The Sumbawa Forest Honey as Preventive Agent for Organ Damaged Caused by Environmental Pollution

Anna Roosdiana1*, Fajar Shodiq Permata2, Putri Stefy Graf2
1Department of Chemistry, Faculty of Mathematics and Natural Sciences, University of Brawijaya, Malang, Indonesia
2Faculty of Veterinary Medicine, University of Brawijaya, Malang, Indonesia
*Corresponding author: aroos@ub.ac.id

Abstract. Forest honey is a major commodity that contributes to the communities’ economies and many indigenous communities preserve their forests for sustainable honey production. Sumbawa forest honey, natural product resulted from Sumbawan forest, contains flavonoid with high concentrations, thus, this forest product can be used as antioxidant. The level pollution in our environment in the last twenty years has been a source of concern which needs a serious and tactical approach. One of the most important toxic pollutants in environment that can be accumulated in the body is lead (Pb). The lead poisoning leading to body organ damage. This research aimed to investigate the preventive effects of Sumbawa forest honey towards lead poisoning to the kidney damage, using animal models. The damaged on the kidney of rats as animal models, induced by lead acetate. This research used 20 rats, divided into 5 groups, which were negative control, positive control which received lead acetate 10 mg per rat/day for 14 days, and preventive therapy groups which administered the Sumbawa forest honey with 25, 50, and 75 mg/kg bw doses for 28 days, and simultaneously received lead acetate with 10 mg per rat/day on day 15th-28th. Levels of BUN (blood urea nitrogen) were measured using spectrophotometry methods, and kidney histopathological features were stained using HE which then observed using microscope. Results showed that the administration of Sumbawa forest honey reduces BUN levels and repaired the kidney damage, with the best dose of 75 mg/kg bw. These show that natural forest products can be utilized as natural cure for diseases related to the environmental pollution. Furthermore, results in the paper are intended to provide farmers and forestry stakeholders (including policy-makers) with the necessary information and motivation to consider forest honey as a forest product integrated in national forest programmes.

1. Introduction

Lead (Pb) is the most important toxic heavy element in the environment. Lead pollutants come from various sources like industrial processes like smelting of lead and its combustion, pottery, boat building, lead based painting, lead containing pipes, battery recycling, grids, arm industry, pigments, printing of book, and gasoline. Human and animals are exposed to lead via ingested food and drink, by breathing polluted air and dermal exposure. Lead is a highly poisonous element affecting almost every organ in the body [1].

Lead poisoning in livestock due to contaminated feed and drinking still often occur in Indonesia. The clinical symptoms of poisoning lead in animals includes gastroenteritis, anemia and encephalopathy [2]. lead content in chicken carcasses and also in meat carcasses beef cattle are still quite high, which is more than three times safe limit values [3,4]. Lead is mostly accumulated in liver,
kidney and meat tissue. White mice receiving lead at a dose of 10 mg orally for 14 days resulted in changes on the histopathologic features of liver [4].

Lead in cellular level is known to cause excessive production of reactive oxygen species (ROS) leading to cell oxidative stress [5]. Reactive oxygen species cause high reactions of lipid peroxidation, lipid membranes, proteins, and DNA. Human and animal body has multiple mechanisms especially enzymatic and non-enzymatic anti-oxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage. However, the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in the body and to protect from various diseases [6]. Honey is one of the good antioxidants which consists of phenolic acids and flavonoids compounds [7]. Honey has been used to prevent diseases, but no evidences to support their properties in detoxification of lead poisoning. Therefore, this research has been conducted to determine the preventive effect of honey towards the increase of the BUN levels and the damaged kidney histopathology of rats induced by lead acetate.

2. Experimental Methods

The animal model of male Wistar white rats were purchased from Institute of Biosains Brawijaya University, Malang. The protocols for animals' study were approved by Research Committee of Brawijaya University with a certificate of ethics No: 790-KEP-UB-2017. The materials used in this research were Sumbawa forest honey which purchased from Materia Medica, Batu City, East Java. Other materials were purchased from Sigma-Aldrich: ethyl alcohol (pure, d = 0.789 g/mL), glacial acetic acid (pharmaceutical secondary standard), HCl (37%, analytical grade), H$_2$SO$_4$ (99.99%, analytical grade), Pb(CH$_3$COO)$_2$.3H$_2$O (powder, 99.999%, analytical grade), NaCl (powder, ≥99.5%, analytical grade), sodium citrate solution, citrate buffer solution pH 4.5. Other material, PBS solution (Phosphate Buffer Saline), Hematoxylin, Eosin, 1% PBS-azide solution, BUN colorimetric detection kit (Thermofisher). Instruments used was Olympus BX51 microscope, microtome, UV-Vis spectrophotometer (1601, Shimadzu).

