

Mung Bean Sprout Extracts Suppress The Effects of *Monosodium Glutamate* (MSG) on The Ovarian Follicular Development of Female Wistar Rats (*Rattus norvegicus*)

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Abstract

Objective: The purpose of this study was to analyze the effects of mung bean sprout extracts on follicular development as indicated by the number of secondary follicles, Graafian follicles, and atretic follicles in female Wistar rats (*Rattus norvegicus*) exposed to monosodium glutamate (MSG). **Samples and Methods:** This true experimental study was conducted by using post-test only control group design in the laboratory for animal experimentation of Faculty of Veterinary Medicine - Airlangga University in which Wistar rats (*Rattus norvegicus*) aged 2 months weighing 150-200 grams were used in this experiment. The samples comprised of 5 rats distributed in each group, totaling 7 groups. I Control Group (P1) was provided with Aquades for 37 days; II (P2) was provided with Aquades for 7 days + MSG 0.03 mg/g of weight on day 8-37; III (P3) was provided with extract of mung bean sprouts 72mg/200g of weight on day 1-37 + MSG 0,03mg/g of weight; IV (P4) was provided with extract of mung bean sprouts 144mg/200g of weight on day 1-37 + MSG 0.03mg/g of weight; V (P5) was provided with Aquades for 7 days + MSG 0.7 mg/g of weight on day 8-37; VI (P6) was provided with extract of mung bean sprouts 72mg/200g of weight on day 1-37 + MSG 0.7mg/g of weight; VII (P7) was provided with extract of mung bean sprouts 144mg/200g of weight on day 1-37 + MSG 0.7mg/g of weight. After 37 days of treatment, the rats were euthanized during estrous period to collect both right and left ovaries, the microscope slide was prepared for histopathology by hematoxylin-eosin staining, and the number of secondary follicles, Graafian follicles, and atretic follicles were counted. To find out the differences between groups, the data were analyzed by using the one-way Anova statistic test. **Results:** The results of this study revealed that the exposure to MSG at dose of 0.03mg /g of weight showed a significant difference in the number of atretic follicles ($p = 0.023$). Whilst, the exposure to MSG at dose of 0.7mg/g of weight showed a significant difference in the number of Graafian follicles and atretic follicles ($p = 0.000$). **Conclusion:** Mung bean sprout extracts affected the number of Graafian follicles and atretic follicles; however the number of secondary follicles showed an insignificant difference.

Key words: Mung bean sprout extracts, Monosodium glutamate (MSG), Follicles, Wistar rats.

I. INTRODUCTION

Infertility is one of the reproductive health problems which can cause not only medical problems, but also economic and psychological problems. It may also bring big effects to a husband-wife relationship eventually resulting in a divorce. One of the risk factors that affects infertility is lifestyle. The lifestyles developing in the society recently also affect changes in food consumption pattern, such as the use of flavor enhancer or MSG (Monosodium Glutamate) in foods. ^{1,2,3}

The preliminary data collected from the infertility polyclinic of RSUD Dr. Soetomo Surabaya in 2017 in 2 weeks reveal that most of the patients with infertility problems have a cooking habit of adding MSG to their foods. Besides, they also have a habit of buying street foods in which nearly all of the food sellers most definitely add MSG to enhance the taste of their foods for more consumers. The use of MSG in foods sold on streets causes uncontrollable MSG consumption.

Both national and international organizations have recommended that MSG is a safe flavor enhancer for daily consumption, maximally 6 mg/kg of adult weight (not more than 2 grams/day). Long-term consumption of MSG in large doses will bring negative effects to the health. ^{3,4} Over-consumption of MSG will increase cell metabolism which creates reactive oxygen species (ROS). The increasing ROS with the imbalanced level of anti-oxidants will trigger the increase of oxidative stress. ⁵ The oxidative stress will trigger cell damages in the nucleus *arcuatus hypothalami* that affect the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) by hypophysis gland. The decreased secretion of FSH will cause disturbances of follicular development.

Mung bean sprouts, food ingredients, which are mostly consumed by Indonesian people contain high vitamin E and C functioning as antioxidants as evidenced by their effectiveness to suppress oxidative stress. The consumption of mung bean sprouts will increase the level of antioxidants in the body, thus suppresses the negative

effects of free radicals resulted from MSG consumption.^{6,7,8} Based on those arguments, this study was purposed to analyze the effects of mung bean sprouts on the follicle maturation of Wistar rats exposed to MSG.

