Preclinical Trial of Propolis Extract in Prevention of High Salt Diet-Induced Hypertension

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ABSTRACT

Background: Propolis has been widely reported as having various biological activities. However, Indonesian propolis seems to be less explored. Objective: The present study aimed to analyze the antihypertensive activity of Indonesian propolis in rats. Materials and Methods: Hypertension was induced by high-NaCl (8%) diet for 3 weeks. A total of 36 rats were divided into 6 groups, including standard diet group (SD), high-NaCl diet group (NaD), high-NaCl diet group + captopril (25 mg/kg) (PD), high-NaCl diet + propolis from Riau Archipelago (NaDP1), high-NaCl diet + propolis from Lampung (NaDP2) and high-NaCl diet + propolis from South Sulawesi (NaDP3). Propolis was daily administered at dose of 200 mg/kg on hypertensive rats for 1 week. Blood pressure and body weight were weekly measured. Moreover, routine urine analysis, haematological parameters and lipid profiles at week 4 were determined. Results: The results showed that high-NaCl diet successfully induced hypertension in rats after 3 weeks of intervention. However, the diet did not cause weight gain (p>0.05). All Indonesian propolis samples significantly reversed either systolic or diastolic blood pressure of hypertensive rats. From urine analysis, propolis from Riau Archipelago and Lampung showed diuretic effect. The haematological analysis mainly showed no significant difference compared standard diet group. Furthermore, LDL and HDL concentrations were significantly improved by propolis from Lampung and South Sulawesi, respectively (p<0.05). In addition, we only found significant decrease in relative weight of liver in all groups administered with high-NaCl diet (p<0.05). Conclusion: The present study suggests that all Indonesian propolis possessed antihypertensive activity.

Key words: Antihypertension, Blood pressure, High-NaCl diet, Stingless bee propolis.

INTRODUCTION

Hypertension is a serious public health problem and the major cause of premature death worldwide.1 WHO (2019) reported around 1.13 billion people had hypertension and mostly lived in low-middle income countries. Hypertension is risk factor for several diseases, including heart attack, stroke, kidney damage, diabetes and even cancer.2-6 In addition, hypertension also may reduce cognitive performance and cause economic lost in either micro or macro scale.7-8

Prevention of hypertension by controlling modifiable risk factors would have beneficial effect on the public health.9 High intake of salty food is one of the important risk factor for developing hypertension.10,11 The estimated salt intake is about 9-12 g per day in most countries which is higher than WHO recommendation of less than 5 g.11 High salt intake causes the impairment of renin angiotensin-aldosterone (RAA) system and oxidative stress leading to the elevation of blood pressure.12,13

The management of hypertension is currently by pharmaceutical therapies, however, it is costly, has adverse effect, and requires medical intervention.14 The triple therapies, including diuretics, a calcium channel blocker and an angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, cannot reduce the risk for resistant hypertension due to high salt intake.15 In addition, the current management seems to be not effective in controlling hypertension, thus it needs another therapies, including dietary, lifestyle and nutritional supplement strategies.14 Meanwhile, the study on bioactive natural components has been increased describing the mechanism of action in the prevention of hypertension.15

Propolis is a natural product manufactured by bee from plant resins.16 The biological activities of propolis have been widely studied, namely antioxidant, antibacterial, antiviral, antifungal, anti-inflammatory, anticancer, anti-diabetes, immunomodulatory, antiemetic properties, and also to show antihypertensive property.16-20 The previous research showed that the constituents of Brazilian propolis ameliorated the hypertension in rats.21 However, there are some challenges on propolis research due to its diverse chemical constituents which depends on botanical origin, bee species and the preparation of extract.22 Therefore, each region possibly has their unique properties.

Indonesia is a country with high biodiversity which has the potential to produce a great variety of propolis. Several studies on Indonesian propolis have showed that Indonesia propolis possessed antioxidant, immunomodulator, anti-inflammatory, and anticancer activities.23-24 However, there has
not been study to investigate the antihypertensive activity of Indonesian propolis. Therefore, this study aimed to analyze the effect of Indonesian propolis from 3 provinces of Indonesia on NaCl-induced hypertension in rats.

