ANALYSIS OF THE EFFECT OF EXPOSURE TO CHLORPYRYPHOS, CARBOFURAN, AND CYPERMETHRIN ON SPERMATOGENESIS IN ADOLESCENT RAT

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ABSTRACT

Objective: *To investigate the effect of exposure to chlorpyryphos, carbofuran, and cypermethrin on spermatogenesis in adolescent rat.* ***Material & Methods:*** *This study is an experimental study with a post-test only control group design. In this study, 30 adolescent rats aged 30-45 days were randomly divided into 5 groups. Group I was a normal control or without treatment for 21 days (K0), group II was a solvent control group, namely DMSO 5% which was administered subcutaneously for 21 days (KI), group III was a group that received chlorpyrifos 20 mg/kgBW dissolved with DMSO 5% subcutaneously for 21 days (P1), group IV was the group that received carbofuran 0.2 mg/kgBW dissolved with DMSO 5% subcutaneously for 21 days (P2), group V was the group that received cypermethrin 20 mg/kg. kgBW dissolved with 5% DMSO subcutaneously for 21 days (P3). Ipsilateral testicular orchidectomy was performed on all rats to remove the left testicular organ for preparation with Haematoxylin Eosin staining. The spermatogenesis score was calculated based on the Johnsen score in 5 seminiferous tubule visual fields. Data were analyzed using Kruskal Wallis followed by Mann Mitney test. The difference was considered significant at p<0.05.* ***Results:*** *There was a significant decrease in spermatogenesis scores between P1, P2, and P3 against K0 and K1 (p<0.05). On the other hand, there was no significant difference in the decrease in spermatogenesis scores between P1 and P2, P1 and P3, P2 and P3 and K0 and K1 (p>0.05).* ***Conclusion:*** *Exposure to chlorpyrifos, carbofuran, and cypermethrin had an effect on reducing spermatogenesis scores in adolescent rats*

Keywords: *Chlorpyryphos, carbofuran, cypermethrin, spermatogenesis.*

ABSTRAK

Tujuan: *Membuktikan pengaruh paparan klorpirifos, karbofuran, dan sipermetrin terhadap spermatogenesis pada tikus remaja.* ***Bahan & Cara:*** *Penelitian ini merupakan penelitian eksperimental dengan desain post-test only control group. Pada penelitian ini sebanyak 30 ekor tikus remaja yang berumur 30-45 hari secara random dibagi menjadi 5 kelompok. Kelompok I adalah kontrol normal atau tanpa perlakuan selama 21 hari (K0), kelompok II adalah kelompok kontrol pelarut yaitu DMSO 5% yang diberikan secara subkutan selama 21 hari (KI), kelompok III adalah kelompok yang mendapat klorpirifos 20 mg/kgBB yang dilarutkan dengan DMSO 5% secara subkutan selama 21 hari (P1), kelompok IV adalah kelompok yang mendapat karbofuran 0.2 mg/kgBB yang dilarutkan dengan DMSO 5% secara subkutan selama 21 hari (P2), kelompok V adalah kelompok yang mendapat sipermetrin 20 mg/kgBB yang dilarutkan dengan DMSO 5% secara subkutan selama 21 hari (P3). Dilakukan orkidektomi testis ipsilateral pada semua tikus untuk mengambil organ testis sebelah kiri untuk dilakukan pembuatan preparat dengan pewarnaan Haematoxylin Eosin. Skor spermatogenesis dihitung berdasarkan skor Johnsen pada 5 lapang pandang tubulus semineferus. Data dianalisa menggunakan Kruskal Wallis diikuti dengan tes Mann Mitney. Perbedaan dianggap signifikan pada p<0.05.* ***Hasil:****Terdapat penurunan skor spermatogenesis yang bermakna antara P1, P2, dan P3 terhadap K0 dan K1 (p<0.05). Sebaliknya tidak terdapat perbedaan penurunan skor spermatogenesis yang bermakna di antara P1 dan P2, P1 dan P3, P2 dan P3 dan K0 dan K1(p>0.05).* ***Simpulan:*** *Paparan klorpirifos, karbofuran, dan sipermetrin berpengaruh menurunkan skor spermatogenesis pada tikus remaja*

