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Histopathological Study of Renal Hypertension with Avocado Leaf Extract and Fractional in Male Wistar Rats

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Abstract

Background: The first of the cardiovascular diseases that may turn deadly is hypertension, which has elevated blood pressure. The causes of hypertension, which causes an average rise of 140/90 mmHg, are based on epidemiological factors such as stroke, heart failure, myocardial infarction, arterial fibrillation, and peripheral artery disease. Hypertension is also known as the silent killer.

Objective: Known histopathology in kidney cells and avocado leaf compounds that could potentially inhibit hypertension.

Methods: Methods that can be used in histopathological testing with hematoxylin and eosin (HE) dying and compound determination by analysis *Gas Chromatography-Spectrometry Mass* (GC-MS)

Results: The study obtained GC-MS (Gas Chromatography-Spectrometry Mass) analysis results at an area of 36.50%, molecular weight 17,03 with 9-Octadecenoic acid compounds, and an area of 10,18%, molécular weight 2,54 with a compound of 9-Oktadecenosic acid (z)-methyl esters (CAS).

An extract of 250 mg/kgBW has a slightly low renal cell damage value (138), and an effective avocado leaf fraction test is ethanol acetate 250 mg/kgBW, with a small renal cell damage value (146), where as a negative control has a high renal cell damage value (189).

Keywords: Hypertension, Avocado leaves, Histopathology, GC-MS

BACKGROUND

Cardiovascular disease is a dangerous disease that can't cause transmission, one of the cardiovascultural diseases is hypertension or can be called by increased blood pressure (1)**.** Factors leading to epidemiological roles include stroke, heart failure, myocardial infarction, arterial fibrillation, and peripheral artery disease with a degree of mild increase in hypertension (140/90 mmHg).

Hypertension is found in all populations, with varying incidence rates. This is caused by several factors, namely genetic, racial, regional, sociocultural, and different influences on lifestyle changes, including food and beverages (2). Changes in people's lifestyles and the consumption of unhealthy foods and drinks lead to increased risk factors for hypertension.

As for drugs that can be used to inhibit hypertension with some treatments, among others are angiotensin converting enzyme inhibitors (ACEI) such as captopril, lisinopril, and angiotensin receptor blockers (ARB), for example, losartan, candesartan, and irbesartan. Calcium channel blockers (CCB) with amlodipine, nifedipine, and beta blockers (BB) with propranol, atenolol, bisoprolol, and diuretic drugs (3).

Alternative treatments of natural materials can be empirically supported by society. Generally speaking, people consume some natural ingredients, one of which was

believed by ancestors to be that avocado leaves (Persea Americana) can lower hypertension. Boiling seven avocado leaves and three glasses of water will reduce hypertension. But there's not much research done about the content of the compounds found in avocado leaves, which can inhibit hypertension (4).

Previous research has investigated the hypertensive activity of extracts and fractions of avocado leaves that have several compounds potentially inhibiting hypertension: flavonoids, alkaloids, tannins, terpenoids, and saponins. But a compound positive in phytochemical screening tests (KLT, IR, and GC-MS) is a flavonoid using standard quercetin as a comparator (5),(6).

Considering the results of previous studies, the activity of the extract and fraction of avocado leaves has been shown to inhibit hypertension, which makes the researchers interested in continuing the study with histopathological tests of the kidneys. Where the kidney organs are the main organs in the external stool, through the urine, the kidneys excrete substances that are no longer needed by the body. The urine is the main pathway to the excretion of toxic substances, as a result of which the kidneys have a high volume of blood flow (7).

METHODS

Materials and Equipment

The samples for the study were 40 white males of Wistar with a healthy condition and a weight of 200–250 grams. The used in the research were equipments for reaction tubes, tube shelves, beaker glass, pipet, petri cups, digital scales, microtoms, glass objects, pinsets, skulls, and microscopes. The materials used were Nacl and prednisone, vocal leaf extract, water fraction, ethyl acetate and n-hexane, 10% formalin buffer solution (NBF), liquid paraffin, 70% alcohol, 80% alcohol, 90% alcohol, and absolute alcohol (8),(1).

GC-MS test avocado extract.

