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Comparative effectiveness of the ethanol extract and infusion of green tea leaves (*Camellia Sinensis* L.) as a diuretic in male swiss mice

Agustina Susilowati*, Sista Nanda Indratika
Academy of Pharmacy Indonesia Yogyakarta
Jl. Veteran Gang Jambu, Pandeyan, Umbulharjo, Yogyakarta, Indonesia

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ABSTRACT

Green tea (*Camellia sinensis* L.) contains flavonoid compounds and *methylxanthine* and has proven efficacy as a diuretic. The dosage form is among the factors determining the biological effects of a drug. This experimental study set out to determine the effectiveness of the ethanol extract and infusion of green tea leaves as a diuretic in male Swiss mice. A total of 20 male Swiss mice were divided into four groups receiving different daily treatments: group I (given 1% Na-CMC), group II (furosemide at a dose of 5.2 mg/kg BW), group III (ethanol extract of green tea leaves at a dose 70 mg/kg BW), and group IV (green tea leaf infusion at a dose of 70mg/kg BW). Before the treatment, all groups were given warm water orally (0.4 mL/20g BW), then the effects of diuretics were measured from urine volume for six hours. The data were analyzed statistically by one-way analysis of variance (ANOVA), followed by LSD comparison tests, with statistically significant $p<0.05$. Based on the phytochemical screening results, the ethanol extract and infusion of green tea leaves contained alkaloids, flavonoids, and tannins. The mean cumulative urine volume in group I was 0.260±0.185 mL, group II 0.869±0.162 mL, group III 0.866±0.197 mL, and group IV 0.642±0.187 mL. Compared to furosemide, the diuretic activity of the ethanol extract of green tea leaves (0.997±0.182) was not significantly different ($p>0.05$) from that of the green tea leaf infusion (0.739±0.182). In conclusion, green tea leaves can be used as diuretics in two dosage forms, namely extract and infusion.

Keywords: diuretics, ethanol extract, infusion, green tea leaves

Corresponding author:
Agustina Susilowati
Academy of Pharmacy Indonesia Yogyakarta
Jl. Veteran Gang Jambu, Pandeyan, Umbulharjo, Yogyakarta, Indonesia
Email: agustinasusilowati@afi.ac.id

INTRODUCTION

Thiazide diuretics are at least as effective as β-blockers, calcium antagonists, and ACE inhibitors in reducing cardiovascular outcomes. They are also particularly useful in preventing stroke and heart failure in hypertensive patients (Grossman et al., 2011). The green tea leaf extract, when combined with hydrochlorothiazide, has been proven to significantly increase diuretic activity (Chakraborty et al., 2014). Regular consumption of green tea may also provide some protection against hypertension in humans (Negishi et al., 2004).

Green tea (*Camellia sinensis* L.) is among the plants that can be used as a traditional diuretic (Susilowati, 2019). Its leaves contain polyphenol catechins, which are flavonoid compounds consisting of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG). EGCG constitutes 50-80% of the total amount of catechins. Also, green tea contains caffeine, vitamin K, flavanols, alkaloids, saponins, proteins, nucleic acids, minerals, and fluoride (Dewi, 2008), as well as three main components beneficial for human health, such as xanthic bases (caffeine and theophylline), essential oil, and polyphenolic compounds. Theophylline induces psychoactive activity and has a slightly inotropic and vasodilatory effect and a much higher diuretic effect than caffeine (Cabrera et al., 2006).

Green tea is more effective as a diuretic than black tea (Actis-Goretta et al., 2006). Diuretics decrease plasma volume, cardiac workload, and oxygen demand, decreasing blood pressure. Diuretics play an essential part in dealing with fluid overload in salt-sensitive hypertensive patients (Jabeen and Aslam, 2013). The main flavonols and purine alkaloids present in green tea are responsible for inhibition of angiotensin-converting enzyme (ACE) activity, lowering blood pressure (Persson et al., 2006).

