Histopathological effectiveness of vitex agnus castus in treating polycystic ovaries induced by letrozole in female rabbits

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Abstract

Female fertility is significantly impact by the common endocrine health issue known as polycystic ovarian syndrome (PCOS) in the childbearing years**,** Due to their ease of use and reduced cost-effectiveness when compared to chemical medicines generated via synthesis, therapeutic wild plants and herbs have been prized agents in the management of different illnesses for ages. Premenstrual syndrome, mastalgia, infertility, and menstrual cycle control are among the numerous conditions and diseases that can be treated with Vitex agnus-castus, a well-known medicinal plant that is native to the Mediterranean region and is widely used in Europe, Asia, and North Africa. The purpose of the study is to learn more about Vitex agnus catus and evaluate its efficacy in treating polycystic ovaries induced by letrozole in comparison to clomiphene citrate therapy. Since the effects of herbal activity are assessed by, observing histopathological changes in rabbit ovaries. The incidence of subcapsular ovarian cysts, capsular thickness, incomplete luteinization, and the quantity of corpora lutea were all higher in the research groups' ovaries. Compared to the group with a positive control (treated with normal saline only and administered orally). Histopathological study of the rabbit ovaries for both treatment groups revealed hyperplasia of the surface epithelium and congested blood vessels of the ovaries.

Keywords: Vitex agnus-castus, polycystic Ovaries, clomiphene citrate

Introduction

Owing to its most prevalent appearances of infertility and menstrual irregularity, polycystic ovarian syndrome (PCOS) has historically been thought of as a condition of the reproductive system [1,2, 3]. Vitex agnus-castus fruit is a commonly used phytotherapeutic for a variety of female reproductive disorders. Vitex agnus castus is a shrub that is indigenous to western Asia and the Mediterranean region [4]. It is also known as the "chaste tree," "chasteberry," or "Monk's Pepper" (common names). The plant has a long history of use for treating the symptoms of various ailments, including those

connected to PMS [5]. Premenstrual syndrome (PMS) and accompanying cyclic mastalgia, premenstrual dysphoric disorder (PMDD), current lactation issues, and decreased fertility are among the conditions that Vitex agnus-castus is used to treat. It is commonly used in Europe [5]. The berries, which are the part of the plant that is most frequently used, contain a number of potentially healthy components, including essential oils, iridoids, and flavonoids. Studies on humans, animals, and in vitro cells have been done to investigate the mechanism of action [6 , 7]. It has been demonstrated that it binds to dopamine receptors and functions as an agonist in vitro, activating the receptor. The substance has been observed to increase luteinizing hormone (LH) and decrease follicle-stimulating hormone (FSH) levels in humans, which increases progesterone and prolactin levels and decreases oestrogen levels [8 , 9]. It is hypothesised that Vitex agnus castus works by lowering prolactin, which reverses LH suppression, allowing the corpus luteum to fully develop, raising progesterone levels, and alleviating PMS symptoms [10]. Instead of using Vitex agnus castus extract as traditional herbalists would, the trials in this study will look at how it affects cystic ovaries caused by letrozole.

Materials and Methods

Experimental design: Rabbits weighing 3 kg initially hospitalised for 10 days to help them adapt to the same environmental conditions. The current experiment designed by using two groups of adult female rabbits, the first group included four female rabbits that received D.W. as a positive control, the second group included twelve female rabbits that received letrozole 1 mg/kg for 21 days [11, 12], and the third group included four female rabbits received aqueous vitex leaf extract for 15 days and four female rabbits where received clomiphene citrate 50 mg per kg [2].

