

## Original article

# Toxic Effects of Deltamethrin on the Histological Structure of the Urogenital System in Adult Male Rabbits and the Protective Role of Folic Acid Antioxidants

Najat Mohammed<sup>1</sup> , Huda Hassan<sup>2</sup> , Alnagy Ali<sup>3</sup> , Fayrouz Khaled<sup>4</sup> 

<sup>1</sup>Department of Medical Laboratory, Faculty of Health Sciences, Omer AL-Mukhtar University, Libya

<sup>2</sup>Department of Pathology, Faculty of Medicine, Omer AL-Mukhtar University, Libya

<sup>3</sup>Department of Nursing, Faculty of Nursing, Omer AL-Mukhtar University, Libya

<sup>4</sup>Department of Chemistry, Faculty of Science, Omer AL-Mukhtar University, Libya

Corresponding Author. [Najat.mohammed@omu.edu.ly](mailto:Najat.mohammed@omu.edu.ly)

## Keywords.

Kidney, Testicular Tissue, Deltamethrin Toxicity, Rabbits, and Folic Acid.

## ABSTRACT

Deltamethrin insecticide has been widely applied for agricultural and veterinary purposes. However, exposure to it is attributed to several deleterious impacts on animals and human health. The study aimed to evaluate the negative impacts of deltamethrin on the normal architecture of kidney and testes of adult male rabbits on long-term exposure and manifested the possible protective effect of folic acids against this insecticide toxicity. Twenty adult male rabbits were divided into four groups: group 1 (control group), group 2 administered 1.28mg/Kg BW of deltamethrin, group 3 received 5mg/kg BW of folic acid, and group 4 was given a combination of deltamethrin and folic acid. The treatment was applied once daily for 12 weeks. The specimens were processed and stained with H&E and PAS stain for light microscopic examination. Deltamethrin-treated rabbits showed tubular injury in the form of loss of brush border, dropping off nuclei, and karyolysis of tubular cells. In addition, flattening of the tubular epithelium and dilatation of the renal tubules were also observed. A glomerulus appeared with mesangial cell expansion and capillary tuft congestion. The peritubular lymphocytic infiltration and areas of hemorrhage were noticed in the interstitium. Kidney sections stained with PAS demonstrated a marked decrease in carbohydrate content of tubular epithelium and disruption of membrane structures, which are induced by deltamethrin toxicity. The same findings were also seen in the combination group, indicating no role of folic acids as antioxidants. Regarding testicular tissue, deltamethrin induced hydropic degeneration of seminiferous tubules, leading to destruction of stratified germinal layers and a series of spermatogonia with focal loss of spermatid production. The interstitial cells of Leydig between tubules revealed a marked proliferation. In spite of treated animals, the combination group showed a focal improvement of spermatogenesis with a return to the normal length of spermatogonia layers. Deltamethrin induced obvious structural changes to the kidneys and testes of rabbits; therefore, it should be carefully limited at farms and animal houses to avoid serious impacts on human health. Application of antioxidants such as folic acid could be useful to ameliorate testes damage while showing a beneficial effect on renal structures.

## Introduction

Application of insecticides in public health, forestry, and on farms has increased recently because of their roles enhancing the quality of vegetable crops and their effects eradicating white fly, mites, and ants [1-3]. However, the intensive use of these insecticides negatively impacts on environment and human lives as they can pollute soil and reach water resources [4]. Different types of insecticides vary in their toxicity and potency. Among common pesticides are synthetic pyrethroids, which have been widely introduced since they are less toxic to humans and highly potent against unwanted pests [5-7]. Deltamethrin (DLM) is the most accepted type of synthetic pyrethroid (class II) that is commonly utilized for agricultural purposes [8, 9]. While several publications have highlighted its negative effects on the human body, causing nephrotoxicity [10], immunosuppression [6], genital inability, and neurological symptoms [11,12]. DLM is a lipophilic substance that acquires its ability to attach to the lipid membranes, leading to lipid peroxidation and subsequently overwhelming free radicals production (ROS)[5,13]. The latter puts the body under oxidative stress and the consumption of natural antioxidant enzymes, all these events participating in the main mechanism of deltamethrin toxicity. Poonam had reported that exposure to deltamethrin led to a decrease in the organ weight of Wister rats, including testes and epididymis, as well as affecting the morphological

