International Journal of Herbs and Pharmacological Research
IJHPR, 2019, 8(1): 2 - 7
ISSN: 2315-537X www.arpjournals.com; www.antrescentpub.com E- ISSN: 2384-6836

RESEARCH PAPER

EFFECT OF DIETARY *XYLOPIA AETHIOPICA* (DUNAL, A. RICH) ON OVULATION, ESTROUS CYCLE, AND FERTILITY IN CYCLIC WISTAR RATS.

[1]Onyebuagu P. C. and [2]Agbai E. O.
[1&2]Department of Human Physiology, School of Basic Medical Sciences, Federal University of Technology, Owerri, Nigeria.
Corresponding author: peteronyebuagu@gmail.com; Co-author: emmanuelagbai7@gmail.com

Endorsed By: *Innovative Science Research Foundation (**ISREF**) and International Society of Science Researchers (**ISSCIR**).* ***Indexed By****: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society; and IRMS Informatics India (J-Gate)*

ABSTRACT

The search for a cheap, readily acceptable effective contraceptive to meet the increasing need for population control was the focus of this study. The study was designed to investigate the effects of dried fruits of *Xylopia aethiopica* on ovulation, estrous cycle, and female fertility in Wistar rats. Forty Wistar rats (38 females, weighing 140 – 165g, and two males, weighing 175 – 185g) were used in this study – designed in three experiments: (I) Five female Wistar rats received 50g/kg of *Xylopia aethiopica* for 21 days and the effects of the treatment on estrous cycle were assessed. (II) Two groups of 5 female rats each received 50g/kg of *Xylopia aethiopica* from 8am to 12noon and from 1 – 5pm respectively on proestrus, to assess the effect on number of ova shed on morning of estrus. (III) Five female Wistar rats received 50g/kg of *Xylopia aethiopica* from Days 1 to 7 postcoitus to assess the effect on implantation and pregnancy. All the groups were control-matched. The results show alterations in the estrous cycle, specifically, with prolonged diestrus, reductions in ovulation levels and implantation, as well as, abortifacient effect. Dietary *Xylopia aethiopica* could be a good candidate for consideration as an oral contraceptive agent.

Key words: *Xylopia aethiopica*, dietary intake, ovulation, estrous cycle

INTRODUCTION

The human population is evidently growing beyond its carrying capacity, as the number of people on earth is expected to exceed 4 billion by 2030. This situation is worst in countries in West Africa - with a population estimate of 392 million as at April, 2019 (WPP, 2017) - where the leaders are, obviously, overwhelmed by efforts to raise the population out of poverty. This state of affairs, therefore, calls for concerted efforts to curb the rate of population expansion, including by the use of less invasive, more acceptable method such as oral contraceptive. This subtle approach is desirable in view of the cultural and religious barriers towards these interventions in the region.This demand for locally acceptable contraceptive was the stimulus for the interest in this study using *Xylopia aethiopica*, a common food spice used in making soups, especially, in the south-eastern parts of Nigeria.

Xylopia aethiopica, commonly called African pepper, or Ethiopian pepper, or "uda"in Igbo language, "urien" in Bini language, "erunji" in Yoruba language, and "kimba" in Hausa language, is commonly used as spice to prepare soup dishes, such a pepper soup, due to its high pungent and peppery properties. It is, extensively, used to prepare diets for women, as a postpartum tonic to stimulate appetite, prevent postpartum depression, heal afterbirth wounds, reduce pain, and aid lactation

(Murray, 1995). It is also believed to be used by herbalist to induce labour, and to terminate unwanted pregnancy (Muanya, 2011). There are scientific reports suggesting the analgesic and anti-inflammatory properties of *Xylopia aethiopica* (Nwafor *et al*., 2012), which are supported by other report of the antioxidant activity of this spice by virtue of the presence of flavonoids and phenolic compounds in this spice (Nworah *et al*., 2012). *Xylopia aethiopica* has also been reported by several researchers to possess antifertility properties (Nwafor and Gwotmot, 2006; Onyebuagu *et al*., 2013).

