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Effect of Ethanol Extract from Semangkuk Fruits (Scapium affine) on Histological Image of Liver Male Mice

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Abstract:

The use of traditional medicine by drinking will go through a digestive process that involves absorption by the intestines, the intestines as a digestive tract supply blood to the liver so that the food and drinks consumed by an individual are closely related to liver histology. The liver as a detoxifier of toxins works by breaking down toxic compounds into several compounds such as urea, ammonia, and uric acid. Damage to liver organs includes necrosis, steatosis, and inflammation. One of the plants that can be used in traditional medicine is a fruit or a semangkuk plant, a semangkuk fruit is a type of plant that is efficacious as a decrease in heat. Testing related to the histological picture of the liver organs on a bowl of fruit has never been carried out so this test aims to find out about the histological picture of animal liver-induced extract at doses of 100 mg / KgBB, 150 mg / KgBB, and 200 mg / KgBB, with positive control given acetaminophen and normal control, in the extraction method carried out by maceration. The test animals used were 30 male white mice and were divided into 5 groups. Mice are surgically taken from the liver organs, then staining is carried out using hemotoksilin eosin and observed using a microscope to see whether or not there is damage to the liver organs. Data analysis using one-way ANOVA. From the results of the study, it was found that there was damage to the liver organs such as damage to necrosis, steatosis, and inflammation with the highest dose of 200 mg / KgBB, so a semangkuk of fruit is not safe to use if at high doses.

Keywords: Semangkuk Fruit, Extract, Liver Organ Histology

Introduction

The use of traditional medicines derived from plants continues to increase, plants as efficacious traditional medicines have long been consumed by the community by boiling the plants first and then drinking the boiled water directly, after the boiled water of the medicinal ingredients is drunk then it goes through a digestive process which involves absorption by the intestine, the intestine acts as the digestive tract supplying blood to the liver so that the food and drink consumed by an individual is closely related to the histology of the liver (Fitmawati *et al* ., 2018).

The liver is one of the organs in the body that has an important role as a neutralizer of toxins and as metabolism, the liver is responsible for the biotransformation of harmful substances into harmless substances. This process causes liver cells to be easily damaged, both in the form of damage to cell structure and impaired function of the liver (Nugraha *et al* ., 2018). The liver acts as a poison detoxifier in the body which works by breaking down toxic compounds into several compounds such as urea, ammonia, and uric acid (Tia Pramesti *et al* ., 2017).

Damage to the liver organs includes necrosis, steatosis, and inflammation. The liver plays an important role as a detoxification organ, so hepatocyte cells experience damage such as necrosis due to the large space in the liver for the bioactivation of various reactions related to the

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elimination of various toxic substances (Zamrodah, 2016). Steatosis is an intracytoplasmic accumulation of triglycerides that can occur due to increased free fatty acids, reduced free fatty acid oxidation, and decreased triglyceride exports due to a deficiency of fat-binding apoproteins. early and will develop into a larger vacuole (macrovesicular) thereby pressing the nucleus to the edge (Zamrodah, 2016). And inflammation is an inflammatory process that occurs in an injured liver, characterized by infiltration of lymphocyte inflammatory cells in the central vein caused by damage to endothelial cells, inflammation of the liver begins in the central vein as a reservoir for blood originating from the hepatic artery and portal vein (Sijid *et al* ., 2020).

One of the plants that can be used in traditional medicine is a fruit or bowl plant which can be found in Semambu village, Sumay District, Tebo Regency, Jambi Province, based on research by Asridawati *et al* (2020) bowl fruit is a type of plant that is efficacious as a heat reduction by processing it pounded first and then soaked in water and after that it is drunk, its availability is still very limited so it needs to be maintained in nature (Anjeli, 2019). The content of secondary metabolites that act as antipyretics in the bowl of fruit is flavonoids which are natural antioxidants capable of acting as reducers of hydroxyl radicals, superoxide, and peroxyl radicals (Tatukude *et al* ., 2014).

