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HEPATOPROTECTIVE ACIVITY OF THE ETHANOLIC EXTRACT OF DOG FRUIT RIND (*Pithecellobium lobatum* Benth.)

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ABSTRACT

The present study was conducted to investigate the hepatoprotective activity of ethanolic extract of dog fruit rind (*Pithecellobium lobatum* benth.) against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats. Male Wistar albino rats were randomly divided into four groups and administered orally with 50 mg/200 g body weight of dog fruit rind extract (K1), 100 mg/200 g body weight (K2) of dog fruit rind extract, 5 mg/200 g body weight of silymarin (K3/positive control), and 0.4 mL/200 g body weight of distilled water (K4/negative control), for seven days The levels of alanine transaminase (ALT) of each 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 aspartate transaminase (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). Group K2 showed better histological pattern than other groups with 60% of mild and 40% of moderate liver damage. Our findings revealed the hepatoprotective activity of the ethanolic extract of dog fruit rind.

Key words: CCl_4, hepatoprotective, *Pithecellobium lobatum* Benth.

ABSTRAK

Penelitian ini bertujuan mengetahui aktivitas hepatoprotektor ekstrak etanol kulit buah jengkol (Pithecellobium lobatum Benth.) pada tikus yang diinduksi karbon tetraklorida (CCl_4). Ekstrak etanol kulit buah jengkol dosis 50 mg/200 g bobot badan (K1) dan dosis 100 mg/200 g bobot badan (K2) diberikan per oral selama tujuh hari berturut-turut. Pembanding positif adalah silymarin dosis 5 mg/200 g bobot badan (K3), sedangkan pembanding negatif adalah akuades dosis 0,4 ml/200 g bobot badan (K4). Kadar enzim alanin transaminase (ALT) dan aspartat transaminase (AST) pada K1; K2; K3; dan K4 masing-masing adalah 143,40±83,75 U/l dan 304,20±128,67 U/l; 94,80±93,77 U/l; dan 213,20±88,93 U/l; 130,20±58,54 U/l AST 333,00±128,31U/l; dan 147,25±107,97 U/l dan 239,25±94,90 U/l) (P>0,05). Gambaran histopatologis setelah pemberian ekstrak etanol kulit buah jengkol dosis 100 mg/200 g bobot badan menunjukkan hasil yang lebih baik dibanding kelompok lainnya yang ditandai dengan 60% kerusakan dengan derajat ringan dan 40% dengan derajat sedang. Disimpulkan bahwa ekstrak kulit buah jengkol memiliki aktivitas hepatoprotektor.

Kata kunci: CCl_4, hepatoprotektor, Pithecellobium lobatum Benth.

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 (CCl_4), 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), 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 Jagadeeshwar, 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 Jagadeeshwar, 2014; Singh *et al*., 2014).

Dog fruit (*Pithecellobium lobatum* Benth.) or *Pithecellobium jiringa* (Areekul *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), anti-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 kaempherol 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

hepatoprotective activity of ethanolic extract of dog fruit rind on CCl_4 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. On 8^{th} day, as much as 0.02 mL/200 g of CCl_4 was administered to all group. Twenty-four hours after CCl_4 administration (the 9^{th} day), blood and liver organ were obtained from all groups. Parameters analysed were ALT and AST levels, as well as liver histopathology.

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 (Analyticon®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).

Data Analysis

The ALT and AST data were analyzed using analysis of variance then followed by Duncan test. Liver histophatology was analysed descriptively.

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±88.93U/L, 333.00±128.31U/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 CCl_4 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 damaged, 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 (CCl_4) induced hepatotoxicity. Domitrović *et al*. (2011) stated that CCl_4 induced liver damaged involved

Table 1. Liver damage scoring system

Score	Evidence
0	No specific alteration
1	Evenly mild hydrophobic and fat degeneration
2	Focal moderate fat degeneration and steatosis
3	Multifocal severe fat degeneration, steatosis, and dystrophy

Table 2. Mean ALT and AST levels in blood serum of male albino rats administered with ethanolic extract of dog fruit rin (n= 5) ($P>0.05$)

Parameters	Treatment			
	A	B	C	D
ALT (U/L)	130.20±58.54	143.40±83.75	94.80±93.77	147.25±107.97
AST (U/L)	333.00±128.31	304.20±128.67	213.20±88.93	239.25±94.90

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 aquadest (negative control)

