

# HISTOLOGICAL ANALYSIS OF AQUEOUS EXTRACT OF *NIGELLA SATIVA* AND *PIPER GUINEENSE* ON REPRODUCTIVE HORMONES OF WISTAR RATS.

## 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 method:** Thirty male and female Wistar rats of average weight (100g) 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 uziza group were treated with 2ml of uziza aqueous leaf extract, while the rats in the black seed group were treated with 2ml of black seed aqueous extract. The rats in the black seed & uziza leaf group were treated with 2ml 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 to the control ( $p=0.05$ ). The testosterone levels (mIU/ml) showed for the, positive control ( $2.28 \pm 0.08$ ), uziza leaf ( $6.45 \pm 0.01$ ), black seed ( $2.85 \pm 0.01$ ), black seed & uziza ( $8.47 \pm 0.08$ ). The increase for estrogen levels (mIU/ml) showed for the positive control ( $99 \pm 0.82$ ), uziza leaf ( $98 \pm 1.63$ ), black seed ( $100 \pm 1.63$ ), black seed & Uziza ( $113 \pm 0.82$ ). 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 (mIU/ml)) showed for the positive control ( $0.23 \pm 0.01$ ), uziza leaf  $0.25 \pm 0.01$ ), black seed ( $0.54 \pm 0.02$ ), black seed & uziza ( $0.58 \pm 0.01$ ).

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. 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 dyspareunia [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 as influencing the increase in steroidogenic pathways that mediated testosterone production [7, 8].

Agbai *et al.* [9] also reported that the aqueous extract of *P.guineense* leaf was capable of influencing follicle stimulating hormone production during diestrus 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 *et al.*, [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 METHOD

### 2.1.Experimental Animals

Thirty (30) Wistar rats (15 males and 15 females) weighing about 100g were purchased from Choba 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.

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176 **Preparation of Uziza leaf (*Piper guineense*) extract.**

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

180 **Preparation of Black seed (*Nigella Sativa*)**

181 One hundred grams (100g) of black seed was broken down to a smooth powder with a blender,  
182 50g of the seed powder was weighed and submerged in 500ml of distilled water. The mixture  
183 was stirred properly and allowed to stand for an hour, after which, it was stirred and kept in an  
184 air tight container.

185 **Administration of the Treatment**

186 **Group 1** control group

187 **Group 2** Induced without treatment

188 **Group 3** Treated with the aqueous extract of *Nigella sativa* with a dose of 2ml/kg body  
189 weight respectively

190 **Group 4** Treated with the aqueous extract of *Piper guineense* with a dose of 2ml/kg  
191 body weight respectively

192 **Group 5** Treated with the mixture (1ml each of *Nigella sativa* extract and *Piper*  
193 *guineense* extract) with a dose of 2ml/kg body weight respectively.

194 Administration of extract was carried out orally by the use of syringes in order to determine the  
195 actual dose. The animals received their doses seven (7) times weekly for a period of three (3)  
196 weeks. At the end of each week, the rats from each group was sacrificed through the means of  
197 cervical strangulation and blood samples were collected. Testosterone level, Follicle stimulating  
198 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].

#### **2.5.: 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 et al., [19] was used for follicle stimulating hormones, and the method of Hall [20] and Ratcliffe et al., [21] was used for estrogen.

#### **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  $\pm$  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. RESULT S

**TABLE 2: Pilot Study Result on Lipid Parameters**

TEST	NON INDUCED	INDUCED
CHL(mmol/L)	$6.28 \pm 0.0002$	$11.75 \pm 0.0016$
HDL(mmol/L)	$3.93 \pm 0.0006$	$8.33 \pm 0.0009$
TRI( mmol/L)	$2.48 \pm 0.3600$	$4.05 \pm 0.0016$
LDL( mmol/L)	$11.33 \pm 0.0002$	$21.92 \pm 0.0024$
VLDL (mmol/L)	$1.12 \pm 0.0002$	$1.82 \pm 0.0007$

Results are expressed as Mean  $\pm$  Standard Deviation, n=3, CHL=Total Cholesterol, HDL= High Density Lipoprotein, TRI= Triglyceride, LDL= Low Density Lipoprotein, VLDL= Very Low Density Lipoprotein.

**TABLE 3: 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	143	186	179	232
Induced	143	250	230	166	186
<i>N.sativa</i>	96	200	125	195	180
<i>P.guineense</i>	60	200	179	189	158
<i>N.sativa</i> And	108	198	157	163	175

<i>P.guineense</i>					
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**TABLE 4: Body Weight Result for Female Albino Rats**

<b>Group</b>	<b>Before Inducement (g)</b>	<b>After Inducement (g)</b>	<b>After one week of treatment (g)</b>	<b>After Second week of Treatment (g)</b>	<b>After third week of treatment (g)</b>
Control	73	143	135	156	167
Induced	143	250	170	178	198
<i>N.sativa</i>	111	200	125	166	210
<i>P.guineense</i>	87	200	138	136	143

