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Authors' Affiliation:
1. Department of Biology, College of Education for Pure Science, University of Diyala – Iraq
2. Environmental Engineering Department, College of Engineering, Mustansiriyah University – Iraq

***Corresponding Author:**
Mohammed Nsaif Abbas
Email: mohammed.nsaif.abbas@gmail.com

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Revealing Potential Histological Changes of Deltamethrin Exposure on Testicular Tissue in Albino Rabbits (*Oryctolagus cuniculus*)

Fatima Salman Abd Al-Latif[1], Thekra Atta Ibrahim[1], Mohammed Nsaif Abbas[2]*

Abstract

Background: The pesticide's broad-spectrum nature raises concerns about its impact on biodiversity, potentially disrupting delicate ecological balances and endangering various species across different trophic levels. Deltamethrin, a widely used synthetic pyrethroid, poses significant risks to both the environment and animals. Its persistence in soil and water can lead to the contamination of ecosystems, affecting non-target insects, aquatic life, and birds.

Methods: This study aims to detect the impact of deltamethrin on the testicular tissues of white rabbits treated by 0.1 and 0.3 mg/kg/day for 30 days. Thirty rabbits were divided into three groups equally. The first group was the control group and was administered distilled water, while the second group experimental groups 1 and 2 received deltamethrin at a concentration of 0.1 and 0.3 mg/kg/day respectively. Tissue sections were prepared, stained and tested via a light microscope equipped with a camera.

Result: The results obtained revealed that all groups of animals treated with deltamethrin experienced disintegration in the germinal cell layer, detachment of the germinal epithelium from the basal membrane, and slight distortion in spermatozoa. The damage was more severe with increasing the concentration, Moreover, there was an increase in the contraction of some seminiferous tubules, resulting in their irregular and wavy appearance, and many cellular changes were observed, in addition to absence of spermatozoa in some seminiferous tubule lumina and Leydig cell hyperplasia.

Conclusion: The treatment with deltamethrin at different doses for one month caused severe pathological tissue damage in the testes, characterized by congestion, hemorrhage, vacuolation, and detachment of a portion of the germinal epithelium from the basement membrane.

Introduction

Preserving the delicate balance of our environment and safeguarding its well-being necessitates the remediation of environmental pollution. Pollution, in its various forms, wreaks havoc on ecosystems, wildlife, and human life [1]. Among these menacing pollutants, pesticides claim a significant role, being widely employed to combat agricultural pests and harmful insects [2]. Various methods for environmental remediation exist, each with varying levels of efficiency, cost, and requirements for specialized equipment or preliminary treatments [3]. One particularly promising method for treating diverse environmental contaminants, such as heavy metals [4], dyes [5], organic matter [6], inorganic toxins [7], hardness [8], eutrophication [9], organic acids [10], and pesticides [2], from water [11], soil [12], and crude oil [13], is adsorption [14]. Amid the arsenal of potential adsorbents, activated carbon stands out as a highly efficient material with unparalleled properties [15]. However, recent attention has shifted towards agricultural and industrial waste materials rather than activated carbon [16]. Surprising contenders such as rice husks [17], watermelon rinds [18], banana peels [19], pomegranate peels [20], orange peels [21], lemon peels [22], waste tea leaves [23], eggshell [24], algae [25], water hyacinth [26], tree leaves [27] and even aluminum foil [28] have taken center stage, what makes these waste materials more alluring. Their abundance, affordability, and negligible toxicity make them intriguing alternatives [29]. Moreover, unlike activated carbon, they require no complicated manufacturing processes [30]. However, here lies a crucial challenge: the concept of Zero Residue Level (ZRL) that has proven successful in laboratory waste management is yet to be implemented on a larger scale [31-34]. These seemingly unremarkable waste materials, which hold potential as eco-friendly adsorption materials, may inadvertently become an environmental problem instead of a solution [35]. The residues and pesticides they adsorb might resurface in new, more hazardous forms, undoing any positive progress made [36]. Despite the array of treatment methods, guidelines, and recommendations, pesticide residues persist in the environment, contaminating its elements and posing risks to human health and wildlife [37]. Among the concerning pesticides is deltamethrin, widely used for its exceptional pest control efficacy in agriculture, industries, and households [38]. However, the frequent and unregulated use of deltamethrin has sparked concerns about its impact on the environment and living organisms, including humans [39]. Confronting pesticide pollution, particularly deltamethrin, demands relentless research to understand its effects on non-target organisms [40] and find ways to mitigate its harmful impacts while balancing its intended purposes [41]. In pursuit of this endeavor, histological studies of living organisms' organs (especially those in direct contact with pesticides or their residues) play a pivotal role in unraveling effects of deltamethrin on the environment and living beings [42, 43]. Although previous histological studies have focused on mice [44] and rats [45], there remains a knowledge gap concerning the histological effects of deltamethrin on rabbits [46]. Shedding light on this uncharted territory holds the potential to unlock vital insights into how deltamethrin interacts with the environment and living organisms, steering us towards sustainable solutions for a harmonious coexistence between humans and nature. The testes, a crucial organ in the physiology of living organisms, plays a vital role in maintaining biological homeostasis and supporting essential functions. Consequently, any detrimental effects on the testes can have far-reaching implications on the overall health of organisms and the delicate environmental balance [47]. As research on this specific subject remains limited, there arises a pressing need to embark on further investigations to comprehensively understand the consequences of deltamethrin exposure, both on rabbits in general and particularly on male rabbits. This endeavor is essential in formulating effective strategies that not only meet agricultural demands but also safeguard non-target organisms, thus ensuring a harmonious coexistence between human activities and the environment [48]. With all the aforementioned points in mind, the primary objective of the current study is to investigate the impact of deltamethrin on the histological structure of the testes in white rabbits, to identify the effect of pesticides as one of the chemicals at the level of cells and tissues accurately.

