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Hepatoprotective and antioxidant activity of the ethanol extract of *Cassia fistula* L. Barks

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Abstract

Cassia fistula L., usually used as an alternative medicine in Indonesia, is one of medicinal plants indicated for various efficacies. Pharmacological studies on the leaves of this plant have been carried out and they indicated that the leaves have several pharmacological activities, one of which is hepatoprotective activity against hepatoxicity induced by isoniazid and rifampicin or paracetamol. This study was carried out to investigate hepatoprotective activity of the ethanol extract of barks of this plant against hepatoxicity induced by paracetamol on rats and antioxidant activity of the extract by the DPPH radical scavenging method. In addition, acute toxicity of the extract was also examined. The results indicated that the ethanol extract at doses of 150 and 300 mg/kg of body weight gave hepatoprotective effects with SGPT levels of 60.83 and 56.95 IU/L, respectively, and SGOT levels of 134.30 and 110.17 IU/L, respectively, significantly different from the control. The extract had radical scavenging activity with IC_{50} of 10.613 µg/ml lower than that of ascorbic acid (4.716 µg/ml). The acute toxicity test revealed that the ethanol extract had LD_{50} values of 14.52 g/kg in male mice and 16.14g/kg in female mice, indicating that the ethanol extract of *C. fistula* barks belonged to practically non-toxic category according to the criteria of toxicity. These results provide evidence of the potential of *C. fistula* barks as hepatoprotector.

Keywords: *Cassia fistula* L., hepatoprotector, acute toxicity, ethanol extract.

INTRODUCTION

The liver plays a very important role in converting and clearing chemicals entering the blood circulation in the body, but it is also susceptible to the toxicity from these agents. Certain agents, when taken in overdoses, may injure the organ and lead to liver diseases. These diseases are associated with cellular necrosis, increase in tissue lipid peroxidation, and depletion in the tissue GSH levels, which are indicated by the elevation of serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, and alkaline phosphatase (Mascolo et al., 1998). Hepatoprotective agents are available and are used clinically, but no agent is really effective to protect liver from the injury caused by toxic chemicals. So, use of medicinal plants as alternative drugs for the hepatoprotective purpose has long been applied and search for effective hepatoprotective agents from natural sources are continually conducted.

Cassia fistula L. belonging to the family of Leguminosae is usually used in traditional medicine for various indications. Some investigations revealed that all parts of the plant have various pharmacological activities. It has been reported that this plant has wound healing (Bhakta *et al*., 1998), antifertility (Yadav and Jain, 1999), antitumor (Gupta *et al*., 2000), antioxidant (Siddhuraju *et al*., 2002; Ramma *et al*., 2002), and hepatoprotective properties (Bhakta *et al*., 1999; Jehangir *et al*., 2010). The n-heptane extract of Cassia fistula leaves was investigated in rats by inducing hepatotoxicity with carbon tetrachloride:liquid paraffin (1:1). The extract has been shown to possess significant protective effect by lowering the serum levels of transminases (SGOT and SGPT), bilirubin and alkaline phosphatase (ALP). The extract of C. fistula at a dose of 400 mg/kg showed significant hepatoprotective activity which was comparable to that of a standard hepatoprotective agent. The n-heptane extract of Cassia fistula leaves was investigated in rats by inducing hepatotoxicity with carbon tetrachloride:liquid paraffin (1:1).

Concerning the hepatoprotective activity, the n-heptane extract of the *C. fistula* leaves at a dose of 400 mg/kg shows significant protection against hepatotoxicity induced by carbon tetrachloride or paracetamol in rats (Bhakta *et al.* 1999; Bhakta *et al*. 2001). Further study carried out by Jehangir et al. (2010) revealed that the ethanol extract of the *C. fistula* leaves also prevents hepatotoxicity induced by isoniazide and rifampicine in rats. The constituents of this plant especially flavonoids and anthraquinones have strong antioxidant activity which give protection against drug-induced hepatotoxicity. Furthermore, hepatoprotective effects of the ethanol extract of the *C. fistula* barks at the doses of 200 and 400 mg/kg of body weight are evidently shown in animals treated with CCl4 as an hepatotoxicity inducing agent (Patwardhan, et al., 2009).

