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DIURETICS EFFECT OF AVOCADO LEAF (PERSEA AMERICANA MILL.) ETHANOL EXTRACT ON THE GASTRIC HISTOLOGY OF WISTAR MALE RATS (RATTUS NORVEGICUS)

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Abstract

Irregular urine output triggers kidney stone disease. One way to cure it is by using synthetic diuretic compounds. The use of synthetic diuretic drugs for a long time can cause side effects, namely gastric damage. Therefore, research is needed to determine the diuretic effect of ethanol extract of avocado leaves (Persea americana) and its effect on the histological structure of the gastric of male rats. Avocado leaf extract is made by maceration method, using 70% ethanol and then evaporated. Wistar male rats were divided into 5 treatment groups namely negative control (aquadest), positive control (furosemide), avocado leaf extract dose of 50 mg / kg body weight, 100 mg / kg body weight, and 150 mg / kg body weight with oral administration for 15 days. The volume of urine was measured for 24 hours, then the mice were sacrificed on the 16th day. Gastric organs were taken for histological observation by the paraffin method and Hematoxylin and Eosin (H&E) staining. The results showed that the optimum avocado leaf ethanol extract was a dose of 100 mg/kg showed a diuretic effect but did not affect the histological structure of the gastric.

Keywords: diuretics, ethanol extract of leaves of avocado, gastric histological structure

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INTRODUCTION

Smoothless urinary output triggers the onset of kidney stone disease. Increasment the rate of urine output is one way to cure kidney stone disease. Diuretic drugs use to increase water expenditure with unabsorbed solutes such as sodium ions and chlorides (Guyton & Hall, 2014).

Currently there are many synthetic diuretics that have been used to increase the rate of urine output, including furosemide. The use of synthetic diuretics in a long period cause side effects such as impaired kidney function and stomach damage (Siswandono and Soekardjo, 2008).

Stomach is a digestive organ that functions to store food, secrete HCl and enzymes for protein digestion. Anatomically the stomach is divided into four parts respectively cardia, fundus, corpus, and pylorus (Mescher, 2017). Disturbances in the stomach are caused by an imbalance between the gastric juice secretion and protection by the gastroduodenal mucosa and neutralization of gastric acid by duodenal gum (Sherwood, 2012). Phylorus is the part of the stomach that is often disturbed (Puspitasari, 2008).

Instead of using synthetic diuretic, it can be used natural ingredients, namely

avocado leaf extract (*Persea americana* Mill) to avoid disruptive effects.

Avocados are traditional medicines that have the ability to treat diseases such as mouth sores, bladder stones, toothaches, dry faces, swelling due to inflammation and also diabetes (Katja *et al*, 2009). Avocado leaves contain chemical compounds including flavonoids, saponins, tannins and steroids (Rahman *et al*, 2018).

Flavonoids are potent antioxidants that can bind free radicals. Flavonoid antioxidant activity is more effective than vitamins C, E and carotenoids (Dai and Mumper, 2010). Tannins function to accelerate wound healing and inflammation of the mucous membrane (Arukwe *et al*, 2012)

Avocado leaves are reported to be effective, among others, as analgesic drugs, hypertension, hypoglycemia, diarrhea, hemorrhage, anti-inflammation and diuretics (Kurniawan, 2014). According to Adha (2009), treatment of avocado leaf extract containing flavonoids at a dose of 100 mg / kg bb in Sprague-Dawley male white rats produce higher total urine yields than furosemide which is usually used as a diuretic drug.

Long term effect using syntetic diuretic cause interference the gastric structure. Therefore, it is necessary to conduct research that aims to determine

the diuretic effect of ethanol extract of avocado leaves as a natural diuretic on the histological structure of the stomach.

MATERIALS AND METHODS

The study was conducted at the Zoology Laboratory and the Botany Laboratory of the Department of Biology FMIPA, University of Jember. The study was conducted using a Completely Randomized Design (CRD), with the implementation stages, namely:

Preparation of Avocado Leaves Ethanol Extract

Dark green avocado leaves are obtained from the Tegalboto Kidul Jember area, then cleaned and separated with leaf bones. The leaves are roasted at 40 ° C for 4 days to dry, then ground and sieved (60 mesh) to produce powder. Avocado leaf extract is made by maceration method, using 70% ethanol with a ratio of 1:10, for 2x24 hours. Avocado leaf maceration results are evaporated with a *rotary evaporator* at a temperature of 70 ° C and a speed of 50 rpm, so that the avocado leaf extract is produced.

Fitochemical assay of Avocado Leaves

A. Flavanoid Test

As much as 0.1 gram of avocado leaf extract mix with methanol until

submerged and then heated. The filtrate was added with concentrated H₂SO₄. The formation of red colour indicates flavonoids.

B. Tanin Test

A total of 0.1 grams of ethanol extract of avocado leaves mix with 5 ml of distilled water then heated to boiling for 5 minutes and filtered. After that, the filtrate was added with 5 drops of FeCl₃ 1% (w / v). The presence of tannin is indicated by the formation of dark blue or greenish black colour .

