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Original Research Article

Diuretic Activity of Water Fraction of Avocado (*Persea Americana* Mill.) Leaves Extract in Rat

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Abstract: Currently available diuretics are not completely free from side effects such as electrolyte imbalance and metabolic alterations. Avocado (*Persea americana* Mill.) contained many type of bioactive substances that related to diuretics. This study aimed to reveal and verify the effect of water fraction of avocado leaves extract against output of rat urine. Three different doses of leaf extract of avocado prepared i.e., 91.125mg/kg BW, 182.25 mg/kg BW and 364.5 mg/kg BW. The urinary parameters assessed were the urine volume hourly for 6 hours, urine color density and urine pH. The results showed water fraction of avocado at the dose of 182.25 and 364.5 mg/kg significantly increase urine volume in comparison to the negative control. Urine color density scores varied but did not have a dependent manner that follows the dose levels of diuretics given. However all treatments showed no statistical difference in the urine pH. Thus, it can be conclude that the water fraction of avocado leaves extract potentially for diuretic herb without make significant changes on pH.

Keywords: Avocado, *Persea mericana*, Diuretics, Diuresis.

1. INTRODUCTION

Diuretics are a class of drugs consisting of substances that can promote the flow of urine and the loss of excessive body fluids, salts, and other metabolic products such as urea. That's why diuretics are often prescribed for people suffering from kidney problems such as nephritis, toxemia of pregnancy, hypertension and heart failures. Unfortunately, currently available diuretics are not completely free from side effects such as electrolyte imbalance and metabolic alterations. Therefore, the search for diuretics made from natural ingredients that are safe and more efficacious needs to be continued (Sayana *et al.,* 2014, Welu *et al*., 2020)

Among the natural bioactive substances that are widely recommended as alternative diuretics are plant-derived chemical compounds—the phytochemicals. Plant bioactive substances extracted from *Podocarpus falcactus* that are known to have diuretic properties are alkaloids, flavonoids, phenolics, and tannins (Meharie and Tunta, 2020). Other plants that have also been proven to have diuretic properties are *Mentha viridis* Linn, *Cyclea peltata*, *Allium sativum, Stauntonia brachyanthera, Coriandrum sativum.*

Phytochemical screening of *Mentha viridis* plants showed the presence of alkaloids, flavonoids, tannins and saponins (Aziz *et al*., 2014). Screening of *Cyclea peltata* leaf extract indicated the presence of alkaloids, flavonoids, tannins, diterpenes and saponins (Hullatti *et al*., 2011). Phytochemical screening of *Allium sativum* plant extracts which were proven to have diuretic properties revealed the present of steroids, triterpenoidal saponins and carbohydrates (Tiwari *et al*., 2012). From stem extract of *Stauntonia brachyanthera,* there were identified triterpenoids, flavonoids, lignanoids, and phenylethanoid glycosides (Liu *et al*., 2015). Next, a plant that is also known to have diuretic properties is *Coriandrum sativum*. Phytochemical screening of the leaf extract of this plant indicated the content of flavonoids, glycosides and saponins (Thuraisingam *et al*., 2019).

One type of plant that is considered to have nutritional and health benefits is avocado (*Persea americana* Mill.). This plant was known to contain *fat-soluble vitamins besides high levels of protein, potassium and unsaturated fatty acids (Duarte et al., 2016).* In addition avocado is a fruit plant that is known to contain bioactive substances related to diuretic function as mentioned above. Abd Elkader *et al*., (2022) in their study of the antioxidant properties of avocado leaf extract succeeded in screening phytogenic compounds i.e., polyphenols and flavonoids. Next, still regarding the study of the antioxidant effects of avocado leaf extract, Rahmah *et al*., (2023) revealed the presence of alkaloids, flavonoids, tannins, steroid saponins, and glycosides (Rahmah *et al*., 2023).

Considering that avocado contains active substances that can be associated with diuretic activity, we tested the diuretic effect of the water fraction of avocado leaf extract on albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Sample and Extraction

The fresh samples of avocado leaves were taken from East Hadimulyo Village, Central Metro District, Metro City, Lampung Province. The botanical determination of the plant was carried out by plant taxonomist at the Laboratory of Botany, University of Lampung, Indonesia. The leaves samples were cleaned of dirt and then washed with running water until clean. After that, the leaves are coarsely chopped and then dried under indirect sunlight using a black cloth cover to block ultra violet rays.

Extraction was carried out using a maceration technique by soaking 500g of simplicia in 70% ethanol solvent for 72 hours. The solvent was replaced every 24 hours with repeated stirring. The final residue removed by filtration and the filtrate was concentrated to be a viscous extract using a rotary evaporator until 150 g of thick extract was obtained.

