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Hepatoprotective effect of ethanol extract of matoa leaves (*Pometia pinnata*) against paracetamol-induced liver disease in rats

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ABSTRACT

The hepatoprotective effects of matoa leaves were evaluated by paracetamol-induced injury in rat's hepatocytes. The ethanolic extract of matoa leaves (EEML) at doses of 200, 300, 500 mg/kg, po and silymarin at dose of 100 mg/kg, po were given for seven days. Silymarin was given as the reference drug. Hepatoprotective effect was studied by measuring the level of AST, ALT, ALP and total protein in serum. In vivo, oral treatment with EEML at dose of 500 mg/kg significantly reduced AST, ALT, ALP in serum whereas total protein was not significantly reduced in each groups. These results indicate that the hepatoprotective action of EEML is likely related to its potent antioxidative activity. Neutralizing reactive oxygen species enhancing the activity of original natural hepatic-antioxidant enzymes may be the main mechanisms of EEML against paracetamol-induced injury.

Keywords: *Matoa Leaves (Pometia pinnata), Paracetamol, Silymarin, Hepatoprotection*

1. INTRODUCTION

Liver is the vital organ that has major roles in metabolism, detoxification and elimination of toxic substances and Liver disease is still a major health problem (Adewusi and Afolayan, 2010). Free radicals are the major risk factor which lead to liver disease (Valko, et al., 2007). Paracetamol is a safe and effective analgesic and antipyretic drug. However, an overdose can cause hepatotoxicity in experimental animals and humans. Paracetamol hepatotoxicity is the most frequent cause of acute liver failure of any etiology in the western world (Larson et al., 2005; Larson, 2007).

Paracetamol toxicity is due to the formation of *N*-acetyl *p*-benzoquinone imine (NAPQI) when a part of it is metabolized by cytochrome P450. NAPQI is a reactive metabolite of paracetamol, detoxified by glutathione (GSH) resulting in extensive hepatic GSH depletion. An overdose of paracetamol causes increasing amount of NAPQI and reacts with protein sulfhydryl groups, causing the covalent adduction of cellular proteins and causes oxidative stress and glutathione (GSH) depletion (Knight et al., 2001). Synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects (Prakash, et al., 2008). So, we attempted to evaluate alternative medicines as hepatoprotector from medicinal plants. One of the most popular models to evaluate the hepatoprotective potential of natural products is paracetamol-induced liver injury (Jaeschke, et

al., 2013).

Matoa leaves (*Pometia pinnata*) contains antioxidant. Previous study by Suedee et al, 2013 succeed to isolate epicatechin, kaempferol-3-O-rhamnoside, quercetin-3-O-rhamnoside, glycolipid, 1-O-palmitoyl-3-O-[α -galactopyranosyl-(1 \rightarrow 6)- β -galactopyranosyl]-sn-glycerol, steroid glycosides, stigmasterol-3-O-glucoside and triterpenoid saponin pentacyclic, 3-O- α -arabinofuranosyl-(1 \rightarrow 3)-[α -rhamnopyranosyl-(1 \rightarrow 2)]- α -arabinopyranosyl hederagenin from matoa leaves extract. A potential of hepatoprotective property underlying matoa leaves may be attributed to the antioxidative constituents (Akachi, et al., 2010). The bioactive compounds that are responsible for relieving oxidative stress are usually indistinctly ascribed to polyphenols and flavonoids compounds (Li, et al., 2015). Hence an attempt has been made to assess the hepatoprotective role of Matoa leaves.

2. EXPERIMENTAL SECTION

2.1. Chemicals

Paracetamol (acetaminophen), Hepa-Q[®] capsule (silymarin), and standard assay kits of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), bilirubin and total protein was obtained from Health Laboratory in Palembang. All other reagents were analytical grade.

2.2. Plant materials

The matoa leaves were collected from Indralaya, Ogan Ilir Dis-

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Table 1. The result of phytochemical screening

No	Screening	Simplex	EEML
1	Alkaloids	-	-
2	Flavonoids	+	+
3	Saponins	+	+
4	Tannins	+	+
5	Triterpenoid/ steroid	+	+

trict South Sumatra, Indonesia. The sample was determined in Herbarium Departement of Biology, Faculty of Mathematics and Natural Science Andalas University. Sample was thoroughly washed with tap water, sorted while wet, cut dried at 50°C for 5 days, and grinded into powder (Purwidyaningrum, et al., 2017).

