The Effect of Ethanol Extract from Binahong Leaves (Anredera Cordifolia (Ten.) Steen) on Histopathology Changes in Female Wistar Rats

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The Effect of Ethanol Extract from Binahong Leaves (*Anredera Cordifolia* (Ten.) Steen) on Histopathology Changes in Female Wistar Rats

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ABSTRACT

The aim of this research is to study of the effect of ethanol extract of binahong leaves *(Anredera cordifolia* (Ten.) Steen) on histopathology changes in female wistar rats. Animal tests were selected using a complete randomized block design. The method to determine the dose of the extracts was according to the OECD guideline 420. Doses used in the sighting study were 300 mg/kg and 2000 mg/kg, in this test there is no death in animal test trials. Animal test in main study divided into 2 groups. Each group consisted of 5 animals tested, which is the treatment group was given a dose of 2000 mg/kg and the control group was given distilled water per oral. Each of the liver tissues and kidneys tissues were processed by paraffin block-embedded and hematoxylin eosin staining method. There were biochemical test (ALT. AST. urea) and organ weights relative to support histopathology data. The results analysis Independent T test show that ethanol extract of binahong leaves did not affect the value of histopathology (liver and kidneys). Histopathology showed the presence of fat degeneration in liver tissues on all of the treatment group and control group, whereas the results histopathological in kidneys tissues on all of the treatment group and control group were normal. The conclusion is ethanol extract of binahong leaves (*Anredera cordifolia* (Ten.)Steen) between dosage 300 mg/kg to 2000 mg/kg no significance toxicity effect on the liver and kidneys of rat (wistar).

Key words: *Anredera cordifolia* (Ten.) Steen, ethanol extract, histopathology, toxicity.

INTRODUCTION

Binahong leaves (*Anredera cordifolia* (Tenore) Steen.) is one of the plants that can be used as a medicine. According Manoi, benefits binahong leaves can be used to cure several diseases including for the treatment of burns, typhus, colitis, ulcers, vaginal discharge, swelling of the liver, heart swelling, increase vitality and endurance (Manoi, 2009). Wardhani and Sulistyani (2012) has also proved that the ethyl acetate extract of the binahong leaves contains polyphenols, which have antibacterial effect against *Shigella flexneri* 8% w/v. Ointment of extract binahong leaves been investigated by Paju et al. (2013), can also heal wounds infected by the bacteria *Staphylococcus aureus* at concentrations of 20% and 40%, and produced more effective healing. Extract of binahong leaves at a dose 50, 100, and 200 mg/kg body weight can reduce levels of creatine that can improve renal failure (Sukandar et al., 2010), in addition to the concentration of 4.25 mmol/100 g (fresh) and 3.68 mmol/100 g (dried) leaves this binahong have antioxidant activity (Selawa et al., 2013). Binahong leaves activity has been widely reported as already described above, but the scientific information regarding the dosage and safety of ethanol extract of binahong leaves not reported. Observations histopathology and blood biochemistry is part of

the oral toxicity test method is based on OECD. Therefore, researchers interested in examining changes in hematology of test animals after administration of ethanol extract of binahong leaves orally.

MATERIALS AND METHODS

The materials needed in this observation were binahong leaves. The chemical materials needed were: ethanol 70%, aquadest, NACl 0,9%, Na-CMC, ether, formaline 10%, rats food, EDTA, reagent enzyme AST/SGOT (buffer Tris pH 7,8, L-aspartat, 2-oksoglutarat, laktat dehidroginase, malat dehidrogenase, and NADH), reagent enzyme ALT/SGPT (buffer Tris, L-alanin, 2-oksoglutarat, LDH, NADH), reagent urea (buffer Tris pH 7,8, 2-oksoglutarat, ADP, Urease, GLDH, and NADH), hematoxylin eosin. The instrument used in this observation was laboratory glass instrument (pyrex), a set of surgical rotary evaporator, animal pair of scales, oral hypodermic needle 1 ml and 5 ml with sensitively 0,1 ml, sifter no 20, hot plate, and BA-80 semi-auto the mistry analyzer, sentrifuge (eppendorf), micropipette. The animal observation used was female wistar rats age 3-4 month with weight 160-200 g and in health condition.

