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Effect of *Xylopia aethiopica* Fruit Extract on Liver Functions in Pregnant Female Wistar Rats

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ABSTRACT: This study investigated the influence of aqueous fruit extract of *Xylopia aethiopica* on the liver function in apparently healthy pregnant Wistar rats. Twenty Wistar rats weighing 140-210 g were divided into four groups (n = 5). Group 1 was the control group and received only distilled water orally, while groups 2, 3 and 4 served as the test groups and each received orally 250 mg/kg, 500 mg/kg, and 1000 mg/kg of the aqueous extract, respectively for 7 days from Day 7 of pregnancy to Day 14. On the 15th day, the animals were sacrificed, the liver was harvested, processed histologically and blood samples were also taken for biochemical assays. There was a significant decrease in AST levels compared with control in rats administered 250 mg/kg aqueous extract of the fruit of *Xylopia aethiopica*. There was an increase in ALP at 1000 mg/kg dose. There was a significant increase in albumin levels compared with control in rats administered 250 mg/kg aqueous extract of *Xylopia aethiopica* fruit. There was no significant difference observed in the other doses and parameters in comparison with control. Thus, administration of aqueous extracts of the fruit of *Xylopia aethiopica* may not be toxic to the liver during pregnancy, except if ingested at high dose.

Keywords: *Xylopia aethiopica*, Liver function, Pregnancy, Toxicity.

Introduction

Xylopia aethiopica (*X. aethiopica*) is a tree that may reach heights of 20 meters and is evergreen and it belongs to the Annonaceae family. It is endemic to wet fringe forests and lowland rainforests of Africa's Savanna zones (Fetse *et al.,* 2016). The fruit is popularly known as “Africa pepper,” “Ethiopian pepper,” or “Guinea pepper”. It's called "Uririen" in the local Urhobo tongue of Delta State, Nigeria. Nutritionally and medicinally, the fruits are regarded as being rather valuable. Preliminary phytochemical analysis of *Xylopia aethiopica* fruit revealed the presence of flavonoids, steroids, phenol, anthraquinones, phlobatannins, saponin, tannins and cardiac glycosides, but the absence of alkaloids, and terpenoids (El Astal, 2005). Igwe *et al*. (2003) stated that it is effective in treating rheumatism, headache, neuralgia, and colic discomfort. For treating coughs, as well as for their carminative and stimulant properties, the fruits are often employed in Nigerian. A lot of people in many parts of Africa rely on traditional medicine for their health care needs because the costs of conventional drugs increase and are becoming unaffordable by many in rural communities (Ngbolua *et al.,* 2011). Despite its socio-economic importance, the cultivation of *Xylopia aethiopica* is not popular owing to the difficulty in seed germination (Kanmegne *et al.,* 2017).

In a study by Ogbuagu *et al.* (2021), there was a significant increase observed in the activities of Alanine transaminase (ALT), Alkaline phosphatase (ALP) and aspartate transaminase (AST) in animals administered higher doses of *Xylopia aethiopica* fruit extract when compared with those in the control group. Their study showed that extract of *Xylopia aethiopica* fruit is hepatotoxic especially at high dosage. Therefore, its use in folklore medicine should not be encouraged. According to a study done by Obodo *et al.* (2013), the *Xylopia aethiopica* extract causes a significant decrease in the level of ALT but there was no significant effect on the

levels of AST, ALP and Gamma-glutamyltransferase (GGT). It is known that an increase in the enzymatic activity of AST, ALT and ALP in the serum indicates hepatocellular damage (Omeodu *et al.,* 2008). Results of the enzyme analysis therefore suggest that extract of *Xylopia aethiopica* has no hepatotoxicity with reference to the doses used in the study.

Pascual and Langaker (2023) described pregnancy as a state of having implanted products of conception located either in the uterus or elsewhere in the body. It is a period when the mother's body undergoes a series of changes that involves all organ systems with the aim of sustaining the growing fetus (Pascual and Langaker, 2023). Pregnant women's exposures to chemicals are especially important, because many chemicals may be transferred from mother to child across the placenta and via breast milk (Mitro *et al.,* 2015), endangering both mother and child. The liver is a significant organ unique to vertebrates that serves several important biological purposes, including detoxification and the production of proteins and biochemicals required for digestion and development (Abdel-Misih *et al.,* 2010). Hepatocytes, hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells are the four main kinds of liver cells (LSECs). Aspartate and alanine amino transferases in addition to alkaline phosphatase have been established as marker enzymes for the liver and variations in the serum/plasma levels of these enzymes give indications of the 'health' of the liver of the individual/experimental animal (Omeodu *et al.,* 2008). Numerous data exist on the effects of *Xylopia aethiopica* extracts on various organs of the body, including the liver, but only few studies have attempted to investigate its effect in the pregnancy state. Owing to the high application of *Xylopia aethiopica* in folkloric medicine during pregnancy, childbirth and postpartum period, and the extremely vital role that the liver play in riddling the body of toxic substances, it became necessary to determine its effects on the liver function in pregnant female Wistar rat. Therefore, the aim of this study was to investigate the effect of *Xylopia aethiopica* fruit extract on liver function in pregnant female Wistar rat.

Materials and methods

Plant material: Dried fruits of *Xylopia aethiopica* bought from Oba market in Edo state, Nigeria were identified and authenticated at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin.