MATERIALS AND METHODS

Preparation of propolis extracts

The raw propolis samples were collected from Riau Archipelago, Lampung, and South Sulawesi in 2018. The extraction of raw samples was performed using ultrasound-assisted extraction. The samples were ground into small pieces (0.5 - 1 cm) and dissolved in 70% ethanol for 24 h (1 : 10). Furthermore, the mixtures were filtered using Whatman No 1 filter paper. The residues were then re-dissolved in 70% ethanol following the previous procedures. The filtrate from the 1st and 2nd extraction were combined. The combined filtrates were kept for 1 day to precipitate the wax and subsequently evaporated to obtain dry extracts. The dry extracts were kept under -20 °C until use.  

Animals and diets

A total of 36 Sprague Dawley rats (all 250-300 g; 7-8 weeks of age) were obtained from Biopharmacare Research Center, IPB University, Bogor, West Java. The hypertension was induced by sodium chloride (NaCl) (Sigma–Aldrich, Germany)-enriched diet for 3 weeks. The animals were divided into 6 groups (n = 6), namely standard diet group (SD), high (8%) NaCl diet group (NaD), high (8%) NaCl diet group + captopril (25 mg/kg) or positive control group (PD), high (8%) NaCl diet + propolis from Riau Archipelago (NaDP1), high (8%) NaCl diet + propolis from Lampung (NaDP2), high (8%) NaCl diet + propolis from South Sulawesi (NaDP3). NaD has been reported to induce hypertension in rats for 3 weeks.  

All propolis samples were given at dose of 200 mg/kg. The prior literatures showed that the administration of propolis at dose of 200 mg/kg was effective in rats.  

The animals were acclimatized for 2 weeks and kept under standard laboratory condition (temperature of 22-25 °C; 12:12 light: dark cycles). The rats were categorized to have hypertension when systolic blood pressure (SBP) ≥ 140 mmHg. The hypertensive rats then administered with propolis for 1 week, while high-NaCl diet was continued. Body weight and blood pressure was measured from week 0 until week 4. In addition, the rats were euthanized at week 4 to collect urine and blood samples for analysis. Heart, liver, kidney, spleen, lungs and urinary bladder were harvested to measure the weight. All experimental procedures have been approved by the Animal Care and Use Committee, IPB University (No. 176-2020 IPB).

Blood pressure measurement and urine collection

The procedures of blood pressure measurement referred to Malkoff et al. with slight modification. The blood pressure was weekly measured through a tail cuff in the morning using non-invasive blood pressure system (CODA, Kent Scientific, USA). The animals were placed in a holder for acclimatization for 10-15 minutes, whereas room temperature was kept at 26°C. Blood pressure was read 10 times and the means were determined.

Biochemical analysis

Blood lipid profiles, including triglyceride, cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL), were determined using Cholesterol Oxidase-Phenol Aminophenazone (CHOD-PAP) standard methods. Blood samples were also used to analyse the routine haematological analysis on an Auto Hematology analyzer (Mindray BC-2800Vet, Shenzhen Mindray Bio-Medica; Electronics Co., Ltd, China). The haematological analysis included white blood cells (WBC), lymphocytes, monocytes, granulocytes, red blood cells (RBC), haemoglobin (Hb), haematocrit (Hct), mean corpuscular volume (MCV), mean corpusular haemoglobin (MCH), mean corpusular haemoglobin concentration (MCHC), red cell distribution width (RDW), platelets (PLT), mean platelet volume (MPV). Meanwhile, the urine volume was collected for 24 h at the last day of intervention. The quantitative (pH, glucose, and urine density) and qualitative (colour, consistency, leukocytes, nitrite, protein, ketone bodies, urobilinogen, bilirubin, erythrocytes and Hb) analysis of urine samples were performed using urinalysis reagent strips (Helath Mate CYBOW REF 0004 reagent strips for Uryanalisis. IVD. DFI Co.Ltd)  

Statistical analysis

Data were expressed as mean ± standard deviation. The differences between groups were analysed with ANOVA followed by post-hoc Tukey's test. The significance was considered at p-value < 0.05.