Based on previous research conducted by Kurniawan et al ., (2014) in the liver histology of mice (*Mus musculus L.*) who were given lamtoro leaf extract (*Leucaena leucocephala*) it is known that lamtoro leaf extract does not affect damage to the liver of mice. In the liver histology of the ddw strain of mice (*Mus musculus L.*) after administration of N-hexane extract of andaliman fruit (*Zanthoxylum acanthopodium DC.*) during the pre-implantation and post-implantation periods, it was found that the ddw strain gave discoloration and damage to the liver of the mice, but in the fruit bowl There is no further research related to histology, therefore I am interested in conducting research related to the effect of a bowl of fruit extract (*Scaphium affine*) on the appearance of necrosis, steatosis and histological inflammation of the liver of male mice.

Methods

The research method used is experimental, starting from the collection of materials, manufacture of Simplicia, extraction, phytochemical screening tests, Liver harvest, and histological examination of the liver.

Tools and materials

The tools used are Rotary Evaporator (BUCHI@), Analytical Scales (Ohauss@), Porcelain Cups, Glassware, Mortar and Stampfer, Sonde, Spuit, Filter Paper, Microscope, Microtome, Paraffin Oven, Freezer, Waterbath, Shaker, Section set, Cassette tissue, Section Set, Mice, Preparation glass, Cover glass.

The materials used were Fruit Bowl (*Scaphium affine*), 1% Na CMC, 70% Ethanol, Aquadest, Hematoxylin Eosin Staining, Exylol (90.5% carbon and 9.5% hydrogen), Bouin solution (75 ml Picrid acid, formaldehyde 25 ml, and 5 ml acetic acid), Paraffin, Magnesium, Amyl Alcohol, Mayer's Reagent, Dragendorf's Reagent, Wagner's Reagent, Chloroform, $FeCl_3$ 1%, HCL 2 N, H_2SO_4 Concentrated, Mice.

Samples

The sample used was a bowl of fruit obtained from Tebo District, Jambi Province. Determination of plant samples was carried out at the Taxonomy Laboratory, Department of Biology, FMIPA UNPAD with species number No.36/HB/11/2021.

Ethical Clearance

The ethical clearance of this research has been approved by the ethics study division of the Jambi Ministry of Health Poltekkes with the number LB.02.06/2/28/2022.

Phytochemical Screening Test

Phytochemical screening tests performed included alkaloids, flavonoids, tannins, steroid saponins, and terpenoids.

Animal Treatment, Calculation of Dosage and Preparation of Extract Solutions

Animal treatment:

- Normal Control: Nothing is given
- Positive Control: Given acetaminophen
- Dosage 100 mg
- Dosage 150 mg
- Dosage 200 mg

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Preparation of Bouin's Solution
Bouin solution contains:

- Picid acid: 75 ml
- Formaldehyde 25 ml
- Acetic acid 5 ml
- Formalin concentration 40%
- Mix all the ingredients carefully, this solution is made as a preservative for the liver.

Preparation of preparations

a. Network Preparation
After all, animals had been dissected and their livers were taken, histological preparations were made using the paraffin method, which started with material selection, fixation, dehydration, clarification, paraffin infiltration, embedding, cutting, attachment, deparaffinization, dehydration, coloring, sealing and labeling. . (Nisfa Lailatun, 2020) .

b. Fixation
The liver is first put into the fixative (boudin solution), and the resulting boudin solution is yellow. Then look at the fixative volume section, the recommended volume is 15 to 20 times, while the recommended tissue volume is 10 to 20 ml for 1-2 cm tissue and the recommended fixation time is 3 hours (Nisfa Lailatun, 2020).

c. washing
Washing, the liver is then washed many times using 70% alcohol until the color is no longer yellow (until clear) and after that, the liver is placed in 70% alcohol (Nisfa Lailatun, 2020).

d. Dehydration
The tissue is placed in alcohol with graded concentrations, namely 70%, 80%, 96% alcohol, and absolute alcohol for 60 minutes (Nisfa Lailatun, 2020).

e. Dealcoholization
The tissue is put into a bottle containing toluol, if the tool solution undergoes a milk-like change, it means that the dehydration step is not correct, then the organs need to be returned to the bottle containing absolute alcohol for complete tissue alcohol substitution by toluol. 2020).

f. Infiltration
Infiltration was carried out in an oven with a temperature of 60ºC, then the organ tissue was inserted sequentially into Toluol paraffin for up to 30 minutes, pure paraffin I for up to 60 minutes, pure paraffin II for up to 60 minutes, and pure paraffin III for up to 60 minutes, after which all the infiltration material was placed in a glass container (Nisfa Lailatun, 2020).

