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Sub-chronic Toxicity of *Ficus benjamina* L. Leaves Ethanol Extract on The Liver Function of White Mice

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**Abstract**

Sub-chronic toxicity studies of ethanol extracts of *Ficus benjamina* L. leaves on liver function of white mice at doses of 200, 400 and 800 mg/kg body weight had been done. The weight ratio of liver, the serum alanine aminotransferase as well as aspartate aminotransferase activities were evaluated on day 61st after being treated daily for 60 days with the ethanol extract. The treatment was divided into four groups of white mice. The results showed that ethanol extract of *Ficus benjamina* L. leaves could increase the serum aspartate aminotransferase activity significantly (p<0.05) 60 days at doses of 400 and 800 mg/kg body weight and could increase the serum alanine aminotransferase activity significantly (p<0.05) after 60 days at doses of 800 mg/kg body weight. The weight ratio on liver of white mice were not significantly (p>0.05) affected by this extract after 60 days application.

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1. **Introduction**

Leaves of *Ficus benjamina* L. has been demonstrated containing some chemical components such as cinnamic acid, naringenin, and quercetin lactose. Its fruit contains caffeic acid, its bark contains stigmasterol, while its root bark contains benjaminamide. The chloroform extract of *Ficus benjamina* L. leaves (var *comosa*) was reported containing serrat-3-one, pentacontanyl decanoic, friedeline, β-sitosterol, (9,11), (18,19)-disecoolean-12-en-28-oic acid and β-amyrin. Flavonoids, including quercetin 3-O-rutinoside, kaempherol 3-O-rutinoside and 3-O-kaempherol robinobioside were isolated from its chloroform extract.

*Ficus benjamina* L. has been used traditionally in Indonesia as medicine for influenza, inflammation of the airways (bronchitis), whooping cough (pertussis), malaria, acute enteritis, dysentery, and hot seizures in children.
Biological activities of fruit extract of *Ficus benjamina* L. showed cytotoxic against T-Lymphoblastic Leukemic (CEM-SS) cell line, antitumor, and antibacterial activities\(^1^,\)\(^6\). The leaves extract showed antibacterial, antiviral against Virus Herpes Simplex 1 and 2\(^3^,\)\(^4\), antinociceptive and analgesic activities\(^7^,\)\(^8\). LD\(_{50}\) Test Delayed 24 hours and toxicity assay showed that the 24-hour LD\(_{50}\) value of ethanol extract of *F. benjamina* L. leaves was > 16 g/kg bw and classified as practically non-toxic\(^8\). In consideration of various biological activities of this plant, continued sub-chronic toxicity tests need to be investigated which were conducted for 60 days. Research of its sub-chronic toxicity on kidney function has been conducted, and the result showed that ethanol extract of leaves of this plant inhibited no toxicity effect on kidney function of animal model used\(^9\). In this study sub-chronic toxicity were performed to observe the toxicity of ethanol extract of *Ficus benjamina* L leaves on liver function of white mice.

2. Experiment

2.1. Material

Adult white mice strain DYY 2-3 months old, weighing between 20-30 grams were used for the study. The materials used: *Ficus benjamina* L leaves, ethanol, NaCMC and commercial kits for alanine aminotransferase (ALT) and aspartate aminotransferase AST (Diasys\(^\text{®}\))

2.2. Preparation of extract

*F. benjamina* L. leaves were collected from Pekanbaru, Riau Province, Indonesia. 1 kg of samples were extracted by maceration method using 96 % ethanol in a dark bottle. The sample was soaked for 5 days at a place protected from light while stirring repeatedly. The extract was filtered and the residue of sample was re-macerated in the same way until macerate produced translucent color. Furthermore the filtrate was concentrated using a rotary evaporator. The yield of the extract was 96.108 g based on dry weight. The ethanol extract obtained was then subjected to sub-chronic toxicity test.

2.3. Sub-chronic Toxicity Test

The mice were weighed and randomly assigned into four groups (5 per group). Ethanol extract of *F. benjamina* L leaves was prepared as suspension in 1 % solution of Na CMC. The suspension was administered orally to each group of mice with doses of 200, 400, and 800 mg/kg bw. The injection volume of suspension given was 1 % of body weight of animal and administered daily for 60 days. As for control group, animals was given only 1 % solution of Na CMC. The weight ratio of liver, serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were evaluated on day 61\(^9\).

Determination of AST and ALT activities was performed accordingly the method of International Federation of Clinical Chemistry (IFCC) using spectrophotometer (Microlab 200) at wavelength of 340 nm at temperature of 37° C. The treated and control animals were sacrificed on day 61st by cutting blood vessel on the neck. The blood was then collected into test tube and allowed to stand for 15 minutes. The blood sample was centrifuged for 20 minutes with speed of 3000 rpm. The serum was measured using spectrophotometer in order to determine their activity (IU/L) levels of ALT and AST. Liver of mice were taken out, weighed and then ratio of organ weight of liver was calculated.

