LETTERS IN BIOMATHEMATICS
An International Journal

RESEARCH ARTICLE

OPEN ACCESS

Impact of 5-Methoxy-N-Acetyltryptamine on the Developmental Toxicity Induced by Deltamethrin in Mice

Baidaa Abdulaziz Mohamed Salah[1*], Mohammed Khalid Shindala[2], Mahaa A. Al-Obaidi[3]

[1]Department of Biology, College of Education for Pure Science, University of Mosul, Mosul, Iraq
[2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Mosul, Iraq
[3]University of Al-Hamdaniya, Al-Hamdaniya District, Iraq

ABSTRACT

Background: decamethrin (synthetic pyrethroid deltamethrin) and other similar compounds in the gestational period have become more worrisome because they cause neurodevelopmental problems in fetuses through three main mechanisms, which include oxidative imbalance, apoptotic signaling, and neuroinflammatory activation. The body produces the 5-methoxy-N-acetyltryptamine (melatonin) as an antioxidant, which has shown potential to protect against pesticide-caused developmental harm to the fetus. They also affect maternal brain architecture. **Aim:** The research investigated how deltamethrin exposure during pregnancy affects mouse brain development. The ability of melatonin to protect against oxidative stress and Bax-induced cell death in fetus brain and CD45-related maternal brain inflammation.

Methods: The study used thirty-two pregnant albino mice. Animals were split into four groups containing eight pregnant mice each. Group I received distilled water, group II dosed orally with 1 mg/kg of deltamethrin, group III injected with 12.5 mg/kg of melatonin intraperitoneally (IP), and the groupIV administrated of both melatonin (12.5 mg/kg) and deltamethrin (1 mg/kg. Both chemicals were given to animals during the period 7-17 days of gestation. The research investigated fetal growth markers together with maternal oxidative stress indicators (MDA and GSH), routine tissue analysis results, and Bax and CD45 protein expression through immunohistochemical testing. Furthermore, some other parameters. **Results:** The exposure to deltamethrin caused significant fetal growth problems and decreased brain weight of embryos, depleted glutathione levels, severe brain tissue damage, increased Bax protein levels, and elevated CD45 immune responses in the maternal brain, which activated cell death mechanisms and neuroinflammatory responses. The combination of melatonin with other toxicants like deltamethrin resulted in a substantial reduction of pathological changes through enhanced antioxidant defense and decreased apoptosis and inflammation but did not restore all affected parameters to normal levels.

Conclusion: Deltamethrin causes developmental neurotoxic effects during pregnancy through multiple pathways, which include oxidative stress, apoptotic cell death, and neuroimmune system disruption. Melatonin shows restricted neuroprotective effects, which make it a potential therapeutic agent for pesticide-induced brain damage that affects fetal brain development.

ARTICLE HISTORY
Received September 25, 2026
Accepted October 30, 2026
KEYWORDS
Deltamethrin, Melatonin, Developmental Neurotoxicity, Oxidative Stress, Neuroinflammation, Pregnancy.

1. Introduction

Synthetic pyrethroid insecticides, which farmers use extensively in agriculture, have raised concerns about their ability to damage human reproductive systems and their potential to cause birth defects in developing fetuses. During vital pregnancy stages, when organ formation occurs and is considered to be a sensitive period

CONTACT Baidaa Abdulaziz Mohamed Salah: baidaamohammed@uomosul.edu.iq

of pregnancy, and the three germ layers are formed from the gastrula, exposure to any physical or chemical factor may cause a defect in the development of vital organs and an imbalance in the percentage of cell proliferation (Priyadarshini and Pandey, 2019). The agricultural and public health sectors use deltamethrin as their primary insecticide because this Type II alpha-cyano pyrethroid compound shows strong insecticidal properties and appears to be safe for use)Rehman et al., 2014). Findings from laboratory and population-based studies showed that exposure to deltamethrin during pregnancy is associated with health risks in both mothers and the developing fetus, mainly through disruption of redox homeostasis, Caspase-dependent cell death, and neuroimmune signaling dysregulation (Huang et al., 2025).

The biological state of pregnancy makes women more vulnerable because their bodies need precise control of cell growth and movement, and scheduled cell elimination to develop embryonic organs and form neural tissue (Kazma et al. 2020; Bradshaw et al.,2024). Outside influences, such as insecticides, directly harm the brain and spine and affect general and occupational health. Male offspring's neural growth may be hampered by the mother's exposure to DM throughout gestation and nursing (Hao et al.,2024).

Previous studies showed that women who experience deltamethrin exposure during their pregnancy and delivery will develop redox instability, because their bodies generate elevated ROS levels while their antioxidant defenses, including superoxide dismutase and catalase, become less effective, which results in tissue damage through lipid peroxidation and mitochondrial destruction (Jia et al., 2019; Hao et al., 2024). Deltamethrin during gestation dramatically changes the mRNA expression of developmental heart ion channels, which alters the developing heart's functional parameters. Pregnant mice that were exposed to deltamethrin had higher morbidity rates and fewer surviving fetuses (Luo et al., 2019).

Melatonin (5-methoxy-N-acetyltryptamine) functions as a pleiotropic neuroendocrine hormone that possesses strong antioxidant, anti-apoptotic, and inflammatory capabilities. The amphiphilic structure of melatonin allows it to pass through biological barriers, which include the placenta and blood–brain barrier, thus it protects both maternal and fetal tissues directly. Previous studies have shown that melatonin acts as a free radical scavenger, which protects endogenous antioxidant systems and controls mitochondrial function to reduce oxidative damage, which safeguards cell structure during embryo development (Reiter et al.,2017; Huang et al., 2025).

Melatonin has proven to affect neurodevelopmental processes that extend past its redox regulatory functions by controlling neuronal cell growth, cell type development, and cell maintenance. Melatonin showed regulatory properties that affect apoptotic mediators and glial activation patterns, which could help protect neurodevelopmental homeostasis when the body faces both environmental and chemical stresses. Melatonin metabolites effectively eliminate free radicals like OH, O_2 , and NO in cellular and embryonic structures of animals and humans (Yurtcu et al., 2007). As an antioxidant that prefers lipids, melatonin is twice as potent as vitamin E. Recently discovered that melatonin enhanced the fetal live ratio, fetal weight, and placental weight of mice during pregnancy. It has been reported that melatonin indirectly influences the health of pregnant women by promoting restful sleep (Nagasawa et al.,2021). Melatonin can restore the gut microbiota in mice exposed to heat stress. This is due to its ability to cross the placental barrier by changing its permeability. In this way, it protects the fetus from inflammation and oxidative stress and improves the skeletal formation of the fetus through a clear improvement in their weight (Wu et al.,2025). It is worth mentioning how melatonin induces implantation, which has been linked to its effects on uterine responsiveness specification in prenatal mice and the formation of the corpus luteum, which begins just before gestation, as well as the treatment with melatonin enhanced the amount of expression of Isolated hypogonadotropic hypogonadism (IHH) in the maternal endometrium and the genes responsible for progesterone production in the female reproductive system (Guan et al., 2017). The present study aimed to determine the fetal damage in pregnant mice caused by deltamethrin pesticide and to identify the possibility of melatonin to reduce the morphological and structural deformities of the fetal bodies, organs, and the maternal brain. It also aimed to determine the effect of the above pesticide on the severity of structural changes represented by cell death and inflammation in the maternal and fetal brain, as well as the role of melatonin in alleviating these lesions by measuring the immunoreactivity of proteins PAX3 and Cd45 in the selected organs.

