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RESEARCH ARTICLE

Histopathological and Epigenetic Alterations in Rat Ovaries Induced by Tamoxifen and Phytoestrogens: Implications for Tumorigenic Risk

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Abstract: ***Background:*** Estrogenic compounds from plant sources have raised growing concern regarding their potential involvement in hormone-dependent tumorigenesis. Elevated estrogen levels are associated with pathological changes, including tumor formation and fibrosis, particularly in estrogen-sensitive tissues such as the ovaries. ***Materials and Methods:*** This experimental study investigated histopathological and epigenetic alterations in ovarian tissue induced by tamoxifen and phytoestrogens under elevated estrogen conditions. Thirty healthy adult female rats were randomly divided into five groups. The control group received no treatment, while the treated groups were administered flaxseed oil (0.5 ml), corn oil (0.2 ml), or their combinations with tamoxifen (1 mg/kg) orally for six weeks. Ovarian tissues were examined histologically and analyzed for DNA methylation in the estrogen receptor alpha (ERα) promoter region. ***Results:*** Exposure to tamoxifen and phytoestrogens caused distinct histopathological and epigenetic alterations. A significant increase ($P \leq 0.01$) in granulosa cell layer thickness was observed in all treated groups, while changes in theca cell layer thickness were not significant. DNA methylation of the ERα promoter region was markedly reduced compared to controls, indicating altered epigenetic regulation. ***Conclusion:*** Phytoestrogens may modulate tamoxifen activity by competing for estrogen receptor binding, leading to structural and epigenetic alterations that could contribute to tumorigenic processes in ovarian tissue. Continuous monitoring of estrogenic compound exposure is essential to assess their potential impact on reproductive health and cancer risk.

Keywords: Tamoxifen; Phytoestrogens; Ovarian histopathology; Estrogen receptor alpha (ERα) methylation ; Tumorigenesis.

INTRODUCTION

Dietary components play a crucial role in modulating cancer risk and progression. Increasing attention has been directed toward phytoestrogens—plant-derived compounds with estrogenic activity—due to their potential impact on hormone-dependent malignancies. Corn oil has been used in the preparation of extracts to assess estrogenic activity, with certain supplements demonstrating measurable estrogenic effects in bioassays [1].

A growing body of evidence highlights the link between phytoestrogens and epigenetic regulation, particularly DNA methylation, suggesting that these compounds may influence the expression of genes involved in estrogen metabolism, inflammation, and cell cycle control through epigenetic mechanisms. Lignans, in particular, have been associated with alterations in DNA methylation patterns, potentially modulating cancer-related pathways. Moreover, the consumption of flaxseed and corn oil has been correlated with improved hormonal balance, a reduced risk of hormone-dependent cancers, and enhanced cardiovascular health—effects that may be partly mediated through epigenetic modulation [2,3].

Tamoxifen, a selective estrogen receptor modulator (SERM), represents a cornerstone in the prevention and treatment of breast cancer due to its dual agonist–antagonist activity on estrogen receptors. It inhibits estrogen-driven proliferation in breast tissue while mimicking estrogenic effects in other tissues, thereby reducing recurrence risk and offering additional therapeutic benefits, including the treatment of infertility in anovulatory women and the prevention of osteoporosis [4].

Understanding the interplay between dietary phytoestrogens, epigenetic modifications, and endocrine modulators such as tamoxifen is essential for elucidating their roles in the development and progression of hormone-related cancers.

This study aimed to investigate the histopathological and epigenetic alterations in rat ovarian tissue induced by tamoxifen and phytoestrogens (flaxseed and corn oil), and to explore their potential implications for tumorigenic risk.

MATERIAL AND METHODS

Study Design

Animal Husbandry

This study was conducted in accordance with ethical guidelines and approved by the Thi-Qar Ethical Committee for Animal Research (Issue 7/54/338, Date: 8/3/2024; eleven members in the committee). Thirty healthy adult female rats aged 3–4 months, with an average weight of 200 g, were used. The animals were

housed in plastic cages in the animal facility of the College of Education for Pure Sciences, Thi-Qar University, under standard laboratory conditions: temperature (22 ± 25°C) and a 12:12-hour light–dark cycle. The rats were acclimatized for two weeks before the start of the experiment and maintained under these conditions throughout the study period. Feed and tap water were provided ad libitum.

