ISSN NO: 2771-8840
Date of Publication: 06-07-2022

Cypermethrin Insecticide Induced Histopathological Alteration in Testis and Ovary of Males and Female Mice

Alaa S.Kathim[1*], Saphia Al –Aitte[2]
[1]Department of biology, College of Education of Al Qurna, University of Basrah, Iraq
[2]Department of health community, college of Medical Technical, University of Southern Technical, Iraq
Corresponding author: Alaa S.Kathim (Ph.D)
Department of biology, College of Education of Al Qurna, University of Basrah, Iraq
Alaa.kathim@uobasrah.edu.iq

Abstract
Background: The present study sought to verify the effect of chemical pesticide Pyrethriod, cypermethyreien on the histopathological alteration in reproductive system such as testis and ovary organs of both sex, males and female mice.
Materials & Method: Muc muscular males and female mice (n=30) were used. To determine the effects of cypermethyreien on the testis and ovary organs, mice receiving a low dose (2.5mg/kg) and high dose (4.75 mg / kg) from cypermethyreien continuously for a period of five weeks. Hematoxeline and eosin stain were utilized to evaluate the histopathological alteration in testis and ovary.
Result: Microscope images showed pathological alteration in two condition (low dose and high dose) represented by separation of cells, sperm-generating layer from the rest of the tubule parts, as well as decreasing in wall thickness, congestion of vein, damage to part of the seminiferous tubule wall and decomposition of most stages of sperm formation compared to control group. Ovary organs after treated with low and high doses of cypermethrin, showed changes represented by hyperplasia, hyperemia, accumulation of inflammatory cells and necrosis of ovarian cells, less of the numbers of primary and primordial follicles and low numbers of corpus luteum.
Conclusion: low and high doses of cypermethrin toxicity used in male and female mice caused pathological alteration of tissues structure in testis and ovary organs.

Keyword: Mice, histological, testis, ovary, Cypermethrin , Toxicity.

Introduction

One of the commonly used pesticides is cypermethrin in order to their high efficiency against of insects, biodegradation, minimal mammalian toxicity, and target-oriented mechanism of action, these insecticides have become more popular in recent decades than organochlorines, organophosphates, and carbamates[1] Cypermethrin is a popular synthetic pyrethroid used in agriculture, foresight, and other applications [2].The studies of cypermethrin's reproductive toxicity are cause for concern because human spermatogenesis may be sensitive to persistent chemical exposure at very low levels[3] Previous studies inducted that a cypermethrin has been demonstrated to be harmful to the reproductive system in male rats [4, 5]. Both androgen receptor levels [4] and blood testosterone levels increased after 15 days of continuous medication [5].Long-term cypermethrin treatment during adulthood causes dopaminergic neurodegeneration in rats, and postnatal cypermethrin exposure increases the vulnerability of animals to dopaminergic neurodegeneration when re-exposed during adulthood [6] To ensure that spermatogenic and steroidogenic activities are not disrupted by persistent xenobiotic exposure [3, 7]. The testicular tissue has a complex array of antioxidant enzymes and free radical scavengers. Peroxidative damage is thought to be the most common cause of decreased testicular function [7] , hence these antioxidant defense systems are critical. Despite the fact that the testes have enough of endogenous antioxidants to scavenge free radicals [8].Phytochemicals have beneficial therapeutic characteristics and are used to treat a variety of ailments with minimal adverse effects [9, 10]. Resveratrol has been shown to have a number of biological actions, including a powerful antioxidative impact via LPO prevention[10]. Pesticides can reproductive toxicity through a number of mechanisms, including a damage to cell body, mediates with biochemical processes required for natural cell

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

function, and biotransformations that result in toxic metabolites [11].Cypermethrin is highly toxic to humans, and has harmful effects on human health, as it causes skin itching and eye irritation , In addition to its effect on animals, including experimental mice, which were used to show the effects of the pesticide as a vital sign [12-14].

The current study aims to investigate the risks of cypermethrin on pathological alternation of reproductive organs of female and male mice exposed to semi-lethal doses, as many studies did not address the study of histological changes for both sexes to determine their importance.

