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Singular and synergistic effect of five medicinal plants on the ovary Histology of wistar rat induced PCOS using Letrozole

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Abstract

The ovary which is a ductless essential reproductive female organ that is held by the fallopian tube that attaches itself to the uterus and which perform the sole responsibility of releasing ova for fertilization which occur in the fallopian tube can be deformed by polycystic ovarian syndrome in such a way that it will not be able to perform its functions again. Polycystic ovarian syndrome, just as the name goes causes the growth of numerous cysts (fluid filled sacs) in the ovary. These cyst are not harmful but causes hormonal imbalance in polycystic ovarian syndrome patient. The five medicinal plants use are *Trigonella feonum granum*, *Glycine max*, *Sesanum indium*, *Glycyrrhiza glabra* and *Lepidium meyenii*. Forty three female wistar albino rats weighing about 200g – 230g was used in the experiment. In the first stage of the experiments, the experimental animals were grouped into twelve, each containing 3 rats selected at random. After their acclimatization for two weeks they were administered letrozole for twenty one days before the administration of the plant extract. Ten of these twelve groups of rats were administered the five plant extract separately at either 300mg/kg or 100mg/kg the eleventh group was administered only letrozole without any plant extract and this group is referred to as the PCOS group, while the twelve group was administered only water and this group is referred to as the normal control group. After fifteen days administration of the plant extract, the experimental animals were then injected ketamine to sedate them and their ovaries harvested for histology. In the second stage of the experiment, the experimental animals were then grouped into six groups containing three rats each; they were administered letrozole for twenty one days followed by the mixed plant extract for fifteen days. Three groups were administered the mixed plant extract at either 500mg/kg, or 200mg/kg or 50mg/kg respectively, the fourth group administered clomid 1mg/kg and merformin at 200mg/kg for five days, fifth group administered only letrozole and the sixth group is the normal control group for the second stage of the experiments. Just as it was in the first stage of the experiment the ovaries of the experimental animals were also harvested after sedation with ketamine. One rat was randomly selected from each group and was taken to School of Vetenary Medicine, Ahmadu Bello University Zaria for the histology studies. The animals were sedated with ketamine, after which the ovaries were removed and preserved in 10% formaline until ready to use. The tissues were then serially dehydrated in graded ethanol and xylene. The specimens was then embedded in paraffin block and sections of 4 -5 um thickness was then

cut and stained with hematoxylin and eosin stain, after which the stained slides can now be viewed under a light microscope, after allowing to dry properly for two days. In the first stage of the experiment, of the five plants used only *G. glabra* at 100mg/kg that show no ability to control cyst formation in the ovary and in the second stage of the experiment (synergistic stage) 500mg/kg was the overall best even better than the clomid and metformin group in controlling the growth of cyst.

Keywords: medicinal plants, Polycystic ovarian syndrome, Ovary histology.

Introduction

The ovary is the female reproductive organ that houses about 500000 ova at birth of any female child, but the ovary releases only about 34000 of these ova from the time of puberty to menopause. The ovary also performs the function of releasing the female hormone; Estrogen and Progesterone (Telfer 2017 and Aurora 2018). The process by which these ova are release is called ovulation. The ovum can grow up to 30millimeter in diameter at maturity before been release from the ovary. Apart from Estrogen and Progesterone, the ovary produces another hormone called Androgens. The ovary which is a ductless essential reproductive female organ that is held by the fallopian tube that attaches itself to the uterus and which perform the sole responsibility of releasing ova for fertilization which occur in the fallopian tube can be deformed by polycystic ovarian syndrome in such a way that it will not be able to perform its functions again. Polycystic ovarian syndrome, just as the name goes causes the growth of numerous cysts (fluid filled sacs) in the ovary. These cyst are not harmful but causes hormonal imbalance in polycystic ovarian syndrome patient (Sanyal, 2018). Virtually all the hormones in the females with PCOS are increased. This hormonal imbalance is the major cause of infertility and irregular menstruation in PCOS patient. The gonadotropin releasing hormone, in patients with PCOS, always gives signals for high pulse level, this high level of LH stimulate the release of androgen (male hormone), high level of androgens and low level of FSH is responsible for amenorrhea and lack of ovulation in PCOS female (Ricard lucida and Center for Reproduction 2019).This is also responsible for hirsitism (excessive hair growth in special part of the body), infertility, ache and other symptoms of PCOS (Rojas *et al* 2014). Moreso, in females with

PCOS, the activities of the enzymes aromatas (conversion of androgens to estrogens) is high in females with PCOS and this increases the level of LH thereby decreasing FSH causing anovulation. Whenever the pulse received by the Gonadotropin realasing hormone(GnRH) is low it signal for the release of FSH (responsible for the development and release of mature eggs or ova in the ovary).However, in patient with PCOS, GnRH do not receive low Pulse rate, this is responsible for the low level of FSH in female with PCOS this is the course of lack of menstruation and ovulation (Richard lucido 2019 and Center for Reproduction, 2019). The diagonosistic criterian for polycystic ovarian syndrome in the following; Check for signs of hirsutism; hirsutism is the abundant to moderate presence of coarse hair on the face, lower abdomen, back,legs, hands, chest in the female as a result of high androgen level OMD (2010). Baldami *et al*, (2012), reported that out of 365 women examined for PCOS, 75% have hirsutism. Transvaginal Ultrascan; This is a type of scan done by passing a scanning stick already worm condom into a female vaginal. This method is more preferably than pelvic scan as it review the ovaries very well for correct diagnosis. When the ultra sound shows the ovaries with more than 12 follicles in each ovary (left and right), increase ovarian volume, cyst etc PCOS confirm OMD (2010). Halm *et al*, (2006), reported that about 83% of women with PCOS shows polysistic ovarian syndrome through ultra scan. Oligomenorrhea and amenorrhea; these are types of ovulation. oligomenorrhea is irregular menstruation while amenorrhea is the absent of menstruation, both of them are diagnostic symptoms of PCOS about 74% women with PCOS show oligomenorrhea and about 34% show amenorrhea OMD, (2010). ALOPECIA; this is the thinning of hair in female with PCOS. Ozdemir *et al*, (2010), in their work reported 34%

occurrence of alopecia in female with PCOS. OBESITY; Obesity in PCOS female has been reported by Ehrmann *et al*, (2006), to be as a result of excess androgen secretion. Obesity is common among US women and can be as high as 80% Sam, (2007). Insulin resistance; Insulin is an hormone produced by the pancreas, it perform the primary function of regulating the sugar level in humans. However, Female with PCOS are resistant to this hormone and this result to high level of sugar and high level of insulin in the blood as they tend to produce more insulin to control the high sugar level in the body. This high level of insulin causes high level of androgen which causes anovulation in females with PCOS (Mayo foundation 2019).

Materials and Methods

The plants *Trigonella feonum graenum*, *Sasame indicum*, *Glycine max*, *Glycyrrhiza glabra* and *Lepidium meyenii* extracts were administered singly and it was then administered as a multi herbal preparation.

Kaetamine and Letrozole were purchased from a pharmacy shop in Kaduna. The plant extract were purchase from herbal market in Kaduna.

Experimental animals; Forty three female wistar albino rats weighing about 200g – 230g was used in the experiment. In the first stage of the experiments, experimental animals were grouped into twelve each containing 3 rats selected at random. After their acclimatization for two weeks they were administered letrozole for twenty one days before the administration of the plant extract. Ten of these twelve groups of rats were administered the five plant extract separately at either 300mg/kg or 100mg/kg the eleventh group was administered only letrozole without any plant extract and this group is referred to as the PCOS group, while the twelve group was administered only water and this group is referred to as the normal control group. After fifteen days administration of the plant extract, the experimental animals were then injected ketamine to sedate them and their ovaries harvested for

histology. At the end of the first stage of the experiments, the second stage commenced, in this stage the rats were grouped into twelve each containing three rats. The experimental animals were then grouped into six groups containing three rats each; they were administered letrozole for twenty one days followed by the mixed plant extract for fifteen days. Three groups were administered the mixed plant extract at either 500mg/kg, or 200mg/kg

Or 50mg/kg respectively, the fourth group administered clomid 1mg/kg and merformin at 200mg/kg for five days, fifth group administered only letrozole and the sixth group is the normal control group for the second stage of the experiments. Just as it was in the first stage of the experiment the ovaries of the experimental animals were also harvested.