In this study, we examined hepatoprotective activity against paracetamol overdose-induced hepatotoxicity in rats, free radical scavenging activity, and acute toxicity in mice of the extract of *C. fistula* barks.

MATERIALS AND METHODS

Plant materials and preparation of the extract

The barks of *Cassia fistula* used in this experiment were collected in Manoko plantation, West Java, Indonesia. The barks were air-dried away from direct sunlight. The dried barks were crushed into a coarse powder. The powdered barks of *C. fistula* (1.4 kg) were extracted with ethanol 70 % (three times, each 24 hr) by a maceration method. The solvent of the extract was then evaporated under reduced pressure to yield a concentrated extract (360 g).

Animals

Rats used in the hepatoprotective examination were male rats of Wistar strains weighing 150-200 grams each. The toxicity test used male and female mice of Swiss Webster strains weighing 20-25 grams each. The animals were kept for one week in an air-conditioned room at a temperature of 22°C and received nutritionally standard diet and tap water.

Phytochemical screening

The extract obtained was subjected to a screening procedure to identify secondary metabolites contained in the extract by a means of Farnsworth method (1966). The metabolites screened were alkaloid, flavonoid, tannin, saponin, quinone, monoterpene, sesquiterpen, triterpenoid, and steroid.

Experimental procedure

Rats were devided randomly into control, paracetamol, and experimental groups. Each group consisted of 5 rats. In seven consecutive days, rats in the control and paracetamol groups received a 1 % Arabic gum suspension orally and those in the experimental group were given the extract orally at doses of 150 and 300 mg/kg of body weight. In days eight and nine, all animals in the paracetamol and experimental groups were administered paracetamol orally at a dose of 1500 mg/kg of body weight, except those in the control group were fed on standard diet and tap water only.

Assay of serum GOT and GPT activities

In days 10, all animals were anaesthetized under ether vapours and the bood samples were collected through a venous vessel of tail. Blood samples were allowed to cloth and serum was separated after centrifugation at 3000 rev/min for 10 min. The serum samples were used for the bioassay of marker enzymes, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvic transaminase (SGPT). The serum GOT and GPT activities were measured according to the enzyme kinetic method of International Federation of Clinical Chemistry (IFCC) using a UV spectrophotometer at maximum absorption of 340 nm.

Evaluation of antioxidant activity by DPPH radical scavenging method

Free radical scavenging activity of the extract of *C. fistula* barks was measured by a modified DPPH method as described by Molyneux (2004). Samples of the extract were prepared in a series of dilution in ethanol (50, 100, 150, 200, 250, and 300 µg/ml).Then, 1.5 ml of 0.1 mM DPPH solution was added to 1.5 ml of each extract sample. The mixture was shaken vigorously and incubated at room temperature for 30 min. Absorbance of the mixture was then measured at 516 nm by using spectrophotometer (UV-VIS Shimadzu). The control group contained only 2 mL of 0.1 mM DPPH and 2 mL of 70% ethanol without extract, and ascorbic acid was used as a reference standard compound. Each sample was measured in triplicate and expressed in mean average. The radical scavenging activity or percent inhibition was calculated by the following equation:

$$\text{Radical scavenging effect or \% inhibition} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

$A_{Control}$ = Absorbance of the control
A_{Sample} = Absorbance of the sample

The IC_{50} value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was obtained from the % inhibition versus concentration plot. Lower absorbance of the reaction mixture indicates higher free radical activity.

Toxicity test

Male and female mice were devided to five groups according to the doses of the extract (1000, 2000, 3000, 4000, and 5000 mg/kg of body weight). Each group consisted of 10 mice. All mice in each group were given the extract according to the related doses. Mortality of mice was observed daily for a period of seven days. In addition, behavioral pattern, changes in physical appearance, and other physiological activities were observed during the period of treatment.

Statistical analysis

Data were analyzed by one way analysis of variance and significance of difference was calculated according to the Student't t-test. P values < 0.05 were considered significant.

RESULTS

Secondary metabolites

Phytochemical screening on the bark extract of *C. fistula* was conducted to identify secondary metabolites contained in the extract. The secondary metabolites identified were tannin, flavonoid, polyphenol, quinone, triterpenoid, and saponin (Table 1).

