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# Histopathological Changes in Testicular Tissues of Male Rabbits by Trimetion

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**Abstract**
This study was conducted to investigate pathological lesions associated with Trimetion intoxication on testes and epididymis and fertility in adult male rabbits. Totally, 24 local male rabbits were purchased, prepared and randomly divided equally into four groups; (G1) negative control group, (G2) drenched Trimetion at 10 ml/Kg daily for two months; (G3) drenched Trimetion at 25 ml/Kg daily for two months; and (G4) drenched Trimetion at 50 ml/Kg daily for two months using of oral gavage. In contrast to control group, the testes of the treated groups had atrophy. The primary histopathological lesions of drenched rabbits revealed that Trimetion causes a dose-related damage to testes as evidenced by mild hydropic degeneration, sloughing of cell lining and by partial suppression of spermatogenesis in G1; while in G2, epididymis appears to be lack of sperm. In G3, there were more severe testicular damages with little hydropic degeneration, spermatogonial inhibition, and severe lack of spermatogenesis. The findings of G4 had aggressive, severe, and widespread lesions in all testicular tissue, severe necrosis and full spermatogonial suppression with no spermatogonia. In conclusion, the current study showed that the Trimetion is caused different pathological lesions in male testicular and epididymal tissues of adult rabbits which appeared microscopically sever necrosis and degenerations and complete suppression of spermatogenesis with vaculation of spermatogonia. However, moreover experiments are required to identify other toxicopathological changes in other body tissues.

**Keywords**: Necrosis, Degeneration, Reproductive system, Iraq.

## Introduction

Any material intended for eradicating or repelling pests is considered a pesticide. (1). Pesticides were a large and varied class of chemicals that were once employed to eradicate and kill insects, rodents, and fungi (2, 3). Additionally, pesticides are to blame for a number of illnesses and conditions, such as cancer, neurobehavioral disorders, retrograde reproductive outcomes, peripheral neuropathies, impaired immunological functions, and allergic sensitization reactions, particularly of the skin (4). Organophosphate (OP) chemicals, including Trimetion (DM), are among the most widely used groups of insecticides in the world for a variety of purposes on fields, agricultural crops, and ornamentals. It is also used indoors to control houseflies (5, 6). Because Trimetion persists in crops and soil, its widespread use could pose a health risk to most animals and people (7). Results from earlier studies on the effects of Trimetion on endocrine and reproductive function suggested that it may have an impact on the serum concentration of hormones involved in the reproductive and metabolic systems (8). In contrast to the controls, Trimetion at 28 mg/kg of body weight was associated with a clear decrease in sperm count, motility, and viability as well as a considerably higher percentage of morphologically aberrant spermatozoa (9). Trimetion produced developmental toxicity, which included decreased live births with the occurrence of resorptions, decreased fetal body weights, decreased fetal implantation rates, and impaired fertility in mice following repeated exposures (10). Both male and female adult reproductive

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systems were shown to be chronically toxic to Trimetion, and the female's estrous cycle was found to be erratic. In addition, there were changes in the females' serum gonadotrophin levels (11). For male infertility, decreased *libido*, deterioration in the semen quality, also altered in the testosterone levels with degeneration of testes are (12-15). The goal of the current study to investigate pathological lesions associated with Trimetion intoxication on testes and epididymis and fertility in adult male rabbits

## Materials and Methods

### Ethical approval

This study was conducted under the license of the Scientific Committee of the College of Veterinary Medicine, University of Wasit (Wasit, Iraq).

### Experiment

24 local male rabbits, 6 months old, weighing between 1500 and 2000 grams, obtained from the local market, were kept in cages in an animal house where they were exposed to 12 hours of light and 12 hours of darkness at a temperature of 22 to 25 degrees Celsius. Rabbits were randomly divided into four equal groups, each with six animals; Group (G1) was considered the control group and only received water; Group (G2) was exposed to Trimetion at a dose of 10 milligrams per kilogram per day for two months; Group (G3) was given Trimetion at a dose of 25 milligrams per kilogram per day for two months; and Group (G4) was given 50 milligrams per kilogram per day for two months using of oral gavage (16).

