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Original Research Article

Percentage Fertility of Female Wistar Rats Treated with Extracts of *Persea americana*

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Abstract

The purpose of this research study was to investigate the effects of ethanolic stem bark extract of *Persea americana* on some reproductive parameters of female Wistar rats. In this study, female Wistar rats were randomly assigned into three (3) groups of 5 rats each. The control group (Group 1) was given distilled water. Group 2 received 200mg/kg of the extract and group 3 received 400mg/kg of the extract daily for a period of 21 days following acclimatization. The female rats were cohabited with males in the ratio 2:1 (M:F) for 10 days. Blood samples were collected and some tissues harvested at sacrifice. The statistical analysis was done using Statistical Package for Social Sciences (SPSS). One-way analysis of variance (ANOVA) test was used to compare groups followed by post-hoc testing to determine whether there were significant differences between the control and treatment groups. P-value less than 0.05 was considered significant and result presented as Mean±SEM. The result showed that the extract of *Persea americana* caused stimulatory effect on anterior pituitary synthesis of luteinizing hormone (LH) and follicle stimulating hormone (FSH) and may have also caused changes in the gonads leading to decreased gonadal hormone synthesis (estrogen and progesterone). The percentage fertility of the female Wistar rats was significantly reduced suggesting that the stem bark extract of *Persea americana* possess antifertility effects.

Keywords: Reproductive parameters, Persea americana, percentage fertility, female Wistar rats.

INTRODUCTION

Medicinal plants have long been used to prevent and cure a variety of disorders in African traditional medicine. A high percentage of the world's population, mostly in Africa and other underdeveloped countries, still rely solely on traditional or herbal medicine to manage diseases. The most effective medicinal plants have very few harmful effects on humans, however some are extremely toxic to both humans and animals, possessing the ability to harm certain organs in the body. This calls for carefulness when using medicinal plants, which are becoming more popular due to their easy availability, affordability, accessibility and promising efficacy compared to the expensive cost and side effects of synthetic therapeutic agents (Okoye *et al.*, 2014).

African medicinal plants possess a diverse range of biological and pharmacological qualities that need to be identified, investigated and documented. In terms of existing pharmacopoeias on medicinal plants, Africa has just a few volumes that contain basic useful information.

Persea americana is a medicinal plant which was first domesticated in tropical America, where they were grown as individual seedling trees. In 2011, the top producers in the world were Mexico, Chile, the Dominican Republic, Indonesia, and Colombia. Commercially, the fruits are grown in Florida, California, Hawaii, South Africa, Brazil, and Australia, as well as on a few Pacific islands and in several Mediterranean countries, including Israel (Jamshidi-Kia *et al.*, 2018). The pharmacological actions of the different parts of the *Persea americana* plant including the leaf, fruit, pulp, peel and seed has been previously studied. Some reports indicates that the extracts possess anti-cancer (Bhuyan *et al.*, 2019) and anti Inflammatory effects (Adeyemi *et al.*, 2002). These effects has been

attributed to their possession of biologically active secondary metabolites such as phenolic compounds (including phenolic and hydroxycinnamic acids, flavonoids, and condensed tannins), carotenoids, and -tocopherols, acetogenins etc. (Boyadzhieva, *et al.*, 2018). However, there is a dearth of data on the effects of *Persea americana* on reproduction. Hence, this study was carried out to investigate the effects of extract of *Persea americana* on some reproductive parameters of female Wistar rats.

MATERIALS AND METHODS

Animal Models

Fifteen (15) adult female Wistar rats used for this study were bred in the animal house of the Faculty of Basic Medical Sciences, Rivers State University, Nigeria. They were placed in standard cages and allowed 2 weeks to acclimatize while they were maintained in standard environmental conditions with proper ventilation and humidity. The animals were also given free access to food and water. Generally, the procedures conformed to the established principles for the care and use of laboratory animals published by the National Institute of Health, USA (National Institutes of Health, 1985). Appropriate institutional approval was obtained for this study.