RESULTS

To confirm whether high-NaCl consumption affects body weight, we weekly measured the weight of animals. The weight changes during the administration of high-NaCl diet and propolis are shown in Table 1. However, the weight of animals during administration of a high-NaCl diet for 3 weeks did not change statistically (p>0.05). After propolis administration for 1 week, the weight of animals was also not affected (p>0.05). A high-NaCl diet successfully caused hypertension in rats after 3 weeks of intervention. Either systolic or diastolic blood pressure was statistically raised at week 3 in all high-NaCl groups compared to standard diet group (p<0.05). All propolis samples remarkably reduced SBP and DBP compared to high-NaCl only group after 1 week of administration (p<0.05). The biggest changes were found in groups administered with propolis from Riau Archipelago and South Sulawesi, where the effect seemed to be similar with positive control group. The blood pressure of animal during the treatments can be seen in Table 2 and Table 3. Result of urine test showed that propolis seems to increase the 24-h urine volume. Propolis from Riau Archipelago and Lampung increased significantly the 24-h urine volume compared to standard diet group (p<0.05). It was confirmed by lighter urine colour in both groups. However, the other parameters (density, pH, consistency, leukocytes, nitrite, glucose, ketone, urobilinogen, bilirubin, erythrocytes, and haemoglobin) showed not statistically significant difference. Nevertheless, protein were relatively higher in all propolis groups. The result of urine test can be seen in Table 4.

Table 5 shows lipid profiles of rats at the end of intervention. The present study found no differences in triglyceride and cholesterol concentrations between the groups. However, among the groups with high-NaCl diet, LDL concentrations were significantly decreased in group administered with propolis from Lampung compared to high-NaCl only group (p<0.05). In addition, HDL concentrations significantly increased in group administered with propolis from South Sulawesi compared to standard diet (p<0.05). Result of haematological analysis is presented in Table 6. Almost all haematological parameters did not differ compared to standard diet group, except lymphocytes levels. The concentration of lymphocytes increased significantly in several groups, including positive control group, group administered with propolis from Riau Archipelago and Lampung compared to standard diet group. Nevertheless, we found relatively low levels of WBC, monocytes, granulocytes, RBC, haemoglobin, HCT, MCHC, PLT and PCT in high-NaCl only group compared to propolis group(s). The weight of organs was measured to see the possibility of pathological
changes. Relative weight of organs is depicted in Table 7. Heart seemed to be altered during the treatments. Decreased relative weight of heart was found in all groups administered with high-NaCl. Meanwhile, the relative weight of liver, spleen, lungs and urinary bladder did not differ significantly between the groups.

DISCUSSION

Obesity has been confirmed as one of the main risk factors for developing hypertension. In the other hand, high consumption of salty food is strongly associated with obesity and probably due to increase in leptin production, leptin resistance and endogenous fructose production. However, the present study found no significantly change in weight of animals after high-NaCl diet intervention and propolis administration. Consumption of salty diet is associated with osmotic stress and will be compensated with increase in water consumption which may limit food intake. The present result is also in line with the prior study.
Table 5: Urine profile.