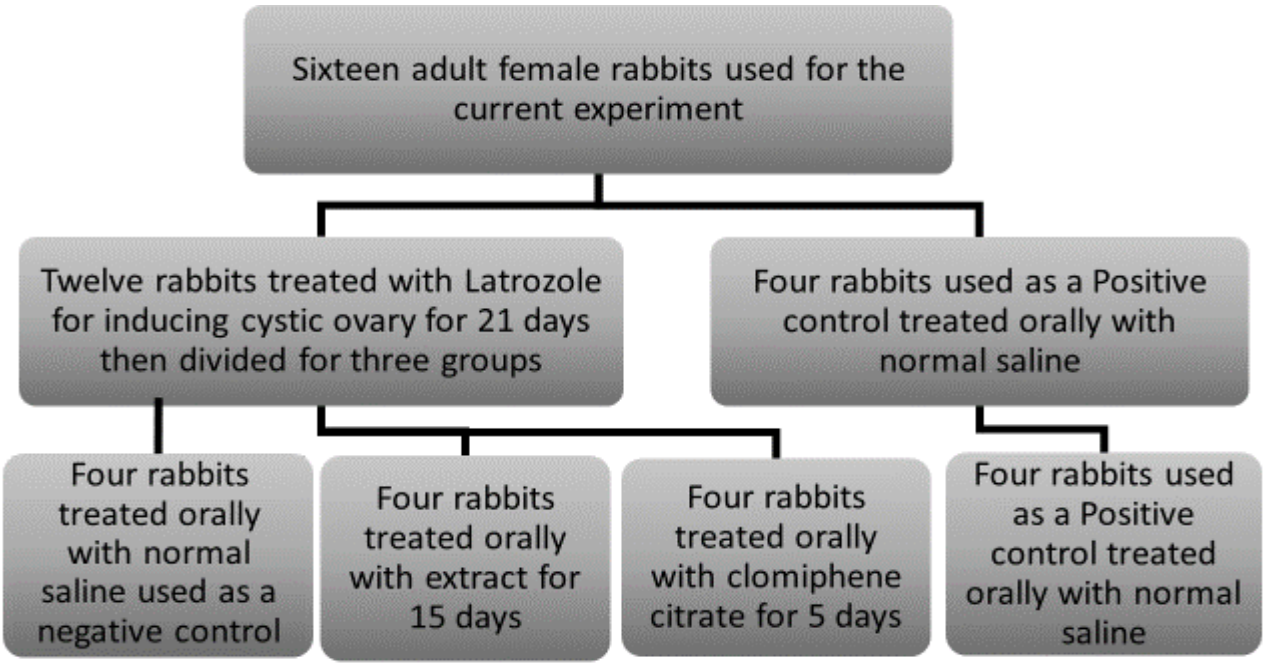

Figure (1): shows the experimental design of current study

Prepare the extract

After the vitex leaves were dried and weighed at 200 mg/kg using a sensitive scale, they were smashed in a mortar, 600 ml of distilled water was placed for soaking, the beaker was located on a stirring machine for 48 hours, the plankton (insolvable leaves parts) were removed by utilizing filter paper, the extract was placed in an oven at 45 ° C for evaporation and separation of the active substance for the whole week, and the harvest was ultimately obtained [2].

Histological samples collections and Examination

The specimens were processed and sectioned utilizing microtome at a diameter of 3-5 um after the animals were sacrificed. Samples were then fixed in neutral formalin solution 10% for 72 hours. Hematoxylin and eosin were used to stain tissue sections, while light microscopes and digital cameras used for recording histopathological image [13].

