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Histological Structure of Mice (Mus Musculus L.) Liver after Administration of Ethanol Extract and Spinasterol from Senggugu (Clerodendron Serratum L) Leaves

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Abstract. Senggugu (Clerodendron serratum) is a plant that used as traditional medicine. Additionally,C. serratum contains several compounds that are classified into natural antifertility products, namely: apigenin, alkaloids, flavonoids, steroids, and triterpenes. This study was conducted to investigate the toxic effect of ethanol extract and spinasterol from C. serratum L. leaves on the mice liver. The study was used completely randomized design with 7 treatments and 6 replications.The mice was administrated the ethanol extract by dosages 250, 500 and 1000 mg/kg bw, whereas spinasterol dose were 26 and 52 mg/kg bw, and for control were used distilled water and DMSO. The treatment was given orally by gavage for 9 and 18 constitutive days. The morphological and histopathological parameters were observed at the end of experimental period. The observation of liver morphology showed no significantly difference between all treatment groups compared control, while the liver histopathological showed significantly difference between the all treatment groups compared to control ($p<0.05$), except for the ethanol extract by dosage 250 mg/kg bw. The highest score of liver histopathology was shown by ethanol extract of 1000 mg/kg body weight. The score was based on the degeneration of parenchyma, as well as karyolisis and necrosis of hepatocytes. It can be concluded that the highest dose of ethanol extract and spinasterol of senggugu leaves was toxic against the liver histological structure of mice.

Keywords: Clerodendron serratum, spinasterol, ethanol extract, liver morphology, histopathology.

Introduction

The plant species *Clerodendron serratum* locally known as senggugu is an important medical species used as a herbal drug in traditional medicine, and showed a major role in the treatment of human and animal diseases. Roots and leaf extracts of *C. serratum* have been used for the treatment of rheumatism, anti-nociceptive (Grainge and Ahmed, 1988), anti-inflamatory, anti-pyretic, asthma and other inflammatory diseases, pest-control antifertility (Narayanan et al., 1999), anti-fertility (Pokharkar *et al.,* 2010), anticancer (Zalke et al., 2010) and anti-proliferation (Nagdeva et al., 2012).The leaves of *C. serratum* contain apigenin, alkaloids, flavonoids, steroid, triterpenoid, phenolic acids (Rastogi et al., 1999), tannins and saponins (Dalimartha, 2002).

The herbals have been usually considered to be safe and nontoxic compared to synthetic compounds, however, the use of herbal medicines must be tested for the safety. The organ that is commonly used as a test for testing the toxicity of a substance is liver. The liver performs a number of functions some of which are plasma protein synthesis, production of bile and detoxification of most substances, however, at the levels of specific toxicant can cause liver damage. The aims of this research were to investigate and examine the hepatic histological structure after administration of ethanol extract and spinasterol derived from *C. serratum* leaves in mice. Observations were carried out on the morphology (i.e. color and surface) and histology (the damage of the central vein and sinusoid, as well as hepatocytes, including nucleus and cytoplasm) of the liver.

Materials and methods

Plant material and extraction procedure

The leaves of *C. serratum* were obtained from Research Institution of Spices and Medicinal Plant, Bandung. The leaves were air-dried for a week and powdered mechanically. Powdered material was extracted using Soxhlet apparatus with 70% ethanol for about 48 hours. The extract were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator till the complete evaporation of the solvent. The spinasterol was isolated from ethanol extract following the methods of Julaeha et al. (2008).

Test animals

A total number of 84 adult male mice were used in this research. The mice were in good health, approximately having the same weight (30±5 gm) and at age of 45 days. They were obtained from the Central Animal House, ITB and were housed in wire mesh cages under standard environmental conditions with the provision of 12 h light and 12 h darkness. They were fed with commercials diet and tap water *ad libitum* during experiment. The animals were acclimated for one week before the treatment.

Experimental protocol

The study was used completely randomized design with 7 treatments and 6 replications. The mice were given the ethanol extract by dosage 250, 500 and 1000 mg/kg body weight, whereas spinasterol dose were 26 and 52 mg/kg body weight, and as a control agent were used distilled water and DMSO. The treatment was given orally by gavage for 9 and 18 constitutive days. The animals were weighed daily throughout the duration of the study.

