

## Original Article

# Effect of black garlic (*Allium sativum*) on gonadosomatic index, follicle-stimulating hormone level and spermatozoa quality: A study in monosodium glutamate-exposed rat model

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## Abstract

Infertility rates have risen significantly, one of which is due to monosodium glutamate (MSG) consumption. Recent studies have shown that flavonoids in black garlic (*Allium sativum*) act as antioxidants. The aim of this study was to assess the effect of black garlic extract (BGE) on gonadosomatic index, follicle-stimulating hormone (FSH) levels, and spermatozoa quality in rats exposed to MSG. Twenty-five healthy rats, aged ten to twelve weeks, were divided equally into five experimental groups: (1) negative control (NC), no intervention; (2) positive control (PC), fed with MSG 8 mg/kg; and (3) fed with MSG + BGE 200 mg/kg; (4) fed with MSG + BGE 400 mg/kg; and (5) fed with MSG + BGE 600 mg/kg. Oral MSG was administered once a day for two weeks before BGE administration was started for two weeks. The measured endpoints were gonadosomatic index, FSH levels, and spermatozoa concentration and quality (spermatozoa motility and abnormality). Analysis of variance (ANOVA) followed by Duncan's post hoc analysis was used to assess the measurement differences. The result suggested that the administration of BGE did not significantly affect the gonadosomatic index ( $p=0.513$ ). Significant decreases in FSH levels ( $p=0.005$ ) and spermatozoa concentration were observed in the PC group compared to other groups ( $p<0.001$ ). Additionally, spermatozoa motility was significantly lower in the PC group compared to NC, BGE200, BGE400, and BGE600 ( $p<0.001$ ), with higher motility noted in BGE200, BGE400, and BGE600 compared to PC ( $p<0.001$ ). Furthermore, PC had significantly higher spermatozoa abnormalities compared to NC, BGE200, BGE400, and BGE600 ( $p<0.001$ ). In conclusion, administration of BGE had a significant effect on the improvement of FSH levels and the quality of spermatozoa in rats exposed to MSG.

**Keywords:** Black garlic, MSG, gonadosomatic index, FSH, spermatozoa quality

## Introduction

Infertility rates increased annually by 12–15% globally. In Indonesia, 10–15% of 39.8 million childbearing-age couples faced infertility issues [1,2]. Oxidative stress contributes to infertility, with approximately 30–80% originating from the environment and dietary lifestyles involving



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monosodium glutamate (MSG) consumption [3,4]. Developed countries consume MSG at 0.3–1.0 g/day, while developing countries, such as Indonesia, have an intake of 0.6 g/day [5,6].

Glutamate in MSG has a direct free radical effect at a cellular level, causing damage to testicular organs and spermatozoa cells [7]. MSG-induced oxidative stress leads to deoxyribonucleic acid (DNA) damage, cell membrane peroxidation, and abnormal spermatozoa morphology, eventually leading to cell death [8,9]. In addition, excessive MSG-induced lipid peroxidation damages mitochondrial DNA, reducing spermatozoa motility and causing infertility by hindering penetration of sperm cells into ovum cells [10,11].

Free radicals from MSG can also affect pituitary cells, disrupting reproductive and endocrine systems [12]. Pituitary cell damage inhibits the production of gonadotropin-releasing hormone (GnRH), leading to decreased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels [12]. Reduced FSH levels hinder spermatogenesis, leading to decreased spermatozoa numbers [13]. Furthermore, diminished LH and FSH impact Leydig and Sertoli cell activity, causing atrophy in testicles and a decline in testicular weight and gonadosomatic index over time [12,14].

Recent studies have shown that antioxidants effectively inhibit oxidative stress, promoting and sustaining male fertility [15-17]. Extensive studies have been conducted on herbal plants as a source of antioxidants, including the utilization of black garlic (*Allium sativum*) [15-18]. Black garlic, produced by heating garlic at 70°C for 15, 25, and 35 days, has higher antioxidant content than raw garlic due to the presence of phenolic compounds of flavonoids and S-Allylcysteine [16]. The aim of this study was to assess the effect of black garlic extract (BGE) on gonadosomatic index, FSH level, and spermatozoa quality in rats exposed to MSG.

