

Influence of mulberry leaf extract (*Morus alba* L.) on diuretic activity of male white Wistar strain rat

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ABSTRACT

Background: The mulberry leaf (*Morus alba* L.) is a traditional medicinal plant that can be used as a diuretic. Aim: This research aimed to know the diuretic effect of mulberry leaf ethanol extract in male white Wistar strain rats using Lipschitz method and ethanol extract optimum dose of mulberry leaf as diuretic. **Methods:** The rats were divided into five groups; each group is consisted of 5 rats and then given treatment. The categorization of groups is as follows: Normal PGA group 2%, furosemide compound group 3.6 mg/kg body weight (BW), dose 1 group extract 140 mg/kg BW, dose 2 group extract 240 mg/kg BW, and dose 3 group extract 420 mg/kg BW. Each group is put in a metabolism cage, and its urine was collected for testing; diuretic effect was done by measuring urine volume for 6 h. **Results:** The data were analyzed by one-way analysis of variance. The results showed that giving mulberry leaf can produce the biggest urine volume with dose 420 mg/kg BW and approach urine volume of the comparison group (furosemide). Statistically, it was different if it was compared to normal control group volume with level $\alpha = 0.01$. **Conclusion:** Mulberry leaf extract had diuretic effect with mechanism increase extractions Na and Cl (Saluretic) effective dose this extract was 420 mg/Kg Bb.

KEY WORDS: Diuretic, Extract, *Morus alba* L., Mulberry leaf

INTRODUCTION

Hypertension or high blood pressure is a condition that is often found in primary health services. Increased blood pressure that lasts for a long time (persistent) can cause damage to the kidneys (kidney failure), heart (coronary heart), and brain (stroke) if not detected earlier and receive adequate treatment. Based on the WHO data in 2013, there were 9.4 million people of the world population died every year and continued to grow due to hypertension. In addition, in 2008, there were >1 billion people who were suffer from hypertension with age above 25 years reaching 40%. In 2013, it was also reported that there were 36% of the population of Southeast Asia with adulthood suffered hypertension. The Data and Information Center of Indonesian Ministry of Health (2014) stated that, in 2013, there were 25.8% of Indonesia's population suffering from hypertension, even West Java was the province with the highest number of people suffering from hypertension with a total of 13,612,359 people or around 29.4% of the total population of West Java province.^[1]

Mulberry (*Morus alba* L.) is a traditional medicinal plant that is efficacious as a diuretic. Mulberry leaves are used to overcome fever, flu, cough, malaria, rheumatism, elephantiasis, red eye inflammation, breast milk booster, high cholesterol, and difficulty of urinating^[2] and can reduce blood pressure for those who are suffered hypertension.^[3] Chemical content of these plants include alkaloids, flavonoids, polyphenols, tannins, saponins, triterpenoids, and steroids.^[4] The method used in this study is by comparing of volume excretion the urine of the test animal with urea control which is called the Lipschitz method.^[5]

There is no research on the diuretic effect of ethanol extract from mulberry leaves; we are interested in researching medicinal plants which might have a diuretic effect, namely the ethanol extract of mulberry leaves which is empirically efficacious and compared with furosemide.

MATERIALS AND METHODS

Materials

Furosemide tablets, prostaglandins, 96% ethanol, concentrated ammonia, chloroform, hydrochloric acid, Mayer's reagents, amyl alcohol, 1% FeCl₃,

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gelatin, ether, Mg powder, Libermann–Bucharat reagents, and distilled water were used.

Collection and Extraction of Plant Materials

Mulberry leaves (*M. alba* L.) were obtained from Manoko Lembang Experimental Plantations, West Bandung, West Java. Determination was carried out at Herbarium Jatinangor, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Sumedang.

Mulberry leaves that have been dried and mashed are then extracted by maceration using a macerator for 3 × 24 h with 96% ethanol solvent. The extraction results are then concentrated using a rotary evaporator at a temperature of 40–50°C. Then, evaporation continued on the water bath at a temperature of 40–50°C until a thick extract with a constant weight was obtained.

Testing of Diuretic Effects

A total of 25 male rats were divided into five groups (every group consisted of 5 rats). Each rat was marked and fasted for 12 h, but drinking was still given. Group 1 was only given distilled water, Group 2 was given furosemide suspension, and Groups 3, 4, and 5 were the groups that given mulberry leaf extract at a dose of 140 mg/kg body weight (BW), 280 mg/kg BW, and 420 mg/kg BW. Each group was treated and put into the metabolic cage. The urine was collected with a container that had a measurement scale. Urine volume was recorded every hour for 6 h.^[5] Urine mice was obtained from the treatment then measured sodium and potassium ion levels using the Flame Photometer.

Data Analysis

Observations were presented in tables and diagrams. Evaluation of observations in the five experimental animal groups for the resulting urine volume was evaluated statistically using analysis of variance.

