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Dietary Xylopia Aethiopica Reduces Fertility Capacity of Male Wistar Rats

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Abstract
The study assessed the effect of dietary intake of whole fruits of *Xylopia aethiopica* on the gonads of male Wistar rats. Ten (10) mature male Wistar rats, weighing 220-240g, were used in this study. Five (5) rats received the 5 % w/w dietary dosage of *Xylopia aethiopica,* and 5 others received the control diet, with clean drinking water, *ad libitum,* for 21 days. Thereafter, the rats were weighed, sacrificed and the gonads were isolated for use in determining testicular weight, semen parameters and histology of the testes. *Xylopia aethiopica* caused significant decreases in ratio of testicular weight to body weight, sperm motility, sperm count, and alterations in the histology of the testes. High intake of whole fruits of *Xylopia aethiopica* has adverse effects on semen quality parameters and gonadal histology, and may compromise male fertility.
Keywords: Xylopia aethiopica, fertility capacity, Wistar rats.

Introduction

The dried fruits of *Xylopia aethiopica*, commonly called Ethiopia pepper or Negro pepper, is used in preparing special soup called "pepper soup", due to its high pungent and aromatic quality, especially in south-eastern Nigeria. Traditionally, this spicy soup is served to women immediately after child birth, because the spice is believed to enhance the recuperation of the women after delivery, and the healing of after-birth wounds. It is also reported to be used to induce labour Muanya (2011), and as a postpartum tonic to stimulate appetite, promote healing, reduce pain, and as lactation aid (Murray, 1995). Muanya (2008) reported that the dried fruits of *Xylopia aethiopica* are used by herbalists for increasing menstrual blood flow, and for terminating unwanted pregnancy. The spice is reported to have several biologic actions, including promotion of prostaglandin synthesis (Ezekwesili *et al* 2010), hypotensive and diuretic effects (Somova *et al.,* 2001), antimicrobial, (Iwu, 1993), anti-malarial, (Eftkin, 1997), anti-parasitic, (Tairu *et al*.,1999), etc, activities. Presently, however, the spice is added to meat and fish sauces which are served as "pepper soup" in restaurants, bars, and at social gatherings. The spice is also added, as a stimulant, to several other herbal preparations by traditional herbalists, for treating such conditions as stomach ache, bronchitis, dysentery, neuralgia, biliousness, etc. Some of these reports, coupled with several experimental evidences (Gwotmut *et al.,* 2002; Onyebuagu *et al*., 2013), point to the antifertility effects of *Xylopia aethiopica*. Consequently, the possibility of the use of the antifertility property of *Xylopia aethiopica* in fertility regulation (contraception) has been suggested. In view of the concerns on male infertility in our society, the scope of the biologic actions of this commonly consumed food spice needs to be assessed. The aim of this study, therefore, was to determine the effect of the spice on the gonads and semen parameters of male Wistar rats.

Materials and Methods

Preparation of Dietary Dosages

The dietary dose of 5% w/w of the dried whole fruits of *Xylopia aethiopica* was prepared as test dosage for use in this experiment. This dosage was prepared and selected as antifertility dosage in Wistar rat in a previous experiment (Onyebuagu *et al*., 2013). The control group received the control diet made of normal rat chow and edible starch only.

Animal Studies

The Wistar rats were bought from the Animal House Unit of Ambrose Alli University, Epkoma, and were kept in laboratory cages to acclimatize for 14 days, while feeding on normal rat chow and clean drinking water, *ad libitum*. All the animals were handled in line with the NAS (National Academy of Science) guidelines for the care and use of laboratory animals, and permission for their use was obtained from the Research Ethics Committee of the university.

Experimental Protocol

Ten **(**10) randomly selected male Wistar rats, weighing 220 - 240g, were used in this experiment. The rats were divided into two groups – control group and group I, of five rats per group. The Group I rats were fed the 5% w/w dietary treatment dosage, while the control group received normal rat feed and edible starch only. All the

rats were fed their respective diets and clean drinking water, *ad libitum,* for 21 days. At the end of the treatment period, the body weight of the rats in the test group and the control group were measured. The rats were then sacrificed by stunning, and dissected to isolate the testes. The testes were weighed, and then used to determine the testicular weight, the semen quality parameters, and to assess the histology of the testes

Semen Analysis

The male rats were sacrificed by stunning, and then pinned astride on the dissecting board. A clean incision was then made on the scrotum with a clean surgical blade. The testes of the rats were exposed using dissecting instruments and isolated with their caudal epididymis. The testes were then removed, weighed together with the epididymis, and put into petri dish containing 50ml of physiological salt solution, which was maintained at 35^0C on a water bath.

