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Evaluation of the Oxytocic and Safety Profile of Ethanol Fruit Extract of *Xylopia aethiopica* in Female Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The goal of this study was to investigate the oxytocic effects of ethanol fruit extract of Xylopia aethiopica (*X. aethiopica*). Pregnancy describes the period of fetal development in the uterus until delivery. Pregnancy, labour, and puerperium present challenges that encourage the use of medications to relieve symptoms, terminate the pregnancy and prevent primary postpartum haemorrhage. Some pregnant women still rely on herbal remedies for the treatment of pregnancy-related problems. *X. aethiopica* is a natural spice used in preparing soup for women after delivery to prevent primary postpartum haemorrhage. Sixty-six adult Wistar rats comprising 12 males for mating and 44 females that weighed 150–180g were used in this study. Acute toxicity test, qualitative phytochemical analysis, abortifacient, and oxytocic studies were all done. Statistical analysis was done using IBMSPSS version 26. The results were presented as mean ± standard deviation while comparison between groups was done using One-Way Analysis of Variance ANOVA with subsequent analysis using Post Hoc Test. A P value of < 0.05 was considered statistically significant. The Median LD50 was established to be 1703 mg/kg in rats. The phytochemical analytes were found to be Flavonoids, phenols, cardiac glycosides, and steroids. There was a dose-dependent decrease in the body weight of the animals treated with *X. aethiopica*. The extract did not have an oxytocic effect on the postpartum uterus like oxytocin. It rather caused relaxation of the uterus. Ethanol fruit extract of X. aethiopica did not exert an oxytocic effect on female Wistar rats. It is, therefore, not recommended in the prevention of primary postpartum haemorrhage.

Keywords: Oxytocin; postpartum; haemorrhage; pregnancy.

1. INTRODUCTION

1.1 Uterus, the Site of Pregnancy

The female uterus, also known as the womb, is a hollow, muscular organ in the pelvis that plays a crucial role in mensuration, pregnancy, and childbirth [1]. It forms between the fifth and sixth weeks of pregnancy through the fusion of Mullerian or paramesonephric ducts, forming the fallopian tube and the uterus, and the upper section of the vagina. The absence of circulating testosterone and Anti Mullerian Hormone causes uterine development in female embryos [2]. The uterus undergoes significant changes throughout pregnancy, including hypertrophy and hyperplasia, and is responsible for fetal protection and expulsion at term. Ligaments and folds are used to support and maintain the uterus, with two types: the anterior Uterovesical fold of the Peritoneum and the posterior retro-vaginal fold [3].

1.2 Gross Features and Location of the Uterus

The uterus is divided into four parts: fundus, corpus, cervix, and cervical canal. The fundus is the upper part above the insertion of tubes, while the corpus connects fallopian tubes and extends downward to the cervix and isthmus [4]. The uterus is located behind the bladder and in front of the rectum.

1.3 Histology Perspective

The uterus is a muscular structure with three layers: the endometrium, myometrium, and perimetrium. The endometrium is the inner lining of the uterus and requires the ability to change across the menstrual cycle to regenerate, decidualize, and shed. It supports implantation and pregnancy when necessary [6]. In the absence of implantation, shedding of the luminal two-thirds of the endometrium occurs during menstruation, under the control of endocrine, immune, vascular, and coagulation systems [7].

The endometrium is composed of connective tissue stroma surrounding glands and surface epithelium. It serves as the implantation site for a fertilized egg and plays a crucial role in its nourishment and development [8]. Estrogen primes the endometrium by inducing a proliferative response with increased mitotic

activity in glands and the stroma. High progesterone levels decrease the number of estrogen receptors in endometrial cells to shift toward secretory differentiation.

The perimetrium is the outer lining of the uterus, consisting of a thin layer of loose connective tissue lined by squamous mesothelium. The myometrium is the muscular layer between the endometrium and perimetrium, providing the bulk of the uterus. The inner and outer myometrial layers are mostly associated with the initial layers of the paramesonephric ducts [9].

The uterine system is composed of several layers, including the middle intermediate layer, which contributes to the uterine thickness and provides numerous blood vessels for nutritional and nervous supply. The myometrium, a smooth muscle, expands and contracts to facilitate parturition [10].

1.4 Blood Supply

The uterine artery, which originates from the internal iliac artery, is the major contributor to the blood supply reaching the uterus. The uterine vein accompanies the artery and drains into the internal iliac vein [11].

