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Research Article

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Investigation of sub-acute toxicity and hypolipidaemic effect of aqueous and methanol fruit extract of *Xylopia aethiopica*

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ABSTRACT

This work investigated the effect of aqueous and methanol friut extracts of Xylopia aethiopica on the serum cholesterol level and some liver marker enzyme activities on wistar albino rats. The animals were randomly selected and divided into seven groups (A-G) of four rats per cage with 3 replicates. Group A served as control, administered commercial feed and water only, while group B-D were administered different concentrations (50 mg/kg, 100 mg/kg and 150 mg/kg) of aqueous extract of Xylopia aethiopica respectively. Rats in groups E-G were treated orally with 50 mg/kg, 100 mg/kg and 150 mg/kg concentration of methanol extract of X. aethiopica respectively. The study lasted for 6 weeks with weekly collection of blood samples for the determination of serum levels of the biochemical parameters. Results showed significant (P<0.05) increase in alkaline phosphatase (ALP) activity from week 1-3 and no difference from week 4-6 across different concentrations of extracts when compared to the normal control. Similarly there was no significant (P>0.05) difference in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities from week 1-6 compared to the normal control. Significant (P<0.05) decrease in cholesterol level of rats was observed in Week 1 and Week 3 in both extracts while no significant difference was seen in the other weeks compared to the normal control. The result of this study was similar for both methanol and aqueous extracts and suggests that the extracts may have hypolipidaemic effect and does not confer toxicity even with prolonged use.

Key words: *Xylopia aethiopica*, cholesterol, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase.

INTRODUCTION

The use of plants for the treatment of various diseases has always found patronage among the populace especially in developing countries such as Nigeria (17, 8). This interest has grown with time as more emphasis is laid on the benefits of natural products as against synthetic ones (9). Limited access to orthodox medicine in Africa and other poor continents of the world has contributed to increased patronage of herbal remedy.

In Nigeria and other developing countries, traditional medicine accounts for more than 80% of rural populace health needs (12). Herbs and medicinal plants have found use in the treatment of an array of ailments and conditions which include immune system disorder, oxidative stress, stomach disorder, diabetes, hypertension, infertility among others (5).

Xylopia aethiopica is a tropical West American evergreen tree bearing aromatic seeds usually used as condiment. The fruit decoction is used to treat bronchitis, asthma and rheumatism (2). *X. aethiopica* is used in many herbal preparations to produce xylopic acid, a substance which has been found to have antimicrobial effects (10). It has a wide spectrum of biological activities and has played a crucial role in traditional medicines because of their physiological and pharmacological properties (14).

The objectives of the study were to determine the effect of aqueous and methanolic extracts of *Xylopia aethiopica* on the enzyme levels; serum alanine aminotransferase (ALT) alkaline phosphatase (ALP), aspartate aminotransferase (AST) and cholesterol levels which are indicators of possible modulatory effect on the liver.

EXPERIMENTAL SECTION

Plants materials

Dried fruits of *Xylopia aethiopica* were bought from orie Orba market, Orba, Nsukka, South Eastern Nigeria. Its botanical identification and authentication was done at the Department of Botany, University of Nigeria, Nsukka, were voucher specimen already exist.

Preparation of *Xylopia aethiopica* Fruit Extract

The fruits were washed with clean tap water and sun-dried. The sample was made into a powder with a grinding machine. The method of extraction followed that of (3). 135g sample of the powdered material and 500 ml of 80% analytical methanol was added into a flask and left for 48 hours with an occasional shaking to increase the extraction capacity, thereafter the soaked sample was filtered, concentrated to dryness in a rotary evaporator and weighed. Solution of the extract was prepared by dispersing 1g of the dried extract with 1 ml of 2% Tween 80 solutions for oral administration, this formed the methanolic extract. The aqueous extract was obtained by soaking 135g of the powdered plant material in 500 ml of distilled water in a flask for two days with an occasional shaking to increase the extraction, the mixture was filtered at the end of the extraction period with a whatmann (No. 1) filter paper.

Procurement, management and morphometric indices of Albino rats/Experiment Design.

