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ORIGINAL RESEARCH

Acute toxicity evaluation of ethanolic extract of the leaves of *Anredera cordifolia* in wistar rats (*Rattus norvegicus*)

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Abstract. *Anredera cordifolia* (Binahong) is one of the medicinal plants that has been widely used for its properties of inhibiting the growth of microorganisms, decreasing blood uric acid, and healing wounds. This study was conducted to evaluate the acute toxicity effects of ethanolic extract of binahong to support its use as a medicinal plant. Ethanolic extracts of binahong leaves were prepared. Twenty-five white male rats (*Rattus norvegicus*) were divided into 5 groups and administered with a single dose of Na-CMC suspension or a dose of 300, 600, 1200, or 2400 mg/kg BW of binahong ethanolic leaf extract, respectively. Toxicity symptoms were monitored at three-time intervals: 30-60 minutes, 90-120 minutes, and 180-360 minutes after the administration. A macroscopic evaluation of the rat's liver was carried out to identify any lesion. The rat's liver and whole-body weights were determined to assess the weight reduction. The results showed that there was no dead rat in any of the groups, indicating that no LD_{50} was identified. Also, there were several toxic effects observed including a decrease in movements, somatic response, insensitivity, and stool consistency. There was no evidence of a lesion on liver macroscopy. However, the weights of rats' livers increased in tandem with the reduction in their body weights. The findings from this study reveal that single dose of binahong ethanolic extract ranging from 300 to 2400 mg/kg BW are safe, while minimal toxic effects were observed.

Keywords: Binahong, LD_{50}, leaf extract, toxicity symptoms

INTRODUCTION

Traditional medicine is one of the alternative treatments chosen by the world community. According to the WHO (World Health Organization), around 80 % of the world's population uses traditional medicines. Traditional medicine is a type of treatment that is based on natural ingredients, especially plants, for medicinal purposes and disease prevention. Although the efficacy of traditional medicines has not been scientifically tested, most modern medicines come from active ingredients developed from plants [1]. Indonesia is the second largest biodiversity country in the world after Brazil. Indonesia has about 25,000-30,000 plant species which constitute 80 % of the world's plant species and 90 % of the plant species in Asia. The use of traditional medicine in Indonesia has been going on for thousands of years before modern medicine was discovered [2]. One of the medicinal plants widely used in Indonesia is binahong (*Anredera cordifolia*). Several studies have investigated the therapeutic effects of its leaves. The ethanolic extract of binahong leaves has shown the antibacterial activities against *Staphylococcus aureus*, Methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus cereus* [3]. Binahong leaves have shown several effects including antihypertension, antihyperlipidemic and antiobesity [4–6].

Research on binahong leaves continues to grow in terms of assessing its benefits; to obtain the best therapeutic dose so that it can be used in treating diseases. In addition to research on

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therapeutic doses, research into the toxicity of binahong leaf extracts has also been investigated. A study conducted to examine the toxic effect used a dose of 15 g/kg body weight on animals. At this dose, there was no death or toxic effect on organs in the animals [7]. Referring to this, further research is needed so that a lethal dose of binahong leaf extract is determined and to discover the effect of acute toxicity on animals. In a preliminary investigation, the toxic effect on rats after administration of a single dose of leaf extract was observed. The present study was therefore conducted to evaluate the acute toxicity effects of ethanolic extract of the leaves of *Anredera cordifolia* in Wistar rats (*Rattus norvegicus*).

MATERIALS AND METHOD

Experimental animals

Twenty-five healthy male Wistar white rats (*Rattus norvegicus),* aged 2-3 months, and bodyweight of 150-250 grams were obtained from the Faculty of Veterinary, Universitas Syiah Kuala, Indonesia. The animals were placed in cages with rice husks that were replaced every 3 days. Food (pellets) and aquades (*ad libitum*) were given. The animals were randomly divided into 5 groups and acclimatized for 7 days before treatment. Before administering the test preparations, the animals were weighed and then fasted for 12 hours, but still given a drink. Pellets were given hours after administering the test preparations.

Preparation of ethanolic extract of *Anredera cordifolia*

Leaves of *Anredera cordifolia* were obtained from Cot Ba'u, Sabang, dried for 5 days, and then blended into a powder. The extraction was done using the maceration method with 70 % ethanol. In an Erlenmeyer, the powder was dissolved in a 1:10 solvent, which was then evaporated using a vacuum rotary evaporator. The solid extract was diluted in a suspension containing 1 % Na-CMC with several doses; 300, 600, 1200, and 2400 mg/kg.

