

ETHANOL EXTRACT OF BINAHONG LEAVES EFFECT (*Anredera cordifolia* (Ten.) Steen) ON HEMATOLOGICAL CHANGES IN FEMALE WISTAR RATS

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2 ETHANOL EXTRACT OF BINAHONG LEAVES EFFECT (*Anredera cordifolia* (Ten.) Steen) ON HEMATOLOGICAL CHANGES IN FEMALE WISTAR RATS

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Abstract

Binahong (*Anredera cordifolia*(Teen.)Steen) is one of the plant that can be used for several diseases. 8 is research has been conducted which explore the effect of ethanol extract of binahong leaves (*Anredera cordifolia* (Ten.)Steen) on hematological changes in female wistar rats. Animal tests are selected using a complete randomized block design, which is the test animals were randomly selected and have the same opportunity to be treated. Sighting study were conducted to determine the dose used in 12; main study. Doses used in the sighting study was 300 mg/kg and 2000 mg/kg, in this test there is no death in animal test trials. Animal test divided into 2 groups: the treatment and the control group. 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. results analysis Independent T test show that ethanol extract of binahong leaves did not affect the value of hematological (erythrocytes,leukocytes). On the other hand the results analysis used Dependent T test show that there is changes in the number of hematological (erythrocytes and 1 leukocytes) in the treatment group on D-1 versus D+1, D-1 versus D+7 and D-1 versus D+14. That different might not by administration of test preparation, because the control group also show changes the number of erythrocytes and leukocytes at the same time measurement.

Keywords: hematology, ethanol extract, binahong leaves (*Anredera cordifolia*(Teen.)Steen), white female rats

INTRODUCTION

Binahong Leaves (*Anredera cordifolia*) is one of the plant which can be used as medicine. According to Manoi, the benefits of binahong leaves can be used to a cure several diseases such as

for burns treatment, typhoid disease, colitis, thrush, vaginal discharge, swelling of the liver, heart swelling, increase vitality and endurance (Manoi, 2009). Binahong leaves have several active substances, such as 8.96% total flavonoids (Depkes RI, 2011), an alkaloid (betanidin) (Titis, 2013), phenolic acids (kumarat acid) (Eka 2 antiwi, et al, 2011) and saponins (28,14±0.22). Ethanol extract of binahong leaves at dose of 50, 100 and 200 mg/kgBW can reduce levels of creatine, thereby improving re 7 failure (Sukandar, et al., 2010), in addition at 4.25 mmol/100g (fresh) and 3.68 mmol/100g (dried) binahong leaves has antioxidant activity (Selawa, et al, 2013), Hermila also reported in the Kill minimum levels (KBM) at 40% ethanol extract of binahong leaves has antifungal activity to *Candida albicans* (Hermila, 2011). Activities of binahong leaves has been reported in many times, but the scientific information about dosage and safety of ethanol extract of leaves binahong was not reported yet. Hematology and blood biochemistry Observation is a part of the oral toxicity test according to OECD method. Therefore, researchers interested in studying hematological changes from animals test after oral administration ethanol extract of binahong leaves.

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MATERIALS AND METHODS

Materials

Materials used in this study is binahong leaf extract ethanol, 70% ethanol, distilled water, rats food, EDTA, turk reagent, hayem reagent.

Instrument

Instrument used in this study is a test animal cages, gloves, drink bottles, glass instrument (pyrex), vacuum evaporator, water bath, hemacytometer (ASSISTENT Germany), (eppendorf), micropipette, analytical balance (Shimadzu), syringe injection of 1 ml and 5 ml oral syringe with a sensitivity of 0.1 ml, stop watch, electric microscope (Olympus).

The study design

Design of the study is a randomized block design, which is the test animals were randomly selected and have the same opportunity to be treated.

Step of research

Extracts preparation. Binahong leaf samples were taken from the garden of Medicinal Plants Faculty of Pharmacy, University of Muhammadiyah Purwokerto, Banyumas, Central Java Province. Binahong leaves that have been obtained, washed, drained with aerated and performed followed by drying in the oven at 40°C until dry. Binahong powdered leaves with engine extracts were calculated using remaseration (repeated immersion). Binahong leaf powder was soaked in 70% ethanol with comparison 1:10 (BPOM, 2004). White female rat Wistar strain were used as the animal in this research.

The design of dosage and course of study

Based on Acute Oral Toxicity Organization for Economic Co-operation and Development (OECD) Guidelines For Chemical Testing of number 420, the dose used is based on research Sighting Study 5 mg/kg, 50 mg/kg, 300 mg/kg and 2000 mg/kg (Anonymous, 2001).