II. METHODS

This study was true experimental using post-test only control group design. It was conducted in March-April 2017 in the laboratory for animal experimentation of Faculty of Veterinary Medicine - Airlangga University. The experimental animals used in this study were Wistar rats (*Rattus norvegicus*) aged 2 months weighing 150-200 grams. The samples comprised of 5 Wistar rats distributed in each group, in which 7 groups were involved in this study, namely I Control Group (P1) was provided with Aquades for 37 days; II (P2) was provided with Aquades for 7 days + MSG 0.03 mg/g of weight on day 8-37; III (P3) was provided with mung bean sprout extracts 72mg/200g of weight on day 1-37 + MSG 0,03mg/g of weight; IV (P4) was provided with mung bean sprout extracts 144mg/200g of weight on day 1-37 + MSG 0.03mg/g of weight; V (P5) was provided with Aquades for 7 days + MSG 0.7 mg/g of weight on day 8-37; VI (P6) was provided with mung bean sprout extracts 72mg/200g of weight on day 1-37 + MSG 0.7mg/g of weight; VII (P7) was provided with mung bean sprout extracts 144mg/200g of weight on day 1-37 + MSG 0.7mg/g of weight.

Mung bean sprout extracts and MSG were given to the Wistar rats in this experiment for 37 days. After the treatment, the rats were euthanized during estrous cycle to collect both right and left ovaries, the microscope slide was prepared for histopathology by *hematoxylin-eosin* staining, and observation was done using a compound light microscope to count the number of secondary follicles, Graafian follicles, and atretic follicles. To identify the difference between the treatment groups, the data were analyzed by using the one-way Anova statistic test; and when the significant (real) difference occurred between the treatments, the data were continued to be analyzed by using the Duncan's post-hoc test with the significance level of 5% ($\alpha = 0.05$).

III. RESULTS AND DISCUSSION

In this study, the follicular development was observed by focusing on the number of secondary follicles, Graafian follicles, and atretic follicles.

Table 1. The average number of secondary follicles, Graafian follicles, and atretic follicles in the ovaries of Wistar rats \pm SD of all groups after the consumption of mung bean sprout extracts and exposure to MSG.

Group	Secondary follicles	Graafian follicles	Atretic follicles
P1 (negative control)	22.6 \pm 3.36	5.6 \pm 1.67	3.4 \pm 2.07
P2 (positive control A : MSG 0.03 mg/g of Weight)	18.6 \pm 9.07	3 \pm 1.58	3.4 \pm 2.19
P3 (Extract 72mg/200g of Weight + MSG 0.03mg/g of weight)	28 \pm 10.51	3.6 \pm 1.34	2.2 \pm 0.44
P4(Extract 144mg/200g of Weight + MSG 0,03mg/g of weight)	31.4 \pm 16.28	3.4 \pm 1.51	2 \pm 0.70
P5 (positive control B : MSG 0.7 mg/g of weight)	18.6 \pm 9.07	0.8 \pm 0.44	2.8 \pm 1.64
P6 (Extract 72mg/200g of Weight + MSG 0.7mg/g of weight)	24.8 \pm 11.88	1.8 \pm 1.48	1.6 \pm 1.51
P7(Extract 144mg/200g of Weight + MSG 0.7mg/g of weight)	26.8 \pm 10.40	3.8 \pm 1.09	0.2 \pm 0.44

Secondary follicles

The result of statistic test using the one-way Anova showed no significant difference in the number of secondary follicles with the value of $p = 0.440$ ($p > 0.05$).

