KUFA JOURNAL FOR VETERINARY MEDICAL SCIENCES

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Deltamethrin Induces Apoptosis in Adult Male Rats Model and the Role of Melatonin

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ABSTRACT

Deltamethrin is considered one of the widely used insecticides in the agricultural and veterinary fields, its toxicity (apoptosis) is linked to oxidative stress, so melatonin was chosen as a potent antioxidant. To conduct this study twenty adult male rats were divided into 4 groups (A, B, C and D) (5 animals /group). Groups (A, B and C) were treated with distilled water (2 ml/kg, orally (P.O.), deltamethrin (25 mg/kg, P.O.), and melatonin (12.5 mg/kg, intraperitoneal, (I.P.) respectively, while group (D) was injected with melatonin (12.5 mg/kg, I.P.) 15 minutes before deltamethrin (25 mg/kg, O.P.), the duration of the treatment in all groups extended to five consecutive days. Then, on the sixth day the rats were sacrificed for brain, heart and liver harvesting to detect apoptosis using (TUNEL assay). The group (B) recorded a positive result for TUNEL stain, represented by the appearance of a high number of positive brown stained apoptotic bodies in the three organs. While group (D) recorded a clear decrease in the positive brown stained apoptotic bodies in the three tissues compared to the group (B). We conclude from our current study that melatonin has effects against apoptosis which induced by deltamethrin in rats.

KEYWORD: Deltamethrin, Apoptosis, TUNEL, Melatonin, Antioxidant.

ARTICLE INFO
Article history:
Received: 9 March 2025
Accepted: 20 April 2025
Published: 30 June 2025
DOI:
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P- ISSN: 2077-9798
E- ISSN: 2959-8478

INTRODUCTION

Deltamethrin is considered one of the widely used insecticides in the agricultural and veterinary fields, and this increases the chance of human exposure to residues of this insecticide that cause toxicity such as (apoptosis) resulting from its causing oxidative stress [1].

Oxidative stress caused by exposure to the insecticide deltamethrin largely causes severe damage to the internal tissues of the organism through inhibition of the pivotal

endogenous pathway represented by nuclear factor erythroid-2-related factor 2/heme oxygenase-1 (Nrf2/HO-1), because this pathway (Nrf2/HO-1) has many vital functions for the cell, including inhibiting programmed cell death (apoptosis), reducing inflammation, and alleviating endoplasmic reticulum stress by regulating phase II detoxification enzyme. Therefore, inhibition of this pathway (Nrf2/HO-1) by deltamethrin leads to inflammation and programmed cell death of cellular tissues [2].

Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay has been designed to detect apoptotic cells that undergo extensive DNA degradation, the method is based on the ability of TdT to label blunt ends of double-stranded DNA breaks [3].

Melatonin (Nacetyl-5-methoxytryptamine) is a powerful natural antioxidant, this neurohormone is produced by the pineal gland, it is involved in circadian and circannual rhythms [4,5]. The mechanism of melatonin as an effective anti-oxidant is through its molecule's ability to scavenge free radicals of oxygen and nitrogen (such as hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide and peroxynitrite anion) due to its possession of reactive sites through which it binds to free radicals in addition to the presence of methoxy and amide side chains in the melatonin molecule, which contribute to its antioxidant capacity [5]. The effect of melatonin as an anti-oxidant is enhances through its molecular structure (as a lipophilic), which enables it to easily cross all biological barriers and accumulate in large quantities within subcellular organelles, including mitochondria, which are the main sites for the production of reactive oxygen species (ROS) [6-9] these antioxidant effects of melatonin mentioned above are responsible for its anti-apoptotic effects [10]. In addition, melatonin has also anti-apoptotic effects by regulating silent information regulator 1 (SIRT1) [11-13].

Because melatonin has these anti-oxidative stress and anti-apoptotic effects mentioned above, it was chosen in our current study to test its against-apoptosis induced by deltamethrin in adult male rats model.

MATERIALS AND METHODS

Ethical considerations

All procedures were approved by the Committee for the Ethics on Animal Care and Experiments of University Mosul/College of Veterinary Medicine (approval No.UM.VET.2024.053).

