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Effect of Oral Administration of Honey on Hemato-biochemical Parameters of Dogs with Atopic Dermatitis

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ABSTRACT

Honey from *Trigona* species is widely used as herbal medicine in humans due to its antimicrobial, anti-inflammatory, and antioxidant effects as well as the potential to increase body resistance and boost blood formation. The current study aimed to determine the hemato-biochemical profile of dogs with atopic dermatitis treated with *Trigona* honey. The hematology profile included the measurement of erythrocytes, hemoglobin, hematocrit, and erythrocyte index, as well as blood biochemical parameters, including aspartate aminotransferase (AST), alanine transaminase (ALT), and blood sugar. A total of 12 local dogs aged 4 months old were divided into two treatment groups, namely the control group (G1) and treatment with liquid *Trigona* honey at a dosage of 5 ml/dog/day (G2) for 35 days. Then, blood was collected and tested for routine and chemical blood assay. The results showed that the administration of fresh *Trigona* honey (5 ml/day for 5 weeks) exhibited a significant increase in most of hematological variables of dogs with atopic dermatitis, compared to G1. The results of blood biochemical profiles (AST, ALT, and blood glucose) remained unaffected by the treatment of *Trigona* honey. It can be concluded that honey from *Trigona* spp. was safe to be given to the dogs with dermatitis and no adverse physiological effects were observed during the present study.

Keywords: Blood, Dermatitis, Dog, Hemato-biochemical, *Trigona* species honey

INTRODUCTION

Honey from *Trigona* spp. is widely used as herbal medicine in humans. It was reported to have antimicrobial, anti-inflammatory, and antioxidant effects, as well as the potential to increase body resistance and boost blood formation (McLoone et al, 2016; Baby et al., 2018). It has no or very low side effects (Erejuwa et al., 2012; Chuttong et al., 2015). Honey from *Trigona* has strong antioxidant potential because it contains polyphenols in the form of phenolic acids (chlorogenic, ferulic, caffeic, ellagic, vanillic, benzoic, cinnamic, and coumaric acids) and bioflavonoids (pinocembrin, apigenin, hesperetin, chrysin, quercetin, luteolin, myricetin, pinobanksin, galangin, and kaempferol) to scavenge free radicals (Nayik and Nanda, 2016; Baby et al., 2018). High levels of antioxidants also have a hepatoprotective function (Visweswara et al., 2016) that may be evident by hemato-biochemical alterations. Antioxidant activity is related to the breakdown of free radicals (Ahmend and Rao, 2013), and has an ameliorative effect on the clinical signs of canine atopic dermatitis (De Santiago et al., 2021). In addition, *Trigona* honey is rich in organic acids, proteins, amino acids, minerals, and Vitamin C. This type of honey is also reported to function as antibiotics, antitoxins, anti-inflammatory (Criner et al., 2014), immunostimulants, and blood-forming (Baby et al., 2018).

There are a few reports on the use of honey for the treatment of dermatitis in dogs. The use of honey as alternative medicine in dogs is effective to manage otitis externa (Maruhashi et al., 2016). However, the use of honey from *Trigona* spp. for the nutrition and treatment of diseases in dogs has not been widely investigated. Stray dogs are reported to have a very high incidence of dermatitis due to poor maintenance, irregular food, and high chances of infection from the environment (Purnama et al., 2019). Infections on external parts of the body can also impact physiological changes in animals, which can be seen from the hematological and biochemical profiles of the blood. Therefore, the current study aimed to determine the effects of oral administration of honey from *Trigona* spp. on hematological and biochemical profiles of blood as liver function indicators in dogs with atopic dermatitis.

MATERIALS AND METHODS

Ethical approval

The procedures performed in this study were guided by the principles of animal welfare, the Animal Welfare Act of the Faculty of Veterinary Medicine of Udayana University, Bali, Indonesia (No B/81/UN14.2.9/PT.01.04/2021).

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Research design

This study used 12 local dogs (2 males and 10 females) aged 2-6 months old diagnosed with atopic dermatitis with clinical signs of itching, redness, and swelling on the skin (Junaedi et al., 2020). Two dogs were served as a control group (G1) while 10 dogs were treated orally with fresh honey from *Trigona* spp. (G2, 5 ml/dog/day for 5 weeks). The honey was collected from a local farmer in Badung regency, Bali, Indonesia. All dogs were placed in separate cages and adapted to the study environment with a room temperature of 26-30°C and relative humidity of 71-85% for 14 days before the treatment. The dogs were given commercial dog dry food (PEDIGREE® Puppy, United Kingdom) for 14 days. Dogs were bathed regularly every week. The hemato-biochemical investigations were carried out three times on day 1 as well as on weeks 3 and 5.