The course of study

1. Sighting study

In the observations test ethanol extract of leaf binahong to rats started from dose 300 mg/kg orally, then the observed toxic effects for 30 minutes followed every hour up to 8 hours during the first 24 hours. If the test animals showed no symptoms of toxic effects, do the next dose is 2000 mg/kgBW orally to rats and

observed another rats like procedure at dose 300 mg /kgBW.

2. Main study

Animals Test were divided into 2 groups, control group and the treatment group. Each group consisted of 5 rats. The treatment group was given 2 ml ethanol Extract of binahong leaf 2000 mg/ml. During test period all rats are given food and water each 2000 mg and 2000 ml of water for 1 week.

3. Hematological analysis

Hematological analyzes were performed using Hemacytometer. Blood taken from the vein rats tail. Blood inserted into a test tube along with EDTA. The measured Parameter were white blood cells and red blood cells. Erythrocyte counting: 5 µL of blood was mixed with EDTA, added with 1000 µL hayem reagent, then dropped into the room count, and wait 2 minutes.

The number of erythrocytes per mm³=number of erythrocytes in 5 small field of x 10000. Counting the number of white blood cells: 10 µL of blood was mixed with EDTA, added with 200 µL Turk reagent, then dropped into count room, and wait 2 minutes. Leukocytes counting: the number of leukocyte per mm³=amount of leukocytes in the 4 field x 50 field.

RESULT AND DISCUSSION

Observations of acute toxicity test was conducted for 14 days and the observations showed no mortality was found in rats.

Table 1. Hematology measurements in the treatment group and the control group, values shown are average values ± standard deviation, P> 0.05 which means that there are no significant differences in hematological values between the control group to the treatment group

Hematological Parameters	D-1	D+1	D+7	D+14
Erythrocyte				
treatment (10 ⁶ /mm ³)	8.02±1.03	8.08±2.93	14.56±5.88	7.36±1.43
control (10 ⁶ /mm ³)	7.93±1.63	10.16±2.39	16.06±7.18	6.40±2.46
P value	0.921	0.254	0.727	0.473
Leukocyte				
treatment (10 ³ /mm ³)	4.52±1.645	7.62±1.071	8.07±1236.2	7.93±1150.8
control (10 ³ /mm ³)	5.36±0.552	7.71±0.855	8.60±1200.00	7.680±1242.2
P value	0.897	0.330	0.365	0.078

