# ANTIOXIDANT POTENTIAL OF FERMENTED GARLIC (Allium sativum L.) ON SPERMATOGENIC AND SERTOLI CELLS COUNT OF MICE (Mus musculus) EXPOSED TO HEAT

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# ANTIOXIDANT POTENTIAL OF FERMENTED GARLIC (*Allium sativum* L.) ON SPERMATOGENIC AND SERTOLI CELLS COUNT OF MICE (*Mus musculus*) EXPOSED TO HEAT

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## ABSTRACT

This research aimed was to know whether fermented garlic (*Allium sativum* L.) extract could protect spermatogenic and Sertoli cells of mice *(Mus musculus)* exposed to heat. Mice were exposed to a temperature of 40°C, 45 minutes daily, for 54 days. This study used 25 mice, which were divided equally into five groups. C(-), the negative control group was not exposed to heat and only given distilled water. C(+), the positive control group was exposed to heat and given distilled water. T1, T2, T3 were given fermented garlic extract with doses of 125, 250, and 500 mg/kg BW, respectively 5 minutes after heat exposure. All of the treatments were given orally. The observations showed significant different ($p<0.05$) in all variables. Results showed a significant difference in spermatogenic and Sertoli cell between C(-) and C(+). C(-) and T2 showed unsignificant differences in spermatogenic and Sertoli cells. It could be concluded that giving fermented garlic extract *(Allium sativum* L.*)* could protect spermatogenic and Sertoli cells of mice *(Mus musculus)* exposed to heat.

***Keywords :*** *Allium sativum* L., heat exposure, spermatogenic cells, Sertoli cells, *Mus musculus*

## Introduction

Disease or disability conditions as a function of the reproductive system can lead to infertility (Zegers-Horchschild *et al*., 2017). Infertility can be caused by damage to spermatogenesis triggered by chemical agents (metal substances, pesticides, industrial chemicals) or physical agents such as ionizing radiation and heat (Sheiner *et al*., 2003).

Spermatogenesis is the process of spermatozoa cell formation that occurs in the testes. Exposure to heat in the testes affects the spermatogenesis process because mammalian testes must be at a temperature of 2-8 °C lower than body temperature so that the spermatogenesis process is optimal (Chen *et al*., 2008).

Hotter testicular conditions trigger oxidative stress, its a disproportionate increase in the production of Reactive Oxygen Species (ROS) accompanied by a decrease in the production of antioxidants in the body (Agarwal *et al*., 2012). Uncontrolled increase of ROS accompanied by Reactive Nitrogen Species (RNS) causes chain reactions mediated by free radicals and damages proteins, lipids, polysaccharides, and DNA (Slimen *et al*., 2014). This condition triggers apoptosis through the intrinsic pathway or necrosis due to lipid peroxidation in spermatogenic and Sertoli cells. Damage due to apoptosis and lipid peroxidation can cause infertility due to decreased spermatogenesis activity, which is mostly supported by these cell.

Exposure to heat affects Sertoli cells (Chen *et al*., 2008). The Sertoli cells have a role as nurse cells, are responsible for caring for and regulating the development of spermatozoa in the testes (Depamede, 2010). Oxidative stress in these two cell types caused by exposure to heat can be prevented and treated with antioxidants.

Antioxidants are substances that play a role in delaying, preventing, or eliminating damage due to oxidative processes on molecular targets (Halliwell, 2007). Antioxidants act to stabilize free radicals by donating electrons as reducing agents so that they can inhibit chain reactions (Windono *et al*., 2001; Vaya *et al*., 2001). The presence of antioxidants is needed by the body both from natural and synthetic sources.

Antioxidants from herbal ingredients are widely used because it is recognized to have cytotoxic effects and lower residues than synthetic antioxidants (Rong-zhen *et al*., 2013). Several compounds that can be used as a source of antioxidants include phenolics, betalain (Nurliyana *et al*., 2010) vitamin A, vitamin C, vitamin E, beta-carotene, lutein, lycopene, and polyphenols (Sayuti *et al*., 2015) which are commonly found. One of the herbal ingredients is fermented garlic. Fermented garlic showed more potent antioxidants compared to fresh garlic (Kimura *et al*., 2016; Jeong *et al*., 2016). Based on the background description, the researchers were interested in researching the antioxidant potential of fermented garlic (*Allium sativum* L.) on spermatogenic and Sertoli cells count of mice *(Mus musculus)* exposed to heat.

