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Effects of Aqueous Extract of *Cissus populnea* Stem on Function Indices of the Ovary and Uterus of Female Wistar Rats

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ABSTRACT: *Cissus populnea* stem has a long indigenous use as an aphrodisiac in males but there is dearth of information on the effect of the plant on the ovarian and uterine function indices of females.

This study was aimed at evaluating the effect of aqueous extract of *Cissus populnea* stem on some function indices of the ovary and uterus of female Wistar rats.

Twenty female rats (187.30 ± 5.85 g) were completely randomized into four groups: A, B, C and D and respectively administered orally 0.5 ml of distilled water and same volume corresponding to 18, 36 and 72 mg/kg body weight of the extract once daily for 17 days. The biochemical parameters of the ovary and uterus were determined using standard methods.

The extract significantly increased ($P < 0.05$) the concentrations of ovarian total protein and cholesterol, uterine cholesterol and glucose as well as the activities of acid and alkaline phosphatase in the ovary and uterus of the female rats. The extract also significantly increased ($P < 0.05$) the concentrations of serum progesterone, estrogen, follicle stimulating and luteinizing hormones whereas the serum prolactin levels decreased significantly. There was well defined zona granulosa surrounding the oocyte, compact theca folliculi, corpora lutea and developing graffian follicles in all the extract treated animals.

The totality of the biochemical and histological data in this study indicates that the aqueous extract of *C. populnea* stem at 18, 36 and 72 mg/kg body weight could promote the normal functioning of the ovary and uterus and consequently would enhance the reproductive capacity of the female rats.

Keywords: *Cissus populnea*, Function indices, Ovary, Hormones, Uterus, Vitaceae

Introduction

Cissus populnea Guill. and Perr. (family: *Vitaceae*) commonly known as food gum (English), ogbolo (Yoruba - Western Nigeria) and dafaaraa (Hausa - Northern Nigeria), is a strong woody vine, 8–10 m long, 7.5 cm in diameter and widely distributed across the tropics in West Africa (Burkill, 2000). *C. populnea* has been claimed to be used in the management of infertility in males, skin diseases, sickle cell and as antimicrobial, anti-helminthic and diuretics (Moody, Ojo, Omotade, Adeyemo, Olumese & Ogundipe, 2003; Ojekale, Lawal, Lasisi & Adeleke, 2006). Studies previously reported in the open scientific literature on *C. populnea* includes the effect of long term use of *C. populnea* stem bark on biochemical parameters of rabbits which indicated that there was no treatment related effect on the liver and kidney (Ojekale, Ojaiko, Saibu, Lala & Olodude, 2007), preservation of the cellular morphology and reproductive function of the testes in flutamide-induced testicular defect in pre-pubertal rats by *C. populnea* and *Panax ginseng* (Oremosu, Arowosaye, Akang & Bassey, 2013), phytochemical profile of *C. populnea* leaves indicating that its very rich in saponins (2.85 ± 0.35 mg/g), anthraquinones (2.00 ± 0.14 mg/g) and flavonoids (0.39 ± 0.03 mg/g) (Soladoye and Chukwuma, 2012). The stem bark has also been reported to contain carbohydrates, tannins, cyanogenic glycosides, anthraquinones, saponins, cardiac glycosides and flavonoids (Ibrahim, Rai & Bangudu, 1993). Despite all these, there has not been any scientific documentation on the effect of aqueous extract of *C. populnea* stem on the ovarian and uterine function indices of female Wistar rats. Hence, this study was carried out to evaluate the effect of aqueous extract of *C. populnea* stem on some function indices of the ovary and uterus of female Wistar rats.

Materials and methods

Plant material

The plant samples were obtained from a farmland in Afon, Ilorin, Kwara State, Nigeria. The authentication was done at the Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria, where a voucher specimen (U.I.H. 1078) was deposited.

Chemicals and reagents

The assay kits for acid phosphatase, alkaline phosphatase, cholesterol and glucose were products of Randox Laboratory Ltd, Co. Antrim, United Kingdom while those of estrogen, progesterone, prolactin, follicle stimulating hormone (FSH) and luteinizing hormone (LH) were from Fortress Diagnostics Ltd, Antrim, United Kingdom. Other reagents used were of analytical grade and were supplied by Sigma-Aldrich Inc., St. Louis, USA.

