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Histopathological changes in the testes of Albino rat exposed to Endosulfan

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ABSTRACT: Despite its extremely harmful effects, endosulfan, an organochlorine insecticide, is a commonly used pesticide in agriculture. Consequently, the study's goal was to ascertain how endosulfan affected the albino rats' testes' histology and the effect of that pesticide on the levels of testosterone. The present study was conducted on 32 male albino rats that divided into four groups, control group and three other groups who were administered endosulfan orally at low, medium, and high doses (3.5, 7, and 10.5 mg/kg), respectively for a duration of 15, 30, and 45 days. Results obtained in the current research revealed that there was a significant reduction in the levels of testosterone with an increase in the dose and the duration of exposure to endosulfan with a generation of macrovaculation germinal epithelial cells, degeneration, necrosis, atrophy of certain tubules revealing aspermia and edema, and seminiferous tubule lining cell sloughing. Hormonal properties were also affected by endosulfan treatment. In conclusion, endosulfan after 15, 30 and 45 days of exposure caused significant damage and was harmful to testicular tissue. The current result concludes that hormonal imbalances and oxidative stress may be involved in the harmful effect's process.

KEYWORDS: Endosulfan; histological changes; pesticides; testes; testosterone.

1. INTRODUCTION

Pesticides are a broad category of chemicals that are extensively used in public health, and agriculture to prevent and control pests. It is believed that these chemicals may have negative health consequences [1]. Many pesticides are suspected of acting as endocrine disruptor chemicals (EDCs) and potentially dangerous to those exposed, even at extremely low exposure levels [2, 3]. Exposure to EDCs during the prenatal and postnatal phases of development until puberty can have a negative impact later in life by interfering with the physiology of normal endocrine-regulated events [4, 5].

Endosulfan, an organochlorine (OC) insecticide, is a widely used agricultural pesticide, despite its life- threatening toxic effects [6]. This insecticide is applied to control a wide variety of pests that infect of crops including hazelnut, tea, and a wide group of fruits, as well as cereals, maize, and else grains [7]. Despite that, the endosulfan utility has been reduced because of its lengthy stability in the agricultural fields, as well as, it strongly toxic to fish in the aquatic ecosystems, and harmful effects to farmers [8-10].

The male reproductive system's dysfunction is a major problem for the livestock industry. The main causes of insecticide-induced male infertility include spermatogenesis impairment, anti-androgenic effects, modifications to reproductive enzyme pathways, and lower sperm quality and motility [11]. The insecticides have a harmful effect on the male reproductive system by directly impacting reproductive organs (testis, Leydig cells, Sertoli cells) and germ cells or impairing hormonal balance in the secondary endocrine system [12,13].

Endosulfan has been linked to several negative effects on male reproductive parameters in rats, including fertility. These effects include degeneration of the seminiferous tubule epithelium, increased abnormal sperm, reduced sperm count, altered spermatogenesis, and testicular necrosis [14, 15]. In another study, DNA damage was discovered in Sertoli cells, primary spermatocytes, and mother cells of spermatogonia through the use of the TUNEL assay. Regarding the induction of DNA damage and weakened DNA repair mechanisms, the effect of ES on germ cells is critical to the maintenance of fertility and stable propagation of species. Concerning outcomes that were noted in male mice, 33% of the animals

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administered a sublethal dose of endosulfan experienced a testicular shrinkage, decreased sperm count, and increased mortality at a 24-hour serum concentration of 23 µg/L [16].

Based on the above, the current study aimed to determine the endosulfan effect on Histology and Physiology of albino rats and its consequences on the levels of testosterone.

.2. RESULTS

2.1. Testosterone level

Results illustrated in Table 1 showed that the levels of testosterone were reduced significantly (P≤0.05) in all treated groups that received different doses of endosulfan in comparison with that in controls. Results also revealed that within each treated group there was a significant (P≤0.05) decrease in the testosterone levels with an increase in the duration of administration, especially between the exposure to endosulfan for 15 days in comparison with the levels after the exposure for 45 days. On the other hand, the levels of testosterone reduced non-significantly (P>0.05) with an increase in the doses of endosulfan administration from 3.5-10.5 ng/ml

Table 1. Effect of doses and period in Testosterone

Groups	15 days	30days	45days	LSD value
Control	5.90 ±0.32	6.26 ±0.41	6.18 ±0.37	0.673 NS
	A a	A a	A a	
3.5mg/kg	2.22 ±0.08	2.13 ±0.12	1.37 ±0.10	0.839 *
	B a	B ab	B b	
7mg/kg	2.07 ±0.08	1.39 ±0.07	0.785 ±0.03	0.902 *
	B a	BC ab	B b	
10.5mg/kg	1.22 ±0.05	1.04 ±0.08	0.437 ±0.03	0.811 NS
	B a	B a	B a	
LSD value	1.074 *	0.984 *	1.195 *	---

Means with different big letters in the same column and small letters in the same row are significantly different.* (P≤0.05).