## Materials and Method

This research used male mice *(Mus musculus)* aged 2-3 months with an average body weight of 30 grams totaling 25 individuals were bought from Pusat Veterinaria Farma (PUSVETMA), Surabaya.

Fermented garlic processed into fermented garlic extract by cold maceration using distilled water. The extract was diluted with distilled water according to each dose. The fermented garlic extract was given using oral gavage once a day for 54 consecutive days for treatment group (T1, T2, and T3) after being given heat exposure treatment.

Heat exposure was given for 54 days using an incubator with a temperature of 40°C for 45 minutes. The samples except from negative control group were exposed to heat using artificial incubators with 4 pieces of 100-watt incandescent lamps.

After 54 days of treatment, all mice were euthanized using ether and then dissected to extract the testes from the mice. The testes were taken and put in a tissue

storage pot containing 10% formalin buffer before the histopathological preparations was made.

**Result and Discussion**

The results of spermatogenic and Sertoli cells count originating from both testes can be seen on Table 1 and Figure 1. Research data was processed used the One-Way ANOVA test and continued with the Duncan test.

Table 1 Spermatogenic (spermatogonia, spermatocytes, spermatids) and Sertoli cells count (Mean ± Standard Deviation) of mice exposed to heat at 40°C for 45 minutes then given fermented garlic extract for 54 days.

| | Spermatogonia | Spermatocytes | Spermatids | Sertoli |
|---|---|---|---|---|
| Contol Negative | $36.54^{c} \pm 1.69$ | $45.72^{b} \pm 1.24$ | $78.00^{c} \pm 9.70$ | $5.34^{b} \pm 0.56$ |
| Control Positive | $24.20^{a} \pm 0.95$ | $32.04^{a} \pm 3.81$ | $37.74^{a} \pm 3.00$ | $3.08^{a} \pm 0.35$ |
| Garlic extract 125 mg | $32.03^{bc} \pm 4.08$ | $41.13^{b} \pm 5.23$ | $66.27^{b} \pm 2.31$ | $4.85^{b} \pm 0.67$ |
| Garlic extract 250 mg | $33.36^{bc} \pm 7.39$ | $44.92^{b} \pm 5.16$ | $73.92^{bc} \pm 9.62$ | $5.12^{b} \pm 0.68$ |
| Garlic extract 500 mg | $29.24^{ab} \pm 1.02$ | $35.28^{a} \pm 1.96$ | $43.80^{a} \pm 2.52$ | $3.60^{a} \pm 0.40$ |

Different superscripts in one column showed significant differences ($p < 0.05$),

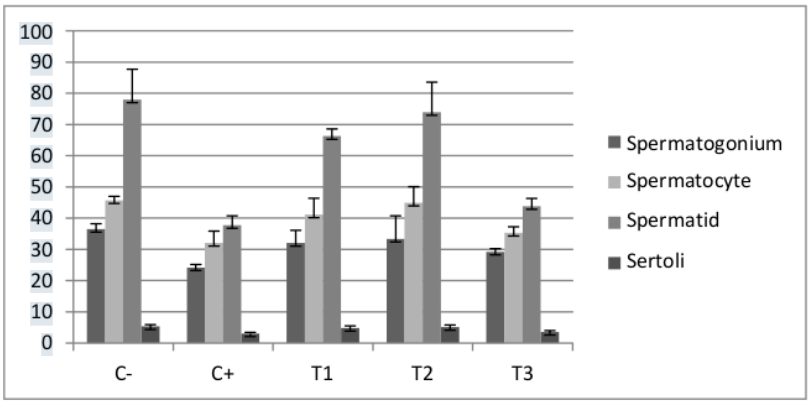


Figure 1 Spermatogenic and Sertoli cells count (mean ± SD) of mice (*Mus musculus*) given fermented garlic extract (*Allium sativum* L.) after given heat exposure treatmen

The highest number of spermatogenic and Sertoli cells was in the negative control group (C(-)). This indicates that giving distilled water for 54 days did not affect the increase or decrease in the number of spermatogenic and Sertoli cells. Observation of the histopathological picture of spermatogenic and Sertoli cells using 400x magnification with Hematoxylin Eosin (HE) staining is presented in Figure 2.

