Original Research Article

Xylopia aethiopica Fruit increased Prothrombin time, activated partial thromboplastin time, and Erythrocyte Sedimentation Rate of Wistar Rats

ABSTRACT

Background: Traditionally, the use of *Xylopia aethiopica* fruit for therapeutic purposes is on the increase without any consideration of its safety and toxicity.

Aim: This study was therefore designed to examine its effect on coagulation factors 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 female Wistar rats were divided into five groups of six rats each. Animals in groups A, B, C, and D were treated with 130, 259, 389 and 518 mg/kg body weight of *X. aethiopica* fruit extract respectively, while those in group E received normal animal feeds and water only. The administration was done once daily for 28 days via oral route. Prothrombin time, activated partial thromboplastin time, and erythrocyte sedimentation rate were determined using standard methods

Results: The extract of *Xylopia aethiopica* fruit was observed to show a significant ($p< 0.05$) increase in prothrombin time, activated partial thromboplastin time and erythrocyte sedimentation rate when compared with those of the control group.

Conclusion: *Xylopia aethiopica* significantly prolongs prothrombin time (PT) and activated partial thromboplastin time (aPTT) and in addition has thrombocytopenic property. These anticlotting properties have clearly elucidated and unraveled mechanisms of action which have laid credence to its use by women in flushing out remnants of product of conception post-delivery. Increase in ESR by *Xylopia aethiopica*l may result from an inherent property in this fruit that is yet to be identified.

Keywords: Prothrombin time, activated partial thromboplastin time, and erythrocyte sedimentation rate *Xylopia aethiopica* fruit

1. INTRODUCTION

Xylopia aethiopica possesses great nutritional and medicinal values in traditional medicine [1]. Almost all parts of *Xylopia aethiopica* are very useful medicinally, but the fruits are most commonly used for therapeutic purposes. Extracts of the fruits are used in the treatment of

cough, biliousness, bronchitis, rheumatism, dysentery, malaria, uterine fibroid and amenorrhea [2,3]. It can also be taken as a decoction, concoction or even chewed and swallowed for the management of various aches and pains [4]. It has also been shown experimentally, that the seeds possess good anthelminthic activity against *Nippostrongylus brasiliensis* and as such, its use in man as an anthelminthics, may be investigated [5].

Various extracts of *Xylopia aethiopica* have also demonstrated some promise in its employment as an adjunct therapy in the management of sickle cell disease [6]. An oily extract of the seeds is used as a lotion for boils and eruptions, and as a liniment for lumbago. Traditional medical practitioners and birth attendants use a decoction of the seeds to induce placental discharge postpartum due to its abortificient effect [7].

The roots of *Xylopia aethiopica* are employed in tinctures, administered orally as an anthelminthic, or in teeth-rinsing and mouth-wash extracts against toothache. Aqueous concoction of the root is administered after child birth as an anti-infective agent [8]. The powdered root is also employed as in dressing of wounds and in the local treatment of cancer [1]. The leaves and bark are used in traditional medicine to manage boils, sores, wounds and cuts [9]. A decoction of the leaves is used as an anti-emetic. Powdered leaves are also taken as snuff for the treatment of headaches [10]. It was revealed in a survey conducted by Kadiri *et al*. [11] that the stem bark of *Xylopia aethiopica* is used in combination with other medicinal plants as an alcoholic decoction that is applied topically in the treatment of postpartum breast infections.

Xylopia aethiopica is also used locally as carminative, stimulant and adjunct to other remedies for the treatment of skin infections [12]. In a review, Van-Hai [13] suggested that the seeds of *Xylopia aethiopica* could potentially be used to boost fishes' immunity against various infections. This is of particular interest to scientists in the field of aquaculture as these natural products, unlike antibiotics, pose minimal risk to the fishes and also to the environment. The ability of *Xylopia aethiopica* to retain the antioxidant properties of tomatoes creates opportunities for the investigation of its candidacy as a stabilizer in the food industry.

Xylopia aethiopica is characterized with numerous chemical components with various medicinal potentials [14]. 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 [15,16], alkaloids, rutin and fixed oils [17,18]. 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 [16,18]. The impact of the fruit on body weight and glucose concentration [19] as well as lipid profile [20] of animals has been reported. The fruit has also been reported to induce hepatotoxicity [21], renal toxicity [22] as well as oxidative stress [23]. Recently, Ogbuagu et al. [24] reported that the fruit extract of *Xylopia aethiopica* adversely perturbed sperm qualities in male Wistar rats. This study was therefore designed to examine its effect on coagulation factors and erythrocyte sedimentation rate of Wistar rats.

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. [25]. 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. [26]. 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 female 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 Estimation of Prothrombin Time (PT)

Prothrombin time (PT) was determined according to the method described by Onyebuagu [27]. 0.1 mL of platelet poor plasma (PPP) sample from each animal was dropped into test tubes numbered 1-30. Thereafter, 0.1 mL of commercially prepared control sample was dropped into tube no. 31. All the tubes were warmed at 37 ^{o}C for 3 minutes using water bath. Then 0.2 mL of prothrombin-thromboplastin reagent was added to each test tube and the clotting time was recorded for each tube.

2.6 Estimation of Activated Partial Thromboplastin Time (aPTT)

Activated partial thromboplastin time (aPPT) was determined according to the method described by Onyebuagu [27]. 0.1 ml of aPTT reagent (Darkez Ltd) was added to a pair of test tubes containing 0.1 ml of the test samples. The tubeswere incubated at 37 ^{o}C for 5minutes, before adding 0.1 mL of freshly prepared $CaCl_2$ solution. The clotting time was recorded using stop watch.