2.2. Histopathological changes

2.2.1. Control group

The results showed that the testes of the control group were surrounded by connective tissue called a capsule. The testicular parenchyma is composed of groups of seminiferous tubules. The seminiferous tubules were separated from each other by interstitial tissue which contains Leydig cells, Spermatogenesis contains the various stages of sperm formation (Germinal epithelium) within the wall of each seminiferous tubules. The germinal epithelium showed variable stages of spermatogenesis, starting with primordium germ cells being divided to give rise to spermatogonia, the precursor of sperm that develops to primary spermatocytes, and then into spermatids and spermatozoa (Figures 1 and 2).

2.2.2. Treated groups for two weeks:

The first signs which were seen under light microscopy, represented a marked reduction in the thickness of germinal epitheliums that revealed marked macrovaculation of spermatogenic cells with marked sloughing detachment of the basal lamina in treatment group 3.5mg/kg and the damage increased to be severe marked hypoplasia of germinal epithelium, associated with severe macrovaculation (cellular degeneration) of spermatogenic cells and degeneration of interstitial tissue involved Leydig cells, and amyloid deposition which was seen in treatment group 7mg/kg (Figure 3).

In the treatment group of 10.5 mg/kg, marked hypertrophy of seminiferous tubules associated with hyperplasia of the germinal epithelium and severe thickening of interstitial tissue had been observed.

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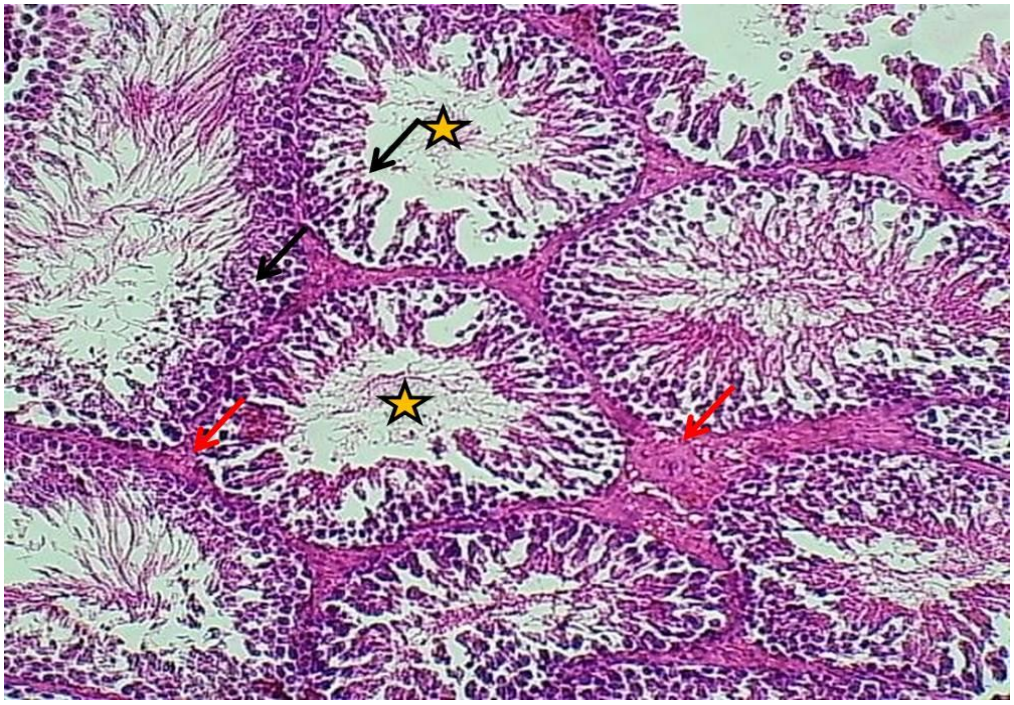

Figure 1. Section of testes (Control) shows: normal seminiferous tubules (asterisks), germinal epithelium (Black arrows) & interstitial tissue (Red arrows) .H&E stain, 100x.