Research and development of medicinal plants with diuretic potentials are a priority in the development and utilization of Indonesian medicinal plants owing to the extensive and frequent use of diuretic (Lingga et al., 2014). Green tea has been reported as an effective diuretic (Chakraborty et al., 2014). At the dose of 70 mg/kg BW, the aqueous extract of its leaves possesses a diuretic property equivalent to furosemide (Susilowati, 2019). The dosage form determines whether or not the absorption process of a drug is optimal and, therefore, can decide the biological effects of said drug. Therefore, it is necessary to test the effectiveness of ethanol extract and infusion of green tea leaves (*Camellia sinensis* L.) in male Swiss mice to find which dosage form is as effective as a diuretic. Research on the effects of this diuretic is expected to contribute to the development of traditional medicines that have diuretic properties.

MATERIALS AND METHODS

Materials

The materials used in this study were dried green tea leaves (*Camellia sinensis* L.) obtained from Nglinggo, Kulon Progo, Yogyakarta.

Methods

Determination of samples

Samples of green tea leaves were first authenticated in the Biology Laboratory of Ahmad Dahlan University (No. 141/Lab.Bio/B/XII/2018).

Sample preparation

The shoots of fresh green tea leaves were picked, washed with water, and dried by aeration. Afterward, these dried samples were grounded and sifted using a 40 mesh sieve (Andaryekti et al., 2015). Finally, the resultant powder was weighed (final weight of the dried samples) and stored in a dry and clean container.

Preparation of green tea leaf extract

Two hundred grams of the powdered dried samples were placed in a vessel, added with 750 mL of 70% ethanol, and stirred using an electric stirrer for one hour. During the extraction, the vessel was tightly closed, left for 2x24 hours, and protected from light while occasionally stirred for ±15 minutes. Afterward, the extract was filtered to obtain filtrate 1, which was later re-macerated for 2x24 hours with 750 ml of 70% ethanol and filtered to obtain filtrate 2. Both filtrates were collected and evaporated in a rotary evaporator at 60^{o}C, then thickened using a water bath until a thick extract was formed (Anisah et al.,2017).

Preparation of green tea leaf infusion

Fifty grams of the powdered dry samples was added with 500 mL of distilled water at 90^{o} C for 15 minutes while stirred occasionally. Then, this mixture was filtered using a flannel cloth to obtain 500 ml of infusion (Irianti et al., 2016).

Phytochemical estimations of the ethanol extract and infusion of green tea leaves

The ethanol extract and infusion of green tea leaves were analyzed qualitatively for the presence of these organic plant constituents: alkaloids, flavonoids, saponins, tannins, dan triterpenoids**.**

Preparation of 1% Na-CMC Solution

One gram of Na-CMC was weighed and allowed to expand in 20 ml of warm water for 30 minutes. Afterward, it was crushed until homogeneous, put in volumetric glassware and added with aquadest up to 100 ml mark line. For every 20 g of mouse weight, 2 mL of 1% Na-CMC solution was given.

Preparation of furosemide suspension

The recommended dose of furosemide for adult humans is 40 mg. When converted to 20 g of mouse BW with a conversion factor of 0.0026 (Syamsudin and Darmono, 2011), the dose of furosemide for the test mice is 5.2 mg/kg BW. In this research, furosemide was administered orally with a volume equal to 1% of the body weight of test animals (Nessa et al., 2013). Therefore, a mouse weighing, for instance, 20 g received 0.2 mL of furosemide. Also, the concentration of furosemide solution for a dose of 5.2 mg/kg BW was 0.52 mg/mL. A total of 20 furosemide tablets (40 mg/tablet) were weighed (the average weight of each tablet was calculated), put into a mortar, and ground until smooth. A total of 22.3405 mg of tablet powder (equivalent to 5.2 mg of furosemide) put in volumetric glassware and dissolved with 1% Na-CMC up to 10 ml mark line (Herman et al., 2012).