Kata Kunci: *Klorpirifos, karbofuran, sipermetrin, spermatogenesis.*

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INTRODUCTION

Pesticides are toxic chemical substances or mixtures of biological substances or agents used to prevent, control and kill insects, weeds, rodents, fungi, and other harmful pests. The use of pesticides is not only limited to agriculture, but is also widely used in homes to control cockroaches, mosquitoes, rats, fleas, and other harmful insects.[1] The use of pesticides has increased in recent years. According to FAO data (2019), about 4.2 billion tons of pesticides are used worldwide per year. Chlorpyrifos, carbofuran, and cypermethrin are pesticides that are quite widely used. Chlorpyrifos was used as much as 19%, carbofuran as much as 11%, and cypermethrin as much as 5% of pesticide use.[2] This increased use of course also increases human exposure to these pesticides. The use of pesticides is not entirely beneficial for humans. So far, based on various existing studies, the use of pesticides also causes various side effects that are harmful to human health, for example, neurotoxicity, DNA damage, cancer, cardiovascular disorders, respiratory disorders, and infertility.[3]

Infertility is a condition when a woman is unable to get pregnant after 12 months or more of sexual intercourse without using contraception. Infertility affects 8-12% of couples worldwide, with male factors contributing to about 50% of couples. Male infertility is one of the big problems in society.[4] Male infertility can also cause depression. About 15% of men diagnosed with infertility experience depression that interferes with their lives.[5]

The causes of male infertility vary widely, ranging from congenital, acquired, or idiopathic factors that interfere with spermatogenesis.[6] Various previous studies have shown that the use of environmental pollutants such as pesticides has side effects on male fertility.[7] Pesticide exposure is one of the causes that are known to be able to inhibit spermatogenesis. Spermatogenesis is the formation of spermatozoa cells in the seminiferous tubules of the testes.[8] Spermatozoa cells are needed in the fertilization process of the egg to produce a zygote which eventually develops into a fetus. Spermatogenesis normally begins when men enter adolescence.[9] Although there are many theories about how pesticides affect spermatogenesis, one of the main theories is that pesticides cause an increase in the production of free radicals that inhibit spermatogenesis.[10]

OBJECTIVE

This study aims to investigate the effect of exposure to chlorpyryphos, carbofuran, and cypermethrin on spermatogenesis in adolescent rat.

MATERIAL & METHODS

The present study was experimental study with post test only control group design. 30-45 day-old Wistar male rats (60-100 g) were maintained under controlled room temperature with food and water ad libitum. 30 Wistar male rats aged 30 - 45 days were grouped into 5 groups. Group I was the normal control/without treatment (K0), group II was solvent control, DMSO 5 % (K1), group III was the group that received chlorpyryphos 20 mg/kgBW dissolved in 5% DMSO subcutaneously (P1), group IV was the group that received carbofuran 20 mg/kgBW dissolved in 5% DMSO subcutaneously (P2), group V was the group that received cypermethrin 20 mg/kgBW dissolved in 5% DMSO subcutaneously (P3).

The rats were sacrified at 22nd days of treatment. These rats were first anesthetized by ether

Table 1. Johnsen Spermatogenesis Score.

Score	Histological Criteria
10	Complete spermatogenesis with well-arranged germinal epithelium
9	Complete spermatogenesis with poorly arranged germinal epithelium
8	Complete spermatogenesis, found spermatozoa cells <15
7	No spermatozoa cells were found, but many spermatid cells were found
6	Found <10 spermatid cells
5	There are no spermatozoa or spermatid cells, but there are many spermatocytes
4	Few spermatocytes were found <5
3	Only found spermatogonia
2	Only found sertoli cells
1	No cell seen in the tubular epithelium

and then euthanized by cervical dislocation. Orchidectomy was performed to get left testis used for histopathological evalutaions. The testis tissue was fixed in 10% neutral buffered formalin solution and then blocked using paraffin by standard technique. Then testis tissue was cutted with size 5 pm then it was stained by haematoxylin and eosin (HE) staining. Histological tissue was investigated using a microscope with 400x magnification. For the histological evaluation of spermatogenesis, we evaluated 5 seminiferous tubules per testis using Johnsen'sscoring system from 10 to 1 points, as shown in Table 1.

Data was analyzed using SPSS 26 for windows. Data were analyzed Kruskall-Wallis test and followed by Post Hoc Mann-Whitney test. Data was considered significant if p-value <0.05.

RESULTS

The level of damage to the heaviest rat testicular spermatogenesis was in the P2 group with a score of 7.5 shown in Figure 1. Based on the average histopathological scoring data of adolescent rat testicular spermatogenesis presented in Table 2, it is known that there is a difference in the average histopathological scoring of spermatogenesis in experimental animals. The lower the value, the more severe the damage to spermatogenesis that occurs. The lowest histopathological score of rat kidney was in group P2, which was 7.5, while the highest average histopathological score of spermatogenesis in adolescent rats was in group K0 which was 9.16.