| Parameters                  | SD                | NaD               | PD                | NaDP1              | NaDP2              | NaDP3              |
|-----------------------------|-------------------|-------------------|-------------------|--------------------|--------------------|--------------------|
| 24 h urine volume           | 14.42 ± 2.95      | 18.42 ± 4.17      | 13.38 ± 2.05      | 34.67 ± 13.26      | 29.67 ± 10.84      | 21.04 ± 4.53       |
| Density                     | 1002.14 ± 2.67    | 1004.17 ± 3.76    | 1005.00 ± 3.16    | 1005.00 ± 0.00     | 1005.00 ± 2.58     | 1004.00 ± 2.24     |
| Ph                          | 8.14 ± 0.24       | 8.00 ± 0.32       | 8.00 ± 0.32       | 8.10 ± 0.22        | 8.17 ± 0.26        | 7.90 ± 0.22        |
| Color                       | Light brown       | Light brown       | Light brown       | Yellowish-light brown | Yellowish-light brown | Light brown |
| Consistency                 | Thin              | Thin              | Thin              | Thin               | Thin               | Thin               |
| Leukocytes (leu/µl)         | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Nitrite                     | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Protein                     | (+) (+) (+)       | (+) (+) (+) (+)   | (+) (+) (+) (+)   | (+) (+) (+) (+)    | (+) (+) (+) (+)    | (+) (+) (+) (+)    |
| Glucose (mg/dl)             | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Ketone                      | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Urobinigen                  | Normal            | Normal            | Normal            | Normal             | Normal             | Normal             |
| Bilirubin                   | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Erythrocytes                | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |
| Haemoglobin                 | Negative          | Negative          | Negative          | Negative           | Negative           | Negative           |

Data are mean ± standard deviation (n=6)
The different superscript in the same column are statistically significant (p<0.05)

SD : standard diet group
NaD : high-NaCl diet group
PD : high-NaCl diet + captopril (25 mg/ kg)
NaDP1 : high-NaCl diet + propolis from Riau Archipelago (200 mg/ kg)
NaDP2 : high-NaCl diet + propolis from Lampung (200 mg/ kg)
NaDP3 : high-NaCl diet + propolis from South Sulawesi (200 mg/ kg)

Table 6: Haematological analysis.

| Parameters          | SD            | NaD           | PD            | NaDP1          | NaDP2          | NaDP3          |
|---------------------|---------------|---------------|---------------|----------------|----------------|----------------|
| WBC (1000/µL)       | 4.9 ± 1.8 b   | 2.1 ± 0.5 a   | 5.7 ± 1.4 a   | 6.1 ± 2.0 a    | 7.0 ± 2.9 a    | 4.1 ± 0.9 b    |
| Lym (1000/µL)       | 1.5 ± 1.2     | 3.2 ± 0.5 a   | 4.1 ± 1.2     | 4.5 ± 1.7 a    | 4.8 ± 2.0 a    | 2.6 ± 0.5 b    |
| Mono (1000/µL)      | 0.16 ± 0.10 b | 0.10 ± 0.03 a | 0.15 ± 0.05 b | 0.16 ± 0.05 b  | 0.23 ± 0.12 b  | 0.12 ± 0.04 b  |
| Gran (1000/µL)      | 1.6 ± 0.7 b   | 0.6 ± 0.2 a   | 1.5 ± 0.3 a   | 1.4 ± 1.2 b    | 2.0 ± 1.2 b    | 1.3 ± 0.5 b    |
| RBC (1000/µL)       | 7.1 ± 1.5 b   | 6.3 ± 1.8 b   | 7.7 ± 0.6 b   | 8.6 ± 0.7 a    | 7.7 ± 1.0 b    | 8.0 ± 0.8 b    |
| HGB (g/dL)          | 13.1 ± 3.0 b  | 11.45 ± 3.4 a  | 13.7 ± 1.2 b  | 15.9 ± 1.5 a   | 14.2 ± 2.3 b   | 14.5 ± 1.1 b   |
| HCT (%)             | 40.9 ± 8.7 b  | 36.6 ± 10.3 a  | 42.8 ± 3.5 b  | 49.2 ± 4.6 a   | 44.4 ± 6.6 b   | 45.4 ± 3.5 b   |
| MCV (fL)            | 57.9 ± 0.9 b  | 58.4 ± 1.5 a   | 55.7 ± 1.0 b  | 57.1 ± 1.5 b   | 57.5 ± 1.1 b   | 57.0 ± 1.6 b   |
| MCH (pg)            | 18.3 ± 0.5     | 18.0 ± 0.8     | 17.8 ± 0.3     | 18.4 ± 0.6     | 18.4 ± 0.5     | 18.1 ± 0.5     |
| MCHC (g/dL)         | 31.8 ± 0.7 b  | 31.0 ± 1.1 a   | 32.0 ± 0.5 b  | 32.2 ± 0.4 a   | 32.0 ± 0.5 b   | 3.84 ± 0.1 b   |
| RDW (%)             | 14.6 ± 0.9     | 15.2 ± 0.5     | 14.9 ± 0.5     | 15.3 ± 0.6     | 15.2 ± 0.4     | 15.2 ± 0.9     |
| PLT (1000/µL)       | 808.1 ± 430.9 b| 285.5 ± 62.7 a | 728.2 ± 217.9 b| 1231.4 ± 338.2 a| 971.3 ± 382.1 a| 925.6 ± 303.7 a|
| MPV (fL)            | 5.9 ± 0.5      | 6.0 ± 0.3      | 5.8 ± 0.3      | 5.7 ± 0.4      | 6.0 ± 0.3      | 5.6 ± 0.1      |
| PDW                 | 16.0 ± 0.1     | 16.2 ± 0.3     | 16.0 ± 0.2     | 16.0 ± 0.1     | 16.0 ± 0.1     | 16.0 ± 0.1     |
| PCT (%)             | 0.4 ± 0.2      | 0.17 ± 0.0     | 0.4 ± 0.1 a    | 0.6 ± 0.0      | 0.5 ± 0.2      | 0.5 ± 0.2      |

