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Phytochemical, Hematologic and Histopathologic Evaluation of Male Albino Rats Treated With Peanut (Arachishypogaea) Extract

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***Abstract*— This experiment was designed to evaluate the phytochemical, hematologicaland histopathological effects of peanuts when administered to male albino rats. Before the commencement of the experimentation, phytochemical screening of the extract was done.Thirty two albino rats comprising of 8 males and 24 females were used for in vivo experiment. The 8 males were further divided into two groups (A & B). Group A served as control while group B was given 800mg/kgpeanut extract (PE) for 21 days after which 3 female rats selected at random were introduced for each male to breed. The females were left with the males for ten days. After, the males were sacrificed and blood collected for hematology while testes and brain were collected for histopathology. The result obtained showed that peanuts contain among others, flavonoids, steroids, glycosides and carbohydrates. Hematologic profile of male rats treated for 31 days showed that PE treated rats had lower hemoglobin, PCV, RBC but increased WBC all at P≤ 0.05. However differential WBC of the albino rats revealed that PE treated rats had lowered neutrophils, lowereosinophils but increased monocytes and lymphocytes all at P≤ 0.01. The lymphocyte value varied from 42.25±0.559 in treated to 27.00±2.435 in control (n=4).Histopathologic lesions revealed that PE treated rats had densely populated spermatozoa in the seminiferous tubules than control. Conclusion: The research shows that peanut extract contains flavonoids which implies that peanuts have anti-inflammatory, anti-oxidant, anti-bacterial, anti-diabetic, anti-viral and anti-cancer potentials. Peanuts also boost the over-all immunity due to the increased lymphocyte and monocytes observed in the treated rats. It is then suggested that peanut diet be recommended among others for immune compromised persons.**

***Index Terms*— anti-oxidant, flavonoids, immunity, lymphocytes, peanuts.**

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I. INTRODUCTION

Peanuts are touted to be full meal when consumed. The question which this research sought to find answers for were: 'What does peanuts contain phytochemically, How does peanut affect the hematology of persons or animals that consume them and How is the histopathologic lesions of the reproductive organs in the treated persons or animals?' To answer these questions, eight male albino rats were used to experiment and three females per male were made available for each male to cross in order to maintain active reproductive performance.

Recent research on peanuts and nuts in general has found antioxidants and other chemicals that may provide health benefits. Research shows that peanuts rival the antioxidant content of many fruits. Roasted peanuts rival the antioxidant content of blackberries and strawberries and are far richer in antioxidant than apples, carrots or beets. Peanuts contain high concentrations of antioxidant; polyphenols, primarily a compound called P – Coumaric acid which is a substance known for its antioxidant, antimicrobial, antimutagenic, anxiolytic, analgesic, sedative and immunoregulatory effects and roasting can increase peanut's P- coumaric acid levels, boosting their overall antioxidant content by as much as 22% [1].

1.1.Peanuts as a source of resveratrol

Peanuts are a significant source of resveratrol, a chemical studied for potential anti – aging effects and also associated with reduced cardiovascular disease and reduced cancer risk [2]. It has been found that the average quantity of resveratrol in one ounce of commonly eaten peanuts without the skin (15 whole peanut kernels) is 73µg. This means that ounce for ounce; peanuts contain almost 30 times as much resveratrol as grapes, which often are touted as being one of the few good sources of the antioxidant [3].

1.2. Distribution of peanuts

Peanuts are 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 (⅜– 2 ¾ in) long and 1 – 3cm (⅜– 1 inch) broad. The flowers are a typical pea flower in shape, 2 – 4 cm (⅜– 11/2 in) across, yellow with reddish veining. After pollination, the fruit develops into a legume 3 – 7cm (1 – 2 in) long containing 1 – 4 seeds, which forces its way underground to mature [4].

The plants name derives from a combination of the

morphemes pea and nut, causing some confusion as to the nature of the fruit. The 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 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 [5].

Other names ascribed to Arachishypogaea include; groundnut (earthnut), goobers, goober peas, pindas, jack nuts, pinders, manila nuts and monkey nuts. The last of these names is often used to mean the entire pod [6], [7].

1.3. Peanuts and Aflatoxin

Aflatoxins are a family of toxins produced by certain fungi that are found on agricultural crops such as maize (corn), *peanuts*, cottonseed, and tree nuts. The main fungi that produce *aflatoxins* are Aspergillusflavus and Aspergillusparasiticus, which are abundant in warm and humid regions of the world. Aflatoxins are produced by both Aspergilusflavus and Aspergilusparasiticus which are common forms of 'weedy' molds widespread in nature [8]. The presence of those molds does not always indicate that harmful levels of aflatoxin are present, but does indicate a significant risk [9]. The molds can colonize and contaminate food before harvest or during storage, especially following prolonged exposure to a high-humidity environment, or to stressful conditions such as drought [10]. Peanuts may be contaminated with *Aspergillusflavus* which produces a carcinogenic substance called aflatoxin. Peanuts are therefore an important source of aflatoxin in both human and animal diet [11].