Preparation of Plant Extract

A mature *Persea americana* (avocado) stem bark sample was collected from a tree in the Rivers State University in Port Harcourt, Rivers State, Nigeria. The bark was removed from the stem, rinsed, and dried at 40°C in the oven. The taxonomist in the Department of Plant Science and Biotechnology, Rivers State University identified and authenticated the plant (voucher number RSUPb041). Ground stem bark of *Persea americana* was packed into a tiny bag weighing about 40g of each sample and placed into the thimble of the soxhlet apparatus using the soxhlet method of extraction. A round-bottom flask containing around 250 mL of solvent (ethanol) was treated to minimal heat for 3 hours using a heating mantle. The resulting solvent-oil mixture was put through a large condenser that was cooled by a constant flow of fresh water. The extract was then decanted into sample bottles after being separated using a rotary evaporator. The process was repeated until a sufficient amount of extract was recovered for analysis.

Experimental Design/Procedure

After a two-week acclimatization period, the 15 female Wistar rats were placed into three groups. Group one served as control and received distilled water, whereas groups 2 and 3 were experimental groups and received 200mg/kg and 400mg/kg of *Persea americana* ethanolic stem bark extract, respectively. Extract administration was carried out with an animal gavage tube. The animals remained on standard pelleted feeds and clean water while extract administration lasted for 21 days. However, in the final ten days of extract administration, the female rats were cohabited with male rats. At the end, animals were sacrificed under chlorofoam anaesthesia. Blood was collected for assay of some reproductive hormones including follicle stimulating hormone (FSH), luteinizing hormone (LH), Estrogen (E), Progesterone (P) while laparotomy was done to deliver the fetus and weigh them.

STATISTICAL ANALYSIS

The differences between the treatment and control groups were calculated using the SPSS (Statistical Package for Social Sciences) software program for Windows XP (version 21.0). The analysis of variance (ANOVA) test was used to compare groups. Least significant differences (LSD) and post hoc testing were used to determine whether there were significant differences between the control and treatment groups. P-values less than 0.05 were considered significant and result presented as mean±SEM.

RESULTS

The result for the study is presented in Tables 1-5.

Table 1: Effect of extract of *Persea americana* on follicle-stimulating hormone level

Groups (mg/kg)	Mean ± SEM (m/µ/ml)	Relative change (%)	Level of significance
Control	0.32 ± 0.07	0	-
200	0.29 ± 0.02	-9.38	0.91
400	1.48 ± 0.29*	362.50	0.00

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Table 2: Effect of extract of *Persea americana* on luteinizing hormone level

Groups (mg/kg)	Mean ± SEM (m/µ/ml)	Relative change (%)	Level of significance
Control	0.54 ± 0.17	0	-
200	1.02 ± 0.21	88.89	0.22
400	1.48 ± 0.37*	174.07	0.03

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Table 3: Effect of extract of *Persea americana* on estrogen level

Groups (mg/kg)	Mean ± SEM (pg/ml)	Relative change (%)	Level of significance
Control	71.80 ± 10.63	0	-
200	55.80 ± 6.38	-22.28	0.19
400	42.80 ± 6.76*	-42.62	0.03

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Table 4: Effect of extract of *Persea americana* on progesterone level

Groups (mg/kg)	Mean ± SEM (ng/ml)	Relative change (%)	Level of significance
Control	170.40 ± 5.10	0	-
200	139.40 ± 9.23*	-18.19	0.04
400	103.60 ± 12.10*	-39.20	0.00

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

Table 5: Effect of extract of *Persea americana* on weight of ovary, uterus, fetus and the number of fetus

Groups (mg/kg)	Weight of ovary (g)	Weight of uterus (g)	Weight of fetus (g)	Number of fetus (n)	Percentage fertility (%)
Control	0.14 ± 0.06	1.25 ± 0.16	5.74 ± 3.83	3.80 ± 2.33	100
200	0.15 ± 0.03	0.97 ± 0.20	0.00 ± 0.00	0.00 ± 0.00	0*
400	0.26 ± 0.09	1.25 ± 0.10	3.98 ± 2.44	2.20 ± 1.36	57.89

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 compared to control.