If *Xylopia aethiopica*, is found to be an effective oral antifertility agent, its wide availability and acceptability as a food supplement may qualify it as a veritable contraceptive candidate for the battle against overpopulation, at least, in this region. Though several reports have stated the abortifacient property of *Xylopia aethiopica* (Nwafor and Kalio, 2006; Onyebuagu *et al*., 2013), another report demonstrated that this spice diminished reproduction through reduction in the plasma lipids and, consequently, testosterone levels in male rats (Onyebuagu *et al*., 2014*).* Whatever the properties of this spice, the corollary is that dietary contraception will be preferred to abortifacient in the efforts to curb human population. This study was, therefore, carried out to determine the effect of dietary intake of *Xylopia aethiopica* on ovulation and pregnancy in Wistar rats.

MATERIALS AND METHODS

Procurement of Animals and Fruits of *Xylopia aethiopica*: A total of 40 female Wistar rats, weighing 160 – 180g, procured from the Animal House Unit of Department of Pharmacology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, were used in this study. All the rats were kept in rat cages under standard laboratory conditions for temperatures of between 26 - 28^{o}C and humidity, with photoperiodicity of 12:12 hours light: darkness. They had unhindered access to clean drinking water and rat chow *ad libitum*. The animal feed (chow) was purchased from Pfizer Co. feed Depot, Port Harcourt, Nigeria. The fresh fruits of *Xylopia aethiopica* were purchased from Iruekpen market, in Esan West L.G.A., Edo State, Nigeria, and authenticated by Professor E. E. Okoegwale, Botany Department, Ambrose Alli University, Ekpoma, Edo State, Nigeria. The fruits were sun-dried for 14 days and then ground into powdered form. 50g of powdered fruits of *Xylopia aethiopica* was mixed with 1 kg of feed and powdered cooked edible starch as binder for the mixture. Note that 50g/kg as effective dietary *Xylopia aethiopica* treatment dosage had been selected in a previous study (Onyebuagu *at al*., 2013).

Test on Estrous Cycle: Two groups of 5 female Wistar rats each were used in this experiment, treated Group I, and control Group II. The rats in Group I received the treatment dietary dose of 50g/kg of feed and edible starch mixture, while control Group II rats received rat chow and edible starch mixture only. The rats in both groups had free access to their respective diet and clean drinking water *ad libitum* for 21 days. The stages of the estrous cycle in the female rats were determined by the method of simple vagina swap, in which by 8am-10am daily, the small suction pipette and normal saline were used to gently collect vaginal lavage from each of the cycling female rats. The collected samples were each placed on clean slides and covered, and then examined under the light microscope at 10x and 40x to determine the stages of the estrous cycle via the proportion of the three types of cells observed in the vaginal samples (Marcondes *et al*., 2002). Weekly body weights of all the rats were measured throughout the duration of the experiment.

Ovulation Studies: Twenty 4-days cycling female rats (selected over a period of 14 days of examination), were used in this experiment. The rats were divided into four groups of 5 rats per group. The test Group IA rats were fed with dietary dose of 50g/kg of feed from 8am to 2pm on proestrus, following overnight fast. Test Group IIA rats received dietary dose of 50g/kg of feed from 1pm to 7pm on proestrus, following a six-hour fasting period. The control Group IB received rat chow only, from 8am to1pm on proestrus following overnight fasting period. The control Group IIB received rat chow only, from 1pm to 7pm on proestrus following a six-hour fasting period. All the rats had free access to clean drinking water *ad libitum*. The rats were all sacrificed the next day using chloroform anesthesia, dissected, and the upper third of the oviducts were identified, harvested and examined histologically under the light microscope to record the number of ova that were shed in each group.

Fertility Studies: Ten female Wistar rats, divided into two groups (test Group I, and control Group II) of 5 rats each, were used in this experiment. Rats in both groups were mated with male rats of proven fertility status, and the presence of copulation plugs was taken as Day I of the rats' pregnancy. The test Group I rats received dietary 50g/kg of feed from Day 1 – Day 7 post coitus, and then continued feeding with normal rat feed, while control Group II female rats received rat feed all through for 21 days. All the rats had free access to clean drinking water *ad libitum*. On the morning of Day 20, all the

rats were sacrificed using chloroform anesthesia, and then dissected for analyses. The number of fetuses in each rat was counted and weighed individually, and the sites of resorption of embryos were noted. The placenta of each rat was harvested and weighed, and the umbilical cord and crown rump lengths were measured.

