Biotechnology Journal International

23(1): 1-14, 2019; Article no.BJI.47857
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

The Effect of *Nigella sativa* and *Piper guineense* Aqueous Extracts on the *in Vivo* Expression Profile of Lipid and Sex Hormones

Okoye Ngozi Franca[1*] and Uvbiekpahor Rume[1]

[1]Department of Biochemistry, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between both authors. Author ONF designed the study, performed the statistical analysis, wrote the first draft of the manuscript. Author UR managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2019/v23i130069
Editor(s):
(1) Dr. Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
Reviewers:
(1) Dennis, Amaechi, Veritas University, Nigeria.
(2) Siva Rami Reddy, E Tantia University, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/47857

Original Research Article

Received 22 December 2018
Accepted 04 March 2019
Published 12 April 2019

ABSTRACT

Aim: The study investigated the effect of aqueous extract of *Piper guineense* and *Nigella sativa* on Testosterone, Estrogen and Follicle stimulating hormone levels in male and female rats induced by diets supplemented with high dose of sucrose and margarine for four (4) week.
Materials and Methods: Thirty male and female Wistar rats of average weight (100 g) were used for the study. The rats were arranged into five groups with six rats in each of the groups. The rats had access to their normal feed but sucrose and margarine were used to induce hyperglycemia and hyperlipidemia respectively on the rats with the exception of the rats in the positive control group. The rats in the negative control were induced using the sucrose and margarine but were not treated using the aqueous extracts. The rats in the *Piper guineense* group were treated with 2 ml of *Piper guineense* aqueous leaf extract, while the rats in the *Nigella sativa* group were treated with 2 ml of *Nigella sativa* aqueous extract. The rats in the *Nigella sativa* and *Piper guineense* group were treated with 2 ml of the combined aqueous extract.
Results: The results showed that the extracts had an increasing effect which was time dependent on the hormones. The highest increase was obtained on the third week of feeding when compared

**Corresponding author: E-mail: francaokoye1@yahoo.com;*

to the control. The testosterone levels (mlU/ml) showed for the, positive control (2.28 ± 0.08), uziza leaf (6.45 ± 0.01), black seed (2.85 ± 0.01), black seed &uziza (8.47 ± 0.08)($p<0.05$). The increase for estrogen levels (mlU/ml) showed for the positive control (99 ± 0.82), uziza leaf (98 ± 1.63), black seed (100 ± 1.63), black seed & uziza (113 ± 0.82). Furthermore, the extracts also had an increasing effect on the follicle stimulating hormone levels with the highest increase obtained on the third week ($p>0.05$). The FSH levels (mlU/ml)) showed for the positive control (0.23 ± 0.01), uziza leaf 0.25 ± 0.01), black seed (0.54 ± 0.02), black seed & uziza (0.58 ± 0.01)($p<0.05$).
Histopathological findings also suggest that treatment with the aqueous extracts of *P.guineense* and *N .sativa* after inducement salvaged the testes and ovaries when compared to the induced group after the second week of administration. Although the combined mixture *N.sativa* and *P.guineense* extract was more effective.
Conclusion: The results of the study indicate that the aqueous extract of a combined mixture of *Nigella sativa* and *Piper guineense* might be an effective plant in indigenous medicine, which may be used to combat infertility caused by hormonal imbalance.

Keywords: Estrogen; follicle stimulating hormone; Nigella sativa; Piper guineense; testosterone.

1. INTRODUCTION

The existence of life without procreation is impossible; without doubt reproduction is a basis of life. Life is a self sustained chemical system that can undergo Darwanian evolution [1,2]. During the reproductive phase of an organism, all reproductive organs and hormones needed for reproduction to occur are developed and synthesized. Therefore, at reproductive maturity an organism is deemed fit biologically to reproduce [1,2].

Nevertheless studies have shown that reproduction may be greatly impaired by the following:

Chronic hyperglycemia (Diabetes Mellitus). Diabetic males were associated with the following conditions, such as testicular dysfunction, impotence, decreased fertility potentials, retrograde ejaculations, erectile dysfunction, poor semen quality, decreased sperm motility and concentration. Also abnormal morphology, increased seminal plasma abnormalities and lastly decreased serum testosterone due to impair leydig function [2,3]. While in diabetic females conditions such as neuropathy, vascular impairment and psychological complaints were suggested in the pathogenesis of decreased libido, decreased vaginal lubrication, orgasmic dysfunction and dyspereumia [3].

Hyperlipidemia (hypercholesterolemia and hyperglyceridemia). It was also reported that endoplasmic reticulum stress, due to hyperlipidemia, reduce blastocytes development and alters oocyte metabolism [4]. High lipid concentration affects semen parameters, specifically sperm head morphology, cholesterol and lipid homeostasis for male fecundity [5,6].

Hormones are coordinated by the endocrine system, without proper endocrine function, our bodies cannot maintain proper hormonal balance. Hormonal level may be influenced by stress, fluid change in the body, poor nutrition, vitamins and mineral levels, infection, exposure to environmental toxins [5,6]. Each endocrine gland plays a specific role in accordance with the maintenance of the bodily functions. If the gland is not functioning properly, hormonal imbalance occurs and the ability of the body to function effectively will be altered. That is, the ability to reproduce may be impaired.

In recent years, researchers have focused on *Nigella sativa* commonly known as black seed and *Piper guineense* commonly known as Uziza whose aqueous extracts were reported to have a positive influence on the reproductive profile; *Piper guineense* was found to have exerted hypolipidemic effects by reducing the cholesterol induced body weight and increased the body's anti-oxidant defense system, as well asinfluencing the increase in steriodogenic pathways that mediated testosterone production [7,8].

Agbai. [9] also reported that the aqueous extract of*P.guineese* leaf was capable of influencing follicle stimulating hormone production during dietrus phase without affecting the luteinizing hormone and estrogen levels. Therefore haltering the early follicular phase which resulted to a decreased population of follicles in the ovary.

Nevertheless, Mbongue [10] reported that the ethanol extract of *Piper guineense* had a negative effect on mice reproduction. This is in fact opposite to the effect of the same leaf when used in aqueous solution.

Nigella sativa was observed to have increased relative weight and size of productive organs (Testes, epididymis, seminal vesicle, ventral prostate) due to an elevated level of androgen. This suggests that *Nigella sativa* had a positive effect on fertility and reproductive system in adult male rats [11]. *Nigella sativa* was reported to have hypoglycemic effect related to the significant increase in the level of testosterone in the serum [11,12].

