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# PREVENTION EFFECTS OF METHANOLIC EXTRACT OF *Eurycoma longifolia* ROOTS ON CARBON TETRACHLORIDE-INDUCED HEPATOTOXICY IN RATS

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## ABSTRACT

The objective of this study was to investigate preventive effects of methanolic extract of *Eurycoma longifolia* roots (MEEL) against carbon tetrachloride-induced hepatotoxicity in rats. A total of 25 rats were randomly divided into five groups (n=5). Group I was treated with Sodium CMC 1% w/v (peroral, 6 days), and group II was treated with single dose of $CCl_4$ in olive oil (1:1) 2 mL/kg (intraperitoneal injection). Groups III-V received 75, 150, and 300 mg/kg BW of MEEL (peroral, 6 days), respectively, and administration of $CCl_4$ in olive oil (1:1) 2 mL/kg (intraperitoneal injection) on the seventh day. The hepatoprotective potential was estimated by measuring serum activity of biochemical parameters. Further, liver weight ratio and histopathological changes were determined. The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) were significantly ($p$<0.05) increased and albumin decreased significantly ($p$<0.05) in the $CCl_4$ treated group, but treatment with MEEL 75 mg/kg significantly ($p$<0.05) prevented the elevation of ALT, AST, and LDH. Histopathological changes also indicated hepatic protection of the MEEL at 75 mg/kg. This finding suggested that methanolic extract of *Eurycoma longifolia* roots at 75 mg/kg was enough to give a protective effect against $CCl_4$-induced hepatotoxicity.

**Keywords:** carbon tetrachloride; *Eurycoma longifolia*; hepatotoxicity; protective

## INTRODUCTION

The liver is a highly complex organ with regards to various physiological functions in the body (Joshi *et al*., 2015). An endogenous antioxidant system has been developed to maintain redox homeostasis in the liver. Homeostasis will be disturbed because of the excess of free radicals, resulting in oxidative stress, which plays a critical role in liver diseases and other degenerative disorders (Li *et al*., 2015).

Non-alcoholic fatty liver disease (NAFLD) is defined by the presence of liver fat accumulation exceeding 5% of hepatocytes, in the absence of alcohol intake and any other cause of liver injury (Musso *et al*., 2011). NAFLD comprises a wide spectrum of liver damage, ranging from benign hepatocellular steatosis to non-alcoholic steatohepatitis, fibrosis, and cirrhosis (El-Kader and Ashmawy, 2015). Prevalence of NAFLD in Asia is around 25%, like many Western countries (Fan *et al*., 2017). Currently, NAFLD has become a worldwide problem and is associated with significant morbidity and mortality.

Carbon tetrachloride ($CCl_4$) administration to rodents is a widely used model for hepatotoxicity study (Riordan and Nadeau, 2014). $CCl_4$ is activated by

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cytochrome P450 2E1 to form trichloromethyl radicals ($CCl_3\cdot$) and trichloromethyl peroxyl radicals ($CCl_3OO\cdot$). Free radical chain reactions lead to lipid peroxidation and finally produce reversible liver damage (Jayesh *et al*., 2017; Weber *et al*., 2003).

Medicinal plants contain a variety of secondary metabolites that can act as antioxidants. Antioxidants are vital substances which can protect the body from damage caused by oxidative stress induced by free radicals (Jothy *et al*., 2011). *Eurycoma longifolia*, which belongs to the Simaroubaceae family, is one of the most well-known herbal medicines in Southeast Asia (Khanijo and Jirangkoorskul, 2016; Rehman *et al.*, 2016). The root possesses strong antioxidant properties (Varghese *et al*., 2013). Previous research has reported anti-hypertriglyceridemia, anti-inflammatory, analgesic, and anti-obesity activities in its methanolic extract (Hendra *et al.*, 2017; Lahrita *et al.*, 2017). Therefore, we aimed to investigate preventive the effects of *Eurycoma longifolia* roots extract against carbon tetrachloride-induced hepatotoxicity in rats.

## METHODS

### Plant material and chemicals

*Eurycoma longifolia* roots were collected from Kalimantan, Indonesia and supplied by Merapi Farma Herbal Co. (Yogyakarta, Indonesia). Plant material was identified and authenticated at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Gadjah Mada University, Yogyakarta, Indonesia. A voucher specimen was deposited in the Laboratory of Pharmaceutical Biology, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia. Sodium carboxymethyl cellulose (Sodium CMC) was supplied by Brataco Chemika, Indonesia. Olive oil was supplied from Bertolli, Italy. Methanol and carbon tetrachloride as hepatotoxins were from E. Merck (Darmstadt, Germany). Diagnostic kits for the estimation serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin were purchased from Abbott Laboratories, Illinois, USA. Diagnostic kit for estimation of lactate dehydrogenase (LDH) in the serum was purchased from Thermo Fisher Scientific, Massachusetts, USA. All other chemicals were of analytical grade and were purchased from E. Merck, Darmstadt, Germany.

