ANTHYPERLIPIDEMIC AND HEPATOPROTECTIVE STUDIES ON LEAVES OF MACARANGA TANARIUS

PHEBE HENDRA*, OKTARIANI AURELIA JAMIL, DIAN AYU MAHARANI, MARIA ANGELIKA SUHADI, CYNDI YULANDA PUTRI, FENTY, JEFFRY JULIANUS

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Sanata Dharma University, Jogjakarta, Indonesia.

Received: 04 September 2016, Revised and Accepted: 17 September 2016

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, 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.

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. NAFLD is associated with metabolic risk factors such as obesity, diabetes mellitus, and dyslipidemia [1]. Global prevalence of NAFLD is 25.24%, which was highest in the Middle East and South America and the lowest prevalence in Africa. Metabolic comorbidities associated with NAFLD included obesity, type 2 diabetes, hyperlipidemia, hypertension, and metabolic syndrome. As the global epidemic continues to fuel development of metabolic disorders, NAFLD will create a massive clinical and economic burden [2].

Carbon tetrachloride (CCL₄) 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 animals 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 CCL₄-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 anti hyperlipidemic 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 Bertoli, 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%).
**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, 34.2, 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.

**Hepatoprotective 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 HF 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/kg BW and Group 4 were given UHEM 34.3 mg/kg BW. All treatments were fed HF diet and continued for 10 days following oral administration. Blood for biochemical analysis from all groups was obtained by sinus orbitals after 24 hrs administration. The blood serum was used to measured serum cholesterol, TG, HDL-c, and fasting blood glucose. 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, HDLc, total bilirubin in CCl intoxicated rats was summarized in Table 2. The biomarkers enzyme levels are mainly increased in the hepatic damage by the CCl, SGPT, SGOT, LDH, ALP, and total bilirubin increased significantly (p<0.05) and albumin decreased significantly (p<0.05) with CCl treated. Administration of HEM 68.6 mg/kg BW 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 anti-diabetic and hypolipidemic effect 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**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol (mmol/l)</th>
<th>Triglyceride (mmol/l)</th>
<th>HDL-c (mmol/l)</th>
<th>LDL-c (mmol/l)</th>
<th>Fasting blood glucose (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>1.70±0.14</td>
<td>0.07±0.10</td>
<td>0.81±0.02</td>
<td>0.23±0.01</td>
<td>3.21±0.16</td>
</tr>
<tr>
<td>GF</td>
<td>1.80±0.15</td>
<td>2.98±0.25</td>
<td>0.65±0.05</td>
<td>0.35±0.04</td>
<td>4.97±0.29</td>
</tr>
<tr>
<td>GF + HEM 34.3 mg/kg BW</td>
<td>1.18±0.19</td>
<td>1.25±0.49</td>
<td>0.80±0.08</td>
<td>0.25±0.03</td>
<td>3.81±0.61</td>
</tr>
<tr>
<td>GF + UHEM 34.3 mg/kg BW</td>
<td>1.92±0.13</td>
<td>2.12±0.14</td>
<td>0.76±0.03</td>
<td>0.25±0.01</td>
<td>3.83±0.26</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD of five animals in each group; p<0.05 vs normal diet; 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 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**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SGPT (U/l)</th>
<th>SGOT (U/l)</th>
<th>Albumin (mg/dl)</th>
<th>LDH (U/l)</th>
<th>ALP (U/l)</th>
<th>Total Bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>55.3±4.45</td>
<td>144.0±26.5</td>
<td>3.47±0.15</td>
<td>1021.2±277.0</td>
<td>177.8±29.0</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>Standard CCl1</td>
<td>156.1±16.9</td>
<td>674.3±12.0</td>
<td>2.95±0.12</td>
<td>1948.0±106.8</td>
<td>24.4±30.6</td>
<td>0.22±0.15</td>
</tr>
<tr>
<td>HEM 34.3 mg/kg BW</td>
<td>72.4±10.0</td>
<td>510.4±17.6</td>
<td>3.39±0.19</td>
<td>968.4±119.0</td>
<td>150.0±18.2</td>
<td>0.22±0.06</td>
</tr>
<tr>
<td>HEM 68.6 mg/kg BW</td>
<td>57.3±10.7</td>
<td>170.0±39.1</td>
<td>3.27±0.09</td>
<td>875.0±43.3</td>
<td>18.0±12.1</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>HEM 137.1 mg/kg BW</td>
<td>157.4±20.3</td>
<td>639.4±35.6</td>
<td>3.09±0.11</td>
<td>835.8±54.0</td>
<td>199.6±21.5</td>
<td>0.24±0.02</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD of five animals in each group; p<0.05 vs normal diet; 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 Kawabata, 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 of 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 hepatoprotective 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 CCl₄-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 CCl₄ toxicity.

CONCLUSION

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

ACKNOWLEDGMENT

This research project was supported by a grant from Ministry of Research, Technology and Higher Education of the Republic of Indonesia with contract number 010/HB-LIT/III.2016.

REFERENCES

3. Riordan JD, Nadeau JH. Modeling progressive non-alcoholic fatty liver disease in the laboratory mouse. Mammm Genome 2014;25(9-10):473-86.