Preparation of Xylopia Aethiopica fruit Aqueous extract (aqueous form of Xylopia Aethiopica): The authenticated fruits of *Xylopia aethiopica* were washed, oven dried at a temperature of 40 °C for 24 hours and then ground to coarse powder. The powder (1 kg) was macerated in 3.2 litres of distilled water and allowed to stand for 24 hours with frequent stirring. After filtration was done with a muciline cloth, the resultant filtrate was freeze dried at Trigas Lab, Medical Complex, University of Benin for 3 days at a temperature of -4 °C. The concentration gave a yield of 48 g. The percentage yield was calculated to be 4.8 %.

The various doses of extract were administered via oral route by means of an oral intubation tube. The animals were observed for 24 hours after administration. None of the rats given the aqueous extract died after 24 hours. The result was subjected to probit log analysis and the LD_{50} was determined to be 5,000 ml/kg. One-tenth of the LD_{50} was used as the highest dose. A stock solution was made using 1 g of aqueous extract dissolved in 10 ml distilled water at every administration time..

Experimental animals: Twenty (20) female adult Albino Wistar rats with weights between 140 and 210 g were procured from the Department of Anatomy animal house of the University of Benin, Ugbowo campus, Edo State, Nigeria, where they were allowed two (2) weeks acclimatization. They were kept in wire mesh cages with a tripod that separates the animal from urine and feaces in order to prevent contamination. During the period of acclimatization in laboratory condition, the rats were fed with grower's mesh and water provided *ad libitum*. The animals were maintained and utilized in accordance with the standard guide for the care and use of laboratory animals.

Animal grouping: The rats were divided into four groups (n = 5). Groups 2, 3 and 4 were administered *Xylopia aethiopica* aqueous extract orally while Group 1 (control) was administered distilled water orally.

Experimental procedure: After two weeks of acclimatization, the rat's vaginal smears were taken to determine the phase of their respective oestrous cycles. Those in oestrus phase were taken for mating. The ratio of male rats to female rats was 1:2. The next day (Gestational Day 0), vaginal smear of the female rats was taken using a pipette and saline solution. The smears for each rat were placed on numbered microscope slides and viewed under the microscope. The smears were checked for presence of spermatozoa. Those that had spermatozoa in them were taken and grouped into groups of three (3) and administered the *Xylopia aethiopica* extracts on Day 7 to Day 14 of pregnancy.

Using sterile 1ml syringes, group 1 (control) received only distilled water orally; Group 2 received 250 mg/kg aqueous extract orally. Group 3 received 500 mg/kg aqueous extract orally while Group 4 received 1000 mg/kg

aqueous extract orally. Five (5) animals from each group were sacrificed on the fifteenth (15^{th}) day of the experiment, after being treated with the extract for 7 days duration. Whole blood (5 ml) was collected from each animal into lithium heparinised sample bottles for liver enzyme analysis.

Sample collection: At the end of the experiment, animals were sacrificed under chloroform anesthesia and blood samples were collected through the heart using a hypodermic syringe (5 ml) and transferred to an anticoagulant bottle (Lithium Heparin). The blood samples were separated at 3000 rpm using a centrifuge (10 minutes) to obtain plasma for the estimation of the biochemical parameters. The liver was harvested and processed histologically.

Estimation of biochemical parameters: Estimation of the activities of ALT, AST and ALP was done using Randox Laboratory test kit (Antrim, UK). Specifically, ALT and AST activities were estimated using the colorimetric method, while ALP was done using the kinetic photometric method.

The estimation of Total serum protein and serum Albumin was done using Cromatest Laboratory test kit (Spain). Serum Albumin (ALB) and total protein (TP) were determined by spectrophotometry method. Globulin was then calculated using the formula described by Ochei and Kolhatkar (2000) as:

Serum Globulin = Total protein – Serum albumin (TP-ALB).

Statistical analysis: Data obtained from biochemical estimations were expressed as mean ± SEM. Statistical analysis was done using one-way analysis of variance (ANOVA). A p value of <0.05 was considered to be statistically significant.

Results

The following are results obtained for liver function markers and plasma proteins.

Alkaline phosphatase (ALP): Figure 1 shows the alkaline phosphatase (ALP) plasma level following 7 days administration of aqueous extract of *Xylopia aethiopica* on Wistar rats. Analysis of this result reveals that there was a significant increase at a dose of 1000 mg/kg of aqueous extract compared with control. But there were no significant changes at 250 mg/kg and 500 mg/kg doses compared with control, respectively ($p > 0.05$).

