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Histological and Hormonal Alterations in the Ovaries of Rats Treated with Estradiol and Corn Oil

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Abstract

Increasing estrogen levels beyond the normal limit can be an indicator of a serious health problem which can be the cause of fibrosis or tumor development in the ovary tissue. In the current study, we seek to understand the mechanism of estrogen and whether the exposure of estradiol and phytoestrogens together increases the risk of developing ovarian cancer. Here, we used 32 healthy adult female rats. They were randomly divided into four groups. The first group (the control group) and the remaining groups were dosed orally with estradiol (30 micrograms/kg) and corn oil (0.5 ml) for six weeks. Our study showed a significant increase in the progesterone, estrogen, and their receptors levels within the treated groups. Further, the histological examinations indicate an increase in ovarian follicles at different stages of development and numerous mature Graafian follicles. We noticed that there were more corpora lutea and the granulosa cell layer was thicker in the treated groups compared to the control group.Taken together, our data suggested that phytoestrogens have a mechanism of action that mimics the body's internal (endogenous) estrogens, especially in the growth and development of ovary tissue. Therefore, adopting a diet rich in plant estrogens for a long period can cause an increase in the level of internal estrogens and disrupt their function.

Keywords: Ovary, estradiol, and corn oil.

I. Introduction

Estrogen and its receptors, including ERα, ERβ, and GPER, also play a role in ovarian diseases such as polycystic ovary disease (PCOS), ovarian cancer, and premature ovarian failure (POF). Ovarian diseases like endometriosis, adenomyosis, leiomyoma, polycystic ovary syndrome (PCOS), and ovarian cancer are intricately linked to progesterone signaling mechanisms and hormone imbalances, impacting women's health significantly (MacLean and Hayashi, 2022 ; Xu *et al*.,2022).

The association between diet and health has been a vital subject of the scientific inquiry for a long time. In particular, the impact of phytoestrogens, plant-derived compounds with estrogen-like properties, on human physiology. Among these phytoestrogens, corn oil was used to prepare extracts for assessing estrogen activity, with some supplements inducing estrogenic effects in bioassays (Furr and Kennedy, 2020;Li *et al*., 2023).

The relationship between phytoestrogens and estradiol involves shared structural similarities and interactions with estrogen receptors, influencing various health aspects (Molina *et al*., 2018).

These phytoestrogens in corn oil have been associated with various health benefits, including anti-inflammatory, antiatherogenic, and potential hormone-mimicking effects. Studies have shown that consuming corn oil can positively influence lipid profiles, especially in menopausal and postmenopausal women, due to its phytoestrogen content. Therefore, corn oil can be considered a source of phytoestrogens with potential therapeutic implications for conditions related to hormonal balance and menopausal health (Takenaka *et al*.,2020; Pourjafari *et al*.,2023).

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II. Materials and Methods

Animal Husbandry: 32 female rats aged (3 - 4) months with an average weight of 200 grams were used. They were housed in plastic cage in the animal facility at the College of Education Pure Sciences, Thi-Qar University, under standard conditions of temperature (22 ± 25°C) and lighting (12:12-hour light-dark cycle) during the study period. Throughout the study, the rats were provided with ad libitum access to feed and tap water. The 32 rats were then randomly assigned into four groups:

1. Group A (Negative Control) received 0.5 mL of distilled water for a period of 28 days.
2. Group B2 received 0.2 ml of corn oil orally over a period of six weeks (Almudhaffer and Ziedan, 2024).
3. Group D (Positive control) received 30 µg/kg of estradiol over a twelve-week period (Ke et al., 1997).
4. Group F2 received a combined treatment of estradiol and corn oil in proportions analogous to those administered to the previous groups.

Biochemical analysis: Rat estrogen receptor alpha, estrogen and progesterone hormone levels were determined using the previously described method (Al-Hamdany *et al*., 2019).

