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Assessing the Toxic Effects of Topik80ec on Rabbit Testicular Morphometry and Histology

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Abstract: This study aimed to investigate the toxic impact of Topik80ec on rabbit testes through morphometric and histological analyses. Twenty-four adult male domestic rabbits (Oryctolagus cuniculus) were used, where three experimental groups, G1, G2, and G3, were assigned and provided escalating dosages of Topik80 EC for a duration of four weeks, while one group served as the control group and received distilled water. The morphometric characteristics, including weight, length, breadth, and thickness, of the testicles were evaluated. Histological examinations were performed on the testicular specimens, which were subjected to staining procedures using the Hematoxylin and Eosin (H&E) technique, as well as Azan's trichrome staining method. The results revealed a significant decline ($p<0.05$) in weight, length, and width in the treated groups compared to the control group, notably in group 3. Furthermore, significant alterations in the structural composition and tissue pathology of the testes in the rabbits subjected to treatment were detected compared to the control group, characterized by the degeneration of seminiferous tubules, as evidenced by the presence of atrophy, deformation, uneven epithelium, and a reduction in germinal cells, with an excessive build-up of collagen fiber within the testicular interstitial tissues resulting in the development of testicular interstitial fibrosis. These changes were particularly noticeable in the lower counts of spermatozoa at higher dosages (G3). The experimental compound Topik80 EC exhibited considerable toxicity towards the testes of male domestic rabbits, resulting in a significant reduction in fertility due to profound structural and functional harm.

Keywords: Atrophy, Histology, Morphometry, Rabbits, Reproduction, Spermatozoa, Testis, Topik80EC

Introduction

Annually, the agricultural chemical sector manufactures a wide array of goods, including pesticides, with the objective of mitigating the negative impact of pests and enhancing food production (Solomon and Schettler, 2000; Severi-Aguiar *et al*., 2014). Globally, the estimated annual use of pesticides is around 2.5 million tons, resulting in approximately three million instances of pesticide poisoning and an estimated 220,000 fatalities. These adverse effects are especially prevalent in less developed countries (World Health Organization, 2000). Studies by Clementi *et al*. (2008); Foster *et al*. (2008); Roeleveld and Bretveld (2008) provide evidence indicating possible reproductive complications associated with recurrent pesticide exposure. In Algeria, the annual use of pesticides exceeds 30,000 tons, including over 400 authorized varieties, of which only about forty are actively utilized by farmers. Herbicides, which fall under the category of pesticides, are used to manage undesired plant proliferation within agricultural environments. However, the overutilization of these substances can lead to water and soil pollution, presenting substantial hazards to the well-being of humans, animals, and plants (Bordjiba and Ketif, 2009).

Several studies have highlighted the harmful properties of herbicides, which have been shown to induce genotoxicity and negatively impact reproduction. These consequences include disturbances in spermatogenesis, decreased sperm motility and viability, and alterations in the estrus cycle among females (Benbrook, 2016; Ferdinand *et al*., 2017; Al-Hamdani and Yajurvedi, 2017). These outcomes have been attributed to herbicide exposure. Chen *et al*. (2005) reported that topik80ec, a herbicide classified as an aryloxyphenoxypropionate and comprising Clodinafop propargyl, is effective in managing grassy weeds in cereal crops. Despite its extensive use and significant commercial success, concerns persist regarding its potential adverse effects on the environment and human health (Stoytcheva, 2011). The potential environmental and health implications of inhibiting the enzyme acetyl-coenzyme-A-carboxylase using clodinafop-propargyl and related chemicals are a subject of concern, as they may affect fatty acid synthesis. Numerous investigations on several animal species have shown significant levels of toxicity, characterized by phenomena such as hepatocyte hypertrophy, tissue necrosis, thymic atrophy, and a plausible association with carcinogenicity in humans (Bhushan *et al*., 2003; US EPA, 2024).

