Serum enzymes activity and liver histopathological changes of lactating mice treated with *Eurycoma longifolia* Jack. roots

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*Eurycoma longifolia* Jack. (*pasak bumi*) is a species that has been used as a traditional medicine. The aim of this research is to evaluate the administration effect of *pasak bumi* roots on liver lactating mice. Each mice was administered roots of *pasak bumi* methanol-water fraction at dose 14 mg/20 g mice body weight for 7 consecutive days. Positive control group received Curcuma at dose 0.16 mg/20 g mice body weight and negative control (placebo) group received 0.06 mL/20 g mice body weight of aquadest daily for 7 consecutive days. Compared to aquadest and Curcuma, the administration of *pasak bumi* slightly increase alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (p < 0.05). However, histopathological studies shown congestive and mild degree of fatty change after the administration of aquadest, roots of *pasak bumi* methanol-water fraction, and Curcuma. It is concluded that the administration of roots of *pasak bumi* methanol-water fraction to lactating mice could not decrease the liver function.

**Keywords**: *Eurycoma longifolia* Jack roots, Liver histopathological studies, Lactating mice

**IPC Int. Cl.** A61K 36/00, C12N 9/00, C12N 11/00, A61K 38/43, A61K 35/15, A01D 7/31, A01D 7/54, A61P 5/14

In the body, liver is the most important organ which plays a pivotal role in regulating various physiological processes. It is the vital organ for metabolism of endogenous and exogenous agent, and also plays an important role in detoxification and drugs elimination. Liver damage may be caused by xenobiotics, alcohol consumption, infection, and medication. Liver disease is still a worldwide health problem. Numerous medicinal plants such as *Glycyrrhiza glabra* L., *Ricinus communis* L., *Aloe vera*, *Eclipta alba*, *Phyllanthus niruri*, *Moringa oleifera*, *Silybum marianum*, and *Flemingia macrophylla* were evaluated their activity as hepatoprotective. *Eurycoma longifolia* Jack. (*pasak bumi* in Indonesian language) is one of plants which was reported as hepatoprotective for male rats. This plant was reported has ability to protect and restore the hepatocytes which was damaged by carbon tetrachloride. *E. longifolia* Jack. is a medicinal plant belonging to Simaroubaceae family. It is found in the forests of Indonesia, Malaysia, Philippines, Burma, and Vietnam. This plant is very popular in many treatments. It was reported has activity as antibacterial, antimalarial, a complementary treatment for male osteoporosis, antioxidant and anti-inflammatory, and hepatoprotective. So far, according to Minorsky (2004) side effects caused by the consumption of this roots plant are difficult to sleep, restless, and can lead the increase body temperature. But, the proper dose to these effects occurred is unknown. Furthermore, Panjaitan *et al.* (2016) reported that the administration of *pasak bumi* roots extract for seven consecutive days in pregnancy period could decrease the liver function of pregnant mice. Lactating is milk secretion process controlled by hormones. During lactation period, maternal nutritional requirements have to be prepared because maternal needs nutrient to produce breast milk. The nutritional requirements during lactation are greater than during pregnancy. Moreover, the quality of breast milk is affected by maternal nutritional status. In Indonesia, empirically, postpartum maternals usually used *pasak bumi* roots to maintain their health. However, they have to remember that in lactation period the maternal have to in healthy condition to produce qualified breast milk and also to nurse their babies. Therefore, the consuming *pasak bumi* roots must not affect the organ which plays in metabolism process, but protect the organ as hepatoprotector. According to Panjaitan *et al.* (2013) enzyme activities such as ALT and AST have been found to be of great value in the assessment of...
clinical and experimental liver damage. This research was done to evaluate the effect of administration of *pasak bumi* roots on liver of lactating mice.

**Methodology**

**Collection of plant material**

*Pasak bumi* roots were collected from National Park in West Kalimantan, Indonesia. Then, the plant specimen was authenticated by Herbarium Bogoriense LIPI Bogor, Indonesia.

**Animals**

Balb/c mice (27-33 g) were provided with standard rodent pellet diet, the food and water were allowed *ad libitum*. Before the treatment, male mice mated with female mice with a ratio one male to four females. Treatment was started since the mice pups were born. In this study, handling the animal fully follows the ethical requirement in our institution and has been done with high intention on animal welfare of involved (ACUC No: 10-2012 RSHP-IPB).