Result and Discussion

Histopathology analysis

The pathophysiology of PCOS is mostly unknown in terms of the processes affected. In addition, it is important to develop fresh treatments for PCOS patients that require early curative intervention. The outcomes demonstrated typical histological ovarian PCOS characteristics. Our findings were remarkable because they demonstrated for the first time that clomiphene citrate and vitex agnus castus could limit the pathological alterations that result in the occurrence of experimental PCOS induced by letrozole. The majority of an ovulatory women seeking infertility therapy have PCOS, which is the most frequent cause of this condition. In the last four decades, fertility treatment has increasing and ovulation-inducing drugs are widely used as independent therapies or during in vitro fertilization cycles [14 , 15]. Consequently, current study looked at the different histological variations caused by both agents deployed in treatment groups, as well as the effectiveness of vitex agnus castus extract agonist clomiphene citrate therapy in compare with the control group (letrozole-induced PCOS) which agree with a previous study [11], when groups were compared based on ovarian histopathology, it was observed that the control group's rabbit expressed normal simple cuboidal epithelium characteristics without any pathological findings on the ovarian surface epithelium, as well as smaller ovarian diameters, thickness of the ovarian surface epithelium, primordial and preantral follicle counts, and corpus luteum counts in the ovarian cross sections (Figures 2 , 3). These groups' ovaries exhibited corpora lutea clarity and normal follicular growth distribution with incidental cystic follicles. The freshly created corpora lutea were composed of massive, polygonal, and finely vacuolated luteal cells and they were situated superficially in the ovary. All the ovaries in the letrozole-induced PCOS groups receiving no treatment were acyclic and featured numerous large cystic follicles lined by one to various layers (1–4 cellular sheets) of cuboidal or flattened granulosa cells lying on a thin wall and surrounded by theca cells. Moreover, ovulation detention was evident in the absence of freshly produced corpora

lutea in the ovaries, which showed a much higher number of corpora lutea and a considerably lower number of cystic follicles in compared to control group (figure 2). The ovarian surface epithelium of the rabbits in both groups of treatment (extract and clomiphene citrate) was shown to have hyperplasia, and their blood vessels were congested (figures 5, 6). Incredibly many benefits, including its high effectiveness in inducing ovulation, relative safety, economy, and convenience of oral administration, clomiphene citrate is one of the most often used ovulation inducing medications in the management of infertility [16]. According to the results of our histological examinations, the severity of the hyperplasia on the ovarian surface epithelium in the clomiphene citrate treated groups increased according to the dose of the drug that was administered which disagrees with some researchers [17]. Antral follicle counts, corpus luteum thickness, and corpus luteum counts in the clomiphene citrate group were lower than those in the control group (negative control) which supporting our findings, this could be explained by the possibility that large doses of clomiphene citrate cause ovarian tissue to undergo apoptotic changes[18]. Although the potential mechanism by which clomiphene citrate exerts its antiestrogenic action at the level of the ovary is poorly known, the mechanism responsible for these apoptotic effects may be caused by clomiphene citrate's antiestrogenic activities [18]. In contrast, researchers proposed that after getting clomiphene citrate medication, oocytes would not develop and mature properly due to low levels of estradiol in the ovary, which would then cause apoptosis 19], and they suggested clomiphene citrate in high dosages could have a toxic effect on growth and development of follicles and luteal cell populations.