Animal and Treatment

The animals used in the study were male rats (*Rattus norvegicus*) Wistar strain aged 2 months and body weight 200g. Rats were obtained from PUSVETMA Surabaya and adapted in a metabolic cage for 7 days, and given feed in the form of pellets and drinking aquadest on an *ad libitum* basis. Rats were divided into 5 treatment groups respectively negative control (distilled water), positive control (5.04 mg / kg body weight furosemide), ethanol extract of avocado leaves at a dose of 50 mg / kg body weight, 100 mg / kg body weight and 150 mg / kg body weight.

The treatment of test animals in this study using the *Lipschitz* method, before the treatment of rats fasted for ± 18 hours.

After that the rats were given a loading dose of 1 ml of warm aquadest and left for 30 minutes. Furthermore, rats were treated orally according to the dosage using gastric sonde for 15 days (Wientarsih et al, 2012).

On the first day after the last treatment, the rats were not given food and drink, then a diuretic test was performed and the total urine volume was measured for 24 hours. Mice were killed on day 16 by being *anesthetized* using 0.1 ml of *Ketamine* and 0.02 ml of *Xyla* per mouse, then dissected. Furthermore, the gastric organs are taken part of the pylorus to make histological preparations with the paraffin block method and Hematoxylin and Eosin staining.

Urine Volume Measurement

Rats were placed in a metabolic cage equipped with a urine container, the urine obtained was measured with a measuring cup. Measurement of total urine volume for 24 hours after the last treatment.

Preparations of Gastric Histology

Gastric organs is carried out for the preparation of gastric histology preparations of the pylorus. The method used is the paraffin method and the staining of HE (*Haemotoxylin-Eosin*). Paraffin methods include *fixation*, *dehydration*, *clearing*, *infiltration*,

embedding, *sectioning*, *affixing*, *staining*, and *mounting*.

Observing Gastric Histology

A. Measurement of Gastric Mucosa Thickness

Measurement of the thickness of the gastric mucosa was carried out using a 400x magnification microscope.

B. Calculation of the Number of Parietal Cells

Calculation of the number of parietal cells is carried out qualitatively in the epithelial section. The number of parietal cells is counted in 10 fields of view (Puspitasari, 2008). The results of the calculation of the number of parietal cells are divided into 3 groups, namely:

- (+) : Number of parietal cells 20-39
- (++) : Number of parietal cells 40-59
- (+++): Number of parietal cells ≥ 60

Data Analysis

Data on urine volume and thickness of the gastric mucosa obtained from the results of the study were analyzed using the One Way ANOVA test with a confidence level of 99% or $\alpha = 0.01$ followed by the Duncan Multiple Range Test (DMRT) to see significant differences between dose treatment groups.

RESULTS AND DISCUSSION

Chemical content analysis of the ethanol extract of avocado leaves in this study using a qualitative phytochemical screening test to detect the presence of flavonoids and tannins. The results of the chemical assay or the ethanol extract of avocado leaves can be seen in Table 1.

Table 1. Qualitatively chemical compounds in the ethanol extract of avocado leaves.

Secondary metabolites	Results
Flavonoid	Positive
Tanin	Positive

Based on Table 1, avocado leaf extract contains flavonoids (positive) which are marked by the formation of red color, and contains tannins (positive) characterized by the formation of greenish black color. This is similar with the research of Adha (2009) that avocado leaves contain flavonoid compounds and tannins. Flavonoids are potent antioxidants, and are diuretic (Nessa et al, 2013) while tannins serve to accelerate wound healing and inflammation of the mucous membrane (Arukwe et al., 2012).

Avocado Leaves Extract Diuretic Activity Test

Diuretic test is done by measuring the total urine volume of male white rats (*Rattus norvegicus*) for 24 hours. The observation of total urine volume for 24 hours can be seen in Table 2.

Table 2. Total urine volume of white rats after administration of ethanol extract for avocado leaves during 24 hours

Dose	Urine Volume (ml)
Treatment	(X± SD)
Negative Control	1,87 ± 1,04 ^a
Positive Control	1,96 ± 0,92 ^a
50 mg/kg bb	2,53 ± 1,60 ^{ab}
100 mg/kg bb	2,54 ± 1,17 ^{ab}
150 mg/kg bb	3,32 ± 1,61 ^b

Note: numbers followed by the same letters in the same column show no significant difference in the DMRT test with a significance level $\alpha = 0.01$.

Based on the *Anova* test, the avocado leaf ethanol extract treatment had a very significant effect on the increase in the total urine volume of white rats compared to the negative and positive controls. *Duncan* test results of 24-hour total urine volume showed that the treatment group of 150 mg / kg bw ethanol extract of avocado leaves was significantly different from the negative control group and the positive control group. The total urine volume of 150 mg / kg bw (3.32 ml) was highest compared to the other groups. Chemical compounds that are thought to have a diuretic effect on ethanol extract of avocado leaves are flavonoids.

Flavonoids increase urine volume expenditure and increase excretion of Sodium, Potassium and Chlorid in urine (Omodamiro & Jimoh, 2016). This is consistent with the results of the study of Adha (2009) administration of ethanol extract of avocado leaves containing

flavonoids at a dose of 100 mg / kg bw resulting in more urine volume compared to the control group urine volume and administration of furosemide in Sprague-Dawley male rats.