Morphology examination and histological preparation procedures of liver

The animals of the entire group were sacrificed on 9^{th} and 18^{th} day under fewer ether anesthesias. The liver samples were excised from the animal of each group after drained the blood and washed with normal saline. The liver morphology was observed by color and smoothness of the surface of the liver. Furthermore, the livers were fixed in 10% buffered neutral formalin for 48 hours and after that were processed for paraffin embedding. The section were taken at 5 µm thickness, processed in alcohol-xylene series and were stained with haematoxyline and eosin. The sections were examined microscopically for evaluation of histological changes.

Data Analysis

The data of morphology and histopathology examinations of liver were analyses by ANOVA at 95% confidence level and if there were any significance different between groups, the data were further analyses with Duncan's multiple range test.

Results

Effect on morphology of mice liver

Based on observations conducted on the liver, there was no significant difference for morphological of treated mice compared with control ones. The surface of liver looked fresh red with smooth and there were no speckled (Figure 1). Treatment accorded did not give effect to the liver morphology.

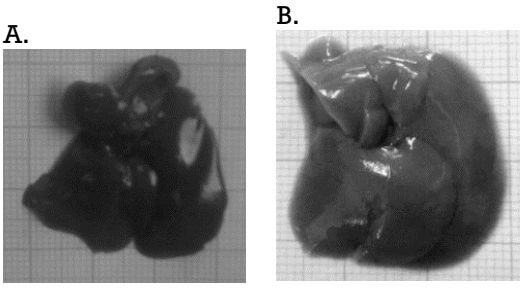

Fig1. The morphology liver of control mice (A) and treated mice (B)

Effect on histological structure of mice liver

Based on statistic analyses, the result showed that all treatments of ethanol extract and spinasterol from *C. serratum* leaves give significant effect against the structure of liver histology compared to control (Table 1).

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Tabel 1 Score of Histological Structure of Mice (*Mus musculus* L.) Liver after Administration of Ethanol Extract and Spinasterol from *Cledodendron serratum* Leaves

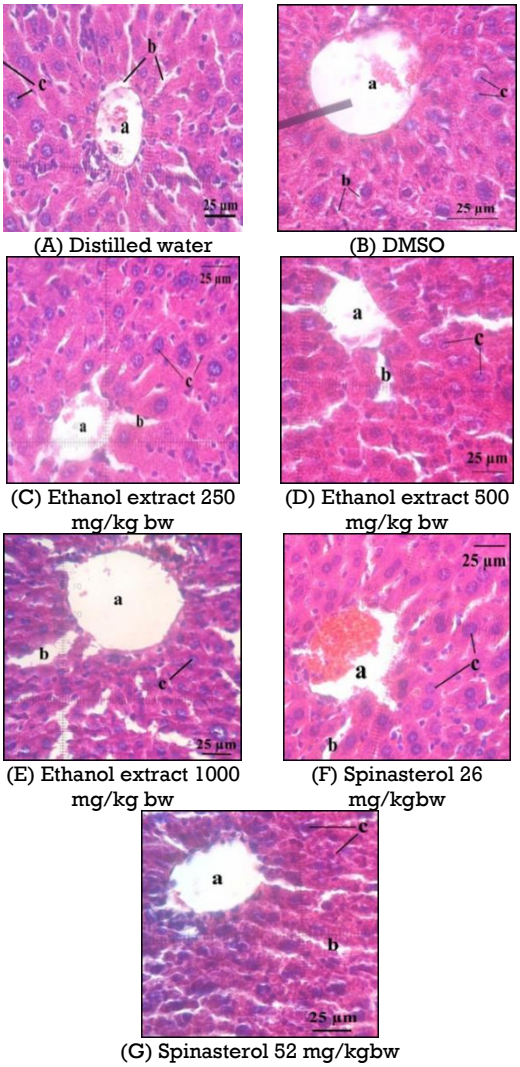
Treatments	Duration of exposure (days)	Score of Liver Histopathology
Distilled water (negative control)	9	0,00(a)
	18	0,000(a)
DMSO (negative control)	9	0,000(a)
	18	0,000(a)
Ethanol extract 250 mg/kg bw	9	0,833(bcd)
	18	1,000(cd)
Ethanol extract 500 mg/kg bw	9	1,167(de)
	18	1,833(fg)
Ethanol extract 1000 mg/kg bw	9	1,833(fg)
	18	2,167(g)
Spinasterol 26 mg/kg bw	9	0,667(bc)
	18	0,500(b)
Spinasterol 52 mg/kg bw	9	1,000(cd)
	18	1,500(ef)

*The data was analyzed by ANOVA and Duncan's Multiple Range Test at 95% significance level. Different letter at the same column was significantly different ($p<0.05$).