It was suggested that the lipid homeostasis that occurs in male reproductive system is performed by the testicular and post - testicular mechanism and the contraction of serum lipid was not corresponding with the quality of sperm parameter [6,13].However, an impairment in the serum total testosterone, FSH, and LH due to the significant increase in triglycerides was observed, with an alteration in testicular development with sabotage of spermatogenic epithelium [13,14].

Traditionally, the *Nigella sativa* seed and its oil are used in the treatment of several diseases. The seeds are found to be bitter, pungent, aromatic, appetizer, stimulant, diuretic, anthelmintic, acrid, thermogenic, carminative, anodyne, deodorant, digestive, constipating, sudorific, febrifuge, expectorant, purgative, abortifacient. They are used in cases of, cough, jaundice, hydrophobia, fever, paralysis, conjunctivitis, piles, skin diseases, anorexia, dyspepsia, flatulence, abdominal disorders, diarrhea, dysentery, intrinsic hemorrhage and amenorrhea. The seed oil is a local anaesthetic [12,15,16,17].

Piper guineense, which is also known as African black pepper or hot leave is widely consumed in some part of West Africa especially Nigeria and Ghana on account of its nutritional and medicinal properties. In traditional herbal medicine, the seeds are put into a variety of uses, for instance, in some parts of Nigeria, the seeds were consumed by women after child birth, to enhance uterine contraction for the expulsion of placenta and other remains from the womb, as an adjuvant in the treatment of rheumatic pains and as an antiasthmatics and also for the control of weight [12].

The aim of this research is to study the effect of the aqueous extract of *Nigella sativa and Piper guineense*on the Testosterone, Estrogen and Follicle Stimulating hormone levels of hyperlipidemic induced wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Thirty (30) Wistar rats (15 males and 15 females) weighing about100g were purchased fromChoba animal house, Department of Biochemistry, University of Port Harcourt. They were fed with growers mash and water ad libitum for seven (7) weeks. One week prior to start of experiment, they were held in experimental room in close observations to acclimate them with experimental room.

Table 1. Feeds formulation

Ingredient	% Composition	Composition by Weight (g)
Normal feeds (growers mash)	50	500
Sucrose	25	250
Margarine	25	250

2.2 Animal Feeding

After one week of acclimatization to the environment, the animals were divided into two main groups. The first group (6 rats) was fed growers mash (100%) throughout the experimental period and it was called control group. The second main group (24 rats) was fed growers mash (50%) supplement with sucrose (25%) and margarine (25%) and it was called Hyperlipidemic group. The animals were allowed free access to food and water for four (4) weeks. At the fourth weeks post feeding and before treatment administration, a pilot study was conducted, the animals were sacrificed by the means of cervical strangulation and blood samples were collected from a rat in each main group via cardiac puncturing. Plasma cholesterol and triglycerides levels were measured.

2.3 Collection and Identification of Plant Materials

Fresh leaves of Uziza (*Piper guineense*) were obtained from Choba Market, Obio-Akpor Local

Government and the black seeds (*Nigella sativa*) were obtained from Barki-dogo market, Kaduna state. The leaves and seeds were identified as *Piper guineense*leaves and *Nigella sativa* seeds by staff of Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba.

2.3.1 Preparation of uziza leaf (*Piper guineense*) extract

Fifty grams (50g) of fresh leaves of *Piper guineense* was grinded with 1000ml of water using a blender. The extract was squeezed out from the grinded leaves and filtered and kept in an air tight container.

2.3.2 Preparation of black seed (*Nigella sativa*)

One hundred grams (100 g) of black seed was broken down to a smooth powder with a blender, 50 g of the seed powder was weighed and submerged in 500 ml of distilled water. The mixture was stirred properly and allowed to stand for an hour, after which, it was stirred and kept in an air tight container.

Administration of extract was carried out orally by the use of syringes in order to determine the actual dose. The animals received their doses seven (7) times weekly for a period of three (3) weeks. At the end of each week, the rats from each group was sacrificed through the means of cervical strangulation and blood samples were collected. Testosterone level, Follicle stimulating hormone level and Estrogen level were determined with Elisa method. The testes and ovaries were also removed and submerged in 10% formalin which was further subjected to histological analysis.

2.4 Lipid Profile Assay

The lipid profile was determined using Mindray test kits. The procedure described in assay kit was used according to the principle highlighted by Tietz [18].

2.4.1 Cholesterol test procedure

Three (3 ml) of blood samples were collected and poured in lithium heparin bottle, and spun to separate the blood while two test tube were labeled “standard” and “sample”, after which a 1000 µl of the cholesterol reagents were pipette into both test tubes of the sample and standard , 10µl of samples and standard reagents were also pipette respectively and it was incubated for 10minutes. The absorbance were read with a colorimeter under the wavelength range of 540 mm.

Calculation

Conc. of Cholesterol = (absorbance of sample X concentration of standard reagent/ Absorbance of standard)

Conc. of standard Reagent = 5
.17mm (The color of the end point is pink-red)

2.4.2 Triglyceride test procedure

Three (3ml) of blood samples were collected and poured in lithium heparin bottle, and spun to separate the blood, after which two test tubes were labeled “standard” and “sample” and 1000ul of the triglyceride reagents were pipette into both test tubes of the samples and standard test tube, 10ul of samples and standard reagents were also pipette respectively and incubated for 10minutes. The absorbance was read at 540nm wavelength.

Calculation

Concentration of triglyceride = (absorbance of sample X concentration of standard reagent/ Absorbance of standard)

Conc. of Standard Reagent = 2.29 mm.

Table 2. Administration of the treatment

Group 1	control group
Group 2	Induced without treatment
Group 3	Treated with the aqueous extract of *Nigella sativa* with a dose of 2ml/kg body weight respectively
Group 4	Treated with the aqueous extract of *Piper guineense*with a dose of 2ml/kg body weight respectively
Group 5	Treated with the mixture (1ml each of *Nigella sativa* extract and *Piper guineense*extract) with a dose of 2ml/kg body weight respectively.

2.4.3 High Density Lipoprotein (HDL) test procedure

Three (3 ml) of blood samples were collected and poured in lithium heparin bottle, and spun to separate the blood, two test tubes were labeled "standard" and "sample" after which 500 µl of the HDL reagents were pipette into both test tubes. samples and standard test tube, 200 µl of sample and standard cholesterol reagents were also pipette respectively and incubated for 10 minutes. The absorbance were read with a colorimeter under the wavelength range of 530 mm-560 mm.

Calculation

Calculations of the concentration were done using 246 X absorbance of the sample

2.4.4 Specimen collection and preparation

Three (3 ml) of blood samples were collected in a plain redtop plain bottle. The blood samples were allowed to clot for serum samples. The clotted blood samples were centrifuged to separate the serum from the cells. The serum samples were refrigerated at 2-8°C for a maximum period of five (5) days.