Materials and Methods

Ethical Approval

All experimental procedures were conducted in accordance with internationally accepted guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee of the College of Veterinary Medicine, University of Mosul, Mosul, Iraq, in reference number UM.VET.2025.013 on 7/1/2025. Every effort was made to minimize animal suffering and to reduce the number of animals used while maintaining scientific validity.

Experimental Animals and Housing Conditions

This study is the first to jointly assess Bax-mediated apoptosis and CD45 neuroinflammation during

gestational duration (GD)7–17 .Thirty-two adult female albino mice (*Mus musculus*) and ten adult males were used in this study. Females weighed 27–31 g., and they were housed under standard laboratory conditions (22 ± 2 °C; 50–60% relative humidity; 12 h light/dark cycle) with ad libitum access to food and water (Salah, 2021). To reduce stress, the animals were given a week to acclimate to the laboratory environment before mating and starting research.

Mating Procedure and Confirmation of Pregnancy

To ensure successful mating, females were housed overnight with males at a ratio of 3:2. The presence of a vaginal plug the following morning was considered indicative of successful copulation and was designated as gestational day 0. Pregnant females were separated into housing spaces because stress during pregnancy could lead to miscarriage, and they needed to deliver treatments correctly. The study included only female mice who had proven vaginal plug presence (Salah,2021).

Chemicals and Reagents

Deltamethrin (2.5% emulsifiable concentrate) was obtained from Vapco Company (Amman, Jordan). Melatonin (5-methoxy-N-acetyltryptamine) capsules were purchased from Green Field Nutrition (USA). All other chemicals and solvents used in biochemical and histological procedures were of analytical grade and obtained from standard commercial suppliers.

Experimental Design and Group Allocation

Thirty-two pregnant albino mice were randomly assigned to four experimental groups (n = 8 per group). The research uses this specific number of animals because it works well for gestational neurotoxicity models and enables researchers to detect developmental oxidative and immunohistochemical changes without using more animals than needed.

The experimental groups were organized as follows:

Group I (negative Control): received distilled water orally. Group II received melatonin (positive control) at 12.5 mg/kg/day through IP injection. Group III received deltamethrin (1 mg/kg/day) through oral administration . Group IV received melatonin (orally)and deltamethrin (injection IP) at the same previous doses. Melatonin was administered 15 minutes before they received deltamethrin. The treatment duration covered gestational day 7 through day 17, which included the critical time for organ development and brain formation in early fetal development.

Scientific Justification of Dose and Route of Administration

The authors used oral administration of 5-methoxy-N-acetyltryptamine (Melatonin) to study animal exposure patterns, because this method represents their main environmental contact route through food and water contamination. The researchers used intraperitoneal melatonin administration to achieve equal systemic drug levels, as this method provides stable drug absorption that bypasses the unpredictable absorption patterns that occur with oral administration. The authors established a particular time frame between melatonin and deltamethrin administration in the combined-treatment group to enable melatonin to execute its antioxidant and cytoprotective actions before toxicant exposure for proper assessment of its protective effects.

Preparation of 5-methoxy-N-acetyltryptamine (Melatonin) and Deltamethrin Solutions

The deltamethrin solution was freshly prepared each day by diluting the commercial 2.5% emulsifiable concentrate (Vapco, Jordan) with distilled water to achieve the required oral dose of 1 mg/kg body weight. Melatonin was first dissolved in a small amount of ethanol before adding it to physiological saline to create a solution with 1% ethanol concentration. 1% ethanol solution was used, because this concentration level does not interfere with biological processes in experimental pregnancy studies (Reiter et al., 2017; Hardeland, 2018). The oral LD50 of albino mice was 15.71 mg/kg (Mustafa and Al-Baggou,2020). The dose of deltamethrin (1mg/kg) represent 8% of the LD50. The melatonin dose was selected depending on preliminary studies (Mercan and Eren, 2013). Both solutions were prepared immediately before administration to ensure chemical stability and reproducibility throughout the treatment period.

Samples Collection and Morphological Assessment

Maternal blood samples were obtained from the retro-orbital venous plexus of pregnant females under light anesthesia (ketamine 2–3 mg and xylazine 0.125–0.3 mg per animal) on gestational day 17. A capillary tube was used, and serum was extracted by centrifugation at 3000 × g for 12 minutes (Salah, 2021). Euthanasia was performed on pregnant mice to obtain their uterine horns, which were weighed and measured with a digital vernier caliper. The authors performed a detailed examination of each fetus, including individual weight measurements and microscopic inspection for any visible developmental defects. The researchers removed fetal brains for examination, weighed them, and then rinsed them with isotonic saline solution before beginning the fixation procedure.

Tissue Sectioning and Staining Workflow

The maternal and fetal brain samples were preserved in 10% neutral-buffered formalin for 72 hours before starting the processing sequence, which used graded ethanol solutions, xylene clearing, and paraffin wax embedding. Traditional H&E staining was used to perform standard histopathological assessments on their 5-µm-thick serial sections. Sections were mounted with a suitable medium (Luna, 1962). The photographing for the histological and immunohistochemical sections was done by using a digital camera that was connected to the Olympus CX31 microscope. The camera was supplied with powerful imaging evaluation software (OMAX Toup View 3.7). The objective lenses for the program have been checked with a stage micrometer for measurement (Salah and Sadoon, 2021).

Immunohistochemical Analysis

Bax and CD45 antibodies (BAX histology Assay Kit, Cat No. IIS-11-22-2p404, Creative Bio Labs, USA; CD45 histology assay kit, CCAT #: IIS-1122-2P44400, CreativeBio Labs, USA) were used to perform immunohistochemical staining. The tissue sections needed to be deparaffinized, followed by rehydration, before the technicians used heat-induced antigen retrieval through citrate buffer at pH 6.0. Technicians used 3% hydrogen peroxide to stop endogenous peroxidase activity, while they employed normal serum for reducing non-specific binding occurrences. The sections underwent 4 °C overnight storage before Bax and CD45 primary antibodies were applied, followed by HRP-conjugated secondary antibodies for additional incubation. The authors used diaminobenzidine (DAB) as a chromogen. Hematoxylin was applied as a counterstain for counterstaining selected sections. Semi-quantitative scoring, immunoreactive areas were digitally documented to allow subsequent quantitative image-based analysis of staining intensity and positive area fraction.

Two independent observers who did not know the group assignments conducted a semi-quantitative evaluation by scoring immunoreactivity. For each animal, at least five non-overlapping high-power fields (400×) were randomly selected from standardized anatomical regions of the brain section. The researchers used a four-point system to assess Bax and CD45 staining patterns, which rated samples from 0 (absent) to 3 (strong) before they calculated animal median scores for the statistical group. The researchers used this method to reduce observer bias while obtaining improved results between different experimental groups.

