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Unripe-pulp *Persea amercicana* Extract Possibly Induces Folliculogenesis

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Abstract

Background: *Persea americana* pulp has monounsaturated fatty acids (MUFAs), which are associated with improved reproductive health.
Objectives: To determine the result associated with *Persea americana* methanol unripe-pulp extract on the ovaries and ovarian cycle of adult female albino Wistar rat.
Study design: Twenty (20) mature female albino rats with regular ovarian cycle, weighing 150-200g were divided into 4 groups of 5 animals, each group received; Group 1 (control) received 5 mg/kg body weight of distilled water, while Group 2, Group 3 and Group 4 received 380 mg/kg, 760 mg/kg and 1,140mg/kg body weight of *Persea americana* methanol unripe-pulp extract respectively. Administration was intraperitoneally at the proestrus phase for 12 days with simultaneous examination of the oestrus cycle which continued for 12 more days after cessation of administration, followed by sacrifice. Blood was collected for analyses of oestrogen, LH, FSH and progesterone while organ weight was measured and subsequently histological analysis. Dosages were from acute toxicity studies performed using the Up and Down method.
Result: There was a proportional decrease in weight gain with increasing dosages and no significant difference in organ weight except for the heart. There was an increase in concentration of estradiol, LH and FSH and a reduction in the concentration of progesterone with increased doses. The frequency of the oestrus phase was increased though not significant. Histologic analysis revealed normal histo-architecture across all groups with apparently more follicles in the experimental groups.
Conclusion: There was an increase in frequency of oestrus phase, concentration of estradiol, LH and FSH levels with apparently more developing ovarian follicles.

Keywords: Oestrus cycle, Fertility, Ovulation, *Persea americana* pulp, Reproductive hormones, Weight gain. Ovarian follicles

Introduction

Infertility, a public health issue with a myriad of negative implications on both sexes and the society at large, is ranked the 5th most common global disability. However, the social burden is more on women than men.[1] The prevalence of infertility cases has increased from 42 million to 48.5 million between 1990 and 2010 respectively, with about 0.37% increase in global disease burden yearly.[2,3] In addition, World Health Organization (WHO) has estimated that approximately 186 million individuals live with infertility globally and 1 in 6 couples worldwide is affected by infertility.[4,5] Diseases like premature ovarian failure, endometriosis, ovarian germ cell tumours, ovarian low malignant potential tumours, ovarian cyst, ovarian cancer, polycystic ovarian syndrome (PCOS) could result in infrequent and/or irregular ovulation, as well as anovulation thereby leading to infertility and ovulatory disorders is responsible for infertility in

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40% of women with the disability.[6,7]

The consequences of infertility are diverse and can include societal discrimination and personal distress. Dieting (nutrition), improved lifestyle and habits, as well as advances in assisted reproductive technologies (ART), such as in-vitro fertilization (IVF) has offered hope to many affected couples but barriers exist in terms of availability and affordability.[4] Currently, many researchers are interested to investigate the use of natural remedies for preventive and curative purposes of infertility which could be economical and convenient.[8]

Traditional and complementary medicine is prevalent and accepted in Africa because it aligns with patient's cultural and religious belief, in addition to being accessible and in-expensive. It is also thought to be efficient and safer compared with the controversies of conventional healthcare.[9] Various studies have shown the role of micronutrients found in plant extracts and plant products in the treatment of female infertility either alone and in combination with other treatments.[5]

Persea americana, better known as avocado pear or alligator pear, is generally ingested for its high nutritional value and health-promoting benefits.[10,11] The seed, pulp, and peel of *Persea americana* has been scientifically investigated for several properties including analgesic, anti-atherosclerotic, anti-cancers, anti-diabetic, anti-hypertensive, anti-inflammatory, anti-mycobacterial, anti-obesity, anti-oxidants, anti-thrombotic and anti-viral effects.[12,13,14,15,16,17,18,19] *Persea americana* pulp is endowed with monounsaturated fatty acids (MUFAs), which are associated with lower rates of ovulatory infertility. In addition to MUFAs, *Persea americana* also contains folate, potassium, and vitamin A, which are all important for reproductive health.[20]

In an in vivo study on the effect of *Persea americana* pulp on reproductive hormones including follicle stimulating hormone (FSH) and progesterone, the result showed that the extract may contain some bioactive constituents which could cause alteration in the hormonal profile and hence can change reproductive functions[21]. However, research into the specific effects on the ovary is necessary to elucidate more on the type of reproductive changes the seed can cause.

