



REVIEWER'S REPORT

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Title: Histopathological Toxicity Evaluation of Deltamethrin on the Integrity of Ovarian Follicles on Mice (*Mus musculus*)

Recommendation:

Accept as it is

Rating	Excel.	Good	Fair	Poor
Originality		√		
Techn. Quality			√	
Clarity			√	
Significance		√		

Reviewer's ID: JPR- 006

Detailed Reviewer's Report

Objective of the Study

The main objective of this investigation was to evaluate the effect of deltamethrin insecticide on ovarian follicle development in mice. Researchers aimed to determine whether exposure could alter the number of primordial, primary, secondary, Graafian, and atretic follicles. The study also intended to compare the effects of two different dose levels. By analyzing follicular changes, the researchers could identify signs of ovarian degeneration and reproductive toxicity. Another purpose was to understand the relationship between oxidative stress and follicular damage. The study further attempted to support previous findings related to pyrethroid-induced reproductive disorders. Experimental evidence from animal studies can help predict possible health risks in humans. Hence, the work provides important insight into pesticide-induced reproductive abnormalities.

Deltamethrin Toxicity

Deltamethrin is a synthetic pyrethroid insecticide commonly used in agriculture and pest control due to its strong insecticidal activity. Although effective against pests, prolonged exposure can also harm non-target organisms such as mammals. The study explains that deltamethrin may damage several organs, especially the reproductive system. Exposure can occur through ingestion, inhalation, or skin contact. After entering the body, deltamethrin is metabolized into toxic compounds that produce reactive oxygen species (ROS). Excessive ROS generation causes oxidative stress and disturbs normal cellular functions. This oxidative stress damages lipids, proteins, and DNA within cells. Consequently, continuous exposure to deltamethrin can seriously affect ovarian health and reproductive function.

Importance of Studying Ovarian Damage

The ovary is one of the most sensitive reproductive organs in females because it controls hormone production and follicle development. Damage to ovarian follicles can interfere with fertility and reproductive health. The study focused on understanding how deltamethrin affects ovarian structures in mice. Follicles are essential for the maturation and release of healthy oocytes. Any disturbance in follicular growth may reduce reproductive efficiency. Earlier studies mainly used purified deltamethrin, but this research examined the effects of commercially available insecticide formulations. Understanding ovarian toxicity is important because environmental exposure to pesticides is increasing worldwide. The

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study therefore contributes valuable information regarding reproductive risks caused by insecticide exposure

Experimental Animals Used

The experiment used fifteen healthy female mice (*Mus musculus* L.) as the test organisms. The mice were approximately 12 weeks old and had an average body weight of 26.7 grams. Before the experiment, the animals were acclimatized for one week under laboratory conditions. Proper acclimatization helped reduce stress and ensured stable physiological conditions. Food and drinking water were supplied freely throughout the experiment. Healthy animals were selected to avoid interference from pre-existing diseases or abnormalities. Female mice were preferred because their ovarian follicular changes can be clearly studied histologically. Using laboratory mice also provides reliable and reproducible biological results. Thus, the chosen animal model was suitable for evaluating reproductive toxicity.

Treatment Groups and Dosage Design

The mice were divided equally into three experimental groups consisting of control and treatment sets. The control group received only corn oil without deltamethrin exposure. The first treatment group (D1) received deltamethrin at a dose of 18 mg/kg body weight. The second treatment group (D2) received a higher dose of 36 mg/kg body weight. Treatments were administered intraperitoneally with a daily volume of 1 ml for fourteen consecutive days. This dosage design allowed researchers to compare dose-dependent toxic effects. Using two concentrations helped determine whether higher exposure produced greater ovarian damage. The control group served as a baseline for comparison. Overall, the experimental design was systematic and scientifically appropriate.

Histological Examination Procedure

On the fifteenth day, the mice were anesthetized and dissected for ovary collection. The ovaries were carefully removed and processed for histological examination using the paraffin embedding technique. Tissue sections were stained with Hematoxylin and Eosin (H&E), a standard staining method in histopathology. This staining method clearly differentiates ovarian structures and follicular cells. Microscopic observations were carried out using a binocular microscope at 400× magnification. Researchers identified and counted different stages of follicles in ovarian tissues. Histological analysis provided direct evidence of structural changes caused by deltamethrin exposure. The procedure ensured accurate observation of follicular degeneration and atresia. Therefore, microscopic evaluation formed the core of the experimental analysis.

Types of Follicles Observed

The study examined five major types of ovarian follicles. Primordial follicles represent the earliest stage of follicular development and contain immature oocytes. Primary follicles develop from primordial follicles and begin active growth. Secondary follicles show further maturation and development of granulosa cells. Graafian follicles are fully mature follicles ready for ovulation. Atretic follicles are degenerated follicles that fail to mature properly. By studying these stages, researchers could determine how deltamethrin interfered with ovarian development. Changes in follicle numbers provide an indication of reproductive health and fertility potential. Therefore, follicular analysis is an important marker of ovarian toxicity.

