Original Research Article

Effect of allethrin exposure on the expression of YBX2 and JHDM2A genes in spermatogenesis of male rats (*Rattus novergicus*) strain wistar

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ABSTRACT

Background: YBX2 and JHDM2A gene is important in spermatogenesis which acts as biomarkers of infertility. Allethrin chemicals widely used in mosquito and potentially toxic drugs that can damage DNA. The purpose of this study was to decide the effect of allethrin on decreasing YBX2 and JHDM2A gene expression on spermatogenesis of male white mice.

Methods: This research is an experimental post-test randomized control group design. Twenty-eight rats were given exposure according to the experimental group is K (control), P1 (exposure 4 hours), P2 (exposure 8 hours), and P3 (exposure 12 hours) for 30 days. Examination of YBX2 and JHDM2A gene expression from testicular tissue using real-time PCR with a relatively quantitative calculation method. Research implementation at Animal House and Biomedical Laboratory. Data analysis using one way ANOVA with a significant level of p<0.05.

Results: The results of this study were found mean differences amount of YBX2 and JHDM2A gene expression. The mean expression of the control group YBX2 gene is 1.1376, P1: 0.8976, P2: 0.5504, and P3: 0.4512. While in JHDM2A gene control group was 1.7033, P1: 1.7025, P2: 0.6863, and P3: 0.4077. Results of statistical tests using one-way ANOVA is YBX2 (p = 0.010) and JHDM2A (p = 0.000).

Conclusions: Based on the results of this study it was concluded that there was an allethrin effect on the decrease in YBX2 and JHDM2A gene expression in the Wistar Albino Rattus novergicus strain.

Keywords: Allethrin, JHDM2A gene, Rat (*Rattus novergicus*), YBX2 gene

INTRODUCTION

Infertility is the inability of a person to reach more than one pregnancy.¹ Worldwide infertility affects about 15%, equal to 48.5 million pairs. Men contribute to the cause of infertility is about 20-30% of cases and 50% of infertility cases overall.²

Based on the Indonesian Demographic and Health Survey (SKDI), in Indonesia, the fertility rate from 1971-2012 has decreased. Data from the Central of Statistics concluded that there was a decrease in fertility rates in the province of West Sumatra, namely from the 1971 fertility rate of 6.18, and in 2012 the fertility rate became 2.80.

Male infertility can be seen based on sperm count of less than 10 million, the volume of 1-5 ml of accumulation, abnormal sperm motility, abnormal morphology, and sterility (lack of sperm production).³ One of the causes of male infertility is a disorder of spermatogenesis.⁴ The process of spermatogenesis occurs in the seminiferous tubules of the testes. This process involves mitosis,
meiosis, and differentiation which depend on the regulation of gene expression. YBX2 and JHDM2A genes are molecules that can act as biomarkers and inactivation of both genes will cause infertility.

YBX2 gene is a transcription factor and acts as a stabilizer in the level of mRNA transcription and translation of specific genes in the testes such as protamine which plays a role in compaction of sperm chromatin. JHDM2A gene is a regulatory cause to regulate the expression of protamine (PRM) and transition nuclear protein 1 (TNP1). DNA packaging in sperm nucleus depends on the composition of protamine which is more dominant than histones. Failure of histone replacement process to protamine will result in the inability of compaction of chromatin in sperm and this is related to the occurrence of male infertility.

Genetic abnormalities are one of the testicular causes that can affect the process of spermatogenesis. Genetic abnormalities can be caused by gonadotoxic substances such as pesticide ingredients. One of the most widely used allethrin pyrethroid chemicals in mosquito repellent. Potentially toxic and free radical chemicals can cause oxidative stress. Oxidative stress causes various changes in DNA resulting in DNA damage. Despite its widespread use throughout the world, there have been relatively few reports of pyrethroid poisoning in humans. Less than ten deaths have been reported from consumption or after exposure. There are at least seven deaths among the 573 cases.

Public perception that the use of electric anti-mosquito drugs is still considered safe. Public perception about the type of mosquito repellent that is safe to use is lotion (31.3%), electrical (31.3%), spray (15.2%) spray/liquid (12.1%) and fuel (1%). Allethrin exposure can damage the testicles. based on the research of Naim (2016) that allethrin affects the quality of spermatooza. The longer usage of electric anti-mosquito drugs will decrease the number of spermatooza in test animals.

Research by Madhubabu and Suresh (2017) in India where the use of allethrin as a pesticide in agriculture. In his research stating that the toxicity of allethrin was given orally 100-150mg/kg in test animals cause decreased levels of YBX2 and JHDM2A, testosterone levels and sperm count is less than normal. Thus, providing further support for evidence of increasing toxicity.