<i>N.sativa</i>					
And	101	198	126	132	176
<i>P.guineense</i>					

**TABLE 5: 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 (mlU/ml)	After Second week of Treatment (mlU/ml)	After third week of treatment(mlU/ml)
Control	2.10 ± 0 . 0 8	2.27 ± 0 . 0 8	2.28± 0 . 0 8
Induced	1.90 ± 0 . 0 8	1.02 ± 0 . 0 1	2.00± 0 . 0 8
<i>N.sativa</i>	2.00 ± 0 . 0 8	2.52± 0 . 0 1	2.85± 0 . 0 1

<i>P.guineense</i>	3.02± 0 . 0 1 <sup>a</sup>	4.98± 0 . 0 1 <sup>b</sup>	6.45± 0 . 0 1 <sup>c</sup>
<i>N.sativa</i> And <i>P.guineense</i>	4.07± 0 . 0 1 <sup>a</sup>	6.50± 0 . 0 1 <sup>b</sup>	8.47± 0 . 0 8 <sup>c</sup>

280 Results are means of three determinations ± standard deviation.

281 <sup>abc</sup> Different letters in a given row denote significant difference, p=0.05.

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289 **TABLE 6: Effect of first, second & third week oral administration of Uziza leaf and black seed on**  
290 **Estrogen level of female Wistar rats.**

<b>Group</b>	<b>After one week of treatment (mIU/ml)</b>	<b>After Second week of Treatment (mIU/ml)</b>	<b>After third week of treatment (mIU/ml)</b>
Control	93 ± 0 . 8 2	99 ± 0 . 0 8 2	99 ± 0 . 8 2
Induced	99 ± 0 . 8 2	102 ± 0 . 8 2	98 ± 0 . 8 2
<i>N.sativa</i>	98± 1 . 6 3 <sup>a</sup>	94± 0 . 1 . 6 3 <sup>b</sup>	98 ± 1 . 6 3 <sup>c</sup>

<i>P.guineense</i>	$98 \pm 1.63^a$	$99 \pm 1.63^b$	$100 \pm 1.63^c$
<i>N.sativa</i>			
And	$101 \pm 0.82$	$112 \pm 0.82$	$113 \pm 0.82$
<i>P.guineense</i>			

291 Results are means of three determinations  $\pm$  standard deviation.

292 <sup>abc</sup> Different letters in a given row denote significant difference,  $p=0.05$ .

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299 **TABLE 7: Effect of first, second & third week oral administration of Uziza leaf and black seed on**  
300 **Follicle Stimulating Hormone 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	$0.22 \pm 0.01$	$0.23 \pm 0.01$	$0.23 \pm 0.01$
Induced	$0.18 \pm 0.01$	$0.28 \pm 0.01$	$0.22 \pm 0.01$

<i>N.sativa</i>	0.51± 0 . 0 2 <sup>a</sup>	0.53± 0 . 0 2 <sup>b</sup>	0.54± 0 . 0 2 <sup>c</sup>
<i>P.guineense</i>	0.23± 0 . 0 1 <sup>a</sup>	0.24± 0 . 0 1 <sup>b</sup>	0.25± 0 . 0 1 <sup>c</sup>
<i>N.sativa</i> And <i>P.guineense</i>	0.55± 0 . 0 1 <sup>a</sup>	0.56± 0 . 0 1 <sup>b</sup>	0.58± 0 . 0 1 <sup>c</sup>

301 Results are means of three determinations ± standard deviation.

302 <sup>abc</sup> Different letters in a given row denote significant difference, p=0.05.

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306 **TABLE 8: Effect of first, second & third week oral administration of Uziza leaf and black seed on**  
307 **follicle stimulating hormone level of female Wistar rat**

<b>Group</b>	<b>After one week of treatment (mIU/ml)</b>	<b>After Second week of Treatment (mIU/ml)</b>	<b>After third week of treatment (mIU/ml)</b>
Control	0.75 ± 0 . 0 1	0.10 ± 0 . 0 1	0.21± 0 . 0 1