2.5 Hormone Assay

The levels of hormones were measured in serum by ELISA testosterone, FSH and estrogen standard kits (Biocheck, Inc. Foster City CA, USA). The procedure described in the hormone assay kits was used according to the principle highlighted by Tietz [18] for testosterone while the method of Uotila, [19] was used for follicle stimulating hormones, and the method of Hall [20] and Ratcliffe., [21] was used for estrogen.

2.5.1 Test procedure for testosterone

Nineteen (19) micro plates' wells were formatted for each serum reference, control and serum specimens to be assayed in duplicate. 0.010 ml (10 µL) of the appropriate serum reference, control and specimens were pipetted into the assigned wells. 0.050 ml (50 µl) of the working Testosterone Enzyme Reagents were added to all wells. The micro plates were swirled gently for 20-30 seconds to mix and 0.050 ml (50 µl) of Testosterone Biotin Reagent was added to all wells. The micro plates were swirled gently for 20-30 seconds to mix and the micro plates were covered and incubated for 60 minutes at room temperature. The contents of the micro plates were discarded by decantation by blotting the plates dry with absorbent paper and 350 µl of wash buffer was added, decanted (tap and blot). Two (2) additional times was repeated for a total of three (3) washes and 100 ml (100 µl) of working substrate solution was added to all wells and the reagents in the same order were always added to minimize reaction time differences between wells. The micro plates were incubated at room temperature for fifteen (15) minutes and 0.050 ml (50 µl) of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a micro plate reader. The results were read within thirty (30) minutes of adding the stop solution.

2.5.2 Test procedure for follicle stimulating hormone

Thirty eight (38) micro plate wells were formatted for each serum reference, control and specimens to be assayed in duplicate. 0.050 ml (50 µl) of the appropriate serum reference, control and specimens were pipetted into the assigned well. 0.100 ml (100 µl) of FSH-Enzyme Reagent solution was added to all wells. The micro plates were swirled gently for 20-30 seconds to mix and covered and the micro plates were incubated for 60 minutes at room temperature. The contents of the micro plates were discarded by decantation, by blotting the plate dry with absorbent paper. 350µl of wash buffer was added, decanted (tap and blot). Two (2) additional times was repeated for a total of three (3) washes with a manual plate washer. 0.100 ml (100 µl) of working substrate solution was added to all wells. Reagents in the same order were always added to minimize reaction time differences between wells. The micro plates were incubated at room temperature for fifteen (15) minutes and 0.050ml (50 µl) of stop solution was added to each well and gently mixed for 15-20 seconds. The absorbance in each well was read at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfections) in a micro plate reader and the results were read within thirty (30) minutes of adding the stop solution.

2.5.3 Test Procedure for Estrogen

Nineteen (19) micro plates' wells were formatted for each serum reference, control and specimens to be assayed in duplicate. 0.025 ml (25 µL) of

the appropriate serum reference, control and specimen were pipetted into the assigned well. 0.050 ml (50 µl) of the Estradiol Biotin Reagent was added to all wells and the micro plates were swirled gently for 20-30 seconds to mix. The micro plates were covered and incubated for 30 minutes at room temperature and 0.050 ml (50 µl) of Estradiol Enzyme Reagent was added to all wells. The micro plates were swirled gently for 20-30 seconds to mix and the micro plates were covered and incubated for 90 minutes at room temperature. The contents of the micro plates were discarded by decantation, blotting the plate dry with absorbent paper and 35 0µl of wash buffer was added, decanted (tap and blot). Two (2) additional times were repeated for a total of three (3) washes with a manual plate washer. 0.100 ml (100 µl) of substrate solution was added to all wells and the micro plates were incubated at room temperature for twenty (20) minutes. 0.050 ml (50 µl) of stop solution was added to each well and gently mixed for 15-20 seconds and the absorbance in each well was read at 450 nm (using a reference wave- length of 620-630 nm. The results were read within thirty (30) minutes of adding the stop solution.

2.6 Statistical Analysis

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean ± SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is $p<0.05$.

3. RESULTS

The results obtained in this study are as presented below

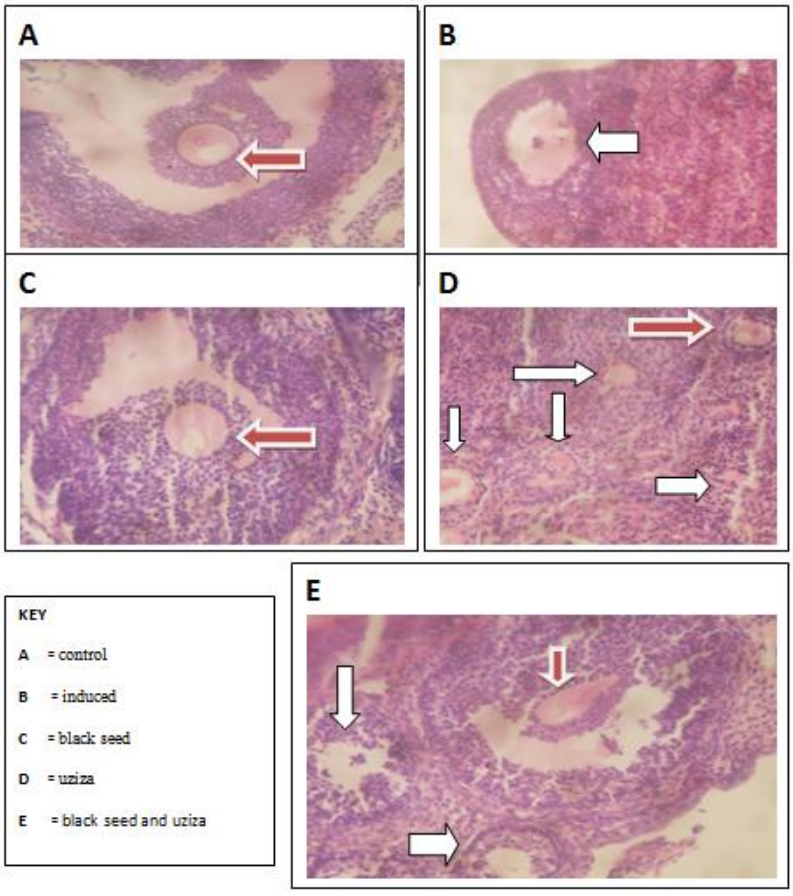

Plate 1. Photomicrographs of the ovaries stained H and E, magnification X400 (week One)