Thirty rats were randomly divided into five groups, with some groups further subdivided as follows:

- **Group I (Negative Control):** Received no treatment [5].
- **Group II (Flaxseed Oil):** Received 0.5 ml of flaxseed oil orally for six weeks [6].
- **Group III (Corn Oil):** Received 0.2 ml of corn oil orally for six weeks [6].
- **Group IV (Flax + TAM):** Received 0.5 ml of flaxseed oil orally for six weeks, followed by tamoxifen treatment (1 mg/kg) for an additional six weeks [7].
- **Group V (Corn + TAM):** Received 0.2 ml of corn oil orally for six weeks, followed by tamoxifen treatment (1 mg/kg) for an additional six weeks [8].

Histopathological Study

The ovary tissues were transversely sectioned and placed in labeled histology cassettes. Each specimen was trimmed to a thickness of approximately 5 mm and immediately fixed in 10% neutral buffered formalin for 48 hours. Samples were then processed through a standard histological procedure involving water washing, dehydration through a graded ethanol series, and embedding in paraffin wax at 70°C.

Paraffin-embedded blocks were sectioned, mounted on glass slides, and stained with hematoxylin and eosin (H&E). The prepared slides were examined microscopically for histopathological alterations under a light microscope [9].

DNA Methylation Analysis

The methylation status of the **estrogen receptor alpha (ERα)** gene was determined following the previously described method [10]. In summary, tissue samples were immediately preserved in DNA stabilizing agents after collection. DNA was extracted using a phenol–chloroform or silica column-based extraction kit. Extracted DNA was then treated with sodium bisulfite (HSO_3^-), which converts unmethylated cytosines (C) to uracil (U), while methylated cytosines remain unchanged.

This was followed by PCR amplification of the **ESR1 promoter** region. Post-bisulfite treatment, sequence differences reflecting methylation status were amplified and analyzed. The methylation profile was assessed using gel electrophoresis, where bands corresponding to methylated and/or unmethylated alleles were visualized.

Statistical Analysis

The obtained data were analyzed using **one-way analysis of variance (ANOVA)**. Statistical computations were performed with **SPSS software version 21** (SPSS Inc., Chicago, IL, USA). Data are presented as **mean ± standard deviation (SD)**, and differences were considered statistically significant at **$P \leq 0.01$**.

RESULTS AND OBSERVATIONS:

Study of epigenetic regulation via dna methylation mechanisms

The current study demonstrated a significant decrease ($P \leq 0.01$) in total region (T) methylation in the experimental groups compared to the negative control group (NC-) at the specified probability level (Figure 1and Table 1).

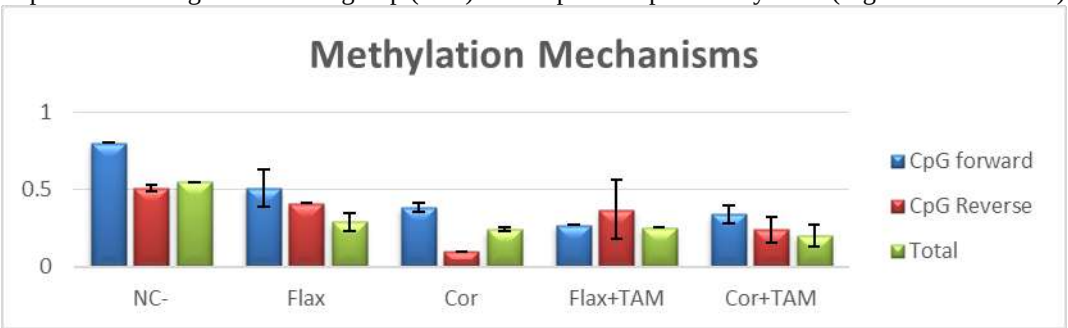

Figure (1): Percentage of ERα promoter methylation in experimental groups for forward (F), reverse (R), and total (T) DNA strands , the data indicates a significant decrease in methylation in the flaxseed oil group, corn oil group, and tamoxifen groups when **compared to the negative control group**