Statistics: The data collected in the experiments are presented as Means ± SD, and were subjected to statistical analyses, using SPSS, and Student's t-test. Statistical significance of the difference between test and control groups data was tested at $p<0.05$.

RESULTS

Results of the morphological data showed that there was reduction in the mean body weight of the Group I (treated) female Wistar rats, compared to the female rats in the control Group II (Table 1). The weight loss was, however, not significant – representing only 5%, compared to the control, which gained 6.2% in mean body weight.

Table 1: Effect of Dietary Intake of 50g/Kg of Dried Fruits of *Xylopia aethiopica* on Mean Body Weight of Female Wistar Rats.

Days	Group I	Group II
Day 1	148.25 ± 5.1	142.40 ± 7.4
Day 20	136.12 ± 2.88	158.74 ± 8.2

n = 5; Mean ± SEM

Analyses of the female estrous cycle showed that intake of dietary dose of 50g/kg of dried fruits of *Xylopia aethiopica* exhibited irregular pattern in the treated rats, with alteration in the cycle pattern by the 4th day of administration. The test female rats in Group I demonstrated prolonged diestrus pattern in each cycle, while on the other hand, there was significant decreases in the duration of the other phases of the cycle (see Table 2)

Table 2: Effects of Dietary Intake of 50g/Kg of Dried Fruits of *Xylopia aethiopica* on Estrous Cycle of Female Wistar Rats.

Estrous Cycle	Group I (%)	Group II (%)
Normal	00	100
Irregular	100	00
Proestrus	10.66	23.16
Estrus	10.54	23.89
Metestrus	9.69	23.97
Diestrus	62.18	28.97

n = 5

The results of the ovulation experiment show that dietary administration of 50g/kg of *Xylopia aethiopica* to test Group 1A female rats from 8 am – 2 pm on proestrus, produced significant reduction ($p<0.05$) in the number of ova shed in the morning of estrus, compared to the control Group IB female Wistar rats. On the other hand, the number of ova shed on the morning of estrus by the test Group IIA female Wistar rats which received dietary intake of 50g/kg of *Xylopia aethiopica* from 1 pm – 7 pm of proestrus, and those of the control Group 1B and control Group IIB female rats which received the placebo were not significantly ($p<0.05$) different.

Table 3: Effects of Dietary Intake of 50g/Kg of Dried Fruits of *Xylopia aethiopica* from 8am to 2pm, and from 1 - 7 pm of Proestrus on Number of Ova Shed by Female Wistar Rats.

Time of Treatment	Animal Groups	Number of Ova shed
8am -2pm	Test (Group IA)	3.48 ± 2.72
	Control (Group IB)	7.80 ± 1.85
1pm – 7pm	Test (Group IA)	7.88 ± 1.27
	Control (Group IB)	7.48 ± 1.64

n = 5; Mean + SEM

The result of the experiment on effect of dietary intake of 50g/kg of dried fruits of *Xylopiaaethiopica*on pregnancy and fetal parameters shows that administration of the spice from Day 1 to Day 7 post-coitus (equivalent to first trimester of pregnancy) had anti-implantation/abortifacient effects on the pregnant female rats. There were visible signs of reddish vaginal discharge which was observed by Day 5 post-coitus. From the "autopsy" performed on Day 20 of gestation, there were significant differences in the litter size and placental weight, compared to the control, while the crown-lump length, fetal weight and umbilical cord length were not significantly different from same parameters in the control rats.

Table 4: Effects of Dietary Intake of 50g/Kg of Dried Fruits of *Xylopia aethiopica* on Pregnancy and Fetal Parameters in Female Wistar Rats.