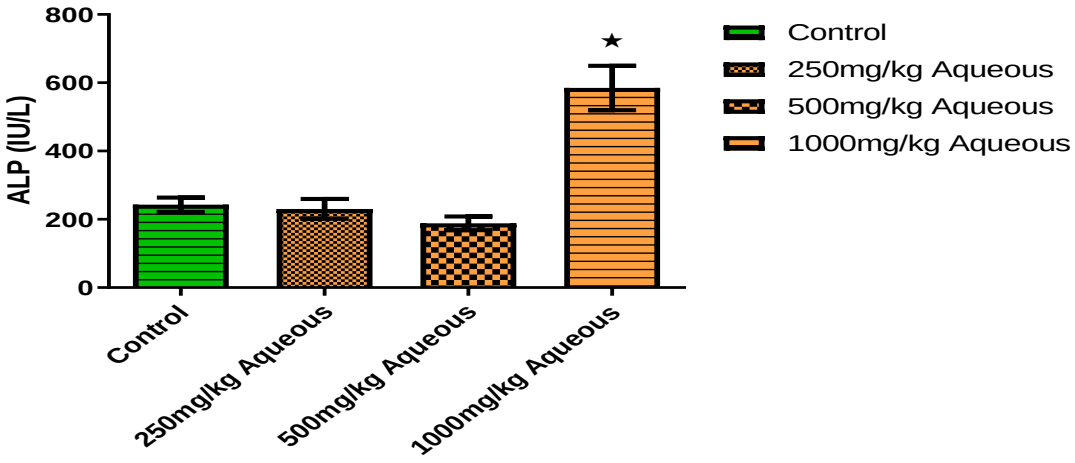

Figure 1: Plasma Alkaline phosphatase **(**ALP) level following an administration of aqueous extract of *Xylopia aethiopica* fruits on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5. *$P < 0.05$ indicates significant difference, when treated groups are compared with control.

Alanine transaminase (ALT): The plasma level of alanine transaminase (ALT) following the treatment of Wistar rats for 7 days, with different doses of the aqueous extract of *Xylopia aethiopica* is given in Figure 2. The result shows that there were no significant differences at 250 mg/kg, 500 mg/kg and 1000 mg/kg compared with control respectively ($p > 0.05$).

Aspartate transaminase (AST): The plasma level of aspartate transaminase (AST) following the treatment of Wistar rats with different doses of the aqueous extract of *Xylopia aethiopica* for 7 days is presented in Figure 3. There was a significant decrease at 250mg/kg of aqueous extract compared with control ($p <. 0.05$). However, at moderate and high doses of 500mg/kg and 1000mg/kg respectively of *Xylopia aethiopica* aqueous extract, there were no significant changes in comparison with control ($p > 0.05$).

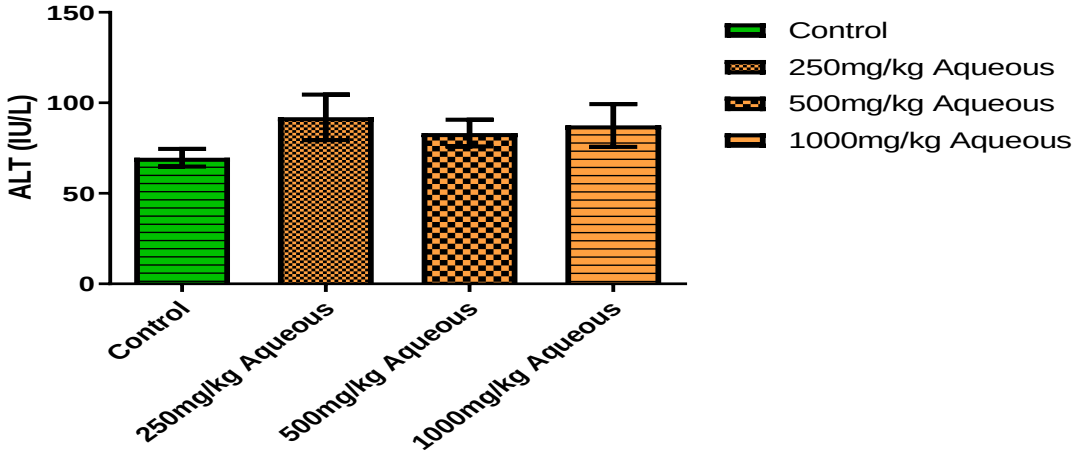

Figure 2: Plasma Alanine transaminase ALT level following an administration of aqueous extract of *Xylopia aethiopica* fruits on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5.

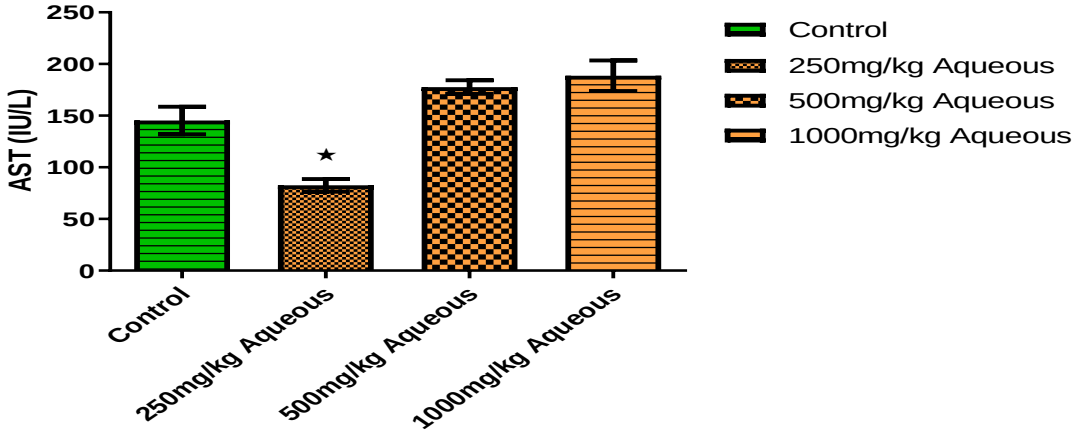

Figure 3: Plasma aspartate transaminase (AST) level following an administration of aqueous extract of *Xylopia aethiopica* fruits on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5. **P* < 0.05 indicates significant difference, when treated groups are compared with control.

