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Effect of Persea americana unripe pulp methanol extract on pregnancy in laboratory animals

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Abstract

Context: Persea americana unripe pulp may induce various hormonal changes in female rats.
Objective: To investigate the effect of Persea americana unripe pulp methanol extract on conception in female Wistar rats
Materials and Method: Forty (40) mature female Wistar rats, divided into 2 groups of 20 rats each were used for preimplantation and post-implantation experiments with mating done before and after administration, respectively. Doses were percentages of LD-50, given intraperitoneally for 12 days and mating was done at late proestrus in the female: male ratio of 2: 1. Control received 1 ml/kg body weight; experimental groups received 380 mg/kg, 760 mg/kg, and 1140 mg/kg body weight of Persea americana unripe pulp methanol extract. Animals were sacrificed on day 5 of pregnancy, day 15, day 20, and day 21 of pregnancy, while the rest were allowed to experience pregnancy outcome before sacrifice under general anesthesia.
Result: Pregnancy was unsuccessful; weight decreased with increasing doses; FSH and progesterone increased, while estrogen and LH decreased. Histological findings showed apparent endometrial thickening in all doses of extract.
Conclusion: The study showed that Persea americana unripe-pulp methanol extract inhibits conception, promotes contraception, and thus should be used with caution in women intending to conceive.

Keywords: Persea americana unripe pulp, uterus, contraception, reproductive hormones.

Introduction

Reproductive health and fertility control are critical in developing countries such as Nigeria, where population growth is out of proportion to available healthcare outlets and people's socioeconomic empowerment.[1] Medicinal plants are the most commonly used form of traditional and complementary medicine in Africa, and their acceptability can be attributed to their affordability, accessibility, and alignment with patients' cultural and religious beliefs.[2]

The sustainable development goals (SDGs) that replaced the Millennium Development Goals (MDGs) by 2015, has its third goal- target aimed at achieving equal and universal access to sexual and reproductive health (SRH), including family planning, information, and education[3,4]. Timely and proper family planning coverage would contribute and hasten the achievement of other sustainable development goals, especially SDG 1, 2, 3, 4, 5 and 10.[5]

Despite the availability of modern contraception, only 12.2% of women use it, while 16.6% prefer traditional methods.[6] Uptake is seemingly poor due

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to several reasons, including the strong influence of cultural norms, values, and belief systems.[6] Aversion to ingesting artificial hormones or devices implanted in their bodies, dread of side effects including rashes, loss of libido, discoloration of the skin, changes in weight or appetite, nausea, migraines, aggravation of varicose veins, and vitamin deficiencies are also compounding factors.[7,8] Lack of use of either modern or indigenous contraceptives results in high rates of unwanted pregnancies, unplanned deliveries, unsafe abortions and unacceptably high maternal mortality ratios in sub- Saharan Africa.[9] The global search for an effective, safe, and reversible anti-fertility agent is continuous, as its needed to face the problem of population explosion, which in turn hampers economic growth and breeds negative health impacts on families and society at large.[10]

Other researchers had shown that ethanolic Persea americana extracts (seed and bark) may induce various hormonal changes in female rats, and these changes may include the inhibition of uterine endometrial hyperplasia proliferation, prolongation of the estrous phase, increase estradiol and LH levels which act to protect the uterus from oxidative stress.[11] Moreover, ethanolic Persea americana extracts were demonstrated to induce endometrial implant regression.[12] Administration of avocado extract to male Wistar rats for 57 consecutive days improved conception in female Wistar rats.[13] In vivo studies on Persea americana investigating its effects on conception in female Wistar rats are lacking, thus the need for this research.

Materials and method

Identification and authentication of plant material

Persea americana unripe fruits procured from Itam market, Uyo in Akwa Ibom State were ascertained by an authorized taxonomist of the Department of Botany, University of Uyo, with specimen number UUPH 16(a) given..

Extraction and sample preparation

The fresh and unripe pulp of Persea americana fruits were squashed into paste after removal of the epicarp and seed. Exactly 200 g of the paste of Persea americana pulp was soaked in 100% methanol for 72hours. The extract was strained using Whatman No. 5 filter paper and the filtrate was refined using a rotator evaporator at 50°c and preserved in opaque glass bottles at 4°C.

