TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 8, Issue 4, Autumn 2023

Original Research

Contraceptive Efficacy of Aqueous Extract of *Xylopia aethiopica* (Dunal) A.Rich. Fruit in Female Sprague Dawley Rats

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Received: 2 May 2023 **Revised:** 8 Sep 2023 **Accepted:** 9 Sep 2023

Abstract

Globally, herbal contraceptives remain a viable option for women in rural settlements with unmet contraceptive needs. Pharmacological reports of the male contraceptive potential of *Xylopia aethiopica* (Dunal) A.Rich. fruit exist in literature, but there is a paucity of information on its female contraceptive potential. This study evaluated the efficacy of aqueous extract of *X. aethiopica* fruit (AEXAF) as a reversible contraceptive remedy in female Sprague Dawley rats against a combined oral contraceptive drug containing ethinyl estradiol and levonorgestrel (COC-EEL). AEXAF was obtained by boiling air-dried pulverized fruit samples in water for 15 minutes. Phytochemical screening of AEXAF was carried out. Mature female rats (30) were assigned into six groups, five per group, with ±20 g weight difference within each group. Group A received water; Groups B, C, D and E received 50, 100, 200, and 300 mg/kg/B.W. doses of AEXAF, respectively; Group F received 3.6 µg/kg/B.W. dose of COC-EEL. COC-EEL and AEXAF were administered orally, once daily, for 21 days. After 14 days of treatment, mature male rats were introduced to the females, two males per group, for 7 days. Litter size was recorded after delivery. Rats that did not produce pups were immediately re-introduced to male rats for 7 days and sacrificed after another 7 days; the number of fetuses in their uteri was determined. The data obtained was analyzed using Unpaired-t test. Phytochemical screening of AEXAF revealed the presence of alkaloids, flavonoids and saponins. High contraceptive efficacy (80%) with 100% reversibility was observed at 50 and 300 mg/kg/B.W. doses of AEXAF; whereas COC-EEL showed 60% efficacy and 100% reversibility. The 100 and 200 mg/kg/B.W. doses of AEXAF did not protect against conception. *X. aethiopica* possesses contraceptive potential worthy of further scientific consideration.

Keywords: *Xylopia aethiopica*; Herbal contraceptives; Ethinyl estradiol; Levonorgestrel

http://doi.org/10.18502/tim.v8i4.14484

Citation: Ajayi BE, Odueke YA, Ibrahim FM, Ighodaro M, Bamgbose JT. **Contraceptive Efficacy of Aqueous Extract of *Xylopia aethiopica* (Dunal) A.Rich. Fruit in Female Sprague Dawley Rats.** Trad Integr Med 2023;8(4):362-369. http://doi.org/ 10.18502/tim.v8i4.14484

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Introduction

Contraceptives are devices, drugs and practices that are used to prevent pregnancy in sexually active women of child-bearing age. Though unintended pregnancy rate is globally believed to be in decline, a recent report show that about 121 million unintended pregnancies were recorded yearly between 2015 and 2019, and about 73.3 million of these pregnancies were aborted [1]. Contraceptives reduce maternal and child mortality by directly reducing the number of unintended pregnancy and unsafe abortions [2]. Oral contraceptive (pill) is the most commonly used reversible contraceptives in the US and it is one of the top modern contraceptive methods used in Sub-Saharan Africa [3,4]. Combined oral contraceptives containing ethinyl estradiol and levonorgestrel are considered first-line, but have been found to be the least cost-effective [5,6]. People in high-income countries have better access to contraceptives than those in low-income countries; globally, 162·9 million women had unmet contraceptive needs in 2019 and 29.7% of these women were in Sub-Saharan Africa [6]. Herbal contraceptives provide a cheaper, more accessible and effective alternative for people in rural communities. Several plants, including *Xylopia aethiopica*, have been reported in African folklore medicine to possess contraceptive or abortifacient activity [7].