g. Embedding
Liquid paraffin from the oven is poured over the block that has been provided, then the tissue is inserted in the desired position, then paraffin blocks are made (planting the tissue in a cassette) and stored in the refrigerator (Nisfa Lailatun, 2020).

h. Microtomy and Affixing
In making paraffin blocks, the first step is to cut the organs 5 to 6 mm thick using a microtome. After the cut organs are floated in warm water at 60°C to stretch so that the tissue does not fold, then the preparation is removed and placed on a glass slide that has been smeared with glycerin. (Nisfa Lailatun, 2020) .

i. Deparaffination
Deparaffinization process using xylol for 2x2 minutes, then hydration using alcohol 100% for (2x2 minutes), 95% for (2 minutes), 90% for (2 minutes), 80% for (2 minutes), 70% for (2 minutes) then cleaned with running water for (2 minutes) (Nisfa Lailatun, 2020).

j. Staining
Coloring uses Hematoxylin Eosin (HE), the first step is to soak the glass slide in a solution of hematoxylin for 5 minutes and then wash it using running water, then dip the slide into 1% acid alcohol and wash it using running water then dehydrate it using 80%, 90% alcohol and absolute alcohol 2 times up to 1 minute and dried, then the slide is put into 1% eosin solution for up to 1 minute, the slide is put into

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96% alcohol solution and absolute twice each for up to 1 minute and dried. Next, the glass slide is put into the xylol solution for up to 1 minute and the process is continued using the mounting process (Nisfa Lailatun, 2020).

k. mounts

The slide was covered with a cover glass and given Canada Balm, then labeled. Then it was observed under a microscope (Nisfa Lailatun, 2020).

Data analysis

The results of the research on the effect of the ethanol extract of a bowl of fruit (*Scaphium affine*) on the histological appearance of the liver of male mice that had been carried out were analyzed using Oneway Anova and continued with the Tukey test.

The values analyzed include:

1. Liver Damage Parameter Score

In the score table, the parameters of liver tissue damage are divided into 3 categories of damage namely necrosis, steatosis, and inflammation, which are marked with a % value marked with a score, and marked with the degree of damage, which can be seen in table I.

Table I. Liver Damage Parameter Score

Skor	Parameter Pengamatan			Derajat Kerusakan
	Nekrosis Hepatosit	Steatosis Hepatosit	Inflamasi Sel	
0	Tidak ada	<10%	Tidak ada	Normal
1	<10%	10-30%	Tersebar masing-masing	Rusak ringan
2	10-50%	31-60%	Berkumpul	Rusak sedang
3	>50%	>60%	Tersebar berkelompok	Rusak parah

