Preventive potency of sumbawa forest honey on rats exposed by lead acetate based on liver histopathology and AST-ALT level

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Abstract. Many cattle are kept in near garbage dump and eat waste product. The cattle will exposure free radical of lead. Sumbawa forest honey contains flavonoids as antioxidant that neutralize the excess free radicals in the body. This research aimed to examine the preventive potency of Sumbawa forest honey toward liver histopathology and AST-ALT level on rats induced by lead acetate. The subject research were rats Wistar, male and age 8-12 weeks. The rats divided in 5 different groups, those were negative control group; positive control group that exposed by lead acetate; and preventive therapy groups with Sumbawa forest honey dose of 25 mg/kgBW, 50 mg/kgBW and 75 mg/kgBW for 28 days and administered with lead acetate dose of 10 mg/day for 14 days. AST-ALT levels were measured by spectrophotometer then analyzed statistically by One Way ANOVA and Tukey test (p<0.05). Liver histopathology was examined descriptively on HE stained. The result showed the forest honey Sumbawa dose 75 mg/kg BW was able to prevent ALT-AST elevation significantly (p<0.05) and reduced the injury of liver tissue on rats exposed lead. The conclusion was the Sumbawa forest honey can be used as a preventative to inhibit the liver injury because of Lead exposure.

Keywords: Lead intoxicity, liver histopathology, AST-ALT, Sumbawa forest honey

1. Introduction
In modern era, free radicals are spread everywhere because of combustion such as smoking, cooking, burning fuel on engines and motor vehicles which containing lead [(1)]. The previous report showed regarding the case of heavy metal pollution such as lead (Pb) was carried out in 2006 on beef cattle in Semarang city landfill [(2)]. Whereas in 2013 Kafiar et al conducted research on the analysis of heavy metal pollution on beef cattle in the Putri Cempo Surakarta waste disposal site [(3)]. Both studies concluded that the Pb content was in small (low) concentrations in feed. According to Azwan, et al, feed containing heavy metals if consumed for a long time, there will be accumulation and stored in the body [(4)]. Excessive accumulation of heavy metals in the body of organisms can cause toxic effects in the long term.

Gildow states that the toxic and accumulative nature of lead can cause disorders in the body’s organs [(5)]. Lead can settle in the tissues of organs and cause interference in these organs. One of them is the liver which is an organ with a high metabolic rate in the human body, giving each other substrate and energy from one metabolic system to another, processing and synthesizing various substances that are transported to other areas of the body. The mechanism of liver damage caused by lead is that lead can
induce a certain level of free radical formation and decrease the ability of the antitoxin system so that oxidative stress can occur [(6)]. Extensive cell necrosis, will reduce the ability of the liver in the synthesis of enzymes and enzyme levels have an impact on Aspartat transaminase (AST) and Alanine transaminase (ALT).

Therefore it would be required to exogenous antioxidants (derived from food consumed) in greater amounts to reduce and neutralize the effects of increased free radicals in the body. Various natural ingredients native of Indonesia contain many antioxidants with various active ingredients. The use of natural ingredients such honey as antioxidants is needed to improve the quality of public health at a relatively affordable cost [(7)]. Sumbawa forest honey is one of the best forest honey in Indonesia. Sumbawa has a dry and hot geographical location which makes the water content contained in Sumbawa forest honey lower than other regions honey and has high activity as antioxidant [(8)].

The research aims were to examine the effect of Sumbawa forest honey to prevent liver damage and elevation of AST-ALT level of rats exposed lead.

2. Methods

2.1. Experimental Animal
Experimental animals in this study were male rats (*Rattus norvegicus*), Wistar strain, as many as 20 animals, weighing 150-200 grams. Animal feed and drink was given an ad libitum. The maintenance of test animals was carried out in a laboratory under controlled and constant conditions. The study animals were adapted for 1 week, then grouped into 5 groups and each group consisted of 4 rats. The groups were Group 1: negative control, Group 2: only lead exposed group, Sumbawa forest honey prevention groups 25 mg/kgBW (Group 3), 50 mg/kg (Group 4), 75 mg/kg BW (Group 5) continued lead exposure. The use of animals got certificate of ethical clearance from the research ethics commission of Universitas Brawijaya with No : 790 -KEP-UB..

2.2. Dose and Administration of Sumbawa forest Honey
Sumbawa forest honey was got from Sumbawa city. Administration of Sumbawa forest honey was conducted by a gastric tube. The first treatment group was given a dose of 25 mg / kg body weight, the second treatment group was given a dose of 50 mg / kg body weight and the third treatment group was given a dose of 75 mg / kg body weight, given for 28 days in a row start in the first day. Honey was diluted with distilled water until 1mL for each administration.

