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CONTRACEPTIVE EFFICACY OF HYDRO-METHANOLIC FRUIT EXTRACT OF XYLOPIA AETHIOPICA IN MALE ALBINO RATS

[*]***Ologhaguo M. Adienbo*; Arthur Nwafor. and Ronami S. Ogbomade.**

Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Port Harcourt, Port Harcourt Nigeria

ABSTRACT

The rising human population, particularly in the developing countries, and the attendant socioeconomic effects necessitates the need for its effective regulation. This study aims at exploring the contraceptive efficacy of the fruit extract of Xylopia aethiopica (Dunal) A Rich, a plant with wide ethnomedicinal, pharmacological and social values, in combating this public health challenge. Forty eight adult male and 48 female wistar rats were used for the study. The males, randomly selected into two groups: 30 days treatment and 30 days reversal groups; each further divided into 3 test and 1 control group of 6 animals each. Daily oral doses of 0.5, 2.0 and 10.0mg/kg body weight respectively were given to the test groups for 30 days followed by 30 days of extract withdrawal. 6 animals were sacrificed from each treatment group animals on day 31 and on day 61 for the reversal group animals. Body weight of the animals were measured at beginning of study and before each sacrifice. Fertility test was done after 30 days of extract treatment and after 30 days withdrawal while testicular and epididymal weight, testosterone as well as sperm parameters were assessed on the day of each sacrifice. Results show a reversible dose dependent negative effect in body and organ weight, sperm parameters and in fertility parameters. Therefore, xylopia aethiopica has antifertility potentials which could be explored for contraceptive purposes.

Key words: xylopia aethiopica, contraceptive, antifertility, reproductive function.

INTRODUCTION

Procreation and/or sexual fulfillment of both partners which are the main essence of marriage in humans is initiated by the mating of a male with a female in sexual intercourse; resulting in rise in human population. An explosion of this population is a leading cause of poverty and pollution especially in developing countries. The need for an effective regulation on human fertility is therefore becoming an obvious challenge for policy formulators, health administrators and medical researchers. Though considerable progress has been made in the development of highly effective, acceptable and reversible methods of contraception among females, such is not the case among males. The only male-specific contraceptive methods currently available are withdrawal, condoms, and vasectomy which have limited

Corresponding Author E-mail: ologhaguo.adienbo@uniport.edu.ng

acceptance because of concerns regarding their side effects and convenience. (Beckman and Harvey 1996). Recently, efforts are being made to explore the hidden wealth of medicinal plants for contraceptive use. In Nigeria, there are several medicinal plants associated with antifertility properties, yet scientific screening and evaluation of fertility – regulating potentials of these phytomedicines is not fully explored in spite of the vast ethno botanical heritage. This study aims at exploring the contraceptive efficacy of the fruit extract of *Xylopia aethiopica (Dunal) A Rich*; a plant with wide ethno medicinal, pharmacological and social value. It is a West African ‘’pepper tree’’ used in decoction to treat dysentery, bronchitis, ulceration, skin infection and female sterility. Several studies have shown that *Xylopia aethiopica* extracts possess haematopoietic (Nwafor et al 2009), antibacterial (Asekun and Adeniyi,2004; Fleischer et al,2008; Konning et al 2004; Kuete 2010), antifungal (Tatsadjieu et al 2003) and anti-plasmodial (Boyom et al 2003) and antigonadotropic (Adienbo et al 2011) activities. The dried fruits are also used as spices in the preparation of two special local soups named “Obe ata” and isi-ewu taken widely in the Southern parts of Nigeria. (Tairu et al, 1999).

MATERIALS AND METHODS

Animal models: Forty eight adult male and 48 female wistar rats weighing between 160-220g each were bred in the Animal House unit of the Department of Human Physiology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria. The rats were maintained in a well ventilated animal house under standard condition of humidity, temperature and a constant 12 hour light: 12 hour dark lighting schedule. The animals were housed in clean wooden cages lined with wood chip beddings. Standard pellet diet (Livestock Feeds, Sapele Nigeria) and water were given ad libitum. Generally, the study was conducted in accordance with the recommendations from the declaration of Helsinki on guiding principles in care and use of animals.