Biochemical Assessment of Redox-mediated cellular stress Markers

The ELISA kits from commercial sources (Rabbit malonaldehyde ELISA kit ccat No. MBS2602584, MyBiosource.com, and Rabbit Glutathione (GSH) ELISA kit, ccat No.MBS52602347, My BioSource, Inc., USA) allowed researchers to measure (malonaldehyde (MDA) and Glutathione (GSH) levels in serum samples. Tests were done following the instructions found in the manufacturer's documentation. The microplate photometer measured absorbance values, which scientists used to determine concentrations through their analysis of standard calibration curves. The ELISA device was manufactured by Biosan Hipo, MPP.96, Microplate photometer, Riga, Latvia. The parameters studied were shown as graphs using the mean ± SD. Comparisons between groups were assessed with appropriate statistical tests selected according to the nature of the data. A threshold of $P \leq 0.001$ was used to determine significance.

Statistical Analysis

The research team conducted statistical analysis through GraphPad Prism version 5(USA). Normally distributed variables were expressed as mean ± standard deviation (SD). Comparisons among multiple groups were conducted using one-way analysis of variance (ANOVA). Dunnett's multiple comparison test was used. The authors performed a non-parametric Kruskal–Walli's test to analyze semi-quantitative histopathological and immunohistochemical scores.

Results

Effects of 5-methoxy-N-acetyltryptamine and Deltamethrin on Fetal Morphology

The gross morphological assessment of day 17 fetuses showed distinct developmental changes that depended on the treatment type. Group I fetuses displayed normal external structures because their bodies developed normally without showing any birth defects (Figure 1A&B). Group II showed normal fetal development patterns because melatonin treatment did not create any negative effects that impacted fetal growth or body structure, but there was little bleeding between cerebral hemispheres (Figure 1C&D). The fetuses from group III showed severe developmental issues, which included smaller body size, thinner skin, and blood vessel problems in the spine and eyes, and bleeding in specific areas of the brain. The bleeding between both hemispheres became more severe (Figure 1E, F, G, & H). The observed abnormalities show that vascular stability has broken down, while neurodevelopmental processes faced disruptions that affected their development during this critical period of organ formation. In group IV, there was a substantial decrease in both the extent of cell damage and the number of cells that became affected. The combined-treatment group showed better external development and decreased cerebral hemorrhages and better body structure preservation than the deltamethrin-only group, which suggests melatonin provides some defense against deltamethrin's developmental harm (Figure 1, I, J, K, & M).

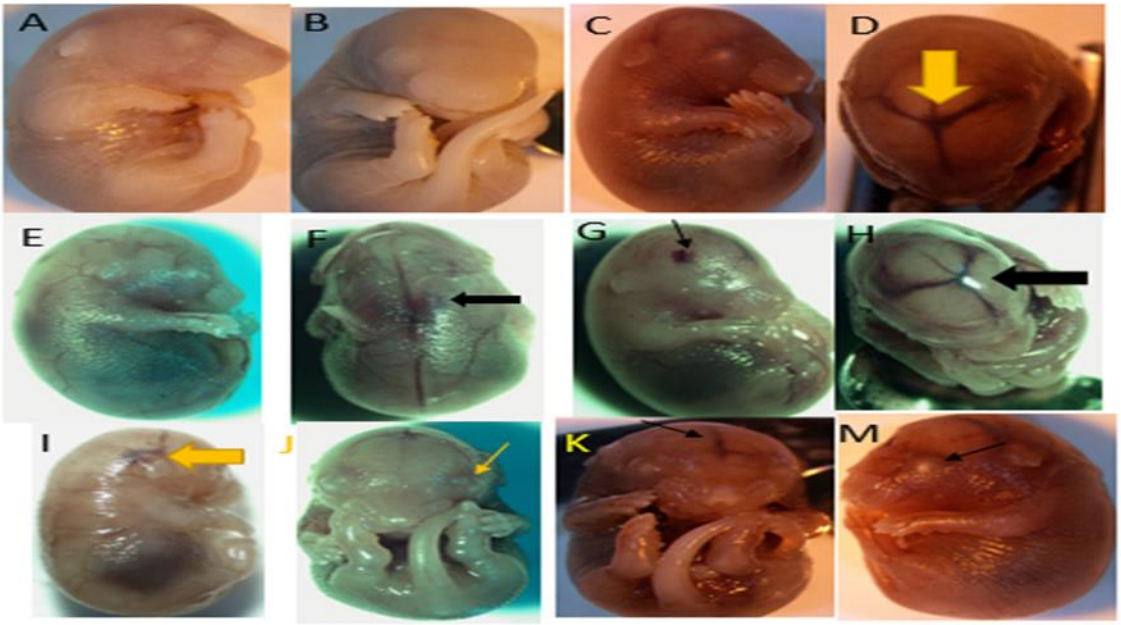


Figure 1: Shows the congenital abnormalities in the studied groups (20x).

Changes in the weight of the Embryonic Brain

Brain weight assessment of embryonic brains (embryo age 17 days) showed that different groups had major weight differences. The research found no statistically significant difference between groups I and II ($p \leq 0.001$), which showed that melatonin treatment did not affect typical brain development. Group III embryo brains weighed significantly more after mothers received deltamethrin during pregnancy compared to group I ($p \leq 0.001$).

The brain weight of group IV embryos became statistically higher than that of group III ($p \leq 0.001$). The partial recovery shows that melatonin protects neurodevelopmental growth from deltamethrin damage, but it does not bring back the original development pattern **(Figure 2).**

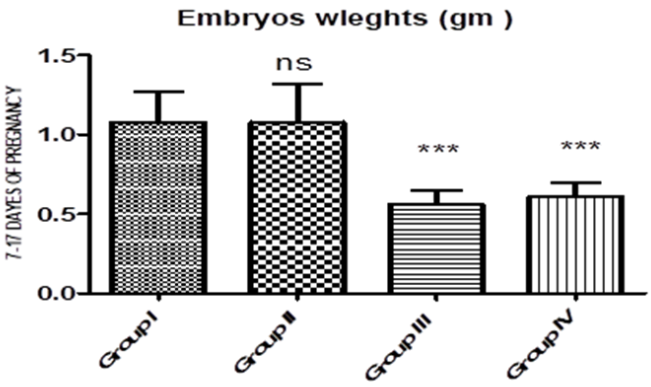


Figure 2. Shows the impact of studied treatments on embryo (17-day age) brain weights (gm) of albino mice. The weights were expressed as (average ± mean). Ns: not significant. n=8. *** means very highly significant $p \leq 0.001$

Uterine Horn Weight Alterations

Measurement of uterine horn weights revealed a significant decrease in the deltamethrin-treated group

relative to the negative control group ($p \leq 0.001$). A comparable reduction was also observed in group IV. In contrast, no statistically
significant change was detected in group II compared with group I (Figure 3).

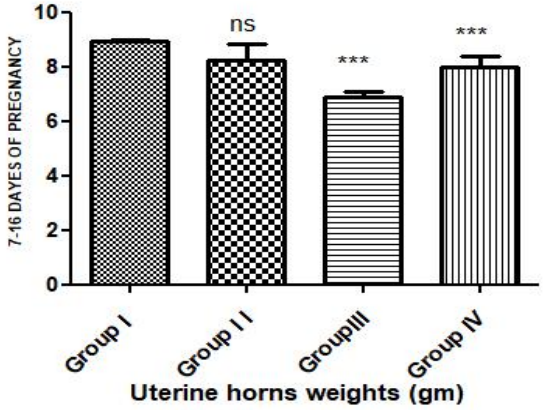


Figure 3: Shows the antioxidant effect of melatonin on the Uterine horns weights(gm) of pregnant albino mice treated with deltamethrin (average ± SD). * significant at P ≤ 0.05, ns: no: significant.**

Effect of Treatments on Fetal Body Weight

Mean fetal body weight was significantly reduced in Group III compared with Group I ($p \leq 0.001$), indicating generalized growth retardation following gestational deltamethrin exposure.