Eighty four male albino rats of the wistar strain of known weight were purchased from the breeding and genetics unit of Department of Zoology University of Nigeria, Nsukka. The animals were kept in well ventilated stainless steel cages. They were handled with care and housed in the experimental house Department of Zoology for one week acclimatization, and fed commercial rat feed (vital growers mash) and clean tap water ad libitum. The rats were randomly selected and divided into seven groups (A-G) of four rats per cage with 3 replicates.

Group A served as control, administered commercial feed and water only, while group B-D were administered different concentrations (50 mg/kg, 100 mg/kg and 150 mg/kg) of aqueous extract of *Xylopia aethiopica* respectively. Rats in groups E-G were treated orally with 50 mg/kg, 100 mg/kg and 150 mg/kg concentration of methanolic extract of *X. aethiopica* respectively.

Acute Toxicity Test (LD_{50}) of *Xylopia aethiopica* (aqueous and methanolic extracts).

This was determined by the method of (11), where 50 albino rats were grouped into ten of five rats per cage. Five groups were given aqueous extract of *Xylopia aethiopica* of different concentrations, 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg. Control group was administered distilled water only while the remaining five groups were given methanolic extract of *Xylopia aethiopica* of different concentration, 5 mg/kg, 50 mg/kg, 300 mg/kg, 2000 mg/kg and control (distilled water)**.**

The various doses of extract were administered via oral route by means of an oral intubation tube. The animals were observed for 24 hrs after administration. Two died under 2 hours and the remaining three died the following day from the group that was administered 2000 mg/kg aqueous extract while none died in the other groups. The results were subjected to probit log analysis and (LD_{50}) were determined to be 1,474 mg/kg concentration for aqueous extract. One animal died after 30 minutes in the group given 300 mg/kg of methanolic extract of *Xylopia aethiopica* while four rats died after five minutes in the group administered with 2000 mg/kg concentration, but no death of animals was observed in other groups as well as the control group. It was also subjected to probit log analysis and the (LD_{50}) was determined to be 458 mg/kg.

Biochemical Analysis

Serum cholesterol was determined according to (7), Alkaline phosphatase (ALP) was determined according to (15). Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) was determined according to (16).

Statistical Analysis

Data collected were analyzed for significant differences for mean± STD ($P \leq 0.05$) compared to respective controls by one way ANOVA using the statistical package for social sciences (SPSS) version 17 mean of groups were separated using Duncan Multiple Range Test. LD_{50} was determined using probit log analysis.

RESULTS

Table 1: Changes in biochemical parameters of rats administered different concentration of methanolic and aqueous extracts of *Xylopia aethiopica* in Week 1

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg`	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	80.50±8.50^{a}	8200±2.00^{a}	90.50±1.50^{a}	76.50±2.50^{a}	77.00±9.00^{a}	79.00±5.00^{a}	80.00±2.00^{a}
ALT (U/L)	21.00±3.00^{a}	43.00±5.00^{bc}	43.00±9.00^{bc}	52.00±4.00^{c}	37.00±7.00^{abc}	30.00±2.00^{ab}	48.00±4.00^{bc}
AST (U/L)	41.00±5.00^{a}	54.00±6.00^{a}	50.00±8.00^{a}	54.00±0.00^{a}	43.00±5.00^{a}	36.00±12.00^{a}	35.00±13.00^{a}
Cholesterol (mmol/L)	3.45±0.25^{b}	2.35±0.35^{ab}	2.00±0.20^{a}	2.50±0.10^{ab}	2.90±0.20^{ab}	3.10±0.60^{ab}	3.40±0.50^{b}

The ALP and AST values of rats administered methanolic and aqueous extracts produced no significant ($P > 0.05$) change when compared with the control and when the extract groups were compared among themselves. Significant increase ($P < 0.05$) was seen in ALT values (43.00 ± 5.00)(43.00 ±9.00) and (52.00 ±4.00) of aqueous e xtract when compared with the control (21.00 ±3.00).