Experimental animals

Twenty female Wistar rats (*Rattus norvegicus*) weighing 187.30 ± 5.85 g used for this study were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Nigeria. All the animals were housed in clean aluminium cages placed in well-ventilated house conditions (temperature: $23 \pm 2^{\circ}\text{C}$, photoperiod: about 12 hours natural light and dark cycle and humidity: 45-50%). The animals were allowed free

access to rat pellets (Premier Feeds, Ibadan, Nigeria) and clean tap water. The cages were cleaned of wastes on a daily basis.

Preparation of extract

C. populnea stem was washed under running tap, cut into pieces and oven dried (Uniscope Laboratory Oven, SM9053, Surgifriend Medicals, England) at 40°C for 48 hours until a constant weight was obtained. Thereafter, the dried stem was pulverized using an electric blender (Super Master Co., Ltd, Osaka, Japan) and stored in an air-tight container prior to extraction. A known amount (60.00 g) of the powder was extracted in 1500 ml of distilled water for 48 hours with constant shaking. The resulting filtrate was lyophilized to give 11.42 g corresponding to a percentage yield of 19.03%. This was reconstituted separately in distilled water to give the required doses of 18, 36 and 72 mg/kg body weight used in this study. The dose of 36 mg/kg body weight corresponds to a teaspoonful of the plant powder, estimated to be consumed to improve sexual function in a 70 kg man, from the ethnobotanical survey conducted where the plant samples were obtained while the doses of 18 and 72 mg/kg body weight represented half and double the ethnobotanical dose of 36 mg/kg body weight.

Animal grouping and extract administration

The animals were completely randomized into four groups (A, B, C and D) of five animals each such that rats in group A (control) were orally administered 0.5 ml of distilled water once daily for 17 days while the animals in groups B, C and D received equal volume of the aqueous extract of *C. populnea* stem at the doses of 18, 36 and 72 mg/kg body weight respectively.

Preparation of serum and tissue supernatants

Twenty four hours after the last daily administration (day 18) of the extract and distilled water, the animals were sacrificed by anesthetization using diethyl ether fumes. The neck area was quickly shaved to expose the jugular veins which were cut with a sterile scalpel blade. Blood was collected into sample bottles, allowed to clot for 20 minutes and then centrifuged at 894 x *g* for 10 minutes. The sera obtained were used within 12 hours of preparation for various biochemical assays. The ovaries and uterus were also removed, blotted in blotting paper, and separately homogenized in iced-cold 0.25M sucrose solution (1:5 w/v). The homogenates were centrifuged at 1340 x *g* for 15 minutes to obtain the supernatants which were stored frozen and used for biochemical analysis within 24 hours of preparation.

Determination of biochemical parameters

The concentrations of total protein, cholesterol as well as the activities of alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined using standard procedures described by Gornall, Bardawill & David (1949), Roeschlau, Bernt & Gurber (1974) and Wright, Leathwood & Plummer (1972a, 1972b) respectively while the levels of glucose, estrogen, progesterone, prolactin, FSH and LH were assayed according to the procedures outlined in their respective manufacturers' protocols. Histological study was carried out on the ovaries following the procedures described by Krause (2001).

Statistical analysis

Data were expressed as the means $\pm$ SEM of 5 determinations. Statistical analysis was performed using One-way Analysis of Variance and Duncan Multiple Range Test. The data were considered statistically significant at $P < 0.05$.

Results

Administration of the aqueous extract of *C. populnea* stem significantly increased ($P < 0.05$) the levels of total protein and cholesterol as well as the activities of ACP and ALP in the ovaries of the female rats when compared with the distilled water treated rats (Table 1). The increase in the levels of the ovarian total protein and activity of ALP were dose dependent, whereas the cholesterol content and activity of ACP were not (Table 1). The extract significantly and dose dependently increased the concentrations of cholesterol and activities of ACP and ALP in the uterus of the female rats; the increase in uterine glucose content was not dose related when compared with the distilled water treated female rats (Table 2).

The aqueous extract of *C. populnea* stem at the doses of 18, 36 and 72 mg/kg body weight significantly elevated the levels of FSH, LH, progesterone and estrogen in the serum of the female rats whereas the levels of prolactin decreased significantly following the administration of the extract (Table 3). The histoarchitecture of the ovaries of the distilled water treated rats illustrated a well defined zona granulosa surrounding the oocyte, compact theca folliculi, corpora lutea and graffian follicles while the rats treated with 18, 36 and 72 mg/kg body weight of the extract also presented all the features of the distilled water treated animals in addition to some developing graffian follicles (Plates A-D) which was most pronounced in the ovarian tissues of the rats treated with 72 mg/kg body weight of the extract.