Semen analysis was done manually by the method of Cheesebrough, (2000), to analyze the semen for motility, as follows: The caudal epididymis of the right testis was immediately exposed. Then a tiny incision (of~1.5mm) was made in the caudal epididymis, from where a drop of semen was expressed onto a slide. The semen sample on the slide was then covered with a cover slide, and viewed for motility with a microscope at x40 magnification. The number of spermatozoa that were motile and the nature of such motility were noted (WHO, 1999), out of a population of 20 sperm cells in a unit area. The sperm motility was then determined by calculating the motile spermatozoa (forward progression) per unit area, and expressed in percentage. The counts obtained of the motile sperm cells were multiplied by 5, to obtain the percentage sperm motility (Nwafia *et al*., 2006, Ogli *et al*., 2009).

The sperm count was assessed by first homogenizing the caudal epididymis in 5ml of physiological saline. The sperm count was carried out with the aid of a counting chamber in a haemocytometer (Adeko and Dada, 1998). First, the semen was diluted 1:20 with a diluting fluid using micropipette. The diluting fluid consists of 5g sodium bicarbonate ($NaHCO_3$), 1ml formalin and 100ml of distilled water. The semen was drawn to the "0.5" mark, and then diluted to the "11" mark with the diluting fluid - which serves to immobilize and preserve the sperm cells. The Neubauer counting chamber was prepared and charged with the diluted seminal fluid and allowed to stand in a moist chamber for 15-20 minutes. Complete mature spermatozoa were counted. The Neubauer counting chamber has a grib containing 1-5 large squares (of $1mm^2$ each). The central square is subdivided into 25 smaller squares. The depth of the chamber is 0.1mm. The volume of fluid between the cover slip and chamber in the large square is $0.1mm^3$. To calculate the number of spermatozoa per ml counted in the chamber, a multiplication factor was used. The multiplication factor of square 5 = 10,000; for large square 1-5, the factor = 2000; and for the smaller squares the multiplication factor = 50,000.

Morphological examination of the semen was done by performing different counts of the morphologically normal and abnormal sperm cell types on stained preparations. Smears were made by placing a drop of well mixed seminal fluid in a clean slide and smeared to a feather edge, and then quickly fixed, while still wet, in a cytological fixative, namely 95% alcohol or ether-alcohol mixture. The smear was then stained with dilute (1:20) carbol fuchsin. The semen smear sample was fixed in the fixative of 95% alcohol for 5-10 minutes, and then washed with sodium bicarbonate-formalin solution to remove any adhering mucus. The smear was covered with dilute carbol fuchsin for 3 minutes, and then washed with distilled water, and then counter-stained with polychrome methlylene blue for 2-3 minutes. Finally, the smear sample was washed with distilled water and air-dried. The stained smear was then examined under the oil immersion objective of the microscope. The complete spermatozoa were counted, while the abnormal forms were also noted.

Histological Studies

Tissue Processing:

The testes of the male rats were harvested and fixed in 10% formal saline for 48 hours, and processed for paraffin wax embedding with a Shandon Duplex automatic tissue processor by dehydrating through 70%, 90% and two changes of absolute alcohol for 120 minutes each. Clearing was achieved through two changes of xylene for two hours each; and impregnation with two changes of paraffin wax for 2 hours each. The impregnated tissues were then embedded in molten paraffin wax and allowed to cool to get solidified. Then sections were cut at 5 microns with a rotatory microtome. The cut sections were stained by hematoxylin and eosin (H&E) method.

Results

Figure 1 shows the histogram of the ratio of mean testicular weight to body weight in the control rats, and test rats treated with 5% w/w of *Xylopia aethiopica* diet dose.