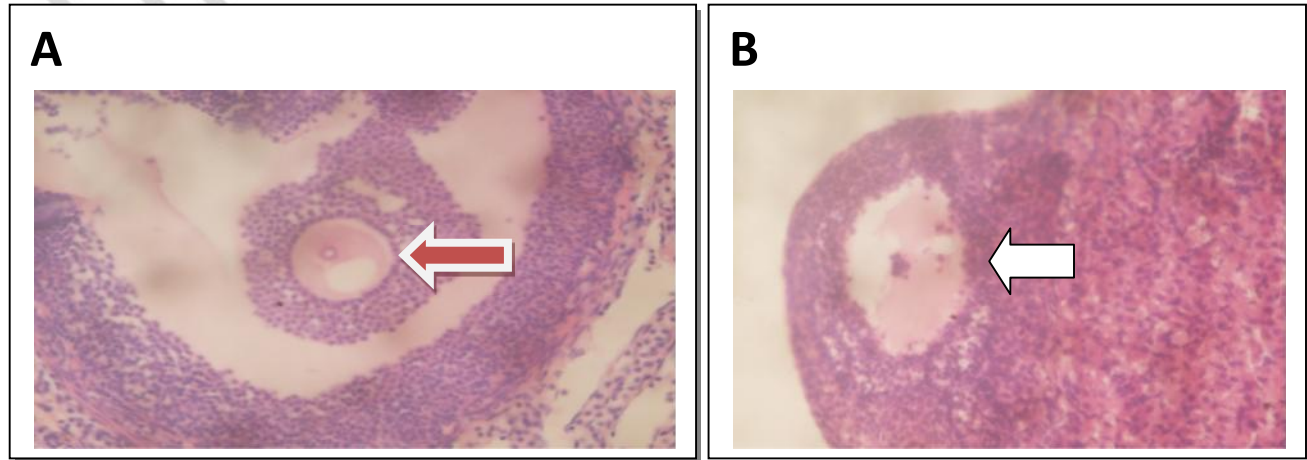


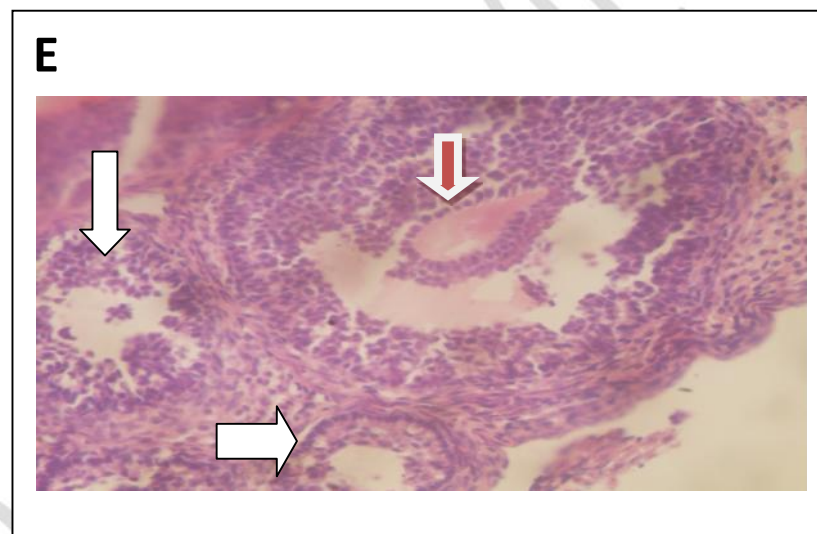
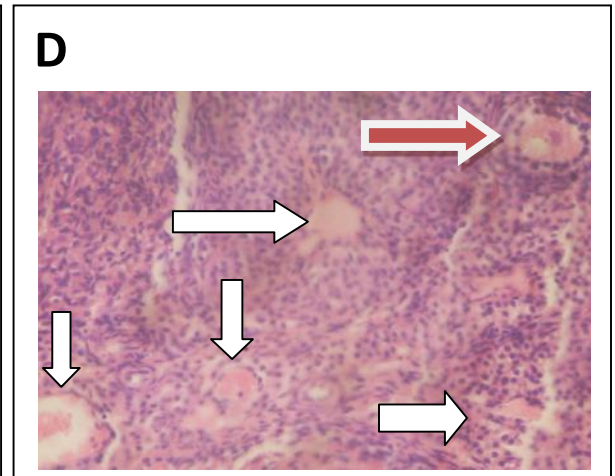
Induced	$0.10 \pm 0.01$	$0.31 \pm 0.01$	$0.22 \pm 0.01$
<i>N.sativa</i>	$0.20 \pm 0.02^a$	$0.33 \pm 0.02^c$	$0.24 \pm 0.02^b$
<i>P.guineense</i>	$0.25 \pm 0.01^a$	$0.69 \pm 0.01^c$	$0.19 \pm 0.01^a$
<i>N.sativa</i> And <i>P.guineense</i>	$0.16 \pm 0.01^a$	$0.11 \pm 0.01^b$	$0.99 \pm 0.01^c$

Results are means of three determinations  $\pm$  standard deviation.

<sup>abc</sup> Different letters in a given row denote significant difference,  $p=0.05$ .

**Plate 1: Photomicrographs of the ovaries stained H and E, magnification X400 (Week One)**



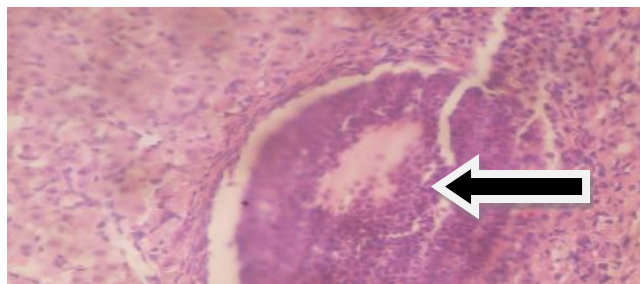


**KEY**

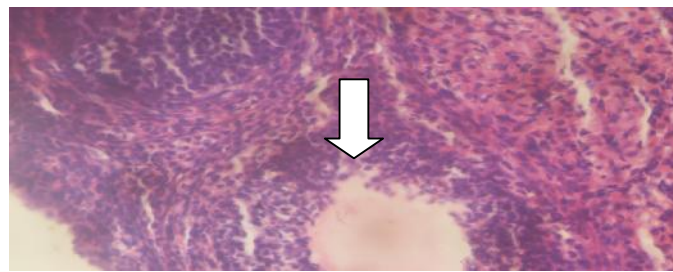
- A** = control
- B** = induced
- C** = black seed
- D** = uziza
- E** = black seed and uziza