Administration of melatonin in Group II did not result in a statistically significant alteration in fetal body weight relative to groups I and II ($p \leq 0.001$).

In Group IV, fetal body weight was significantly higher than that observed in the deltamethrin-treated group ($p \leq 0.001$), although it did not fully reach the values of groups I and II. These findings indicate that melatonin partially counteracts deltamethrin-induced fetal growth restriction (Figure 4).

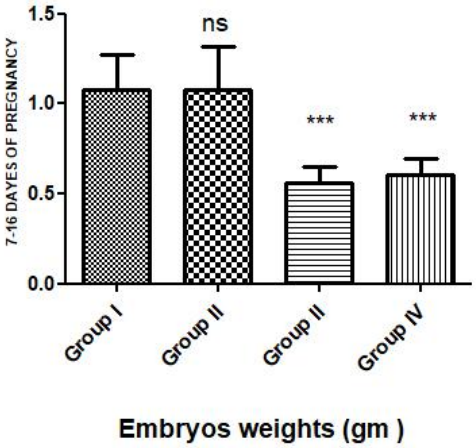


Figure 4: Shows the antioxidant effect of melatonin on the embryo weights(gm) of pregnant albino mice treated with deltamethrin (average ± SD). * significant at P ≤ 0.05, ns: no: significant.**

Biomarkers of Mother's Oxidative Stress

Malondialdehyde (MDA)

Group III showed higher maternal serum malondialdehyde (MDA) levels than groups I & II, but the findings did not reach statistical significance ($p \leq 0.001$). The research shows that lipid peroxidation began systemically at the selected gestational stage, yet the difference in serum concentration was not sufficient to be detected. The study shows that reduced glutathione levels decreased simultaneously with MDA, which confirms that oxidative stress occurred before it caused significant damage to cells.

The study shows that melatonin treatment in group II led to non-significant ($P \leq 0.001$) decrease in MDA levels when compared to deltamethrin treatment, because it indicates its protective role against lipid peroxidation induced cellular damage cells from lipid peroxidation damage. The research data indicate that oxidative stress operations function with limited control, but the biochemical system shows only minimal ability to recover from damage (Figure 5).

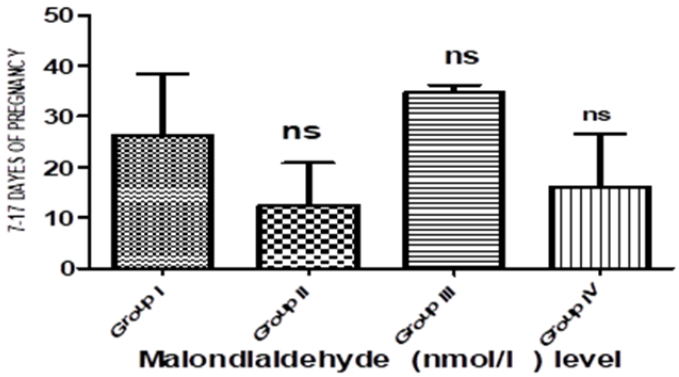


Figure 5: Shows the impact of treatments studied on the maternal serum malondialdehyde levels in albino pregnant mice. Data were shown as mean ± SD. ns: non-significant. n:8.

Reduced Glutathione (GSH)

Group III displayed decreased maternal serum reduced glutathione (GSH) levels when compared to the group I, which showed that endogenous antioxidant defenses became depleted following gestation. The single dose of group II did not result in any substantial change in GSH levels when it was compared to group I samples (p>0.05). Suggesting that the melatonin did not influence the body's typical redox balance during its normal functioning.

The GSH levels in group IV exceeded those of group III, but the difference did not achieve statistical significance. The body system of pregnant mice shows partial recovery of antioxidant defenses, but it remains in an incomplete state of recovery **(Figure 6).**

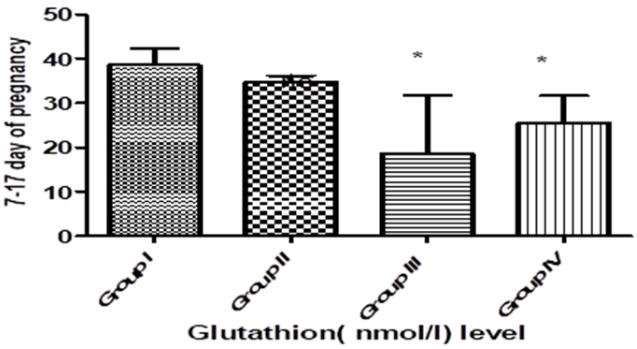


Figure 6. Shows the Impact of studied treatments on the maternal serum reduced glutathione (GSH) levels in albino pregnant mice. Data were shown as mean ± SD. ns: non-significant, while those labelled with * mean significant at p ≤ 0.001. n: 8.

Histopathological Findings in Embryonic Brain

The embryonic brain sections (from embryos aged 17 days) underwent histological analysis, which showed that the neural tissue architecture underwent specific changes due to the treatments. The brains from group I showed well-defined neuroepithelial layers, intact ventricular zones, and normal cellular organization, indicating typical neurodevelopment (Figure 7A). The histological patterns of group II brain showed similar results to the previous results, which demonstrated that melatonin treatment did not harm the brain structure of developing embryos (Figure 7 B).

Brain sections of Group III demonstrated neurodegenerative alterations, programmed cell death, suggesting that there is an increase in the apoptotic activity in the primitive neuronal tissue in a harmful way that caused the previous lesions, as well as nuclear condensation and breakdown of neuroepithelial tissue structure (necrosis), also observed (Figure 7 C). The brain sections from group IV showed better maintenance of their natural structure and less cell damage and programmed cell death than what had been seen in the deltamethrin-only treatment group (Figure 7D).