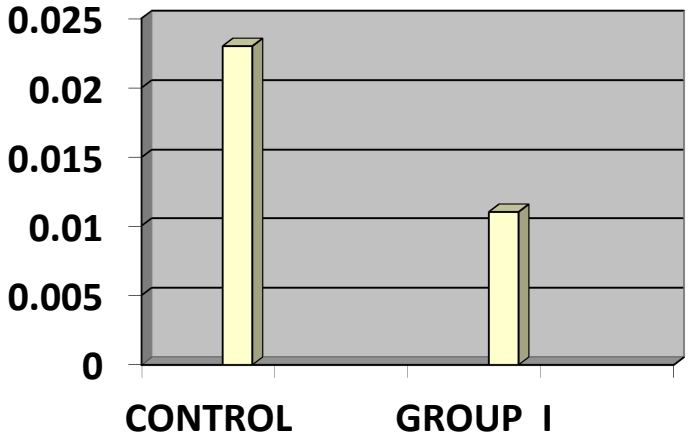

Figure 1: Histogram of the Ratio of Testicular Weight to Body Weight of the Control and Treated Male Wistar Rats.

The mean of the ratio of the testicular weights to body weights of the rats at the end of 21 days treatment for test Group and control Group rats were 0.011±0.00lg and 0.023±002, respectively. The result shows that there was significant ($p<0.01$) decrease of 52.2% in the testicular weight to body weight ratio in male test Group, compared to the control Group rats.

The mean values of the sperm quality parameters in male Wistar rats following 21 days of dietary treatment with the 5% w/w dietary dosage of whole fruits of *Xylopia aethiopica* are shown in Table 1. The mean values for progressive sperm motility of the males rats in the test Group and the control Group were 42.60±8.60% and 68.15±4.85%, respectively. There was a significant ($p<0.05$) decrease in sperm motility of the test Group rats by 25.55%, compared to the value for the control Group rats. The mean value of the sperm count of the male rats in test Group and control Group were 18.14±6.48 $x10^6$/ml, and 44.2±3.81 $x10^6$/ml, respectively, showing significant decrease ($p<0.05$) in the sperm count of 58.96% in the test Group I rats, compared with the control Group Wistar rats. The mean values of the percentage of sperm with normal morphology in the test Group I male rats was 47.40±11.64%, while that for the control Group rats was 58.64± 8.40%, showing 19.20% decrease in the percentage of sperm with normal morphology in the test Group I rats, compared with the control, but the difference was not significant.

Table 1: Mean values of Semen Quality Parameters in Male Wistar Rats after Dietary Intake of Whole Fruits of *Xylopia aethiopica* on.

GROUPS	Sperm Motility (%)	Sperm Count (10-6/ml)	Sperm Morphology (%)
Control	.68.15±4.55	44.20±3.81	58.64±8.40
Group I (5% w/w)	42.60±8.60*	18.14±6.48*	47.40±11.64
Percentage Change	-25.55%	-58.96	19.20%

Values are expressed as means ±SD ; n = 5 ; *Significant Difference at $p< 0.05$, compared with Control.

