Aphrodisiac Effect of Peanut Extract in Male

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Article

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Abstract

Peanut is a legume and contains L-3, 4-Phenyl Alanine which is a precursor for Dopamine. Dopamine is a prolactin inhibitor. A slight increase in prolactin leads to infertility.

Objective: To investigate the effect of peanut extract on the reproductive performance of males.

Methods: Thirty two adult albino rats comprising of 8 males and 24 females were used. The 8 males were divided into two groups (A and B) of 4 rats each. Group A (control) was given 2ml/kg of distilled water (DW), B was given 800mg/kg of Peanut Aqueous Extract (PAE) for 30 days. At day 21, 3 females were introduced to each male for impregnation. At day 31, the males were sacrificed. Blood was collected for hematology and serology. The females carried their pregnancy to term.

Result: PAE treated male rats had significant ($P \leq 0.05$) increase in testosterone, FSH and LH secretions. There was significant ($P \leq 0.05$) increase in sperm concentration in PAE treated males than in control. Fertility indices showed that PAE treated male rats' had 75% impregnation success while DW treated males had 42%. The results confirmed PAE as an aphrodisiac for male and for the treatment of hyperprolactinemia-induced-infertility and early stages of Parkinson's disease.

1.0. Introduction

Reproductive hormones seem to be grossly affected by nutrition. The consumption of some plants or plant products such as peanuts seem to boost the hormone profile of some vertebrates [1].

Vertebrate reproduction is usually sexual. Sexual reproduction is the joining of gametes during fertilization to produce genetically viable offspring. Terrestrial species such as rats and primates have internal fertilization. They are viviparous; the embryo develop and are nourished within the mother's body [2].

Prolactin is secreted in both male and females. The basal serum prolactin concentration is increased by increased duration of daylight (photoperiod) with normal secretion during late spring and early summer [3] [4].

The normal plasma prolactin concentration is approximately 5–20µg/ml in males and 8–25µg/ml in female primates [5]. Hyperprolactinemia suppresses Gonadotropin Releasing Hormone (GnRH), causing a decrease in Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) ultimately leading to decrease in serum testosterone levels and hypogonadism [6] [7].

1.1. Dopamine as a prolactin Inhibitor

Dopamine belong to a class of neurotransmitters known as catecholamine. Structurally, Dopamine has a catechol ring and an amine side chain. Dopamine is synthesized primarily in the Central Nervous System (CNS) and Adrenal Medulla through the electron and enzymatic activation of Tyrosine to L-3, 4-

Dihydroxyphenylalanine (L-DOPA) [8]. The L-DOPA is catalyzed by DOPA decarboxylase to Dopamine and carbon dioxide. The site of action of Dopamine is the pituitary. There, Dopamine inhibits Prolactin Releasing Lactotroph (PRL) by controlling calcium fluxes [9].

In fact Dopamine and Prolactin maintain homeostasis in the body. Unfortunately, the presence of some drugs or stress conditions, or even prolonged fasting of persons or animals easily block Dopamine release from the hypothalamus. When this happens, there is hyperprolactinemia which cannot easily be reversed back at the same ease with which it was formed [10].

Most of the conventional drugs (Dopamine agonists) used to treat hyperprolactinemia induced infertility as well as Parkinson's disease leaves the patience with schizophrenia as side effect [11]. Therefore peanut which does not have any such side effect could be a better option for the treatment of hyperprolactinemia induced infertility and Parkinson's disease.

Peanuts (*Arachis hypogaea)* is a species in the legume fabaceae native to South America, Mexico and Central America [12]. Peanut plant is a woody, indehiscent legume and not a nut. The word pea describes the edible seeds of many other legumes in the fabaceae family, in that sense peanut is a kind of pea [13].

Peanut is rich in protein, carbohydrate, fats, minerals and vitamins. In fact, United States' agricultural data base 2009 [14] revealed that 100g of raw Valencia variety of peanuts contains: Thiamin (Vit B_1) 0.6mg, Riboflavin (Vit B_2) 0.3mg, Niacin (Vit.B_3) 12.9mg, Pantothenic acid (B_5) 1.8mg, Vitamin (B_6) 0.3mg, Folate vit (B_9) 246µg. It does not contain Vitamin C. One hundred grams (100g) of raw Valencia also contain the following minerals: Calcium 62mg, Iron 2 mg, Magnesium184 mg, Phosphorus 332mg and Zinc 3.3mg.

Furthermore, USDA 2014 [15] revealed that peanut all type contain in addition; omega 3 fatty acids, omega 6 fatty acids, potassium; 1029mg which is 29% of daily requirements, very little sodium ;26.3mg which 1% of daily requirements and manganese; 2.8mg which is 141% of daily requirements.

Potassium maintains the cells at normal tone. Therefore peanut being rich in potassium is an indication of its effect in maintaining cardiac tone. In other words peanut is anti-hypertensive in activity [16].

Peanut being rich in manganese is an indication that peanut consumption leads to enhanced metabolism of glucose and lipids. Manganese is an essential element in the body mainly obtained from food and water. Manganese is involved in the metabolism of glucose and lipids. It also activates antioxidants [17]. Manganese absorbed in diets is less than 4%. Deficiency of manganese can cause skeletal abnormalities, depressed reproduction and neonatal abnormalities. Manganese toxicity is not likely to occur since toxicity can only occur at values greater than 1000 mg/kg body weight [18].

However, peanut seed coat should be avoided since it causes hypertrophy and hyperplasia of the thyroid gland [19].

1.2. Distribution of Peanuts

Peanut is world -wide in distribution. It is an annual herbaceous plant growing to 30–50cm (1–1.5ft) tall. The leaves are opposite, pinnate with four leaflets (two opposite pairs; no terminal leaflet). Each leaflet is 1–7cm long and 1–3cm broad. The flowers are a typical pea flower in shape, 2–4 cm across, yellow with reddish veining. After pollination, the fruit develops into a legume 3–7cm long containing 1–4 seeds, which forces its way underground to mature [20].

The plants name derives from a combination of the morphemes pea and nut, causing some confusion as to the nature of the fruit. The word pea describes the edible seeds of many other legumes in the fabaceae family and in that sense peanut is a kind of pea. Although peanut is not a nut, in the culinary arts peanuts are utilized similarly to nuts [21].

Peanut and its products are grossly consumed by humans and animals. This research was designed to investigate the effect of peanuts in male reproduction using albino rats. To do this, libido, gonadosomatic index, analysis of spermatozoa, hormonal profile, hematology, histology of the testis, hypothalamus and pituitary of the animals treated were considered necessary.

All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) FV-U -IACUC-2020-0262 of University of Nigeria, Nsukka and the approved guideline stipulates alleviation of pain in animals used for research purposes.