Effects of the extract of *C. fistula* barks on the serum GOT and GPT levels

Table 2 shows that administration of paracetamol induced hepatotoxicity that was indicated by a marked incease in serum GOT and GPT levels in rats. The hepatoxicity of paracetamol was prevented by the ethanol extract of C. fistula barks. At doses of 150 and 300 mg/kg, the extract decreased the GOT and GPT levels in serum significantly different from those induced by paracetamol.

Table 1. Secondary metabolites contained in the ethanol extract of *C. fistula* barks

Secondary metabolites	Identification
Alkaloid	-
Tannin	+
Polyphenole	+
Flavonoid	+
Quinone	+
Steroid	-
Terpenoid	+
Saponin	+

+ : Identified + : Not identified

Table 2. Effect of the ethanol extract of *C. fistula* barks on serum GOT and GPT levels in paracetamol-induced hepatotoxicity.

Treatment	Dose (mg/kg, bw)	SGOT (IU/L)	SGPT (IU/L)
Control	-	106.65 ± 14.52	49.51 ± 5.92
Paracetamol	1500	210.89 ± 32.56^{+}	141.54 ± 15.69^{+}
ECFB + Paracetamol	150 ± 1500	134.30 ± 29.50*****	60.83 ±12.42*****
	300 ± 1500	110.17 ± 20.20******	56.95 ± 8.26******

ECFB: Extract of *C. fistula* barks
Control group: Rats received a 1 % Arabic gum suspension.
Paracetamol group: Rats received a 1 % Arabic gum suspension and paracetamol (1500 mg/kg)
ECFB group : Rats received the extract of *C. fistula* barks and paracetamol (1500 mg/kg)
$^{+}$ Significantly different from the normal control group (Group A), $p<0.05$
* Significantly different from the paracetamol group (Group B), $p<0.05$
** Significantly different from the paracetamol group (Group C), $p<0.01$

Table 3. Absorbance of the ethanol extract of *C. fistula* barks and the standard ascorbic acid at 517 nm by uv visible spectrophotometer

Concentration (µg/ml)	Absorbance		% Inhibition	
	Ascorbic acid	ECFB	Ascorbic acid	ECFB
2	0.625	0.738	31.694	19.344
4	0.548	0.713	40.109	22.077
6	0.301	0.603	67.104	34.098
8	0.259	0.539	71.694	41.093
10	0.166	0.503	81.858	45.027
12	0.79	0.395	91.366	56.831

ECFB: Extract of *C. fistula* barks

DPPH radical scavenging activity

Absorbance and % inhibition of the ethanol extract of C. fistula barks and the standard ascorbic acid are shown in Table 3, and their IC50 values were obtained from the % inhibition versus concentration plot (Figure 1). The ethanol extract of C. fistula barks inhibited DPPH radical reaching up to 56.831% at the concentration of 12 µg/ml , and its IC50 was 10.613 µg/ml higher compared with that of ascorbic acid (4.716 µg/ml).

Acute toxicity of the extract of *C. fistula* barks

The results of the acute toxicity test are shown in Table 4. Single oral doses of crude ethanol extract of C. fistula barks did not cause death of any animal (0 % mortality) during the experimental period up to the highest dose of 5000 mg/kg of body weight. Moreover, no other signs or symptoms of toxicity were found, except for increasing motoric activity, and no other adverse effect was noted.

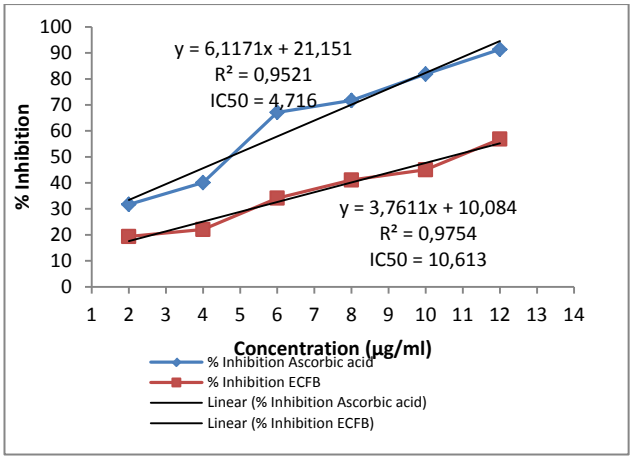

Figure 1

Table 4: Acute toxicity of the extract of *C. fistula* barks in all groups of mice (For a period of 7 days)

