HEPATOMO

INTRODUCTION

Liver is the most important vital organ in the human body. It plays major role in metabolism of nutrition i.e. carbohydrate, protein, and lipid. In addition, drugs and xenobiotic are also metabolized and excreted via liver, making it vulnerable to damage. Some studies reported that acetaminophen, carbon tetrachloride (CCL4), and alcohol produced liver damage (Dash et al., 2007; Arun and Balasubramanian, 2011; Panjaitan et al., 2013). Liver damage is indicated by changes of alanine transaminase (ALT), aspartate transaminase (AST), damage is indicated by changes of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), alkaline phosphatase, bilirubin, total protein levels, a necrotic, and inflammation microanatomy pattern of the liver (Dash et al., 2007; Arun and Balasubramanian, 2011; Domitrović et al., 2011; Sengupta et al., 2011; Eidi et al., 2012; Panjaitan et al., 2013).

Various plants were reported for their protective and regenerative property in liver cells, including Dillenia indica Linn. (Padhya et al., 2008), Eurycoma longifolia Jack (Panjaitan et al., 2011; Panjaitan et al., 2013), Moringa oleifera Lam (Singh et al., 2014), and Pithecellobium dulce Benth. (Raju and Jagadeeswar, 2014). The protective effect of the plant is not only limited in their leaves but also in their roots and fruits which was associated with their chemical compound (Padhya et al., 2008; Panjaitan et al., 2013; Raju and Jagadeeswar, 2014; Singh et al., 2014).

Dog fruit (Pithecellobium lobatum Benth.) or Pithecellobium jiringa (Arekeul et al., 1976 cit. Bakar et al., 2012) is family of Leguminosae (Martin et al., 1987). This plant has potential as antimicrobial (Bakar et al., 2012), anti-ulcer (Ibrahim et al., 2012), angiogenesis (Muslim et al., 2012), and anti-oxidant (Muslim et al., 2012; Yanti et al., 2015). Their leaves (Bakar et al., 2012), seeds (Bakar et al., 2012; Ibrahim et al., 2012; Yanti et al., 2015), and rinds (Bakar et al., 2012; Yanti et al., 2015) can be used for medicinal purposes.

Panjaitan et al. (2013) stated there was a relationship between hepatoprotective effects and antioxidant properties. So far, recent studies have shown that flavonoid, total phenol, β-sitosterol, quercetin, and kaempferol can generate hepatoprotective effect (Singh et al., 2014). Moreover, Eidi et al. (2012) reported that there was a relationship between the chemical compounds (flavonoid, glycoside, coumarin, alkaloid, anthraquinone, steroid, tannin, and terpenoid) contained in ethanolic cinnamon rind extract (Cinnamomum zeylanicum L.) and its hepatoprotective effect. In fact, dog fruit rind have also been reported contained flavonoid compound (Lim, 2012 as cited in Yanti et al., 2015). Therefore, the present study was conducted to investigate the

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hepatoprotective activity of ethanolic extract of dog fruit rind on CCl₄ induced hepatotoxicity in rats.

MATERIALS AND METHODS

Experimental Animals
The study was carried out using male Wistar albino rats (200-250 g) aged 2-2.5 months old. The animals were acclimated for seven days before the experiment. We observe the rat health by weighing their body weight and feeding them ad libitum.

Extraction and Partition
Five kg of dog fruits were collected from traditional market in Pontianak and the seed was separated from the rind. A total of 2.43 kg of the rind was obtained and dried under the sunlight until the weight was 630 g, and it was then crushed into pieces. The pieces of dog fruit rind were macerated for 48 h in ethanol 96% at room temperature based on Harborne (1998). Maceration was performed twice by adding new ethanol. The extract filtrate was concentrated until it weighs 72.75 g.

Hepatoprotective Activity Assessment
The rats were randomly divided into four groups of five animals per each group. Treatment was carried out according to the following groups for seven days: Group K1-K2 (the extract groups) received 50 mg/200 g body weight (K1) and 100 mg/200 g body weight (K2) of ethanolic extract of dog fruit rind; Group K3 (positive control) received silymarin at the dose of 5 mg/200 g body weight; and Group K4 (negative control) received 0.4 mL/200 g body weight of distilled water.

Biochemical Liver Function Analysis
The blood samples were collected from the heart. Serum was separated by centrifugation at 4000 rpm for 5 min and collected into eppendorf tube for further biochemical parameters analysis using a certain kit (Analyticontm Biotechnologies AG, Germany).

Histological Studies
The rats were sacrificed by cervical dislocation and their liver was carefully removed followed the routine process. The slides were stained with hematoxylin and eosin for pathological analysis (Kiernan, 1990). The score was examined under light microscope (Table 1).