### Preparation of plant extract

The dry powder of *Eurycoma longifolia* roots (1.2 kg) was extracted with methanol (95% v/v) in a mechanical shaker for 48 h at room temperature. The mixture was filtered, then concentrated by using a rotary evaporator at 60$^o$C to yield a semi solid residue (1.89% w/w). This methanolic extract of *Eurycoma longifolia* roots (MEEL) was kept in a desiccator and used for further experiments.

### Phytochemical screening

The MEEL was screened for various chemical constituents (flavonoids, alkaloids, tannins, phenolic compound, alkaloids and terpenoids) by using established methods (Harborne, 1973; Evans and Trease, 1989).

### Experimental animals

Adult male Wistar rats weighing 150-250 g were used for this experiment. The animals were procured from Department of Pharmacology and Therapy, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia. Five rats were kept in one cage and acclimatized to the surroundings (Pharmacology and Toxicology Laboratory, Faculty of Pharmacy, Sanata Dharma University, Yogyakarta, Indonesia) for 1 week before starting the experiment. All animals were housed under standard laboratory conditions (temperature 22±3$^o$C and 12 h light/dark cycle), fed with rodent pellet diet and water ad libitum. The study protocol was approved by Medical and Health Research Ethics Committee, Faculty of Medicine, Gadjah Mada University, Yogyakarta, Indonesia (KE/FK/0794/EC/2017).

### Animal grouping and treatment

The animals were randomly divided into five groups with 5 rats in each group. Group I (normal control): animals were treated with

Sodium CMC 1% w/v p.o., for 6 days. Group II (toxic control): animals were treated with $CCl_4$ in olive oil (1:1) 2 mL/kg, i.p. Groups III-V (test groups): animals were treated with 75, 150, and 300 mg/kg of MEEL p.o., for 6 days, respectively, and a single dose of $CCl_4$ in olive oil (1:1) 2 mL/kg, i.p. was given on the seventh day. Blood samples from all groups were obtained directly through retro-orbital plexus venous puncture 24 h after the last administration (Dongare *et al*., 2013; Hendra *et al*., 2017). The blood was allowed to coagulate and centrifuged at 3000 rpm for 10 min. Serum samples were used for determination of some biochemical parameters.

**Biochemical determinations**

All biochemical parameters were determined in a Cobas C501 analyzer using commercial kits following standard procedures.

**Histopathological assessment**

Rats were euthanized by cervical dislocation at the end of the experiment. Rats were dissected, livers were collected and weighted. The liver weight ratio is expressed as follows: (liver weight/body weight) x 100% (Li *et al*., 2017; Ekeanyanwu and Njoku, 2014). Then the organs were fixed in neutral buffered formalin. The tissues were embedded in paraffin, cut in 5 μm sections and stained with hematoxylin and eosin for histopathological findings.

**Statistical analysis**

All the data were expressed as mean±SEM. Groups were compared by Kruskal-Wallis test, followed by Mann-Whitney test. P values less than 0.05 were considered. Statistical analysis was done using IBM SPSS Statistics 22.

**RESULTS AND DISCUSSION**

This study was designed to investigate the activity of methanolic extract of *Eurycoma longifolia* roots (MEEL) on some biomarker enzymes and histopathological changes of hepatic lesions indicating hepatocellular injury induced by $CCl_4$ in rats.

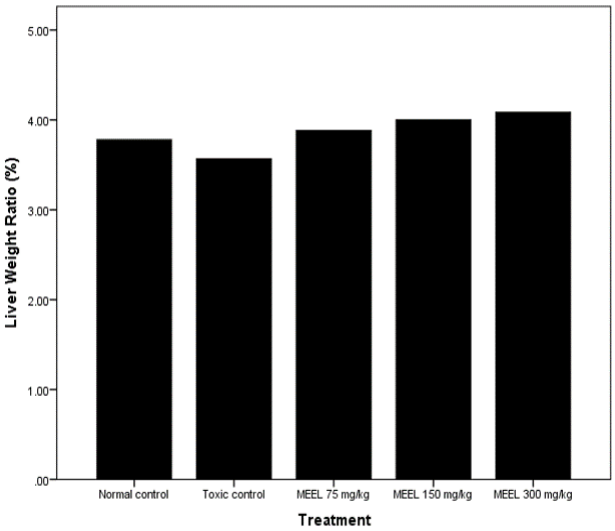


**Figure 1.** Effect of MEEL on liver weight ratio

**Table I.** Effect of MEEL on liver biochemical parameters

| Treatment | ALT (U/L) | AST (U/L) | LDH (U/L) | Albumin (mg/dL) |
|---|---|---|---|---|
| Normal control | 52.8±2.5[b] | 140.0±7.7[b] | 529.0±27.1[b] | 3.49±0.06[b] |
| Toxic control | 220.5±25.0[a] | 839.3±16.5[a] | 1511.6±136.1[a] | 3.15±0.05[a] |
| MEEL 75 mg/kg | 146.9±18.6[a,b] | 566.8±63.6[a,b] | 851.0±31.5[a,b] | 3.01±0.05[a] |
| MEEL 150 mg/kg | 134.9±11.5[a,b] | 581.9±41.3[a,b] | 1247.6±165.9[a] | 3.05±0.06[a] |
| MEEL 300 mg/kg | 222.2±24.8[a] | 743.7±38.8[a,b] | 1158.6±102.7[a] | 3.12±0.08[a] |