1.3. Hematology

Hematological parameters are very important in determining health and physiological status of animals. These parameters reflect the changes in the organism correctly and play an important role in the detection of disease. Haematological indices such as the number and morphology of erythrocytes, leucocytes, and thrombocytes are useful in disease diagnosis and monitoring [12], [13], [14].

According to Etim et al. (2014) [15], the examination of blood provides the opportunity to clinically investigate the nutritional, physiological and pathological status of an animal. The role of nutrition as a key modulator of blood haematology is increasingly being recognized. A number of studies have revealed that different diets could exert diverse effects on haematological indices of animals and humans[16], [17], [18], [19], [20].

Within the scope of the study, hematological parameters such as red blood cells count (RBC), white blood cell count (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) and differential (WBC) leukocyte count were analyzed.

II. Materials and Methods

2.1. Materials

2.1.1. Research sample: the research sample used was Valencia variety of peanuts commonly known as grade 1 in Nigerian markets.

2.1.2. Equipment: the equipment used were electric blender (Corona glass jar blender with stainless steel blades, made for Landers, Columbia; Linea 018000 9 47203), Crude weighing balance (CAMRY EMPERORS), Sensitive weighing balance (Metler Balance), Sart Fax2100 (Awareness Technologies), Freezer dryer: (Christ; Alpha1-2 LDplus 1.5, serial No: 16508, Made in Germany), Stainless steel animal cages with drinkers, centrifuge, PCV reader, VIS-UV spectrophotometer, Neubauer counting chamber and automatic counter.

2.1.3. Consumables:

The consumable used include: heparinized bottles for collecting blood for hematology, 5ml syringe and needles, capillary tubes, Bouin's fluid, 70% alcohol, Xylene, paraffin wax, oil immersion oil (Hopkin and Williams, England), thumb forceps, scissors, clamping pins, dissecting board and plastic containers for collecting organs for histopathology, lead acetate, sulphuric acid, chloroform,iodine, Drabkin's solution, Giemsa and Pasteur pipette.

2.1.4. Animals: Male and female Albino rats were used. The males were 12-14 weeks of age and weighed between 215g-294g while the females were 10-12 weeks of age and weighed between 146g-198g. The rats were procured from the Departmental animal house of the Department of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike. The rats were kept in standard cages and fed Topfeed chick marsh ad libitum and given water through nipples.

All the experimental procedures were performed according to the ethical guidelines for research using animal studies established by the Ethical Committee of University of Nigeria, Nsukka which is 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.

2.2.0. Methods

2.2.1. Sample Collection:*Arachishypogaea*, Valencia variety (grade 1) was procured from OrieUgbaa Market, Umuahia North LGA, Abia state, Nigeria. The nuts were washed, spread and the spoilt nuts were sorted out and the good ones allowed to dry on top of the laboratory bench

2.2.2. Extraction:

Cold maceration extraction procedure was used in this study in Step B laboratory, Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka.

3.328g was soaked in a 2.5 litre Winchester bottle for 5 minutes. The extract was sieved using Mounilex sieving cloth.

The filtrate was lyophilized using freeze dryer (Christ; Alpha1-2 LDplus 1.5, serial No: 16508, Made in Germany. The percentage yield was calculated using the method below:

$$\frac{\text{Weight of the extract}}{\text{Weight of pulverized peanuts}} \times \frac{100}{1}$$

The yield was named Arachis Lyophilized Aqueous Extract (ALAE) and stored in the freezer until needed.

2.2.3. Phytochemical testing of *Arachishypogaea*

Qualitative phytochemical screening was carried for: tannins, saponins, phlobatanins, flavonoids, terpenoids steroids, alkaloids glycosides and total carbohydrate. However the methods for positive phytochemical is discussed in this manuscript which include: flavonoids, steroids, glycosides and carbohydrates. The said screening of ALAE was done using standard procedures [21], [22].

2.2.3.1. Test for flavonoids

One milliliter of ALAE was added to 1ml of 10% lead acetate solution. The formation of a yellow precipitate was taken as a positive reaction for flavonoids.

2.2.3.2. Test for steroids

Two milliliters of ALAE was dissolved in 2ml of chloroform. Then 2ml of sulphuric acid was added. A red color produced in the lower chloroform layer was an indication for the presence of steroids.

2.2.3.3. Test for glycosides

Two milliliters of the ALAE was dissolved in 2ml of chloroform. 2ml of sulphuric acid was added carefully and shaken gently. A reddish brown colour indicated the presence of a steroidal ring; glycine portion of glycoside (Salkowski's test) [23].