Histology staining of the ovary; Histology staining of the sections of the ovaries was carried out according to the method described by (Hazam *et al.*, 2019). Few minutes immediately after dissection, the dissected ovaries were then fixed in 10% formalin. The tissues were then serially dehydrated in graded ethanol and xylene. The specimens was then embedded in paraffin block and sections of 4 -5 um thickness was then cut and stained with hematoxylin and eosin stain, after which the stained slides can now be viewed under a light microscope, after allowing to dry properly for two days.

Results

Histology of the ovary after administration of Letrozole

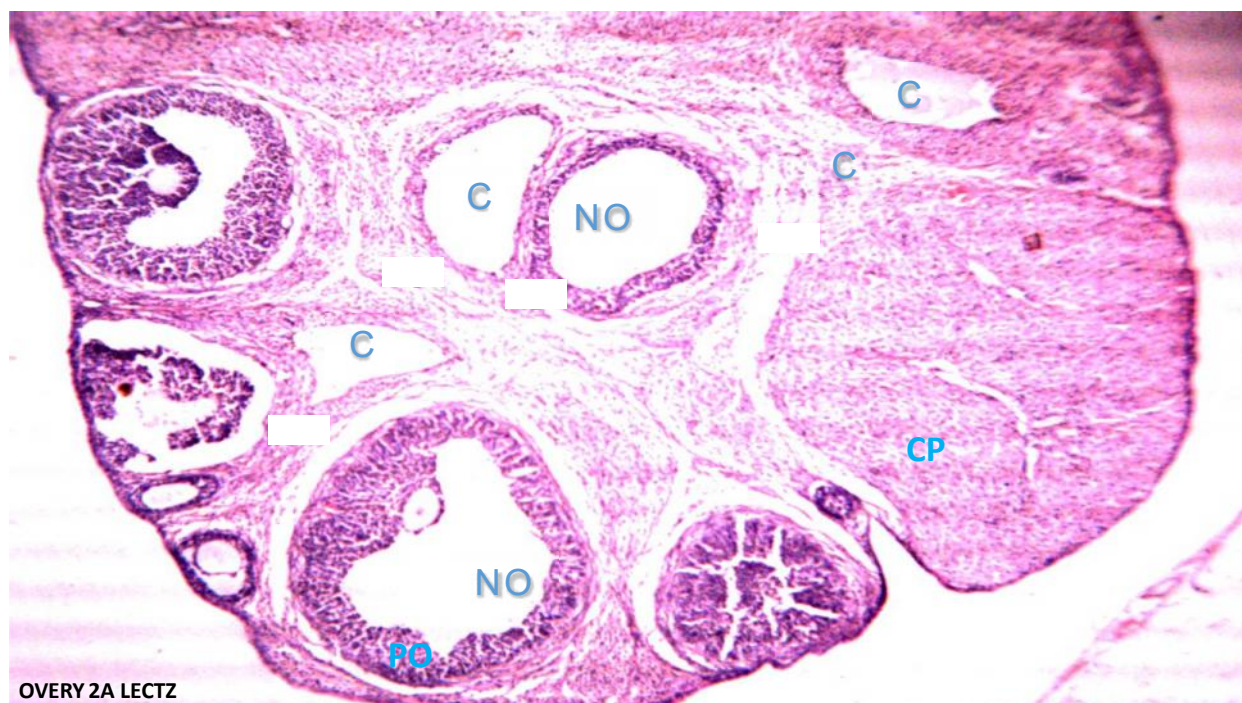


Plate 4.1 The ovary of one of the rats administered letrozole. Letter C is the cyst on the ovary and they are about four of them, letter PO is the primary oocyte of a secondary follicle while letter CP is the corpus luteum of a released matured egg.

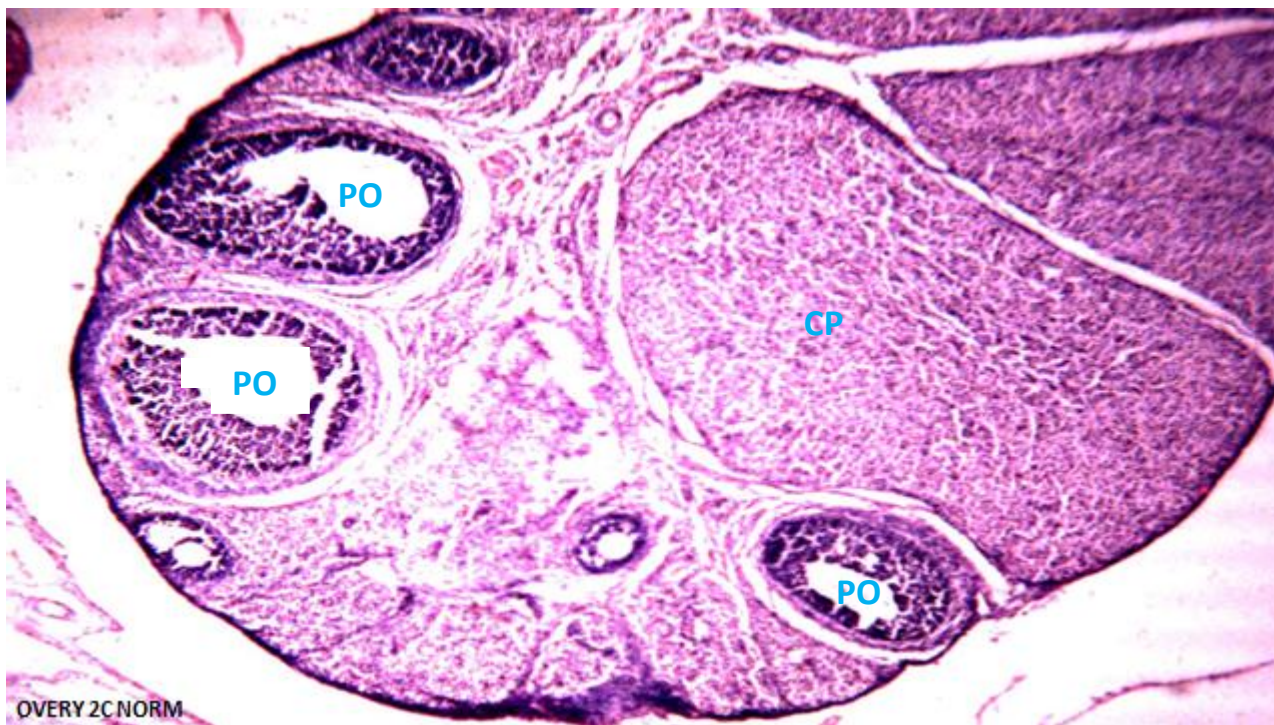


Plate 4.2 The ovary of a rat from the normal control group, this photograph does not show the presence of any cyst in the ovary but rather shows several follicles at different developmental stages. Letter PO indicate the primary oocyte of the several follicles at various developmental stages while letter CP is the corpus luteum of a released matured egg.