Animals

This study used adult albino male rats that were raised in the animal house of the College of Veterinary Medicine/University of Mosul, and their weight was limited to between (195-280) grams at the age of 3 months and taking into account that the weights of the rats were similar in the experiment. The animals were raised in special laboratory conditions characterized by a photoperiod of (12) hours of light and (12) hours of darkness, and the laboratory temperature was (22±2) °C and rats were acclimatized for two weeks prior to the start of experiment. The rats were placed in plastic cages 41x24 x19.5 cm, an average of 5 rats in one cage, food and water were available ad libitum.

Preparing doses of deltamethrin and melatonin

Deltamethrin dose (25 mg/kg, P.O.) was prepared using the original insecticide solution (Deltarin, 2.5%, Emulsifiable - Concentrate, Vapco-Jordan). While melatonin (10 mg/equivalent per capsule- green field nutrition's company -USA) dose (12.5 mg/kg,

I.P.) were prepared by dissolving melatonin in a 1% vehicle solution (ethanol in physiological saline solution) [14].

Study design

To conduct this study, twenty adult male rats were divided into 4 groups (5 animals/group) (A, B, C and D). Group (A) was considered control and was given distilled water (2 ml/kg, P.0.), group (B) was dosed with deltamethrin alone (25 mg/kg, P.O.) which represents (23.4%) of (LD50-106.6 mg/kg, P.O.) [15]. Group (C) was injected with melatonin alone (12.5 mg/kg, I.P.) Which dose was chosen based on preliminary experiments, and group (D) was injected with melatonin at a dose of (12.5 mg/kg, I.P.) 15 minutes before giving daltamethrin at a dose of (25 mg/kg, O.P.). The treatment period of animals in groups (A, B, C and D) extended for five consecutive days.

Detection of the effect of melatonin against apoptosis induced by deltamethrin using immunohistochemistry technique [3]**.**

After 24 hours from the end of the treatment of male rats in groups (A, B, C and D) for five consecutive days, the animals were sacrificed for brain heart and liver harvesting to detect DNA fragmentation (apoptosis) using terminal deoxynucleotidyl transferase dUTP nick end labeling_assay - TUNEL assay.

TUNEL assay the sections were prepared according to kits instructions (TUNEL Apoptosis Assay kit- Elabscience - USA) starting from dewaxing, hydration and cell permeability, adding TUNEL reaction solution, then adding converter-POD reagent, DAB color, hematoxylin redyeing, and sealing. After the sealed sections were dried, sections were observed under an optical microscope (400 ×), apoptotic cells will appear brown, while non-apoptotic cells remain unstained and finally images were collected [3]. It is worth noting that the brain, heart and liver were selected to evaluate the effect of melatonin against deltamethrin-induced apoptosis in rats based on [2].

RESULTS

Dosing adult male rats with the insecticide deltamethrin alone at a dose of (25 mg/kg, P.O.) for five consecutive days led to tissue changes in the brain, heart and liver in the form of programmed cell death, represented by the appearance of positive brown stained apoptotic bodies which were detected by (TUNEL) staining compared to the control group treated with distilled water (Fig. 1,2,3).

It is worth noting that the severity of tissue changes represented by the appearance of programmed cell death induced by the insecticide in the brain, heart and liver of adult male rats was measured based on the numbers of positive brown stained apoptotic bodies in the tissues. Based on this rule, brain cells were considered to be more sensitive to programmed cell death compared to the heart and liver. These results confirm that deltamethrin has neurotoxicity, because the central nervous system was its target organ (brain cells; Fig. 4).