The genetic cause of male infertility is still not recognized and in diagnosing male infertility, identification of genes that play a role in spermatogenesis needs to be studied. In addition, the public perception that the use of electric mosquito repellents that are still considered safe is the basis of this study. Based on this background the authors conducted a study to decide the effect of exposure to allethrin electric repellent active ingredients on the expression of YBX2 and JHDM2A genes in male spermatogenesis (Rattus norvegicus) Wistar strains.

METHODS

This type of research is experimental with a randomized control group design post-test research design. Maintenance of animal induction and allethrin exposure at Animal House of the Faculty of Medicine, Andalas University. Examination of YBX2 and JHDM2A genes at Andalas University Biomedical Laboratory, Padang. This research will be conducted from October 2017 to August 2018.

The population in this study was male Wistar white rats (Rattus norvegicus) obtained from Bungus rat farmers, Padang. The inclusion criteria are samples of white male rats aged 2-3 months, the body weight of 150-200 grams, activity, and normal behavior, and in good health. While the exclusion criteria are sick mice before by exposure to mosquito repellent and a dead rat after being exposed to the allethrin mosquito repellent drug during the study period. Adlibitum feeding and drinking and cage with a length of 170 cm, width 70 cm, and height 60 cm. Mice were acclimatized, then male and female rats were mated with the aim of seeing the pregnancy in female rats or male rats have done copulation indicating that mice are healthy and capable of fertilization.

Twenty-eight male rats were exposed to the experimental group, namely K (control), P1 (exposure to 4hours), P2 (exposure to 8 hours), and P3 (exposure to 12 hours) for 30 days. The dose of exposure at 45mg allethrin/mat and 4mg transfluthrin/mat. The expression of YBX2 and JHDM2A genes was examined using the real-time PCR method, total RNA was isolated from testicular tissue using TRizol® reagent and PureLink® RNA Mini Kit.

Twenty-eight male rats given the proper exposure experimental groups is K (control), P1 (exposure to 4 hours), P2 (exposure to 8 hours), and P3 (exposure to 12 hours) for 30 days. The dose of exposure at 45mg allethrin/mat and 4mg transfluthrin/mat.

The expression of YBX2 and JHDM2A genes was examined using the real-time PCR method, total RNA was isolated from testicular tissue using TRizol® reagent and PureLink® RNA Mini Kit. Preparation of cDNA using cDNA Synthesis Kit iScriptTM. Design of primers for YBX2 gene expression analysis (F: TCTTTGTTCACCGACAGCTATTA, R: CCCAGGCCCAGTTACATTAG), gene JHDM2A (F: GAAGCAGTAATAAAACCCAGACTCC, R: TGTGAAATTGTAACTCCTGAAGTG and Housekeeping gene (GADPH) (F: CATGGCCTTCCGTGTCCCCTA, R: CCTGCTTACCACCTTCTTGAT) as an internal control for quantification of target gene expression.
Data obtained by the normality test data and the expression analysis of YBX2 and JHDM2A genes used One Way ANOVA method with 95% confidence level where the value of p<0.05% (significant), then continued with multiple (post hoc test) types of Bonferroni forsee significance between groups.

RESULTS

Data of YBX2 gene expression amount was analyzed by using One Way ANOVA and Post Hoc test Bonferroni (Table 1).

Table 1: The mean of YBX2 gene expression of control group and treatment group on Rattus norvegicus wistar strain (n = 27).

| Subject of group | n  | YBX2 gene expression (R) mean±SD | P value |
|------------------|----|--------------------------------|---------|
| Control          | 7  | 1.1376±0.342                   | 0.010   |
| P1               | 6  | 0.8976±0.407                   |         |
| P2               | 7  | 0.5504±0.256                   |         |
| P3               | 6  | 0.4512±0.467                   |         |

The Table 1 shows there is a difference YBX2 gene expression between the control group and the treatment group. It can be seen that the level of gene expression in the treatment group is lower than the control group, and each treatment group showed a decrease in gene expression. To see the difference of YBX2 gene expression between the control group and the treatment group in detail, it is necessary to do further testing with Bonferroni Post Hoc Test.

Table 2: The result of Post Hoc Test Bonferroni on YBX2 gene expression in each research group.

| Group | YBX2 gene expression |
|-------|----------------------|
|       | Control | P1 | P2 | P3 |
| Control | -      | 1.000 | 0.043* | 0.018* |
| P1      | 1.000   | -   | 0.637 | 0.292 |
| P2      | 0.043*  | 0.637 | -   | 1.000 |
| P3      | 0.018*  | 0.292 | 1.000 | -   |

The Table 2 shows changes mean gene expression between the control group and the treatment group. The decrease gene expression can be seen from each treatment group. To see the differences of gene expression between the control group and the treatment group in detail, it is necessary to have further testing with the Bonferroni Post Hoc test.