Total bilirubin: Figure 4 presents the plasma level of total bilirubin, following the administration of aqueous extract of *Xylopia aethiopica* on Wistar rats for 7 days. The analysis of this result shows that there were no significant differences at low (250 mg/kg), moderate (500 mg/kg) and high doses (1000 mg/kg) of *Xylopia aethiopica* aqueous extract compared with control respectively ($p > 0.05$).

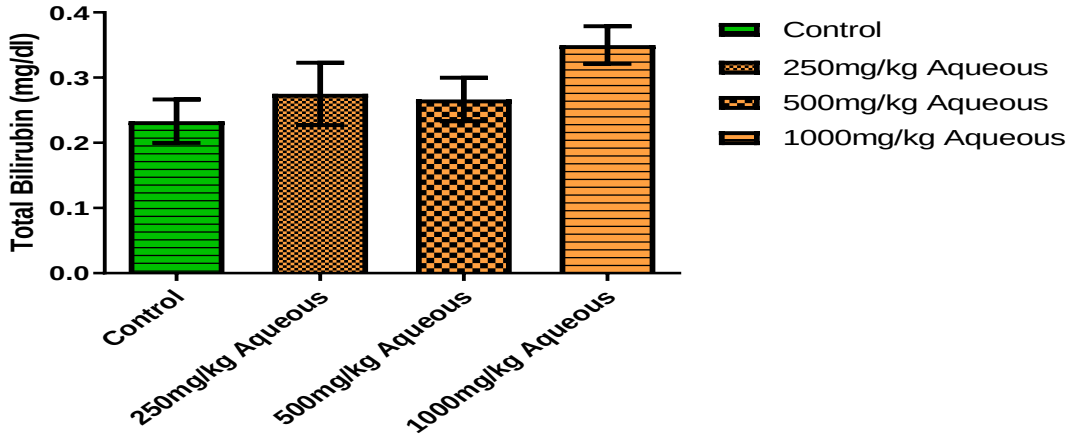

Figure 4: Plasma total bilirubin level following an administration of aqueous extract of *Xylopia aethiopica* fruits on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5.

Direct bilirubin: Figure 5 presents the blood level of direct bilirubin following an administration of the different doses of aqueous extract of *Xylopia aethiopica* on Wistar rats for 7 days. Analysis of this result shows that there

were no significant differences at 250mg/kg, 500mg/kg and 1000mg/kg compared with control respectively ($p > 0.05$).

Total protein: Figure 6 shows the plasma level of total protein following an administration of aqueous extract of *Xylopia aethiopica* on Wistar rats for 7 days. Analysis of this result shows that there were no significant differences at 250mg/kg, 500mg/kg and 1000mg/kg compared with control respectively ($p > 0.05$).

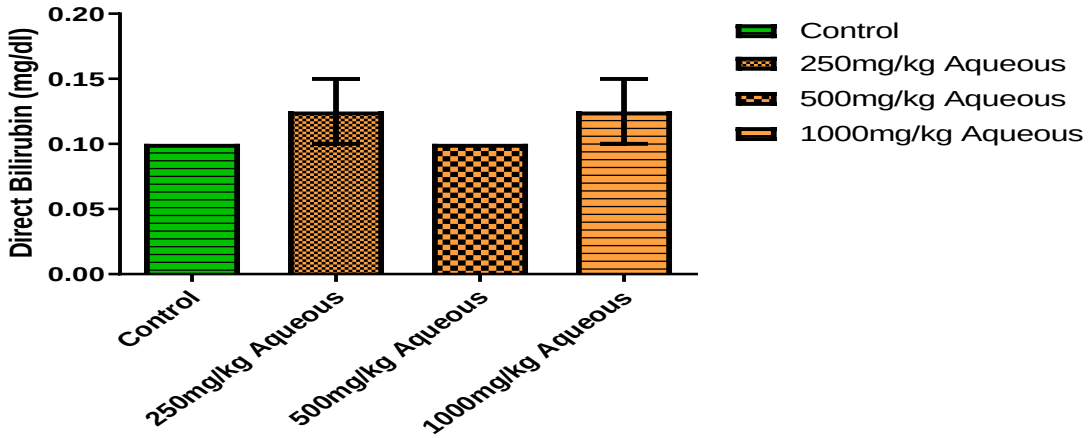

Figure 5: Plasma direct bilirubin level following an administration of aqueous extract of *Xylopia aethiopica* fruit on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5.

