

# Effect of Trigona Honey to mRNA Expression of Interleukin-6 on *Salmonella Typhi* Induced of BALB/c Mice

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**Abstract** Weak inflammatory response after *Salmonella* infection can cause persistent infection and facilitate the long survival of pathogens. Honey can induce key immunomodulators such as TNF- $\alpha$ , interleukin-6 (IL-6) and IL-1, that it can be used in the treatment of bacterial infectious diseases caused by *Salmonella typhi*. The purpose of this study is to determine the effect of honey on the mRNA expression of IL-6 in *Salmonella enterica* Typhi induced of BALB/c mice. The study used experimental pretest-posttest control design. Honey treatment was given for 7 days commencing after the induction of *Salmonella* bacteria. 20 BALB/c males mice whose weight 25-29 grams, were divided into four groups where 5 mice per group within; the negative control group was given regular feed without bacteria induction, the positive control group was given regular feed with bacteria induction, 0.27 ml/kg-weight honey group and 0.27 ml/kg-weight of Propolis honey group. Blood samples for examination of mRNA expression was examined three times that prior to the induction, 24 hours after induction and 72 hours after induction of *Salmonella*. The results showed that 0.27 ml/kg-weight of Propolis honey group showed the highest mRNA expression ( $p = 0.000$ ) for both after 24 hours after induction of *Salmonella typhi* ( $p = 0.000$ ) and 72 hours after induction of *Salmonella typhi* ( $p = 0.000$ ). We conclude that there was effect of honey on the mRNA IL-6 expression in *Salmonella typhi* induced of BALB/c mice.

**Keywords:** Interleukin-6, Honey, *Salmonella typhi*, Balc/c mice

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## 1. Introduction

Honey is rich in phenolic content such as quercetin, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin [1] which serves as an anti-inflammatory and immunomodulatory, that can be used to treat several inflammatory diseases [2,3].

Honey possesses the ability to induce some key immunomodulators such as TNF- $\alpha$ , IL-1, IL-6, IL-10, NO. All types of honey significantly increase TNF- $\alpha$ , IL-1 $\beta$  and IL-6 [3]. The ability of honey to induce the activation and proliferation of peripheral blood cells includes lymphocytic and phagocytic activity, such as its role in combating infections by stimulating anti-inflammatory and immunomodulatory [4,5].

Honey stimulates monocytes in cell culture to release cytokines TNF- $\alpha$ , IL-1 and IL-6, cells that activate the

immune response to infection [3]. When there is a stimulation of leukocytes, honey provides a steady supply of essential glucose and glycolysis for macrophages to produce hydrogen peroxide, in performing the function of destroying bacteria (phagocytosis) [5]. In another study in vivo showed that Manuka honey is used as a potent anti-typoid activity [6].

## 2. Materials and Method

### 2.1. Materials

Trigona honey obtained from beekeeping in the Masamba Regency, South Sulawesi, then through the honey processing of Prof. Mappatoba. In this place, process was through a settling, filtering, and then obtained to be Trigona honey. First used honey has to do testing in the Medicinal Plants Laboratory of Hasanuddin University Research Centre using UV spectrophotometry. Honey

dose of 0.27 ml/kg-weight honey and Propolis ratio 0.17 honeys and Propolis 0.1, the dosage of 0.27 ml/kg.

## 2.2. Bacterial

*S. typhi* bacteria used is derived from the Biomolecular and Immunology Laboratory, Faculty of Medicine, University of Hasanuddin. The amount was 103 CFU/mL (Mc Farland Standard).

## 2.3. Animals

BALB/c mice male whose weight was 25-29 grams were obtained from maintenance in Molecular Biology and Immunology Laboratory, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. Mice were adapted for 7 days before intervene, stored in a standard cage, divided into 4 groups (n = 5 /group). Group 1 was standard diet without *Salmonella typhi* induction (negative control), group 2 was standard diet and with induction of *Salmonella* bacteria without the intervention of honey (positive control), group 3 intervention 0.23 ml/kg-weight honey, group 4 was intervention of 0.27 ml/kg-weight of Propolis honey. Mice that had been adapted for 7 days were injected intravenously with a bacterium 103 CFU/mL (Mc Farland Standard). Honey intervention was given at the time after injection of *Salmonella* until the seventh day.

## 2.4. PCR Examination

Blood samples were taken for 3 times: before induction of *Salmonella typhi* (S<sub>0</sub>), 24 hours (S<sub>1</sub>) and 72 hours (S<sub>2</sub>) after induction of *Salmonella typhi*. Total mRNA was isolated from blood samples obtained by using Boom protocol methods. Quantitative *real-time polymerase*

*chain reaction* used BRILLIANT II SYBR® by following product instructions. Primary Interleukin-6 synthesized by using Macrogen (Korea), the primary CCA CAC CTT AAG GAG TCG GCTTA and CCA GTT TGG TAG CCAT CAT CAT TTC.