Statistical Analysis of Data

The collected data were statistically analyzed using One-Way Analysis of Variance (ANOVA). This method helped determine whether significant differences existed among the treatment groups. A significance level of 95% ($p < 0.05$) was selected for analysis. Duncan's Multiple Range Test (DMRT) was further applied to identify differences between specific groups. Statistical analysis increased the reliability and validity of the findings. Results showed that some follicular changes were statistically significant while others were not. Proper statistical evaluation minimized random experimental errors. The use of ANOVA and DMRT strengthened the scientific credibility of the research. Hence, the conclusions were supported by quantitative evidence.

REVIEWER'S REPORT***Effect on Primordial Follicles***

The results showed that deltamethrin exposure did not significantly affect the number of primordial follicles. Statistical analysis revealed a p-value greater than 0.05 for this follicular stage. Primordial follicles are relatively inactive and may therefore be less sensitive to toxic compounds. The similarity between control and treated groups suggests that early-stage follicles were preserved. However, slight numerical variations were still observed among the groups. The lack of significant change indicates that short-term exposure may not directly damage dormant follicles. Despite this, later stages of follicular development were clearly affected. Thus, primordial follicles appeared more resistant compared to developing follicles.

Effect on Primary Follicles

The study also found no significant difference in the number of primary follicles among treatment groups. Primary follicles continued to exist in relatively similar quantities in control and treated mice. Statistical analysis again produced a p-value greater than 0.05. This suggests that deltamethrin toxicity may not strongly influence the early transition from primordial to primary follicle stages. However, toxic stress could still affect cellular metabolism within these follicles. Researchers observed that advanced follicular stages were more sensitive to pesticide exposure. The findings indicate that follicular maturation becomes increasingly vulnerable as development progresses. Therefore, primary follicles showed limited but not complete resistance to toxic effects.

Reduction in Secondary Follicles

A significant decrease in the number of secondary follicles was observed in the higher dose group. The D2 treatment group showed much lower secondary follicle counts compared to the control group. Statistical analysis confirmed that the reduction was significant ($p < 0.05$). Secondary follicles are metabolically active and highly dependent on hormonal regulation. Deltamethrin-induced oxidative stress may disrupt granulosa cell function and follicular growth. The reduction indicates impaired follicular maturation and developmental arrest. Lower numbers of secondary follicles suggest reduced reproductive capability. This finding demonstrates that deltamethrin adversely affects ovarian follicular progression. The toxic effect became more severe with increasing dosage.

Decrease in Graafian Follicles

Graafian follicles showed a marked decline in both treatment groups compared to controls. These follicles represent the mature stage necessary for ovulation and successful reproduction. The significant reduction indicates impaired follicular maturation caused by deltamethrin toxicity. Graafian follicles are highly sensitive to hormonal imbalance and oxidative damage. Disruption of estrogen signaling may prevent proper follicular growth and ovulation. Reduced Graafian follicle numbers can lead to decreased fertility and reproductive failure. The findings suggest that deltamethrin interferes with the final stages of follicular development. Therefore, mature ovarian function was seriously affected by insecticide exposure.

Increase in Atretic Follicles

One of the most important findings of the study was the significant increase in atretic follicles. Atresia refers to follicular degeneration and cellular breakdown. The highest number of atretic follicles was observed in the D2 treatment group receiving 36 mg/kgBW. Increased atresia indicates severe ovarian damage and follicular death. Oxidative stress and hormonal disruption are believed to accelerate follicular degeneration. Damaged follicles lose their ability to mature into functional oocytes. The progressive increase in atresia with higher doses demonstrates dose-dependent toxicity. Thus, deltamethrin exposure clearly promoted ovarian degeneration.

Metabolism of Deltamethrin

After entering the body, deltamethrin undergoes metabolism mainly in the liver through microsomal enzymes. Several toxic metabolites are formed during this process, including 3-phenoxybenzoic acid (3-PBA). Other metabolites such as hydroxylated derivatives are also produced. Studies cited in the paper

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indicate that some metabolites may be more toxic than deltamethrin itself. These metabolites circulate throughout the body and contribute to cellular damage. The metabolic process enhances oxidative stress by increasing free radical formation. Rodent studies have shown rapid metabolism and widespread tissue distribution. Therefore, metabolite toxicity plays a major role in reproductive damage.

Role of Reactive Oxygen Species (ROS)

Reactive oxygen species are chemically reactive molecules produced during normal metabolism. Under excessive toxic exposure, ROS levels increase abnormally and cause oxidative stress. Deltamethrin metabolites stimulate the formation of hydroxyl radicals, superoxide ions, and hydrogen peroxide. Excess ROS damages cellular membranes, proteins, and genetic material. In ovarian tissues, ROS accumulation affects follicular fluid and granulosa cells. Moderate ROS levels are necessary for physiological functions such as oocyte maturation. However, excessive ROS impairs meiosis and disrupts follicular development. Persistent oxidative stress eventually results in apoptosis and follicular atresia. Hence, ROS generation is a key mechanism of deltamethrin toxicity.

