International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Trigona Honey, a Natural Bee Product Promotes mRNA Foxp3 Expression in Healthy in Mice Balb/c Strain

Andi Nilawati Usman[a*], Muhammad Hatta[b], Rosdiana Natsir[c], Sutji Pratiwi Rahardjo[d], Yuyun Widaningsih[e], Yuliana Syam[f], Ainurrafiq[g], Hariati Lestari[h], Hartati Bahar[i]

[a]*MedicalScienceofPostgraduateProgram,FacultyofMedicine,HasanuddinUniversity,Makassar,Indonesia; Health College of RSU Daya, Makassar,Jakarta, Indonesia*

[b,c,d,e,f]*Faculty of Medicine, Hasanuddin University, Makassar, Indonesia*

[g,h,i]*Faculty of Public Health, Haluleo University, Makassar, Indonesia*

[a]*Email:nilawatiandi@gmail.com*

Abstract

Transcription factor, *Foxp3 Treg* plays an important role in the balance of the immune system, diets containing polyphenols and flavonoids could increase the expression of *FOXP3mRNA* although some studies have contrary results. Trigona honey is a specific honey from Trigona bees containing polyphenols and could influence the immune homeostasis. There have never beenstudies provingits effectson the expression of*Foxp3mRNA* as the transcription factor of Regulatory TCell. It was a laboratory research; mice Balb/c were divided into the reference, positive and treatment groups. The reference group was only given standard feed and positive control was intraperitonially injected with *Salmonella Enterica serovarTyphi*. The treatment group was divided into 2 groups and given Trigona honey using canule with both doses of 0.23 ml/20 g Bw and 0.27 ml/20 g Bw daily for 10 days respectively. *Foxp3 mRNA* expression was examined by real-time RT-PCR. Repeated Anova and One Way Anova were used as the statistical methods, a *p*-value of less than 0.050 at the final analysis was considered indicating statistical significance. Results indicated *Foxp3mRNA*expression of the groups given by honey was higher than the control group.

---

* Corresponding author.

The highest Foxp3 mRNA expression in Trigona honey was the group given with a dose of 0.27 ml/20 g Bw (p=0.000, however, the group given Trigona Honey with a dose of 0.23 ml/20 g Bw also had moderate *Foxp3 mRNA* expression. These data suggested that Trigona honey could induce *Foxp3mRNA*expression. The higher dose given, the higher *Foxp3 mRNA* expression.

***Keywords*:** Trigona; Honey; Foxp3; Salmonella typhi.

1. Introduction

Regulatory T cells (Treg) have pivotal roles in immune homeostasis, protein Foxp3 as both marker and transcription factor of Treg cell needs stable expression to maintain normal function of Treg. [1,2] . Some diseases with immunological self-tolerance disorders, such as rheumatoid arthritis, multiple sclerosis, arthritis and encephalomyleietis have close links with imbalance function of *Foxp3* Treg. Results of the advanced studies showing that *Salmonella* have been attenuated by making a vaccine for severe cases of arthritis and encephalomyelitis that indicate its capability to induce *Foxp3* regulatory T cells. Despite its success in inducing Foxp3 Teg, the use of pathogenic bacteria are still being debated [3-5]. Bacterial infections as *Salmonella leishmania* and *Mycobacterium tuberculosis* also need the balance of Foxp3 expression to control homeostasis between immunosuppressive and immunopathology [6-11].

Potency of natural products to induce T Reg cells continue to be studied and also their important compounds contributed to the induction of *Foxp3* expression. Several studies have found that polyphenols could induce Treg cells through the expression of *Foxp3* transcription factors [12]. Nevertheless, some studies have shown polyphenols and flavonoids actually inhibited Treg activity and decreased the expression of Foxp3 [13, 14].

Trigona honey contains polyphenols and flavonoids compounds and it is always available and use daily as a natural product, product of Trigona bees from Masamba, South Sulawesi contained total phenols and quercetin[15-17]. Compounds of Honey has various roles as both antibacterial and anti-inflammatory and could affected cytokines interleukin 6 (IL-6) and interleukin-10 (IL-10 10 [18]. This study aimed to analyze the effect of Trigona honey from Masamba district, South Sulawesi Province, Indonesia to the increase of *Foxp3 mRNA* expression and to test whether there are different conditions among healthy mice subjects based on doses given.