Animal handling

Forty (40) adult female Wistar rats weighing between 150 g and 250 g, used for the experiment, were obtained from the Animal House of the Faculty of Pharmacy of Pharmacology, University of Uyo, Uyo, Akwa Ibom State. They were housed in 8 cages (40 cm × 35 cm) with adequate space to encourage free movement. The animals were allowed 12 hours of light and dark cycles, respectively, at a room temperature of 25°C - 29°C. They were fed a standard rat pelletized diet (Vital Feed, Grand Cereals Limited Nigeria) and water ad libitum. Animals were cared for accordingly.[14]

Acute toxicity

The Up and Down method, as documented and adjusted, was used to investigate the acute toxicity of the extract.[15,16] Twelve (12) mature female Swiss albino mice of 25g each, obtained from the Faculty of Pharmacy, University of Uyo, Uyo, were used after being acclimatized for one week. Animals were dosed with the test extract intraperitoneally one at a time and observed for a minimum of 48 hours, starting with 100mg/kg of body weight as the first dose. Subsequent doses depended on the outcome of the previous dosing. If the previous animal survived, the next animal received a higher dose, but if the animal died, the next animal received a lower dose. The animals were monitored for a total of 14 days for the long-term possible lethal outcome.

Experimental design

The experimental design was carried out according to documented experimental protocol but with slight modification.[17,18,19] After acclimatization for one week, animals with at least 2 successive oestrous cycles were divided into 2 groups of 20 rats each for preimplantation and post-implantation experiments.[20] Doses were calculated as 10%, 20%, and 30% of LD-50, given intraperitoneally for 12 days, and mating was done at late proestrus in the female: male ratio of 2: 1. Presence of vaginal plug was taken as conception and pregnancy day 1

Pre-Implantation: Extract was administered

before mating as follows:
Control – 1 ml/kg body weight of normal saline
Low dose group: 380 mg/kg body weight of Persea americana unripe-pulp methanol extract
Middle dose group: 760 mg/kg body weight of Persea americana unripe-pulp methanol extract
High dose: 1140 mg/kg body weight of Persea americana unripe-pulp methanol extract
Post-implantation: Extract was administered after mating was confirmed, and doses were the same as in pre-implantation experiments.
Animals were sacrificed on day 5 of pregnancy, day 15, day 20, and day 21 of pregnancy, while the rest were allowed to experience pregnancy outcome before sacrifice under general anesthesia. Uteruses were harvested and appropriately fixed for histological analysis, while the kidney, liver, heart, and kidneys were weighed and the organosomatic index calculated. Percentage embryonic loss after implantation (post) was calculated as

Number of implantation - number of embryos in development x 100 / Number of implantation ..... Equation 1

Histological analysis
Procedures for histological analysis were conducted with slight changes as researched.[21] Harvested and weighed, organs were fixed in 10% buffered formal saline, dehydrated through various concentrations of alcohol, i.e., 70%, 95%, and 100% for 20 mins per step for 6 times. Dehydrated tissues were cleared using xylene, impregnated with paraffin wax, and then embedded. The paraffin tissue blocks were trimmed, placed on an ice bar for cooling to enhance the plasticity and tissue-paraffin homogeneity for proper sectioning. The tissues were sectioned at five micrometres (5μm), and ribbons were gently picked with a Carmel brush and dropped in a Floating water bath at 60°C to enable ribbons to float, expand, and flatten out.
Tissue sections were deparaffinized and stained in Haematoxylin for ten minutes (10 min), sections were rinsed and differentiated in one percent (1%) acid alcohol and blue in running water using saturated lithium carbonate solution until sections appeared sky blue. The blue-stained section was counterstained in Eosin solution for three minutes (3 min). Tissues were washed well in water and dehydrated in ascending grade alcohol, cleared in xylene, and mounted in DPX covered with cover-slips.

