



ASN-PH-020919
ISSN: 2315-537X

International Journal of Herbs and Pharmacological Research

IJHPR, 2013, 2(4): 48 – 53.

www.arpjournals.com

RESEARCH PAPER

XYLOPIA AETHIOPICA LOWERS PLASMA LIPID PRECURSORS OF REPRODUCTIVE HORMONES IN WISTAR RATS

¹Onyebuagu P.C., ²Aloamaka C.P. ²Igweh J.C.

Department of ¹Human Physiology, College of Health Sciences, Niger Delta University, Wilberforce Island, Nigeria; ²Human Physiology, College of Medical Sciences, Delta State University, Abraka, Nigeria.

Correspondence: peteronyebuagu@gmail.com

Received: 3rd September, 2013

Accepted: 24th October, 2013

Published: 31st October, 2013

ABSTRACT

This study investigated the effects of dietary *Xylopiya aethiopic*a on reproductive hormones and plasma lipids in rats. 10 male and 10 female Wistar rats weighing 200-220g and 120-150g respectively, and subdivided into two respective groups of 5 rats each (M1 and F1 as control; M2 and F2 as test), were used for this study. For 21 days, the control rats received normal feed and clean water *ad libitum*, while test rats received 50g/kg of feed diet. Blood was collected from the rats and used to determine the reproductive hormone profile and plasma lipids concentration. Hormonal analyses were performed by the ELISA method, while lipid analyses were done using enzymatic saponification for total cholesterol, and enzymatic hydrolysis by lipases for the triglyceride. Results showed that there was a significant decrease ($p < 0.05$) in plasma testosterone concentration ($2.70 \pm 0.82 \text{ ng/ml}$) of the treated male rats. The plasma concentrations of estradiol ($13.80 \pm 2.84 \text{ pg/ml}$) and progesterone ($2.85 \pm 0.64 \text{ ng/ml}$) in the treated females were also significantly reduced. Lipid analyses showed significant reduction in total plasma cholesterol in the treated male rats ($1.20 \pm 0.63 \text{ mmol/l}$) and female rats ($1.75 \pm 0.55 \text{ mmol/l}$). Our results suggest that dietary *Xylopiya aethiopic*a can induce a reduction in plasma levels of steroid reproductive hormones, possibly through reduction in plasma cholesterol.

Keywords: *Xylopiya aethiopic*a, reproductive hormones, plasma lipids.

INTRODUCTION

The dried fruits of *Xylopiya aethiopic*a is a common spice known for its strong aromatic quality, and used in the preparation of two special local soups in south-eastern Nigeria, called “isi ewu” and “obe nta”. *Xylopiya aethiopic*a is also used as a postpartum tonic in alleviating after-birth wounds and as lactation aid (Murray, 1995). Others include usage in the termination of unwanted pregnancy (due to its abortifacient properties), when administered in combination with the root of *Blighia sapida* (Sapindaceae) (Muanya, 2008), and to increase menstrual flow, when administered in combination with the leaves of *Newbouldia laevis* (Bignoniaceae) or chieftaincy leaf, as well as the induction of labour to achieve delivery, when seven dried fruits of *Xylopiya aethiopic*a and 21 leaves of *Rouwulfiya vomitaria* are orally administered in combination (Muanya, 2011).

However, several researchers have reported that the intake of *Xylopiya aethiopic*a causes diminished reproductive performance in experimental animals, with decreases in litter size in females (Nwafor, 2013), and reduction in sperm count in males (Onyebuagu, 2012; Nwafor, 2013). Histological studies of the gonads in the rats have also demonstrated changes in the testicular and ovarian architecture in male and female rats, respectively, following the intake of *Xylopiya aethiopic*a. This study was therefore, embarked on to assess the effects of dietary *Xylopiya aethiopic*a on the reproductive hormone profile of the rats, since reproduction is largely governed by the hormonal milieu, among other factors.

MATERIALS AND METHODS

Experimental Animals: The Wistar rats were bought from the Animal House Unit of Ambrose Alli University, Ekpoma, and were housed in the Human Physiology laboratory of Ambrose Alli University, Ekpoma, in standard rat cages. They were maintained for 2 weeks prior to the study, to allow for acclimatization and uniform husbandry conditions, feeding on normal rat chow and clean drinking water, *ad libitum*.