2. Material and Methods

Study was conducted at Microbiology Laboratory, Immunology and Biomolecular Laboratory and Biopharmacology Laboratory of Hasanuddin University. Ethical procedure has been approved by Ethical Commission of Hasanuddin University. Samples were 20 mice BALB/c and categorized into four groups, where each group consisted of 5 mice. Trigona honey was compiled from Masamba, a district in South Sulawesi Province of Indonesia. In Indonesia, besides Kalimantan Island, one of the well-known regions as the producer of Trigona Honey is Masamba district, located in South Sulawesi Province [19].

2.1 Materials

Samples of honey were taken from Masamba, North Luwu District, South Sulawesi Province, Indonesia. Afterward, samples were precipitated in dark period for 72 hours in hygienic condition, and then they were stored into a heating/drying oven to decrease their water contents.

2.2 Experimental animals

Male mice Balb/c with 25-27 gr were kept in individual cages under standard condition (12-h light and 12-h dark condition). They were given water and chow diet *ad libitum* after 7 days adaptation to environmental condition. The experimental protocol was approved by Ethical use of Animal in Ethical Commission of Hasanuddin University.

2.3 Honey intervention

Male Mice Balb/c were divided into four groups (each group consisted of 5 mice) and received different intervention, the first group as the reference group was not given any intervention, but only standard diet. The second group was positive control, induced intraperitoneally by *Salmonella typhi* 10^3, group 2 and 3 were given honey with both 0.23 ml/20 g Bw and 0,27 ml/20 g Bw for 10 days respectively.

2.4 RNA isolation and real-time RT-PCR

Blood samples were taken in the tail, total mRNA was isolated from blood samples obtained using the Boom protocol methods. Quantitative *real-time polymerase chain reaction* used BRILLIANT II SYBR® by following product instructions. Primary *Foxp3*synthesized using Macrogen (Korea), Primer sequences wasFW-TTTACTCGCATGTTCGCCTACTT, RV-TCAAATTCATCTACGGTCCACAC [12, 20].

2.5 Statistical analysis

Data were presented with figures and tables and expressed as means and standard deviation (SD). Statistical test was one- way ANOVA. All p-values ≤ 0.05 were considered significant.

3. Results

Data showed the increase of *Foxp3 mRNA* expression in healthy mice treated by honey, the higher dose of honey, the higher *Foxp3 mRNA* expression. Healthy mice induced by *Salmonella typhi* also showed the increase of *Foxp3 mRNA* expression, but still lower than the groups treated by honey (Figure1 and Table 1).

The increase of *Foxp3 mRNA* expression of healthy mice given Trigona Honey was significance compared to the control groups, both negative and positive controls and all groups significantly difference (Figure1 and Table 1).

Table 1: The Foxp3 mRNA Expression of Healthy Mice and Control Groups

Group	*Foxp3 mRNA* Expression (Mean±SD)	*p**
Negative Control	11.04±0.13	
Positive Control	11.07 ±0.24	0.000[a]
Honey 0.23 ml/20 gBw	12.14±0.20	
Honey 0.27 ml/20 gBw	12.37±0,13	

***** *One-way ANOVA Statistical Test*
[a] *Significant (P≤0.05)*

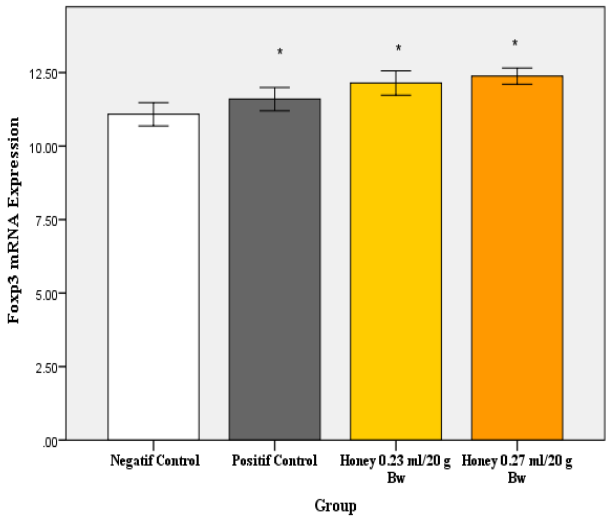

Figure 1: Comparison of mean values for the *Foxp3 mRNA* Expression among groups given both Trigona Honey and Control groups. Mice Balb/cwere divided into 4 groups (n=5), one group as the reference and one group as the positive control (Induced Salmonella), 2 groups were given Trigona honey for 10 days with different doses. *Values are significantly different from the control group at p≤0.05.