2.0. Materials And Methods

Peanut was purchase from Orie Ugba market in Umuahia North and taken to the Department of Botany, University of Nigeria Nsukka and was identified as Valencia variety of peanuts (Fig. 1). Corona glass jar blender (Linea 018000 9 47203, Columbia) was used for the maceration of the sample while Mounilex sieving cloth was used for the aqueous extraction. Lyophilization of the extract was done using a freeze dryer (Christ; Alpha1-2 LDplus 1.5, serial No: 16508: Germany) and the sample was labelled Peanut Aqueous Extract (PAE) and stored in a freezer at -4^0 C to be used when needed.

2.1. Toxicity testing and minimum effective dose of PAE

These were done according to the method described by Nwankudu et al., (2020) [22]. Thirty albino rats grouped into six of 5 rats per group were used for the toxicity testing. Group 1 was control given 2ml/kg of DW while groups 2–6 were given PAE in the following order: 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg and 5000mg/kg.

Minimum effective dose was done due to the fact that there was no toxicity observed in PAE treated rats. The minimum effective dose was done using 15 mature female rats grouped into five of 3 rats per group. The animals were fasted for 12 hours. Group 1 was control and given 2ml/kg of DW. Groups 2–5 were treated with graded doses of PAE; 200, 400, 800 and 1,600 mg/kg. The experiment was replicated. The group that came to estrus within 24 hours showed a positive response to the treatment.

2.2. Peanut aqueous extract on rabbit jejunum

The effect of PAE on rabbit jejunum was done according to the method described by Ijioma et al., (2014) [23]. The rabbits were starved for 24 hours before treatment. The rabbits were sacrificed by stunning to reduce pain. Exsanguination was done and the jejunum exteriorized and isolated. The isolated jejunum was put into a beaker containing Tyrode solution that was continuously bubbled with Oxygen (95%) and Carbon iv oxide (5%) and maintained at 37^0 C with pH value of 7.4. About 2-3cm of the isolated jejunum was cut and suspended vertically in a 35m ml organ bath by means of ligature attached at one end to a tissue holder and to the other end to an isometric force displacement transducer attached to a Physiograph (Medicaid Physiopac, India). The Physiograph was connected to a computer screen for displaying isometric contractions. Resting tensions on the muscle strip was readjusted to remove slack and preparation was allowed to equilibrate for 20 minutes. Graded doses of PAE were administered. Thirty seconds was allowed for tissue response before being washed 2–3 times with Tyrode solution after each dose.

2.3. Peanut aqueous extract on uterine smooth muscle

This was done according to the method described by Nwankudu et al., (2014) [24] but with little modification. Matured non pregnant female rats were primed 24 hours before the experiment with stilbesterol (0.1mg/kg). They were sacrificed using mild ether for sedation and cervical dislocation to eliminate pain. They were put in dissecting board and four pins were used to fasten each one to the board. Evisceration of the lower abdomen to carefully expose and isolate the two uterine horns was done. The isolated horns were transferred into De Jalon (Physiological saline) solution that was continuously bubbled with air. The physiological saline was maintained at 37^0C (pH 7.4). One horn at a time was trimmed of fatty tissue and 2–3 cm cut out and mounted in a 35ml organ bath containing De Jalon solution. The mounted tissue was allowed to equilibrate for 30 minutes after which dose response relationships were established.

2.4. In-vivo experimentation

Thirty two albino rats comprising of 8 males of 14 weeks of age weighing between 215-294g and 24 females of 12 weeks of age weighing between 146-198g were used. The rats were procured from the animal house of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike (MOUAU). The rats were kept in standard cages and fed Topfeed chick marsh ad libitum. The rat had access to clean water. The 8 males were divided into two groups of 4 rats per group. Group A served as control and was treated with 2ml/kg DW but group B was given 800mg/kg PAE. The female rats were divided into two groups of 12 rats per group (groups C and D) and housed in larger cages. The females were not treated. The treatments of the males were done through the oral rout for 30 days. However at day 21 of treatment, 3 females picked at random from group C were introduced into each cage containing the group ‘A’ male while 3 females picked at random were introduced to the cages where group ‘B’ males were housed for impregnation. Mating was confirmed through vaginal cytology.

Libido in the rats was determined by physical observation. This was done by observing the male rats' reaction and sexual behavior or response when a female rat on estrus was introduced, such sexual behavioral reactions include; general grooming, sniffing, mounting and thrusting on the female rats. Based on these reactions, scores were allocated following the scoring pattern described by Chibundu, (2013) (Table 1) [25] but with little modification.

Table 1
Libido Grading for Experimental Rats

Sexual Behavior	Score	Grading
Grooms, Sniffs and attempt to mount	5	Very high libido
Grooms, sniffs but no attempt to mount	4	High libido
Sniffs only	3	Moderate
Grooms only	2	Low
Does not pay attention to the female rat	1	Poor libido
Source: Chibundu (2013)		

At day 31, all the males were sacrificed using mild ether for sedation and cervical dislocation to avoid pain. Exsanguination and evisceration was done. Blood was collected for hematology Nwankudu et al., (2020) [22] and serology in test tubes through cardiac puncture.

The blood for serology was kept for 24 hours post collection to allow the natural separation of serum through the coagulation of the blood cells. But the one that did not separate well were centrifuged at 2500 rotations per minute and the serum collected with pipette into a clean non-heparinized bottles. The sera collected were sent to the laboratory in Federal Medical Centre (FMC), Umuahia for analysis of testosterone, follicle stimulating hormone, luteinizing hormone and prolactin using Elisa kits specific for each hormone. Thereafter, a graph for known quantities of these hormones was used to extrapolate the levels of the individual hormones found in each rat.

Concurrently, after evisceration, the testes were exteriorized and the caudal epididymis lacerated. Spermatozoa concentration were scoped into bijou bottle containing 1ml of phosphate buffered saline (PBS) to keep the sperm alive. The contents of the bottles were gently mixed to form caudal epididymis sperm suspension (CESS). A drop of the suspension was then put in a clean slide, covered with cover slip and viewed using research microscope DN: 10 connected to a laptop and the sperm motility were observed in waves and captured.

Further examination of the caudal epididymis sperm suspension (CESS) were done and the color, motility, cell viability, sperm concentration and sperm morphology were done using standard methods.

Gonadosomatic index of the testis was done using the formula: $\frac{\text{Weight of Testis (g)}}{\text{Live Weight (g)}} \times \frac{100}{1}$

2.4.1. Fertility indices of rats treated with PAE

The fertility indices of male albino rats were done by introducing three female rats per male for impregnation. Male and female rats were left together for ten days to factor in 2 estrous cycles for the females. On day 31, the males were sacrificed while the females were left till twenty days post mating. At day 20 post mating, each female rat was sedated and evisceration was done. Gravid uteri were exteriorized and the following parameters taken: fetal size, fetal weight, crown-rump length of fetuses, corpora lutea per ovary, resorbed embryo and females pregnant serviced by a male in percentages.

The fertility indices of the male rats were judged by their ability to impregnate the three females introduced to them, the number of fetuses alive at the point of evisceration of the females, weight of the fetuses and the cumulative crown-rump length of fetuses sired by the male.

