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The Abortifacient Effect of the Ethanol Fruit Extract of *Xylopia Aethiopica* on Female Wistar Rats

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Abstract

The fruit extract of Xylopia aethiopica (X. aethiopica) is a common spice used in preparing soup for women immediately after delivery as it is believed to cause uterine contraction and involution. Therefore, this study aimed at investigating the abortifacient effects of X. aethiopica on adult female wistar rats and also, to histologically examine the uterus of the experimental animals. 66 adult wistar rats comprising of 12 males for mating and 44 females that weighed 150 – 180g were used. Thirty pregnant rats were divided into five groups. Group 1 was negative control, groups 2, 3 and 4 received 100mg|kg, 200mg|kg and 400mg|kg of the extract while group 5 was the positive control and received 4microgram/kg of misoprostol (cytotec) as the standard drug. They were weighed and treated daily every morning for 14 days. After the experiment, the rats were sacrificed by dislocating their cervical spines and dissecting them using a midline incision on the anterior abdominal wall. A dose-dependent decrease in body weight in rats was seen. X. aethiopica's mechanism of abortion was to prevent early pregnancy implantation and pregnancy resorption, unlike cytotec, which caused thrombosis and necrosis. Extract showed abortifacient effect on female wistar rats. Extract of X. aethiopica was shown to possess abortifacient effects on pregnant female wistar rat as it prevents implantation at early stages and pregnancy resorptive at a later stage. It therefore has emergency contraceptive property.

Keywords: Abortion, pregnancy, Xylopia aethiopica, abortifacient

Introduction

The female uterus, sometimes referred to as the womb, is a muscular, hollow organ that is situated in the pelvis between the rectum and the bladder in people who are assigned as female at birth. It affects menstruation, getting pregnant, and giving birth [1]. The Mullerian or paramesonephric ducts fuse to form the uterus between weeks five and six of pregnancy. The cervical canal has differentiated and the uterine cavity is visible by 30 weeks. Throughout pregnancy, the uterus experiences major changes, such as hypertrophy and hyperplasia, and is in charge of protecting and expelling the fetus at term[2]. The uterus is supported and kept in place by ligaments and folds, which are divided into peritoneal folds and fibromuscular ligaments. The uterus is encircled by the anterior uterovesical fold of the peritoneum, whereas the posterior ligament consists of a retro-vaginal fold [3].

Gross Features and Location of the Uterus

Anatomically, the uterus is divided into four parts which are the fundus, corpus, cervix, and cervical canal [4]. The term fundus refers to the upper portion of the uterus above the insertion of the tubes. The corpus is the wide area where the fallopian tubes join the uterus (body), it extends downward and continues as the cervix and the isthmus. It extends from the isthmus downstream and joins to Vagina. The uterus is situated behind the bladder and in front of the rectum[5].

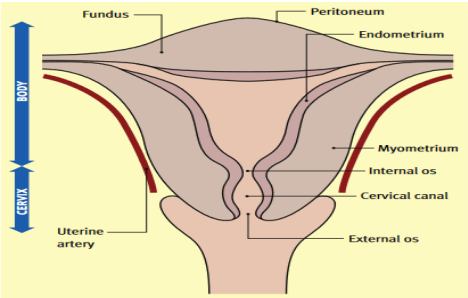


Figure 1: Showing the features of the uterus[5]

Histology perspective

The uterus is a muscular structure with three layers: endometrium, myometrium, and perimetrium. The endometrium is the inner lining of the uterus and requires changes during the menstrual cycle to regenerate, decidualize, and shed[6]. It supports implantation and pregnancy. The perimetrium is the outer lining, consisting of a thin layer of loose connective tissue. The myometrium, between the endometrium and perimetrium, is the muscular layer that provides the bulk of the uterus. It consists of three layers of smooth muscle fibers and neurovascular tissues. The inner and outer myometrial layers are mostly associated with the initial layers of the paramesonephric ducts [7].

The middle intermediate layer contributes to the uterine thickness with many blood vessels providing nutritional and nervous supply [7]. Functionally, the myometrium expands and

contracts to facilitate parturition. The muscular wall of the uterus, the myometrium, is an example of smooth muscle and possess the ability to generate contractions autonomously[8].

Blood Supply

The uterine artery arises from the internal iliac artery and it is the major contributor to the blood supply reaching the uterus[8]. Uterine artery anastomoses with terminal branch of the ovarian artery and the cornual part of the uterus may receive blood from both arteries. The uterine vein accompanies the artery and drains into the internal iliac vein [9].

Lymphatics

The fundal area of the uterus chiefly drains into para-aortic lymph nodes along with the ovarian and fallopian tube lymphatic drainage. Some of it also drains into superficial inguinal lymph nodes along the round ligament. The lower portions of the uterus drain along uterine blood vessels into external and internal iliac lymph nodes [1].

Nerve supply

The uterus is innervated via the inferior hypogastric plexus. This plexus receives post-ganglionic sympathetic fibres from the inferior hypogastric nerves (branches of the superior hypogastric nerve) and preganglionic parasympathetic fibres from pelvic splanchnic nerves from S2-S4. Most of the parasympathetic nerves will synapse in ganglion at the level of the uterus. Visceral afferent fibres pass within the pelvic splanchnic nerves [10].

Clinical Anatomy

Uterine atony after delivery

Uterine atony is characterized by inadequate contraction of the myometrial cells of the corpus uteri while responding to endogenous oxytocin, which is released during delivery[11].