2.3. Administration Lead Acetate
For making lead intoxicity, we used Lead acetate (Pb acetate). The administration of Lead acetate to rats was carried out by gastric tube. Pb acetate was dissolved with 1 mL distilled water. Exposure with Pb acetate was 10 mg / rat/day for 14 days, from 15th until 28th day. The dose was reported could affect liver tissue becoming necrosis and degeneration [(9)]

2.4. Liver and blood collection
Rats were euthanized by cervical dislocation on the 29th day. Rat liver was collected and immersed in formaldehyde solution 4%. Rat blood collected by intracardial and centrifuged 15,000 rpm 15 minutes to separated serum and cells. Serum was preserved in -20°C until measurement of AST-ALT serum level.

2.5. Examination of Liver Histopathology
Liver tissue was processed to be histopathology slide with Hematoxylin-Eosin staining. The tissue processing were conducted by Paraffin method, and sectioned in 5µm thickness. Examination of Liver histopathology was observed descriptively comparing among groups about inflammation, degeneration and necrosis using 40x objective magnification.

2.6. Measurement of AST-ALT serum level
Measurement of AST and ALT serum levels was carried out by using the Horiba Pentra-C400 Auto Analyzer using AST and ALT reagent (ReiGed Diagnostics) respectively.

2.7. Data analysis
Quantitative data including AST and ALT level were analyzed statistically with One Way ANOVA and continued with Tukey Test (p<0.05) comparing among groups. The liver histopathology was analyzed descriptively comparing among groups.

3. Result and Discussion
3.1. Liver histopathology
Rat liver of negative control group showed the hepatocyte normal with polygonal shape and round nucleus. There was looked normal sinusoid also among hepatocyte (Figure 1).

Figure 1. Rat liver of negative control groups showed normal liver tissue. A hepatocytes were formed radially from centralis vein (blue arrow) and there was sinusoid among hepatocyte (green arrow). B. Hepatocyte was looked polygonal shape and round nucleus (HE, A. 400x magnification, B. 1000x magnification).

While giving Lead exposure, liver tissue got inflammatory cell and hepatocytes became hydrophic degeneration and coagulative necrosis (Figure 2)
Figure 2. Liver rat of Pb exposed showed hepatitis and abnormalities of hepatocyte. A. There were infiltration of inflammatory cells to liver (purple arrow), hepatocyte were hydrophic degeneration (red arrow) and nucleus hepatocytes were coagulative necrosis. B. Hepatocyte showed clear cytoplasm and nucleus looked fragmentation (karyorhexis), C. Infiltration of mononuclear inflammatory cells; (HE, A. 400x magnification, B and C. 1000x magnification)

Rat of Prevention of Sumbawa forest honey dose 25 mg/kg BW groups has a liver which still showed hydrophic degeneration (clear cytoplasm) and coagulative necrosis but there was not inflammation (Figure 3).

Figure 3. Liver tissue Group 3 showed that still has hydrophic degeneration with clear cytoplasm (red arrow), and coagulative necrosis (orange arrow, A) which signed karyorhexis (B) and karyopiknosis (C) (HE, A. 400x magnification, B and C. 1000x magnification)

The giving Sumbawa forest honey dose 50 mg/kg BW as prevention before Pb exposure showed that there were coagulative necrosis but reduce to amount of hydrophic degeneration of hepatocytes (Figure 4).
Figure 4. Liver tissue of group 4 showed the reduction of hydrophic degeneration (A, green arrow) and still many coagulative necrosis (B, blue arrow) (HE, A. 400x magnification, B. 1000x magnification)

The liver tissue of group 5 (prevention of Sumbawa forest honey 75 mg/kg BW) showed the repair of hepatocytes becoming normal, although a few of them still showed hydrophic degeneration, but the nucleus looked normal (Figure 5).

Figure 5. Liver tissue of group 5 showed hepatocyte back to normal (red arrow), and there was sinusoid among hepatocytes (green arrow) (HE, A. 400x magnification, B. 1000x magnification)

3.2. AST and ALT serum level
The administration of Pb acetate induced significant elevation of AST-ALT level (p<0.05) (Table 1 and 2). Otherwise, the prevention using Sumbawa forest honey before and along Pb exposure could prevent the elevation AST-ALT level. The significant reductions (p<0.05) of AST-ALT level were found in Group 4 (14.12% and 23.18%) and group 5 (23.91% and 48%). However, none of prevention group could make AST-ALT level become normal level (Table 1 and 2).

Table 1. Average AST serum level among the groups (Means±SD)

| Groups                                   | AST blood level (U/L) | AST blood level % Elevation Reduction |
|------------------------------------------|-----------------------|--------------------------------------|
| Negative control (Group 1)               | 53.50 ± 1.29a         | 62.14                                |
| Positive control (Group 2)               | 86.75 ± 1.71d         |                                      |
| Preventive 25 mg / kg BW of Sumbawa forest honey (Group 3) | 84.25 ± 1.71d         | -                                    |
| Preventive 50 mg / kg BW of Sumbawa forest honey (Group 4) | 74.50 ± 1.29c         | -                                    |
| Preventive 75 mg / kg BW of Sumbawa forest honey (Group 5) | 66.00 ± 1.83b         | -                                    |