Experimental protocol

This study has received ethical approval from the research ethics committee of Ahmad Dahlan University (No.021907007). A total of 20 male Swiss mice aged 2-3 months, with an average weight of 20-35g, were divided into four groups, each consisting of 5 mice.

Group I : Negative control group was given 1% Na-CMC
Group II : Positive control group received furosemide at a dose of 5.2 mg/kg BW
Group III : Ethanol extract of green tea leaves at a dose of 70 mg/kg BW
Group IV : Infusion of green tea leaves at a dose of 70 mg/kg BW

The test animals were acclimatized for seven days in laboratory conditions, then fasted for 12 hours from food but still given access to water until two hours before receiving the treatment. Afterward, they were weighed and given warm water orally as much as 0.4 ml/20g BW (Purwidyaningrum et al., 2016). In Hour 0, all groups received their respective treatment orally. The urine produced by every test mouse was collected in a container and observed for any diuretic effects hourly for six hours. The measurement results in Hours 1, 2, 3, 4, 5, and 6 were calculated to obtain the mean cumulative urine volume during the six hours. The diuretic action and diuretic activity were

calculated, and the diuretic effects of the treatment groups were compared to each other. Formulas for the calculations of diuretic action and diuretic activity are as follows:
Diuretic action = urinary excretion of the treated group/urinary excretion of the control group.
Diuretic activity = diuretic action of the tested drug/diuretic action of standard drug.

Data Analysis

The mean cumulative urine volume was analyzed statistically by one-way analysis of variance (ANOVA), followed by LSD comparison tests. $P<0.05$ indicates significantly different means.

RESULTS AND DISCUSSION

Preliminary phytochemical investigation results

Phytochemical screening was conducted to screen and identify medicinally active compounds found in the ethanol extract and infusion of green tea leaves. It included tests of alkaloid, flavonoid, saponin, tannin, and triterpenoid detection with three replicates for each test. Table 1 summarizes the phytochemical screening results of the two tested dosage forms.

Table 1. Phytochemical screening test results of the ethanol extract and infusion of green tea leaves

Tested Compounds	Treatments	Results	
		Infusion of green tea leaves	Ethanol extract of green tea leaves
Alkaloid	+ HCl 2M + Mayer reagents (Marliana *et al*., 2005)	+	+
	+ HCl 2M + Wagner reagents (Marliana et al., 2005)	+	+
Flavonoid	+ Mg powder + HCl (p) + amyl alcohol (Andaryekti et al., 2015)	+	+
	+ 10% NaOH (Rahayu et al., 2015)	+	+
Saponin	+ warm water (shaken for 1 minute) (Andaryekti et al., 2015)	-	-
Tanin	+ $FeCl_3$ 1% solution (Andaryekti et al., 2015)	+	+
Triterpenoid and steroid	+0.5 mL chloroform + 0.5 mL acetic acid anhydride + 2 mL concentrated sulfuric acid (Sembiring et al., 2018)	-	-

The phytochemical screening results showed that the ethanol extract and infusion of green tea leaves were positive for alkaloids, flavonoids, and tannins. These findings are consistent with research conducted by Andaryekti et al., (2015), which affirmed that ethanol extracts of green tea leaves contain alkaloids, flavonoids, and tannins. Also, using a qualitative test, Susilowati (2019) found that the aqueous extract of green tea leaves contains polyphenols, tannins, alkaloids, and flavonoids.

Diuretic effects

This study set out to compare the effectiveness of the ethanol extract and infusion of green tea leaves *(Camellia sinensis* L.*)* as diuretics in male Swiss mice. The diuretic test results of both dosage forms are presented in Table 2.

