Lawsonia inermis linnaeus leaf ethyl acetate extract evaluation on the kidneys of rats

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Abstract. Leaf extract of Lawsonia inermis Linn. leaf extract (EAE) showed the ability to decrease the high of blood glucose level and to protect the hepar of diabetic rats at dose 1 g/kgbw. The aim of the present study was to evaluate the effect of EAE on normal rats organs, namely the kidneys. EAE was obtained by serial extraction using n-hexane and ethyl acetate (EAE). 10 rats were divided into two groups that treated with EAE 1.25 g/kgbw (Group I) and distilled water (DW) 10 ml/kgbw (Group II) as control group, orally, once daily for 14 days. After 14 days the rats were sacrificed for histopathological evaluation of kidney using hematoxylline-eosine staining. Grossly, no difference was found on the kidney of DW- or EAE-treated groups. However, microscopically, kidney of DW-treated group showed normal structure of glomerulus and tubulus renalis, whereas EAE-treated group showed the congestion of tubulus renalis. The present study concluded that EAE 1.25 g/kgbw caused the inflammation of the kidney.

1. Introduction
Our previous study showed the efficacy of Lawsonia inermis Linnaeus leaf ethyl acetate extract (EAE) at dose 1 g/kgbw in alleviating the hyperglycemia in diabetic rats. To be used as medicinal plants, a herb must be supported not only by the efficacy but also it’s safety. However, ingestion of herbs may be toxic to body organs including kidneys [1, 2]. This study examines the kidneys after treated by EAE at higher dose 1.25 g/kgbw.

2. Materials and methods

2.1. Ethyl Acetate Extraction Process
Medan Johor, Indonesia (Coordinate: 3.526093, 98.684528) was the place to obtain the L. inermis Linn leaf. The plant was identified at “Herbarium Bogoriense”, Bogor, Indonesia and given a
herbarium identification number - No.924/IPH.1.01/If.07/III/2017. Dry simplicia were produced by drying the fresh leaves under shade. To obtain the extract, the powdered leaf was extracted serially by maceration using \( n \)-hexane and ethyl acetate (EAE). Before used, the freeze-dried extracts were stored in the refrigerator (minus twenty degrees).

2.2. Animals
The male Wistar rats (180-250 g), normal condition, were obtained from Universitas Sumatera Utara’s animal house. Acclimatization of the rat by condition at normal temperature of room. Before being used for experimentation, they were allowed to access food and water \textit{ad libitum} for one week. The study was conducted after Animal Research Ethics Committees (AREEC), Mathematics and Natural Sciences Faculty (FMIPA), Universitas Sumatera Utara approval (N0.00132/KEPH-FMIPA/2018).

2.3. Procedure of experiment.
Group I (EAE-treated group) were given EAE 1.25 g/kgbw, while group II were given distilled water 10 ml/kgbw (DW-treated group). The treatments were administered orally, at single dose and followed for 14 days. Each group consists of 5 rats.

2.4. Histopathological evaluation
The carbogen gas (95% O2 and 5% O2) were used to sacrifice the rats and to excise their kidneys. The kidney was prepared in 10% formaldehyde solution formaldehyde (10 %). Afterwards serial process was carried out using alcohol (70%, 96%, absolute), xylene and paraffin. The tissue was processed using tissue processor (Thermo Scientific STP 120-3) and then was embedded with paraffin (Thermo Scientific Microm EC 350-1). Sectioned paraffin-embedded tissues, 5 μm of each, were prepared using a microtome (Leica RM 125RTS) and mounted on a slide of microscope. Finally, the slides observed to evaluate the histopathological appearance using hematoxyl-eosin (H-E) staining technique.

2.5. 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) Laboratory of Anatomic Pathology, Department of Anatomic Pathology, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia.

3. Results and Discussion

3.1. Gross appearance
Macroscopically, the kidney mass of EAE-treated rat showed capsula renalis intact, tan to red and smooth that similar to DW-treated rat as shown at Figure 1.
3.2. **Microscopic appearance**

The histopathological appearance of DW- and EAE-treated rat’ kidneys were shown in Figure 2. Normal configuration of proximal and distal tubule were found on normal kidney. The glomerulus shown intact architecture. Conversely, the kidney of EAE-treated rat showed glomerular inflammation, vascular dilatation and congestion and interstitial hemorrhage peritubular.

![Figure 2. The photomicrographs of kidney section of EAE-treated rat](image)

(Glomerulus=G; Proximal Tubule=PT; Distal Tubule=DT; Blue arrow: congestion and dilatation of vascular; Black arrow: interstitial hemorrhage peritubular; 100x)

The use of herbs or medicinal plants may lead to renal injury as one of elimination organs. This unwanted effect due to an unspecified drug content, toxic non-herbal compounds contamination, pharmacokinetic interaction among concomitant medicines and or plant species that unidentified [2-5]. Generally, the high level of oxidative stress related with the destruction of kidney. Thus, this can be alleviated by antioxidants. High level of antioxidant and its activities usually reduce kidney injury [6,7].

The histopathological evaluation of the current study showed that EAE dose 1.25 g/kgbw affected kidney structure. It was found clearly by the glomerulus, vascular and peritubular alteration which
describes an inflammatory state. The higher dose of EAE than previous study may have the role of this adverse effect. However, it is suggested to study further the optimum dose that showed EAE’ efficacy and safety.

4. Conclusion
Ethyl acetate extract of Lawsonia inermis Linnaeus leaf at dose 1.25 mg/kgbw caused the inflammation of the kidney.

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