Extraction of plant material: Dry fruits of *xylopia aethiopica* purchased at Choba market in Port Harcourt were identified and authenticated at the Department of Forestry and Wildlife Management, Faculty of Agriculture, University of Port Harcourt (Voucher No. UUH1819). The identified fruits were washed, oven dried at 40^{o}c to a constant weight. 2kg of the dried fruit was then ground to coarse powder form. Each 500g of dried and ground fruits was then refluxed in 2 liters of hydro-methanol solvent (1:4) at 60–70^{0}C for 36 hrs in a continuous extraction (soxhlet) apparatus. The extract was filtered and concentrated under reduced pressure at 60^{0}C using a rotary evaporator. The net yield was 174.6g. This was then preserved in a refrigerator at -4^{o}c. When required the residue was suspended in olive oil to prepare fresh stock solutions of 0.1mg/ml and 0.5mg/ml respectively. The volume of solution corresponding to the dose administered to each animal was obtained using the formula: Volume (ml) = (Dosage ($mg.kg^{-1}$) x Body weight (kg)) / (Concentration($mg.ml^{-1}$))

Experimental Design: Following 10 days of acclimatization, the Forty eight male rats were randomly divided into two groups: (a) 30 days treatment group and (b) 30 days reversal group. Each group was further divided into one control group and three test groups of 6 animals each. During the first 30 days of the study, all the test groups animals were each treated, for 30 consecutive days, with group-specific single daily doses of the hydro-methanolic extract of *X. aethiopica* at the doses 0.5mg/kg body wt, 2mg/kg body wt, and 10mg/kg body wt respectively, each in 1ml volume of the vehicle (olive oil) using animal-feeding intubation needles (Popper and Sons, New Hyde Park, NY). Similarly, animals in all the control groups each received gastric infusion of 1 ml olive oil in the same way as the experimental rats for

same duration. Initial body weight of all the animals was noted at the starting day of the experiment and continued weekly throughout the period of the study. Six rats from each group in the 30 day treatment were randomly sacrificed after 30 days of extract administration and twenty four hours after the last dose. The remaining 6 rats from each of the groups for the 30 days reversal were allowed to recover for another 30 days before sacrification. During the recovery period food and water were supplied ad libitum, but extract and vehicle administration were discontinued. Also, weekly measurement of the body weight of the animals was continued throughout the recovery period.

Body/organ weights: Initial body weight of all the animals was measured at the beginning of the experiment, continued weekly throughout the period of the study, and before scarification; while the weights of right testes and cauda epididymides were measured after scarification.

Serum Testosterone: Blood was obtained by cardiac puncture from the rats in each study group after sacrification. Each blood sample was spun at 2500 rpm for 10 minutes in a desktop centrifuge at 10-25 ^{o}C. Serum samples were assayed for testosterone using the enzyme linked immunoassay (EIA) technique.

Sperm Quality Analysis: At the end of 30 days of treatment, 24 male rats (6 from each group) were anaesthetized with chloroform for about 5 minutes, an abdominal incision was made and the scrotum was dissected to expose the testis and epididymis. The testicles were surgically removed. The epididvmides were trimmed off the testes and semen was collected by maceration from the cauda epididymis through an incision made with a scalpel. Semen examination to evaluate spermatozoa characteristics such as motility, count, viability and morphology were done. The sperm count was determined by routine procedure using an improved Neubauer haemocytometer, while viability was assessed by eosin-nigrosin dye exclusion test. Quantitative epididymal sperm motility was assessed by calculating motile spermatozoa per unit area and was expressed as percent motility. Sperm morphology was done using the Wells and Awa stain and examined under the microscope as described earlier in literatures.

Fertility Testing: To evaluate the fertility, rather contraceptive potency of *xylopia aethiopia*, both the control and the test group rats were mated with parous untreated female rats of the same strain in the ratio of 1:1, for 10days. Cohabitation commenced on the first day of the last 10 days of extract treatment and the first day of the last 10 days of the recovery periods respectively. The animals were left together to mate freely for 10 days, a period corresponding to at least two oestrous cycles. One week after removal of the males, the females were sacrificed by cervical dislocation under light chloroform anesthesia, and the number of pregnant females and viable fetuses counted. A male rat was considered fertile if he impregnated the female rat with which he cohabited. Fertility rates and fertility indices were calculated.

Statistical Analysis:

Statistical analysis was performed with the Statistical Package for Social Sciences Software (SPSS; version 17.0, USA). Differences between groups were examined by ANOVA test (analysis of variance of means) with mean and standard error of mean (M±S.E.M). P value ≤ 0.05 was considered as statistically significant.