Methods

Animals

Thirty albino rabbits were used in this study; their average weight was ranged between 1.0-1.5 kg.

Chemicals

The pesticide Deltamethrin used in the current study was of 25 g/l concentration as an active ingredient and supplied by the Jordanian company MedMAC in the form of a yellowish-white liquid that is soluble in water. Formalin solution of 10% concentration was prepared by adding 90 ml of distilled water to 10 ml of formaldehyde solution, while the formation of eosin stain was conducted via dissolving of one gram of eosin in 99 ml of ethyl alcohol of 70% concentration before adding 0.2 ml glacial acetic acid. Finally, the blended then filtered carefully. Harris's hematoxylin stain was prepared by mixing two solutions, the first one was

dissolving 2.5 g of hematoxylin powder in 25 ml of ethyl alcohol. The other solution prepared by dissolving 50 g of alum in one-half liter of distilled water according to the method described by [43].

Experimental design

The rabbits used in this study were randomly divided into two groups, the details of which were as follows: The first group is the control group contains 10 rabbits, and the second group is the test group includes 20 rabbits, this group, in turn, was divided equally into two secondary groups (10 rabbits per group). The rabbits of test group were injected with deltamethrin, by doses of 0.1 and 0.3 mg/kg of body weight daily for 30 days. The selection of the aforementioned dosages was predicated on the lethal dose of the pesticide, established at 2000 mg/kg of body weight in rabbits.

Methods for estimation of various histological changes

When the experiment was finished, the rabbits were anesthetized with chloroform, then the animals were dissected, and the liver was removed from its site. After that, the testes samples were fixed with formalin solution for 24 hours, and then the histological sections were prepared.

Results

The current study revealed significant histological changes in the testes of treated adult male rabbits with a concentration of 0.1 mg/kg of deltamethrin over a period of 30 days.

Dose-dependent effect of deltamethrin on Sertoli cells and blood vessels

It was observed that most of the seminiferous tubules exhibited disorganized germ cell layers, and their lumen was filled with germ cells, along with occurrence of Sertoli cell vacuolation. Additionally, congestion of blood vessels between the seminiferous tubules was evident, and there was an increase in Leydig cell numbers with reduced distance between adjacent seminiferous tubules in certain histological sections, as illustrated in Figure (1).

Dose-dependent effect of deltamethrin on Leydig cells

In some other sections, shrinkage of the seminiferous tubule lumen was observed, along with dissociation of the germinal lineage cells and separation of the germinal epithelium from the basement membrane. These cells accumulated in the central lumen of the seminiferous tubule. Additionally, evidence of degeneration and disintegration in the germinal layer and Leydig cells was also noted, as shown in Figure (2).