Materials and method

Fruit identification and authentication

The *Persea americana* was purchased from Itam market, Uyo in Akwa Ibom State and identified and authenticated by a certified taxonomist of the department of Botany, University of Uyo, Uyo. Specimen Voucher number UUPH No. 16(a)

Preparation of methanolic extract of *Persea americana* unripe pulp

The fresh unripe *Persea americana* fruits was washed thoroughly under running clean tap water to remove dirt and debris. The fresh unripe *Persea americana* fruits was sliced open with a clean sharp knife. The seed and peel were discarded. The pulp was chopped into bits to increase surface area and make the extraction faster. Methanolic extract of *Persea americana* pulp was prepared using exactly 500 gm of *Persea americana* pulp added to 2,000 ml of 100% methanol and macerated for 3 days. Following this time period, the resulting homogenate was filtered using Whatman No. 5 filter paper thereafter, the crude extract of *Persea americana* pulp was concentrated in a rotary evaporator (at 60°C) and then stored in an opaque glass bottle at until use.

Acute toxicity

Acute toxicity studies were done following the Up and Down method as documented[22,23] and a total of 13 mice weighing 25g each were used. After acclimatization, a stat dose of 100mg/kg was given intraperitoneally and the animal was observed for signs of toxicity within 48hrs. The next dosage depended on the outcome of the animal, that is, dose would be increased if there were no mortality and vice versa. Monitoring lasted for a total of 14 days for the animals that survived.

Animal management and handling

Twenty (20) female albino rats of Wistar strain with weight ranging between 150-250g were procured from the Animal House of the Faculty of Pharmacy, University of Uyo, Uyo, Nigeria. Animals with regular and consecutive oestrus cycles were randomly sorted into groups and housed in well-ventilated and spaced cages at a room temperature of 27°C-30°C and humidity. The rats had free access to water and feed (Vital feed, Grand Cereals Limited Nigeria) and were exposed to 12 hours light and dark cycles. Care for animals were based on international recommendations of National Research Council.[24]

Preparation of test sample and dosing

Stock solution of the extract was prepared based on

the LD50 using a clean dried beaker and normal saline. These solutions were kept in dark glass vials and administered to the subjects daily intraperitoneally between 6a.m. and 10 a.m.

Experimental design

The experiment was performed according to the procedure as described with slight modifications.[25] Twenty (20) albino Wistar rats with regular and consecutive cycle were divided into 4 groups as follows:

Group 1 (control) received 1ml/kg body weight of distilled water.

Group 2 received 380 mg/kg body weight of *Persea amercicana* methanol unripe-pulp extract

Group 3 received 760 mg/kg body weight of *Persea amercicana* methanol unripe-pulp extract

Group 4 received 1,140 mg/kg body weight of *Persea amercicana* methanol unripe-pulp extract

Administration was for 12 days. Vaginal smears were taken for during the administration of the extract and 12 days after between 8a.m. and 10 a.m. daily.

Examination of vaginal smears

Vaginal secretions were collected daily (between 8a.m. and 10a.m.) using a glass pipette for 3 successive cycles as described but with slight modifications.[26] The different phases of the oestrous cycle were determined by analyzing the proportion of the three distinct cell types present on the slide under an optical microscope (at magnifications of 100 and 400). The distinguishing phases of the cycle was classified into oestrus (E), metoestrus (M), dioestrus (D) and proestrus (P). A typical regular cycle lasts for 4 days. Oestrous cycles lasting <4 days or >5 days cycle was marked as irregular.[27]

Sacrifice

After the last day of observation, the animals were fasted overnight and sacrificed on the next day under general anaesthesia (0.7ml of ketamine). Blood samples were collected by cardiac puncture into plain sterile sample collection tubes with appropriate labels after which the animals were dissected and the ovaries were harvested and weighed. The average weight of the left and right ovaries was recorded for each animal and used to determine the organ weight.