1.5 Lymphatics

The fundal area of the uterus drains into para-aortic lymph nodes, ovarian and fallopian tube lymphatic drainage, and superficial inguinal lymph nodes along the round ligament. Lower portions of the uterus drain along uterine blood vessels into external and internal iliac lymph nodes [12].

1.6 Nerve Supply

The uterus is innervated via the inferior hypogastric plexus, which receives post-ganglionic sympathetic fibers from the inferior hypogastric nerves and preganglionic parasympathetic fibers from pelvic splanchnic nerves from S2-S4. Visceral afferent fibers pass within the pelvic splanchnic nerves [13].

1.7 Clinical Anatomy

1.7.1 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 [13].

1.7.2 Uterine fibroma

Uterine fibroma, or leiomyoma, is the most common benign tumor of the female genital tract, affecting around 50% of women during their fertile life [14].

1.8 Primary Postpartum Haemorrhage (PPH)

Postpartum hemorrhage (PPH) is a leading cause of global maternal morbidity and mortality, accounting for approximately 30% of all pregnancy-related deaths in Asia and Africa. Any delay in achieving hemostasis after birth can result in a major loss of maternal blood volume, leading to hypotension, hypoxia, acidosis, renal failure, and even disseminated intravascular coagulation (DIC) [15].

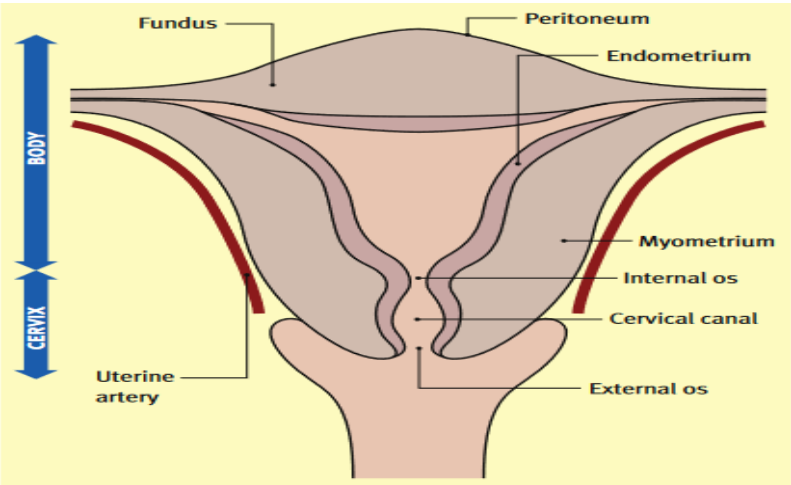


Fig. 1. Showing the features of the uterus [5]

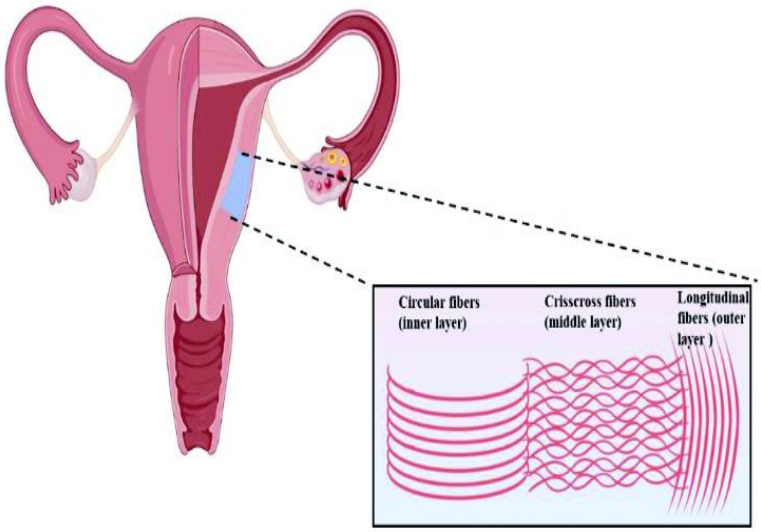


Fig. 2. Showing the muscle arrangement of the myometrium of the uterus [9]

1.9 Oxytocin and Uterus

Oxytocin, also known as α-Hypophamine, is a non-peptide hormone containing nine amino acids and plays a pivotal role during human labor and birth [16]. It is produced in neurons that originate in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus and is transported to the posterior pituitary, where it is stored. During labor, oxytocin is released in pulses from the pituitary into the circulation to induce uterine contractions [16]. It performs a wide variety of functions, with pituitary gland secretions responsible for its peripheral functions and centrally projecting oxytocin neurons responsible for its behavioral effects [17].