2.2 Fractionation of Extract

The thick extract was added with 50 ml of water and 3x150 ml of hexane, then shaken with a separating funnel to obtain the hexane fraction and the water fraction. The remaining water fraction was added with 3x150 ml of chloroform, then shaken with a separating funnel and the chloroform fraction and water fraction were obtained. The water fraction was then evaporated using vacuum distillation until a condensed water fraction was obtained. The viscous fraction was then dried in an oven at a temperature of less than 60° C until a dry water fraction was obtained.

2.3 Experimental Animals and Treatment

Twenty-five albino male Wistar rats (*Rattus norvegicus*) weighing 200-250g, aged 3-4months, obtained from Lampung Veterinary Office, Indonesia. Before treatment animals were allowed to acclimatize for a week, during which the rats placed in cages (one animal per cage) at room temperature, 12/12 hour light/dark cycle, and given water and food *ad libitum.* All the animal treatment procedure in accordance with the Ethical Research Committee, Faculty of Medicine, University of Lampung, Indonesia.

By using a completely randomized design, the animals were divided into five groups consisted of 5 rats. Group 1 received distilled water as a negative control; group 2 given 3.16 mg/kg body weight of furosemide as a positive control; group 3 treated with 91.125mg/kg body weight of extract of avocado leaves; group 4 fed with 182.25 mg/kg of the extract; group 5 treated with 364.5 mg/kg of the extract. The substances were orally administered by gavage using a Sonde feeding needle. All rats treated twice during the day of experiment, one in the morning and one in the afternoon.

2.4 Study Parameters and Statistical Analysis

Urine samples were collected at the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, 5^{th}, and 6^{th} hours. After 6 hours the total urine of each rat was assessed for volume, colors, and pH. The scale of urine colors were assessed qualitatively based on the Armstrong's urine color chart which consists of 8 levels (scores) of urine color density (Armstrong *et al*., 1998). Both parametric and nonparametric statistics were used for data analysis. Parametric statistic, one way ANOVA and LSD test applied for dependent variables, volume and pH of the urine. While nonparametric statistics, Mann-Whitney Test, was applied for qualitative parameters of urine colors.

3. RESULTS AND DISCUSSION

3.1 Urine Volume

Administration of the water fraction of avocado leaf extract at a dose of 182.25 mg/kg BW and 364.5 mg/kg BW significantly increased the urine volume of rats when compared to the negative control treatment (Table 1). However, the diuretic effect of both doses of extract on the urine volume did not exceed effects of the standard diuretics of furosemide.

Table 1: Diuretic effects of avocado extract on urine volume of rat

Treatment	Urine volume (ml)					Mean
	1	2	3	4	5	
Distilled Water	0.283	0.250	0.167	0.250	0.317	0.253^{a}
Furosemide	0.417	0.433	0.467	0.33	0.533	0.436^{c}
Extract 91.125mg/kg	0.300	0.283	0.367	0.283	0.350	0.317^{ab}
Exr\tract 182.25 mg/kg	0.300	0.300	0.383	0.350	0.400	0.347^{b}
Extract 364.5 mg/kg	0.317	0.383	0.383	0.433	0.467	0.397^{bc}

*Mean values followed by the same superscript are not significantly different at α=0.05

Water fraction of leaf extract of *P. americana* is diuretics which by definition mean able to increase urine volume (Johnson *et al*., 1999). Saponins, tannins, flavonoids, phenols, and alkaloids contained in avocado leaf extract may make this plant a diuretic (Gupta *et al*., 2011). As in diuretic *Flueggea leucopyrus* (Phyllanthaceae) that has also revealed to increase volume of urine output contained alkaloids, triterpenoids, and tannins (Ellepola *et al*., 2015). The mechanism is that these bioactive compounds can promote the kidney to excrete urea, water, K^{+}, Na^{+}, Cl^{-}, and NaCl (Ellepola *et al*., 2015).

3.2 Urine Color Density

The diuretic effect of avocado leaf extract on the color of rat urine based on the color density scale on the Armstrong Chart is shown in photographs (Fig. 1). The results of non-parametric analysis on urine color density scale using the Mann-Whitney test are presented in Table 2.