Table (1): CpG island methylation status of estrogen receptors

```
CCTCGGGAATGGCCTTGTTGCACCAGATCCAAGGGAACGAGCTGGAGCCCCTCAATCGCC
::|++|||||||::|||||||:|::|||||::|||||||++||:|||||::::|:|||++::
TTTCGGGAATGGTTTTGTTGTATTAGATTTAAGGGAACGAGTTGGAGTTTTTTAATCGTT
                    >>>>>>>>>>>>>>>>>>>>>>>>>

CGCAGCTCAAGATGCCCATGGAGAGGGCTCTGGGCGAGGTGTACGTGGACAACAGCAAGC
++:|||:|:|||||||:::|||||||||||:|:||||++|||||||++||||:|||:|||:
CGTAGTTTAAGATGTTTATGGAGAGGGTTTTGGGCGAGGTGTACGTGGATAATAGTAAGT


CCGCCGTGTTCAACTACCCCGAGGGCGCCGCCTACGAGTTCAACGCCGCCGCCGCCGCCG
:++:++||||:||:||:::++||||++:++::||++|||||:||++:++:++:++:++:++
TCGTCGTGTTTAATTATTTCGAGGGCGTCGTTTACGAGTTTAACGTCGTCGTCGTCGTCG


CGGCCGCCGCCGCCTCGGCTCCGGTCTATGGCCAGTCGAGCATCACCTACGGTCCCGGGT
++|:++:++:++::|++|:|:++||:||||||::|||++||:|||:|::||++||::++|||
CGGTCGTCGTCGTTTCGGTTTCGGTTTATGGTTAGTCGAGTATTATTTACGGTTTCGGGT


CCGAGGCGGCCGCCTTTGGTGCCAATAGTCTGGGGGCTTTCCCCCAGCTCAACAGCGTGT
:++|||++|:++::|||||||::|||||||:|||||||:|||:::::::||:|:||:||++|||
TCGAGGCGGTCGTTTTTGGTGTTAATAGTTTGGGGGTTTTTTTTTTAGTTTAATAGCGTGT
                    <<<<<<<<<<<<<<<<<<<<<<<<<
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Upper row: Original sequence
Lower row: Bisulfite modified sequence
assume all CpG sites are methylated
++ CpG sites
:::: Non-CpG 'C' converted to 'T'
CpGs in product: 29

Measurement of ovarian follicular cell thickness

The results presented in the figures demonstrated a significant increase ($P \leq 0.01$) in the thickness of the granulosa cell layer in the experimental groups compared to the negative control group (NC-) at the specified probability level. The findings also revealed a decrease in the thickness of the theca cell layer in the experimental groups (Figures 2,3); however, this decrease was not statistically significant when compared to the negative control group.

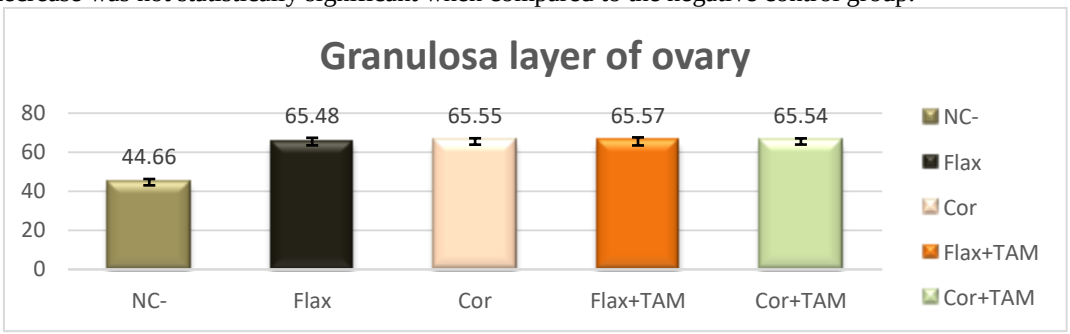

Figure (2): Measurement of the thickness of the granular layer in all experimental groups of rats ,the data indicates a significant increase in thickness of the granular layer in the positive control group, flaxseed oil groups, corn oil groups, and tamoxifen groups when **compared to the negative control group**

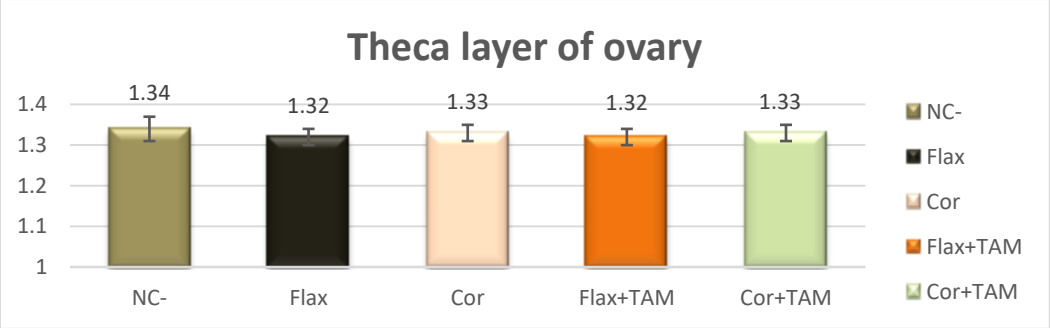

Figure (3): Measurement of the thickness of the theca layer in all experimental groups of rats ,the data indicates a significant increase in thickness of the theca layer in the positive control group, flaxseed oil groups, corn oil groups, and tamoxifen groups when **compared to the negative control group**

The histological examination of the ovaries in the negative control group (NC–) revealed a normal architecture, characterized by a well-defined outer cortex and an inner medulla containing follicles at different stages of growth and development, as well as a corpus luteum (Figure 4).