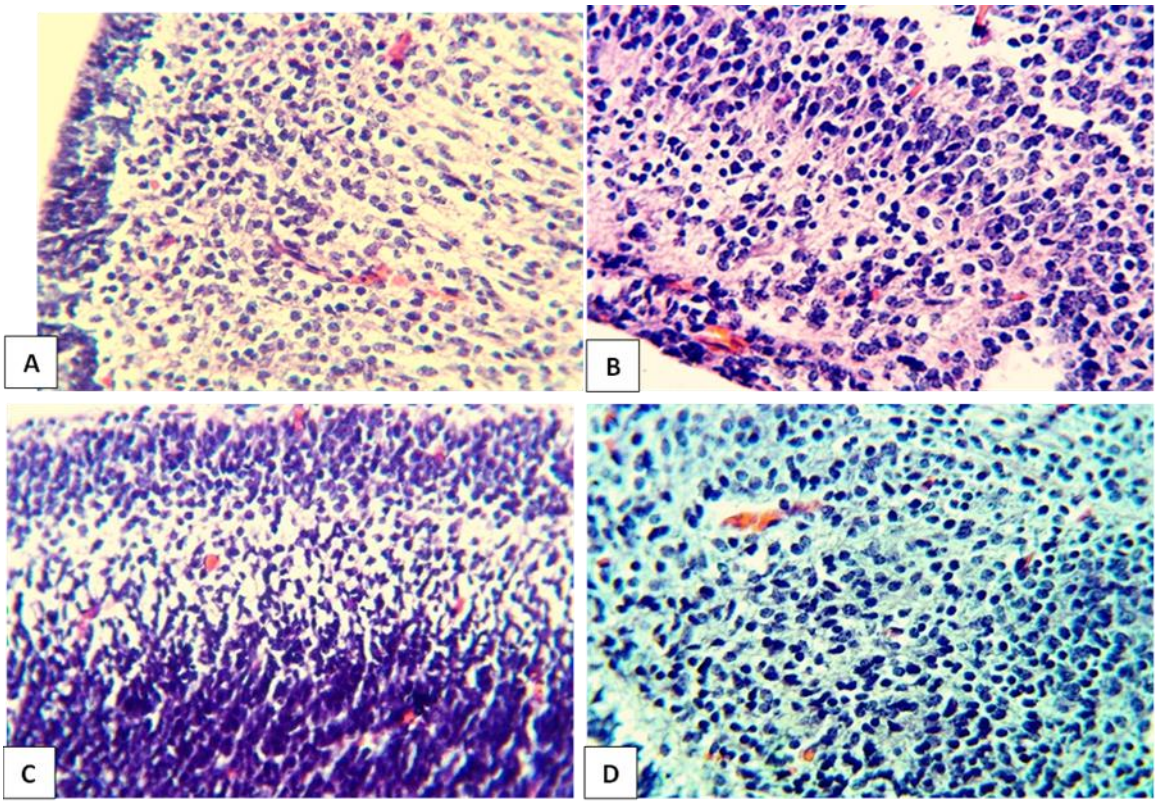


Figure 7: Photomicrograph of developing cerebral neuroepithelial tissue from embryonic albino mice brain following deltamethrin and melatonin exposure. (A) Group I: showing normal neuroepithelial layer organization (N) and intact ventricular zone (V). (B) Group II displays preserved brain architecture. (C)Group III showing neuronal degeneration (red arrow), necrosis (black arrow), tissue disorganization, and nuclear condensation (green arrow). (D) Group IV demonstrates reduced cellular degeneration (yellow arrow) and partial preservation of primitive neuroepithelial cells (blue arrow) of the cerebral cortex, as well as in the germinal region(thick orange arrow). Neuronal degeneration was also reduced in structure with reduced cellular degeneration compared to sections of group III, indicating a protective. H&E stain. 400 x.

Immunohistochemical Expression of Bax in Developing Neuroepithelial Tissue

Bax expression levels in embryonic cerebral cortex tissue showed distinct patterns among treated groups. The Bax immunoreactivity in group I remained low, as few neural cells showed only weak cytoplasmic staining, which is consistent with the normal level of programmed cells observed in typical neuronal primitive tissue. The Bax expression in scattered cells of the group II sections was weak to moderate, but there was no indication of tissue damage. Group III neuroepithelial developing tissue, including both ventricle and germinal zones, showed an intense reaction to the antibodies to the BAX protein. The reaction was detected by noticing widespread strong cytoplasmic staining throughout the selected tissue. This indicates that the intrinsic mitochondrial apoptotic pathway becomes active after deltamethrin during pregnancy, according to the observed pattern. There was a substantial reduction of Bax protein expression in the primitive neuronal tissue of group IV when compared to what was observed in group III (Table 1; Figure 8).

Scoring criteria were adapted from established immunohistochemical and toxicopathological assessment methods (Remmele & Stegner, 1987; Fedchenko & Reifenrath, 2014; Frazier et al., 2012).

•Immunohistochemical specificity was confirmed by appropriate positive and negative controls included in each staining run.

•Scores represent median values derived from multiple non-overlapping microscopic fields per section.

Table 1. Semi-quntitative assessment of Bax immunoreactivity and associated histopathological alternations in developing neuronal epithelial tissue of albino mice

Experimental group	Bax immunoreactivity Score (0-3)	Histopathological injury Score (0-3)	Overall interpretation
Group I	0 (absent to very weak)	0(none)	Basal Bax expression is consistent with apoptotic activity in structurally intact neuronal

			tissue
Group II	1 (weak to moderate)	0(none)	Mild Bax expression within preserved tissue architecture indicates the absence of cytotoxic injury
Group III	3(strong)	3(severe)	Marked Bax overexpression is associated with pronounced cellular degeneration and apoptotic injury
Group IV	1-2(mild to moderate) Attenuation of Bax expression and cellular injury , indicating partial effect		

Scoring criteria were adapted from established immunohistochemical and toxicopathological assessment methods (Remmele & Stegner, 1987; Fedchenko & Reifenrath, 2014; Frazier et al., 2012).

•Immunohistochemical specificity was confirmed by appropriate positive and negative controls included in each staining run.

•Scores represent median values derived from multiple non-overlapping microscopic fields per section.