Tabel 2. The average cumulative urine volume of mice given the ethanol extract and infusion of green tea leaves during 6 hours of observation

Treatment Groups	Average Cumulative Urine Volume (ml)	Diuretic Actions	Diuretic Activities
Negative Control (1% Na-CMC)	0.260±0.185	-	-
Positive control (Furosemid at 5.2 mg/kg BW)	0.869±0.162	3.342±0.501	-
Ethanol Extract of Green Tea Leaves at 70 mg/kg BW	0.866±0.197[a]	3.331±0.501	0.997±0.182
Infusion of Green Tea Leaves at 70 mg/kg BW	0.642±0.187[a,b]	2.469±0.501	0.739±0.182

Description: a = significantly different from the negative control, b = significantly different from the positive control

Diuretics are effective in lowering blood pressure by reducing excess fluid volume and can lessen the workload of the heart, justifying their vital role in hypertensive patients (Muthia et al., 2017). The diuretic effect test was carried out using 1% Na-CMC as the negative control. According to Herman et al. (2012) and Muthia et al. (2017), 1% Na-CMC can serve as a negative control because it does not produce diuretic effects. Meanwhile, the positive control used was furosemide at a dose of 5.2 mg/kg BW. Based on Table II, the positive control group (Group II) showed the highest average cumulative urine volume compared to other groups. In this study, furosemide was selected as a positive control because it is a potent loop diuretic and, following its use, diuresis begins in 0.5-1 hours and lasts for ± 6-8 hours (Siswandono and Soekardjo, 2008).

Groups III (ethanol extract of green tea leaves) and IV (green tea leaf infusion) showed a higher mean cumulative urine volume than Group I (negative control). The statistical analysis in the SPSS program also revealed that the cumulative urine volumes of Groups III and IV were not different from each other, but the diuretic activity of the former was higher than the latter. The diuretic effects of the ethanol extract and infusion of green tea leaves, which contribute to relatively high urine output (diuretic), are attributable to the presence of flavonoid compounds. As a diuretic agent, flavonoids work with the mechanism of inhibiting the reabsorption of Na^+, K^+, and Cl^-, resulting in increased electrolytes in the tubules and, finally, diuresis (Nurihardiyanti et al., 2015). Furthermore, other studies state that flavonoids can increase urine volume as these compounds increase the rate of glomerular velocity (Jouad et al., 2001).

Chakraborty et al. (2014) prove that the flavonoids contained in green tea are responsible for inhibiting the activity of the angiotensin-converting enzyme (ACE). Green tea can inhibit ACE activity through the mechanism of mixed inhibition, and ACE inhibition is one of the main reasons for diuretic activity and increased Na^+ concentrations in urine. Moreover, it can increase the glomerular filtration rate by increasing renal blood flow and cardiac output, which are contributors to diuretic activity. According to Susilowati (2019), flavonoids and alkaloids in the aqueous extract of green tea leaves (70 mg/kg BW) have been shown to exhibit diuretic activities.

In addition to measuring urine volume, this study observed the urine pH of the test mice to determine the effects of administrating the ethanol extract and infusion of green tea leaves on this variable. The results of this observation are presented in Table 3.

Table 3. Urine pH of mice given the ethanol extract and infusion of green tea leaves during 6 hours of observation

Treatment groups	Urine pH
Negative Control (1% Na-CMC)	7
Positive Control (Furosemide at 5.2 mg/kg BW)	7
Ethanol Extract of Green Tea Leaves (at 70 mg/kg BW)	7
Green Tea leaf Infusion (at 70 mg/kg BW)	7

The urine test results showed that the administration of the ethanol extract and infusion of green tea leaves did not affect the urine pH of the test mice, as evident from the same urine pH (i.e., 7) among the treatment groups. Normal urine pH ranges from 7.3 to 8 (Nurihardiyanti et al., 2015), meaning that the urine pH of all treatment groups in this study approaches the normal urine pH in mice.

CONCLUSION

The diuretic activity of the ethanol extract of green tea leaves (0.997±0.182) is not significantly different ($p>0.05$) from the leaf infusion (0.739±0.182). Therefore, green tea leaves made into both dosage forms (extract and infusion) can function as diuretics.

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