Preparation of Plant Material and Treatment Diets: The whole dried fruits of *Xylopia aethiopica* were purchased from the local market in Ekpoma, Nigeria, washed in clean water and air dried for 10 hours prior to drying in the oven at 40°C for 12 hours.

The dried fruits were then ground into powdered form. This was achieved by first pounding the whole dried fruits into small pieces using wooden mortar and pestle, and then grinding the pieces into powdered form using mechanical grinding machine. The desired amount of the powdered *Xylopia aethiopica* was measured using a sensitive digital weighing balance (Zohaus, Model CS 200, N.J. USA). The eventual prepared diet dose also contained cooked starch as binder. The idea to formulate the dietary treatment into crumbs came from the observation that Wistar rats prefer to hold their food with both hands to eat, while crouching on their hind limbs.

Two categories of treatment diets was made available for use in this study: the control diet containing normal rat chow and edible cassava starch only; and treatment diet that contained the test material at 50g/kg of feed, with cassava starch as binder.

Experimental Protocol: Ten (10) male rats weighing 220-240g and 10 female rats weighing 150-180g were used in this study. The male and female rats were each divided in two groups – I and II of five rats per group. The rats were allowed to acclimatize for 14 days, while feeding on normal rat feed mixed with cassava starch, and clean drinking water, *ad libitum*. Thereafter, M2 and F2 rats were fed with the 50g/kg treatment diet, while M1 and F1 (controls) received rat feed and edible cassava starch only. All the rats were fed with their respective diets and clean drinking water, *ad libitum*, for 21 days. At the end of the treatment period of 21 days, the rats were sacrificed by stunning and their blood samples were collected for use in the determination of the reproductive hormone profile and plasma lipid concentrations.

Collection and Handling of Blood Samples: The animals were anaesthetized in a chloroform chamber at the end of treatment period of 21 days, and the blood was obtained through cardiac puncture. Blood sample from each animal was put in well-labeled heparinized sample tubes. The blood samples were centrifuged at 1500rpm for 10 minutes. The plasma was then collected and stored at -2°C until ready for use in hormonal assay and lipid determinations.

Biochemical Analyses: The hormonal analyses were performed using the ELISA (a solid base enzyme-linked immunosorbent assay) method, which is based on the sandwich principle (Engvall, 1980). The plasma concentrations of total cholesterol was determine by the method of Roeschlau et al., 1974, using reagent kits from Randox, UK, which involves enzymatic hydrolysis and oxidation (enzymatic saponification). The triglyceride concentration was determined using reagent kits from Randox, UK, for the GPO-PAP colorimetric method for the quantitative *in vitro* determination of triglycerides in plasma, involving enzymatic hydrolysis by lipases, as described by Tietz, 1990. The absorbance was read using spectrophotometer.

Data Analysis: Data were presented as means \pm SD, and as percentages. Data of test groups and control groups were analyzed statistically using Student's t-test. The level of significance of the difference between test groups and control data were determined. Values of $p < 0.05$ were considered to imply statistical significance.

RESULTS

The result of the experiment on effect of *Xylopia aethiopica* on plasma levels of reproduction hormones in male Wistar rats are shown in Table 1. In the treated male Wistar rats (Table 1), the plasma FSH level was 2.60 ± 1.15 mIU/ml (Group II), and 2.50 ± 1.10 mIU/ml for the male control Group I, - representing a percentage change of 3.80%. The plasma LH concentrations for the two groups were similar: 2.50 ± 0.71 mIU/ml for test Group II, and 2.50 ± 0.58 mIU/ml for the control. The plasma levels of LH and FSH in the test group were, not significantly different from the respective value for the male control group. For prolactin, the plasma level in the male Wistar rats was 3.35 ± 0.57 ng/ml for the test Group II, and 4.10 ± 0.81 ng/ml for the male control Group I. There was 18.32%

decrease in the plasma level of prolactin in the male test group, compared with the control, though the difference was not significant ($p < 0.05$). The mean plasma levels of testosterone in the male rats for the groups were $2.70 \pm 0.82 \text{ ng/ml}$ for test Group II, and $4.60 \pm 2.06 \text{ ng/ml}$ for the control group, showing percentage decrease of 41.30%. There was a significant decrease ($P < 0.05$) in the plasma level of testosterone in the test Group II, compared with the control Group I.