The literature suggests a correlation between herbicide exposure and a range of reproductive complications, including reduced sperm quality and motility, abnormalities in reproductive structures and tissues, and disturbances in hormone regulation (Roeleveld and Bretveld, 2008; Clementi *et al*., 2008; Foster *et al*., 2008). The available scientific evidence establishes a connection between herbicide exposure and reproductive issues. However, there is a scarcity of scholarly inquiry focused on the precise impacts of Topik80ec on the morphology and histology of the testes in domestic rabbits. This study aims to examine the impact of TOPIK80EC on the reproductive system of male rabbits, including assessments of the size, weight, width, and thickness of the testes, as well as histological analyses.

Materials and Methods

Anima and Experimental Condition

The study consisted of a total of twenty-four adult male rabbits, each with a weight of 1.9-2.5 kg and an estimated age of 5-6 months. The rabbits were housed in regulated conditions, residing in galvanized cages. Environmental conditions were carefully controlled, ensuring a temperature range of 22±2 degrees Celsius and a humidity level of 60±5 percent. The photoperiod was meticulously managed, providing 12 h of illumination followed by an equal duration of darkness. The rabbits were given unrestricted access to a purified rabbit-conglomerated diet made from cereals, plants, minerals, and vitamins twice a day with fodder once a day and ad libitum access to water. A three-week adaptation period was conducted. Afterward, the rabbits were divided into four groups through a random process, with each group comprising six animals.

The Control Group, a group of six rabbits was administered simply physiological water and functioned as the untreated control group for a duration of four weeks.

In group I, a total of six rabbits were subjected to a daily exposure of 1 mg/kg.

In group II, a total of six rabbits were subjected to a daily administration of 4 mg/kg.

Group III, consisted of six rabbits that were administered a dosage of 20 mg/kg/day utilizing a gavage instrument for a duration of four weeks. The experiments were conducted by a veterinarian and validated by the Research Laboratory of Science and Techniques for Living at the Institute of Agriculture and Veterinary Sciences at the University of Souk Ahras.

Tissue Collection and Morphometric Evaluation

Following the last day of exposure, the rabbits were humanely killed in accordance with halal practices. Firstly, a thorough dissection was conducted on their reproductive systems, with particular attention given to the removal and measurement of the testis's weight using a precise electronic balance. Further morphometric measures, including the dimensions of length, breadth, and thickness of the testes, were acquired using vernier calipers.

Histological Analysis

The testicular tissues were preserved in a 10% neutral buffered formalin solution for histological analysis. After preservation, the tissues were sectioned and embedded in paraffin wax, resulting in slices that were 5 µm thick. The staining procedure used for these slices included the application of Hematoxylin and Eosin (H&E), as well as Masson's trichrome, following the methodology described by Cardiff *et al*. (2014). Subsequently, the stained sections were examined using a light microscope to identify any histological changes. The histological analysis was done at the histology and microscopy laboratory, at Constantine Biotechnology Research Center in Algeria.

Statistical Analysis

The assessment of group differences was conducted using a two-way Analysis of Variance (ANOVA). Post hoc Tukey's Honestsly Significant Difference (HSD) testing for intergroup comparisons was performed using the R program. Statistical significance was considered when the p-value was ≤ 0.05.

Results

Macroscopic Study

According to macroscopic observation, a marginal decrease in the size of the testes was observed in groups I and II in comparison to the control group. A significant reduction in testis size, characterized by atrophy, was observed in group III compared to the control group Fig. (1).

The Morphometric Study

Values are expressed as means ± SD.

The results demonstrated that the testis weight, Length, Width, and Thickness of rabbits were significantly ($p<0.05$) decreased obviously following periods of administration. where we found highly significant differences among the four groups .

To compare inter-groups we used post hoc Tukey HSD test based on the data in Table (1).

Testicular Weight

A highly significant difference was observed among the four groups ($p<0.01$). An insignificant decrease ($p>0.05$) in the testicular weight was observed in experimental group I in comparison to the control group. However, there was a highly significant decrease ($p<0.01$) in testicular weight in groups II and III compared to the control, as well as in group I compared to group III and in group II compared to group III. Additionally, there was a significant decrease ($p<0.05$) in testicular weight in group I compared to group II Fig. (2).