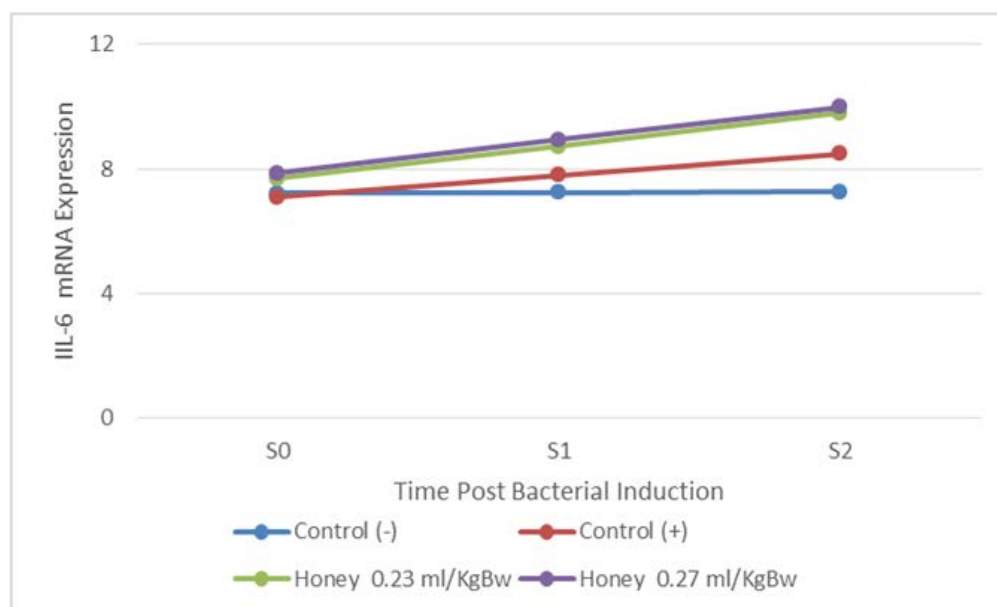
## 2.5. Statistical Analysis

Data are presented mean ± standard deviation and analyzed using Repeated ANOVA non-parametric test to analyze the significance of each intervention group, One-way ANOVA test to analyze significance of each inspection, and LSD (Least Significantly Difference) test to be as an advanced analysis to examine the major differences between the mean and the probability.

## 3. Result

### 3.1. Differences in mRNA Expression of IL-6 in *Salmonella typhi* Induced Mice after the Intervention of Propolis Honey

There was an increase in mRNA expression of IL-6 in BALB/c mice linearly to 3 times inspection before and after being given Propolis honey within 4 groups. Trend graphs mRNA expression of IL-6 shows the negative control has the lowest graphic expression (7.27) compared with positive controls (8.49). The highest increase with trends that form the linear graph since the first 24 to 72 hours after induction is in the group who was given 0.27 ml/kg-weight of Propolis honey (10.96), followed by the group who was given 0.27 ml/kg-weight honey (10.94) (Figure 1).



**Figure 1.** Trend mRNA expression of IL-6 in the control group and the group that was provided honey and Propolis honey after *S. typhi* induction, S<sub>0</sub>: time before *S. typhi* induction, S<sub>1</sub> and S<sub>2</sub>: respectively 24 and 72 hours after *S. typhi* induction

### 3.2. Effect of Honey to mRNA expression of Interleukin-6 after *Salmonella* Induction

Anova Repeated test results showed no significant difference in mRNA expression of IL-6 in the negative control group ( $p = 0.093$ ) since before to 72 hours post

infection, while positive controls showed no significant difference between before *S. typhi* induction and after *S. typhi* induction ( $p = 0.001$ ). The intervention group of 0.27 ml/kg-weight honey and 0.27 ml/kg-weight of honey Propolis showed a significant difference on the expression of mRNA of IL-6 expression after being given the honey intervention ( $p = 0.001$ ) and ( $p = 0.001$ ).

One-way ANOVA analysis test for comparison between treatment groups based on the time of treatment showed that there were statistical differences between all

treatment groups at before *S. typhi* induction ( $p = 0.003$ ), the first 24 hours ( $p = 0.000$ ) and 72 hours post infection ( $p = 0.000$ ).

**Table 1. Average of mRNA Expression in BALB/c Mice which Given Honey after *S. typhi* Induction**

Treatment Group	Baseline	24 hours	72 hours	<i>p</i> *
	Mean±SD mRNA expression of IL-6	Mean±SD mRNA expression of IL-6	Mean±SD mRNA expression of IL-6	
Control (-)	7.22±0.41	7.25±0.43	7.27±0.36	0.093
Control (+)	7.08±0.27	7.79±0.25	8.49±0.29	0.001
0.27 ml/kg-weight honey	7.69±0.08	9.03±0.17	10.42±0.31	0.001
0.27 ml/kg-weight of Propolis honey	7.73±0.20	9.37±0.29	10.96±0.43	0.001
<i>p</i> **	0.003	0.000	0.000	

\*Repeated Anova test

\*\* One-way Anova test.