The mean values of reproductive hormones in female Wistar rats following dietary treatment with whole fruits of *Xylopiya aethiopic* are shown in Table 2. The reproduction hormone profile of the female Wistar rats shows that the values for the plasma level of follicle stimulating hormone (FSH) were $2.50 \pm 0.34 \text{ mIU/ml}$ for test Group II, and $2.60 \pm 0.50 \text{ mIU/ml}$ for control Group I, showing percentage decrease of 3.9 %. The mean plasma level of luteinizing hormone (LH) for the female test Group II was $2.50 \pm 0.55 \text{ mIU/ml}$, and $2.50 \pm 0.50 \text{ mIU/ml}$ for the female control Group I. For prolactin, the mean plasma level was $2.95 \pm 0.38 \text{ ng/ml}$ for the control Group I, and $3.45 \pm 0.65 \text{ ng/ml}$ for the test Group II, with a percentage increase of 14.50%. The above results show that the treatment diet had no significant effect on the plasma levels of FSH, LH and prolactin in the female rats. The plasma level of estradiol in the test group was $13.80 \pm 2.84 \text{ pg/ml}$ for test Group II rats, while the value for the control Group I rats was $23.20 \pm 12.44 \text{ pg/ml}$, representing a percentage decrease of -40.50%. For progesterone, the mean value in the test Group II was $2.85 \pm 0.64 \text{ ng/ml}$, which was significantly less by 81.30% than the plasma progesterone level for the female control Group ($15.25 \pm 7.86 \text{ ng/ml}$). Overall, the hormone profile in the female Wistar rats showed significant ($p < 0.05$) suppressions in the plasma levels of estradiol and progesterone in the test group, compared to the respective control plasma levels of these two hormones. The effect on the other hormones assessed was not significant.

The mean values of total cholesterol and triglyceride concentrations in plasma of male Wistar rats, following treatment with 50g/kg dietary dose of *Xylopiya aethiopic* for 21 days, are shown in Table 3. In the male Wistar rats, the mean plasma total cholesterol concentration of the test Group II rats was $1.20 \pm 0.63 \text{ mmol/l}$, while the cholesterol concentration for the male control Group rats was $3.10 \pm 0.58 \text{ mmol/l}$. There was a significant decrease ($p < 0.05$) in the plasma cholesterol concentration in male test Group II rats by 61.30%, compared with the value for the control. The plasma triglyceride concentration in the test Group II rats was $1.77 \pm 0.23 \text{ mmol/l}$, while the plasma triglyceride concentration in the control Group II rats was $2.11 \pm 0.47 \text{ mmol/l}$. The result show that there was a decrease in the plasma triglyceride concentration in the male test Group II rats of 16.11%, compared with the control, but the decrease was not significant ($p < 0.05$).

Table 4 shows the mean values of plasma total cholesterol and triglyceride concentrations in female Wistar rats, following treatment with 50g/kg dietary dosage of whole fruits of *Xylopiya aethiopic* for 21 days. In the female Wistar rats, the mean plasma total cholesterol concentration of the test Group II rats was $1.75 \pm 0.55 \text{ mmol/l}$, while the value for the female control Group I rats was $3.15 \pm 0.50 \text{ mmol/l}$. There was a significant ($p < 0.05$) decrease in the total cholesterol concentration in test Group II rats by 44.40%, compared with the female control. The plasma triglyceride concentration in the test group II was $1.28 \pm 0.04 \text{ mmol/l}$, while the plasma triglyceride concentration for the control Group I was $1.49 \pm 0.38 \text{ mmol/l}$, showing a percentage decrease of 14.10%. There was also no significant difference ($P < 0.05$) in the plasma levels of triglyceride between the female test group and the female control rats.

Table1 1: Mean Values of Reproduction Hormones in Male Wistar Rats following Dietary Treatment with Whole Fruits of *Xylopiya aethiopic*.

