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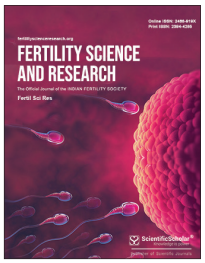

Original Article

Hydro-Ethanol Extracts of Xylopia *aethiopica* Enhanced Serum Testosterone Levels in Male Adult Wistar Rats

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ABSTRACT

Objectives: To investigate the effects of hydro-ethanolic extracts of leaves and fruit with or without seeds on male sex hormones.

Material and Methods: Hydro-ethanolic extracts of the leaves, fruits with seeds, and fruits without seeds were processed using the Soxhlet extraction technique. Wistar rats were administered 125, 250, and 500 mg/kg of the extracts by oral gavage for 30, 60, and 90 days while the control group received feeds and water *ad libitum*. After the treatment periods, rats were sacrificed by cervical dislocation, and blood was collected by cardiac puncture. Serum follicle-stimulating hormones (FSHs), luteinising hormone (LH), progesterone, prolactin, and testosterone levels were determined using an enzyme-linked immunosorbent assay technique. Data were compared using analysis of variance (ANOVA), and a p-value of 0.05 was considered significant.

Results: Serum testosterone, FSH, and LH levels in rats treated with 125, 250, and 500 mg/kg were significantly increased ($p < 0.05$) in a dose-dependent manner compared with controls.

Conclusion: The consumption of this commonly used food spice appears to have beneficial effects on the male sex hormones.

Keywords: Rats, Male, Wistar, *Xylopia*, Testosterone, Ethanol

INTRODUCTION

The world over from creation is blessed with medicinal plants whose extracts are used in the treatment, management, and prevention of diseases. They are also used traditionally to treat human body system disorders in most African countries. They serve as sources of food, clothing, fuel, and shelter.[1,2] A large number of plants exhibit beneficial therapeutic and pharmacological properties due to a variety of metabolically synthesised organic compounds called phytochemicals contained in them, which have specific physiological effects in humans. Examples of these bioactive substances are tannins, alkaloids, carbohydrates, terpenoids, steroids, flavonoids, and phenols.[3] Among these plants with great therapeutic potential is *Xylopia aethiopica*.[4] In English, it is called Negro pepper, Kani pepper, moor pepper, Ethiopian pepper, and Senegal pepper. In Arabic, it is called hab-zelim, in French, it is Noir de Guinee and Poivre de Senegal while in German, it is called Mohrenpfeffer, Kannniiipfeffer,[5] and in Benin, it is called Unien.

Ethnobotanical survey of *X. aethiopica* reveals that the different parts of the plant are used in herbal medicine for the treatment of several illnesses and a wide variety of applications, such

as soup ingredients. This prized medicinal plant is found in the rainforest of Africa, spanning from Senegal to Angola. *Xylopia aethiopica* is cultivated mainly for fruits and has an aromatic, pungent taste. The dried fruits with seeds, when crushed, are used as a pepper substitute.[6] They are used in traditional medicine as a carminative cough remedy, to treat stomach aches and dysentery, as a postpartum tonic in alleviating after-birth wounds, and as a lactation aid.[7] The effects of *X. aethiopica* consumption in experimental studies on sexual behaviour and reproductive properties have not been consistent.[8-11] A dose-dependent significant decrease in the plasma levels of LH, FSH, and testosterone was reported upon administration of an aqueous extract of fruits of *X. aethiopica* for 7 days and 14 days in guinea pigs.[8] A dose-dependent increase in ejaculation latency and post-ejaculatory interval with a significant decrease in ejaculation frequency and serum testosterone levels were reported in Wistar rats when treated with hydro-ethanolic extract of the fruits for 60 days.[9] Onyebuagu *et al.*[10] reported that consumption of high concentrations of whole fruits of *X. aethiopica* has adverse effects on semen quality and reproductive potency. Significant dose and time-dependent decreases in semen quality, luteinising hormone (LH), follicle-stimulating hormone (FSH), and testosterone levels were reported in Wistar rats administered with *X. aethiopica*. They concluded that the use of *X. aethiopica* may have adverse effects on male reproduction function.[11] Conversely, a dose-dependent significant increase in sperm concentration and live sperm cells was reported in animal models administered with *X. aethiopica*.[12,13] Following this conflicting information, it is imperative to use extracts of different components of the plant for a long duration of time to validate the various claims. The objective of this study, therefore, was to investigate the effects of hydro-ethanolic extracts of leaves and fruit with or without seeds on male sex hormones in Wistar rats.