### Histopathology

The rabbits were scarified after two months of the trial, and all test animals had necropsies. After being removed, specimens including testicles and epididymis were stored in 10% neutral buffered formalin. After the first 48 hours of preservation, the specimens were processed by increasing the alcohol concentrations, embedding the tissue slice in paraffin blocks, and cutting the tissues into 5 m sections with a microtome. Finally, the tissues were stained with hematoxylin and eosin stain (H and E stain), and a light microscope was used to detect microscopic lesions (17).

## Results and discussion

Trimetion poisoning in rabbits caused variable microscopic and gross pathological alterations to be found in treated groups, however group four (G4) showed more severe lesions. For control untreated animals (G1), there were no notable gross or microscopic lesions (Figure 1A). In contrast to control group, the testes of the treated groups had atrophy. The primary histopathological lesions of the treated rabbits were only visible at a light microscopic level, but they revealed that the Trimetion caused dose-related damage to the testes, as evidenced by mild hydropic degeneration in the germinal layers lining the seminiferous tubules with sloughing of their cell lining and by partial suppression of spermatogenesis, while the epididymis appears microscopically to be empty and lacking sperm in case of G2. While the primary microscopic changes in the G3 stage were more severe than those in the G2 stage in

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terms of testicular damage, there was little hydropic degeneration in the germinal layer of the seminiferous tubules' cell lining along with the inhibition of spermatogenesis. Additionally, there was a complete lack of spermatogenesis and the epididymal tubules were empty and devoid of spermatozoa (Figure 1B). In contrast to the other groups (G2, G3), G4 had aggressive, severe, and widespread lesions in all testicular tissue, as well as severe necrosis in the germinal layers of the lining of the seminiferous tubules and full suppression of spermatogenesis with no spermatogonia (Figures 1C, 2A). Additionally, the epididymal tubules had completely stopped spermatogenesis, were empty, and did not contain spermatozoa (Figures 2B and C). These findings are in agreement with findings of other study that described similar histopathological lesions in the treated rats, including Trimetion-induced damage to the male testes, including sloughing, atrophy of the seminiferous tubule's epithelial lining, degeneration and necrosis of the germ cell, and partial to complete spermatogenesis arrest (18). Microscopic lesions in the testis of rabbits given Trimetion (10, 25 and 50 mg/kg BW for two months) in our investigation showed that the Sertoli cells' span was also suppression and deterioration of spermatogenesis. These results are consistent with the findings of other researchers who obtained identical results, but in testis of rats which exposed to nickel (19).

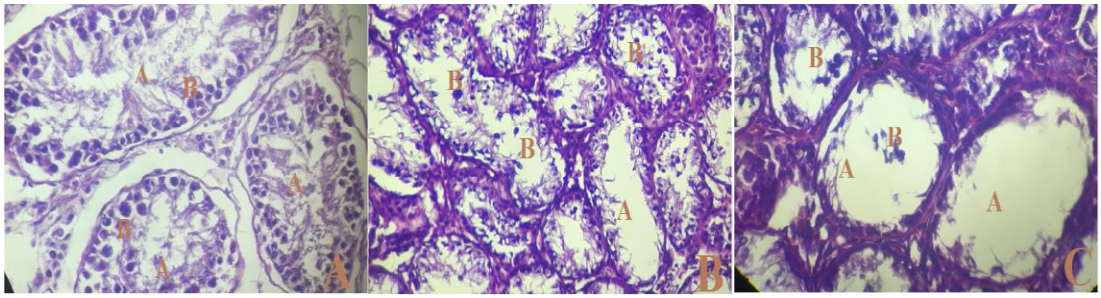


**Figure (1): [A]:** Normal structure of testis showed somniferous tubules; A: spermatozoa, B: Spermatogenia (X 40 H and E). **[B]:** Testis of rabbits which exposed to 25 mg/ kg of BW per day from Trimetion appeared; A: Suppression of spermatogenasis; B: Reduced numbers of spermatozoa in lumen (X 20 H and E). **[C]:** Testis of rabbits which exposed to 50 mg/ kg of BW per day from Trimetion showed; A: Sever suppression of spermatogenasis; B: Complete reduction numbers of spermatozoa in lumen (X 40 H and E).