activities of the sperm [14]. Others revealed severe degenerative changes on renal tubules and glomerular tuft necrosis in rats after oral administration of DLM [15]. Deltamethrin, similar to other synthetic pyrethroids, has oxidative toxicity as seen after exposure to cypermethrin, which induces DNA damage and blocks normal cell divisions [16]. Some experimental studies have confirmed that dietary supplementation of antioxidants like vitamin C or Vitamin E plays a vital role in preventing lipid peroxidation, oxidative stress, and protein oxidation in rat tissue exposed to insecticides [8,17,18]. Folic acid, which is a synthetic form of folate and considered a member of the vitamin B group (B9), is usually recommended to enhance male and female fertility [19, 20]. It stimulates DNA synthesis and promotes a proper nuclear cell division as well as stimulates cellular redox hemostasis [21]. It is involved in converting toxic accumulation of homocysteine into methionine [22]. It is commonly prescribed to reduce the risk of neural tube defects in newborns [23,24]. Thus, this study aimed to determine the antioxidant activity of folic acid on the kidney and testicular tissue of male rabbits induced by deltamethrin toxicity.

## Materials and Methods

### Chemicals

Commercial formulation of deltamethrin (25% EC), of high purity (98%) product of DuPont, was obtained from Kafr El-Zayat Pesticides & Chemicals Company, Egypt. Folic acid (pteroylglutamic acid) tablets (5mg), which is a water-soluble vitamin, were purchased from Sigma Aldrich, India.

### Animals

The animal work followed guidelines of laboratory animal care principles (NIH publication No 85-23, revised 1985). As well as the study protocol was granted by the Bioethics Committee at the Biotechnology Research Center (BEC-BTRC) (Ref No./NBC: 001.A.24.05). Twenty adult male rabbits aged nearly 6 months, weighing ( $27.6 \pm 1.891$  Kg), were involved in this work. They were divided into four groups with five animals in each. All animals were housed in separate cages, and they acclimatized for five days in a laboratory environment before the experiment under standard laboratory conditions with free access to food and water. Cleanliness and hygiene of the rabbits were checked regularly, and cages were also cleaned of waste every day.

### Experimental design

Animals were divided into four experimental groups. The control group received only distilled water. The deltamethrin-treated group was administered orally with 1.28 mg/kg body weight (BW) of the insecticide [12,25]. The folic acid group received folic acid alone at a dose of 5 mg/kg BW. The combination group was co-administered deltamethrin (1.28 mg/kg BW) and folic acid (5 mg/kg BW). All treatments were delivered once daily in the morning following a food supplement, for a duration of 12 weeks, via gastric gavage.

