paired comparison between ALT and AST in all the observed groups. The serum level of ALT was not statistically different from the serum level of AST in the control group and 400 mg/kg b.w treated group *(P>.05)*. Contrarily, AST activity was statistically higher than ALT activity in the PCM-IHT (group B) *(P>.001)* and 200 mg/kg b.w treated group *(P<.01)*.

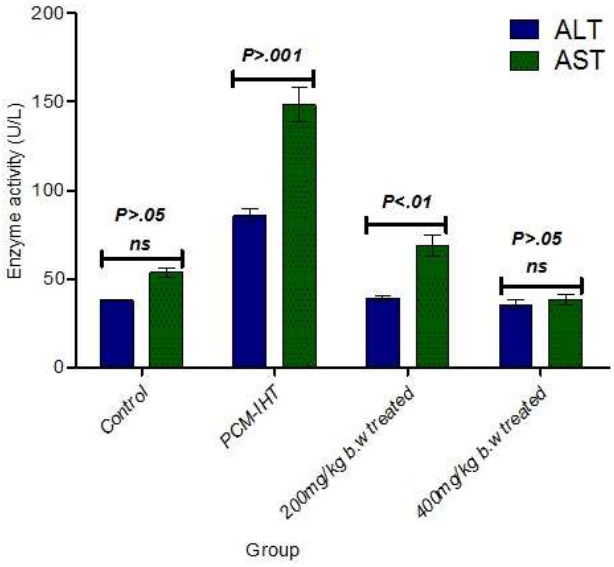


Fig. 5. Comparison between ALT and AST in the control induced and treated stages. Each bar represents enzyme activity (mean±SEM, n = 3)

Fig. 6 shows the comparison between the serum activities of ACP and ALP in acetaminophen-induced hepatotoxic male Wistar rats. There was no significant difference in the activities of these enzymes in the serum of all the observed and groups whether induced or treated *(P<.001)*.

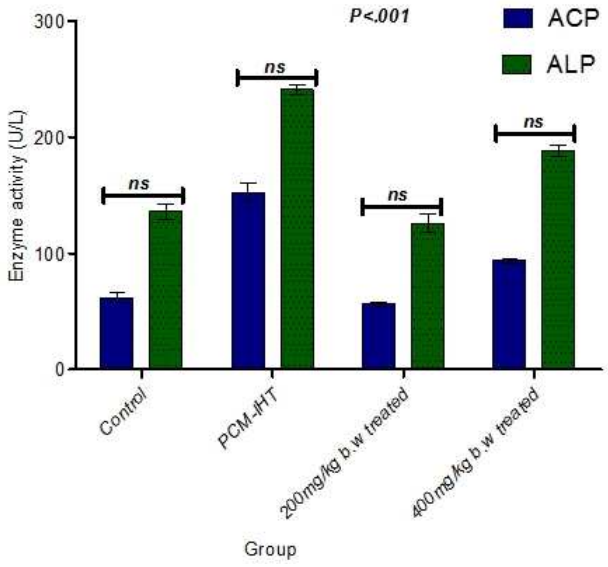
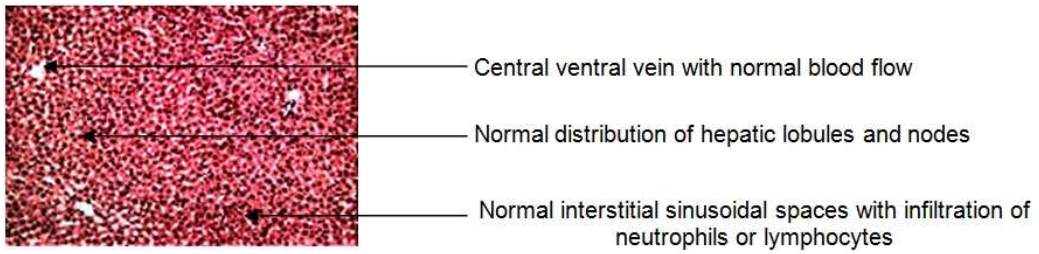
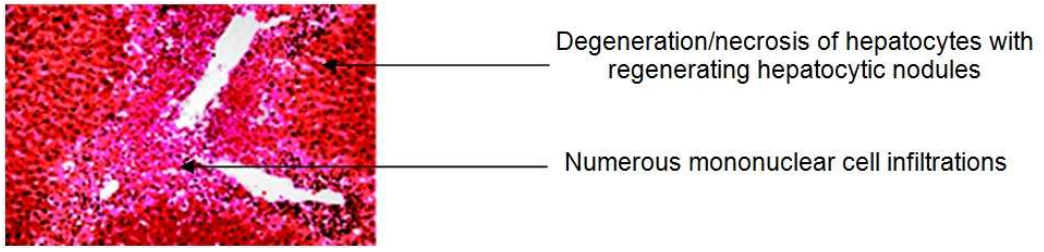