Oxidative Stress and Antioxidant Imbalance

The study emphasized that oxidative stress results from an imbalance between oxidants and antioxidants. Normally, antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione S-transferase protect cells from free radical damage. Deltamethrin exposure decreases the activity of these protective enzymes. Reduced antioxidant defense allows ROS to accumulate within tissues. Lipid peroxidation increases, leading to membrane instability and cell injury. Such biochemical disturbances impair ovarian cellular functions. Oxidative stress also triggers inflammatory and apoptotic pathways. Therefore, weakened antioxidant protection contributes significantly to ovarian degeneration caused by deltamethrin.

Endocrine Disrupting Activity of Deltamethrin

Deltamethrin and its metabolites are considered endocrine-disrupting compounds. These chemicals can interfere with hormone receptors and alter reproductive hormone balance. Research cited in the study showed that deltamethrin behaves similarly to estrogen. It may bind directly to estrogen receptors and disturb normal hormonal signaling. Such interference disrupts communication between the ovaries and pituitary gland. Hormonal imbalance affects follicular maturation and ovulation processes. Endocrine disruption may therefore contribute to infertility and reproductive dysfunction. This mechanism explains why follicular development decreased significantly after exposure. Thus, hormonal disturbance is another major toxic pathway.

Influence on Estrogen, FSH, and LH Regulation

Estrogen plays a central role in regulating female reproductive physiology. It is produced mainly by granulosa cells within ovarian follicles. Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) coordinate follicular growth and ovulation. Deltamethrin may disrupt these hormonal pathways by interacting with estrogen receptors. Such disruption can inhibit estrogen synthesis and impair gonadotropin regulation. Reduced hormonal signaling affects follicular proliferation and maturation. Consequently, developing follicles fail to reach the Graafian stage. The study therefore links pesticide exposure with endocrine imbalance and reproductive impairment. Proper hormonal coordination is essential for healthy ovarian function.

Dose-Dependent Ovarian Toxicity

The study clearly demonstrated a dose-dependent relationship between deltamethrin exposure and ovarian damage. Lower doses caused mild follicular changes, while higher doses produced severe degeneration. Secondary and Graafian follicles decreased progressively with increasing dosage. At the same time, the number of atretic follicles increased dramatically. This pattern confirms that toxicity becomes more severe with higher pesticide concentrations. Dose-dependent responses are important indicators of chemical toxicity in experimental studies. Such findings help establish safe exposure limits for humans and animals. Therefore, the study strongly supports the harmful reproductive effects of excessive deltamethrin exposure.

REVIEWER'S REPORT***Histopathological Changes in Ovarian Tissue***

Microscopic examination revealed clear structural abnormalities in ovarian tissues of treated mice. Degenerated follicles and increased atresia were commonly observed in exposed groups. Granulosa cell damage and follicular shrinkage indicated impaired follicular health. Some follicles displayed disrupted architecture and abnormal cellular arrangement. These pathological changes confirmed the toxic impact of deltamethrin on ovarian tissue integrity. Histological findings correlated well with the statistical reduction in healthy follicles. Tissue damage became more pronounced at higher doses. Therefore, histopathological analysis provided visual evidence of reproductive toxicity.

Comparison with Previous Studies

The findings of this study were consistent with earlier research on pyrethroid toxicity. Previous investigators also reported reductions in healthy follicles and increased follicular atresia after deltamethrin exposure. Similar oxidative stress and endocrine disruption mechanisms were identified in other rodent models. The study reinforced evidence that pyrethroid insecticides negatively affect female reproductive organs. However, this work expanded earlier findings by using commercially available insecticide formulations. Consistency with previous studies increases confidence in the observed toxic effects. The study also highlighted the importance of dose-dependent reproductive toxicity. Thus, the results contribute valuable support to existing toxicological knowledge.

Final Conclusion and Significance

The study concluded that deltamethrin exposure caused significant ovarian damage in mice. Administration of 18 and 36 mg/kgBW reduced the number of secondary and Graafian follicles while increasing atretic follicles. The toxic effects were mainly associated with oxidative stress, ROS generation, and endocrine disruption. Higher doses produced more severe reproductive damage, demonstrating dose-dependent toxicity. The findings suggest that prolonged exposure to pyrethroid insecticides may impair female fertility. Histological and statistical analyses strongly supported the conclusions. This research highlights the need for careful pesticide handling and exposure control. Overall, the study provides important insight into the

Recommendations

The use of deltamethrin insecticide should be carefully controlled to reduce harmful effects on reproductive health and non-target organisms. Protective measures such as gloves, masks, and proper handling procedures must be followed during pesticide application. Further studies should be conducted to investigate the long-term reproductive effects of deltamethrin exposure at different dosage levels. Research on antioxidant therapies, such as vitamin C or natural antioxidants, may help reduce oxidative stress caused by deltamethrin toxicity. Environmental monitoring and public awareness programs are also recommended to minimize unnecessary pesticide exposure and protect human and animal health.