1.9.1 Brain secretion of the oxytocin

Brain secretion of oxytocin involves transporting it from magnocellular neurons from the SON and PVN to the posterior pituitary, where it is released into the circulation. It is released into many areas of the brain from axon collaterals emanating from the axons of the magnocellular neurons from the SON and PVN, projecting to the posterior pituitary [18].

1.9.2 Functions of oxytocin

Oxytocin is a hormone that plays a crucial role in the body's physiological functions, including mood enhancement, social interaction promotion, anxiety reduction, and stress reduction. It is commonly used in maternity care to induce labor and prevent postpartum haemorrhage. However, the increased use of synthetic oxytocin raises questions about its potential impacts on endogenous oxytocin levels and its effects on mothers and babies [20].

1.9.3 Oxytocin levels during labour and birth

During labor and birth, oxytocin levels gradually rise, reaching maximum frequency just before the baby is born. Some peaks may be spontaneous and centrally induced, while others may be induced by the Ferguson reflex, which is stimulated by uterine contractions [21]. This reflex activates afferent sensory nerve fibers, releasing oxytocin into the brain and circulation. The maximum expression of this reflex during birth corresponds to a 3- to 4-fold rise in oxytocin levels during and immediately after the baby's birth. Sympathetic afferents from the myometrium are activated by myometrial contractions, reducing the release of oxytocin [21].

1.9.4 Connection between synthetic oxytocin and contraction of the uterus

The connection between synthetic oxytocin and uterine contraction has been well-established for over four decades [22]. The uterine muscles are highly sensitive to oxytocin during labor and birth due to the upregulation of oxytocin receptors by high oestrogen elevations in late pregnancy. Oxytocin release in labour promotes prostaglandin release, further strengthening uterine contractions and labour progress [23].

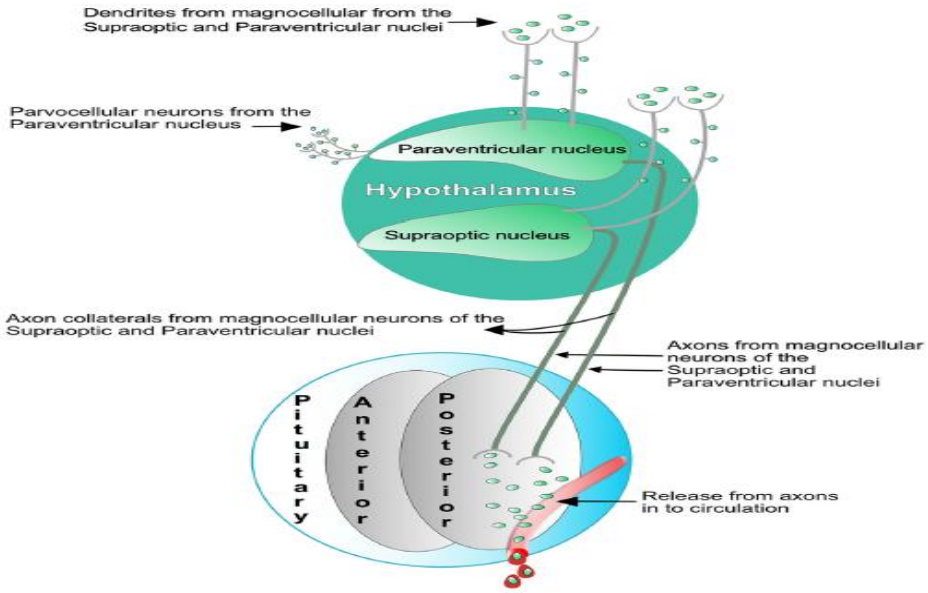


Fig. 3. Displaying the Brain Secretion of Oxytocin [19]

1.9.5 Levels and effects after infusion of synthetic oxytocin

Administrative of synthetic oxytocin produces flat oxytocin levels in maternal blood, which may influence the pattern of uterine contractions and contribute to hyperstimulation that can result from high doses of synthetic oxytocin [24]. Excessive uterine activity may also compromise fetal blood supply, causing hypoxia. Prolonged exposure to synthetic oxytocin may eventually lead to reduced contractility of the uterine muscles due to desensitization of oxytocin receptors. The decreased efficacy of oxytocin may also increase the risk of postpartum haemorrhage [25].