4. Discussion

Polyphenols (gallic acid) and flavonoid (quercetin) compounds of Trigona Honey are higher than Trigona Carbonaria derived from from Australia [15]. In this study, the data suggested that honey could increase *Foxp3 mRNA* expression, the higher dose given, the higher *Foxp3* expression. *Salmonella* induction also enhanced the expression of FOXP3, but still much lower than honey.

One of the theories underlying the mechanism of polyphenols affecting the immune system is the epigenetic mechanism, such as DNA methylation [21]. DNA methylation is controlled by DNA methyltransferase (DNMT)

[22]. *Foxp3* expression epigenetically regulated by DNA methylation, a diet suggested containing polyphenols as a DNA methyltransferase inhibitor (DNMT) that enhances demethylation and can induce the expression of Foxp3 [23,24, 25].

Other mechanisms are regarded play important roles through cytokine transforming growth factor β (TGF-β) because this cytokine is considered one of the factors which necessary in both initiating and maintaining the expression of Foxp3. Flavonoids, quercetin may affect the alteration of TGF-β and although research is still lacking, results of some studies indicate that honey can increase TGF-β [26-29]. Increased systemic TGF-β will raise the frequency of TREQ, a mechanism of TGF-β triggering the FOXP3 gene expression that involves the induction of Smad3 (pSmad3). Induced Smad3, initially binds to the enhancer site of *Foxp3* in intron 2 and interacts with nuclear factor-kB, NFATc2 and CREB that binds with *Foxp3* promoter [30].

Results of this study indicated that honey could increase the expression of *Foxp3 mRNA*, but did not examine the mechanisms involved in the process, so it could be investigated in future studies. It is also interesting to search the benefits of honey in auto-immune diseases considering it has been proven able to induce the expression of *Foxp3 mRNA*.

5. Conclusion

In this study, the data showed that honey could increase *Foxp3 mRNA* expression; the higher the dose given, the higher *Foxp3 mRNA*expression. Induction of *Salmonella* also enhanced the expression of *Foxp3*, but still much lower than honey.

Competing interest

The authors declare that they have no competing interests.

References

[1] Wohlfert, E. and Y. Belkaid, Role of endogenous and induced regulatory T cells during infections. J Clin Immunol, 2008. **28**(6): p. 707-15.

[2] Katoh, M., et al., Cancer genetics and genomics of human FOX family genes. Cancer Lett, 2013. **328**(2): p. 198-206.

[3] Kochetkova, I., et al., Segregated regulatory CD39+CD4+ T cell function: TGF-beta-producing Foxp3- and IL-10-producing Foxp3+ cells are interdependent for protection against collagen-induced arthritis. J Immunol, 2011. **187**(9): p. 4654-66.

[4] Ochoa-Reparaz, J., et al., Regulatory T cell vaccination without autoantigen protects against experimental autoimmune encephalomyelitis. J Immunol, 2007. **178**(3): p. 1791-9.

[5] Zozulya, A.L. and H. Wiendl, The role of regulatory T cells in multiple sclerosis. Nat Clin Pract

Neurol, 2008. **4**(7): p. 384-98.

[6] Merrell, D.S. and S. Falkow, Frontal and stealth attack strategies in microbial pathogenesis. Nature, 2004. **430**(6996): p. 250-6.

[7] Monack, D.M., A. Mueller, and S. Falkow, Persistent bacterial infections: the interface of the pathogen and the host immune system. Nat Rev Microbiol, 2004. **2**(9): p. 747-65.

[8] Scott-Browne, J.P., et al., Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. J Exp Med, 2007. **204**(9): p. 2159-69.

[9] Belkaid, Y., et al., CD4+CD25+ regulatory T cells control Leishmania major persistence and immunity. Nature, 2002. **420**(6915): p. 502-7.