## Methods

### Study design

An *in vivo* experimental study was conducted from August 2022 to September 2023 at the Laboratory of Experimental Animal and Physiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Federer equation was employed for sample size calculation, resulting in 25 rats for five experimental groups—divided equally to each group. Randomization was carried out using a completely randomized design method with a unidirectional pattern. Each experimental unit was homogeneous and each unit had the same opportunity for each intervention.

### Eligibility criteria

Wistar rats (*Rattus norvegicus*) were obtained from the Laboratory of Experimental Animal and Physiology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia. Healthy rats, aged ten to twelve weeks and weighing 150–200 grams, were included. Exclusion criteria were rats with ongoing infection and the presence of structural or functional abnormalities. Drop-out criteria included the presence of any complication, infection, and death during the study period.

### Animal preparations

Rats were caged for seven days in a consistent environment, well-shaded, and tranquil rooms. The animals were provided with the same quantity and type of food, with a tray beneath the cages to collect urine and feces, cleaned once daily. Each rat was accommodated in ventilated cages maintained at a temperature of 22°C and humidity ranging from 50–60%. A regular diurnal lighting cycle (12:12 light-dark) was maintained, with lights turning on at 5:30 AM. Rats were fed commercial ratio brand 552 at 10% of the rat's average body weight, approximately 15–20 grams per day. Rats were given uncontaminated clean water *ad libitum*.

### Study groups and interventions

Rats were randomly grouped into five groups: (1) negative control (NC), no intervention; (2) positive control (PC), provided with MSG 8 mg/kg only; (3) BGE200, provided with MSG 8 mg/kg and BGE 200 mg/kg; (4) BGE400, MSG 8 mg/kg and BGE 400 mg/kg; and (5) BGE600, MSG 8 mg/kg and BGE 600 mg/kg. MSG was administered for two weeks, of which it was

dissolved in 10 mL of 0.5% carboxymethyl cellulose (CMC) and administered once a day orally. BGE200, BGE400, and BGE600 groups received BGE with specific doses once a day orally for two weeks. All animals were then cervical decapitated.

### Outcome endpoints

The endpoints of the study were the gonadosomatic index, FSH level, and spermatozoa quantity and quality (spermatozoa motility and abnormality). The gonadosomatic index was calculated by weighing both testicular organs, dividing by the rat's body weight, and multiplying by 100%. Serum FSH level was measured using enzyme-linked immunosorbent assay (ELISA) using Rat Follicle-Stimulating Hormone Competitive Elisa Kit (BZ-08185100-CPEB, Biozatic Indonesia, Indonesia). The blood was collected using a cardiac puncture. The absorbance was read on a spectrophotometer at a wavelength of 450 nm [19].

Spermatozoa were obtained from epididymal secretions in 0.9% NaCl to assess the spermatozoa quality. Spermatozoa concentration and motility were assessed using wet preparations in a Neubauer counting chamber (400× magnification) and recorded with an OptiLab camera on a microscope. The concentration value of spermatozoa was expressed in mm<sup>3</sup>. Evaluation of spermatozoa motility was carried out by observing sperm movement directly under a microscope and grouped into moving groups (both fast and slow moving) and groups that did not move at all. Spermatozoa morphology (spermatozoa abnormality) was analyzed from photographs of smears stained with eosin 2%. The percentages of normal and abnormal spermatozoa were counted from every 200 spermatozoa observed. Spermatozoa cells with a head without an acrosome, a neck that bends and/or folds, and a tail that bends and/or twists were considered abnormal as recommended [20,21].

### Statistical analysis

Shapiro-Wilk test was employed for assessing the normality of data distribution. To assess differences in gonadosomatic index, FSH level, spermatozoa concentration and quality (motility and abnormality), the analysis of variance (ANOVA) test followed by Duncan's post hoc test was used. Statistical analysis was conducted using SPSS version 25.0 software (IBM SPSS, Chicago, IL, USA), with  $p < 0.05$  was considered statistically significant.

## Results

### Effect of black garlic on gonadosomatic index

Our data suggested that the administration of BGE did not significantly affect the gonadosomatic index of rats ( $p=0.513$ ) (Table 1). The MSG-treated group (positive control) had the smallest gonadosomatic index, while the negative control group had the highest. The negative control had 15.2%, 2.2%, 8.4%, and 10.1% higher gonadosomatic indexes compared to the positive control, BGE200, BGE400, and BGE600 groups, respectively.