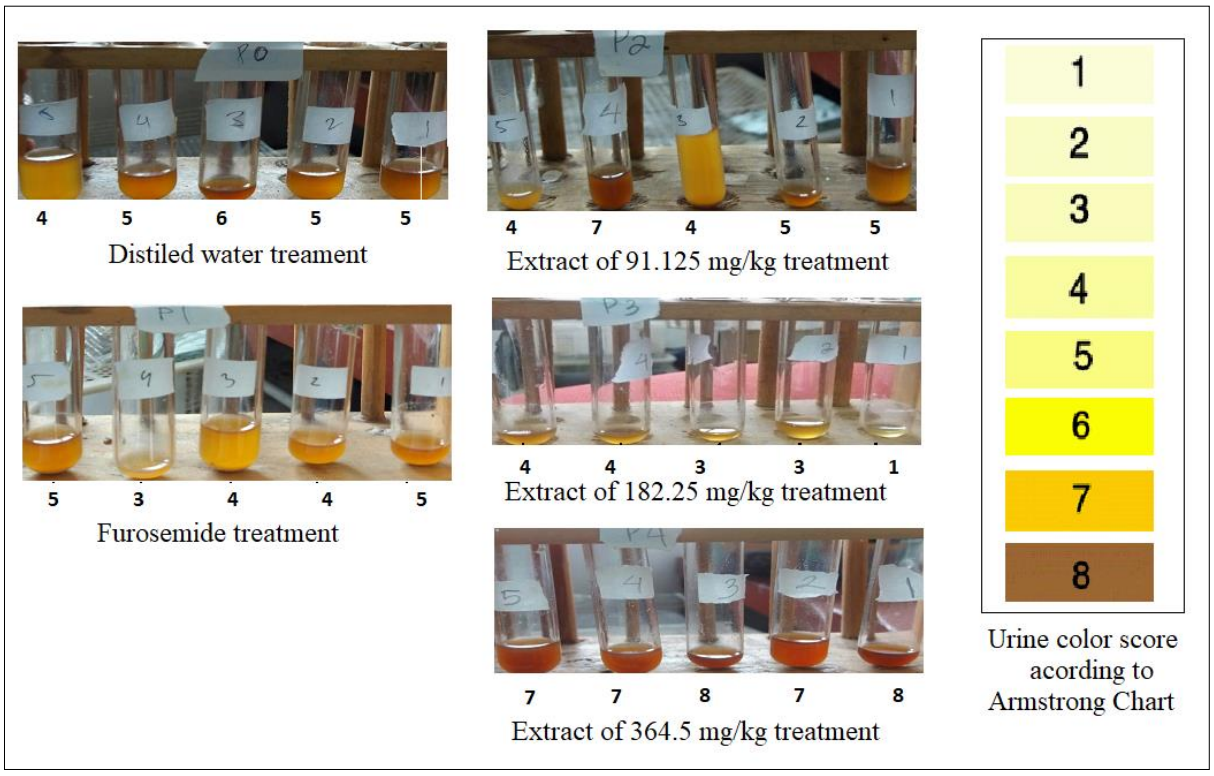

Fig. 1: Photographs showing urine color density and scores in rats given different treatment based on Armstrong Chart of urine color density

Table 2: Diuretic effects of avocado extract on urine color density scale in rat

Treatment	Urine color density score					Mean
	1	2	3	4	5	
Distilled Water	5	5	6	5	4	5^{b}
Furosemide	4	4	4	3	5	4^{cd}
Extract 91.125mg/kg	5	5	4	7	4	5^{bc}
Exr\tract 182.25 mg/kg	1	3	3	4	4	3^{d}
Extract 364.5 mg/kg	8	7	8	7	7	7.4^{a}

*Mean values followed by the same superscript are not significantly different at α=0.05

The group of rats given avocado leaf extract at a dose of 364.5 mg/kg produced significantly more concentrated urine than the other groups. However, the average urine color density score does not have a dependent manner that follows the dose levels of diuretics given. Actually, the relationship between diet intake and urinary pigment has been reported long ago in 1927. Urine color, according to the report, is determined by some factors including fasting, acid and base treatment, calorigenic stimulation, and diuresis. Urinary pigment increase up to of 46.4% by fasting treatment, 34.6% by the administration of hydrochloric. Diueresis—the increase of urine volume, almost invariably occurred simultaneously with increased pigment output (Drabkin, 1927).

3.3 Urine pH

Administration of avocado leaf extract did not show any significant different effect on the urine pH of rats (Table 3).

Table 3: Diuretic effects of avocado extract on urine pH of rat

Treatment	pH of urine					Mean
	1	2	3	4	5	
Distilled Water	7.88	7.21	7.54	7.35	7.73	7.542[a]
Furosemide	7.36	7.42	7.11	7.23	7.65	7.468[a]
Extract 91.125mg/kg	7.94	7.58	7.37	7.84	7.16	7.578[a]
Exr\tract 182.25 mg/kg	6.65	8.55	7.69	8.66	7.33	7.776[a]
Extract 364.5 mg/kg	7.45	6.83	7.26	6.48	7.57	7.118[a]

*Mean values followed by the same superscript are not significantly different at α=0.05

The findings of this study showed all urine produced by rats have pH of more than 7 (alkaline). Additionally there is no significant difference in the pH of urine between treated rats. There were factors known to affect the level of urine pH of a subject such as alcohol consumption (Eggleton, 1946) and acid-base load of the diet (Welch, 2008).

4. CONCLUSION

Water fraction of leaf extract of avocado (*P. americana*) potentially for diuretic ingredient without make no significant changes on urine pH.

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