The cholesterol level of rats that received aqueous extract was significantly ($P < 0.05$) lower in 100 mg/kg b.w. group (2.00 ±0.20) compared with control (3.45 ±0.25). The cholesterol levels of rats administered methanolic group did not change significantly ($P > 0.05$) from control.

[1]Mean ±S.E values in a row for a given group of extracts compared to control with different superscripts are significantly different ($P < 0.05$).

Table 2: Changes in biochemical parameters of rats given different concentrations of methanolic and aqueous extracts of *X. aethiopica* in Week 2

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg`	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	97.50±1.50^{a}	94.00±1.00^{a}	88.00±11.00^{a}	82.50±7.50^{a}	92.00±0.00^{a}	92.50±4.50^{a}	87.00±6.00^{a}
ALT (U/L)	26.00±2.00^{a}	22.00±2.00^{a}	23.00±3.00^{a}	27.00±1.00^{a}	24.00±0.00^{a}	26.00±0.00^{a}	22.00±4.00^{a}
AST (U/L)	39.71±6.00^{a}	41.00±1.00^{a}	45.00±3.00^{a}	42.00±4.00^{a}	39.00±3.00^{a}	37.00±3.00^{a}	36.00±4.00^{a}
Cholesterol (mmol/L)	3.85±0.07^{a}	4.15±0.45^{a}	3.80±0.10^{a}	4.10±0.20^{a}	3.80±0.10^{a}	4.05±0.15^{a}	4.10±0.20^{a}

[1]Mean ±S.E values in a row for a given group of extracts compared to control with different superscripts are significantly different ($P < 0.05$). The ALP levels of different extract groups did not differ from control and from each other significantly ($P > 0.05$). ALT values of aqueous extract increased slightly as the dose increased, non-significantly. No significant difference was observed in the AST values of the different groups. Cholesterol value of rats administered extracts did not differ from control values and the extract group did not also differ from each other.

Table 3: Changes in biochemical parameters of rats given different concentrations of methanolic and aqueous extracts of *X. aethiopica* in Week 3

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg`	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	88.50±0.50^{a}	87.00±2.00^{a}	87.00±4.00^{a}	89.00±2.00^{a}	87.00±300^{a}	85.00±1.00^{a}	82.50±2.50^{a}
ALT (U/L)	49.00±1.00^{ab}	41.00±1.00^{ab}	35.00±5.00^{a}	43.00±1.00^{ab}	54.00±2.00^{b}	50.00±4.00^{b}	40.00±8.00^{ab}
AST (U/L)	55.00±1.00^{a}	38.00±8.00^{a}	51.00±1.00^{a}	59.00±1.00^{a}	54.00±4.00^{a}	58.00±2.00^{a}	53.00±1.00^{a}
Cholesterol (mmol/L)	4.10±0.10^{bc}	3.00±0.30^{a}	3.30±0.020^{ab}	2.95±0.15^{a}	3.10±0.20^{a}	3.40±0.30^{ab}	4.30.030^{c}

[1]Mean ±S.E values in a row for a given group of extracts compared to control with different superscripts are significantly different ($P < 0.05$). In the third week of administration of the aqueous and methanolic extracts of Xylopia aethiopica, ALP and AST values of extract groups were not significantly different from those of the control. Comparing the extract groups, no significant change was observed.

There were significant ($P < 0.05$) decreases in the cholesterol values of 100 mg/kg aqueous (3.30 ±0.02) and 100 mg/kg methanolic (3.40 ± 0.30) extract groups compared to control (4.00 ±0. 10).