2.2.3.4. Test for carbohydrates

Three milliliters of the ALAE was added to 1ml of iodine solution. A purple colouration at the interphase indicated the presence of carbohydrates.

2.2.4. Toxicity Testing: Oral acute toxicity test

Oral acute toxicity tests was done using 35 albino rats grouped into seven. The rats were both male and females and were picked at random comprising of young, mature and old. Each group according to age range or weight comprised of five rats each. Acute toxicity evaluation of the extract was done according to the method used by Chinedu*et al*., (2013) [24], but with little modification. The groups were given graded doses of *Arachis*lyophilized aqueous extract ALAE in the following order: 500mg/kg, 1000mg/kg, 2000mg/kg, 3000mg/kg, 4000mg/kg, 5000mg/kg and 6000mg/kg. When no toxicity was observed, the rats were further kept for seven days.

2.2.5. Minimum effective dose

Minimum effective dose was done because the sample did not cause any toxicity even at highest dose of 6000mg/kg.

Thirty mature female albino rats of the same age range were used. The experiment was carried out in two stages.

Stage 1 experiment 1: fifteen female rats of twelve weeks of age were grouped into 5 of 3 rats per group. The rats were fasted for twelve hours. Group 1 served as control and was given 2ml/kg of distilled water. Groups 2, 3, 4 and 5 were treated with graded doses of: 200, 400, 800, 1,600mg/kg body weights with ALAE having the same dilution factor of 1gram dissolved in 10ml of distilled water. The experiment was replicated.

After treatment all the animals (Grps 1-5) were left for one hour before they were fed with Topfeed finisher and given water through nipple.

After twenty four hours, the vaginal swab of all the rats were taken and the histology of the estrous cycle determined.

The group that all came to estrus within twenty four hours is accepted as a positive response to the dose administered.

2.2.6. Experimental Design: 32 adult albino rats comprising of 8 males and 24 females were used. The males were divided into two groups (A and B). Group A was treated with 800mg/kg *Arachis*Lyophilized Aqueous extract (ALAE) while group B which served as control was treated with 2ml/kg of distilled water.

2.2.7. Treatment: All treatment was done through oral route of administration for 31 days.

After 21 days, three females picked at random were introduced per male to cross in both treated and control groups. Treatment of males was continued until after ten days post introduction of females to allow two estrous periods for the females to have chance of being mated after which the males were sacrificed using mild ether soaked in cotton wool as sedative and cervical dislocation was done before the internal organs were exteriorized. Blood was collected from the heart through cardiac puncture for hematology.The testicles were exteriorized and the two testes harvested for histopathology

2.2.8.0. Haematology of male albino rats treated with ALAE

2.2.8.1 Blood sample collection

The male rat for data collection was sacrificed through the use of mild sedation with mild ether soaked in cotton wool and cervical dislocation. The blood for hematology was collected from the heart with an 18 gauge needle and 5ml syringe and the blood collected was put in heparinized bottles. The blood analyzes was carried out manually within 24 hours post collection. The following were analyzed.

2.2.8.2 Haemoglobin (Hb)

The haemoglobin concentration in the blood was determined by Cyanomethemoglobin method. The blood sample (0.2 ml) was mixed with 4 ml of Drabkins solution in a test tube and allowed to stand for 15 minutes at room temperature. The absorbance of the mixture was read at 540 nm against reagent blank using a spectrophotometer. The Hb was obtained by multiplying the absorbance of the sample with a calibration factor (36.8) derived from the absorbance and concentration of the standard [25].

2.2.8.3 Packed cell volume (PCV)

The PCV was determined by microhaematocrit method. This was done using standard technique as described by Coles (1986) [26]. Blood samples were collected into heparinized capillary tubes. One end of the tubes was sealed with plasticine and centrifuged for 5 minutes at 2500 r.p.m, using hematocrit centrifuge. The levels of the packed red blood cells in the capillary tubes were read by means of a PCV hematocrit reader.

2.2.8.4 White blood cell (WBC)

The white blood cell count was determined by Hemocytometer method. The blood sample was diluted (1:20) using Turks solution (2% glacial acetic acid). The diluted

sample was loaded into a Neubauer counting chamber with the aid of a Pasteur pipette. The WBC was determined by counting the required number in the appropriate squares on the counting chamber under a microscope. The number of cells counted for each blood sample was multiplied by 50 to obtain the total white blood cell count per microlitre of blood described by Schalm*et al*., (1975) [27].

2.2.8.5 Red blood cell (RBC)

This was also determined using Hematocrit method [27].

Blood sample (0.02 ml) was collected with a pipette and added to 4 ml of red blood cell diluting fluid in a clean test tube to make 1:200 dilution of the blood sample. The diluted blood sample was loaded into a Neubauer counting chamber and counted using a light microscope.