Figure 1. *Xylopia aethiopica* fruit

Xylopia aethiopica (Dunal) A. Rich is a tropical tree crop belonging to the family Annonaceae. It is widely distributed in the rain forests along the coastal regions of Africa [8]. Fruits of *X. aethiopica* (Figure 1) bear seeds that are used as spice for making soups because of their characteristic aroma and extracts of the fruit have been reported to be used in traditional treatment of several ailments and diseases, including bronchitis, diabetes, dysentery and cough [9,10]. The extract of the fruit is also used by lactating women to encourage the flow of breast milk. Phytochemical analysis revealed the presence and abundance of alkaloids, flavonoids, saponins, and tannins in the fruit extracts [11,12]. Extracts of *X. aethiopica* fruit have been reported to possess antibacterial, antifungal, cytotoxic, antiproliferative and antioxidant activities [13,14,15,16,17]. Pharmacological evidence supporting the contraceptive use of *X. aethiopica* in traditional medicine has been limited to studies on the potential of the plant in male contraception. It was reported that ethanol extract of *X. aethiopica* fruit reduced semen quality and induced testicular degeneration in male Wistar rats [18], but another study found the ethanol extract to improve sperm quality and increase serum testosterone and luteinizing hormone levels in male Sprague Dawley rats [19]. Adienbo et al. reported that the hydromethanol extract of *X. aethiopica* fruit enhanced sexual performance in male Wistar rats [20].

Research on the contraceptive potential of fruit extracts of *X. aethiopica* has been limited to application in male gender with dearth of information on the efficacy of extracts as female contraceptive remedies. This study therefore examined the contraceptive efficacy of aqueous extract of *X. aethiopica* fruit in female Sprague Dawley rats with comparison to a combined oral contraceptive drug, Levofem®, which contains ethinyl estradiol and levonorgestrel. This study reports the first pharmacological evidence of the strong potential of aqueous extract of *X. aethiopica* as a reversible female herbal contraceptive remedy.

Methods

Plant Sample Collection

Dried fruits of *Xylopia aethiopica* were purchased in May, 2022 from Oke-Aje market, Ijebu-Ode, Ogun State, Nigeria and the sample was identified and authenticated by Mr D. Esimenhhuai of the herbarium, Department of Botany, University of Ibadan.

Preparation of Aqueous Extract

The *Xylopia aethiopica* fruits were air-dried under shade for two weeks and were cut into small bits before being blended with an electric blender. From the pulverized fruit material, 30 g was put into 400 mL of

distilled water and heated until it boiled for about 10 to 15 min. After boiling, the mixture was allowed to cool for an hour, it was filtered and the filtrate (stock) was stored in the fridge at a temperature of 5 °C. A fresh stock solution of the extract was prepared every 48 h.

Phytochemical Screening of Aqueous Extract

Phytochemical screening of the aqueous extract was carried out using standard protocols as previously reported [21].

Preparation of Oral Contraceptive Solution

Levofem® is a known contraceptive pill that is taken daily to prevent pregnancy. Levofem® contains levonorgestrel, ethinyl estradiol and ferrous fumarate. The pills (levonorgestrel and ethinyl estradiol components only) were crushed and properly mixed, 0.6 g of it was weighed, dissolved in 2.0 mL of dimethylsulfoxide in a beaker and 8.0 mL of distilled water was added. The solution was thoroughly mixed, stored in an amber-colored sample bottle and kept in the fridge (5 ℃) as stock. A fresh stock solution of the drug was prepared every week.

Experimental Animals

Matured females Sprague Dawley rats (30) weighing between 100 to 120 g and 12 matured male Sprague Dawley rats were used for the experiment. The rats were kept in standard plastic cages with five females in a group, making six groups. The male and female rats were kept apart, allowed to acclimatize separately for three weeks in the animal house with the ambient temperature of 24 – 28 ℃ and 12 h light-dark cycle. The animals had free access to solid pellet diet and clean water throughout the study.

Experimental Design

The female rats were weighed and divided into six groups, five per group, according to their weights and labeled A, B, C, D, E and F. The extract was administered to the animals via oral route, once daily for 21 days. Group A, the negative control, received distilled water only. Group B received 50 mg/kg/B.W. of the extract. Group C received 100 mg/kg/B.W. of the extract. Group D received 200 mg/kg/B.W. of the extract. Group E received 300 mg/kg/B.W. of the extract. Group F received Levofem (ethinyl estradiol and levonorgestrel components) only, a 3.6 µg/kg/B.W. dose (this dose is equivalent to the dosage for a 50 kg female human). The contraceptive was administered once daily for 21 days via oral route. The weight of each rat was monitored weekly. The males were introduced, two per cage on the 15th day of drug and extract administration for 7 days. To ensure uniformity and control for impotence or rejection of a male, the males in the cages were rotated daily for the 7 days. Mounting was observed in each of the groups during the 7 days and the males were withdrawn on the 21st day of the treatment. The female rats were observed for pregnancy for 21 days after the last week of treatment. The litter size for each of the pregnant rats was recorded. The rats that did not get pregnant were separated and observed for a week and the next week the mating exercise was repeated with them for another 7 days, and the rats were sacrificed after another 7 days to determine if they are pregnant from the numbers of fetus present in their uteri. Histological analysis was carried on the uteri and ovaries of rats that were not pregnant after both periods of mating. The volume of extract or drug administered was determined using the formula below:

$$\text{Volume (mL)} = \frac{\text{Average Weight of rats in Group (g)} \times \text{Dosage (mg/kg)}}{\text{Concentration of Extract / Drug (mg/mL)} \times 1000}$$