Hormonal analysis
About 5 ml of blood was collected by cardiac puncture from each animal into clean and dry test tubes. The blood in the clean, dry test tubes were allowed to stand for about 15 minutes to clot and was further spun in a Westerfuge centrifuge (Model 1384) at 10,000g for 5 minutes; serum was separated from the clot with a Pasteur pipette into sterile sample tubes for the measurement of hormonal profile. Analysis for follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, and estradiol was carried out using Format microplate wells with kits from Monobind Inc.
Estimation of the concentration of follicle-stimulating hormone and luteinizing hormone was done with established methods[22], and the same for estradiol (E2) and progesterone were done as documented.[23] Absorbance at 450nm (using a reference wavelength of 620 - 630nm to minimize well imperfection) was read within 15 minutes with a microplate reader for all hormones.

Statistical analysis
Results were expressed as mean ± standard error (mean ± SE) of five observations.
Means were analyzed 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.

Ethical approval
The ethical approval was gotten from the Ethical Review Committee of the Akwa Ibom State Ministry of Health, Uyo with number AKHREC 29/03/22/088.

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

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

Results

The result of acute toxicity test done using the Up and Down method for the Persea americana unripe-pulp methanol extract showed that the extract caused no mortality at dose level of 100mg/kg - 3500mg/kg as in Table 1, because no lethal effects were noted throughout the short and long-term observation period except for toxic symptoms of tremor and recumbency at doses of 3500mg/Kg body weight. At doses of 3800 mg and 4000 mg/Kg body weight, there was 50% and 100% mortality, respectively.

Table 2: Effect of Persea americana unripe pulp methanol extract on weight of laboratory animals

Groups	Initial weight (g)	Final weight (g)	Weight difference (g)
1 ml/rat/day, normal saline (Control)	161.70 ± 4.81	195.70 ± 4.06	33.33 ± 7.86
380 mg/kg body weight of *Persea americana* unripe pulp methanol extract	172.00 ± 2.30[a]	188.00 ± 2.89[a]	16.00 ± 0.58[a]
760 mg/kg body weight of *Persea americana* methanol unripe-pulp extract	160.00 ± 7.51	170.00 ± 6.43[a]	10.00 ± 1.53[a]
1140 mg/kg body weight of *Persea americana* unripe-pulp methanol extract	167.70 ± 6.17	168.70 ± 9.87	7.00 ± 2.05[a]

Significance = P<0.05

Key: a = Shows a significant difference between the Control Group and any other group

Table 3: Pre-implantation effect of Persea americana unripe-pulp methanol extract on female albino Wistar rat on different pregnancy days

Groups	Label	Day 5 (n)	Day 10 (n)	Day 15 (n)	Day 21 (n)	Degree of protection (%)
1	1 ml/rat/day Normal saline (Control)	5.67 ± 0.88	8.67 ± 0.33	8.0 ± 1.15	6.33 ± 1.15	Nil
2	380 mg/kg body weight *Persea americana* unripe pulp methanol extract	0.0 ± 0.00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3
3	760 mg/kg body weight of *Persea americana* unripe pulp methanol extract	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3
4	1140 mg/kg body weight *Persea americana* unripe pulp methanol extract	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3

Post-Implantation

Weight difference significantly decreased with increase in concentrations of extract given showing that the amount of weight gained was inversely proportional to the quantity of extract as in Table 2.

The control group showed various number of implantation sites at different stages of pregnancy, but groups administered Persea americana unripe-pulp methanol extract had no implantation sites. Pregnancy was not achieved for the period of 3 oestrus cycles, as in Table 3.

Table 4: Mean weight of pups excised from control group in post-implantation experiment

Groups	Animal A(g)	Animal B(g)
Normal saline 1 ml/rat/day (Control Group)	6.816 ± 0.36	5.96 ± 0.35

Table 5: Effect of Persea americana unripe-pulp methanol extract on weight of organs on the 21day of pregnancy