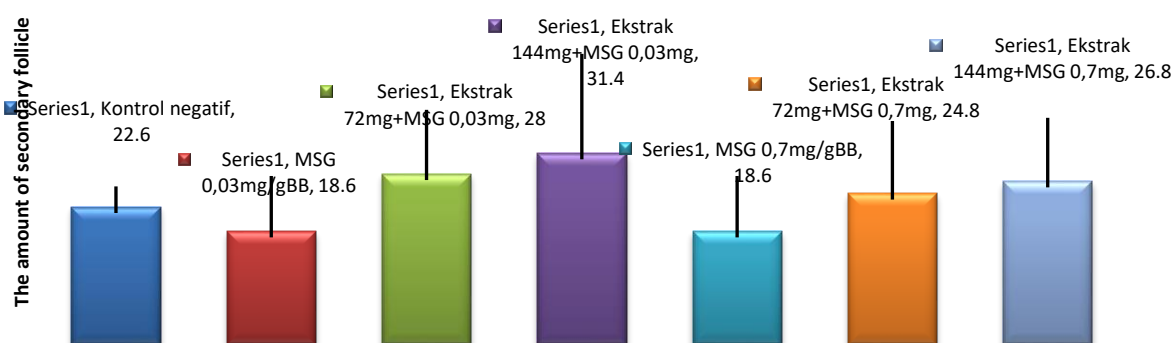


Figure 1. The diagram showed the average number of secondary follicles of the Wistar rats after consuming mung bean sprout extracts and exposed to MSG

Graafian follicles

The result of statistic test by using the one-way Anova showed a significant difference in the number of Graafian follicles with the value of $p = 0.000$ ($p < 0.05$). To find out the difference between groups, the data were analyzed by using The Duncan's post-hoc test.

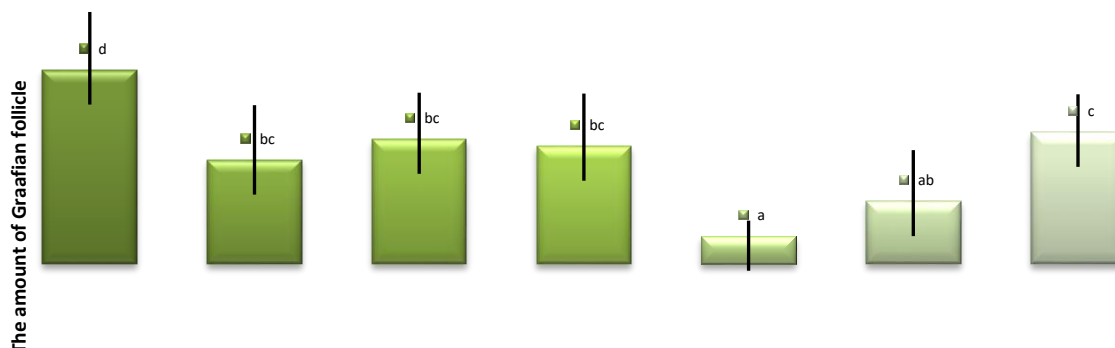


Figure 2. The diagram showed the average number of Graafian follicles and the result of the Duncan's post-hoc test done to Wistar rats was different with $\alpha = 0.05$ after consuming mung bean sprout extracts and exposed to MSG.

A. Atretic Follicles

The result of statistic test by using the one-way Anova showed a significant difference in the number of atretic follicles with the value of $p = 0.023$ ($p < 0.05$). To find out the difference between groups, the data were analyzed by using the Duncan's post-hoc test.

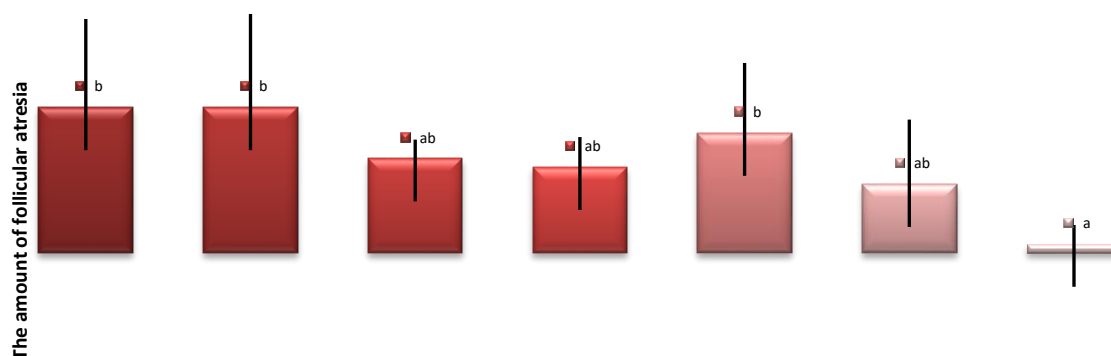


Figure 3. The diagram showed the average number of atretic follicles and the result of statistic test by using the Duncan's post-hoc test done to Wistar rats with $\alpha = 0.05$ was different after consuming mung bean sprout extracts and exposed to MSG.