2.2.8.6. The Mean Corpuscular Volume (MCV)

The MCV was calculated by dividing the PCV by erythrocyte count then multiplied by a constant of 10. The values obtained were expressed in femtolitres:

MCV = PCV x 10/ RBC

2.2.8.7. Mean Corpuscular Haemoglobin (MCH)

The MCH was calculated by dividing the haemoglobin concentration by the erythrocyte count, already determined and then multiplied by a factor of 10. The values were expressed in picogram.

MCH = Hb (mg/dl) x 10/RBC

2.2.8.8. Mean corpuscular haemoglobin concentration (MCHC)

The MCHC was calculated by dividing haemoglobin concentration by the PCV value already obtained and then multiplied by 100. The values were expressed in grams per litreas described by Emberth, (1986) [28].

2.2.9. Differential Leukocyte count of male albino rats treated with ALAE

Differential leukocyte count was done using Neubauer and automatic counter according to May-Grunewald Giemsa technique. Two hundred cells were then counted and classified.

Slide preparation for microscopic viewing:

A thin film of blood smear was made on clean microscopic slide. It was fixed in pure methanol for 30 seconds by immersing the slide or putting a few drops of methanol on the slide. The slide was them immersed in a freshly prepared 5 % Giemsa stain solution for 20-30 minutes. Flushed with water and left to dry. The slide was viewed through a microscope at X_{100} magnification.

2.2.9.1 Neutrophils has granular cytoplasm which stains pink while the nucleus consists of two to five lobes and stains blue.

2.2.9.2 Eosinophils also has granular cytoplasm but the granules stain red or orange and are closely packed but the nucleus is bi-lobed.

2.2.9.3 Basophils: has large cytoplasmic granules which stains blue. The nucleus is kidney shaped. The granules sometimes obscure the nucleus.

2.2.9.4 Lymphocytes: the cytoplasm does not have granules. They may be large or small depending on the age. In the small (mature) lymphocytes, the cytoplasm is small and stains blue while the nucleus is round and stains deep purple, while the large (young) lymphocytes has larger cytoplasm which stains pale blue and the nucleus also stains pale purple.

2.2.9.5 Monocytes: they are the largest in size among the leukocytes. The cytoplasm stains pale gray while the nucleus is large, kidney shaped and centrally placed within the cell. The cytoplasm does not have granules.

After identifying the cells, count was started from one margin and spread across the entire field.

2.2.10. Structural/functional characteristics of cells in the reproductive organs of male albino rats treated with ALAE and Distilled water

In the male albino rats treated with 800mg/kg of ALAE and 2ml/kg of distilled water in control rats for 31 days, the following organs were collected for histopathology (Testes, Hypothalamus and Pituitary). The organs were fixed in Bouin's fluid for 48 hours.Subsequently, 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.

STATISTICAL ANALYSIS

The data was subjected to analysis of variance using Statistical Package for Social Sciences (SPSS) version 20. Differences between means were compared using students T-test. Values of $P \leq 0.05$ and $P \leq 0.01$ were considered significant.

III. RESULTS

3.1. Percentage yield

Percentage yield of *Arachishypogaea*

$\frac{659g}{1,328g} \times \frac{100}{1}$ =49.62% approximately 50%

The extract was labeled as *Arachis*Lyophilized Aqueous Extract *(ALAE) and* was stored in a freezer until needed.

3.2. Phytochemicals in ALAE

The result of qualitative phytochemical screening of ALAE is presented in Table 1. It shows that the plant extract contains flavonoids, steroids, glycosides and carbohydrates.

3.3. Result of acute toxicity studies: The result shows that there were no deaths at 500, 1000, 2000, 3000, 4000, 5000 and 6000mg/kg body weight. When the animals were left for extra seven days, there was no sign of any toxicity noticed in all the groups (Table 2).

3.4. Minimum effective dose

The result of the minimum effective dose in adult female rats showed that the extract in the dose ranges of 400-1600mg/kg body weight resulted in estrus in all the experimental animals (This is because ALAE is found to synchronize estrus within 24 hours therefore the estrous synchronizing potential was used a s a measure to determine effectiveness) [29]whereas all the animals that received the

lowest concentration of 200mg/kg body weight did not show any sign of estrus rather the animal were in different stages of estrous cycle namely; proestrus, diestrus and metestrus (Table 3, figs:1).

