Effect of Mung Bean Sprouts Extract to the Morphology and Motility Spermatozoa in Mice Exposed Monosodium Glutamate

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ABSTRACT

Background: The prevalence of male infertility in 30% of the case is considered idiopathic infertility that is infertility cases the cause cannot be found with certainly but on examination showed increased levels of reactive oxygen species (ROS) in seminal liquid. Increased level of ROS caused by the presence of free radical. Monosodium glutamate (MSG) is a food additive that is used for flavoring food, but MSG has the effect of free radicals when usage exceeds normal limits. Mung bean sprouts are natural antioxidants because it contains vitamins E and C and zinc. This study aimed to analyze the effect of mung bean sprouts extract to the morphology and motility spermatozoa in mice exposed MSG.

Materials and Methods: This research is an experimental research with post-test only control group design. 30 male mice divided into five group control negative, control positive (Monosodium glutamate [MSG] 4 mg/g BB), P1 (MSG 4 mg/g BB + 108 mg extra), P2 (MSG 4 mg/g BB + 216 mg extra), and P3 (MSG 4 mg/g BB + 432 mg extra). Data were analyzed with one-way analysis of variances (ANOVA) and followed by Duncan.

Result: ANOVA one-way test showed that there were significant differences among the groups with P = 0.000. Further tests by Duncan test showed that there are significant differences in each group because they are on a different subset group with significant < 0.05.

Conclusion: The results showed that the extract of mung bean sprouts can increase the percentage of morphology and motility of spermatozoa.

Key words: Infertility, Monosodium glutamate, Mung bean sprouts extract, Morphology spermatozoa, Motilitas spermatozoa

INTRODUCTION

Infertility is one important component of reproductive health but efforts in resolve infertility are often neglected.1 Infertility is the inability to get pregnant after sexual intercourse without protection for a period of 1-year and at least three times a month.2 The prevalence of infertility in the world currently estimated at 8-12% or about 60-80% million couples in the world do not have children, and this failure is expected to appear about 2 million annually.3

The problem of infertility in men is basically caused by sexual disorders and fertility disorders.4 A man was called as a cause of infertility if when the results of the analysis of spermatozoa found at least one change in the spermatozoa are either on the number, morphology, motility or viability based on the guidelines of laboratory examination of human semen analysis.

Male infertility is characterized by a low number of spermatozoa and a high percentage of slowing moving spermatozoa and morphology abnormal. The prevalence of male infertility in 30% of case is considered idiopathic infertility that is infertility cases the cause cannot be found with certainly either with general clinical methods or laboratory examination.

Men with idiopathic infertility generally show increased levels of reactive oxygen species (ROS) in the seminal liquid.
Increased levels of ROS caused by the presence of free radical in tissues that produce spermatozoa. Free radical can damage important compounds for maintaining integrity and fluidity of cells and spermatozoa are cells that are highly sensitive to ROS because spermatozoa membrane rich in unsaturated fatty acids so easily oxidized.

Monosodium glutamate (MSG) is natural salt compounds derived from L-glutamic acid, MSG in the form of a white crystalline powder and has long been used as a flavor enhancer in a variety of food products. In general, MSG has been accepted as one of the food additives are safe, but many studies have shown that consumption exceeds safe limits may increase health risks.

Indonesian people who consume MSG is currently at 50.3%. MSG consumption may be higher than that because Indonesian people like to consume cooked foods that would increase the risk of consumption MSG because for many foodstuff are not mentioned MSG as the manufacture. Consumption of MSG in large doses and in a long time will have a negative impact on health.

Consumption of MSG in toxic doses will cause glutamate in the body increased. The high of glutamate levels in the blood will increase glutamate receptor activity, and an increase in this activity will spark the opening of Ca\(^{2+}\) channels. The opening of Ca\(^{2+}\) channels will lead ions calcium enter and activate the enzymes such as protease, lipase, and endonuclease that will affect the phospholipids which are building blocks of cell membranes.

Increased cell metabolism caused by toxic doses of MSG will increase the production of ROS and increased ROS will lead to high oxidative stress. Oxidative stress itself will spark the disruption of spermatogenesis process by two ways that are hormonal pathways and testicular pathways. Hormonal pathways oxidative stress causes an injury in Arkuatus nucleus hypothalamus which is hormones that play a role in the production of spermatogenesis.

On testicular pathways, oxidative stress causes a decrease in spermatozoa formation by two mechanisms that are membrane cells damage and DNA damage. Oxidative stress can decrease the role of antioxidant in the body and decreased role of antioxidant will increase lipid peroxidation which will lead to decrease integrity (wholeness) and fluidity (spasticity) spermatozoa cells membrane.

Decreased of morphology and motility spermatozoa will increase the likelihood of a man becomes infertile and one step that can be done to inhibit the negative effect of MSG on male reproductive function is the antioxidant therapy because the presence of radical scavenger (antioxidant) are expected to help clean up the free radicals on tissues of producing spermatozoa.

Mung bean sprouts have a lot of good nutritional content of vitamins and minerals. From some of the nutrients in the sprouts are several vitamins and minerals that are believed as antioxidants and also substances essential nutrients that can help the process of establishing spermatogenesis, namely vitamins E and C and zinc.