GROUPS	FSH (mIU/MI)	LH (mIU/MI)	PRL (ng/MI)	Testosterone (ng/MI)
Group I (M1; Control)	2.50 ± 1.10	2.50 ± 0.58	4.10 ± 0.81	4.60 ± 2.06
Group II (M2; 50g/kg)	2.60 ± 1.15	2.50 ± 0.71	3.35 ± 0.57	$2.70 \pm 0.82^*$
Percentage Change	+3.80%	-	-18.32%	-41.3%

Values are expressed as Mean \pm SD; n=5; *Significantly different at $p < 0.05$, compared with control.; FSH: Follicle stimulating hormone; LH: luteinizing hormone; PRL: prolactin

Table 2: Mean Values of Reproductive Hormones in Female Wistar Rats following Dietary Treatment with Whole Fruits of *Xylopi aethiopia*

GROUPS	FSH (mIU/mL)	LH (mIU/mL)	PRL (ng/mL)	Estradiol (pg/mL)	Progesterone (ng/mL)
Group I (F1,Control)	2.60 ± 0.50	2.50± 0.50	2.95± 0.38	23.20±12.44	15.25± 7.86
Group II (F2; 50g/kg)	2.50± 0.34	2.50± 0.55	3.45± 0.65	13.8±2.84*	2.85± 0.06*
Percentage Change	-3.90%	-	+14,5%	-40.5%	-81.30%

Values are expressed as Mean ± SD; n=5; *significantly different at p<0.05, compared with control. FSH: Follicle stimulating hormone; LH: luteinizing hormone; PRL: prolactin

Table 3: Mean Values of Plasma Lipids Concentrations in Male Wistar Rats following Dietary Treatment with Whole Fruits of *Xylopi aethiopia*

Groups	Total cholesterol (mmol/l)	Triglyceride (mmol/l)
Group I (control)	3.10±0.58	2.11±0.47
Group II (50g/kg)	1.20±0.63*	1.77±0.23
Percentage Change	-61.30 %	- 16.11 %

Values are presented as means ±SD; n=5 ; * Significantly different at P< 0.05, compared with control.

Table 4: Mean Values of Plasma Lipids Concentrations in Female Wistar Rats following Dietary treatment with Whole Fruits of *Xylopi aethiopia*.

Groups	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)
Group I (control)	3.15±0.50	1.49±0.38
Group II (50g/kg)	1.75±0.55 *	1.28±0.04
Percentage Change	-44.40%	-14.10%

Values are presented as means ± SD; n = 5; *significantly different at p< 0.05, compared with control.

DISCUSSION

This study has shown that the dietary treatment of the male and female Wistar rats with *Xylopi aethiopia*, significantly reduced the plasma levels of testosterone in the males, and estrogen and progesterone in the females. In a similar study, Nwafor (2013) reported that *Xylopi aethiopia*, *in vivo* caused significant declines in all sex hormones (LH, FSH, PRL, estrogen and progesterone) in female albino rats, as well as significant declines in sperm parameters in male albino rats.

Furthermore, the observation that reproductive hormones of the Wistar rats (especially steroid hormones) were affected by the dietary treatment with *Xylopi aethiopia*, might be due to the route of administration and/or duration of treatment. It also suggests that the mechanism by which *Xylopi aethiopia* affected the plasma level of reproductive hormones in this study may be related to the mechanism by which the steroid hormones are produced in the animals. More so, the low plasma testosterone level in the treated male Wistar rats suggests that *Xylopi aethiopia* may influence changes at the sites of steroidogenesis, specifically the gonads. The same explanation may also apply for the low plasma estradiol and progesterone levels in the treated female Wistar rats. These speculations are supported by the fact that the plasma levels of LH and FSH, which are derived from the pituitary gland, were not

significantly altered in the treated male and female rats, while the reproductive hormones derived from the gonads were significantly decreased.