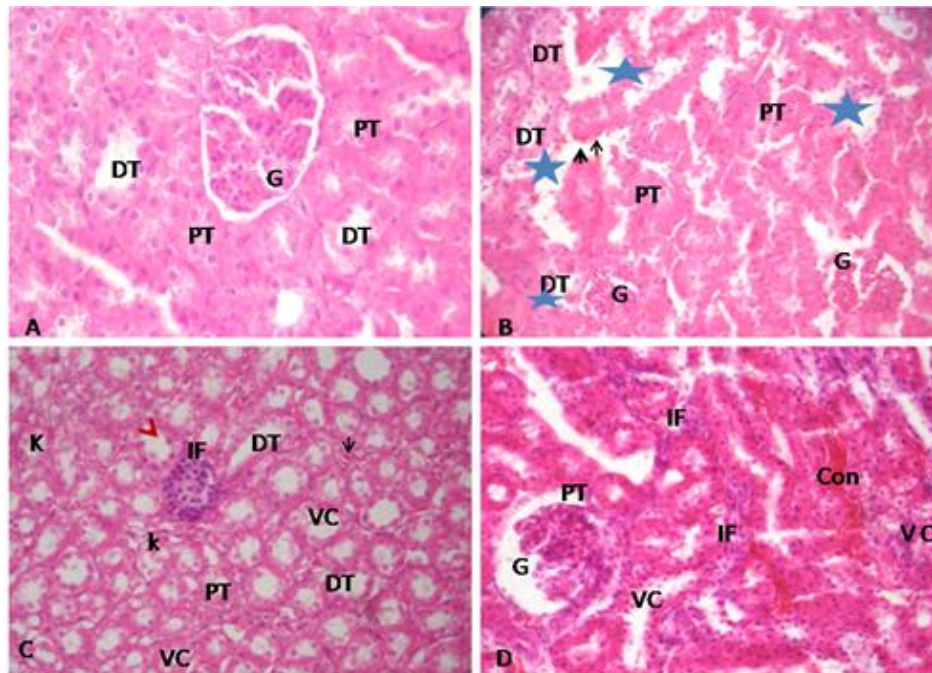
At the conclusion of the experiment, animals were anesthetized with chloroform and sacrificed. The kidneys and testes were excised from each animal, fixed in 10% buffered formalin, dehydrated through graded concentrations of absolute ethanol (70–100%), cleared with xylene, and subsequently embedded in paraffin blocks [26]. Tissue sections of 5  $\mu$ m thickness were prepared and stained with Harris Hematoxylin and Eosin (H&E) as well as Periodic Acid–Schiff (PAS). The slides were examined microscopically to assess histopathological alterations in renal and testicular tissues.

## Results

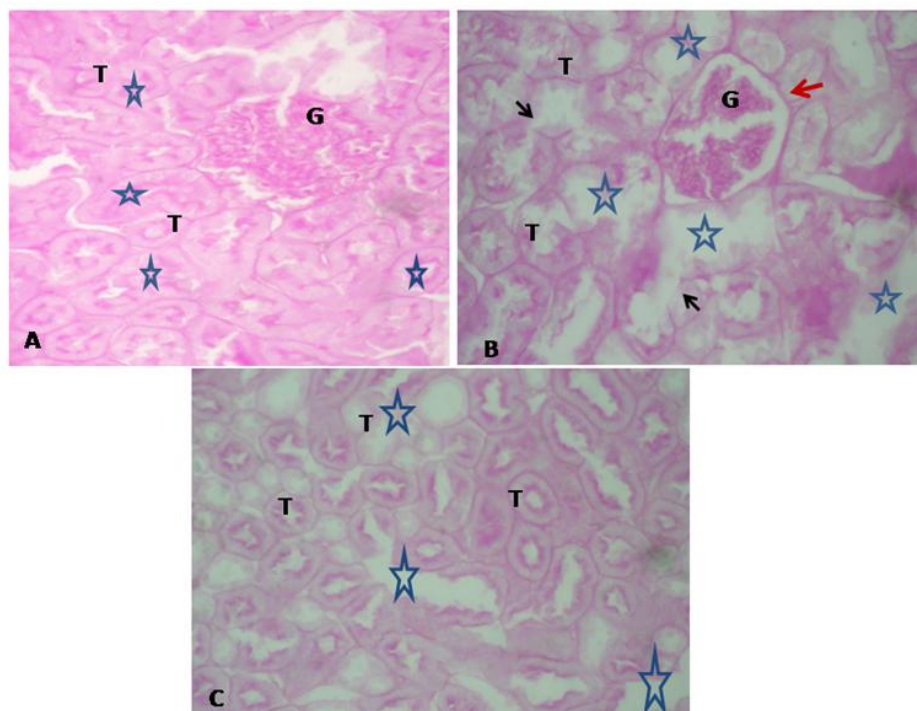
Control section of the kidney rabbit's stained with H&E, showed normal architecture in terms of epithelium of renal tubules; proximal and convoluted tubules (PT&DT) as well as normal structure of renal corpuscles with a two-layered Bowman's capsule and glomerular capillaries (Figure 1A). Examination of kidneys of deltamethrin-treated rabbits showed tubular injury in the form of loss of brush border, dropping off nuclei, and karyolysis. In addition, flattening of tubular epithelium and dilatation of renal tubules were also observed. The renal glomeruli exhibited hypercellularity, revealing mesangial cell expansion and capillary vascular congestion were also noticed. Interstitium showed peritubular lymphocytic infiltration along with peritubular congestion (Figure 1B and C). Similarly, the same changes seen were also manifested in folic acid-treated animals with prominent leukocytic infiltration and vascular congestion (Figure 1D).