Table 4: Changes in biochemical parameters of rats given different concentrations of methanolic and aqueous extracts of *X. aethiopica* in Week 4

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	61.50±3.50^{a}	59.50±4.50^{a}	66.50±0.50^{a}	58.50±3.50^{a}	80.50±2.50^{b}	62.00±1.00^{a}	66.50±0.50^{a}
ALT (U/L)	24.00±2. 00^{b}	15.00±1.00^{a}	15.00±1.00^{a}	19.00±3.00^{ab}	22.00±4.00^{ab}	23.00±3.00^{ab}	19.00±1.00^{ab}
AST (U/L)	41.00±700^{ab}	36.00±8.00^{a}	60.00±6.00^{b}	47.00±5.00^{ab}	33.00±3.00^{a}	35.00±3.00^{a}	57.00±3.00^{b}
Cholesterol (mmol/L)	3.60±0.10^{a}	3.45±0.55^{a}	4.40±0.20^{a}	4.85±0.75^{a}	4.60±0.60^{a}	4.15±0.85^{a}	4.60±0.00^{a}

[1]Mean ±S.E values in a row for a given group of extracts compared to control with different superscripts are significantly different (P < 0.05). In the fourth week, the ALP activity of rats given 50 mg/kg methanolic extract showed significant (P < 0.05) increase when compared with the control.

ALT activity of the rats in the control group (24.00 ±2.00) was significantly increased (P < 0.05) compared with the ALT of rats administered the lower doses of aqueous extract (50 mg/kg and 100 mg/kg) (15.00 ±1.00 and 15.00 ±1.00 respectively).

Cholesterol levels of rats administered different concentrations of both extracts did not differ from control values and the extract groups did not also differ statistically (P > 0.05) from each other.

Table 5:Changes in biochemical parameters of rats given different concentration of Methanolic and Aqueous extracts of *X. aethiopica* in Week 5

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg`	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	83.00±1.00^{a}	88.00±0.00^{b}	88.50±0.50^{b}	87.50±1.50^{b}	90.00±1.00^{b}	87.50±0.50^{b}	89.00±0.00b
ALT (U/L)	25.00±1.00^{a}	33.00±1.00^{a}	29.00±3.00^{a}	39.00±7.00^{a}	46.00±16.00^{a}	25.00±1.00^{a}	26.00±2.00^{a}
AST (U/L)	54.00±2.00^{b}	55.00±15.00^{b}	69.00±3.00^{b}	59.00±1.00^{b}	25.00±9.00^{a}	53.00±9.00^{b}	54.00±0.00^{b}
Cholesterol (mmol/L)	5.40±0.00^{a}	4.50±0.20^{a}	4.75±0.05^{a}	4.85±0.05^{a}	5.00±0.20^{a}	5.20±0.20^{a}	5.00±0.60^{a}

[1]Mean ±S.E values in a row for a given group of extracts compared to control with different superscripts are significantly different (P < 0.05). In the fifth week, the ALP activity of rats given the different concentration of both extracts (aqueous and methanolic) was significantly (P < 0.05) increased when compared with the control group.

Statistically, AST levels of rats administered different concentrations of aqueous extract were not different from the control. However, cholesterol levels of all the groups did not differ significantly (P > 0.05) with the Control

Table 6: Changes in haematological and biochemical parameters of rats given differentconcentrations of methanolic and aqueous extracts of *X. aethiopica* in Week 6

	EXTRACTS[1]						
	Control	Aqueous			Methanolic		
Parameters[2]	Distilled water	50 mg/Kg	100 mg/Kg	150 mg/Kg	50 mg/Kg	100 mg/Kg	150 mg/Kg
ALP (U/L)	65.00±5.00^{a}	85.00 ± 5.00^{b}	81.50± 0.50^{b}	80.00 ± 2.00^{b}	88.00 ±0.00^{b}	84.00 ± 1.00^{b}	86.00 ± 6.00^{b}
ALT (U/L)	70.00 ± 2. 00^{ab}	46.00 ± 2.00^{a}	64.00±1600^{ab}	57.00 ± 5.00^{ab}	68.00 ±14.00^{ab}	81.00 ± 5.00^{b}	73.00 ± 1.00^{ab}
AST (U/L)	21.00 ± 1.00^{a}	22.50 ± 0. 50^{a}	21. 00± 0.00^{a}	20.50 ± 0. 50^{a}	25.50 ± 2.50^{a}	25.50 ± 3.50^{a}	22.00 ± 1.00^{a}
Cholesterol (mmol/L)	4.40 ± 0.20^{ab}	415 ± 0.25^{a}	410 ± 0.30.^{a}	4.10 ± 0.10^{a}	4.60 ±0.00^{ab}	4.15 ±0.05^{a}	4.80 ± 0.00^{b}

In the sixth week of the study (ie post administration of extract), results of effects is as shown in Table above. The ALP activity of rats in all the extract groups significantly increased (P<0.05) compared with the control. There was no significant (P > 0.05) difference in ALT activity when the different concentrations of groups of aqueous extract of X. aethiopica was compared with the Control group.

