



# Antibacterial Activity of Single Garlic Against *Salmonella typhi* Lipopolysaccharide in Vivo in *Mus musculus*

Lia Siti Halimah<sup>1</sup>(✉), Luthfi Nurlela<sup>2</sup>, Eka Noneng Nawangsih<sup>1</sup>, Reni Farenia<sup>3</sup>, and Dhisa Ridzkianursepta<sup>4</sup>

<sup>1</sup> Department of Microbiology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

lia.dio28@gmail.com

<sup>2</sup> Department of Parasitology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

<sup>3</sup> Department of Physiology, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

<sup>4</sup> Medical Study Program, Faculty of Medicine, Universitas Jenderal Achmad Yani, Cimahi, Indonesia

**Abstract.** Black garlic is a product of fermented garlic. According to previous research, black garlic has anti-inflammatory properties such as against *Salmonella typhi*. *S. typhi* has lipopolysaccharide (LPS) which will activate inflammatory cytokine mediators such as Tumor necrosis factor-alpha (TNF- $\alpha$ ). TNF- is one of the major cytokines in the acute inflammatory response. This study aims to determine the anti-inflammatory properties and concentration of single black garlic extract against *S. typhi* lipopolysaccharide in vivo in mice. This study used an experimental method with a posttest only research design with control group, using BALB/C white male mice which were divided into 5 groups, namely negative control (NC), positive control (PC), treatment 1 (T1) was given 25% extract. Treatment 2 (T2) was given 50% extract, and treatment 3 (T3) was given 75% extract. Lipopolysaccharide induction of 0.5 mg/BW was carried out for 3 days after 14 days of extract administration. Blood samples were taken 24 h after LPS induction. The average result of TNF- levels was obtained in the PC group with the largest 8.43 pg/ml, the lowest NC group 4.69 pg/ml, of all treatment groups, the lowest T3 group 6.08 pg/ml. The results of data analysis showed  $p = 0.000$  ( $p < 0.05$ ). This indicates that there are significant differences in TNF- $\alpha$  levels in all study groups. It was concluded that a single black garlic extract has anti-bacterial properties because it can reduce TNF- levels in LPS-induced mice with the most effective concentration of 75% .

**Keywords:** Antibacterial · Black garlic · Lipopolysaccharide · TNF- $\alpha$

## 1 Introduction

Black garlic is obtained by fermenting garlic which is heated at a temperature of 60–90 °C with a humidity of 70–80% for 12–14 days without the addition of any ingredients so

that the water content decreases [1, 2], Black garlic has a fine texture. Chewy and soft, the aroma is not too strong, black in colour, has a little water content, and a slightly sweet and tasty [3–5]. In the processing of black garlic can be affected by humidity and high temperature without the addition of other substances that called the aging process, in this aging process the Maillard reaction occurs. This Maillard reaction causes a change in colour to black, the pungent odour no longer exists, and the taste will become sweet in black garlic [6–8]. In addition, bioactive compounds such as S. allyl cysteine (SAC), flavonoids, and polyphenols will increase as described in the Maillard reaction [9, 10].

Salmonella typhi is described as a bacterium that has a size of  $0.7\text{--}1.5\text{ }\mu\text{m} \times 2.0\text{--}5.0\text{ }\mu\text{m}$ , gram-like negative, rod-shaped, and belongs to the Enterobacteriaceae family which is unable to ferment lactose (non-lactose fermenter) [11]. These include facultative anaerobic bacteria, which grow optimally at temperature of  $36\text{--}37\text{ }^{\circ}\text{C}$  [12, 13].

Having lipopolysaccharide containing endotoxin will activate the immune response in the form of macrophages, which in turn activates proinflammatory cytokines such as IL-1, IFN- $\alpha$ . And TNF- $\alpha$ . Tumor necrosis factor-alpha (TNF- $\alpha$ ) is one of the main cytokines in the acute inflammatory response. Function from TNF- $\alpha$  which is for enhance the pro-thrombotic role and stimulate adhesion molecules from leukocytes and induce endothelial cells, regulate macrophage activity and immune responses in tissues [14].