**Plate 2: Photomicrographs of the ovaries stained H and E, magnification X400 (Week two)**

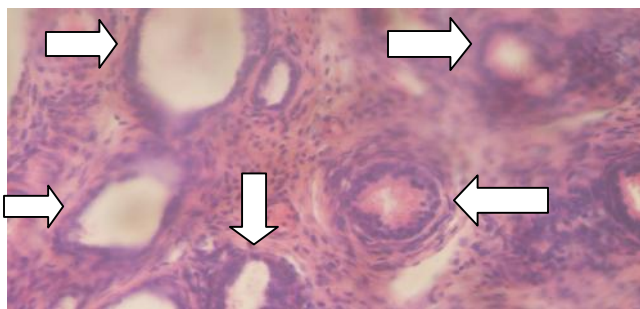
**A**



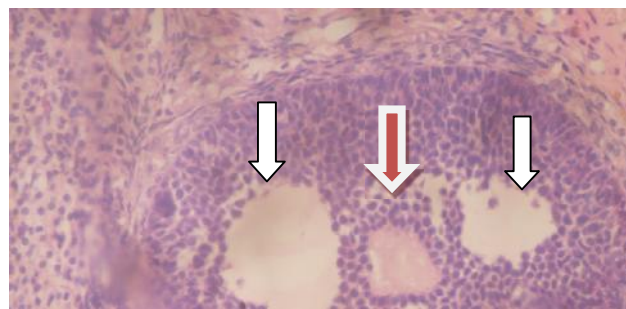
**B**



**C**



**D**



**KEY**

**A** = control

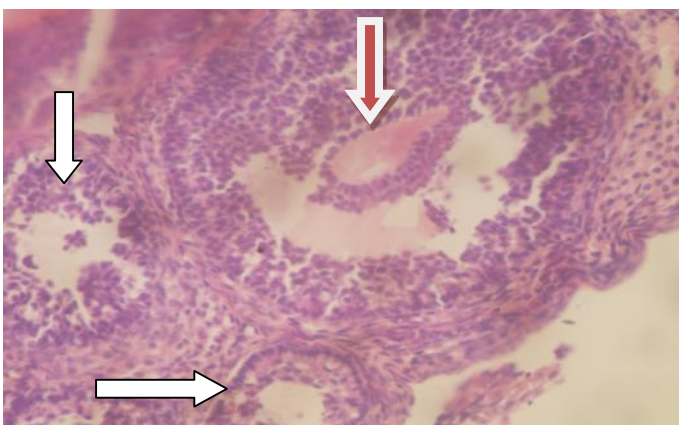
