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Original Research Article

Effect of exposure to active allethrin on expression of HSF2 and Ovol1 genes in a rat spermatogenesis

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ABSTRACT

Background: Heat shock factor protein 2 (HSF 2) and Ovo like transcriptional repressor 1 (Ovol1) genes are found in germ cells that control spermatogenesis. One reason is exposure to mosquito repellent drugs from allethrin. Allethrin is one of the causes of male reproductive dysfunction that affects male reproduction resulting in infertility.
Methods: This study was an experimental post-test randomized control group design. Twenty-eight rats given exposure according to the experimental group were K (control), P1 (4 hours exposure), P2 (8hours exposure), and P3 (12hours exposure) for 30 days. Examination of HSF 2 and Ovol1 genes from testicular tissue using real-time PCR with a relatively quantitative calculation method. Implementation of research at animal houses and biomedical laboratories. Data analysis using one-way ANOVA with a significant level of $p < 0.05$.
Results: The results of this study found differences in the average number of expressions of the HSF2 and Ovol1 genes. The average expression of the HSF2 control group gene was 3.50, P1: 2.99, P2: 0.62, and P3: 0.49. Whereas in the Ovol1 gene control group were 1.17, P1: 0.80, P2: 0.57, and P3: 0.65. The results of statistical tests using one-way ANOVA are HSF2 ($p = 0{,}000$) and Ovol1 ($p = 0.045$).
Conclusions: Based on the results of this study, it was concluded that there was an allethrin effect in decreasing the expression of the HSF 2 and Ovol1 genes in Wistar albino Rattus Novergicus strain.

Keywords: Allethrin, Heat shock factor protein 2 gene, Ovo like transcriptional repressor 1 gene, Rat (Rattus Novergicus)

INTRODUCTION

Infertility is a condition of the inability of couples of childbearing age (PUS) to get off spring after having sexual intercourse regularly and correctly without prevention efforts of more than one year.[1] With in a set period of time, usually 85% of couples already have offspring and as many as 15% of couples of childbearing ages have infertility problems.[1] Infertility is an inability of sexually active couples to have offspring without the use of contraception for 12 months or more on a regular sexual relationship.[2] The World health organization (WHO) estimates the incidence of infertility in 8-10% of partners. Based on the picture of the world with a population of 50-80 million couples and there are 2 million new infertile couples every year and this number continues to increase.[3] According to the American society for reproductive medicine the prevalence of couples in America suffers from infertility as much as 14% of couples and 30% to 40% of cases are influenced by malefactors. Infertility in developed countries is 5-8% while in developing countries the infertility rate is much

