

Hepatoprotective Effect of Extract of Black Cincau (*Mesona palustris BL*) on Paracetamol-Induced Liver Toxicity in Rats

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Abstract: Black cincau (*Mesona palustris BL*) or Grass black jelly is a traditional Indonesian food that has been used as a folk medicine and is effective against heat-shock, hypertension and diabetes. Therefore, the aim of this research was to determine the hepatoprotector effects of ethanol extracts of black cincau in protecting the liver induced by high doses of paracetamol. The ethanol extracts of black cincau were studied for their hepatoprotective and antioxidant effects on paracetamol (750 mg/kg BW) induced acute liver damage on Wistar rats and treatment during 4 weeks. The degree of protection was measured by using biochemical parameters such as Serum Glutamate Oxalate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (SGPT) and Alkaline Phosphatase (ALP) were estimated. The result showed that ethanol extracts of black cincau IC₅₀ value of 46.92 ppm and 829.7 ppm for total phenols. The ethanol extracts of black cincau at a dose level of 500 and 1000 mg/kg BW produce significant ($p < 0.05$) hepatoprotection by decreasing the activity of SGOT, SGPT and ALP). From this study, it can be concluded that the ethanol extracts of black cincau is not only an effective hepatoprotective agent, but also possesses significant ($p < 0.05$) antioxidant activity.

Keywords: Ethanol extract of Black Cincau (*Mesona palustris BL*), hepatoprotective, IC₅₀, paracetamol, SGPT, SGOT and ALP

INTRODUCTION

Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. Paracetamol (acetaminophen) is widely used as an antipyretic and analgesic and it produces acute liver damage if administered in excess (Ahmed and Khater, 2001). In the absence of a reliable liver protective drug in modern medicine there are a number of medicinal preparations in Ayurveda recommended for the treatment of liver disorders (Chatterjee, 2000). In view of severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity.

Black cincau (*Mesona palustris BL*) or Grass black jelly is a traditional Indonesian food that has been used as a folk medicine and is effective against heat-shock, hypertension and diabetes. Black cincau as herbal drink is also known in Asian countries, in China and Taiwan similar black cincau is called *hsian-tsao* (*Mesona procumbens* Hems). *Hsian-tsao* is also used as a herbal remedy in the folk medicine of China and is effective

against heat-shock, hypertension, diabetes, liver disease and muscle and joint pains. Many compounds, such as sterol compounds, stigmasterol, $\hat{\alpha}$ -sitosterol, tripterene compounds, oleanolic acid and ursolic acid, have been isolated from *Hsian-tsao* (Hung and Yen, 2001). Some studies have indicated that oleanolic acid and ursolic acid showed many biological effects including hypoglycemia, anti-inflammatory and hepatoprotective effects and relief of acute and chronic hepatitis (Yen *et al.*, 2004). In our previous study, we found that phenolic compounds extracted from *hsian-tsao* and black cincau significantly contributed to the antioxidant activity and free radical scavenging effects (Yen and Hung, 2001; Widyaningsih, 2010). Water extracts of black grass jelly were also immunomodulatory and cancer chemo preventive against mice induced benzo(a)piren (Widyaningsih *et al.*, 2012). The objective of this study was to hepatoprotective effect of ethanolic extract of Black cincau (*Mesona palustris BL*) on paracetamol-induced liver toxicity in rats.

MATERIALS AND METHODS

Materials: The dried plant bulbs of black cincau were collected from the farm in Magetan, East of Java, Indonesia. The herb was ground into a fine powder with

a hammermill. The powder was passed through an 80-mesh sieve, collected and sealed by a polyethylene plastic bag and then stored at 0-4°C for further use.