Table 3. Pilot study result on lipid parameters

Test (Mmol/L)	Non Induced Wistar Rats	Induced Wistar Rats
Cholesterol	6.28 ± 0.0002[a]	11.75 ± 0.0016[b]
High density lipoprotein	3.93 ± 0.0006 [a]	8.33 ± 0.0009[b]
Triglyceride	2.48 ± 0.3600 [a]	4.05 ± 0.0016[b]
Low density lipoprotein	11.33 ± 0.0002 [a]	21.92 ± 0.0024[b]
Very low density lipoprotein	1.12 ± 0.0002 [a]	1.82 ± 0.0007[b]

Results are means of three determinations ± standard deviation.
[ab] *Different letters in a given row denote significant difference, p<0.05*

Table 4. Body weight result for male albino rats

Group	Before Inducement (g)	After Inducement (g)	After one week of treatment (g)	After Second week of Treatment (g)	After third week of treatment (g)
Control	57± 0 . 0 1 [a]	143± 0 . 0 1 [b]	186± 0 . 0 1 [c]	179± 0 . 0 1 [d]	232± 0 . 0 1 [e]
Induced	143± 0 . 0 1 [a]	250± 0 . 0 1 [b]	230± 0 . 0 1 [c]	166± 0 . 0 1 [d]	186± 0 . 0 1 [e]
N.sativa	96± 0 . 0 1 [a]	200± 0 . 0 1 [b]	125± 0 . 0 1 [c]	195± 0 . 0 1 [d]	180± 0 . 0 2 [e]
P.guineense	60± 0 . 0 1 [a]	200± 0 . 0 1 [b]	179± 0 . 0 2 [c]	189± 0 . 0 1 [d]	158± 0 . 0 1 [e]
N.sativa and *P.guineense*	108± 0 . 0 1 [a]	198± 0 . 0 1 [b]	157± 0 . 0 1 [c]	163± 0 . 0 1 [d]	175± 0 . 0 2 [e]

Results are means of three determinations ± standard deviation.
[abcde] *Different letters in a given row denote significant difference, p<0.05*

Table 5. Body weight result for female albino rats

Group	Before Inducement (g)	After Inducement (g)	After one week of treatment (g)	After Second week of Treatment (g)	After third week of treatment (g)
Control	73 ± 0 . 0 1 [a]	143± 0 . 0 1 [b]	135± 0 . 0 1 [c]	156± 0 . 0 1 [d]	167± 0 . 0 [e]
Induced	143± 0 . 0 1 [a]	250± 0 . 0 1 [b]	170± 0 . 0 1 [c]	178± 0 . 0 1 [d]	198± 0 . 0 [e]
N.sativa	111± 0 . 0 1 [a]	200± 0 . 0 1 [b]	125± 0 . 0 1 [c]	166± 0 . 0 1 [d]	210± 0 . 0 [e]
P.guineense	87± 0 . 0 1 [a]	200± 0 . 0 1 [b]	138± 0 . 0 2 [c]	136± 0 . 0 1 [d]	143± 0 . 0 [e]
N.sativa And *P.guineense*	101± 0 . 0 1 [a]	198± 0 . 0 1 [b]	126± 0 . 0 1 [c]	132± 0 . 0 1 [d]	176± 0 . 0 [e]

Results are means of three determinations ± standard deviation
[abcde] *Different letters in a given row denote significant difference, p<0.05*

Table 6. Effect of first, second & third week oral administration of uziza leaf and black seed on testosterone level of male wistar rat

Group	After one week of treatment (mIU/ml)	After Second week of Treatment (mIU/ml)	After third week of treatment (mIU/ml)
Control	2.10 ± 0 . 0 8 [a]	2.27 ± 0 . 0 8 [b]	2.28± 0 . 0 8 [c]
Induced	1.90 ± 0 . 0 8 [a]	1.02 ± 0 . 0 1 [b]	2.00± 0 . 0 8 [c]
N.sativa	2.00 ± 0 . 0 8 [a]	2.52± 0 . 0 1 [b]	2.85± 0 . 0 1 [c]
P.guineense	3.02± 0 . 0 1 [a]	4.98± 0 . 0 1 [b]	6.45± 0 . 0 1 [c]
N.sativa and *P.guineense*	4.07± 0 . 0 1 [a]	6.50± 0 . 0 1 [b]	8.47± 0 . 0 8 [c]

Results are means of three determinations ± standard deviation.
[abc] *Different letters in a given row denote significant difference, p<0.05*

Table 7. Effect of first, second & third week oral administration of uziza leaf and black seed on estrogen level of female wistar rats

Group	After one week of treatment (mIU/ml)	After second week of treatment (mIU/ml)	After third week of treatment (mIU/ml)
Control	93 ± 0 . 8 2 [a]	99 ± 0 . 0 8 2 [b]	99 ± 0 . 8 2 [c]
Induced	99 ± 0 . 8 2 [a]	102 ± 0 . 8 2 [b]	98 ± 0 . 8 2 [c]
N.sativa	98± 1 . 6 3 [a]	94± 0 . 1 . 6 3 [b]	98 ± 1 . 6 3 [c]
P.guineense	98 ± 1 . 6 3 [a]	99 ± 1 . 6 3 [b]	100 ± 1 . 6 3 [c]
N.sativa and *P.guineense*	101 ± 0 . 8 2	112± 0 . 8 2	113 ± 0 . 8 2

Results are means of three determinations ± standard deviation.
[abc] *Different letters in a given row denote significant difference, p<0.05*

Table 8. Effect of first, second & third week oral administration of uziza leaf and black seed on follicle stimulating hormonelevel of male wistar rat

Group	After one week of treatment (mIU/ml)	After Second week of Treatment (mIU/ml)	After third week of treatment (mIU/ml)
Control	0.22± 0 . 0 1 [a]	0.23 ± 0 . 0 1 [b]	0.23± 0 . 0 1 [c]
Induced	0.18± 0 . 0 1 [a]	0.28 ± 0 . 0 1 [b]	0.22± 0 . 0 1 [c]
N.sativa	0.51± 0 . 0 2 [a]	0.53± 0 . 0 2 [b]	0.54± 0 . 0 2 [c]
P.guineense	0.23± 0 . 0 1 [a]	0.24± 0 . 0 1 [b]	0.25± 0 . 0 1 [c]
N.sativa and *P.guineense*	0.55± 0 . 0 1 [a]	0.56± 0 . 0 1 [b]	0.58± 0 . 0 1 [c]

Results are means of three determinations ± standard deviation.
[abc] *Different letters in a given row denote significant difference, p<0.05*

