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To cite this article: E Sabri *et al* 2018 *J. Phys.: Conf. Ser.* **1116** 052056

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The effect of n-hexane extract of andaliman (*Zanthoxylum acanthopodium* DC.) fruit in the liver tissues of mice during post implantation of pregnancy

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Abstract. The aim of this research was to analyze the effect of n-hexane extract of andaliman (*Zanthoxylum acanthopodium* DC.) fruits in the liver tissues of mice during post implantation of pregnancy. This study used female *Mus musculus* strain DDW of pregnant which were divided into 5 groups: (i) negative control group (K0), (ii) solvent control treatment carboxyl methyl cellulose (CMC) 1% (K+), (iii) n-hexane extract of andaliman fruit 2% (P1), (iv) 4% (P2), and (v) 6% (P3). The treatments were conducted for 6-14 days during post implantation of pregnancy. At the end of treatment, the liver tissues were collected and analyzed morphologic and histology. The result showed that a pale and spotted on liver surface found in treatment groups. The P1, P2, and P3 treatment increased of hepatocyte damage ($p > 0.05$) than in K0 and K+ treatment groups. From the result of this analysis, it could be then concluded that the n-hexane extract of andaliman fruit has negative impact on liver histology of treated mice.

1. Introduction

Andaliman fruits (*Zanthoxylum acanthopodium* DC.), known as the spice in Batak society to eliminate the smell of fish and raw meat. It had been reported to have anti-inflammatory activity [1] and antioxidant activity [2, 3]. Even Batak people use this plant as a spice of traditional cuisine and used it as a tuba to catch fish [4]. Andaliman fruit contains terpenoid compounds [5] that is used as antioxidant and antimicrobial, as well as phenolic and steroidal contents [6] that have toxic properties against predatory animals [7].

In some studies reported that Andaliman fruit may affect the development of rodents, where these plants have anti-inflammatory effects that can inhibit prostaglandin synthesis [7]. Even in the study of [8] reported that the extract of n-hexane from Andaliman fruit increases the incidence of microphthalmia and hydrocephalus in house mouse embryos.

Based on several reports of the study, it is expected that the liver does not work optimally in the detoxification function of compounds that enter the body. Liver plays an important role in the function of metabolism and some other physiological functions of the body [9].



Liver diseases are considered serious problems which can be caused by toxic chemicals, drugs, and virus infiltration through ingestion or infection [10]. These toxins induce the production of reactive oxygen species (ROS), which can attack hepatic tissue and cause serious injury [11].

2. Materials and methods

This study used 30 pregnant female mice (*Mus musculus*) strain DDW. The mice were divided into 5 groups: (i) negative control group (K0), (ii) solvent control treatment carboxyl methyl cellulose (CMC) 1% (K+), (iii) n-hexane extract of andaliman fruit 2% (P₁), (iv) 4% (P₂), and (v) 6% (P₃). The treatments were conducted for 6-14 days during post implantation of pregnancy. At the end of treatment, the mice were anesthetized using a combination of ketamine (75 mg/kgBW) and silacin (8 mg/kgBW). Mice liver was taken in order to analyzed morphologic and histology technique.

2.1 n-hexane Extract of Andaliman Fruits Processing

1000 grams of dried Andaliman fruit is blended until become a powder. Furthermore, an extract was made by maceration method by soaking in 1,000 ml of n-hexane for 1 night. The maceration results are isolated until a clear liquid is obtained. The percolation result is concentrated with the evaporator until the viscous extract is obtained. Andaliman extract is insoluble in water, so to obtain a homogeneous mixture, a carboxy methyl cellulose (CMC) solvent with concentration of 1% (1 mL CMC is dissolved in 100 mL aquadest) is used to obtain the desired extract. Then, the researcher made a modified dose of 2%, 4%, and 6%.