Materials And Methods

Animals

A total of thirty of male and female mus musculus mice between (10-12weeks) of age (22-25) was used. We selected female mice after ending from the estrous cycle, animals were purchased from Animal Center Laboratory of biology department –faculty of Education/Al-Qurna/ university of Basra. The animals were housed in special cages and maintained at a controlled environment with 12:12 h light, dark cycle at 22–24 oC, with standard rodent chow and water available ad libitum. Subsequent, animals randomly assigned to one of sex groups (5 mice pre group). Experiments were performed according to the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, National Research Council, Washington, DC, National Academy Press, no. 85-23, revised 1996).

Cypermethrin administration

Five male and five female mice were injected with low doses (2.5mg/kg) of cypermethrin C22H19Cl2NO3. likely, rest animals of (males=5) and (female=5) of mice were received high dose (4.75 mg / kg) of cypermethrin C22H19Cl2NO3. Ten mice consider as control group and only treated with an equal volume of normal saline. Cypermethrin C22H19Cl2NO3 was given subcutaneously in 2-3 times per week and disconnected till 5 weeks. The dose level was selected based on positive outcomes of previous experiment [15].

Euthanasia and Histological examination

In day 16 post experimental, mice were anesthetized with ketamine/xylazine (3/1). Animals sacrificed, testes and ovary organs were immediately removed and dehydrated with graded concentrations of alcohol for embedding in paraffin. Thereafter paraffin-embedded tissues were sectioned on a microtome (Leica, Wetzlar, Germany) into 5-7-µm coronal sections, and they were mounted into silane-coated slides. The sections were stained with hematoxylin and eosin (H&E) according to genera protocol.

Results

Testis evaluation

(i) Control group: The results of the microscopic examination of the sections of the control testes revealed that they consist of a seminiferous tubule containing cells that are spermatogonia, primary spermatocytes, and spermatids (Fig.1). (ii) Low dose group: Injections with a low dose of Alpha-Cypermethrin showed pathological histological changes in the testes of mice, these changes were represented by the separation of cells from each other and the separation of the sperm-generating layer from the rest of the tubule parts, as well as a decrease in wall thickness (Fig.2,3) and congestion of vein (Fig.4).

(iii) High dose group: The microscopic examination showed changes of different intensity in the high dose group represented by damage to part of the seminiferous tubule wall, especially the testis circumference the seminiferous tubules of high dose rats shows complete lysis of primary and secondary sperm layers, disappearance of Sertoli cells and lysis of spermatogona , As the cytoplasm and the plasma membrane appear degraded, and the nucleus suffers from major changes, the nucleus appears in the form of large balls containing a clear nucleus (Fig.5), and the disappearance and decomposition of most stages of sperm formation and the seminiferous tubules of the high-dose group shows the lack of primary and secondary sperm cells, and necrosis is noted in some of their cells (arrows) (Fig. 5 , 6). The enlargement of the spermatozoa cells and their nuclei clogged (Fig.7) and congestion of the blood vessels (Fig.8).

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

Ovary evaluation

(i) Control group: Fig.9. Shows the ovary of the control group consisting of primary follicles, secondary follicles, Garaffian follicles, and Corpus leutus.

(ii) Low dose group: The histological changes of the ovaries of the low dose group were represented by hyperplasia, hyperemia, and accumulation of inflammatory cells and necrosis of ovarian cells (Fig. 10, 11, 12, 13, 14).

(iii) High dose group: The changes in the ovaries of the high dose included in the less of the numbers of primary and primordial follicles (Fig.15), the absence of secondary follicles, low numbers of corpus luteum and hyperplasia ,Fibrosis and venous congestion ,inflammatory cell collection, lysis and loss of ovarian cells (Fig.16).

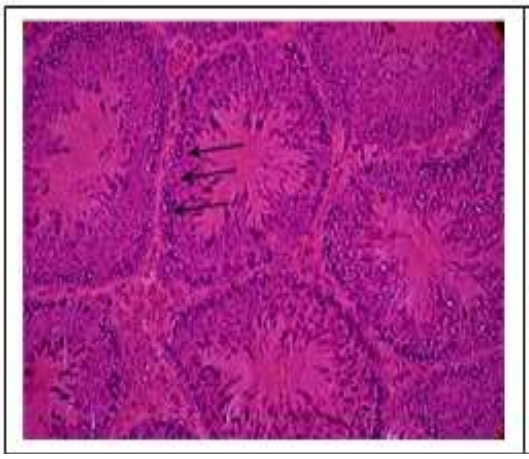

Figure (1) A cross section of the tubules of the control group illustrating the residual stages of spermatogenesis, the spermatozoa, the primary spermatocyte and the elongated sperm, H&E (400X)