**Preparation of plant extract and partitions**

The preparation of plant extract and partitions process follows Harborne (1987)\(^29\). The first process, the roots of *pasak bumi* were dried and made into a coarse powder. The second, the powdered material was dissolved in methanol to maceration process. Then, the extract was filtrated with evaporated under reduce pressure and vacuum-dried. Finally, methanol extract was partitioned with *n-*hexane, chloroform, and ethyl acetate. The residue of partitions process is named methanol-water fraction, and this fraction which was used in this experiment.

**Evaluation of the administration of *pasak bumi* roots on liver of lactating mice**

Mice were divided into three groups that each groups has three mice. Group I (placebo) were administered aquadest 0.06 mL/20 g mice body weight, group II were administered roots of *pasak bumi* methanol-water fraction at dose 14 mg/20 g mice body weight, and group III were administered Curcuma at dose 0.16 mg/20 g mice body weight. The treatment was given for 7 consecutive days, and at the 8\(^{th}\) day blood samples from mice sinus orbitalis were collected, then the mice were sacrificed by cervical dislocation. Blood serum was obtained by centrifugation. The activities of ALT and AST were determined using assay kit Diasys Diagnostic Systems. The method is optimized UV-Test according to IFCC (International Federation of Clinical Chemistry). To 200\(\mu\)L serum was added 1000\(\mu\)L GOT (ASAT) or GPT (ALAT) and vortexed until homogen then incubated. Absorbance was read at 334 nm after 1 min, read absorbance again after 2 and 3 min. Activities were expressed as U/L.

**Histopathological examination**

The mice liver was dissected out and fixed in 10 % formalin, then dehydrated in gradual ethanol, cleared in xylol, and embedded in paraffin. The paraffin sections were prepared and stained with haematoxylin and eosin, and examined using light microscope. The procedure of histopathological studies was conducted by Kiernan (1990)\(^30\). Scoring lessio of liver is presented in Table 1.

**Statistical Analysis**

Statistical analysis was performed by using SPSS for Windows version 13. The data of ALT and AST serum enzymes were evaluated statistically using the one-way analysis of variance followed by the Tukey to correct multiple comparison treatments. Statistical significance was set at the p < 0.05 level.

**Results**

This research was conducted to evaluate the administration of *pasak bumi* roots methanol-water fraction on liver of lactating mice. In lactating period the mother acts as the only provider energy for her pups. So that, during this period, health and nutritional status of maternal need to be considered because the healthy mother will produce breast milk which is qualified. In this research, the result showed that the adminstration of methanol-water fraction could slightly increase (p < 0.05) ALT and AST serum enzymes (Table 2). Moreover, histopathological study in this study showed congestion and mild fatty liver degeneration on liver of lactating mice.
transaminase enzyme of lactating mice after administration roots of pasak bumi methanol-water fraction for 7 consecutive days in lactating period (n=3).

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<td>Methanol-water fraction</td>
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Each value represents the mean±SD for 3 mice per each group. a,b,c p < 0.05 significantly different compared between groups.