Table 2 shows the results of data analysis using the Kruskall-Wallis test. From the results of the Kruskall-Wallis test, it was found that there was a significant difference in spermatogenesis scores in at least two groups being compared (p <0.05). After the Kruskall-Wallis test was carried out, it was continued with the Mann-Whitney post hoc test to determine the significance value between groups. If the data from the Post Hoc Mann-Whitney test result is <0.050, then there is a significant decrease in spermatogenesis scores between the groups being compared. The results of the Post Hoc Mann-Whitney test can be seen in Table 3.

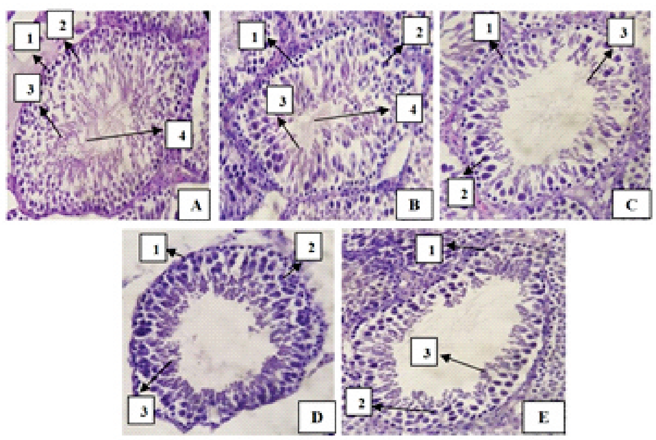

Figure 1. Description of spermatogenesis of adolescent male rats treated for 21 days with Hematosilin Eosin (HE) staining (microscope magnification 400x); A. Normal control; B. Solvent control DMSO 5%; C. Treatment with chlorpyrifos 20 mg/kgBB : 50 mg/kg; D. Treatment of carbofuran 0.2 mg/kgBB ; E. Treatment of cypermethrin 20 mg/kgBW; 1. Spermatogonia; 2. Spermatocytes; 3 Spermatids; 4. Spermatozoa.

Table 2. The average score of spermatogenesis for each treatment group.

Group	N	Treatment	Score (Mean) ± SD	p
K0	5	No Treatment	9.160 ± 0.380	0.00*
K1	6	DMSO 5 %	8.730 ± 0.300	
P1	6	Chlorpyryphos 20 mg/ kgBW	7.760 ± 0.400	
P2	6	Chlorpyryphos 20 mg/ kgBW	7.500 ± 0.300	
P3	5	Chlorpyryphos 20 mg/ kgBW	7.760 ± 0.660	

SD = Standart Deviation ; *p<0.05

Table 3. Comparison of the spermatogenesis score in each group by Mann-Whitney post hoc test.

Comparison between groups	Mean ± SD	Vs	Mean ± SD	p*
K0 vs K1	9.160 ± 0.380	Vs	8.730 ± 0.300	0.084
K0 vs P1	9.160 ± 0.380	Vs	7.760 ± 0.400	0.006
K0 vs P2	9.160 ± 0.380	Vs	7.500 ± 0.300	0.006
K0 vs P3	9.160 ± 0.380	Vs	7.760 ± 0.660	0.009
K1 vs P1	8.730 ± 0.300	Vs	7.760 ± 0.400	0.003
K1 vs P2	8.730 ± 0.300	Vs	7.500 ± 0.300	0.002
K1 vs P3	8.730 ± 0.300	Vs	7.760 ± 0.660	0.005
P1 vs P2	7.760 ± 0.400	Vs	7.500 ± 0.300	0.113
P1 vs P3	7.760 ± 0.400	Vs	7.760 ± 0.660	0.570
P2 vs P3	7.500 ± 0.300	Vs	7.760 ± 0.660	0.139

SD = Standart Deviation ; *p<0.05

In this study, the K0 group was the reference for the normal value of the spermatogenesis score. The K1 group did not show a significant decrease in spermatogenesis scores when compared to the K0 group (p=0.084). The P1 group showed a significant decrease in spermatogenesis scores when compared to groups K0 (p=0.006) and K1 (p=0.003), but the P1 group did not show a significant decrease in spermatogenesis scores when compared to groups P2 (p=0.113) and P3 (p =0.570). The P2 group showed a significant decrease in spermatogenesis scores when compared to groups K0 (p=0.006) and K1 (p=0.002), but the P2 group did not show a significant decrease in spermatogenesis scores when compared to groups P1 (p=0.113) and P3 (p = 0.139). The P3 group showed a significant decrease in spermatogenesis scores when compared to groups P1 and P2, but did not show a significant decrease in spermatogenesis scores when compared to groups P1 (p=0.570) and P2 (p=0.139).