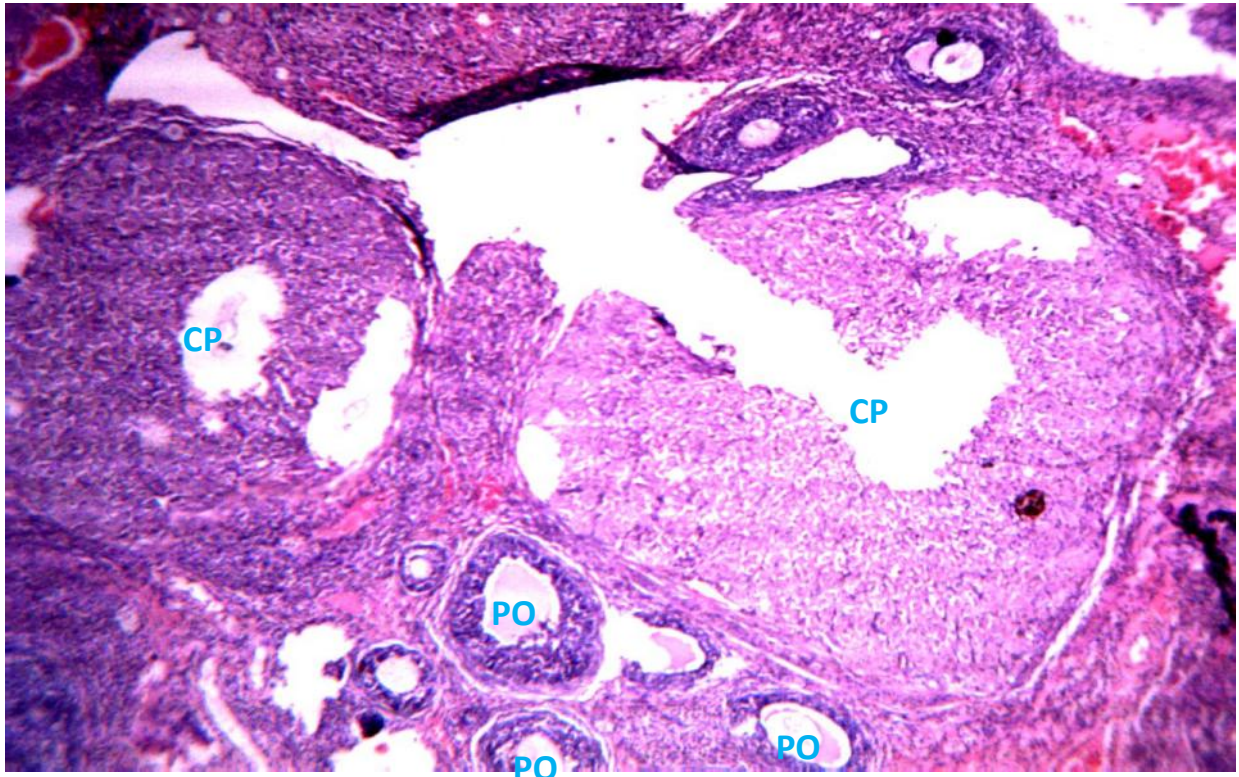


Plate 4.3 The ovary of the group treated with *Trigonella* spp at 300mg/kg. CP is degenerating corpus letuem of a released secondary follicle and PO are primary oocytes of follicle at different developmental stages. No cyst found.

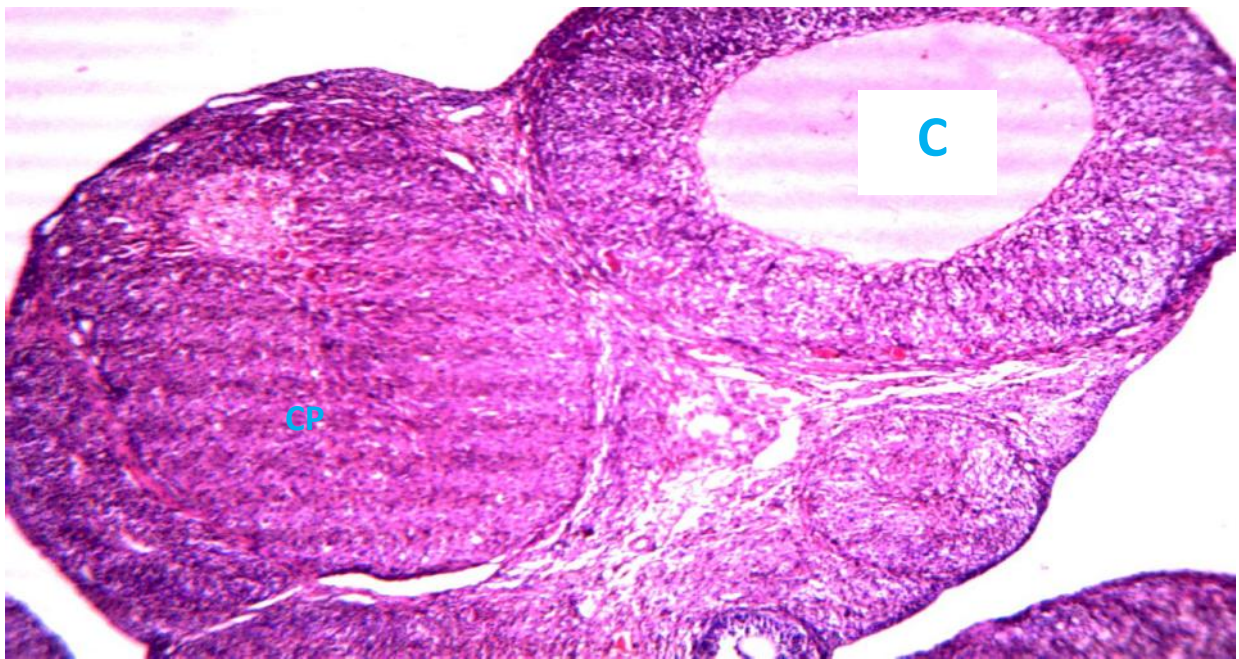


Plate 4.4 The ovary of the group treated with *Trigonella* spp at 100mg/kg. C is an enlarge cyst, CP is a corpus leteum of a released secondary follicle that is about degenerating. One cyst found.

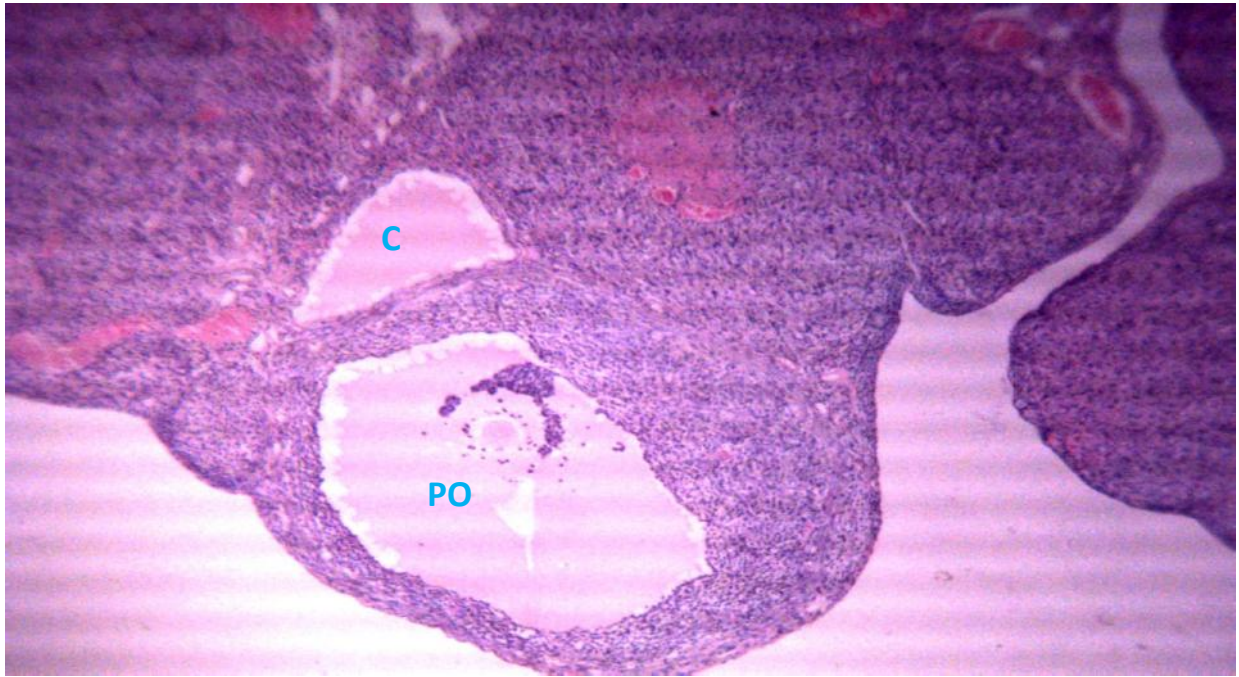


Plate 4.5 The ovary of the group treated with *Glycine max* at 300mg/kg. PO is the primary oocyte of a secondary follicle, C is a singular cyst. The presence of a singular cyst shows that the plant extract was able to reduce the number of cyst from four to one.