Groups	Litter size (n)	Fetal Weight(g)	Placental Weight(g)	Crown-rump length (cm)	Umbilical cord length (cm)
I (Test)	3.75 ± 1.51	3.96 ± 0.23	1.81 ± 0.26	3.11 ± 0.14	2.77 ± 0.33
II (Control)	7.00 ± 1.81	3.36 ± 0.38	2.61 ± 0.26	3.47 ± 0.14	3.12 ± 0.23

n = 5; Mean ± SEM

DISCUSSION

The results of this study have demonstrated that dietary intake of 50g/kg of *Xylopia aethiopica* caused alterations of the estrous cycle in Wistar rats, through increases in the duration of the diestrus phase of the cycle, and consequently reducing the frequency at which the estrus phase occurs. This effect translates to reduction in the frequency of ovulation in the treated animals, and ultimately lower fertility in the animals. This findings compare favorably with other reports on the effect of *Xylopia aethiopica* on reproduction in laboratory animals (Nwafor and Gwotmot, 2006; Nwafor and Kalio, 2006; Onyebuagu *et al*., 2013). The administration of *Xylopia aethiopica* may alter ovulation through its effects on the hormonal milieu, since the physiological events during ovulation are mediated by female reproductive hormones such as the steroid hormones, estrogen and progesterone Adienbo*et al*. (2011). Nwafor *et al*., (2011) also demonstrated the contraceptive effect of aqueous extract of *Xylopia aethiopica* in female Wistar rats. There are reports suggesting that intake of *Xylopia aethiopica* reduces steroid hormone production via its lowering effect on plasma level of cholesterol, which is the precursor for the steroid hormones biosynthesis (Mill. 1998; Vander *et al.*, 2001; Onyebuagu *et al*., 2013).

Physiologically, a luteinizing hormone surge during proestrus is responsible for the events leading to, and following ovulation. This rapid rise in plasma level of LH begins at about 2-3 pm on proestrus, and eventually reaches peak levels at about 5 – 7 pm of same day (Okanlawon and Ashiru, 1992). Some researchers have demonstrated that administration of chloroquine or sodium pentobarbital at 9 am on proestrus blocked ovulation completely, but same agents had no effect on

ovulation when administered at 6 pm (Okanlawon and Ashiru, 1992; Van der Schoot *et al*., 1982). This suggests that the mechanism of action may involve blocking of the rise in plasma level of LH during early proestrus phase of the estrous cycle.

The effect of the dietary intake of 50g/kg of *Xylopia aethiopica* on pregnancy outcome in the female Wistar rats shows significant ($p<0.05$) reduction in the litter size of the offspring and placental weight from the treated rats, which appear to be indicative of incomplete abortion of the pregnancy. The abortifacient property of *Xylopia aethiopica* in this study was suggested by Nwafor and Kalio (2006), who demonstrated the strong contractile effect of aqueous extract of fruit of *Xylopia aethiopica* on uterine smooth muscles *in vitro*. This result was also corroborated by the presence of reasorption sites in the uterine walls of the treated female rats, which were not observed in the control group. However, the dietary *Xylopia aethiopica* did not seem to have affected the fetal weight, crown-rump length and umbilical cord length, as these were not significantly different between the test and control groups.

In conclusion, long term dietary use of *Xylopia aethiopica* could be a promising candidate for consideration as an oral contraceptive agent. Further investigations, however, are recommended using other animal species to determine if this spice will exhibit similar effects as in the Wistar rats, as well as, to determine the mechanisms underlying the observed effects.

ACKNOWLEDGEMENT

We wish to acknowledge the tremendous assistance of two scientists: Dr. Oboma, Y. of Histopathology Department, and Dr. Onyinje Felix, of Human Anatomy Department, both of Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The efforts of other colleagues in this work are also highly appreciated

REFERNCES

Adienbo, O. M., Nwafor, A. and Iwuji, S. (2011). Effect of Aqueous Extract of *Xylopia aethiopica*on Reproductive Hormones in Male Guinea Pigs. *Global J. Pure and Appl. Sc. 17(2): 137 – 139.*

Marcondes, F.K., Bianchi, F.G., and Tanno, A.P. (2002). Determination of Estrous Cycle Phases of Rats: Some Helpful Considerations. *Braz. J. Biol. Vol 62 (4a).*

Mill, W. L. (1998). Molecular Biology of Steroid Hormone Biosynthesis. *Endocr. Rev. 9: 295.*

Muanya, C. (2008). How Plants can Induce Labour and Heal Afterbirth Wounds. *The Guardian Newspaper, 17th Jan. 2008.*

Muanya, C. (2011). Natural Health: Walking, Sex and Spicy Foods to Induce Labour. The Guardian Newspaper, 23rd June 2011.

