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Research Article

ANTIHYPERLIPIDEMIC AND HEPATOPROTECTIVE STUDIES ON LEAVES OF *MACARANGA TANARIUS*

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ABSTRACT

Objective: This study investigated the antihyperlipidemic and hepatoprotective effects of the hexane-ethanol fraction of methanol extract of *Macaranga tanarius* (HEM) in rats.

Methods: The hexane-ethanol fraction was screened for toxicity by oral acute toxicity study. The antihyperlipidemic effect of the hexane-ethanol fraction and the unsolved of the hexane-ethanol fraction is measured against Wistar rats induced by glucose-fructose diets for 42 days through measuring serum cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and fasting blood glucose. The hepatoprotective effect of the hexane-ethanol fraction is determined against Wistar rats with liver damage induced by carbon tetrachloride through measuring serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), albumin, lactate dehydrogenase (LDH), and total bilirubin.

Results: There is no toxic effect that was observed on acute toxicity study. The TG, LDL-c, and fasting blood glucose levels were significantly ($p<0.05$) reduced after both of treatment the hexane-ethanol fraction and the unsolved HEM. Administration of the hexane-ethanol fraction 68.6 mg/kgBW significantly ($p<0.05$) prevented elevation of SGPT, SGOT, LDH, ALP, and decreasing of albumin level.

Conclusion: The study showed antihyperlipidemic and hepatoprotective activities of the HEM in animal models.

Keywords: *Macaranga tanarius*, Antihyperlipidemic, Hepatoprotective.

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INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes. NAFDL is associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia [1]. Global prevalence of NAFDL is 25.24%, which was highest in the Middle East and South America and the lowest prevalence in Africa. Metabolic comorbidities associated with NAFDL included obesity, type 2 diabetes, hyperlipidemia, hypertension, and metabolic syndrome. As the global epidemic continues to fuel development of metabolic disorders, NAFDL will create a massive clinical and economic burden [2].

Carbon tetrachloride (CCL_4) is one of the most commonly hepatotoxic agents that have been reported to show steatosis, centrilobular necrosis, inflammation, fibrosis, and liver cancer in the experimental animals [3,4]. Administration of high carbohydrate diet in animal models is frequently induced obesity, insulin resistance, impaired glucose tolerance, hyperinsulinemia, hypertension, and hyperlipidemia [5-10].

Macaranga tanarius is a pioneer species in disturbed rainforest areas. It has great potential in scavenging free radical [11,12] and can be a vital source of antioxidant phytochemicals [13]. Anti 2,2-diphenyl-picrylhydrazyl radical-scavenging activity of the isolated compounds of *M. tanarius* also been reported previously [14-16]. These activities are effective in treating CCL_4 induced toxicity.

The isolated ellagitannin and chebulagic acid of *M. tanarius* was found to inhibit α-glucosidase and intestinal maltase that may benefit diabetes treatment [17]. Although the earlier study has demonstrated that the hexane-ethanol fraction of methanol extract of *M. tanarius* (HEM) for 5 days has not antidiabetic and antihyperlipidemic in rats feed with high glucose-fructose (GF) diet [10], a prolonged administration of HEM remains to be established.

In this present study, the HEM was interesting to observe for their hepatoprotective and antihyperlipidemic anti-inflammatory activities. Carbon tetrachloride was chosen as model study for hepatotoxicity and administration of high GF to induce hyperlipidemia.

METHODS

Plant material and chemicals

The fresh leaves of *M. tanarius* were collected from Sleman, Yogyakarta, Indonesia and were identified and authenticated using descriptive literature. A voucher specimen was deposited in the Laboratory of Pharmaceutical Biology, Pharmacy Faculty, Sanata Dharma University, Yogyakarta, Indonesia. Sodium carboxymethyl cellulose (CMC) was supplied by Brataco Chemika, Indonesia. Glucose, fructose, methanol, and carbon tetrachloride as hepatotoxin were from E. Merck (Darmstadt, Germany). Olive oil was supplied by Bertolli, Italy. Diagnostic kit for the estimation of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), albumin, lactate dehydrogenase (LDH), total bilirubin, glucose, cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and triglyceride (TG) kits were purchased were from Roche Diagnostics GmbH, Mannheim, Germany. All other chemicals were of analytical grade and were purchased from E. Merck, Darmstadt, Germany.

Preparation of plant extract

Dried leaves of *M. tanarius* were extracted with 50% aqueous methanol for 24 hrs at room temperature. The crude extract was extracted with hexane-ethanol 50:50 for 24 hrs at room temperature. The resulting suspension was filtered and was evaporated by vacuum rotary evaporator at 50°C to yield a solid residue of soluble HEM (yield 21%) and unsoluble of HEM (UHEM) (yield 79%).