Histological analysis

The histological analysis was carried out as stated but with a little change.[28] The ovaries were extracted, weighed and fixed in 10% buffered formal saline for histological analysis. The fixed tissues were dehydrated through changes of graded alcohols. This was done to remove excess water inherent in tissues as follows; two changes of 70% and 95% alcohol for a period of 15 minutes each, three changes of absolute alcohol for a period of 15 minutes. Dehydrated tissues were cleared using xylene (2 changes). Tissues were impregnated with two changes of paraffin wax all the procedures done in Histoline Automatic tissue processor set at the temperature of 60^{o}C at thirty minutes (30mins) each to enable embedding. After infiltration tissues cassette were transferred from the final wax bath. The tissues were taken out of the cassette placed in proper anatomical orientation inside the mould filled with molten wax, and covered with the corresponding cassette. The embedded tissue blocks were allowed to solidify. The paraffin tissue blocks were trimmed, and the trimmed surfaced were placed on ice bar for cooling to enhance the plasticity and tissue-paraffin homogeneity for proper sectioning. The tissues were sectioned at five micronmetres (5μm), and ribbons were gently picked with Carmel brush and dropped in a floating water bath at 60^{o}C to enable ribbons float, expand and flatten out. Slides were rubbed with thymol containing egg albumen, and gently dipped into the bath to pick up the flattened-out tissue ribbons.

Tissue sections were taken to water by deparaffinising in two changes of xylene and hydrated through graded series of alcohols in descending order rinse in water and stain in Haematoxylin for ten minutes (10mins), sections were rinsed and differentiated in one percent (1%) acid alcohol and blue in running water using saturated lithium carbonate solution until sections appear sky blue. The blued section was counterstained in Eosin solution, for three minutes (3mins). Tissues were washed well in water and dehydrated in ascending grade alcohol, cleared in xylene and mounted in DPX covered with coverslips.

Hormonal analysis

The blood was collected blood in a sterile plain specimen container under room temperature before centrifugation in a refrigerated centrifuge and the extraction of the serum. Concentrations of follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, and estradiol were determined.

Determination of concentration of follicle stimulating hormone and luteinizing hormone in

serum

This was conducted using assay kits from Monobind Inc.[29] The desired number of coated wells was secured in a holder. Fifty microlitre (50 µL) of standards, specimens and control were dispensed into appropriate wells. FSH and LH enzyme reagent solution (100 µL) was dispensed into each well, swirled thoroughly and allowed to mix for 23 seconds. The mixture was allowed to incubate for 60 minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked three times with wash buffer (350 µL).

Working substrate solution (100 µL) was dispensed to each well. The mixture was incubated at room temperature for 15 minutes. The reaction was stopped by addition of stop solution (50 µL) to each well and gently mixed 15 - 20 seconds to ensure a complete colour change. Absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfection) was read within 30 minutes with a microplate reader.

Determination of concentration of progesterone hormone in serum

This was conducted using assay kits from Monobind Inc. The desired number of coated wells was secured in a holder. Twenty-five microlitre (25 µL) of standards, specimens and control were dispensed into appropriate wells. Progesterone enzyme reagent solution (50 µL) was dispensed into each well, swirled thoroughly and allowed to mix for 20 - 30 seconds.

Progesterone biotin reagent (50 µL) was also dispensed into each well, swirled thoroughly and allowed to mix for 20 - 30 seconds. The mixture was allowed to incubate for 60 minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked three times with wash buffer (350 µL). Substrate solution (100 µL) was dispensed to each well. The mixture was incubated at room temperature for 20 minutes. The reaction was stopped by addition of stop solution (50 µL) to each well and gently mixed for 15 - 20 seconds to ensure a complete colour change. Absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfection) was read within 15 minutes with a microplate reader.[30]

Determination of concentration of estrogen (E2) in serum

This was conducted using assay kits from Monobind Inc. The desired number of coated wells was secured in a holder. Twenty-five microlitre (25 µL) of standards, specimens and control were dispensed into appropriate wells. Estradiol biotin reagent (50 µL) was dispensed into each well, swirled thoroughly and allowed to mix for 20 - 30 seconds. The mixture was allowed to incubate for 30 minutes at room temperature. Estradiol enzyme reagent (50 µL) was also dispensed into each well, swirled thoroughly and allowed to mix for 20 - 30 seconds. The mixture was allowed to incubate for ninety minutes at room temperature. The contents of the micro wells were discarded by decantation, then rinsed and flicked 3 times with wash buffer (350 µL). Substrate solution (100 µL) was dispensed to each well. The mixture was incubated at room temperature (18-22°C) for 20 minutes. The reaction was stopped by addition of stop solution (50 µL) to each well and gently mixed for 15 - 20 seconds to ensure a complete colour change. Absorbance at 450 nm (using a reference wavelength of 620-630 nm to minimize well imperfection) was read within 30 minutes with a microplate reader[30]

Ethical approval

The experimental protocols for this study were approved by the Ethical Review Committee of the Akwa Ibom State Ministry of Health, Uyo with no AKHREC/29/03/088. The study was conducted in strict accordance with the nationally accepted standard ethical guidelines for laboratory animal use and care as described.[24]

Statistical analysis

Results was expressed as mean ± stand error (mean ± SE) of six observations. Means were analysed using a one-way ANOVA followed by Student's test to compare the difference between the control and treated values and $P<0.05$ were considered significant.