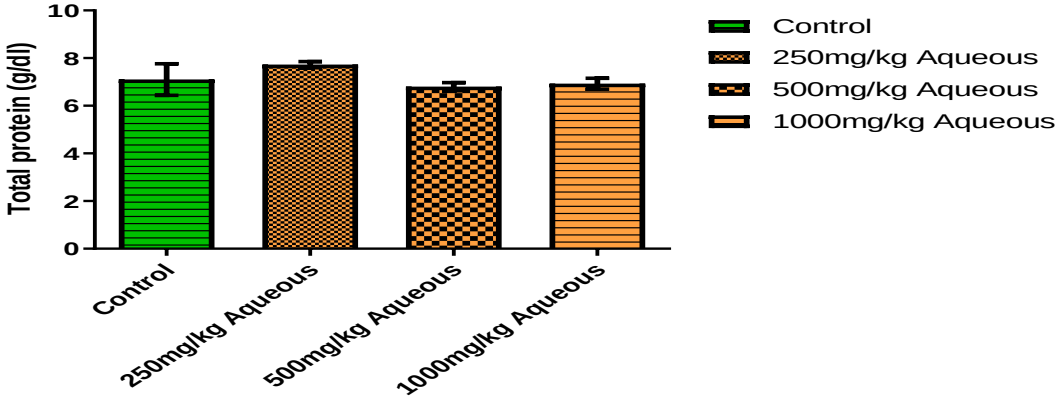

Figure 6: Plasma total protein level following an administration of aqueous extract of *Xylopia aethiopica* fruit on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5.

Serum albumin: The plasma albumin concentration following an administration of aqueous extract of *Xylopia aethiopica* on Wistar rats for 7 days is presented in Figure 7. Analysis of this result shows that there was a significant increase at a dose of 250 mg/kg of aqueous extract compared with control ($p < 0.05$). However, administration of the same extract at higher doses of 500 mg/kg and 1000 mg/kg produced no significant change in comparison with control respectively ($p > 0.05$).

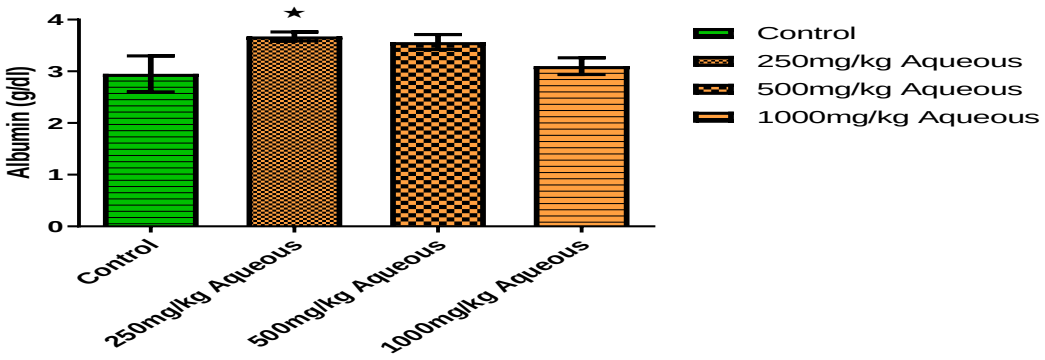

Figure 7: Plasma albumin level following an administration of aqueous extract of *Xylopia aethiopica* fruits on Wistar rats for a period of 7 days. The results are presented as Mean ± SEM, n= 5.

**P < 0.05 indicates significant difference, when treated groups are compared with control.*

Serum globulin: The plasma globulin concentration following an administration of aqueous extract of *Xylopia aethiopica* on Wistar rats for 7 days is given in Figure 8. The analysis of this result shows that there were no significant differences at the different doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg compared with control respectively ($p > 0.05$).

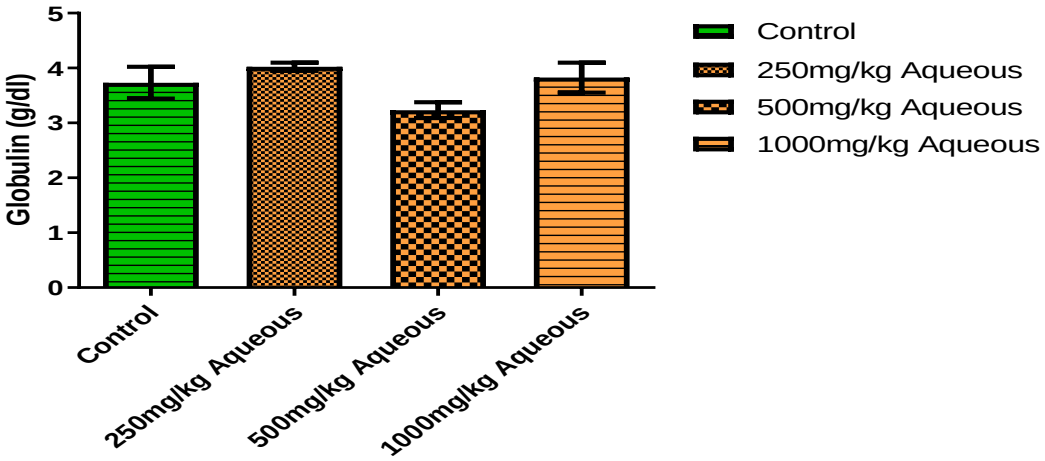

Figure 8: Plasma Globulin level following an administration of aqueous extract of *Xylopia aethiopica* on Wistar rats. The results are presented as Mean ± SEM, n= 5.

Histology of the liver: Plate 1 shows a **s**ection of the liver of control rats which consists of hepatocytes with nuclei having inconspicuous nucleoli (A), sinusoids (B), portal vein (C) and bile duct (D) (H&E x 100), which are all consistent with the normal morphology of a healthy liver section. The sections were stained with hemotoxylin and eosin (H&E) and interpreted with the light microscope.