Collecting blood samples

The blood samples were collected from the cephalic vein in a non-EDTA vial for routine hemato-biochemical examination without using any medication. The collected blood samples were stored in a cooler box and transported to the laboratory for blood examination on the same day within 30 minutes.

Routine blood tests

Routine blood tests were performed using an automated hematology analyzer (BC-2800Vet Mindray, Nanshan, Shenzhen, China). The hematologic investigation includes red blood cell count, hemoglobin, hematocrit, white blood cell count, lymphocytes, monocytes, and neutrophils.

Blood serum biochemical assay

Blood biochemical assays for aspartate aminotransferase (AST), and alanine transaminase (ALT) were performed using an automated chemistry analyzer (chemray 120; Rayto Co., Shenzhen, China). Evaluation of Blood glucose was performed using glukometer GlucoDr^{TM} (All Medicus Co., Ltd., Anyang, South Korea). Albumin levels were determined using a Spectrophotometer (Rayto life and AnalyticalScience Co., Ltd, Shenzhen, China). All tests were done in the Laboratory of Veterinary Internal Medicine, Faculty of Veterinary Medicine, University of Udayana, Bali, Indonesia.

Statistical analysis

All data were analyzed by using SPSS for windows 25 (SPSS Inc., Chicago, IL, USA). In order to investigate the statistical difference between the treatment and control group, Student's T-test was applied. The t-test probability result values greater than 0.05 were considered non-significant.

RESULT AND DISCUSSION

The results showed that the hematology profile values for all parameters were below the normal value range (Table 1). This means that the hematological profile, including red blood cells, hemoglobin, and hematocrit values, of dogs who suffered from atopic dermatitis (control group), was below the normal range for healthy dogs. The Hemoglobin and red blood cell (RBC) values below normal range indicated that dogs were anemic. This result indicated that the control dogs with atopic dermatitis had anemia at the beginning of the study. It could result from receiving no treatment and the presence of atopic dermatitis at the beginning of the study (Figure 1a). Atopic dermatitis usually causes anemia because the dogs feel uncomfortable with the itching leading to stress. Widyanti et al. (2018) reported that most dogs with dermatitis had anemia. Stress conditions also trigger anemia due to problems in the erythropoiesis process. Iron deficiency can be caused by ectoparasite infection, leading to low hemoglobin conditions which were previously reported by Sakina and Mandial (2013) in dogs with scabies and demodicosis. Bacterial infections also play a role in the occurrence of anemia, as bacteria use heme as a source of iron in their growth (Widyanti et al., 2018). This anemia condition was also confirmed by the hematocrit value below the normal range. Hematocrit value can be used to measure the degree of anemia or polycythemia.

The administration of honey from *Trigona* improved the hematological profile of dogs with dermatitis although the increase was not statistically significant ($p > 0.05$). The improvement of the hematological profile of dogs treated with honey indicated that honey from *Trigona* spp. contains 85% glucose and fructose, which can be used as a ready-to-use energy source in the body. This type of honey is rich in organic acids, proteins, amino acids, minerals, and vitamin C, which triggers the formation of erythrocytes and is easily absorbed into the gastrointestinal tract (Criner et al., 2014: Baby et al., 2018).

In addition, the high polyphenol content in honey from *Trigona* spp. did not damage internal organs, such as the liver and pancreas (Nayik et al., 2016; Baby et al., 2018). This is evident from AST, ALT, and blood glucose values which were recorded within the normal range (Table 1).

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Table 1. The hematological parameters of dogs with dermatitis and treated with *Trigona* spp. honey

Parameter	Treatment	Day 1	Week 3	Week 5	Normal Range*
RBC ± SD (x10^6/ µL)	G1	6.21 ± 2.56	4.10 ± 1.27	4.51 ± 1.09	5.5-8.5
	G2	4.60 ± 1.39	4.75 ± 0.39	4.84 ± 0.59	
Hemoglobin ± SD (g/dL)	G1	11.2 ± 2.26	8.9 ± 2.82	8.5 ± 1.55	12-18
	G2	8.98 ± 1.67	10.3 ± 0.92	9.35 ± 1.10	
PCV ± SD (%)	G1	30 ± 4.24	26 ± 8.48	30.35 ± 6.4	37-55
	G2	25.16 ± 6.49	30.8 ± 2.22	30.85 ± 3.4	
WBC± SD (x10^3/ µL)	G1	31.15 ± 251[a]	32.5 ± 0.98	28.39 ± 6.93	6.0-17.0
	G2	19.76 ± 7.45[b]	19.58 ± 6.44	15.93 ± 7.3	
Monocyte (%)	G1	8.00 ± 0.00	7.5 ± 3.53	7.01 ± 1.4	3-10
	G2	9.16 ± 6.61	6.33 ± 4.08	8.50 ± 2.73	
Eosinophil (%)	G1	3.50 ± 3.53	10.5 ± 2.12	9.23 ± 3.1	2-10
	G2	5.33 ± 5.68	12.6 ± 12.19	11.6 ± 5.46	
Lymphocyte (%)	G1	14.00 ± 2.82	19 ± 1.41	33.4 ± 6.27	12-30
	G2	12.66 ± 4.17	14.33 ± 5.85	26.8 ± 11.4	
Neutrophil (%)	G1	74.5 ± 0.70	60.5 ± 3.53	48.1 ± 13.9	60-77
	G2	72.83 ± 8.9	65.83 ± 10.9	53.00 ± 5.00	