Results

Acute toxicity studies of *Persea americana* methanol unripe-pulp extract

Table 1: Acute toxicity studies of *Persea americana* methanol unripe-pulp extract

Dose (mg/kg body weight)	No. of deaths recorded
100 mg/kg	0/1
500 mg/kg	0/1
1000 mg/kg	0/1
2000 mg/kg	0/1
3500 mg/kg	0/4
3800 mg/ kg	2/4
4000 mg/kg	1/1

The acute toxicity test using the Up and Down method of the methanolic extract of *P. americana* caused no mortality in the mice at dose levels 100 mg/kg - 2000 mg/kg as in Table 1 and no lethal effects were noted throughout the short and long-term observation period. At dose level 4000 mg/kg mortality was recorded. At dose level of 3500mg/kg no mortality was recorded but toxic symptoms of tremor and recumbency was noted. Finally at dose level 3800mg/kg, Mortality was recorded leading to the LD50 at 3800mg/kg.

Table 2: Effect of *Persea americana* methanol unripe-pulp extract on body weight of female albino Wistar rat

Group	Sample size	Initial Weight (g)	Final Weight (g)	Weight Difference (g)
Normal Saline 1 ml/kg body weight	5	172.2 ± 2.87	183.3 ± 2.83	13.10 ± 1.87
380 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	5	167.0 ± 4.90	173.3 ± 6.69	6.25 ± 2.18
760 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	5	175.5 ± 8.57	179.3 ± 6.03	3.75 ± 2.50
1140 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	5	165.5 ± 7.24	168.5 ± 11.20	3.00 ± 10.06

Values represent Mean ± SEM; P < 0.05

Table 3: Effect of *Persea americana* methanol unripe-pulp extract on organ weight of female albino Wistar rat

Group	HW(g)	KW (g)	LW (g)	LU(g)	OW (g)	UW (g)
Normal Saline 1 ml/kg body weight	0.62±0.06	1.00±0.01	5.28±0.07	1.54±0.20	0.16±0.01	0.88±0.07
380 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	0.66±0.06	1.21±0.05	6.78±0.16	1.45±0.11	0.17±0.02	0.98±0.07
760 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	0.79±0.03	1.16±0.03	6.42±0.27	1.53±0.19	0.16±0.02	0.88±0.06
1140 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	0.78±0.04	1.11±0.08	5.87±0.36	1.55±0.17	0.15±0.01	0.86±0.05

Values represent Mean ± SEM; P < 0.05
Key:
HW – Heart Weight
KW – Kidney Weight
LW – Liver Weight
LU – Lung Weight
OW – Ovarian Weight
UW – Uterine Weight

Table 4: Effect of *Persea americana* methanol unripe-pulp extract on hormonal parameters

Group	E2 (pg/mL)	FSH (mIU/mL)	LH (mIU/mL)	PROG (ng/mL)
5 ml Kg b. wt Normal Saline	48.75±1.75	0.2125±0.013	0.5925±0.020	28.35±0.92
380 mg/Kg b. wt of the extract	56.25±2.39^{b}	0.3700±0.045$^{a\ b}$	0.5503±0.044^{b}	27.85±1.26^{c}
760 mg/Kg b. wt of the extract	74.00±5.67$^{a\ b}$	0.9125±0.019^{bc}	0.8950±0.070$^{a\ b}$	25.75±3.69^{c}
1,140 mg/Kg b. wt of the extract	82.10±2.677^{a}	0.5050±0.047$^{a\ c}$	0.8075±0.096	16.53±0.466$^{a\ c}$

Values represent Mean ± SEM; P < 0.05
Key:
E2 – Estradiol
PROG - Progesterone
FSH - Follicle Stimulating Hormone
LH - Luteinizing Hormone

a = significance between the control group and any other group
b = significance between low dose and any other group
c = significance between middle dose and any other group
d = significance between high dose and any other group

Effect of *persea americana* methanol unripe-pulp extract on body and organ weight of female albino wistar rat

There was a gradual reduction in weight difference in the experimental groups with increase in dose of extract administered to the test groups as compared to the control group. With the experimental group treated with 1,140 mg/kg body weight of extract having the lowest weight difference value of 3.00 ± 10.06g.