Kidney of treated rabbits by carbohydrate staining techniques (PAS reaction) demonstrated loss of brush border of tubular epithelium and disruption of membrane structures, which are induced by deltamethrin toxicity (Figure 2B). The same findings were also seen in the combination group, indicating no role of folic acids as antioxidants (Figure 2C). Light microscopic examination of control testes showed normal structure of seminiferous tubules with normal maturation stages of spermatogenic cells, as well as interstitial cell Leydig (Figure 3A). While deltamethrin induced hydropic degeneration of seminiferous tubules, which leads to a decrease of spermatogenic layers with focal loss of spermatid production. The interstitial Leydig cells revealed a marked proliferation (Figure 2B). Interestingly, in the combination group, there was a focal

improvement of spermatogenesis with returning back to the normal length spermatogonia layers (Figure 3 C).

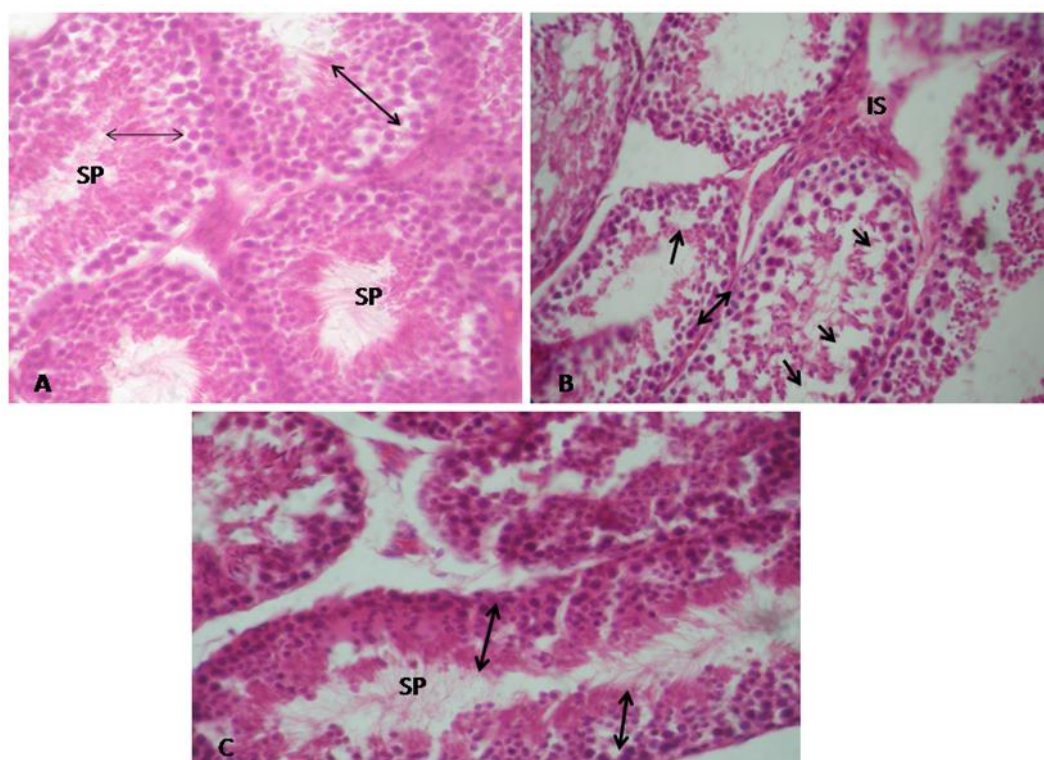


**Figure 1. Light micrographs of kidney of rabbits showing: A). Normal renal corpuscles of the control group with a tuft of glomerular capillaries(G), cytoplasm, and nuclei of epithelium lining proximal and distal tubules appeared in normal morphology (PT&DT). B&C). The DLM-treated group reveals tubular injury (Star) in the form of loss of brush border, dropping off nuclei (Red arrow), vacuolated cytoplasm (VC), karyolysis (K), and peritubular congestion (black arrow). Interstitium showed lymphocytic infiltration (IF). The similar pathological changes were also manifested in the combination group, with prominent leukocytic infiltration and vascular congestion in section (D) ( x400 H&E)**



**Figure 2. Kidney tissue of rabbits with PAS reaction showing: A). The control group appeared with normal carbohydrate contents of brush borders and intact cell membranes of tubular epithelium (star). B). DLM-treated rabbits showed the adverse effect of insecticide in terms of loss of brush borders (star) and distortion of renal membrane tubules (T) ( black arrow). C). DLM with folic acid group also presented a lack of carbohydrate content of renal tubules (star). (x400(PAS satin)).**





**Figure 3.** A). Seminiferous tubules of control rabbits with normal height of germinal epithelium and maturation layers of spermatogonia (arrow). B). Section of testicular tissue after exposure to DLM (arrow refers to the area of decreasing spermatogenic series) and proliferation of Leydig cells were also seen (IS). C). Testicular tissue of rabbits giving folic acid along with deltamethrin showing alleviation of insecticide toxicity and returning height of the germinal (arrow) and lumen filled with sperm (SP). (X400 H&E).