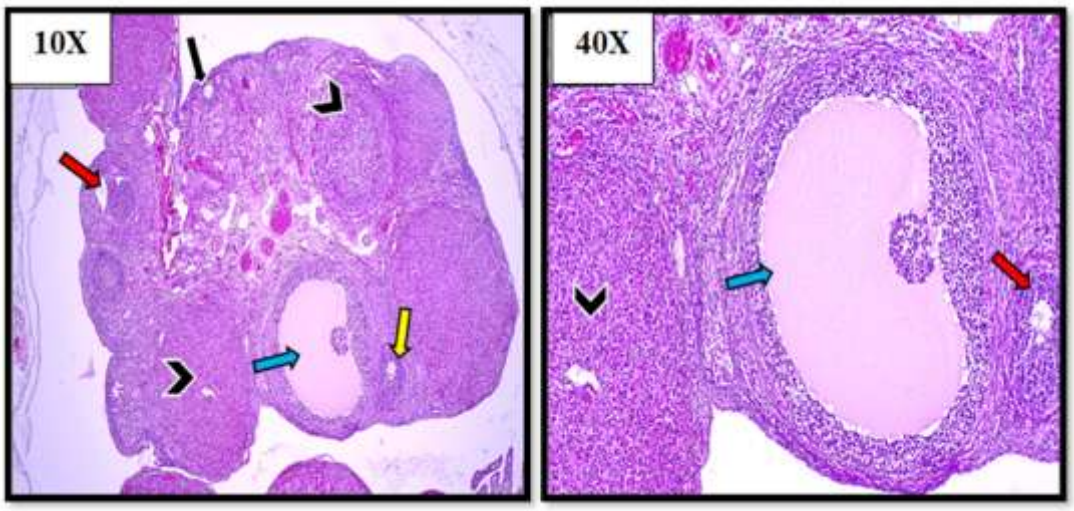

Figure (4): A cross-section view of the ovary of a negative control rat. Normal histological architecture of the ovary was observed. Ovarian follicles at different developmental stages, including primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), Graafian follicle (blue arrow), and corpus luteum (arrowhead), were noted. Approximately five ovarian follicles were observed within the ovarian tissue. **H&E staining. (10X , 40X)**.

Microscopic examination of ovarian sections from the positive control group (PC+) revealed a marked proliferation of ovarian follicles at distinct stages of maturation, accompanied by the presence of multiple mature Graafian follicles and the development of ovarian cystic formations.

In the groups treated with flaxseed oil or corn oil, histopathological analysis demonstrated the occurrence of follicle-like cystic structures characterized by the absence of oocytes and the formation of small cystic follicles. These alterations were associated with an overall increase in the number of ovarian follicles at different stages of development.

Similarly, co-administration of tamoxifen with either flaxseed oil or corn oil induced notable histopathological alterations, reflected by a higher density of follicles at varying maturation stages in most treatment groups. Interestingly, in these combination groups, a considerable portion of the ovarian parenchyma was predominantly replaced by corpus luteum structures, suggesting that tamoxifen may modulate follicular dynamics and promote luteal tissue dominance (Figures 5–8).

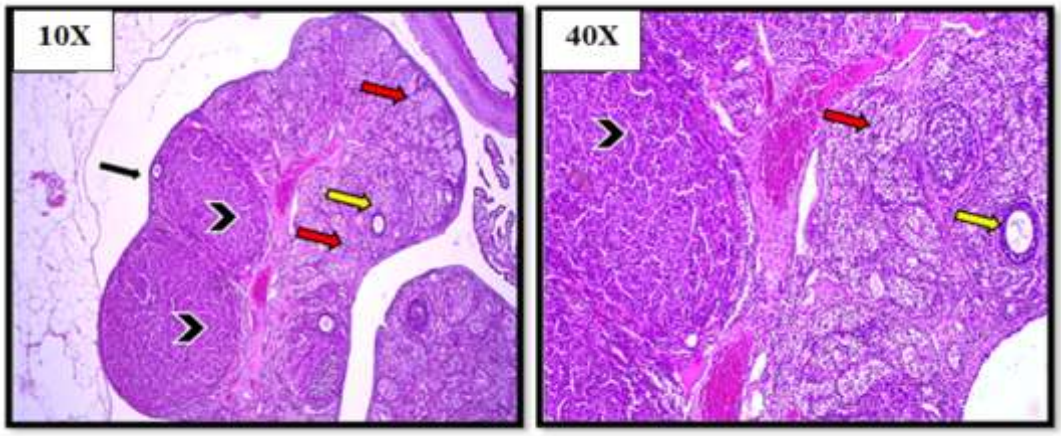

Figure (5): A cross-section view of the ovary of a flaxseed oil-treated rat. An increase in the number of ovarian follicles at various maturation stages was observed compared to the negative control group. Notable structures include primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), Graafian follicle (blue arrow), a follicle-like cyst with a degenerated/disappeared oocyte (green arrow), and corpus luteum (arrowhead). **H&E staining. (10X , 40X)**.

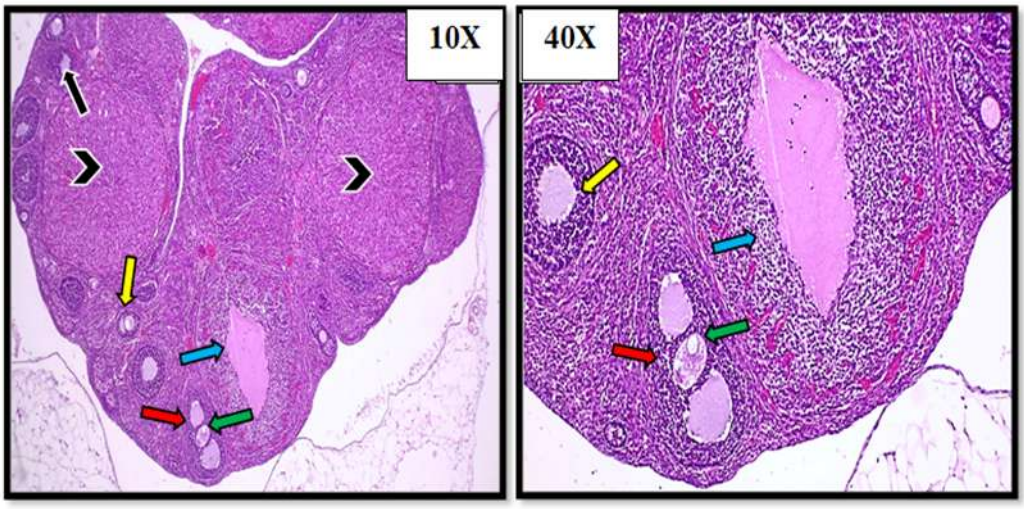

Figure (6): A cross-section view of the ovary of a corn oil-treated rat.
An increase in the number of ovarian follicles at various maturation stages was observed compared to the negative control group. Notable structures include primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), an ovulated Graafian follicle transitioning to corpus luteum (blue arrow), a small cystic follicle (green arrow), and corpus luteum (arrowhead). **H&E staining. (10X , 40X)**.