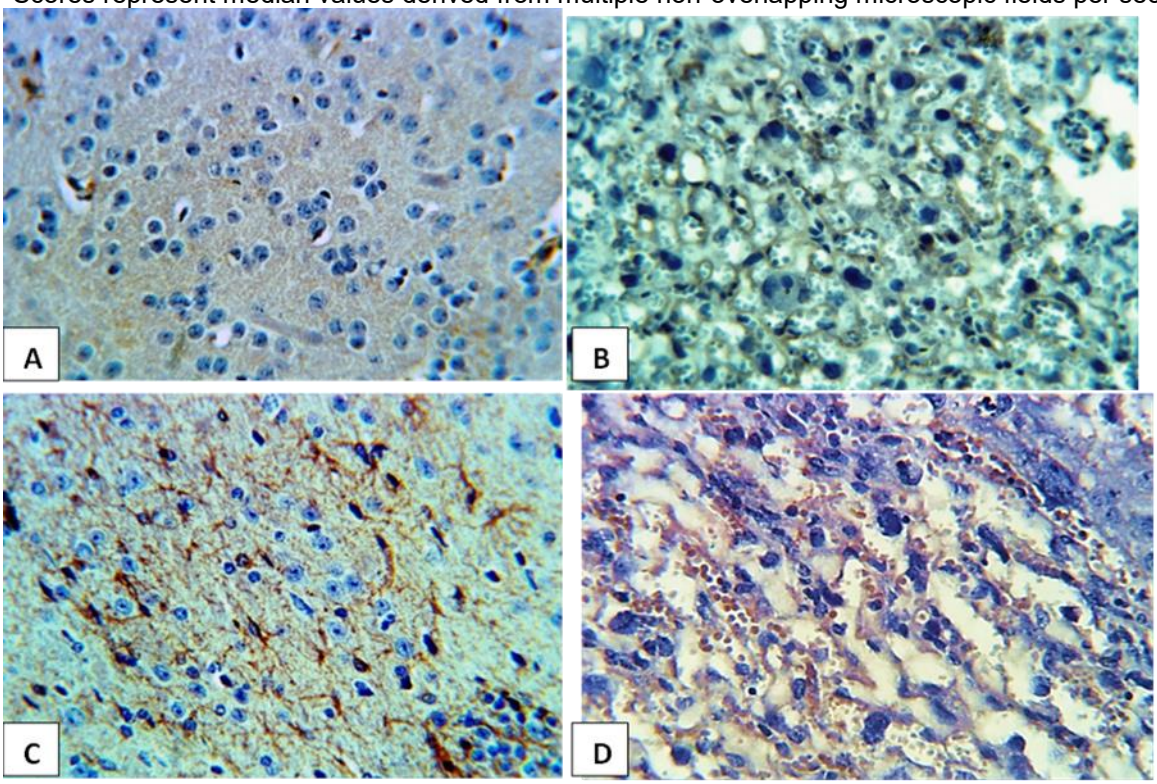


Figure 8: Photomicrograph Immunohistochemical localization of Bax protein expression in embryonic brain tissue of different experimental groups. (A) Group I showing absent to very weak Bax immunoreactivity. (B) Group II exhibited weak Bax expression without associated tissue damage. (C) Group showing strong and widespread cytoplasmic Bax immunoreactivity, indicating activation of the intrinsic apoptotic pathway. (D) Group IV showing reduced Bax expression with weak to moderate staining intensity compared to the deltamethrin-treated group, reflecting partial attenuation of apoptosis by melatonin. Brown staining indicates positive Bax immunoreactivity; sections counterstained with hematoxylin (400X).

Immunohistochemical Expression of CD45 in Maternal Brain

The CD45 protein expression in brain tissues underwent specific changes, which immunohistochemical analysis revealed about pregnancy-related inflammation treatment responses. The brain sections of the negative control group (group I) displayed either no CD45 staining or very little CD45 staining, which indicated that their neuroimmune system remained inactivated. Group II showed weak and restricted CD45 staining,

suggesting limited immune cell activation, which indicated that their parenchymal microglia did not activate strongly.

In contrast, maternal brains were highly activated with microglial cells and infiltrating macrophages from group III exhibited strong and extensive CD45 immunoreactivity, characterized by widespread brown staining. This pattern reflects robust neuroinflammatory activation following gestational deltamethrin exposure.

CD45 expression was markedly reduced in both intensity and spatial distribution of mildly activated microglia in group IV compared with the Group III. The observed weak-to-moderate immunoreactivity suggests attenuation of neuroinflammatory activation in microglia by melatonin rather than complete suppression (Table 2; Figure 9).

Table 2. Semi-quantitative assessment of CD45 immunoreactivity as an indicator of neuroinflammatory activation in maternal brain tissue

Experimental group	CD45 immunoreactivity score (0–3)	Neuroinflammatory interpretation
Group I	0 (absent)	Absence of CD45-positive staining, indicating a non-activated neuroimmune state
Group II	1 (weak)	Minimal and localized CD45 expression, consistent with physiological immune surveillance (Resident microglia was active)
Group III	3 (strong)	Intense and widespread CD45 immunoreactivity, indicating robust neuroinflammatory activation(high activated microglia and infiltrating macrophages)
Group IV	1–2 (weak to moderate)	Reduced CD45 expression relative to deltamethrin alone, reflecting attenuation of neuroinflammatory response(low and high activated microglia)

•CD45 immunoreactivity was evaluated using a semi-quantitative visual scoring system ranging from 0 (absent) to 3 (strong), a method widely applied in experimental neuroinflammation and toxicologic pathology studies.

•Scores represent the median staining intensity and distribution assessed across multiple non-overlapping microscopic fields per section.

•Immunohistochemical evaluation was performed under identical staining and imaging conditions for all experimental groups to ensure comparability.

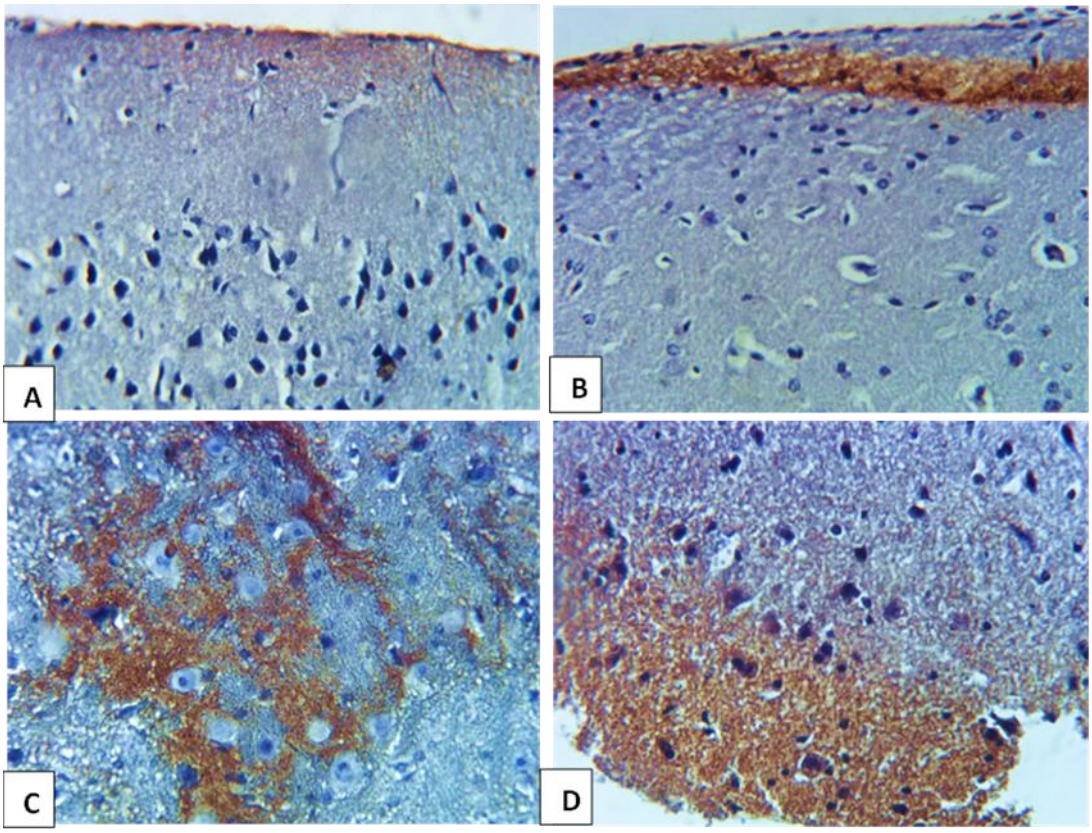


Figure 9: Immunohistochemical expression of CD45 in maternal brain tissue of pregnant albino mice from different experimental groups.

(A) Group I showing absence of CD45 immunoreactivity, indicating a non-activated neuroimmune state.

(B) Group II displaying weak and localized CD45 staining consistent with physiological immune surveillance.

(C) Group III exhibiting strong and diffuse CD45 immunoreactivity, indicating pronounced neuroinflammatory activation.

(D) Group IV showing reduced CD45 expression with weak to moderate staining intensity, demonstrating attenuation of neuroinflammation by melatonin.

Brown staining represents CD45-positive immune cells; sections counterstained with hematoxylin. 400X.

Discussion

The present study provides integrated biochemical, histopathological, and immunohistochemical evidence that gestational exposure to deltamethrin induces measurable developmental neurotoxicity, while melatonin exerts a partial, but biologically meaningful protective effect. The data collectively support a mechanistic framework in which oxidative imbalance represents an early initiating event that subsequently triggers apoptotic signaling and neuroinflammatory activation within embryonic and maternal brain tissues during critical windows of neurodevelopment. Those findings were similar to the previous toxicological and antioxidant research, like those done by Jassim &Shindala 2025; Al-Sowyan &Al-Hussain 2018).