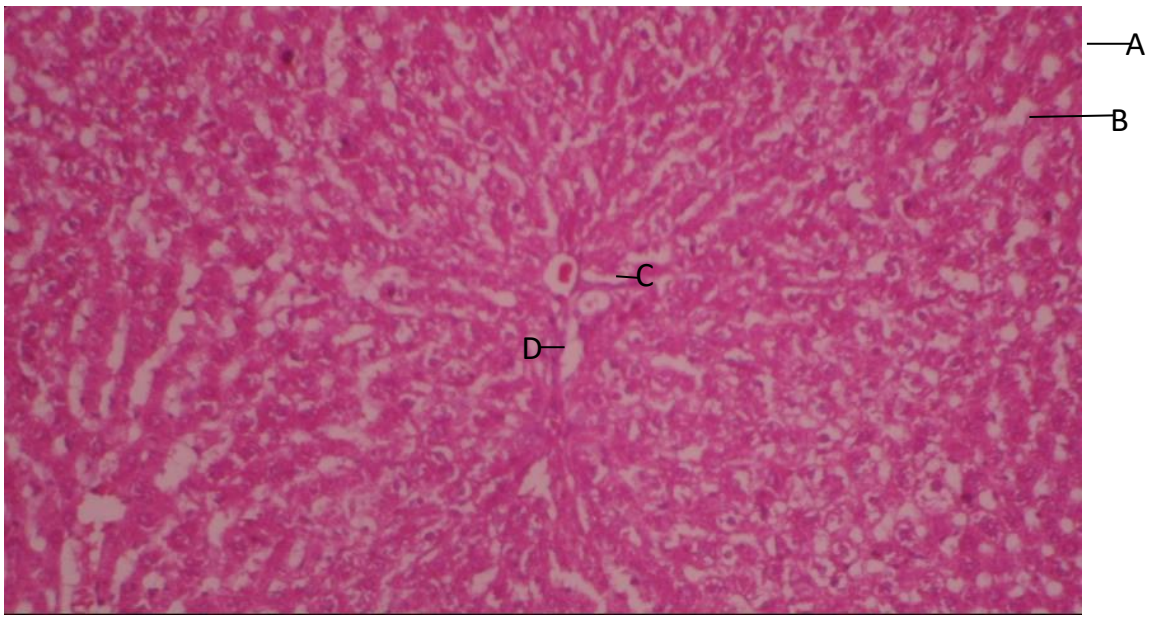

Plate 1: Section of the liver of control rats (H&E x 100).

Plate 2 which shows a section of the liver of rats given 250 mg of aqueous extract of *Xylopia aethiopica* fruit for a period of 7 days also follows a similar pattern of the normal architecture of the liver, with the hepatocytes (A), having a conspicuous nucleolus, portal vein (B) and bile duct (C). However, Plates 3 and 4 shows some deviation. Although Plate 3, which displays a section of the liver of rats given 500 mg aqueous extract of *Xylopia aethiopica* for a period of 7 days shows the normal architecture of hepatocytes with conspicuous nucleoli (A), there are additional presence of periportal mobilization of lymphocytes (B) and Kupffer cells activation (C). Also, Plate 4 which contains a **s**ection of the liver of rats treated with 1000 mg aqueous extract of *Xylopia aethiopica* for a period of 7 days, shows normal architecture with the hepatocytes (A) having conspicuous nucleoli, hepatic artery (B) and Kupffer cell activation (C) present.

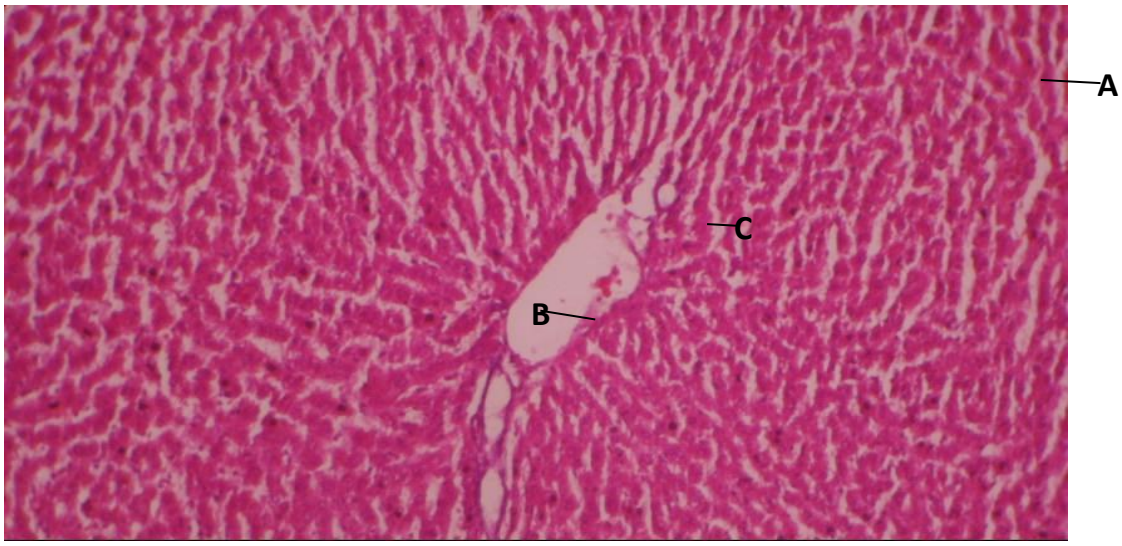

Plate 2: Section of the liver of rats given 250 mg of aqueous extract of *Xylopia aethiopica* fruit for a period of 7 days (H&E x 100).