The highest score of liver histopathology caused by administration ethanol extract at a dose 1000 mg/kg bw for 18 constitutive days. This score were significantly higher than the other treatments, except for treatment with ethanol extract at a dose of 500 mg/kg bw for 18 days and 1000 mg/kg bw for 9 days. The histological damage of liver was increased along with the increased of dose and duration of exposures.

Microscopic examination on the liver sections of the control groups showed normal histological structure, such as hepatic lobules formed of hepatocytes that in radial form from central vein to the peripheral of lobules (Fig. 2 A, B). Exposured of lower dose of ethanol extract caused loss of hepatocyte architecture, central vein congestion, damage on endothelium of sinusoids i.e. dilation, as well as formation of pyknotic nuclei and hepatocellular necrosis, while the treatment with higher dose of ethanol extract induced more degenerative changes in the liver (Fig. 2 C-E). Whereas exposure of spinasterol at a dose 26 and 52 mg/kg bw caused similarly adverse effect on liver histology as lower doses of ethanol extract (Fig. 2 C-E). Mitchell and Cotran (2003) described that damaging in the cells depends on dose amount of the toxic material administration. The plant contains tannin, phenol, flavones, as found in C. serratus leaves extract, if used with high concentration will produced toxic effect and caused necrosis on the liver cells.

Due to its unique metabolism and close relationship with the gastrointestinal tract, the liver is susceptible to injury from drugs and other substances. In this experiment, the ethanol extracts and spinasterol from *C. serratus* leaves were treated orally and will be entered into the digestive tract and are absorbed by the epithelial cells of the small intestine, so then transported via the hepatic portal vein to the liver lobes. In the liver, the compounds in the extract were circulated via intralobular vein and entered sinusoid in order to supply the hepatocytes. If any toxicant compound in the extract or spinasterol, then can caused hepatocytes damage, i.e. hepatocytes become irregular, the cell membrane disappears so that the boundary between cells was not clear, vacuolization in the cytoplasm, the nucleus begins to diminish and finally lyses, as well as cell necrosis. Necrosis in the liver tissue begins with swelling of the cytoplasm and dilatation of endoplasmic reticulum (Fawcett, 2002). Necrosis can be seen by the reduction in the number of nuclei in the cells or karyolitic and vacuolization of cytoplasm (Damjanov, 2000). In addition, the treatments were also caused lyses of endothelial cells in the central vein so that the boundary of the vein was discontinued. The damage of central vein was related to its role in circulation, where the vein received blood from sinusoids, so that toxic and nontoxic compound would be accumulated in this vein. Sinusoid lumen began to swell because the distribution of blood via strong perfusion and caused hepatocytes damage. The toxicant that damaged hepatocytes will easily come into contact with the sinusoid and the high concentration of the toxicant caused damage to the sinusoid (Junqueira, 1991). In conclution, it was demonstrated that *C. serratum* extract could produce adverse effect and toxic on mice if administered at high concentration.



(A) Distilled water (B) DMSO

(C) Ethanol extract 250 mg/kg bw (D) Ethanol extract 500 mg/kg bw

(E) Ethanol extract 1000 mg/kg bw (F) Spinasterol 26 mg/kgbw

(G) Spinasterol 52 mg/kgbw

Fig 2. Histological structure of mice liver after the treatment of ethanol extracts and spinasterol from *Cladodendron serratus* leaves.

(A, B): Transverse section in the liver of a mice control showed normal structure, normal sinusoidal space (b), and normal central vein (a), normal hepatocyte with polygonal shape (c); (C, F, G): light damage; (D, E): serious damage in the hepatic structure administered of ethanolic exctract of *C. Serratum* or Spinesterol.

Conclusion

In summary, it was demonstrated that *C. serratum* extract could produce disturbance effect and toxic on mice by using high concentration.

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