Test animals and housing

Adult male rats of Wistar strain weighing 150-250 g were selected for this experiment. The animals were obtained from the Imono Laboratory of Pharmacy Faculty of Sanata Dharma University, Indonesia. The animals were maintained under standard laboratory condition. They were housed in standard cages (five animals per cage) at temperature 22±2°C and 12:12 hrs light dark cycle. The animals were provided with pelleted diet as normal diet or GF-enriched diet and water *ad libitum*. The experimental protocol and procedures used in this study were approved with approval number KE/FK/551/EC/2016 by The Medical and Health Research Ethics Committee Faculty of Medicine Gadjah Mada University.

Acute oral toxicity study

Acute toxicity study was performed according to the acute toxic conventional method. Adult female rats of Wistar strain weighing 150-250 g were used for acute toxicity study. The animals were kept in fasting condition for overnight providing only water, and then, the HEM was administered orally at the doses of 34.2, 342, and 3420 mg/kg BW. The animals were closely observed for any signs of toxicity during the first 3 hrs, and the number of dead animals was recorded at 24 hrs.

Antihyperlipidemic study

Healthy rats were weighed and randomly divided into 4 groups of 5 animals in each. Group 1 as normal healthy control rats was fed normal diet for 52 days. Group 2-4 were fed GF diets for 42 days [9,10]. After 42 days, Group 2 were received CMC as negative control, whereas Group 3 were administered HEM 34.3 mg/kgBW and Group 4 were given UHEM 34.3 mg/kgBW. All treatments were fed GF diet and continued for 10 days following oral administration. Blood for biochemical analysis from all groups was obtained by sinus orbitalis after 24 hrs administration. The blood serum was used to measured serum cholesterol, TG, HDL-c, LDL-c, and fasting blood glucose. All tests were estimated in a Cobas C501 analyzer using commercial kits (Roche Diagnostic GmbH, Germany) following standard procedures.

Hepatoprotective study

Healthy rats were weighed and randomly divided into 5 groups of 5 animals in each. Group 1 were treated with carbon tetrachloride (2 ml/kg, i.p.), in a 1:1 solution with olive oil [18,19]. Group 2 received olive oil (2 ml/kg, i.p) as negative control. Group 3-5 received HEM at doses 34.3, 68.6, and 134.1 mg/kg BW orally once and after 6 hrs, respectively, received treated carbon tetrachloride (2 ml/kg, i.p.). Blood sample from all groups was obtained by retro-orbital sampling after 24 hrs application [19-22]. The blood serum was used to measured SGPT, SGOT, ALP, albumin, LDH, and total bilirubin. All tests were estimated in a Cobas C501 analyzer using commercial kits (Roche Diagnostic GmbH, Germany) following standard procedures.

Statistical analyses

The results are expressed as mean±standard deviation. Data were analyzed using Kolmogorov-Smirnov test, followed by analysis of variance with 95% confidence interval. Scheffe test was used for significantly different results using the IBM SPSS 22. Differences were regarded as statistically significant with $p<0.05$.

RESULTS

Acute toxicity study in rats indicated that oral administration of HEM produced no toxic effect. No unusual changes in behavior or in locomotor activity and no signs of intoxication were observed.

The administration of GF diet exhibited a significant ($p<0.05$) rise in TG, LDL-c, and fasting blood glucose. This was significantly ($p<0.05$) reduced after both of treatment the HEM and the UHEM as shown in Table 1.

The effect of HEM on SGPT, SGOT, ALP, albumin, LDH, total bilirubin in CCl_4 intoxicated rats was summarized in Table 2. The biomarker enzyme levels are mainly increased in the hepatic damage by the CCl_4. SGPT, SGOT, LDH, ALP, and total bilirubin increased significantly ($p<0.05$) and albumin decreased significantly ($p<0.05$) with CCl_4 treated. Administration of HEM 68.6 mg/kgBW significantly ($p<0.05$) prevented elevation of SGPT, SGOT, LDH, ALP, and decreasing of albumin level, thus signifying its hepatoprotective effect.

DISCUSSION

For the acute toxicity study, the results suggest that the HEM is not toxic after an acute expose to the dose of 3420 mg/kg.

After 42 days of GF diet, the elevation of TG, LDL-c, and fasting blood glucose levels were recorded in this study indicated that GF diet caused hyperlipidemia and hyperglycemia in rats. Our previous study demonstrated that the HEM for 5 days has not antidiabetic and antihyperlipidemic in rats feed with high GF diet [10]. However, in this study, concurrent administration 10 days both of HEM markedly decreased the release of TG, LDL-c, and fasting blood glucose levels. This indicates that the prolonged administration of fraction possesses an antihyperlipidemic and antihyperglycemic effect against GF diet.

