Effects of Piperine on Relative Tissue Weight Index of Ovaries and Gonadotropin Levels in Female Albino Rats.

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ABSTRACT:

Objective: To evaluate the effects of piperine on ovarian relative tissue weight index and serum gonadotropin levels in adult female albino rats.

Methodology: This experimental study was conducted at the department of Anatomy, Niazi Medical and Dental College, Sargodha, over a period of four-month period from September 2025 to December 2025. Thirty adult female Wistar albino rats were divided into three groups (each group 10 rats): Group A (control; normal saline), Group B (piperine 5 mg/kg/day) and Group C (piperine 10 mg/kg/day). Drugs were given orally for 30 days daily. Body and ovarian weights were recorded to calculate RTWI. Serum levels of FSH and LH were quantified by enzyme-linked immunosorbent assay (ELISA). One-way ANOVA with Tukey's post hoc test were used to measure the group differences.

Results: A significant increase in ovarian Relative Tissue Weight Index (RTWI) was observed after piperine treatment, with the highest values recorded in the 10 mg/kg group ($p < 0.001$). Serum FSH and LH levels were significantly increased in piperine-treated groups, particularly in Group C ($p < 0.001$).

Conclusion: Piperine induces dose-dependent ovarian toxicity with altered RTWI, structural ovarian damage and significant disruption of gonadotropin secretion. Therefore, prolonged or high-dose piperine exposure can disturb female reproductive function.

Keywords: Follicle-Stimulating Hormone; Luteinizing Hormone; Ovaries; Piperine; Relative Tissue Weight Index.

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Introduction:

Piperine is the major bioactive alkaloid found in black pepper (Piper nigrum) and long pepper (Piper longum), contributing to both their characteristic pungent flavor and medicinal value. For centuries, black pepper has held an important place in traditional medicines, especially Ayurveda and Unani, where it has been widely utilized not only as an aromatic ingredient but also for the management of digestive, respiratory and metabolic disorders.[1] These properties of piperine has increased the concern of researchers as the major active constituent to explore its pharmacological actions at molecular and cellular levels.[2] The modern sciences has investigated that piperine influences on multiple cellular signaling mechanisms, including inflammatory pathways, oxidative stress responses and regulation of neurotransmitters.[3] Piperine has neuroprotective, cardioprotective, antioxidant, anti-inflammatory and anticancer properties.[4] Due to its antioxidant nature piperine decreases the release of reactive oxygen species with increasing the formation of antioxidant enzymes. Reduction in the activity of pro-inflammatory cytokines and their signaling pathways such as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) mediates its anti-inflammatory effects.[3] Because of all these health benefits specially to prevent the chronic illness, piperine is now commonly used in our diet as a compulsory supplement.[5] From the pharmacological aspect, piperine optimizes the bioavailability of various drugs.[6] All those drugs that are poorly absorbed from oral route including anticancer agents, some antibiotics and herbal medicines, piperine helps to increase their absorption by modulating drug-metabolizing enzymes, inhibiting P-glycoprotein-mediated efflux and increasing intestinal permeability, thereby enhancing systemic availability of co-administered drugs. It has also shown to affect the cytochrome P450 enzyme activity leading to change the metabolism and half-life of various circulating compounds.[3] As the uncontrolled use of every supplement and drug is very dangerous for our health, same for piperine, it's unethical use can be very dangerous for the normal physiological mechanisms and systems of our body including liver, brain and reproductive system.[7,8] While lower doses are generally considered beneficial but too much exposure in higher concentrations can disturb the cellular homeostasis and produce tissue-specific toxicity.[9] According to new studies, piperine is producing oxidative stress, mitochondrial dysfunction and apoptosis in multiple tissues due to its prolonged exposure.