Dose-dependent effect of deltamethrin on the walls of the seminiferous tubules

Moreover, the current study revealed significant histological alterations in the seminiferous tubules. These alterations were characterized by irregular and undulating appearance of the tubule walls, as well as atrophy in certain sections of the seminiferous tubules. Additionally, there was irregularity observed in the germinal epithelium, and intriguingly, vacuolation was observed between some regions of the germinal layer, as illustrated in Figure (3).

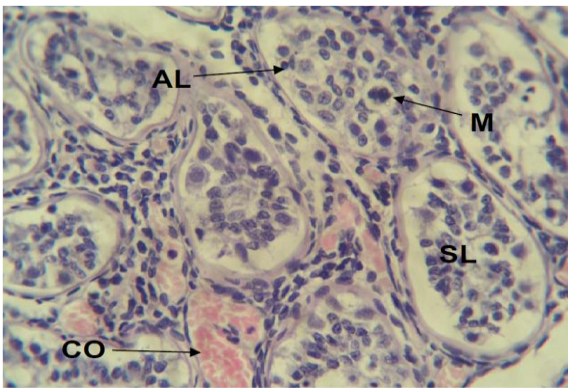

Figure 1: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.1 mg/kg/day for 30 days. The section demonstrates irregular germinal layers (AL), the seminiferous tubule lumen filled with germinal cells (SL), congestion of blood vessels between the seminiferous tubules (CO), stained using Hematoxylin and Eosin (H&E), at ×40 magnification.

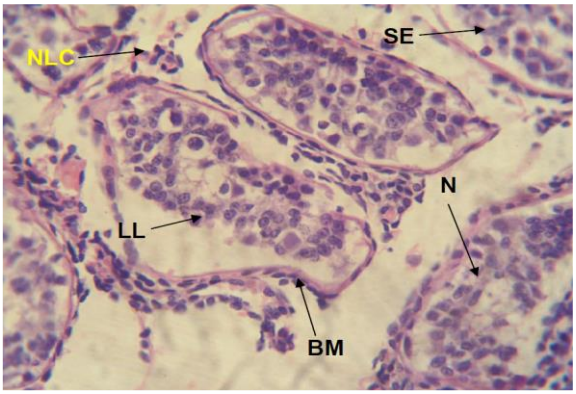

Figure 2: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.1 mg/kg/day for 30 days. The section shows epithelial separation and its accumulation in the central lumen of the seminiferous tubule (LL), necrosis (N), disintegration in the germinal layer (SE), necrosis in Leydig cells (NLC), the basement membrane (BM), stained using Hematoxylin and Eosin (H&E), at ×40.

Dose-dependent effect of deltamethrin on macrophages and inflammatory cells

Furthermore, the study demonstrated the presence of large multinucleated cells within the seminiferous lumen, as depicted in Figure (4). Concurrently, notable congestion of blood vessels and infiltration of inflammatory cells near the vascular structures were also observed, as illustrated in Figure (1). These

findings indicate the occurrence of inflammation and vascular changes within the testicular tissue.

The effect of increasing the dose of deltamethrin on testicular tissue

In a related context, the histological results of the testes in rabbits treated with 0.3 mg/kg concentration of deltamethrin for a duration of 30 days revealed more pronounced tissue alterations compared to the previous groups.

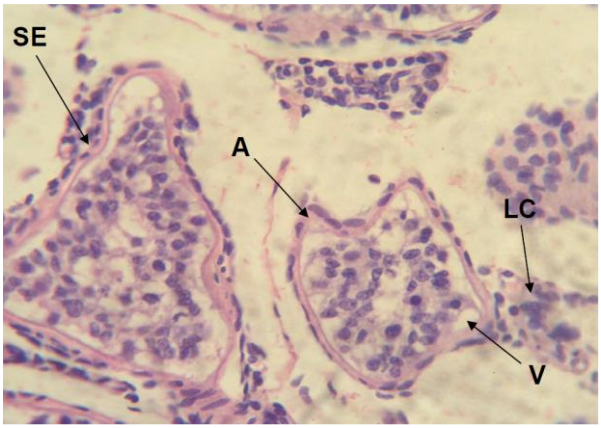

Figure 3: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.1 mg/kg/day for 30 days. The section shows vacuolation between the germinal layer cells (V), shrinkage of the walls of the seminiferous tubules (SE), atrophy in some of the seminiferous tubules (A), and Leydig cells (LC). The section is stained using Hematoxylin and Eosin (H&E), at ×40.