G1: Control, G2: Treatment *Trigona* spp. honey, SD: Standard deviation, [a,b]: Different letters in column mean significant ($p < 0.05$), RBC: Red blood cell, PCV: Packed cell volume, WBC: White blood cell. *Reference: Weiss et al. (2010).

Table 2. The blood biochemical parameters of dogs with dermatitis and treated with honey from *Trigona* spp.

Parameter	Treatment	Day 1	Week 3	Week 5	Normal Range*
AST ± SD (u/L)	G1	55.0 ± 1.41	53.5 ± 7.7	44.20 ± 10.18	10-62
	G2	63.3 ± 23.61	51.83 ± 8.54	51.66 ± 13.86	
ALT ± SD (u/l)	G1	33.5 ± 3.53	31 ± 4.24	30.25 ± 8.84	10-94
	G2	35 ± 10.05	26.83 ± 7.19	30.16 ± 5.56	
Albumin (g/dL)	G1	2.75 ± 0.70[a]	1.9 ± 0.56[b]	2.20 ± 0.56[b]	3.2-4.7 (g/dL)
	G2	2.11 ± 0.37[a]	1.88 ± 0.19[b]	2.08 ± 0.14[b]	
Glucose (mg/dL)	G1	105.5 ± 9.19	105.5 ± 9.19	71.36 ± 9.00	53-117
	G2	93.5 ± 31.3	93.5 ± 31.3	72.83 ± 17.12	

G1: Control, G2: Treatment *Trigona* spp. honey, SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, [a,b]: Different letters in a row mean significant ($p < 0.05$). *Reference: Willard and Tvedten (2012)

Figure 1. Balinese local dog with atopic dermatitis before and after treatment with honey from *Trigona* spp.

A: Dog before treatment with the lesion of alopecia and visible erythema at the beginning of the study;
B: Three weeks after treatment, the fur starts to grow and the lesion of alopecia is smaller than before, the erythema is not as visible as day 1;
C: Five weeks after treatment, the soft fur is fully grown and no sign of erythema

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The administration of *Trigona* honey in dogs with dermatitis had a significant effect on the total WBC, monocytes, eosinophils, basophils, lymphocytes, and neutrophils ($p < 0.05$). However, there was an increase in eosinophils and lymphocytes and a decrease in total white blood cells (WBC) and neutrophils, which indicated a healing response in dogs suffering from dermatitis (Ferrer et al., 2014). An increase in WBC number has many potential etiologies. The Increase of WBC (Leukocytosis) is a common sign of bacterial infection, chronic inflammatory conditions, or some medications. In the acute stage of several bacterial infections, primarily mature and immature neutrophils can be found. The obtained results of the current study indicated an increased number of WBC in the first week of the study in both groups. Stressors also can trigger acute leukocytosis. The most common type of leukocytosis is neutrophilia, which can be due to infections, stress, chronic inflammation, and medication use. Leukocytosis occurs due to hypersensitivity reactions, immune dysregulation, and/or bacterial and fungal infections on the skin surface. The total WBC value at the next week of honey administration leads to normal values. In other words, honey as an anti-inflammatory agent also has the potential to increase leukocytes (Majtan, 2014).

An increase in monocytes (monocytosis) followed by neutropenia indicates recovery from inflammation and infection. Honey plays an important role in activating macrophages, which play an active role in wound healing due to inflammation in the skin (Majtan, 2014). Although uncommon, eosinophilia may suggest allergic conditions such as dermatologic conditions and parasitic infections (Alangari et al., 2017). Lymphocytosis can occur in patients with viral infections and hypersensitivity reactions. The administration of honey could increase lymphocytes which act as the immune system of the body (Ahmed et al., 2018). Flavonoids from honey stimulate antibody production and antibacterial activity (Yaghoobi and Kazerouni, 2013). In patients with dermatitis, there is a strong corticoid reaction with systemic stress affecting lymphopenia and eosinophilia as well as decreasing neutrophil values (Dulman et al., 2015). Neutrophilia can occur because a secondary pathogen infects the dog's skin (Breathnach et al., 2011).