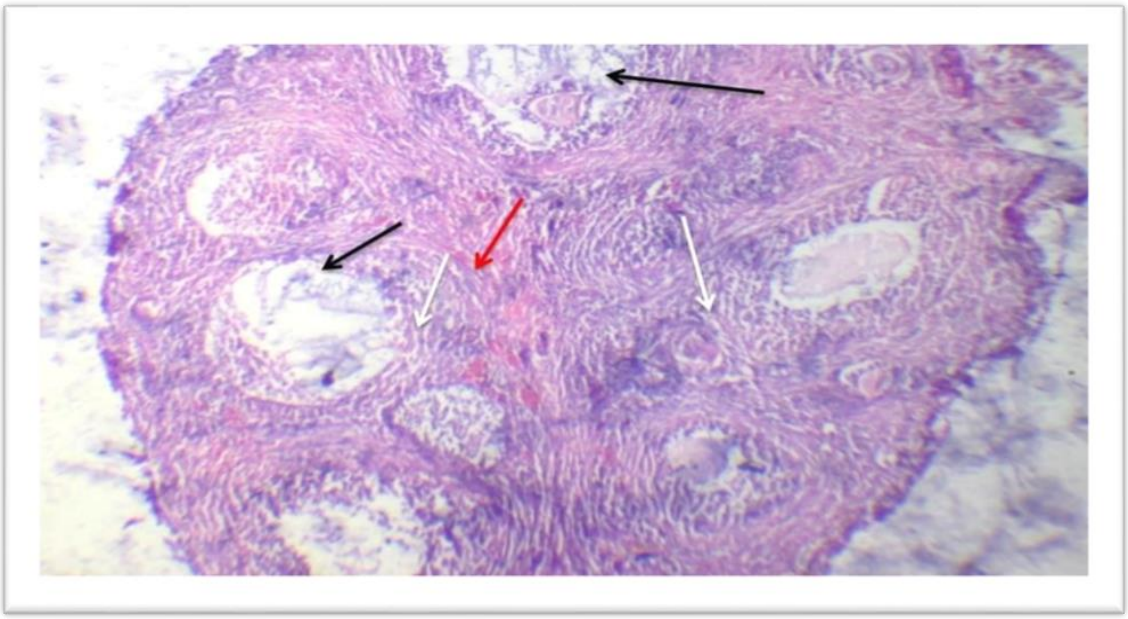

Figure (2): Photomicrograph of an ovarian section of a negative control animal showing follicles in different stages of maturation, mainly enlarged graffian follicles (black arrow), normal stroma cells histology (white arrow) and slight con gestion in medulla (red arrow). (H and E, 4X).

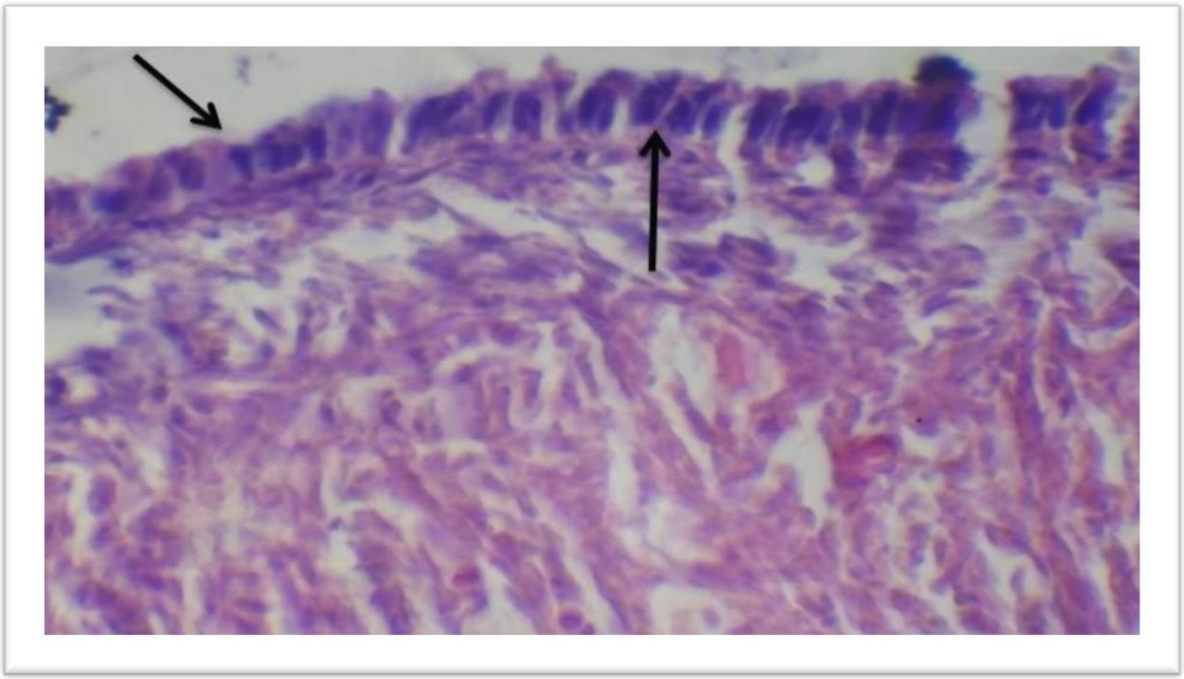

Figure (3):Photomicrograph of an ovarian section of letrozole treated group animal showed hyperplasia of ovarian surface epithelium (thickness) (black arrow) . (H and E, 40X).