2.1. Preparation of Lead Poisoning Rats

The rats were adapted in the animal house for a week prior to experiment. Twenty rats were divided into five groups: (I) negative control group (without any intervention); (II) lead poisoning group; and (III, IV, V )lead poisoning and preventive therapy groups, 4 rats in each group. Lead acetate 1.6 g was dissolved in 16 mL H$_2$O. Group III, IV, V were administered Sumbawa forest honey in the doses of 25, 50, and 75 mg/kg bw doses, respectively for 28 days, and simultaneously administrated 1 mL lead acetate with 10 mg/rat/day on day 15$^{th}$ until 28$^{th}$. Lead poisoning group or positive control group was administered with 1mL lead acetate 10 mg/kg bw by gavage for 14 days. At the end of the assay, all rats were sacrificed, and the kidney organ was collected for further analysis.

2.2. Measurement of BUN levels

Blood samples were collected from the rats' hearts, then put into a 3 mL whole blood tube (plain tube). After that it was centrifuged with 5000 rpm for 10 minutes. Serum is taken using a 100 μl micropipette and inserted into the microtube. Next, an observation and measurement of Blood Urea Nitrogen (BUN) levels were used spectrophotometry [8].

2.3. Kidney histopathological observation

Histopathologic preparations of the rats’ kidney were made using the HE staining method. Histopathologic features of the kidney were visualized using the Olympus BX51 microscope with 400x magnification to observe the Bowman capsule widening, pyknosis and lysis of glomerular and interstitial oedema. Histopathology images were captured using a digital camera.
2.4. Statistical Analysis
The data of BUN levels were analyzed by Analysis of Variance (ANOVA), followed by Tukey test with $\alpha = 0.05$ using Microsoft Office Excel and Statistical Package for The Social Science (SPSS) version 21.0 for windows, while the observation of kidney histopathology changes were descriptive analyzed.

3. Results and Discussion
Urea is an end product of protein and amino acid catabolism that produced in liver and distributed through intra and extracellular liquid to blood circulation and followed filtration in Glomerular [9]. Measurement of serum urea is useful to evaluate the kidney function, hydration status, nitrogen equilibrium, progressivity of kidney disease and haemodialysis results. Generally, the increase of BUN levels indicates the decrease of kidney function. Table 1 present the BUN levels on rats in all groups in the end week of assay.

| Group                        | BUN levels (mg/dL)* |
|------------------------------|---------------------|
| I. Healthy rats              | 17.05 ± 1.72        |
| II. Pb poisoning rats        | 21.50 ± 0.76        |
| III. Pb + honey 25 mg/kg bw  | 20.75 ± 0.64        |
| IV. Pb+ honey 50 mg/kg bw    | 19.85 ± 0.73        |
| V. Pb +honey 75 mg/kg bw     | 17.60 ± 1.40        |

*different notations show a significantly different effect in each group ($p < 0.05$)

Based on the data on Table 1, it can be seen that BUN levels in group I was at a range of normal rats, 17.05 ± 1.72 mg/dL, while BUN levels in rats exposed to lead acetate were 21.50 ± 0.76 mg/dL. These show an increase of 26.10% when compared with healthy rats. The lead acetate enters the body via digestive tract and absorbed in small intestine entering blood circulation. Lead ion in blood circulation bind to erythrocytes and inhibit activity of oxidase enzymes. Lead is toxic to enzymes rich in sulphhydryl groups like ALA-dehydratase (ALA-D), ALA synthetase and heme synthetase. The inhibition of main enzymes of heme biosynthesis, delta aminolevulenic acid dehydrogenase (δ-ALAD) cause the increase of ALA substrates levels (aminolevulinic acid) in blood and either urine leading to produce reactive oxygen species (superoxide free radical and hydrogen peroxide [10]. The increase of ROS can activate NF-kB that can stimulate transcription of inflammatory gene in endothelial cells, smooth muscle and macrophage. Moreover, the increase of ROS lead to stress oxidative conditions and lipid peroxidation which trigger cell degeneration and organ damage especially kidney. Consequently, kidney releases a high concentration of urea to blood circulation.

The levels of BUN on rat receiving Pb and honey 25 mg/kg bw and 50 mg/kg bw (Group III and IV) did not show significant different compared to Pb poisoning rats (Group II). In contrast, BUN levels on rats receiving Pb and honey 75 mg/kg bw (Group V) showed significant different compared to Group II and closed with Group I.