**B** = induced

**C** = black seed

**D** = uziza

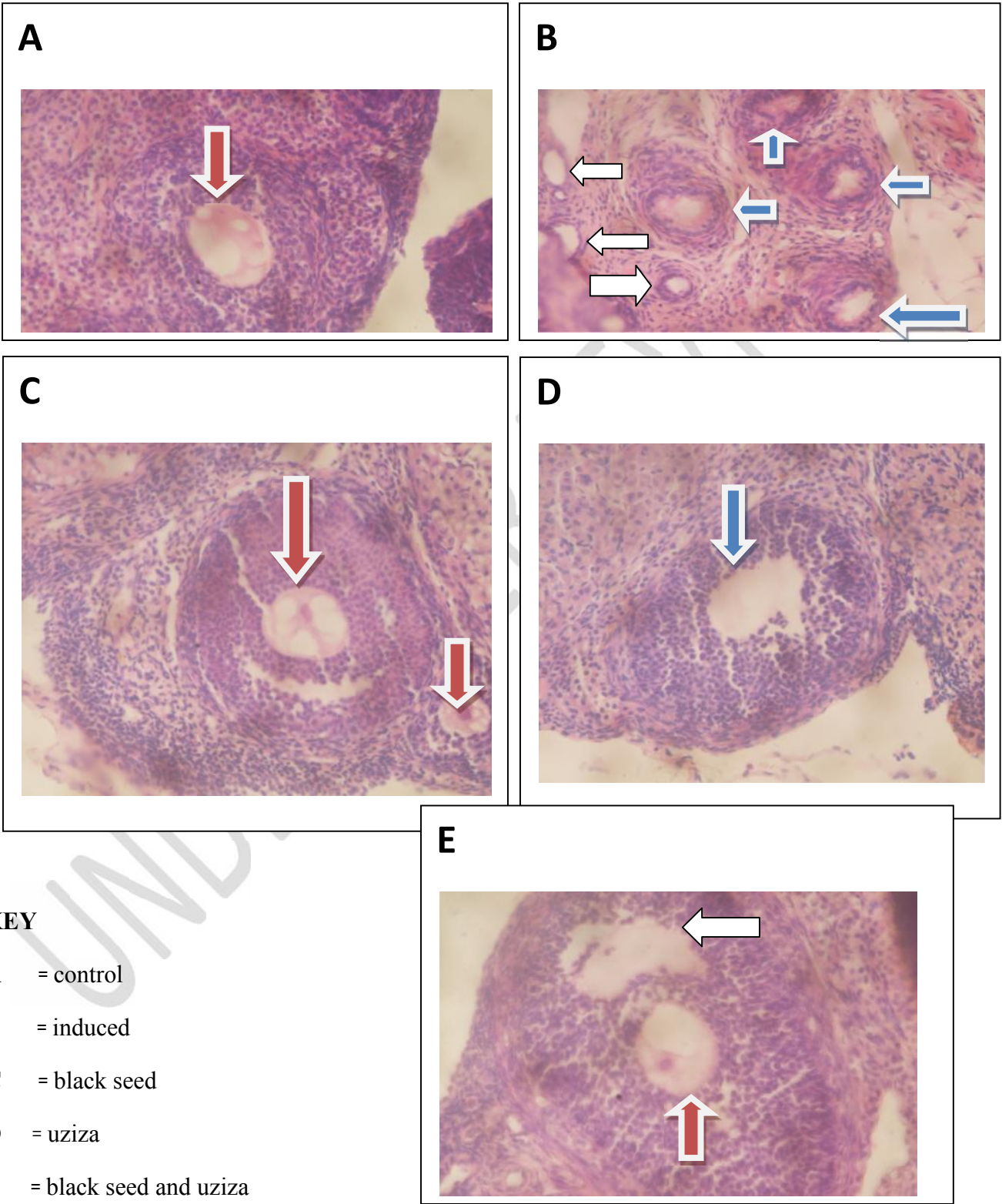
**E** = black seed and uziza

**E**

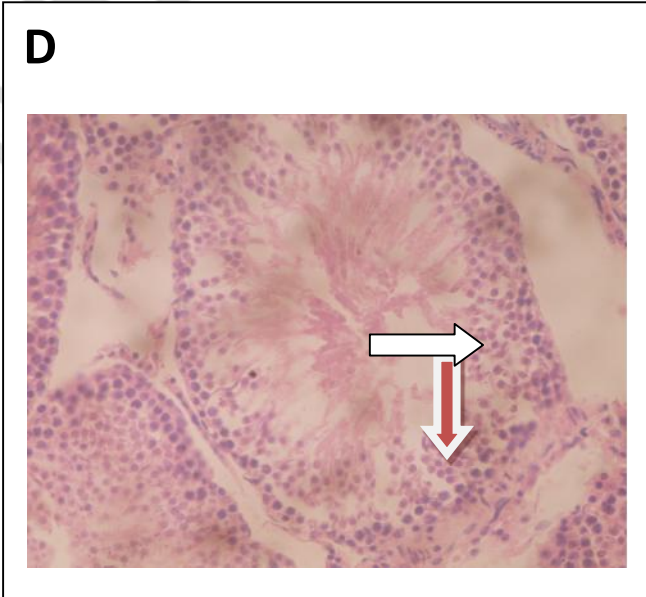
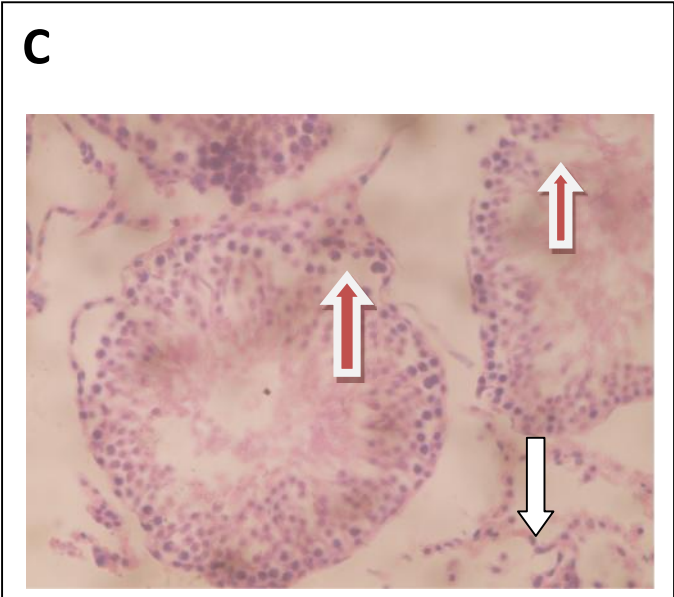
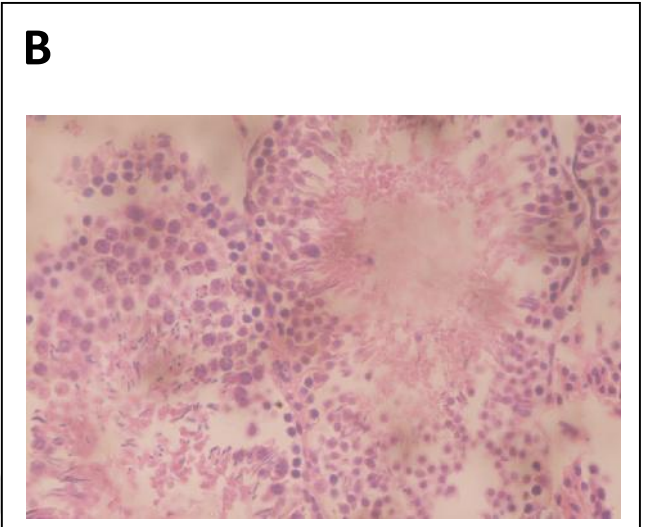
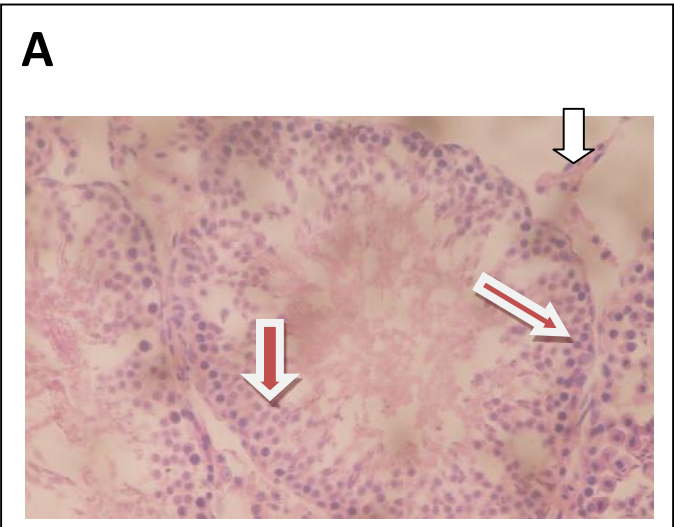