2.5. Structural/functional characteristics of cells in the reproductive organs of rats treated with PAE

The skull and the testes were collected from the males treated with 2ml/kg body weight of DW as control and 800mg/kg of PAE respectively. The organs were fixed in Bouin's fluid for 48 hours. The skull was cracked and the brain containing hypothalamus and pituitary separated. Thereafter, the organs were fixed in 70% alcohol and cleared with Xylene. The tissues were embedded in paraffin wax, sectioned with microtome, stained with hematoxylin and Eosin (H&E), covered with cover slips and mounted in a Canada balsam. Examination of slides were under light microscope ($X_{40,}$ $X_{100,}$ X_{400} and X_{1000}) magnifications. Photomicrographs were taken using research microscope DN-10, DC 7.5 V Made in China.

All the experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) FV-U -IACUC-2020-0262 of University of Nigeria, Nsukka and the procedure approved were in compliance with guide for the care and use of laboratory animals, (eighth edition) which upholds recognition and alleviation of pain in laboratory animals according to the Institute for Laboratory Animal Research publication, (2009).

3.0. Statistical Analysis

Statistical package for social sciences (SPSS) version 20 was used for the data analysis. Student's T-test was used to compare differences between means and probability values at ninety five percent confidence interval ($P \leq 0.05$) was considered significant.

4.0. Results

4.1 Toxicity testing

There was no toxicity (salivation, sleepiness, lethargy diarrhea or death) observed even in the highest dose of 5000mg/kg body weight treatment [26].

4.2 Minimum effective dose

The minimum effective dose was observed to be 400mg/kg body weight. A dose of 200mg/kg body weight did not induce estrus in the treated female rats [22]. However, in this research double the minimum dose was used for treatment.

4.3.0. In vitro results

4.3.1. Effect of PAE in rabbit jejunum

The result obtained showed that PAE treatment does not lead to significant contraction in the digestive system (Fig. 2)

4.3.2. Effect of PAE in non-pregnant albino rat uteri

The result obtained shows that PAE does not contract uterus (Fig. 3).

4.4.0. Libido of males treated with PAE

The libido of the male rats treated with peanut aqueous extract was observed to be enhanced when compared to DW (control) treated rats (Table 2).

4.4.1 Table 2. Libido of rats treated with PAE

Treated rats	A_1 DW	A_2 DW	A_3 DW	A_4 DW	B_1 PAE	B_2 PAE	B_3 PAE	B_4 PAE
Score	3	1	2	3	5	5	4	5
Reaction time	10 sec.	20 sec.	15 sec.	10 sec.	2 sec.	2 sec.	4 sec.	2 sec.
Grading	Moderate	Poor	Low	Moderate	Very high	Very high	High	Very high

The scoring is reflected in column. The scoring system is according to Chibundu, 2013. However reaction time was considered necessary and added. Sec. = seconds. DW=Distilled water treated as control. PAE = Peanut aqueous extract.

4.5. Sperm motility in PAE treated male rats (Wet mount)

The result of the wet mount showed that PAE treated male rats had faster spermatozoa motility in micro seconds with strong wave-like movement up to 4 waves while control had only single wave and bunting movement with no wave (Figs. 4a, 4b, and 4c).

4.6.0. Spermatozoa analysis is PAE treated male rats

The color observed was milky in both treated and control. Spermatozoa was significantly ($P \leq 0.05$) highly concentrated in PAE treated than in control. There were significantly more motile sperm in PAE treated rats than in control. Also, the percentage of live sperm (Viability) was higher in PAE treated rats than in control. Furthermore, several aspect of spermatozoa morphology were considered; sperm cell without head, sperm cell with small head, sperm cell with twisted head, sperm cell with short tail, sperm cell with bent mid-piece and sperm cell with cytoplasmic droplets. In all the aspects of morphology, PAE treated male rats had significantly ($P \leq 0.05$) lower numbers except in sperm cell with twisted head where PAE had significantly higher numbers than control. However there were no sperm cells with short tail in PAE treated rats (Table 3).

4.6.1 Table 3. Spermatozoa analysis in PAE treated rats

S/No.	Spermatozoa parameters	PAE treated	DW treated (Control)
1	Sperm. Count ($\times 10^6$/CE)	174.92±6.51	138.78±12.050
2	Mass motility (%)	88.61±1.143	66.26±2.213
3	Live sperm proportion (%)	90.26±0.914	72.69±2.000
4	Sperm. cell without head (↓)	0.35±0.095	0.50±0.080
5	Sperm cell with small head (↓)	0.06±0.041	0.15±0.098
6	Sperm cell with twisted head (↑)	0.60±0.645	0.27±0.108
7	Sperm cell with short tail	0.00±0.000	0.21±0.139
8	Sperm cell with bent mid-piece (↓)	0.28±0.068	0.42±0.092
9	Sperm cell with cytoplasmic droplets (↓)	0.43±0.049	0.58±0.104
10	Total abnormalities (%) (↓)	1.72±0.180	2.12±0.11

Superscript indicate significant ($P \leq 0.05$) difference in a row. Numbers 4-10 shows sperm morphology. Down arrow shows positive response due to PAE treatment, up arrow shows negative response and no arrow shows extra positive response due to the treatment.

4.7.0. Result of gonadosomatic index of the testis (relative organ weight)

Gonadosomatic index is an indicator of reproductive activity. The gonadosomatic indices of albino rats treated with PAE show that PAE treated male rats significantly ($P \leq 0.05$) had higher testicular weight than control (Table 4)

4.7.1. Table 4. Gonadosomatic indices of albino rats treated with PAE

S/No	Animal	PAE Treated (mean)	DW treated Control (mean)
1	Animal live weight (g)	261.31±7.192	234.94±8.295
2	Paired testicular weight (g)	3.37±0.090	2.52±0.168
3	Relative testicular weight (%)	1.29±0.005	1.07±0.043

Superscripts show significant ($P \leq 0.05$) difference in a row.

4.7.0. Result of fertility indices of male albino rats treated with PAE

As earlier expressed, fertility indices of the male rats were judged by their ability to impregnate the three females introduced to them, the number of fetuses alive at the point of evisceration of the females, weight of the fetuses and the cumulative crown-rump length of fetuses sired by the male.

The result obtained showed that PAE treated male rats impregnated females had (75%) conceptions than DW (control) treated males which had (42%) conceptions. Peanut aqueous extract treated males impregnated females had significantly ($P \leq 0.05$) more fetuses alive than control. The weight of fetuses sired by PAE treated male impregnated females were significantly heavier than in control. Also the cumulative crown-rump length of fetuses kidded by females impregnated by PAE treated males were significantly ($P \leq 0.05$) longer than in control. There were significantly more fetuses, heavier weight of fetuses and longer crown-rump length in the right fallopian tubes in both PAE treated male impregnated females and control (Table 5).

