Effect of Lawsonia inermis Linnaeus Leaf Ethyl Acetate Extract on Liver in Normal Rat

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Abstract: Previous study revealed the antihyperglycemic activity and hepatoprotective effect of *Lawsonia inermis* Linn. leaf extract (EAE) dose 1 g/kgbw in streptozotocin-induced diabetic rats. The present study was conducted to evaluate the toxic effect of EAE on hepar in normal rat. EAE was obtained by serial extraction using n-hexane and ethyl acetate (EAE). Two groups of Wistar rats (n=5) were treated with EAE (1.25 g/kgbw) and distilled water (NC: 10 ml/kgbw), orally, daily for 14 days, respectively. After 14 days the rats were sacrified for histopathological evaluation of liver using hematoxyilline-eosin staining.The result showed normal apperance of liver in NC-treated rat, contrarily in EAE-treated rats showed the hydrophic degeneration, sinusoid and central vein congestion. Multiple nodules with strict lines, consisted of fat cells undergoing proliferation, monocytes and limphocytes infiltration were also found. These results suggest that EAE dose 1.25 g/kgbw toxic to liver and potentially destroy its function.

1 INTRODUCTION

Our previous study showed that ethyl acetate extract of *Lawsonia inermis* Linnaues (EAE) leaf which obtained by serial extraction (nhexane-ethylacetate-ethanol-water) was the most active as antihyperglycemic in streptozotocin-induced diabetic rats at dose 1 g/kgbw. It was also demonstrated the hepatoprotective effect of this extract. Qualitative chemical screening of EAE traced the presence of flavonoid, tannin, saponin and glycoside (Widyawati et al, 2018). These chemical compounds were suggested contributed to its benefit pharmacological activities. In order for medicinal plants to be utilized, it must be supported not only the efficacy, but also its safety through toxicity test. Liver is one of organ that have important role to detoxify the xenobiotic including herbs (Gao et al, 2008; Nasri, 2013; Teschke, 2015). The purpose of this study is to evaluate the effect of EAE at higher dose 1.25 g/kgbw on liver in normal rat.

2 MATERIAL AND METHODS

2.1 Plant Material Collection and Preparation of Ethyl Acetate Extract

*L. inermis*Linn. leaves were collected from TitiKuning, Medan, Indonesia (Coordinate: 3.526093, 98.684528). The plant was identified at "Herbarium Bogoriense", the Research Centre for Biology-Indonesian Institute of Science, Bogor, Indonesia and given a herbarium identification number - No.924/IPH.1.01/If.07/III/2017. The fresh leaves were dried under shade and ground into powder. About 1.5 kg of the powdered leaf was extracted serially by maceration in *n*-hexane and ethyl acetate (EAE). The freeze-dried extracts were kept in the freezer (-20C) before used.

2.2 Animals

Healthy male Wistar rats weighing between 180-250 g were obtained from animal house of Universitas Sumatera Utara. The animals were acclimatized at room temperature and a 12-h dark/light cycle, and

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were allowed to access food and water ad libitum for one week before being used for experimentation.The study was performed after approved by Animal Research Ethics Committees (AREEC), Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatera Utara.

2.3 Experimental Procedure

Rats were divided into two groups, n=5, respectively. Group I (EAE-treated group) were given EAE 1.25 g/kgbw, while group II were given distilled water 10 ml/kgbw (NC-treated group). The treatments were administered orally, at single dose and followed for 14 days.

2.4 Preparation of Liver for Histopathological Examination

The rats were sacrified with the carbogen gas (95% O2 and 5% O2) and the liver was excised. The liver was fixed in 10% buffered formaldehyde for 24 hours, followed by dehydrationusing 70% alcohol (60 min), 96% alcohol (45 min), and absolute alcohol (2 h). The clearing phase of the samples was made by repeated xylene immersions, followed by paraffin wax infiltrations.The samples were then automatically processed with tissue processor Thermo Scientific STP 120-3 and paraffin embedding was prepared using modular tissue embedding center Thermo Scientific Microm EC 350-1. The parafffin-embedded tissues were sectioned into 5µm using the Leica RM 125RTS microtome and mounted on a microscope slides. The mounted slides were stained with hematoxylline (H) and eosin (E) according to H&E staining technique. The stained sections were then mounted in DPX mounting medium with cover slide.