The current findings delineate a coordinated pathogenic sequence linking redox disruption to Bax-mediated mitochondrial apoptosis and CD45-associated neuroimmune activation. The integrated pattern demonstrates that deltamethrin causes brain development problems through various connected biological systems, which increase fetal brain vulnerability to damage during pregnancy.

The observed developmental toxicity of deltamethrin appears to stem from oxidative cellular injury, which acts as the main cause of this effect. The elevation of maternal malondialdehyde levels did not achieve statistical significance, but the simultaneous decrease in reduced glutathione levels shows that antioxidant defenses are weakened, which makes cells more vulnerable to lipid peroxidation. The body experiences harmful redox imbalance when pregnancy occurs because fetal neural tissues lack built-in antioxidant protection and need their mother to maintain redox equilibrium Hussain et al. (2021).

The recent findings showed no significant statistical difference in maternal malondialdehyde levels. The results indicate that lipid peroxide occurs as a limited process that does not produce enough damage to be detected through serum tests during this specific period of pregnancy. The breakdown of cellular redox

protection begins when reduced glutathione levels fall to a dangerous level, making cells more susceptible to damage before lipid peroxidation products accumulate. The present model shows glutathione depletion as its main redox-related trigger. Still, MDA functions as an indicator for oxidative stress that supports oxidative processes instead of determining the outcome, and those results align with prior studies of (Huang et al., 2025; Zhang et al., 2018; Hussain et al., 2021.

The findings also revealed depletion of maternal glutathione levels in group III. This deficiency leads to the dysregulation of mitochondrial redox balance, leading to increased ROS and activation of intrinsic apoptotic signaling, including upregulation and mitochondrial translocation proteins such as BAX, which promotes cytochrome C release and caspase activation (Chen et al., 2024)

The observed pattern matches a previous study, which showed deltamethrin produces increased reactive oxygen species, but weakens natural antioxidant systems in the body of pregnant women (Kazma et al.,2020; Jai et al.,2019).

The deltamethrin-induced oxidative stress caused cell death patterns in the developing neuroepithelial tissue of the cerebral cortex, and that was confirmed in the current study. The previous findings were similar to those of El-Shazly et al., 2018; Chen et al., 2025 on programmed cell death, which researchers confirmed through histological and immunohistochemical studies of embryonic brain samples. Group III showed intense Bax immunoreactivity, and this was confirmed by Li, J et al. (2021). The process of mitochondrial membrane permeabilization through Bax functions as a survival mechanism for neurons during brain development, but its increased expression results in abnormal neuronal death and brain cell formation problems (Wu et al.,2025; Blaschke et al., 1996). Pyknosis and nuclear fragmentation in group III developing neuroepithelial tissue, confirming that deltamethrin causes abnormal apoptotic events instead of creating random cell death according to immunohistochemical results. This was in line with what was found by Khalil et al. (2023). The findings revealed that deltamethrin exposure during pregnancy caused a severeneuroinflammatory response, which scientists identified through strong CD45 protein staining in maternal brain cerebral tissue.

The inflammatory condition leads microglia to express CD45, but this expression also indicates the presence of blood-borne leukocytes when the blood-brain barrier becomes disrupted. CD45 functions as a neuroimmune activation marker, but it does not specifically identify resident microglia because it can also detect peripheral leukocytes that enter the brain when the blood–brain barrier becomes compromised (Zrzavy et al., 2022).

The expression of CD45 indicates that immune-related glial cells have become activated while being recruited to the site, which shows a shift from normal immune monitoring to an active inflammatory response (Prinz et al., 2019). Recent findings showed that the high expression of CD45 in mothers brain related to the neuroimmune activation functions, that consider as a main factor which causes developmental neurotoxicity when it happens together with oxidative stress and apoptotic signaling Research studies showed that pyrethroid-induced oxidative stress triggers microglial cell activation which results in pro-inflammatory cell behavior that makes neurons more vulnerable through their production of cytokines and reactive oxygen species and apoptotic secondary mediators (Youle et al. 2008; Al-Sowyan, 2018). The study data show Bax expression levels stay elevated while CD45 immunoreactivity increases, which proves that apoptosis and neuroinflammation function as connected disease mechanisms. The gestational environment enables these two elements to create a cycle that intensifies neural damage through sequential events of oxidative damage and inflammatory activation, and programmed cell death (Bradshaw et al., 2024).

Maternal exposure to environmental toxicants during pregnancy triggers neuroinflammatory responses in leukocytes, which result in higher CD45 protein levels throughout maternal and fetal brain tissues, thus making their brains more susceptible to oxidative damage and apoptosis during development (Luo et al., 2019).

The pathological process triggered by deltamethrin exposure underwent significant changes through melatonin treatment, which resulted in decreased redox imbalance and blocked Bax-induced cell death, and decreased CD45-mediated neuroinflammation. Melatonin protects the nervous system of pregnant women through its various mechanisms, which involve direct free radical elimination, body defense activation, and mitochondrial stability preservation (Reiter et al. , 22017; Slotkin ,2021).

Notably, the protective effect of melatonin observed in the present study was partial rather than complete. The incomplete normalization process does not indicate reduced effectiveness because deltamethrin-induced developmental toxicity results from various overlapping mechanisms that surpass cellular oxidative challenge as the sole cause of damage. The compound causes developmental toxicity through its interference with ion channels and its effects on neurotransmission and endocrine signaling systems (Hardeland et al.,2018; Huang et al.,2025). Melatonin works to reduce redox-dependent and inflammation-driven pathways, but it does not completely stop toxic substances from disrupting neurodevelopmental processes (Reiter et al., 2017). The study results gain additional strength from their biological basis because the study participants received only partial protection from the treatment.

The pathological cascade underwent major changes because of melatonin treatment. The MDA levels decreased, while glutathione levels showed some improvement in group II, which suggests enhanced antioxidant function throughout the body. The research data show that melatonin functions as an established

free radical scavenger, which protects mitochondria and controls oxidative degradation of lipids to maintain cellular redox equilibrium (Reiter et al., 2017). The antioxidant properties of melatonin became most apparent through histological and immunohistochemical assessments instead of serum biomarker measurements, which proved its value in developmental toxicology studies.

The cellular analysis showed that melatonin reduced Bax protein levels in developing neuroepithelial tissue, and the number of CD45- positive inflammatory cells decreased in the maternal brain tissue. The dual effect demonstrates that melatonin stops both apoptotic and inflammatory signaling pathways, which protects neural tissue from damage. Research indicates that melatonin regulates mitochondrial apoptotic regulators and decreases abnormal glial activation to defend neurons during times of excessive developmental stress (Soderlund, 2012; Huang et al.,2025). Group IV showed partial normalization instead of achieving complete normalization, which requires special attention. The treatment with melatonin reduced multiple harmful effects of deltamethrin exposure, but it failed to bring all measured parameters back to normal values. The incomplete recovery of the animal's results from multiple factors underlying deltamethrin toxicity, including oxidative stress, impaired neurotransmission, and alteration of endocrine signaling during development

(Al-Sowyan and Al-Hussain, 2018). The range of toxic effects will decrease when antioxidants are used for treatment, but these effects will not disappear completely.