## Discussions

The use of insecticides could have an inhibitory effect on cell function and promote organ damage. Our study found that exposure to deltamethrin resulted in pathological alterations in the kidney tissue of rabbits, including signs of tubular cell injury, flattening of tubular epithelium, dilatation, and peritubular congestion. The interstitium showed lymphocytic infiltration spread between renal tubules. These findings are in corroboration with other research that manifested degenerative changes on the cells of the renal tubules with glomerular congestion and focal leukocytic infiltration induced by the toxic effect of methomyl on kidneys of rats [22]. Likewise, other studies reported the noxious effect of DLM on the kidney's cortex of Wistar rats [27]. The degenerative changes observed on glomerulus and tubules due to the deltamethrin cytotoxicity induced cellular dysfunction and further caused bio-accumulation of ROS, depletion of glutathione (GSH), and other antioxidant enzymes [11,18,28].

Oxidative stress is attributed to an increase in cell permeability, which leads to a hydropic cytoplasmic appearance of the damaged cells [5,22]. Additionally, mesangial cells expansion was exhibited in our findings, which may result from cell proliferation. This coincides with Abdel Razik et al work on the effects of lufenuron and profenofos insecticide on the kidney of albino rats [29], as well as reported by other investigators [3]. A destructive renal system is strongly related to impaired renal excretion, and its function, which was assessed through marked elevation of blood urea and creatinine in the experimental modules and this coincided with results obtained by Ali Khaled in his research [8,16]. Moreover, leukocytes are attracted to the site of tubular injury, promoting an inflammatory reaction and inducing an inflammatory cascade. El-Gerbed et al had confirmed elevation of cytokines, primarily IL6, IL1 $\beta$ , and TNF $\alpha$  in kidney rats exposed to DLM [15,30]. Renal toxicity had been reported in the previous studies through elevation of kidney Malondialdehyde (MDA) tissue level, which indicates the oxidation of polyunsaturated fatty acid of lipid membrane and perturbation of kidney antioxidant activity [8,22,31]. Nearly similar changes were also seen in the group that co administrated of folic acid and deltamethrin, indicating that folate has no protective effect against nephrotoxicity induced by DLM.