2.5 Histopathological Interpretation

Histopathological appearance of the liver was evaluated by macroscopic and microscopic.
Degree of liver destruction were determined as follow:
0 = normal or no destruction
+= one of these criteria was found; fatty degeneration, or congestion of central vein and sinusoid, focal necrosis, benign cysts with fat
++= two of these criteria were found; fatty degeneration, or congestion of central vein and sinusoid, focal necrosis, benign cysts with fat
+++= 3-4 criteria: fatty degeneration, or congestion of central vein and sinusoid, focal necrosis, benign cysts with fat

2.6 Photomicrography and Image Analysis

Records of the histopathological results were obtained by photomicrography using digital photomicrographic microscope (®Olympus BX 41 and ® Olympus DP25 video camera) at the Anatomic Pathology Laboratory, Department of Anatomic Pathology, Universitas Sumatera Utara.

3 RESULTS

Table 1 showed the degree of liver destruction in NC- and EAE-treated rats. No destruction (Degree 0) were found in normal rat. Generally, EAE-treated rats showed level 2 of liver destruction.

Table 1: Liver destruction degree macroscopically.

Group	Degree
NC	0
EAE1	+++
EAE2	++
EAE3	+
EAE4	++
EAE5	++

NC-treated group as control showed normal liver, red to tan, well defined and soft consistency (Figure 1-NC), while grossly, the liver of EAE-treated group showed pale tan to red, smooth and multiple cystic (black arrow)appearance with diameter 0.2 cm. The cysts relatively well-demarcated, white and smooth (Figure 1-EAE).

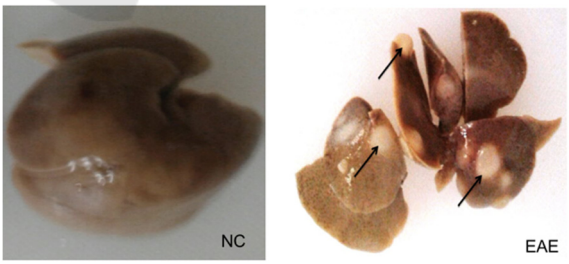

Figure 1: Gross appearance of hepar NC- and EAE-treated group (black arrow: cyst).

In normal hepatocyte (Figure 2-NC) showed normal architecture with regular hepatocyte cells, round nucleus, fine-chromatin cytoplasm eosinophilic; normal sinusoid and central vein. Contrarily, the liver cells of EAE-treated group showed congestion in central vein and sinusoidal,enlargement

of*sinusoid*, focal inflammation (black arrow) and benign cysts contained proliferation of the fat cells (red arrow) (Figure 2-EAEa-b).

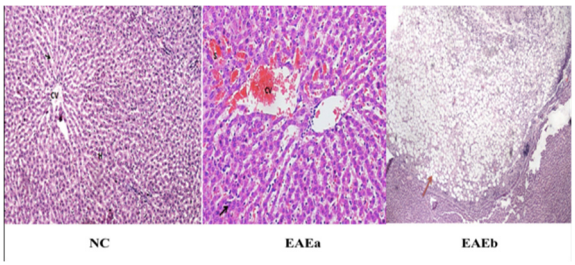

Figure 2: The photomicrographs of liver section of NC- and EAE-treated group (NC: 100x; EAE: 400x; Hepatocyte cell (H); sinusoid (arrow); central vein (CV).

Herbal medicine derived from plant extracts are being increasingly used to treat various of disease (Seif, 2016). Some plant extracts and natural compounds were found as hepatoprotective active principles, while others adversely induced liver toxicity (Manfo et al, 2016). The liver represents the key "metabolic factory" is the most exposed organ to xenobiotics including medicinal plant extracts. This may be modulated by any compound irrespective to the purpose of use. The histopathological evaluation of the present study showed that EAE dose 1.25 g/kgbw affected the structure of liver. It was found clearly by the changing of gross appearance of liver ie pale tan to red and multiple cystic. This result contradictive with our previous study that showed the hepatoprotective effect of EAE dose 1 g/kgbw in streptozotocin-induced diabetic rats. The higher dose of EAE may have the role of this unwanted effect.
The action mechanisms involved in the hepatoprotection or hepatotoxicity by the medicinal plants are still not well elucidated.Herb induced liver injury can be caused by the chemical compounds as their causative agents. Elimination process for metabolic degradation may yield hepatotoxic metabolites that causing liver injury (Manfo et al, 2016; Frenzel and Teschke, 2016).

4 CONCLUSIONS

Ethyl acetate extract of *Lawsonia inermis* Linnaeus leaf at dose 1.25 mg/kgbw toxic to the liver.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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