**Plate 3: Photomicrographs of the ovaries stained H and E, magnification X400 (Week three)**

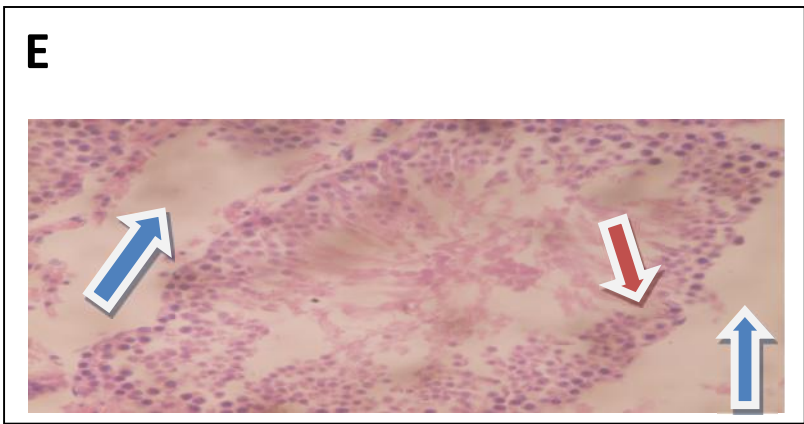


**Plate 4: Photomicrographs of the testes stained H and E, magnification X400 (Week one)**



**KEY**

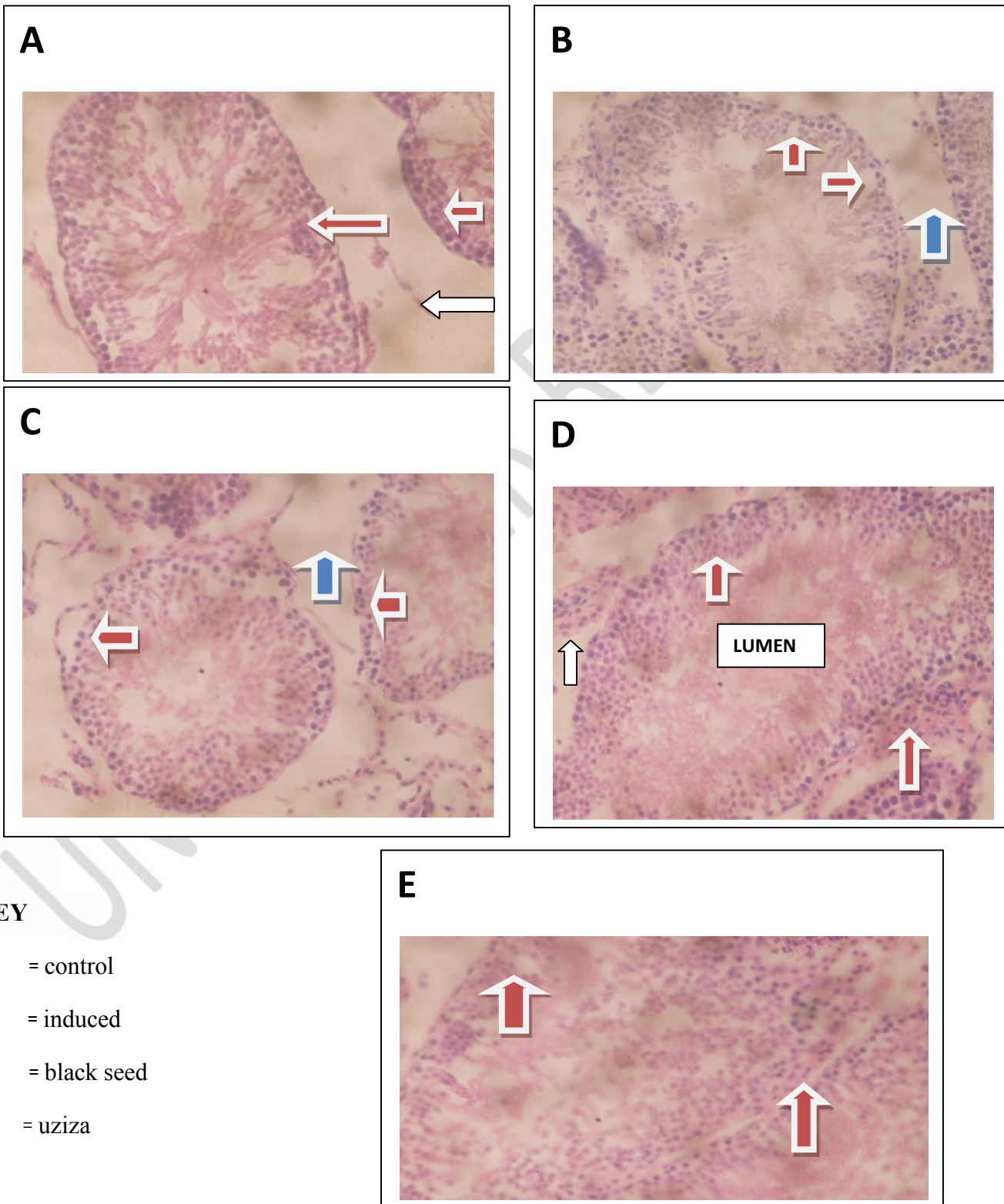
- A** = control  
**B** = induced  
**C** = black seed  
**D** = uziza