Testicular Length

Highly significant differences were observed among the four groups ($p<0.01$). An insignificant decrease ($p>0.05$) in the testicular length was observed in experimental group I compared to the control group, as well as between groups I and group II. However, we found a highly significant ($p<0.01$) decrease in testicular length in group II and group III compared to the control group, as well as in group I in comparison to group III and group II and group III Fig. (3).

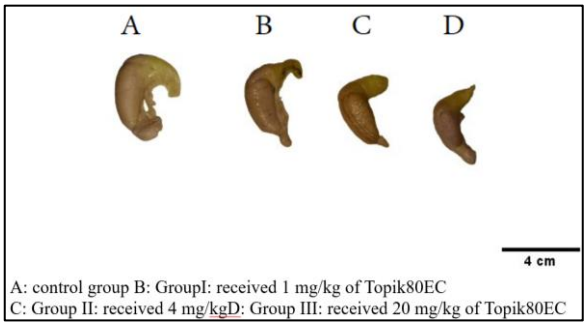


Fig. 1: Comparison of the macroscopic appearance of testicles in rabbits control vs. treated group

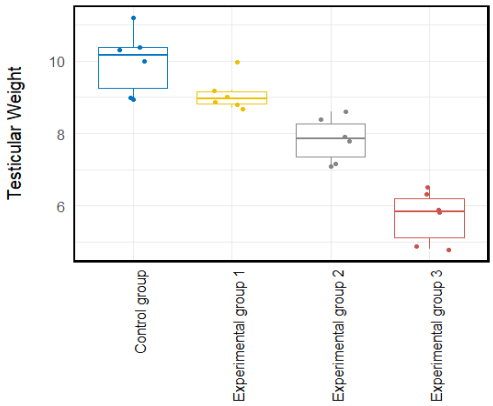


Fig. 2: Distribution of testicular weight values according to treatments

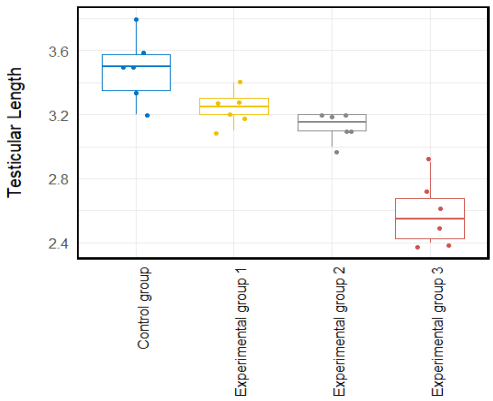


Fig. 3: Distribution of testicular length values according to treatments

Width of Testis

The results demonstrated a highly significant difference in testicular width among the four groups ($p<0.01$). An insignificant decrease ($p>0.05$) was observed in the testicular width of experimental groups I and II compared to the control group, as well as between groups I and II. However, a highly significant ($p<0.01$) decrease in testicular width was observed in experimental group III compared to the control group, as well as between groups I and III and groups II and III Fig. (4).

Microscopic Results

A highly significant difference was observed among the four groups ($p<0.01$). There was an insignificant decrease in the testicular thickness of experimental group I compared to the control group ($p>0.05$). Conversely, the results demonstrated a significant decrease in testicular thickness between experimental groups II and III ($p<0.05$). Furthermore, a highly significant decrease in testicular thickness was observed in experimental groups II and III compared to the control group ($p<0.01$), as well as between experimental group I and group II ($p<0.01$) and between group I and group III ($p<0.01$) Fig. (5).

Table 1: The impact of topik 80EC on the morphometric parameter of the testicle in rabbit

Morphometric parameters testicle	Control group	Experimental group 1	Experimental group 2	Experimental group 3
Weight	9.9667±0.88242	9.1000±473290	7.8333±0.60882	1.0333±0.70711
Length	3.4833±0.21370	3.2500±0.10488	3.1333±0.08165	2.5833±0.19408
Width	1.3500±0.10488	1.2833±0.07528	1.2333±0.05164	1.0333±0.08165
Thickness	1.6667±0.14024	1.6250±0.10840	1.3917±0.08010	1.2167±01.2167