Group	Final Weight(g)	Uterus and Ovaries (g)	Liver (g)	Heart (g)	Lungs (g)	Kidney (g)
(Control Group) Normal saline 1 ml/rat/day	225.3 ± 5.90	4.80[a] ± 0.15	6.67 ± 0.09	0.58 ± 0.02	1.47[a] ± 0.09	1.19 ± 0.02
(Group 2) 380 mg/kg body weight of *Persea americana* unripe pulp methanol extract	194.7[a] ± 1.67	0.62[a] ± 0.02	6.61 ± 0.09	0.67 ± 0.01	1.18[ab] ±0.02	1.19 ± 0.03
(Group 3) 760 mg/kg body weight of *Persea americana* unripe pulp methanol extract	196.3[a] ± 2.03	0.67[a] ± 0.05	6.14 ± 0.26	0.65 ± 0.03	1.18[ab] ± 0.02	1.13 ± 0.07
(Group 4) 1140 mg/kg body weight of *Persea americana* unripe pulp methanol extract	189.0[a] ± 5.77	1.07[a] ± 0.18	6.64 ± 0.05	0.76 ± 0.02	1.43[ab] ± 0.07	1.23 ± 0.03

Significance=P<0.05

There was a significant decrease in the uterine and ovarian weight of all groups compared to control. However, the increase in the weight of lungs, heart, liver and kidney was not significant in all groups.

Organosomatic indexes of the liver, heart and lungs increased with increasing dose of extract. Significance was in the high dose group of Persea americana.

Pre-Implantation

The effect of Persea americana on weight difference of animals showed an inverse proportionality. This implies that body weight gained decreased with

increasing dosage in all groups compared to the control. However, there was weight gain in all experimental groups compared to the control.

Control group had no degree of protection against conception, however, the groups administered low, middle and high doses of Persea americana unripe pulp methanol extract had protection for 3 cycles as in Table 4.

There was significant decrease in the uterus weight of all groups compared to control. However, the increase in the weight of the lungs, heart, liver, and kidney were nonsignificant in all groups, as in Table 6.

Table 6: Organ-somatic Index of Organs on 21 days of pregnancy

Groups	Label	Uterus/ Ovaries(g)	Liver (g)	Heart (g)	Lungs (g)	Kidney (g)
1	Normal saline 1 ml/rat/day	0.33 ± 0.03	2.96[a] ± 0.05	0.24 ± 0.00	0.59 ± 0.02	0.52 ± 0.00
2	380 mg/kg body weight of *Persea americana* unripe pulp methanol extract	0.34 ± 0.01	3.15 ± 0.09	0.28 ± 0.03	0.56 ± 0.04	0.58 ± 0.04
3	760 mg/kg body weight of *Persea americana* unripe pulp methanol extract	0.35 ± 0.28	3.12 ± 0.10	0.32 ± 0.01	0.60 ± 0.02	0.57 ± 0.03
4	1140 mg/kg body weight of *Persea americana* unripe pulp methanol extract	0.57 ± 0.11	3.52[a] ± 0.13	0.40 ± 0.00	0.76 ± 0.01	0.67 ± 0.02

Significance: P<0.05

Table 7: Effect of Persea americana unripe pulp methanol extract on weight of animals

Treatment groups	Initial Weight (g)	Final Weight (g)	Weight Difference (g)
Normal Saline1 ml/rat/day (Control)	161.70 ± 4.81	195.70 ± 4.06	33.33 ± 7.86
380 mg/kg body weight of *Persea americana* unripe pulp methanol extract	177.70 ± 10.11	196.3 ± 4.39	12.00 ± 5.57
760 mg/kg body weight of *Persea americana* unripe pulp methanol extract	173.70 ± 6.84	180.00 ± 2.51	10.00 ± 6.00
1140 mg/kg body weight of *Persea americana* unripe pulp methanol extract	183.70 ± 5.36	187.00 ± 2.88	5.67 ± 1.45

Significance P<0.05

Follicle stimulating hormone and progesterone increased with higher doses of extract given. All result were statistically significant at $P < 0.05$.

The result of concentration of luteinizing hormone showed a slight but not significant decrease in treatment group compared to control. The lowest concentration of 1.134 seen in the medium dose group while the highest as in the high dose 1.378 ± 0.219.