Uterine fibroma

Uterine fibroma (or leiomyoma), is the most common benign tumor of the female genital tract: worldwide, around 50% of women present it during their fertile life. Clinically, these tumors manifest with chronic pelvic pain, abnormal bleeding, spontaneous abortions, and infertility, all associated with deterioration in the quality of life[12].

Methods of abortion

Abortion is the termination of pregnancy before the age of viability. This can be medical or surgical. Various medical methods of termination of pregnancy exist including the use of mifepristone and misoprostol[13]. This two drugs are licensed for that purpose. However, several agents including herbs have been tried by many with varying results[14].

Abortifacient effect of xylopia aethiopica

X. aethiopica which is taken in some parts of Africa to encourage fertility and ease child birth is suspected to have abortifacient property since it induces strong regular uterine contraction at high dose[15].

Herbal consumption in Pregnancy

Shinde *et al*., 2012, stated that herbs are part of whole plants, whose effects have to do with the synergistic action of nature's formulation. They further reported that some herbs are unsafe during pregnancy because they can induce uterine contractions and high blood pressure that could lead to miscarriage, a premature birth or injury to the fetus or even death.

In their study, Jahan *et al*., 2022 stated that a large majority of pregnant women (71.80%) consumed herbs, which is similar to a former study performed in Bangladesh in 2018, which reported that 70% of expectant mothers took herbs[16]. In the United Kingdom, Holst *et al*. (2009) found that among 578 mothers between November 2007 and February 2008, 334 (57.8%) self-reported use of herbal remedies during pregnancy[17]. In another study, the percentage among a group of women in Norway was 22.5%[18]. In a regional hospital in Nigeria, herbal medicine consumption was found to be 36.8% among pregnant and lactating mothers[19]. In Kenya, about 12% of women took herb throughout their pregnancy cycle[20]. *Zingiber officinale Roscoe* (ginger), *Nigella sativa* (black seed), *Citrus limon* (L.), *Prunus domestica* (prune), and *Allium sativum* L. (garlic) were the most commonly mentioned herbs in this study, which is similar to another study performed in Bangladesh[16]. Ginge, garlic, peppermint, and Chinese Okra have been the most prevalent herbs in other studies [21]. In the case of *X. aethiopica*, little information about its uses during pregnancy is availableand even when it is available, its roles as abortifacient remains unclear, yet herbs are consumed based on several reasons.

The higher prevalence of herb use during the third trimester (50.91%) could be due to mothers' increased concern for the development of the baby's body structure and organ system during this period. The majority of users (71.8%) believed herbs were safer than medicines, which may be relevant considering that 91.03% of them reported no negative effects from any herb. Informal sources of information, such as their own views and friends/family, were cited by women as being critical in their decision to explore herbal treatment, which is consistent with previous findings[22].

Xylopia Aethiopica

X. aethiopica, a family of Annonaceae is a tall, slim, aromatic, evergreen tree that grows to 15–30 m high and 60–70 cm in diameter. The natural habitat of *X. aethiopica* include Nigeria, Ghana, Ethiopia Cameroon, and Senegal all of which are countries found in Savanna region of Africa[15].

Fruits of *X. aethiopica* represent small, twisted bean-pods; they are dark brown in color, cylindrical in shape, 2.5~5.0 cm in length, and 4~6 mm in thickness (plate 1). Each pod contains 5 to 8 kidney-shaped seeds, each of which are approximately 5 mm in length [23].

Figure 2:Showing the picture of the dried fruit of XA[24]

Taxonomy

X. aethiopica, also known as Negro pepper, is an angiosperm found in tropical and subtropical Africa. It belongs to the Annonaceae custard apple family and is a member of the Magnoliopsida superorder [24].

Its medicinal use includes aqueous concoction of the root for anti-infective purposes after childbirth, a decoction of the leaves for anti-emetic purposes, and powdered leaves for headache treatment. The stem bark of *X. aethiopica* is used in combination with other medicinal plants for postpartum breast infections[25].

In Nigeria, *X. aethiopica* fruits and seeds are used to prevent fever, cough, and postpartum bleeding, and facilitate post-natal recovery[26]. Previous studies have reported antioxidant, hypolipidemic, antifungal, and antibacterial effects of whole *X. aethiopica* fruits, as well as their preventive effects against dysentery and fertility challenges[27].

Phytochemical Constituents of the *X. aethiopica*

According to Amajor *et al*., 2014, XA phytochemical content include alkaloid, tannins, flavonoids, phenols and saponin[28]. In their study, Ibe *et al*. 2023, agreed with Amajor *et al*., 2014, on the five phytochemical constituents mentioned in their study, also stated the presence of oxalate, anthraquinone and cardio glucoside. Ibe *et al*. 2023, further reported that there are presence of vitamin A, C and E in *X. aethiopica*[22].

Phenol

Phenols have been reported to have smooth muscle contractile and cytotoxic properties that are connected to their uterine contractile and abortifacient properties (Ijioma *et al*., 2020). Phenolic compounds particularly 2-methoxy-4-vinyl phenol identified in the GC-MS analysis of E. heterophylla extract have been identified in various abortifacient plants such as Balanites aegyptiaca (Murthy *et al*., 2021). Exposure to phenolic compounds has been associated with spontaneous abortion (Chen *et al*., 2013). Thus, phenols may majorly contribute to the abortifacient properties of E. heterophylla.