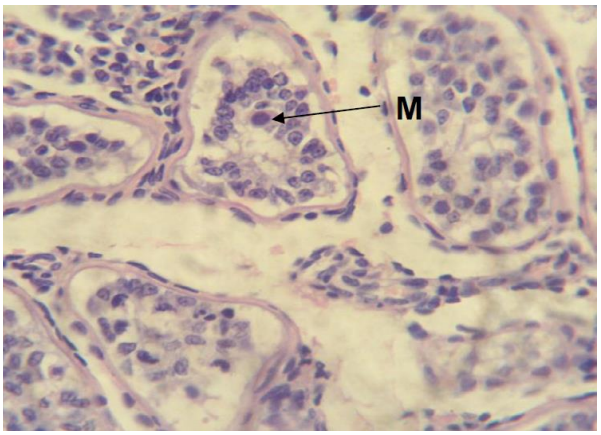

Figure 4: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.1 mg/kg/day for 30 days. The section illustrates the presence of macrophages (M). The section is stained using Hematoxylin and Eosin (H&E), at ×40.

Dose-dependent effect of deltamethrin on the lumen of seminiferous tubules and the basement membrane

These alterations were characterized by the occurrence of dilation in some of the seminiferous tubules and the presence of small spaces between Sertoli cells and the detachment of the germinal epithelium from the basal membrane. Additionally, vacuolation was observed in certain regions of the seminiferous tubules, as well as the occurrence of vacuoles between adjacent Sertoli cells, as depicted in Figure (5). Furthermore, there was noticeable occurrence of disorganization in the Sertoli cells, leading to an increase in intercellular space between neighboring Sertoli cells, as illustrated in Figure (5). These findings indicate significant disruptions in the testicular tissue structure and function due to exposure to the deltamethrin pesticide at the specified concentration and duration.

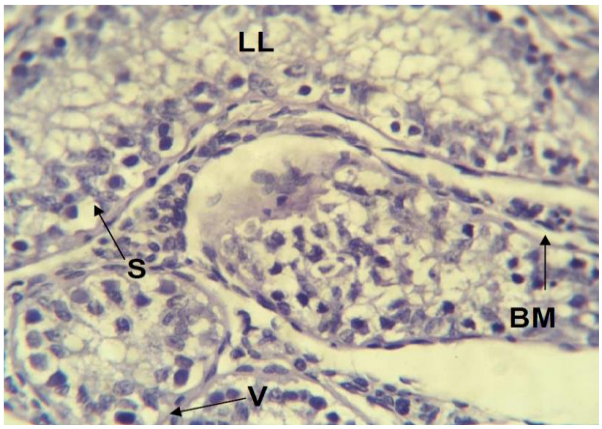

Figure 5: Cross-sectional view of the testes of adult male rabbits treated with 0.3 mg/kg/day deltamethrin for 30 days. Note: the enlargement of the seminiferous tubule (LL) and the appearance of spaces between the germ cells (S). Additionally, it shows the detachment of the germinal layer from the basement membrane (BM), vacuolation (V), and a decrease in the size of the Sertoli cells (SC). Furthermore, there are signs of necrosis (N) and vacuolation (V). (H&E), at ×40.

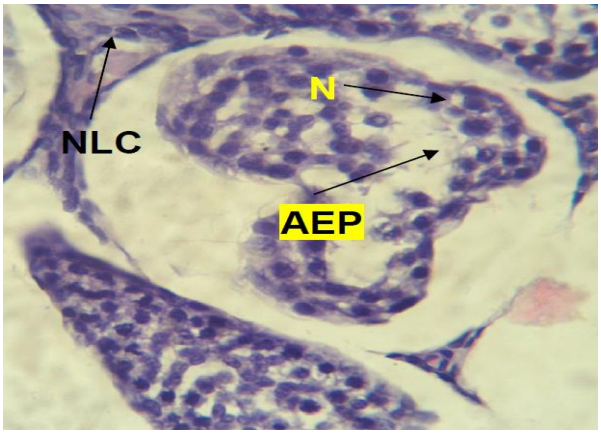

Figure 6: Cross-sectional view of the testes of adult male rabbits treated with 0.3 mg/kg/day deltamethrin for 30 days. The section shows the detachment of some germ cells from the germinal layer and their accumulation in the lumen of the seminiferous tubule. Additionally, it illustrates the separation of the spermatozoa from the basement membrane (AEP). Necrosis is observed in Leydig cells (NLC) and germ cells (N). (H&E), at ×40.