RESULTS

Body weight and organ weight: Results of the effect of the extract on body weight and reproductive organ weight of the rats (table 1) shows that there was an absolute increase in rat body weights in all the test groups (0.5, 2 and 10 mg/kg). However, the increase in body weight (g) in each of the test groups is

significantly (P<0.05) lower when compared with that of the control group. This implies that there was a relative reduction in body weight by 30.68%, 31.83% and 30.85% in the 0.5mg/kg, 2mg/kg and 10mg/kg test groups respectively, relative to the control group. This relative weight loss was however, reversed in the recovery groups as there was no significant difference in the body weight of both the test and control groups. Further, the testicular weight, showed a progressive dose dependent decrease, in 0.5mg/kg (p>0.05), 2mg/kg (p>0.05) and 10mg/kg(P<0.05) test groups when compared with the control group; while the epididymal weight, decreased significantly (p<0.05) in the 2 and 10 mg/kg test groups respectively, when compared with the control group. After the withdrawal of the extract administration, there was no observed difference in the body and organ weight of experimental animals compared to their respective control groups.

Serum Testosterone: Figure1 shows the result for testosterone. There was a dose dependent significant (p<0.05) decrease in testosterone concentration in all the treatment groups when compared with the control group. The decrease was reversed in all the test groups.

Sperm Quality Analysis: The results of the sperm motility, sperm count, sperm viability and abnormal sperm morphology, for the 30 days treatment and 30 days reversal of hydromethanolic extract of *xylopia aethiopica* on the rats are as presented in table 2. It was observed that during the treatment period, the extract caused highly significant (P<0.01) decline in the percentages of sperm motility in all the test doses as compared to the control group. Also, a significant reduction was noticed in the sperm count at doses of 0.5mg/kg (p< 0.05), 2mg/kg (P < 0.01), and10mg/kg (P < 0.01), as compared to the control group; while the percentage of viable sperm were decreased significantly (P < 0.01) in rats treated with 2 and 10 mg/kg doses of the extract when compared to the control group. However, a significant increase in sperm abnormality (%) for all the test groups of the extract was observed for the 0.5mg/kg (P < 0.05), 2mg/kg (p<0.01) and 10mg/kg (p<0.01) respectively, when compared to the control group. After the 30 days withdrawal, there was an improvement in the percentage of motile spermatozoa resulting in an insignificant (p>0.05) reduction in all the test groups, when compared with the control group. The sperm count became insignificantly (P>0.05) decreased in the 0.5mg/kg and 2mg/kg test groups, while the 10mg/kg group remained significant (P<0.05), when compared with the control group. The percentage of spermatozoa with abnormal morphology was improved in the 0.5mg/kg test group (P>0.05), while the higher dose groups (2mg/kg and 10mg/kg) respectively remained significantly (P<0.05) increased when compared with the control group. It was further observed that the percentage of viable spermatozoa in all the test groups were insignificantly (P>0.05) decreased when compared with the control group.

Fertility Test: Results of the fertility parameters for the 30 days treatment groups (Table 3) showed a dose dependent decrease in the number of pregnant females in all the test groups, which was significant (p<0.05) in the 2.0mg/kg and 10mg/kg treatment groups as compared to the control group. The number of viable fetuses also showed a dose dependent decrease in the 0.5mg/kg (p>0.05), 2mg/kg (p<0.01) and 10mg/kg (p<0.01) treatment groups as compared to the control group; while the fertility rate (%) showed a significant reduction in the 2mg/kg (P< 0.05) and 10mg/kg (p<0.01) treated groups when compared with the control. During the reversal period, there was no improvement the number of pregnant rats, number of viable fetuses as well as the fertility rate, respectively as they all maintained significant ($p \leq 0.05$) reduction in the test groups treated with 2.0mg/kg and 10 mg/kg b. wt. respectively, when compared with their respective control groups.