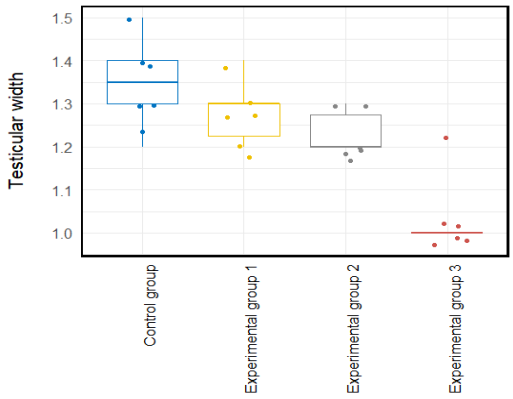


Fig. 4: Distribution of testicular width values according to treatments for testicular thickness

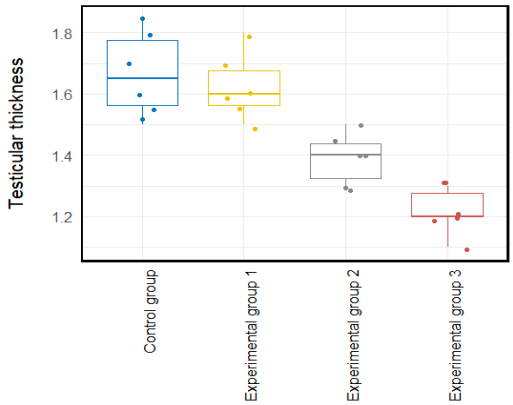
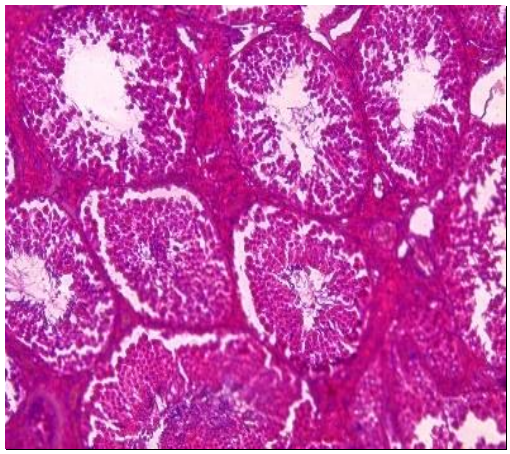


Fig. 5: Distribution of testicular thickness values according to treatments

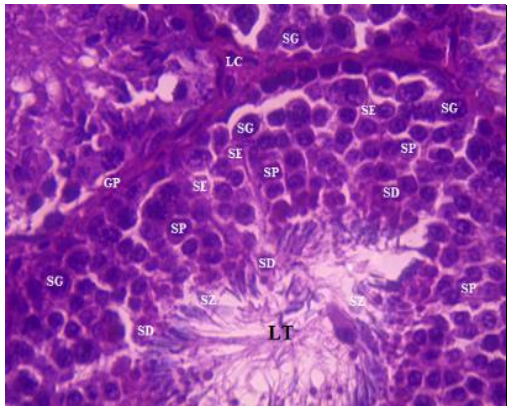
Microscopic Results

The control group was characterized by the presence of fully developed spermatozoa inside the seminiferous tubules. These tubules displayed a well-organized epithelial lining, minimal intertubular gaps, and healthy interstitial tissue containing Leydig cells. The seminiferous tubules consisted of germinal cells at different stages within an exposed lumen, with a notable quantity of spermatozoa present in Fig. (6).

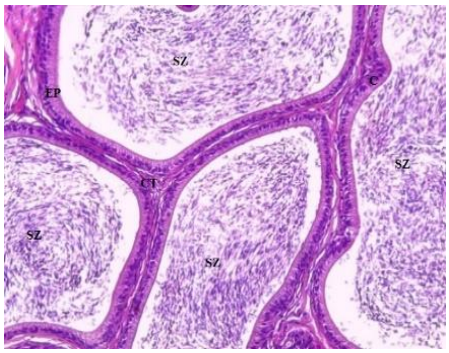
Structural modifications were observed in the testicular tissues of treated rabbits. These changes were severe in group 3, characterized by a disorganized state of the interstitial tissue and distortion of the seminiferous tubules. The spermatogenic epithelium exhibited a distorted structure, as seen in Fig. (6) (H&E, 40X). Additionally, there was detachment of germ cells from the underlying basement membrane, with cytoplasmic vacuolization within seminiferous tubules, as seen in Fig. (7) Also, degeneration of spermatogenic components led to reduced intensity in germ cells. Additionally, necrotic cells were frequently observed within the seminiferous tubules. The lumens of the seminiferous tubules appeared empty in some cases, indicating a decrease in diameter leading to their shrinking. There was a complete absence of stored spermatozoa in the epididymis Fig. (8). Disorganized structure and deformed connective tissue, lined by disorganized epithelium and cell layers, with a few stored spermatozoa sometimes absent in the epididymal lumen Fig. (9). Severe accumulation of collagen fibers in the interstitial space, with abundant necrotic cells within the seminiferous tubules Fig. (10).