Alkaloids

Alkaloid constituents in plants can cause neonatal heart failure, myocardial infarction, and stroke[29]. Extracts of Graptophyllum pictum have been found to suppress uterine contraction and have anti-implantation activity [30]. Studies have shown that plant extracts can lower

progesterone levels in mice, indicating anti-progestogenic potential[31]. Progesterone is crucial for ovulation, fertilization, implantation, pregnancy maintenance, and lactation. Alkaloids containing plants can impair progestogenic activities, facilitating abortion or miscarriage. Flavonoids have biological functions such as protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses, and tumors. They reduce the risk of estrogen-induced cancers by interfering with estrogen-producing enzymes. Prostaglandins, hormone-like compounds, stimulate uterine contractions and can promote pain reduction. Flavonoids can also protect against inflammatory disorders and reduce edema formation[31].

Tannic acid

Tannin is a common constituents of medicinal plant crude extracts. It has been shown to antagonize contractions evoked by a variety of agonists in isolated rat uterus, likely by affecting calcium availability for contraction. Tannic acid has also been shown to reduce smooth muscle contractility in vascular tissue [31].

Saponins

Saponins are naturally occurring bioorganic compounds having at least one glycosidic linkage (C-O-sugar bond) at C-3 between aglycone and a sugar chain[32]. Saponins exhibit a biological role and medicinal properties such as hemolytic factor anti-inflammatory, antibacterial, antifungal, antiviral, insecticidal, anticancer, cytotoxic and molluscicidal action[32]. In addition, saponins are reported to exhibit cholesterol-lowering action in animals and human. Saponins on the other have been shown to possess uterine stimulating.

Materials and methods

Materials

Study location

This experiment was carried out at the Pharmacology Departments of both Ebonyi State University Abakaliki and University of Nigeria Teaching Hospital Enugu.

Collection of plant materials

Dry fruit of *X. aethiopica* was collected from the local forest together with its cobs.

Identification and Authentication of the plant material

The plant was identified and authenticated by Mr Nwankwo in Applied Biology Department of Ebonyi State University with taxonomy no: 1317910

Animals used for the study

A total of sixty six (66) wistar rats weighing 150 to 180g were used for this study. The animals were procured from the animal house of the Faculty of Medicine/Pharmaceutical Sciences of Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. The rats were separated into male and females during the period of acclimatization in the pharmacology Laboratory, Ebonyi State University, Abakaliki that lasted for two weeks. Twelve (12) of the 66 wistars were males, and were used for mating. Twelve female wistars were used for the acute toxicity test. Thirty pregnant rats were divided into 5 groups of 6 rats each and were used to study the abortifacient effect of the extract. Twelve pregnant rats were used to study the oxytocic effect of the extract.

Drugs/chemicals/Reagents

This included ethanol, cytotec, oxytocin, tween 80 and De Jalon solution

Equipments/instruments

Clean glass tube, filter paper, stainless plates, water bath, organ bath, refrigerator, cages and kymograph.

Ethical approval

Before the commencement of this study, ethical approval was sought for and obtained. Following the approval of this study, the Directorate of Research, Innovation and Commercialization of Ebonyi State Research Ethics Committee gave this study an ethical code which was **EBSU/DRIC/UREC/Vol 08/001**

Methods

Extraction technique

The dry fruits of *X. aethiopica* was washed and air dried at room temperature. It was ground into powdered form and weighed. Five hundred and forty grams (540g) of the powdered *X. aethiopica* fruit was macerated in 2 litres of ethanol. The extract was shook and starred intermittently for 24 hours, after which it was sieved into a clean glass tube using the filter paper. The filtrate was poured into stainless plates and dried on a water bath at a reduced temperature of 45^{o}C to recover the extract. The final extract was 27% w/w Semi-solid brown powder. The dried extract was stored in airtight sterile containers in a refrigerator until the experimental period.
Before the administration to the experimental animals, 1000mg was dissolved in 2 ml of tween 80 since the extract was not soluble in water and dissolving it in ethanol would result in making the animals drowsy and possibly unfit for the study. After which 8 ml of distilled water was added to make it to 10 ml for easy calculation.

Phytochemical Screening of X. *aethiopica*

Qualitative phytochemical screening was done to identify the analytes present in the plant of interest since it shows which chemical dominates and is very helpful in the synthesis of useful new drug.

Preparation of fat free sample:

Two (2g) of the sample was de-fatted with 100ml of diethylene using a Soxhlet apparatus for 2 hrs.

Alkaloid determination:

The sample was weighed into a 250ml beaker, and then added to a 250ml beaker with 10% acetic acid in ethanol. The extract was concentrated, then ammonium hydroxide was added drop-wise until precipitation was complete. The solution was allowed to settle, and the decimate was collected, washed, and filtered. The crude alkaloid residue was then weighed.

Determination of total phenols by Spectrophotometric method

The fat free sample was boiled with 50ml of ether for 15minutes for the extraction of the phenolic component. A 5ml portion of the extract was pipette with 50ml flask, then 10ml of distilled water was added, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were made up to the mark and left for 30mins for colour development. The absorbance of the solution was read at 505nm wavelengths using a spectrophotometer[33].

Tannin Determination

A500mg of the sample was weighed into 100ml plastic bottle. A 50ml of distilled water was added and shaken for 2 hours in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtrate was pipetted out into a tube and mixed with 3ml of 0.1M FeCI2 in 0.1N HCI and 0.008M potassium ferocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelength within 10mins. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid and obtained 100ppm measurement[34].

Saponin determination

A 20g of each plant sample were dispersed in 200ml of 20% ethanol. The suspension was heated over a hot water bath for 4hrs with continuous stirring at 55^0C. The mixture was filtered and the residue re-extracted with another 200ml of 20% ethanol. The combined extracts were reduced to 40ml over the water bath at 900C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discharged. The purification process was repeated; 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the

samples were dried in the oven to a constant weight. The saponin content was calculated in percentage[35].

Flavonoid determination

A 10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper, no 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight[36].

