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The Effect of Giving Trigona Honey and Honey Propolis Trigona to the mRNA Foxp3 Expression in Mice Balb/c Strain Induced by Salmonella Typhi

Andi Nilawati Usman¹, Yuliana Syam², Rosdiana Natzir³, Sutji Pratiwi Rahardjo⁴, Mochammad Hatta⁵*, Ressy Dwiyanti⁶, Yuyun Widaningsih⁷, Ainurafiq⁸, Prihantono⁹

¹Department of Epidemiology, Health College of RSU Daya, Makassar, Indonesia
²Department of Nursing, Hasanuddin University, Makassar, Indonesia
³Department of Biochemistry, Hasanuddin University, Makassar, Indonesia
⁴Department of ENT, Faculty of Medicine, Hasanuddin University, Indonesia
⁵Department Molecular Biology and Immunology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
⁶Department of Microbiology, Tadulako University, Palu, Indonesia
⁷Department of Clinical Pathology, Hasanuddin University, Makassar, Indonesia
⁸Department of Chemistry, Hasanuddin University, Makassar, Indonesia
⁹Department of Oncology, Hasanuddin University, Makassar, Indonesia

*Corresponding author: hattaram@indosat.net.id

Abstract Immune balance during infection is important to support both the defense of body immune system and prevent excessive immune response. Protein Foxp3, a transcription factor of regulatory T cell has pivotal roles in balancing body immune system. Honey and Propolis have proved their effects to both the proinflammatory and anti-inflammatory responses but their effects to the Foxp3 expression need to be investigated. This study was investigated the effect of giving Trigona honey and honey propolis Trigona to the mRNA Foxp3 expression in Balb/c mice induced Salmonella typhi. Results of the study indicated that honey propolis Trigona had the highest effect to the mRNA Foxp3 expression followed by Trigona honey. Both Trigona honey and honey propolis had immunomodulatory effects through the Foxp3 mRNA expression.

Keywords: Trigona, Honey, Honey Propolis, Foxp3


1. Introduction

Several studies have been conducted to analyze the role of regulatory T cells (Treg) during the infection process because they have important roles in immune homeostasis. Induction of Treg cells are the response of hosts either to maintain or restore immune homeostasis during the infection, because body requires the control of immune response that recognize and control the microbial attack while preventing damage of tissues due to excessive immune responses [1,2].

Development and function of Treg cells require a stable and continuous expression of Foxp3 as a transcription factor (Abbas, 2011 [3,4,5]. Foxp3 insufficiency affects the development of Treg and Induction of transcription factor Foxp3 could change naïve CD4⁺ T cells into CD4⁺ CD25⁺ Treg [6,7].

Honey and propolis have been shown affecting the immune system, both pro-inflammatory and anti-inflammatory. Intravenous injection of honey in rats induced LPS showed effects on TNF-α, IL-1β, IL-6 and IL-10 [8]. Honey and Propolis, natural chemical products of bees contain polyphenols and flavonoids, Evidence also indicates that flavonoid content of honey and propolis could serve as both therapeutic and effective agents against bacterial pathogens including salmonella [9-14]. However their effects on the balance of immune homeostasis during infection have not been studied previously, particularly on the expression of the transcription factor of Foxp3.

Flavonoids components can be found in propolis and honey [11]. Existing study regarding the effects of flavonoids on Foxp3 have different results. Bicacin, flavon components found at the root of Scutellaria Baicalensis Georgi has proved induced the expression of Foxp3 Treg [15]. Polyphenols, naringenin had also proved induced by Foxp3 Treg in the presence of TGF-β through the activation of transcription factor of Aryl hydrocarbon receptor [16]. Contrary results shown to others studies, giving total flavonoids isolated from the roots of Radix tetrastigmae T. hemsleyanum Diels et Gilg able to suppress the development of regulatory T cells in mice [17]. Experiments in mice by giving flavonoids from...
Scutellaria Ocmulgee leaf extract (Socl) showed significant inhibition against Treg frequency [18]. This study aimed to determine the effect of honey and honey propolis Trigona on the expression of Foxp3 mRNA expression in Balb/c mice induced Salmonella typhi.

2. Material and Methods

2.1. Honey and Honey Propolis Trigona

Compilation and processing of both honey and propolis were conducted in collaboration with Professor Mappatoba Trigona Sila, the Indonesian expert for both bee honey and propolis. Both honey and propolis produced by bees Trigona types were obtained from Masamba district, South Sulawesi Province.

Honey was deposited and filtered for 3 days. Propolis was dissolved in 1000 mL of distilled water and heated, homogeneous solution was cooled to float on the surface of the solid wax, this solid wax then removed and the propolis solution was filtered. Honey and propolis then stored in the refrigerator. Honey propolis made by mixing 85% honey with 25% propolis (85 gram honey and 15% propolis Trigona).

2.2. Experimental Animals

Ethical use of Animal in Ethical Commission of Hasanuddin University and Immunology and Biomolecular Laboratorium have approved the protocol of study. Male Balb/c mice with weighing 25-27 g were housed under both 12-hour light and 12-hour dark periods, they were fed a chow diet and water ad libitum. Mice Balb/c were divided into four groups (n=5/group) after 7 days of adaptation to environmental condition.

Two of groups served as control group, both the negative control and positive controls, while others as the intervention group. Negative control group was not given any intervention but only standard diet, positive control was injected Salmonella typhi \(10^3\), one of treatment group was intraperitoneally injected with Salmonella typhi \(10^3\) and also treated with Trigona honey 0.27 ml/20 g Bw and other groups also injected Salmonella typhi \(10^3\) intraperitoneally and given honey propolis 0.27 ml/20 gr Bw. Salmonella typhi injected at the first day and honey and honey propolis were given for 3 days after salmonella induction. All groups were taken 100 µL via blood vessels in the tail before injected Salmonella typhi (baseline), 24 hours and 72 hours after injected Salmonella typhi.