In a related study, Ugwoke *et al.* (2005) also reported a steroidogenic disruption in male albino rats that exhibited low serum testosterone levels following exposure to environmental endocrine disruptors via inhalation of gasoline vapour. In this study, the disruption of steroidogenesis by *Xylopiya aethiopica* in the treated rats is supported by the observed significant reduction in the plasma concentration of total cholesterol in the animals. The biosynthesis of steroid hormones in the gonads depends on the plasma concentration of total cholesterol (Mill, 1998). Mayes and Law (2000), had earlier reported that the immediate precursor for steroid biosynthesis is cholesterol, with the limiting step being the delivery of cholesterol by the transport protein called "STAR" (Steroidogenic Acute Regulatory Protein). Once delivered, cholesterol is acted upon by side chain cleavage enzyme P450 (Mill, 1998). Both the male and female gonads use the same pathway for steroid hormone biosynthesis in the gonadal tissues, starting with cholesterol from plasma and *in situ* cholesterol from acetyl co-enzyme A.

In line with the above report, was the observation in this study that the intake of *Xylopiya aethiopica* decreased the plasma concentrations of total cholesterol and triglyceride; both in the male and female rats. The mechanism by which *Xylopiya aethiopica* caused the decrease in plasma total cholesterol concentration may include inhibition of intestinal cholesterol absorption by its phytosterol content (Ostlund et al, 2003). Other reports suggests however, that *Xylopiya aethiopica*-induced decreases in plasma cholesterol may occur through binding of the test material with bile acids (needed for intestinal absorption of cholesterol), and thus, reduce the absorption of dietary cholesterol in the gut (Ezeilo, 2009).

Conclusion

Our results suggests therefore, that dietary intake of *Xylopiya aethiopica* induced decreases in steroid reproductive hormones in male and female rats via mechanisms that may be related to reduction in plasma total cholesterol concentration in the treated animals. Further research however, is required so as to fully understand the underlying processes involved in the observed reduction in plasma steroid reproductive hormones and total cholesterol by *Xylopiya aethiopica*.

ACKNOWLEDGEMENT

We are very grateful to Mr. Patrick Ogarah, Ambrose Alli University, Ekpoma, Edo State, and Mr. Oboma K. P. of the Department of Pathology, Niger Delta University, for their technical assistance. We also give thanks to Mr. Frank Opuene of the Department of Mathematics and Statistics, Niger Delta University, for his assistance with the statistical work.

REFERENCES

- Engvall, E. (1980). Methods in Enzymology. Vanvunakis, H. and Langone, J.J. (Eds). Vol. 70. Academic Press New York. P. 418.
- Ezeilo, G.C. (2009). Textbook of Physiology. Oxford University Press, New Delhi, India.
- Mayes, P.A. and Law, M. (2000). Plant sterol and stanol margarines and health. *Br. Med. J.*; 320:72-83.
- Mill, W.L. (1998). Molecular biology of steroid hormone biosynthesis. *Endocr. Rev.*; 9:295.
- Muanya, C. (2008). How plants can induce labour, heal after-birth wounds. The Guardian Newspaper. 17th Jan. 2008.
- Muanya, C. (2011). Natural Health: Walking, Sex and Spicy Food to Induce Labour. The Guardian Newspaper, 23rd June, 2011.
- Murray, M. (1995). The 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.

Onyebuagu, P.C. (2012), Assessment of the Effects of *Xylopiya aethiopica* on Reproductive Performance in Wistar Rats. PhD Thesis. Delta State University, Abraka.

Ostlund R.E Jr. (2007) Phytosterols, Cholesterol Absorption and Healthy Diets. *Lipids* 42(1) 14-45.

Ostlund R.E, Racette B. and Stenson W.F (2003) Inhibition of Cholesterol Absorption by Phytosterol-replete wheat germ compared with Phytosterol-depleted wheat germ. *Am J Clin Nutr* 77(6): 1385-1389.

Roeschlau P., Bemt, E. and Gruber, J.W. (1974) *Clin Chem Clin Biochem.* 12:403

Tietz N.W, Ed (1995) Clinical Guide to Laboratory Tests 3rd Edn. WB Saunders Co. Philadelphia, USA

Ugwoke C.C, Nwobodo E.D, Uneke P, Odike M, Chukwuma S.T and Amilo G (2005). The Reproductive Dysfunction Effects of Gasoline Inhalation in Albino Rats, *Nig. J. Physiol. Sc*, 20(1-2): 54-57.

AUTHORS' CONTRIBUTIONS

All authors (Onyebuagu P.C., Aloamaka C.P., Igweh J.C.) were actively involved in the conception, development and actual animal experiment of this study and approved the final draft of this article.