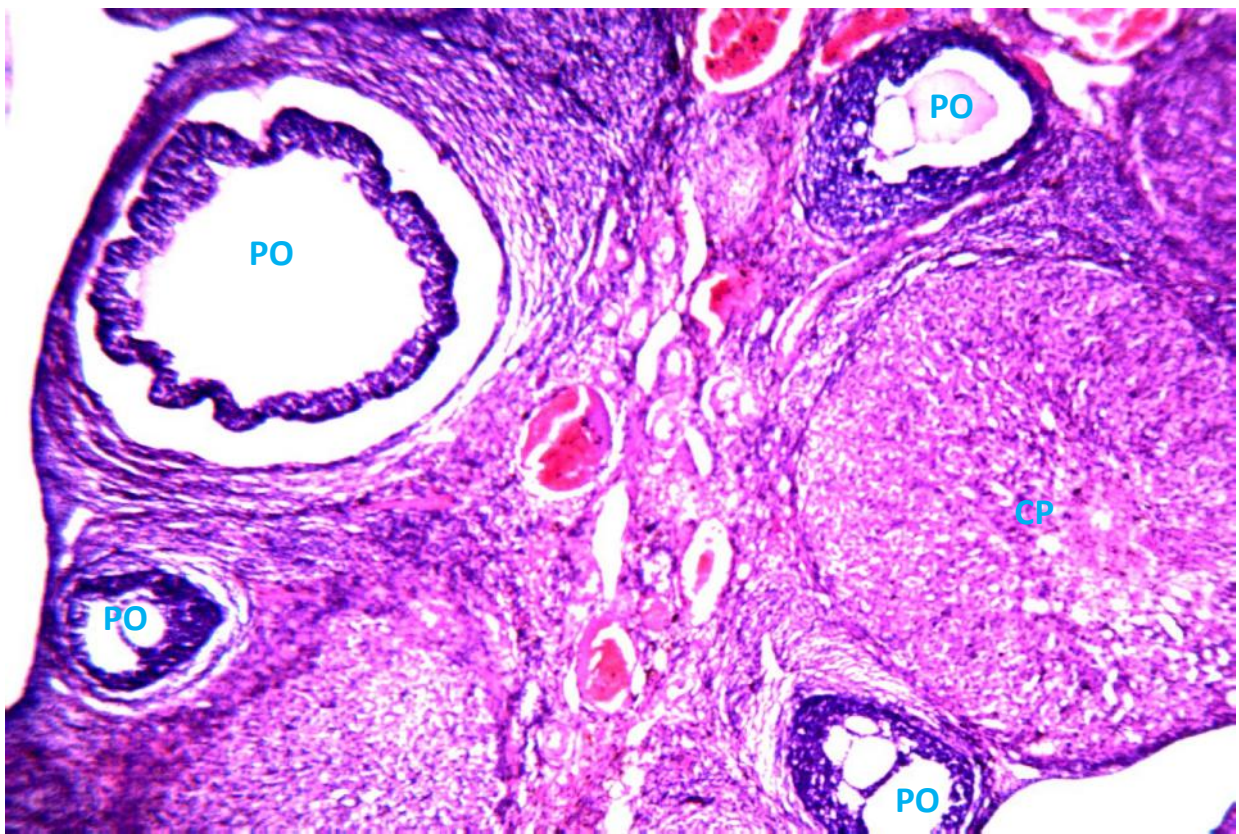


Plate 4.6 The ovary of the group treated with *Glycine max* at 100mg/kg. CP is the corpus luteum of a released secondary follicle, PO primary oocyte of various follicles at different developmental stages. No cyst found

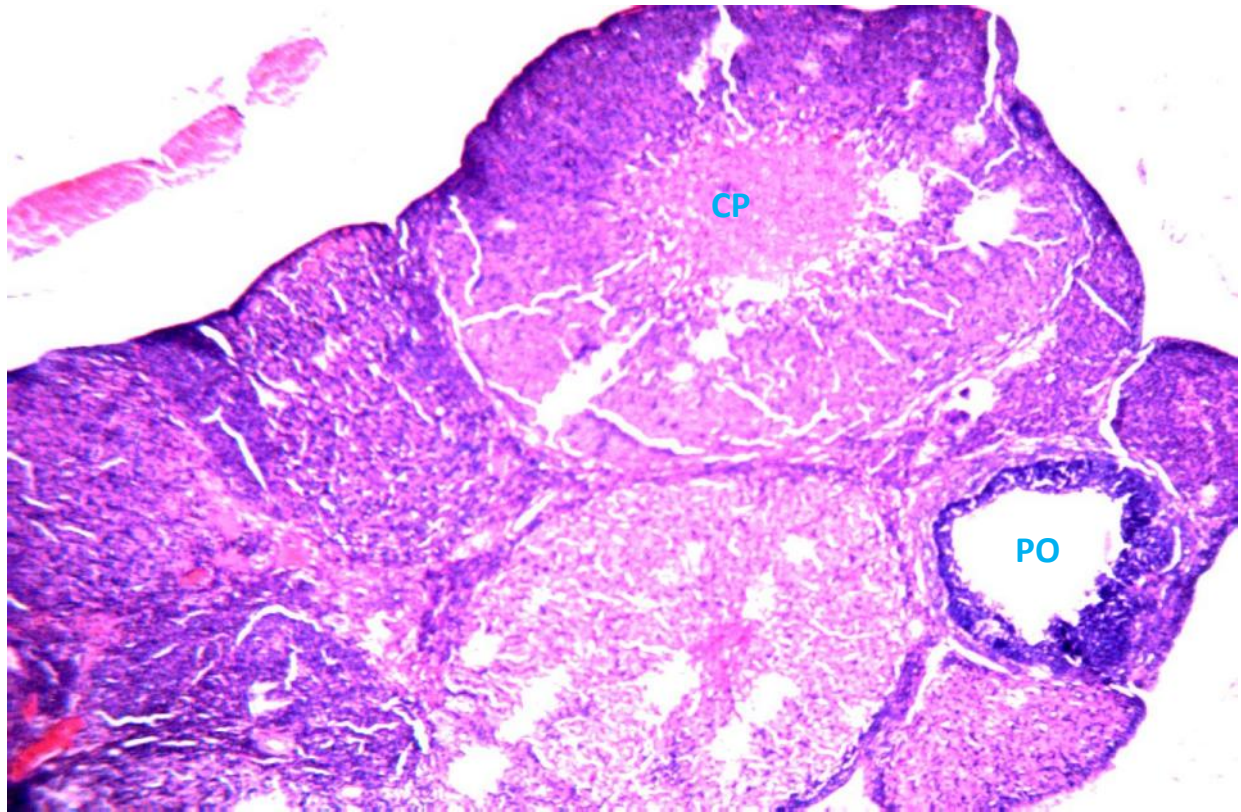


Plate 4.7 The ovary of the group treated with *Sesame indicum* at 300mg/kg. PO is the primary oocyte of a follicle. No cyst found.