Histopathological study: Ovaries were sectioned transversely and entirely submitted in a labeled histology cassette. Each specimen was cut into a thickness of 5 mm, immediately fixed in 10% formalin solution for 48 hours, processed through water wash, a graded ethanol series, and then embedded in paraffin wax at 70°C. The paraffin-embedded blocks were sectioned to prepare slides and stained with hematoxylin and eosin. All sections were examined for histopathological changes under a light microscope (Sumaya and Saleh,2023).

Statistical analysis: The study findings underwent evaluation utilizing one-way analysis of variance (ANOVA) test. Statistical computations were performed using SPSS V. 21 (SPSS Inc.). Data presentation included mean values ± standard deviation.

III. Results

Estrogen Hormone Assessment in all Treated Groups

The current study demonstrated a significant increase ($P \leq 0.01$) in the estrogen hormone concentration in female laboratory rats in the groups (positive control group D, B2 and F2 groups) compared to the negative control group A, at the specified probability level. Figure (1)

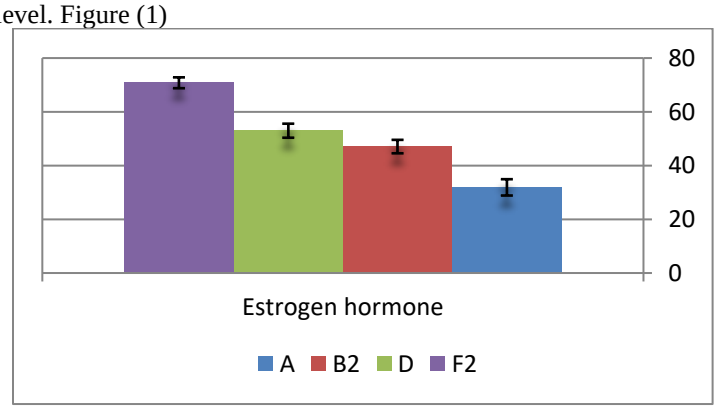

Figure 1: It shows an increase in estrogen hormone in the estradiol group, the corn oil group, and the corn oil and estradiol groups together compared to the negative control group

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Estrogen receptor alpha Assessment in all Treated Groups

The current study demonstrated a significant increase (P≤0.01) in the estrogen receptor concentration in female laboratory rats in the groups (positive control group D, B2 and F2 groups) compared to the negative control group A, at the specified probability level .Figure (3)

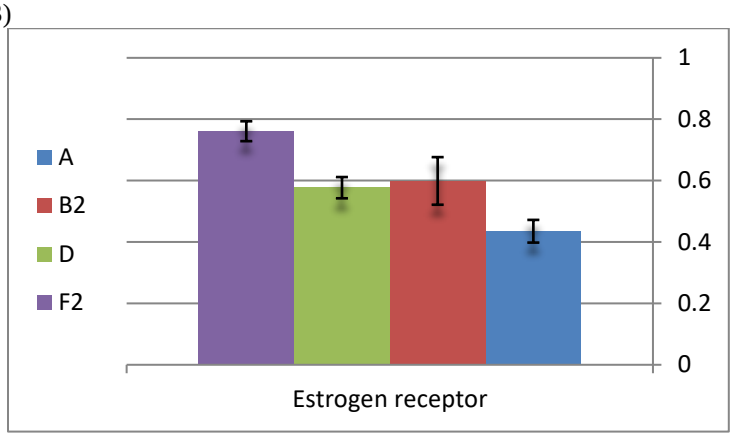

Figure 2: It shows an increase in estrogen receptor in the estradiol group, the corn oil group, and the corn oil and estradiol groups together compared to the negative control group

Progesterone Hormone Assessment in all Treated Groups

The current study demonstrated a significant increase (P≤0.01) in progesterone hormone concentration in female laboratory rats in the positive control groups D and B2, compared to the negative control group A, as well as a significant decrease in group F2 compared to the positive control groups D and B2 at the specified probability level. Figure (3)