RESULTS AND DISCUSSION

Diuretic effect testing was carried out by measuring the volume of urine released for 6 h. Observations were made on the volume of urine released at 1, 2, 3, 4, 5, and 6 h after treatment [Table 1].

Table 1 and Figure 1 show that, in the normal group (aquades), the results of urine volume obtained were

low compared to other treatment groups. This is because aquades does not contain active substances which can increase urine volume. The highest diuretic activity to the lowest sequentially was Group 5 with a dose of 420 mg/kg BW of rat obtained an average yield of 1.24 mL, Group 4 with a dose of 280 mg/kg BW of rat obtained an average yield of 1.11 mL, and Group 3 with a dose of 140 mg/kg BW of rat obtained an average yield of 1 mL. The diuretic effect of the best ethanol has extracted of mulberry leaves and the average volume of the urine that was close to comparison (furosemide) where in mulberry leaf extract dose 3 with an average of 1.24 mL while the average yield of the comparison (furosemide) has been obtained 1.3 mL. Increased doses of mulberry leaf extract had an effect on increasing urinary volume expenditure on male white Wistar strain rat.

The mulberry leaf thick extract produced has a yield of 13% and meets the standard.^[6] The providing of extracts of doses 1, 2, and 3 experienced an increase in urine volume caused by the presence of flavonoids in the ethanol extract of mulberry leaves which played a role in increasing urine expenditure (diuresis)^[7] so that the greater the dose given, it showed the greater level of flavonoids on the ethanol extract of mulberry leaves. The mechanism of action of flavonoids as a diuretic was by inhibiting the reabsorption of Na⁺, K⁺, and Cl⁻, causing an increase in Na⁺ and water in the tubules. Thus, there was an increase in the volume of water in the tubules so that diuresis occurred.^[7] The types of flavonoids that act as diuretics were polymethoxy flavone^[8] and anthocyanin.^[9] This result when compared with previous studies by Susilo *et al.*^[10] results of mulberry leaf infusion (*M. alba* L.)

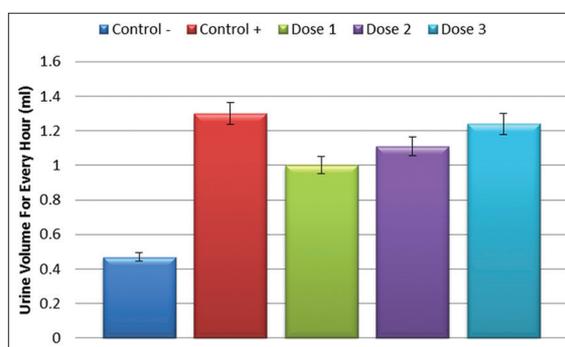


Figure 1: Results of measurement of urine volume in rat for 6 h

Table 1: Results of average measurement of rat urine volume for 6 h

Treatment	Urine volume for every hour (ml)						Amount
	1	2	3	4	5	6	
Negative control	0.34	0.4	0.48	0.54	0.56	0.55	0.47
Positive control	1.21	1.25	1.3	1.33	1.35	1.36	1.3
Dose 1	0.94	0.97	1.01	1.03	1.04	1.06	1
Dose 2	1.05	1.05	1.07	1.12	1.16	1.21	1.11
Dose 3	1.15	1.21	1.26	1.26	1.29	1.29	1.24

Table 2: Average results of sodium measurement in mice urine

Sample	Average (mEq/L)
Negative control	18
Positive control	180*
Dose 1	55.2*
Dose 2	81*
Dose 3	90.6*

*Different meaningful compared to normal group ($P < 0.05$)

Table 3: Average results of kalium measurement in mice urine

Sample	Average (mEq/L)
Negative control	393.2
Positive control	596.4*
Dose 1	322.6*
Dose 2	472.6*
Dose 3	547.2*

*Different meaningful compared to normal group ($P < 0.05$)

levels of 20% b/v had a diuretic effect comparable to furosemide.

The measurement of the electrolyte of sodium and potassium ions in the urine of the rat aims to determine whether the test preparation is saluretic. In Table 2, the measurement of sodium ion levels in the negative control group is 18 mEq/L, positive control is 180 mEq/L; in the extract group, the dose of 1, 2, and 3 is 55.2 mEq/L, 81 mEq/L, and 90.6 mEq/L. Potassium ion measurements according to Table 3 are negative control 393.2 mEq/L and positive control 596.4 mEq/L, in the extract group the dose of 1, 2, 3 in a row are 322.6 mEq/L, 472.6 mEq/L, 547.2 mEq/L. These results indicate that all doses in the test preparation are

saluretic compared to the control group as indicated by the excretion of high sodium and potassium.^[8,9]

CONCLUSION

Mulberry leaf extract had diuretic (saluretic) effect with effective dose of 420 mg/kg BW.

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