4.7.1 **Table 5. Showing fertility indices of PAE treated male rats**

S/No	Fetal characteristics	PAE treated males sired females' fetuses N=12	DW treated males sired females' fetuses N=12
1	Percentage pregnant (%)	75.03±5.450	41.68±10.453
2	Corpora lutea left ovary	5.33±0.590	5.42±0.716
3	Corpora lutea right ovary	6.00±0.620	6.33±0.833
4	Fetal Number, left tube	2.58±0.596	1.63±0.596
5	Fetal Number, right tube	4.50±0.764	3.00±0.749
6	Fetal weight left fallopian tube (g)	6.28±2.135	1.63±0.646
7	Fetal weight right fallopian tube (g)	9.08±2.584	2.14±0.918
8	Crown-Rump length left fallopian tube (cm)	10.42±2.378	2.14±0.918
9	Crown-Rump length right fallopian tube(cm)	14.33±3.208	4.81±1.963
10	Resorbed embryo in left fallopian tube n=8	2.75±0.675	3.79±0.675
11	Resorbed embryo right fallopian tube n=8	1.50±0.779	3.25±1.118

The superscript indicate significant ($P \leq 0.05$) difference per row. Corpus luteum develop in an ovary after an ovum has been released. In a poly-gravidae like albino rats, corpora lutea which are yellowish protrusions at the surface of an ovary is an indication of the number of fetuses conceived. Number of corpora lutea minus fetal size equals the number of resorbed embryo. The parameters displayed in Table 4 shows that PAE treated male impregnated females are significantly ($P \leq 0.05$) more productive than DW treated male impregnated females.

4.8.0 Reproductive hormone profile of male albino rats treated with PAE

Albino rats administered with PAE for 30 days had significantly ($P \leq 0.05$) higher testosterone, higher follicle stimulating hormone and higher luteinizing hormone but significantly lower prolactin (Table 6).

4.9.1. Table 6. Reproductive hormones in albino rats administered with PAE

S/No	Hormones	PAE treated males	DW treated males
1	Testosterone (ng/ml)	3.78±1.436	2.22±0.595
2	FSH (nmol/ml)	0.64±0.147	0.45±0.086
3	LH (nmol/ml)	0.68±0.161	0.50±0.052
4	Prolactin (ng/ml)	0.65±0.042	0.89±0.205

The superscript shows significant (P≤ 0.05) difference in the same row. PAE treated males had higher testosterone, FSH and LH levels but lower Prolactin level than DW (control) treated males. These indicate higher fertility in PAE treated males.

4.10.0 Hematology of male albino rats treated with PAE

Peanut extract treated male rats had significantly (P ≤ 0.05) lower hemoglobin, lower packed cell volume, lower number of red blood cells but higher number of white blood cell. Further analysis of the differential white blood cells count revealed that PAE treated male rats had significantly higher lymphocytes and higher monocytes but the control rats had higher neutrophils and eosinophils (Tables; 7 and 8)

4.10.1. Table7. Hematology of male albino rats treated with PAE

S/No	Blood parameters	PAE treated males	DW treated males
1	Hemoglobin (g/dl)	15.60±0.424	16.80±0.441
2	Packed cell volume (%)	39.00±0.845	43.25±0.901
3	RBC ($\times 10^6 mm^3$)	5.27±0.135	5.87±0.164
4	WBC ($\times 10^3 mm^3$)	5.86±0.633	5.84±0.284
5	MCV (fl)	78.60±0.469	76.79±0.389
6	MCH (pg)	29.98±0.222	28.30±0.188
7	MCHC (g/dl)	39.98±0.365	38.83±0.298

Superscript shows significant (P ≤ 0.05) difference in a row. Table 7 shows that PAE treated male rats had lower hemoglobin, lower PCV and lower RBC count but MCV in both PAE and (DW) treated male were within normal range (<) lower than 80 (fl) which means that PAE treatment does not cause anemia.

4.10.2 Table 8. Differential Leukocytes in albino rats treated with PAE

S/No	Blood parameters	PAE treated males	DW treated males
1	Neutrophils	29.13±0.227	44.00±3.937
2	Lymphocytes	42.25±0.559	27.00±2.435
3	Monocytes	11.50±0.779	8.50±0.681
4	Eosinophils	17.00±0.267	20.50±0.982

Superscripts indicate significant ($P \leq 0.05$) difference in a row. Table 8 shows higher lymphocyte and monocyte count in PAE treated males which means that PAE treated males had higher immunity than Distilled water (DW) treated males

4.11. Result of Structural/functional characteristics of cells in testes, hypothalamus and pituitary of PAE treated male rats.

Testis: The testes of PAE treated rats for 30 days showed many seminiferous tubules (ST) with clear cut lumen at ×40 and ×100 magnifications. The clarity of the lumen increased as magnification increased. However, at ×400 and ×1000 (oil immersion) magnifications, there were evidence of spermatid and spermatozoa which were densely populated in PAE treated rats and sparsely populated in control rats (Figs. 5a and 5b).

Also, the hypothalamus of PAE treated rats showed acidophilic staining nuclei which are small round nuclei with definite shape and deep staining. They secrete prolactin releasing hormone and growth hormone releasing hormone. There were also basophilic staining nuclei with lacy chromatin in the cytoplasm and irregular in shape. They secrete gonadotropin releasing hormone (GnRH) and Thyrotropin releasing hormone [27]. Peanut extract treated male rats had more of the basophils than acidophil. Also in the pituitary, there were more basophilic cells secreting FSH, LH and Thyroxin than acidophil secreting prolactin (figs. 6a, 6b, 7a and 7b).

5.0. Discussion And Conclusion

The result of the toxicity testing shows that peanut extract is safe even at highest dose of 5000mg/kg. Signs of toxicity include but not limited to: hyperactivity, abnormal gait, spasms in rear legs, salivation, lethargy, sleepiness, diarrhea and death. None of these signs were observed in PAE treated male rats even after 7 days post treatment which proves that PAE which contains L-DOPA in high and tolerable doses [24] is safe and cannot lead to schizophrenia. Therefore, PAE is better option for the treatment of Hyperprolactinemia induced infertility and Parkinson's disease. This agrees with Ecker et al., 2009 [11] who stated that there is a positive correlation between psychotic episodes and dementia in pergolide (dopamine agonist) intake.

The result of the in-vitro experiments showed that PAE is neither uterotonic nor does it have contractile effect in rabbit jejunum. This shows that PAE is safe but since peanuts contain L-DOPA which is a precursor for Dopamine mediated through DOPA decarboxylase enzyme and inhibits prolactin leading to luteolysis and estrous induction; women in reproductive age should be careful with peanut consumption especially in empty stomach Prolactin maintains corpus luteum and corpus luteum secretes progesterone which maintains pregnancy. This agrees with Bachelot et al., 2009 [28] who stated that the most important regulatory protein (hormone) necessary for luteal cell function is prolactin. Therefore, care should be taken by pregnant women especially during first trimester not to consume peanut at a dose above 200mg/kg body weight in order to avoid embryo resorption or abortion. This agrees with Yahi et al 2017 [29] who hypothesized that reduced progesterone in blood of pregnant animals lead to abortion.