Table 8: Anti-conceptive effect of Persea americana unripe pulp methanol extract on female albino Wistar rats on different pregnancy days

Group/Treatment	Day 5	Day 10	Day 15	Day 21	Degree of Protection
Control (1 ml/kg body weight normal saline)	4.00 ± 1.0	7.33 ± 1.5	7.33 ± 2.08	8.33 ± 0.58	0
380 mg/kg body weight *Persea americana* unripe pulp methanol extract	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3
760 mg/kg body weight of *Persea americana* unripe pulp methanol extract	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3
1140 mg/kg body weight *Persea americana* unripe pulp methanol extract	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3

Table 9: Mean weight of pups excised from control group

	Animal A(g)	Animal B	Animal c(g)
Control Group	7.816 ± 0.36	6.08±0.44	5.96 ± 0.35

Significance = P<0.05

Table 10: Post implantation activities of Persea americana unripe pulp methanol extract on hormonal parameters of female albino Wistar rat

Group	FSH	LH	E	Progesterone
1 ml/kg body weight Normal saline (Control)	0.804 ± 0.01	1.398 ± 0.097	83.809 ± 3.313	18.40 ± 1.722
380 mg/kg body weight of *Persea americana* unripe-pulp methanol extract	1.140 ± 0.0259	1.175 ± 050	60.009 ± 5.541	28.14 ± 1.393
760 mg/kg body weight of *Persea americana* unripe-pulp methanol extract	1.232 ± 0.322	1.134 ± 0.247	63.80 ± 8.45	27.62 ± 2.76
1140 mg/kg body weight of *Persea americana* unripe-pulp methanol extract	1.682 ± 0.5536	1.378 ± 0.214	48.20 ± 2.375	37.3716 ± 1.509

Significant P<0.05

There is a significant decrease in the level of estrogen concentration when compared with the control group. The amount of estrogen is inversely proportional to concentrations of extract given. The lowest value of E2 (48.20 ± 2.88) was in the high dose group and the highest was in the middle dose group with 63.80 ± 8.45 respectively.

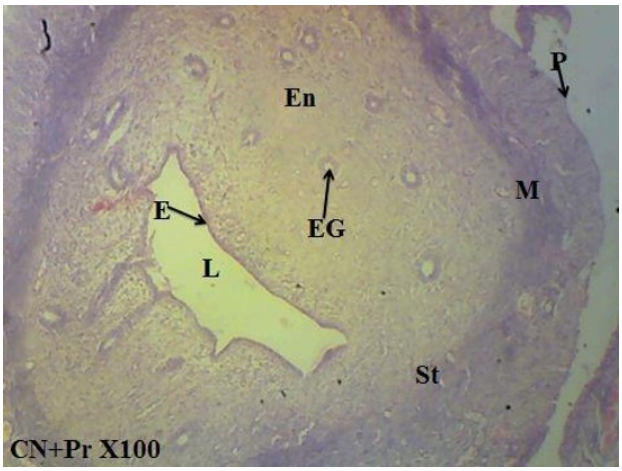


Figure 1: Photomicrograph of uterus histology of control group at preimplantation showing normal features; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, P= Perimetrium, L= Lumen, M= Myometrium. H&E, 100 x.

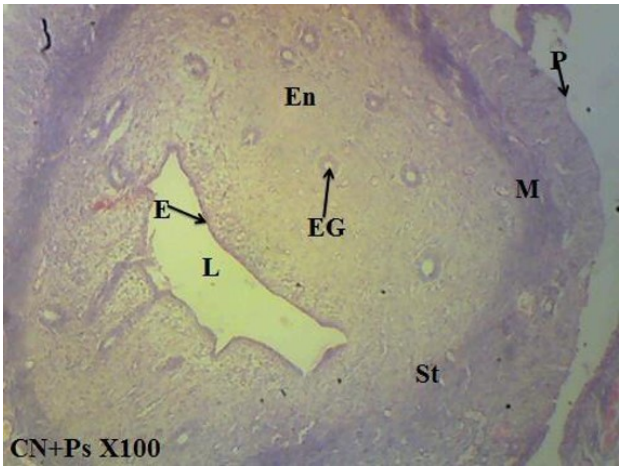


Figure 2: Photomicrograph of uterus histology of control group at post-implantation showing normal features; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, P= Perimetrium, L= Lumen, M= Myometrium. H&E, 100x