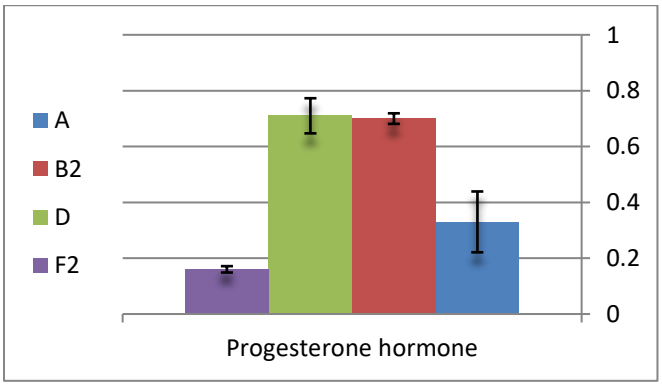

Figure 3 It shows an increase in progesterone receptor in the estradiol group and the corn oil group, as well as a significant decrease in the corn oil and estradiol groups together compared to the negative control group

Haematoxyln-eosin stained sections The examination of the ovaries in the negative control group showed a normal structure, with an outer layer called the cortex and an inner part called the medulla, containing follicles at various stages of growth and development Figure 4. Microscopic examination of ovarian sections in the treated groups and the positive control group showed an increase in ovarian follicles at different stages of development and numerous mature Graafian follicles Figure 5-7.

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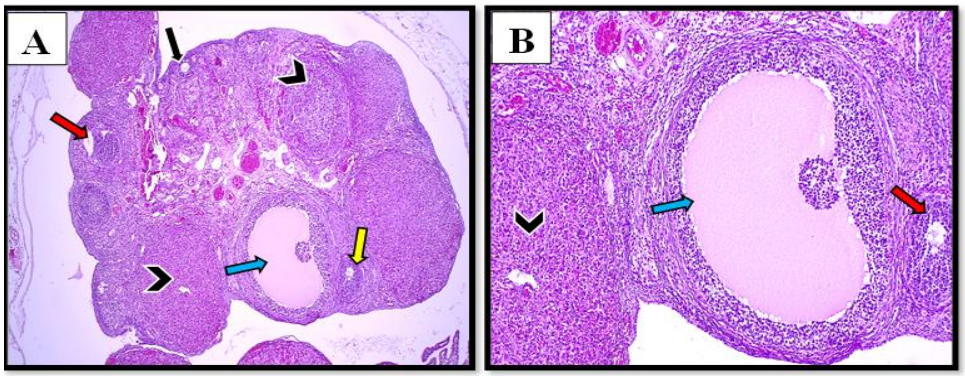

Figure 4: Photomicrograph of the ovary of control group rat.

A& B/ Normal histological architectures of ovary. Note the ovarian follicles at different development stages, including primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), graafian follicle (blue arrow) and corpus luteum (arrowhead). However, about 5 ovarian follicles were observed in the ovarian tissue **H&E, A: 100x and B: 400x**

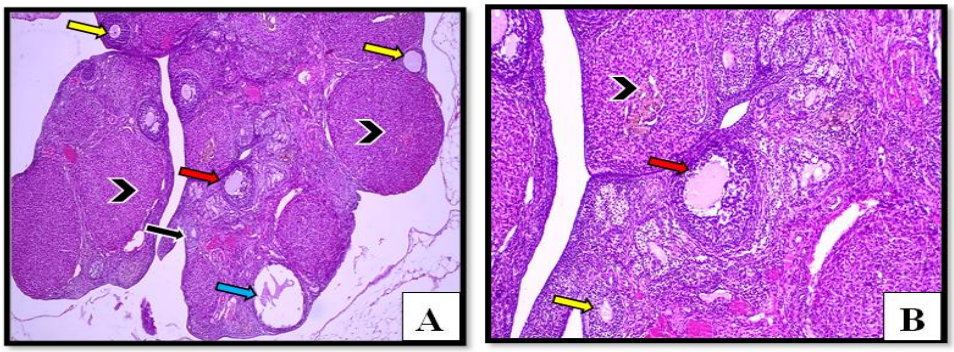

Figure 5: Photomicrograph of the ovary of estradiol treated rat.