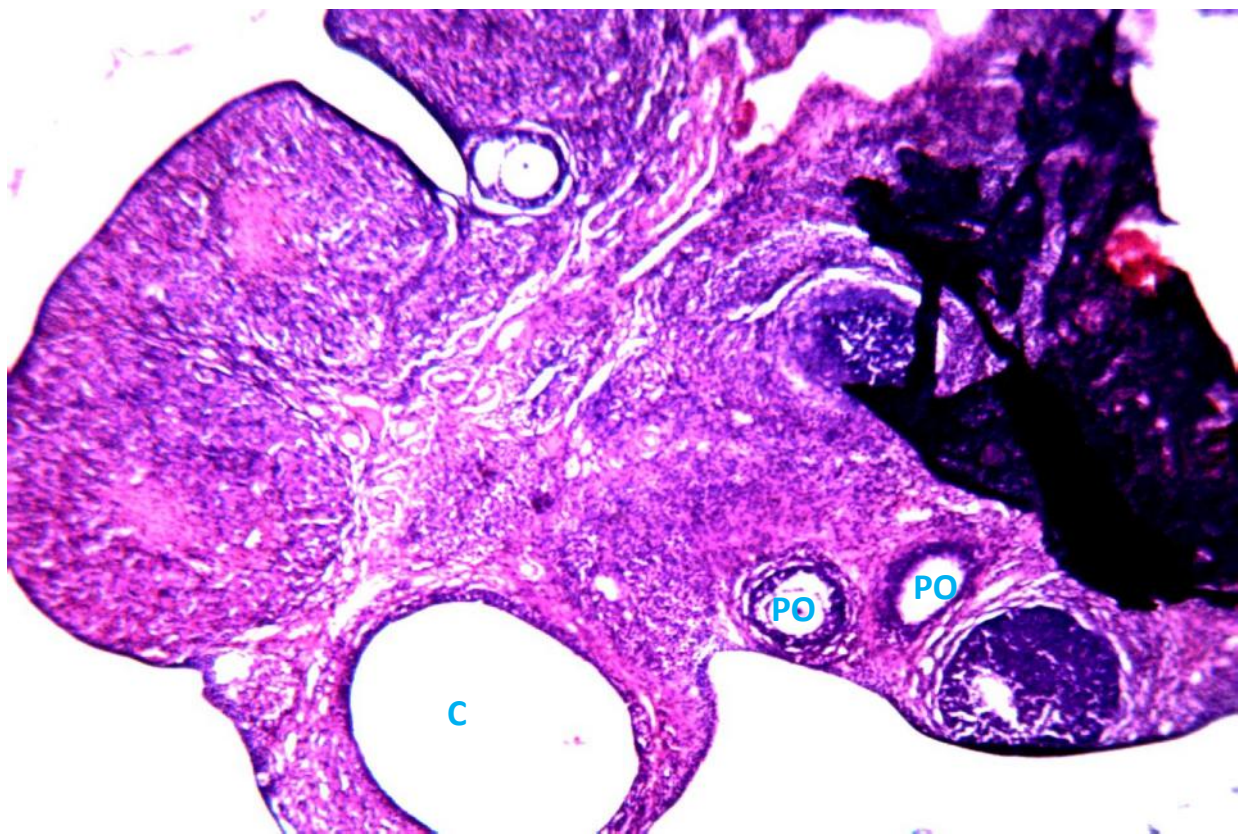


Plate 4.8 The ovary of the group treated with *Sesame indicum* at 100mg/kg. C is a singular cyst found, PO is the primary oocyte of primary follicle.

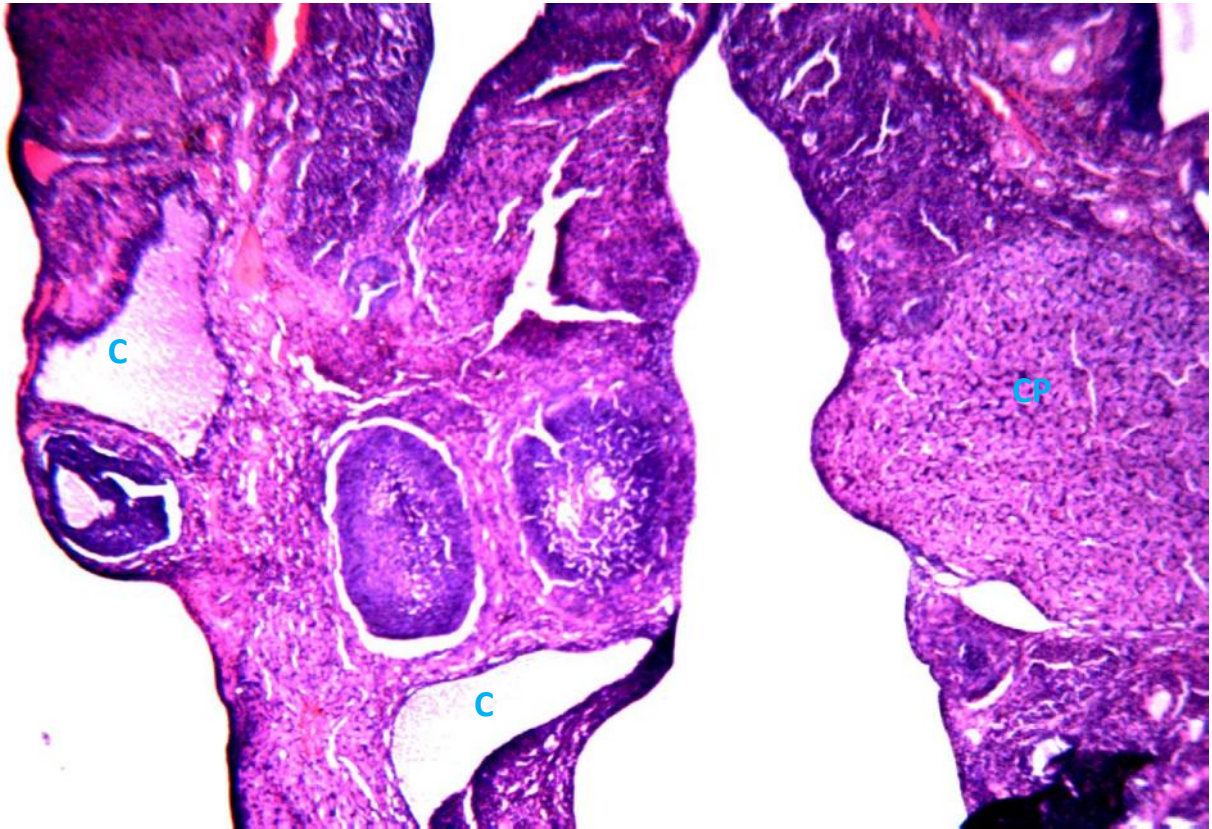


Plate 4.9 The ovary of the group treated with *Glycyrrhiza* at 300mg/kg. The two C found stand for the two cyst found on the ovary. CP is the corpus luteum of a released secondary follicle.



Plate 4.10 The ovary of the group of the rats treated with *Glycyrrhiza* spp at 100mg/kg. C is cyst, they are about 10 of them. The cyst increase from four to ten, this is indicating that the treatment cannot control cyst development.