Regarding kidney tissue with PAS reaction, the use of DLM led to a marked decrease in polysaccharide content in kidney tissue. Roa elucidated that a reduction in glycogen contents in kidney tissue probably reflects the consumption of carbohydrates in response to the toxic effects of pesticide exposure, mainly by the oxidative stress mechanisms [29,32,33]. Others explained that depletion of carbohydrates under insecticide therapy is related to the lysosomal destruction and rupture of hydrolytic enzymes [34].

Our findings also showed that deltamethrin inhibits spermatogenesis and sloughed off the germinal epithelium, which is strongly supported by other works reporting a decrease in testes weight and an increase of testosterone levels, perhaps DLM destroys germinal layers, leading incomplete series of spermatogenic cells with distortion of seminiferous tubules [14,35]. Abdel-Rahman et al reported that administration of deltamethrin caused inhibition of hypothalamus-releasing hormone, mainly LHRH, which in turn blocks the release of LH and subsequently affects testosterone production [36]. In addition, the hydrophilic nature of DLM causes its harmful effect on testes structure since the sperm is coated with high levels of fatty acids, thus it simply enters peroxidation processes. On the contrary, there was an improvement in the histological features of seminiferous tubules in the combination group (deltamethrin plus folic acid) in comparison with the deltamethrin group, indicating the critical role of folic acid in supplying one methyl group for DNA methylation and preventing genetic defects during neonatal life [20]. Others highlighted its role in scavenging of reactive oxygen species through induction of apoptosis-related gene [22]. A group of workers noted that folic acid alleviates reproductive toxicity through elevation of serum testosterone level in the combination group when compared to the treatment group (carbufuran) [37]. In vitro animal studies on the deleterious impact of carbamate on testicular tissue showed that this pesticide causes overexpression of apoptosis genes and inhibits proliferation-related genes at the spermatogonia level, also led to downregulation of Leydig cells function through inhibition of testosterone producing [22,38]. However, damage to the testicular cell was diminished to a certain degree after oral administration of folic acid, which is in agreement with our work [22].

## Conclusion

Exposure to deltamethrin exerts marked toxic effects on renal and testicular tissues, as evidenced by tubular injury, glomerular congestion, oxidative stress, and impaired spermatogenesis. These alterations highlight the insecticide's capacity to disrupt cellular function through reactive oxygen species accumulation, antioxidant depletion, and inflammatory cascades. While folic acid supplementation showed partial improvement in testicular histology, it did not provide significant protection against deltamethrin-induced nephrotoxicity. Overall, the study underscores the potential hazards of deltamethrin exposure and emphasizes the need for further investigation into effective protective strategies.

## Acknowledgements

The authors are grateful to the Department of Medical Laboratory of AL-Bayda Medical Center for their medical assistance and support to carry out this research work.