Acute toxicity test on rats

a. Determination of LD_{50}

The experimental procedure started on the 8th day after acclimatization. The ethanolic extract of binahong was administered orally to each group according to the following treatment; standard feed and distilled water (NT group); standard feed and binahong leaf extract at a dose of 300 mg/kgBW dissolved in 1 % CMC (B300 group); standard feed and binahong leaf extract at a dose of 600 mg/kg BW dissolved in 1 % CMC (B600 group); standard feed and binahong leaf extract at a dose of 1200 mg/kg BW dissolved in 1 % CMC (B1200 group); and standard feed and binahong leaf extract at a dose of 2400 mg/kg BW dissolved in 1 % CMC (B2400). Observations for determining the LD_{50} were made by counting the number of test animal deaths from each group for 24 hours after delivering the test preparation. The LD_{50} value obtained was then used to determine the toxicity category, according to Loomis criteria [8].

b. Evaluation of toxicity symptoms

Toxicity symptoms were observed at three-time intervals: 30-60 minutes, 90-120 minutes, and 180-360 minutes after the administration of binahong extract suspensions. The rats were evaluated to determine an increase or decrease in 14 parameters: locomotor activity, sensitivity to pain, aggressiveness, somatic response, awareness, tremor, eyes, defecation, salivation, breathing, nose, piloerection, tail, and reflexes [9]. Percentage of rat number experiencing toxic effect calculated as follows (Table 1).

$$\frac{n}{\text{the number of experimental animals per group}} \times 100\%$$

n= Number of experimental animals showing symptoms of toxicity

c. Macroscopic evaluation of rat liver

The rats were allowed to live for 14 days after single-dose administration of binahong extract. On the 15th day, the animals that did not die were surgically operated. The liver was then removed and examined macroscopically for identification. Any lesions including consistency, surface, and color that occurred on the liver were investigated [10].

d. Evaluation of rat's liver and body weights

The measurement of liver and body weights was conducted using the Mettler toledo analytical balance. The organs were dried with absorbent paper, then weighed to get absolute organ weights. The relative organ weight was calculated as follows [11] .

$$= \frac{\text{absolute weight of liver}}{\text{body weight}}$$

e. Change in body weight

The measurement of body weight was carried out on days 1, 3, 5, 7, and 14 to identify the changes in body weight. The change in body weight on a monitored day (X) was calculated as follows [11]:

$$= \text{body weight on day } X - \text{ initial body weight day}$$

Data analysis

Observations of liver weight and body weight of experimental animals were tested for homogeneity using the Levene test. ANOVA test was used to determine the significant differences between treatment groups followed by the Post Hoc Duncan test to determine the most significant difference. Data are shown as mean ± standard deviation of the mean.

Ethical approval

Ethical approval of this study was obtained from the Ethics Committee of the Medical Faculty of the University of Syiah Kuala, Darussalam, Banda Aceh, Indonesia.

RESULTS AND DISCUSSION

After 24 hours of monitoring, binahong leaf extract at doses ranging from 300 to 2400 mg/kg BW did not cause death in experimental rats. There were changes in locomotor activity as measured by the decrease of spontaneous movement when touched in groups administered with 1200 and 2400 mg/kg BW (Table 1). Dose-related symptoms were also observed in somatic response, pain sensitivity, and defecation as shown in Table 1. The observations on the macroscopic liver (Table 2) revealed that there was no lesion on any groups (also shown in figure 1). The only difference found among the groups was the color of the liver. The rats administered with binahong leaf extract showed blackish red. Table 3 indicated that the absolute and relative weights of the white rats' livers increased in the three highest doses. From the third to the fourteenth day, the rats' body weight decreased (Table 4).

The effect of single-dose administration of binahong ethanolic leaf extract on rats at doses ranging from 300 to 2400 mg/kg BW was investigated. There was no treatment dose resulting in mortality in rats. Experts agreed that if the maximal dose administered does not induce mortality in experimental animals, the test preparation is included in the category of practically non-toxic [8]. The results of detecting symptoms of toxicity indicated changes in locomotor activities, pain sensitivity, somatic response, and defecations. Locomotor activity is a movement arising from changes in electrical activity in the central nervous system. These changes are caused by changes in post-synaptic membrane permeability and the release of neurotransmitters by presynaptic neurons. Increased locomotor activity suggests central nervous system stimulation, whereas decreased locomotor activity indicates central nervous system depression [11]. Changes in pain sensitivity in experimental animals can occur due to analgesic effects of binahong leaf extract. A study revealed the influence of binahong leaf extract on analgesic activity in male mice infused at a dose of 400 mg/Kg BW [12]. The compounds in binahong leaves that might implicate to act as analgesics are flavonoid glycosides, saponins, vitamin C, and flavones [13]. The mechanism of action of flavonoids as analgesics is by inhibiting the enzyme cyclooxygenase (COOX), thereby reducing the production of prostaglandins by arachidonic acid which causes pain.

