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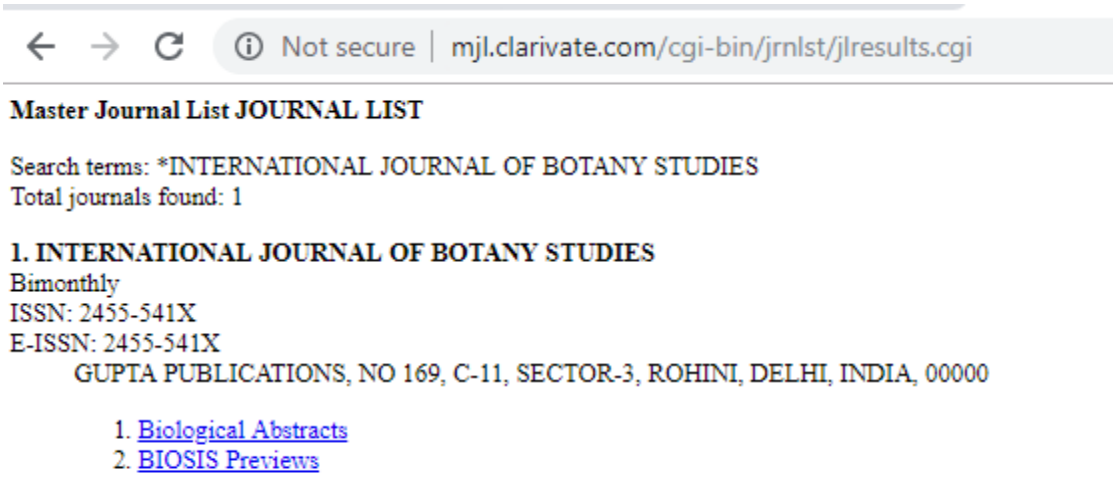

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The antihyperlipidemic activity of *Apis dorsata* Binghami nesting extract in atherogenic diet-induced hyperlipidemic rats

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Abstract
Apis dorsata Binghami is one of the endemic species of Sulawesi, Indonesia. *Apis dorsata* Binghami has not yet been cultured, but produces more honey and is richer in secondary metabolites than *Apis mellifera*. Empirically, the people of Minahasa, North Sulawesi, Indonesia have long been using honey bee nest as medicine. Research has been conducted to determine the antihyperlipidemic activity of infusion extract of *Apis dorsata* Binghami nest. *Apis dorsata* Binghami nest has obtained from natural forests, Minahasa, North Sulawesi, Indonesia. Extraction was done by the infusion method. Antihyperlipidemic analysis using pathogenic free white mice. Blood lipid profiles measured were total cholesterol, low-density lipoprotein (LDL) and triglycerides. This study used a completely randomized design with five treatments and three replications. Data from the study were analyzed variance. The results of this research showed that the treatment of 5% and 10% infusion extract *Apis dorsata* Binghami nest, significantly reduce total cholesterol, LDL and rat blood triglycerides. Further research on the antihyperlipidemic activity of *Apis dorsata* Binghami nest extract has the potential to produce antihyperlipidemic herbal drugs.

Keywords: *Apis dorsata* Binghami, nest, antihyperlipidemic

Introduction
Sulawesi Island is a biogeographic transition area for Asian and Australian flora and fauna (Hadisoesilo 2001; Mokosuli, 2013) [11, 22]. One of Sulawesi's endemic species are giant forest honey bees, *Apis dorsata* Binghami. This honey bee was studied and reported by Alfred Russel Wallace in the 18th century (Otis 1991; Raffiudin, 2002; Mokosuli, 2013) [30, 22]. *Apis dorsata* Binghami has yet to be cultivated, living naturally in the forests of Sulawesi (Otis 1991; Hadisoesilo 2001) [11]. However, *Apis dorsata* Binghami produces more honey than *Apis mellifera*. In addition, the diversity of plant sources of nectar, pollen, and propolis *A. dorsata* Binghami is higher than *A. mellifera* (Hadisoesilo 2001; Mokosuli, 2013) [11, 22]. Previous research has been carried out, it is known that *Apis dorsata* Binghami worker bees visited more than 700 plant species. *Apis dorsata* makes some Sulawesi endemic plants a source of nectar, pollen and propolis. Sulawesi endemic plants as a source of nectar and pollen include *Ficus minahasae*, *Elmerelelia celebica* L. and *Lansium minahase* L. (Mokosuli, 2013) [22].