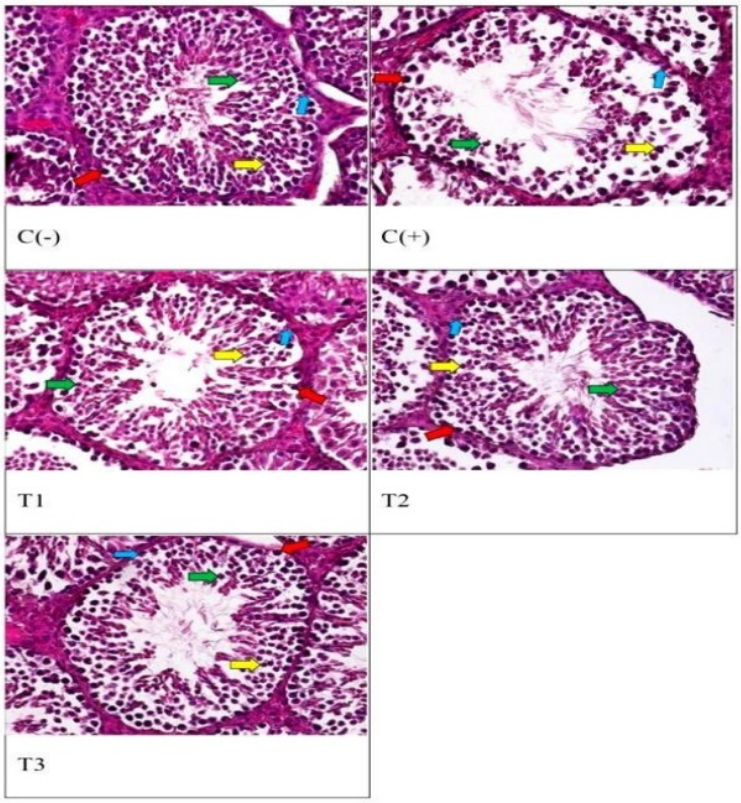


Figure 2 Histopathological preparation of seminiferous tubules of mice (*Mus musculus*) exposed to heat and given fermented garlic extract (*Allium sativum* L.). Red arrows = spermatogonia, yellow = spermatocytes, green = spermatids, and blue = Sertoli cells. C(+): Heat exposure 40°C, Heat exposure 40°C for 45 minutes and garlic extract 125 mg/kg BW (T1), 250 mg/kg BW (T2), 500 mg/kg BW (T3).

Exposure to heat 40°C with a duration of 45 minutes for 54 days without giving fermented garlic extract *(Allium sativum* L.) given to the positive control group (C(+)) showed the lowest number of spermatogenic cells and Sertoli cells among all groups.

Giving exposure to heat 40ºC with a duration of 45 minutes for 54 days causes oxidative stress caused by an increase in free radicals in the body. Increase in free radicals in the form of Reactive Oxygen Species (ROS) in the testes. The increase in ROS is triggered by dysfunction in mitochondrial activity as a cell organelle in charge of metabolizing oxygen to produce energy for the body (Slimen *et al*., 2014). The testes are organs that have a small amount of the Superoxide Dismutase enzyme so that excessive oxidative stress can easily damage cells in the tissue (Ishii *et al*., 2005). In male animals, heat stress has the most influence on the meiosis phase (Sun *et al*., 2019). The effect can be in the form of DNA damage in spermatogenic cells, especially spermatocytes or damage due to apoptosis (Perez-Crespo *et al*., 2008; Paul *et al*., 2008).

Apoptosis is the programmed death of a cell under certain conditions. Reactive Oxygen Species are one of the causes of apoptosis through the intrinsic pathway. The result of this event is the active pro-apoptotic protein, which makes the outer membrane of the mitochondria permeable and triggers the release of the second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pI (Smac/DIABLO) and cytochrome C to the cytosol. Smac/DIABLO then binds directly to cytosolic IAP (inhibitor of apoptosis protein) and removes it so that the caspase enzyme can be active and break down the substrate (Kulbacka *et al*., 2012).

Increased levels of ROS in the testes also can lead to lipid peroxidation. Lipid peroxidation occurs as a result of the meeting of free radicals with Polyunsaturated Fatty Acids (PUFA), which are mostly contained in spermatogenic cell membranes.This lipid peroxidation causes changes in membrane fluidity and permeability, disrupts ion transport, and inhibits metabolic processes. The disruption of the membrane causes the antioxidant system to be ineffective (Desai *et al*., 2014).