2.2 Liver Tissue Processing

Prior to the process of analyzing the hepatocyte damage level with histology techniques, the liver tissue has been sampled fixed in Bouin fixation solution for 24 hours. After that the next process was dehydrating the tissue using alcohol with stratified concentration (70%, 80%, 90%, 95% until absolute alcohol I, II, III) and continued with tissue clearing stage with xylene solution (xylene I, II, III). Next, the tissue was infiltrated with paraffin (paraffin I, II, III) carried out in the oven and followed by embedding the tissue in paraffin mold. Then, the tissue block was cut (sectioning) thickness 4 μ m using microtome. The pieces of tissue in the form of ribbons were attached to a glass object that had been given glue (0.2% neofren[®] in toluene). Furthermore, the histology staining stage was performed. Liver tissue that had been deparaffinized and then visualized using hematoxylin-eosin. The tissue then dehydrated with alcohol and cleansed with xylene. After the process, the tissue being mounted with entellan[®]. Quantitative observation were performed by counting the amount of hepatocytes damage in the observed tissue (Histopathology Manja Roenigk scoring model). The number of hepatocytes damage was analyzed using a SPSS software program.

Table 1. Criteria for degree assessment of liver histopathology, model Scoring Histopathology Manja Roenigk.

Cell type	Score
Normal	1
Parenchymal degeneration	2
Hydrophic degeneration	3
Necrosis	4

3. Result and discussion

The morphology of mouse liver (**Figure 1, Table 2**) showed that K and K+ did not show any changes neither in color and liver surface. Conversely, in the treatment of P1, P2, and P3 were found changes in the color become pale with spotted in the surface. According to [12], a normal liver has a flat and smooth surface with maroon color, whereas abnormal liver has a surface like a form of connective tissue, cysts and spots with discoloration. The morphological changes of the liver that occur are thought to be caused by terpenoid and steroids compounds contained in the n-hexane extract of Andaliman fruit. As [13] stated that many steroids are found in nature; in plants and animals. Steroids in plant tissues are called sitosterols, which are usually found in layers of wax leaves that serve as a protective plant from insect attack (insecticide).

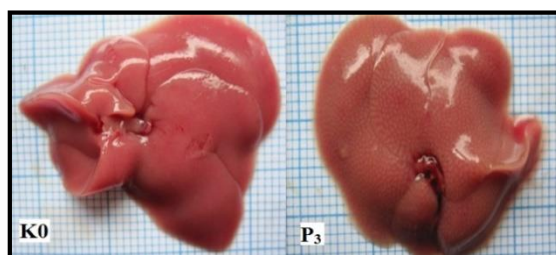


Figure 1 House Mouse Liver Morphology.(a) Normal liver with maroon color
(b) Abnormal liver with pale color and spotted surface

Table 2. The effect of N-Hexane Extract of Andaliman Fruit (*Zanthoxylum acanthopodium* DC.) in the changes on liver color and texture of mice

Groups	Observation	
	Colour (%)	Texture (%)
K	100 (N)	83,33 (N)
		16,67 (A)
K+	83,33 (N)	83,33 (N)
	16,67 (A)	16,67 (A)
P1	50 (N)	50 (N)
	50 (A)	50 (A)
P2	50 (N)	66,66 (N)
	50 (A)	33,33 (A)
P3	33,33 (N)	33,33 (N)
	66,66 (A)	66,66 (A)

The result of hepatocyte damage rate analysis showed significant increase in hepatocyte damage. The damage values of each treatment P1 (239.05), P2 (242.49), and P3 (265.89) showed an increase in hepatocyte damage higher than K + (174.50) and K (119.83).

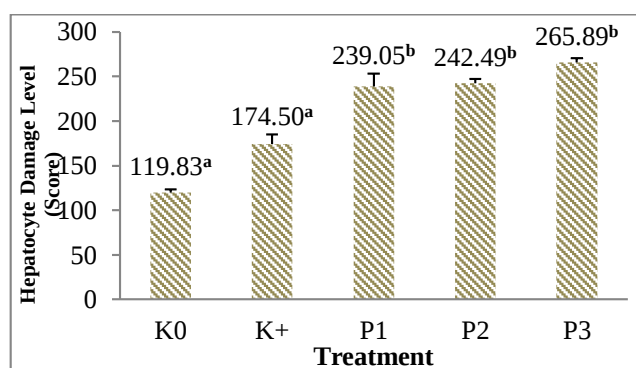


Figure 2 Hepatocyte Damage Level. K0=negative control group, (K+) solvent control treatment carboxyl methyl cellulose (CMC) 1%, n-hexane of Andaliman fruit extract 2% (P₁), 4% (P₂), and 6% (P₃). The same letter on the different treatments states no significant difference at the 5% level. (tn = p > 0.05)