Discussion

Pasak bumi is one of famous plants which used as medicinal plant, specially as a prodisiac. Moreover, according to Panjaitan et al. (2011) the oral administration of 500 mg/kg body weight roots of pasak bumi methanol extract and its derived fraction (n-hexane, chloroform, ethyl acetate, and methanol-water) for seven consecutive days had no significant effects on liver of male rats, so assumed that their compound relative non toxic to consume. Kuo et al. (2004) reported that they have isolated totally 19 quassinoids that were distributed in non polar until polar fraction of pasak bumi roots. Panjaitan et al. (2011) also reported that the administration roots of pasak bumi methanol extract and its derived fraction at dose 500 mg/kg BB possess hepatoprotective activity that evidence by histopathological of liver and the significant inhibition in the elevated levels of ALT and AST serum enzymes activities induced by CCl4. According to Panjaitan et al. (2013) the administration of selected fraction of pasak bumi roots could protect hepatocytes which were attacked by CCl4. The selected fraction inhibited lipid peroxidation and recovered the decrease hepatic glutathione level towards normalization. Moreover, declared that the mechanism liver protection of selected fraction related by glutathione-mediated detoxification as well as free radical suppressing activity. So, pasak bumi roots was stated as hepatoprotector. Related to the aims of this research, preliminary study showed that pasak bumi roots has potency to optimize metabolism process of lactating mice. The administration of pasak bumi roots methanol extract and its derived fraction on lactating mice could increasing body weight of mice pups. Moreover, Panjaitan & Zulfan (2015) reported that the administration of methanol extract of pasak bumi roots and its derived fractions in lactation period could not influence the body weight of lactating mice. Panjaitan et al. (2009) also assumed that the compound of methanol extract of pasak bumi roots and its derived fraction not toxic to lactating mice, on the contrary the compound of methanol extract and its derived fraction could maintain the quality of breast milk during lactating period. However, result study of Panjaitan & Zulfan (2015) showed that the administration of pasak bumi roots methanol-water fraction for 21 consecutive days during lactating period could slightly increase leukocytes counts, although the value of leukocytes differentiation between groups is not significant statistically. Moreover, the erythrocytes count, haemoglobin, and haematocrit between groups also showed not significance statistically. According to Panjaitan et al. (2013) ALT is a specific cytosol liver enzyme, and its increase in the blood serum is specific for changes in the liver function. Aspartate aminotransferase is widely distributed enzyme, which is found in many tissues and organs, with high activity in the liver. Moreover, Domitrovic et al. (2011) said that the decreased of enzymes ALT and AST in serum indicates the capability to preserves the structural integrity of hepatocellular membrane, which was supported by the histological finding. Related to it, Panjaitan et al. (2013) also state when the liver cell plasma membrane is damaged, various of enzymes

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Fig. 1 — Liver section of lactating mice treated aquadest at dose 2 mL/kg body weight (A) showed congestion (white arrowhead) and fatty liver degeneration (black arrowhead); methanol-water fraction of pasak bumi roots at dose 14 mg/20 g body weight (B) showed fatty liver degeneration (black arrowhead); Curcuma at dose 0.16 ml 20 g body weight (C) showed infiltration of inflammatory cells (white arrowhead) and fatty liver degeneration (black arrowhead). HE. 40X.
normally located in the cytosol are released into the blood stream. The liver damaged can caused serious ultrastructural changes in the hepatocytes. Ultrastructural studies of liver damaged confirmed the prominent changes were organelle abnormality and focal cytoplasmic degeneration. Mitochondria abnormalities were observed, including variation in size and shape, and reduction of cristae. Then, the rough endoplasmic reticulum was disarranged and apparently degenerated. Actually, value range of ALT and AST serum enzyme between indivual is wide, for example Domitriović et al. (2011) reported that the value of ALT and AST serum enzymes in control group is 34.9 ± 3.1 and 52.3 ± 4.2, by given berberine at dose 10 mg/kg is 32.9 ± 2.3 and 49.4 ± 3.1, by given CCl₄ at dose 2 ml/kg BB is 7765 ± 875 and 3329 ± 355, by given CCl₄ + berberine at dose 5 mg/kg is 7750 ± 686 and 3050 ± 392, by given CCl₄ + berberine at dose 10 mg/kg is 444.5 ± 86.3 and 261.5 ± 49.3, then by given CCl₄ + silymarin at dose 100 mg/kg is 7012 ± 480 and 2849 ± 160. They also state that control mice and berberine alone had no significant effect on liver. The histopathological change such as mainly pericentral necrosis with loss of hepatic architecture, vacuolar fatty change, and mild inflammatory cell infiltration, comprised predominantly of mononuclear cells and macrophages have been found in group treated with CCl₄, also the presence of necrotic area in group treated with CCl₄ + berberine at dose 5 mg/kg, and silymarin at that dose stated have weak hepatoprotective activity. But, in group treated CCl₄ + berberine at dose 10 mg/kg which was only showing minimal hepatic damage and completely prevented liver necrosis declared dose-dependent protective effects against CCl₄-induced liver damage also seems to be more potent hepatoprotective drug. Related to this research, we assume that the variation in the value of ALT and AST serum enzyme also the histopathological studies is still normal range. So that, we assume that the consumption of methanol-water fraction of pasak bumi roots in lactating period could not decrease liver function.

Acknowledgement
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References