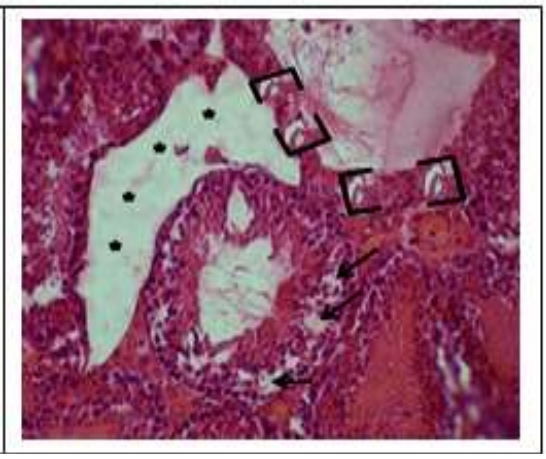

Figure (2) a cross section of the tubules of the low dose group showing the separation of the seminiferous epithelium layer (arrows and stars) and the low number of sperm cells (arches) H&E (400).

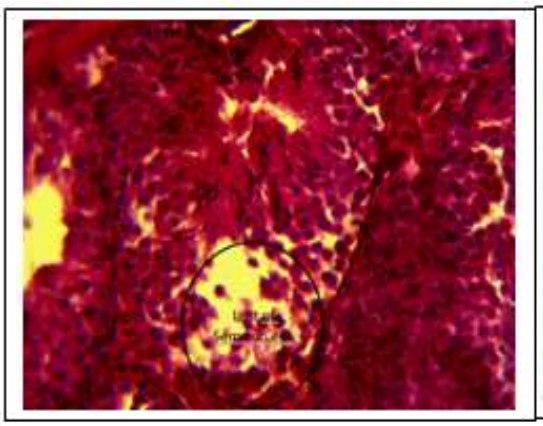

Figure (3) A cross section of the tubules of the low dose group showing the loss of the endothelium of the generative sperm cells lacling Spermatogonic epithelialum , H&E (400X).

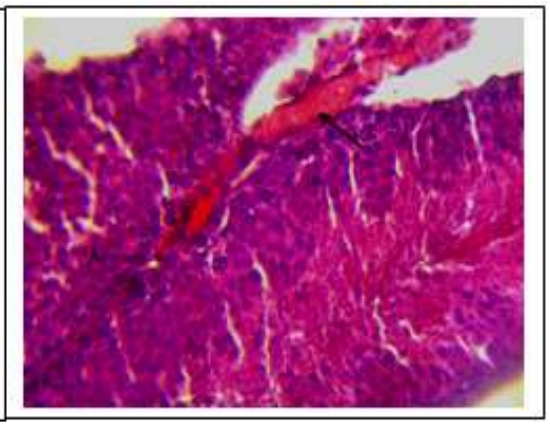

Figure (4) A cross section of the tubules of the low dose group showing the congestion of (vein H&E), (400).

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

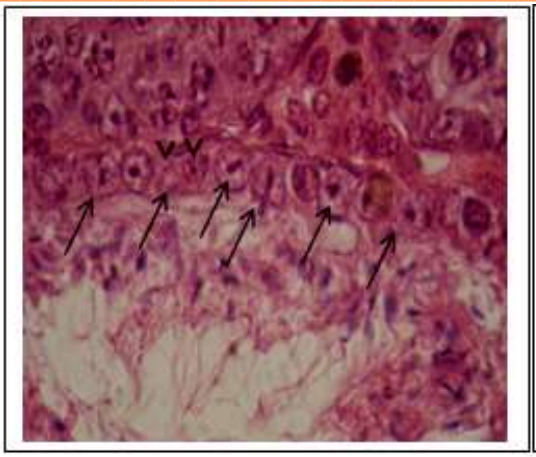

Figure (5) A cross section of the seminiferous tubules of high dose rats shows complete lysis of primary and secondary sperm layers, disappearance of Sertoli cells and lysis of spermatogona (arrows)As the cytoplasm and the plasma membrane appear degraded, and the nucleus suffers from major changes, the nucleus appears in the form of large balls containingaclearnucleus(arrowheads).H&E,(1 000X).