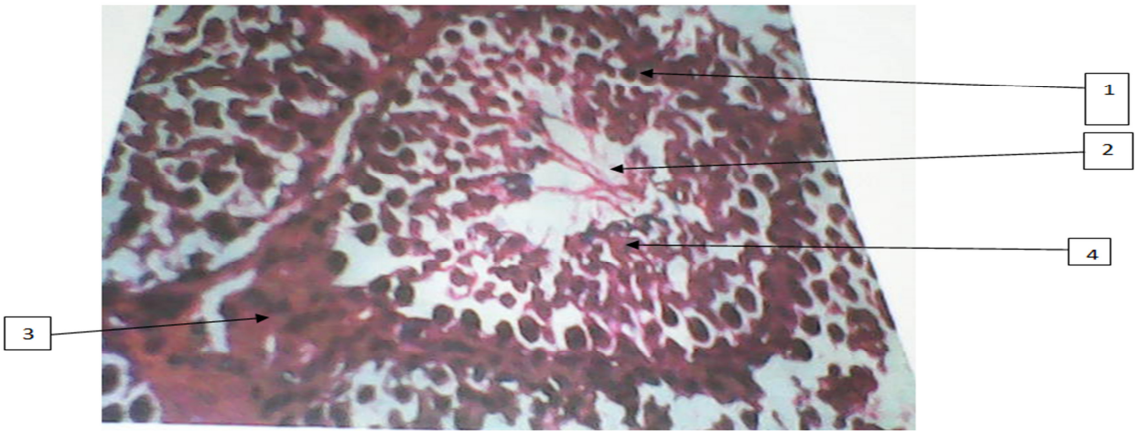

Figure 2 A : Photomicrograph of cross-section of testis of the **Control** male Wistar rat, showing seminiferous tubules with uniform cell-to-cell cohesion and density of the developing germ cells, normal size of lumen of seminiferous tubule. 1 = Germ cell; 2 = Lumen of seminiferous tubule; 3 = Leydig cell; 4 = Sertoli cell. (Magnification x 40).

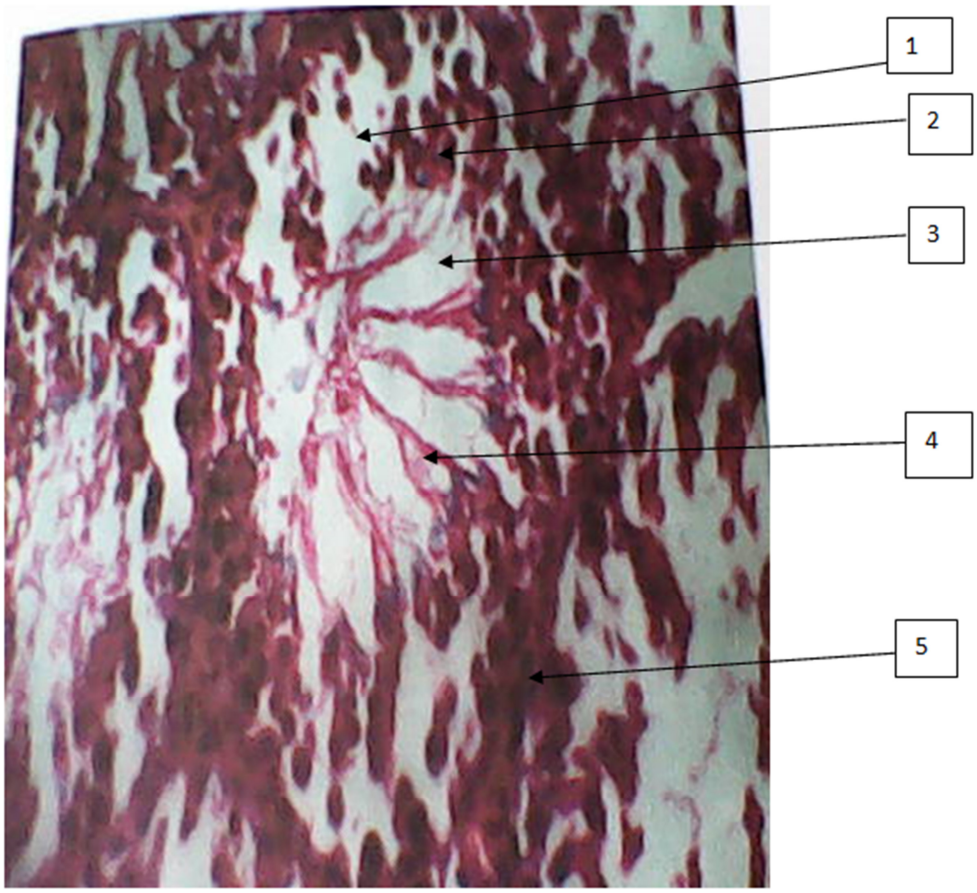

Figure 2 B : Photomicrograph of cross-section of testes of **treated** male Wistar rats, showing seminiferous tubule with reduction in the number and density of the developing sperm cells, vacuolation between the sertoli cells, and enlargement of the seminiferous tubules lumen; 1 = Germ cell; 2 = Vacuole; 3 = Lumen of seminiferous tubule; 4 = Sertoli cell ; 5 = Leydig cells. (Magnification x 40)