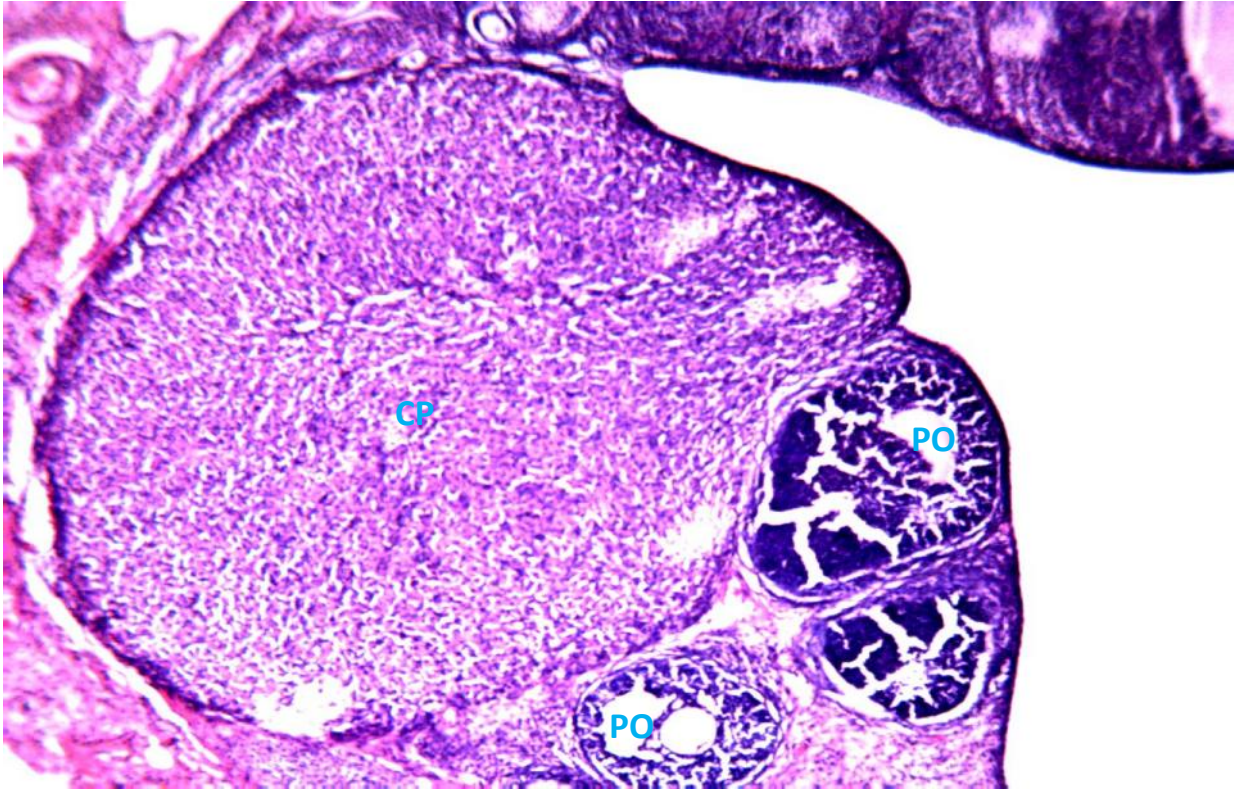


Plate 4.11 The ovary treated with *Lepidium meyenii* at 300mg/kg. PO is the primary oocyte of the different follicle at various developmental stages. CP is the corpus luteum of a released secondary follicle. No cyst found.

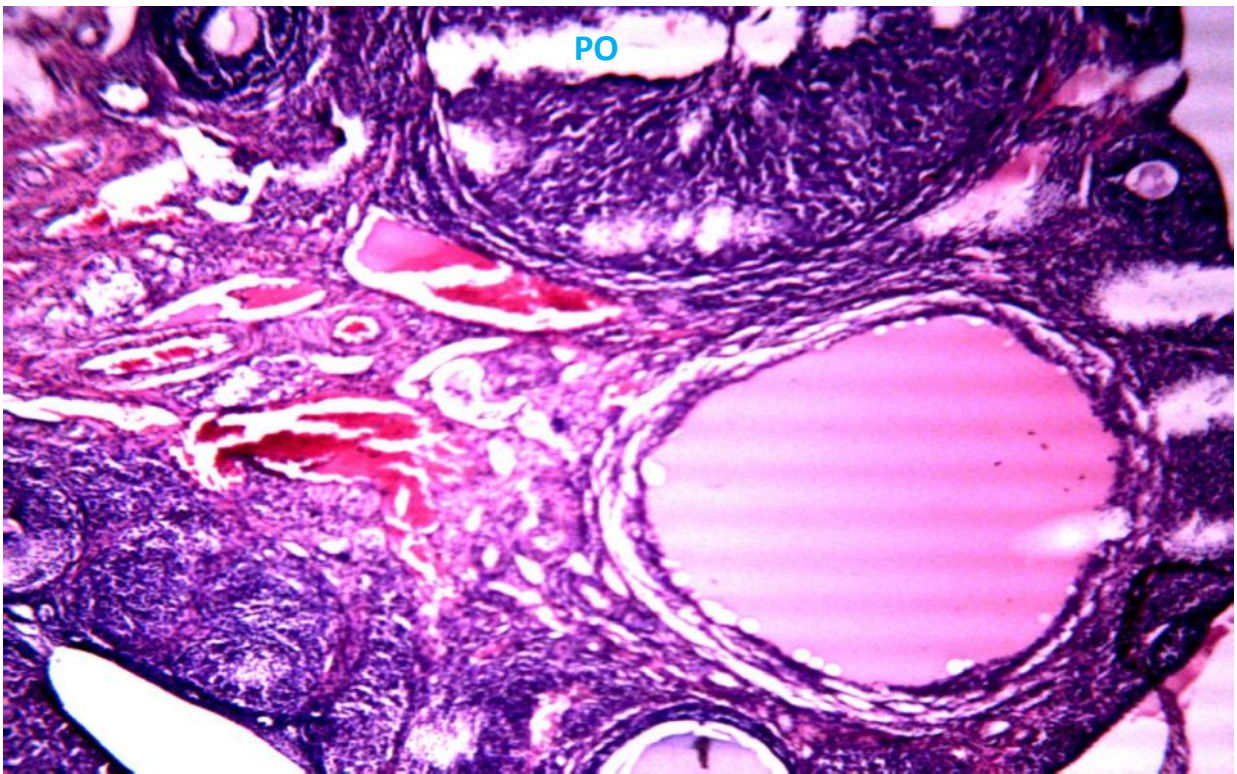


Plate 4.12 The Ovary of the group treated with *Lepidium meyenii* at 100mg/kg. No cyst found on the ovary of the rats.

Histology results of the second stage of the research



Plate 4.13 The ovary of the normal control group (Group K x 40). The ovary section with normal ovarian cytoarchitecture; Pf: Primary follicle, GE: Germinal epithelium, M: Inner medullary region