Ethical approval (FNS/ERC/2022/120EC) for the study was obtained from The Research Ethics Committee, Faculty of Natural Sciences, Ajayi Crowther University, Oyo State, Nigeria.

Data Analysis and Presentation

One tail Unpaired (Independent two-sample) t test with selection of parametric test (for Gaussian distribution) and 95% confidence limit was used to determine statistical significance of differences between means, using the GraphPad Prism Software (Version 9.0) (GraphPad Software Inc., CA, USA). A p value$<$ 0.05 was considered statistically significant. Results are presented as mean and standard error of the mean (Mean ± SEM) and other values are presented either as tables and bar charts.

Results

Phytochemical Screening

The phytochemical screening of the aqueous extract of *X. aethiopica* fruits shows the presence of alkaloids, flavonoids, tannins and saponins, but cardiac glycosides and anthraquinones were not present in the extract. Table 1 shows the observation from the phytochemical screening of the extract.

Effect of Treatment on Body Weight

Relative to the negative control group, treatments of the experimental rats with the combined oral contraceptive and the plant extract did not cause any adverse effects on the body weight of the animals (Figure 2).There was significant weight gain ($p< 0.001$) in all the groups, the average weight gain in the groups were 34.35 ± 5.4 g, 39.36 ± 2.8 g, 42.8 ± 7.1 g, 37.92 ± 4.4 g,

32.44 ± 3.6 g and 32.66 ± 4.5 g for Group A to Group F, respectively. The differences in average weight gain among the groups are not statistically significant.

Contraceptive Efficacy of X. aethiopica Extract

The treatment of the rats with the aqueous extract of *X. aethiopica* showed strong contraceptive effect at the 50 mg/kg/B.W. dosage and the 300 mg/kg/B.W. dosage with reduced effect at the intervening dosages: 100 mg/kg/B.W. and 200 mg/kg/B.W. (Table 2). Of the four rats in Group A, the negative control, one rat did not get pregnant and the three pregnant rats produced a total of 21 pups. In Group B, the 50 mg/kg/B.W. treatment group, only one rat got pregnant and it produced 3 pups. The same trend was observed in Group E, the 300 mg/kg/B.W. treatment group in which only one rat got pregnant and it produced 6 pups. In Group F, the Levofem-treated group, only two rats got pregnant and they produced a total of 14 pups. Rats in Groups C and D produced a total of 50 pups with all the rats in Group C getting pregnant; while all the rats in Group D got pregnant save one. Two rats, one each in Groups C and D, produced 2 pups each, a very small litter size. There was significant difference, $p< 0.01$ and $p< 0.05$, between the total number of pups in Group A (negative control) and the number of pups produced Group B (50 mg/kg/B.W.) and Group E (300 mg/kg/B.W.), respectively. The difference between the total number of pups produced in the Levofem-treated group and the negative control group was not statistically significant.

To determine the duration of the contraceptive effect

Table 1. Phytochemical constituents of aqueous extract of *X. aethiopica* fruit

Class of Phytochemical	Observation
Alkaloids	+
Anthraquinones	–
Cardiac Glycosides	–
Flavonoids	+
Saponins	+
Tannins	+

+: Present; –: Absent

Table 2. Litter Size of Rats Treated with Aqueous *Xylopia aethipica* Extract and Levofem

Groups	Litter Size					
	R1	R2	R3	R4	R5	Total
A	4	7	10	NP	-	21
B	NP	NP	3	NP	NP	3**
C	9	6	5	2	4	26
D	5	13	4	NP	2	24
E	NP	NP	NP	6	NP	6*
F	NP	6	8	NP	NP	14

Group A was not treated (negative control); Groups B – E were treated with 50, 100, 200 and 300 mg/kg/B.W. doses, respectively; and Group F was treated with ethinyl estradiol and levonorgestrel (3.6 µg/kg/B.W. dose). **p<0.01 and *p<0.05 represent significant difference the total number of pups produce between the treatment group and the negative control group (Group A). NP: Not Pregnant, R1, R2, R3, R4 and R5 represent individual rat in each group. Group A has only four rats, R1 to R4.