Murray, M. (1995) Healing Powers of Herbs. Primes Publishing, Rocklin, CA.

Nwafor, A. (2013). Life Under Assault: No Where to Hide. *Inaugural Lecture Series No.102nd. University of Port Harcourt*, Port Harcourt.14th March, 2013.

Nwafor, A. and Gwotmut, M. D. (2006). In-vitro Effect of the Extract of *Xylopia aethiopica* on uterine smooth muscle. *African J. Appl. Zool. And Environ. Biol. 8: 130 – 132*

Nwafor, A., Egberike, E. F. and Nworah, D. C. (2011). Assessment of Contraceptive Effect of Aqueous Fruit Extract of *Xylopia aethiopica* on Female Wistar Rats. *J. Advanced Med. and Pharm Sc. 5(1): 114 – 117.*

Nwafor, A. and Kalio, I. D. (2006). Physiological Effects of *Xylopia aethiopica*on Pregnancy in albino Rats. *J. Med.and Pharm. Sc. 2(1): 1 – 4.*

Nwafor, A. , Nworah, D. C., Ukpuho, E. I., and Aniyeloye, M. A. (2012). In-vivo Studies on the Analgesic and Anti-inflammatory Potentials of Novel *Xylopiaa ethiopica* formulations. *Am. J. Pharm. Techn. Res. 2(5): 354 –362.*

Nworah, D. C., Nwafor, A. and Bekinbo, M. T. (2012). Comparative Characterization of Phytomedicinal Constituents of *Xylopiaa ethiopica .Am. J. Pharm. Techn. Res. 2(2): 706 – 712.*

Okanlawon, A. O. and Ashiru, A. A.(1992). Effect of Chloroquine on Estrous Cycle and Ovulation in Cyclic Rats. *Applied Toxicol. 12: 45 – 48.*

Onyebuagu, P. C., Pughikumo, D. T. and Aloamaka, C. P. (2014). Effects of Dietary *Xylopiaa ethiopica* on Hematological Parameters and Plasma Lipids in Male Wistar Rats. *Int. J. Basic and Innovative Res. 3(1): 29 – 34.*

Onyebuagu, P. C[1], Pughikumo, D. T. and Aloamaka, C. P.. (2013). Antifertility Effects of Dietary *Xylopia aethiopica* ((Dunal) A. Rich) in Female Wistar Rats. *Nigerian Annals of Natural Sciences, 13(1): 017 – 025.*

Onyebuagu, P. C[2]., Aloamaka, C. P. and Igweh, J. C. (2013). *Xylopia aethiopica* ((Dunal) A. Rich) Lowers Plasma Lipid Precursors of Reproductive Hormones in Wistar Rats. *Int. J. Herbs and Pharmacological Res.2(4):48 – 53.*

Vander, A. J., Sherman, J. and Luciano, D. (2001). Human Physiology: The Mechanisms of Body Function. The McGraw-Hill Companies. Eight Edition.

Van der Schoot, P., DenBensten, D., and Vilen Brock, J. (1982). Atresia of Preovulatory Follicles in Rats Treated with Sodium Pentobarbital. *Bio. Reprod. 29: 189 – 199*.

World Population Prospects, (2017). United Nations Department of Economic and Social Affairs, Population Division. 2017 Revision. www.Worldmeters.info.

AUTHORS' CONTRIBUTIONS

The authors collaborated in carrying out this study, both in the design and execution of the experiments. Author PCO designed the study and managed the literary aspect of the work. Author EOA took part in the protocol and the analyses. Both authors read and approved the manuscript.