## References

1. Gurgel GLdA, D'Amico FAM, Mangrich RM, Gaberz Kirschnik P, Weber SH, Turra Pimpão C. Determination of lethal and sub-lethal concentrations of deltamethrin in Jundiá (Rhamdia quelen). *Rev Agrar Acad.* 2013;1(2):125-30.
2. Ullah MS, Amjad A, Ahmad N, Khan MZ, Ahmad I. Toxic Effects of Cypermethrin in Female Rabbits. *Pak Vet J.* 2006;26(4):193-6.
3. Bouzar AC, Benyagoub B, Bitam A. A low-dose protective effect of phycocyanin on the toxicity of deltamethrin to vital organs in rats: in vivo study. *J Fundam Appl Sci.* 2020;12(1):149-66.
4. Korkmaz N, Erdoğan K, Örün GN, Erkmen B, Doğru Mİ, Doğru A, et al. Determination of acute toxicity of sodium pyrethrin on common carp and its effects on some hormones and hematological parameters. *KSU Tarım ve Doğa Derg.* 2022;25(5):1027-38.
5. Chargui I, Grissa I, Bensassi F, Hrira MY, Haouem S, Haouas Z, et al. Oxidative Stress, Biochemical and Histopathological Alterations in the Liver and Kidney of Female Rats Exposed to Low Doses of Deltamethrin (DM): A Molecular Assessment. *Biomed Environ Sci.* 2012;25(6):672-83.
6. Tewari A, Gill JSPS. Assessment of hemato-biochemical parameters on exposure to low level of deltamethrin in mouse model. *Vet World.* 2014;7(3):152-7.
7. Ananda SS, Hainest Wendy T, Muralidhara S, Fisher W Jeffrey, Padilla Stephanie. Characterization of deltamethrin metabolism by rat plasma and liver microsomes. *Toxicol Appl Pharmacol.* 2006;212(2):156-66.
8. Amin KA, Hashem KS. Deltamethrin-induced oxidative stress and biochemical changes in tissues and blood of catfish (*Clarias gariepinus*): antioxidant defense and role of alpha-tocopherol. *BMC Vet Res.* 2012;8:45.
9. Atamanalp M, Yanik T. Alteration in hematological parameters of rainbow trout (*Oncorhynchus Mykiss*) exposed to Mancozeb. *Turk J Vet Anim Sci.* 2003;27(5):1213-7.
10. Mansour SA, Mossa A-TH. Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pestic Biochem Physiol.* 2010;96(1):14-23.
11. Abdel-Daim MM, Abuzead SM, Halawa SM. Protective role of *Spirulina platensis* against acute deltamethrin-induced toxicity in rats. *PLoS One.* 2013;8(9):e72991.
12. Yousef MI, Awad TI, Mohamed EH. Deltamethrin-induced oxidative damage and biochemical alterations in rat and its attenuation by Vitamin E. *Toxicology.* 2006;227(3):240-7.
13. Rehman H, Aziz AT, Saggu S, Abbas ZK, Mohan A, Ansari AA. Systematic review on pyrethroid toxicity with special reference to deltamethrin. *J Entomol Zool Stud.* 2014;2(6):60-70.

