Original Research Article

Effect of reduced glutathione on follicle stimulating hormone levels and spermatozoon glutamate administration

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ABSTRACT

Background: Excessive consumption of Monosodium Glutamate (MSG) causes oxidative stress of hypothalamic cells and testicular cells thus affecting the process of spermatogenesis. Reduced Glutathione (GSH), as an antioxidant can reduce oxidative stress. This study aimed to see the effect of GSH on Follicle Stimulating Hormone (FSH) levels and the quality of spermatozoon in male rats given MSG.

Methods: This research used experiment with post test only control group design. The samples used were 27 male rats divided into 3 groups. The negative control group (KN) was given a standard diet, the positive control group (KP) was given MSG 144mg/200grbw, the treatment group (P) was given a standard diet and MSG dose was 144mg/200grbw and continued with GSH dose 25.2mg/200grbw. On the 52nd day, FSH levels were analyzed using ELISA, and the number, morphology and motility of spermatozoon were examined using a digital microscope.

Results: The results showed an average FSH level in the KN group (105.42ng/ml SD±64.44), KP (58.88 ng/ml SD±22.1), P (64.57ng/ml SD±35.8) with a value of p = 0.072. The average number of spermatozoon in the KN group (43.59 million/ml SD±14.06), KP (26.37 million/ml SD±12.7), P (43.26 million/ml SD±76.15) with a value of p = 0.007. The average normal morphology of spermatozoon in KN group (21.63% SD±10.9), KP (4.65% SD±4.38), P (23.18% SD±12.59) with p = 0.001. The average motility of spermatozoon in the KN group (57.58 million/ml SD±11.24), KP (34.29 million/ml SD±21.60), P (72.33 million/ml SD±4.072) with a value p = 0.000.

Conclusions: Results of this study concluded that the administration of GSH did not significantly increase FSH levels. There is an effect of GSH on improving spermatozoon quality of rat given MSG.

Keywords: Follicle stimulating hormone levels, Monosodium glutamate, Reduced glutathione, Spermatozoon quality

INTRODUCTION

Monosodium glutamate (MSG) is an additive in foods that can improve the taste. MSG is widely available in packaged foods but the dosage is not listed on the label. The absence of MSG written doses in these packaged foods causes people to consume MSG in high concentrations. MSG is consumed by 77.3% of Indonesia's population aged more than 10 years.

Excessive MSG consumption is feared to have an impact on organs including reproductive organs.1-3

MSG consists of sodium and glutamate. Glutamate is a nonessential amino acid that can be synthesized from other amino acids in the body. It has been reported that excessive administration of MSG can damage a large proportion of the liver lobules severely, the inability of liver cells to metabolize excess glutamic acid will increase plasma glutamic acid levels.4
Glutamic acid in MSG can be classified into D-glutamic acid and L-glutamic acid. The increase of glutamic acid levels in the body, especially glutamic acid-D causes impaired protein synthesis from glutamic acid, thus becoming free radicals in the body. Increased free radical levels will cause oxidative stress in cells.5

To minimize cell damage due to free radicals, antioxidants are needed. The human body does not have an excess amount of antioxidants, so if a lot of radicals are formed, the body needs more antioxidant. Reduced glutathione (GSH) is an antioxidant that functions as a co-substrate for the glutathione peroxidase enzyme. GSH is facilitated by sulfidyl groups from cysteine. Reduced glutathione is also called a master of antioxidants, because other antioxidants depend on the presence of this GSH to function properly. Reduced glutathione is one of the antioxidants that cannot act as prooxidants. Reduced glutathione can also easily convert antioxidants that have been oxidized to reduced forms so they can function again in eliminating free radicals.6

Reduced glutathione can function as an antioxidant through various mechanisms. GSH can chemically react with singlet oxygen, superoxide radicals and hydroxyl. Reduced glutathione can also minimize the formation of acyl peroxide in the lipid peroxidation reaction.6

METHODS

This type of research was post test only control group design with an experimental approach. The study was conducted in Immunology Laboratory Faculty of Pharmacy Andalas University Padang for maintenance, rat surgery and examination of sperm quality using digital microscopes. Follicle Stimulating Hormone levels were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) conducted at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. The research was conducted from August 2017 to December 2017.

The population of this study was adult male rats (Rattus norvegicus), with the consideration that rats were mammalian experiments that were often used in biological research. The samples used were adult male rats aged 2-3 months, weight 160-240 grams and healthy, while the exclusion criteria were that the rats did not want to eat and were sick, the drop out criteria were male rats that experienced a decline in physical condition and died when the research was being carried out. Rats were fed daily on an ad libitum basis.