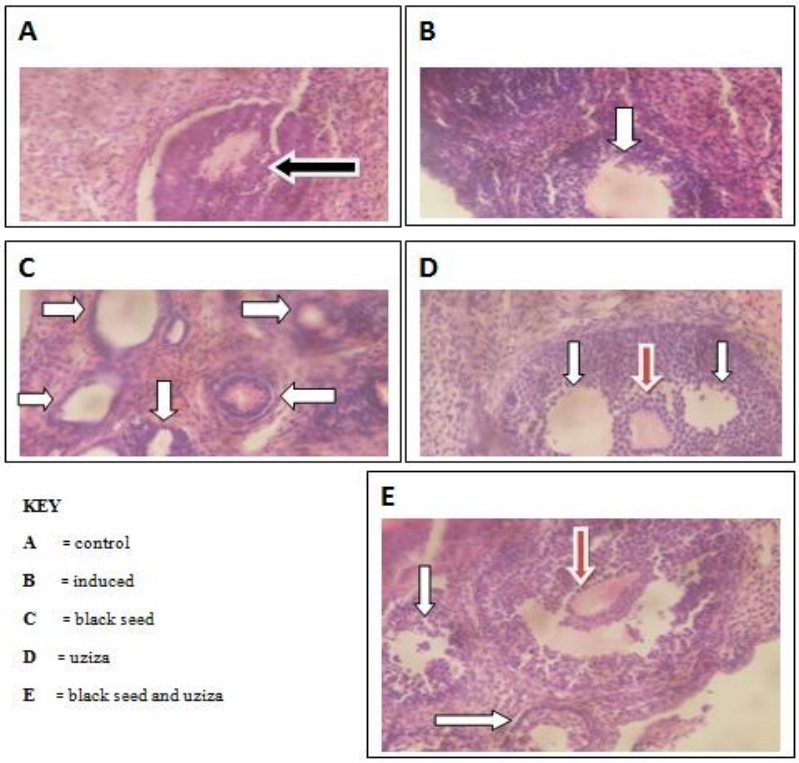

Plate 2. Photomicrographs of the ovaries stained H and E, magnification X400 (week two)

Table 9. Effect of first, second & third week oral administration of uziza leaf and black seed on follicle stimulating hormone level of female wistar rat

Group	After one week of treatment (mIU/ml)	After Second week of Treatment (mIU/ml)	After third week of treatment (mIU/ml)
Control	0.75 ±0.01	0.10 ±0.01	0.21±0.01
Induced	0.10 ±0.01	0.31 ±0.01	0.22±0.01
N.sativa	0.20±0.02 [a]	0.33±0.02 [c]	0.24±0.02 [b]
P.guineense	0.25±0.01 [a]	0.69±0.01 [c]	0.19±0.01 [a]
N.sativa And *P.guineense*	0.16±0.01 [a]	0.11±0.01 [b]	0.99±0.01 [c]

Results are means of three determinations ± standard deviation.
[abc] Different letters in a given row denote significant difference, p<0.05

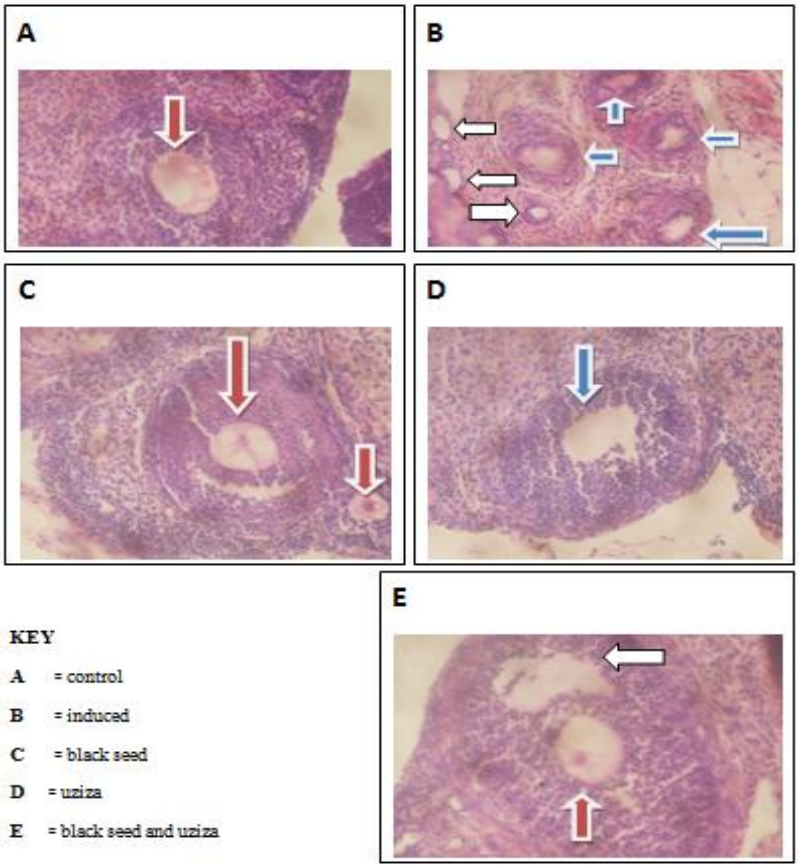

Plate 3. Photomicrographs of the ovaries stained H and E, magnification X400 (week three)

4. DISCUSSION

In the present study, the result obtained from the pilot study of the male and female albino rats, showed an increase in the lipid parameters (mmol/l) of the induced group when compared to the non induced group. This increase maybe as a result of an extreme intake of a diet containing high sucrose with margarine which resulted to an alteration in lipid metabolism that led to a disorder. This disorder is a key factor for the development of atherosclerosis and subsequent coronary heart diseases. The increase in LDL concentration indicates the consumption of a diet rich in saturated fatty acid that decreased the LDL receptor mediated catabolism, a decrease in the LDL receptors in the cell membrane affects the uptake of LDL by target cells, thereby increasing the concentration of LDL at the intracellular space [3].