Table 1. Effects of black garlic extract on gonadosomatic index of rats (n=25)

Group	Gonadosomatic index (%) ( $\pm$ SD)	<i>p</i> -value
Negative control	1.78 $\pm$ 0.24	0.513
Positive control	1.51 $\pm$ 0.19	
BGE200	1.74 $\pm$ 0.33	
BGE400	1.63 $\pm$ 0.30	
BGE600	1.60 $\pm$ 0.21	

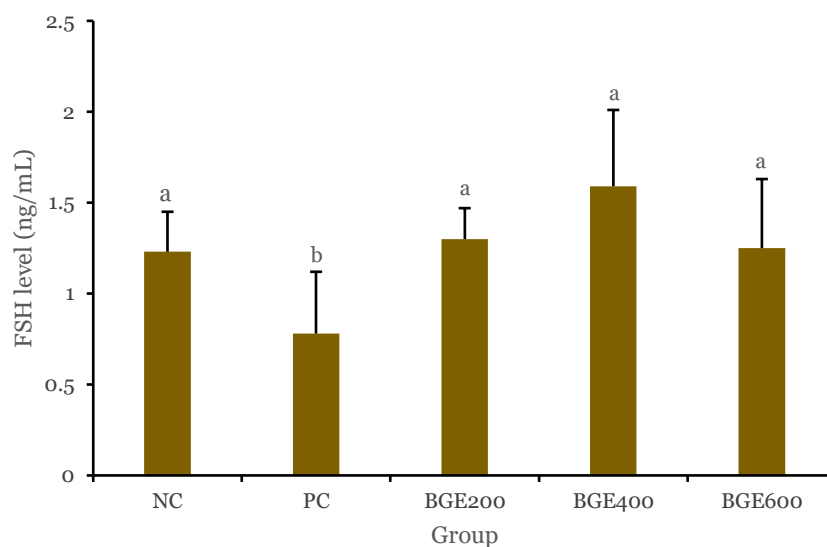
### Effect of black garlic on FSH level

Our data indicated that the MSG-treated only group (positive control) had lower FSH levels compared to the non-treated group (Table 2). Administration of BGE could increase FSH levels in rats exposed to MSG (Table 2). There was a significant difference in FSH levels among groups ( $p=0.005$ ). The FSH level in the negative control group was 36.6% lower compared to the positive control group, while the FSH concentration in groups BGE200, BGE400, and BGE600 was higher by 5.7%, 29.3%, and 1.63% compared to the positive control group, respectively. Post-hoc

analysis indicated that the positive control group had lower FSH levels compared to other groups significantly (**Figure 1**).

**Table 2.** Effect of black garlic on follicle-stimulating hormone (FSH) level of rats (n=25)

Group	FSH level (ng/mL) ( $\pm$ SD)	<i>p</i> -value
Negative control	1.23 $\pm$ 0.22	0.005
Positive control	0.78 $\pm$ 0.34	
BGE200	1.30 $\pm$ 0.17	
BGE400	1.59 $\pm$ 0.42	
BGE600	1.25 $\pm$ 0.38	



**Figure 1.** Post-hoc analysis showing the comparison of follicle-stimulating hormone (FSH) levels between groups of animals. Different superscripts indicate significant differences ( $p < 0.05$ ). BGE: black garlic extract; NC: negative control; PC: positive control.

### Effect of black garlic on spermatozoa quantity and quality

Our data suggested that administration of BGE could increase spermatozoa concentration in rats exposed to MSG (**Table 3**). The positive control group had the lowest spermatozoa concentration, while the BGE600 group had the highest concentration. There was a significant difference in rat spermatozoa concentration among groups ( $p < 0.001$ ) (**Table 3**). Post-hoc analysis indicated that spermatozoa concentration in the positive control group was significantly 72.27% lower compared to negative control ( $p < 0.001$ ). The level of spermatozoa in groups BGE200, BGE400, and BGE600 was lower at 40.23%, 13.51%, and 2.73% compared to the negative control, BGE200, and BGE400 groups that were significantly lower than the negative control, respectively (**Figure 2A**).