DISCUSSION

The serum level of follicle-stimulating hormone, luteinizing hormone, estrogen and progesterone were assessed in this study in addition to changes in some other parameters after administration of ethanolic extract of *Persea americana*. Some of these hormones play major roles in stimulating secondary sexual characteristics, stimulating maturation of follicles, triggering ovulation, and maintaining the functionality of the reproductive organs during ovarian, menstrual and gestational cycles. Thus, these hormones play major roles in fertility in females of reproductive age.

The administration of *Persea americana* caused significant increase in levels of follicle stimulating hormone and luteinizing hormone and significant decrease in levels of estrogen and progesterone. The extract of *Persea americana* may affect fertility majorly by altering the levels of specific reproductive hormones.

The follicle stimulating hormone (FSH) level increased significantly in the group that took the higher dose (400mg/kg) of the extract. The level of the FSH affects the quality and quantity of eggs in the ovaries and can directly affect the chances of conceiving and sustaining pregnancy. When the levels are too high, achieving conception becomes very difficult in that, it will affect both the menstrual cycle and the process of ovulation. The body's hormonal regulation system compensates for this by producing more FSH in order to stimulate ovarian function (American Pregnancy Association, 2020).

FSH levels are also used to evaluate whether in vitro fertilization (IVF) treatment or fertility drugs by injecting could be effective (Gurevich, & Odunsi, 2021). In a report by Orabueze *et al.*, (2021), *Persea americana* seed extract significantly increased FSH on day 60 and 90 of administration. Physiologically, a surge in LH causes the ovary to release a mature egg in the second (2nd) week of each menstrual cycle. A high LH level around this time indicates a very fertile period in females. High level of LH indicates that the reproductive organs are not producing enough steroid hormones needed for the process of reproduction to occur. That is, it causes a decline in reproductive function, and ultimately reduced fertility. Disorders associated with this in females include: Turner Syndrome and Polycystic ovarian Syndrome ('Cleveland Clinic', 2022).

The result from this study showed that LH was increased in the animals treated with higher dose of the extract when compared to control. In a study by Balen, *et al.*, (1993), hypersecretion of LH during the follicular phase results in an elevated concentration of intrafollicular LH which in turn results in premature oocyte maturation. This in turn causes the problem with fertilization and miscarriage (Homburg, 1998).

Persea americana had an effect on estrogen (or estradiol) levels of female Wistar rats. It showed a significant (p<0.05) decrease in estrogen in the group that took the higher dose of 400mg/kg of the extract. Estradiol is the most important hormone during a female reproductive years and is required for reproductive and sexual function as well as having an impact on the health of other organs and tissues (Mandal, 2019). Low estrogen may lead to missed or irregular periods. It can prevent ovulation and make getting pregnant difficult, leading to infertility (Pietro & Cobb, 2018).

The findings in this study is in aggreement with a report in a study by Essono *et al.,* (2020) on the effects of avocado seeds on implant regression. The result showed that the ethanol extract of *P. americana* seeds has a potent inhibitory effect on development of endometriotic implants by decreasing serum levels of estradiol and progesterone. The extract of *Persea americana* had an effect on progesterone level of female Wistar rats by significantly ($p<0.05$) decreasing the hormone level in both test groups.

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 (Roche, 2020). In a study by Essono *et al.,* (2020), ethanol extract of avocado [*Persea americana*- Mill (Lauraceae)] seeds successfully induced impact regression in a rat model of endometriosis where serum level of progesterone were significantly decreased. In this study, the ovarian and uterine weights were not significantly altered. In terms of the number of fetus and fetal weights, there were no significant differences between treated rats and control. But, the percentage fertility was statistically reduced in treated female rats suggesting that the stem bark extract of *Persea americana* demonstrated overall antifertility effects.