Bioactive research from *Apis dorsata* Binghami for the purpose of utilizing the pharmaceutical field has not been widely used. Bioactive research from *Apis dorsata* Binghami that has been done is an analysis of antioxidant activity of bee venom (Mokosuli et.al., 2013) [22], analysis of anticancer and antibacterial activity of bee venom (Mokosuli et.al., 2016) [22]; analysis of antioxidant activity of ethanol extract of *Apis dorsata* Binghami nest (Mokosuli et.al., 2018) [24]. Nevertheless, there have not been reports of research on the utilization of *A. dorsata* Binghami nest as a bioactive source of drugs for diseases caused by hyperlipidemia conditions.

Honey bees produce several important products that are very beneficial for human life. Natural products produced by honey bees include honey, royal jelly, bee pollen, beeswax, bee venom and propolis (Sawaya *et.al.* 2009; Chen *et al.*, 2018) [35, 40]. The product is a complex biomolecule that is very rich in secondary metabolites because it is sourced from hundreds or even thousands of plants around the honeycomb. Honey bee nest is specific according to its habitat so that it has the potential to find medicinal bioactive (Raffiudin, 2002; Mokosuli, 2013) [30, 22].

Honey bee nest is a structure used by bees as a place of refuge for colonies from attacks of bacteria, fungi, viruses, and predators (Kumazawa *et. al.*, 2004; Raffiudin, 2002) [19, 30]. The inside of the nest is a collection of hexagonal structures made of wax and propolis compounds. Honey bee nest are used to store honey, pollen, bees, eggs, larvae, and pupae of bees. *Apis dorsata* nests in the form of a single comb are found in an open place, with a large size, the area can reach 1- 1,5 m2 (Mokosuli, 2013; Hadisoesilo, 2001) [22, 11]. The bioactive composition of nest is very dependent on the types of plants that are food sources (Sawaya *et.al.*, 2009) [35].

Empirically, the people of Minahasa, North Sulawesi, Indonesia have used *Apis dorsata* nest as degenerative medicine. Interviews conducted (n = 50) in people aged 40-65 years in Minahasa were found that beehives consumed

directly and boiled could reduce blood lipid content. However, there have been no research reports on the antihyperlipidemic activity of *Apis dorsata* Binghami honeycomb extract. On the other hand, the prevalence of diseases caused by hyperlipidemia in Indonesia is high (BPPS, 2019). Furthermore, many heart diseases and strokes caused by the condition of hyperlipidemia are still diseases with the highest mortality rates in the world today (WHO, 2019). Exploration of new bioactive sources that have antihyperlipidemic activity is very necessary. Research has been conducted to determine the antihyperlipidemic activity of *Apis dorsata* Binghami nest extract.

Materials and Methods

Sample collection

Apis dorsata Binghami's nest was obtained directly in the Minahasa forest, North Sulawesi, Indonesia. The analysis was carried out in the bioactivity and molecular biology laboratory, department of biology, state university of Manado.

(a) (b)

Fig 1: (a). *Apis dorsata* Binghami nesting at Raringis forest, Minahasa, North Sulawesi, Indonesia. (b). *Apis dorsata* Binghami nest.

Animals

The test animals used were white rats (Rattus norvegicus) pathogenic free. Before being used in research, rats were adapted for 7-14 days. In the adaptation stage, the rat was given standard feed. The rat used were healthy with an average body weight of 230 gr.

Methods

Simplisia preparation from *Apis dorsata* Binghami nest

Apis dorsata Binghami nest samples obtained were air dried for 3 days at room temperature. The nest was blended to form a powder. The powder was stored in a sterile plastic container and stored in the refrigerator.

Preparation of *Apis dorsata* Binghami nest infusion extract

Apis dorsata Binghami nest powder is used for making infusion extracts. Each of 5 grams and 10 grams of powder is boiled with distilled water for 15 minutes, at a temperature of 80^0C. The results of the decoction were filtered using filter paper. Furthermore, the filtrate obtained was called *Apis dorsata* Binghami nest infusion extract. The infusion extract was made when it was given to the test mice.