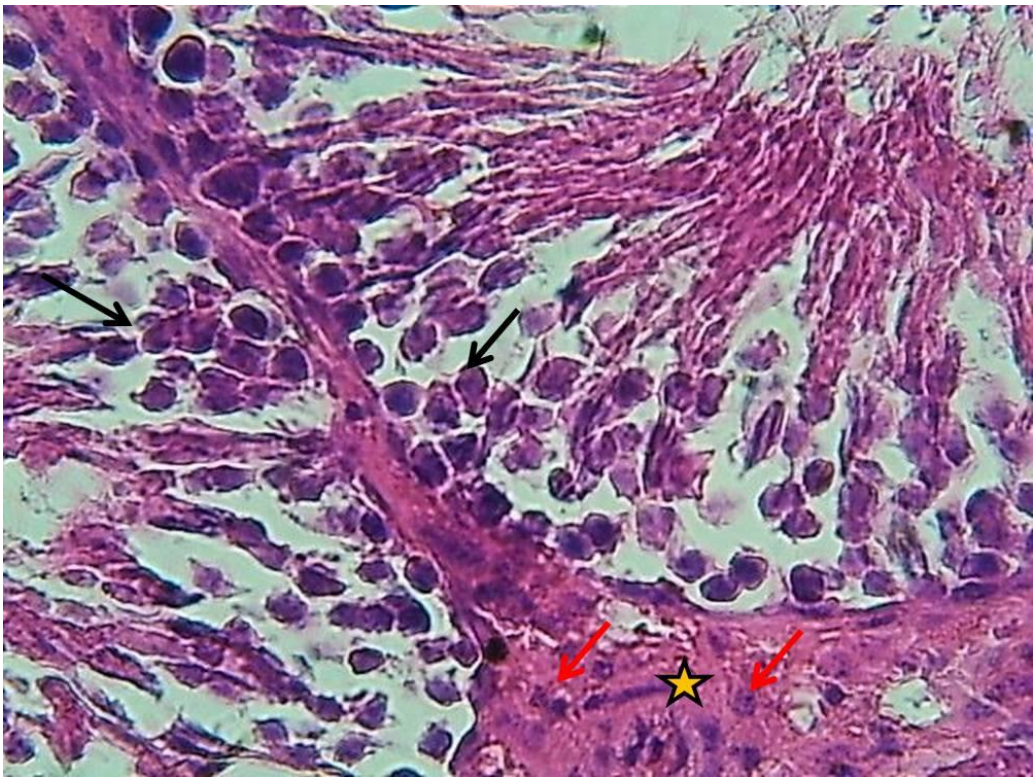

Figure 2. Section of seminiferous tubule (Control) shows: normal spermatogenic cells (Black arrows), interstitial cells (Red arrows) & thickness of interstitial tissue (Asterisk) .H&E stain, 400x.

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2.2.3. Treated groups for four weeks

At this period, the transmission of the damage caused by the pesticide that showed moderate hyperplasia and marked thickening of interstitial tissue in section of testis (3.5mg/kg). Group with 7mg/ kg revealed severe deterioration of seminiferous with necrosis of germinal epithelial cells and cellular depletion and interstitial tissue with marked irregular outline associated with hyperplasia of germinal epithelium mild thickening of interstitial tissue. While Section of testis 10.5mkg/mg shows marked hypertrophy of seminiferous tubules with marked irregular outline associated with hyperplasia of the germinal epithelium mild thickening of interstitial tissue (Figure 4 and 5)

2.2.4. Treated groups for six weeks

In this period, all groups exposed to the three concentrations (3.5, 7.0, 10.5 mg/kg) showed advanced signs of macrovaculation with necrosis of germinal epitheliums and marked degeneration of interstitial tissue with necrosis, severe atrophy of some tubules that revealed aspermia and edema of interstitial tissue (Figure 6, 7).

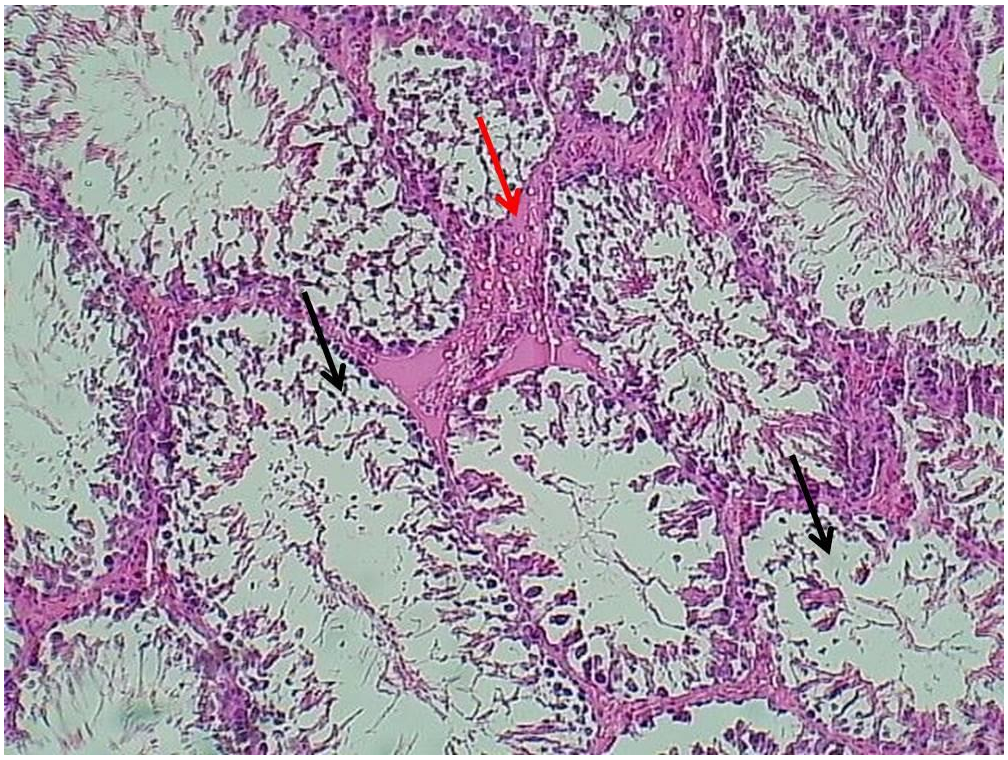

Figure 3. Section of testis (7 mg/kg-15 days) shows: severe macrovaculation of germinal epithelial cells (Black arrows) degeneration of interstitial tissue (Red arrows). H&E stain.100x.