3.5. Hematology of male albino rats treated with ALAE

Arachis lyophilized aqueous extract treated rats had lower hemoglobin, lower packed cell volume, lower number of red blood cells but higher number of white blood cell at $P \leq 0.05$. Further analysis of the differential white blood cells count revealed that ALAE treated male rats had higher lymphocytes and higher monocytes but the control rats had higher neutrophils and eosinophils (Tables; 4 and 5)

3.6.0. Structural and functional characteristics of the testes, hypothalamus and pituitary in albino rats treated with ALAE and distilled water as control

3.6.1. Testis: Hematoxylin and eosin (H&E) was used for the staining. The testes of Arachis lyophilized aqueous extract (ALAE) treated rats for 31 days showed many seminiferous tubules (ST) with clear cut lumen at X_{40} and X_{100} magnifications. The clarity of the lumen increased as magnification increased. However at X_{400} and X1000 (oil immersion) magnifications, there were evidence of spermatid and spermatozoa which were densely populated in ALAE treated rats and sparsely populated in control rats (Figs.5, 6, 7, 8, 9, and 10).

3.6.2. Hypothalamus: Some nuclei of the hypothalamus are characterized with lacy chromatin and dense nucleoli [30]. Those nuclei with lacy chromatin in the cytoplasm are irregular in shape. There are also small round nuclei with definite shape and deep staining. In fact, many nuclei exist in the hypothalamus and they produce compounds that affect the release of hormones from the pituitary gland [31].

3.6.3. Pituitary: the pituitary is also characterized by well demarcated acini that usually contain a mixture of different hormone producing cells. The gonadotrophs secreting FSH and LH are cells diffusely scattered throughout the gland [32].

However, the two types of nuclei observed in the hypothalamus just like in the pituitary stains acidophilic and basophilic. The acidophils are small round cells with definite shapes.

In the hypothalamus, acidophilic nuclear secret growth hormone releasing hormone and prolactin releasing hormone while in the pituitary, the acidophils secrete growth hormones (somatostacin) and prolactin while the basophilic nuclei secrete Gonadotropin Releasing Hormone (GnRH) [33].

The basophils in the pituitary has irregular shapes and lacy cytoplasmic appearance just as in hypothalamic nuclei. The basophils also function in two ways: as thyrotrophs secreting thyroxin (T_4) and triiodothyronin (T_3) and gonadotrophs which secretes follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Inferentially however, one may deduce that the nuclei in the hypothalamus with structural similarity to the cells in the pituitary could be performing synergistic function. In the hypothalamus, the nuclei could be involved with the synthesis of the releasing hormones which passes through the portal system to stimulate the synthesis of the tropic hormones.

In this research, the hypothalamus of ALAE treated rats showed acidophilic staining nuclei (a) and basophilic staining nuclei (b). Subjectively, ALAE treated rats had more of the basophils than acidophil which secretes GnRH and Thyrotropin releasing hormone. Also in the pituitary, there were more basophilic cells secreting FSH, LH and Thyroxin

IV. DISCUSSION AND CONCLUSION

4. 1. Discussion

4.1.1.0. Phytochemicals: The phytochemicals found in *Arachis* lyophilized aqueous extract (ALAE) are flavonoids, steroids, glycosides and carbohydrates (Table 1).

4.1.1.1.Flavonoids: Flavonoids are a diverse group of phytonutrients (plant chemicals) found in almost all fruits and vegetables. Flavonoids are the most important plant pigment for flower coloration. The catechins are the most frequent flavonoid found in human diet. They are poorly absorbed in the human body with most of what is absorbed being quickly metabolized and excreted [34]. Flavonoids have the following effects in vitro and in vivo: anti-inflammatory [35], anti-oxidant [36], anti-diabetic [37], anti-bacterial [38], antiviral [39], anti-cancer [40], and also effective against hyperlipidemia and atherosclerosis [41].

[1] From the above scientific revelations, it implies that ALAE has anti-inflammatory, anti-oxidant, anti-bacterial, anti-diabetic, anti-viral and anti-cancer effects. This agrees with (Timothy et al., 2000) peanuts contain polyphenols which are antioxidants that contain P – Coumaric acid; a substance known for its antioxidant, antimicrobial, anti-mutagenic, anxiolytic, analgesic, sedative and immunoregulatory effects [42].

4.1.1.2.Steroids: Steroids are natural or synthetic organic compounds with 17 carbon atoms arranged in four rings. Synthetic steroids of therapeutic value are a large number of anti-inflammatory agents, anabolic or growth-stimulating agents and oral contraceptives. Steroids in plants are called Phytosterols [43].The absorption of dietary plant sterols and stanols in humans is low about 0.02-3.5%when compared to cholesterol [44].Also, dietary supplements of plant sterols have been reported to have anti-cancer effects and also reduces low density lipoprotein (cholesterol) absorption from food [45]. Although sterols are not efficiently absorbed, 7-keto-sitosterol and 7-keto-campesterol have been detected in human plasma and have the potential to exert a variety of biological effects. For example, they have pro-atherogenic and pro-inflammatory properties in animal models [46], [47].