Final weight of PAE treated male rats was within the range of 215-294g which was the weight at the commencement of experimentation. This implies that PAE treatment does not lead to increase in weight. This shows that peanut is a safe snack for weight watchers. Also, peanut extract treated male rats had higher testicular weight than control which shows that PAE treatment leads to improved reproductive activity and fertility. This agrees with Ramadan et al., (2021) [30] whose research shows that there is a positive relationship between increased testicular weight and increased fertility.

When the females were introduced to males for impregnation, result obtained showed that PAE treated male rats had mostly very high libido and got 75% of the females introduced to them pregnant as against 42% seen in DW treated males (control) which shows that PAE is an effective aphrodisiac. This agrees with Iddi et al., 2013 [31] who inferred that groundnut may be a potential solution to age related decline in testosterone production. Increased secretion of testosterone leads to increased spermatogenesis and increased testicular weight. This also agrees with Preston et al., (2011) [30] who stated that spermatozoa production is dependent on high levels of testosterone.

Spermatozoa analysis in PAE treated males revealed that PAE treated male rats had significantly more motile sperms which were wave-like in movement having 4 waves in split seconds in wet mount while the control had only one wave. Sperm concentration and live sperm proportion were also higher in PAE treated males. In sperm morphology; sperm cell without head, sperm cell with small head, sperm cell with bent mid-piece and sperm cell with cytoplasmic droplets were significantly lower in PAE treated males. Also, there were no sperm cells with short tail. All these indicate positive energy of the spermatozoa and reproductive efficiency. However, sperm cells with twisted head were significantly present in PAE treated males. This could be attributed to processing defect. This agrees with Franken and Henkel, (2012), [32] who described sperm head defects as large head, small head and elongated head but no twisted head was mentioned as defect.

Furthermore, PAE treatment had strong effect on the females impregnated by treated males such that PAE treated males impregnated females had 75% conception rate and had significantly more fetuses alive at the point of evisceration than DW (control) treated male impregnated females. There were significantly lower embryo resorption in PAE treated male impregnated females. These show among

others that rate of pregnancy and viability of fetuses has direct relationship with the nutritional status of the male than the female. Recall that only the males were treated with PAE, the females were not treated.

Reproductive hormone profile of male albino rats treated with PAE showed that PAE treated rats had significantly high testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) but lower prolactin. Increased level of testosterone, LH, FSH and reduced level of prolactin leads to increased fertility and fecundity. Prolactin is a stress hormone. Elevated level of prolactin no matter how minute at the other hand leads to hyperprolactinemia induced infertility which depressed the synthesis of gonadotropin releasing hormone (GnRH) synthesis from the hypothalamus which leads to depressed synthesis of FSH and LH from the anterior pituitary leading to depressed synthesis of testosterone from the gonads cumulating in infertility. This agrees with Chun et al., 2014 [7] who stated that hyperprolactinemia induced infertility suppresses GnRH causing a decrease in LH and FSH, ultimately leading to decrease in serum testosterone and hypogonadism. But, PAE treated males at the other hand had significantly higher fertility indices than DW (control) treated males.

From the research, the hematological findings in PAE treated rats showed significantly ($P \leq 0.05$) lower hemoglobin, lower packed cell volume, lower number of red blood cells but higher number of white blood cell. However, there was no evidence of anemia since the mean corpuscular volume is higher in PAE treated males than control and all were within the same range of slightly lower than 80 fl and this agrees with Zivot et al., 2017; [33] shortening of red cell survival does not always cause anemia since there is a compensatory increased rate of erythropoiesis by the bone marrow.

Histology of testes, hypothalamus and pituitary revealed that PAE treated male rats had comparatively higher population of spermatozoa released from the seminiferous tubules when viewed at ×1000 (oil immersion) magnification using hematoxylin and eosin staining. The increased number of sperm cells seen in seminiferous tubule of PAE treated rats was confirmed by increased number of basophilic nuclei in the hypothalamus which secretes GnRH and basophilic cells in the pituitary which secretes FSH and LH which might have led to the increased secretion of testosterone cumulating in heightened spermatogenic activity and increased fertility observed in PAE treated male rats. This agrees with Kerringan et al., (2017) [27] who stated that basophilic nuclear in the hypothalamus characterized by lacy chromatin in the cytoplasm and irregular in shape secretes Gonadotropin releasing hormone and Thyrotropin releasing hormone. Increased GnRH leads to increased serum testosterone as stated earlier cumulating in heightened spermatogenesis leading to increased fertility and fecundity which was observed in PAE treated males.

5.1. Conclusion

From the research, peanut aqueous extract (PAE) treatment in males leads to significant ($P \leq 0.05$) increase in libido, testicular size which leads to increased fertility. It also leads to significant increase in spermatozoa concentration. In fact, Peanut extract stimulates gonadal steroidogenesis through the pituitary-gonadal axis due to significant increase in FSH and LH secretion leading to increase in

testosterone secretion. Also, PAE treated males had relative increase in number of spermatids and spermatozoa in the seminiferous tubules of the testes, relative increase in basophilic nuclei secreting GnRH in the hypothalamus and relative increase in the number of basophilic cells secreting FSH and LH in the pituitary. All these confirm that PAE is an aphrodisiac. Peanut as a legume contains L-DOPA which is a precursor for Dopamine; a prolactin inhibitor. Therefore, peanut or its extract can be used in the treatment of hyperprolactinemia induced infertility and early stages of Parkinson's disease. Normally, hyperprolactinemia and Parkinson's disease are treated with pharmaceutical dopamine agonists and they have side effect of schizophrenia but PAE does not have such side effect.

Declarations

The research was approved by institutional animal care; FV-U -IACUC-2020-0262 of University of Nigeria, Nsukka and the reporting of the research was thorough and conforms to the ARRIVE guidelines for in vivo experimental animal reporting (PLOS BIO. B (6), E1000412 2010).

Availability of data and materials: Data is available upon request from the corresponding author

Conflict of interest: There are no conflict of interest. The three authors consented to the publication.There were no funding received from internal or external sources. This work is a part of PhD thesis of the corresponding author.

Author contribution:

ONN; Put down the research topic, garnered information about the topic from available literature, designed and performed the experiments.

CNU; Adjusted the experimental design, supervised the research, rearranged the manuscript and proofread the manuscript

RIO; Corrected the document.