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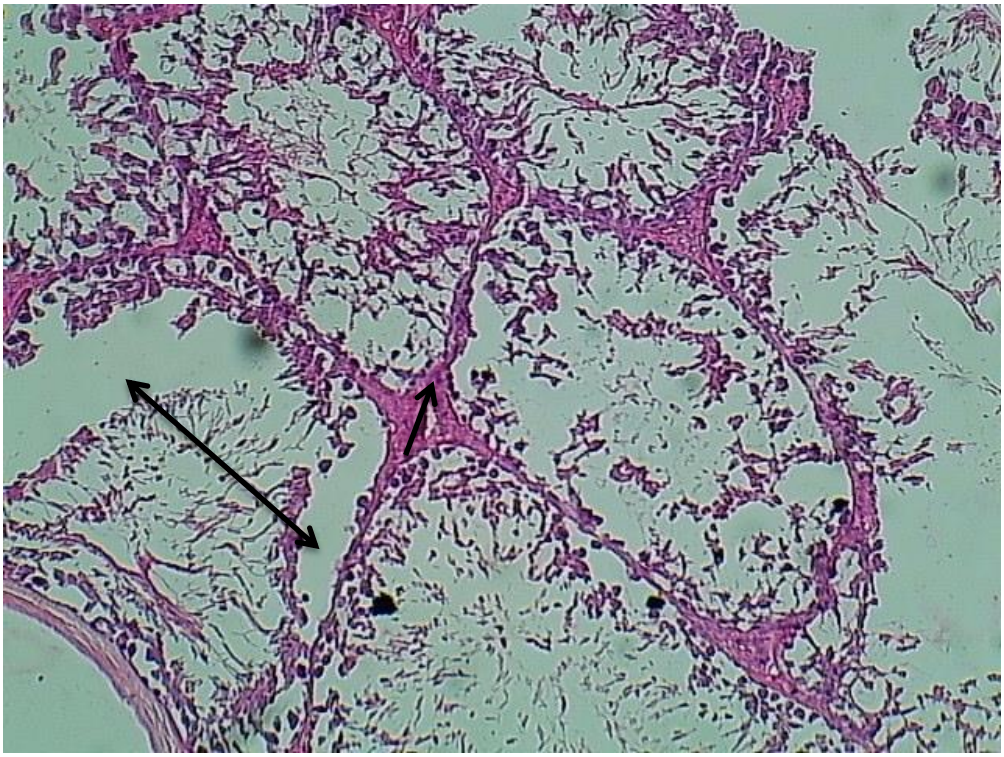

Figure 4. Section of testis (7mg/kg-30 days) shows: severe deterioration of the seminiferous tubules and cellular depletion (Reverse arrow) & interstitial tissue (Black arrow). H&E stain, 100x.