higher at 30%.[4] The prevalence of infertility in Asia consists of 30.8% in Camboja, 10% in Kazakhstan, 43.7% in Turkmenistan and 21.3% in Indonesia.[5] Based on the prevalence of infertility couples in Indonesia is 15-25% of all existing partners as much as 98.3%.[6] The central statistics agency recorded an increase in the number of infertility in Indonesia from 1971 to 2012, and in the province of West Sumatra the increase that occurred was very significant from 1971 to 2012.WHO believes infertility originating from male factors was around 40% Male infertility is a common problem throughout the world, one of which is exposed to the environment.[7] One that is exposed to the environment is exposure to mosquito repellents almost every home uses mosquito repellent drug because they are easily available, many are sold and are effective in repelling mosquitoes.[8] Electricity mosquito repellent drugs have many advantages compared to other methods, which are easy to apply, relatively simple, active substances spread constantly, save costs and energy.[9] The electric mosquito repellent drugs are a substitute for the use of mosquito repellent drugs that do not produce smoke, are durable, fragrant and easy to use.[10] Pyrethroid is an antinyamuk compound whose first derivative is that it is useful as a mosquito repellent poison.[11] Allethrin is the first derivative compound of pyrethroid compounds which contains many active ingredients of electric mosquito repellents.[12] Exposure to allethrin is one of the causes of male reproductive dysfunction that affects male reproduction resulting in infertility.[13] Allethrin can cause testicular histological changes, decrease testicular weight and reduce the diameter of the seminiferous tubules.[14] It can show if the testis is damaged then spermatogenesis will be disrupted, so that the spermatozoa produced will decrease.[15] The effects on male reproduction from allethrin exposure can reduce the quality of spermatozoa as in concentration, motility, and morphology.[16] The influence of allethrin on concentration, motility, morphology, and molecularities is related to the failure of detectable sperm production in the HSF2 gene and Ovol1 gene. The HSF2 gene and the Ovol1 gene are located on the Y chromosome. [17] Expressions of the HSF2 gene and Ovol1 gene affect the decrease in testicular regulation of mice due to allethrin influence.[13] The HSF2 gene and the Ovol1 gene are present in germ cells that function to control spermatogenesis.[18] The HSF2 gene is in the meiotic process at the beginning of the pakhiten process in the formation of spermatogenesis.[19] The Ovol1 gene functions in the formation of spermatogenesis. If the deficiency in the Ovol1 gene will reduce the pakhiten process in miosis 1.[20] Increased use of allethrin-based electric mosquito repellent drugs which can damage the mitochondrial membrane of Leydig cells which have a disturbing effect on the process of spermatogenesis, so accurate information is needed about the effects of the HSF 2 gene and Ovol1 gene that has been exposed to allethrin-based anti-mosquito drugs. Therefore, the researchers were interested in conducting a study to determine the effect of mosquito repellent based exposure to allethrin on the expression of HSF2 and Ovol1 genes in spermatogenesis of male Wistar (Rattus novergicus) white mice.

METHODS

This type of research was experimental with a randomized design post-test control group design. maintenance of animal induction and allethrin exposure in animal houses at the animal house of the faculty of medicine, Andalas university, examination of the HSF2 and Ovol1 genes in the biomedical laboratory of Andalas University, Padang. This research will be conducted from October 2017 to December 2018. The population in this study were male Wistar rats (Rattus Novergicus) obtained from Bungus rat farmers, Padang.

Inclusion criteria

- Samples of male white mice aged 2-3 months, body weight 150-200grams, activity, and normal behavior, and in good health.

Exclusion criteria

- Rats that were previously ill because of exposure to dead mosquito and rat drugs after being exposed to the allethrin mosquito repellent drug during the study period.

Adlibitum feeds and drinks and the cage is 54 cm long, 36.5 cm wide and 28.5 cm high. The mice were acclimatized, then male and female mice were mated with the aim of seeing pregnancy in female rats or male mice that had carried out copulation which indicated that the mice were healthy and able to be fertilized. Twenty-eight male rats were exposed in the experimental group, namely K (control), P1 (exposure to 4hours), P2 (exposure to 8hours), and P3 (exposure to 12hours) for 30 days. Exposure dose at 45 mg allethrin/mat and 4mg transfluthrin /mat. Expressions of the Hsf 2 and Ovol1 genes were examined using the real-time PCR method, total RNA was isolated from testicular tissue using the TRIzol® reagent and MiniL Pure Link® RNA mini kit. Twenty-eight male rats given exposure to the right experimental group were K (control), P1 (4 hours exposure), P2 (8 hours exposure), and P3 (exposure to 12 hours) for 30 days. Dosis pajanan pada 45 mg allethrin/mat dan 4 mg transfluthrin / mat. Expressions of the HSF2 and Ovol1 genes were examined using the real-time PCR method, total RNA was isolated from testicular tissue using the TRIzol® reagent and MiniL Pure Link® RNA Mini Kit. Preparation of cDNA using the cDNA Synthesis Kit I Script TM. Primary design for HSF2 gene expression analysis (F:TAAATGGCTCCTCCAACCTG, RCCAACAGCTCAACCTTTCCTA:), Ovol1 gene (F:GACCTCAAGAGACATGTCC,R:GGCGCTCCTTA TAGCATAC and Housekeeping genes (GADPH) (F: CATGGCCTTCCGTGTTCCTA,R:CCTGCTTCACCAC CTTCTTGAT) As internal controls for quantification of the expressions target gene.