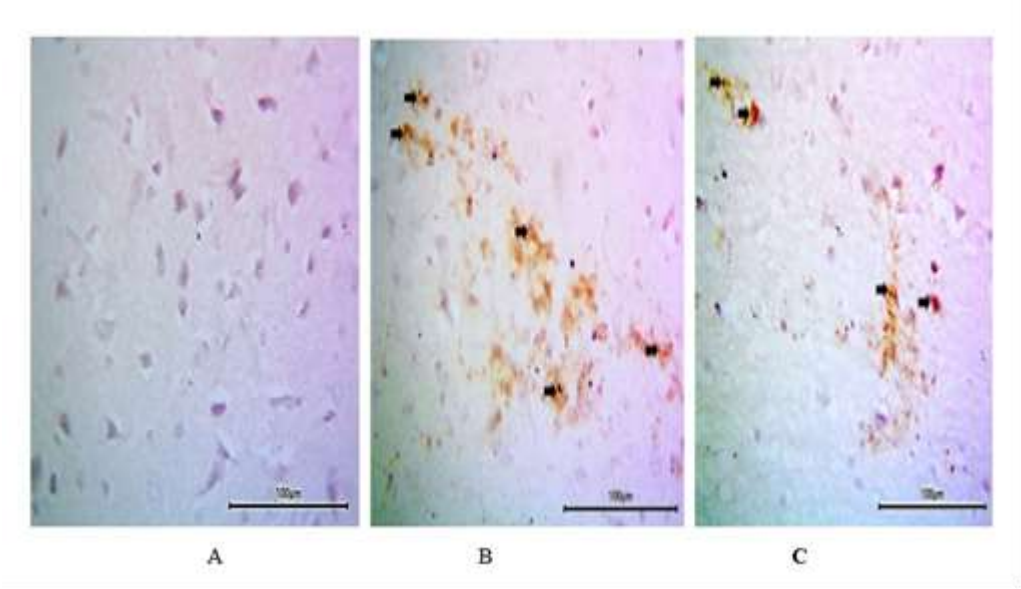

Fig. 1. Effect of melatonin against apoptosis in brain tissue induced by the insecticide deltamethrin in adult male rats

A- Showing negative for TUNEL stain, NO brown stained apoptotic bodies were observed in brain cells of adult male rats treated with distilled water (2 ml/kg, P.O.; control) for five consecutive days TUNEL stain, 400×.

B- Showing very high number of positive brown stained apoptotic bodies (black arrows) of brain cells of adult male rats treated with deltamethrin alone (25 mg/kg, P.O.) for five consecutive days using the TUNEL stain, TUNEL stain, 400×.

C- Showing clear decrease in number of positive brown stained apoptotic bodies (black arrows) of brain cells of adults male rats injected with melatonin (12.5 mg/kg, I.P.) 15 minutes before deltamethrin dosing (25 mg/kg, O.P.) for five consecutive days in compared to the group treated with deltamethrin alone (B), using the TUNEL technique, TUNEL stain, 400×.

Pre-treatment of adult male rats with melatonin at a dose of (12.5 mg/kg, I.P.) led to a reduction in the severity of programmed cell death represented by a clear decrease in the number of positive brown stained apoptotic bodies in the brain, heart and liver tissues compared to the group treated with deltamethrin alone (Figure 1,2,3). This result proves that melatonin has effects against apoptosis induced by deltamethrin.

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It is important to mention that the group injected with melatonin alone (12.5 mg/kg, I.P) for consecutive days did not show any histological changes (negative for TNEL stain) in the tissues of the brain, heart and liver, identical to the negative control group treated with distilled water (2 ml/kg, O.P.) for 5 consecutive days