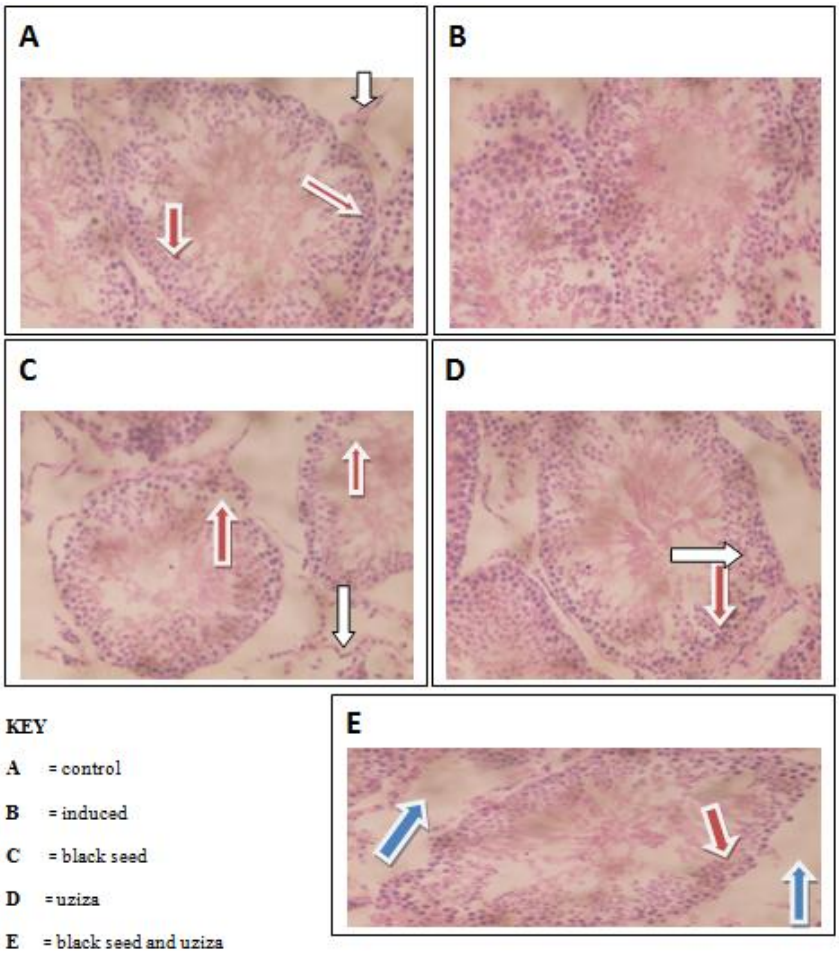

Plate 4. Photomicrographs of the testes stained H and E, magnification X400 (week one)

The increase of HDL was in contrast with the findings of Doubrain, [17] in which a moderately high fat diet (32%) KCal from fat significantly increased the serum total cholesterol and the triglyceride levels but decreased the HDL level in Sprague Darwley rats as compared with control group of 10 weeks. Their results suggested that hypercholesterolemia was linked to their diet, so the difference between the used diet, sucrose, breeding of Wistar rats and duration of feeding can be considered as possible reasons for the result obtained in this present study.

The Results obtained also for the body weight of the male and female rats in the induced group fed with growers mash (50%) supplemented with sucrose (25%) and margarine (25%) for a period of four weeks increased when compared with those of the control. The body weight of the male / female wistar rat in the induced groups were 250g while that of the control were 143g. This may result from the inducement of hyperlipidemia which caused elevated lipid levels in the lipid stores [15].

After the treatment, the body weight (125 g and 125 g) of the male and female wistar rats in the group treated with 2ml of aqueous extract of *N. sativa* and the body weight (157 g and 126 g) of the male and female wistar rats treated with 2ml of the aqueous extract of *N. sativa* and *P.guineense* were decreased significantly. The body weight (125 g and 125 g) of the male and female wistar rats in the *N.sativa* group and the body weight (157g and 126 g) of the male and female wistar rats in the *N.sativa* and *P.guineense* group were decreased as compared to the body weight (186 g and 135 g) of the male and female wistar rats in the control. This decrease maybe associated to the *N.sativa* and

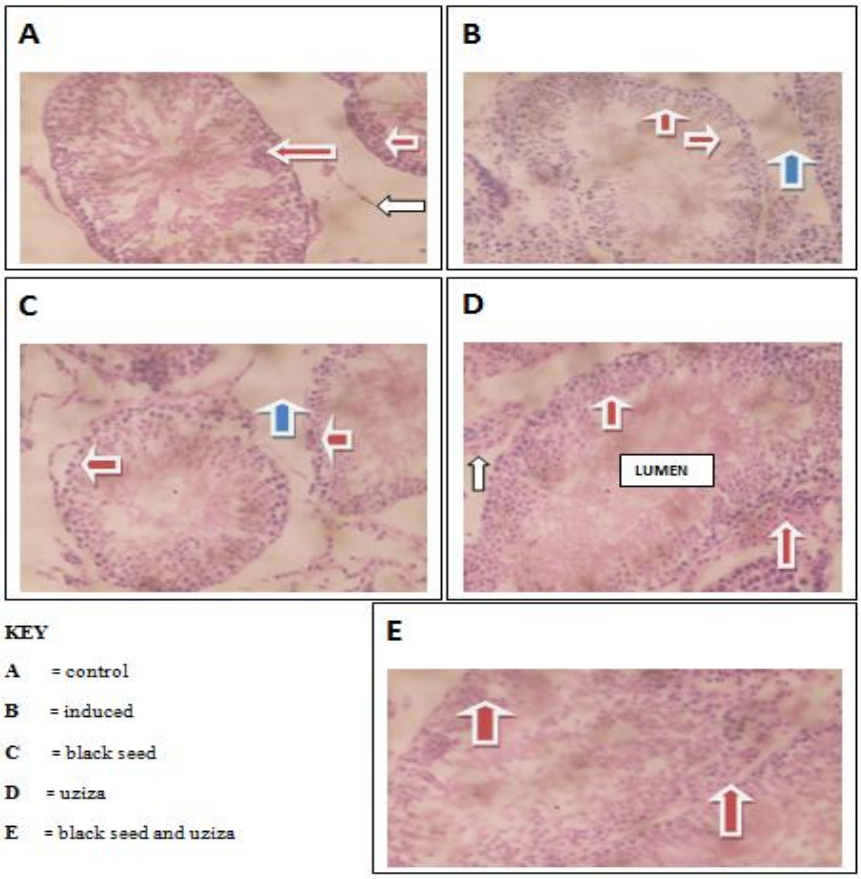

Plate 5. Photomicrographs of the testes stained H and E, magnification X400 (week two)

*P.guineense*anti obesity effect [10,18,19,]. Nevertheless, the body weight (125 g and 125 g) of the male and female wistar rats in *N. sativa* group were decreased as compared to the body weight (157 g and 126 g) of the male and female wistar rats in the *N.sativa* and *P.guineense* group which may results from.