MATERIAL AND METHODS

This experimental study was conducted following the ethical approval obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria, with reference number EC/FP/018/19 issued on 04 April 2018.

Plant Material (Collection, Authentication and Processing)

The leaves and fruits of mature *X. aethiopica* were harvested during the daytime from cultivated trees growing in Uhe village in Ovia North-East Local Government area of Edo state, Nigeria. They were authenticated by a plant taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, and assigned a voucher number of UBH_X 348.

Garbling was done on the harvested leaves and fruits of *X. aethiopica* to remove extraneous matters and adulterants. Each plant part was then packed into different sacks and transported to the laboratory. In the laboratory, they were spread out on very clean benches for 2 weeks at room temperature (25–27°C) to allow for proper air drying. The leaves were further dried in a thermostatically regulated oven at 60°C for 30 min while the fruits were further dried at 60°C for 1 h before milling with an electric miller (Kenwood, UK). The powdered samples were packed into air-tight amber-coloured glass bottles and stored until required for the work.

Extraction Process

The soxhlet extraction method was used to obtain hydro-ethanol extracts of the leaves and fruits (with and without seeds) of *X. aethiopica*. A measured quantity of 2.5 kg of the powdered leaves and fruits (with and without seeds) were each extracted separately with 7.5 l of hydro-ethanol (20% ethanol in distilled water). Each of the extracts obtained was concentrated separately in a vacuum using a rotary evaporator and reduced to dryness in a thermostatically regulated electric oven maintained at a temperature of 20°C. They were packed separately into screw-capped bottles, labelled and preserved in the refrigerator at 4°C for biological evaluations.

Acute Toxicity Tests and Efficacy Tests of Extracts

The hydro-ethanol extracts of the leaves and fruits (with and without seeds) of *X. aethiopica* were used in the acute toxicity and efficacy tests.

Experimental Animals

Healthy male Wistar rats (160–180 g) were used for the tests. They were procured from the College of Medicine, Ambrose Ali University, Ekpoma, Edo State, Nigeria, and kept in well-ventilated plastic cages. They were transported to the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. The Wistar rats were kept at room temperature of 25–27°C and served a standard diet (Premier Feed Mill Ltd, Edo State) and water *ad libitum* for 2 weeks so that they could acclimatise.

Acute Toxicity Test

The acute toxicity test was done using Miller and Tainter (1944) method. Thirty male Wistar rats were used. Before the experiment, the animals were given only water *ad libitum*. The experimental animals were divided into five groups (A–

E) of six rats per group. Group A, which served as the control, received 10 ml/kg of 2% Tween-80 solution, while groups B, C, D, and E were served 0.5, 1, 2, and 5 g/kg of the hydro-ethanol extract of the leaves and fruits (with and without seeds) in 2% Tween-80, respectively, orally by oral gastric administration. Adverse side effects like weakness, aggressiveness, refusal to eat, diarrhoea, eye discharge, ear discharge, noisy breathing, morbidity, and mortality due to the administration of the plant extracts to the Wistar rats were observed for 24 h for immediate effects after dosing and subsequently for 2 weeks (14 days), for delayed effects post-drug administration.

Sub-Acute Toxicity Test

The sub-acute toxicity test was done in compliance with OECD (2001) guidelines. Twenty-four (24) Wistar rats were used. They were divided into four groups (I–IV) of six rats per group. Group I served as the control group and was served 5 ml/kg 2% Tween-80 solution, while groups II-IV were served 125, 250, and 500 mg/kg of the hydro-ethanol extract of the leaves and fruits (with and without seeds) of *X. aethiopica* in 2% Tween-80, respectively, as a single dose daily for 1 month (30 days).

Sub-Chronic Toxicity Test

For the sub-chronic toxicity test, 24 Wistar rats were used. They were divided into four groups (I–IV) of six rats per group. Group I served as a control group and was served 5 ml/kg 2% Tween-80 solution, while groups II-IV were served 125, 250, and 500 mg/kg of the hydro-ethanol extract of the leaves and fruits (with and without seeds) of *X. aethiopica* in 2% Tween-80, respectively, as a single dose daily for 2 months (60 days).