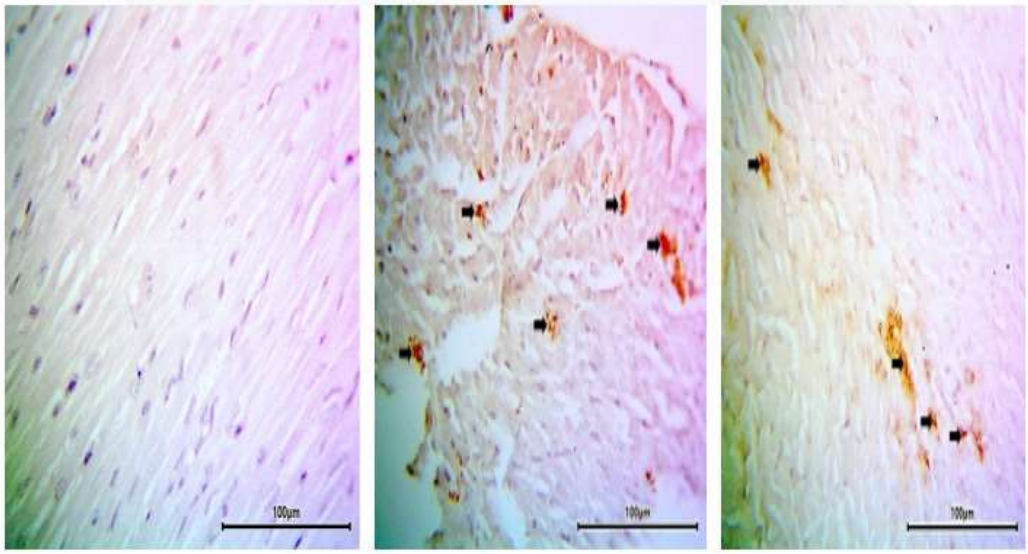

Fig. 2. Effect of melatonin against apoptosis in heart tissue induced by the insecticide deltamethrin in adult male rats

A- Showing negative for TUNEL stain, NO brown stained apoptotic bodies were observed in cardiac cells of adult male rats treated with distilled water (2 ml/kg, P.O.; control) for five consecutive days TUNEL stain, 400×.

B- Showing very high number of positive brown stained apoptotic bodies (black arrows) of cardiac cells of adult male rats treated with deltamethrin alone (25 mg/kg, P.O.) for five consecutive days using the TUNEL technique TUNEL stain, 400×.

C- Showing clear decrease in number of positive brown stained apoptotic bodies (black arrows) of cardiac cells of adults male rats injected with melatonin (12.5 mg/kg, I.P.) 15 minutes before deltamethrin dosing (25 mg/kg, P.O.) for five consecutive days in compared to the group treated with deltamethrin alone (B), using the TUNEL technique, TUNEL stain, 400×.

DISCUSSION

The group of adult male rats treated with deltamethrin alone showed tissue changes in the brain, heart and liver represented by the occurrence of programmed cell death (apoptosis), which was detected by the (TUNEL) technique. This can be explained based on what was reached by [16] in a study on the effect of chronic exposure to deltamethrin on the occurrence of oxidative stress and programmed cell death (cerebral injury) in the brain of quail. These researchers [16] attributed these tissue changes in the brain to the inhibitory effect of deltamethrin on antioxidant mechanisms through both down-regulation of gene expression of the factor nuclear factor erythroid-2-related factor 2 (Nrf2), which is responsible for transcription factor that regulates the expression of various antioxidant enzymes [17-19] and anti-apoptotic factor (B-cell lymphoma gene 2 (Bcl2) level), While deltamethrin stimulated the apoptosis-inducing factors (Jun N-terminal kinase3, caspase-3, and Bcl-2-associated X protein levels). Accordingly, the histological changes (apoptosis) in the brain, heart, and liver of adult male rats recorded in our current study can be attributed to the same causes as those reached by [16].