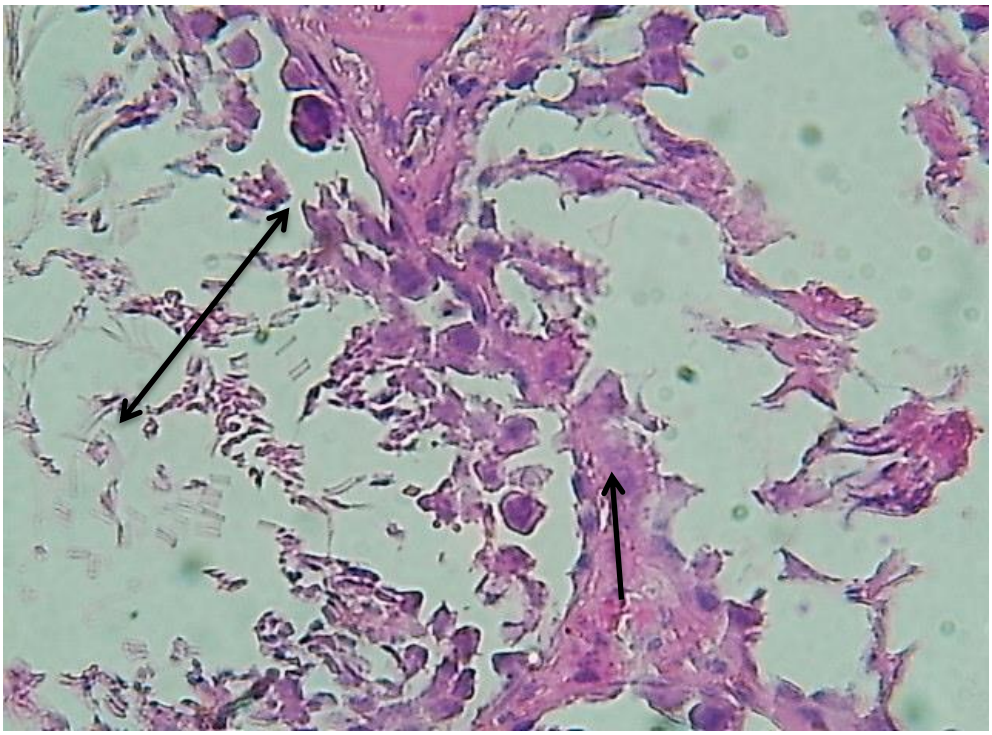

Figure 5. Section of testis (7mg/kg-30 days) shows: severe deterioration of the seminiferous with necrosis of the germinal epithelial cells (Revers arrow) and cellular depletion & interstitial tissue (Black arrow). H&E stain, 400x.

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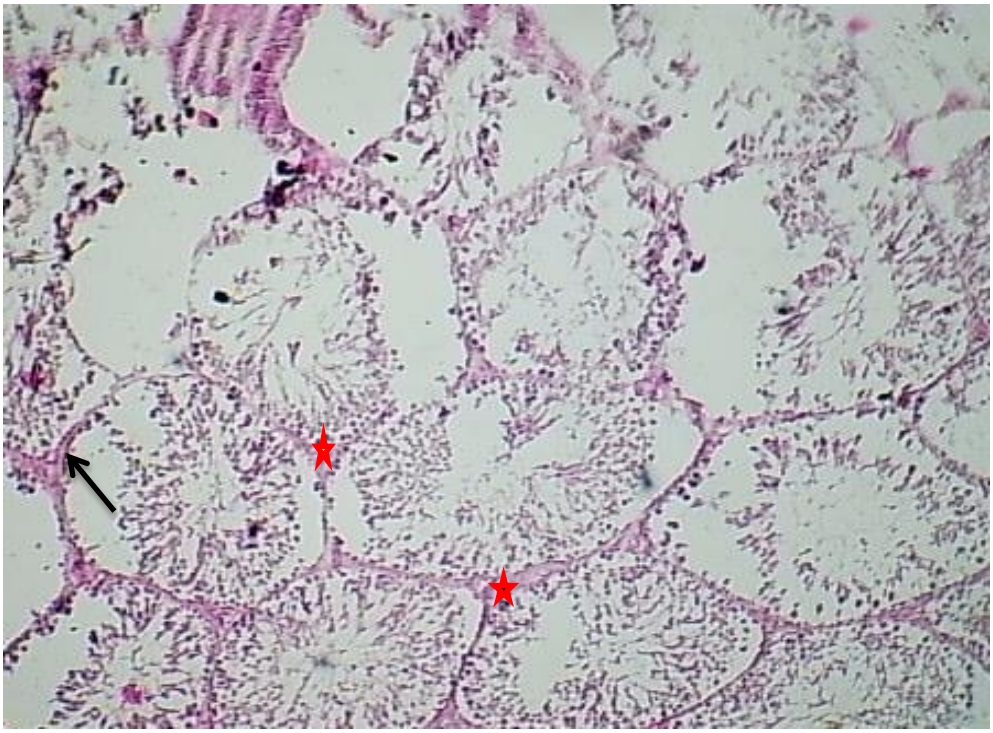

Figure 6. Section of testis (10.5mg/kg - 45 days) shows: marked deterioration of the seminiferous tubules with marked damage outline (Black arrow) and hypoplasia of germinal epithelium and tissue depletion (Asterisks). H&E stain, 100x