Source from Padjadjaran University Research Laboratory

2. Liver Damage Score Chart

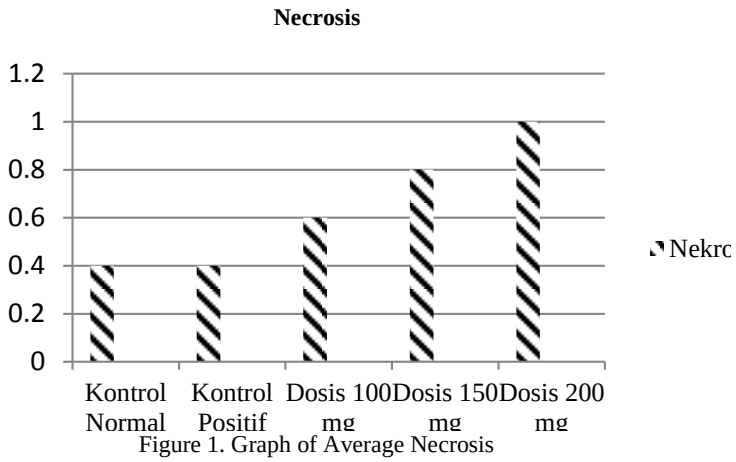

Figure 1. Graph of Average Necrosis

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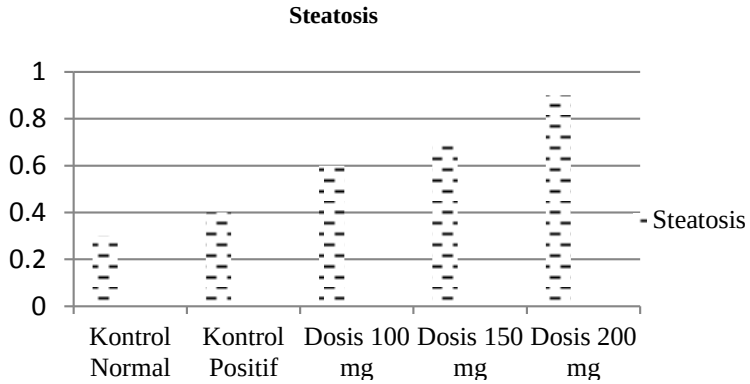

Figure 2. Graph of Average Steatosis s

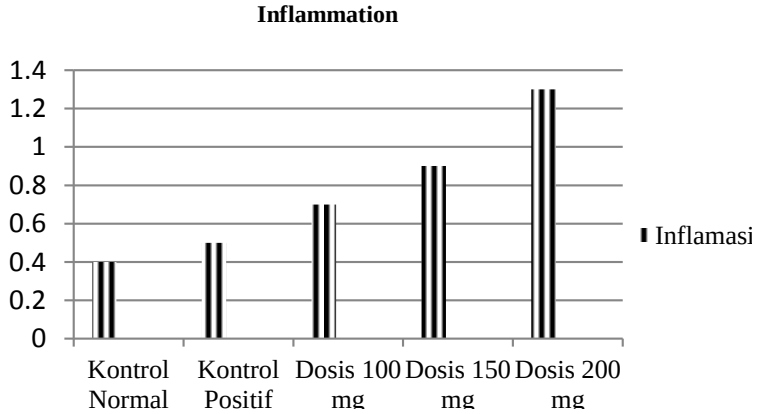

Figure 3 . Graph of Average Inflammation

Information:
0-1.4: Average Value of Liver Damage
Normal-Dose Control 200 mg: Treatment of Mice

Results and Discussion

Bowl Fruit Extraction

Extraction simplicity use method maceration. Election method maceration because it can be used for easily decomposed compounds in filtering by heating, besides that this method is simple both in the process and the equipment used in the extraction process. The solvent used is 70% ethanol

Table II. Results Bowl Fruit Extraction

Type	Results
Bowl of Fruit	2 kgs
Early Simplicia	500g
Extract a thick 70% bowl of fruit	23,4 g
yield extract	4.68 %

Phytochemical Screening Test

The results of the phytochemical screening aimed to find out what secondary metabolites are present in the ethanol extract of a bowl of fruit (*Scaphium affine*).

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Table III. Results of Phytochemical Screening

Compound Metabolites Secondary	Results screening
Alkaloids	+
Flavonoids	+
Saponins	+
Tannins	-

Liver Damage Picture

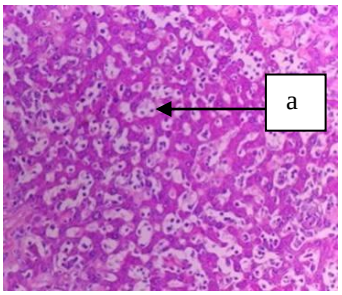

Figure 4 . Normal Cells (P 400)

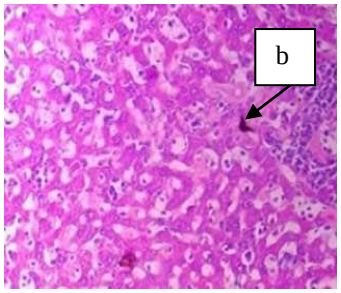

Figure 5. Necrosis (P 400)

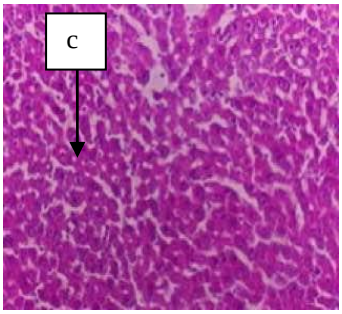

Figure 6. Steatosis (P 400)