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Figures

Figure 1

Valencia variety of peanuts

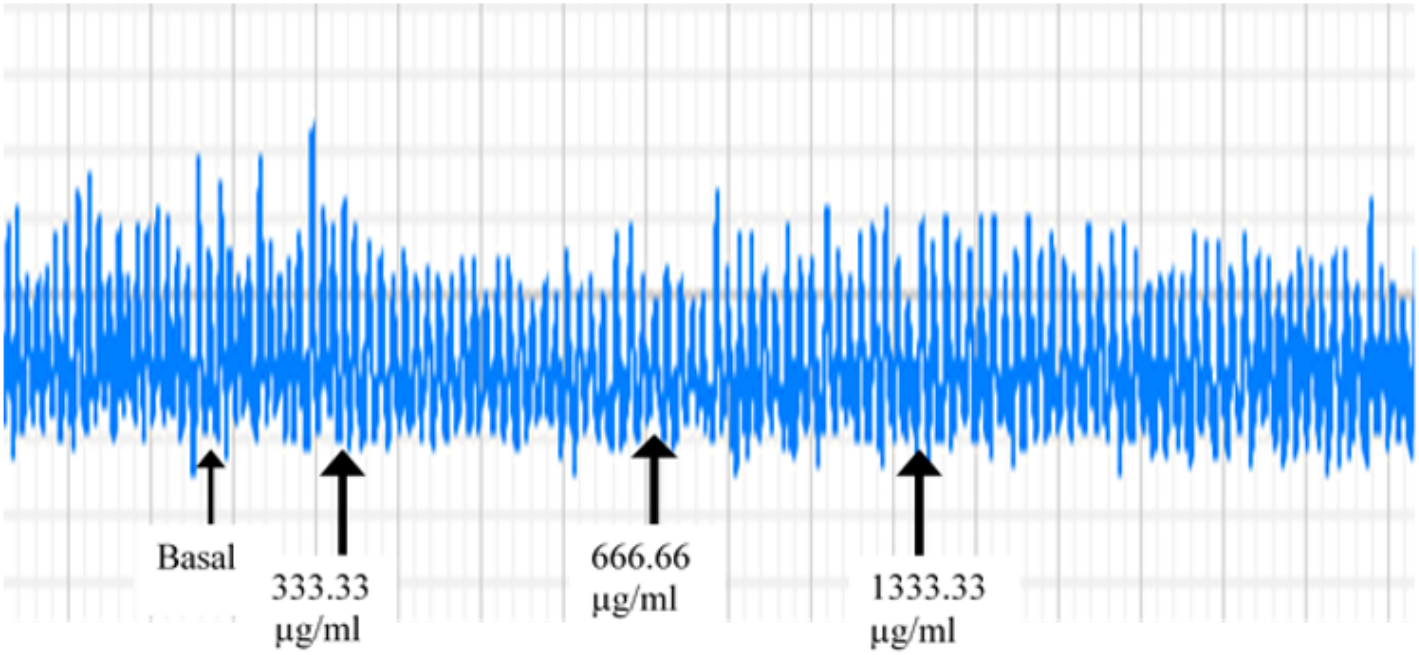

Figure 2

Rabbit jejunum showing no significant difference in contraction between basal and treatment with varying concentration of Peanut Aqueous Extract.

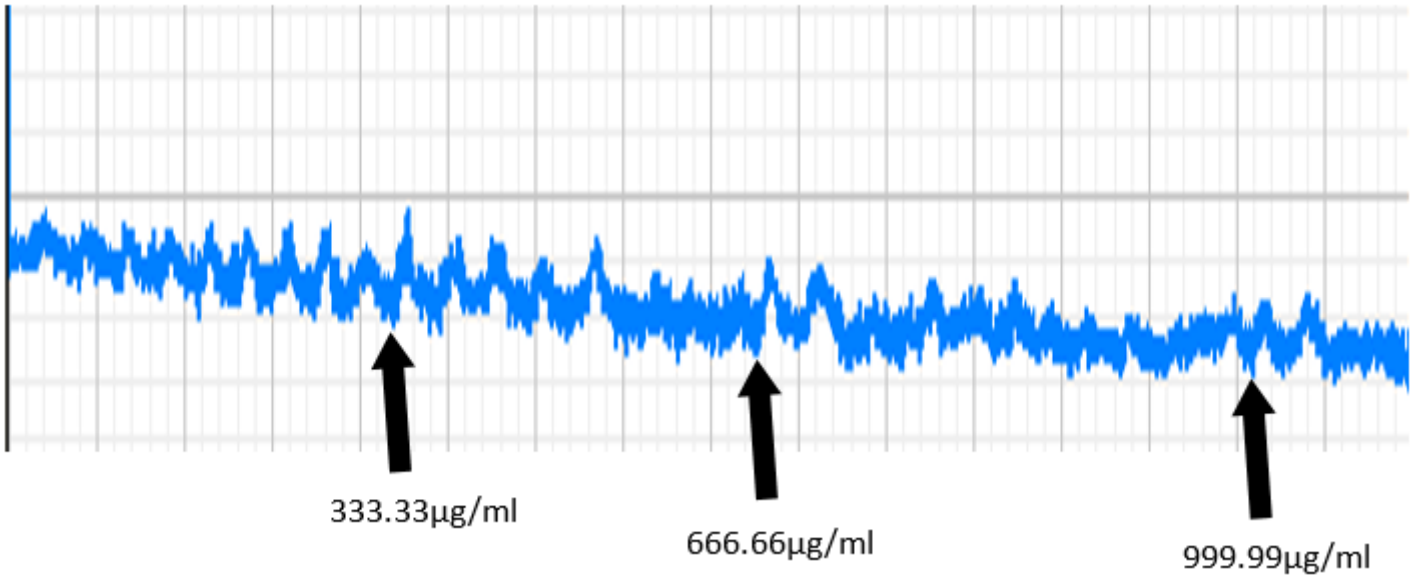

Figure 3

Albino rat uterus showing no significant difference in contraction between basal and treatment with varying concentration of Peanut Aqueous Extract.