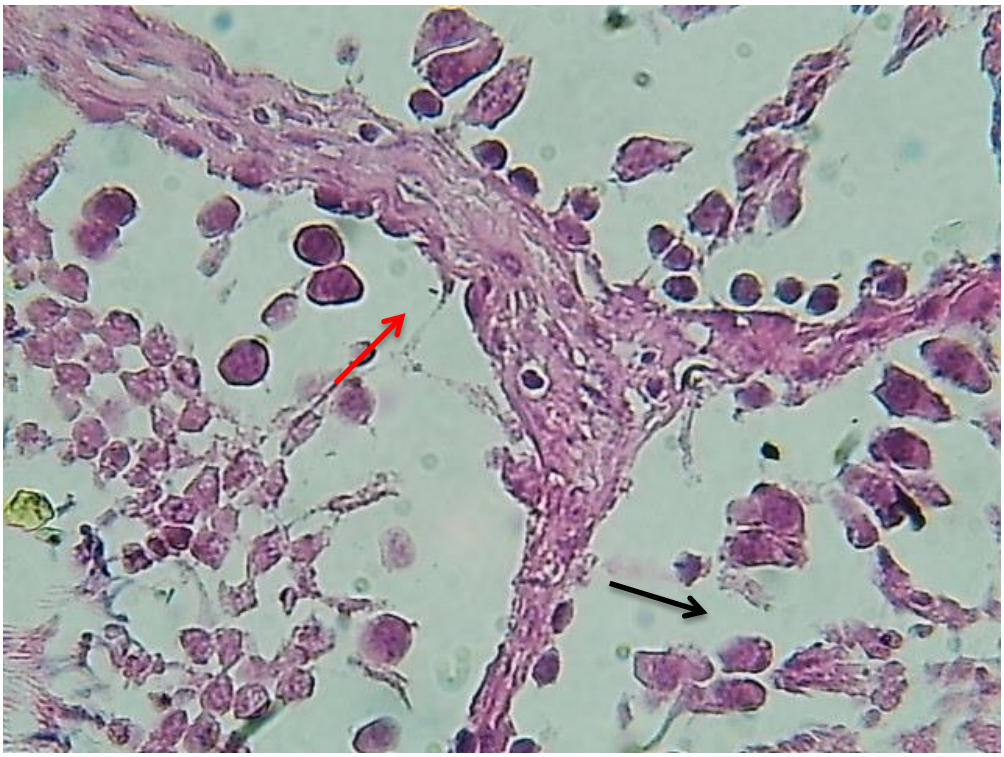

Figure 7. Section of testis (10.5ml -45 days) shows: marked hypoplasia of germinal epithelium (Red arrow) and tissue depletion associated with necrosis of germinal cells (Black arrow) H&E stain.400x.

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3. DISCUSSION

The World Health Organization (WHO) categorized Endosulfan as a moderately hazardous technically product [17]. Endosulfan has been shown in experiments to have negative effects on the histological structure of testes and sex hormone synthesis. The findings agree with Nath *et al.* [18] in that the treatment of endosulfan causes a drop in testosterone levels in Swiss albino male mice exposed to 3mg/kg of endosulfan. Since testosterone which is main circulating androgen is synthesized from cholesterol [19], a drop in plasma cholesterol levels may lead to a decrease in plasma testosterone [20]. It was discovered that there is a significant dose-dependent decline in plasma cholesterol levels. Moreover, endosulfan disrupts the function of the rat testicles by being toxic to spermatogonia and mature spermatids, as well as somatic (Leydig and Sertoli) cells [21]. Globally, long-term health impacts are not adequately researched, tested, or recorded [22, 23].

This study has demonstrated that endosulfan has a long-term impact on male rats' testes. According to a study using light microscopy, the spermatogenic cells, intertubular space, germinal epithelium, and seminiferous tubules saw the majority of the alterations. Following endosulfan therapy in rats, the testes showed several notable alterations, including edema, degeneration, necrosis, atrophy of certain tubules revealing aspermia, and macrovaccination of germinal epithelial cells. A majority of the testes from rats treated with endosulfan had damage to the seminiferous tubules. It was seen that the seminiferous tubule-lining cells were desquamating or sloughing. The degenerative condition seen in this study is in line with what Singh *et al*. [24] found after subjecting male of mice to the pesticide endosulfan at 2 mg/kg. I also concur with Nath *et al*. [19], who discovered that endosulfan exposure causes to degeneration of spermatogenesis, and induce oxidative stress in Swiss albino male mice exposed to 3mg/kg of endosulfan. Similar to Koç *et al*. [21], we also observed endosulfan-induced oedema and desquamation of the seminiferous tubule lining cells in this investigation. When he treated wistar albino male rats with endosulfan at 30ml/day, 50ml/day.

Endosulfan has been reported to cause oxidative stress in a variety of organs, including the liver and kidneys of many species [18]. It has been demonstrated that oxidative stress damages sperm DNA, proteins, and lipids and triggers apoptosis. As is well known, these pathways may contribute to sperm count reduction and sperm function impairment [25]. It is well recognized that one of the main causes of testicular dysfunction, which results in infertility, is oxidative stress [26]. Oxidative damage and the destruction of spermatogenesis and steriodogenesis are caused by the generation of reactive oxygen species (ROS) in the testis [27]. Endosulfan-induced elevation of MDA levels in rat liver and kidney tissues could be a sign of the presence of free radicals in metabolism and the lipid peroxidative damage they cause [18]. This could be the reason for testicular injury (damage to the seminiferous tubules) and atrophy in certain seminiferous tubules.

4. CONCLUSION

It was concluded that endosulfan may be hazardous to albino rats' male reproductive systems in all doses examined which caused a harmful effect with a significant damage to the testicular tissue that was appear clearly by this histological study, and this effect on testicular tissue leads to a significant reduction in the levels of testosterone hormone. Testicular oxidative stress that generated by endosulfan may be connected to the underlying mechanisms of its toxic effects.