In reproduction, phytosterols which are precursors for cholesterol are not coordinately expressed during spermatogenesis as is the case in other tissues where they provide a housekeeping role in cholesterol synthesis [48]. In other words, spermatogenesis is controlled by hypothalamo-pituitary- gonadal hormones. Since the hypothalamo-pituitary hormones are proteins, consumption of the protein rich diet (peanuts) could have led to the increased production of FSH, and LH. Increased secretion of FSH and LH stimulates the secretion of the steroids in the gonads (Testosterone in the male and estrogen in the female).

From the research, it is possible that consumption of peanut could lead to lowered absorption of cholesterol in human and animal diets since it contains phyto-sterols. This is advantageous since excessive cholesterol in the body could lead to heart related diseases. Also, muscle response to phytosteroid according to Nolan et al., (2017) [49] is increase in protein synthesis which leads to increase in muscle protein. This knowledge can be used in treating the aged whose rate of apoptosis is greater than rate of cellular replacements. This agrees with Muradian and Schachtschabel, (2001) [50]; "Aging increases the rate of apoptosis".

4.1.1.3.Glycosides: A glycoside is a molecule in which sugar is bound to a non-sugar functional group through a bond often called glycosidic bond. Cardiac glycosides can be found in leguminosae of which peanut belong. Cardiac glycosides are used to treat cardiac failure [51]. Many plants store chemicals in the form of inactive glycosides. These glycosides are not biologically available for use by the animal [52]. However Lactobacillus acidophilus a pro-biotic domiciled in the digestive tract of the animal possess transport proteins that import the plant glycoside into the cell of the bacteria where hydrolytic enzymes that cleave off the sugar moieties and frees aglycone out of its cell are domiciled. The freed aglycone is released into the general circulation of the host where it is absorbed and the bioeffect of the aglycone established [53], [54]. Therefore, it is possible that regular consumption of peanuts could function as prophylaxis against heart failure. This assertion is supported by peanut institute, Albany Georgia "About a handful peanuts eaten five or more times a week can cut the risk of heart disease in half".

4.1.1.4.Carbohydrates: Carbohydrates are one of the three macronutrients in human and animal diets which provides energy. Classes or forms of carbohydrates include: monosaccharides (glucose, fructose and galactose), disaccharides (sucrose, lactose and maltose), oligosaccharides (fructo-oligosaccharides and malto-oligosaccharides), polyols (isomalt, maltitol, sorbitol, xylitol and erythritol), starch polysaccharides (amylose, amylopectin and maltodextrin) and non-starch polysaccharides otherwise known as dietery fiber (cellulose, Pectins, hemicellulose, gums and inulin) [55]. During digestion, complex carbohydrates are broken down into monosaccharides by digestive enzymes and are absorbed causing a glycemic response. The body uses glucose for energy and stores the excess from diet in the form of glycogen by the interaction of insulin. However, some of the carbohydrates cannot be broken down and they get either fermented by gut bacteria or they transit through the gut without being changed [56]. The research therefore highlights the potential of peanuts as energy source for humans and other vertebrates [57]. This agrees with Food and Agricultural Organization (FAO) and World Health Organization (WHO), (1991) quoted by Schaafsma, 2000 [58]; Protein Digestibility Corrected Amino Acid Score (PDCAAS) of peanut protein and other legume protein such as soy protein are the nutritional equivalent of meat and eggs for human growth and health.

4. 1. 2.Acute toxicity test: The result of acute toxicity studies shows that there were no deaths at 500, 1000, 2000, 3000, 4000, 5000 and 6000mg/kg body weight. When the animals were left for extra seven days, there was no sign of any toxicity noticed in all the groups (Table 2). This shows that consumption of peanuts is safe. However, it is necessary to physically observe peanuts for any sign of molds to avoid toxicity due to aflatoxicosis caused by *Apergillus* species of fungus. This agrees with Lien et al., (2019) [59]: "Afflatoxins are highly toxic and cause disease in livestock and humans. But, Zhang et al., (2018) [60] postulated that high heating rates of temperature above 80°C is lethal to *Aspergillusflavus* in peanuts. However surface roasting does not suffice as lethal temperature, but surface roasting combined with oven roasting is recommended [61].

4.1. 3.Minimum effective dose: The result of the minimum effective dose in adult female rats showed that the extract in the dose ranges of 400-1600mg/kg body weight resulted in estrus in all the experimental animals which shows 100% effectiveness in estrous synchronization whereas all the animals that received the lowest concentration of 200mg/kg body weight did not show any sign of estrus rather the animal were in different stages of estrous cycle namely; proestrus, diestrus and metestrus (Table 3, figs:1, 2, 3 and 4). This shows that the receptors for the compounds that cause estrus which is found in Arachis lyophilized aqueous extract (ALAE) are continuously available even at high doses. However, more work should be done to ascertain whether there is down-regulation of the receptors at any level of high dosage.