In spite of the safe limit of MSG consumption recommended by FAO, the use of MSG still affected the follicular development, especially to the number of secondary follicles and Graafian follicles. It was evidenced by the result of this study showing that the groups consuming MSG both at dose of 0.03mg/g of weight and 0.7mg/g of weight had a lower number of secondary follicles and Graafian follicles compared to the negative control group. However, the significant difference between groups only occurred in the number of Graafian follicles.

The formation of ROS resulted from the exposure to MSG causes damages to the nucleus arcuatus in hypothalamus⁹, thus affects the *gonadotropin releasing hormone* (GnRH) to secrete FSH. FSH and estrogen increase the content of FSH receptor in the follicles and stimulate proliferation of granulosa cells.¹⁰ The disturbed mechanism of follicular development causes the formation of ROS which can support the incidence of apoptosis.¹¹

Mung bean sprout extracts preventively brings effects to the follicular development resulted from the exposure to MSG. Statistically, the number of Graafian follicles and atretic follicles showed a significant difference. The group receiving mung bean sprout extracts at dose of 72mg/200g of weight along with the exposure to MSG 0.03mg/g of weight had a higher number of secondary follicles and Graafian follicles and a lower number of

atretic follicles compared to the group which was only exposed to MSG without receiving mung bean sprout extracts.

The groups receiving mung bean sprout extracts at dose of 72mg/200g of weight and exposed to MSG 0.7mg/g of weight also had a higher number of secondary follicles and Graafian follicles and a lower number of atretic follicles compared to the groups who were exposed to MSG at dose 0.7mg/g of weight and received no mung bean sprout extracts.

The groups exposed to MSG at dose 0.03mg/g of weight with the consumption of mung bean sprout extracts at higher dose (144mg/200g of weight) showed no significant difference in the number of Graafian follicles and atretic follicles compared to the groups receiving mung bean sprout extracts at dose 72mg/g of weight; however, they had an increasing number of secondary follicles and Graafian follicles and a decreasing number of atretic follicles. Whilst, the groups who were exposed to MSG at dose of 0.7mg/g of weight and consuming mung bean sprout extracts at dose of II (144mg/200g of weight) showed a significant difference in the number of Graafian follicles and atretic follicles compared to the groups receiving mung bean sprout extracts at dose of 72mg/g of weight in which they experienced an increasing number of secondary follicles and Graafian follicles and a decreasing number of atretic follicles.

Mung bean sprout extracts work as antioxidants which suppress oxidative stress resulted from the exposure to MSG. The antioxidant effects of mung bean sprouts suppress the oxidation of lipid and protein as well as decrease the deoxyribose damages by binding the iron and the activities of free radicals.¹² Mung bean sprouts contain some nutrients which play their roles as antioxidants, such as vitamin E and vitamin C.

Vitamin E (α -tocopherol) is a fat soluble vitamin which has antioxidant activities. It functions as an antioxidant to cut the chain reaction of oxidative stress. α -tocopherol donates hydrogen ions to fatty acid which experiences peroxidation.¹³ α -tocopherol and tocotrienols have big roles in antioxidant activities because they react with lipid radicals produced during lipid peroxidation. This reaction produces α -tocoperoxide oxidized radicals which can transform back to the actively reduced form which reacts with other antioxidants, such as ascorbate, retinol or ubiquinol.¹¹

Vitamin C (acid ascorbate) is well-known as redox catalysts which can decrease and neutralize ROS. Its decreasing form is maintained while reacting with GSH and can be catalyzed by protein disulfide-isomerase and glutaredoxin (Agarwal, 2012). It also has an effect as antiapoptosis in oocyte granulosa cells. Supplementation of Vitamin C affects the oocyte to fight oxidative damages of hydrogen peroxide and slow down the DNA damages resulting from the increasing ROS.¹⁴

IV. CONCLUSION

Consuming MSG at dose of both 0.03 mg/g of weight and 0.7mg/g of weight affects the follicular development, mainly the number of Graafian follicles. On the other hand, providing mung bean sprout extracts at dose of both 72mg/200g of weight and 144mg/200g of weight affects the follicular development with the exposure to MSG at dose of 0.7mg/g of weight, mainly the number of Graafian follicles and atretic follicles.

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