Fig. 6. Comparison between ACP and ALP in normal, induced and treated stages, each bar represents enzyme activity (mean±SEM, n = 3)

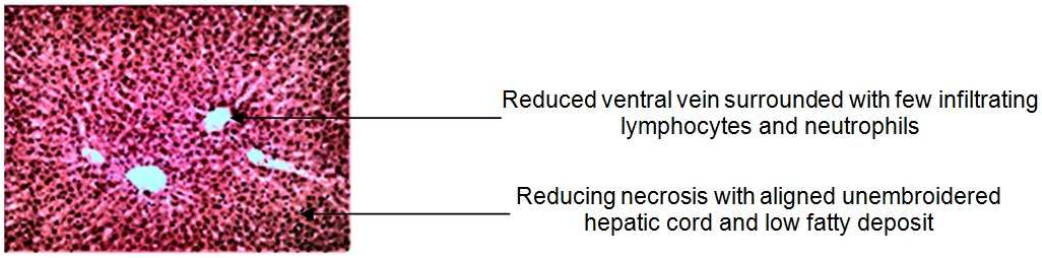
Furthermore, AST/ALT ratio (De Ritis Ratio), which assists in differentiating the site of biliary obstruction, was employed to predict the nature of the hepatotoxic damage incurred by acetaminophen. In this work, the AST:ALT ratio was >1.5 and this indicated intrahepatic fibrosis characterized by the formation of Hepatomegaloventralnecrotic veins and necrosis [33,34,35]. By observation, the ratio of ALP/ACP or *vice versa* remained fairly constant and equally affected in acetaminophen-induced liver damage.



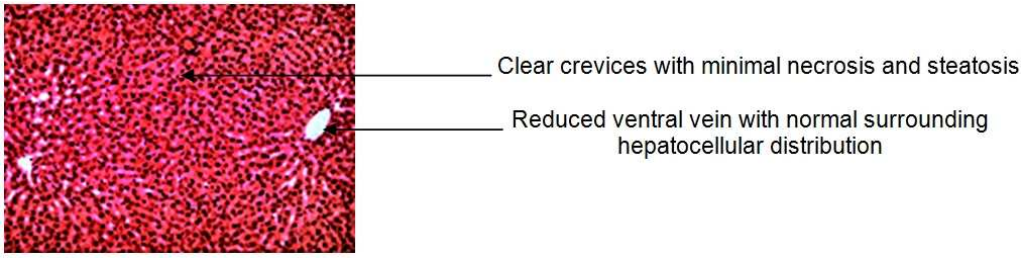
(a) Photomicrograph of liver section of rats in control group (A), which were not received paracetamol *(H and E mag x100)*



(b) Photomicrograph of liver section of rats in PCM-IHT (Group B) administered with 350 mg/kg b.w. This histoarchitecture was characterized by the formation of fibronecrosis *(H and E mag x100)*



(c) Partial normalization of the histoarchitecture of hepatocyte after 200 mg/kg b.w treatment (Group C) of the methanolic extract of *Xylopia aethiopica* *(H and E mag x100)*



(d) Normalization of the necrotic hepatocytes after 400 mg/kg b.w treatment (Group D) of the methanolic extract of Xylopia eathiopica (H and E mag x100)

Fig. 7. Histoarchitecture photomicrographs of the control, PCM-IHT, 200 mg/kg b.w treated and 400 mg/kg b.w treated groups. H and E staining

There was a formation of large ventral vein and steatosis with infiltration of lymphocytes and neutrophils in the 350 mg/kg b.w induced hepatotoxic male Wistar rats and this was gradually ameliorated after a week of treatment with the methanolic extract of *Xylopia eathiopica* characterized with reduced ventral vein and normal cellular distribution (Fig. 7a-d). The average value of lesions caused by hepatic necrosis in Group A, the control (0.01±0.003), Group C, 200mg/kg b.w treated (38.53±3.10) and Group D, 400g/kg b.w treated (15.77±2.84) were lesser than that of Group B, hepatotoxic-induced (74.80±4.51).