DISCUSSION

In the present study, *xylopia aethiopica* fruit extract significantly decreased sperm motility, count and viability with corresponding increase in percentage of sperm with abnormal morphology, which were reversible following withdrawal of extract administration. The deleterious effect of the extract on sperm quality could be attributed to unfavourable and decreased spermatogenic activities as results of the effect of the extract in all the test groups. Decrease in epididymal sperm count and increase in morphologically abnormal sperm, as observed in this study, have been associated with testosterone deficiency (Matsumoto 1990, Islam and Trainer 1998). In this study, a significant dose dependent reduction in plasma testosterone, similar to earlier reports of Woode et al (2012) and Adienbo et al (2010), was induced by *Xylopia aethiopica.* Testosterone is known to be critically involved in the development of sperm cells (spermatogenesis) and therefore, derangement in testosterone level results widely from leydig cell dysfunction and testicular steroidogenic disorder (Zhang et al, 2001). This suggests that *Xylopia aethiopica* interferes with testicular steroidogenesis and hence, spermatogenesis. It is known that a major function of the epididymis is sperm maturation which leads to the acquisition of fertilizing ability, motility and viability of spermatozoa. Therefore, the decrease in sperm motility and viability observed in this study suggests alteration of sperm maturation in the epididymides due to the impact of the extract in the activities of the epididymides. The reduction in the sperm motility observed in this study suggests that the mitochondrial activity in the spermatozoa may be reduced (Breitbartet al 1984; Randel et al 1992), with possible impairment of dynein- ATPase system on microtubular dynein arms so leading to restriction of sliding movement of flagellar fiber (Teng, 1995). This implies that *xylopia aethiopica* may inhibit cAMP formation which subsequently decreases sperm motility [Zavos and Zarmakoupis-Zavos, 1996]. The reduction in sperm motility, count and viability observed in this study are consistent with earlier reports (Eze 2012; Woode et al 2012, and Burkhills 1985) but however, contradicts those of Abaidoo et al (2011) and, Oguike and Archibong (2011) who rather observed an improvement in sperm quality. Also, while the sperm motility was observed to significantly decrease, there was an associated increase in percentage of morphologically abnormal sperm in rats from all the test groups. This agrees with earlier reports (Shi et al, 2003) that the abnormal morphological changes in spermatogenic cells and spermatozoa induced by plant extract, gossypol, is related to the reduction in motility and therefore may be due to decreasing the amount of tubulin and dynein in spermatocyte and spermatid thereby inhibiting the normal transformation of mature spermatids to spermatozoa. This implies that *xylopia aethiopica* may inhibit cAMP formation which subsequently decreases sperm motility [Zavos and Zarmakoupis-Zavos, 1996].
Reduced sperm count, motility and viability as well as increased sperm abnormality, as observed in this study, have each been associated with reduced fertility (Chauhan and Dixit 2008; Raji et al 2003; Adeeko and Dada 1998; Liu and Baker 1992) and are often used as a measure of sperm production, testicular function and / or male fertility. This may therefore explain the significant reduction in the number of pregnant female rats, viable fetuses and fertility rate respectively observed in rats in the test groups, in this study. The decrease in the absolute and relative difference in body weights of rats in the treatment groups could be due to the activities of diterpene kaurene derivatives of *Xylopia aethiopica* which have earlier been implicated in the diuretic and hypotensive properties of *Xylopia aethiopica* (Somova et al, 2001). The diuretic activities of these constituents could have resulted in loss of water and electrolytes and consequently, loss in body weights. Androgens have been shown to be necessary for the development, growth and normal functioning of the testes and male accessory reproductive glands, and studies have shown that the level is positively correlated with the weight of testis and epididymis (Setty et al, 1997; Prins et al, 1991). Therefore, the observed significant reduction in the weight of the testis and

epididymis, which is in line with the earlier reports of Ameyaw and Owusu-Ansah (1998) and Eze (2012), could be due to decreased androgen biosynthesis as evidenced by the significant decrease in serum testosterone levels in the extract treated test groups.

Conclusion: The results suggest that hydromethanolic extract of xylopia aethiopica could have reversible deleterious effects on sperm function and fertility in male rats. The adverse effects were probably mediated through reductions in testicular steroidogenic and spermatogenic activities; which could be harnessed for possible use in male contraception.

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Table 1: Effect of hydro-methanolic extract of *xylopia aethiopica* on the absolute and relative body weights, testicular weight and epididymal weight of male albino rats.

Dose (mg/kg)	**Study Group**	**Body weight of Rats (g)**			**Organ weight (g)**	
		Initial Weight	**Final Weight**	**Difference (% rel. diff.)**	**Testis (% rel. diff.)**	**Epididymis (%rel. diff.)**
Control	Treatment	203.08±7.11	271.54 8.54	68.46± 7.58 (0)	1.27±0.03 (0)	0.82±0.06 (0)
	Reversal	250.00± 13.15	262.00± 12.81	12.00 0.02 (0)		
0.5 mg/kg	Treatment	208.57± 6.19	255.71± 5.00	47.14± 3.04** **(-30.68)**	1.20±0.06 (-5.51)	0.77±0.07 (-6.50)
	Reversal	250.00± 15.74	265.00± 7.03	15.00 0.32 **(25)**		
2.0 mg/kg	Treatment	208.00± 6.98	254.67± 5.24	46.67± 3.03** **(-31.83)**	1.18±0.02 (-7.09)	0.60±0.05* (-26.83)
	Reversal	273.33± 10.49	287.00± 16.31	13.67± 1.04 (14.75)		
10.0 mg/kg	Treatment	213.33± 6.38	260.67± 6.13	47.34± 4.73** **(-30.85)**	1.06±0.04* (-16.54)	0.56±0.05** (-31.71)
	Reversal	263.67± 12.81	274.0 10±.25	10.33 1.00 **(-13.92)**		

significant difference *($p \leq 0.05$), **($p \leq 0.01$). rel, diff (relative difference).