Chronic Toxicity Test

The chronic toxicity test was done using 24 Wistar rats. They were also divided into four groups (I–IV) of six rats per group. Group I was the control group and was served 5 ml/kg 2% Tween-80 solution, while groups II-IV were served 125, 250 and 500 mg/kg of the hydro-ethanol extract of the leaves and fruits (with and without seeds) of *X. aethiopica* in 2% Tween-80, respectively, as a single dose daily for 3 months (90 days).

At the end of each of the study periods above, the rats were sacrificed by cervical dislocation and dissected. Blood samples were collected from each of the animals via carotid cannulation. The samples so collected were put in plain containers for hormonal assays.

Hormonal Assays

Quantitative Determination of Serum Testosterone, LH, FSH, Progesterone, and Prolactin in Wistar Rat

Principle

The sex-measured hormones were assayed by the Enzyme-Linked Immunosorbent Assay (ELISA) technique using reagents supplied by Inteco Diagnostics, UK Ltd, England. It detects the presence of specific proteins (testosterone, LH, FSH, progesterone, and prolactin) in the serum. It does this by using antigen-antibody reaction where the mouse monoclonal antibody is immobilised on a micro-well solid surface. The specific hormone in the serum is bound to the mouse monoclonal antibody in the micro-well. After incubation, the unbound antigen or antibody is removed by washing, and then a mouse-monoclonal antibody linked to an enzyme (horseradish peroxidase conjugate solution) is added. After incubation, the enzyme substrate is added to the complex. After another round of washing, the resultant product is measured spectrophotometrically using a plate reader. The concentrations of the hormones are extrapolated from the calibration curves previously prepared.

Statistical Analysis

The data were statistically analysed using SPSS Software (IBM) version 23.0. The results obtained were expressed as mean ± standard deviation. The differences between the groups were determined by one-way analysis of variance (ANOVA), and a p-value less than 0.05 was considered statistically significant.

RESULTS

The effect of hydro-ethanol extracts of the leaves, fruits with seeds, and fruits without seeds of *X. aethiopica* on sex hormones of male Wistar rats after 1, 2, and 3 months of oral administration are shown in Tables 1–6.

Effects of *X .aethiopica* Leaves Extract on Serum Testosterone and FSH Levels

Table 1 indicates that serum testosterone significantly increased in a dose-dependent manner following 1 month ($p < 0.03$), 2 months ($p < 0.03$), and 3 months ($p < 0.01$). Serum FSH levels also significantly increased ($p < 0.02$) following administration of 125, 250, and 500 mg/kg for 3 months [Table 1].

Table 2 shows that there was a significant increase in serum LH following treatment with leaves extract of *X. aethiopica* for 1 month ($p < 0.02$), 2 months ($p < 0.01$), and 3 months ($p < 0.03$), respectively. There were, however, no significant changes in the serum levels of progesterone and prolactin following treatment with leaves extract of X. *aethiopica* throughout the study.

Table 1: Comparison of the fertility hormone levels of male Wistar rats on *X. aethiopica* leaves for 3 months

Parameter/ Units	Duration in months	Treatment doses				*p* -value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		LEAVES				
Testosterone (ng/ml)	1	0.98 ± 0.62 (-0.74–2.70)	3.01 ± 0.45* (1.76–4.26)	3.12 ± 0.38* (2.07–4.16)	3.04 ± 0.63* (1.30–4.79)	0.03
	2	1.09 ± 0.32 (0.21–1.97)	4.08 ± 0.90* (1.57–6.59)	4.17 ± 1.08* (1.19–7.15)	4.27 ± 0.46* (2.99–5.54)	0.02
	3	1.12 ± 0.24 (0.44–1.80)	4.06 ± 0.78** (1.88–6.23)	4.34 ± 0.56** (2.77–5.91)	4.21 ± 0.26** (3.49–4.92)	0.01
FSH (mIU/ml)	1	0.70.04 (0.61–0.83)	0.95 ± 0.08 (0.72–1.17)	1.07±0.10* (0.81–1.34)	1.05 ± 0.06* (0.87–1.23)	0.02
	2	0.76 ± 0.05 (0.61–0.90)	0.98 ± 0.06* (0.81–1.14)	0.97 ± 0.07* (0.79–1.16)	1.01 ± 0.04* (0.90–1.12)	0.02
	3	0.79 ± 0.05 (0.65–0.93)	0.98 ± 0.06* (0.82–1.14)	1.00 ± 0.04* (0.88–1.11)	1.02 ± 0.05* (0.89–1.15)	0.02

FSH: Follicle-stimulating hormone, *p<0.02, **p<0.01.