Induced hyperlipidemia

The high-fat feed used to induce hyperlipidemia consists of a mixture of chicken egg yolks, quail egg yolks, fine corn, pellets, and beef fat. The feed has made by heated beef fat and then quail egg yolks, fine corn, pellets and mixed evenly. This study used a completely randomized design, carried out in five treatment groups. Each treatment consisted of three test rats (Table 1.)

Table 1: Experimental design

Group	Replication	Treatment
N	3	Feed standard 15-20 gr / day and drinking water with ad libitum.
K+	3	Feeded with 15-20 grams of hyperlipidemia induction feed / day for 30 days, after which simvastatin was given for 7 days.
K-	3	Feeded with 15-20 gr hyperlipidemia induction feed / day for 30 days.
P1	3	Feeded with 15-20 grams of hyperlipidemia induction feed / day for 30 days, after which the nest infusion extract was given for 7 days.
P2	3	Feeded with 15-20 grams of hyperlipidemia iduction feed / day for 30 days, followed feeded *Apis dorsata* Binghami nest infusion extract was given for 7 days.

Evaluation of antihyperlipidemic activity

Rat for normal control was only given standard feed and drinking water, while the negative control group rats were given high-fat feed for 30 days. The positive control group was given high-fat feed for 30 days after which it was followed by standard feeding and simvastatin for 7 days. The dose I test group was given a high-fat diet for 30 days, after which it was followed by the provision of standard food and 5 g / kg body weight honey bee nest infusion extract for 7 days. The test group dose II was given high-fat feed for 30 days, after which it was followed by administration of standard food and 10 gr/kg honey bee nest infusion extract for 7 days.

Analisis of lipid profile

Measurements of blood lipid levels were carried out three times: after adaptation, after induction of hyperlipidemia and after induction of infusion extract of *Apis dorsata* Binghami nest. The parameters measured were levels of total cholesterol, low-density lipoprotein (LDL) and triglycerides in the blood. Rat blood was taken through the tail, before taking rat blood was cleaned with alcohol. The tail of the mouse has cut with sterile scissors until it bleeds out. The blood coming out has taken using a blood spoon, then dropped on the test strip on the Lipid Pro tool.

Statistical analysis

The results of the study were expressed as mean± S.E. Data was analyzed by using one-way analysis of variance test (ANOVA) followed by the Least Significance Different test for multiple comparisons. Values with $p < 0.05$ were considered as significant. Statistical analysis was used SPSS IBM 20.

Results and Discussion

The infusion extract of *Apis dorsata* Binghami nest

The nest of *Apis dorsata* Binghami was obtained from the Minahasa region, North Sulawesi, Indonesia. The color of the nest was golden yellow. The nest used has around 30 days old. Honey and nest were clove-flavored, due to the large number of cloves that bloom around the nest of *Apis*

dorsata Binghami. The nest infusion extract was light yellow, both extracts with a concentration of 5% (P1) and 10% (P2). Nevertheless, the extract of P2 was more yellow than the extract of P1 (Figure 2).

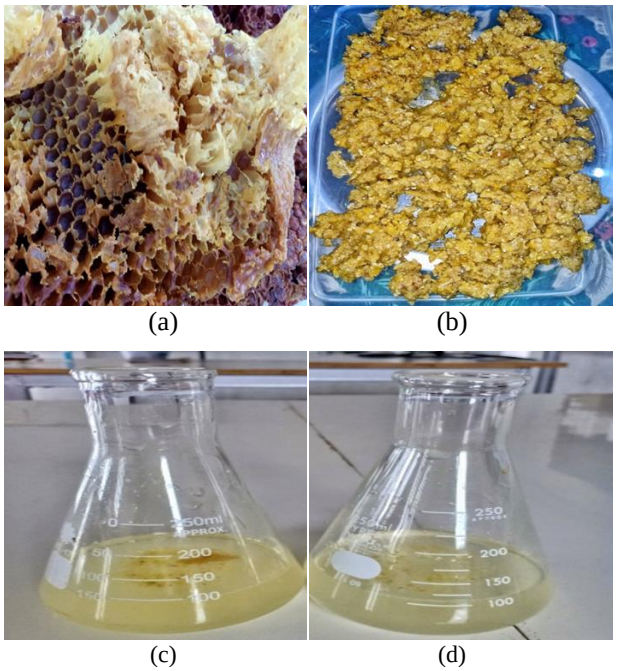

Fig 2: a. *Apis dorsata* Binghami nests from Minahasa b. The nest after drying and smoothing c. Infusa extract 10%. d. Infusion extract 5%.