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Ultimately leading to morphological and biochemical changes and decrease functioning of organs.[10] Piperine is affecting the reproductive organs even on very low doses and within very short time period leading to impaired gametogenesis, steroidogenesis and endocrine regulation. In females, oxidative stress can disturb the maturation of follicles, quality of oocytes and alter the synthesis of hormones thereby affecting the outcomes of fertility.[11] On males there are so many studies discussing the alterations in spermatogenesis, testosterone synthesis and steroidogenic gene expression due to piperine exposure.[9] But data concerning its effects on female reproductive organs remain comparatively scarce and fragmented that need to be focused specially the ovarian morphology and endocrine responses.

The relative tissue weight index (RTWI), which is determined by comparing organ weight with total body weight, is considered a sensitive and reliable indicator in experimental toxicology. Due to exposure to xenobiotics RTW1 is used to identify the alterations on organs even on early stages along with hyper and hypotrophy of organs.[12] Despite of apparently normal organ weight the variations in RTWI may reflect pathological conditions such as follicular degeneration, stromal damage, vascular congestion, inflammatory infiltration and cellular atrophy.[13] Hypothalamic pituitary ovarian axis (HPO) regulates the normal ovarian physiology by stimulating the anterior lobe of pituitary gland to produce gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in pulsatile manner. Leads to development of follicles, ovulation and formation of corpus luteum by positive feedback mechanism.[14] If at the start or at any stage the structure of ovarian follicles is damaged and steroidogenic pathways is disturbed it will impair the negative feedback mechanisms and causes alterations in concentrations of circulating gonadotropins. Such endocrine disturbances may manifest as irregular estrous cycles, anovulation, or hormonal imbalance, all of which are indicators of impaired ovarian function. As hormonal deregulation serves as a functional indicator of ovarian toxicity and reproductive endocrine imbalance.[15]

Because piperine as black pepper is commonly used as a nutraceutical and drug enhancer, there is growing concern about its possible effects on female reproductive health. For this reason, it is important to carefully study its safety. This study was conducted to investigate the dose-dependent effects of piperine on ovarian relative tissue weight index, ovarian histology and hormone levels (FSH and LH) in adult female albino rats in order to clarify its potential toxic effects on the ovaries and overall female reproductive function.

Methodology:

A laboratory based experimental study was carried out over a four-months period from September 2025- December 2025 at the animal house and histopathology laboratory of Niazi Medical and Dental College, Sargodha after taking Ethical permission from the Institutional Ethical Review Committee (Approval No: NMDC-ERC/IER/31/25). Thirty adult healthy female Wistar albino rats, ranging in age from 3 to 4 months and body weight 200-250 g, with no prior exposure to experimental drugs or chemicals were obtained for experiment. Rats that were pregnant or lactating, showed signs of illness, injury or abnormal behavior, fell outside the specified age or weight range were excluded from the experiment. The sample size was chosen based on prior experimental research on ovarian histology and hormonal alterations in rodents, in which 8–10 animals per group were found adequate to identify biologically relevant differences among treatment groups.[16] Before starting the experiment, the animals were housed in the institutional animal house. The rats were allowed to acclimatize for one week in a controlled laboratory environment maintained at 25 ± 2°C with alternating 12-hour light and dark cycles, while receiving standard laboratory chow and distilled water ad libitum.

Rats were weighed before starting the experiment. They were allocated into three groups (n = 10 per group) by using a convenient sampling method. Group A (control) was administered normal saline (10 mL/kg body weight) orally via orogastric gavage once daily for 30 consecutive days. Group B (experimental group 1) was given piperine dissolved in normal saline at a dose of 5 mg/kg body weight by orogastric gavage for 30 days. Group C (experimental group 2) received piperine at a dose of 10 mg/kg body weight in normal saline through the same route and duration. Throughout the study period the animals from all groups were provided with free access to diet and distilled water.