There was an increase in the weight of the heart in the experimental groups with the group treated with 760mg/kg having the highest value of 0.79 ± 0.03g as compared to the control group. The weight of kidneys increased in all the experimental groups with the groups treated with 380 mg/kg having the highest weight increase with the values of 1.21 ± 0.05g compared to the control. There was an increase in the weight of the liver in the experimental groups with the group treated with 380 mg/kg having the highest value of 6.78 ± 0.16g compared to the control. The weight of the lungs was reduced in the groups treated with 380mg/kg and 760mg/Kg of extract with the values of 1.45 ± 0.11g and 1.53 ± 0.19g respectively and increased in the groups treated with 1140mg/kg of extract with values of 1.55 ± 0.17g, compared to the control. There was an increase in weight of the ovaries in the group administered with 380 mg/kg of extract with the values of 0.17 ± 0.02g and a reduction in weight of the ovaries in the groups administered with 1140 mg/kg with values of 0.15 ± 0.01g as compared to the control group. There was an increase in the weight of the uterus in the group treated with 380 mg/kg and a decrease in weight of the uterus in the group treated with 1140mg/kg with values of 0.98

± 0.07g and 0.86 ± 0.05g, respectively, compared to the control. These changes were not significant.

Effect of Methanolic Extract of *Persea americana* Pulp on Hormonal Parameters of Female Albino Wistar Rat

Oestrogen was increased in all the test groups compared to control with increasing dose of extract administered. There was an increase in the concentration of FSH with increasing dose but paradoxically with the highest concentration in the middle dose group (760mg/kg dose body weight of extract) with 0.9125 ± 0.019 mIU/mL. LH was reduced in low dose group (380mg/kg dose body weight) with values of 0.5503 ± 0.044 mIU/mL but increased in the middle dose and high dose group with value of 0.8950 ± 0.070 mIU/mL and 0.8075 ± 0.096 mIU/mL respectfully, compared to control. There was a dose dependent decrease in progesterone in the experimental groups with the lowest concentration in the high dose group (1,140 mg/kg dose weight of the extract) with 16.53 ± 0.466 ng/mL.

Table 5: Effect of *Persea americana* methanol unripe-pulp extract on the frequency of the oestrus cycle phases of female albino Wistar rat

Group	Dioestrus	Proestrus	Oestrous	Metoestrus
Normal Saline 1 ml/kg body weight.	18.34±1.67	25.03±0.24	28.33±2.04	26.67±1.67
380 mg/kg body weight *Persea americana* methanol unripe-pulp extract	18.33±3.12	21.67±2.04	28.33±2.04	28.33±3.33
760 mg/kg body weight *Persea americana* methanol unripe-pulp extract	20.10±3.33	16.67±2.64	40.0±4.86	19.00±5.64
1140 mg/kg body weight *Persea americana* methanol unripe-pulp extract	20.10±3.33	18.33±3.12	38.33±5.65	25.10±4.56

Values represent Mean ± SEM; $P < 0.05$

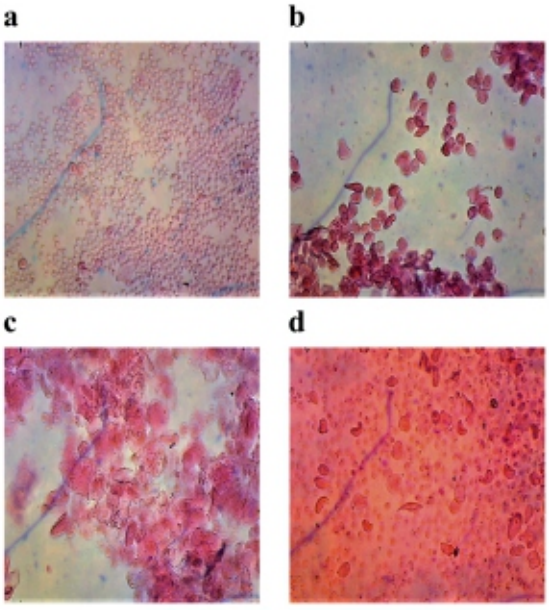

Figure 1: Phases of the Oestrous Cycle of Rats

Showing;
a – Diestrous
b – Proestrous
c – Oestrous
d – Metestrous

Effect of *Persea americana* methanol unripe-pulp extract oestrus cycle of female albino wistar rat