Identification was done to determine the profile of the compound extract of avocado leaf with the GC-MS test that can be assumed to have the effect of a compound potentially inhibiting hypertension. The result obtained is a chromatogram by showing a graph and several peaks; each one of the peaks that appear is a type of compound. The sample is injected into a 30 m x 0.25 mm i.d. column with a 0.25 µM thin film, the carrier gas uses 1 ml/min helium, the injector is pressed at 200ºC, and the column temperature is set in the program at 50–250ºC at a speed of 10ºC/min injection method. At MS applied ionization voltage 70 eV temperature 250ºC mass range 50-600. Thus obtained chromatograms and mass spectra of unknown compounds were later compared to the spectra of known compounds (9).

Group of test animals

The test animals were grouped into 7 groups consisting of negative groups (Nacl and prednisone), extracts of 75, 150, and 250 mg/kgbw, water fractions, ethyl acetate, and n-hexane..(1)

Histopathological tests

The trial animals were tested with hypertension treatment for 29 days, divided into several parts, namely, given Nacl and prednisone as animal induction for 21 days. Subsequently, they were treated with both extracts and fractions. The study was gradually tested to determine the effective dosage of the extract. Once the effective dose was proven, the same treatment was continued at fractional doses (1).

Each group of test animals had been treated for 29 days, then determined by disorting the neck and then taking the right kidney. The kidney organ to be tested is cut to size 1x1x1 cm. Subsequently, it was submerged in a neutral formalin buffer solution of 10% (NBF). Then proceeded with the dehydration process with alcohol concentration levels of 70%, 80%, and 90% absolute alcohol 1 and absolute II 2 hours, respectively.

The process of cleaning with xylol and printing paraffins is fine-tuned so that the preparations printed on paraffin blocks are then stored in the refrigerator (10).

The resulting slice is wrapped in a water bath at a temperature of 60ºC. The preparation is then removed and placed in a glass of the object for hematoxylin and eosin (HE) dyeing.

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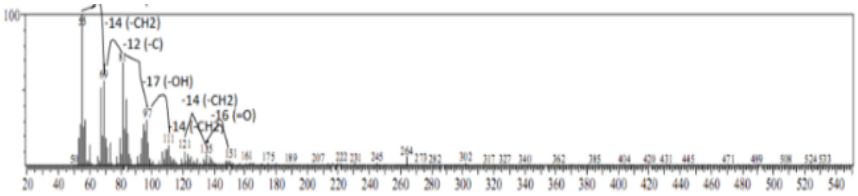
Each preparation reads two layers of proximal tubulus and glomerulus with a magnification of 400x. Renal tubulus damage in the animal test mice is examined by counting the lumen of the proximal tube that closes and the proximal necrosis of the tubulus at 5 areas of view for each treatment groups (7),(11).

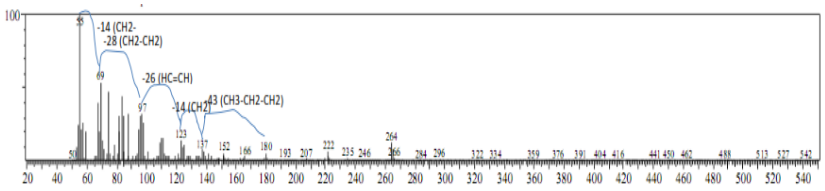
RESEARCH RESULT

GC-MS Avocado Leaf Extract Test Results

The results of the Gas Chromatography-Mass Spectrometry test of avocado leaf extract can be explained in figures 1 and 2, the histopathological test results can be explained in table 2, figures 3 and 4.This will be explained in more detail on the discussion page.



Gambar 1. 9-Octadecenoic acid



Gambar 2. 9-Octadecenoic acid (z)- methyl ester (CAS)

Table 1. GC-MS Avocado Leaf Extract Test Results

No	Time (Minutes)	Area (%)	Molecular Weight	Compound
1.	24,50	36,18	17,03	9-Octadecenoic acid
2.	23,78	10,18	2,54	9-Octadecenoic acid (z)- methyl ester (CAS)

Table 2. Histopathology test results

Treatment group	Damaged cell	SD
Negative	189	14
Extract 75 mg/kgbw	184	9
Extract 150 mg/kgbw	175	20
Extract 250 mg/kgbw	138	12
Fraction n-hexsane	179	9
Fraction ethyl acetate	146	6
Fraction water	179	16