5. MATERIALS AND METHODS

5.1. Methods

A case control study was conducted on thirty - two sexually mature laboratory males Albino rats, aged 6-8 weeks with an average weight of about 200 - 230 g. The rats were divided into four groups; each one of them contains eight rats. Animals were bought from the Biotechnology Research Center/Al-Nahrain University, and housed in the animal house of the Collage of Science for Women, University of Baghdad. They were kept in plastic cages (40x25x15 cm) with a controlled room temperature of 25 °C, under laboratory conditions, and a 12:12 dark and light cycle. Rats were provided with water and food ad libitum.

5.2. Groups of Experimental Animals:

The animals were divided into four groups according to the doses of endosulfan given and duration (15, 30, 45 days) were treated as follows:

5.2.1. First Group *(G1)*

The group included eight animals as a control, and each of them was dosed with one ml of normal saline solution only for 45 days- via daily oral administration.

5.2.2. Second *group (G2)*:

The group included eight animals each of them was dosed with one ml at a concentration of 3.5mg/kg for 45 days- via daily oral administration.

5.2.3. Third group *(G3)*:

The group included eight animals that were dosed with the insecticide at a dose of one ml at a concentration of 7mg/kg for 45days-via daily oral administration.

5.2.4. Fourth group (G4):

The group included eight animals that were dosed with the insecticide at a dose of one ml at a concentration of 10.5mg/kg for 45days- via daily oral administration.

At the end of each period (after 15, 30, and 45 days), male rats have been sacrificed, and the testis obtained from each group were fixed with10% buffered formalin, testis tissues embedded with paraffin. After routine processing, paraffin sections were cut into 5 µm thickness and stained with haematoxylin and eosin [28].

5.3. Estimation of Testosterone level:

Testosterone level of all groups and each period (15, 30, 45 days) of rat in the serum samples were determined by using testosterone ichroma ™ 11 kit.

5.4. Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significant compare between means in this study. ($P < 0.05$) was considered statistically significant [29].

Author contributions: Concept – M.H.; Design – B.Y., N.M., M.H.; Supervision – M.H.; Resources – N.M.; Materials – N.M.; Data Collection and/or Processing – N.M.; Analysis and/or Interpretation – N.M., M.H.; Literature Search – N.M.; Writing – N.M.; Critical Reviews – M.H.

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REFERENCES

[1] Mostafalou S, Abdollahi M. Pesticides and human chronic diseases: evidences, mechanisms, and perspectives. Toxicol Appl Pharmacol. 2013 ;268(2):157-177. https://doi.org/10.1016/j.taap.2013.01.025

[2] Kim KH, Kabir E, Jahan SA. Exposure to pesticides and the associated human health effects. Sci Total Environ. 2017;575:525-535. https://doi.org/10.1016/j.scitotenv.2016.09.009

[3] Combarnous Y. Endocrine Disruptor Compounds (EDCs) and agriculture: The case of pesticides. C R Biol. 2017;340(9-10):406-409. https://doi.org/10.1016/j.crvi.2017.07.009

[4] Crain DA, Janssen SJ, Edwards TM, Heindel J, Ho SM, Hunt P, Iguchi T, Juul A, McLachlan JA, Schwartz J, Skakkebaek N, Soto AM, Swan S, Walker C, Woodruff TK, Woodruff TJ, Giudice LC, Guillette LJ Jr. Female reproductive disorders: The roles of endocrine-disrupting compounds and developmental timing. Fertil Steril. 2008;90(4):911-940. https://doi.org/10.1016/j.fertnstert.2008.08.067.

[5] Varayoud J, Ramos JG, Muñoz-de-Toro M, Luque EH. Long-lasting effects of neonatal bisphenol A exposure on the implantation process. Vitam Horm. 2014;94:253-275. https://doi.org/10.1016/b978-0-12-800095-3.00010-9

[6] Ghuman SPS, Ratnakaran U, Bedi JS, Gill JPS. Impact of pesticide residues on fertility of dairy animals: A review. Indian J Anim Sci.2013;83(12):1243-1255. http://dx.doi.org/10.56093/ijans.v83i12.35789