Blood lipid levels of rats before and after giving induction of hyperlipidemia feed.

The average total cholesterol content of white rats measured by auto check was below 100 mg/dl, both in positive controls, normal and treatment groups (Appendix 1). After the treatment of induction of hyperlipidemia, there was an increase in lipid levels in the blood of white mice. The highest total cholesterol level in P2 group was 224 mg/dl; the highest triglyceride level in the K-group was 236 mg/dl, while the highest LDL level in the P2 group was 166 mg/dl (table 4.).

Table 4: Average rat lipid levels after administration of hyperlipidemia induction feed.

Group	Lipid profile		
	Total cholesterol	Trigiserida	LDL
*N	104,67	105,67	35,33
K+	193,67	227,67	144,67
K-	217,33	236,00	143,00
P1	217,33	226,33	157,67
P2	224,00	227,33	166,00

*No treatment for hyperlipidemia induction feed was given.

Blood lipid levels of rats after administration infusion extract of *Apis dorsata* nest

The highest total cholesterol level was found in the K-group was 231, 33 mg/dl, while the lowest in the P2 group was 104.67 mg/dl. The highest triglyceride level in the K-group was 251 mg/dl, while the lowest in the N group was 105.67 mg/dl. The highest LDL level in the K-group was 162 mg/dl while the lowest in the N group was 35, 33 mg/dl. The average total cholesterol, triglyceride, and LDL in the infusion extract of *Apis dorsata* nest treatment group (P1 and P2) were still better than the negative controls (Table 5 and Figure 3).

Table 5: Average rat blood lipid levels after administration of infusion extract of *Apis dorsata* Binghami nest (mg / dl).

Group	Lipid profile		
	Total cholesterol	Trigiserida	LDL
*) N	104,67	105,67	35,33
*) K+	137,33	131,33	101,67
*) K-	231,33	251,00	162,00
P1	188,33	163,67	111,33
P2	167,00	138,33	110,67

*No treatment for hyperlipidemia induction feed was given.

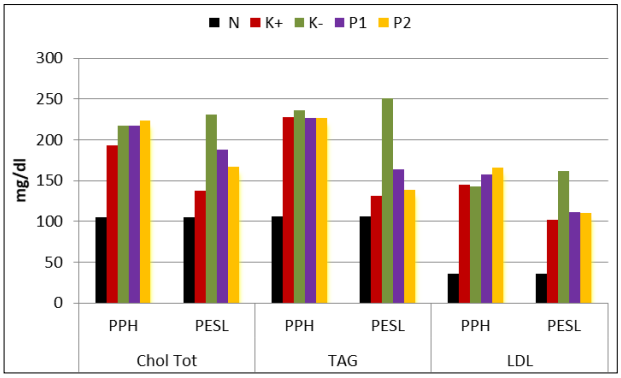

Fig 3: Histogram of blood lipid profile of test rats.

Description Feeding induction of hyperlipidemia (PPH), Application of infusion extract of *Apis dorsata* nest (PESL), Normal control group, not given induction of hyperlipidemia feed (N), Positive control group, administration of simvastatin (K+), negative control group (K-), test group, administration 5 gram of infusion extract *Apis dorsata* Binghami nest (P1), test group, administration 10 gram of infusion extract *Apis dorsata* Binghami nest (P2).

The results of analysis of variance showed that there was an effect of infusion extract of *Apis dorsata* Binghami nest on total cholesterol, triglyceride and LDL levels ($p > 0.05$) (Appendix 2). Because the treatment of infusion extract significantly affected the three parameters of lipids measured, it was followed by the LSD test.