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Histopathology test results

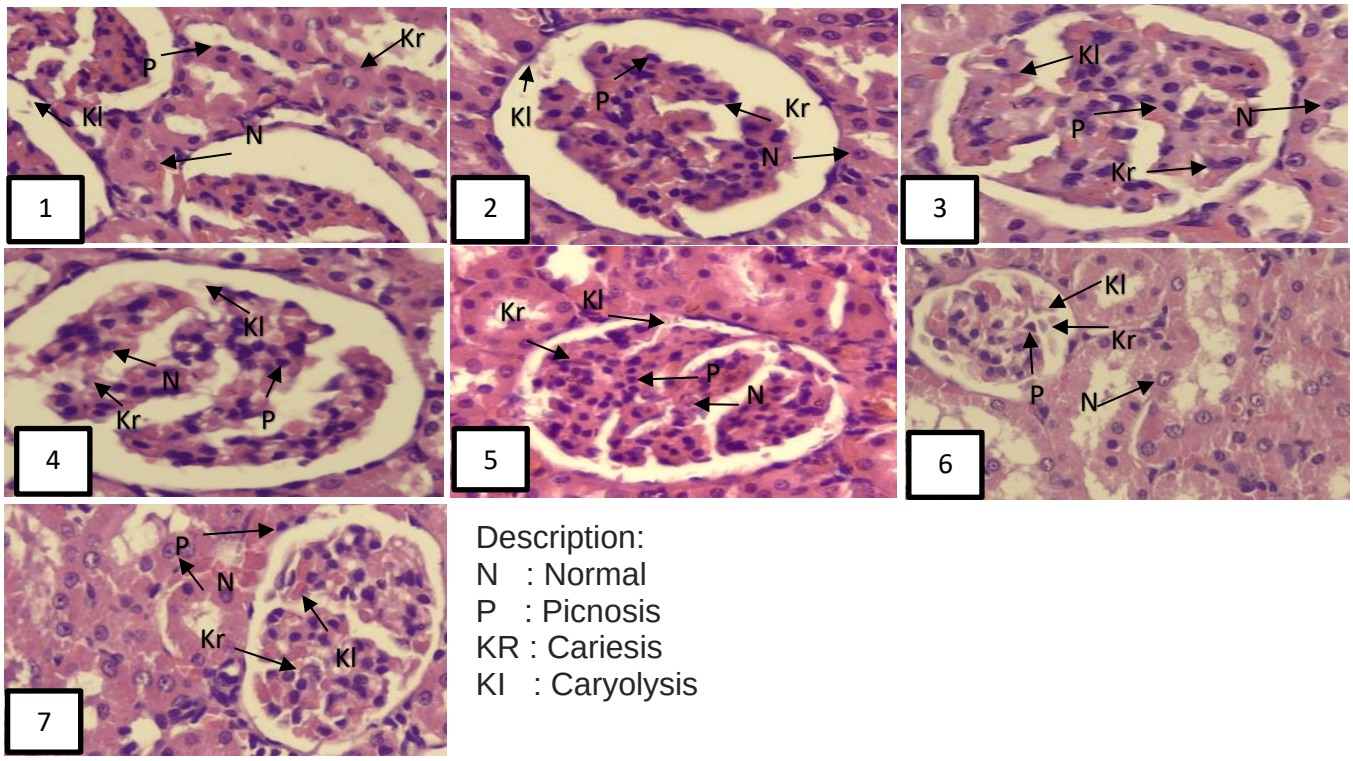

Figure 3. Renal histopathology in male white rats by mouth. The visible lumen of the proximal tubulus and the glomerulus part of the kidney cells are in three categories: picnosis, caryorexia, and caryolysis. Number 1 (negative), 2 (extract of 75 mg/kgbw), 3 (extract of 150 mg/kgbw), 4 (extract of 250 mg/kgbw), 5 (water fraction), 6 (ethyl acetate fraction), and 7 (fraction of n-Heksana).