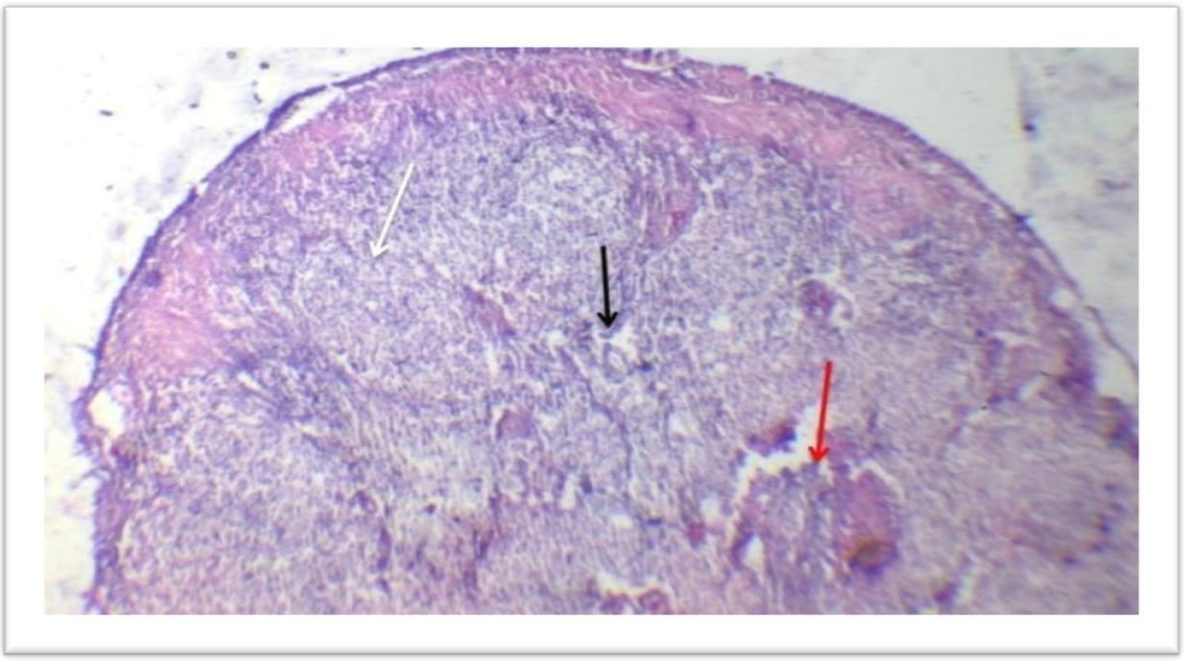

Figure (4): Photomicrograph of an ovarian section of clomiphene citrate treated group animal revealed degenerated follicles (black arrow), corpus luteum (white arrow) and congested blood vessles (red arrow). (H and E, 4X) .