The results of the AST and ALT values showed an average decrease after being given honey from *Trigona* spp. for 5 weeks, but the decrease was still within the normal range (Table 2). The administration of fresh *Trigona* honey to dogs with dermatitis had no significant effect ($p > 0.05$) on AST and ALT activity. The decrease in AST and ALT values showed that honey does not cause interference with liver function. Factors that cause increased AST activity are tissue inflammation, toxicity, malnutrition, and hypo-albumin. Therefore, at the beginning of the study, the AST value was higher than the weeks after the administration of honey. This is because the dogs used in this study were stray dogs without owners and roamed in the wild, with a high level of stress. With the administration of honey, skin tissue damage was reduced, leading to a decrease in AST values at week 5. The clinical sign showed that the fur started to grow, erythema decreased, and dogs did not show any itching signs after the treatment (Figures 1b and 1c). This condition shows that honey from *Trigona* contains high levels of antioxidants and has a hepatoprotective effect (Visweswara et al., 2016) by scavenging free radicals (Nayik et al., 2016; Baby et al., 2018) because it contains hydroxyl groups that act as reducing agent and as a hydrogen donor against free radicals (Palupi and Martosupono, 2009). Honey from *Trigona* contains small amounts of sucrose, so it does not increase AST values (Botezelli et al., 2012), and triggers inflammation in the liver (Fu et al., 2010; Sanchez-Lozada et al., 2010).

The ALT activity of dogs with dermatitis after administration of *Trigona* honey for 5 weeks was 30.16 ± 5.564 (u/L). This value was still within the normal range for healthy dogs (5-60 u/l). An increase in ALT and AST activity up to 2-4 times the normal value indicates liver damage. ALT enzymes are known to be found in liver cells, and used as an indicator of hepatocellular destruction. If the ALT value is higher than the AST, it indicates acute liver parenchymal damage, while the chronic process shows the opposite result. The increase in ALT in this study did not show mild or severe damage because it was still within normal limits. Polyphenol compounds as antioxidants effectively maintain enzyme activity in the blood (Kikuzaki and Nakatami, 1993). Likewise, honey from *Trigona* spp. did not trigger an increase in the amount of blood glucose as an indication that there was no disruption of insulin hormone activity in glycogenesis. This is also due to the high fructose content in *Trigona* honey.

Fructose stimulates glucokinase enzymes to increase glucose uptake by the liver, glycogen storage, and stimulates hexokinase enzymes that play a role in the phosphorylation of glucose by adenosine triphosphate into glucose-6-phosphate when blood sugar levels are high (Erejuwa et al., 2012). Fructose has an important role in producing energy in the liver. Fructose does not require insulin to enter the cells. Furthermore, the metabolic process of fructose is also different from that of glucose. The rate of absorption of fructose is slower than that of glucose. If fructose is not absorbed completely, then fructose is fermented by fungi or bacteria and then converted into ethanol and carbon dioxide (Prahastuti, 2011). The content of flavonoids lowers blood sugar levels by donating one electron to an unpaired electron in free radicals formed through the pathway of glucose metabolic activity. The antioxidant content works to fight against free radicals and oxidative stress by providing one Hydrogen atom to convert free radicals into neutral and non-destructive compounds.

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CONCLUSION

Dogs with dermatitis were found with anemia and had a low number of erythrocytes, hemoglobin levels, and PCV values. The administration of fresh *Trigona* honey resulted in a numerical increase in the number of erythrocytes, hemoglobin, and hematocrit. The administration of *Trigona* honey for 35 days exhibited no significant changes in the values of AST, ALT, and blood glucose. Thus, administration of *Trigona* honey in liquid form at a dosage of 5 mL daily for 5 weeks is safe to give to dogs with atopic dermatitis. Further research is needed to find the most optimal dosage of *Trigona* honey to treat atopic dermatitis in dogs.

DECLARATIONS

Authors' contributions

I Nyoman Suartha designed the study, analyzed it, and wrote the manuscript. Luh Made Sudimartini and Putu Devi Jayanti collected the samples and analyzed the data. Ni Putu Ayu Dewi Wijayanti supports the conduct of the study. All authors have read and approved the data and final draft of the manuscript.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

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Competing interests

The authors declare that there are no competing interests.

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