Similarly, observations revealed the detachment of some germinal epithelial cells and their accumulation within the lumen of the seminiferous tubules, leading

to the separation of the spermatic epithelium from the basal membrane (Epithelial separation), as depicted in Figure (6).

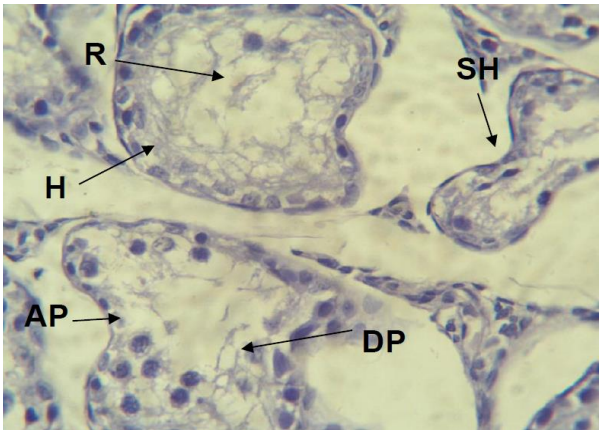

Figure 7: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.3 mg/kg/day for 30 days. The section reveals several observations: reduced histological structure (H), depletion of germ cells in some seminiferous tubules (DP), shrinkage of the seminiferous tubules (SH), retraction of spermatozoa (R), and apoptosis of primary germ cells (AP). The section is stained using Hematoxylin and Eosin (H&E), at ×40.

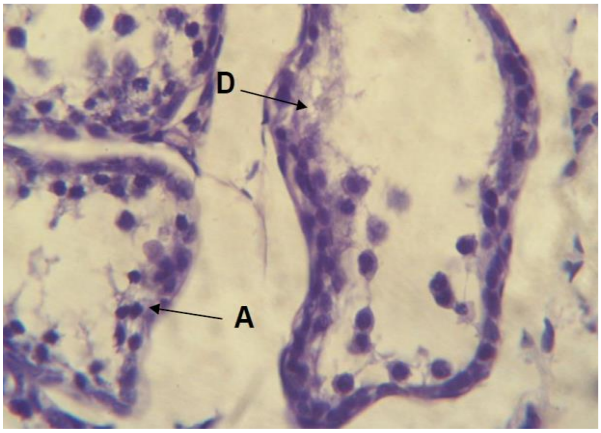

Figure 8: Cross-sectional view of the testes of adult male rabbits treated with deltamethrin at a concentration of 0.3 mg/kg/day for 30 days. The section illustrates the following findings: absence of spermatozoa in some seminiferous tubules, degeneration of germ cells (D), atrophy in some seminiferous tubules (A), and presence of giant cells (A). The section is stained using Hematoxylin and Eosin (H&E), at ×40.

Dose-dependent effect of deltamethrin on sperm cells

Furthermore, there was an increase in the contraction of the seminiferous tubules and depletion in certain germinal cell layers, resulting in degeneration, vacuolation, and apoptosis of spermatogonia, Primary sperm cells, spermatids, and mature spermatozoa, with the retraction of spermatids and mature spermatozoa back into the seminiferous tubules, as illustrated in Figure (7). On the other hand, the study indicated the absence of spermatozoa in some seminiferous tubule lumens and revealed the presence of Sertoli cells within the lumen of the seminiferous tubules, along with an increase in fibrosis between the seminiferous tubules, as demonstrated in Figure (8). These findings collectively highlight significant disruptions in spermatogenesis and impaired sperm production due to the effects of the deltamethrin pesticide on the testicular tissue.

Discussion

The current study revealed noticeable histological changes in rabbits exposed to concentrations of 0.1 and 0.3 mg/kg of deltamethrin. These changes include alterations in the thickness of the seminiferous tubule walls, dissociation of Sertoli cells, separation of the germinal epithelium from the basal membrane, and their shrinkage. The results of this study are consistent with the findings of Aboelwafa *et al.*, [49] in their research on the preventive effects of melatonin supplementation against taxol-induced testicular cytotoxicity in adult rats. Aboelwafa *et al.* reported that the basal lamina plays a crucial role in maintaining the transportation of materials between interstitial tissues and the spermatogenic germinal epithelium, thus preserving the structure and function of these tissues. In their study, Ghanami Gashti *et al.*, [50] indicated that the thickness of the seminiferous tubule wall weakens its relationship with the interstitial tissue. Moreover, an increase in the wall thickness leads to the manifestation of several pathological disturbances within the testis, particularly affecting the function of Sertoli cells. These disturbances have an impact on the differentiation of germ cells and inhibit the formation of spermatozoa. In their study on mice, Wang *et al.*, [51] demonstrated that Sertoli cells secrete Collagen fibers IV, which lead to thickening of the walls of the seminiferous tubules. Consequently, this process results in impaired spermatogenesis and a reduction in sperm formation.