Pregnancy confirmation

The animals were paired for mating in the ratio of 3 female rats to 1 male rat. After the period of acclamatization, the male and female rats were placed together in a large mating cage. Pregnancy was confirmed with the aid of vaginal plug in the females' vagina clearly seen with the help of a ×5 magnifying hand lens and weight gain. Thirty pregnant rats divided into 6 groups were used to study the abortifacient effects of the extract.

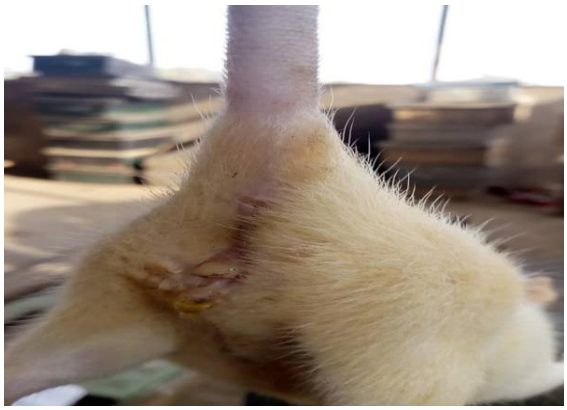


Figure 3:Showing vaginal plug in the females' vagina clearly seen with the help of a ×5 magnifying

Acute toxicity study

This study was performed following the method described by Lorke (1983). This study was conducted in two phases using 12 adult female rats with the weight mentioned above. In the first phase, 3 groups of rats with each containing 3 female rats in each group received 10mg/kg, 100mg/kg and 1000mg/kg of ethanol extract of *X. aethiopica* via orogastric administration. The

rats were observed for signs of toxicity such as hyper activity, salivation, Paw-licking, writhing, muscle paralysis, respiratory distress and mortality within the first 4 hours and after 24 hours. In the second phase, three groups of animals with one animal in each group were orogastrically given ethanol extract of *X. aethiopica* of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. The rats were allowed with the similar conditions as that observed in phase 1 and observed for signs of toxicity as stated above and mortality at first 4 hours, 24 hours and 72 hours. Mortality was observed at the dosage of 2900 mg/kg and 5000 mg/kg[37].

Preparation of fresh DeJalon solution

Twenty litres of De Jalon Solution were used in this study. It was freshly prepared and used for the organ bath experiment with each liter constituted with 90 grams of NaCl, 42 ml of 10% of KCl solution, 5 g of KH_2PO_4 in 10% glucose solution, 5 g of $NaHCO_3$, 2.7 ml of Cacl solution, aerating gas of O_2 + 5% CO_2 and 20 liters of distilled water[38].

Experimental assessment of abortifacient effect

Thirty pregnant rats were divided into 5 groups of 6 rats each and were used to study the abortifacient effect of the extract. Group 1 was negative control and received no treatment, groups 2, 3 and 4 received 100mg|kg, 200mg|kg and 400mg|kg of the extract while group 5 was the positive control and received 4microgram/kg of misoprostol (cytotec) as the standard drug. They were weighed and treated daily every morning for 14 days. Treatment was commenced from day 5 of pregnancy confirmation. The animals were examined for bleeding using X5 magnification lens. They were examined 4 times daily from 8am to 6pm. At the end of the experiment, the animals were sacrificed by dislocating their cervical spines. This was done by holding the rat at the neck and tail the base and stretched it to dislocate the cervical vertebrae. They were dissected on a dissecting board by midline incision on the anterior abdominal wall.

Histological examination

The uterine horns were identified and examined noting the implantation and pregnancy resorption sites. The number of fetuses in the horns were also noted. The endometrial samples were taken for histology. The tissue was preserved with formalin and taken to histopathology laboratory of Anatomy department of EBSU. The tissue was fixed, embedded, sectioned and stained with hematoxylin and eosin. It was examined using light microscope.

Statistical analysis

The data obtained from this study was carefully documented and entered into the International Business Machine Statistical Package for Social Sciences (IBMSPSS) version 26, Chicago II, USA. The result of this study was presented as mean ± standard deviation (SD). Comparison between groups as done using One-Way Analysis of Variance with subsequent analysis using *Post Hoc* Test since the comparison was more than 3 groups. The significance difference in this study was set at $P < 0.05$. The qualitative components of the study were analyzed manually.

Results

Acute Toxicity Studies

The acute toxicity of ethanol fruit extract of *X. aethiopica*, mortality was determined after oral administration of the ethanol fruit extract of at the dual doses' of 2900 mg/ kg and 5000 mg/kg. The Median LD_{50} was established to be 1703 mg/kg in rats.

Qualitative Phytochemical Analysis of XA Fruits

The evaluation of the phytochemical constituents of the ethanol extract of *X. aethiopica* fruits showed the presence of ten (10) secondary metabolites. These secondary metabolites were alkaloids, flavonoids, cardiac glycosides, phenol and phlobatannins. Other detected were terpenoids, tannins, steroids, saponins and anthraquinones as shown in the table 1,

Table 1:Showing Outcome of the Phytochemical Screening of the Ethanol Extract of *X. aethiopica* Fruit

Phytochemical Constituents	**Designation**
Alkaloids	+
Flavonoids	++
Cardiac glycosides	++
Phenols	++
Phlobatannins	+
Terpenoids	++
Tannins	+
Steroids	++
Saponins	+
Anthraquinones	+

Keys: + and ++ denoted less and more presence

Weight of the Pregnant Rats after 7 and 14 Days and Comparison between Groups

This study revealed mean ± SD of 184.50±14.50, 171.80±16.84, 172.67±18.03, 168.17±17.02 and 157.67±11.88 for the female pregnant wistar rats in groups 1, 2, 3, 4 and 5 respectively after 7 days. This study further indicated that 14 days, female pregnant wistar rats in groups 1, 2, 3, 4 and 5 presented mean ± SD of 210.67±14.22, 171.00±18.93, 180.83±16.63, 166.50±19.38 and 149.17±25.69 correspondingly.