No significant (P > 0.05) difference was seen in AST values of all groups.

Cholesterol levels of rats administered the different concentrations of aqueous extract of X. aethiopica showed no significant difference compared with Cholesterol levels of rats given 100 mg/kg methanolic extract decreased significantly compared with 150 mg/kg methanolic extract groups.

DISCUSSION

This study showed that initially from Weeks 1-3, there was no significant increase in ALP values of rats fed with aqueous and methanolic extracts of *Xylopia aethiopica* until in Week 4 where there was significant increase in ALP value of rats administered 50 mg/kg methanolic extract compared to control. This was followed by significant increases of ALP values of rats in all the experimental groups both aqueous and methanolic extract groups compared to the control in Week 5 and 6 respectively. It is known that an increase in the enzymatic activity of ALT, AST and ALP in the serum directly reflects hepatocellular damage. The result of ALP analysis therefore, suggests that extract of *Xylopia aethiopica* could have hepatotoxicity with prolonged use. This finding is in contrast with the work of (6) in which extract of *X. aethiopica* caused no significant effect on ALP. Comparing the effect of both aqueous and methanolic extract of *Xylopia aethiopica* on the ALP of rats, none of them seemed significantly different from the other.

ALT showed increases in values initially in Week 1 with all the different concentrations of aqueous extract of *X. aethiopica* compared with the control. Methanolic extract did not produce significant effect except in higher dose (150 mg/kg) which produced significant increase, indicating some form of liver damage. There seem to be no definite trend in the effect of the extracts on the animals as from Weeks 2-6. It does appear that the animals adjusted to the effects of the extract on the ALT after Week 1. This is in contrast to the work of (6) in which the ethanolic extract caused a significant reduction in ALT of rats over 60 days of exposure to *Xylopia aethiopica.*

AST activity showed no significant change in all the groups compared with control from week one to six. AST is found mainly in liver, kidneys, cardiac muscles and skeletal tissues. Liver and heart release AST and ALT; an elevation in plasma concentration is an indicator of liver and heart damage (18). This result is in agreement with the work of (14) in which there was no significant increase in AST in the animals treated with lower doses of *X. aethiopica* compared with the Control.

In this study, when compared with the Control, 100 mg/kg aqueous extract caused a significant decrease in cholesterol of rats in Week 1 and Week 3. Significant decrease in cholesterol levels were observed with 50 mg/kg, 150 mg/kg aqueous extract and 50 mg/kg methanolic extract. This is in agreement with the work of (13) in which plasma cholesterol and LDL Cholesterol were decreased when compared with the Control. This observed decrease could be associated with the presence of hypolipidemic component of the extract. This showed that the extract has some beneficial effects which could reduce cardiovascular risk factors. The levels of serum lipids are usually elevated in cardiovascular diseases; such an elevation represents a risk factor for coronary heart diseases. Comparing the effect of aqueous and methanolic extracts of *Xylopia aethiopica* on the cholesterol levels, aqueous extract seem to perform better than methanolic extract.

CONCLUSION

Since increase in the enzymatic activity of ALT, AST and ALP in the serum reflects hepatocellular damage, results of ALP analysis which showed significant increases compared to control in weeks 5-6 suggests that X. *aethiopica* could have hepatoxicity effect when use is prolonged from 4-6 weeks. AST did not show significant change while cholesterol had significant decreasing effect, which could be associated with hypolipidemic component of the extract. The results show that aqueous extract had a boosting effect on the hematological parameters, RBC, WBC, and PCV (aqueous and methanolic) while both extracts had similar effect on the biochemical parameters studied- ALT, AST and ALP.

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