Table 3. Fetuses Produced on Re-exposure to Males after Initial Treatment with Aqueous *Xylopia aethipica* Extract and Levofem

Groups	Number of Fetus in Uteri					
	R1	R2	R3	R4	R5	Total
A	--	--	--	0	-	0
B	8	8	--	10	8	34
C	--	--	--	--	--	--
D	--	--	--	0	--	0
E	12	11	8	--	0	31
F	8	--	--	9	9	26

R1, R2, R3, R4 and R5 represent individual rat in each group. (--) is used to indicate that the rats were not included in the re-exposure because they were pregnant at the initial exposure.

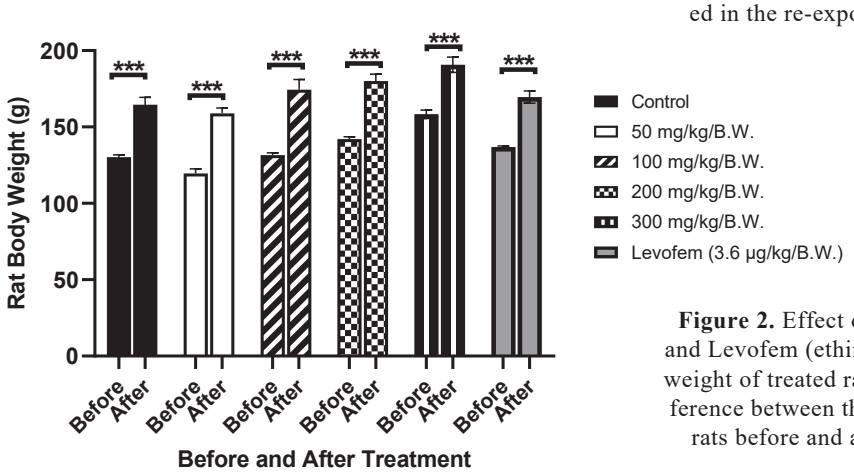

Figure 2. Effect of aqueous extract of *X. aethiopica* fruit and Levofem (ethinyl estradiol and levonorgestrel) on body weight of treated rats. ***p<0.001 represent significant difference between the average body weights of experimental rats before and after treatment, once daily for 21 days.

as well as its reversibility, the rats that did not produce pups after the mating exercise were again exposed to the males for mounting for seven days. The numbers of fetus in their uteri were determined seven days after the mating period. Table 3 shows the number of fetus found in the uteri of the rats after re-exposure to males for mounting. The only rat (R4) in the negative control group (Group A) that was not pregnant after the initial mating exercise was again not pregnant after the second mating exercise. In Group B, the four rats that were not pregnant after treatment with the extract were all pregnant after the second mating exercise; these rats had a total of 34 fetuses in their uteri. In Group E, three of the four rats that were not pregnant after the first mating exercise were pregnant after the second mating exercise; these three rats had a total of 31 fetuses in their uteri. In Group F, the three rats that were not pregnant after initial treatment with ethinyl estradiol and levonorgestrel were all pregnant after the second mating exercise; they had a total of 26 fetuses in their uteri. The only rat in Group D that was not pregnant after the initial mating period was again not pregnant after the second mating period.

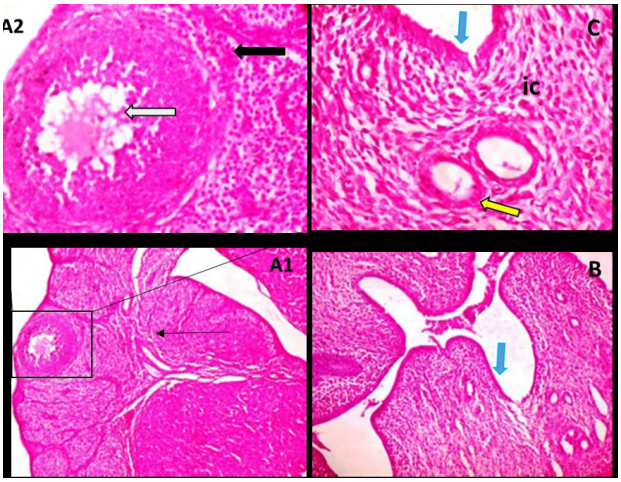

Figure 3. Photomicrographs of sections of the ovary and uterus of R4 in Group A stained with hematoxylin and eosin. (A1): a section of the ovary (×100), the stroma appears normal with normal connective tissues (slender black arrow). (A2) is a magnified section (×400) of A1, showing normal antral follicles (white arrow) with normal theca cells (thick black arrow) within the ovarian cortex. (B) (×100) and (C) (×400) are sections of the uterus showing eosin normal endometrium epithelial layer (blue arrow), normal endometrial gland (yellow arrow), and moderate infiltration of the endometrial stroma by inflammatory cells (ic).

