Hepatoprotective activity of *Eurycoma longifolia* Jack. roots

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The hepatoprotective activity of *Eurycoma longifolia* Jack. roots methanol-water fraction on therapeutic dose was evaluated in carbon tetrachloride (CCl4)-induced rats. Hepatic cells were still normal after three months administration of methanol-water fraction at therapeutic dose. The administration of methanol-water fraction on therapeutic dose prior to CCl4-induced or CCl4-induced prior to methanol-water fraction resulted in suppression of ALT and AST. Histopathological and ultrastructure studies confirmed that methanol-water fraction protected hepatic cells. It is concluded that the methanol-water fraction of *pasak bumi* roots has a hepatoprotective activity.

**Keywords:** *Eurycoma longifolia* Jack., Therapeutic dose, Hepatoprotective

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Hepatic damage is associated with distortion of metabolic function. Liver disease is still a world problem, because liver is a regulator of various important metabolic functions. Liver diseases are mainly caused by toxic chemicals, excess of consumption of alcohol, infections, drugs, and autoimmune disorders1-3.

Carbon tetrachloride (CCl4) is an established hepatotoxin. It is believed to require activation by hepatic microsomal mixed function oxidase to trichloromethyl free radical (·CCl3), and the presence of oxygen trichloromethylenperoxy radical (·CCl3O2) which is more reactive. Trichloromethyl free radical binds to lipids and protein components of the membrane leading to covalent binding, while trichloromethylperoxy radical interacts with polyunsaturated fatty acids and causes lipid peroxidation4.

Natural products recently attracted attention as health beneficial foods (physiologically functional foods) and as source materials for drug development. Herbal medicines derived from plant extracts are increasingly being utilized to treat a wide variety of clinical diseases5. Many traditional remedies employ herbal drugs for treatment of liver diseases6-7.

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*Eurycoma longifolia* Jack. (*pasak bumi* in Indonesian language), a medicinal plant belonging to Simaroubaceae family, is cultivated widely in some Southeast Asia regions such as Indonesia, Malaysia, Thailand, and Vietnam. The roots of this plant contains a series of quassinoids8-15, canthin-6-one alkaloids, β-carboline alkaloids14,15, tirucallane-type triterpenes15, squalene derivatives15,16, and biphenylenoignans15. Some of these constituents were shown to possess cytotoxic15, antimalarial14,15,17, and aphrodisiac activities19-21, but no information in literature was found concerning its possible hepatoprotective. Therefore, to investigate the in vivo hepatoprotective activity, we assessed the hepatoprotective activity of *E. longifolia* Jack. methanol-water fraction. Prior to conducting the study, we established the safety level of the fraction by determining median lethal dose (LD50) and subchronic toxicity of the fraction to determine the safety of the fraction.

**Methodology**

**Animals**

Sprague-Dawley rats (225 ± 25 gm) and DDY mouse (30 ± 5 gm) were purchased from the Faculty of Husbandry, Bogor Agricultural University,
Indonesia. Animals were provided with standard rodent pellet diet, the food and water were allowed ad libitum.

Collection of plant material
The fresh roots of *E. longifolia* Jack. were collected from Betung Karuhun National Park and Gunung Pulung Ketapang National Park, West Kalimantan. The plant specimen was authenticated by Herbarium Bogoriensis LIPI Bogor, Indonesia (348/IPH.1.02/If.8/2004).

Preparation of plant extract and partitions
The process first, the air-dried roots (12.5 kg) of *E. longifolia* Jack. were made into a coarse powder. The second, the powdered material was dissolved in methanol and subjected to maceration process. The extract was filtrated with evaporated under reduce pressure and vacuum-dried (2.75%). Finally, 95% of methanol extract was partitioned with *n*-hexane, chloroform, and ethyl acetate. The fractions obtained were as follows *n*-hexane fraction (4.34%), chloroform fraction (28.79%), ethyl acetate fraction (7.22%), and residue (methanol water fraction) (53.74%).

Determination of oral median lethal dose (LD₅₀), hepatoprotective median effective dose (ED₅₀), and sub chronic toxicity liver of methanol-water fraction of *Eurycoma longifolia* Jack. roots
Median lethal dose was determined according to Weil method. This experiment used DDY mouse (30 ± 5 gm) that were purchased from Faculty of Husbandry, Bogor Agricultural University, Indonesia. Animals were divided into four groups based on dose level to determine LD₅₀ value. The toxic symptoms and mortality rate were recorded after 24 hrs of per oral administration of the methanol-water fraction. Moreover, to determine ED₅₀ hepatoprotective the animals were divided into four groups based on dose level. The hepatoprotective activity was recorded at the ninth day, after seven consecutive days of per oral administration of the methanol-water fraction and CCl₄ at dose 0.1 ml/kg body weight intraperitoneal on the eighth day. The hepatoprotective activity of *E. longifolia* Jack. compared to silymarin.