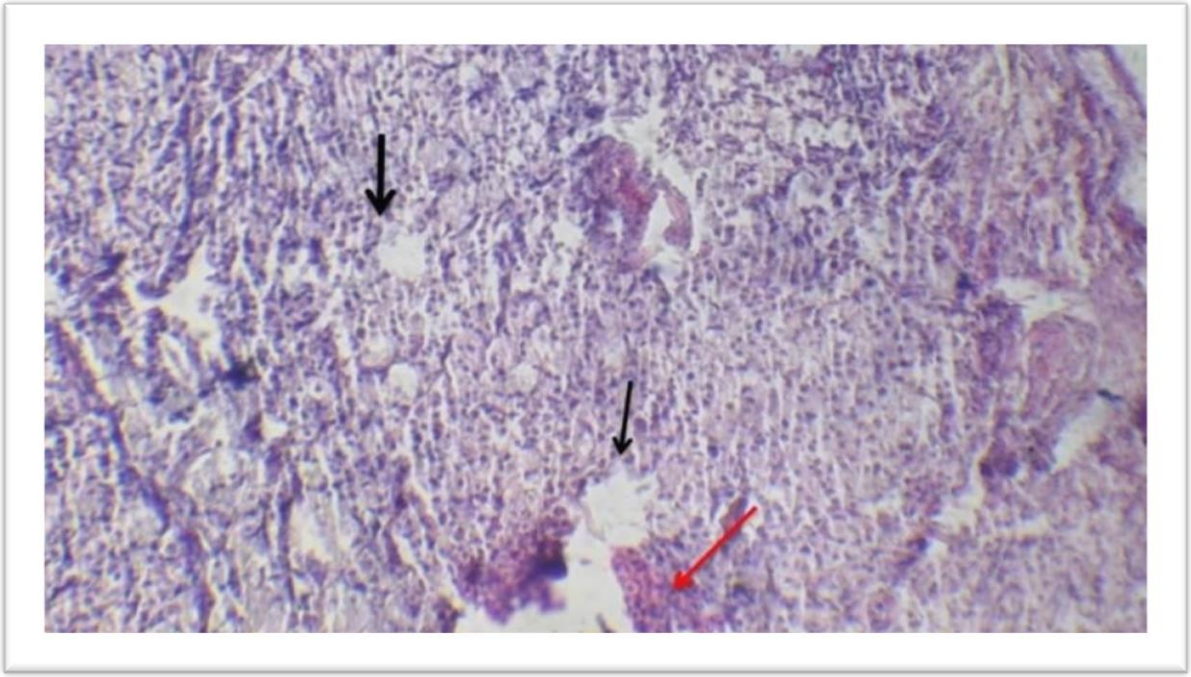

Figure (5):Photomicrograph of an ovarian section of clomiphene citrate treated group animal revealed atretic follicles , number of vacuoles (black arrow), and congested blood vessels (red arrow). (H and E, 10X).