The results of the LSD test for the parameters of rat blood cholesterol levels showed that the K-group was significantly different from the P2 and K + groups ($p > 0.05$). In the group, P1 showed a significant difference with the K-group ($p > 0.05$). Group P1 showed a not significant difference with the P2 group. The K + group did not show a significant difference with the P2 group ($p > 0.05$) (Table 5). The results of the LSD test for triglyceride parameters, K-group differed significantly from group P1, P2 and K + groups ($p > 0.05$). P2 group did not show significant differences with groups P1 and K + ($p > 0.05$). The LSD test results for LDL parameters, K-group differed significantly from K + group, group P1 and group P2 ($p > 0.05$). Group P1 and group P2 were not significantly different from group K + ($p > 0.05$).

Discussion

The application of 10% infusion extract *Apis dorsata* Binghami nest (b/v) showed a decrease in total cholesterol, triglycerides, and LDL. The application of 5% infusion extract has not significantly affected total cholesterol but has a significant effect on LDL and triglycerides. However, the K + group that used the standardized drug Simvastatin (Kalbe Farma), had a lower average cholesterol level

(137.33 mg/dl) compared to P2 (167.00 mg/dl).

Based on the content analysis of phytochemical groups, the ethanol extract of *Apis dorsata* Binghami nest from Minahasa contains flavonoids (+++), alkaloids (+), steroids (++), triterpenoid (++). Furthermore, based on the results of HPLC analysis and UV Vis spectrophotometer, it was known that the ethanol extract of *Apis dorsata* Binghami honeycomb from Minahasa contained 20 types of flavonoids (Mokosuli et.al. 2018) [24]. Analysis of the content of phenol compounds with a UV Vis spectrophotometer found 5 types of phenolic compounds in the ethanol extract of *Apis dorsata* Binghami nests from Minahasa, North Sulawesi, Indonesia (Mokosuli et.al. 2018) [24]. However, the composition of the bioactive content in honey bee nest depends on the type of honeybee, plant biodiversity and geographical location (Sawaya et.al. 2009; Mokosuli, 2013) [35, 22]. In this study, *Apis dorsata* Binghami nest from Minahasa has a distinctive aroma, namely the aroma of cloves. This means that many worker bees visit and take nectar, plant resins (propolis) and pollen from clove plants around their nests. Honey bees are composed of biomolecules of wax, propolis, royal jelly, pollen, and honey. Each of these biomolecules contains specific compounds and has been studied with many medicinal properties. Flavonoids found from honey bee nest in this study are thought to originate from propolis. Propolis is a resin with a mixture of bee enzymes, essential oil, organic minerals, candles, and honey. Propolis is the main type of flavonoids from honey bee propolis (Chen et. Al. 2018; Kumasawa et. al. 2004; Sawaya et. al. 2009) [5, 35].

Flavonoids play a role in reducing blood cholesterol levels by reducing the activity of HMG-CoA reductase, decreasing the activity of the enzyme acyl-CoA cholesterol acyltransferase (ACAT) and reducing the absorption of cholesterol in the digestive tract. The ability of anti-hyperlipidemic honey bee propolis plays a role in reducing the risk of cardiovascular disease and other complications of the disease (Rumanti, 2011) [32]. Flavonoids can inhibit LDL oxidation in blood vessels (Orsolic *et al*. 2019; Miura *et al*., 2000; Zhu et. al. 2000) [29, 25, 40]. In previous studies, it was known that crude extract of *Apis dorsata* Binghami nest from Minahasa had strong DPPH free radical scavenger activity (IC50: 6.69 mg / L) compared to the positive control of vitamin C (IC50: 6.73 mg / L) (Mokosuli et.al. 2018) [24].

In addition to flavonoids, steroid phytochemical groups can mimic cholesterol in the formation of lipoprotein and kilomicron. This can cause a decrease in blood cholesterol content (Kumar and Devanna, 2016). Saponins can bind LDL cholesterol to blood vessels, thus preventing the process of atherosclerosis (Mokosuli, 2008) [24]. Honey bee nest extract of *Apis dorsata* Binghami contains high intensity saponins and steroids.

Conclusion

The nest infusion extract of *Apis dorsata* Binghami has antihyperlipidemic activity. The P2 extract (10%) has a greater effect on the reduction of total cholesterol, LDL and triglycerides than the P1 extract (5%).

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