Discussion

The ovary and uterus are the primary reproductive organs in females. The ovaries which contain eggs are situated at either side of the uterus and are connected to it by the fallopian tubes. They produce hormones such as progesterone and estrogen that control the menstrual cycle (maturation of the ovaries and ovulation).

Cholesterol is the major precursor in the biosynthesis of steroid hormones like estrogen and progesterone and are required for normal ovulation and folliculogenesis (Yakubu, 2012). The increase in the level of cholesterol in both the ovary and uterus may account for the increase in the levels of estrogen and progesterone in the female rats following the administration of the aqueous extract of *C. populnea* stem.

Limiting the blood glucose or long-chain fatty acid oxidation potently inhibits gonadotropin secretion, ovulatory potential and reproductive behaviour (Nagatani & Bucholtz, 1996; Schneider, 2004). Unequivocally, it is the availability of metabolic fuels and not the presence of energy depot that is responsible for reproduction (Loucks, 2003). Therefore, the increase in the concentration of glucose in the uterus implies that the extract probably enhanced the availability of metabolic fuel for ovulation and reproduction, possibly via activation of glycogen phosphorylase that catalyses the breakdown of glycogen to glucose. Such increase in the glucose concentration might also

be responsible for the elevations in the levels of gonadotropic hormones (LH and FSH), which in turn enhanced the secretion of estrogen and progesterone as evidenced in the present study. The extract treatment related enhancement in the production and or release of glucose and the reproductive hormones could promote the process of ovulation in the female rats.

ACP is a lysosomal enzyme that contributes to ovarian metabolic functions like oocyte maturation, resumption of mitotic divisions, germinal vesicle breakdown and ovulation (Rosales, Avalos, Vergara, Hernandez, Ballesteros & Garcia, 2000; Bull, Murray, Thomas, Fraser & Nelson, 2002). ALP on the other hand, is a major sialoglycoprotein, localized on the basal and apical cell membrane, cytoplasm, glandular epithelium and play a role in the attachment of blastocyst to the endometrium (Buxton & Murdoch, 1980). The activity of ALP may alter the composition and the volume of luminal secretion, which is essential for development and adhesion of embryo. ACP and ALP are more active in the luteal phase, mostly visible at the apical portion of the epithelial cells in the endometrium (Wordinger, Dickey & Hill, 1972). Therefore, the increase in activities of ACP and ALP in the ovaries and uterus of the female rats following the administration of aqueous extract of *C. populnea* stem indicates that the extract could enhance the development of fertilized eggs and provide a niche for its maturation.

A number of proteins reside in follicular fluid and surrounding granulosa cells; therefore, the increase in ovary proteins might enhance ovarian cell proliferation possibly induced by estrogenic effects of the plant extracts.

Progesterone and estrogen are the most vital hormones known for the maintenance of pregnancy and implantation of blastocyst in humans and other mammals (Singh, Chadhry & Asselin, 2011; Hamid and Zakaria, 2012). Progesterone is responsible for stimulating sexual desire (Montaserti, Pourheydar, Khazaei & Ghorbani, 2007) and for maintaining the uterus during luteal phase of menstruation, thus preparing it for implantation of the fertilized egg (Mboso, Eyong, Ebong, Iwara & Odey, 2013). The increase in the level of serum progesterone by the aqueous extract of *C. populnea* stem suggests that it could support implantation or the normal maintenance of early pregnancy (Bran, Mills & Mahesh, 1995). Estrogen is responsible for the maturation of female sexual organs and could also be used to monitor the induction of ovulation and differential diagnosis of amenorrhea (Egba, Udom & Okonkwo, 2014). Elevation in the level of serum estrogen following the administration of aqueous extract of *C. populnea* stem could be an indication that it supports maturation of the ovaries and uterus or induction of ovulation in the female rats. Our finding suggests that *C. populnea* stem extract could enhance the normal functioning of the ovary and uterus.