Mung bean sprouts are a plant that is almost there throughout the regions in Indonesia; the price is also relatively cheap with huge benefits but its weakness for storage which can not last long and sometimes less precise cooking process will cause a decline in the nutritional content of Mung bean sprouts.

MATERIALS AND METHODS
This study is an experimental research with post-test only control group design because in this study researchers give MSG to exposure mice and then give mung bean sprouts extract with graded doses for therapy. This research was conducted in two places, the first in the Laboratory of the Faculty of Medicine, the University of Airlangga for maintenance of mice and the second is at the Faculty of Science and Technology Laboratory of the University of Airlangga. This research was started in April until June 2016.

This study used 30 male mice Balb/c were divided into 5 groups. Group 1: Negative control, Group 2: Positive control (MSG 4 mg/g BB), Group 3: First treatment group (MSG 4 mg/g BB + extract 108 mg), Group 4: S treatment group (MSG 4 mg/g BB + extract 216 mg), and Group 5: Third treatment group (MSG 4 mg/g BB + extract 432 mg). MSG granted for 35 days from 1st day until 35th day and extract of mung bean sprouts granted for 15 days from 21st day until 35th day.

The independent variables in this study were of mung bean sprouts, whereas the dependent variable is the morphology and motility of mice. For the control variables are the provision of MSG. Data morphology and motility of mice collected by microscopic laboratory examinations. Processing data by two stages. The first stage is through descriptive analysis to look at the frequency distribution, mean and standard deviation. The second stage for analytical analysis, to know normality used the Kolmogorov-Smirnov test and for homogeneity used Levene test and for to know the effect of treatment use analysis of variances (ANOVA) one way followed by a further test of Duncan.

RESULTS

Morphology Spermatozoa
Based on the results of the study showed a decrease in the average of morphology spermatozoa from the group that was not exposed to the group exposed MSG 4 mg/g BW. The increase in the percentage of spermatozoa morphology seen in the extract of mung bean sprouts at a dose of 108 mg, 216 mg, and 432 mg. Increasing the highest number is in the group with a dose of 432 mg, equivalent to 33.2 kg of fresh sprouts (Figure 1).

The one-way ANOVA test result show obtained significant 0.000, the value of P < 0.05 so that it can be concluded that Ho is rejected, or there are significant differences between the groups. The letter above diagram shows the results of different test Duncan, based on test morphology of spermatozoa expressed into five groups. The division of this group indicates that each group has a significant difference.

Motility Spermatozoa
Based on the results of the study showed a decrease in the average of motility spermatozoa from the group that was not exposed to the group exposed MSG 4 mg/g BW. The increase in the percentage of spermatozoa morphology seen in the extract of mung bean sprouts at a dose of 108 mg, 216 mg, and 432 mg. Increasing the highest number is in the group...
The content of vitamin E is considered as the antioxidant effects that can be caused. As an antioxidant, vitamin E function as a hydrogen ion donor is able to transform into a radical peroxyl radical tocopherol less reactive, so it is not capable of damaging chain fatty acids. The content of vitamin E is considered as the greatest antioxidant capacity levels in bean sprouts if evaluated antioxidant effects that can be caused.

Improvement of existing improvements cannot match the results of the group not exposed, it is associated with high doses of MSG presented are spread 4 mg/g, equivalent to 38.7 g/day in humans with a body weight of 70 kg. The maximum increase is likely to occur at exposure levels of MSG with the lower dose and extract a longer time.

Motility Spermatozoa

The results showed a decrease in the percentage of normal sperm morphology in the group that was exposed to MSG. Morphology or shape of abnormal spermatozoa will increase the possibility of infertility. Morphological abnormalities according are basically caused by disturbance in the process of spermatogenesis, especially on spermatogenesis stages: Stage of maturation. MSG causes deformities of the spermatozoa through testiskular. On track testiskular, MSG disrupts the formation of spermatozoa by higher activity of oxidative spark to the formation of reactive compounds caused by MSG.

High oxidative stress can cause a decrease in membrane fluidity and integrity of spermatozoa and can also damage directly to the deoxyribose purine and pyrimidine nucleic acid (DNA). Increased membrane damage and DNA spermatozoa will increase the likelihood of abnormal sperm morphology. In this study, the majority of sperm morphological abnormalities occur on the tail and found many spermatozoa are attached to one another or spermatozoa are still many having cytoplasm so that sticking together.

In the group of mice was exposed to MSG 4 mg/g BW for 35 days and given mung bean sprouts extract of 108 mg, 216 mg, and 432 mg for 15 days showed an increase in the percentage of normal sperm morphology. Repair morphology of spermatozoa was given extracts of mung bean sprouts due mung bean sprouts extract contains vitamins C and E and zinc can help enhance the role and function of antioxidants in testis decreased. Besides vitamin C and E and zinc can prevent DNA damage due to oxidative stress.