The phases of the oestrous cycle of the rats identified during the experiment were; Diestrous, Proestrous, Oestrous and Metestrous. These phases were identified as the images below;

In the dioestrus phase, the frequency is increased in the groups treated with 760mg/kg and 1140mg/kg with the value 20.10 ± 3.33 as compared to control group. In the proestrus phase, there is a reduction in the frequency in the experimental groups with the lowest being the group treated with 760mg/kg with the value 16.67 ± 2.64 as compared to the control group. In the oestrus phase, there is an increase in the frequency in the experimental groups with the highest frequency being the group treated with 760mg/kg with the value 40.0 ± 4.86 as compared to the control group. In the metoestrus phase, there is an increase in the frequency in the experimental group treated with 380mg/kg with the value 28.33 ± 3.33 and a decrease in the frequency with the groups treated with 760mg/kg and 1140mg/kg with the values 19.00 ± 5.64 and 25.10 ± 4.56 respectively as compared to the control group.

Ovarian sections demonstrate typical primordial and other developing follicles within the cortex. The histomorphology of the ovary in the control group revealed primordial cells and corpus luteum, indicating normal ovarian microarchitecture. When compared to the control group, all rats in the experimental groups had more follicles. Their ovarian follicles were seen at various stages of maturation

Histomorphologic effect of *Persea americana* methanol unripe-pulp extract on the ovary of female albino Wistar rat

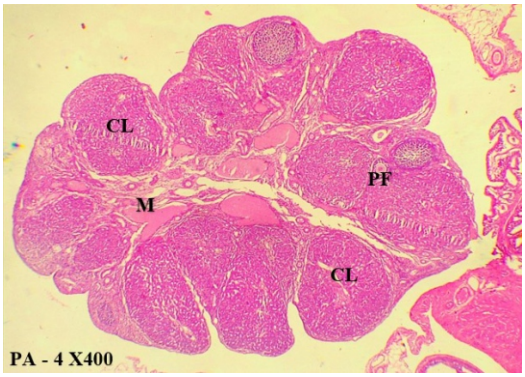

Figure 2: Photomicrograph of ovary histology with distilled water (5 ml/Kg b wt) showing PF= Primary Follicle, CL= Corpus Lutuem, M= Medulla. H&E, 40x

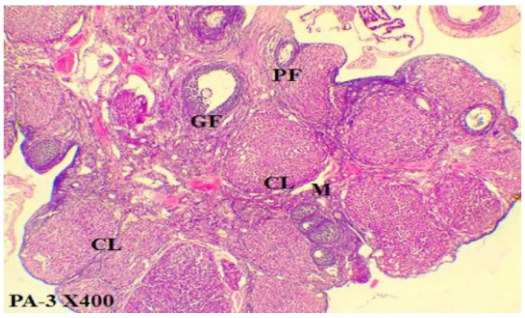

Figure 3: Photomicrograph of ovary histology with *P. Americana* (380mg/kg) showing PF= Primary Follicle, CL= Corpus Lutuem, GF- Graffian Follicle, M= Medulla. H&E Mag 40x

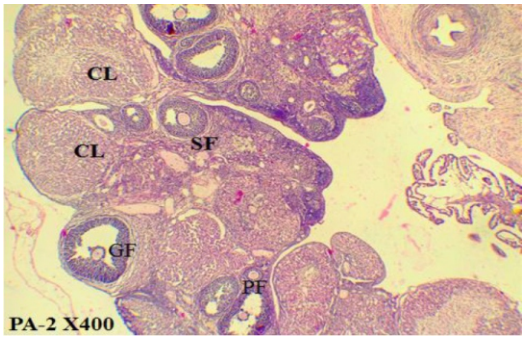

Figure 4: Photomicrograph of ovary histology with *P. Americana* (740mg/kg) showing PF= Primary Follicle, CL= Corpus Lutuem, GF- Graffian Follicle, SF= Secodary Follicle. H&E, x40