Black pepper was subjected to Soxhlet extraction with ethanol to obtain piperine, which was subsequently purified by gas chromatography-mass spectrometry (GC-MS). Before administration, the compound was suspended in normal saline. On day 30 of the experiment, the final body weight of animals was measured. The rats were rendered unconscious using chloroform inhalation anesthesia at the last day. Blood was withdrawn directly from the heart with sterile disposable syringes, and approximately 3-4 mL of blood was collected from each animal. Clot activator tubes were used to place the samples and left undisturbed at room temperature for 30 minutes to allow coagulation. After separating the serum the concentrations of serum FSH and LH were determined.[17] Following sacrifice, both ovaries were excised and weighed. The ovarian relative tissue weight index (RTWI) was calculated using the formula[18]:
RTWI = (Ovary weight / Body weight) × 100

Statistical Analysis:

Statistical analysis was done by SPSS version 24. Quantitative variables, including biochemical parameters and RTWI, were presented as mean ±standard deviation. Study groups differences were evaluated by one-way ANOVA and pairwise comparisons were carried out with Tukey's post hoc test. A p-value of ≤0.05 was taken as statistically significant.

Results:

Relative Tissue Weight Index:

An increase in ovarian RTWI was seen among the study groups ($p < 0.001$). The highest RTWI values were recorded in Group C (10 mg/kg/day), followed by Group B (5 mg/kg/day), in comparison with control group (Table 01). One-way ANOVA showed a highly significant difference among the three groups (Table 02).

Table No1: Relative tissue weight index (RTWI) of ovaries after piperine administration.

Group	Treatment	RTWI (Mean ± SD)
A	Control (Saline)	0.05 ± 0.004
B	Piperine 5 mg/kg/day	0.07 ± 0.003
C	Piperine 10 mg/kg/day	0.09 ± 0.005

One-way ANOVA

Table No 2: Inter-group comparison of Relative Tissue Weight Index by Post hoc Tukey's Analysis.

(I) Group	(J) Group	Sig. (p-value)
Group A (Control)	Group B (Piperine 5 mg/kg)	< 0.001
Group A (Control)	Group C (Piperine 10 mg/kg)	< 0.001
Group B (Piperine 5 mg/kg)	Group C (Piperine 10 mg/kg)	< 0.001

Serum Gonadotropin Levels:

Administration of piperine resulted in a dose-related rise in serum FSH and LH concentrations across the treated groups (Table 03). The highest levels of both hormones were observed in Group C, level were significantly higher than the control group ($p < 0.001$).

Table No 3: Serum gonadotropin levels (FSH and LH) in control and piperine-treated rats.

Groups	Treatment	FSH (mIU/ml) Mean ± SD	LH (mIU/ml) Mean ± SD
A	Control (Saline)	3.2 ± 0.4	2.8 ± 0.3
B	Piperine 5 mg/kg/day	4.1 ± 0.5	3.6 ± 0.4
C	Piperine 10 mg/kg/day	5.6 ± 0.6	4.9 ± 0.5

One-way ANOVA.

Table No 4: Intergroup comparison of serum FSH and LH by Tukey's post-hoc test.

(A) FSH

(I) Group	(J) Group	Sig. (p-value)
Group A (Control)	Group B (5 mg/kg)	< 0.05
Group A (Control)	Group C (10 mg/kg)	< 0.001
Group B (5 mg/kg)	Group C (10 mg/kg)	< 0.01

(B) LH

(I) Group	(J) Group	Sig. (p-value)
Group A (Control)	Group B (5 mg/kg)	< 0.05
Group A (Control)	Group C (10 mg/kg)	< 0.001
Group B (5 mg/kg)	Group C (10 mg/kg)	< 0.01

Discussion:

Current study gives the combined dose dependent changes on morphology and endocrine system of ovaries following piperine administration in adult female albino rats. Experimental studies have shown that before producing any structural change piperine initially disrupts the hormonal regulation in ovaries then damages the tissues. Our results showed the increase in RTW1 of ovarian tissue along with marked alterations in serum FSH and LH levels suggesting disruption of the hypothalamic-pituitary-ovarian (HPO) axis. This axis is integral to maintain reproductive homeostasis by regulating the hormonal feedback mechanisms.[14] While considering the excessive use of piperine in our daily food and using is as herbal supplements, these findings are very important.[19]