The study achieves its main strength through its combination of biochemical, morphological, and immunohistochemical assessments, which took place during a specific gestational period. The research provides scientific evidence about how melatonin could protect developing brains from environmental toxins.

Conclusions

The developmental neurotoxic effects of deltamethrin exposure during pregnancy occur in a particular order, which starts with antioxidant depletion, followed by Bax-induced mitochondrial apoptosis, and ends with CD45-mediated neuroinflammation that affects both fetal and maternal brain areas. The therapeutic effects of melatonin supplementation help reduce disease progression through its ability to enhance redox equilibrium and block both apoptotic and inflammatory responses, but it does not fully restore normal conditions. The research confirms that melatonin functions as a natural substance that affects pesticide-induced fetal brain development problems instead of completely blocking their effects, so scientists need to study protective pregnancy interventions through multiple approaches.

Acknowledgments

The authors of the study would like to express their sincere thanks to the University of Mosul and the College of Education for Pure Sciences for their substantial scientific support, which made the research much easier to finish.

Conflict of interest

The researchers declare there are no competing interests connected to this work.

Authors' contributions

All three researchers actively participated in preparing and completing the research. The first researcher wrote the research paper, performed the statistical analysis, and carried out part of the laboratory work. The second researcher designed the research topic and concept. The third researcher handled the laboratory work and collected blood and embryo samples.

Funding

No specific grant was received for this research.

Data Availability

If requested, the accompanying author can provide the data supporting the study's findings.

References

Al-Sowyan, N.S., Al-Hussain SM. Neuroinflammatory responses following pyrethroid exposure.2018. Neurochem Int. *120,121-133.*

Blaschke, A.J., Staley, K, Chun ,J. Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. 1996.*Development*. 122,1165–1174.

Bradshaw, J. L., Wilson, E. N., Gardner, J. J., Mabry, S., Tucker, S. M., Rybalchenko, N., Vera, J. E., Goulopoulou, S., and Cunningham, R. L. 2024. Pregnancy-induced oxidative stress and inflammation are not associated with impaired maternal neuronal activity or memory function. American Journal of Physiology – Regulatory, Integrative and Comparative Physiology, 327(1), R35–R45.

Chen, T. H., et al. 2024 . Mitochondrial glutathione in cellular redox homeostasis and cell death. International Journal of Molecular Sciences, 25(2), Article 1314.

Chen, Y., et al. 2025. Selenium nanoparticles ameliorate apoptotic pathways and DNA neural damage in

deltamethrin-treated rats. Egypt J Vet Sci . Retrieved from https://ejvs.journals.ekb.eg/article_457486.htm

El-Shazly, A. M., et al. 2018. Ameliorative effect of pomegranate molasses on deltamethrin-induced neurotoxicity in adult albino rats: biochemical, histopathological & immunohistochemical study.Egypt J Forensic Sci Appl Toxicol. Retrieved from https://ejfsat.journals.ekb.eg/article_26920.html

Hao, F., Bu, Y., Huang, S., Li, W., Feng, H., Wang, Y. 2024. Maternal exposure to deltamethrin during pregnancy and lactation impairs neurodevelopment of male offspring.Ecotoxicology and Environmental Safety , 274:116196. doi:10.1016/j.ecoenv.2024.116196 .

Hardeland R.2018 .Melatonin and redox regulation. *Antioxidants & Redox Signaling*, 29(12):123–145.

Huang, S., et al. 2025. Maternal exposure to deltamethrin during pregnancy and lactation impairs hippocampal learning and memory. Ecotoxicol Environ Saf. 290:117729.

Hussain ,T., Murtaza G., Metwally E., Kalhoro D. H., Kalhoro, M. S., Rahu, B. A., Tan B. 2021 . The role of oxidative stress and antioxidant balance in pregnancy. Mediators of Inflammation. 2021:9962860.

Jia, Z.Z., Zhang, J.W., Zhou, D., Xu , D.Q., Feng, X.Z. 2019 . Deltamethrin exposure induces oxidative stress and affects meiotic maturation in mouse oocytes. *Chemosphere*. 223:704–713.

Kazma, J.M., Van den Anker ,J., Allegaert, K., Dallmann, A., Ahmadzia, H.K. 2020.Anatomical and physiological alterations of pregnancy. *Journal of Pharmacokinetics and Pharmacodynamics*, 47(4):271–285.

Li, J., Yu, Z., Han, B., et al. 2021. Chronic deltamethrin exposure induces oxidative stress, inflammation, and apoptosis in quail cerebrum with increased Bax, caspase-3, and related pathways. Mol Neurobiol. 58(8),4054-4066.

Luo, D., Masika, J., Guan, X.W., Nie, L., Ao, D.H., Qi, Y., Shi, R. , Hescheler, J., Zeng, Y. 2019.Pyrethroid-induced microglial activation and neuronal apoptosis. *Toxicology*. 423:152–161.

Priyadarshini, A., Upreti, P., Nautiyal, R., and Goyal, M. 2019 . Placental location and development of preeclampsia: a longitudinal study. International Journal of Reproduction, Contraception, Obstetrics and Gynecology, 8(4), 1283–1287.

Rehman, H., Aziz, A.T., Saggu, S.H.A., Abbas, Z.K., Mohan, A., Ansari, A.A.2014 .Systematic review on pyrethroid toxicity with special reference to deltamethrin. *Journal of Entomology and Zoology Studies*. 2(6):60–70.

Reiter, R.J., Tan, D.X., Galano, A.2017 .Melatonin as a mitochondrial protector. *Journal of Pineal Research*. 62(2):e12394.

Reiter, R. J., Mayo, J. C., Tan, D. X., and Sainz, R. M. 2016. Melatonin as an antioxidant: under promises but over delivers. Journal of Pineal Research, 61(3), 253–278. (Mechanistic review of antioxidant/inflammatory modulation by melatonin.

Salah, B. A. 2021. Effect of boron on some organs of pregnant BALB/c mice. Iraqi Journal of Veterinary Sciences, 35(4), 633–642.

Slotkin, T.A. 2021.Developmental neurotoxicity of pesticides: A mechanistic framework linking oxidative stress, neuroinflammation, and apoptotic signaling. *Neurotoxicology*. 83:101–112.

Soderlund, D.M.2012.Pyrethroids, sodium channels, and neurotoxicity. *Toxicology*. 290(1):1–12.

Wu, J.J., Zheng, X., Wu, C., Ma, W., Wang, Y., Wang, J., Wei,,Y., Zeng, X., Zhang, S., Guan, W., Chen, F. 2025.Melatonin alleviates high-temperature-induced fetal growth restriction via the gut–placenta–fetus axis in pregnant mice. *Journal of Advanced Research*. 68:131–146.

Youle, R.J., Strasser, A. 2008. The BCL-2 protein family: opposing activities that mediate cell death. *Nature Reviews Molecular Cell Biology*. 9(1):47–59.

Zhang, C., Xu, Q., Xiao, X., Li, W., Kang, Q., and Zhang, X. 2018. Prenatal deltamethrin exposure-induced cognitive impairment in offspring and associated mechanisms. Frontiers in Neuroscience, 12, 720.

Zrzavy, T., Machado-Santos, J., Christine, S., et al. 2022. Dominant role of microglial activation in neuroinflammation. Frontiers in Immunolog