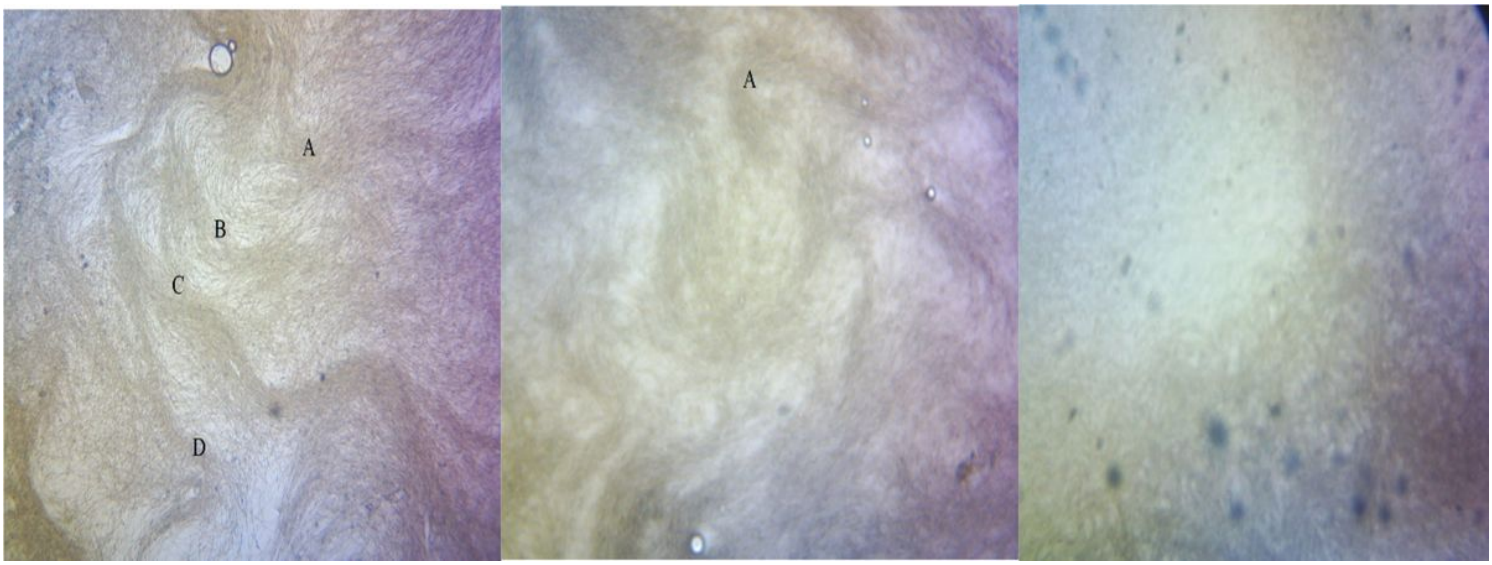

Figure 4

a. PAE ×40 b. DW ×40 c. DW ×40

a. PAE treated rat with strong wave-like movement; A, B, C and D of the spermatozoa.

b. Distilled water treated male rat (control) with single wave-like movement; A.

c. Distilled water treated (Control) showing immotile movement of the spermatozoa.

These shows that peanut aqueous extract treatment leads to increased motility of the spermatozoa which leads to increased fertility.

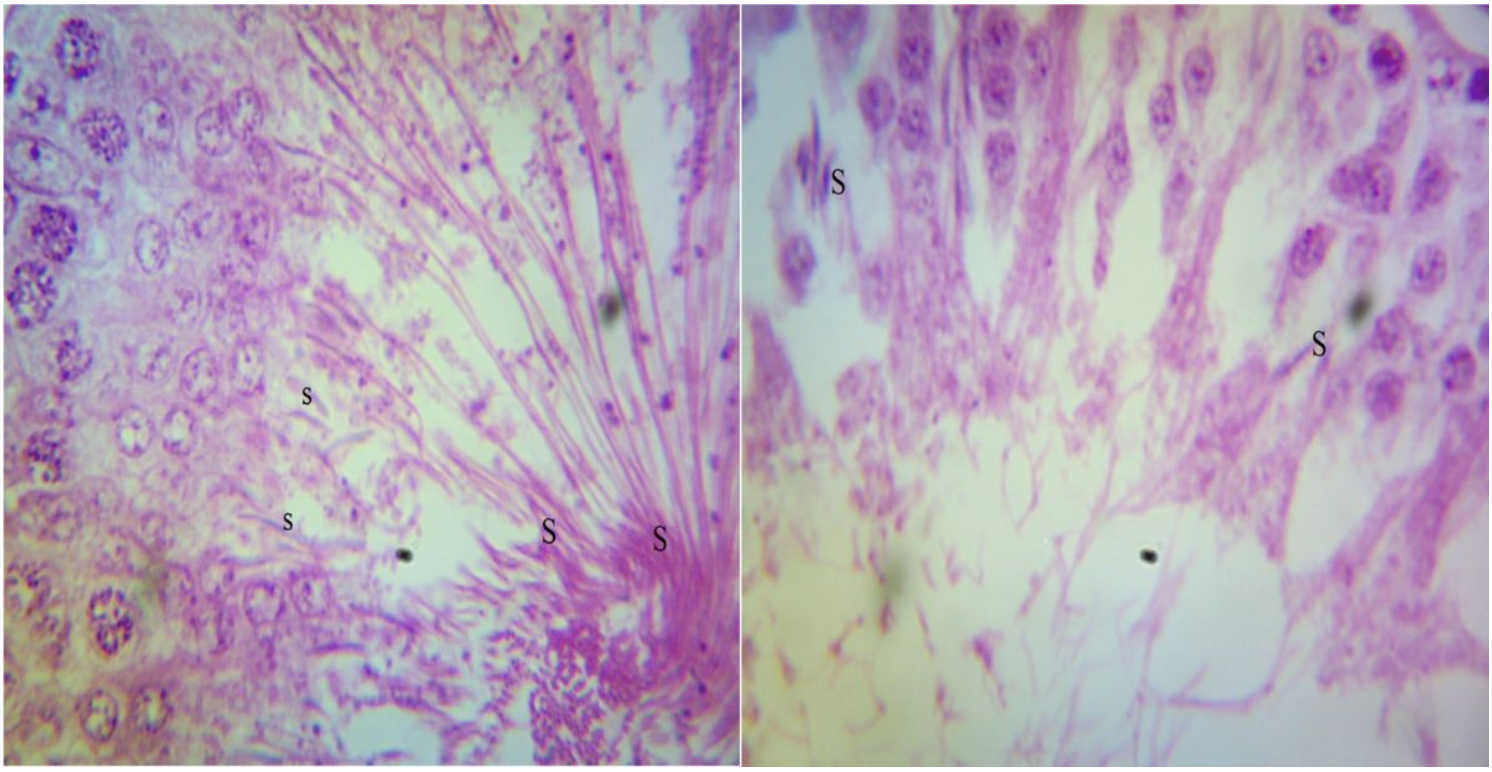

Figure 5

a. ×1000 (oil immersion) H&E b. ×1000 (oil immersion) H&E

a. PAE treated male rats showing many S=spermatozoa and s=elongated spermatids which shows that PAE treatment leads to increased fertility.

b. DW treated (control) show few S=elongated spermatids.