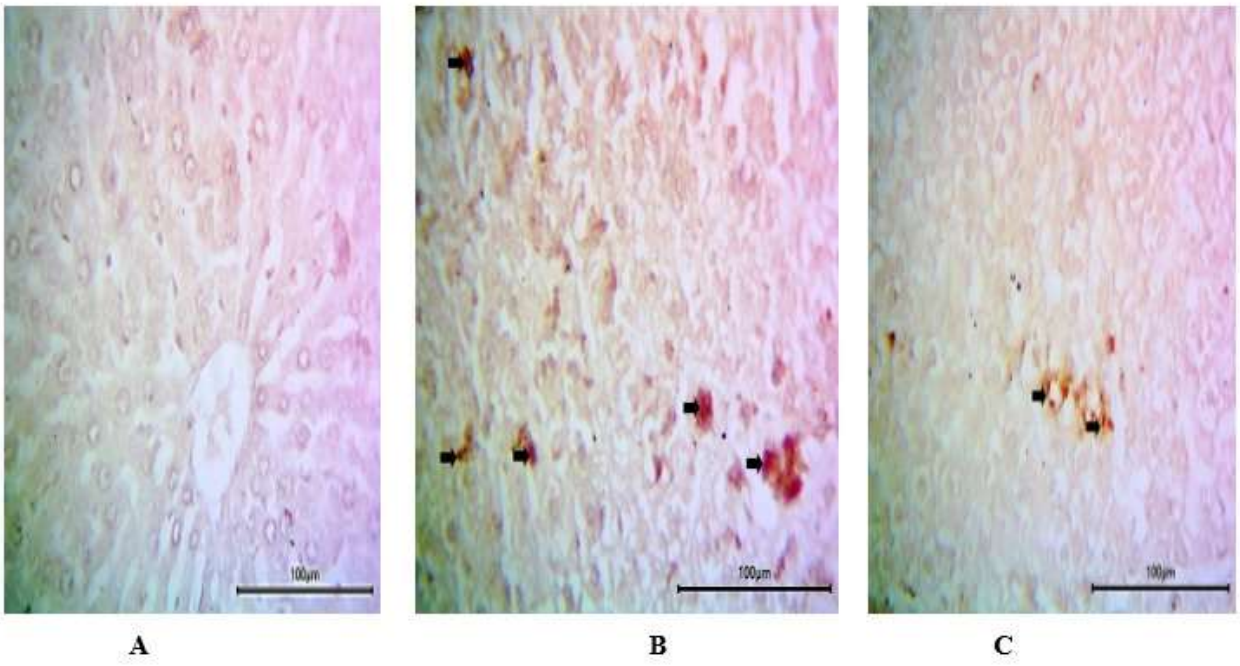

Fig. 3. Effect of melatonin against apoptosis in liver tissue induced by the insecticide deltamethrin in adult male rats

A- Showing negative for TUNEL stain, NO brown stained apoptotic bodies were observed in liver cells of adult male rats treated with distilled water (2 ml/kg, P.O.) (control) for five consecutive days (TUNEL stain, 400X).

B- Showing very high number of positive brown stained apoptotic bodies (black arrows) of liver cells of adult male rats treated with deltamethrin alone (25 mg/kg, P.O.) for five consecutive days using the TUNEL technique (TUNEL stain, 400X)

C- Showing clear decrease in number of positive brown stained apoptotic bodies (black arrows) of liver cells of adults male rats injected with melatonin (12.5 mg/kg, I.P) 15 minutes before deltamethrin dosing (25 mg/kg, P.O.) for five consecutive days in compared to the group treated with deltamethrin alone (B), using the TUNEL technique, TUNEL stain, 400X

Oxidative stress caused by exposure to the insecticide deltamethrin largely causes severe damage to the internal tissues of the organism through inhibition of the pivotal endogenous pathway represented by nuclear factor erythroid-2-related factor 2/heme oxygenase-1 (Nrf2/HO-1),because this pathway (Nrf2/HO-1) has many vital functions for the cell, including inhibiting programmed cell death, reducing inflammation, and alleviating endoplasmic reticulum stress by regulating phase II detoxification enzyme,therefore, inhibition of this pathway ((Nrf2/HO-1) by deltamethrin leads to inflammation and programmed cell death of cellular tissues [2].

Based on what was mentioned above about the ability of delta to inhibit the (Nrf2/HO-1) pathway (2) responsible for the response against oxidative stress, and because of this inhibition, the insecticide will induce oxidative stress. Thus, this can be used to interpret tissue changes (apoptosis) in the brain, heart and liver of rats treated with deltamethrin alone in our current study may be related to the accumulation of the insecticide in these tissues and the generation of reactive oxygen species (ROS), which in turn damage proteins, nucleic acids and lipids of the cells of these tissues [20-23].