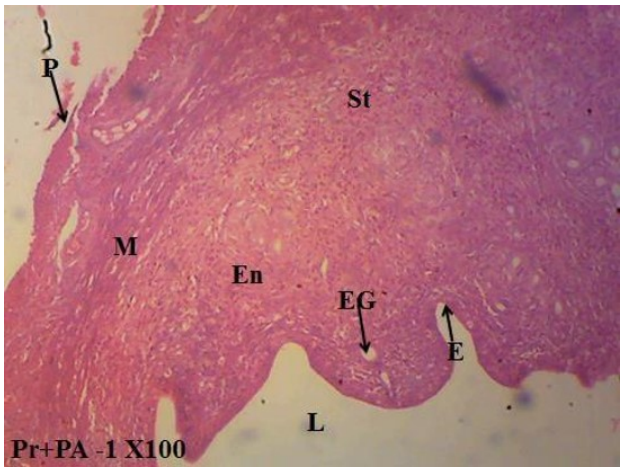


Figure 3: Photomicrograph of uterus histology with P. americana (380 mg/kg) treated group at pre-implantation showing; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, L= Lumen, M= Myometrium, P= Perimetrium. H&E Mag 100x.

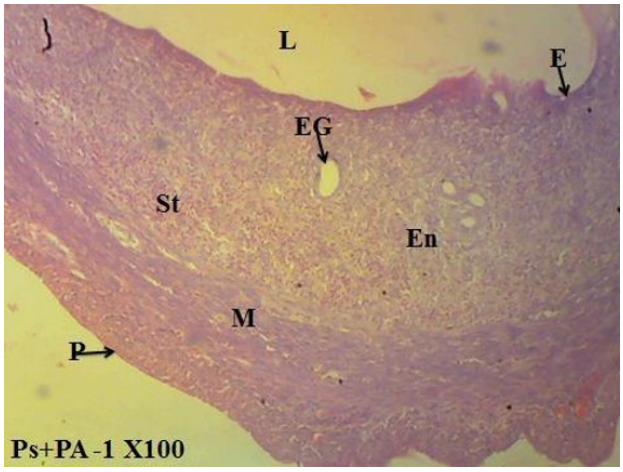


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Histology

Histology of the Uterus

The histology of the uterus from the rats in control group showed large lamina content, healthy endometrium with rounded endometrial gland. Lumen also appeared normal and healthy. Uterus from rats in treated groups showed endometrium

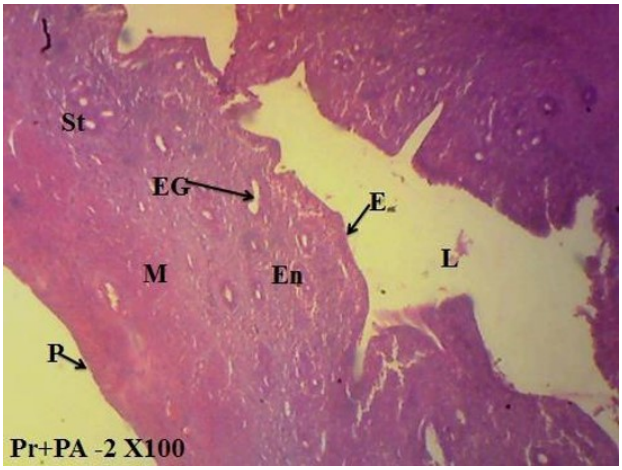


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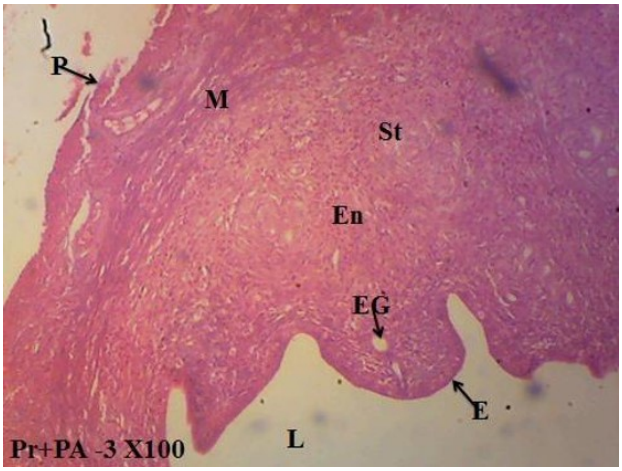