Table 2: Comparison of the fertility hormone levels of male Wistar rats on *X. aethiopica* leaves for 3 months

Parameter/Units	Duration in months	Treatment doses				*p*-value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		Leaves				
LH (mIU/ml)	1	0.84 ± 0.14 (0.46–1.22)	1.42 ± 0.15 (1.01–1.84)	1.66 ± 0.29* (0.86–2.47)	1.86 ± 0.24* (1.19–2.53)	0.02
	2	1.10 ± 0.12* (0.76–1.44)	1.80 ± 0.22* (1.20–2.40)	1.84 ± 0.20* (1.28–2.24)	1.99 ± 0.16** (1.54–2.43)	0.01
	3	1.18 ± 0.20 (0.64–1.72)	1.86 ± 0.14* (1.46–2.26)	1.98 ± 0.14** (1.58–2.38)	2.05 ± 0.11** (1.74–2.36)	0.03
Progesterone (ng/ml)	1	1.74 ± 0.20 (1.18–2.30)	1.90 ± 0.26 (1.18–2.62)	2.08 ± 0.33 (1.17–2.99)	1.80 ± 0.55 (0.28–3.32)	0.91
	2	0.94 ± 0.06 (0.77–1.10)	1.02 ± 0.13 (0.67–1.38)	1.06 ± 0.16 (0.62–1.50)	1.11 ± 0.07 (0.93–1.29)	0.72
	3	0.88 ± 0.05 (0.74–1.01)	1.06 ± 0.84 (0.82–1.29)	1.11 ± 0.10 (0.83–1.40)	1.10 ± 0.02 (1.03–1.17)	0.11
Prolactin (ng/ml)	1	1.98 ± 0.29 (1.16–2.79)	2.08 ± 0.22 (1.46–2.70)	2.00 ± 0.29 (1.21–2.80)	2.31 ± 0.37 (1.29–3.33)	0.85
	2	1.39 ± 0.14 (1.01–1.77)	1.51 ± 0.24 (0.83–2.19)	1.79 ± 0.29 (0.99–2.59)	1.67 ± 0.31 (0.82–2.53)	0.70
	3	1.64 ± 0.12 (1.31–1.97)	1.84 ± 0.17 (1.38–2.30)	2.09 ± 0.25 (1.40–2.77)	1.81 ± 0.17 (1.34–2.28)	0.40

LH: Luteinising hormone, *p<0.02, **p<0.01.

Effect of *X. aethiopica* Fruit Without Seeds Extract on Sex Hormones

There was a significant increase in the serum levels of testosterone following administration of *X. aethiopica* for 1 month ($p < 0.03$), 2 months ($p < 0.02$), and 3 months ($p < 0.01$). Similarly, serum FSH level was significantly increased following exposure to *X. aethiopica* for 1 month ($p < 0.02$), 2 months ($p < 0.01$), and 3 months ($p < 0.01$) [Table 3].

Table 4 indicates a significant increase in the levels of serum FSH in rats administered with extract for 1 month ($p < 0.04$), 2 months ($p < 0.03$), and 3 months ($p < 0.01$). There was no significant alteration ($p > 0.05$) in serum levels of progesterone and prolactin throughout the study.

Effect of *X. aethiopica* Fruit with Seeds Extract on Sex Hormones

Table 3: Comparison of the fertility hormone levels of male Wistar rats on *X. aethiopica* fruits without seeds for 3 months.