Sub chronic toxicity was conducted for three months. Group I (positive control) animals were administered aquadest (2 ml/kg body weight, per oral) and group II (test group) animals were received methanol-water fraction of *E. longifolia* Jack roots at therapeutic dose. Animals were sacrificed 24 hrs after the last administration, blood was collected allowed to clot and serum separated. Liver was dissected out for histopathological study.

Hepatoprotective activity of methanol-water fraction of *Eurycoma longifolia* Jack. roots
Rats were divided into three groups that each group has three rats. Group I (negative control) animals were administered aquadest (2 ml/kg body weight, per oral) and group II (positive control) animals were administered silymarin at dose 25 mg/kg body weight, per oral. Animals group III were administrated methanol-water fraction at therapeutic dose per oral. Aquadest, silymarin, and methanol-water fraction were administered orally daily for seven consecutive days, and at the eighth day, the experimental rats were injected CCl₄ at dose 0.1 ml/kg body weight. Animals were sacrificed 24 hrs after CCl₄ injection, blood was collected allowed to clot and serum separated. Liver was dissected out for histopathological study.

Curative effect of methanol-water fraction of *Eurycoma longifolia* Jack. roots
Rats were divided into three groups that each group has three rats. Group I (negative control) animals were administered aquadest (2 ml/kg body weight, per oral) and group II (positive control) animals were administered silymarin at dose 25 mg/kg body weight, per oral. Animals group III were administrated methanol-water fraction at therapeutic dose per oral. At the first day, the experimental animals injected CCl₄ at dose 0.1 ml/kg body weight. Then, at the second until eighth day, aquadest, silymarin, and methanol-water fraction were administered orally daily for seven consecutive days. Animals were sacrificed 24 hrs after the last administration, blood was collected allowed to clot and serum separated. Liver was dissected out for histopathological and ultrastructure study.

Biochemical estimations
The blood samples from rat heart were collected, and then the rats were sacrificed by cervical dislocation. Blood serum was obtained by centrifugation. The activities of serum alanine transaminase (ALT) and aspartate transaminase (AST) were determined using assay kit ST. Reagensia.

Histopathological examination
The rat 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.

**Transmission electron microscope**

The rat liver was dissected out and fixed in 5% glutaraldehyde, then dehydrated in gradual ethanol, and embedded in propylene oxide. The ultrathin sections were prepared and stained with uranyl acetate and triple lead, and examined using electron microscope.

Statistical analysis

The data were expressed as mean (n=3). Results were analyzed statistically by one-way ANOVA followed by Tukey’s multiple comparison using SPSS 11.5 version for Windows. The difference was considered significant at p<0.05.

**Results**

**Determination of oral median lethal dose (LD₅₀), hepatoprotective median effective dose (ED₅₀), and subchronic toxicity liver of methanol-water fraction of Eurycoma longifolia Jack. roots**

The result showed that the oral LD₅₀ value has more than 15 gm/kg body weight and categorized practically non toxic. ED₅₀ value has not more than the range of lethal dose, and base on the ED₅₀ value we determine the therapeutic dose. Therapeutic dose was used for sub chronic toxicity. Compared to aquadest (ALT enzymes on 0 and 3rd months are 134.70±11.97 U/L and 137.42±29.63 U/L, and AST enzymes on 0 and 3rd months are 437.66±98.94 U/L and 320.10±72.46 U/L), there were no significant differences in ALT (0 and 3rd months are 123.39±22.25 U/L and 100.90±24.94 U/L) and AST (0 and 3rd months are 384.05±122.38 U/L and 299.52±15.66 U/L) enzymes concentrations. Histopathological studies confirmed that the hepatic morphology in male rat was still normal after administration of methanol-water fraction on therapeutic dose for three months.

**Hepatoprotective activity of methanol-water fraction of Eurycoma longifolia Jack. roots**

Compared with aquadest (ALT 161.70±7.37 U/L and AST 330.67±42.00), the therapeutic dose administration of methanol-water fraction prior to CCl₄-induced resulted in suppression of ALT (91.78±9.63 U/L) and AST (249.50±20.5 U/L) enzymes as well as silymarin (ALT 105.09±21.62 U/L and AST 310.25±2.45 U/L) after CCl₄-induced. Histopathological studies confirmed that the methanol-water fraction gave similar results to silymarin (Fig.1).

**Curative effect of methanol-water fraction of Eurycoma longifolia Jack. roots**

The results demonstrated that compared with aquadest (ALT 231.57±67.66 U/L and AST 303.87±105.04 U/L), the CCl₄-induced prior to therapeutic dose administration of methanol-water fraction resulted in suppression of ALT (136.97±46.19 U/L) and AST (322.80±112.89 U/L) enzymes as well as silymarin (ALT 143.57±37.00 U/L and AST 321.33±25 U/L). Histopathological studies confirm that the methanol-water fraction restored hepatic cells as well as silymarin (Fig. 2). After 0.1 ml/kg body weight CCl₄, compared with methanol-water fraction on therapeutic dose (Fig. 3 A), the administration of 2 ml/kg body weight aquadest (Fig. 3 B) caused serious ultrastructural changes in the hepatocytes of rats. The prominent changes were organelle abnormality and focal cytoplasmic degeneration. Mitochondria abnormalities were observed, including variations in size and shape, and reduction of cristae. Then, the rough endoplasmic reticulum was disarranged and apparently degenerated.