(A)



(B)



(C)

Fig. 6: Photomicrographs of cross sections of the testis of the control group; (a) Seminiferous Tubules (ST), Intertubular Gaps (IG) (H & E, 40X magnification); (b) Spermatogonia (SG), Secondary spermatocytes (SP), spermatids (SD) and mature spermatozoa (SZ) in the Lumen of the Tubule (LT) (H&E, 100X magnification); (c) Proper connective tissue (CT), Pseudostratified Epithelium (EP), and Cells (C) with mature spermatozoa (SZ) (H&E stain, 40X magnification)

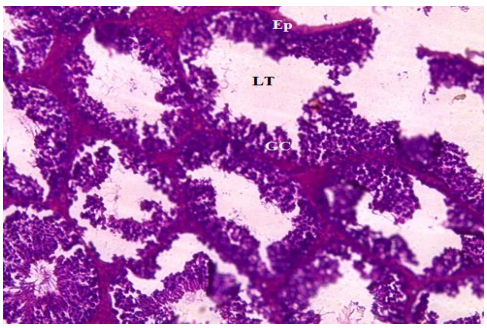


Fig. 7: A photomicrograph of the testisin-treated rabbits showing distortion of the Seminiferous Tubules (ST) with a disorganized state of the interstitial tissue and abnormal peritubular sheath, along with the absence of sperm in the tubule lumen (H&E, 40X)

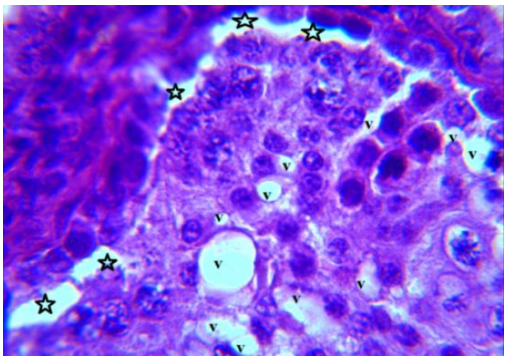


Fig. 8: A photomicrograph of the testis in treated rabbits showing detachment of germ cells from the underlying basement membrane, along with cytoplasmic Vacuolization (v) and necrotic cells within seminiferous tubules, with irregular empty spaces between cells (H&E, 100X)

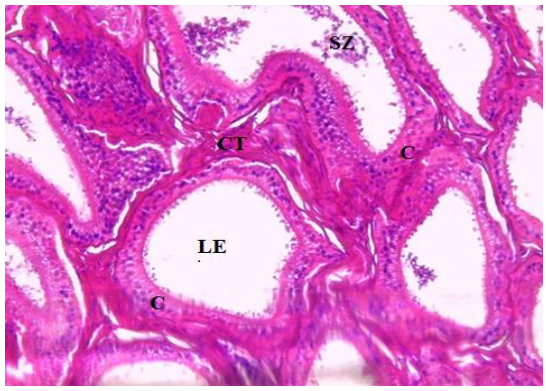


Fig. 9: A photomicrograph of the epididymis in treated rabbits showing a disorganized structure and deformed Connective Tissue (CT), lined by disorganized epithelium and Cell layers (C), with a few stored spermatozoa and sometimes absence in the epididymal lumen (H&E, 40X)