Preparation of extracts of black cincau: Three 10 g samples of black cincau powder were soaked for 24 h at room temperature with 200 mL of either ethanol, respectively, followed by filtration through Whatman #1 filter paper. The filtrates were then evaporated in vacuo to dryness and weighed to determine the yields. Water extract of black cincau was prepared with 20-times volume of 100°C boiling water for 2 h. The extraction was evaporated under reduced pressure (34-etha 36 kPa) using a rotary vacuum-evaporator at 40°C and the contents were freeze-dried. The dried extract was pulverized, sieved (100 meshes), sealed in zip plastic bags for future use.

Determination of Antioxidant Activity by IC 50 method: Antioxidant activity was determined according to the method of Okawa *et al.* (2001) with slight modification. Sample was diluted to distilled water with concentration 20, 40, 60 and 80 ppm, respectively. Each solution samples (4 mL) was added to 1 mL DPPH solution (0.2 mM). The reduction of DPPH was measured at 517 nm against a blank assay for 30 min. The percentage of radical inhibition in medium is calculated as the different of absorbance of the blanko and absorbance of the sample divided by that of DPPH control at the same time multiplied by 100. It can be calculated by this equation:

$$\% \text{ inhibition} = \frac{(A \text{ blanko} - A \text{ sample}) \times 100\%}{A \text{ blanko}}$$

The value of sample concentration and inhibition percentage was graphically plotted for equation of linear regression ($y = ax + b$). The equation was used for calculate the IC₅₀ value (inhibitor concentration 50%) from each sample. The IC₅₀ value is defined as the concentration of antioxidant (sample or control) necessary to decrease the initial DPPH concentration (50%) and is expressed as mg/mL.

Experimental animals: Male Wistar rats (150-200 g) were used for the experiments. They were obtained from the animal house, Food Nutrition Laboratory Brawijaya University Malang Indonesia. They were kept in polyacrylic cages in group of 5 and maintained under standard laboratory conditions (temperature 20±5°C) and humidity 60-65%) with 12 h light and dark cycle. The food and water were available ad libitum. All the animals were acclimatized to laboratory condition for a week before commencement of experiment. All procedures described were reviewed and approved by the University Animals Ethical Committee.

Paracetamol-induced hepatotoxicity in rats: Wistar rats (150-200 g) were divided into five groups each containing six rats taken for assessing hepatoprotective activity of ethanol extract of black cincau. Paracetamol induced hepatotoxicity rat model. The groups were treated as follows:

- Group 1 :** Control group
- Group 2 :** Positive control (Paracetamol treated 750 mg/kg BW)
- Group 3 :** Paracetamol + ethanolic extract of black cincau (500 mg/kg BW)
- Group 4 :** Paracetamol + ethanolic extract of black cincau (1000 mg/kg BW)
- Group 5 :** Paracetamol + water extract of black cincau (1000 mg/kg BW)

The animals in group I served as normal and was given distilled water for 10 days in succession. The group II rats served as control and were administered with distilled water by oral administration of Paracetamol @ 750 mg/kg body weight, 1 h after distilled water administration. The animals in group III and IV served as experimental and were treated orally with ethanolic extract of black cincau 500 mg and 1000 mg/kg body weight, once in a day for 10 days followed by a single oral administration of Paracetamol (750 mg/kg body weight), 1 h after extract administration. After 24 h of Paracetamol administration rats of all groups were sacrificed by decapitation and the blood was collected by cutting the jugular vein. Blood samples collected in heparinized tubes were centrifuged at 3000×g at 4°C for 10 min to obtain serum. On the other hand, the liver of each rat was promptly removed histopathological study.

Estimation of biochemical parameters: Activities of Serum Glutamate Oxaloacetate Transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) and Serum Alkaline Phosphatase (ALP) were estimated by using standard biochemical diagnostic kit obtained from Merck, Germany.

Histopathological studies: Small pieces of liver tissues in each group were collected in 10% neutral buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5-6µm in thickness were cut and stained with Hematoxylin and Eosin (H&E). These sections were examined photo microscopically for necrosis, steatosis and fatty changes of hepatic cells.