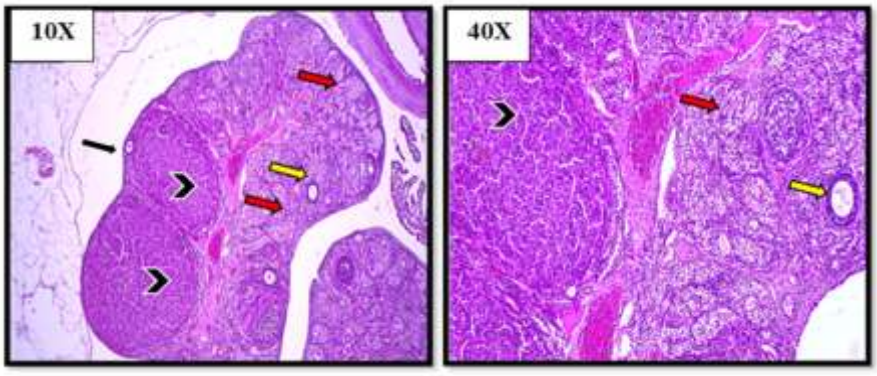

Figure (7): A cross-section view of the ovary of a flaxseed oil- and tamoxifen-treated rat.
A decrease in the number of ovarian follicles at various maturation stages was observed compared to the flaxseed oil-only treated group. No tertiary or Graafian follicles were identified in the ovary tissue. Notable structures include primary

follicle (black arrow), secondary follicle (yellow arrow), corpus albicans (red arrow), and corpus luteum (arrowhead). **H&E staining. (10X , 40X).**

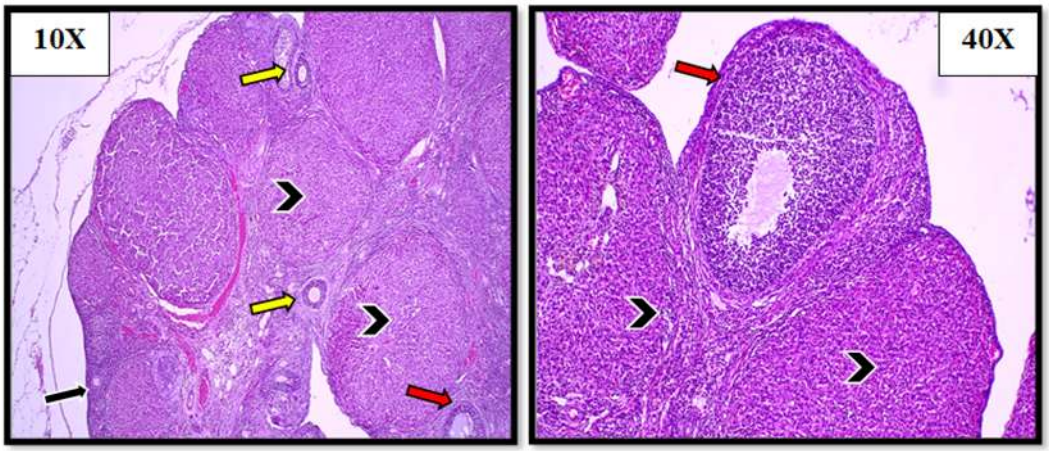

Figure (8): A cross-section view of the ovary of a corn oil- and tamoxifen-treated rat. A decrease in the number of ovarian follicles at various maturation stages was observed compared to the corn oil-only treated group. No Graafian follicles were identified in the ovary tissue. Notable structures include primary follicle (black arrow), secondary follicle (yellow arrow), tertiary follicle (red arrow), and corpus luteum (arrowhead). **H&E staining. (10X , 40X).**

DISCUSSION

This study highlights the potential health implications arising from the structural and functional resemblance between endogenous estrogens and phytoestrogens found in flaxseed and corn oils. Such similarities may constitute an important risk factor due to their capacity to modulate estrogenic pathways at multiple biological levels, including biochemical, histopathological, and epigenetic processes. The findings revealed a marked stimulation of ovarian follicle and oocyte growth in treated rats, accompanied by improved fertility outcomes. These effects are primarily attributed to the diverse bioactive phytochemicals in flaxseed and corn oils—particularly phenols, flavonoids, and dietary fibers—which have been previously associated with enhanced ovarian health and endocrine balance [11]. These results are consistent with previous reports emphasizing the role of phytoestrogen-rich dietary sources in promoting reproductive and hormonal functions.

Phytoestrogen-rich oils also contain essential amino acids such as aspartic acid and arginine, both of which play critical roles in regulating gonadotropin secretion and ovarian physiology [12]. Aspartic acid has been shown to stimulate gonadotropin release , whereas arginine supports follicular growth and maintenance through nitric oxide production—a key mediator of gonadotropin secretion. This pathway underlies the synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both indispensable for ovarian steroidogenesis, follicle development, and oocyte maturation [13]. Additionally, phytoestrogenic flavonoids such as quercetin—abundant in flaxseed and corn oils [14]—can bind to estrogen receptors and mimic estrogenic signaling, thereby enhancing FSH secretion [15]. This mechanism contributes to