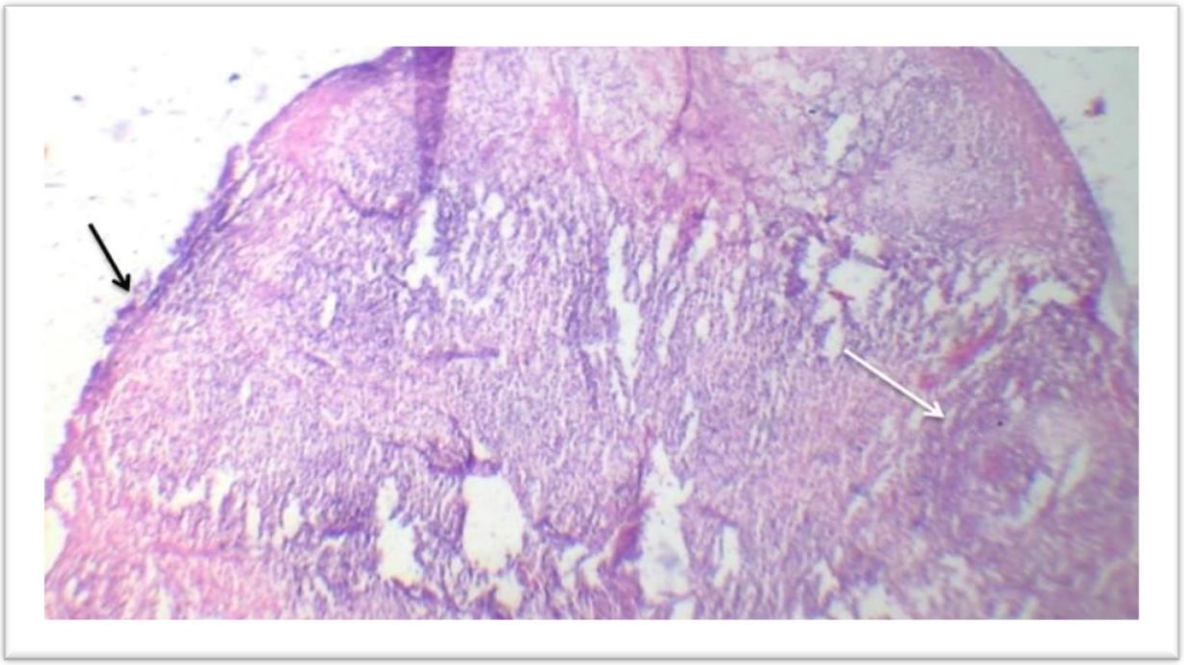

Figure (6) :Photomicrograph of an ovarian section of clomiphene citrate treated group animal showed hyperplasia of ovarian surface epithelium (thickness) (black arrow) . (H and E, 40X).

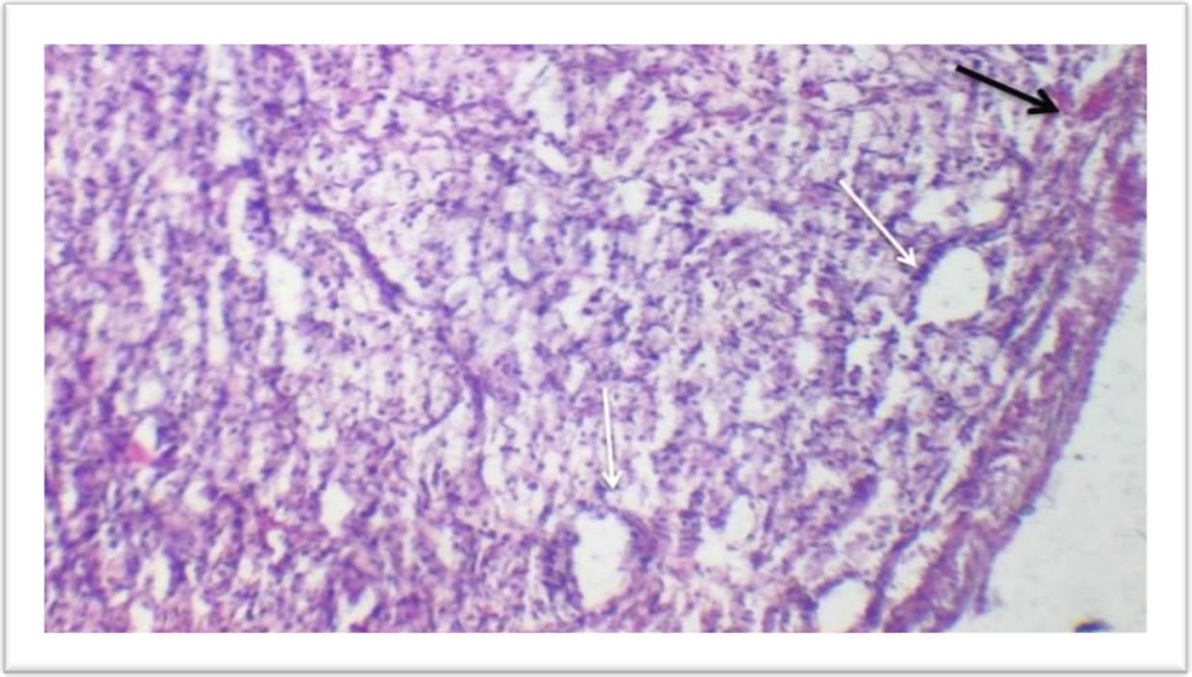

Figure (7): Photomicrograph of an ovarian section of Vitex extract treated group animal revealed some atretic follicles (white arrow) and congestion of ovarian blood vessles (black arrow). (H and E, 10X).