The operational definition of research was Monosodium Glutamate which is given orally for the first 20 days, the dose was given based on body weight. GSH was given intraperitoneally on day 21-31 after administration of monosodium glutamate, with dose based on body weight. FSH levels were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method, measuring instrument with spectrophotometer, with the results of measuring in ng/ml and interval scale. The number of spermatozoa was measured by a digital microscope and a measuring device with a measurement result of million/milliliters and ratio scale. Then for normal morphology and progressive motility the spermatozoa were measured with a digital microscope and a counter with the percentage and ratio scale results.

In the experiment, the rats divided into 3 groups, the negative control group (KN) was placed in a cage without treatment, the positive control group (KP) given monosodium glutamate and the treatment group (P) given monosodium glutamate in the first 20 days followed by GSH 31 days later. The data obtained were analyzed using One Way Anova with a significance level of p < 0.05.

RESULTS

Data on FSH levels and quality of rat spermatozoon were obtained by statistical tests to assess normality using the Shapiro-Wilk test. While to see the effect of GSH on FSH levels and spermatozoon quality of male mice given MSG, one-way ANOVA test was used.

Table 1 showed that the mean FSH level of rats in the negative control group was 105.42-ng/ml. The mean FSH level of rats in the positive control group was 58.88-ng/ml. The mean FSH levels of rats in the treatment group were 64.57-ng/ml. It can be concluded that there was a decrease in the mean of MSG administration in the positive control group compared to the negative control group, and there was an average increase in FSH levels after GSH administration at a dose of 25.2mg/200 gr bw but was not significant (p = 0.072).

Table 1: Mean FSH levels in each group after treatment.

| Subject group | Follicle stimulating hormone levels (ng/ml) | n | Mean±SD | P |
|---------------|--------------------------------------------|---|---------|---|
| KN            | 105.42±64.4                                | 9 |         |    |
| KP            | 58.88±22.19                                | 9 |         | 0.072 |
| P             | 64.57±35.87                                | 9 |         |    |

Table 2: Mean spermatozoon count in each group after treatment.

| Subject group | Spermatozoon count (million/ml) | n | Mean±SD | P |
|---------------|--------------------------------|---|---------|---|
| KN            | 43.59±14.06                    | 9 |         |    |
| KP            | 26.37±12.7                     | 9 |         | 0.007 |
| P             | 43.26±7.6                      | 9 |         |    |

Table 2 showed that the mean spermatozoon count in the negative control group was 43.59 million/ml, the mean spermatozoon count in the positive control group was 26.37 million/ml, the average spermatozoon count in the treatment group was 43.26 million/ml. The results of data
analysis with one-way ANOVA statistical test showed the value of $p = 0.007$ ($p \leq 0.05$) which can be concluded that there was an significant effect of GSH on the spermatozoon count in rats given MSG.

**Table 3: Mean percentage of normal morphology spermatozoon in each group after treatment.**

| Subject group | Normal Morphology Spermatozoon (%) | Mean±SD | P      |
|---------------|-----------------------------------|---------|--------|
| KN            | 9                                 | 21.63±10.9 |       |
| KP            | 9                                 | 4.6±4.38 | 0.001 |
| P             | 9                                 | 23.18±12.59 |      |

In Table 3, the mean percentage of normal spermatozoon morphology in the negative control group was 21.63%, the mean percentage of normal spermatozoon morphology in the positive control group was 4.6% and the mean percentage of normal spermatozoon morphology in the treatment group was 23.18%. The results of the one-way ANOVA statistical test obtained $p = 0.001$ ($p \leq 0.05$) so that it can be concluded that there was an significant effect of GSH on the percentage of normal spermatozoon morphology in male rats given MSG.

**Table 4: Mean progressive motility spermatozoon percentage in each group after treatment.**

| Subject group | Progressive Motility Spermatozoon (%) | Mean±SD | P      |
|---------------|-------------------------------------|---------|--------|
| KN            | 9                                   | 57.58±11.24 |       |
| KP            | 9                                   | 34.29±21.6 | 0.000 |
| P             | 9                                   | 72.33±4.07 |       |

Table 4 showed that the mean percentage of progressive spermatozoon motility in the negative control group is 57.58%, the mean percentage of progressive motility of spermatozoon in the positive control group is 34.29%, and the average percentage of progressive motility of spermatozoon in the treatment group is 72.33%. The results of data analysis using one-way ANOVA test showed that $p = 0.000$ ($p \leq 0.05$), which can be concluded that there was a significant effect of GSH on the progressive motility of spermatozoon in male rats given MSG.

**Table 5: Multiple comparison post Hoc Bonferroni test in spermatozoon count.**

| Subject group | Significance level of spermatozoon count (p) | KN | KP | P   |
|---------------|---------------------------------------------|----|----|-----|
| KN            | -                                           | 0.015* | 1.000 |
| KP            | 0.015*                                      | - | 0.017* |
| P             | 1.000                                       | 0.017* | -   |

Based on the results of the post Hoc Bonferroni test in Table 5 it can be concluded that, there was a significant difference in the number of negative control spermatozoon with the treatment group, and the positive control group with the treatment group because of the $p \leq 0.05$ value. While other groups did not show a significant relationship ($p \geq 0.05$).