Table 2. Ratios of transaminases-phosphatases in acetaminophen-induced liver damage

Enzyme ratio	AST	ALT	ALP	ACP	Condition
AST	1.0	0.5**	0.8	0.9	Induced
	1.0	1.4*	2.7	3.1	Control
ALT	1.9**	1.0	1.5	1.7	Induced
	0.7	1.0	1.9	2.1	Control
ALP	1.3	1.3^{+}	1.0	1.2^	Induced
	0.4	0.5	1.0	1.2^	Control
ACP	1.1	1.1	0.9^	1.0	Induced
	0.3	0.5	0.9^	1.0	Control

**Normal, ALT/AST >1; **Acetaminophen-induced liver damage was similar to Liver Cirrhosis (1 > AST/ALT < 2); $^{+}$Formation of acute cholestatic damage caused by acetaminophen toxicity (ALT/ALP< 5); ^ALP = ACP, evidence that phosphatases were equally affected in cirrhosis-like acetaminophen-induced liver damage.*

4. DISCUSSION

The phytocomponents of medicinal plants are fundamentals to the pharmacological effects elicited by these plants against pathogenic-infectious diseases. In addition, medicinal plants had been employed to treat, cure or manage numerous ailments even terminal diseases like cancer and cardiovascular diseases [7,12]. The results obtained in this work have buttressed the pharmacological impacts of *Xylopia aethiopica* especially as one of either hepato-protective/reparative agent [36,37,38]. The elevated levels of serum ALT, AST, ALP and ACP observed in the induced group indicated that paracetamol at 350 mg/kg b.w was able to cause acute toxicity in the liver leading to hepatonecrosis and hepatodegenerative effects

characterized by high ratio level of AST:ALT [35,39]. Previous studies have shown elevated activities of transaminases as a result of tissue leakages, which has made these enzymes to find their ways into the blood stream [40,41]. An increased in the percentage proportion by mass of the liver to body weight indicated the occurrence of steatosis and this invariably led to hepatomegaly due to the fatty deposit and formation of fibrous tissue at the crevices of hepatocyte nodules (Fig. 7b). This, in addition, proved that acetaminophen induced hepatic-damage.

Following damages, extrusion of intramolecularly located proteins/enzymes and other biomolecules occurred [42]. Serum levels of these enzymes beyond threshold have been undoubtedly attributed to liver degenerative [43] such as infectious hepatitis, myocardial infarction, obstructive jaundice and intra-hepatic cholestasis [44], though there could be cross-link of these enzymes in multiple tissue necrosis. Generally, treatment of induced hepatotoxic group with methanolic extract of *Xylopia aethiopica* showed a concentration dependent activity especially in aminotransaminases (Figs. 1 and 2) unlike the phosphatases (Figs. 3 and 4). This observation may have been possible because of the nature of these enzymes and their functions; aminotransaminases are constitutive and cytoplasmic located [45] while phosphatases are inductive and membrane attached [42]. Though, AST was more affected *(P<.001)* by hepatic damages than ALT (Fig. 5) as a result of multivariant copies of this enzyme; yet, ALT is a more liver-specific marker enzyme than AST [46]. AST was earlier released during liver damages than ALT, but phosphatases were equally affected (Fig. 6). Though, both treatments have been found to be effective in reducing the activities of ALT and AST to comparable values with the control but 400 mg/kg b.w treatment appeared to be more effective.

Contrarily, higher concentration of the methanolic extract of this plant may not play a conspicuous significant effect in reducing the ACP and ALP serum activities because 200 mg/kg b.w treatment was effective enough to reduce the high level of activities of these enzymes (Figs. 3 and 4). Therefore, while transaminases responded quite well to 400 mg/kg b.w treatment, phosphatases required lower concentration to elicit similar pharmacological effect. As shown in Fig. 6, both ACP and ALP were equally affected in acetaminophen-induced hepatotoxic male Wistar rats *(P<.001)*. It could be envisaged that probably higher concentration of the methanolic extract of *Xylopia eathiopica* may be not elicit any significant pharmacological action but rather posed to be toxic and interfered with other normal cellular activities.