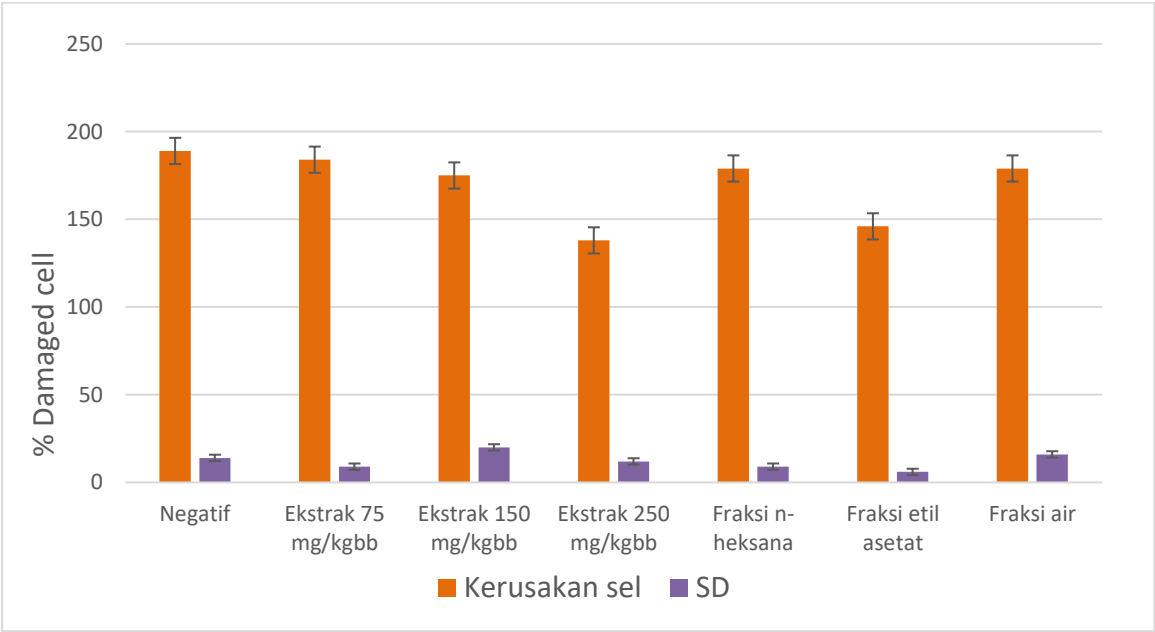

Figure 4. Histopathological test chart

DISCUSSION

GC-MS Avocado Leaf Extract Test Results

The avocado leaf extract has analyzed by measuring the GC-MS (Gas Chromatography Spectrometry Mass) analysis at an area of 36,50%, molecular weight 17,03 with 9-Octadecenoic acid compounds, and an area of 10,18%, molecular weight 2,54 with a compound of 9-Oktadecenosic acid (z)-methyl esters (CAS).

This could be used as a basis for further pharmacological testing to determine whether the compound has potential as a hypertension inhibitor. In the case of previous research or related literary sources (1).

Histophatology Test Results

Histopathological test results on Figure 3 and Figure 4 an effective test of avocado leaf extract is an extract of 250 mg/kgbw with a low renal cell damage value (138) and an effective avocados leaf fraction test is 250 mg /kgbw ethyl acetate with a small renal cells damage value (146) while on a negative control has a high renal cells damage value (189) this is based on the results of the study.

The damage is slightly lowered by the role of the chemical compound from the avocado leaf extract, the flavonoid (9-Octadecenoic acid (z)-methyl ester (CAS)) that can inhibit hypertensive activity so that systolic and diastolic blood pressure decreases by 120/80 mmHg.

Decreased hypertensive activity can affect renal cell repair. Where the kidney organs are the main organs in the excretion phase, through the urine the kidney excretion substances that are no longer needed by the body. The urine is the main pathway to the excretion of toxic substances, as a result of which the kidneys have a high volume of blood flow (11), (7).

CONCLUSIONS

The conclusion of this study is the analysis of the GC-MS (Gas Chromatography-Spectrometry Mass) test at an area of 36.50%, molecular weight 17,03 with compound 9-Octadecenoic acid, and area of 10.18%, molecular weight 2,54 with composition 9-Oktadecenosic acid (z)-methyl ester (CAS).

250 mg/kgbw extract with a slightly low renal cell damage value (138), and an effective avocado leaf fraction test is 250 mg/kgbw ethyl acetate with a small renal cell damage value (146), while a negative control has a high renal cell damage value (189).

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