The current study also revealed the absence of sperm in some seminiferous tubules. Moreover, the seminiferous tubules exhibited areas of detachment within the interstitial tissue, along with an increase in the distance between germ cells. Additionally, germ cells were found to detach and accumulate within the lumen of the seminiferous tubules. Indeed, these results are consistent with what Mostafa *et al.*, [52] reported in their study on the effects of permethrin, a pesticide, on the testes of adult albino rats. The findings in both studies indicate similar alterations in the seminiferous tubules, such as detachment and accumulation of germ cells, as well as changes in the interstitial tissue, which align with the toxic effects of

permethrin on the testicular structure. Ghanami Gashti and others [50] highlighted in their study that the occurrence of a disorder in Sertoli cells will undoubtedly affect germ cells and eventually lead to tissue abnormalities in the testes. These findings are consistent with the results of Wang and others [51] regarding the impact of Sertoli cell syndrome on the testicular histology and genetic mechanisms in male albino mice. Washburn *et al.* [53] stated in their study that Sertoli cells play a crucial role in the development of germ cells by forming the blood-testis barrier, which protects and nourishes the germ cells and facilitates the transport of nutrients and hormones to them. It is believed that all these pathological signs are due to a dysfunction in the structure and function of Sertoli cells. The results of this study also showed the presence of large phagocytic cells within the lumen of the seminiferous tubules. Researchers have differed in identifying the origin of these cells. Some have suggested that they are true phagocytes, while others have attributed them to lymphoid cells or identified them as Sertoli cells with phagocytic activity, which engulf residual bodies from spermatozoa under normal conditions. The current study revealed that deltamethrin has an effect on Leydig cells. Indeed, the results of the current study are consistent with the findings of Zirkin and Papadopoulos [54] in their research on the immune effects of Sertoli cells. They indicated that Leydig cells serve as a center for regulating fertility by producing the hormone testosterone. They also clarified in their study that Leydig cells are stimulated by luteinizing hormone (LH), which in turn stimulates the production of arachidonic acid and male hormones. The results of the current study revealed that injecting rabbits with a concentration of 0.3 mg/kg of deltamethrin led to an increase in degenerative changes in the epithelium of seminiferous tubules, shrinkage of the spermatic tubules, depletion of certain germinal cell layers in the seminiferous tubules, and programmed cell death in germ cells. Moreover, the study showed a retraction of spermatogonia and mature sperm back into the seminiferous tubules. Additionally, numerous seminiferous tubules were found to be devoid of germ cells, indicating that the effect of the pesticide is dose-dependent. With an increase in the concentration of the dose, the degenerative changes were more pronounced. This effect is believed to be associated with a disruption in Sertoli cells, which, in turn, affects the crucial proteins necessary for the differentiation of germ cells. These proteins are secreted at their highest levels during the differentiation stage of spermatogonia. This result is consistent with what Doyle *et al.*, [55] found in their study regarding the characteristics of sperm and the histological structure of testes in mice after long-term exposure to the compound (Di-(2-ethylhexyl) Phthalate). This result is also consistent with what Moreira *et al.*, [56] mentioned in their study on fertilization and reproductive toxicity mechanisms due to pesticide exposure. They suggested that the retrograde movement of spermatids and mature sperm within the seminiferous tubule wall might be a response to testicular toxicity induced by deltamethrin.

The treatment with deltamethrin at different doses (whether low or high doses) for one month caused severe pathological tissue damage in the testes, characterized by congestion, hemorrhage, vacuolation, and detachment of a portion of the germinal epithelium from the basement membrane.

Author Contributions

T. A. Ibrahim planned the study, T. A. Ibrahim and F. S. Abd Al-Latif executed the experiment and performed lab work, M. N. Abbas contribute to preparations of chemicals and technical writing of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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