In addition, AST:ALT (De Ritis Ratio) has indicated that the hepatic damage offered by acetaminophen could be likened to liver cirrhosis with the formation of intrahepatic fibrosis and hepatomegaloventralnecrotic veins [44], because this ratio was >1.5 in this study (Table 2). De Ritis Ratio may be used in differentiating the sites of biliary obstruction. In liver hepatitis, the ratio of AST:ALT is typically less than 1.0 and this can rise to greater values as fibrosis and cirrhosis developed within the parenchymal layer of the hepatic lobes. Exact mechanism of AST: ALT ratio alteration in monitoring the prognosis of liver disease is vague, and the correlation with and accuracy in predicting degree of fibrosis, steatosis and presence of cirrhosis are controversial. In many forms of acute and chronic liver injury or steatosis (fatty infiltration of the liver), the ratio is ≤1. Wilson's disease can sometime cause the AST/ALT ratio to exceed 4 [46]. AST: ALT ratios are suggestive of certain conditions and there may be some levels of significant overlap between AST:ALT ratios in different conditions. Therefore, this ratio cannot be relied on exclusively when making a diagnosis of the liver [45]. Nevertheless, the result has indicated that both ALP and ACP were equally affected pathologically during liver damage, AST offshoot ALT.

Hepatoarchitecture micrographs of the parenchymal layer of the control, hepato-induced toxicity and treated groups have shown the occurrence of pathological conditions characterized by the formation of centrilobular megaloventral vein, lymphocyte and neutrophil infiltration and formation of fibrous tissue intercalated with bile ducts (Fig. 7b). The formation of reduced infiltration of lymphocytes and neutrophils (Fig. 7c and d) coupled with reduced ventral vein were evidences that the methanolic extract of *Xylopia eathiopica* repaired the hepatic damage caused by acetaminophen. Further analysis, using Shackelford [32] grading system, had revealed that the degree of lesion was observed to be higher in the PCM-IHT group than every other considering groups. This lesion was gradually repaired by the methanolic extract of *Xylopia aethiopica* in a concentration dependent manner (Fig. 7).

Likewise, hepatoprotective activities of *Hedyotis corymbosa* [47]*, Andrographis lineata* [48]*, Acacia catechu* [49]*, Coronopus didymus* [50]*, Chrysophyllum albidum* [51]*, Sarcostemma brevistigma* [37]*, Pterocarpus santalinus* [52] and *Ginkgo biloba* [26] have being documented using aqueous and organic solvents with shared similarities in their effects. Moreover, the extracts of *Xylopia aethiopica* have been found to contain alkaloids, sterols, flavonoids, saponins, terpenoids and the phenolic compounds, which are important bioactive constituents of medicinal plants [53]. Some of these phytochemicals have been linked to protecting/repairing liver damages [54,55,37,38,56]. The reparation of the damages in the liver by these plants has been attributed to their complementary effect in stimulating protein synthesis, biogenesis and as antioxidant [17,26]. Further investigation into the physicochemical properties of this plant extract, among other medicinal plants, revealed that it has a high degree of unsaturated fatty acid components such as omega 3 and 6 and this may enable it to stabilize membrane as stated in the work of Ezekwesili et al. [56] that the methanol extract of this plant prevented haemolysis of red blood cells suggesting that it maintains the integrity of the erythrocytes and lysosomes and possibly acts by enhancing active transport across the membrane of the erythrocytes [57,58,59]. It is therefore envisaged that though pharmacological importance of this medicinal plant could be innumerable, yet clinical investigation of the isolated active component(s) of this plant demands thorough clarification.

4. CONCLUSION

The results obtained in this work have confirmed the hepatoreparative effect of the methanolic extract of *Xylopia aethiopica*. While transaminases responded to both lower and higher concentrations of the extract, phosphatases responded only to lower concentration. Furthermore, hepatotoxicity caused by acetaminophen may have affected AST more than ALT but phosphatases were equally affected. The toxicity study and LD_{50} analysis of the methanolic extract of this plant and their mechanism of actions are paramount to understanding the pharmacological importance of this medicinal plant.

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ETHICAL APPROVAL

All authors hereby declare that all animals were treated in accordance with the recommendations of adopted guidelines of the Committee on Ethical Permit, College of Medicine, Idi Araba, University of Lagos as contained in the Institute for Laboratory Animal Research Guides for the Care and Use of Laboratory Animals (ILAR, 2011; NIH, 1985).

COMPETING INTERESTS

The authors of this review article wish to declare that there was no competing interests among the participating authors.

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