Diuretic Effect of the Aqueous Extract of Green Tea Leaves

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***Abstract*— The use of diuretics is needed in situations that require increased liquid excretory, one of which is hypertension. Green tea leaves are natural ingredients containing catechin polyphenols (flavonoids). This study aims to determine the diuretic effect of the aqueous extract of green tea leaves (AEGTL).**

This experimental study using 35 Swiss male mice was divided into seven groups. Group I was given CMC Na 1%, group II was given a dose of furosemide 5.2 mg/kg body weight, and group III-VII was given an aqueous extract of green tea leaves with doses of 14, 28, 42, 56 and 70 mg/kg body weight. Before treatment, all mice were given lukewarm water orally as much as 0.4 ml/20 kg body weight. Diuretic effects were tested by measuring the urine volume, measurements taken for 6 hours then calculated diuretic activity.

From the results of the study obtained the diuretic activity of furosemide is 1.00, and for groups, III-VII was given AEGTL respectively 0.53, 0.57, 0.60, 0.71, and 0.91. Green tea causes an increase in glomerular filtration rates by increasing blood flow and cardiac output, which may contribute to the diuretic activity.

It can be concluded that AEGTL doses 70mg/kg body weight have a diuretic activity that is equivalent to furosemide ($p < 0.05$).

Keywords*— *diuretic, aqueous extract, green tea leaves

I. INTRODUCTION

Basic Health Research (Riskesdas) 2018 shows that the prevalence of Non-contagious Diseases has increased when compared to the 2013 Riskesdas, including cancer, stroke, chronic kidney disease, diabetes mellitus, and hypertension. The prevalence of hypertension increased from 25.8% to 34.1%. With the increase in the number of hypertension, it can be ascertained the number of accompanying diseases such as stroke, heart disease, and kidney failure will also increase. Besides, according to BPJS Health data, the cost of hypertension services has grown every year, namely IDR 2.8 trillion in 2014, IDR 3.8 trillion in 2015 and IDR 4.2 trillion in 2016 (Wisnubro, 2018). The three main factors that determine blood pressure in hypertension are renal sodium excretion (and the resultant impact on plasma and total body volume), vascular tone and cardiac performance (Gradman et al., 2010).

According to research conducted by Susilowati and Wardani (2018), the highest antihypertensive in Yogyakarta's health centers is amlodipine (66.67%). The study by Rahmawati and Agustina (2010) at the Apotek Sehat Pharma Klaten, revealed that amlodipine (20.5%) and captopril (20.5%) were the most widely prescribed antihypertensive drugs at the pharmacy. The most prescribed group of antihypertensive drugs was outpatient BPJS RSUD KRT Setjonegoro in March 2015-March 2016 namely calcium channel blockers of 35.38%, and the most widely used type of hypertension drug was amlodipine which was 22.17% (Hapsari and Agusta, 2017). According to Kiraz et al., 2017 states that amlodipine is an antihypertensive drug that is cost-effective and uses only once a day so that it is widely prescribed in patients with hypertension. However, this drug has side effects, namely peripheral edema (Khisen et al., 2018). To overcome this edema, a diuretic is needed. The use of diuretics is required in all situations that require increased water expenditure, one of which is hypertension (Tjay and Rahardja, 2007). Hypertension is generally treated using synthetic drugs such as furosemide (Sukandar, 2013). Furosemide is a diuretic that is widely used to reduce edema. However, these drugs have many side effects. The management of hypertension includes necessary lifestyle modifications and pharmacotherapeutic management there of. Thiazide diuretics are still being promoted by most guidelines to be the initial (first-line) drug of choice, with the addition of other suitable antihypertensive agents, if necessary, and according to any relevant co-morbid conditions (Schellack, 2015).

The use of medicinal ingredients derived from plants is increasing because it is safe for consumption and if used appropriately, the use of medicinal plants has relatively smaller side effects compared to synthetic drugs (Sari, 2006). Green tea leaves (Camellia Sinensis) are plants that contain flavonoids and methylxanthine and have properties as diuretics (Syah, 2006). Therefore, this study aims to determine the diuretic effect of green tea leaf extract on swiss strain male mice.

II. METHODOLOGY

II.1. Chemicals

All chemicals used with analytical grades and purchased from the chemical company. Furosemide tablets used are generic from Indofarma pharmaceutical industry.

II.2. Experimental Animals

The test animals used in this study were 35 male Swiss strain mice; each group consisted of five mice. The average weight of the test animals is 20-35g, which is 2-3 months old.

II.3. Plant Materials

Green tea leaves (Camellia sinensis) were purchased in April 2018 from a local product called GREEN TEA which is produced by PT Gondang Sari, Karanganyar, Indonesia which already has a marketing permit with DIN-KES number No2133313490610-21. 20 g of green tea leaf powder boiled with 200 ml of aqua dest for 10 minutes at 70˚C. After heating, then filtered using a flannel cloth. The macerate obtained is evaporated over the water bath until a thick extract is obtained.