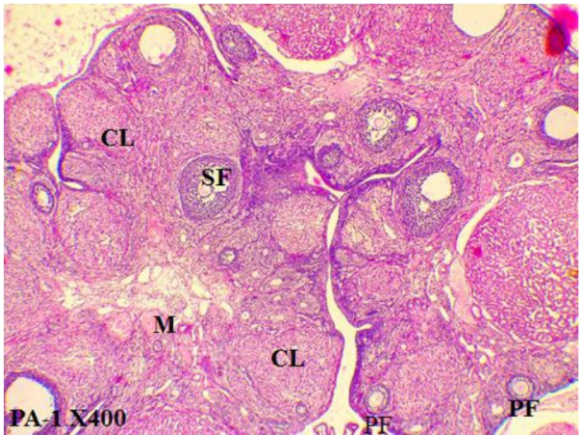

Figure 5: Photomicrograph of ovary histology with *P. Americana* (1140mg/kg) showing PF= Primary Follicle, CL= Corpus Lutuem, GF- Graffian Follicle, M= Medulla, SF- Secondary Follicle. H&E 40×

along with mature follicles and corpus luteum, the medulla composed of connective tissue stroma with blood vessels. The group that received 740 mg/kg body weight seemed to have the most primordial and developing follicles.

Discussion

The acute toxicity tests are used to ascertain the harmful effects of a particular chemical or drug which is absorbed in body by various routes such as through oral, cutaneous, inhalation and intravenous routes.[31,32] Degree of toxicity based on doses; substances with LD50 below 5 mg/kg are considered extremely toxic, substances between 5–50 mg/kg are considered highly toxic, substances between 50–500 mg/kg are considered moderately toxic, substances between 500–5,000 mg/kg are considered lightly toxic, substances between 5000-15,000mg/kg are considered practically non-toxic while substances ranging above 15,000 mg/kg are considered relatively harmless.[31] The LD50 of the methanolic extract of Persea americana pulp was determined at 3,800 mg/kg, thus it is lightly toxic.

Acute oral toxicity of the blended homogenised pulp of *Persea americana* could not be determined as no lethality was observed up to 3000mg/kg. No toxicity symptoms were recorded, only mild diarrhoea was observed.[33] In a toxicity study, ethanolic extract of p. americana pulp was administered orally to Wistar rats. No death occurred even in the highest doses administered, nor were there any signs of toxicity

reported. The LD50 value was determined to be higher than 5000mg/kg body weight. The extract was classified as belonging to the practically non-toxic category.[34] The first pass effect of drugs could reduce the bioavailability of the active drug component at site of action or the systemic circulation if the drug was administered orally.[35] Thus, it could account for the difference in the acute toxicity values.

Persea americana is highly nutrient dense and has high levels of monounsaturated fatty acids (MUFA).36 MUFA diets do not promote weight gain and are more acceptable than low-fat diets for weight loss in obese subjects.[37] This would explain the decrease in weight gain in the experimental groups.

Administration of aqueous extract of *P. americana* pulp on experimental diabetic rats resulted in decrease in weight differences.[38] Similar findings in the present were seen in a Cohort studies performed on the BMI of individuals after the intake of fresh avocados pulp showed a considerable reduction in weight gain compared to the control, which consecutively lowered various cardiovascular problems associated with obesity.[39]

No significant differences ($P > 0.05$) were observed in the liver, lungs, kidney and uterus of all the test groups when compared to the control groups. The weight of the heart was significantly increased across the experimental groups. *Persea americana* pulp contains close to twenty times the amount of fat-soluble phytosterols than other fruits. Phytosterols are plant chemicals considered to have beneficial effects on the heart's health.[40]

The ovaries had highest weight in the group treated with 380mg/Kg b. wt. of extract. It has been suggested that an increase in ovarian weight may be as a result of increase in the number of growing follicles.[41]

The weight of the heart, liver and kidney of male albino Wistar rats were significantly increased at ($p< 0.05$) in the experimental groups when compared with control rats' group in a study where the hepatoprotective activity of avocado oils gotten from the avocado pulp and coconut oils against biochemical alteration and oxidative stress induced by nicotine in male albino rats was studied.[42]

The proestrus phase corresponds to the human follicular stage, oestrous phase correlates with ovulation while the metoestrus and dioestrus phases are homologous to human early and late secretory stages of the reproductive cycle, respectively.[43,44]

The proestrus phase is associated with a rise in circulating oestrogen concentrations which leads to a rise in luteinizing hormone (LH) and follicle stimulating hormone (FSH) release leading to ovulation.[43] The increased FSH and LH level in the study may be due to effect of the extract on the hypothalamus since the gonadotropic releasing hormone secreted by the hypothalamus regulates the secretion of FSH and LH.[45] The significant increase of FSH and LH is an indication that the *P. americana* extract favours the oestrus cycle and ovulation.