Based on **Figure 2** it can be seen that the provision of n-hexane extract of Andaliman fruit during the post-implantation period (6-14 days) has affected the rate of hepatocyte damage. The increase in hepatocyte damage is most likely caused by the chemical compounds contained in the Andaliman fruit n-hexane extract such as steroids, which [13] suggested that many steroids are found in nature; in plants and animals. Steroids also known as sitosterol in plants are usually found in the layer of wax leaves that serve as a protective plant from insect attack (insecticide). This means that the compounds contained in the n-hexane extract of Andaliman fruit are generally steroid and terpenoid compounds, thus allowing hepatic damage, caused by exposure to steroid and terpenoid compounds whose intensity is long enough. [7] stated that terpenoids can be used as insecticides and toxic to high animals.

Damage to the liver due to toxic substances is influenced by several factors, such as the type of chemical substance, the dose given, and the duration of exposure to such substances such as acute, subchronic or chronic. The higher the concentration of a given compound the greater the toxic response is generated. Toxic responses may be necrosis, cholestasis, or the onset of liver dysfunction slowly [14].

Hepatotoxicity due to chemical compounds is a potential complication that is almost always present in every incoming chemical compound, including Andaliman. As suggested by [12] that hepatic cell damage is rarely caused by a substance directly, but often by toxic metabolites of the substance in question. Because drug metabolism / various compounds are mainly occur in the liver, so the likelihood of the damage occurrence to these organs becomes very large [15]. If the metabolic process does not run normally, it will cause various diseases, one of which is a disease that occurs in the liver. The cells which stay in the liver will be deposited so that it will change [16].

Liver also has the ability to excrete toxicants with higher capacity in the toxic biotransformation process. However, exposure by a variety of toxic materials in excess can cause hepatic damage. It was concluded that the n-hexane extract of Andaliman fruit (*Zanthoxylum acanthopodium* DC.) given during post-implantation period 6-14 days had negative effect on the liver of mice (*Mus musculus* L.) strain DDW which are the changes of color and hepatic surface texture, also the increased of hepatocyte damage.

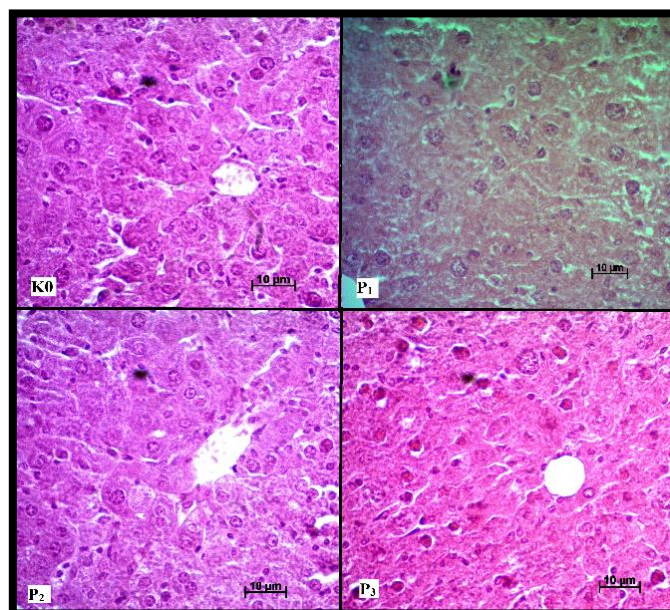


Figure 3 Microscopic Images of hepatocytes. After N-hexane extract of andaliman fruit (*Zanthoxylum acanthopodium* DC.) administered with HE Staining and 400 times magnification

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