RESULTS AND DISCUSSION

The levels of ALT and AST of rats in K1, K2, K3, and K4 is presented in Table 2. Both ALT and AST levels were not significantly different among treatments (P>0.05). The ALT levels in K1, K2, K3, and K4 were 143.40±83.75 U/L, 94.80±93.77 U/L, 130.20±58.54 U/L, and 147.25±107.97 U/L, respectively, while the AST levels were 304.20±128.67 U/L, 213.20±88.93 U/L, 333.00±128.31 U/L, and 239.25±94.90 U/L, respectively (P>0.05).

As illustrated in Figure1, histological assessment of the liver sections revealed that injection of CCl₄ induced pathological changes with different level of damage. About 60% of K4 group showed moderate damage (score 2), whereas the rest 40% showed severe damage (score 3). The rats treated with the extract at dose of 50 mg/200 g produce similar protection as silymarin group, in which mild and moderate damage developed in 40% rats, while 20% of rats showed severe damage. Administration of 100 mg/200 g of ethanolic extract of dog fruit rind attenuate liver damage, and was indicated by mild damage in 60% rats and moderate damage in the other 40% rats. The score was significantly lower than the group given standard drug, silymarin, which generate mild damage only in 20% of rats, whereas 60% had moderate liver damage, and the other 20% had severe liver damage.

Histopathological examination of liver sections confirmed our biochemical finding. This research describes the potential protective effect of ethanolic extract of dog fruit rind against carbon tetrachloride (CCl₄) induced hepatotoxicity. Domitrović et al. (2011) stated that CCl₄ induced liver damaged involved

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<th>Score</th>
<th>Evidence</th>
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<tbody>
<tr>
<td>0</td>
<td>No specific alteration</td>
</tr>
<tr>
<td>1</td>
<td>Evenly mild hydrophobic and fat degeneration</td>
</tr>
<tr>
<td>2</td>
<td>Focal moderate fat degeneration and steatosis</td>
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<tr>
<td>3</td>
<td>Multifocal severe fat degeneration, steatosis, and dystrophy</td>
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<th>Parameters</th>
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<tr>
<td>ALT (U/L)</td>
<td>A 130.20±58.54</td>
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<tr>
<td>AST (U/L)</td>
<td>A 333.00±128.31</td>
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A= Silymarin 5 mg/200 g (positive control), B= 50 mg/200 g ethanolic extract of dog fruit rind, C= 100 mg/200 g ethanolic extract of dog fruit rind, D= 0.4 mL/200 g aquades (negative control)
necrosis and steatosis since the process of CCl₄ biotransformation in the liver by P450 cytochrome reductase, with NADPH as cofactor, formed free radicals i.e. trichloromethyl (CCl₃*) and trichloromethyl peroxyl (CCl₃O₂*). Free radicals bind to hepatocyte membrane and cell organelles leading to lipid peroxidation and unbalanced calcium that further cause cell death. Moreover, Stockham and Scott (2002) revealed one of the techniques to detect, determine the cause, and assessing the severity of liver disease is by analyzing liver biochemical enzyme, including AST and ALT. In addition, liver abnormality can be detected by histopathological examination.

Alanine transaminase is a cytosolic enzyme involved in gluconeogenesis. The increase in ALT levels in the blood indicates hepatocyte damage. Aspartate transaminase is also involved in gluconeogenesis and elevated levels of AST in the blood indicates advanced hepatocyte damage accompanied with necrosis which release the mitochondrial enzyme (Shanmugasundaram and Venkataraman, 2006; Panjaitan et al., 2013). In relation with ALT and AST levels in blood, Panjaitan et al. (2007) revealed that administration of 0.1 mL/kg CCl₄ induce multifocal cells degeneration and necrosis in the liver and hepatocyte alteration is directly proportional to the dose. Moreover, the administration of 0.1 mL/kg CCl₄ produce extensive and severe hepatocyte damage that lead to very low availability of ALT and AST levels in hepatocyte.

Hepatoprotector is an agent that produces protective and regenerative effects on toxic induced liver damage. It has been reported that there is a relationship between hepatoprotective and antioxidant effect. Moreover, the activity of medicinal substances related to their chemical compounds (Panjaitan et al., 2013; Singh et al., 2014). The chemical compounds in dog fruit rind encompass alkaloid, flavonoid, tannin, quinone (Syafnir et al., 2014) and polyphenol (Syafnir et al., 2014; Yanti et al., 2015). Particularly, flavonoid (Syafnir et al., 2014) and polyphenol (Syafnir et al., 2014; Yanti et al., 2015) have antioxidant activity against free radicals. Therefore, the hepatoprotective activity of ethanolic extract of dog fruit rind is associated to the antioxidant activity of the compound they contain.

CONCLUSION

Ethanolic extract of dog fruit rind at dose of 100 mg/200 g have hepatoprotective activity against CCl₄ induced liver damage in rat.

REFERENCES