Statistical analysis: The experimental results were expressed as the Mean±SEM for six animals in each

Sample	IC ₅₀ (ppm)
Ethanolic extract of black cincau	46.92±1.86
Water extract of black cincau	66.67±2.54

Table 2: Effect of ethanolic and water extract of *Black cincau* on liver function marker in paracetamol intoxicated rat rats

Group	SGPT (U/L)	SGOT (U/L)	Serum ALP (U/L)
• Control group	41.50±6.24a	92.00±6.980a	170.00±6.98a
• Control Positive (paracetamol)	78.25±7.97d	135.50±11.73d	224.25±10.53d
• EEBC 500 mg/kg BW+paracetamol	70.00±2.16c	116.75±16.36c	212.00±10.17c
• EEBC 1000 mg/kg BW+paracetamol	52.25±10.37b	109.00±13.14b	184.75±6.29b
• WEBC 1000 mg/kg BW+paracetamol	50.00±6.98ab	99.75±12.09ab	179.25±4.57ab

• The same notation is not significantly different; EEBC = Ethanolic Extract of Black Cincau; WEBC = Water Extract of Black Cincau

group. The biochemical parameters were analysed statistically using one-way analysis of variance ANOVA, followed by Duncan test. p value of <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

- **Antioxidant Activity by IC₅₀ (DPPH radical scavenging activity):** The extracts of black cincau (*Mesona palustris* BL). Examined were found to possess good DPPH radical scavenging activity. The IC₅₀ values as depicted in Table 1.

IC₅₀ water extract of black cincau was higher than the ethanolic extract of black cincau. This showed the antioxidant activity of the ethanolic extract of black cincau was higher.

Serum analysis: In this study, rats subjected to ethanol only, developed significant (p<0.05) hepatocellular damage as evident from significant increase in serum activity of SGOT, SGPT and ALP, in compared to normal control group, which has been used as reliable markers of hepatotoxicity (Table 2):

- The same notation is not significantly different
- EE BC = Ethanolic Extract of Black Cincau
- WEBC = Water Extract of Black Cincau

In the assessment of liver damage by paracetamol, the determination of enzyme levels such as SGOT and SGPT is largely used. Necrosis or membrane damage releases the enzyme in to circulation; therefore, it can be measured in serum. A high level of SGOT indicates liver damage such as that due to viral hepatitis as well as cardiac infarction and muscle injury. SGPT catalyses the conversion of alanine to pyruvate and is released in a similar manner. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Kumar *et al.*, 2012). Serum ALP on other hand are related to the function of hepatic cell. Increase in serum level of ALP is due to increased synthesis, in presence of increasing biliary presence.

The ethanolic and water extract of black cincau was observed to exhibit hepatoprotective effect as demonstrated by a significant decrease in SGOT, SGPT and ALP concentrations and in rats induced with paracetamol hepatotoxicity. Paracetamol is an antipyretic and analgesic drug, which is widely used to

cure fever, headache and other pains and is readily available without prescription. When taken in at toxic doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and experimental animals (Bessems and Vermeulen, 2001). In this present study, the serum level of hepatic enzymes SGOT, SGPT and ALP were increased and reflected the hepatocellular damage in the paracetamol induced hepatotoxicity animal model. The ethanolic extracts of black cincau with dose of 500 and 1000 mg/kg BW, however, could lower the SGOT, SGPT and ALP in these paracetamol intoxicated animals. This showed ethanolic extract of black cincau and water extracts of black cincau can reduce reactive free radicals that might lessen oxidative damage to the tissues and improve the activities of the hepatic antioxidant enzyme.

In this present study, the water extract of black cincau dose of 1000 mg/kg BW can also reduce SGOT, SGPT and ALP. When compared with the ethanol extract of black cincau dose of 1000 mg/kg BW decrease was not significantly different. This may be because was water extract of black cincau in addition to dissolving the bioactive compounds are also dissolving hydrocolloid compound or gum (gel-forming component), while ethanol precipitate hydrocolloid precisely (Haryadi and Bangun, 2002; Widyaningsing, 2011).