Somatic response refers to the rhythmic movements of the body observed in experimental animals. The emergence of somatic response is linked to changes in skeletal muscle tone and contraction-relaxation patterns, meanwhile, the reticular activation system of the medulla oblongata has an impact on skeletal muscle tone. The cerebral cortex and other motor centers control movements resulting from contractions-relaxation. In binahong leaves, the constituent compounds might influence the system and cause changes in the somatic response of experimental animals. Changes in stool consistency indicate gastrointestinal disorders and are often associated with toxic effects. Soft stool and diarrhea are caused by impaired water resorption and intestinal hypersecretion. The cause of diarrhea can be in the form of irritant agents, infections, allergies, and poisoning of other foreign substances. The digestive system of experimental animals may perceive the ethanolic extract of binahong leaves as a foreign substance, causing feces to change consistency. In the current study, the maximal dose employed was 2400 mg/kg BW. The maximum dose for acute toxicity assessment in rats is 5000 mg/kg BW, therefore it has not yet reached the recommended maximum dose and has not killed any experimental animals.

The results of the macroscopic observations of white rats' liver did not reveal any lesions, so the parameters for assessing lesions could not be observed. However, the color parameters were found to change the color of the treatment group's liver compared to the control group's liver, namely a change in color to blackish-red after being given the ethanol extract of binahong leaves, allegedly due to compounds contained in the ethanol extract of binahong leaves. Discoloration of the liver is regarded to be an early sign of acute inflammation. The damaged organ shows circulatory alterations in the early phases of acute inflammation. Vasodilation of the arterioles in the liver, leading to increased blood flow and local obstruction (hyperemia) of capillary blood flow, resulting in red color in the liver [14].

Table 1. Toxicity symptoms of experimental rats

Evaluation components		Percentage of rats experiencing toxic symptoms (*)				
Symptoms	Time (minutes)	NT	B300	B600	B1200	B2400
Locomotor activity	30 - 60	0	0	0	40 %	40 %
	90 - 120	0	0	0	40 %	60 %
	180 -360	0	0	20 %	100 %	80 %
Somatic response	30 - 60	0	20 % scratching	20 % sniffing	20 % sniffing	20 % sniffing
	90 - 120	0	0	20 % sniffing	20 % sniffing	20 % sniffing
	180 -360	0	0	0	40 % sniffing	40 % sniffing
Sensitivity to pain	30 - 60	0	0	0	0	0
	90 - 120	0	0	0	40%	40 %
	180 -360	0	0	0	0	60 %
Defecation	30 - 60	0	0	0	0	0
	90 - 120	0	0	0	0	0
	180 -360	0	0	0	0	20 % soft blobs

Table 2. Macroscopic evaluation of experimental rats' livers

Liver macroscopic	Treatment group				
	NT	B300	B600	B1200	B2400
Lesion size ($cm^{2)}$	0	0	0	0	0
lesion shape	0	0	0	0	0
lesion boundaries	0	0	0	0	0
Lesion distribution	0	0	0	0	0
Consistency	soft	Soft	soft	soft	soft
Surface	flat	Flat	Flat	Flat	Flat
Color	Fresh red	Blackish red	Blackish red	Blackish red	Blackish red

*0 : no lesion

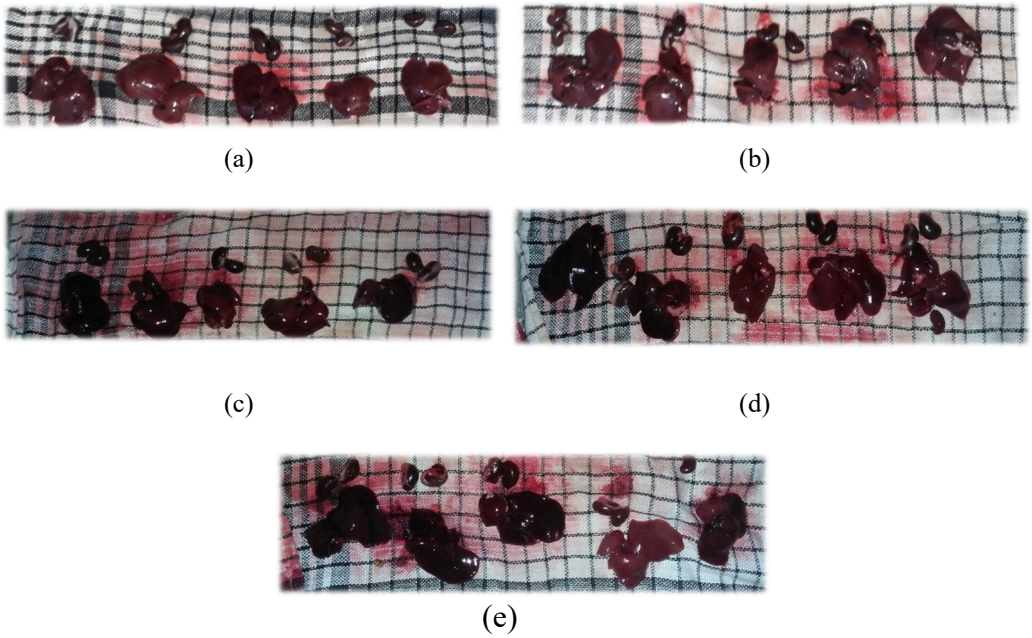

Figure 1. Macroscopic of rats with no treatment (a), B300 (b), B600 (c), B1200 (d), and 2400 (e)