The design of the study is randomized block design, in which test animals were randomly selected and have the same opportunities to be given treatment. The method to determine the dose of the extracts was according to the OECD guideline 420. The method of observation used was experimental method in laboratory with some steps as follow:

a. Preparation of extract was done by using remaserasi (immersion repetitive), the binahong leaves soaked in 70% ethanol with 1:10 comparing.
b. Animal prepare were stable and food. Good condition of stable for experimental was in 22 °C (± 3 °C), 30% - 70% wet and radiance cycles 12 hour (OECD, 2001) total of food consumption 200 g for every rats. During the testing period, all tested animals was given fed and watered each as much as 2000 mg and 2000 mL of water for 1 week (Halim et al., 2011).
c. Preface test (sighting study) with accounted the total of rats death along 24 hours (OECD, 2001).
d. Main study with observation along 14 days (OECD, 2001) Include toxic effect indication, weight, hematology, biochemistry, organ weight, and heart and kidney histopathology test.
e. Data analysis systematically used paired T-test to compare rats in group item and independent T-test to compare control group and the treatment in one time.
f. The method on histopathology in liver tissues and kidneys tissues were processed by paraffin block-embedded and hematoxylin eosin staining method.

RESULTS AND DISCUSSION

Extract ethanol of binahong leaves was made by using the method remaceration with 70% ethanol. The filtrate of maceration results was evaporated using evaporator until viscous and evaporation followed by using the water bath until no smell of ethanol. Extracts obtained viscous greenish-black with a weight of 57.29 g and the final yield obtained 32.198% of the dry powder.

3.1 Sighting Study

There was not information about toxicity binahong leaves, so this sighting study started from dose 300 mg/kg WB with using 3 rats per oral (OECD, 2001). The indication observation of toxic effect in sighting study along 30 minutes followed every hour until 8 hours along 24 first hours (OECD, 2001). It was not found toxic effect indication and animal death test, because there was not death of rats in dose 300 mg/kg WB, so sighting study was done again with dose 2000 mg/kg BW (OECD, 2001) using 3 rats and after it was observed again along 24 hours. It was not found there was death of animal test and toxic effect indication.

3.2 Main Study

From sighting study, it had teen got highest dose was 2000 mg/kg WB that it could not cause of death animal test so that dose was used in main study with observing along 14 days (OECD, 2011), control group Na–CMC 1%, and treatment group binahong leaves ethanol extract dose 2000 mg/kg WB, each of group used 5 rats. Observation along 14 days include weight, biochemical, organ weight, and heart and kidney histopathology.

3.3 Weight

Along of 14 days observation, rats was scaled every day to know the change of weight happened. Based on Table 1, there was not change significant to inter treatment group binahong leaves ethanol extract dose 2000 mg/kg WB.

Table 1. Analysis result independen T-Test of weight control group and treatment group

Time	Weight (g)		P value
	Control	Treatment	
D-1	139.5±16.78	*153.8±22.40*	0.287
D+7	169.8±10.65	*184.1±17.65*	0.162
D+14	168.8±16.57	*188.1±6.73*	0.043

Control group (Na-CMC 1%) and treatment group (ethanol extract of binahong dose 2000 mg/kg WB) store was expressed with average ± deviation standard, n= 5 (p<0.05) significant score.

Table 2. Analysis result paired T-Test of weight inter-treatment

Time	Treatment	P value
D-1 vs D+7	*-30.30±36.09*	*0.134*
D-1 vs D+14	*-34.31±28.36*	0.054

Treatment group (ethanol extract of binahong dose 2000 mg/kg WB) store was expressed with average ± deviation standard, n= 5 (p< 0.05) significant score.