Motility Spermatozoa

The results showed a presentation motility in the group exposed MSG decreased compared to the figures which are not exposed Kelompok MSG. Motility is a picture of the ability of sperm to move properly toward an egg. MSG in toxic doses will affect the movement of sperm because MSG is known to cause a disturbance in the process of sperm formation. Increased sperm shape abnormality Khsusunya on the tail will decrease the speed of movement of spermatozoa.

The tail of the sperm is very strong and serves to move and contain little cytoplasm. The source of energy for the movement of spermatozoa exist in mitochondria located at the base of the head.

Figure 1: The mean of spermatozoa which have normal form (%) and show the result of duncan test. The negative control was not being exposed to monosodium glutamate and extracts. Positive control was exposed to monosodium glutamate 4 mg/g BW and 0 g of extracts. Treatment 1,2 and 3 were exposed monosodium glutamate 4 mg/g BW for 35 days and were given the extract at a dose of 108 mg, 216 mg and 432 mg on day 21st up to day 35th. The letters that are above diagram shows the real difference is based on test Duncan at α=0,05

Figure 2: The mean of spermatozoa which have good ability to move (%) and show the result of duncan test. The negative control was not being exposed to monosodium glutamate and extracts. Positive control was exposed to monosodium glutamate 4 mg/g BW and 0 g of extracts. Treatment 1,2 and 3 were exposed monosodium glutamate 4 mg/g BW for 35 days and were given the extract at a dose of 108 mg, 216 mg and 432 mg on day 21st up to day 35th. The letters that are above diagram shows the real difference is based on test Duncan at α=0,05

with a dose of 432 mg, equivalent to 33.2 kg of fresh sprouts (Figure 2).

The one-way ANOVA test result show that obtained significant 0.000, the value of $P < 0.05$ so that it can be concluded that Ho is rejected, or there are significant differences between the groups. The letter above diagram shows the results of different test Duncan, based on test motility of spermatozoa expressed into five groups. The division of this group indicates that each group has a significant difference.

DISCUSSION

Morphology Spermatozoa

The mice spermatozoa morphological examination is an examination which shows the percentage of spermatozoa form of normal mice in units percent. Morphology of spermatozoa is one of the main factors to determine the quality of spermatozoa because spermatozoa have abnormal morphology at higher risk of experiencing a failure in fertilization. Abnormalities in sperm head shape will make it difficult to penetrate the cell wall of the egg, while the spermatozoa have abnormalities in the tail will be difficult to reach the egg or ovum.

The results showed a decrease in the percentage of normal sperm morphology in the group that was exposed to MSG. Morphology or shape of abnormal spermatozoa will increase the possibility of infertility. Morphological abnormalities according are basically caused by disturbance in the process of spermatogenesis, especially on spermatogenesis stages: Stage of maturation. MSG causes deformities of the spermatozoa through testiskular. On track testiskular, MSG disrupts the formation of spermatozoa by higher activity of oxidative spark to the formation of reactive compounds caused by MSG.

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The tail of the sperm is very strong and serves to move and contain little cytoplasm. The source of energy for the movement of spermatozoa exist in mitochondria located at the base of the head.
Motility describes the ability of sperm to move properly toward an egg, and the success of this movement is one of the quality parameters of spermatozoa, which is a determining factor in the success of fertilization. Spermatozoa produced in the testes but gain the ability to move in the epididymis. So that when the sperm preparation process to move from the seminiferous tubules to the epididymis to occur the damage caused by oxidative stress caused by MSG. Hence, to reduce the ill effects of the influence of MSG is to provide antioxidants.

The research results show there was differences in the percentage of sperm motility in each group as in the treatment group were given extracts of mung bean sprouts increased the percentage of sperm motility indicate where it extracts of mung bean sprouts can reduce the adverse effects of MSG on motility of spermatozoa.

Vitamin E is an antioxidant that provides prevention is protective effects against spermatozoa membrane damage caused by oxidative stress than because ROS itself. Ascorbic acid gives a good effect to integitas of or against the tubular structures that sperm function and can neutralize hydroxyl radicals, superoxide, and hydrogen peroxide and prevent agglutination of spermatozoa. In several studies have demonstrated that ascorbic acid intake can improve sperm quality.

At this stage spermatid, maturation zinc serves to physically and physiologically, in which the zinc will be incorporated into the tail of the spermatid in large numbers. Zinc in the sperm tail control, the use or utilization of energy through the system adenosine triphosphate and the regulation of phospholipid, thus contributing to increasing the motility of spermatozoa. MSG exposure can increase sperm immotile and mung bean sprouts extract showed an improvement in sperm motility, however, the extract with the highest dose belum still be able to match the percentage of sperm motility in the group not exposed to MSG.

CONCLUSION

Based on these results, we can conclude that there are significant differences between the groups. Giving MSG 4 mg/g BW, equivalent to 38.7 g/day in humans with 70 kg body weight for 35 days can decreased morphology and motility of spermatozoa in mice. The extract of mung bean sprouts for 15 days starting from day 21 through to 35 at a dose of 108 mg, equivalent to 8.3 kg of fresh sprouts has been able to boost the number, morphology, motility, and spermatozoa vibilitas mice. At doses of 432 mg, equivalent to 33.2 kg of fresh sprouts showed improvement examination results spermatozoa were highest among other dosing.

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