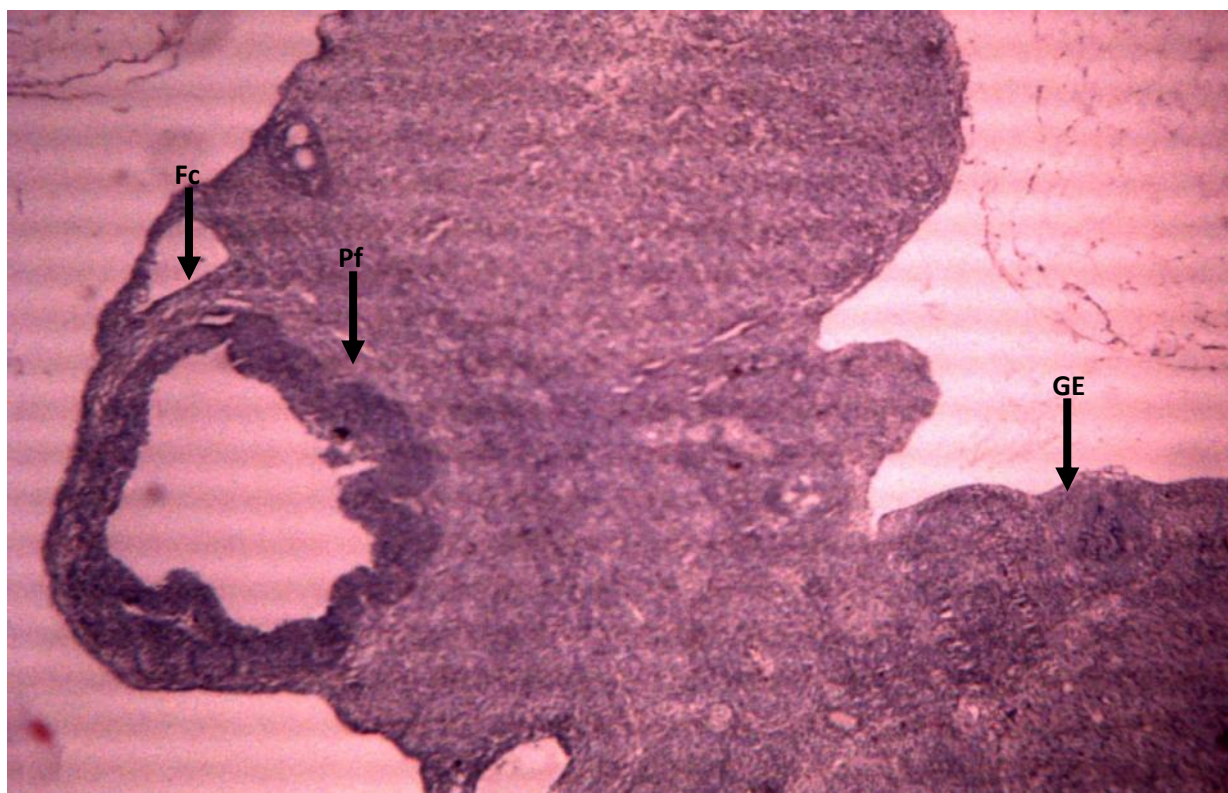
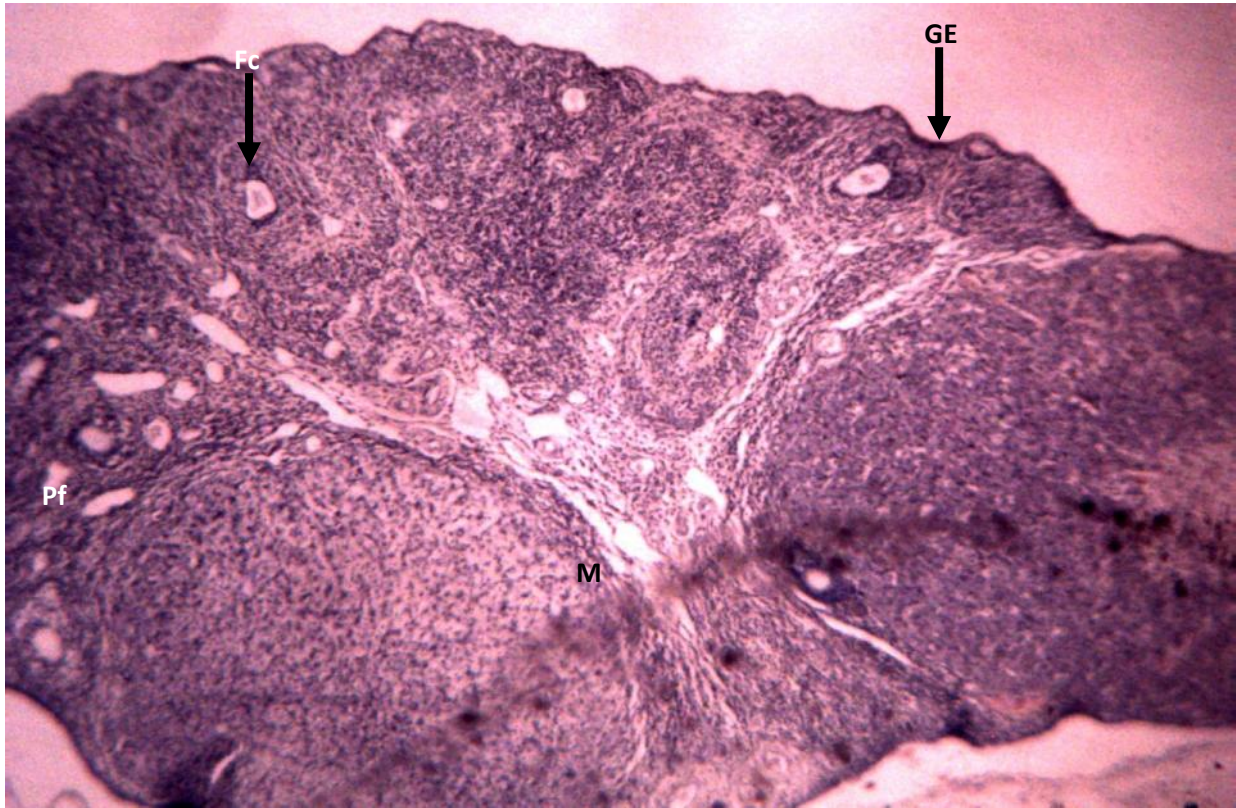
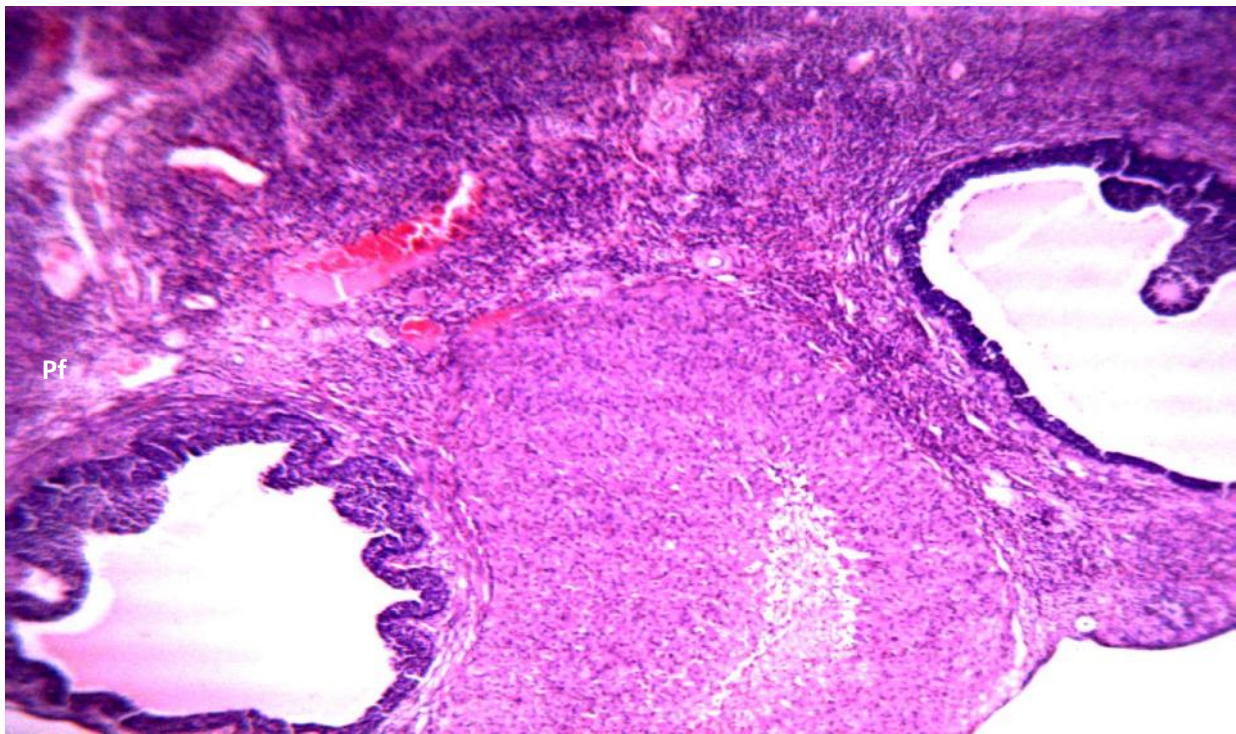


Plate 4.14 The ovary of the group treated with the plant extract at 500mg/kg (Group F x 40). The section of the ovary showing a cytoarchitecture distortion; Pf: Primary follicle, GE: Germinal epithelium, Fc: Follicular cysts (only one cyst found). ▲



Plant 4.15 Photomicrograph of the group treated with the plant extract at 200mg/kg(Group G x 40).The section show ovarian cytoarchitecture distortion; Pf: Primary follicle, GE: Germinal epithelium, Fc: Six very tiny follicular cysts, M: Inner medullary region.