Figure 7: Photomicrograph of uterus histology with P. americana (1140 mg/kg) treated group at pre-implantation showing; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, L= Lumen, M= Myometrium, P= Perimetrium. H&E, 100x

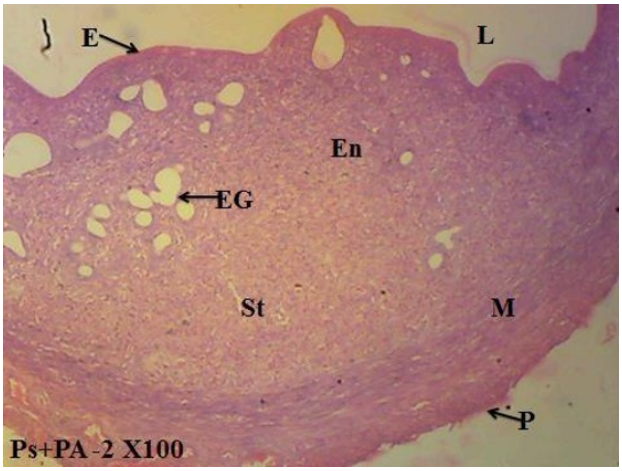


Figure 6: Photomicrograph of uterus histology with P. americana (760 mg/kg) treated group at post-implantation showing; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, L= Lumen, M= Myometrium, P= Perimetrium. H&E, 100x.

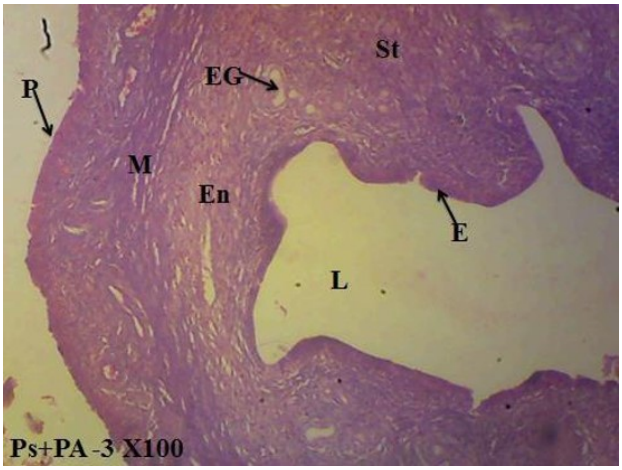


Figure 8: Photomicrograph of uterus histology with P. americana (1140 mg/kg) treated group at post-implantation showing; EG= Endometrial gland, E= Epithelium, En= Endometrium, St= Stroma, L= Lumen, M= Myometrium, P= Perimetrium. H&E, 100 x

with thicker, stratified epithelium. They also showed thin glandular lining of glands that were tortuous unlike the fine rounded glands in the uterus of control group. The changes were dose dependent as the endometrium appeared to be more stratified and the crypt became deeper with increase in dosage.

Discussion

Acute toxicity experiments evaluate the extent of safety of a substance like drugs or chemicals irrespective of the mode of administration.[24] The Up and Down method of determining LD50 was used to access safety profile of Persea americana unripe-pulp methanol extract via the intraperitoneal route at

different dose levels. Within dose 100mg/kg-3800mg/kg, no abnormal changes both in general appearance and behavioral characteristics and mortality were recorded after 48hrs of administration. Higher doses elicited abnormal changes such as diarrhea, tremor, slouching, difficulty in locomotion, heavy breathing and finally, death in experimental animals. The LD50 of Persea americana methanol unripe-pulp extract, investigated to be 3,800mg/kg correlated with other results and classified to be moderately toxic.[25,26]

A change in body weight has always been a common parameter used in evaluating the severity of various health challenges. A reduction in body weight can be related to low nutritional status as a result of decreased food intake or increased metabolism.[27]

The weight gained decreased with increasing concentrations of extract. This corroborates the results in a study on the effects of Persea americana fruit extracts on body weight and liver lipids in rats; that the administration of aqueous and methanolic extracts of Persea americana provoked a reduction in body weight gain compared to the hyperlipidaemic control.[28] It could be that P. americana extracts increased the catabolism of lipids accumulated in adipose tissue resulting in a decrease in mean body weight.