Table 3: The mean of JHDM2A gene expression of control group and treatment group on Rattus norvegicus Wistar strain (n = 27).

| Subject of group | n  | JHDM2A gene expression (R) mean±SD | P value |
|------------------|----|--------------------------------|---------|
| Control          | 7  | 1.7034±0.504                   | 0.000   |
| P1               | 6  | 1.7025±1.340                   |         |
| P2               | 7  | 0.6803±0.198                   |         |
| P3               | 6  | 0.4077±0.317                   |         |

The Table 3 shows changes mean gene expression between the control group and the treatment group. The decrease gene expression can be seen from each treatment group. To see the differences of gene expression between the control group and the treatment group in detail, it is necessary to have further testing with the Bonferroni Post Hoc test. From the Table 4, it can be concluded that there is a significant effect of JHDM2A gene expression between Control group and P2, Control group and P3 and P1 and P3 groups, because of p-value <0.05.

DISCUSSION

Effect of allethrin exposure on the expression of YBX2 gene in spermatogenesis of male rat

The results of the YBX2 gene expression data analysis found that there was an effect of allethrin on YBX2 gene expression. The highest mean is the control group of 1.1376. While the lowest mean was the P3 group which was given 12hours exposure treatment that is 0.4512. From these results, it shows that there is a decrease in gene expression due to exposure to allethrin.

Allethrin toxicity produces down-regulation of genes involved in sperm maturation. Expression of the YBX2 gene is important in the process of spermatogenesis, especially the stages of spermiogenesis when compaction of the sperm nucleus occurs, if there is a decrease it will cause infertility. A several mRNA binding proteins are important in the process of spermatogenesis in producing physically mature spermatozoa. Decrease in YBX2 gene expression will affect the morphology of sperm.

YBX2 gene (Y box binding protein2) is a transcription factor by marking certain mRNAs on the promoter region which is transcribed from the Y-box promoter especially in male germ cells. YBX2 works on testicular specific genes, namely protamine proteins at the level of transcription and translation.

The process of transcription and post-transcriptional on spermatogenesis process controlled by genes is a mechanism that depends on androgens and estrogen. Allethrin acts as an endocrine disruptor and changes hormonal homeostasis in both men and women leading to subfertility.
This is because protamine is important in the structural reorganization of chromatin spermatid during the stages of spermiogenesis in the process of spermatogenesis. So, that the reduction of expression from YBX2 will give to the defect of the condensation process that occurs in spermatid.20

According to Najafipour et al, changes in the YBX2 gene are found in the blood serum of men with severe disabilities in spermatogenesis including azoospermia, oligozoospermia, and abnormal protamine expression. Decreasing YBX2 gene expression can be one of the causes of infertility, because of the role of these genes in spermatogenesis.7

**Effect of allethrin exposure on the expression of JHDM2A gene in spermatogenesis of male rat**

In this study, the results were significant where there was a decrease in the expression of the JHDM2A gene. Data analysis with one-way ANOVA states that p<0.05 (significant). The highest mean was in the control, which was 1.7034 while the lowest average was in the P3 group with allethrin exposure for 12 hours which was 0.4077.

Allethrin does not directly reduce the expression of YBX2 and JHDM2A genes, but damage from DNA itself is due to disruption of the hormonal pathway and cell damage due to oxidative stress induced by ROS. Where these disorders occur because of exposure to allethrin which is cytotoxic, so it can damage cells with the occurrence of oxidative stress.

Non-neutralized free radicals cause damage to cells. Nucleic acids such as DNA and RNA are susceptible to free radical compounds because groups such as aldehydes, ketones, and hydroxyl are easily oxidized.21

Allethrin is neurotoxic which can bind and inhibit the closure of sodium channels in the nerve membrane so that damage to the nervous system and brain will affect the work of the hypothalamus and disrupt the hormonal pathway.16 Neurotoxic effects on the peripheral nervous system result in paralysis.22

Decreasing the expression of JHDM2A gene causes a decrease in sperm count and defects in germinal cells resulting in abnormal sperm.16

JHDM2A (Jumonji Histone Demethylase 2A) has histone demethylase 2A which is important for the process of spermatogenesis. This gene has been shown to have histone demethylase activity.23

The JHDM2A gene contributes to transcription activation in several testicular specific genes such as stimulating TPN1 and PRM1 (protamine 1) by removing H3K9 methylation and binding to the core promoter.7 The lack of the JHDM2A gene in mice shows chromatin defects in the postmeiotic condensation process. Because of the disruption of expression of nuclear protein transitions (Tnp1) and protamine 1 (Prm1) needed in the process of sperm chromatin condensation.23

**CONCLUSION**

There is an influence of allethrin on the expression of YBX2 and JHDM2A genes on spermatogenesis of male white mice. There was a decrease in the mean of YBX2 and JHDM2A gene which is given allethrin starting from 4 hours exposure.

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