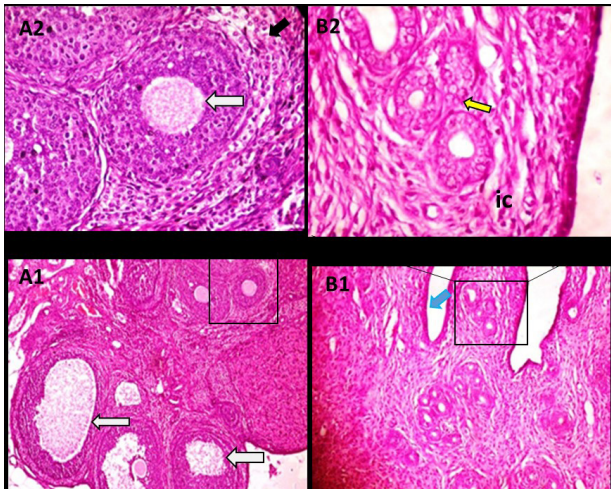

Figure 4. Photomicrograph of sections of the ovary and uterus of R5 in Group E stained with hematoxylin and eosin. A1 is ×40 image of the ovary, A2 is a magnified (×400) section of A1. The images show some normal antral follicles (white arrows) with normal theca cells (thick black arrow) within the ovarian cortex. The ovarian stroma appear normal with normal connective tissues (slender black arrow). B1 (×100) and B2 (×400) are images of a section of the uterus. B1 and B2 show normal endometrium epithelial layer (blue arrow), mild endometrial gland hyperplasia (yellow arrow), and moderate infiltration of the endometrial stroma by inflammatory cells (ic).

The results show the reversibility of the contraceptive effect of aqueous extract of *X. aethiopica* fruit after cessation of treatment. Most of the rats that were initially not pregnant from the first mating exercise due to the treatments with the extract and Levofem, were pregnant after the second mating exercise. The intervening period between the last day of contraceptive treatment and the second mating exercise was 28 days. Comparing the contraceptive performance of aqueous extract of *X. aethiopica* and the combined oral contraceptive containing ethinyl estradiol and levonorgestrel, the aqueous extract showed better contraceptive potential than the pill at 50 mg/kg/B.W. and 300 mg/kg/B.W. dosages. From the treatment of each of the dosages, only one out of five rats was pregnant, a value corresponding to 80% contraceptive efficacy. On the other hand, the group treated with a combination of ethinyl estradiol and levonorgestrel had two out of the five rats to be pregnant after exposure to males, this corresponds to 60% contraceptive efficacy. On re-exposure to males, the three rats that were not pregnant after the first round of mating all produced pups, this shows 100% reversibility of the contraceptive pill.

Histological analyses of the uteri and ovaries of the rats (R4 of Group A and R5 of Group E) that were not pregnant after the first and second mating exercise are presented in figure 3 and figure 4. The images show that the treatment with aqueous extract of *X. aethiopica* fruit did not have negative impact on the architecture of the ovaries and uteri of the rats. Mild endometrial gland hyperplasia and moderate infiltration of the endometrial stroma by inflammatory cells is seen in the uterus of the Group E rat.

Discussion

X. aethiopica fruit is consumed as spice in various

soups, and different parts of the tree are used in treatment of various ailments. The preparation of the aqueous extract of the fruit used in this study mimicked the method of preparing the local "pepper soup" delicacy. Natural products, particularly of plant origin are generally perceived to be safer and more tolerable than synthetic drugs. The toxicity of the aqueous extract of *X. aethiopica* has not been reported in literature. Histological examination of the uteri and ovaries of the rats that did not get pregnant after treatment with the aqueous extract, showed intact histological architecture of the organs, suggesting a non-toxic profile for *X. aethiopica* at the dosages employed. High doses of ethanol extract of *X. aethiopica* fruit (386 mg/kg/B.W. up to 518 mg/kg/B.W.) have been reported to have adverse effect on tissue architecture of the ovary and uterus, including degeneration of endometrial cells, inflammation in epithelial cells, follicle atrophy, development of blood vessel within ovarian cortex and stroma, and development of ovarian cyst [22]. Also, the ethanol extract of the fruit has been reported to induce testicular tissue degeneration and in the process reduce semen quality in male Wister rats [18]. Methanol extract of *X. aethiopica* fruit, however, at 300 mg/kg/B.W. and lower dosages did not have toxic effect on rat liver and kidney [16].