2.4. Data analysis

The ALT, AST activity and ratio of organ weight results were analyzed using One Way ANOVA followed by Tukey HSD Pos Hoc Test. The data were compared with control data and mean difference is significant at \(P<0.05\) level.
3. Result and Discussion

Mice were randomly placed in groups (n=5). After 60 days of treatment, its AST and ALT activity as well as liver weight ratio were determined. The results showed that AST activity of the control group mice was 90 IU/L, while AST activity of group of treated animals with doses of 200, 400, and 800 mg/kg bw was 108; 124.8; and 142.8 IU/L, respectively (Figure 1).

![Figure 1. AST activity of administration of ethanol extract of Ficus benjamina L leaves with various doses.](image_url)

This present study showed that administration of ethanol extract of *Ficus benjamina* L leaves gave an increasing activity of AST significantly \( (P<0.05) \). The AST activity was significantly difference between control group with treated groups with doses of 400 and 800 mg/kgbw. It showed that ethanol extract of *F. benjamina* L. leaves for 60 days application, can increase activity of the enzyme. The increased AST activity also occurred at dose of 200 mg/kgbw, but it was statistically insignificant \( (P>0.05) \). This data suggested that ethanol extract of *Ficus benjamina* L leaves can damage the liver, since the AST activity determination is one of most sensitive assay for assessing liver cell injury. In a state of chronic hepatotoxic, enzymes of AST and ALT can reach 5-10 times the normal state. Increased activity of AST and ALT in serum occurred in the liver tissue, the enzyme is considered as a typical indicator for liver damage. At onset of acute damage of liver, AST and ALT level can increase up to 5 times normal level. Increased levels of AST higher than ALT should be suspected as a sign of malignant transformation.

As for ALT activity, serum of mice of control group showed ALT activity of 37.2 IU/L. The ALT activity of group of treated animals with doses of 200, 400, and 800 mg/kg were 42; 48; and 67.2 IU/L, respectively. The result was shown in Fig. 2. Increased in alanine aminotransferase activity was significantly difference \( (p<0.05) \) between all groups.

![Figure 2. ALT activity of administration of ethanol extract of Ficus benjamina L leaves with various doses.](image_url)

Alanine aminotransferase activity in the blood plasma differs significantly between control and dose of 800 mg/kg. ALT is present in heart muscles, liver, muscles of the body, kidneys and pancreas. This enzyme is mainly localized in the mitochondria and little in the cytoplasm. Number ALT whole lot more than AST and mainly found in the cytoplasm. ALT and AST localization within the cell affect the degree of liver damage. At the heart of damage shown that acute and severe levels of AST and ALT equally increased due to the great damage both to the cell membrane and mitochondria. In light damage mitochondria were intact so that the levels can exceed AST and ALT. AST and ALT activity measurement is method used in detection of abnormalities of liver function. Where the activity of both enzymes is generally increased when there is hepatic necrosis. AST values higher than the results of this study demonstrate the occurrence of damage to the hepatocyte cell mitochondria.

The ratio of liver weight of control group of male white mice was 0.0319. While the ratio of liver weight in the group given the extract at a dose of 200, 400, and 800 mg/kg was 0.0354; 0.0359 and 0.0406 respectively, as shown in Figure 3.
Figure 2. ALT activity of administration of ethanol extract of Ficus benjamina L leaves with various doses.

Figure 3. Ratio of liver weight of administration of ethanol extract of Ficus benjamina L leaves with various doses.

Ratio of liver weight is consistent and highly sensitive value. It is important indicator of toxicity. Liver is often become target organ for most of toxicant enters the body through gastrointestinal system, and after toxicant absorbed carried by the portal vein to the liver. Based on this sub-chronic toxicity test afforded that ethanol extract of Ficus benjamina L leaves did not affect weight of liver and it was statistically significant \((P > 0.05)\). Liver is the organ that has the ability to restore large cell damage. And liver cytochrome P 450 enzymes in large quantities, which can metabolize foreign substances in the body, to make the most of toxicant to be less toxic and more soluble in water.

4. Conclusion

Study on sub-chronic toxicity of ethanol extract of Ficus benjamina L leaves showed that ethanol extract of Ficus benjamina L. leaves could increase the serum aspartate aminotransferase activity significantly \((p<0.05)\) 60 days at doses of 400 and 800 mg/kg body weight and could increase the serum alanine aminotransferase activity significantly \((p<0.05)\) after 60 days at doses of 800 mg/kg body weight. The weight ratio on liver of white mice were not significantly \((p>0.05)\) affected by this extract after application 60 days.

References