2.7 Estimation of Erythrocyte Sedimentation Rate (ESR)

Erythrocyte sedimentation rate (ESR) was determined using Westergreen method described by Onyebuagu [27]. 0.4ml of 2.8% sodium citrate was drawn into a syringe and the anticoagulated blood sample was added to the 2ml mark. The mixture was then drawn by suction up the calibrated Westergreen tube to above the zero mark. The top of the tube was quickly closed with the index finger, and then carefully rotated till the upper level of blood was exactly at the zero mark. Then the clock was started and allowed to run for 1 hour, and the temperature also recorded. The height of the clear fluid above the upper limit of the red cells column after 1hour was recorded in mm/hr.

2.8. Statistical Analysis

Results are expressed as mean ± standard deviation. The levels of homogeneity among the groups were assessed using One-way Analysis of Variance (ANOVA) followed by Tukey's test. All analyses were done using Graph Pad Prism Software Version 8.2 and P values < 0.05 were considered statistically significant.

3. RESULT

3.1 Median Lethal Dose (LD_{50}) Result

The physical signs of toxicity observed in the animals included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death. In the first phase of the median lethal dose determination, no mortality was recorded in the group treated with 1000 mg/kg body weight of *X. aethiopica* fruit extract. However, 100 % mortality was recorded in the groups treated with 2000, 3000, 4000, and 5000 mg/kg body weight of *X. aethiopica* fruit extract respectively. Similarly, in the second phase of medial lethal dose determination, no mortality 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 calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

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

Where a = 1200 mg/kg

b = 1400 mg/kg

LD_{50}= 1296.15 mg/kg

3.2 Effect of ethanol extract of *Xyiopia aethiopica* fruit on Prothrombin time (PT), activated partial thromboplastin time (aPTT), Erythrocyte Sedimentation Rate (ESR) after 28 days of treatment.

The extract of *Xylopia aethiopica* was observed to show a significant increase in prothrombin time, activated partial thromboplastin time and erythrocyte sedimentation rate when compared with those of the control group ($p < 0.05$), as presented in Table 2.

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

Table 2: Effect of ethanol extract of *Xylopia aethiopica* fruit on Prothrombin time (PT), activated partial thromboplastin time (aPTT), Erythrocyte Sedimentation Rate (ESR) after 28 days of treatment.

Group	A	B	C	D	E	
Dose of extract (mg/kg)	**130**	**259**	**389**	**518**	**Control**	**P Value**
PT (Sec)	15.34±2.92*	17.15±3.47*	20.01±3.27*	22.56±2.35*	12.99±2.66	0.03
aPTT (Sec)	36.83±3.37*	38.56±3.93*	39.63±4.27*	42.87±3.24*	32.67±2.78	0.05
ESR (mm/hr)	5.04±0.94	6.97±0.23*	7.56±0.45*	9.67±1.04*	4.67±0.34	0.02

Values are presented as Mean±S.D, where n = 6. Values with * are statistically significant at p value ≤ 0.05 when compared with the control group.

Legend: aPTT = Activated Partial Thromboplastin Time, PT = Prothrombin Time, ESR = Erythrocyte Sedimentation Rate

4. DISCUSSION

The acute toxicity study of the plant extracts recorded 100% mortality at a dose of 1400 mg/kg bodyweight and above (table 1). This shows that the fruit of *Xylopia aethiopica* might be highly toxic. The physical signs of toxicity observed in the animals included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

In this study, administration of ethanol extract of *Xylopia aethiopica* fruit produced a significant increase in prothrombin time (PT) and activated partial thromboplastin time (aPTT) when compared with those in the control group. This increase was most significant at the highest dose. This significant increase appears to be related to the decrease in the platelet count reported by Ogbuagu *et al*. [28] when they exposed animals to *Xylopia aethiopica* fruit for 28 days. This assertion is based on the fact that a reduction in platelet number is associated with disorders of the hemostatic mechanisms. The findings on the effect of dietary *Xylopia aethiopica* on coagulation factors observed in this study is similar to the report by Obembe *et al.,* [29] who reported a significant increase in clotting time when animals were treated with 200 mg/kg body weight of *Xylopia aethiopica*, but contradicted the report by Nwafor [14], who observed a slight decrease in PT and aPTT in albino rats treated with methanolic extract of fruits of *Xylopia aethiopica*. The difference in these results might be due to the different solvents used in the extraction of *Xylopia aethiopica* fruit.

In this study, a dose-dependent increase was observed in the erythrocyte sedimentation rate (ESR) as presented in table 2. This result contradicted the findings of Onyebuagu *et al.,* [27] who investigated the effects of dietary *Xylopia aethiopica* on hematological parameters and plasma lipids in male Wistar rats. Saha *et al.,* [30] reported that increase in ESR could result from inflammation, pregnancy, anemia, autoimmune disorders (such as rheumatoid arthritis and lupus), infections, some kidney diseases and some cancers (such as lymphoma and multiple myeloma). This corroborated the adverse effect of *Xylopia aethiopica* fruit on liver [21] and kidney [22] indices recently reported.

CONCLUSION

Xylopia aethiopica significantly prolongs prothrombin time (PT) and activated partial thromboplastin time (aPTT) and in addition has thrombocytopenic property. These anticlotting properties have clearly elucidated and unraveled mechanisms of action which have laid credence to its use by women in flushing out remnants of product of conception post-delivery. Increase in ESR by *Xylopia aethiopica*l may result from an inherent property in this fruit that is yet to be identified.

CONSENT

It is not applicable.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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