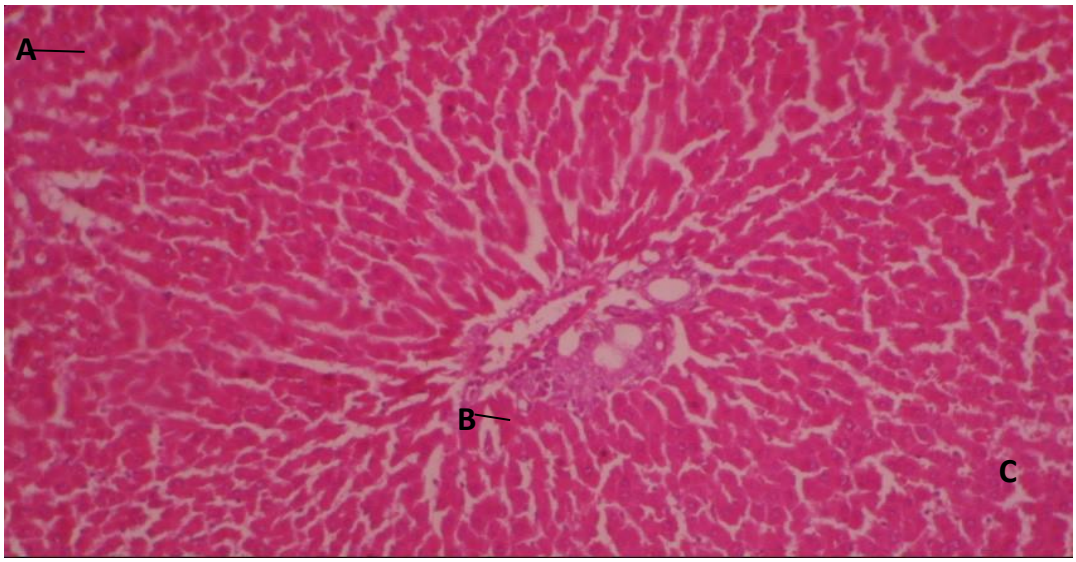

Plate 3: Section of the liver of rats given 500 mg aqueous extract of *Xylopia aethiopica* for a period of 7 days (H&E x 100).

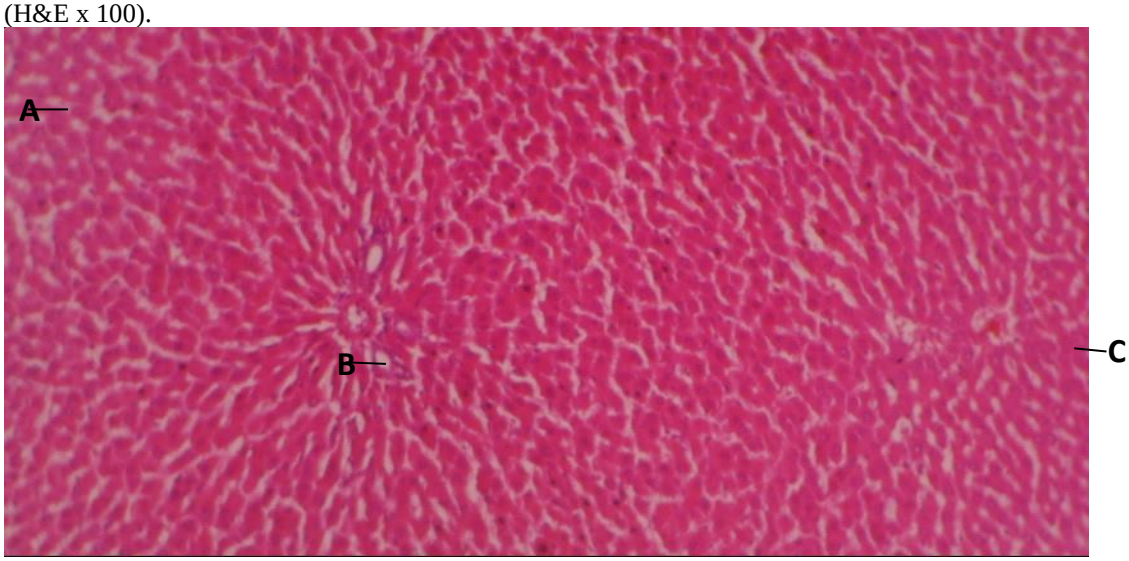

Plate 4: Section of the liver of rats given 1000 mg aqueous extract of *Xylopia aethiopica* for a period of 7 days (H&E x 100).