Our data also suggested that MSG reduced spermatozoa motility, while BGE could increase spermatozoa motility in rats exposed to MSG (**Table 3**). There was a significant difference in rat spermatozoa motility among groups ( $p < 0.001$ ). The motility of spermatozoa in the positive control, BGE400, and BGE600 groups was 34.28%, 15.71%, and 32.85% lower, respectively, compared to the negative control group, while the motility of spermatozoa in BGE200 group was 7.14% higher than the negative control group. Post-hoc analysis indicated that the motility of rat sperm between PC and BGE200 groups had significant differences ( $p < 0.001$ ). The motility of spermatozoa between PC, BGE400, and BGE600 showed no significant difference (**Figure 2B**).

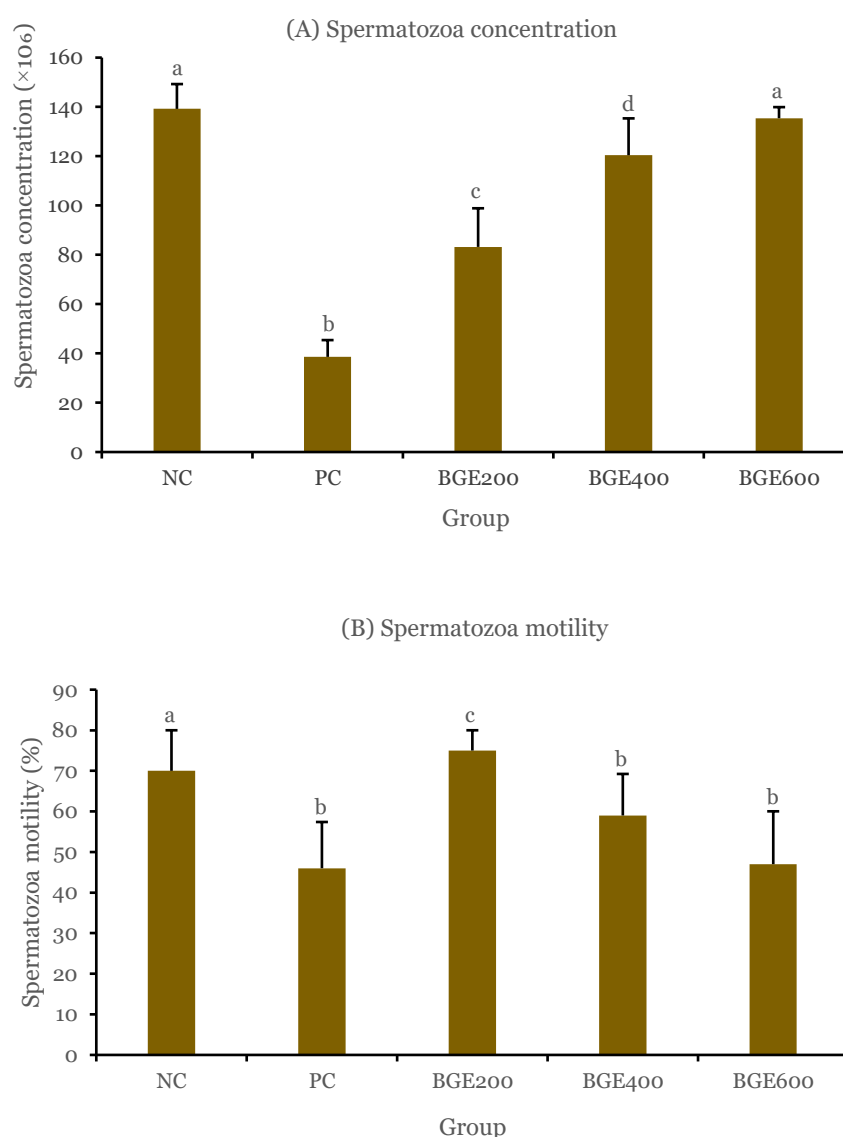
In addition, our data suggested that MSG increased spermatozoa abnormalities, while BGE reduced spermatozoa abnormalities in rats exposed to MSG (**Table 3**). The BGE200 group had the lowest spermatozoa abnormalities, while the positive control group had the highest (**Table 3**). The morphology of spermatozoa abnormalities in the positive control, BGE400, and BGE600 groups were respectively 120.62%, 23.86%, and 81.57% higher compared to the negative control group, while the morphology of spermatozoa abnormalities in the BGE200 group was 10.07%

higher than the negative control group. The results of statistical analysis showed that the morphology of mouse spermatozoa abnormalities between treatments showed very significant differences ( $p < 0.001$ ) (**Table 3**). Post-hoc analyses indicated that the positive control group had significantly higher spermatozoa abnormalities ( $p < 0.001$ ) compared to the negative control, BGE200, BGE400, and BGE600 (**Figure 2C**).