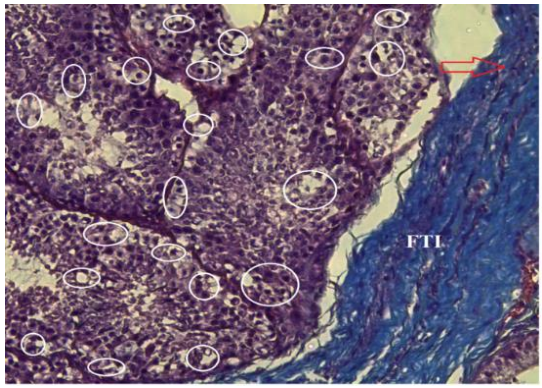


Fig. 10: A photomicrograph of the testis in treated rabbits showing an increased accumulation of collagen fibers in the extracellular matrix in their testicular interstitial tissues (FTI), with abundant necrotic cells within the seminiferous tubules (Azan's trichrome stain, 40X)

Discussion

Our study demonstrates that Topik80EC has a harmful influence on the fertility and reproductive system of male rabbits, leading to a notable decrease in the weights of the testes and potentially causing atrophy, particularly at higher doses. Similarly, a study conducted by European Food Safety Authority *et al*. (2020) showed that clodinafop propargyl led to decreased body and testes weights, as well as testicular atrophy in male mice. Earlier research by Abarikwu *et al*. (2010); Khozimy *et al*. (2022) observed that the application of the atrazine pesticide resulted in a noticeable decline in the overall body mass of laboratory rats and the weight of their reproductive organs after a treatment duration of seven days. In contrast, Oliveira *et al*. (2007) found no significant differences in testicle weight between control animals and

those exposed to different amounts of Roundup herbicide in male ducks. The observed decline in weight may potentially be associated with the process of spermatogenesis. However, it is worth noting that it might also be linked to a disturbance in liver tissue, as shown by the research conducted by Castilla-Cortázar *et al*. (2004); López-Lemus *et al*. (2018). This may also be related to disruptions in hormone levels, as shown by studies by Elsharkawy *et al.* (2019) induced toxicity by mancozeb fungicide in male rabbits showed a significant reduction in serum FSH and serum LH levels, this result may be caused by interference of Mancozeb fungicide with testicular function and act indirectly at the level of the hypothalamus or pituitary gland, or may act directly on the testicles like Carbamates. With also a significant decrease in serum testosterone levels (ng/mL) of male rabbits during the whole period of the experiment in comparison with the control group, The decrease in serum testosterone indicates an inhibitory effect of Mancozeb on the secretion of pituitary gonadotropins (FSH and LH) and the subsequent synthesis of testosterone. Singh and Pandey (1990). Which leads to complications of spermatogenesis were a significant decrease in testicle weight. Also, a histopathological examination of the testis showed an alteration in the structure of the seminiferous tubules.

Microscopic examination revealed histopathological findings, including morphological and histological abnormalities in the testis. Our results indicated a decline in Leydig cells and a decrease in the diameter of the lumen tube. Additionally, our study identified the decrease in luminal diameter as a significant outcome. Similarly, Ahmed *et al.* (2023) observed certain effects following the administration of clodinafop propargyl bait (0.98%) to male rats, specifically noting the degradation of spermatogenic components and the presence of coagulated seminal lumen, accompanied by an abnormal increase in the number of Leydig cells, referred to as hyperplasia. In a study conducted by Mathias *et al*. (2012), it was observed that the administration of the herbicide metolachlor to male Wistar rats resulted in alterations in the morphology of the seminiferous epithelium in the treated subjects.

Furthermore, Moselhy *et al*. (2016) demonstrated a harmful impact on fertility indices and morphological alterations in the reproductive organs (ovarian and uterine tissues) of female rats exposed to the herbicides Glyphosate and Atrazine. In contrast, Williams *et al*. (2000) concluded that chronic and/or subchronic trials did not reveal any detrimental effects on reproductive organs in animals treated with Glyphosate herbicide. However, a study by Romano *et al*. (2010) demonstrated that glyphosate roundup Transorb has harmful impacts on the endocrine reproductive system of rats. Our researchers noted significant stenosis and deformation in the diameter of the tubular lumen in all the groups subjected to treatment, with a particularly pronounced effect observed in the group receiving a higher dosage. It is crucial to emphasize that even at lesser dosages, the herbicide's deleterious effects remained apparent. These changes may be due to decreased activity of the seminiferous tubules.