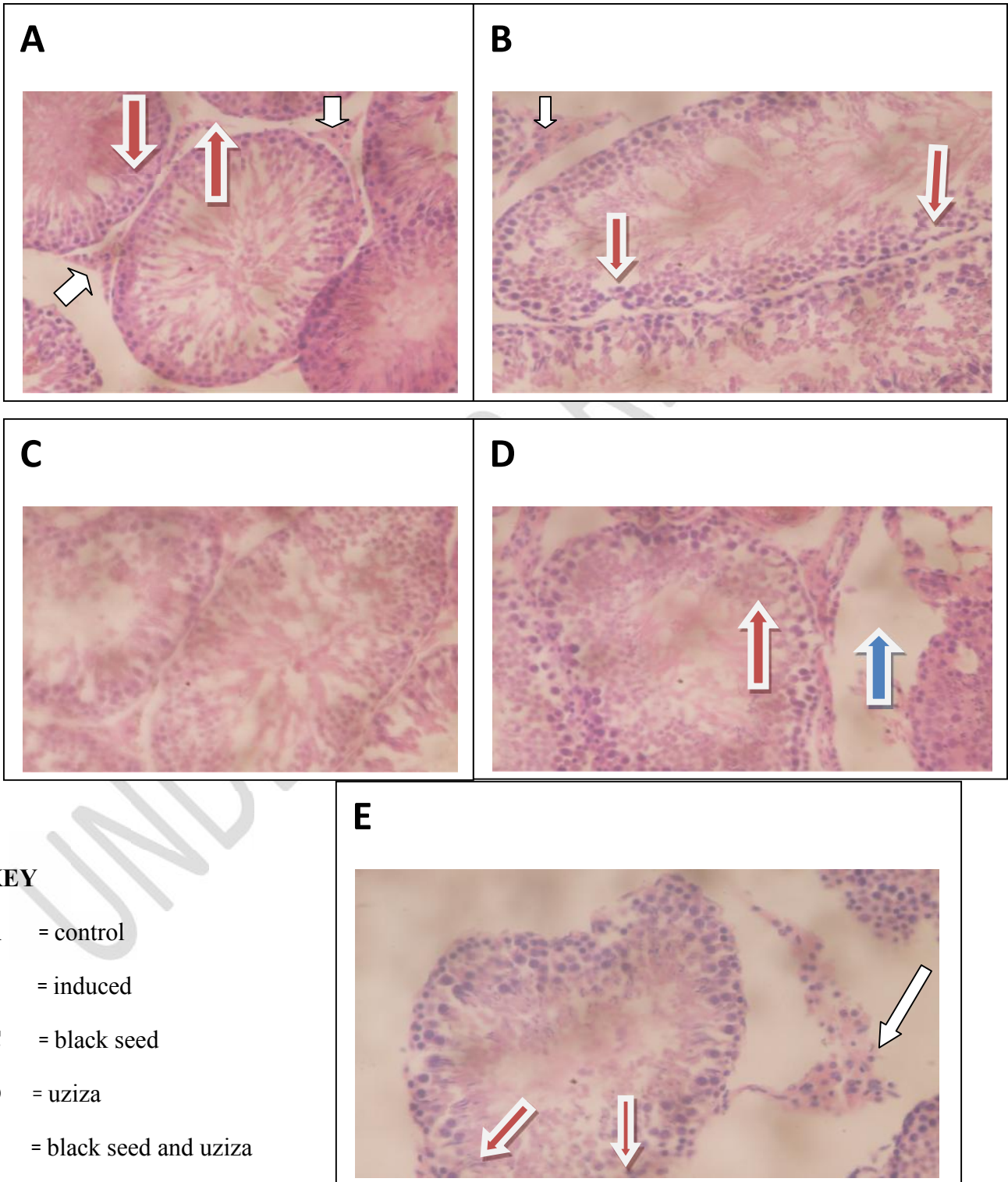
**E** = black seed and uziza

**Plate 5: Photomicrographs of the testes stained H and E, magnification X400 (Week two)**



**E** = black seed and uziza

**Plate 6: Photomicrographs of the testes 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. The lipid parameters such as cholesterol level, HDL level, triglyceride level, LDL level and VDL level of the wistar rat in the induced group were  $11.75 \pm 0.0016$ ,  $8.33 \pm 0.0009$ ,  $4.05 \pm 0.0024$  and  $1.82 \pm 0.0007$  respectively while those in non induced group were  $6.28 \pm 0.0002$ ,  $3.93 \pm 0.0006$ ,  $2.48 \pm 0.36$ ,  $11.33 \pm 0.0002$  and  $1.12 \pm 0.0002$ .

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].

The increase of HDL was in contrast with the findings of Doubrain *et al.*, [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 (125g and 125g) of the male and female wistar rats in the group treated with 2ml of aqueous extract of *N. sativa* and the body weight (157g and 126g) 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 (125g and 125g) of the male and female wistar rats in the *N.sativa* group and the body weight (157g and 126g) of the male and female wistar rats in the *N.sativa* and *P.guineense* group were decreased as compared to the body weight (186g and 135g) of the male and female wistar rats in the control. This decrease maybe associated to the *N.sativa* and *P.guineense* anti obesity effect [10, 18, 19,]. Nevertheless, the body weight (125g and 125g) of the male and female wistar rats in *N. sativa* group were decreased as compared to the body weight (157g and 126g) 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 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 the third week, Also, the extract 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 *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

530 Testosterone are responsible for spermatocytogenesis and spermiogenesis, while Testosterone is  
531 responsible for epididymal function in sperm maturation [6, 14].

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