accelerated follicular maturation and improved fertility. Other amino acids, including asparagine and tryptophan, also present in these oils, modulate gonadotropin activity by influencing FSH and LH secretion, further supporting folliculogenesis and reproductive performance [16,17].

The micronutrient composition of flaxseed and corn oils, encompassing essential vitamins and minerals, further contributes to follicular dynamics by acting as cofactors in steroidogenic enzymatic reactions and promoting cellular growth and differentiation [18]. Histometric analyses confirmed significant increases in the numbers of follicles and corpora lutea in treated groups, emphasizing the stimulatory roles of these bioactive constituents. The effects of quercetin, together with the synergistic activity of arginine and aspartic acid on hypothalamic gonadotropin-releasing hormone (GnRH) secretion [13], underscore the integrated regulatory network governing ovarian function. These findings align with previous research highlighting the pivotal roles of flavonoids and amino acids in follicular growth and oocyte quality [19]. Moreover, the antioxidant properties of flaxseed and corn oils are particularly relevant, as they reduce oxidative stress, elevate estrogen levels, and enhance fertility—especially in conditions such as polycystic ovarian syndrome (PCOS) [20].

A prominent histological observation was the increased thickness of granulosa cell layers and the reduced thickness of the theca layers in treated groups compared with the negative control. This structural alteration reflects the proliferative and differentiative effects of phytoestrogens, flavonoids, vitamins, and minerals present in flaxseed and corn oils, which promote

granulosa cell expansion, facilitate follicular maturation, and attenuate the fibrotic changes typically observed in the theca layer [11]. Similar findings have been reported in recent research [21]. The thickening of the granulosa cell layer indicates enhanced follicular activity and increased estrogen synthesis, as granulosa cells represent the principal site of estradiol production and are stimulated by both FSH and estradiol [22]. Conversely, the reduction in theca layer thickness may indicate a functional redistribution between follicular compartments resulting from granulosa cell hyperactivity [23].