14. Sharma P, Singh R, Jan M. Dose-Dependent Effect of Deltamethrin in Testis, Liver, and Kidney of Wistar Rats. *Toxicol Int.* 2014;21(2):175-81.
15. Gündüz E, Ülger BV, İbiloğlu İ, Ekinci A, Dursun R, Zengin Y, et al. Glutamine Provides Effective Protection against Deltamethrin-Induced Acute Hepatotoxicity in Rats But Not Against Nephrotoxicity. *Med Sci Monit.* 2015;21:1107-14.
16. Alwan AK. Toxic Effects of Cypermethrin on Liver and Kidney of Male Domestic Rabbits [dissertation]. Gaza: The Islamic University of Gaza; 2015.
17. El-Demerdash FM. Antioxidant effect of vitamin E and selenium on lipid peroxidation, enzyme activities and biochemical parameters in rats exposed to aluminium. *J Trace Elem Med Biol.* 2004;18(1):113-21.
18. Raina R, Verma PK, Pankaj NK, Kant V. Ameliorative effects of alpha-tocopherol on cypermethrin induced oxidative stress and lipid peroxidation in Wistar rats. *Int J Med Med Sci.* 2009;1(9):396-9.
19. Yousef MI, El-Demerdash FM, Kamil KI, Elswad FA. Ameliorating effect of folic acid on chromium (VI)-induced changes in reproductive performance and seminal plasma biochemistry in male rabbits. *Reprod Toxicol.* 2006;21(3):322-8.
20. Forges T, Monnier-Barbarino P, Alberto JM, Guéant-Rodriguez RM, Daval JL, Guéant JL. Impact of folate and homocysteine metabolism on human reproductive health. *Hum Reprod Update.* 2007;13(3):225-38.
21. Shalaby MA, El Zorba HY, Ziada RM. Reproductive toxicity of methomyl insecticide in male rats and protective effect of folic acid. *Food Chem Toxicol.* 2010;48(11):3221-6.
22. Sakr S, Hassanien H, Bester MJ, Arbi S, Sobhy A, El Negrish H, et al. Beneficial effects of folic acid on the kidneys and testes of adult albino rats after exposure to methomyl. *Toxicol Res (Camb).* 2018;7(3):480-91.
23. Barrett JR. Folic Acid and ASDs: A Preventive Measure against Potential Effects of Pesticide Exposures? *Environ Health Perspect.* 2017;125(10):104006.
24. Ulaiwi HK. Hemato-biochemical and histopathological alterations induced by acute cypermethrin toxicity in rabbits. *AL-Qadisiya J Vet Med Sci.* 2011;10(2):84-94.
25. Khan AM, Raina R, Dubey N, Singh G, Beigh SA. Toxic Effects of Deltamethrin and Fluoride on Hematological Parameters in Rats. *Fluoride.* 2013;46(1):34-8.
26. Bancroft JD, Gamble M. Theory and practice of histological techniques. 6th ed. Philadelphia: Churchill Livingstone Elsevier; 2008.
27. Abbassy MA, Marzouk MA. Hematobiochemical effect of formulated and technical cypermethrin and deltamethrin insecticides in male rats. *Toxicol Environ Health Sci.* 2012;7(7):312-21.
28. Meng SL, Hu GD, Qiu LP, Song C, Fan LM, Chen JZ, et al. Effects of chronic exposure of methomyl on the antioxidant system in kidney of Nile tilapia (*Oreochromis niloticus*) and recovery pattern. *J Toxicol Environ Health A.* 2013;76(15):937-43.
29. Farrag ARH, Shalby S. Comparative Histopathological and Histochemical Studies on IGR, Lufenuron and Profenofos Insecticide Albino Rats. *J Appl Sci Res.* 2007;3(5):377-86.
30. El-Gerbed MSA. Protective effect of lycopene on deltamethrin-induced histological and ultrastructural changes in kidney tissue of rats. *Toxicol Ind Health.* 2014;30(2):160-73.
31. Sangha GK, Kaur K, Khera KS, Singh B. Toxicological Effects of Cypermethrin on Female Albino Rats. *Toxicol Int.* 2011;18(1):5-8.
32. Rao JV. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. *Pestic Biochem Physiol.* 2006;86(2):78-84.
33. El-durssi ISH, El-Awami IOS, Mahmoud GS, Benkhaial FA. Experimental Studies on the effects of chlorpyrifos on Rats-II. Histochemical changes. *AL-Mukhtar J Sci.* 2006;13:80-8.
34. Shalaby AA. Effect of cyolane on the cytology and histochemistry of the ileum of *Clarias lazera* [MSc Thesis]. Zagazig: Faculty of Science, Zagazig University; 1985.
35. Faddladdeen KAJ. Effect of liver fibrotic changes on testicular histological structure: An updated review. *Middle East Fertil Soc J.* 2019;24(1):24.
36. Abdelrahman M, Arafa MS. Effect of deltamethrin on the release of catecholamines and its related effect on some sex hormones in adult male albino rats. *Isot Radiat Res.* 2005;37(1):89-102.
37. Kobeasy MI, El-Naggar AY, Abdallah AA. A novel methods for protective role against reproductive toxicity of carbofurem in male rats using palm pollen grains and vanadyl (II) folate as a new compound. *J Chem Pharm Res.* 2015;7(4):1142-8.
38. Lu M, Liu Y. Folic acid reduces methomyl insecticide damage to testicular cells by altering the DNA methylation environment. *Toxicol Mech Methods.* 2022;32(4):311-20.