540 The effects of *P. guineense* on male reproductive parameters have been observed either by  
541 extraction of its active compound, its aqueous extraction or combining it with other herbs or  
542 plants. It is often used to improve libido. However, some reports stated that *P. guineense* induced  
543 adverse and detrimental effects on the testicular function and structure (25, 26, 27, 28].

544 In the histological plates as shown above, the ovary in group A (control) had ovarian follicles in  
545 the cortical region with secondary follicle arrowed blue which indicates a normal histological  
546 ovarian section. Group B had ovarian cortex, follicles at different stages of development were  
547 seen (white) similar to control which indicates a normal histological ovarian section. Group C  
548 had ovarian cortex filled with follicles (white) at different stages of development, when  
549 compared to control which indicates a normal histological ovary with Hyperstimulation. Group  
550 D had an ovarian cortex showing follicles at different stages of development (white). A matured  
551 ovarian follicle arrowed red which indicates a normal histological ovarian cortex. Group E had  
552 ovarian cortex filled with follicles (white) and matured ovarian follicles arrowed Red which  
553 indicates historical normal ovarian section.

554 Also the histological analysis showed that the testes in group A (control) had seminiferous  
555 tubules (red) containing spermatogenic cells (spermatogonia, primary and secondary  
556 spermatocytes, spermatids) and mature spermatozoa, arranged from basement membrane to  
557 luminal border respectively. The interstitial spaces containing leydig cells which indicates  
558 normal histological section. Group B had vacuolation of the seminiferous 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 may be 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*.

## Competing Interests

Authors have declared that no competing interests exist.

## 6. ETHICAL APPROVAL:

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

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