2.3. RNA Isolation and Real-time RT-PCR

Total RNA from blood cells was prepared by using the Trizol reagent according to the manufacturer’s protocol (Invitrogen). cDNA was synthesized with the first-strand cDNA synthesis kit and oligo (dT) primers (Fermentas, Hanover, MD), Primer sequences was FW-TTTACTCGCATGTTCGCCTACTT, RV-TCAATTCATCTACCGGTCCACAC. PCR reaction used SYBR® Green PCR Master Mix (Macrogen) and GADPH gene was chosen as an internal standard, normalizing by GADPH preceding calculation of mRNA level [15,19].

2.4. Statistical Analysis

Data presented with figures and tables and expressed as means and standard deviation (SD). All \(p\)-values \(\leq 0.05\) were considered significant.

3. Results

Data indicated no significance difference from baseline until both 24 hours and 72 hours after Salmonella typhi induction for the negative control group (\(p=0.300\)). Data in Table 1 showed the significance difference of Foxp3 mRNA expression from baseline to 24 and 72 hours after Salmonella typhi induction for the positive the control group (\(p=0.020\), where the experimental group was given honey (\(p=0.002\)) and another was given honey propolis (\(p=0.006\)). (Table 1)

Figure 1. Comparison of mean values for the Foxp3 mRNA Expression among experimental groups given by both Trigona Honey and Honey Propolis Trigona with the control group. Mice Balb/c strains were divided into 4 groups (n=5), one group as the reference and the others as the positive control (Induced Salmonella), Both experimental groups were given both honey and honey propolis Trigona. All groups were examined for their Foxp3 mRNA Expression before and after the induction of Salmonella Typhi for 24 and 72 hours. Data were presented as mean±standard deviation. (*\(P\leq 0.05\))
by LPS could be protected from endotoxemia through the (pSmad3). Induced Smad3, in initially binds to the enhancer Treg, its mechanism involves induction of Smad3 systemic rising of TGF-β to affect Foxp3 is investigated through their ability to site increase TGF-β, interleukin-6 (IL-6) [21]. Honey treatment in rats induced interleukin-1β, tumor necrosis Alpha (TNF-α) and Figure 1). The difference of Foxp3 mRNA expression than both negative and positive control groups. The highest Foxp3 mRNA expression was the group given honey propolis Trigona.

After 72 hours of post Salmonella typhi induction, the positive control group still had higher significance in increasing the Foxp3 mRNA expression than the negative control group, and the group given both honey and honey propolis Trigona had higher significance in increasing the Foxp3 mRNA expression than both negative and positive control groups. The highest Foxp3 mRNA expression was the group given honey propolis Trigona.

5. Conclusion
The evidence from this study revealed that honey and honey propolis Trigona could increased the Foxp3 mRNA expression and the effect of honey propolis was higher than honey.

The data in this study simply indicate the potentiality of honey and honey propolis Trigona to increase Foxp3 mRNA expression, but did not study the mechanism. Further study should include the mechanism, either to TGF-β or other potential mechanisms.

4. Discussion
Data from this study showed that the intervention group was given honey and honey propolis had the greater increase of the expression of Foxp3 mRNA than the negative control group. Notwithstanding, both the positive control groups also increased the expression of Foxp3 mRNA but lower than the intervention group.

Acknowledgement
The authors would like to acknowledge the support of the Indonesian honey expert, Professor Mappatoba Sila for his valuable discussions.

Competing Interest
The authors declare that they have no competing interests.

Table 1. Analysis of the Foxp3 mRNA Expression from Baseline to 24 Hours and Post Salmonella typhi Induction for 24 hours ad 72 hours

<table>
<thead>
<tr>
<th>Group</th>
<th>Foxp3 mRNA Expression (Mean ±SD)</th>
<th>Baseline Before Salmonella typhi induction</th>
<th>24 Hours Post Salmonella typhi Induction</th>
<th>72 Hours Post Salmonella typhi Induction</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>11.08±0.19</td>
<td>11.09±0.23</td>
<td>11.12±0.26</td>
<td>0.300</td>
<td></td>
</tr>
<tr>
<td>Positive Control</td>
<td>11.07 ± 0.24</td>
<td>11.59±0.19</td>
<td>12.16±0.09</td>
<td>0.020*</td>
<td></td>
</tr>
<tr>
<td>Honey</td>
<td>11.04±0.13</td>
<td>12.16±0.17</td>
<td>13.31±0.34</td>
<td>0.002*</td>
<td></td>
</tr>
<tr>
<td>Honey Propolis</td>
<td>11.13±0.11</td>
<td>12.67±0.29</td>
<td>14.19±0.39</td>
<td>0.006*</td>
<td></td>
</tr>
</tbody>
</table>

*Repeated ANOVA Statistical Test
'Significant (P≤0.05).

Analysis in Figure 1 showed no significance difference of the Foxp3 mRNA expression between negative and positive control group at baseline. Positive control group had higher significance in increasing the Foxp3 mRNA expression than negative control group at 24 hours of post Salmonella typhi induction and group given honey and honey propolis Trigona had higher significance in increasing the Foxp3 mRNA expression than both negative and positive control groups. The highest Foxp3 mRNA expression was the group given honey propolis Trigona.

Further study should include the mechanism, either to TGF-β or other potential mechanisms.

References

NFATc2 and CREB that would binding with Foxp3 promoter [25].

Our data did not explain the mechanism, so further studies should be conducted to explore more understandings on honey, propolis and Foxp3 Treg.