**Table 6: Multiple comparison post Hoc Bonferroni test in normal spermatozoon morphology percentage.**

| Kelompok Subyek | Significance level of normal spermatozoon morphology percentage (p) | KN | KP | P   |
|-----------------|---------------------------------------------------------------|----|----|-----|
| KN              | -                                                            | 0.004* | 1.000 |
| KP              | 0.004*                                                       | - | 0.002* |
| P               | 1.000                                                        | 0.002* | -   |

Based on the results of the post hoc test of Bonferroni in Table 6 it can be concluded that there were significant differences in the normal morphology of the spermatozoon between the negative control group and the positive control group, the positive control group with the treatment group ($p \leq 0.05$). While the other groups did not show significant relationship.

**Table 7: Multiple comparison post Hoc Bonferroni test in progressive spermatozoon motility percentage.**

| Kelompok Subyek | Significance level of progressive motility spermatozoon percentage (p) | KN | KP | P   |
|-----------------|-------------------------------------------------------------------|----|----|-----|
| KN              | -                                                                | 0.006* | 0.114 |
| KP              | 0.006*                                                           | - | 0.000* |
| P               | 0.114                                                           | 0.000* | -   |

Based on the results of the post hoc test of Bonferroni in Table 7 it can be concluded that there were differences in spermatozoon motility between the negative control group and the positive control group, the positive control group with the treatment group ($p \leq 0.05$). While the other groups did not show significant relationship.

**DISCUSSION**

The results of this study found that in the positive control group given MSG dose 144mg/200 g bw in the first 20 days of the study, there was a decrease in the average FSH level. Glutamate acts as a neurotransmitter in brain cells, but at excessive doses, glutamate can cause disruption of the hypothalamic-pituitary-testicular axis caused by oxidative stress on brain cells even more so on the hypothalamus, which is unprotected by blood brain barrier. Damage to the hypothalamus results in a decrease in the secretion of Gonadotropin Releasing Hormone (GnRH) by the hypothalamus. The decrease in GnRH levels also has an effect on decreasing levels of FSH and LH produced by the anterior pituitary. The treatment group whom given MSG dose of 144 mg/200 g bw in the first 20 days of treatment then continued with GSH administration for 31 days showed an increase in the...
average FSH level. GSH as an antioxidant can minimize cell damage caused by free radicals caused by excessive consumption of MSG.\textsuperscript{7,9}

Based on the results of the study it was found that the count, normal morphology, and progressive motility of rat spermatozoon were decreased in the positive control group compared to the negative control group. This is as a result of a decrease in FSH levels produced by the anterior pituitary, so that the process of spermatogenesis is disrupted. FSH works on sertoli cells which help launch the final stage of spermatozoon maturation. FSH also stimulates the secretion of Androgen Binding Protein (ABP) whose functions to bind to the testosterone.\textsuperscript{9}

Excessive MSG can reduce the number of sertoli cells. Sertoli cells function to support, protect and regulate spermatozoon nutrition. In addition, sertoli cells also function to produce androgen binding protein (ABP). This ABP functions to bind to the testosterone so it is not to easily diffuse out of from seminiferous tubules.\textsuperscript{10}

The radical effects of MSG can be suppressed by antioxidant intake. GSH can be used to restore the condition of unsaturated fatty acids in the spermatozoon cell membrane, to improve the quality of seminiferous tubules so that the process of spermatogenesis occurs well and can produce good spermatozoon.\textsuperscript{11}

In the treatment group there was an increase in the number, normal morphology, and progressive motility of the spermatozoon. GSH can repair damage to the hypothalamus due to free radicals arising from excessive consumption of MSG, chemically can react with singlet oxygen, superoxide radicals, and can directly act as free radical scavenger. GSH can also stabilize the hypothalamic membrane structure by removing or minimizing the formation of acyl peroxide in the peroxide lipid reaction.\textsuperscript{6}

GSH can also stabilize the membrane structure by removing or minimizing the formation of acyl peroxide in the lipid peroxide reaction.\textsuperscript{6} GSH also plays a role in the protection mechanism of reactive oxygen species (ROS), including peroxide produced by cellular oxygen metabolism.

This reactive oxygen compound can be neutralized through reduction by glutathione with the help of glutathione peroxidase enzymes. In this process glutathione will be converted to oxidized glutathione (GSSG).\textsuperscript{12}

CONCLUSION

Administration of GSH can increase the number, normal morphology, and progressive motility of the Rattus norvegicus spermatozoon given MSG.

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