No significance difference in weight was observed in the comparison of the weight of female pregnant wistar in group 1 to weight of female pregnant rats in groups 5 ($P = 0.676$), 2 ($P = 0.694$), 3 ($P = 0.400$), and 4 ($P = 0.050$) as well as the comparison of the weight of the female pregnant rats in group 5 to weight of female pregnant wistar rats in groups 2 ($P = 1.000$), 3 ($P = 0.995$) and 4 ($P = 0.584$) after 7 days.

Significance difference in weight was observed in the comparison of the weight of female pregnant wistar in group 1 to weight of female pregnant rats in groups 5 (P = 0.019), 3 (P = 0.005), and 4 (P = 0.000) but no significance difference was observed in the comparison of the weight female pregnant in group 1 to weight of pregnant female wistar rats in group 2 (P = 0.089). No significance difference in weight was observed in the comparison of the weight of the female pregnant rats in group 5 to weight of female pregnant wistar rats in groups 2 (P = 0.916), 3 (P = 0.995) and 4 (P = 0.364) after 14 days as shown in table 2.

Table 2:Showing Mean Weight of Female Pregnant Wistar Rats after 7 and 14 days and Comparison of Weight of Female Wistar Rats in Group 1 to weights of Pregnant Rats in Groups 5, 2. 3 and 4 and Weight of Pregnant Rats in Group 5 to Weight of Pregnant Rats in Groups 2, 3 and 4

		Weight (g)	**P-values ^{N}**	**P-values ^{P}**
	Groups	**Mean ± SD**		
	1 (Negative Control)	184.50±14.50		
	5 (Positive Control)	171.80±16.84	0.676	
7^{th} Days	2	172.67±18.03	0.694	1.000
	3	168.17±17.02	0.400	0.995
	4	157.67±11.88	0.050	0.584
	1 (Negative Control)	210.67±14.22		
	5 (Positive Control)	171.00±18.93	0.019	
14^{th} Days	2	180.83±16.63	0.089	0.916
	3	166.50±19.38	0.005	0.995
	4	149.17±25.69	0.000	0.364

N and P are the P values when group 1 was compared to groups 5, 2, 3, and 4 as well as when group 5 was compared to group2, 3 and 4 correspondingly.

This table shows that the miscarriage rate in the animals treated with the fruit extract of *X. aethiopica* is comparable with the positive control, although the miscarriage was not statistically significant.

Table 3:The abortificient effect of *X. aethiopica* clinically

Groups	No Bleeding	Bleeding	Total	X^2	P Value

Group 1	6	0	6	2.772*	0.764
Group 2	6	0	6		
Group 3	5	1	6		
Group 4	5	1	6		
Group 5	5	1	6		
Total	27	3	30		

*Fisher's Exact test used

Gross Findings for Abortion

Figure 4:Shows the finding of Group 1which was the negative control. The animals in this group did not receive any treatment. The uterine horns with the fetuses were intact with no resorption site or area of thrombosis. Six fetuses were noted in the uterine horns.

Figure 5:Shows Group 2 that received 100mg/kg of *X. aethiopica*. The animals in this group had one resorption site. The other fetuses

Figure 6:Shows Group 3 that received 200mk/kg of *X. aethiopica*. No resorption site was noted in this group but the gestational sacs appear thickened.

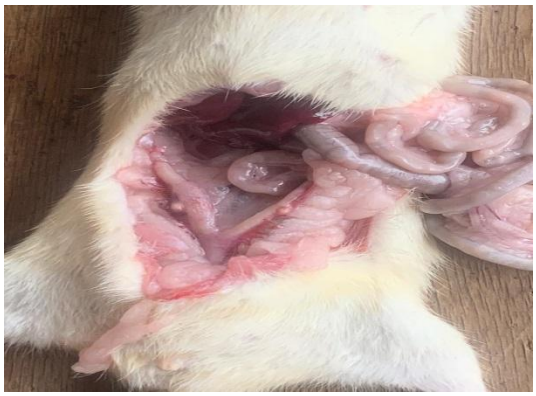
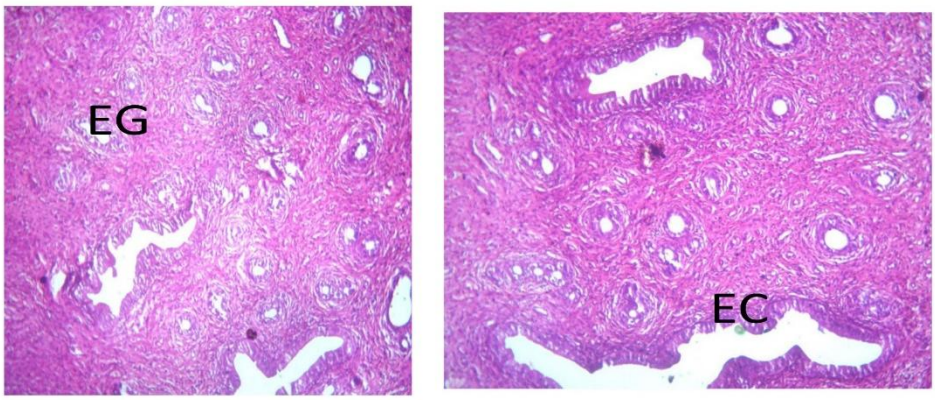


Figure 7:Shows group 4 treated with 400mg of *X. aethiopica*. Three resorption were noted. No fetus developed.