Parameter/ Units	Duration in months	Treatment doses				*p*-value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		Fruits without seeds				
Testosterone (ng/ml)	1	0.76 ± 0.09 (0.52–0.99)	3.13 ± 0.73* (1.11–5.15)	3.53 ± 0.72* (1.53–5.54)	3.48 ± 0.73* (1.46–5.50)	0.03
	2	0.85 ± 0.08 (0.62–1.09)	2.94 ± 0.32** (2.06–3.82)	2.99 ± 0.51** (1.57–4.42)	3.24 ± 0.57** (1.67–4.82)	0.02
	3	0.92 ± 0.08 (0.70–1.14)	2.78 ± 0.22 (2.17–3.40**)	3.03 ± 0.39** (1.95–4.11)	3.72 ± 0.33** (2.80–4.63)	0.01
FSH (mIU/ml)	1	0.64 ± 0.06 (0.46–0.81)	1.04 ± 0.15* (0.62–1.45)	1.07 ± 0.07* (0.88–1.25)	1.05 ± 0.11* (0.74–1.37)	0.02
	2	0.67 ± 0.05 (0.54–0.80)	0.93 ± 0.05* (0.78–1.08)	0.97 ± 0.09* (0.71–1.24)	1.04 ± 0.08** (0.82–1.26)	0.01
	3	0.70 ± 0.05 (0.58–0.83)	0.97 ± 0.05* (0.83–1.10)	0.99 ± 0.09* (0.73–1.26)	1.09 ± 0.07** (0.89–1.29)	0.01

FSH: Follicle-stimulating hormone, *p<0.02, **p<0.01.

Table 4: Comparison of the fertility hormone levels of male Wistar rats on *X. aethiopica* fruits without seeds for 3 months

Parameter/Units	Duration in months	Treatment doses				*p*-value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		Fruits without seeds				
LH (mIU/ml)	1	0.62 ± 0.09 (0.37–0.86)	1.10 ± 0.14* (0.71–1.50)	1.14 ± 0.09* (0.90–1.37)	1.12 ± 0.12* (0.78–1.46)	0.04
	2	0.61 ± 0.05 (0.47–0.74)	1.27 ± 0.09* (1.03–1.51)	1.35 ± 0.16* (0.92–1.78)	1.44 ± 0.27** (0.70–2.18)	0.03
	3	0.68 ± 0.04 (0.56–0.80)	1.28 ± 0.08* (1.07–1.49)	1.40 ± 0.10** (1.11–1.11)	1.81 ± 0.25** (1.11–2.52)	0.01
Progesterone (ng/ml)	1	1.09 ± 0.07 (0.89–1.29)	2.12 ± 0.96 (-0.56–4.79)	1.60 ± 0.86 (-0.79–3.99)	2.04 ± 0.38 (0.99–3.09)	0.69
	2	0.45 ± 0.16 (0.01–0.89)	0.84 ± 0.42 (-0.33–2.02)	0.89 ± 0.46 (-0.38–2.17)	0.58 ± 0.19 (0.06–1.09)	0.75
	3	0.67 ± 0.09 (0.43–0.90)	0.74 ± 0.17 (0.26–1.22)	0.81 ± 0.24 (0.15–1.47)	0.66 ± 0.21 (0.08–1.24)	0.93
Prolactin (ng/ml)	1	2.19 ± 0.32 (1.29–3.09)	2.48 ± 0.71 (0.52–4.44)	2.93 ± 0.43 (1.75–4.11)	3.04 ± 0.97 (0.34–5.74)	0.78
	2	1.74 ± 0.12 (1.41–2.07)	2.43 ± 0.47 (1.13–3.72)	2.42 ± 0.64 (0.63–4.21)	2.26 ± 0.78 (0.10–4.43)	0.80
	3	1.84 ± 0.20 (1.28–2.39)	2.12 ± 0.13 (1.77–2.47)	2.03 ± 0.40 (0.92–3.13)	2.18 ± 0.32 (1.29–3.08)	0.83

LH: Luteinising hormone, *p<0.02, **p<0.01.

Serum testosterone levels were significantly increased following administration with fruit with seeds extract in male Wistar rats for 1 month ($p < 0.01$), 2 months ($p < 0.02$), and 3 months ($p < 0.03$), respectively. Serum FSH levels also increased following treatment for 1 month ($p < 0.01$), 2 months ($p < 0.02$), and 3 months ($p < 0.01$) [Table 5].