The combined effects of estradiol, phytoestrogen-rich oils, and tamoxifen are particularly noteworthy. Tamoxifen's ability to modulate estrogen receptor activity may influence follicular signaling cascades, enhancing granulosa cell proliferation while altering theca layer responses [24]. The observed increase in ovarian follicles, including mature Graafian follicles, can be partly attributed to estradiol-mediated stimulation of folliculogenesis. Estradiol promotes granulosa cell proliferation and differentiation, thereby supporting follicular growth and maturation [24]. These observations align with previous studies reporting enhanced follicular development following exogenous estradiol administration. Furthermore, flaxseed and corn oils—rich in omega-3 and omega-6 fatty acids, respectively—may further regulate ovarian function by improving insulin sensitivity and reducing inflammation. Microscopic evaluation of the positive control group (PC+) revealed pronounced follicular proliferation at different maturation stages, the presence of mature Graafian follicles, and cystic structures, consistent with estrogen-driven folliculogenesis [25]. Treatments with flaxseed and corn oils induced follicle-like cystic formations devoid of oocytes, likely due to phytoestrogen–receptor interactions that modulate follicular viability [26].

From an epigenetic perspective, the decreased methylation levels of the ERα gene observed in the tamoxifen and corn oil-treated groups deserve particular attention. DNA methylation serves as a key epigenetic mechanism that generally suppresses gene expression, including that of estrogen receptor genes. Reduced methylation enhances ERα transcription, thereby amplifying estrogen signaling. This demethylation may result from inhibition of DNA methyltransferases (DNMTs) under high-estrogenic conditions induced by treatment. Estrogen is known to regulate the expression and activity of epigenetic modifiers, and elevated ERα expression could establish a positive feedback loop that intensifies estrogen signaling within target tissues [27, 28, 29].

CONCLUSION

The findings of the present study indicate a potential pharmacodynamic interaction between tamoxifen and dietary phytoestrogens, particularly those derived from corn and flaxseed oils. When co-administered with elevated phytoestrogen levels, the inhibitory action of tamoxifen on estrogen signaling appears to be partially diminished, most likely due to competitive or physicochemical interactions at the estrogen receptor binding sites. These observations emphasize the necessity of considering dietary phytoestrogen consumption during tamoxifen therapy and suggest that tamoxifen administration should ideally occur independently to maintain its therapeutic effectiveness in suppressing estrogen receptor–mediated signaling.

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