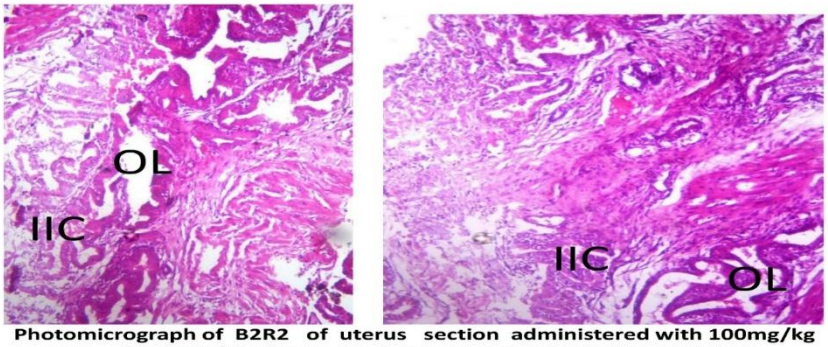
Tissue histology of endometrial plate

Plate 1: Tissue histology of group 1



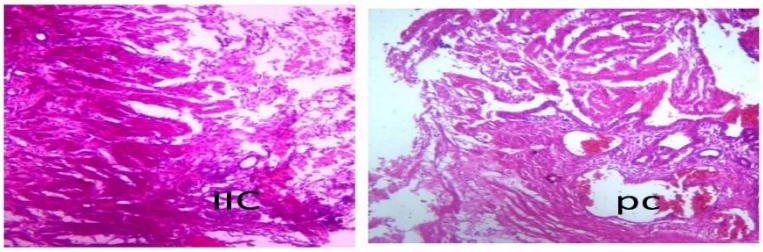
Photomicrograph GP A1 control section of uterus (x400)(H/E) shows normal uterine tissue with numerous active endometrial gland (EG). And active epithelia cell (EC_ .

Plate 2**:** Tissue histology of group 2



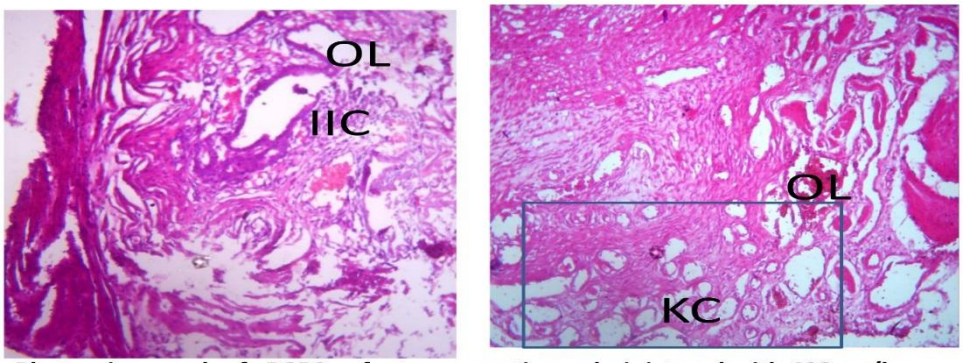
Photomicrograph of B2R2 of uterus section administered with 100mg/kg extract (X400)(H/E) shows moderate degeneration with moderate obliteration of the lumen (OL) with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix.

Plate 3**:** Tissue histology of group 3



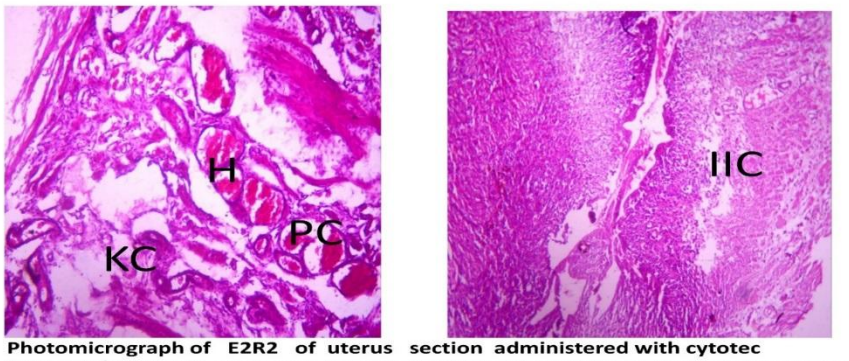
Photomicrograph of C2R2 of uterus section administered with 200mg/kg extract (X400)(H/E) shows moderate degeneration with moderate obliteration of the lumen (OL) with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix and moderate polycystic (PC)area with hemorrhage (H).

Plate 4:Tissue histology of group 4



Photomicrograph of D2R2 of uterus section administered with 400mg/kg extract (X400)(H/E) shows sever degeneration with severe obliteration of the lumen (OL) with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix and severe kilocytic chages(KC) focal areas of hemorrhage (FAH)

Plate 5:Tissue histology of group 5



Photomicrograph of E2R2 of uterus section administered with cytotec (X400)(H/E) shows moderate to severe degeneration with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix and moderate polycystic (PC)area with hemorrhage (H) and severe kilocytic changes

Discussion

Phytochemicals, found in plants, are antinutrients with various nutritional, biological, and pharmacological properties. They play a crucial role in human health, influencing antioxidant activity, hormone mimicking, and disease suppression. Minerals in spices and food products are essential for human health and maintaining certain physicochemical processes [39]. Some secondary metabolite, such as alkaloids, flavonoids, and terpenoids, can be toxic due to their ability to stimulate oxidative stress[39]. A study found that the ethanol extract of *X. aethiopica* fruits had a lower LD50 (1703 mg/kg in rats) than the aqueous LD50 (2154 mg/kg). This study study also found that the weight of female Wistar rats decreased with increasing dosage of the ethanol fruit extract, consistent with previous studies. The ethanol fruit extract caused a dose-dependent reduction in body weight, with death occurring in extreme cases.