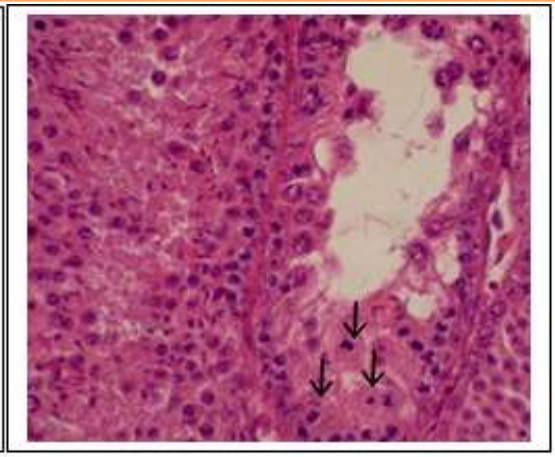

Figure (6) A cross-section of the seminiferous tubules of the high-dose group shows the lack of primary and secondary sperm cells, and necrosis is noted in some of their cells (arrows), H&E (400).

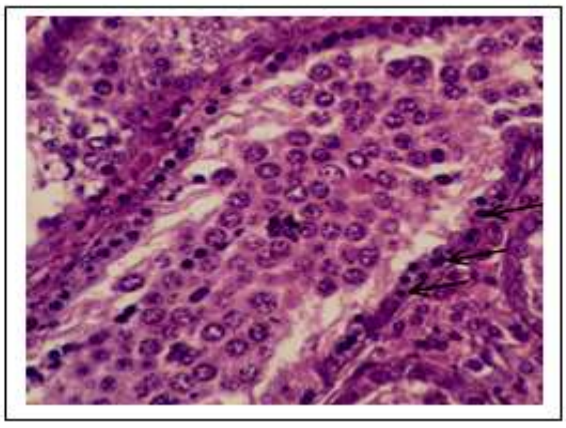

Figure (7) A cross section of the tubules of the high dose group showing thickening of the nuclei of spermatogona (arrows) and separation of sperm cells from them (H&E) 400X.

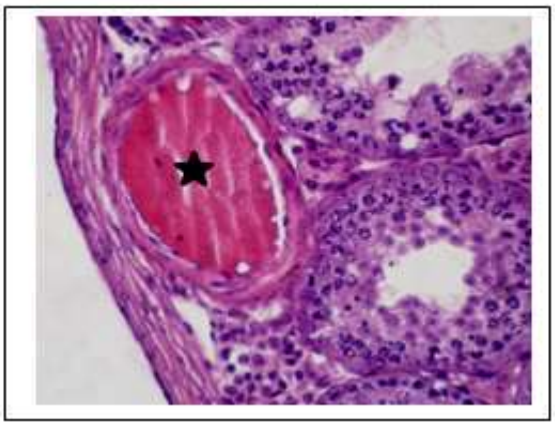

Figure (8) a cross section of the tubules of the high dose group congestion of blood vessels . (H&E)400X.

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

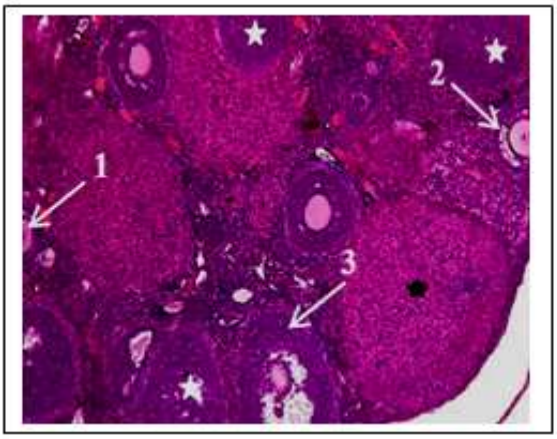

Figure (9) is a cross section of the ovary of the total control, showing the primary ovarian cells (1), secondary ovarian cells (2), and Krave's follicle (3), with a number of arritic follicles H&E (400X).