DISCUSSION

From the results of observations made in the chlorpyrifos, carbofuran, and cypermethrin groups when compared to the control group, both normal control and 5% DMSO solvent, there was a significant decrease in the average spermatogenesis score. So that the pesticides chlorpyrifos, carbofuran, and cypermethrin can cause histopathological damage to spermatogenesis in the testes of adolescent rats. This finding is in accordance with previous studies of pesticides chlorpyrifos, carbofuran, and cypermethrin capable of causing damage to the structure of testicular tissue.[11-13] This is because the pesticides chlorpyrifos, carbofuran, and cypermethrin are known to be able to cause damage to the histopathological score of spermatogenesis of adolescent rat testis with a similar mechanism, namely by increasing the production of free radicals due to accumulation of acetylcholine and hyperexcitation in the central nervous system, peripheral, and neuromuscular junction.[14-15]

Aceticoline also acts on specific receptors on endothelial cell membranes to increase the amount of intracellular calcium that will bind to calmodulin (CaM) which causes an increase in the formation of NO free radicals. NO free radicals will also react with superoxide free radicals (O2-) which is a by-product of mitochondria. The increased production of NO and O2- in the body will be dangerous because it can trigger the formation of bonds between NO and O2- which will produce strong oxidant compounds, namely peroxynitrite. (ONOO-). Peroxynitrite compounds are RNS which are strong oxidants that can damage body tissues and cells.[16]

The more free radicals that are formed, the activity of superoxide dismutase (SOD), glutathione reductase (GR), and catalase as natural antioxidants in the body decreases so that they are unable to neutralize the amount of free radicals and antioxidants. The imbalance between the amount of antioxidants and free radicals will further expand the systemic damage to cells and tissues in the body.[17] Peroxynitrite free radicals also affect the Krebs cycle in the mitochondria of cells in the formation of ATP by inhibiting the aconitase enzyme. The aconitase enzyme in the krebs cycle functions to convert citrate to isocitrate. Inhibition in the krebs cycle will cause a decrease in ATP production.[18]

In addition to increasing the production of free radicals, excessive levels of NO in the body can also result in reduced ATP production by mitochondria. NO can easily diffuse into cells and interact with mitochondria. NO affects mitochondria by interfering with respiratory chain complex I (NADH dehydrogenase) and IV (cytochrome oxidase) which causes disruption of the electron transport process in mitochondria, thereby inhibiting the process of oxidative phosphorylation in mitochondria in the formation of ATP.[19]

A decrease in ATP production will cause a malfunction of the ATP reliant ion transport pump which causes an influx of sodium, H2O, and calcium into the cell, causing cell swelling. Excessive calcium influx will also increase the activation of cellular calcium enzymes (protease, endonuclease, ATPase, and phospholipase) which causes cell damage. The decrease in ATP production will also increase the occurrence of anaerobic metabolism which increases the production of lactic acid. Excessive lactic acid production will lower cell pH, causing chromatin clumps and damage cells.[20]

Cell and tissue damage caused by free radicals due to exposure to pesticides chlorpyrifos, carbofuran, and cypermethrin also affects cells in the testes and the hyphothalamus-pituitary-gonad axis (HPG AXIS).[21-23] Damage to the hypothalamus will inhibit the function of the hypothalamus in secreting gonadotropin releasing hormone (GnRH). The decrease in GnRH will inhibit the function of the anterior pituitary in secreting follicle stimulating hormone (FSH) and luteinizing hormone (LH) to the testes. A decrease in LH will inhibit the work of Leydig cells in producing the hormone testosterone.

The decrease in testosterone will inhibit the process of developing spermatogenic cells into mature spermatozoa.[24] A decrease in FSH will inhibit Sertoli cells from producing androgen binding protein (ABP) which functions to concentrate testosterone levels until they reach the levels required for spermatogenesis.[25] So that through this mechanism it is known that the pesticides chlorpyrifos, carbofuran, and cypermethrin have an effect on reducing spermatogenesis scores in adolescent rats.

CONCLUSION

In the present study suggested that exposure to chlorpyrifos, carbofuran, and cypermethrin had an effect on reducing spermatogenesis scores in adolescent rats.

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