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Table 7: Relative weight (%) of organs.

| Groups              | Heart         | Liver         | Kidney        | Spleen        | Lungs         | Urinary bladder |
|---------------------|---------------|---------------|---------------|---------------|---------------|-----------------|
| Standard diet       | 0.50 ± 0.03   | 3.34 ± 0.14   | 0.38 ± 0.04   | 0.23 ± 0.03   | 0.52 ± 0.06   | 0.07 ± 0.02     |
| High-NaCl diet group| 0.44 ± 0.03 a | 3.05 ± 0.34   | 0.36 ± 0.05   | 0.21 ± 0.02   | 0.47 ± 0.04   | 0.07 ± 0.00     |
| High-NaCl diet + captopril | 0.39 ± 0.03 | 3.45 ± 0.21   | 0.36 ± 0.02   | 0.20 ± 0.02   | 0.47 ± 0.05   | 0.06 ± 0.01     |
| High-NaCl diet + propolis from Riau Archipelago | 0.40 ± 0.02 | 3.20 ± 0.15 | 0.36 ± 0.02 | 0.20 ± 0.04 | 0.46 ± 0.02 | 0.06 ± 0.01 |
| High-NaCl diet + propolis from Lampung | 0.39 ± 0.05 | 3.38 ± 0.33 | 0.35 ± 0.03 | 0.23 ± 0.10 | 0.50 ± 0.05 | 0.05 ± 0.01 |
| High-NaCl diet + propolis from South Sulawesi | 0.39 ± 0.12 | 2.92 ± 0.37 | 0.33 ± 0.02 | 0.21 ± 0.03 | 0.48 ± 0.08 | 0.06 ± 0.02 |

Data are mean ± standard deviation (n=6)
The different superscript in the same column are statistically significant (p<0.05)
associated with hypertension. 

The present study found that Indonesian propolis successfully decreased either SBP or DBP of hypertensive rats. Indeed, SBP was normalized after 1 week of propolis administration. Propolis is rich in phytochemical compounds, such as phenolics, flavonoids, and terpenes. The prior studies also reported the antihypertensive activity of propolis from several regions, including Brazil, Australia, and Tunisia. Several compounds with antihypertensive activity have also been isolated from propolis, including dihydrokaempferide, kaempferide, buteolol, isosakuranetin, and caffeoylquinic acid. Indonesian propolis is a tropical-type propolis containing phenolic and flavonoid compounds and possesses strong antioxidant activity. The antioxidiant activity of Indonesian propolis was proposed to underlie its antihypertensive activity through enhancing bioavailability of NO which responsible for vessel effect vasodilation. Furthermore, low antioxidant levels are associated with hypertension.