Research on black garlic as an antibacterial has been conducted in vitro using the Kirby-Bauer diffusion test and liquid dilution method, which resulted in the bacteria being sensitive to black garlic extract. Previously, research on black garlic extract had the effect of inhibiting the growth of *Pseudomonas aeruginosa* and *Escherichia coli*, *Bacillus subtilis* [1, 15, 16].

Based on background back shown, we are interested in doing research entitled Study Antibacterial Activity of Black Garlic Single Against Lipopolysaccharide Salmonella typhi In vivo In Mus musculus, to know antibacterial activity and concentration the most effective one owned by single black garlic extract against Salmonella lipopolysaccharides typhi in vivo on Mus musculus seen from decreased levels of TNF- $\alpha$  in mice.

## 2 Methods

The subjects in this study are 25 male rats of the BALB/C strain, 2–3 months, 25–30 g, healthy, active move, never experience previous drug treatment. Object of this study is a single black garlic, has a dark brown color, it's sweet, and fermented at  $80\text{ }^{\circ}\text{C}$ , for 15 days. It comes from the Department of Microbiology, Medical Faculty, Jenderal Ahmad Yani University. The design is Experimental research with posttest only with control group. This research was conducted in the Laboratory of Experimental Animal and Department of Microbiology, Faculty of Medicine, Jenderal Ahmad Yani University in November – December 2021. This research has been get ethical approval from Ethics committee of Medical Faculty, Jenderal Ahmad Yani University, number 018/UH1.11/2021.

The mice were divided into 5 groups i.e. Negative Control (NC), Control Positive (PC), Treatment 1 (Extract 25%), Treatment 2 (Extract 50%), and Treatment 3 (75% Extract) by means of simple random sampling, the acclimatization is carried out for 7 days. The treatment group of mice was given single black garlic extract for

14 days. On the next day all treatment groups 1,2,3 and positive control group induced by lipopolysaccharide of *Salmonella typhi* as much as 0.5 mg/kg body weight for 3 consecutive days.

Next on the last day the blood of mice was taken through an orbita vein, which will perform Serum test using the ELISA method. The data obtained were then analysed using normality test and homogeneity by Shapiro Wilk followed by a non-parametric test by Kruskal Wallis and post hoc.

### 3 Results and Discussion

Based on the results of Table 1 in above it can be seen that the NC. Group have a mean level of TNF- $\alpha$  the lowest is 4.69 pg/ml compared to the PC. Group as mice that experienced increased levels of TNF- $\alpha$  with the mean result of the highest TNF- $\alpha$  level i.e. 8.43 pg/ml. Average yield TNF-  $\alpha$  in the P1 group showed the result of 7.49 pg/ml. Results mean TNF-  $\alpha$  levels in the group P2 shows a decreasing result levels of TNF- $\alpha$ , which is equal to 6.55 pg/ml. The results of the mean levels of TNF- in the P3 group has more results small, which is 6.08 pg/ml. Results mean TNF-  $\alpha$  levels in the group P3 has a concentration value of the lowest TNF- $\alpha$  and close to NC group results, and results the mean is furthest from PC group. So that the P3. Group is the group with the most effective in preventing increased levels of TNF-  $\alpha$  in blood compared to P1 and P2 groups.