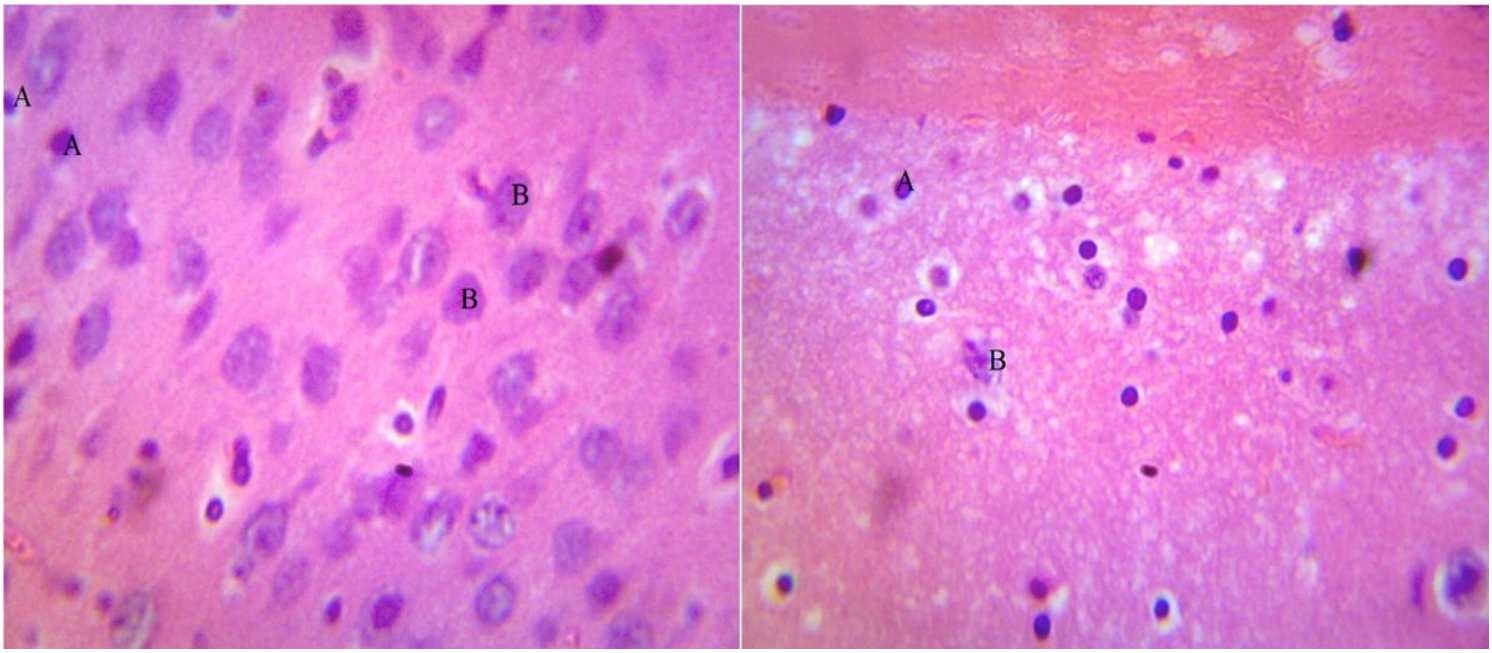

Figure 6

a. Hypothalamus ×1000, H&E b. Hypothalamus ×1000, H&E

a. Hypothalamus in PAE treated male rats showing few A=acidophil and many B=basophil which shows increased secretion of GnRH leading to increased fertility.

b. Hypothalamus in control showing many A=acidophil and few B=basophil which shows increased secretion of prolactin releasing hormone and decreased fertility.

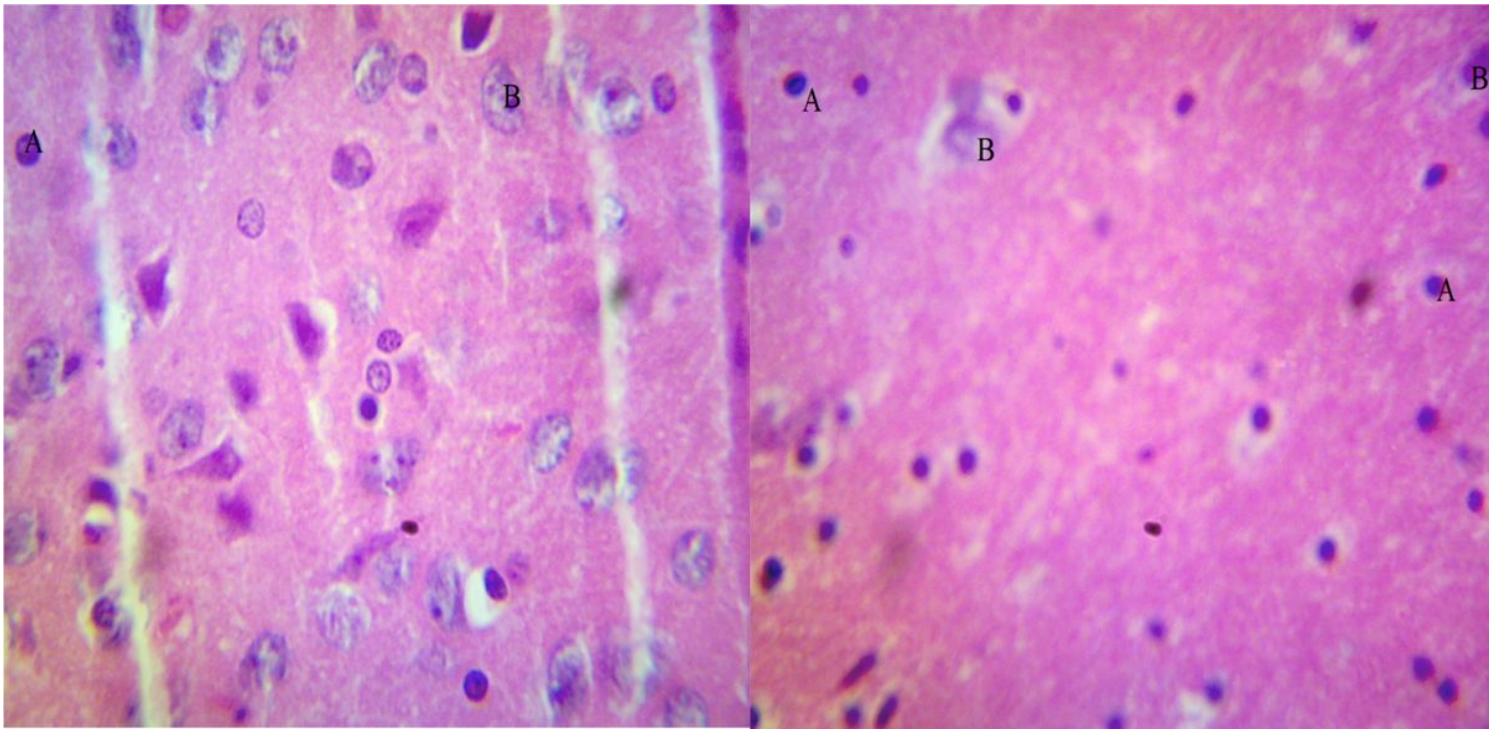

Figure 7

a. Pituitary×1000, H&E b. Pituitary ×1000, H&E

a. Pituitary in PAE treated male rats showing very few A=acidophil and many B=basophil which shows increased secretion FSH and LH leading to increased spermatogenic activity and increased fertility.

b. Pituitary in control rats showing many A=acidophil and very few B=basophil which shows increased secretion of prolactin and decreased fertility.