Badary*et* al., [22] reported a decrease in the concentration of the total cholesterol, low density lipoprotein (LDL) and triglyceride levels accomplished by constant administration of *Nigella sativa.* Although low density lipoprotein (LDL) is a biomarker of cardiovascular disease while high density lipoprotein (HDL) is a the third week, Also, theextract had increasing effects on the Follicle Stimulating Hormone levels of both male and female. Histopathological findings also suggest that treatment with the aqueous extracts of *P. guineense* and *N .sativa* after inducement salvaged the testes and ovaries when compared to the induced group after the second week of administration. Although the combined mixture *N. sativa* and transporter of cholesterol in the body, when HDL content was high and LDL content was low, they were helpful in reducing the risk of cardiovascular disease [4].

From the results obtained for the hormone levels, it showed that the effect of aqueous extract of *Nigella sativa* and *Piper guineense*as a combined mixture produced an elevated level of testosterone which is more evident after the third week of administration. The results also revealed an efficiency of boosting the level of estrogen when *Nigella sativa* and *Piper guineense*were administered in the first week, which progressed to the second week but had a negative effect on *P.guineense* extract was more effective but the histological ovaries and testes section were mildly distorted when compared with that of control. The increased levels of FSH and Testosterone after the third week of treatment may result to an impaired spermatocytogenesis and spermiogenesis in the seminiferous tubules because FSH and Testosterone are responsible for spermatocytogenesis and

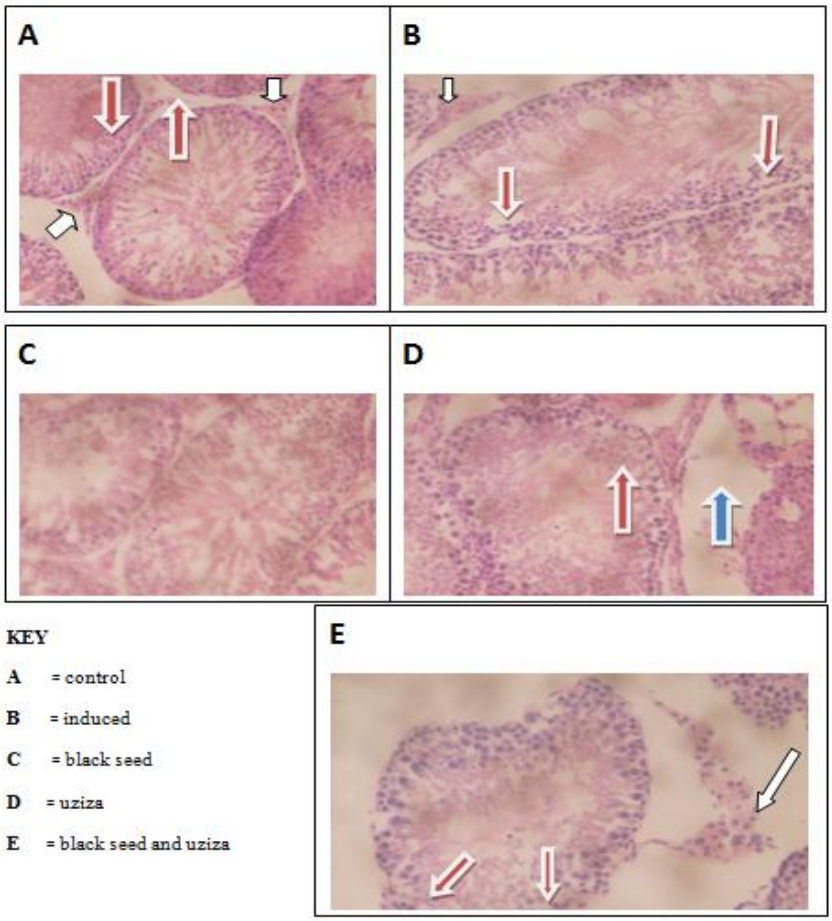

Plate 6. Photomicrographs of the testes stained H and E, magnification X400 (week three)

spermiogenesis, while Testosterone is responsible for epididymal function in sperm maturation [6,14].

The histological results of the extracts on Ovaries and testes of Wistar rats revealed that the effects of the treatments are more effective at the second week of administration. However, there was a significant difference between various groups and the control. Research proved that medicinal properties of *Piper guineense* are due to piperidine and piperine compound. Leaves have been useful in combating female infertility and to boost male fertility [23, 24]. According to Mbongue*et* al., [10] leaves of *P. guineense* have been used by traditional medical practitioners for the treatment of respiratory diseases and correction of female infertility problems, and the seeds as an aphrodisiac.

The effects of *P. guineense* on male reproductive parameters have been observed either by extraction of its active compound, its aqueous extraction or combining it with other herbs or plants. It is often used to improve libido. However, some reports stated that *P. guineense* induced adverse and detrimental effects on the testicular function and structure [25-28].

In the histological plates as shown above, the ovary in group A (control) had ovarian follicles in the cortical region with secondary follicle arrowed blue which indicates a normal histological ovarian section. Group B had ovarian cortex, follicles at different stages of development were seen (white) similar to control which indicates a normal histological ovarian section. Group C had ovarian cortex filled with follicles (white) at different stages of development, when compared to control which indicates a normal histological ovary with Hyperstimulation. Group D had an ovarian cortex showing follicles at different stages of development (white). A matured ovarian follicle arrowed red which indicates a

normal histological ovarian cortex. Group E had ovarian cortex filled with follicles (white) and matured ovarian follicles arrowed Red which indicates historical normal ovarian section.

Also the histological analysis showed that the testes in group A (control) had seminiferous tubules (red) containing spermatogenic cells (spermatogonia, primary and secondary spermatocytes, spermatids) and mature spermatozoa, arranged from basement membrane to luminal border respectively. The interstitial spaces containing leydig cells which indicates normal histological section. Group B had vacuolation of the seminiferious tubules (red) and a reduction in number of matured spermatozoa which indicates histological distorted testicular section. Group C had seminiferous tubules (red) containing decrease number of spermatozoa and spermatids when compared with the control which indicates a distorted histological testicular section. Group D had seminiferous tubules (red) containing decreased number of matured spermatozoa when compared to control. However, the reduction is not as much as those of group C. The interstitial spaces (white) had normal leydig cells which indicates mildly distorted testicular section. Group E had increased number of matured speematozoa on the luminal border when compared to groups B, C, D but disarray/disorganized matured spermatozoa and spermatogenic cells when compared to the control which indicates mildly distorted testicular section [29,30].