However some experiments have shown no significant changes in body weight, body mass index, percent of body fat, or visceral adipose tissue in response to intervention with Persea americana group compared with the control group except for some benefits in metabolic parameters, mainly related to lipid profile.[29]

Another study on the effects of aqueous and ethanolic extracts of Persea americana seed on body weight in rats resulted in 14% and 25% reduction, respectively, in the body weight of the treated rats compared to the hyperlipidemic control[30]. It can be hypothesized that P. americana leaf extracts increase catabolism of lipids accumulated in adipose tissue causing a decrease in body weight but does not influence liver lipid levels in rats. Persea americana could also exhibits hypolipidemic activity probably by increasing the expression of adiponectin and PPAR-γ, which inturn reduces the formation of adipocytes.[31]

The effect of ethanolic extracts of Persea americana seed on the organ weight of albino rats had no significant differences (P> 0.05) in the liver, lungs and heart of all the test groups when compared to the control, except for the spleen which was significantly reduced in the low dose group[24]. This is similar to this study as there were no significant differences in lungs kidney and liver weight except for heart and uterine weight. However organo-somatic indexes of all the organs increased significantly in all the experimental groups. Visceral adiposity is associated with elevated triglycerides and low high-density lipoproteins and Persea americana possibly prevents these.[32] The kidney weight was however significantly increased in the group given highest dose of Persea americana pulp when compared to the control administered normal saline. This could indicate a possible renal toxicity.[33]

Estrogen is the most important hormone during female reproductive years and is required for reproductive and sexual function. It also has an impact on the health of other organs and tissues.[34] Estrogen concentration was inversely proportional to dose of extract in the current study. Other extracts of parts of Persea americana such as the stem bark, seeds also reduced the concentrations of estrogen at higher doses.[12,35] When an egg is released by the ovary each month, progesterone is the hormone that helps in thickening the lining of the uterus, in preparation for a fertilized egg. As progesterone is responsible for preparing the body for pregnancy, healthy progesterone levels are essential to becoming pregnant and maintaining a healthy pregnancy, when progesterone levels drop it can cause some serious complications like infertility and miscarriage.[36] Progesterone increased significantly in all treatment groups, which may impact pregnancy maintenance and reproductive health. High doses of progesterone is implicated in metabolic disruption such as abnormality in the number of secretory vesicles, lipid synthesis, golgi apparatus function leading to poor pregnancy maintenance and outcome.[37,38] Although progesterone is necessary in early pregnancy, it is implicated in poor fetal outcome.[39] FSH (Follicle-Stimulating Hormone) increased significantly in all treatment groups, with the highest dose showing the most substantial rise. This suggests that Persea americana extract may stimulate follicular growth and development. LH (Luteinizing Hormone) decreased in groups 2 and 3, but slightly increased in

group 4. This fluctuation may impact ovulation and reproductive functions. Estrogen decreased significantly in all treatment groups, with the highest dose showing the most pronounced decline. This could be attributed to the extract's potential anti-estrogenic properties. The potential hormonal effects of Persea americana methanolic unripe pulp extract may be attributed to its phytochemical composition, including flavonoids and phenolic acids.[40] Flavonoids, in particular, have been shown to interact with steroid hormone receptors, influencing hormonal activity, supported by Phenolic acids.[41,42] These findings are supported by several studies.[35,43]

Histology allows for the visualization of tissue structure and characteristic changes tissues may have undergone. The changes observed in the histomorphology of the uterus showed that various doses of P. americana may cause the endometrium state to be unfavourable for the implantation of the fertilized ovum hence, their infertility effects. This could be compared to a study on ethanolic extract of P. americana seed where endometrial implant volume and height of the endometrium was decreased.[12]

Conclusion

Though the administration of methanolic extracts of Persea americana pulp provides varying degrees of effects based on doses administered and weight of animals, it can be concluded that Persea americana pulp, when taken, does inhibit conception, promote contraception and thus should be used with caution in women intending to conceive.

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Competing Interest

The authors declare that there are no competing interests.

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