We further analyzed the urine of rats after 1 week of intervention. We found some propolis samples including propolis from Riau Archipelago and Lampung had diuretic effect which might also be responsible for its antihypertensive effect. The evidences have showed the diuretic agents as promising antihypertension by removing any extra fluid and widening blood vessel. Unfortunately, we also found relatively higher concentrations of urine protein in all propolis groups, which in contrast to the current knowledge. However, one toxicological study reported Brazilian propolis induced acute renal failure. Our present results were similar to antihypertensive effect of amlopidine. Therefore, our data suggest the possible mechanism of Indonesian propolis as antihypertension may be similar to the antihypertensive effect of calcium channel blockers (CCBs).

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The other possible mechanism of propolis in lowering blood pressure is probably through lipid metabolism. Propolis from Lampung decreased significantly LDL levels compared to high-NaCl only group. We also found propolis from South Sulawesi increased significantly HDL levels when compared to standard diet group. The previous studies showed that propolis from Nigeria and Brazil could improve LDL and HDL levels.

LDL levels have been reported to correlate with blood pressure in Japanese women that probably due to its association with leptin resistance. In addition, low HDL levels are also associated with reduced kidney function and may contribute to hypertension.

The haematological parameters data mainly showed no significant changes between interventions and control group. Nevertheless, increased lymphocyte concentrations in propolis groups possibly were related to its immunomodulatory effect. In addition, our present study found relatively low concentrations of several haematological parameters in high-NaCl only group compared to propolis group(s). Increase in blood volume due to high-NaCl diet might cause relatively low concentration of several haematological parameters. In fact, the prior study reported protective effect of propolis on haematological alterations.

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**CONCLUSION**

The present study found that high-NaCl diet did not cause weight gain. All propolis samples ameliorated high-NaCl diet-induced hypertension, where the biggest changes found in both group administered with propolis from Riau Archipelago and those with propolis from South Sulawesi. According to 24-h urine volume, propolis from Riau Archipelago and Lampung possessed diuretic effect. Increased urine protein was found in all propolis groups. Nevertheless, the other parameters (density, pH, consistency, leukocytes, nitrite, glucose, ketone, urobilinogen, bilirubin, erythrocytes, and haemoglobin) were not affected. Propolis from Lampung and South Sulawesi are seemed to improve LDL and HDL concentrations, respectively. Moreover, haematological parameters mainly did not change after the treatments.

In addition, we only found decrease in relative weight of liver in all groups administered with high-NaCl diet.

Our study suggests that the antihypertensive effect of Indonesian propolis samples through different pathways. Care should be taken in use of propolis among patients with proteinuria. Histopathological changes need further investigation.

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36 rats were divided into 6 groups (n = 36)

Blood pressure and body weight were weekly measured at baseline

Standard diet (SD) n=6

High NaCl diet (NaD) n=6

High NaCl diet + captopril (25 mg/ kg) (PD) n=6

High NaCl diet + Propolis from Riau (NaDP1) n=6

High NaCl diet + Propolis from Lampung (NaDP2) n=6

High NaCl diet + Propolis from South Sulawesi (NaDP3) n=6

Blood pressure and body weight were weekly measured during the intervention

Routine urine analysis, haematological parameters and lipid profiles at week 4 were determined

24 hours urine collected

Weight of organs Analysis

Haematological parameters and lipid profiles analysis

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SUMMARY

- High-NaCl diet-induced hypertension on Sprague Dawley rats.
- Indonesian Propolis Shown antihypertensive effect through different pathways.

ABOUT AUTHORS

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