1.9.6 Pharmacological agents used as oxytocics

Pharmacological agents used as oxytocics include oxytocin, ergometrin, misoprostol, and their combinations. These agents have been tried in the prevention of postpartum hemorrhage (PPH) with varying safety and effectiveness [26]. Either of the oxytocics or their combination is given at the third stage of labor (after delivery of the baby) to enhance uterine contraction, which is one of the components of active management of the third stage of labour [27].

Oxytocin, ergometrin, and misoprostol have all been successfully used to reduce blood loss following delivery, miscarriage, and induced abortion with varying efficacy. Oral misoprostol has been reported to have a more uterotonic effect on first-trimester pregnancy, as there was less bleeding following a surgical evacuation in the misoprostol group [27].

1.9.7 Non- pharmacological agents used as oxytocics

Non-pharmacological agents used as oxytocics include methanol and ether crude extracts of *Vernonia amygdalina*, *Melaleuca lanceolata*, *Rhynchohyalus natalensis,* Sida acuta plant extract, *Nymphaea alba*, *Piper guineense* seed *Uvariodendron anisatum*, and *xylopia aethiopica* [28].

1.9.8 Oxytocic uses of *xylopia aethiopica*

Xylopia aethiopica is said to possess more uterine contraction properties than *Ocimum gratissium*, comparable to standard oxytocin. However, it is recommended not to be used in early pregnancy as it could cause miscarriage [29].

1.9.9 Herbal consumption in pregnancy

Herbal consumption during pregnancy is a growing concern, with many women using herbs for various reasons. Some herbs can induce uterine contractions and high blood pressure, leading to miscarriage, premature birth, or even death. A study by Jahan et al. (2022) found that

71.80% of pregnant women consumed herbs, similar to previous studies in Bangladesh and the United Kingdom [30]. In Nigeria, herbal medicine consumption was found to be 36.8% among pregnant and lactating mothers [31]. In Kenya, about 12% of women took herbs throughout their pregnancy cycle [31].

The most commonly mentioned herbs in this study include ginger, *Nigella sativa*, *Citrus limon*, *Prunus domestica*, and *Allium sativum* L. Ginger, garlic, peppermint, and Chinese Okra are also common. However, little information about *X. aethiopica*'s uses during pregnancy is available, and its roles as oxytocic and abortifacient remain unclear. The higher prevalence of herb use during the third trimester (50.91%) may be due to mothers' increased concern for the baby's body structure and organ system [32]. The majority of users (71.8%) believed herbs were safer than medicines, with 91.03% reporting no negative effects from any herb. Informal sources of information, such as personal views and friends/family, were cited as critical in women's decision to explore herbal treatment.

1.9.10 *Xylopia aethiopica*

X. aethiopica, a tall, slim, aromatic evergreen tree, grows in the Savanna region of Africa, including Nigeria, Ghana, Ethiopia, Cameroon, and Senegal. Its fruits are small, twisted bean-pods with dark brown color, cylindrical shape, and 5 to 8 kidney-shaped seeds [32].

1.9.11 Taxonomy

Fetse et al. (2016) reported that *X. aethiopica*, also known as Negro pepper, is an angiosperm belonging to the custard apple family, Annonaceae. The genus *X. aethiopica* consists of 150 plants distributed in tropical and subtropical Africa [33].

1.9.12 Medicinal use of *X. aethiopica*

X. aethiopica, also known as Negro pepper, is used medicinally for its anti-infective properties, anti-emetic properties, and headache treatment. The stem bark of *X. aethiopica* is used in combination with other medicinal plants as an alcoholic decoction for postpartum breast infections.

In Nigeria, *X. aethiopica* fruits and seeds are used to prevent fever, cough, and postpartum bleeding and facilitate post-natal recovery [34]. Previous studies have reported antioxidant, hypolipidemic, antifungal, and antibacterial effects of whole *X. aethiopica* fruits, as well as their preventive effects against dysentery and male/female fertility challenges. However, the information on the relative abundances of proximate, mineral, and phytochemical constituents in the different anatomical parts of *X. aethiopica* fruits remains limited [35].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Study location

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

2.1.2 Collection of plant materials

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

2.1.3 Identification and authentication of the plant materials

The plant was identified and authenticated by Mr. Nwankwo in the Applied Biology Department of Ebonyi State University.

2.1.4 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 males and females during the period of acclimatization in the pharmacology Laboratory at Ebonyi State University, Abakaliki, which 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.

2.1.5 Drugs/chemicals/reagents

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

2.1.6 Equipment/instruments

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

2.2 Methods

2.2.1 Extraction technique

The dry fruits of *X. aethiopica* were 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 liters of ethanol. The extract was shaken and starred intermittently for 24 hours, after which it was sieved into a clean glass tube using 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.