Based on Table 1. average score of weight in treatment group and control group (p>0.05), there was not different that full of meaning. This case showed extract of binahong leaves didn't have any influence to the animal test weight. The average store of weight in treatment group and control group got increase the weight in H+1 and H+7 and got decrease with H+7 in control group. Based on Sihombing (2011) that according to correlation analyzing result and linear regression was got by relation between age with the increasing weight of rats. One of the important factors in increasing rats weight except the age was food. Food composition (stock diet) must be controlled well. In faraway it has not found the result about avoiding of ethanol extract of binahong leaves to rats weight.

3.4 Biochemical Test

Before testing of blood biochemical, animal test was fasting first. In scaling of GPT enzyme activity, GOT enzyme and Urea were used blood plasma. Blood was taken from rat tail arthery and was patched in a tube content of EDTA then it was be centrifuge with 3000 rpm speed along 10 minutes. Adding anticoagulant was to avoid shock and after be separated with centrifuge was got plasma.

Table 3 show the average value of GPT test animal control and treatment groups before treatment (H-1), 21.14 U / L and 33.3 U / L, respectively. The average value was still normal as mentioned Fukuda et al. (2004) GPT values in female Wistar rats. Wistar strain aged 2 months to 6 months were 18.7 to 31.6 U / L. Results of statistical test T unpaired on D+ 1 and D+7 in control group can be seen an increase in the average value of GPT enzyme activity, whereas the activity of the enzyme D+14 GPT decreased. Furthermore, in the treatment group D+1 an increase in the average value GPT and D+7 looks for a decline, and its value remains at D+14. Despite the increase and decrease in the average value of GPT, figures show the value of P>0.05. In the paired T test also showed there were no significant changes in enzyme activity (p>0.05). It can be concluded that the increase and decrease in value of GPT is not a result of ethanol extract of binahong leaves.

GOT average value of test animals on D-1 was 66.44 U/l (see Table 3) for control group and 85.94 U/l for the treatment group. According to Fukuda et al. (2004) normal value on the activity of the enzymes GOT wistar strain female rats aged 2-6 months ranged from 51.9 to 97.5 U/l. So the value of GOT is still said to be normal. At D+1 and D+7 control group increased the enzyme activity of GOT and decrease in D+14. Whereas, the treatment group increased GOT activity in D+1 and a decrease in the D+7 and D+14. Despite the increase and decrease in enzyme activity GOT, but according to a statistical calculation, it is not caused by the ethanol extract of leaves binahong (p>0.05), as in the control group also showed a similar thing and the results of paired T test showed there were no changes in the activity of the enzyme GOT significant in both treatment groups before treatment and after treatment (P>0.05). The

increase and decrease in enzyme activity GPT and GOT can be caused by body weight of test animals, the occurrence of hemolysis, biochemical reactions, physics or chemistry, physiology and macro differences of individual enzymes, and the test animals stress from the test preparation pencekokkan (Girindra, 1989),

Table 3. Analysis result *independent T-test* blood biochemical control group and treatment group

	D-1	D+1	D+7	D+14
GPT (U/l)				
control	21.14±2.79	29.9±5.46	35.26±14.5928	20.7±4.17
Treatment	33.3±21.96	38.5±17.81	56±5.08	28.98±8.13
P value	0.285	0.331	0.361	0.078
GOT (U/l)				
control	66.44±14.8	98.16±1.19	139.8±41.91	94.78±18.12
Treatment	*85.94±24.1*	118.2±40.8	107.3±21	99.26±23.32
P value	*0.162*	0.337	0.163	0.743
Urea (mg/dL)				
control	34.80±9.88	31.20±3.67	31.40±3.91	28.20±5.54
Treatment	36.80±14.2	43.40±29.33	27.60±4.00	34.00±5.03
P value	0.803	0.388	0.329	0.862

Control group (Na-CMC 1%) and treatment group (ethanol extract of binahong dose 2000 mg/kg WB) score was expressed by average ± deviation standard, n=5 (p<0.05) significant score.