Histopathological studies: Histopathological studies of rat liver tissue from Group 1 animals show normal hepatic cells with central Vein (V) and sinusoidal dilation (Fig. 1). In paracetamol treated group (Group 2), severe hepatotoxicity was observed by severe Necrosis (N) with disappearance of nuclei (Fig. 2). Mild degree of necrosis (N) with normal Cells (C) was observed in Group 3 and 4. Mild degree of necrosis (N)

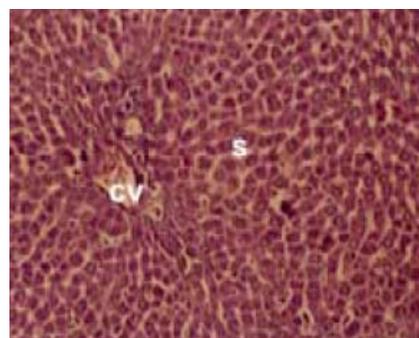


Fig. 1: Group 1. animals show normal hepatic cells



Fig. 2: Group 2. Paracetamol treated hepatotoxicity was observed by severe necrosis

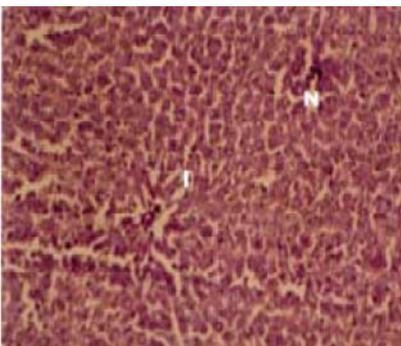


Fig. 3: Group 3. Treated with EEBC at 500 mg/kg BW

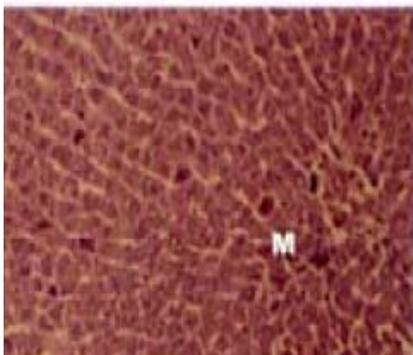


Fig. 4: Group 4. Treated with EEBC at 1000 mg/kg BW

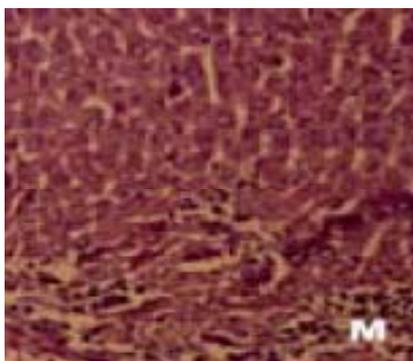


Fig. 5: Group 5. Treated with WEBC at 1000 mg/kg BW

with areas of inflammation adjacent to necrosed area was observed, treated with EEBC (500 mg/kg/BW) respectively (Fig. 3) and while normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M) was observed with Group 4 and 5, normal hepatocytes with mild inflammation of portal triad (M) at the dose of 1000 mg/kg/BW of EEBC and 1000 mg/kg/BW of WEBC respectively (Fig. 4 and 5). Photomicrographs (original magnification 45 \times) of histopathological studies of livers of various groups stained with haematoxylin and eosin.

CONCLUSION

In conclusion, the present study has demonstrated that the Ethanolic Extract and Water Extract of Black Cincau (*Mesona palustris* BL) has hepatoprotective activity against paracetamol induced hepatotoxicity in rats and it may be due to their antioxidant property. Our study also showed that the water extract of black cincau dose of 1000 mg/kg BW and ethanol extract of black cincau dose of 1000 mg/kg BW decrease was not significantly different in reduce SGOT, SGPT and ALP

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