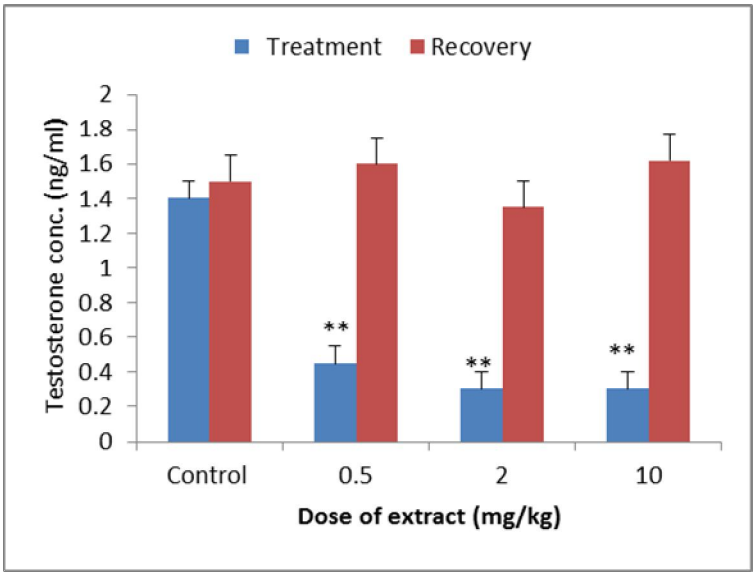

Figure 1: Effect of hydro methanolic extract of *xylopia aethiopica* on plasma testosterone concentration. **significant difference ($p<0.01$).

Table 2: Effect of hydro-methanolic extract of *xylopia aethiopica* on epididymal sperm Parameters (Mean ±S.E.M) of male rats.

SPERM PARAMETER	**STUDY GROUP**	**DOSE OF EXTRACT (mg/kg)**			
		Control	0.5 mg/kg	2 mg/kg	10 mg/kg
Motility (%)	Treatment	79.17±2.39	38.17 ±5.24 **	25.00 ±7.19 **	19.17 ±7.46 **
	Reversal	74.17 ±5.97	64.17± 5.07	55.00 ±5.77	49.17 ±5.83
Count (10^6)	Treatment	74.17± 1.99	48.67 ±5.81	40.17 ±3.25 **	32.00± 4.69 **
	Reversal	70.83± 5.99	61.00± 6.62	50.83± 3.87	44.17± 5.25 **
Abnormal morphology ((%)	Treatment	8.33± 1.67	16.67± 4.01 *	25.00 ±1.83 **	28.33± 5.27 **
	Reversal	13.33± 2.11	20.83 ±2.01	27.50± 2.50 *	28.23 ±2.11 *
Viability (%)	Treatment	77.50 ±1.57	38.42 ±5.54 **	28.83 ±3.50 **	30.00± 1.97 **
	Reversal	69.00 ±6.82	51.83± 8.57	52.75± 3.27	47.33± 6.98

*= $p<0.05$, **= $p<0.01$. 30DT=30 days Treatment ; 30DPT= 30 days post Treatment.

Table 3: Effect of hydro-methanolic extract of *xylopia aethiopica* on fertility parameters (Mean ± S.E) of male rats after mating with female rats.

PARAMETERS	**STUDY GROUP**	**DOSE OF EXTRACT (mg/kg)**			
		Control	**0.5 mg/kg**	**2 mg/kg**	**10 mg/kg**
mated males/females	Treatment	6/6	6/6	6/6	6/6
	Reversal	6/6	6/6	6/6	6/6
Pregnant females	Treatment	6	4	3*	3*
	Reversal	6	4	4	3*
Viable fetuses (n)	Treatment	8.0± 0.52	6.0± 0.45	4.3± 1.5 **	3.50± 0.43 **
	Reversal	7.5± 0.34	5.83± 0.65	4.5± 0.43 **	5.0± 0.73 *
Fertility Rate (%)	Treatment	100	66.67	60.78*	43.14**
	Reversal	100	74.47	57.45*	59.58*
Fertility index	Treatment	100	66.67	66.67	50
	Reversal	100	66.67	50	50

*= $p<0.05$, **= $p<0.01$. 30DT=30 days Treatment ; 30DPT= 30 days post Treatment.