CONCLUSION

Result of the current study showed that the ethanolic stem bark extract of *Persea americana* exhibited stimulatory effects on brain secretion of FSH and LH and inhibitory effects on gonadal secretion of estrogen and progesterone. In addition, there was a statistically significant reduction in percentage fertility of female Wistar rats.

REFERENCES

1. Adeyemi, O. O., Okpo, S. O., & Ogunti, O. O. (2002). Analgesic and anti-inflammatory effects of the aqueous extract of leaves of Persea americana mill (lauraceae). *Fitoterapia*, *73*(5), 375–380. https://doi.org/10.1016/s0367-326x(02)00118-1
2. American Pregnancy Association. (2022). How to Use Ovulation kits & fertility monitors. URL: https://americanpregnancy.org/getting-pregnant/infertility/ovulation-kits/
3. Balen, A. H., Tan, S., & Jacobs, H. S. (1993). Hypersecretion of luteinizing hormone: A significant cause of infertility and miscarriage. *British Journal of Obstetrics and Gynaecology.* 100(12), 1082-1089. https://doi.org/10.1111/j.1471-0528.1993.tb15170.x
4. Bhuyan, D. J., Alsherbiny, M. A., Perera, S., Low, M., Basu, A., Devi, O. A., Barooah, M. S., Li, C. G., & Papoutsis, K. (2019). The Odyssey of Bioactive Compounds in Avocado (*Persea americana*) and Their Health Benefits. *Antioxidants (Basel, Switzerland)*, *8*(10), 426. https://doi.org/10.3390/antiox8100426
5. Boyadzhieva, S., Georgieva, S., & Angelov, G. (2018). Recovery of antioxidant phenolic compounds from avocado peels by solvent extraction. *Bulg. Chem. Commun*, 50, 83-89.
6. Cleveland Clinic. (2022). Luteinizing hormone: Levels, Function & Testing. URL: https://my.clevelandclinic.org/health/body/22255-luteinizing-hormone
7. Essono, S. M., Mvondo, M. A., Ngadjui, E., Nguimatio, F. X. K., & Watcho, P. (2020). The ethanol extract of avocado (*Persea americana* Mill. (Lauraceae)) seeds successfully induces implant regression and restores ovarian dynamic in a rat model of endometriosis. *Evidence – Based Complementary and Alternative Medicine*, https://doi.org/10.1155/2020/8521831
8. Gurevich, R., & Odunsi, T. (2021). Here's why an FSH test is important for monitoring your fertility. *Verywell Family.* https://www.verywellfamily.com/day-3-fsh-levels-test-1960172
9. Homburg R. (1998). Adverse effects of luteinizing hormone on fertility: fact or fantasy. *Bailliere's clinical obstetrics and gynaecology*, *12*(4), 555–563. https://doi.org/10.1016/s0950-3552(98)80051-5 https://doi.org/10.1186|40738-019.0068-6
10. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Kloei, H. (2018). Medicinal Plants: Past history and future perspective. *Journal of Herbmed Pharmacology;* 7(1): 1-7.
11. Mandal, A. (2019). What does estradiol do? *News Medical Lifesciences.* Retrieved: February 17, 2022 URL: https://www.news-medical.net/amp/health/What-does-Estradiol-do.aspx
12. Okoye, T. C., Uzor, P. F., Onyeto, C. A., & Okereke, E. K. (2014). Safe African medicinal plants for clinical use: Toxicological survey of African medicinal plants. #535-555. https://doi.org/10.1016B978-0-12-
13. Orabueze, I. C., Babalola, R., Azuonwu, O., Okoko, I. I., & Asare, G. (2021). Evaluation of possible effects of Persea americana seeds on female reproductive hormonal and toxicity profile. *Journal of ethnopharmacology*, *273*, 113870. https://doi.org/10.1016/j.jep.2021.113870
14. Pietro, M., & Cobb, C. (2018). Low estrogen: Causes, effects and treatment options. *Medical News Today*. Retrieved: March 4, 2022. URL: https://www.medicalnewstoday.com/articles/321064