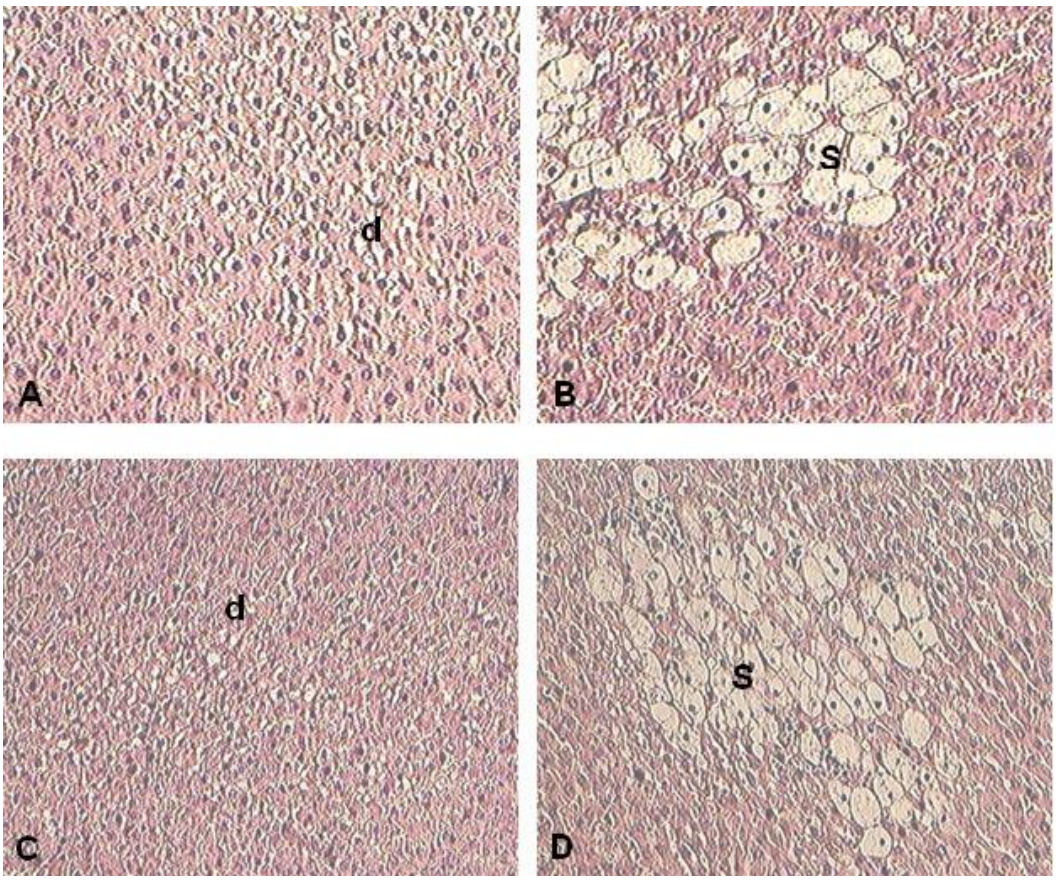

Figure 1. Liver histopathology of albino rats. A= 5 mg/200 g silymarin group, B= 50 mg/200 g ethanolic extract of dog fruit rind group, C= 100 mg/200 g ethanolic extract of dog fruit rind group, D= 0.04 mL/200 g distilled water group, d= Degeneration of hepatocyte, s= Steatosis (HE, 10x).

necrosis and steatosis since the process of CCl_4 biotransformation in the liver by P450 cytochrome reductase, with NADPH as cofactor, formed free radicals i.e. trichloromethyl (CCl_3^*) and trichloromethyl peroxyl ($CCl_3O_2^*$). 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 CCL_4 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 CCl_4 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 CCl_4 induced liver damage in rat.

REFERENCES

Arun, K. and U. Balasubramanian. 2011. Comapartive study on hepatoprotective activity of *Phyllanthus amarus* and *Eclipta prostrata* against alcohol induced in albino rats. **Int. J. Environ. Sci.** 2(1):361-379.

Bakar, R.A, I. Ahmad, and S.F. Sulaiman. 2012. Effect of *Pithecellobium jiringa* as antimicrobial agent. **Bangladesh J. Pharmacol.** 7:131-134.

Dash, D.K., V.C. Yeligar, S.S. Nayak,T. Ghosh, D. Rajalingam, P. Sengupta, B.C. Maiti, and T.K. Maity. 2007. Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. **Trop. J. Pharm. Res.** 6(3):755-765.