Follicle stimulating hormone is a glycoprotein produced in response to gonadotropin-releasing hormone (GnRH) (Hordgen, 1989). In females, FSH acts on immature follicular cells of the ovary and induces development into mature follicle and oocyte capable of fertilization (Litwack and Schmidt, 2001). The significant elevation in the level of FSH by the extract might be responsible for the enhancement in the maturation of follicles, intact zona granulosa surrounding the oocytes which are consistent with the histological findings on the ovaries of the female rats in the present study.

Luteinizing hormone stimulates the secretion of sex steroids (Yakubu, Akanji, Oladiji, Olatinwo, Adesokan, Yakubu, Owoyele, Sunmonu & Ajao, 2008). In females, an acute rise in LH (LH surge) triggers ovulation, growth of corpus luteum and secretion of progesterone (Uotila, Ruoslati & Engvall, 1981). Therefore, the significant rise in the level of LH may also account for the increase recorded in the levels of estrogen and progesterone in the present study.

High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both FSH and GnRH, which are necessary for ovulation (Fitzgerald and Dinan, 2008). Reduction in the levels of serum prolactin in the female rats treated with the extract suggests the extract might be capable of facilitating ovulation and promoting conception probably by inhibiting the synthesis of prolactin.

The histological study on the ovary suggests that the extract had no detrimental effect on the structural integrity of the organ; rather, it enhanced the normal functioning of the organ by developing more graffian follicles which has been supported by the data obtained in this study.

Conclusion

Overall, the aqueous extract of *C. populnea* stem enhanced the normal functioning of the ovary and uterus of female rats and consequently could enhance reproductive capacity in females. The active ingredient and mechanism of action of the aqueous extract of *C. populnea* stem on the selected function indices could be a subject for further studies.

References

- Bran, D.W, Mills, T.M., & Mahesh, V.B. (1995). Female reproduction: the ovulatory cycle. In: Witorsh R.J. (ed.). *Reproductive Toxicology*, NY, Raven Press (pp. 23-44).
- Bull, H., Murray. P., Thomas. D., Fraser. A, & Nelson, P. (2002). Acid phosphatase. *Journal of Clinical Pathology*, 55, 65-72.
- Burkill, H.M. (2000). The Useful Plants of West Tropical Africa, Vol. 5. Royal Botanic Gardens, Kew, pp. 296 - 297.
- Buxton, L.E., & Murdoch, R.N. (1980). Behaviour and properties of membrane bound mouse uterine alkaline phosphatase during early pregnancy. *Australian Journal of Biological Sciences*, 33, 539-548.
- Egba, S.I, Udom, I.D., & Okonkwo, C.O. (2014). Comparative effect of oral administration of some dietary lipids on fertility hormones of female Wistar albino rats. *Global Journal of Biotechnology & Biochemistry*, 9(1), 24-29.
- Fitzgerald, P., & Dinan, T.G (2008). Prolactin and dopamine: What is the connection? A review article. *Journal of Psychopharmacology*, 22, 12-19.
- Gornall, A.C., Bardawill, C.J., & David, M.M. (1949). Determination of serum protein by means of Biuret reaction. *Journal of Biological Chemistry*, 177, 751–756.