**Table 1.** Average concentration of TNF- levels in each group

Grup	Mean	Strd. Deviation	Min-Max
Negative Control	4.69	0.09	4.58–4.82
Positive Control	8.43	0.42	8.08–9.02
Treatment 1	7.49	0.52	6.87–7.93
Treatment 2	6.55	0.28	6.26–7.02
Treatment 3	6.08	0.13	5.97–6.28

**Table 2.** Test for normality and homogeneity between research groups

Grup	Normality tes(p*Value)	Interpretation	Homogeneity test (p**Value)	Interpretation
Negative Control	0.940	Normal	0.000	No Homogeneity
Positive Control	0.202	Normal		
Treatment 1	0.051	Normal		
Treatment 2	0.256	Normal		
Treatment 3	0.409	Normal		

**Table 3.** Concentration comparison results between groups

Grup	Mean $\pm$ SD	P
Negative Control	4.69 $\pm$ 0.09	0.000
Positive Control	8.43 $\pm$ 0.42	
Treatment 1	7.49 $\pm$ 0.52	
Treatment 2	6.55 $\pm$ 0.28	
Treatment 3	6.08 $\pm$ 0.13	

*Kruskall Wallis*,  $p \leq 0,05$  (There is a significant difference)

Based on the results of Table 2 in above it can be seen that all groups has a normal distribution because the p value is greater than 0.05 ( $p > 0.05$ ).

Homogeneity test was carried out and the result is 0.000 ( $p < 0.05$ ) so that the data is expressed as variation between relatively inhomogeneous variables. Next, it will be tested non-parametric using test Kruskal Wallis to find out concentration difference between all research group. Criteria hypothesis on this Kruskal Wallis test is  $H_0$  rejected if p-value  $< 0.05$  (See Table 3).

Based on the results of the study, it was found that there was a significant difference between the NC group and the PC group, in the P1 group there was a significant difference with the NC group and a non-significant difference in the PC group indicating that the P1 group was mice given a single black garlic extract with a concentration of 25%. Unable to reduce the inflammatory reaction because it indicated that TNF- $\alpha$  levels were still high, so there was no significant difference between the P1 group and the PC group. In the P2 group, there were significant differences with the NC group and the PC group, indicating that the P2 group were mice given a single black garlic extract with a concentration of 50% were able to reduce the inflammatory reaction but were less effective because the TNF- $\alpha$  levels in the P2 group were not close to TNF- $\alpha$  levels. in the NC group. In the P3 group, there was a significant difference with the PC group and a non-significant difference in the NC group indicating that the P3 group were mice given a single black garlic extract with a concentration of 75% were able to reduce the inflammatory reaction because it indicated low TNF- levels and approached the NC group.

TNF- as a pro-inflammatory cytokine has an important role in the acute inflammatory response, especially against Gram-negative bacteria and other microbes. When inflammation occurs, macrophages are the first to be activated. - $\alpha$ , IL-1, and IL-6 which are cytokines that can cause fever [14].

Single black garlic contains an active compound in the form of S-allyl cysteine (SAC) which helps the absorption of allicin so that the metabolism of protection against bacterial infections becomes higher. S-allyl cysteine (SAC) which is an organic compound natural element of fresh garlic. It is a derivative of the amino acid cysteine, where an allyl group has been added to a sulfur atom [3, 8]. The antibacterial effect produced by these sulfur compounds changes the reaction of thiol compounds on bacterial enzymes, as well as RNA and DNA polymerases that cause disturbances in bacterial metabolism, bacterial virulence and even bacterial growth itself [16]. Based on the research that has been

carried out, it was found that a single black garlic extract with a concentration of 75% was the most effective concentration as an antibacterial that could reduce TNF-  $\alpha$  levels close to the negative control group, because at a concentration of 75% the content of black garlic extract was thicker, so it contained S- allyl cysteine (SAC) was higher when compared to the concentrations of 25% and 50%, respectively.

## 4 Conclusion

Single black garlic extract has antibacterial activity against *Salmonella typhi* lipopolysaccharide in vivo in *Mus musculus*. Single black garlic extract can reduce TNF-  $\alpha$  levels in mice induced by lipopolysaccharide, with statistical tests that gave significant results ( $p < 0.05$ ). A 75% concentration of single black garlic extract proved to be the most effective in influencing the antibacterial activity of single black garlic against *Salmonella typhi* lipopolysaccharide in vivo in *Mus musculus*.

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