**Table 3.** Effect of black garlic on spermatozoa quantity and quality of rats (n=25)

Parameters	Negative control	Positive control	BGE200	BGE400	BGE600	p-value
Spermatozoa concentration ( $10^6/\text{mm}^3$ )	139.20 $\pm 10.06$	38.60 $\pm 6.80$	83.20 $\pm 15.67$	120.40 $\pm 14.94$	135.40 $\pm 4.51$	$< 0.001$
Spermatozoa motility (%)	70.00 $\pm 10.00$	46.00 $\pm 11.40$	75.00 $\pm 5.00$	59.00 $\pm 10.24$	47.00 $\pm 13.03$	$< 0.001$
Spermatozoa abnormality (%)	31.76 $\pm 21.07$	70.07 $\pm 3.86$	34.96 $\pm 1.44$	39.34 $\pm 14.03$	57.70 $\pm 5.56$	$< 0.001$

BGE: black garlic extract



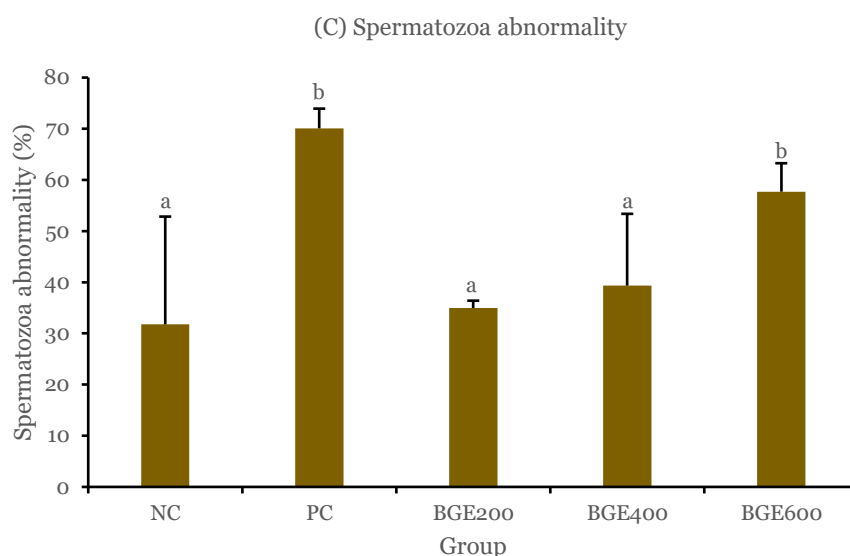


Figure 2. Post-hoc analysis showing the comparison of spermatozoa concentration (A), spermatozoa motility (B), and spermatozoa morphology (C) between groups of animals. Groups with different superscripts indicate significant differences ( $p < 0.05$ ). BGE: black garlic extract; NC: negative control; PC: positive control.

## Discussion

The present study found that the administration of black garlic extract did not significantly affect the gonadosomatic index of rats ( $p > 0.05$ ). This lack of significance in the gonadosomatic index may be attributed to the relatively brief duration of MSG administration, in which the increase in testicular weight in this study was accompanied by an increase in body weight in other groups, resulting in nonsignificant differences in the gonadosomatic index. The gonadosomatic index is influenced by testicular weight and body weight of rat, with testicular weight dependent on spermatogenic cell differentiation [14].

Black garlic has main bioactive components of phenolic organic compounds, especially flavonoids, contributing as antioxidants to minimize damage to Sertoli cells in testicles [14]. However, studies on black garlic and its impact on testicular weight and gonadosomatic index are limited. A study found that oral administration of 5% raw and 15% cooked garlic for 30 days resulted in an increase in Sertoli cell count and testicular weight [18]. In addition, flavonoids in neem seed extract increased testicular weight and gonadosomatic index [22]. In contrast, another study reported a decrease in testicular weight and gonadosomatic index in rats orally administered 0.3 and 0.6 mg/gr BW of MSG for 35 days [23]. Leydig and Sertoli cells play a crucial role in spermatogenesis, and a decrease in testicular weight due to MSG toxicity in previous study may result from a reduction in germ cell and mature spermatid density, along with Leydig and Sertoli cell damage.