[7] Altinok I, Capkin E. Histopathology of rainbow trout exposed to sublethal concentrations of methiocarb or endosulfan. Toxicol Pathol. 2007;35(3):405-410. https://doi.org/10.1080/01926230701230353
[8] Menezes RG, Qadir TF, Moin A, Fatima H, Hussain SA, Madadin M, Pasha SB, Al Rubaish FA, Senthilkumaran S. Endosulfan poisoning: An overview. J Forensic Leg Med. 2017;51:27-33. https://doi.org/10.1016/j.jflm.2017.07.008
[9] Moon YS, Jeon HJ, Nam TH, Choi SD, Park BJ, Ok YS, Lee SE. Acute toxicity and gene responses induced by endosulfan in zebrafish (*Danio rerio*) embryos. Chem Spec Bioavailab. 2016;28(1-4): 103-109. https://doi.org/10.1080/09542299.2016.1198681
[10] Kim EJ, Park YM, Park JE, Kim JG. Distributions of new Stockholm Convention POPs in soils across South Korea. Sci Total Environ. 2014;476-477:327-335. https://doi.org/10.1016/j.scitotenv.2014.01.034
[11] Devi NL, Yadav IC, Raha P, Shihua Q, Dan Y. Spatial distribution, source apportionment and ecological risk assessment of residual organochlorine pesticides (OCPs) in the Himalayas. Environ Sci Pollut Res Int. 2015;22(24):20154-20166. https://doi.org/10.1007/s11356-015-5237-5
[12] Moreira S, Silva R, Carrageta DF, Alves MG, Seco-Rovira V, Oliveira PF, de Lourdes Pereira M. Carbamate pesticides: Shedding light on their impact on the male reproductive system. Int J Mol Sci. 2022;23(15):8206. https://doi.org/10.3390%2Fijms23158206
[13] Moreira S, Pereira SC, Seco-Rovira V, Oliveira PF, Alves MG, Pereira ML. Pesticides and male fertility: A dangerous crosstalk. Metabolites. 2021;11(12):799. https://doi.org/10.3390%2Fmetabo11120799
[14] Dalsenter PR, Dallegrave E, Mello JR, Langeloh A, Oliveira RT, Faqi AS. Reproductive effects of endosulfan on male offspring of rats exposed during pregnancy and lactation. Hum Exp Toxicol. 1999;18(9):583-589. https://doi.org/10.1191/096032799678845124
[15] Sinha N, Adhikari N, K Saxena D. Effect of endosulfan during fetal gonadal differentiation on spermatogenesis in rats. Environ Toxicol Pharmacol. 2001;10(1-2):29-32. https://doi.org/10.1016/s1382-6689(01)00066-7.
[16] Sebastian R, Raghavan SC. Exposure to Endosulfan can result in male infertility due to testicular atrophy and reduced sperm count. Cell Death Discov. 2015;1:15020. https://doi.org/10.1038/cddiscovery.2015.20
[17] Sharma A, Kaninathan A, Dahal S, Kumari S, Choudhary B, Raghavan SC. Exposure to endosulfan can cause long term effects on general biology, including the reproductive system of mice. Front Genet. 2022;13:1047746. https://doi.org/10.3389%2Ffgene.2022.1047746
[18] Nath A, Anshu AK, Priyanka CKS, Behera S, Singh JK. Endosulfan induced oxidative stress and biochemical changes in testes of mice. Eur J Pharm Med Res. 2016; 3: 600-603.
[19] Orta Yilmaz B, Korkut A, Erkan M. Sodium fluoride disrupts testosterone biosynthesis by affecting the steroidogenic pathway in TM3 Leydig cells. Chemosphere. 2018;212:447-455. https://doi.org/10.1016/j.chemosphere.2018.08.112
[20] Eacker SM, Agrawal N, Qian K, Dichek HL, Gong EY, Lee K, Braun RE. Hormonal regulation of testicular steroid and cholesterol homeostasis. Mol Endocrinol. 2008;22(3):623-635. https://doi.org/10.1210/me.2006-0534
[21] Koç ND, Kayhan FE, Sesal NC, Contuk G. Histological and biochemical effects of endosulfan and malathion on rat testis. Fresenius Environ Bull. 2008;17:2262-2265.
[22] Singh SK, Pandey RS. Gonadal toxicity of short term chronic endosulfan exposure to male rats. Indian J Exp Biol. 1989;27(4):341-346.
[23] Sinha P, Verma P, Kumar A, Nath A. Testicular atrophy in mice (*Mus musculus*) under sublethal doses of endosulfan. J Ecophysiol Occup Health. 2004;4(3-4):191-196.
[24] Singh JK, Nath A, Kumar A, Ali MD, M, Kumar R. Study on the effect of endosulfan on testosterone level and seminiferous tubule of testis of mice. World J Envir Poll. 2011;1: 1-4.
[25] Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. Cent European J Urol. 2013;66(1):60-67. https://doi.org/10.5173/ceju.2013.01.art19
[26] Turner TT, Lysiak JJ. Oxidative stress: A common factor in testicular dysfunction. J Androl. 2008;29(5):488-498. https://doi.org/10.2164/jandrol.108.005132
[27] Chainy GB, Samantaray S, Samanta L. Testosterone-induced changes in testicular antioxidant system. Andrologia. 1997;29(6):343-349. https://doi.org/10.1111/j.1439-0272.1997.tb00328.x
[28] Aziz ZW, Saeed MG, Tawfeeq KT. Formalin versus bouin solution for rat testicular tissue fixation: A histochemical and immunohistochemical evaluation. Int J Med Toxicol Forensic Med. 2023; 13(2): 40267. https://doi.org/10.32598/ijmtfm.v13i2.40267
[29] Aldafaay AAA, Abdulamir HA, Abdulhussain HA, Badry AS, Abdulsada AK. The use of urinary α-amylase level in a diagnosis of chronic renal failure. Res J Pharm Technol. 2021; 14(3):1597-1600. http://dx.doi.org/10.5958/0974-360X.2021.00283.3