2.2.2 Phytochemical screening of the *X. aethiopica*

The preliminary phytochemistry of *X. aethiopica* ethanol fruit extract was carried out to determine different secondary metabolites, and these include the following test: for tannins, some quantities of *X. aethiopica* extract about 0.5g by approximation were dissolved in 1ml of distilled water, stirred, and filtered. Some drops of ferric Chloride reagent were introduced into the filtered solution. The presence of blue-black, green, or blue-green precipitate indicates the presence of tannins 36 For alkaloids, 0.5g of the *X. aethiopica* extract was turned into 5 ml of 1% diluted HClaq in a heated water bath. Thereafter, 1 ml of the resulting solution was added with a few drops of Mayer's reagent, Dragendort's reagent, and *picic* acid solution. The presence of precipitates was seen as an indication of the presence of alkaloids in the extract [37]. For saponins, approximately 0.5g of *X. aethiopica* extract was dissolved in water and thoroughly shaken in a test tube. The continuous appearance of frothing upon heating was seen as an indication of the presence of saponin [37]. For steroids, about 0.5g of the *X. aethiopica* was collected and liquefied in water and filtered thereafter. 1 ml of the resulting solution was introduced to 2 ml of H_2SO_4 in a test tube. The steroid was taken to be present so long as a reddish brown ring is seen within the interface [37].

For terpenoids, some portion of *X. aethiopica* extract was dissolved in water, and 5ml of the portion received 2ml of chloroform and subjected to evaporation by means of a water bath. Thereafter, the resulting portion was boiled in 3ml of concentrated H_2SO_4. The appearance of grey colouration was taken to indicate the availability of terpenoids.

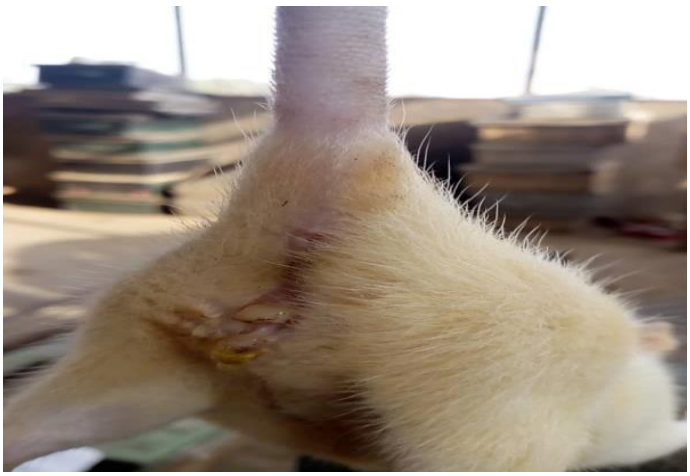


Fig. 4. Showing vaginal plug in the female's vagina clearly seen with the help of a ×5 magnifying

For flavonoids, a lead sub-acetate test was used. 100mg of the extract of *X. aethiopica* was liquefied in 5ml of water and filtered thereafter. A lead *sub-acetate* of about two to three drops was introduced. Precipitation of yellow colouration suggests the availability of flavonoids. For anthraquinones, some quantities of *X. aethiopica* were collected into a conical flask containing 10ml of benzene and were allowed to mix for 10 minutes thoroughly. It was filtered, and 10ml of solution of 10% ammonia was introduced to it and shaken very strongly within 30 seconds. Any appearance of pink, violet, and or red colour suggests the presence of anthraquinones [38].

2.3 Pregnancy Confirmation

The animals were paired for mating in the ratio of 3 female rats to 1 male rat. After the period of acclimatization, the male and female rats were placed together in a large mating cage. Pregnancy was confirmed with the aid of a vaginal plug in the female's 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.

2.4 Acute Toxicity Study

This study followed Lorke's method and involved 12 adult female rats in two phases. In the first phase, three groups of rats were given different dosages of ethanol extract of *X. aethiopica* via orogastric administration. The rats were observed for signs of toxicity, such as hyperactivity, 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 were given different dosages of ethanol extract of *X. aethiopica*. The rats were observed for signs of toxicity and mortality at the first 4 hours, 24 hours, and 72 hours. Mortality was observed at the dosages of 2900 mg/kg and 5000 mg/kg. A fresh De Jalon solution was prepared and used for the organ bath experiment [39].