### 3.3. Effect of Honey to mRNA Expression of Interleukin-6 before and after 24 Hours and 72 Hours Post Induction of *Salmonella* with the Intervention of Honey and Propolis Honey

There were no difference before and after the first 24 hours induction of *S. typhi* in the negative control group ( $p = 0.074$ ) with a 0.24 of mean improvement ( $p = 0.074$ , 95% CI-0.004-0.05), while the positive control group showed an increase in expression significantly, the mean difference was 1.03 ( $p = 0.000$ , 0.8 to 1.25). Both of he 0.27 ml/kg-weight honey and 0.27 ml/kg of Propolis honey intervention group have significant level of  $p = 0.000$ , showed increased significantly compared with the

positive and negative values control group where 0.27 ml/kg-weight honey group mean difference was 1.34 ( $p = 0.000$ , CI95% 1.06 to 1.62), and Propolis honey group has the highest increase of mRNA expression of IL-6 with of 1.63 mean difference ( $p = 0.000$ , 95% CI 1.41-1.85).

There were significant differences between 24 hours to 72 hours after injection of *Salmonella* respectively in the positive control group whose mean difference was 0.69 ( $p = 0.000$ , 95% CI 0.56 to 0.83), the 0.27 ml/kg-weight honey was 1.39 of mean difference ( $p = 0.000$ , CI 95% 1.18 to 1.60) and the 0.27 ml/kg-weight Propolis honey was 1.59 of mean difference ( $p = 0.000$ , 95% CI 1.34 to 1.83). Data show that increased expression of mRNA of IL-6 is the highest for both of the first 24 hours or the next 72 hours of group given 0.27 ml/kg-weight of Propolis honey.

**Table 2. Analysis of mRNA Expression of IL-6 from before and after the First 24 Hours and 72 Hours Post Infection of *Salmonella* with Honey and Propolis Honey Intervention**

Group Intervention	Baseline	24 Hours	Mean Difference (95%CI)	<i>p</i> *	72 Hours	Mean Difference (95%CI)	<i>p</i> *
	Mean±SD mRNA expression of IL-6	Mean±SD mRNA expression of IL-6			Mean±SD mRNA expression of IL-6		
Rerefence	7.22±0.41	7.25±0.43	0,24 (-0,004)-(0,05)	0,074	7.27±0.36	0,29 (0,008)-(0,049)	0,074
Positif kontrol	7.08±0.27	7.79±0.25	1,03 (0,8)-(1,25)	0,000	8.49±0.29	0,69 (0,56)-(0,83)	0,000
Trigona Honey 0.27/kg-weight	7.69±0.08	9.03±0.17	1,34 (1,06)-(1,62)	0,000	10.42±0.31	1,39 (1,18)-(1,60)	0,000
Trigona Honey dan propolis 0.27/kg-weight	7.73±0.20	9.37±0.29	1,63 (1,41)-(1,85)	0,000	10.96±0.43	1,59 (1,34)-(1,83)	0,000

\*Repeated Anova test,

\*\*One-way Anova test.

## 4. Discussion

The same study related to honey investigates the effects of honey on the state of activation of immunocompetent cells, that honey was significantly increase TNF- $\alpha$ , IL-1 $\beta$  and IL-6 when compared to cells not treated ( $p < 0.001$ ) [3]. Donya Nikaein's research results showed that honey treatment can significantly increase the production of IL-6 and IL-1 $\beta$  in infected mice and improve the work of macrophages perform phagocytosis ( $p < 0.05$ ). And mice treated with honey had a greater life than the infected group. This study has shown that honey can boost the immune system [7].

Other study found the fact that the flavonoids (6-dimethoxy tangeretin / 6-DMT), suppress cell activity HMC-1 with PMA through inhibition activity of ALK

(Anafastic Lymphoma Kinase) and Mitogen Activatin Protein Kinases (MAPKs), which eventually suppress the production and gene expression TNF-alpha and interleukin-6. This shows that 6-DMT could play a role in the regulation of mast cells, which mediate the inflammatory response [8].

Honey is also able to reduce IL-10, which is one inhibitor of macrophage activation and proliferation of T cell [2]. Propolis, which is a product of bees and contains flavonoids together with honey although a higher content, was also able to increase the cytokine IFN- $\gamma$  [9].

## 5. Conclusion

Giving honey and Propolis Trigona honey are able to increase the mRNA expression of IL-6 in *Salmonella typhi* induced BALB/c mice.

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## Competing Interest

The authors declare that they have no competing interests.

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