A& B/ Increasing in the numbers of ovarian follicles at different stages of maturation compared with control group, where more than 15 ovarian follicles were observed in the ovarian tissue. Note the primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), ovulated graafian follicle (blue arrow) and corpus luteum (arrowhead). **H&E, A: 100x and B: 400x**

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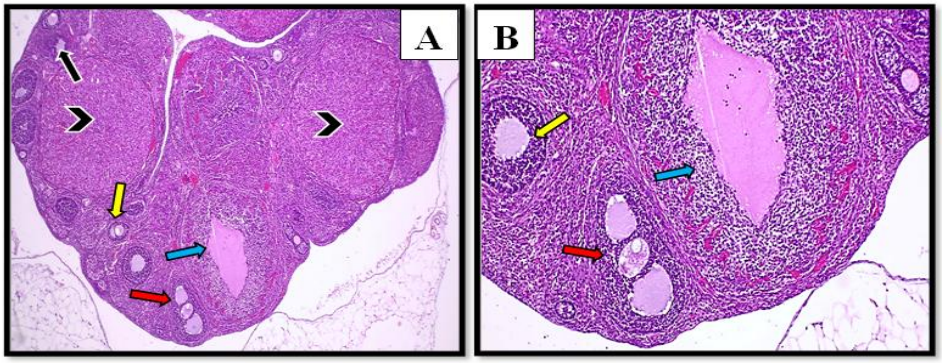

Figure 6: Photomicrograph of the ovary of corn oil treated rat.

A& B/ Increasing in the numbers of ovarian follicles at different stages of maturation compared with control group, where more than 16 ovarian follicles were observed in the ovarian tissue. Note the primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), ovulated graafian follicle transversed to corpus luteum (blue arrow) and corpus luteum (arrowhead). **H&E, A: 100x and B: 400x**

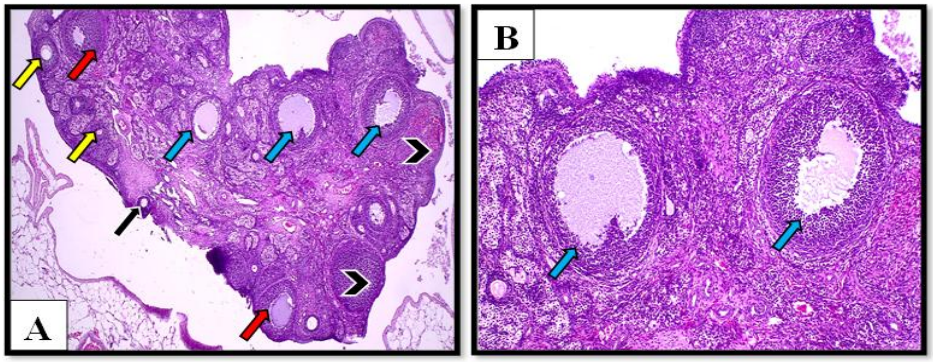

Figure 7: Photomicrograph of the ovary of corn oil and estradiol treated rat.

A& B/ Increasing in the numbers of ovarian follicles at different stages of maturation compared with control group or corn oil only treated group, where more than 20 ovarian follicles were observed in the ovarian tissue. Also, many graafian follicles were observed in the affected tissue. However, decreasing in corpus luteum numbers were observed. Note the primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), graafian follicle (blue arrow) and corpus luteum (arrowhead). **H&E, A: 100x and B: 400x**

IV. Discussion

The increase in ovarian follicles and mature Graafian follicles in the treatment groups may be attributed to the stimulatory effects of estradiol on folliculogenesis. Estradiol, a potent estrogen, is known to promote the growth and maturation of ovarian follicles by stimulating granulosa cell proliferation and differentiation (Chauvin *et al*., 2022). This is consistent with findings from earlier studies that have reported enhanced follicular development in response to exogenous estradiol administration (Chauvin *et al*., 2022). Moreover, the presence of corn oil, a rich in omega-6 fatty acid, may further

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modulate ovarian function. Omega-6 fatty acids have been shown to possess anti-inflammatory properties and may enhance ovarian activity by improving insulin sensitivity and reducing oxidative stress (Komal *et al*., 2020). The combination of corn oil with estradiol may create a synergistic effect, promoting follicular development and oocyte maturation.