Serum LH was significantly increased following administration of fruit with seeds extract for 1 month ($p < 0.05$), 2 months ($p < 0.02$), and 3 months ($p < 0.01$), respectively. No significant difference was observed in the levels of progesterone and prolactin following treatment for 1, 2, and 3 months. [Table 6]

DISCUSSION

Xylopia aethiopica is a plant of great importance in African diets and traditional medicine. It is rich in nutritional and phytochemical compounds. The therapeutic functions of the plant might be attributed to its diverse phytochemical compounds and the plant has been used to alleviate vitamin and mineral deficiencies.[14] In this study, male sex hormones,

Table 5: Comparison of the fertility hormone levels of male Wistar rats on *X. Aethiopica* fruits with seeds for 3 months

Parameter/Units	Duration in months	Treatment doses				*p*-value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		Fruits with seeds				
Testosterone (ng/ml)	1	1.26 ± 0.19 (0.72–1.80)	3.14 ± 0.62* (1.42–4.86)	3.24 ± 0.41* (2.11–4.38)	3.57 ± 0.59* (1.93–5.22)	0.01
	2	1.38 ± 0.14 (0.98–1.78)	2.89 ± 0.41** (1.75–4.02)	3.02 ± 0.36** (2.01–4.02)	3.05 ± 0.13 (2.69–3.41)	0.02
	3	1.50 ± 0.10 (1.22–1.77)	2.99 ± 0.27** (2.25–3.74)	3.05 ± 0.31** (2.19–3.91)	3.04 ± 0.15** (2.61–3.47)	0.03
FSH (mIU/ml)	1	0.67 ± 0.05 (0.52–0.82)	0.99 ± 0.09* (0.75–1.23)	1.04 ± 0.11* (0.75–1.34)	1.08 ± 0.07 (0.88–1.29)	0.01
	2	0.76 ± 0.03 (0.68–0.85)	1.13 ± 0.05** (0.99–1.2)	1.13 ± 0.11** (0.83–1.43)	1.20 ± 0.08** (0.97–1.42)	0.02
	3	0.81 ± 0.03 (0.73–0.89)	1.17 ± 0.03** (1.09–1.24)	1.20 ± 0.09** (0.96–1.44)	1.27 ± 0.05** (1.13–1.41)	0.01

FSH: Follicle-stimulating hormone, *p<0.02, **p<0.01.

Table 6: Comparison of the fertility hormone levels of male Wistar rats on *X. aethiopica* fruits with seeds for 3 months

Parameter/ Units	Duration in months	Treatment doses				*p*-value
		Control (A)	125 mg/kg (B)	250 mg/kg (C)	500 mg/kg (D)	
		Fruits with seeds				
LH (mIU/ml)	1	0.75 ± 0.10 (0.49–1.02)	1.22 ± 0.04 (1.11–1.33)	1.17 ± 0.10 (0.91–1.44)	1.17 ± 0.16 (0.74–1.60)	0.05
	2	0.81 ± 0.10 (0.52–1.09)	1.23 ± 0.04** (1.13–1.33)	1.23 ± 0.08** (1.01–1.44)	1.32 ± 0.05** (1.18–1.46)	0.02
	3	0.86 ± 0.09 (0.62–1.11)	1.25 ± 0.05** (1.11–1.39)	1.27 ± 0.04** (1.16–1.38)	1.37 ± 0.05** (1.24–1.51)	0.01
Progesterone (ng/ml)	1	1.34 ± 0.23 (0.71–1.98)	1.88 ± 0.46 (0.59–3.16)	1.42 ± 0.08 (1.18–1.65)	1.54 ± 0.16 (1.09–2.00)	0.54
	2	0.93 ± 0.11 (0.63–1.23)	0.98 ± 0.11 (0.67–1.29)	1.09 ± 0.13 (0.72–1.45)	1.04 ± 0.21 (0.44–1.63)	0.88
	3	0.94 ± 0.06 (0.78–1.09)	1.01 ± 0.08 (0.78–1.24)	1.06 ± 0.09 (0.81–1.31)	1.09 ± 0.14 (0.69–1.49)	0.71
Prolactin (ng/ml)	1	2.05 ± 0.21 (1.47–2.63)	2.47 ± 0.40 (1.35–3.60)	2.32 ± 0.91 (-0.21–4.85)	2.59 ± 0.35 (1.61–3.57)	0.90
	2	1.92 ± 0.47 (0.60–3.23)	1.92 ± 0.34 (0.97–2.87)	2.01 ± 0.22 (1.39–2.63)	2.15 ± 0.50 (0.77–3.53)	0.97
	3	1.77 ± 0.35 (0.79–2.76)	1.98 ± 0.32 (1.11–2.86)	1.79 ± 0.09 (1.53–2.05)	2.23 ± 0.25 (1.54–2.92)	0.61