Calculate the yield of the thick extract obtained (Chakraborty et al., 2014).

II.4. Phytochemical Estimations of the Extract

The plant and aqueous extract of green tea constituents were subjected to qualitative analysis for following plant constituents: polyphenols, tannins, saponins, alkaloids, terpenoids, steroids, and flavonoids. The qualitative analysis can be seen in Table I.

TABLE I. THE QUALITATIVE ANALYSIS

Plant Constituents	Test
Test for Polyphenols	Ferric chloride test
Test for tannins	Ferric chloride test
Test for saponins	Foam formation test
Test for alkaloids	Mayer's test, Dragendroff's test
Test for terpenoids	Liebermann-Burchard test
Test for steroids	Ferric chloride test
Test for flavonoids	Ferric chloride test

II.5. Experimental Protocol.

Prepare 35 male mice of the Swiss strain with a weight of 20-35 g aged between 2-3 months. Mice were then divided into seven groups, each group consisting of 5 mice. Each group with a different treatment, namely:

Group I: negative control with CMC Na 1%.

Group II: positive control with furosemide dose of 5.2 mg/kg body weight.

Group III: aqueous extract of green tea leaves at a dose of 14 mg/kg body weight.

Group IV: aqueous extract of green tea leaves at a dose of 28 mg/kg body weight.

Group V: aqueous extract of green tea leaves at a dose of 42 mg/kg body weight.

Group VI: aqueous extract of green tea leaves at a dose of 56 mg/kg body weight.

Group VII: aqueous extract of green tea leaves at a dose of 70 mg/kg body weight.

All test animals were adapted for seven days. Before testing, mice were fasted for 12 hours without being fed but still given a drink. Two hours before the study was carried out, the feeding of mice was stopped. The treatment was carried out for 6 hours without being given food and drink. Before the experimental animals were given treatment, warm water was given as much as 4 ml/kg body weight in 200 g rats (Purwidyaningrum et al., 2016), then given to mice 20 g was 0.4 ml/kg body weight. Warm water is given orally. The treatment was carried out at 0 o'clock, and mice were given oral treatment according to their groups. Urine volume is then measured and recorded every 1 hour for 6 hours in each treatment group. Measurements were taken at 1,2,3,4,5, and 6, then percent diuretics were calculated and compared the diuretic effects between treatment groups. Then the data was analysis:

Diuretic action = urinary excretion of treated group/urinary excretion of the control group.

Diuretic activity = diuretic action of test drug/diuretic action of standard drug.

II.6. Statistical Analysis

Statistical significance assessment was made using one-way analysis of variance (ANOVA) followed by LSD comparison tests. $P < 0.05$ was considered significant.

III. RESULTS & ANALYSIS

III.1. Preliminary Phytochemical Investigation

The percentage yield of aqueous extract of green tea leaves (AEGTL) was found to be 90.45%. Compounds taken from the extraction process of green tea leaves are flavonoids. Flavonoids are polar compounds because they have several unsubstituted hydroxyl groups. Polar solvents such as water can be used to extract flavonoids from plant tissue (Rijke, 2005). In this study, to determine the content of chemical compounds found in AEGTL, a qualitative test was performed on the extract by phytochemical screening. The results of the phytochemical screening test can be seen in Table II.

TABLE II. PHYTOCHEMICAL SCREENING RESULTS

Chemical Compounds	Plant Constituent	Extract Constituent
Polifenol	+	+
Tanin	+	+
Saponin	-	-
Alkaloid	+	+
Terpenoid	-	-
Steroid	+	+
Flavonoid	+	+

Description: +: positive and -: negative

From the results of the qualitative test, it is known that AEGTL contains polyphenols, tannins, alkaloids, steroids, and flavonoids, but does not contain saponin and terpenoid compounds. This is in same with research from Chakraborty et al., 2014. The study from Dewi (2008) also states that green tea contains polyphenols which are flavonoid compounds consisting of epicatechin (EC), epicatechin error (ECG), epigallocatechin (EGC), epigallocatechin error (EGCG), besides green tea also contains caffeine, vitamin K, flavanols, alkaloids, proteins, nucleic acids, minerals, and fluoride. The major polyphenols in green tea are flavonoids. The four major flavonoids in green tea are the catechins, epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG) and epigallocatechin gallate (EGCG) (Sinija & Mishra, 2008)

III.2. Diuretic effect

The purpose of this study was to determine the diuretic effect of aqueous extract of green tea leaves (AEGTL) and the effective dose of the extract as a diuretic. The results of the diuretic test of aqueous extract of green tea leaves can be seen in Table III.