Long term (12 months or more) use of hormonal contraceptives has been found to increase body weight significantly relative to non-hormonal contraceptives [23]. In this study, the weight gain by the ethinyl estradiol–levonorgestrel treated rats was not significantly different from the weight gain by rats in the negative control group and the extract treated groups. This suggests that short term usage of aqueous extract of *X. aethiopica* fruit as herbal contraceptive remedy would not have negative impact on body weight. Woode et al. reported that adult male Sprague Dawley rats treated with ethanol extract of *X. aethiopica* showed significant increase in reproductive organ size and body weight, with concomitant increase in serum testosterone and luteinizing hormone levels [19]. According to Nnodim et al., the aqueous extract of *X. aethiopica* obtained by maceration at room temperature reduced the serum levels of estradiol, follicle stimulating hormone, prolactin, testosterone, and luteinizing hormone in male Wister rats [24]. The polarity difference between water and ethanol ensures that more organic phytochemicals are extracted by ethanol, and this may account for the toxicity and contrasting effect on hormonal levels reported for the ethanol extract.

Identification and isolation of compounds from aqueous extracts of *X. aethiopica* have not been reported in literature. From the phytochemical screening of the aqueous extract, flavonoids alkaloids, tannins and saponins were present. Flavonoids and alkaloids are known for their strong antifertility activity, inhibition of embryo implantation in the endometrium and reduction of sex hormone secretion levels [25,26]. The mechanism of action of levonorgestrel includes the inhibition of endometrial cells proliferation in humans and reduction of the frequency of the rhythmic beating of the cilia in the fallopian tubes which hinders the transport and implantation of the embryo in the endometrium [27,28]. Users of combined oral hormonal contraceptives have risk of carcinogenic outcomes, frequent headaches, edema and low libido [29,30,31]. Inferring from previous reports, fertility hormone modulation and embryo implantation disruption are the probable mechanisms of action by which the aqueous extract of *X. aethiopica* fruit exerts its contraceptive effects.

Comparing the contraceptive performance of the aqueous extract of *X. aetheopica* fruit with the performance of the pill containing ethinyl estradiol and levonorgestrel, the aqueous extract showed better contraceptive potential at the lowest and highest dosage administered; only one rat got pregnant in the group treated with the lowest dose and highest dose but two rats got pregnant in the combined oral contraceptive treated group. The litter sizes also show the extract to perform better either in reducing the number of eggs release during ovulation or greater inhibition of embryo implantation. Also, the reversibility of the contraceptive effect after cessation of treatment with the extract was at par with that of the combined oral contraceptive. The number of fetuses in the uteri of the extract-treated rats after the second mating period was within the normal range (8 – 14 litters) in rats. *X. aethiopica* is readily available and affordable to meet the contraceptive needs of several women in the rural environment who do not have access to modern contraceptives and cannot afford the costs. Though the fruit of *X. aethiopica* is used as spice in foods and soups, there is need to investigate the safety of daily usage of the aqueous extract over a longer period of time. Also, there is need to identify the compounds present in the extract with a view to isolate and characterize them. This will provide leads for the development of non-hormonal contraceptive drugs of plant origin.

Conclusion

For women in the rural communities with unmet contraceptive needs, aqueous extract of *X. aethiopica*, which is rich in alkaloids, flavonoids, tannins and saponins, could provide an affordable and accessible alternative to modern contraceptive methods. This study shows aqueous extract of *X. aethiopica* to be efficacious as a reversible contraceptive with results comparable to that of combined oral contraceptives composed of ethinyl estradiol and levonorgestrel. It is also advisable that women who are desirous of getting pregnant should avoid the use of *X. aethiopica* as

spice or condiment in their foods. Clinical studies are needed to further explore the safety and efficacy of this plant in human.

Conflict of Interests

The authors declare that there are no conflicts of interests.

Acknowledgments

None.

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