LH: Luteinising hormone, **p<0.01.

particularly testosterone, FSH, LH, progesterone, and prolactin, were assayed in male Wistar rats administered with leaves, fruit without seed, and fruit with seeds extracts of *X. aethiopica* in acute, sub-acute, and chronic states. This is of public health importance to know whether the administration of extract of *X. aethiopica* boosts or lower sex hormone levels since this plant is increasingly consumed as food and medicines in Africa. Furthermore, several studies reported conflicting findings on the merit or demerit of this plant.[8-15] The need to carefully investigate the several factors responsible for the increasing trend of male infertility and proffer effective therapeutic and preventive measures cannot be exaggerated.[16]

Data from this study indicated that serum testosterone was significantly increased following administration of leaves, fruit without seed, and fruit with seed extracts for up to 90 days in a dose-dependent manner. The consumption of leaves and fruit with or without seeds enhanced the secretion of

serum testosterone, FSH, and LH. Previous study has reported that the analysis of *X. aethiopica* using Gas Chromatography-Mass Spectrometry indicated the presence of L-aspartic acid in the fruits of *X. aethiopica*.[15] Although L-aspartic acid is an amino acid used to synthesise proteins, D-aspartic acid plays a significant role in the synthesis and secretion of hormones, including testosterone, in the body.[17-19] Although D-aspartate is naturally present in the adenohypophysis, hypothalamus, brain, and testes of rats, the administration of exogenous D-aspartate cause the induction of growth hormone and LH. D-aspartate acts on the hypothalamus to release GnRH, which in turn leads to the release of LH.[17] In the testes, D-aspartate present in the Leydiq cells is involved in testosterone synthesis.[18] This finding is consistent with a previous study, which reported a significant dose-dependent increase in serum testosterone levels among Sprague–Dawley male rats administered with ethanolic fruit extract of *X. aethiopica* at the doses of 30, 100, and 300 mg/kg for 60 days.[12] Also, a dose-dependent significant increase in sperm concentration and live sperm cells was reported in rabbits administered with *X. aethiopica*.[13] To clearly understand the level of testosterone observed in this study, FSH and LH levels were also assayed. Testosterone is the principal male gonadal hormone synthesised by Leydig cells in the testis. Testosterone is synthesised by Leydig cells of the testes in response to LH, a process that is regulated by the hypothalamic-pituitary–testis axis. FSH is a major hormone in both male and female reproductive systems. In males, FSH, along with testosterone, is important in stimulating and maintaining spermatogenesis. It acts directly on Sertoli cells to carry out its function and maintenance of spermatogenesis.[20] Conversely, a significant decrease in the plasma levels of LH, FSH, and testosterone was reported by some authors.[8-11] It was concluded that the "use of *X. aethiopica* fruits by human males may have both dose and time-dependent adverse effects on the reproductive hormones, and therefore on their reproductive capacity". The disparity in the findings might be attributed to several factors: differences in potentials of *X. aethiopica*, the concentration used in the experiments, species differences in *X. aethiopica*, site of cultivation, choice of solvent used for dilution, and type of kit used in the assay. For example, Adienbo *et al.*[8] used ELISA Human antibody kits. The source of *X. aethiopica* is a major factor, whereas some obtained their products from the markets (Chioba market in Port Hardcourt [8] and Ama Hausa in Imo[11]), we harvested fresh ripe *X. aethiopica* from the field and processed.

CONCLUSION

The administration of *X. aethiopica* at the concentrations used in this study enhanced the levels of serum testosterone, LH, and FSH in male Wistar rats. The consumption of this commonly used food spice appears to have beneficial effects on the male sex hormones.

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Ethical approval

This experimental study was conducted following the ethical approval obtained from the Ethics Committee of the Faculty of Pharmacy, University of Benin, Benin City, Nigeria, with reference number EC/FP/018/19 issued on 04 April 2018.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of Artificial Intelligence (AI)-Assisted Technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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