The ethanol fruit extract of *X. aethiopica* has been found to have some abortifacient effects, which were increased with increasing doses [40]. The extract was found to cause abortion by resorption, similar to the effects of Graptophyllum pictum, which has suppressant effects on uterine normal contraction and high anti-implantation activity[41]. *X. aethiopica* is also rich in phenolic acids, which have been reported to cause spontaneous abortion.

Conclusion

Ethanol fruit extract of *X. aethiopica* was shown to possess abortifacient effects on pregnant female wistar rat as it prevents implantation at early stages and pregnancy resorptive at a later stage. It therefore has emergency contraceptive property.

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Conflicts of interest

The authors have declared that there is no conflict of interest

References

1. Ameer MA, Chaudhry H, Mushtaq J, Khan OS, Babar M, Hashim T, Zeb S, Tariq MA, Patlolla SR, Ali J, Hashim SN *et al*: **An overview of systemic lupus erythematosus (sle) pathogenesis, classification, and management.***Cureus* (2022) **14**(10):e30330.

2. Ameer MA, Fagan SE, Sosa-Stanley JN, Peterson DC: **Anatomy, abdomen and pelvis: Uterus.** In: *Statpearls.* StatPearls Publishing. Copyright © 2024, StatPearls Publishing LLC., Treasure Island (FL) ineligible companies. Disclosure: Sarah Fagan declares no relevant financial relationships with ineligible companies. Disclosure: Jessica Sosa-Stanley declares no relevant financial relationships with ineligible companies. Disclosure: Diana Peterson declares no relevant financial relationships with ineligible companies. (2024).

3. Clark KM, Pandya AM: **Anatomy, abdomen and pelvis: Cardinal ligaments (mackenrodts, transverse cervical, or lateral cervical ligaments).** In: *Statpearls.* StatPearls Publishing. Copyright © 2024, StatPearls Publishing LLC., Treasure Island (FL) ineligible companies. Disclosure: Ashish Pandya declares no relevant financial relationships with ineligible companies. (2024).

4. Ramanah R, Berger MB, Parratte BM, DeLancey JO: **Anatomy and histology of apical support: A literature review concerning cardinal and uterosacral ligaments.***International urogynecology journal* (2012) **23**(11):1483-1494.

5. Ellis H: **Anatomy of the uterus.***Anaesthesia & Intensive Care Medicine* (2005) **6**(3):74-75.

6. Philip M, Snow RJ, Gatta PAD, Bellofiore N, Ellery SJ: **Creatine metabolism in the uterus: Potential implications for reproductive biology.***Amino Acids* (2020) **52**(9):1275-1283.

7. Han C, Wang C, Han L, Liu G, Li H, She F, Xue F, Wang Y: **Incarceration of the gravid uterus: A case report and literature review.***BMC pregnancy and childbirth* (2019) **19**(1):408.

8. Edwards JK, Solsona B, N EN, Carley AF, Herzing AA, Kiely CJ, Hutchings GJ: **Switching off hydrogen peroxide hydrogenation in the direct synthesis process.***Science* (2009) **323**(5917):1037-1041.

9. Hanuman S, Pande G, Nune M: **Current status and challenges in uterine myometrial tissue engineering.** (2023) **14**(1):2251847.

10. Alkatout I, Wedel T, Pape J, Possover M, Dhanawat J: **Review: Pelvic nerves - from anatomy and physiology to clinical applications.***Translational neuroscience* (2021) **12**(1):362-378.

11. Ullah E, Lail RA, Taj N, Alam MI: **Malignant and benign lesions of female genital tract - an experience at a tertiary care hospital in bahawalpur - pakistan.**

12. Barjon K, Mikhail LN: **Uterine leiomyomata.** In: *Statpearls.* StatPearls Publishing

Copyright © 2024, StatPearls Publishing LLC., Treasure Island (FL) ineligible companies. Disclosure: Lyree Mikhail declares no relevant financial relationships with ineligible companies. (2024).

13. Shakhatreh HJM, Salih AJ, Aldrou K, Alazzam FAF, Issa MSB: **Medico-legal aspects of abortion: Updates of the literature.***Medical archives* (2022) **76**(5):373-376.

14. Berer M: **National laws and unsafe abortion: The parameters of change.***Reproductive health matters* (2004) **12**(24 Suppl):1-8.

15. Anyamele T, Ugbogu EA, Nwankwo VC, Ibe C: **A review of the traditional uses, phytochemistry and toxicological profile of xylopia aethiopica a.Rich.***Pharmacological Research - Natural Products* (2023) **1**(100001.

16. Jahan S, Mozumder ZM, Shill DK: **Use of herbal medicines during pregnancy in a group of bangladeshi women.***Heliyon* (2022) **8**(1):e08854.

17. Holst L, Wright D, Haavik S, Nordeng H: **The use and the user of herbal remedies during pregnancy.***Journal of alternative and complementary medicine (New York, NY)* (2009) **15**(7):787-792.

18. Holst L, Wright D, Haavik S, Nordeng H: **Safety and efficacy of herbal remedies in obstetrics-review and clinical implications.***Midwifery* (2011) **27**(1):80-86.