5. CONCLUSION

In conclusion, the results of the study indicate that the aqueous extract of combined mixture of *N. sativa* and *P.guineense* might be an effective plant in indigenous medicine, which maybe used to combat infertility caused by hormonal imbalance. All these properties can be attributed to the combination of certain compounds in *P.guineense* and *N.sativa.*

ETHICAL APPROVAL

This research work was carried out with the approval of the University of Port Harcourt research ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Luisi PL. About various definitions of life. Orig Life Evol Bios. 1998;28:613–622.
2. Chodasewicz K. Evolution, reproduction and definition of life. Theory in Biosci. 2014; 133(1):39–45.
3. Amaral S, Oliveira P, Ramalho-Santos J. Diabetes and the impairment of reproductive function: Possible role of mitochondria and reactive oxygen species. CurrDiab Rev. 2008;4(1):46-54.
4. Wong S, Linda LW, Rebecca L, Robker M, Jeremy GT, Melanie LS. Hyperglycaemia and lipid differentially impair mouse oocyte developmental competence, Reproduction, Fertility and Development. Ira J of Med Sci. 2015;27(4):583-592.
5. Schisterman E, Mumford S, Chen Z, Browne R, Boyd BD, Kim S, Buck LG. Lipid concentrations and semen quality: the life study. Androl. 2014;2(3):408-415.
6. Jianqing Z, Yousheng J, Jian Z, Bin W, Yin L, Zhaoqiong P, Daokui F, Bin L, Haiyan H, Cai H, Chunlei W, Feina L. Elevated body burdens of PBDEs, dioxins, and PCBs on thyroid hormone homeostasis at an electronic waste recycling site in China, Environ Sci and Tech. 2010;44(10):3956-3962.
7. Memudu A, Akinrinade I, Ogundele O, Dare B. Effects of crude extract of Dry fruits of Piper guineense on male fertility parameters of adult Sprague Dawley rats, Euro J of Med Pla. 2014;5(3):297.
8. Echo IA, Osuagwu AN, Agbor RB, Okpako EC, Ekanem BE. Phytochemical composition of *Aframomum melegueta* and *Piper guineense* seeds. World J of Appl Environ Chem. 2012;2(1):17-21.
9. Agbai EO, Onyebuagu PC, Ekezie J, Eke CC, Nwanegwo CO, Nwafor CA. *Piper guineense* leaf extract elevates serum follicle stimulating hormone level in the dietrus phase in non pregnant female albino Wistar rat. J of Complemen and Alterna Medical Res. 2017;2(4):1-8.
10. Mbongue FG, Kamtchouing P, Essame OJ, Yewah PM, Dimo T, Lontsi D. Effect of the aqueous extract of dry fruits of *Piper guineense* on the reproductive function of adult male rats. Ind J of Pharmacol. 2005; 37:30-32.
11. Mukhallad M, Mohamad MJ, Hatham D. Effects of black seeds (*Nigella sativa*) on spermatogenesis and fertility of male

albino rats. Res J of Med Sci. 2010;4:386-390.
12. Ekanem AP, Udoh FV, Oku EE. Effects of ethanol extract of *Piper guineense* seeds (Schum. and Thonn) on the conception of mice, Afri J of Pharm and Pharmacol. 2010; 4(6):362-367.
13. El-Ameen MH, Taha EM, Abdelwahab IS, Elfatih FK, Sheikh YB. Anti-diabetic properties of thymoquinone is unassociated with glycogen phosphorylase inhibition, Pharmacog J. 2015;7(6):406 - 410
14. Zhu ZP, Huang YF, Pan IJ, Xia YX. The effects of diabetic hyperlipidemia on the development of testes and penis in mole New Zealand rabbits, Zhong Nan Xu. 2005; 11:904-90
15. Bamosa A, Ali AB, Al-Hawsawi AZ. The effect of thymoquinone on blood lipids in rats, Ind J of Physiol and Pharmacol. 2002; 46(2):195-201.
16. Bucar J, Bruits M. Antioxidant activity of *Nigella sativa* essential oil, Phytotherma Resear. 2000;14:323-328.
17. Doubrian A, Davies M, Prewitt R, Lauteries T. Development of hypertension in a rat model of diet-induced obesity, Hypertension in a rat model of diet-induced obesity, Hyperten. 2000;35:1009.
18. Tietz NW. Clinical guide to laboratory tests. 3rd Ed. Philadelphia: W.B. Saunders, Co. 1995;405.
19. Uotila M, Ruoslathi E, Envall E. Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha fetoprotein. J Immunol Methods. 1981; 42:11-15.
20. Hall PF. Testicular steroid synthesis: Organization and regulation. In: Knobil E, Neill JD, Editors. The Physiology of Reproduction. Raven Press, New York. 1988;975-98.
21. Ratcliffe WA, Carter GD, Dowsett M, Hillier SG, Middle JG, Reed MJ. Oestradiol assays: Applications and guidelines for the provision of a clinical biochemistry service. Ann Clin Biochem. 1988;25:466-83.
22. Badary OA, Abdel-Naim BA, Abdel-Wahab MH, Hamada FM. The influence of thymoquinone on doxorubicin-induced hyperlipidemia nephropathy in rats. Toxicol. 2000;143(3):219-226.
23. Al-Audi JA, Al-Khuzai DA, Al-Aydi FN. Effect of alcoholic extract of *Nigella sativa* on fertility in male rats, Iraq J of Vet Sci. 2009;23(2):123-128.
24. Mouafo ET, Tulin A, Victor K, Augustin EN, Barthelemy N. Antibacterial activity of selected Cameroonian dietary spices ethno medicinally used against strains of mycobacterium tuberculosis. J of Ethnopharmacol. 2012;142(2):374-382.
25. Klin KD, Barimalaa I, Achinewhu SC. Adeniji TA. Effects of extracts from 3 indigenous spices on the chemical stability of smoked dried catfish, (*Clariaslazera*) during storage. Afri J of Food Agric. Nutri and Develop. 2011;11(6):72-85.
26. Manta S, Saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants, J of Pharmacog and Phytochem. 2013;1(6):168-182.
27. Okoye EI, Ebeledike AO. Phytochemical constituents of *Piper guineense*(uziza) and their health implications on some microorganisms. Glo Resear J Sci. 2013; 2(2):42-46.
28. Okwu DE. Evaluation of the chemical composition of indigenous species and flavoring agents. Glo J Pure and Appl Sci. 2001;7:455-459.
29. Egba SI, Sunday GI, Anaduaka EG. The effect of oral administration of aqueous extract of *Newbouldia laevis* on fertility hormones of male albino rats. J of Pharm and Bio Sci. 2014;9(3):61 -64.
30. Ukoha U, Egwu OA, Dimkpa U, Maduka SO, Okafor JI, Ndukwe GU. Histological and weight changes in testes of male albino rats fed with diets containing yaji (A local meat sauce). Inter J of Health Sci and Resear. 2014;4(8):118- 126.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/47857