4.1.4. 1.**Hematology of male albino rats treated with ALAE**

Hematology assessments are relevant tools in evaluating the physiological and pathological status of mammals and birds, as they provide information for the proper diagnosis of diseases, making a prognosis, evaluating the efficacy of instituted therapy, and toxicity of drugs and chemical substances [62]. The hematological parameters of utmost clinical importance include the red blood cell (RBC) counts, packed cell volume (PCV), hemoglobin concentration (Hbc), mean corpuscular values, white blood cell (leukocyte) counts and differential leukocyte counts [63].

Furthermore, hematological parameters reflect the changes in the organism correctly and play an important role in the detection of disease. Hematological indices such as the number and morphology of erythrocytes, leucocytes, and thrombocytes are useful in disease diagnosis and monitoring [12], [13], [14].

From the hematological findings during the experimentation, ALAE treated rats had lower hemoglobin, lower packed cell volume, lower number of red blood cells but higher number of white blood cell (Table 4). However, there was no evidence of anemia since the mean corpuscular volume is higher in ALAE treated males than control and all were within the same range of slightly lower than 80 fl and this agrees with Kumar and Clark, (2002) [64], "shortening of red cell survival does not always cause anemia since there is a compensatory increase in red cell production by the bone marrow".

4.1.4.2. Differential leukocyte count: These include:

4.1.4.2.1. Neutrophils: Neutrophils are also known as polymorphonuclear (PMN) leukocytes. They are the most abundant cell type in animal and human blood. They are produced in the bone marrow in large numbers. They have a half-life of 6-12 hours [65]. Neutrophils constantly patrol the organism for signs of microbial infections, and when found, these cells quickly respond to trap and kill the invading pathogens. Three main antimicrobial functions are recognized for neutrophils: phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps [66].

Neutrophils also respond to multiple signals and respond by producing several cytokines and other inflammatory factors that influence and regulate inflammation and also the immune system [67]. From the research, ALAE treated male rats had significantly lower neutrophils. This could be because, ALAE has polyphenols which have antimicrobial activity. It is possible that the whole animals were harboring microbial infections but when treated with ALAE, the microorganisms were destroyed. However, more research should done to confirm the antimicrobial activity of ALAE as well as the mechanism of destruction of the microorganisms.

4.1.4.2.2. Lymphocytes: Lymphocytes are one of the main types of immune cells. Peripheral blood lymphocytes (PBLs) are mature lymphocytes that circulate in the blood rather than being localized to organs. They are one of several types of white blood cells (WBCs) that are crucial for the immune system. Peripheral blood lymphocytes are made up of: B-cells, T-cells, and natural killer cells All PBLs work together to protect the body against bacteria, viruses, and other toxins that cause diseases [68].

B-lymphocytes produce antibodies (gamma globulins) that recognize foreign substances (antigen) and attach themselves to them. Each B lymphocytes is programmed to make one specific antibody [69]. When a B-cell comes across its triggering antigen it gives rise to many large cells known as plasma cells. Each plasma cell is essentially a factory for producing antibody. Whenever the antibody and antigen interlock, the antibody marks the antigen for destruction [70].

T-lymphocytes are leukocytes that penetrate the cell and are programmed to recognize, respond to and remember antigens in cell-mediated immunity. T-lymphocytes contribute to the immune defenses in two major ways: Directing and regulating the immune responses and making lymphokines when stimulated by the antigenic material presented by the macrophages. The lymphokines signal other cells for activity [71].

Natural killer cells provide rapid responses to virus-infected and tumor cells [72].

From the research, ALAE treated rats had significantly high lymphocytes when compared to control. However, there was no separation of the lymphocytes into B or T lymphocytes neither was there any separation to indicate natural killer cells. It is possible that peanuts from which ALAE was gotten enhances immunity in vertebrates. This agrees with Timothy et al., (2000)[41] andLotito and Frei, (2006) [35]; 'Peanuts contain anti-oxidant (P-Coumaric acid) which has immune-regulatory effects'.

4.1.4.2.3. Monocytes: They are the largest type of leukocyte and can differentiate into macrophages and myeloid lineage dendritic cells. They form part of the vertebrate innate immune system and also influence the process of adaptive immunity. They are agranulocytes and composed 2-8% of total white blood cell count [73].

Monocytes are produced by the bone marrow from precursor cells called monoblast and bipotent cells which differentiated from hematopoietic stem cells. Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body where they differentiate into macrophages and dendritic cells [74].Macrophages are the body's first line of defense and have many roles. A macrophage is the first cell to recognize and engulf foreign substances (antigens). Macrophages break down these substances and present the smaller proteins to the T-lymphocytes. Macrophages also produce substances called cytokines that regulates the activity of lymphocytes [75].