The spermatogenic epithelium exhibited a distorted structure and the degeneration of spermatogenic components led to reduced intensity in germ cells. Additionally, necrotic cells were frequently observed within the seminiferous tubules. In some cases, the lumen of the seminiferous tubules appeared empty after treatment, with a notable decrease in diameter leading to their shrinking or even disappearance. These findings resemble the study conducted by Olayinka and Ore (2015), which revealed that the herbicide Haloxyfop P Methyl Ester (HPME) induces diverse morphological degenerations in the testes of rats. The observed alterations include pronounced interstitial edema (OD) and the presence of necrotic and degraded germinal epithelium. Additionally, the examination revealed the existence of immature germ cells and cellular aggregates inside the luminal space of some seminiferous tubules. These findings are similar to those derived from research on AOPP (fluazifop-p-butyl) and other herbicides in the phenoxyacetic acid category by Ore and Olayinka (2017); Joshi *et al*. (2012). Our results also found that the degeneration of spermatogenic components led to reduced intensity in germ cells. Additionally, necrotic cells were frequently observed within the seminiferous tubules and in some cases, the lumen of the seminiferous tubules appeared empty after treatment, with a notable decrease in diameter leading to their shrinking or even disappearance.

Therefore, there was detachment of germ cells from the underlying basement membrane, accompanied by cytoplasmic vacuolization within the seminiferous tubules. In accordance with the findings of Elsharkawy *et al*. (2019), the histological evaluation of testicular tissue in rabbits subjected to a daily dose of 100 mg/kg of Mancozeb revealed the formation of vacuoles resulting from the loss of germ cells and disturbance of the interstitial tissue. Furthermore, a discernible reduction in the quantity of spermatozoa inside the lumen was evident. The Sertoli cells displayed vacuolation, a decrease in the count of spermatogenic cells, and a disturbance in the structure of early sperm cells (spermatogonia) and developing sperm cells (spermatocytes). Additionally, the interstitial cells were impacted, resulting in the development of edema in the interstitial tissue and degeneration of Leydig cells. The demise of spermatocytes was observed, leading to a reduction in germ cells after the spermatocyte stage, accompanied by a gradual increase in apoptosis and a depletion of the elongation phase of spermatid growth.

In addition, Sunder (2002) found that the administration of a combination of Metalaxyl and Mancozeb at a dose of 500 mg/kg for durations of 30, 60, and 90 days led to notable biochemical abnormalities. These phenomena were distinguished by alterations in the histological structure of the testes, including disturbance of the germinal epithelium with vacuoles, depletion of spermatozoa inside the tubular lumen resulting in reduced sperm count (oligospermia), accumulation of fluid under the outer layer of the testes (subcapsular edema) and degeneration of the seminiferous tubules. A reduction in both the dimensions and functionality of Leydig cells was also noted.

Conclusion

Our study conclusively demonstrates that Topik80EC negatively impacts the fertility and reproductive system of male rabbits, causing significant reductions in testicular weight and potential atrophy, particularly at higher doses. Histopathological examinations revealed pronounced degeneration in the testes, including reduced germ cell intensity, necrosis within the seminiferous tubules, and cytoplasmic vacuolization. These results highlight the pervasive and detrimental impact of herbicides on the male reproductive system, emphasizing the need for careful evaluation and regulation of such chemicals to mitigate their potential risks to reproductive health.

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Author's Contributions

Samia Rebahi: Conducted statistical analysis of historical writing experiments.

Alouani Abdelouahab and Meguini Mohamed Nadir: Interpreted the writing data.

Khenenou Tarek: Provided data interpretation.

Fares Mohamed Amine: Performed statistical analysis.

Aidoudi Hafiza: Designed and analyzed writing experiments.

Chikha Maria: Corrected and refined the writing.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all co-authors have reviewed and approved the manuscript. Additionally, there are no ethical issues associated with this publication.

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