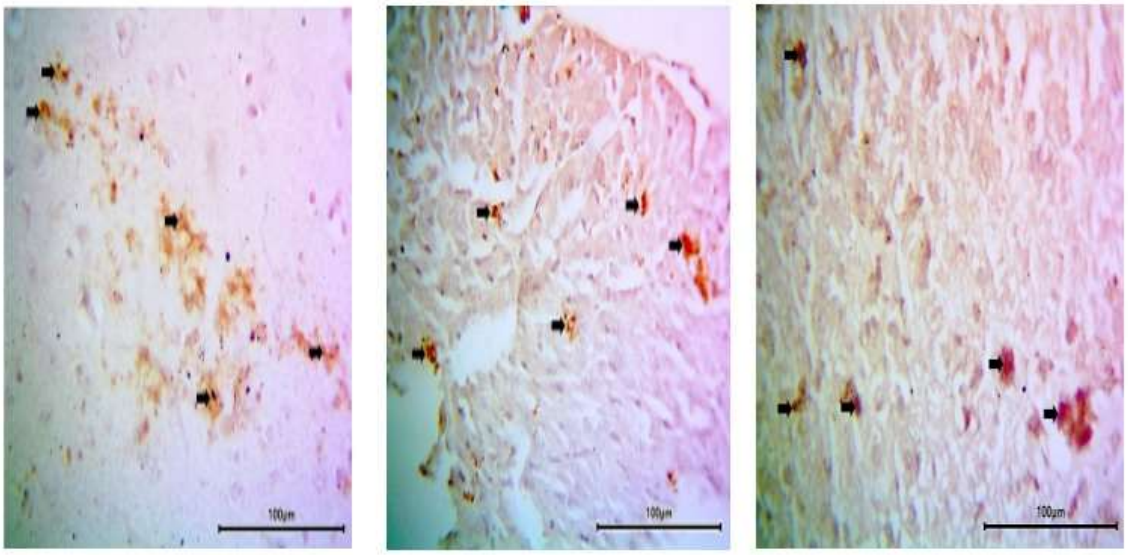

Fig. 4. Showning the brain cells of adult male rats are more sensitive to programmed cell death induced by deltamethrin alone (25 mg/kg, P.O.) for 5 consecutive days, as represented by a high number of positive brown stained apoptotic bodies (black arrows) compared to cardiac and liver cells using the TUNEL technique, (TUNEL stain, 400X)

These active oxygen species also have the ability to activate the classic Nuclear factor-κB (NF-κB) inflammatory signaling pathway, which in turn contributes to the release of many pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, COX-2, and iNOS, which leads to the exacerbation of inflammatory reactions in these tissues and the occurrence of histological changes in rats [16,24,25,26]. In addition, deltamethrin-induced mitochondrial damage may play a role in the onset of apoptosis [27], and what confirms the association of mitochondrial damage with deltamethrin toxicity is the appearance of apoptotic bodies in the heart cells of adult male rats belonging to the group treated with deltamethrin alone in our current study, which were detected in the form of a TNEL (Fig. 2), the reason for this is attributed to the fact that the heart is considered one of the organs very rich in mitochondria [2,27].

The results of our current study showed that brain tissue cells are more sensitive to programmed cell death induced by the insecticide deltamethrin alone compared to heart and liver tissues. This can be explained by the fact that brain tissue is rich in peroxidizable unsaturated lipids with high oxygen utilization and at the same time has relatively low antioxidant potential, making the brain more susceptible to peroxidative damage than other organs [28,29].

Pre-treatment of adult male rats with melatonin 15 minutes before dosing deltamethrin led to a reduction in the severity of apoptosis represented by a clear decrease in the number of positive brown stained apoptotic bodies in the brain (Figure -1). This is because melatonin has effect against apoptosis induced by deltamethrin through inhibition of c-Jun N-terminal kinase (JNK), and p38 mitogen-activated protein kinase (p38 MAP kinase (p38/JNK), which are involved in the induction of the apoptotic pathway and It is accompanied by melatonin stimulating the antioxidant enzyme pathway thioredoxin (Trx) which prevents the occurrence of oxidative stress responsible for

programmed cell death [30].

Another mechanism of melatonin's anti-apoptotic action is its ability to up-regulate the expression of the anti-apoptotic gene B-cell lymphoma-2 (BCL-2). In addition, melatonin simultaneously down-regulated the expression of the gene responsible for activating both the proapoptotic genes BAX (BCL2) associated X (BAX) and the inflammation (Caspase-3) [31].

CONCLUSION

We conclude from our current study that melatonin has effects against apoptosis induced by the deltamethrin in adult male rats model

CONFLICTS OF INTEREST

The authors declare there is no conflict of interest.

ACKNOWLEDGMENTS

This research was supported by the College of Veterinary Medicine, University of Mosul.

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