Dendritic cells are known as the most efficient antigen-presenting cell type with the ability to interact with T-lymphocytes and initiate an immune response. In fact, monocyte, lymphocyte ratio (M:L) is used to predict the prognosis of debilitating diseases like cancer [76]. About half of the body's monocytes are stored as a reserve in the spleen [77]. There are at least three types of monocytes in human blood. They are: the classical monocytes which are characterized by high level expression of the CD14 cell surface receptor ($CD14^{++}CD16^{-}$monocyte), the intermediate monocyte with high level expression of CD14 and low expression of CD16 ($CD14^{++}$CD16 + monocyte) and the non-classical monocytes which show low level expression of CD 14 and additional co-expression of CD 16receptor ($CD14^{+}CD16^{++}$ monocyte) [78].

In mice, monocytes can be divided in two subpopulations. Inflammatory monocytes (CX3CR1low, CCR2+, Ly6Chigh, PD-L1-), which are equivalent to human classical CD14++ CD16− monocytes and resident monocytes (CX3CR1high, CCR2neg, Ly6Clow, PD-L1+), which are equivalent to human non-classical CD14low CD16+ monocytes [79].

Resident monocytes in mice have the ability to patrol along the endothelium wall in the steady state and under inflammatory conditions [80].

In humans, monocyte crawling behavior, similar to the patrolling in mice, has been demonstrated both for the classical and the non-classical monocytes [81].

Monocytes function in both innate immunity and acquired immunity through antigen presentation. To do this monocytes transform into **macrophages** in the tissues gobbling up bacteria, viruses, debris, and any cells that have been infected or are sick while the T-cells and macrophages are more immediately available to recognize and attack a new threat [82].

However, the dendritic cells accumulate debris from the breakdown of bacteria, viruses, and other foreign material and present it to the T-cells so they can see it and form an immune response to the invaders [83].

A monocyte count is part of a complete blood count and is expressed either as a percentage of monocytes among all white blood cells or as absolute numbers. Both may be useful but these cells become valid diagnostic tools only when monocyte subsets are determined [84].

This peanut research shows that ALAE treated male albino rats had significantly high monocyte count than the control. Just like in lymphocytes monocytes are part of the immune regulators in the body. It then implies that peanuts have the potential to enhance the immunity of treated animals.

4.1.4.2.4.Eosinophils: They are a minority circulating granulocyte involved in host defense against parasites and promoting allergic reactions [85].

Again, from the research, ALAE treated rats had significantly lower eosinophil (Table 5). This could be because peanuts contains flavonoids which has anti-parasitic effect. By treating the rats with ALAE, the parasites in the blood were taken care of. This agrees with Martinez-Castillo et al (2018) [60], peanuts contains flavonoids that has anti-parasite activity.

4.1.5. Structural and functional characteristics of the testes, hypothalamus and pituitary in albino rats treated with ALAE and distilled water as control

The testes of Arachis lyophilized aqueous extract (ALAE) treated rats for 31 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 ALAE treated rats and sparsely populated in control rats (Figs. 5, 6, 7, 8, 9, and 10). The research showed that though ALAE treatment did not affect the number of seminiferous tubules in rats' testes, it did positively affect the function of the tubule which is spermatogenesis. Treatment of the rats with ALAE increased the fertility and fecundity of the rats.

However, in the hypothalamus, ALAE treated rats showed acidophilic staining nuclei (a) and basophilic staining nuclei (b). Subjectively, ALAE treated rats had more of the basophils than acidophil which secretes GnRH and Thyrotropin releasing hormone (Figs. 11 and 12). This implies that there was enough gonadotropin releasing hormone (GnRH) in the hypothalamus which led to the synthesis of FSH and LH from the pituitary. Also in the pituitary, there were more basophilic cells secreting FSH, LH and Thyroxin (Figs. 13 and 14) whichprobably led to the improved spermatogenesis in the treated rats observed in the testis (figs. 9 and 10).

4.2. Conclusion

Peanuts (*Arachishypogaea*) though nuts in the culinary sense and viewed as whole food according toProtein Digestibility Corrected Amino Acid Score (PDCAAS) by WHO and FAO 1991, has high medicinal properties as well as immunoregulatory properties which was observed in the treated rats that possessed lower eosinophils and lower neutrophils but higher lymphocytes and monocytes.

Also, higher basophilic nuclei in the hypothalamus and higher basophilic cells in the pituitary may suggest that peanut consumption may lead to improved reproductive efficiency.

V. Declarations

All the experimental procedures were performed according to the ethical guidelines for research using animal studies established by the Ethical Committee of University of Nigeria, Nsukka which is 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.

Consent of publication: All the authors unanimously consent to this publication.

Availability of data and materials: The materials and data are available upon reasonable request from the corresponding author.

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Author contribution:

(1). First author (NN) wrote the topic of the research, sourced available literature for the work, designed the experiment and performed the research.

(2). Second author (UN) co-designed the experiment, supervised the research and proof read the manuscript

(3). Third author (OR) proof read and corrected the manuscript.

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