- Hamid, H.Y., & Zakaria, Z.A.B. (2012). Embryo implantation: shedding light on the roles of ovarian hormones, cytokines and growth factors in the implantation process. *African Journal of Biotechnology*, 11(97), 16297-16304.
- Hordgen, G.D. (1989). Neuroendocrinology of the normal menstrual cycle. *Journal of Reproductive Medicine*, 34(1), 68-75.
- Ibrahim, H., Rai, P.P, & Bangudu, A.B. (1993). Pharmacognostic studies of the stem bark of *Cissus populnea* Guill & Perr. *Glimpses in Plant Research*, 1, 175-180.
- Krause, W.J (2001). The art of examining and interpreting histologic preparations. A student handbook. Partheton Publishing Group, United Kingdom (pp. 9-10).
- Litwack, G., & Schmidt, R.S. (2001). Biochemistry of hormones I: polypeptide hormones. In: Textbook of Biochemistry with Clinical Correlations, 5th ed. (Delvin T. M. ed.) John Wiley and Sons Inc., New York (pp. 905-956).
- Loucks, A.B. (2003). Energy availability, not body fatness, regulates reproductive function in women. *Exercise & Sport Sciences Review*, 31(3), 144-148.
- Mboso, O.E., Eyong. E.U, Ebong, P.E., Iwara, A., & Odey, M. (2013). The effects of the ethanolic extract of *Ereromastax speciosa* leaf on the serum levels of leuteinizing hormone, follicle stimulating hormone, progesterone and estradiol in female pubertal rats. *Annals of Biological Research*, 4(2), 136-140.
- Montaserti, A., Pourheydar, M., Khazaei, M., & Ghorbani, R. (2007). Anti-fertility effects of *Physalis alkekengi* alcoholic extract in female rat. *Iranian Journal of Reproductive Medicine*, 5, 13-16.
- Moody, J.O, Ojo., O.O., Omotade, O.O., Adeyemo, A.A., Olumese, P.E., & Ogundipe, O.O. (2003). Anti-sickling potential of a Nigerian herbal formula (ajawaron HF) and the major plant component (*Cissus populnea* L. CPK). *Phytotherapy Research*, 17(10), 1173-1176.
- Nagatani, S., Bucholtz, D.C., Murahashi, K., Estacio, M.A., Tsukamura, H., Foster, D.L. & Maeda, K.I. (1996). Reduction of glucose availability suppresses pulsatile luteinizing hormone release in female and male rats. *Endocrinology*, 137(4), 1166-1170.
- Ojekale, A.B., Lawal, O.A., Lasisi, A.K., & Adeleke, T.I., (2006). Phytochemistry and spermatogenic potentials of extract of *Cissus populnea* (Guill and Per) stem bark. *TSW Holistic Health Medicine*, 1, 176–182.
- Ojekale, A.B., Ojiako, O.A., Saibu, G.M., Lala, A., & Olodude, O.A., (2007). Long term effects of aqueous stem bark extract of *Cissus populnea* (Guill. and Per.) on some

- biochemical parameters in normal rabbits. *African Journal of Biotechnology*, 6(3), 247-251.
- Oremosu, A.A., Arowosaye, V.O., Akang, E.N., & Bassey, R.B. (2013). Effects of *Cissus populnea* and *Panax ginseng* on flutamide-induced testicular defect in pre-pubertal male rats. *British Journal of Medicine & Medicinal Research*, 3(1), 173-181.
- Roeschlau, P., Bernt, E., & Gurber, W.J. (1974). Enzymatic colorimetric endpoint method with CHOD-POD (CHOD: cholesterol oxidase, POD: peroxidase). *Z Klin Chem Klin Biochem*, 12, 226.
- Rosales, T., Avalos, R., Vergara, O., Hernandez, P., Ballesteros, L., & Garcia, M. (2000). Multi-parametric study of atresia in ewe antral follicle: histology, flow cytometry, internucleosomal DNA fragmentation and lysosomal enzyme activities in granulosa cells and follicular fluid. *Molecular Reproduction and Development*, 55(3), 270-281.
- Schneider, J.E. (2004). Energy balance and reproduction. *Physiological Behaviour*, 81(2), 289-317.
- Singh, M., Chadhry, P., & Asselin, E. (2011). Bridging endometrial receptivity and implantation: network of hormones, cytokines and growth factors. *Journal of Endocrinology*, 210, 5-14.
- Soladoye, M.O., & Chukwuma, E.C. (2012). Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill. & Perr. (Vitaceae) – an important medicinal plant in Central Nigeria. *Archives of Applied Science Research*, 4(1), 200-206.
- Uotila, M., Ruoslati, E., & Engvall, E. (1981). Two-site sandwich enzyme immunoassay with monoclonal antibodies to human alpha-fetoprotein. *Journal of Immunological Methods*, 42(1), 11-15.
- Wordinger, R.J., Dickey, J.F., & Hill, J.R. (1972). Influence of undernutrition on the histology and histochemistry of the bovine endometrium. *Journal of Animal Science*, 34, 453-459.
- Wright, P.J., Leathwood, P., Plummer, D.T. (1972a). Enzymes in rat urine: Alkaline phosphatase. *Enzymologia*, 42, 317-327.
- Wright, P.J., Leathwood, P.D., Plummer, D.T. (1972b). Enzymes in rat urine. Acid phosphatase. *Enzymologia*, 42, 459-462.
- Yakubu, M.T. (2012). Effect of a 60-Day oral gavage of a crude alkaloid extract from *Chromolaena odorata* leaves on hormonal and spermatogenic indices of male rats. *International Journal of Andrology*, 33(6), 1199-1207.