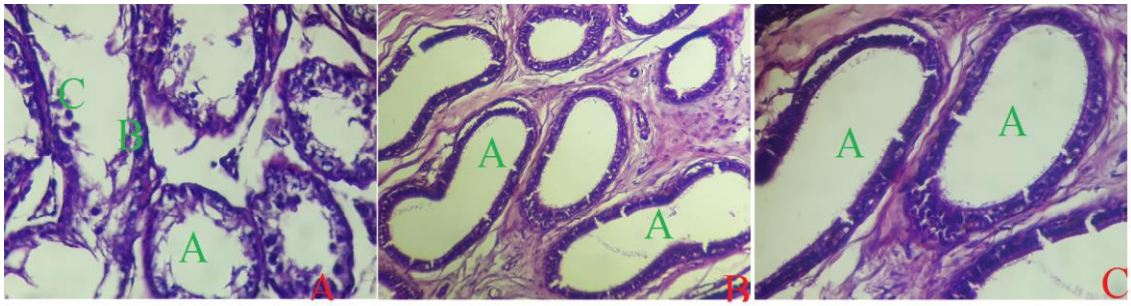


**Figure (2): [A]:** Testis of rabbits exposed to 50 mg/ kg of BW per day from Trimetion show complete destruction of testicular tissue; A: Sever suppression of spermatogenasis; B: Complete reduction numbers of spermatozoa in lumen; C: Decrease in numbers and vaculation of spermatogonia (X 40 H and E). **[B]:** (A): Epididymis of rabbits exposed to 50 mg/ kg of BW per day from Trimetion showed complete loss of spermatogenesis (X 20 H and E). **[C]:** A: Epididymis of rabbits exposed to 50 mg/ kg of BW per day from Trimetion showed majority of tubules empty and no spermatozoa (X 40 H and E).

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A considerable reduce in the relative size and weight in testes of rabbits, our results are agree with 20, the reduce in the size and weight of testes may occur due to spermatogenic arrest and depression in the tubule size, in addition to inhibition in biosynthesis of steroid in Leydig cells, which consider as a site of steroid biosynthesis (21, 22).

In our research there were decreased in testicular and epididymal size and weight, this results are coordinated with results of different studies that showed in adult male rodents due to direct incur to the organophosphorus pesticides and Trimetion and pesticides which posses the antiandrogenic affects (23-28).

In present work, the effects of Trimetion to spermatogenic and steroidogenic compartment of the testes were appearedin its microscopically, reduced in the diameter of seminiferous tubule, damage of the germ cells, absent ofthe sperms in the lumens completely and decreased in the size and number of Leydig cells were showed, and arrest of spermatogenesis completely was noticed in drenched animals with Trimetion, while numbers of germ cells were obviously decreased and lack of spermatozoa completely in several tubules. Similar microscopic lesions in testicular tissues was detected incur and expose to the sublethal doses of the Trimetion and another organophosphates which have been showed the epididymal tubules showed significant atrophy and changes in histo-architecture of the original cells of epididymis was furthermore characteristic for negative effect of the Trimetion on male reproductive system (13, 24-26, 29). Adult male rodent epididymis incur to trichloroethane, lindane dichlorodiphenyl and dieldrin showed similar histopathological finding who approved by (30-32)

## Conclusion

The current study showed that the Trimetion is caused different pathological lesions in male testicular and epididymal tissues of adult rabbits which appeared microscopically sever necrosis and degenerations and complete suppression of spermatogenesis with vaculation of spermatogonia. However, moreover experiments are required to identify other toxicopathological changes in other body tissues.

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