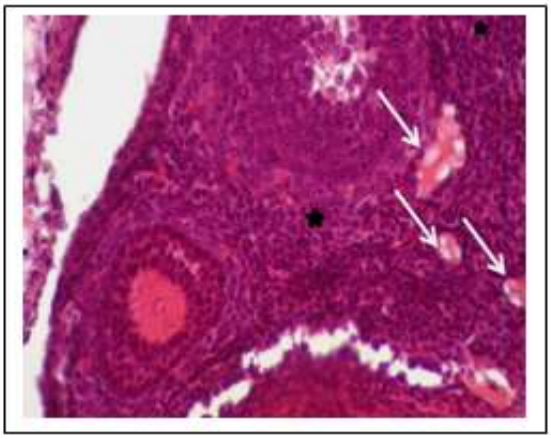

Figure (10) a cross section of the ovaries of the low dose group showing hyperplasia of the seminal tubules (asterisk) and congestion (arrows) H&E (400).

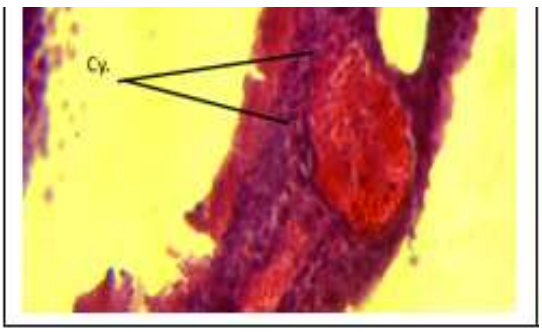

Figure (11) Cross-section of the ovary of the lower group showing cystic endometrial hyperplasia and a collection of inflammatory cells H&E (400).

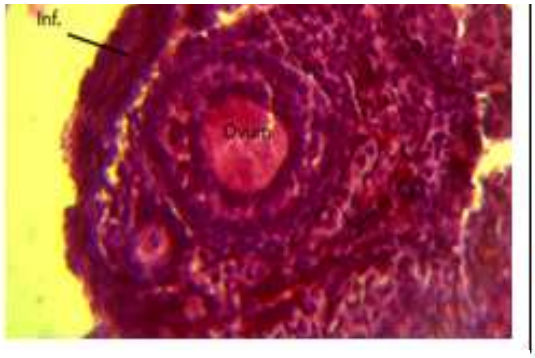

Figure (12) cross-section of the ovary of the lower group showing cystic endometrial hyperplasia, necrosis of ovary cells, and inflammatory cells (Inf.) H&E (400X).

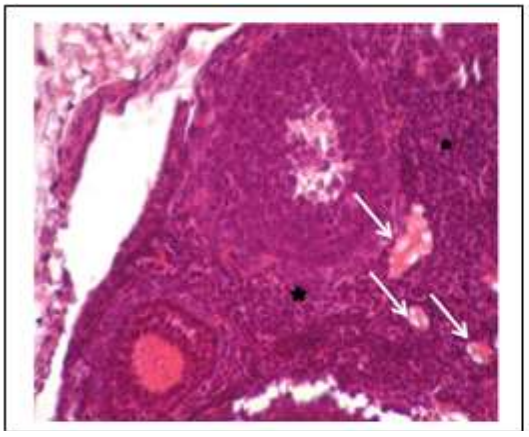

Figure (13) Enlarged portion of low dose ovary showing hyperplasia (stars) and vascular congestion (arrows), H&E (400X).

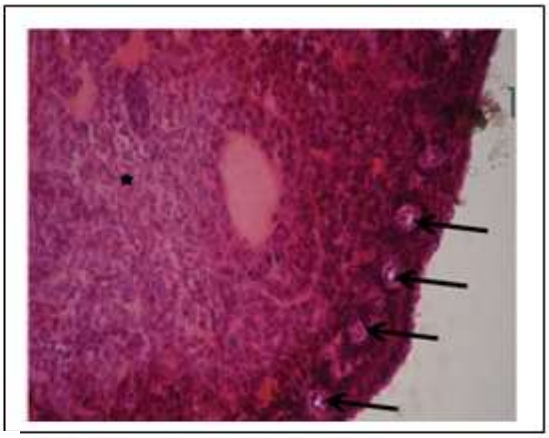

Figure (14) Cross section of low dose ovary showing hyperplasia (asterisk) Note primitive cells (arrows) , H&E (400X).