Group	Dose (mg/kg, bw)	Mortality of male animals[a]	Mortality of female animals[a]
Control	-	0	0
ECFB	1000	0	0
	2000	0	0
	3000	0	0
	4000	0	0
	5000	0	0

ECFB: Extract of *C. fistula* barks. [a]Ten mice were used for each group.

Discussion

In an experimental study to examine hepatoprotective activity of drugs, paracetamol known as an effective analgesic and antipyretic agent is usually used to induce hepatotoxicity in test animals. Hepatotoxicity caused by paracetamol is due to its metabolite produced during metabolism by drug metabolizing enzymes that covalently binds to protein (Hinson et al., 2010). Paracetamol undergoes cytochrome P450-mediated N-hydroxylation to form a highly reactive metabolite, N-acetyl-pbenzoquinone imine. At non-toxic doses, this metabolite reacts with hepatic glutathione and detoxified forming a harmless paracetamol-glutathione conjugate. However, at toxic doses, the metabolite depleted glutathione and subsequently covalently bound to protein, in which the amount of covalent binding correlates with the relative hepatotoxicity (Hinson et al., 2010).

Our experiment revealed that the levels of GOT and GPT in serum of rats administered paracetamol were significantly elevated. This indicated that there was a hepatic demage caused by paracetamol since serum GOT and GPT are known as biochemical markers of the hepatic demage. Treatment of rats with the extract of C. fistula barks at doses of 150 and 300 mg/kg significantly decreased the elevated serum GOT and GPT levels resulting from the administration of paracetamol to the near normal levels in a dose-dependent manner. This result suggests that the ethanol extract of C. fistula barks may have protective activity against toxic effects of hepatic demage-inducing agents. This evidence is in agreement with the previous studies (Bhakta et al., 2001; Jehangir et al., 2010; Patwardhan, et al., 2009).

The DPPH assay was used in this study to measure antioxidant activity of the samples. This assay is based on the ability of the samples or compounds to scavenge free radicals in order to inhibit chain initiation and break chain propagation by donating hydrogen atoms or electrons. The ability of the samples to scavenge DPPH free radicals is visually noticeable as the colour changes from purple to yellow due to hydrogen donating ability, with the consequence of decreasing absorbance (Molyneux, 2004). The more rapid the absorbance decreases, the more potent the primary antioxidant activity. In the present study, the ethanol extract of C. fistula barks at increasing concentrations showed weakening colour intensity of purple, indicating the presence of free radical scavengers which reduced the initial DPPH concentration. In the phytochemical screening, flavonoids and other phenolic compounds were identified in the extract of C. fistula barks, and the previous investigation reported that the main constituents in C. fistula are potent phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives (Bachorun et al., 2005). These compounds might be involved and responsible for the hepatoprotective activity of the extract of C. fistula barks in the paracetamol-induced hepatotoxicity.

Oral acute toxicity testing of substances in mice is commonly done not only to identify the doses that cause mortality and signs or symptoms of toxicity in the tested animals, but also to determine the range of doses that are regarded safe in clinical uses. In this study, oral acute toxicity of the bark extract of the C. fistula was evaluated in male and female mice, and no mortality and toxic symptoms were observed at all doses used (1000, 2000, 3000, 4000, and 5000 mg/kg) in any mice. This indicated that the bark extract of the C. fistula did not cause acute toxicity and its LD50 value might be greater than 5000 mg/kg. It is in line with the previous results of acute toxicity test carried out by Ilavarasan et al. (2005) that the bark extract of C. fistula did not cause any mortality up to 2000 mg/kg, and it is regarded being safe or practically non-toxic according to the study by Kennedy et al. (1986).

Conclusion

These results suggest that C. fistula barks might be a potential hepatoprotector that can be applied as an alternative medicine for management of a liver disease.

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