19. El Hajj M, Holst L: **Herbal medicine use during pregnancy: A review of the literature with a special focus on sub-saharan africa.***Frontiers in pharmacology* (2020) **11**(866.

20. Mothupi MC: **Use of herbal medicine during pregnancy among women with access to public healthcare in nairobi, kenya: A cross-sectional survey.***BMC Complement Altern Med* (2014) **14**(432.

21. Illamola SM, Amaeze OU, Krepkova LV, Birnbaum AK, Karanam A, Job KM, Bortnikova VV, Sherwin CMT, Enioutina EY: **Use of herbal medicine by pregnant women: What physicians need to know.***Frontiers in pharmacology* (2019) **10**(1483.

22. Ibe C, Irondi EA, Ugbogu EA, Anyamele T: **A review of the nutritional and antimicrobial properties of xylopia aethiopica a.Rich.***Food and Humanity* (2023) **1**(1099-1116.

23. Ezeobiora CE, Igbokwe NH, Amin DH, Okpalanwa CF, Stephen CM, Mendie UE: **Phylogenetic insights and antimicrobial biosynthetic potential of serratia sp. Xafb12 and pseudomonas sp. Xafb13 from xylopia aethiopica.***Brazilian journal of microbiology : [publication of the Brazilian Society for Microbiology]* (2024) **55**(1):201-213.

24. Johnson DM, Murray NA: **A revision of xylopia l. (annonaceae): The species of tropical africa.***PhytoKeys* (2018) **97**(1-252.

25. Katawa G, Ataba E: **Anti-th17 and anti-th2 responses effects of hydro-ethanolic extracts of aframomum melegueta, khaya senegalensis and xylopia aethiopica in hyperreactive onchocerciasis individuals' peripheral blood mononuclear cells.** (2022) **16**(4):e0010341.

26. Fategbe MA, Avwioroko OJ, Ibukun EO: **Comparative biochemical evaluation of the proximate, mineral, and phytochemical constituents of xylopia aethiopica whole fruit, seed, and pericarp.***Preventive nutrition and food science* (2021) **26**(2):219-229.

27. Yin X, Chávez León M, Osae R, Linus LO, Qi LW, Alolga RN: **Xylopia aethiopica seeds from two countries in west africa exhibit differences in their proteomes, mineral content and bioactive phytochemical composition.***Molecules (Basel, Switzerland)* (2019) **24**(10).

28. Amajor JU, Oti E, Ekeledo N, Omodamiro R, Amajor EE, Aniedu C: **Studies on the characteristic properties of fermented, sun-dried orange-fleshed sweet potato flour.***Nigerian Food Journal* (2014) **32**(1):45-53.

29. Shah SMA, Akram M: **Cardioprotective potential of plant-derived molecules: A scientific and medicinal approach.** (2019) **17**(2):1559325819852243.

30. Singh P, Khosa RL, Mishra G, Jha KK: **Pharmacognostical evaluation of aerial parts of graptophyllum pictum (l.) griff. (syn: Justicia picta linn.): A well-known folklore medicinal plant.***Ancient science of life* (2015) **34**(4):223-229.

31. Domínguez-López I, Yago-Aragón M, Salas-Huetos A: **Effects of dietary phytoestrogens on hormones throughout a human lifespan: A review.** (2020) **12**(8).

32. Timilsena YP, Phosanam A: **Perspectives on saponins: Food functionality and applications.** (2023) **24**(17).

33. Siddiqui N, Rauf A, Latif A, Mahmood Z: **Spectrophotometric determination of the total phenolic content, spectral and fluorescence study of the herbal unani drug gul-e-zoofa (nepeta bracteata benth).***Journal of Taibah University Medical Sciences* (2017) **12**(4):360-363.

34. Sharma T, Gamit R, Acharya R, Shukla VJ: **Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of byttneria herbacea roxb.***Ayu* (2021) **42**(3):143-147.

35. Lim JG, Park HM: **Analysis of saponin composition and comparison of the antioxidant activity of various parts of the quinoa plant (chenopodium quinoa willd.).** (2020) **8**(1):694-702.

36. Shraim AM, Ahmed TA, Rahman MM, Hijji YM: **Determination of total flavonoid content by aluminum chloride assay: A critical evaluation.***LWT* (2021) **150**(111932.

37. Erhirhie EO, Ihekwereme CP, Ilodigwe EE: **Advances in acute toxicity testing: Strengths, weaknesses and regulatory acceptance.***Interdisciplinary toxicology* (2018) **11**(1):5-12.

38. Vilvanathan S: **Physiological salt solutions.** In: *Introduction to basics of pharmacology and toxicology: Volume 3 : Experimental pharmacology : Research methodology and biostatistics.* Lakshmanan M, Shewade DG, Raj GM (Eds), Springer Nature Singapore, Singapore (2022):151-156.

39. Kumar A, P N: **Major phytochemicals: Recent advances in health benefits and extraction method.** (2023) **28**(2).

40. Obiri DD, Osafo N: **Aqueous ethanol extract of the fruit of xylopia aethiopica (annonaceae) exhibits anti-anaphylactic and anti-inflammatory actions in mice.***J Ethnopharmacol* (2013) **148**(3):940-945.

41. Babalola AS, Idowu OA, Ademolu KO, Olukunle J, Rahman SA: **Antiplasmodial activities and abortifacient properties of three commonly used african indigenous anti-malarial plants in plasmodium berghei infected pregnant mice: Implication for maternal and fetal health.***Bulletin of the National Research Centre* (2020) **44**(1):153.