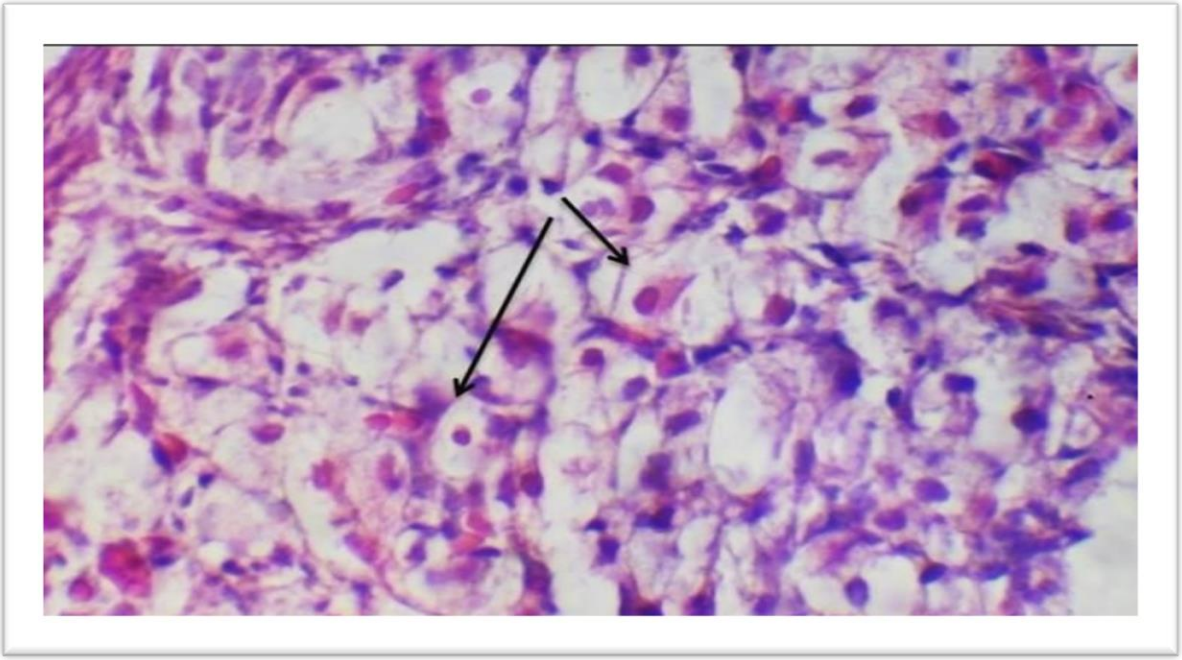

Figure (8):the ovarian section of an animal treated with Vitex extract also shown on a photomicrograph to have extensive stromal cell vacuolation (black arrow). 40X, H, and E.

Vitex might induce a physiologic imbalance in the ovary-pituitaryhypothalamic axis, thus possibly cause a change in the follicular morphology and physiology [20] which through controlling kisspeptin expression would demonstrate positive results in the treatment of PCOS. Studies suggest that vitex extract may reduce the symptoms of PCOS by altering the lipid and hormonal patterns as well as oxidative stress. Moreover, these substances' beneficial effects are equivalent to control [21]. Vitex agnus-castus aqueous extracts at a concentration of (50 mg/kg) had an antioxidant effect and significantly contributed to ovarian repairs with extensive stromal cell vacuolation and atretic follicles (figures 7,8). Vitex in human clinical trials, toxicity, and safety, as well as marketed products and genus-specific patents. While the genus' extracts have been incorporated into numerous commercial items, including as supplements and essential oils, the majority of them are designed for use by women to relieve premenstrual syndrome and enhance menstruation conditions. Interestingly, vitex agnus-castus L. has been known to undergo clinical studies among the species, with the majority of these trials focusing on the use of the genus for the treatment of mastalgia, menstrual bleeding issues, amenorrhea, menorrhagia, luteal insufficiency, and premenstrual syndrome [22]. The article describes current therapy advances and suggests areas for further research that need to be investigated for additional studies.

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