Statistical analysis

Data were obtained with normality test data and expression analysis of the HSF2 and Ovol1 genes using the one-way ANOVA method with a 95% confidence level where the value of p <0.05% (significant), then followed by several (post hoc test) types of LSD significance between the group.

RESULTS

Data of HSF2 gene expression amount was analyzed by using One Way ANOVA and Post Hoc t-test LSD (Table 1).

Table 1: Mean of HSF2 gene expression of control group and treatment group on Rattus Norvegicus Wistar strain (n = 27).

Subject of group	N	HSF2 gene expression (R) Mean±SD	P value
Control	7	3.50±2.23	0.000
P1	7	2.99±1.98	
P2	7	0.62±0.41	
P3	6	0.49±0.17	

The results of the calculation of statistical tests showed differences in the expression of HSF2 genes exposed from allethrin. The decrease in mean HSF 2 gene expression has occurred from the 4-hour exposure treatment group (P1). To see the differences between treatment groups exposure to mosquito repellent drugs made from active allethrin need to be further tested with a Post Hoc test of multiple LSD comparisons.

Table 2: The result of post hoc test Bonferroni on HSF 2 gene expression in each research group.

Group	HSF2 gene expression			
	Control	**P1**	**P2**	**P3**
Control	-	0.574	0.000*	0.000*
P1	0.574	-	0.001*	0.001*
P2	0.000*	0.001*	-	0.825
P3	0.000*	0.001*	0.825	-

From the results of the LSD further test in the table, it can be concluded that there is a significant effect of the expression of the HSF2 gene between the negative control group and the P2 group and the negative group P3, the P1 group with the P2 group and the P1 group with the P3 group.

The results of the statistical test show that there is a difference in the expression of the Ovol1 gene which is exposed from allethrin. The decline in the mean Ovol1 gene expression has occurred from the 4-hour exposure treatment group (P1).

Table 3: Mean of Ovol1 gene expression of control group and treatment group on Rattus norvegicus Wistar strain (n = 26).

Subject of group	N	Ovol1gene expression (R) Mean±SD	P value
Control	7	1.17±0.27	0.045
P1	6	0.80±0.47	
P2	7	0.57±0.32	
P3	6	0.65±0.64	

To see the differences between treatment groups exposure to mosquito repellent drugs made from allethrin-based agents need to be further tested with the Post Hoc test multiple comparisons of LSD results in Table 4 below.

Table 4: The result of post Hoc test LSD on Ovol1 gene expression in each research group.

Group	Ovol1 gene expression			
	Control	**P1**	**P2**	**P3**
Control	-	0.138	0.015*	0.015*
P1	0.138	-	0.329	0.300
P2	0.015*	0.329	-	0.919
P3	0.015*	0.300*	0.919	-

Based on the results of the Post Hoc LSD Test in Table 4, it can be concluded that there is a significant influence on the expression of the Ovol1 gene between the negative control group and the P2 group and the negative control group with the P3 group.

DISCUSSION

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Based on the results of the analysis it was concluded that the highest decrease in the expression of the HSF2 gene in group 3 with a duration of 12hours was 0.49 compared to treatment 1 with a length of exposure of 4hours of 2.99 and treatment 2 with a length of exposure of 0.62. Allethrin exposure through absorption of inhalation results in toxicity to the target organs, namely the testis which results in free radicals.[21] Previously there was an exchange of fat gas which has a double bond on the membrane of lipid bilayer cells which causes a chain reaction of lipid peroxide so that cell membrane damage occurs. The imbalance between free radicals and the ability of antioxidants will cause damage to special cells in the cell membrane.[22]

This is in accordance with the research of Naim F et al, at 2016 which shows that allethrin contained in insect repellent can cause free radicals, so that if free radicals cannot be stopped so that it will damage the mitochondrial cell membrane due to increased oxidative stress.[23] Supported by the research of Atessahim et al, at