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

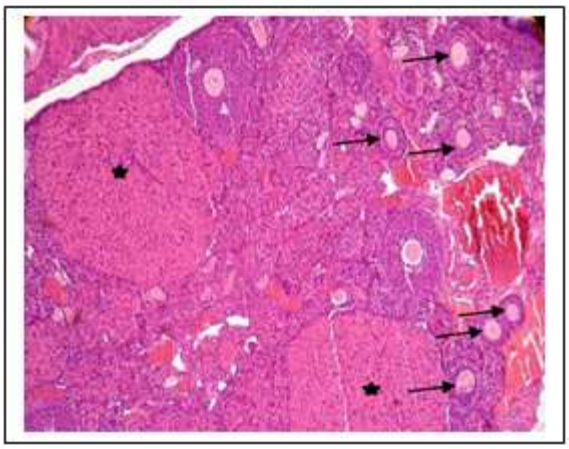

Figure (15) A cross section in the ovaries of the high dose group shows the low numbers of primitive follicles, the absence of Krafe's and secondary follicles, the lack of numbers of the luteal body (stars), and the presence of large numbers of primary follicles (arrows) , H&E , .400X.

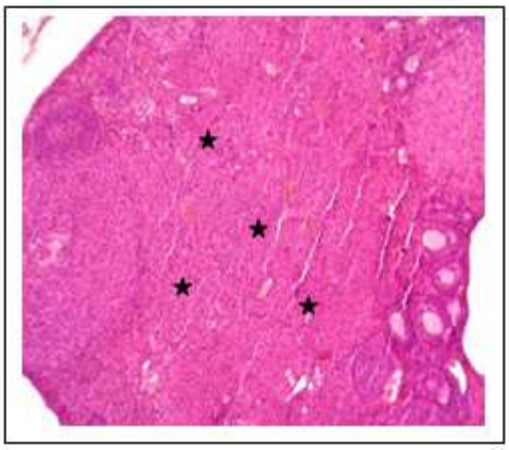

Figure (16) A cross section of the ovaries of Al-Alia group showing the low numbers of primordial follicles and hyperplasia in the ovarian parenchyma (stars),H&E,100X

Discusses

The use of natural plant products is increasing day by day to address human health issues. Thus, this study had the objective of examining the low and high doses of chemical pesticide Pyrethriod, cypermethyreien toxicity on the tissue structure of testis and ovary organs for 5 weeks. Regarding to testis, we observed pathological alteration represented by tissue structure damage in mice receiving low and high doses of cypermethyreien. Although insufficient studies of the effect of the toxicity of cypermethyreien in mice testis tissue , we suggesting that the pathological damage occurred in testicular tissue during different doses may be due to the ability of the doses of pesticide to cross the blood barrier to the testicle which leads to damage to the biological membranes[15] .In contrast , previous study inducted the cypermethrin lead to reduce the weight of testis [16] and that was accompanied with some impartation of testicular function such as decrease in, testicular sperm head counts, sperm motility and live sperm counts and increase in sperm abnormalities[17] . A study of Jin-xia and colleagues [18] used a cypermethrin at doses of 25 and 50 mg kg and found the weights of prostates were significantly decreased and reduction in testicular daily sperm production. they suggested that cypermethrin can induce impairments of the structure of seminiferous tubules and spermatogenesis in the male rats, the impairments can be attributed to the reduced AR expression.

In female mice, we investigated the low and high doses of toxicity of cypermethrin . we found that both low and high doses resulted to destructive the ovarian organs of mice received cypermethrin in 5 weeks. In this study, pesticides have a clear effect on the to the tissues structure of ovarian and is form of inflammation resulting from the effect of toxic substances, as there is an increase in cell proliferation, which leads to an increase in the size of the organ that suffers from hyperplasia and is one of the causes of hormonal imbalances [19]. Other studies concern our data and suggested that the toxicity of cypermethrin cased the ovarian hyperplasia and is also associated with a lack of development of ovarian follicles and is in the form of neoplastic or non-tumorous, and this is associated with an endocrine disorder and steroid hormones, especially androgens[20].Previous studies confirmed this that the pesticide causes a decrease in steroid hormones, including androgens [21]. The tissue damage and tissue changes caused by exposure to pesticides included various degrees of degenerative changes and accumulation of cellular debris in the seminiferous tubules confirmed the toxic ether is on the testicles through DNA damage and the generation of free adicals, and then the death of cells.

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

Conclusion

Low and high dose of cypermethrin induces damaging in tissue structure of testis and ovary mice in five weeks of dose administration.

Acknowledgement

We thankful to Professor Cruyes Jalili from Kermanshah medical science university for his help and support.