2.5 Histological Examination

The uterine horns were examined for implantation and pregnancy resorption sites, and the number of fetuses. Endometrial samples were taken for histology, preserved with formalin, and sent to the Anatomy department of EBSU's histopathology laboratory. The tissue was fixed, embedded, sectioned, stained, and examined using a light microscope.

2.5.1 Experimental assessment of oxytocic effect

The study involved a surgical procedure where rats were sacrificed, and a caesarean section was performed. The uterine horns were dissected and cut into equal halves, and the tissue was aerated with oxygen at 300C. The uterus was suspended in the De Jalon Solution and allowed to equilibrate for 30 minutes before the experiment. The drug introduction was allowed a contact time of 30 seconds before stimulation, and the tissue was washed three times to remove any remnant drug. The kymograph was used for tracing contractions.

The experiment began with 0.1 to 1 international unit of oxytocin, followed by varying concentrations of the extract in the organ bath. The effect of the fractions was also determined in the presence of 0.2 µg of calcium channel blocker verapamil. The procedure was repeated with both oxytocin and the extract, and the effects were recorded.

The data was meticulously documented and entered into the International Business Machine Statistical Package for Social Sciences (IBMSPSS) version 26, Chicago II, USA. Comparisons between groups were made using the One-Way Analysis of Variance and Post Hoc Test, with a significance difference set at $P < 0.05$. The qualitative components of the study were analyzed manually.

3. RESULTS

3.1 Acute Toxicity Studies

The acute toxicity of *X. aethiopica*'s ethanol fruit extract was assessed in rats after oral administration at dual doses (2900 mg/kg and 5000 mg/kg), with the median LD50 being 1703 mg/kg.

3.2 Qualitative Phytochemical Analysis of XA Fruits

The study analyzed the phytochemical constituents of *X. aethiopica* fruits' ethanol extract, revealing ten secondary metabolites, including alkaloids, flavonoids, cardiac glycosides, phenol, phlobatannins, terpenoids,

tannins, steroids, saponins, and anthraquinones, as well as other compounds.

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

3.3 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 significant difference in weight was observed in the comparison of the weight of female pregnant Wistar in group 1 to the 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 the weight of female pregnant Wistar rats in groups 2 (P = 1.000), 3 (P = 0.995) and 4 (P = 0.584) after 7 days.

A significant difference in weight was observed in the comparison of the weight of female pregnant Wistar in group 1 to the 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 the weight of pregnant female Wistar rats in group 2 (P = 0.089). No significant difference in weight was observed in the comparison of the weight of the female pregnant rats in group 5 to the 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.

3.4 Tissue Histology of Endometrial Plate

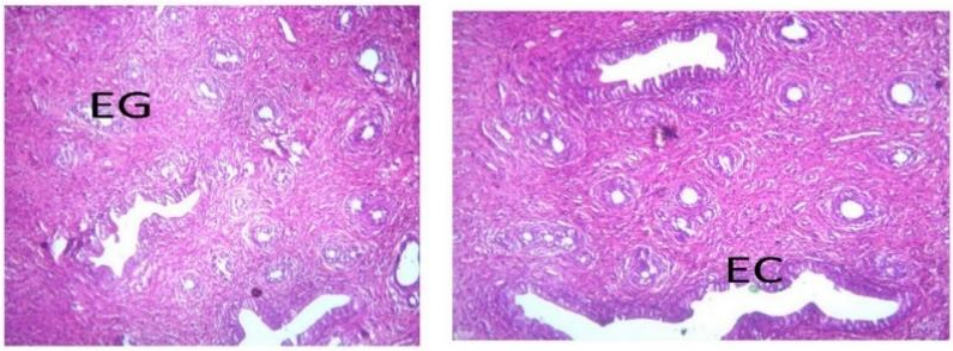
3.4.1 Response of uterus to oxytocin and ethanol extract of *X. aethiopica*

Ethanol extract of XA stimulated uterine contraction to peak once at 0.3cm, which is like a twitch. On the other hand, the administration of oxytocin resulted in uterine contractions peaking at 1.4 cm, decreasing to 0.8 cm, and peaking to 1.3 cm, which is the normal oxytocin curve, as shown in Fig. 5.