The presence of membrane permeability disturbances in cells caused by lipid peroxidation can lead to cell injury. In conditions of injury, cells have a limited response and depend heavily on the type of cell and the nature of the injury. This cell response can be in the form of adaptation, degeneration or cell death. Cells can adapt to sublethal (reversible) injury by increasing efficiency or productivity, or undergo degeneration resulting in reduced functional capacity. The presence of irreversible lesions will lead to cell death (Miller *et al*., 2017).

The morphological changes of cell injury due to reversible and irreversible injuries is certainly different. In reversible lesions, degeneration occurs, namely a state of decreased intracellular biochemistry accompanied by morphological changes due to reversible lesions. Morpholological changes can be in the form of accumulation of fluids or other substances in cell organelles. When the injury is severe enough, the cell will reach the point where the cell reaches the "point of no return" and its called necrosis. Necrosis is presence of cell death in living tissue and metabolism is unable to continue (Miller *et al*., 2017). The presence of damage in the form of irreversible lesions (necrosis) can be the cause of the inability of cells to return to near-normal numbers after being given heat exposure and then given the extract.

The results of the research that have been done showed that giving fermented garlic extract (*Allium sativum* L.) can maintain the number of spermatogenic and Sertoli cells

in mice, after exposed to heat at 40ºC with a duration of 45 minutes for 54 days with an optimum dose of 250 mg/kg BW. The group that was given a fermented garlic dose of 250 mg/kg BW received a spermatogonia count of 36.54, 45.72 of spermatocytes, 78.00 spermatids, and 5.34 Sertoli cells, these results were the highest compared to other groups.

Fermented garlic contains antioxidant compounds, including polyphenols, alkaloids, flavonoids, *S-allyl-cysteine*, and antioxidant products derived from the Maillard reaction (Kimura *et al.,* 2016; Choi *et al.,* 2014). Flavonoids are phenolic compounds and are one of the secondary metabolites in plants that function as a source of antioxidants (Zuraida *et al.,* 2017). Flavonoids are water-soluble, polar, and less reactive antioxidants, and it is reported that during the process of making Fermented garlic, the levels of these antioxidants have increased (Choi *et al.,* 2014; Hwang *et al.,* 2011).

Flavonoids can play a role in inhibiting lipid autoxidation. Flavonoids can transfer electrons to free radicals, act as metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit the oxidation process (Heim *et al.,* 2002). Flavonoids can also act as intracellular antioxidants through inhibition of these free radical-producing enzymes as *xanthine oxidase*, *lipoxygenase*, *protein kinase C, cyclooxygenase, microsomal monooxygenase*, *mitochondrial succinoxidase*, and NADPH *oxidase* (Procházková *et al.,* 2011).

Polyphenols have a role in fighting Reactive Oxygen Species (ROS), including suppressing the formation of ROS by inhibiting enzymes involved in ROS production or increasing regulation or protection of antioxidant defenses (Hussain *et al.,* 2016).

Fermented garlic extract must be given in the right and optimal dosage to get the best results. Mice given Fermented garlic extract at a dose of 125 mg/kg BW experienced an increase in the number of spermatogenic cells (spermatogonia, spermatocytes, spermatids) and Sertoli cells, but still lower than mice given fermented garlic extract at a dose of 250 mg/kg BW. This could be due to the insufficient amount of antioxidants provided by the fermented garlic extract at a dose of 125 mg/kg BW compared to the ROS production caused by exposure to heat. The imbalance of antioxidants and free radicals causes antioxidants to not work optimally so that the number of spermatogenic cells and Sertoli cells has decreased because oxidative stress still occurs.

Mice given Fermented garlic extract at a dose of 500 mg/kg BW experienced a decrease in the number of spermatogenic cells (spermatogonia, spermatocytes, spermatids) and Sertoli cells when compared to mice given fermented garlic extract at a dose of 250 mg/kg BW. Too high levels of antioxidants in the body will be converted into pro-oxidants so that there is a decrease in antioxidant function in counteracting free radicals.

Giving exogenous antioxidants in high doses can also disrupt the balance in redox reactions (Bouayed and Bohn, 2010). This event is called hormesis, which causes the opposite effect between giving a low dose and a high dose of a drug compound or substance (Ray *et al.,* 2014). This condition also known as antioxidant paradox, which

is the condition of the presence of oxidants and free radicals in the body that cause certain diseases, but giving large doses of dietary antioxidants has little or no therapeutic or preventive effect (Halliwell, 2012).

**Conclusion**

Fermented garlic (*Allium sativum* L.) can maintain spermatogenic and Sertoli cells of mice (*Mus musculus*) after given exposure to heat.

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