2005 which states that oxidative stress is a condition of impaired balance between the production of free radicals and antioxidants that cause damage.[24] Oxidative stress that occurs will affect gene expression, especially in the HSF2 gene. The HSF2 gene is the gene most commonly found in the testes. HSF2 works as a regulator of the stress response obtained. HSF2 which is disturbed by allethrin exposure causes HSF2 not work properly to induce the work of) Heat shock protein promoter (HSP) is a family of proteins that are produced by cells as a response to exposure to stress conditions for protein folding. Heat shock protein functions as a molecular companion to stabilize proteins, ensure proper protein folding and, helps to multiply damaged proteins due to exposure to allethrin seen in the transcription process. So that it is very necessary that HSF2 is very stable without the influence of oxidative stress Bettegowda A et al, and Wilkinson FW et al, at 2010 explain in their research that expression of the HSF2 gene moves in germinal cells which are useful for controlling cell spermatogenesis, especially working at the beginning of the pachytene process from spermatocytes to spermatids.[18] The HSF2 gene is an RNA m with a complex pattern of testes and HSF2 proteins to activate HSF2 gene expression.[25] Mutations in HSF2 in male mice result in hypofertil so ensuring the size of the testes and epididymis and increasing apoptosis in the pachiten stage results in reduced sperm formation.[19] The results of the same study were carried out by Madhubabu G et al, and Yenugu S et al, at 2016 by administering allethrin orally to Wistar rats for 60 days at doses of 25, 50, 100, and 150 mg/kg. There is a significant result in a decrease in the regulation of the testicles.[13] This means that allethrin affects the HSF2 gene against white mice to interfere with spermatogenesis. Effect of exposure to an electric mosquito repellent made from activists allethrin against Ovol1 gene expression in male rats' spermatogenesis. Based on the results of the analysis, it was found that the influence exposure made from allethrin active ingredients on the expression of the Ovol1 gene between the control group and the treatment group with different exposures, from the treatment of the three groups there was the highest Ovol1 gene expression in treatment group two compared to treatment 1 and treatment.[3] The difference in the difference in average decline from treatment group 2 to treatment 3 with a difference of 0.08 was due to the genetic variation found in the sample. Sahabuddin et al, at 2014 explains genetically there are no two individuals in one species that are exactly the same.[26] Two individuals who have members of the same species, both can be different because it is a variation of various factors, namely genetic factors, food, and habitat.

Allethrin induces toxicity through oxidative stress by producing free radicals.[27] The free radicals involved are ROS. If the formation of ROS increases, there will be an imbalance in the formation of free radicals that cause oxidative stress.[28] According to Winarsih H et al, at 2007 in the study explained that allethrin induces the occurrence of toxicity through free radicals.[21] The imbalance between free radicals and antioxidant ability will cause damage to specialized cells in the cell membrane.[22] Oxidative stress that occurs will affect gene expression, especially in the Ovol1 gene. The Ovol1 target gene is Id2 which functions to inhibit DNA binding for regulation of transcription. Decreasing the expression of Ovol1 gene has an upregulatory ID2 effect which results in the double helix dimer helix being unable to bind DNA so that the occurrence of encryption and differentiation are not blocked. Disrupted disorders of the Ovol1 gene expression result in testicular abnormalities.[29] Ovol1 deficiency makes abnormalities of proliferative miosis in spermatogonia and approximately 50 percent of spermatocytes are unable to pass through the slow pachytene phase.[15]According to the study of Madhubabu et al, at administration of allethrin at a dose of 25 to 150 mg/kg body weight was given to mice for 60 days.[13] The expression of m RNA factors that are very much needed in the process of spermatogenesis in the Ovol1 gene have significant results to reduce regulation. From these studies, it was found that allethrin affected spermatogenesis and sperm function, so it was seen that allethrin proved that it was toxicity.

CONCLUSION

There was an effect of exposure to mosquito repellent drugs made from active allethrin on the expression of the HSF2 gene and Ovol1 gene on spermatogenesis of male Wistar (Rattus novergicus) white rats. Decreasing HSF2 gene expression and Ovol1 gene starts from exposure for 4 hours.

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