Table 3. Absolute and relative weights of rat's liver

Groups	Absolute weight (grams)	Relative weight
K	5.22 ± 2.10048^{a}	0.0306 ± 0.01101^{a}
P1	4.94 ± 0.82037^{a}	0.0306 ± 0.00730^{a}
P2	7.14 ± 0.82037^{b}	0.0448 ± 0.00593^{b}
P3	7.24 ± 1.05499^{b}	0.0450 ± 0.00648^{b}
P4	6.30 ± 0.86891^{ab}	0.0377 ± 0.0631^{ab}

Table 4. Change in body weights of experimental rats

Group	Reduction in body weight (grams)				
	Day 1	Day 3	Day 5	Day 7	Day 14
K	$8{,}20\pm6{,}38^{a}$	$-24{,}40\pm29{,}26^{c}$	$-28{,}40\pm24{,}46^{d}$	$-27{,}80\pm23{,}94^{e}$	$-24{,}20\pm23{,}69^{f}$
P1	$3{,}40\pm5{,}13^{a}$	$-26{,}80\pm13{,}39^{c}$	$-29{,}80\pm13{,}42^{d}$	$-28{,}20\pm13{,}53^{e}$	$-24{,}20\pm13{,}81^{f}$
P2	$6{,}80\pm6{,}14^{a}$	$-26{,}60\pm33{,}29^{c}$	$-25{,}00\pm33{,}13^{d}$	$-29{,}00\pm36{,}57^{e}$	$-29{,}80\pm36{,}42^{f}$
P3	$-8{,}40\pm10{,}01^{b}$	$-23{,}80\pm14{,}58^{c}$	$-24{,}00\pm13{,}51^{d}$	$-18{,}00\pm10{,}79^{e}$	$-25{,}80\pm16{,}96^{f}$
P4	$-8{,}20\pm9{,}78^{b}$	$-19{,}00\pm14{,}19^{c}$	$-19{,}00\pm14{,}19^{d}$	$-13{,}40\pm10{,}67^{e}$	$-9{,}20\pm11{,}34^{f}$

The average relative weight of treatment group 2 was 4.48 %, and the average relative liver weight of treatment group 3 was 4.5 %, according to the results of the liver weight observation. The normal range for relative liver weight is 3.5 to 4.0 %, hence there was an increase in relative liver weight in this study. An increase in liver weight is thought to be toxic or adaptive to the liver. Increased liver weight with toxic properties, such as hepatotoxicity that will disrupt the structure of the endoplasmic reticulum membrane and decrease the cytochrome P-450 content. Drugs or toxic substances are oxidized by the cytochrome P-450 enzyme, which produces stable metabolites. The rate of biotransformation and metabolism of medicines and other hazardous chemicals in the body can be reduced if the cytochrome P-450 concentration decreases. Adaptive properties of increasing liver weight, such as enhanced cytochrome P-450, higher drug metabolism, and smooth endoplasmic reticulum proliferation [14].

An increase in liver weight is thought to be caused by an increase in vascular permeability in inflammation. Increased vascular permeability occurs in venules due to expansion of the interendothelial cell junction or in endothelial cells directly impacted by acute inflammation, resulting in tissue edema (exudate of extravascular fluid rich in protein) [15]. In comparison to the control group, the findings of the body weight observations of white rats revealed a decrease in bodyweight on day 1 after administration of ethanol extract of binahong leaves in treatment groups 3 and 4. The body weight of white rats in both the control and treatment groups decreased on the third, fifth, seventh, and fourteenth days which is represented as negative value in table 4. The weight of rats that are well maintained and in a balanced condition between consumption and food intake requirements will usually increase at a rate of 5 grams per day, following the pattern of growth, development, and age. The experimental animals given an ethanolic extract of binahong leaves containing flavonoid chemicals, on the other hand, were able to suppress weight gain, and their body weight reduced as compared to the control group after receiving the ethanolic extract of binahong leaves.

CONCLUSION

The findings from this study reveal that administering binahong ethanolic leaf extract had no significant toxic effects on the experimental animals, as measured by liver histology and body weight, implying that using binahong leaves as a treatment is safe. However, further research is needed into the contents of binahong plants as well as the toxicity effects that may result from the long-term administration of binahong leaves.

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