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

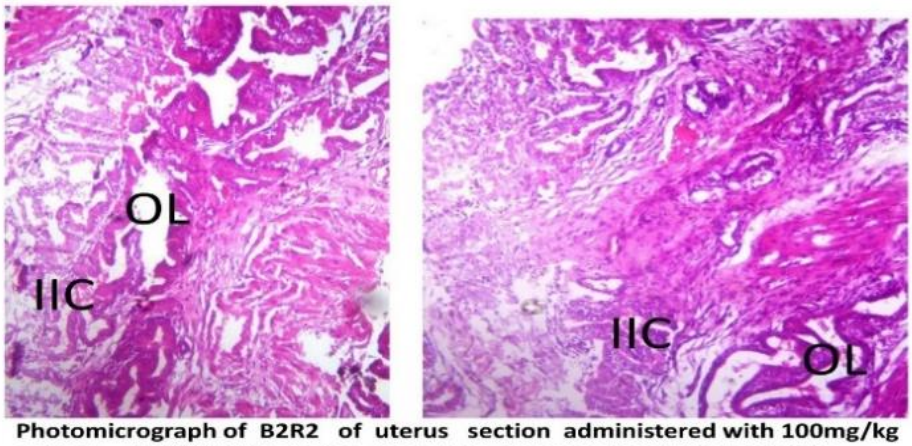
		Weight (g)	P-values ^{N}	P-values ^{P}
	Groups	Mean ± SD		
7th Days	1 (Negative Control)	184.50±14.50		
	5 (Positive Control)	171.80±16.84	0.676	
	2	172.67±18.03	0.694	1.000
	3	168.17±17.02	0.400	0.995
	4	157.67±11.88	0.050	0.584
14th Days	1 (Negative Control)	210.67±14.22		
	5 (Positive Control)	171.00±18.93	0.019	
	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 groups 2, 3, and 4 correspondingly



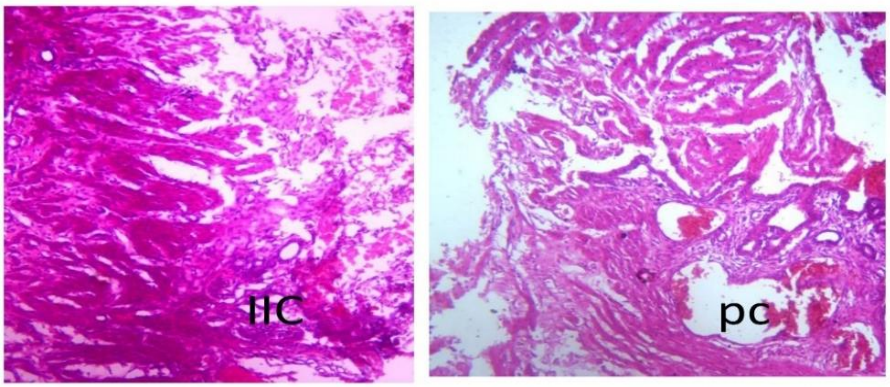
Photomicrograph GP A1 control section of uterus (x400)(H/E) shows normal uterine tissue with numerous active endometrial gland (EG). And active epithelia cell (EC_ .

Plate 1. Tissue histology of group 1



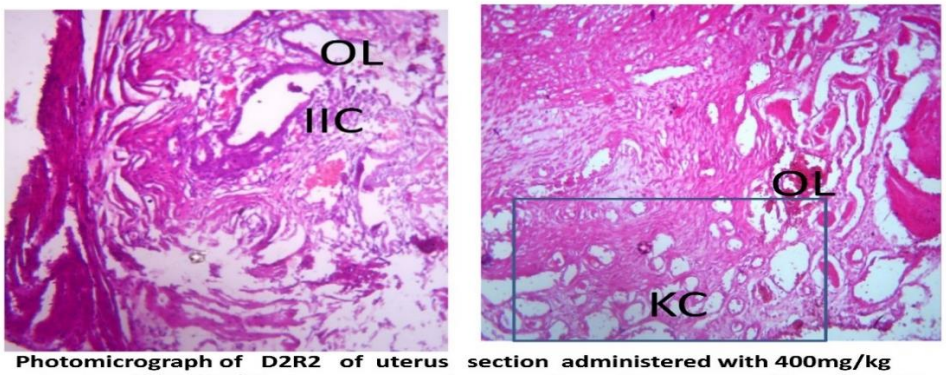
Photomicrograph of B2R2 of uterus section administered with 100mg/kg extract (X400)(H/E) shows moderate degeneration with moderate obliteration of the lumen (OL) with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix.