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Fig. 1—A-C: Liver sections of aquadest 2 ml/kg body weight, silymarin 25 mg/kg body weight, and methanol-water fraction at therapeutic dose prior to CCl₄ 0.1 ml/kg body weight. Fig. 1 A-C showed liver degeneration on difference degree. Bar = 20 µm.
The previous study showed that the oral administration of 500 mg/kg body weight methanol extract and its derived fractions (n-hexane, chloroform, ethyl acetate, and methanol-water) of *E. longifolia* Jack. for seven consecutive days had no significant effects on liver function, so it means that their compound relative non toxic to consume. Furthermore, the investigation to select one fraction of *E. longifolia* Jack. (methanol extract and n-hexane, chloroform, ethyl acetate, and methanol-water fraction of methanol extract), the administration of 500 mg/kg body weight methanol-water fraction of *E. longifolia* Jack. possess hepatoprotective activity that evidenced by the significant inhibition in the elevated levels of ALT and AST serum enzyme activities induced by CCl₄. Moreover, its histopathological study gave similar results to silymarin. According to it has been showed that protective agents exert their action against CCl₄ induced liver injury by impairment of CCl₄-mediated lipid peroxidation and decreased production of free radical derivatives.

Based on the value of LD₅₀ and ED₅₀ of methanol-water fraction, Lu (1995) has categorized an extract with the value of LD₅₀ more than 15 gm/kg body weight in practically non toxic. Furthermore, the sub chronic toxicity showed that the administration of methanol-water fraction at therapeutic dose for three month have no alteration in histopathological studies. The investigation of hepatoprotective and curative effect of methanol-water fraction showed that it has potensial effect as hepatoprotector.

Alanine aminotransferase (ALT) is a specific cytosol liver enzyme, and its increase in the blood serum is specific for changes in the liver function. Aspartate aminotransferase (AST) is widely distributed enzyme, which is found in many tissues and organs, with high activity in the liver. Increased AST enzyme in serum is a sensitive marker of liver damage. Estimating the activities of serum marker enzymes like ALT and AST can make assessment of liver function. When liver cell plasma membrane is damaged, various of enzymes normally located in the cytosol are released into the blood stream. Their estimations in the serum are a useful quantitative marker of the extent and type of hepatocellular damage. Compound which is decreased ALT and AST serum enzyme of course increased the activity of GST, which metabolizes toxic compounds to non

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**Discussion**

The previous study showed that the oral administration of 500 mg/kg body weight methanol extract and its derived fractions (n-hexane, chloroform, ethyl acetate, and methanol-water) of *E. longifolia* Jack. for seven consecutive days had no significant effects on liver function, so it means that their compound relative non toxic to consume. Furthermore, the investigation to select one fraction of *E. longifolia* Jack. (methanol extract and n-hexane, chloroform, ethyl acetate, and methanol-water fraction of methanol extract), the administration of 500 mg/kg body weight methanol-water fraction of *E. longifolia* Jack. possess hepatoprotective activity that evidenced by the significant inhibition in the elevated levels of ALT and AST serum enzyme activities induced by CCl₄. Moreover, its histopathological study gave similar results to silymarin. According to it has been showed that protective agents exert their action against CCl₄ induced liver injury by impairment of CCl₄-mediated lipid peroxidation and decreased production of free radical derivatives.

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toxic ones, means they have an increasing protective activity of the liver. Histopathological and ultrastructure studies confirm that the administration of methanol-water fraction at therapeutic dose preserved the structural integrity of the hepatocellular membrane and restored hepatic cells in a therapeutic dose (Figs. 1,2,3).

Actually, the content of methanol-water fraction that has hepatoprotective activity is not clear, but Kuo et al. (2004) reported that they have isolated totally 19 quassinoids of four types such as eurycomalactone, laurycolactone, klaineanone, and longilactone that were distributed in non polar until polar fraction of E. longifolia Jack. roots. A range of studies has demonstrated that triterpenoid can reduce the amounts of CCl₄ metabolite and thus protect the liver. Therefore, the hepatoprotective activity of methanol-water fraction may be supported by bioactive compound. We assume that the methanol-water fraction inhibited lipid peroxidation and recovered the decrease hepatic GSH level induced by CCl₄ towards normalization. In addition, the mechanism liver protection of methanol-water fraction is related by glutathione-mediated detoxification as well as free radical suppressing activity.

Conclusion
We concluded that methanol-water fraction of E. longifolia Jack roots has potential activity as hepatoprotector at therapeutic dose. As long as it is used based on the therapeutic dose no negative effect on liver function obtained.

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References

22. Weil CS, Tables for convenient calculation of median-effective dose (LD$_{50}$ or ED$_{50}$) and instructions in their use, *Biometrics*, 8 (1952) 249-263.