[10] Johanns, T.M., et al., Regulatory T cell suppressive potency dictates the balance between bacterial proliferation and clearance during persistent Salmonella infection. PLoS Pathog, 2010. **6**(8): p. e1001043.

[11] Guyot-Revol, V., et al., Regulatory T cells are expanded in blood and disease sites in patients with tuberculosis. Am J Respir Crit Care Med, 2006. **173**(7): p. 803-10.

[12] Yang, J., X. Yang, and M. Li, Baicalin, a natural compound, promotes regulatory T cell differentiation. BMC Complement Altern Med, 2012. **12**: p. 64.

[13] Feng, Z., et al., Antitumor activity of total flavonoids from Tetrastigma hemsleyanum Diels et Gilg is associated with the inhibition of regulatory T cells in mice. Onco Targets Ther, 2014. **7**: p. 947-56.

[14] Zheng, Q., et al., Effects of Bu Shen Yi Sui Capsule on Th17/Treg cytokines in C57BL/6 mice with experimental autoimmune encephalomyelitis. BMC Complementary and Alternative Medicine, 2015. **15**(1): p. 1-14.

[15] Usman, N.A., et al., Nutrient Content and pH of Honey Propolis Trigona from Masamba, South Sulawesi Indonesia.IJSBAR, 2016. **26**(3).

[16] Othman, N.H., Does honey have the characteristics of natural cancer vaccine? J Tradit Complement Med, 2012. **2**(4): p. 276-83.

[17] Moniruzzaman, M., et al., Physicochemical and antioxidant properties of Malaysian honeys produced by Apis cerana, Apis dorsata and Apis mellifera. BMC Complement Altern Med, 2013. **13**: p. 43.

[18] Kassim, M., et al., Gelam honey inhibits lipopolysaccharide-induced endotoxemia in rats through the induction of heme oxygenase-1 and the inhibition of cytokines, nitric oxide, and high-mobility group protein B1. Fitoterapia, 2012. **83**(6): p. 1054-9.

[19] Kustiawan, P.M., et al., In vitro cytotoxicity of Indonesian stingless bee products against human cancer cell lines. Asian Pac J Trop Biomed, 2014. **4**(7): p. 549-56.

[20] Takeuchi, T., et al., Differential expressions of toll-like receptor genes in the vagina of pregnant mice. J Vet Med Sci, 2013. **75**(5): p. 561-5.

[21] Ahmed, S. and N.H. Othman, Honey as a potential natural anticancer agent: a review of its mechanisms. Evid Based Complement Alternat Med, 2013. **2013**: p. 829070.

[22] Bestor, T.H., Cytosine methylation and the unequal developmental potentials of the oocyte and sperm genomes. Am J Hum Genet, 1998. **62**(6): p. 1269-73.

[23] Wong, C.P., et al., Induction of regulatory T cells by green tea polyphenol EGCG. Immunol Lett, 2011. **139**(1-2): p. 7-13.

[24] Polansky, J.K., et al., DNA methylation controls Foxp3 gene expression. Eur J Immunol, 2008. **38**(6): p. 1654-63.

[25] Janson, P.C., et al., FOXP3 promoter demethylation reveals the committed Treg population in humans. PLoS One, 2008. **3**(2): p. e1612.

[26] Fu, S., et al., TGF-beta induces Foxp3 + T-regulatory cells from CD4 + CD25 - precursors. Am J Transplant, 2004. **4**(10): p. 1614-27.

[27] Kim, Y.S., et al., Effects of Allium victorialis leaf extracts and its single compounds on aldose reductase, advanced glycation end products and TGF-beta1 expression in mesangial cells. BMC Complement Altern Med, 2013. **13**: p. 251.

[28] Horwitz, D.A., et al., Critical role of IL-2 and TGF-beta in generation, function and stabilization of Foxp3+CD4+ Treg. Eur J Immunol, 2008. **38**(4): p. 912-5.

[29] Majtan, J., et al., Effect of honey and its major royal jelly protein 1 on cytokine and MMP-9 mRNA transcripts in human keratinocytes. Exp Dermatol, 2010. **19**(8): p. e73-9.

[30] Tone, Y., et al., Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol, 2008. **9**(2): p. 194-202.