Plate 2. Tissue histology of group 2



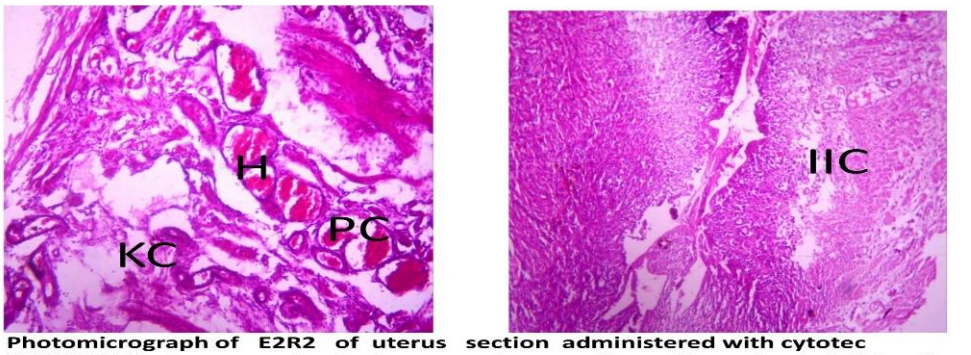
Photomicrograph of C2R2 of uterus section administered with 200mg/kg extract (X400)(H/E) shows moderate degeneration with moderate obliteration of the lumen (OL) with moderate infilteration of inflammatory cell (IIC) within the mucosa of the endocervix and moderate polycystic (PC)area with hemorrhage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

Plate 3. Tissue histology of group 3



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 4. Tissue histology of group 4



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

Plate 5. Tissue histology of group 5

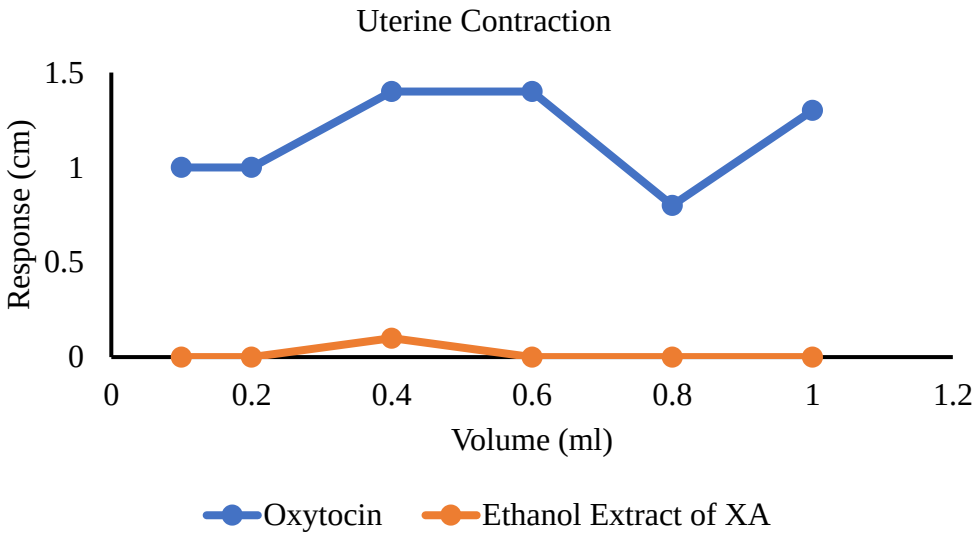


Fig. 5. Showing response of uterine

4. 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. Some compounds, such as alkaloids, flavonoids, and terpenoids, can be toxic due to their ability to stimulate oxidative stress [40]. 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) [41]. This 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* did not show a significant oxytocic effect in the organ bath experiment, as it only showed a twitch that was not enough to sustain uterine activity. This could be linked to the various roles or possible interplay between the phytochemical constituents in causing postpartum uterine relaxation. Tannic acid, flavonoids, alkaloids, and phenols have been shown to possess uterine-stimulating effects.

The N-Haxane fruit extract of *X. aethiopica* showed some oxytocic effect at high doses on the guinea pig uterus more than *Ocimum gratissium*, but this was not statistically significant according to previous findings [42]. Another study by Wood et al. reported that the ethanol fruit extract of *X. aethiopica* caused relaxation of the Wistar rat ileum, similar to the findings of this study [43]. Both the extract studied and oxytocin did not have an effect on the virgin uterus, likely because oxytocin receptors develop in later dates of pregnancy.

5. CONCLUSION

In this current study, the investigation of the oxytocic effect of the *X. aethiopica* extract, it was found to cause postpartum uterine relaxation rather than contraction except the few occasional twitches caused by the extract .

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

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

COMPETING INTERESTS DISCLAIMER

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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