Table 4. Analysis result paired T-Test inter group treatment

	D-1 vs D+1	D-1 vs D+7	D-1 vs D+14
SGPT	*-5.22±20.31*	4.74±24.47	*4.32±24.60*
P value	*0.596*	0.687	*0.715*
GOT	*-3.25±53.30*	-21.44±27.61	*-13.32±39.28*
P value	*0.244*	0.158	*0.491*
Urea	*-6.60±24.52*	*9.20±12.15*	2.80±15.03
P value	*0.580*	*0.166*	0.699

Treatment group (ethanol extract of binahong dose 2000 mg/kg WB), score was expressed by average ±deviation standard, n=5 (p<0.05) significant score.

Levels of urea Wistar strain female rats aged 2-6 months according to Fukuda et al. (2004) is 17 to 19.7 mg / dL. The average of urea levels in treatment group and control group was greater than normal. Paired T test results there is no significant difference in urea concentration between the control group and the treatment group (p>0.05), resulting in increased levels of urea is not caused by the ethanol extract of binahong leaves. Paired t test (P>0.05), which means that the ethanol extract of binahong leaves not affect changes in blood urea values in the test animals before and after treatment. Increased urea in feed test animals suspected of containing a lot of protein, thereby increasing levels of urea. Protein is needed or not needed by the body to be metabolized by the liver. Proteins that are not needed by the

body to be broken down into amino acids. Amino acids are broken down into urea. Amino acids are high because of the high protein consumption then excreted urea levels will increase (Almatsier, 2006).

3.5 *Organ Weight*

After 14 days doing observation, animal test was anaesthetized with ether, and then was surgical using sterile surgical instrument and taken liver organ and kidney to be scaled. Relative organ weight in percentage was accounted by absolute organ weight divided weight. The average weight of the liver in the control group and the treatment group was 6.52 grams and 6.58 grams of the relative liver weight control group was 3.89% and the relative weight of the treatment group was 3.49%. According to Popp and Cattley (1991), normal relative liver weight was 3.5% - 4.0%. so the weight of liver treatment group was still within the normal range. This shows that the ethanol extract of binahong leaves not affect liver weights.

The result of weight analysis in Table 5, showed that relative weight of rats liver and kidney in control and treatment group was still normal (P>0.05), it means that there was no meaning full different between both group.

Table 5. Analysis result independent T-Test weight organ rats in control group and treatment group

Organ	Organ Weight (g)		P value
	Control	Treatment	
Liver (g)	*6.52±0.35*	6.58±1.09	*0.100*
Relative weight (%)	*3.89*	3.49	
Kidney			
Right	*0.55±0.06*	*0.57±0.05*	*0.647*
Relative weight (%)	*0.32*	*0.30*	
Left	*0.52±0.08*	*0.56±0.05*	*0.411*
Relative weight (%)	*0.30*	*0.29*	

Control group (Na-CMC 1%) and treatment group (ethanol extract of binahong dose 2000 mg/kg WB), score was expressed by average ± deviation standard, n=5 (p< 0.05) significant score.

3.6 *Histopathology*

This observation took 3 samples of liver and kidney organ from each treatment and control group as representative to histopathology test. Histopathology of liver tissue and kidney tissue of rat used method hematoxiline and eosin painting. Based on Table 6. it got that control group and treatment didn't happen pathology alteration of kidney. Meanwhile, in liver there was fatty degeneration in both group.

Table 6. Analysis result blood smear histopathology liver organ and kidney

Code	Liver	Kidney
Control Group		
F6	*FD++*	NCP
F7	*FD+*	NCP
F9	*FD+*	NCP
Treatment Group		
F1	*FD+*	NCP
F4	*FD++*	NCP
F5	*FD+*	NCP

Control group and treatment group ethanol extract of binahong leaves dose 2000 mg/kg WB, NCP: No Change of Pathology, FD: Fatty Degeneration.