Plant 4.16 Photomicrograph of the group treated with the plant extract at 50mg/kg(Group H x 40).The section show normal ovarian cytoarchitecture; PO: Primary Ocytes, GE: Germinal Epithelium, M: Inner medullary region.CL; Corpus luteum. No cyst found.

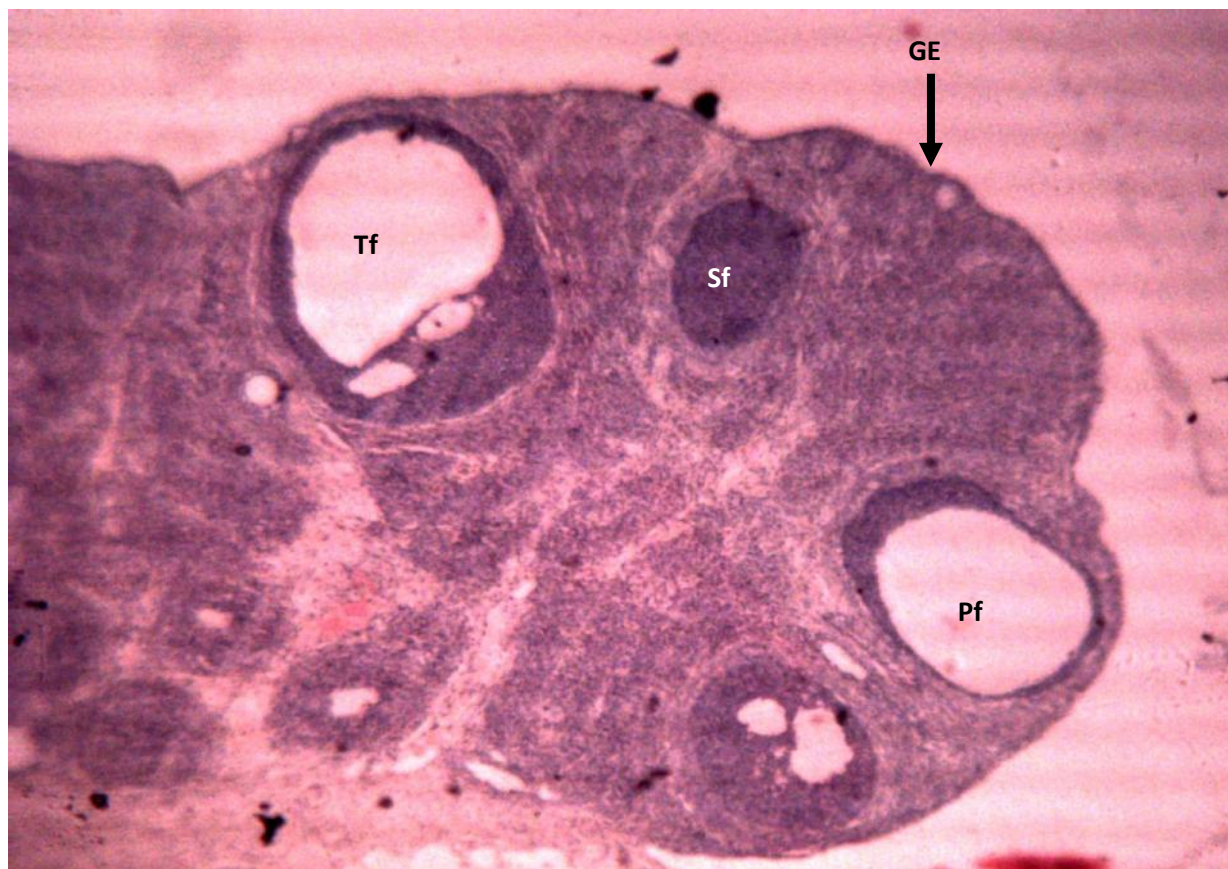


Plate 4. 17 The ovary of the group treated with Metformin and Calomiphene (Group Ix40). The section show relatively normal ovarian cytoarchitecture; Pf: Primary follicle, Tf: Tertiary follicle with well-preserved zona pellucid, Sf: Secondary follicle, GE: Germinal epithelium, M: Inner medullary region and four very tiny follicular cysts (FC).

Discussion

Polycystic ovarian syndrome (PCOS) is the pathology effect of the structure and function of the ovaries (Teeda *et al*, 2010), this syndrome is unique in the sense that it has several parameters which include; high glucose level as a result of insulin resistance, increase in lipid profile as a result of increase androgens level, hormonal imbalance as a result of increase in androgen level, development of numerous cyst in the ovary as a result of hormonal imbalance, irregular and absence of menstruation as a result of hormonal imbalance and infertility. It is a complex endocrine condition which involves disorder in the hypothalamic pituitary and ovarian network, resulting in chronic anovulation and androgen excess (Smeltzer *et al*, 2010). Polycystic ovarian syndrome leads to excess secretion of androgen

hormones which impaired the activities of the ovary (growth and releases of mature follicle), as a result fluid filled sacks (cyst) are formed around the ovary. Clinical presentation of PCOS include infertility, menstrual disorder, baldness, acne (Zehora *et al*, 2018). The ovary is the female reproductive organ that houses about 500000 ova at birth of any female child, but the ovary releases only about 34000 of these ova from the time of puberty to menopause. The ovary also performs the function of releasing the female hormone; Estrogen and Progesterone (Telfer 2017 and Aurora 2018). The process by which these ova are release is called ovulation. The ova can grow up to 30millimeter in diameter at maturity before been release from the ovary. Apart from Estrogen and Progesterone, the ovary produces another hormone called Androgens.

The ovary which is a ductless essential reproductive female organ that is held by the fallopian tube that attaches itself to the uterus and which perform the sole responsibility of releasing ova for fertilization which occur in the fallopian tube can be deformed by polycystic ovarian syndrome in such a way that it will not be able to perform its functions again. Polycystic ovarian syndrome, just as the name goes causes the growth of numerous cysts (fluid filled sacs) in the ovary. These cyst are not harmful but causes hormonal imbalance in polycystic ovarian syndrome patient (Sanyal, 2018). This hormonal imbalance is the major cause of infertility and irregular menstruation in PCOS patient. The ovary in every month releases a follicle which contains estrogens, progesterone and egg. In a normal female at ovulation this follicle releases the egg which then travels to the fallopian tubes for fertilization to occur. Whereas, in females with PCOS on the other hand their follicles keep growing without releasing any egg. There are two major types of functional cyst: follicular cyst and corpus luteum cyst. The follicular cyst is a type of cyst formed during the mid staged of the menstrual cycle, this type of cysts is formed as a result of the continuous growth of a follicle that failed to rupture and releases its egg. Corpus luteum cyst is a type of cysts formed when a follicle releases its egg and then starts accumulating fluid inside its self this then cause the corpus luteum to grow into a cyst. Other type of cyst include Endometriomas cyst: this type of cyst is developed when the endometrial lining of the uterus is formed outside the uterus, Dermoid cyst: this type of cyst is developed from the embryonic cells, another type of cyst is the Cyst adenomas cyst: this is the type of cyst formed from the surface of the ovary and might either be mucus or fluid filled (Mayo Clinic's 2020). Apart from the complications of infertility and irregular menstruation caused by cyst other complications include: Ovarian torsion which can cause decrease or stoppage of blood flow to the ovaries and the possibility of a very big cyst rupturing and causing severe pelvic pain and internal bleeding.

In the first stage of the experiment, of the five medicinal plants used only *Glycyrrhiza glabra* at 100mg/kg that was not able to control nor reduce the cyst formation as the number of cyst the animals treated with it had the highest number of cyst in their ovary. *Trigonella foenum graecum* on the other hand was the overall best plant in controlling cyst formation in the ovaries of the rats treated with them. In the second stage of the experiment, the 500mg/kg was the most effective dose in controlling the development of cyst even more effective than the standard control (clomiphene and metformin).

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