The control group's urine volume was negative for 24 hours at the lowest compared to the other treatment groups, this shows that the treatment of furosemide and ethanol extract of avocado leaves can increase urine volume.

Histological Structure of Rat Stomach After Giving Avocado Leaf Ethanol Extract

Histological structure of the rat's stomach after the ethanol extract of avocado leaves was observed by measuring the thickness of the gastric mucosa and counting the number of parietal cells. Pylorus is more sensitive to acids than other parts of the stomach (Puspitasari, 2008).

The results of the tunica mucosa thickness can be seen in Table 3. *Anova* test shows that the treatment of avocado leaf extract has a very significant effect on the thickness of tunica mucosa. Further tests with Duncan, avocado leaf extract dose of 150 mg / kg bw were very significantly different from the negative control group, positive control and the treatment group were doses of 50 mg / kg bw and doses of 100 mg / kg bw.

Table 3. Average tunica mucosa thickness of gastric male white rat (*Rattus norvegicus*) after administration of ethanol extract of avocado leaves

Treatment	Thickness of Tunica Mukosa (μm)
	15 Days ($\bar{x} \pm \text{SD}$)
Negative Control	296,09 $\pm$ 74,00 ^a
Positive Control	288,04 $\pm$ 16,39 ^a
50 mg/kg bb	297,65 $\pm$ 50,37 ^a
100 mg/kg bb	311,68 $\pm$ 17,35 ^a
150 mg/kg bb	387,70 $\pm$ 14,62 ^b

Note: numbers followed by the same letter in the same column show no significant difference in the DMRT test with a significance level $\alpha = 0.01$

The treatment of avocado leaves ethanol extract tends to increase the thickness of the tunica mucosa at a dose of 50 mg / kg and 100 mg / kg. This is presumably because the ethanol extract of avocado leaves functions to maintain the the mucous membrane integrity. Tannins have an astringent effect that causes precipitation of micro-proteins on the outer surface of epithelial cells in the stomach to form a protective layer that prevents absorption of toxic substances and maintains the gastric mucosal layer against the action of proteolytic enzymes (Salawu *et al.*, 2009). Flavonoids act as antioxidants, compounds that can capture free radicals that can cause damage to the gastric mucosa (Bintari *et al.*, 2014). The thickness of the gastric mucosa after treatment of avocado leaf extract (*Persea americana*) can be observed in the following gastric structure (Picture 1).

Picture 1. Tunica mucosa thickness after avocado leaf extract (*Persea americana*) (400x magnification) A. Negative control, B. Positive control, C. dose of 150 mg / kg

The use of synthetic diuretic drugs causes damage to the stomach. In this study the use of avocado leaf extract did not damage the stomach because of the presence of tannins and flavonoids which function as protectors for the gastric mucosa. The optimum treatment of avocado leaf extract is up to a dose of 100 mg / kgbw, because a dose of 150mg / kgbw shows the thickness of the tunica mucosa significantly increased compared to the control, but the structure is normal. The mechanism of thickened gastric epithelium is unknown.

Based on Table 4, it can be seen that the number of parietal cells in the stomach tends to increase with increasing treatment dose.

Table 4. Qualitatively the number of parietal cells in mucosa of the gastric male white rat (*Rattus norvegicus*) after administration of ethanol extract of avocado leaves

Treatment	Number of Parietal Cells	Scoring
control (-)	44	++
control(+)	37	+
50 mg/kg bb	49	++
100 mg/kg bb	54	++

150 mg/kg bb	69	+++
Notes :		
(+)	: Number of Parietal Cells 20-39	
(++)	: Number of Parietal Cells 40-59	
(+++)	: Number of Parietal Cells ≥ 60	

Positive control had the lowest parietal cells number compared to other treatment groups, this showed that furosemide treatment tended to reduce the number of parietal cells, while the treatment of avocado leaf extract could increase the number of parietal cells. This is presumably due to the flavonoid content in the ethanol extract of avocado leaves.

Flavonoids in addition to playing a role in diuretics and anti-inflammatory, also acts as an antioxidant. Antioxidants can capture free radicals (Omodamiro & Jimoh, 2016). Free radicals are molecules that cause lipid peroxide in the gastric mucosa, so cell membrane permeability increases. Increased permeability will facilitate the diffusion of stomach acid

again so that gastric acidity can increase, which causes damage to the tissue.

As an antioxidant, flavonoids can inhibit lipid peroxidation in the gastric mucosa so as to prevent an increase in cell membrane permeability and do not occur

back diffusion of gastric acid so that gastric acidity does not increase and parietal cells can survive longer (Rostika, 2012). The structure of parietal cells can be seen in Picture 2.

Picture 2. Parietal cells after treatment of avocado leaf extract (*Persea americana*) Black arrow () indicates parietal cell

CONCLUSION

Based on the results of this study, it can be concluded that dose of 100 mg / kg bw show the optimum diuretic effect and has no effect on the histological structure of the stomach.

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