Discussion

The effect of aqueous extract of *Xylopia aethiopica* (*X. aethiopica*) fruit on liver function is presented thus: There was significant increase observed in figure 1 when the activities of ALP in animals treated with 1000 mg/kg was compared with those in the control group. The main physiological action of ALP is to catalyze the hydrolysis and therefore the inactivation of inorganic pyrophosphate (PPi) (Sciacqua *et al.,* 2020). Plasma Alkaline phosphatase (ALP) is an enzyme that is elevated by various hepatic diseases. Although Serum ALP levels have also been reported to increase in pregnancy, especially in the third trimester where it can rise by twofold to fourfold higher as a result of a physiological increase in placental ALP (Shipman *et al.,* 2013), but the likely cause of the raised serum ALP recorded in this study may have been as a result of a toxic effect of *X. aethiopica* on the liver tissues. The fact that this effect was manifested at a high dose of 1000 mg suggests that the toxicity of *X. aethiopica* may be dose-dependent. In addition, a related finding was recorded by Oso *et al.* (2019) who reported a significant increase in the activities of AST, ALT and ALP when they investigated the influence of ethanolic extracts of dried fruit of *X. aethiopica* on haematological and biochemical parameters in healthy Wistar rats. In figure 2, no significant difference was observed when the activities of ALT in animals treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract were compared with those in the control group at $p < 0.05$. Figure 3 presented a significant decrease in the activities of AST in animals treated with 250 mg/kg dose of the extract when compared with those in the control group. The significant decrease observed in the activities of just AST in animals treated with doses of aqueous fruit extracts of *X. aethiopica* when compared to the control groups does not show that *X. aethiopica* is hepatotoxic because low levels of AST is not related to liver damage (Gaze, 2007). This is in support of the findings of Chinedu *et al.* (2018) who reported a significant decrease in levels of AST when they evaluated the effect of ethanolic fruit extracts of *X. aethiopica* on haematological and biochemical parameters in male rats.

However, a significant increase was observed in the concentrations of albumin in animals treated with 250 mg/kg dose of aqueous extract when compared with those in the control group. In this study, the concentrations of total albumin were observed to have significantly increased in animals treated with 250 mg/kg aqueous extract when compared with those of control animals. This elevation could be a symptom of dehydration which is detrimental to cellular homeostasis (Shittu *et al.,* 2015). This will negatively affect the metabolic activities of the liver and consequently, the health of the animals. Albumin binds and transports metal ions, bilirubin, and drugs. Its level is used to assess the synthetic function of the liver. Significant increases in the levels of these parameters is an indication that the extract had stimulated its synthesis in the liver at dosage of 250 mg/kg aqueous extract. Serum protein levels are regulated via synthesis in the liver and its levels thus reflect the synthetic ability of the liver, whereas a decrease in albumin is an indication that the liver may have been negatively affected, since the liver is the only site for albumin synthesis (Bernardi *et al.,* 2012). This result agrees with the findings of Oso *et al.* (2019) who reported a significant increase in albumin concentration when they investigated the influence of ethanolic extracts of dried fruit of *X. aethiopica* (Dunal) on haematological and biochemical parameters in healthy Wistar rats (Oso *et al.,* 2019).

The results of the histopathological investigation of the liver of animals treated with *X. aethiopica* fruit extracts are presented in Plates 1 to plates 4, with Plate 1 showing the control group and Plates 2-4 showing the liver of animals in the treatment groups, but at different doses. Normal histoarchitecture with numerous hepatocytes with inconspicuous nucleotide, well oriented arrays of sinusoids, and a central vein with organized vascular epithelial layer were observed in Plate 1 containing the liver of the control group. The central vein with mild vascular epithelial distortion, hepatocytes with conspicuous nucleotides, and irregular orientation of the arrays of sinusoids were observed in the liver tissue of animals treated with 250 mg/kg of *X. aethiopica* fruit (Plate 2), while Plate 3 containing the liver tissue of animals treated with 500 mg/kg of *X. aethiopica* fruit revealed a central vein with altered vascular epithelium, hepatocytes with conspicuous nucleoli, periportal mobilization of lymphocytes, Kupffer cell activation and abnormal orientation of arrays of sinusoids. Further investigation revealed that the liver tissue of animals treated with 1000 mg/kg of *X. aethiopica* fruit showed the central vein with hepatocytes with conspicuous nucleoli, hepatic artery and Kupffer cells, as seen in Plate 4. The presence of lymphocytes and Kupffer cells in the tissues treated with *X. aethiopica* extract increased with increase in dose of aqueous extract. This finding is in line with the study of Chris-Ozoko *et al.* (2015), who noticed vascular congestion and infiltration of the interstitial space by chronic inflammatory cells in the liver of animals given 200 mg/kg and 300 mg/kg aqueous extract of *X. aethiopica* (Chris-Ozoko *et al.,* 2015). The activation of Kupffer cells and presence of lymphocytes in liver tissues of animals treated with higher doses may have been a response to liver tissue injury triggered by xylopic acid, which is present in *X. aethiopica*. Lymphocytes and kupffer cells (resident liver macrophages) are inflammatory cells which are activated in living tissues in response to injuries, it involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Nguyen-Lefebvre and Horuzsko, 2016: Wafa and Sofiane, 2020). Thus, with increased dose the extract may become toxic to the liver.

Conclusion

The findings of this study suggest that *X. aethiopica* fruit may be hepatotoxic in pregnancy, especially when administered at high doses. This is reflected in the histological examination which showed the presence of altered vascular epithelium, hepatocytes with conspicuous nucleoli, periportal mobilization of lymphocytes, Kupffer cell activation and abnormal orientation of arrays of sinusoids, all of which indicates hepatic cell damage. This effect may have been caused by xylopic acid, a known constituent of *X. aethiopica*.

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