Yakubu, M.T., Akanji, M.A., Oladiji, A.T., Olatinwo, A.W.O., Adesokan, A. A., Yakubu, M. O., Owoyele, B. V., Sunmonu, T. O. & Ajao, M. S. (2008). Effect of *Cnidioscolous aconitifolius* (Miller) I.M. Johnston leaf extract on reproductive hormones of female rats. *Iranian Journal of Reproductive Medicine*, 6(3), 149-155.

Table 1: Ovarian parameters of female Wistar rats treated with aqueous extract of *C. populnea* stem

Parameters	Control	<i>Cissus populnea</i> (mg/kg body weight)		
		18	36	72
Ovarian Total Protein (mg/ml)	0.77±0.02 ^a	0.81±0.02 ^a	0.99±0.01 ^b	1.29±0.02 ^c
Ovary cholesterol (mmol/L)	5.48±0.14 ^a	9.34±0.26 ^b	9.20±0.19 ^b	8.87±0.41 ^b
Ovarian ACP (U/L)	12.03±0.02 ^a	24.32±0.12 ^b	25.14±0.67 ^b	24.70±0.42 ^b
Ovarian ALP (U/L)	16.04±0.23 ^a	19.76±0.02 ^b	24.53±0.17 ^c	26.04±0.50 ^d

Values are mean of 5 replicates ± SEM, values carrying different superscript across the rows for each parameter are significantly (P < 0.05) different.

Table 2: Uterine parameters of female Wistar rats treated with aqueous extract of *C. populnea* stem

Parameters	Control	<i>Cissus populnea</i> (mg/kg body weight)		
		18	36	72
Uterine Cholesterol (mmol/L)	17.11±0.21 ^a	24.94±0.41 ^b	26.88±1.11 ^b	30.51±0.33 ^c
Uterine ACP (U/L)	14.62±0.14 ^a	17.35±0.15 ^b	21.97±0.26 ^c	28.14±0.24 ^d
Uterine ALP (U/L)	13.24±0.13 ^a	15.10±0.34 ^b	15.77±0.26 ^b	16.01±0.51 ^b
Uterine glucose (mmol/L)	3.71±0.83 ^a	4.35±0.50 ^b	4.17±0.28 ^b	7.93±0.95 ^c

Values are mean of 5 replicates ± SEM, values carrying different superscript across the rows for each parameter are significantly (P < 0.05) different.

Table 3: Hormonal profile of female Wistar rats treated with aqueous extract of *C. Populnea* stem

Parameters	Control	<i>Cissus populnea</i> (mg/kg body weight)		
		18	36	72
Follicle Stimulating Hormone (mIU/ml)	2.59±0.03 ^a	3.11±0.12 ^b	3.60±0.22 ^c	3.58±0.06 ^c
Luteinizing Hormone (mIU/ml)	2.95±0.05 ^a	4.89±0.07 ^b	4.85±0.05 ^b	5.17±0.23 ^b
Prolactin (ng/ml)	6.86±0.06 ^a	5.54±0.11 ^b	5.45±0.21 ^b	5.46±0.16 ^b
Progesterone (ng/ml)	19.43±0.45 ^a	26.58±1.02 ^b	31.57±1.26 ^c	37.49±1.06 ^d
Oestrogen (ng/ml)	24.34±1.18 ^a	41.31±0.37 ^b	44.62±0.82 ^c	43.80±0.83 ^c

Values are mean of 5 replicates ± SEM, values carrying different superscript across the rows for each parameter are significantly ($P < 0.05$) different.

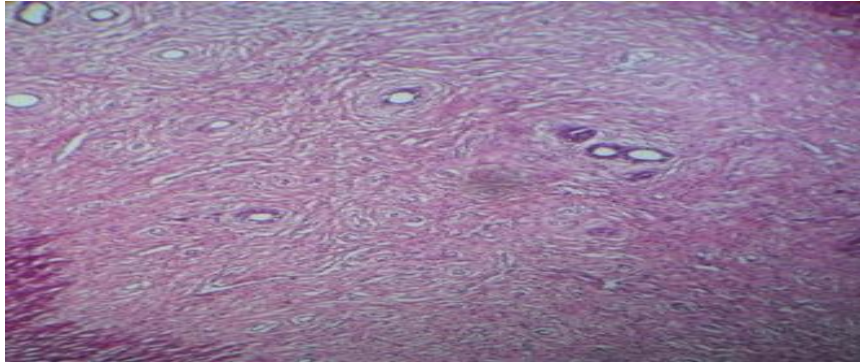


Plate A: Cross section of the ovary of female rats administered distilled water (H & E; x 400)