RTWI is a sensitive marker of early tissue pathology. Instead of only focusing on the weight of organ, RTW1 reflects changes in vascularity, stromal expansion and cellular composition of the tissue. In toxicological research, alterations in RTWI are frequently associated with inflammatory processes, edema, or abnormal proliferation of stromal elements, which may precede detectable microscopic lesions.[20] In current study, an increase in ovarian RTWI was seen in the piperine-treated groups, especially at the higher dose, the reason may be due to follicular enlargement, stromal edema and vascular congestion within the ovary same discussed in study of Francis et al.[18] The study of Wang et al, also demonstrates that increased ovarian size may also reflect the persistence of developing follicles that fail to undergo normal luteinization, resulting in accumulation of immature follicular structures.[17] Such tissue expansion is consistent with the accumulation of growing follicles that fail to progress normally through ovulation, resulting in increased ovarian mass despite of impaired reproductive functions. This phenomenon has been described in models of endocrine disruption, where hormonal imbalance leads to altered follicular dynamics and structural enlargement without functional maturation, Same results were reported by Curi et al., that exposure to chemical agents can disrupt endocrine balance and lead to adverse reproductive outcomes.[21] These findings are also justifying our results, where piperine administration produced marked hormonal imbalance, suggesting its potential to adversely affect female reproductive functions.[14] The resemblance of these results to other xenobiotic exposure models further supports the possibility that higher doses of piperine may exert endocrine-modulating effects capable of disrupting normal ovarian physiology.[18]

During current study the hormonal results revealed following changes i.e.; the animals who were exposed to high doses of piperine have shown elevations in serum FSH and LH level. These increase levels are generally due to reduce response from ovaries, resulting increase hormonal production from pituitary gland to maintain the homeostasis. Due to disturbance of functioning of granulosa cells the synthesis of estrogen and follicular maturation will be compromised, ultimately leading to endocrine imbalance.[22] Similar findings were reported by Wang L et al , who explained that due to follicular atresia, the regulation of ovarian feedback is impaired, leading to malfunctioning of granulosa cells and steroidogenic activity, including decrease production of estrogen affects the negative feedback mechanism of hypothalamus and pituitary gland.[23] Likewise, Choi et al. also observed that when ovarian reserves are less in quantity, LH and FSH levels are raised and fertility is reduced. All these are compensatory mechanisms.

Moreover, due to persistent elevation of gonadotropins the follicular stress increases, leading to hormonal imbalance and structural degeneration of ovarian tissue.[24]

There are different mechanisms that support the ovarian toxicity caused by piperine. One of them is that the high doses of piperine modulate cytochrome P450 enzymes that are involved in production of steroidal hormones thereby, disturbing the production of FSH, LH, estrogen and progesterone. The hormonal balance required for ovulation will be disturbed, leading to abnormal production of follicles and luteal phase of ovarian cycle.[25]

Another mechanism is piperine induced oxidative stress that is increase release of reactive oxygen species with decrease antioxidant capacity. On ovaries it can damage the granulosa cells, oocytes and mitochondrial function. Apoptosis caused by oxidative stress is a very common disorder of female reproductive system leading to follicular atresia and endocrine imbalance.[13]

Conclusion:

At low dietary levels, piperine is very beneficial for health but its high doses have deleterious effects on the morphology and physiology of ovaries in adult female albino rats. Increase in ovarian relative tissue weight index shows the impaired follicular maturation. Along with this the hormonal imbalance was also observed with increased concentrations of gonadotropins.

Limitations and future Recommendations:

This study did not assess estrous cycle patterns, or fertility outcomes as ovulation rate, mating success, and pregnancy were not investigated. Work should be done on this line too. In addition, only two doses of piperine were evaluated, and molecular markers of oxidative stress and steroidogenesis were not assessed that should be investigated.

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Author's Contribution	
Amna Mahmood	Conception, Study Design
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Samankal Sara	Analysis and Interpretation of Data
Rabia Ejaz	Final Approval, Drafting the Article
Saqib Mansoor	Analysis and Interpretation of Data