2.3. Preparation of extract

Sample was extracted by maceration with 96% ethanol . 1 kg of crude extract was maceration with 1.5 L of 96% ethanol for 3 days at room temperature on a shake. Done triplicate. The filtrate was collected, and then evaporated using a rotary vacuum evaporator at 45°C under reduced pressure and speed of 40 rpm to give a viscous extract and then freeze dried to give a dried extract. The extract was called EEML (Sihotang, et al., 2016).

2.4. Phytochemical screening

Phytochemical screening was carried out by standard procedures, as described by Harborne (1998) and Bargah (2015). The color intensity or the precipitate formation was used as analytical responses to these tests.

2.5. Test for Alkaloids

About 3 ml of extract was stirred with 3 ml of 1% HCl on steam bath. 1 ml of mixture was taken separately in two test tubes. Few drops of Dragendorff's reagent were added in one tube and occurrence of orange red precipitated was taken as positive. Two the second tube Mayer's reagent was added and appearance of buff colored precipitate was taken as positive test for presence of alkaloids.

2.6. Test for flavonoids

To 1 ml of extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for presence of flavonoids.

2.7. Test for tannins

About 2 ml of the extract was stirred with 2ml of distilled water and few drops of ferric chloride (FeCl_3) solution were added. Formation of green precipitate was indication of presence of tannins.

2.8. Test for Saponins

About 5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and warmed. The formation of stable foam was taken as an indication of the presence of saponins.

2.8. Test for Terpenoids

About 2 ml of the organic extract was dissolved in 2ml CHCl_3 and evaporated to dryness. 2 ml of conc. H_2SO_4 was then added and heated for about 2 minutes. Development of a grayish color indicates the presence of terpenoids.

2.9. Test for Steroids

Liebermann Burchard test: Development of a greenish color when 2 ml of the organic extract was dissolved in 2 ml of chloroform and treated with concentrated sulphuric acid and acetic acid indicates the presence of steroids.

2.10. Experimental animals

Albino adult Wister male rats (150–200 g) were used for this study. Animals were divided into six groups ($n = 6/\text{group}$) as follows: Group I: normal control rats received Na CMC 1% b/v 5 mL/kg b.w. orally for 7 days. Group II: rats received paracetamol 3 g/ kg b.w orally for 2 days. Group III: rats received the standard drug silymarin 100 mg/kg b.w. orally for 7 days. Group IV: rats received sample EEML 200 mg/kg b.w. orally for 7 days. Group V: rats received sample EEML 300 mg/kg b.w. orally for 7 days. Group VI: rats received sample EEML 500 mg/kg b.w. orally for 7 days.

All the animals in the groups III–VI were pre-treated with EEML from 1st day to 5th day with concurrent administration of paracetamol on 6th and 7th day. During the period of EEML, the rats were maintained under normal diet and water ad libitum. After 2 days of respective drug treatments, animals were anaesthetized using diethyl ether inhalation jar. Blood was collected through retro-orbital plexus under mild ether anesthesia (Prakash, et al., 2008).

2.11. Determination of key liver biochemical markers

Liver biochemical markers such as ALT, AST, ALP, and total protein have been evaluated in the serum. Blood was obtained from all animals by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2.5 x g at 30°C for 15 min and assayed for AST, ALT, ALP, and total protein as shown in Table 2. (Rajasekaran and Periyasamy, 2012; Abirami, et al., 2015).

2.12. Statistical analysis

All data were expressed as mean \pm standard deviation. Statistical analysis was performed using one-way analysis of variance followed by post hoc Tukey. Significant differences were set at values <0.05 .