Fatty degeneration seen in the histopathological results not because the value of GOT and GPT. Because the value of GOT and GPT by Sass et al. (2005) did not significantly affect the fatty degeneration, but occurrence of the effect is the ratio of GOT to GPT. Fatty degeneration is an abnormal accumulation of fat in the parenchymal cells contained in the cell cytoplasm. Fatty degeneration is characterized by the presence of fat vacuoles or water (hydropic) in the cytoplasm with large and small sizes, so that the edge of the cell nucleus to the cytoplasm pressed fat vacuoles that can be seen microscopically (Robbins and Kumar, 1992). Fatty degeneration due to interruption of hepatocytes (diet or toxin) that causes an imbalance of fatty acid absorption rate and secretion as VLDL (Very Low Density Lipoprotein) in the systemic circulation. This causes the inhibition of the formation of lipoprotein accumulation of triglycerides in the liver parenchyma which causes fatty degeneration (Popp and Cattley, 1991). Fatty degeneration caused by a toxin, protein malnutrition, diabetes mellitus, obesity, and anoxia (Suhita, 2013).

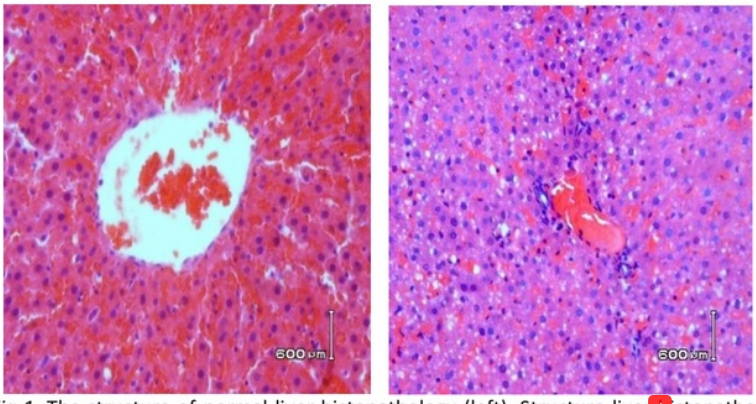

Fig 1. The structure of normal liver histopathology (left). Structure liver histopathology fatty degeneration (right) is characterized by the vacuole-vacuoles of various sizes with clear boundaries cytoplasm and nucleus appears some urgency to the edge.

Ethanol extract of binahong leaves contains flavonoids and saponins. Based on previous studies, these compounds should be able to lower blood cholesterol so it does not cause fatty degeneration, but in this study the treatment group found their formation fatty degeneration. Discovery fatty degeneration is suspected prior to the studies, test animals have suffered fatty degeneration for comparison GOT to GPT>2 from before treatment (D-1) to D+14. Fatty degeneration occurs due to feeding and drinking are not regular, stable conditions are less than ideal, rat stress factors, the influence of substances or other diseases, as well as other internal factors such as the immune system and susceptibility of mice to external influences (Bhara. 2013). Fig 1. shows the results of histopathological on liver tissue.

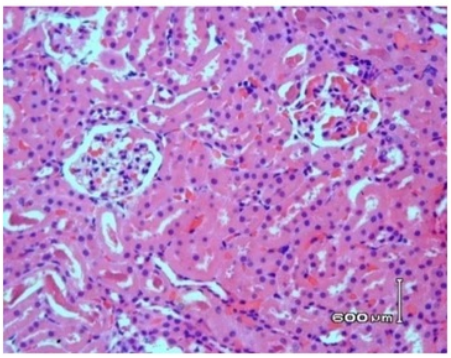

Fig 2. The structure of normal renal histopathology.

Figure 2 shows the results of histopathological on kidney tissue. The results of histopathological on kidney tissue in the control group and the treatment group. there was no pathological changes such as necrosis or degeneration fatty. This shows that the ethanol extract of binahong leaves not affect kidney function and still be safely used up to a dose of 2000 mg/kg.

CONCLUSIONS

a. Ethanol extract of binahong leaves dose 2000 mg/kg WB there was no rats death and didn't create toxic effect indication.
b. Ethanol extract of binahong leaves dose 2000 mg/kg WB could not influence weight, blood biochemical score (GPT enzyme, GOT enzyme, and blood urea,). liver and kidney histopathology in control and treatment group.

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