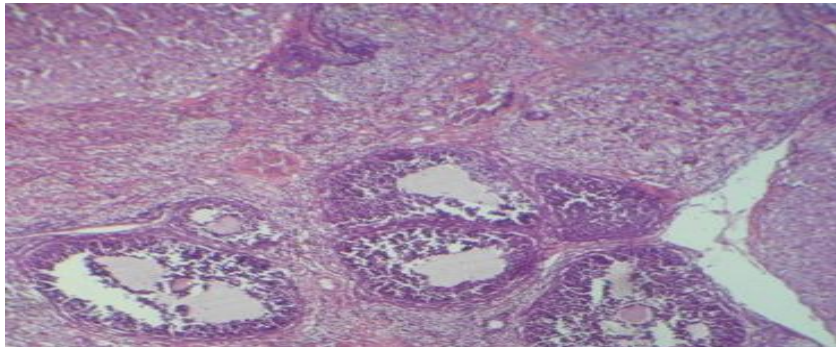


Plate B: Cross section of the ovary of female rats administered 18 mg/kg body weight of aqueous extract of *C. populnea* stem (H & E; x 400)

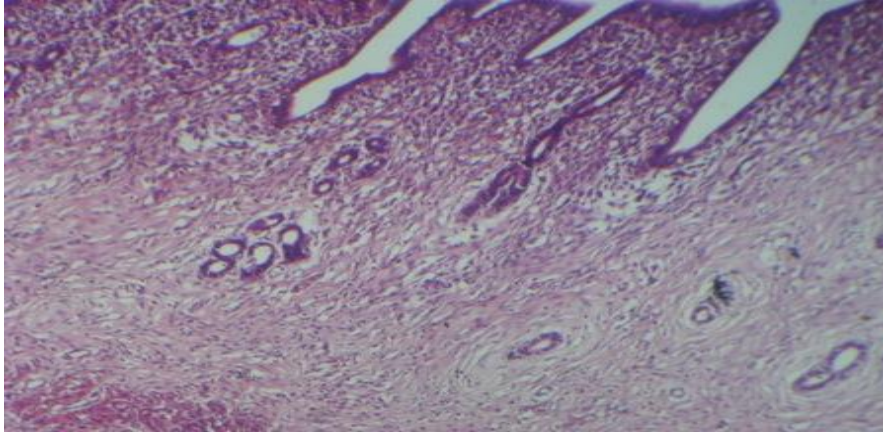


Plate C: Cross section of the ovary female rats administered 36 mg/kg body weight of aqueous extract of *C. populnea* stem (H & E; x 400)

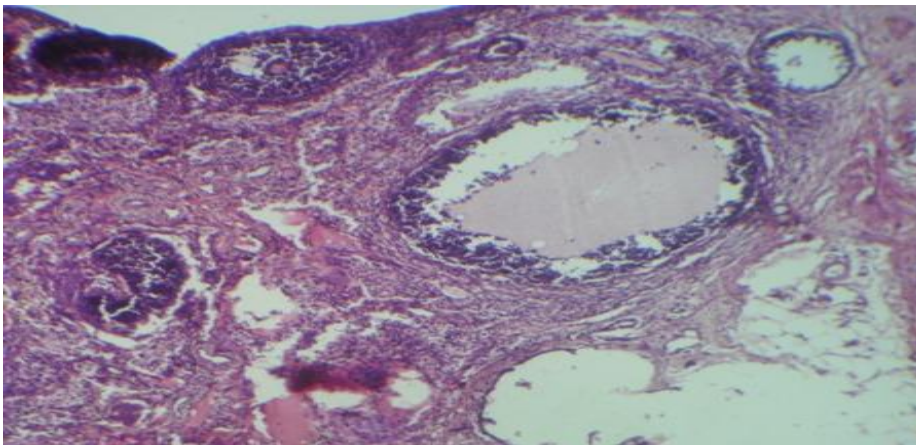


Plate D: Cross section of the ovary of female rats administered 72 mg/kg body weight of aqueous extract of *C. populnea* stem (H & E; x 400)