Figure 2 A and 2 B show the histology the testes of the control and test Wistar rats, following treatment with the control and test diets, respectively. Figure 2A (control) shows uniform cell-to-cell cohesion in the arrangement of the sertoli cells within the seminiferous tubules. The density of the developing germ cells, anchored in the sertoli cells, was normal. There was normal cohesion and triangular arrangement of the leydig cell clusters between adjacent seminiferous tubules. The lumen of the seminiferous tubules, bordered by the apices of the sertoli cells, was normal in form and size. The basement membrane epithelium was also normal in form. The test Group I rats (Figure 2 B), which received the dietary treatment dosage of 5% w/w of feed, exhibited reduction in the number and density of the developing sperm cells anchored in the sertoli cells. The sertoli cells, themselves, appear to be reduced in size, and lacked cohesion within the seminiferous tubules. There was also evidence of vacuolation between the sertoli cells, which suggests a reduction in the relative number of sertoli cells. There was also evidence of enlargement of the lumen of the seminiferous tubules bordering the apical surfaces of the sertoli cells. The basement membrane did not show any distinct alterations, compared with the control.

Discussion

The results of this study showed that the intake of 5% w/w of whole fruits *Xylopia aethiopica* caused significantly decreases in the ratio of testicular weight to body weight, percentage progressive sperm motility, and sperm count, compared with the controls. The decrease in the percentage of sperm with normal morphology was not significant, when compared with the mean control value. The decrease in testicular weight to body weight ratio would only have occurred through an increase in body weight of the male rats, or by decrease in testicular weight during the treatment period. The observed 6.9% decrease in body weight of the male rats following treatment with *Xylopia aethiopica* in this study should give rise to increase in the ratio of testicular weight to body weight. The observation of a decrease in the ratio of testicular weight to body weight, when compared to the ratio for the control male rats, suggests a selectively greater weight loss in the testes than in the body weight, following treatment with *Xylopia aethiopica*. Histological studies of the testes of the treated male

rats in this study revealed that intake of *Xylopia aethiopica* caused reductions in the population and density of the developing germ cells, as well as, in the size/number of the sertoli cells. This loss of testicular tissues may well account for the relatively greater testicular weight loss in the treated male rats. The result on testicular weight and histology, when taken together, would suggest a possible relationship between change in testicular weight and male fertility, as indeed some scientific evidence suggesting such are available (Bailey *et al*., 1998; Jain *et al*., 2008).

The mean values of sperm quality parameters obtained for the treated male rats in this study were observed to below those which are associated with fertility in rats (Ogli *et al*., 2009; WHO, 2000). This observed decrease in sperm quality parameters may well be a reflection of the observed alterations in the testicular architecture in the male rats treated with *Xylopia aethiopica*. Nwafor (2013) also reported similar decline in sperm parameters in albino rats following *in vivo* administration of alcoholic extract of the fruits of *Xylopia aethiopica.* Moreover, previous experiments have shown that the administration of *Xylopia aethiopica* caused decreases in plasma testosterone, estradiol and progesterone in Wistar rats (Onyebuagu *et a*l., 2013; Nwafor, 2013). Testosterone is believed to be required for the completion of meiotic division at the early stages of spermatid maturation, as well as, for the maintenance of spermatogenesis (Sembulingam and Sembulingam, 2010). Consequently, the low sperm parameters may possibly be related to the reported decrease in plasma testosterone caused by *Xylopia aethiopica* intake.

Histological studies of the testes of the treated male Wistar rats in this study exhibited alterations in testicular architecture - indicating that structural changes occurred in the testes following treatment with *Xylopia aethiopica.* The structural alterations may be related to the reported reduction in plasma testosterone concentration caused by *Xylopia aethiopica*, which in turn, may have affected the spermatogenetic process and sperm quality. Moreover, the observed reduction in the relative number of sertoli cells in the treated rats, may also have contributed to the observed decrease in testicular weight and, consequently, sperm count in the treated male rats, since reduction in sertoli cell number is believed to be a major cause of low sperm count (Sharpe and Franks, 2002).

Conclusion

The 5% w/w of the whole fruits of *Xylopia aethiopica* caused significant decreases in the ratio of testicular to body weight, sperm quality parameters and changes in the histology of the male gonads in the Wistar rats. The high intake of this commonly used food spice appears to have adverse effects on the male gonads, and may, possibly, compromise male fertility.

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