All values are expressed as mean±SEM, n=5. [a]$p$<0.05 when compared with normal control and [b]$p$<0.05 when compared with toxic control.

Carbon tetrachloride is extensively known as an inducer for hepatotoxicity models. The elevated of serum enzyme ALT and AST as sensitive markers, are indicative of cellular leakage and loss of functional integrity of cell membranes in liver that was initiated by hepatocellular damages caused by $CCl_4$ (Nirmala *et al*., 2012; Thanh *et al*., 2015). Changes in the activities of serum enzymes (ALT, AST and LDH) and albumin in the serum of $CCl_4$-induced liver damage as evidence from Table I. The level of serum marker enzymes ALT, AST and LDH were found to be significantly ($p$<0.05) increased

and albumin decreased significantly ($p<0.05$) in $CCl_4$ treated group when compared to normal control. In the present study, significant increases in aminotransferase, LDH and decrease of albumin level in the $CCl_4$ intoxicated rats represent hepatic damage, consistent with previous reports.

Treatment with MEEL 75 mg/kg significantly ($p<0.05$) prevented the elevation of ALT, AST, and LDH. MEEL 150 mg/kg reduced levels of ALT and AST significantly ($p<0.05$). Treatment with MEEL 300 mg/kg significantly ($p<0.05$) decreased levels of AST. However, there were no significant ($p>0.05$) increases in the level of albumin of the rats with all of the doses of MEEL.

Figure 1. shows the average liver weight ratio in the five groups animals. No significant differences in livers among all the treatments were observed.

Histopathological observation of liver tissue of the normal control group showed a normal liver architecture of hepatocytes since they were well arranged without any alteration at the central vein, while $CCl_4$ intoxicated hepatocytes showed severe multifocal areas of fat degeneration (steatosis). Steatosis is known as a type of liver injury that may manifest as triglyceride accumulation which leads to either a micro vesicular or macro vesicular fatty liver (Sing *et al*., 2014). The MEEL 75 mg/kg treated group's liver sections showed less hydropic degeneration, while MEEL 150 and 300 mg/kg treated groups' liver sections showed hydropic degeneration with multifocal areas of fat degeneration.

In our study, administration of MEEL 75 mg/kg markedly decreased the elevated aminotransaminase and LDH levels induced by $CCl_4$. A dose-dependent effect was not found in the 150 and 300 mg/kg of MEEL groups when compared to the 75 mg/kg group. These trends were also found in histological observation of liver tissue. Similarly, previous study showed the hepatoprotective effect of water extract of *Eurycoma longifolia* at 1.5 g/kg against $CCl_4$-induced hepatotoxicity (Al-faqeh *et al*., 2010). An in vivo study reported that the methanolic extract of *Eurycoma longifolia* roots reduced triglyceride levels induced by glucose-fructose enriched diet and shown anti-inflammatory activity (Hendra *et al*., 2017). Another in vitro study confirmed that *Eurycoma longifolia* suppressed lipid accumulation in 3T3-L1 adipocytes (Lahrita *et al*., 2015). Hence, the hepatoprotective effect of methanolic extract of *Eurycoma longifolia* roots 75 mg/kg may be achieved by the anti-inflammatory activity which may be associated with scavenging of free radicals responsible for $CCl_4$ toxicity. Moreover, flavonoids are known as hepatoprotectives agents, so the mechanism of hepatoprotective activity of MEEL 75 mg/kg may be due to the presence of flavonoids. Preliminary phytochemical screening conducted in this study indicated MEEL contains flavonoids, tannins, phenolic compound, and terpenoids, while alkaloids were absent.

## CONCLUSION

In summary, the results of this study showed that $CCl_4$ caused hepatotoxicity and the administration of methanolic extract of *Eurycoma longifolia* roots 75 mg/kg to rats offered significant protection from the hepatic damage by $CCl_4$. Evidently, histopathological examination of liver also supported the benefits of the methanolic extract of *Eurycoma longifolia* roots as it helped in improving liver cell architecture damage caused by $CCl_4$. Further study should be done to characterize the hepatoprotective components of methanolic extract of *Eurycoma longifolia* roots.

### Conflict of interest statement

We declare that we have no conflict of interest.

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