Table 1: Effect of HEM on lipid parameters and fasting blood glucose in rats feeds with GF diets

Treatment	Cholesterol (mmol/l)	Triglyceride (mmol/l)	HDL-c (mmol/l)	LDL-c (mmol/l)	Fasting blood glucose (mmol/l)
Normal diet	1.70±0.14	0.87±0.10[b]	0.81±0.02[b]	0.23±0.01[b]	3.21±0.16[b]
GF	1.88±0.15	2.98±0.25[a]	0.65±0.05[a]	0.33±0.04[a]	4.97±0.29[a]
GF+HEM 34.3 mg/kgBW	1.81±0.19	1.25±0.49[b]	0.80±0.08[b]	0.25±0.03[b]	3.81±0.61[b]
GF+UHEM 34.3 mg/kgBW	1.92±0.13	2.12±0.14[a,b]	0.76±0.03[b]	0.25±0.01[b]	3.83±0.26[a,b]

Values are expressed as mean±SD of five animals in each group; [a]p<0.05 vs normal diet; [b]p<0.05 vs GF. GF: Glucose-fructose, HDL-c: High-density lipoprotein cholesterol, LDL-c: Low-density lipoprotein cholesterol, HEM: Hexane-ethanol fraction of methanol extract of *Macaranga tanarius,* UHEM: Unsoluble of hexane-ethanol fraction of methanol extract of *Macaranga tanarius*, SD: Standard deviation

Table 2: Effect of HEM on different biochemical parameters in carbon tetrachloride-induced hepatotoxicity

Treatment	SGPT (U/l)	SGOT (U/l)	Albumin (mg/dl)	LDH (U/l)	ALP (U/l)	Total Bilirubin (mg/dl)
Normal	55.3±4.4[b]	144.0±6.5[b]	3.47±0.15[b]	1021.2±277.0[b]	177.8±29.0[b]	0.16±0.01[b]
Standard CCl_4	156.1±16.9[a]	674.3±12.0[a]	2.85±0,12[a]	1848.8±106.85[a]	244.4±30.6[a]	0.22±0.05[a]
HEM 34.3 mg/kgBW	72.2±10.0[b]	510.4±176.7[a]	3.39±0.10[b]	968.4±119.0[b]	150.8±18.2[b]	0.22±0.06[a]
HEM 68.6 mg/kgBW	57.3±10.7[b]	170.0±39.1[b]	3.27±0.09[a,b]	875.0±43.3[b]	183.0±12.1[b]	0.21±0.02[a]
HEM 137.1 mg/kgBW	157.4±20.3[a]	639.4±35.6[a]	3.09±0,11[a,b]	835.8±54.0[b]	199.6±21.5[b]	0.24±0.02[a]

Values are expressed as mean±SD of five animals in each group; [a]p<0.05 vs normal diet; [b]p<0.05 vs standard. SGPT: Serum glutamate pyruvate transaminase, SGOT: Serum glutamate oxaloacetate transaminase, LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, HEM: Hexane-ethanol fraction of methanol extract of *Macaranga tanarius*, SD: Standard deviation

Puteri and Kawabat, 2010, have investigated the α-glucosidase inhibitor activity of isolated of *M. tanarius* [17]. It is reasonable to suppose the antihyperlipidemic and antihyperglycemic effect of HEM. Similarly, we have shown that the UHEM showed a reduction in TG, LDL-c, and fasting blood glucose levels. In contrast, it can be postulated that the UHEM contained pharmacologically active compound(s) that interfere with the elevation TG, LDL-c, and fasting blood glucose levels.

In this present study, we examined the hepatoprotective effect of HEM against liver damage induced by carbon tetrachloride in rats. Carbon tetrachloride as common hepatotoxin used in the experimental study, induced a significant elevation of blood hydroperoxide and malondialdehyde (lipid peroxidation products in liver, moreover, this toxicant caused a significant decrease in glutathione content in hepatic tissue [23]. Carbon tetrachloride exhibited drastic alterations on liver such as extensive fatty change, fatty degeneration [24], and infiltration by inflammatory cells [25]. Administration of carbon tetrachloride causes severe liver injuries in the present rats. Our results provided evidence for the hepatotoxicity effect of carbon tetrachloride (2 ml/kg) on the liver functions (Table 2). The SGPT, SGOT, ALP, LDH, albumin, and total bilirubin level significantly increased when compared with the normal rats [4,26,27]. This injury is indicated of liver cell damage as steatosis and leakage of enzymes from cell [18,22,28].

The result of this study shows that the HEM has the potential to protect the hepatotoxicity. Since the HEM contains antioxidant compound [11-16], it might effective in treating CCl_4-induced hepatotoxicity. It results the HEM at dose of 68.6 mg/kgBW gave the most potent hepatoprotective effect which decreased the elevation of SGPT, SGOT, LDH, ALP, and increasing of albumin level. The possible mechanism of action may be associated with scavenging of free radicals for CCl_4 toxicity.

CONCLUSION

In conclusion, the study showed antihyperlipidemic and hepatoprotective activities of HEM in animal modes.

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