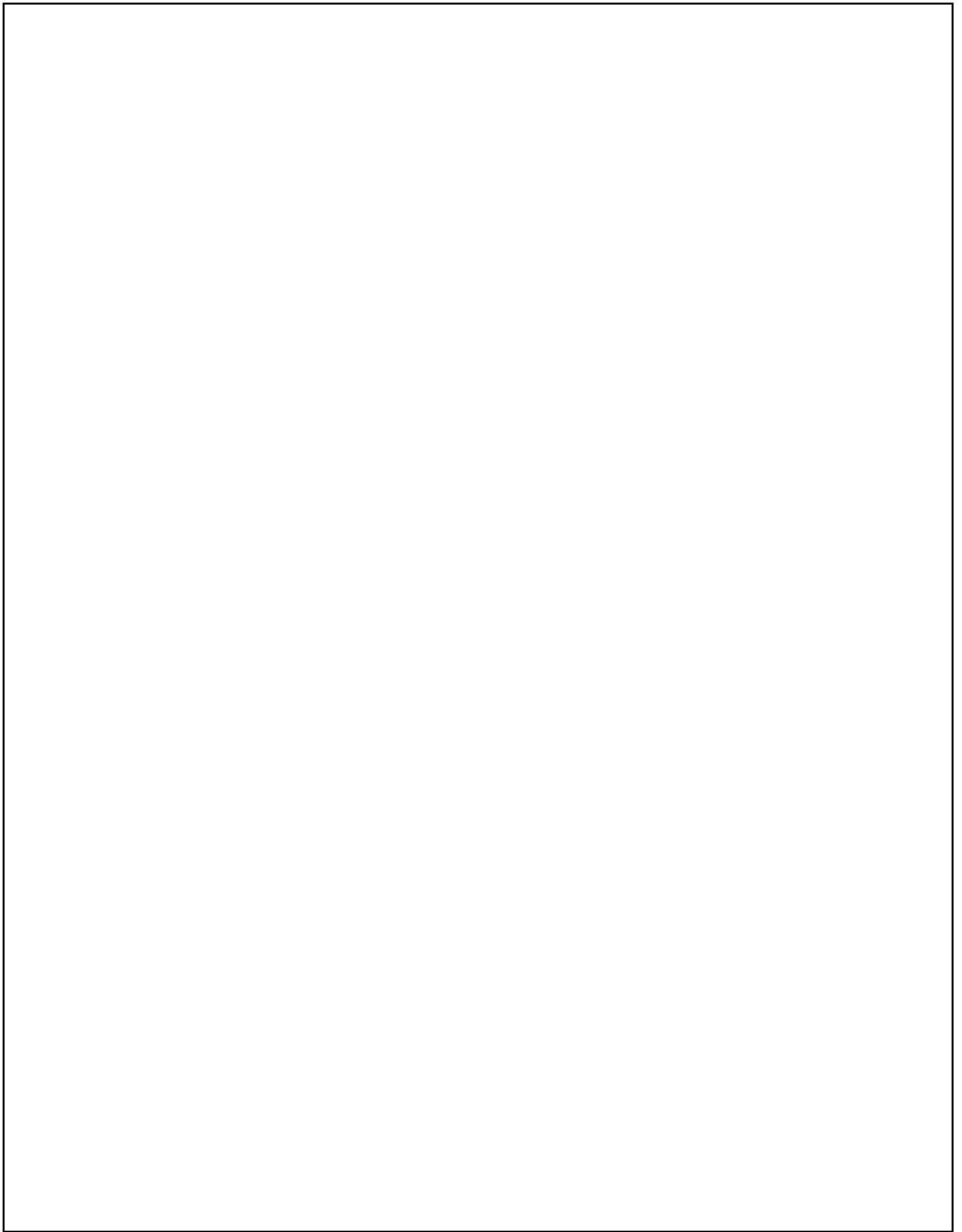
In Table 1. mean number of erythrocytes on D-1 and D+1 each treatment group and control group was 7.93×10^6 and 10.16×10^6 , by Anonymous in 2010 that the number of normal rat erythrocytes ranged 7×10^6 - $13 \times 10^6/\text{mm}^3$ (Anonymous, 2010). In treatment group, the number of erythrocytes in D+1 was increases and D+14 was decreased, it also common in the control group. Generally, the results of the analysis of unpaired T there were no differences mean number of erythrocytes significantly between the control group and the treatment group ($P > 0.05$) in D-1, D+1, D+7 and D+14. Also in D-1 mean value of leukocytes amount for each treatment and control groups were 4.52 ± 1.645 and 5.36 ± 0.551 which is still the normal range for adult rats ie 5000-12000/ mm^3 (Anonymous, 2010). In D+1, D+7, and D+14 in the treatment group and the control group there is an increasing number of leukocytes, but the change of leukocytes is still within the normal range. The Changes of a leukocyte amount can be influenced by physiological or pathological state. the physiology Influence can be muscle activity, fear arousal and emotional disturbance. While the effect of pathological stimuli could be a disease (Ganong, 1999). analysis results of the unpaired t test showed no difference leukocyte number between treatment and control group ($P > 0.05$) in D-1, D+1, D+7 and D+14. From the analysis, it can be concluded that the change of leukocytes and erythrocytes amount in treatment group was not caused by the ethanol extract of binahong leaves, because the control group showed similar changes as in the treatment group. Dependent T Test analysis was also conducted to determine the hematological changes in each treatment by comparing the D-1 versus D+1, D-1 versus D+7 and D-1 versus D+14. The results of the analysis indicate the number of erythrocytes in the D-1 versus D+1 and D-1 versus D+14 not change significantly, but in D-1 versus D+7 there are significant changes in hematological values ($P > 0.05$). In D-1 versus D+1, D-1 versus D+7 and D-1 versus D+14 there were a significant change in the leukocytes number ($P < 0.05$), decline and increase in the number of erythrocytes and leukocytes occurs not only in the treatment group but in the control group also showed the same thing, so it can be concluded that the change is not due to the effect of ethanol extract of binahong leaves but allegedly due to the influence of the un controlled environment. only in the treatment group but in the control group also showed the same thing, so it can be concluded that the change is not due to the effect of ethanol extract of binahong leaves but allegedly due to the influence of the un controlled environment.

CONCLUSION

Results of this research showed that ethanol extract of binahong leaf did not affect changes in hematology rats. The results of the analysis of independent T test showed there was no change in the number of hematological (erythrocytes and leukocytes) are significant in the treatment and control group. However, the analysis using Dependent T test showed a change in the number of hematological erythrocytes and leukocytes in the treatment group D-1 versus D+1, D-1 versus D+7 D-1 versus D+14. It is not due administration of test preparation but because control group also has the same erythrocytes and leukocytes amount changes for each measurement.

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