The increased thickness of the granulosa cell layers observed in the treatment groups suggests enhanced follicular activity and elevated estrogen production. Granulosa cells are critical for the synthesis of estradiol, and their proliferation is stimulated by FSH and estradiol itself (Huang *et al*., 2022). Conversely, the observed decrease in the thickness of the theca cell layers may indicate a shift in the functional dynamics between these cellular compartments, possibly due to increased granulosa cell activity and redistribution of cellular resources within the follicles (Li *et al*., 2020).

Estradiol, a potent form of estrogen, is integral to various physiological processes, including the regulation of the menstrual cycle, maintenance of pregnancy, and modulation of secondary sexual characteristics. The administration of exogenous estradiol has been shown to elevate serum estrogen levels significantly. The introduction of estradiol in postmenopausal women led to increased serum estrogen concentrations, which subsequently activated estrogen receptors (ERs) and influenced gene expression related to reproductive health. This is consistent with the findings of other studies that indicate that exogenous estradiol can upregulate both ERα and ERβ, enhancing the overall estrogenic response in target tissues (Park *et al*., 2017).

In addition to synthetic estradiol, phytoestrogens—plant-derived compounds that mimic estrogen—have also been implicated in modulating hormone levels and receptor activity. Corn oil, rich in phytoestrogens such as coumestrol and isoflavones, has been shown to exert estrogenic effects in various animal models. A study by Chen et al. (2023) demonstrated that dietary supplementation with corn oil led to increased serum estrogen levels in female rats, suggesting that the phytoestrogens present in corn oil can stimulate the endocrine system similarly to estradiol. This finding is further supported by research indicating that phytoestrogens can bind to estrogen receptors, activating signaling pathways that enhance estrogen production and receptor expression (Chen *et al*., 2023).

The mechanisms underlying the increased levels of estrogen and progesterone in response to estradiol and phytoestrogens are multifaceted. One potential pathway involves the regulation of aromatase, the enzyme responsible for converting androgens to estrogens. Estradiol has been shown to upregulate aromatase expression in various tissues, including adipose tissue and the ovaries. This upregulation not only increases local estrogen production but may also impact systemic hormone levels. Furthermore, phytoestrogens have been reported to influence aromatase activity, although the effects can vary depending on the specific compound and concentration used (Van Duursen , 2017).

Additionally, the interplay between estradiol and progesterone is crucial for maintaining reproductive health. Estradiol is known to stimulate the production of progesterone, particularly in the luteal phase of the menstrual cycle. Studies have shown that increased levels of estradiol can lead to a subsequent rise in progesterone secretion from the corpus luteum (Kutlusoy et al., 2014). This relationship underscores the importance of estradiol not only in promoting estrogenic effects but also in facilitating the synthesis and secretion of progesterone.

The implications of these findings extend to various health outcomes, including fertility, menopausal symptoms, and the risk of hormone-related cancers. For instance, the use of estradiol in hormone replacement therapy has been associated with improved reproductive health outcomes in postmenopausal women, yet it also raises concerns regarding the potential for increased breast cancer risk (Criste et al., 2020). Similarly, the consumption of phytoestrogens, while generally regarded as safe, necessitates further investigation into their long-term effects on hormone levels and receptor dynamics, particularly in populations with varying predispositions to hormone-sensitive conditions.

V. Conclusion

The current study concluded that consuming high levels of phytoestrogens, such as corn oil, without adhering to a balanced diet may lead to increased levels of progesterone, estrogen and their receptors in the blood, which act on a similar mechanism to estrogen, which is considered a risk factor for ovary diseases.

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