The higher doses of Sumbawa forest honey as preventive therapy the lower BUN level on rats. The Sumbawa forest honey contains flavonoid as plant secondary metabolites. The flavonoid can act as antioxidant to scavenge free radicals and inhibit the formation of singlet oxygen (O₂⁻). Based on the structure of flavonoid, it can donate one hydrogen atom from hydroxyl group to free radicals to form stable radicals. The depletion of free radical can cause the decrease of oxidative stress that means the decrease of protein and lipid peroxidation leading to decrease of the damage of kidney cells and tissue as same as the BUN levels.

Lead poisoning and Sumbawa forest honey preventive therapy have repaired the damaged kidney as shown in the histopathologic image of rats kidney (Figure 1). Histopathologic changes of kidney features were observed from Bowman’s capsule widening, pyknosis and lysis of glomerulous and interstitial oedema.
Figure 1. Comparison of histopathologic features of rats from group I, II, III, IV and V with HE staining (400 × magnification): (A) group I; (B) group II; (C) group III, (D) group IV and (E) group V. (▲) indicates glomerular pyknosis, (▼) indicates Bowman’s capsule widening, (▶) glomerular lysis (◀) indicates interstitial oedema.

The condition of kidney in Group I (Figure 1A) showed normal arrangement of kidney nephron including glomerular, proximal convoluted tubules and distal convoluted tubules. In normal condition, protein cannot pass through the glomerular, but in pathologic condition, protein can pass glomerular [11]. Tubular cell functions to reabsorb and add chemical substances like uric acid, ammonia, creatine, hydrogen ion and some drugs to urine leading to body free of toxic substances. In glomerular dysfunction, foreign substances in abnormal level reach in tubules through Bowman’s capsule which
can cause degeneration and even death of epithelial cell. Proximal tubules have a main function to reabsorb of sodium, albumin, glucose and water that beneficial to bicarbonate reuse. The proximal tubular epithelium is the most frequently affected by ischemia or damage due to toxins and the increase of metabolic rate. Morphologically, glomerular damage is characterized by necrosis and proliferation of membrane cells and leukocyte infiltration. Functional glomerular damage is characterized by reduced blood flow perfusion, large numbers of protein and macromolecule release in the glomerular filtrate. Glomerular damage can also be secondary atrophy in the renal tubules [12]. Figure 1B is a histological image of a positive group, where the rats received lead acetate induction. At the glomerular there is a widening in the Bowman’s capsule, glomerular pyknosis, and interstitial edema of the renal tubules. The widening of the Bowman’s capsule is due to the occurrence of glomerular atrophy as a result of reduced cell number or reduced cell size. Furthermore, the apparent edema is due to a decrease of protein levels in body that can decrease the plasma osmotic pressure and the fluid can move from intravascular to interstitial.

Figure 1C displayed a histological picture of the first preventive therapy group with a honey dose of 25 mg/kg BW, that can be seen a widening the Bowman’s capsule and glomerular lysis. Lysis is the destruction of cells due to damage to the plasma membrane. Figure 1D is a histological picture of the second preventive therapy group with a honey dose of 50 mg/kg, that can be seen in the Bowman’s capsule widening has been reduced. In the preventive therapy group with a honey dose of 75 mg/kg bw showed improvement in the structure of renal nephrons, namely the glomerulus, proximal convoluted tubules and distal convoluted tubules. In addition, there is the disappearance of the Bowman’s capsule widening and glomerular pyknosis.

The decrease of BUN levels and improvements in kidney profile of rats induced lead acetate, therapies with honey can be associated with the content of antioxidant compounds in honey. Honey is rich in flavonoid compounds like luteolin, quercetin, apigenin, fisetin, kaempferol, ishoramnetin, acacetin, tamarixetin, chrysin, and galangin which can act as antioxidants [7]. These suggests that flavonoid contents in honey enable to suppress the presence of free radicals in order not to bind the lipid component of PUFA in kidney cells by removing the H atoms in the hydroxyl group (-OH). As a result, the free radicals will bind to atoms with high electronegativity. Moreover, flavonoid will balance the amount of oxidants and antioxidants in the body that play the role of protecting cells from free radicals.

4. Conclusion
The current study demonstrated that administration of Sumbawa forest honey that decreased BUN level in lead poisoning rats and improved histopathological profiles on the kidney damage. The flavonoid contained in honey may be responsible for prevention of the lead poisoning. This paper outlines the utilization of forest product, i.e. Sumbawa forest honey, that can contribute significantly to forest conservation. The arguments in the paper are important to provide forestry stakeholders in Indonesia with the information to consider forest honey production for one of the methods for forest conservations.

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