TABLE III. TABLE III. DIURETIC TEST RESULTS OF AQUEOUS EXTRACT OF GREEN TEA LEAVES

Treatment	Average Cumulative Urine Volume (ml)	Diuretic Action	Diuretic Activity
Negative control (CMC Na 1%)	0.320±0.177	-	-
Positive control (Furosemide 5,2mg/kg body weight	1.294±0.264	4.044	-
AEGTL 14 mg/kg body weight	0.682±0.175a,b	2.131	0.527
AEGTL 28 mg/kg body weight	0.742±0.145a,b	2.319	0.573
AEGTL 42 mg/kg body weight	0.782±0.142a,b	2.444	0.604
AEGTL 56 mg/kg body weight	0.916±0.173a,b	2.863	0.708
AEGTL 70 mg/kg body weight	1.176±0.172a,c	3.675	0.909

Description: p = 0.05, a = significantly different from negative controls, b = significantly different from positive controls, c = not significantly different from positive controls

Testing of diuretic effects is done because diuretics have an essential role in hypertensive patients. It is because diuretics are effective in reducing blood pressure by reducing the excessive fluid volume to reduce the workload of the heart (Muthia et al., 2017). EGCG in green tea leaves is believed to be an active component of green tea which is beneficial as an antihypertensive, antioxidant, anti-carcinogenesis, protects the skin from UV light, and anticancer (Fulder, 2004). Presently, many esearch findings suggest the role of tea in mediating the proper functioning of the cardiovascular system, reduction of body mass, and even decreasing the risk of cancer and neurodegenerative diseases (Yang and Hong, 2013).The negative control in this study used was CMC Na 1%. CMC Na does not have a diuretic effect (Herman, 2012; Muthia et al., 2017). The positive control used is furosemide. The mechanism of action of furosemide as a diuretic is to work by inhibiting Na+/ K +/ Cl- co-transport in the Henle (Gunawan et al., 2007). This section has a high NaCl reabsorption capacity so that furosemide has a more significant diuretic effect than other diuretics (Mutschler, 2010).

In Table III, it can be seen that all doses of AEGTL affect a diuretic, but only at a dose of 70 mg /kg body weight, which has a diuretic effect equivalent to furosemide. In green tea leaves are efficacious as a diuretics are flavonoids and alkaloids. The mechanism of action of flavonoids as a diuretic is by inhibiting the reabsorption of Na + and Cl- which can cause an increase in Na+ and water in the tubules, resulting in an increase in the volume of water in the tubules and an increase in urine volume (Hasanudin et al., 2012). The mechanism of action of alkaloids as a diuretic is to work directly in the tubules by increasing the excretion of Na+ and Cl-, so as to increase excretion of water and cause increased urine volume (Nessa et al., 2013). It is consistent with Chakraborty et al., 2014 study which states that extract of green tea leaves combined with HCT can increase the diuretic effect of HCT and can reduce potassium loss compared to HCT alone. Flavanols and alkaloids in green tea can inhibit ACE (angiotensin-converting enzyme) activity (Persson, 2012). Through inhibition of ACE inhibitors, this is an approach that green tea leaves have a diuretic effect and increase the excretion of Na + and Cl- (Rang et al., 2007). Green tea causes an increase in glomerular filtration rates by increasing blood flow and cardiac output, which may contribute to the diuretic activity so that it can be used as an antihypertensive agent. Caffeine can decreases the sensation of fatigue and has a diuretic effect (Miyoshi et al., 2015; Suzuki et al, 2016)

In addition to measurements of urine volume, a urine pH test of mice was also carried out. The results of testing the urine pH of each treatment in mice can be seen in Table III. The pH test results were carried out to see whether the administration of the extract affected the urine pH of the mice. The results of testing the urine pH of mice showed that administration of AEGTL did not affect the urine pH of mice, because between the AEGTL treatment groups had the same urine pH.

TABLE IV. URINE PH TEST RESULTS FOR EACH TREATMENT IN MICE

Treatment	pH
Negative control (CMC Na 1%)	6
Positive control (Furosemide 5,2mg/kg body weight)	7
AEGTL 14 mg/kg body weight	7
AEGTL 28 mg/kg body weight	7
AEGTL 42 mg/kg body weight	7
AEGTL 56 mg/kg body weight	7
AEGTL 70 mg/kg body weight	7

Based on Table IV, the results of testing the urine pH in the negative control group with CMC Na 1% had a urine pH of 6. The results of urine pH testing in the positive control group with furosemide dose of 5.2 mg /kg body weight had seven urine pH equal to the treatment group given AEGTL . While the normal pH of the urine of mice ranges from 7.3-8 (Safitri, 2018). So it can be concluded that EAGTL, as a diuretic, does not affect urine pH.

IV. CONCLUSION & RECOMMENDATION

It can be concluded that the AEGTL doses 70 mg/kg has a diuretic activity that is equivalent to furosemide ($p < 0.05$). This research can be increased the tablet or capsule formulation stage so that it can be used as a medicinal product that is useful as a diuretic or antihypertensive agent.

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