Also, in support of ovulation, the proestrus phase which is characterized by folliculogenesis is supported by high levels of luteinizing hormone and follicle stimulating hormones.[46] FSH enhances the development of primordial follicles until the preovulatory stage.[47] High levels of LH and oestrogen triggers the rupture of a large number of mature follicles thereby increasing ovulation rate.[43] The suppression of the release of FSH and LH from the anterior pituitary inhibits ovulation and oestrogen production in women.[49]

In the experimental group treated with 1,140 mg/Kg, there is a slight decline in the concentration of FSH and LH, although the actual cause of the reduction in concentration is unclear, one would suggest that the decline of FSH and LH concentration may be attributed to the direct negative feedback effect of oestrogen within physiological limits on the pituitary gland which is responsible for FSH and LH secretion.[50] The same study also showed that the negative effect of oestrogen on FSH responsiveness is greater than on LH and this could be the reason for the slight decrease in the concentration of LH in comparison to FSH in the experimental group treated with 1,140 mg/kg body weight.[50]

A comparison of the oestrus cycle of the control and treated groups revealed a mild change in the frequency of the phases of the oestrus cycle. Oestrus cycle in rat is controlled by the ovarian hormones, oestrogen and progesterone, secreted from granulosa cells and corpus luteum respectively. These hormones in turn are regulated by pituitary gonadotropins; follicle stimulation hormone and luteinizing hormone.[51] These hormones govern the stages of oestrus cycle. A change in these hormones results in changes of the oestrus cycle.[45] The cyclic changes of the vaginal smear observed in the oestrus cycle gives a reasonable layout of ovarian activity.[47] The data obtained in the present study reveals that extract of *P. americana* pulp causes an increase in frequency of oestrus stage in all experimental groups. The oestrus phase has a higher frequency than other phases across

all the doses of the extract administered. An increase in LH and estradiol is observed in the experimental groups, high levels of LH and estradiol stimulates the rupture of a large number of mature follicles thereby increasing ovulation rate which falls within the oestrus phase.[48]

In contrast, a study to check the effect of Cleome gynandra leaf extract on the oestrus cycle of albino Wistar rats reported a persistent proestrus, followed by a reduction in oestrus, metoestrus and dioestrus.[52] Similarly, the effects of Cyclea peltata root extracts on reproductive system of female rat induced a significant prolongation of dioestrus and metoestrus phase thereby acting as antifertility agent.[53]

The ovarian histomorphological assessment showed a normal ovarian architecture across all the *Persea americana* pulp treated groups, no pathologic changes were found in the ovary which suggests the non-toxic effects of *Persea americana* unripe-pulp on the ovaries and the formation of the corpus luteum is a direct continuation of preovulatory follicular development.[54] The number of ovarian follicles appeared to have increased and folliculogenesis enhanced in the current study. One of the best indicators for evaluating female fertility is ovarian folliculogenesis.[55] Pituitary gonadotropins (follicle-stimulating hormone (FSH) and luteinizing hormone (LH)) tightly control follicle development and have a stimulatory impact upon follicle growth.[56]

In the ovary, gonadotropins interact with intraovarian factors to regulate steroidogenesis, follicle development, oocyte maturation, ovulation, and formation of the corpus luteum.[56] Oestrogens synthesized in the ovary during folliculogenesis in turn act on the hypothalamic–pituitary (H–P) axis to regulate gonadotropin secretion.[58] While oestrogens generally exert a negative regulatory effect on gonadotropin secretion, a high level of oestrogens during the preovulatory period induces a surge of gonadotropins, which is essential for oocyte maturation and induction of ovulation.[59] Follicle-stimulating hormone (FSH), Luteinizing hormone and oestrogen are critical for ovarian folliculogenesis and female fertility and oestrogen deficiency can eventually lead to follicular apoptosis and atresia.[60,61] The apparent increase in the number of follicles in the presence of methanolic extract of *Persea americana* pulp may have been due to the increase in gonadotropins (FSH, LH, oestrogen) exerted by the extract as observed in the current study.

Conclusion

The results of the present study shows that methanolic extract of *P. americana* pulp causes a positive alteration in the female hormonal profile thereby changing reproductive functions. *P. americana* may be useful in management of female reproductive hormonal oriented health issues.

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