Notes: Different on superscript notation was a significant difference (p<0.05)

Table 2. Average ALT serum level among the groups (Means±SD)

| Groups                                   | ALT blood level (U/L) | ALT blood level % Elevation Reduction |
|------------------------------------------|-----------------------|--------------------------------------|
| Negative control (Group 1)               | 20.75 ± 1.32a         | 89.15                                |
| Positive control (Group 2)               | 39.25 ± 2.22d         |                                      |
| Preventive 25 mg / kg BW of Sumbawa forest honey (Group 3) | 36.12 ± 1.83b         | -                                    |

Notes: Different on superscript notation was a significant difference (p<0.05)
Preventive 50 mg / kg BW of Sumbawa forest honey (Group 4) 30.15 ± 0.06c - 23.18
Preventive 75 mg / kg BW of Sumbawa forest honey (Group 5) 26.52 ± 1.63b - 48.00

Notes: Different on superscript notation was a significant difference (p<0.05)

3.3. Preventive potency of Sumbawa forest honey on Liver Tissue

Lead acetate is an organic salt with the chemical formula Pb will be hydrolyzed when dissolved in water. The hydrolysis process forms positive ions and negative ions. Pb will be ionized in the solution into cation that Pb2+ and anion C2H3O2-. Pb2+ cations have free atoms in their outer layers. Pb2+ turns into free radicals because the free atom is trying to complete the outer layer to be more stable by binding to other molecules of organs. Pb2+ in the liver will try to complete the outer layer by binding to the PUFA (Polyunsaturated Fatty acid) liver cell membrane to initiate cell membrane lipid peroxidation. This peroxidation will affect membrane fluidity, membrane cross-linking, and membrane structure and function [(10)]. After reacted with membrane cell, sodium and potassium canal of membrane cell disrupted and induce water of extracellular goes to intracellular and cause hydrophic degeneration. Lead also induced coagulative necrosis cells which nucleus looked fragmentation and then inflammatory cell would come in necrotic area.

The liver tissue of rats in prevention Sumbawa forest honey groups showed an improvement. The best improvement was in group 5, there was no visible hydrophic degeneration and infiltration of inflammatory cells, but the sinusoid was not fully visible. Although the reduction in damage has not reached the normal histopathological picture, but the results of this study showed improvement. Therefore, administration of Sumbawa forest honey with a dose of 75 mg / kg body weight can reduce and reduce the level of damage to cells caused by exposure to Lead acetate. Administration of Sumbawa forest honey as preventive can reduce and neutralize free radicals in the body. Sumbawa forest honey is an antioxidant that contains polyphenol compounds such as flavonoids [(8)]. Flavonoids are able to inhibit oxidation reactions through the mechanism of radical scavenging by donating one electron to unpaired electrons in free radicals so that the number of free radicals is reduced [(11)]. Flavonoids are also thought to have an effect on inhibiting liver damage by binding to free radicals so that their impact on the liver is reduced. Therefore lead could not react with PUFA so that the degeneration and necrosis was significant reduction in dose of 75 mg/kg body weight.

3.4. Preventive potency of Sumbawa forest honey on AST-ALT level

Based on statistical analysis it is known that the induction of Lead acetate can significantly increase AST and ALT levels (p< 0.05). This is due to an increase in oxidative stress due to exposure to Lead acetate. AST-ALT enzyme is a specific enzyme to detect liver damage. This enzyme normally present in the cytoplasm of liver cells, will but this enzyme would come out to the extracellular fluid when there is disruption of membrane permeability. Membrane leakage can occur due to high concentration between extracellular and intracellular environments [(10)]. Tukey test showed that the AST-ALT levels of the positive control group (Lead exposure) were significantly different from the negative control group. Under normal circumstances the levels of the AST and ALT enzymes in the blood are low because they are present in cells, but if there is tissue damage, the cells will rupture and the enzymes will break down from hepatocytes into the circulatory system, so their levels in the blood will increase compared normal state [(12)].

Sumbawa forest honey contains antioxidants in the form of flavonoids that can reduce levels of AST and ALT in the blood. Flavonoid components from Sumbawa forest honey could be carried in the bloodstream and circulate with plasma, as well as antioxidant components contained in Sumbawa forest.
honey. The antioxidant compounds of this flavonoid group will enter the body's cells, stabilizing free radicals by donating hydrogen atoms [(11)]. The flavonoid of honey could prevent lead to react with PUFA of hepatocyte cell membrane. Therefore, AST-ALT to elevation high in prevention of Sumbawa forest honey.

The administration of Sumbawa forest honey at a dose of 75 mg / kg BW as preventive shows the highest decrease in AST and ALT to near negative control group, so the use of Sumbawa forest honey as a dose of 75 mg / kg BW as a preventive is the best dose of Sumbawa forest honey in this research.

4. Conclusion

The research concluded that the Sumbawa forest honey could to prevent liver damage based on histopathology description and elevation of AST-ALT serum level.

5. References

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