3. RESULT AND DISCUSSION

The results of phytochemical screening of Matoa Leaves (*Pometia pinnata*) is presented in Table 1. The etanol extract of matoa leaves (*Pometia pinnata*) contains flavonoids, saponins, tannins, and triterpenoid/ steroid. Phenolic and flavonoid compounds of plant extracts are recognized as active substances responsible for the antioxidant activity due to their free radical scavenging effects (Li Yin, et al., 2014).

The result of Determination of key liver biochemical markers are shown in Table 2. The effects of the oral treatment of EEML on the serum AST, ALT, ALP, and total protein levels of

Table 2. Effects of EEML on liver biochemical parameters

Groups (n=5)	Treatments	Liver Biochemical Parameters			
		ALT (U/L)	AST (U/L)	ALP (U/L)	Protein Total (U/L)
I	Normal Control	70.44 ± 5.634*	88.96 ± 8.657*	121.53 ± 23.353*	7.10 ± 0.827
II	Paracetamol (PCT)	136.88 ± 20.234	167.66 ± 39.047	198.42 ± 16.257	7.38 ± 0.376
III	Silymarin + PCT	88.30 ± 11.264*	104.98 ± 7.661*	128.82 ± 13.223*	7.02 ± 0.626
IV	EEML 200mg/KgBB + PCT	118.72 ± 10.011	229.42 ± 9.263*	211.10 ± 50.104	7.22 ± 0.228
V	EEML 300mg/KgBB + PCT	111.16 ± 7.717*	203.26 ± 31.446	141.17 ± 45.637	7.6 ± 0.374
VI	EEML 500mg/KgBB + PCT	95.86 ± 3.519*	106.08 ± 5.506*	131.18 ± 27.379*	7.08 ± 0.178

Values are expressed as mean ± S.D (n=5).

Normal group and experimental groups were compared with paracetamol group: *p<0.05. There were values were significantly different (p<0.05).

hepatic-damaged rats are shown in Table 2. The serum AST, ALT, and ALP levels of the paracetamol group were elevated, whereas the total protein of this group were not reduced, thus indicating that liver cell damage was significantly induced. The enhancement of EEML doses significantly attenuated the paracetamol-induced elevation of the AST, ALT, and ALP levels. These data suggest that EEML reduced the paracetamol-induced hepatic damage.

Paracetamol (acetaminophen) is widely consumed as an antipyretic drug that is safe in therapeutic doses but can cause fatal hepatic damage in human and animal at higher toxic doses. Bioactivation of paracetamol by hepatic cytochrome P-450 leads to formation of a highly reactive and toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). NAPQI is normally detoxified by conjugation with reduced glutathione (GSH) to form mercapturic acid which is excreted in urine (Abirami, et al., 2015).

Toxic overdose of paracetamol depletes hepatic GSH content so that free NAPQI binds covalently to cellular mitochondrial proteins and results in massive necrosis and apoptosis of hepatocytes (Chen, et al., 2009., Bhattacharyya, et al., 2013). An obvious sign of hepatic injury is the leaking of cellular enzymes such as ALT, AST and ALP into plasma. ALT is more specific to the liver, and it is a better parameter for analyzing hepatic injury. High levels of AST indicate the cellular leakage as well as loss of functional ability of cell membrane in liver. Serum ALP is also related with liver cell damage. High concentration of ALP cause serious hepatic damage in paracetamol treated rats (Darbar, et al., 2011).

The mechanism of hepatoprotection by EEML is due to their antioxidant potential. This suggests that EEDM can reduce ROS that may lessen the oxidative damage to the hepatocytes and improve the activities of the liver antioxidant enzymes, thus protecting the liver from paracetamol induced damage (Valko, et al., 2007). Also, the possible mechanism could be by the stimulation of hepatic regeneration through an improved synthesis of protein or accelerated detoxification and excretion. (Akachi, et al., 2010).

4. CONCLUSION

Oral treatment with EEML at dose of 500 mg/kg significantly reduced AST, ALT, ALP in serum whereas total protein was not significantly reduce. These results indicate that the hepatopro-

TECTIVE action of EEML is likely related to its potent antioxidative activity. It can be concluded in our study that etanol extract of matoa leaves (*Pometia pinnata*) may have a protective effect against paracetamol induced hepatotoxicity in rats.

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