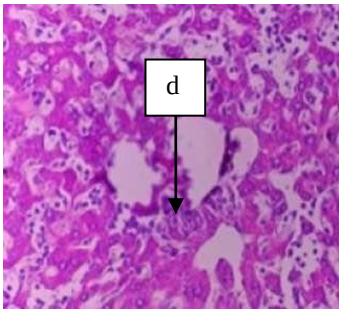

Figure 7. Inflammation (P 400)

Information:

a: Normal cells
b: Cell Necrosis
c: Steatosis cells
d: Inflammatory Cells

Liver Damage Score Difference Test

1. Liver Damage Data Normality Test

   In table IV the normality test for the amount of liver cell damage obtained a value of necrosis 0.200, steatosis 0.200, and inflammation 0.200, with a normal significance level if it is greater than 0.05 (Rusliadi *et al* ., 2016). It can be seen that necrosis, steatosis, and inflammation showed normality test scores which were normality distributed.

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Table IV. Liver Damage Data Normality Test

P- Value			Information
Necrosis	Steatosis	Inflammation	
0.200	0.200	0.200	Normal

2. Liver Damage Data Homogeneity Test

In table V, testing the homogeneity of the amount of liver cell damage, the value of necrosis was 0.103, steatosis was 0.225 and inflammation was 0.582, with a normal significance level of 0.05 (Rusliadi *et al* ., 2016). It can be seen that necrosis, steatosis, and inflammation show homogeneity test scores which are homogeneously distributed.

Table V. Liver Damage Data Homogeneity Test

P- Value			Information
Necrosis	Steatosis	Inflammation	
0.103	0.225	0.582	Normal

3. Oneway ANOVA Test on Liver Damage Data

Based on the results of the homogeneity test which are homogeneously distributed, it is followed by table VI of the one-way test marked by the standard deviation value. -on average, the standard deviation values are well distributed if they are smaller than the mean value (Zein *et al* ., 2019). Researchers got the results of the total standard deviation values obtained were necrosis 4.35107, steatosis 6.28129, and inflammation 5.21289. Can be seen in table VI.

Table VI. Oneway Anova Liver Damage

Category Cell Damage	Total Value Standard Deviation (standard deviation)	Mean Total Value
Necrosis	4.35107	3.2500
Steatosis	6.28129	7,0000
Inflammation	5.21289	11.0833

And based on the one-way results that have been obtained, it is continued with table VII of the ANOVA test which is marked with the P-Value. Researchers got the results of ANOVA data on the amount of damage to liver cells, necrosis 0.737, steatosis 0.124, and inflammation 0.167. Can be seen in table VII

Table VII. Liver Damage Anova

Score P-Value			Information
Necrosis	Steatosis	Inflammation	
0.737	0.124	0.167	Normal

4. Tukey Advanced Test Liver Injury Data

Based on the results of the ANOVA that have been obtained, then it is continued with a follow-up test carried out with the Tukey test or honest real test (Wulandari *et al* ., 2017). Researchers got the results of the data from the follow-up test for the amount of liver damage carried out using the Tukey method, in the necrosis treatment of normal controls, positive controls, doses of 150 mg, and doses of 200 mg there was no significant difference so that a P-Value of 0.735 was obtained. In steatosis, there was no significant difference in the normal control treatment, positive control, doses of 150 mg, and doses of 200 mg, so a P-Value of 0.135 was obtained. Can be seen in table VIII, table IX, and table X.

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Table VIII. Tukey Necrosis test

Group	N	1
Normal control	3	1.0000
Positive control	3	2.6667
Dosage 150 mg	3	4.3333
Dosage 200 mg	3	5,0000
P-Value		0.735

Table IX. Tukey Steatosis test

Group	N	1
Normal control	3	2,0000
Positive control	3	4,0000
Dosage 150 mg	3	9.3333
Dosage 200 mg	3	12.6667
P-Value		0.135

Table X. Inflammatory Tukey Test

Group	N	1
Normal control	3	2,0000
Positive control	3	4.3333
Dosage 150 mg	3	11.6667
Dosage 200 mg	3	16.3333
P-Value		0.188

Discussion

It can be concluded that the extract of a bowl of fruit (*Scaphium affine*) affects the picture related to damage to necrotic cells, steatosis, and inflammation in histology, and with the highest dose of 200 mg, liver damage is more severe.

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