In the present study, administration of black garlic extract can increase FSH concentrations in rats exposed to MSG. The decrease in FSH levels in MSG-treated control in the present study is attributed to direct damage to Sertoli cells by MSG, leading to oxidative stress and membrane damage, thereby interfering with FSH receptors (FSHR) and inhibiting FSH input into Sertoli cells [24]. This finding aligned with a previous study that showed decreased FSH concentrations in rats administered 30 and 60 mg/kg BW of MSG for eight weeks compared to the control group [9]. A previous study also observed decreased testosterone concentrations in rats administered 8 mg/kg BW/day of MSG for 14 days compared to the control group [25].

Flavonoids in black garlic reduce oxidative stress by inhibiting lipid peroxidation and preventing the conversion of androgens into estrogen, ultimately increasing testosterone levels [14,26]. In the present study, an increase in FSH levels was observed in the BGE200 and BGE400 groups, indicating that flavonoid antioxidants can stimulate FSHR. This finding was aligned with a previous study that reported consuming flavonoid in Icarin dietary supplement at 100 and 200 mg/kg BW for 35 days increases testosterone and FSHR levels, and protects the Sertoli cells and

blood-testis barrier [27]. A study found garlic administration at 6 mL/kg BW/day for 65 days could increase androgen receptors [28]. This is consistent with another study that found elevated androgen hormones after administration of garlic powder at 200 mg/kg BW for 42 days [29]. In contrast, a study reported a decrease in androgen levels after administration of garlic extract at 400 mg/kg BW for one month [30]. This finding may be attributed to variation in dosage or administration duration, yet research on the effect of black garlic in androgen levels, especially FSH levels, remains limited.

In the present study, administration of black garlic extract can increase spermatozoa concentration in rats exposed to MSG. Flavonoids in black garlic stabilize radicals, combat superoxide, and inhibit lipid peroxidase, thus enhancing spermatozoa quality [14,26,34]. A previous study demonstrated that the administration of garlic powder 200 mg/kg BW for 42 days could increase spermatozoa concentration and motility, and decrease morphological abnormalities [35]. Consistent findings were observed in another study, which emphasized the positive impact of garlic on spermatozoa concentration, motility, and viability [18,36].

On the other hand, the MSG-treated only group in the present study has the lowest spermatozoa concentration, which might be due to the oxidative stress of MSG. This finding was aligned with a previous study that showed decreased motility yet normal morphology in rats exposed to 4 mg/gr BW of MSG for 35 days [33]. Small cytoplasm content in sperm cells leads to insufficient antioxidant defenses during oxidative stress. Excessive lipid peroxidase formation damages mitochondrial DNA, contributing to decreased spermatozoa motility. This process causes a loss of 60% of polyunsaturated fatty acids, disrupting sperm membrane permeability and depleting sperm cell ATP, affecting flagellum movement. Oxidative DNA damage and peroxidation also increase abnormal morphology and death in sperm cells, ultimately reducing spermatozoa concentration [31,32].

MSG can cause damage to the hypothalamus, leading to pituitary cell damage, inhibition of GnRH production, and a decrease in the production of FSH levels [12,13]. The resulting decrease in FSH levels can affect spermatogenesis and lead to a reduction in spermatozoa concentration [12,13]. Furthermore, a decrease in FSH levels leads to a decline in Leydig and Sertoli cell activity, causing testicular atrophy, decreased testicular weight, and a decline in gonadosomatic index percentage [12,13]. Hence, long, continuous exposure to MSG can be attributed to testicular atrophy and a decrease in gonadosomatic index percentage [12,13].

## Conclusion

Administration of BGE had a significant effect on the improvement of FSH levels and quality of spermatozoa in rats exposed to MSG. However, BGE treatment did not yield a significant effect on the gonadosomatic index.

## Ethics approval

This study protocol was approved by the Committee of Research Ethics, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia (Approval number: 175/KEPH/X/2022).

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## Competing interests

All the authors declare that there are no conflicts of interest.

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This study received no external funding.

## Underlying data

All data underlying the results is available as part of the article and no additional source data is required.

## How to cite

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