Domitrović, R., H. Jakovacb, and G. Blagojević. 2011. Hepatoprotective activity of berberine is mediated by inhibition of TNF-α, COX-2, and iNOS expression in CCl_4-intoxicated mice. **Toxicology**. 280:33-43.

Eidi, A., P. Mortazavi, M. Bazargan, and J. Zaringhalam. 2012. Hepatoprotective activity of cinnamon ethanolic extract against CCl_4-induced liver injury in rats. **EXCLI J.** 11:495-507.

Harborne, J.B. 1987. **Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan**. (Translated by Kosasih, P. and S. Iwang). 2nd ed. ITB Press, Bandung**.**

Ibrahim, I.A.A., S.W. Qader, M.A. Abdulla,A.R. Nimir, S.I. Abdelwahab, and F.H. AL-Bayaty. 2012. Effects of *Pithecellobium jiringa* ethanol extract against ethanol-induced gastric mucosal injuries in Sprague-Dawley rats. **Molecules**. 17:2796-2811.

Kiernan, J.A. 1990. **Histological and Histochemical Methods. Theory and Practice**. 2nd ed. Pergamon Press, Canada.

Martin, F.W., C.W. Campbell, and R.M. Ruberté. 1987. **Perennial Edible Fruits of the Tropics: An Inventory.** U.S. Department of Agriculture, Washington DC.

Muslim, N. S., Z.D. Nassar, A.F.A. Aisha, A.Shafaei, N. Idris1, A.M.S.A. Majid, and Z. Ismail. 2012. Antiangiogenesis and antioxidant activity of ethanol extracts of *Pithecellobium jiringa*. **BMC Complemen. Altern. Med.** 12(210):1-10.

Padhya, I.P., N.S.K. Choudhary, S.K. Padhy, and S. Dash. 2008. Effect of *Dillenia indica* leaves against carbon tetrachloride induced hepatotoxicity. **J. Pharm. Chem.** 2(4):24-27.

Panjaitan, R.G.P., E. Handharyani, Chairul, Masriani, Z. Zakiah, and W. Manalu. 2007. Pengaruh pemberian karbon tetraklorida terhadap fungsi hati dan ginjal tikus. **Makara, Kesehatan.** 11(1):11-16.

Panjaitan, R.G.P., W. Manalu, E. Handharyani, and Chairul. 2011. Aktivitas hepatoprotektor ekstrak metanol akar pasak bumi dan fraksi-fraksi turunannya. **J. Vet.** 12(4):319-325.

Panjaitan, R.G.P., W. Manalu, E. Handharyani, and Chairul. 2013. Hepatoprotective activity of *Eurycoma longifolia* Jack. roots. **Indian J. Tradit. Knowle.** 12(2):225-230

Raju, K. and K. Jagadeeshwar. 2014. Phytochemical investigation and hepatoprotective activity of ripe fruits of *Pithecellobium dulce* in albino rats. **Sch. Acad. J. Pharm**. 3(6):449-454.

Sengupta, M., G.D. Sharma, and B. Chakraborty. 2011. Hepatoprotective and immunomodulatory properties of aqueous extract of *Curcuma longa* in carbon tetra chloride intoxicated Swiss albino mice. **Asian Pac. J. Trop. Biomed.** 1(3):193-199.

Singh, D., P.V. Arya, V.P. Aggarwal, and R.S. Gupta. 2014.Evaluation of antioxidant and hepatoprotective activities of *Moringa oleifera* Lam. leaves in carbon tetrachloride-intoxicated rats. **Antioxidants**. 3:569-591.

Shanmugasundaram, P. and S. Venkataraman. 2006. Hepatoprotective and antioxidant effects of *Hygrophila auriculata* (K. Schum) Heine Acanthaceae root extract. **J. Ethnopharmacol**. 104:124-128.

Stockham, S.L. and M.A. Scott. 2008. **Fundamentals of Veterinary Clinical Pathology**. 2nd ed. Blackwell Publishing Professional, Iowa.

Syafnir, L., Y. Krishnamurti, and M. Ilma. 2014. Uji aktivitas antidiabetes ekstrak etanol kulit jengkol (*Archidendron pauciflorum* (Benth.) I.C.Nielsen). **Prosiding SnaPPSains, Teknologi, dan Kesehatan:**65-72.

Yanti, F. Irnawati, M. Vivian, and Y.R.E. Wulandari. 2015. Extraction yield and fractions antiooxidant activity of biomolecule and bioactive from seed and peel parts of *Pithecellobium jiringa*. **Sch. Acad. J. Biosci.** 3(9):790-795.