Conflict of Interest: There is no any Conflict of Interest.

Reference

1. Muthuviveganandavel, V., et al., A study on low dose cypermethrin induced histopathology, lipid peroxidation and marker enzyme changes in male rat. Pesticide Biochemistry and Physiology, 2008. 91(1): p. 12-16.
2. Donham, K.J. and A. Thelin, Agricultural medicine: rural occupational and environmental health, safety, and prevention. 2016: John Wiley & Sons.
3. Sharma, P., A.U. Huq, and R. Singh, Cypermethrin-induced reproductive toxicity in the rat is prevented by resveratrol. Journal of human reproductive sciences, 2014. 7(2): p. 99.
4. Hu, J.x., et al., Toxic effects of cypermethrin on the male reproductive system: with emphasis on the androgen receptor. Journal of applied toxicology, 2013. 33(7): p. 576-585.
5. Wang, H., et al., Cypermethrin exposure during puberty disrupts testosterone synthesis via downregulating StAR in mouse testes. Archives of toxicology, 2010. 84(1): p. 53-61.
6. Singh, A.K., et al., Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: postnatal exposure enhances the susceptibility during adulthood. Neurobiology of aging, 2012. 33(2): p. 404-415.
7. Aitken, R.J. and S.D. Roman, Antioxidant systems and oxidative stress in the testes. Molecular mechanisms in spermatogenesis, 2009 :p. 154-171.
8. Zhang, Y.-J., et al., Antioxidant phytochemicals for the prevention and treatment of chronic diseases. Molecules, 2015. 20(12): p. 21138-21156.
9. Gülçin, İ., Antioxidant properties of resveratrol: A structure–activity insight. Innovative food science & emerging technologies, 2010. 11(1): p. 210-218.
10. Colborn, T., Endocrine disruption from environmental toxicants. Environmental and Occupational Medicine. Lippicott-Raven Publishers, Philadelphia, 1998: p. 803-812.
11. Agrawal, A. and B. Sharma, Pesticides induced oxidative stress in mammalian systems. Int J Biol Med Res, 2010. 1(3): p. 90-104.
12. López, S.L., et al., Pesticides used in South American GMO-based agriculture: A review of their effects on humans and animal models. Advances in molecular toxicology, 2012. 6: p. 41-75.
13. Abd, R.N. and A.L. Jebur, Effect Of Evisect On Organo-Somatic Index And Pathohistological Changes Of Some Vital Organs In White Mice. Systematic Reviews in Pharmacy, 2020. 11(11): p. 1910-1914.
14. Rodriguez ,H., et al., Cypermethrin effects on the adult mice seminal glands. Ecotoxicology and environmental safety, 2009. 72(2): p. 658-662.
15. Al–Aitte, S., M.H. Zain, and A.S. Kathim, INDUCED THE C22H19CL2NO3 CYPERMETHYREIEN PESTICIDES ON HISTOLOGICAL ALTERNATION FOR THE LIVER OF ALBINO MICE (MUS MUSCULUS). Turkish Journal of Physiotherapy and Rehabilitation. 32: p. 3.
16. Choudhary, N., R. Goyal, and S. Joshi, Effect of malathion on reproductive system of male rats. Journal of environmental biology, 2008. 29 :(2)p. 259.
17. Sharma, P. and R. Singh, Protective role of curcumin on lindane induced reproductive toxicity in male Wistar rats. Bulletin of environmental contamination and toxicology, 2010. 84(4): p. 378-384.
18. Li, Y.F., et al., Effects of cypermethrin on male reproductive system in adult rats. Biomedical and environmental sciences, 2013. 26(3): p. 201-208.

ISSN NO: 2771-8840
Date of Publication: 06-07-2022

19. Bretveld, R.W., et al., Pesticide exposure: the hormonal function of the female reproductive system disrupted? Reproductive Biology and Endocrinology, 2006. 4(1): p. 1-14.
20. Al-Hamdani, N.M. and H. Yajurvedi, Effect of cypermethrin on the ovarian activity and its impact on fertility and pubertal onset of offspring. Beni-Suef University journal of basic and applied sciences, 2017. 6(4): p. 37,38-2.
21. Wang, H., et al., Cypermethrin exposure reduces the ovarian reserve by causing mitochondrial dysfunction in granulosa cells. Toxicology and applied pharmacology, 2019. 379: p. 114693.