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Hepatoprotective effect of chewable tablet of *Centella asiatica* (L.) Urb extractin Wistar rats induced by high fat diets

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ABSTRACT

Gotukola or *Centella asiatica* (L.) Urban contains high flavonoids which well known as fatty liver protector. This study aimed to evaluate the hepatoprotective effect of chewable tablet of *C. asiatica* (L.) Urb extract (CTCE) in Wistar rats induced by high fat diets. Twenty-one Wistar male rats aged 8-12 weeks with body weight ranging from 100-150 g were used in this study. Rats were randomly divided into seven groups i.e. Group 1 as normal control, rats were given standard food, Group 2 as high fat diets control, rats were induced high fat diets (HFD), Group 3 as positive control, rats were induced HFD and given simvastatin, Group 4 as placebo control, rats were induced HFD and given placebo, Group 5-7 as treatment group, rats were induced HFD and given CTCE at doses of 100, 200 and 300 mg/kg BW, respectively. The HFD induction was conducted for five weeks and the CTCE was given for one week in the last week of the induction. At the end of the intervention, blood triglyceride levels and SGPT as well SGOT activities were examined. Analysis of variance (ANOVA) with confidence interval of 95% ($p<0.05$) was applied. The results showed that the HFD induction increased the serum triglyceride levels and SGPT activity. The serum triglyceride levels and SGPT activity of Group 2 were significantly higher than Group 1 ($p<0.05$). Furthermore, the simvastatin and CTCE administration reduced the serum triglyceride levels and SGPT activity. The serum triglyceride levels and SGPT activity of Group 3, 5, 6 and 7 were significantly lower than Group 2 and 4 ($p<0.05$). In addition, the serum triglyceride levels and SGPT activity of Group 5, 6 and 7 were significantly lower than Group 3 ($p<0.05$). In conclusion, CTCE can reduce the serum triglyceride levels and SGPT activity in Wistar rats induced by HFD.

ABSTRAK

Pegagan atau *Centella asiatica* (L.) Urban megandung flavonoid dalam kadar tinggi yang diketahui secara luas sebagai pencegah perlemakan hepar. Penelitian ini dilakukan untuk mengkaji efek hepatoprotektor tablet kunyah ekstrak *C. asiatica* (L.) Urb (TKEC) pada tikus Wistar yang diinduksi lemak tinggi. Dua puluh satu tikus Wistar jantan berumur 8-12 minggu dengan berat badan 100-150 g digunakan dalam penelitian ini. Tikus dibagi menjadi tujuh kelompok yaitu Kelompok 1 sebagai kontrol normal, tikus diberi pakan standar, Kelompok 2 sebagai diet tinggi lemak (DTL), tikus diinduksi dengan DTL, Kelompok 3 sebagai control positif, tikus diinduksi DTL dan diberi simvastatin, Kelompok 4 sebagai control negatif, tikus diinduksi DTL dan placebo, Kelompok 5-7 sebagai kelompok perlakuan, tikus diberi DTL dan TKEC berturut-turut dengan dosis 100, 200 dan 300 mg/kg BB. Induksi DTL dilakukan selama lima minggu dan pemberian TKEC diberikan satu minggu pada minggu terakhir induksi. Di akhir perlakuan, kadar trigliserida darah dan aktivitas SGOT dan SGPT diperiksa. Analisis varian dengan tingkat kepercayaan 95% ($p < 0,05$) digunakan untuk analisis. Hasil penelitian menunjukkan induksi DTL meningkatkan kadar trigliserida darah dan aktivitas SGPT. Kadar trigliserida serum dan aktivitas SGPT Kelompok 2 lebih tinggi secara nyata dari Kelompok 1 ($p<0,05$). Selanjutnya, pemberian simvastatin dan pemberian TKEC menurunkan secara nyata kadar trigliserida serum dan aktivitas SGPT. Kadar trigliserida serum dan aktivitas SGPT Kelompok 3, 5, 6 dan 7 lebih rendah secara nyata dibandingkan Kelompok 2 dan 4 ($p<0,05$). Selain itu, kadar trigliserida serum dan aktivitas SGPT Kelompok 5, 6 dan 7 lebih rendah secara nyata dari Kelompok 3 ($p<0,05$). Dapat disimpulkan, pemberian TKEC dapat menurunkan kadar trigliserida serum dan aktivitas SGPT tikus Wistar yang diinduksi DTL.

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INTRODUCTION

Obesity, one of the public health problems in Indonesia, is a risk factor for cardiovascular and non-alcoholic fatty liver disease (NAFLD).[1,2] Obesity can cause metabolic syndrome which associated with NAFLD, one of the chronic liver. About 90% of people with NAFLD meet one criterion of metabolic syndrome, and about 33% of people with NAFLD meet three or more criteria for metabolic syndrome.[3,4] Hyperlipidemia in obesity causes accumulation of fat in the hepatic cells or fatty liver which characterized by an increase of enzymes serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activities. The increase of the blood SGPT and SGOT is well known as a common sign of the liver damage.[5-7]

A high-fat diet can cause a nutritional problem that is one of the causes of fatty liver and metabolic syndrome. Excessive fat intake causes an imbalance in the formation and remodeling of triglycerides.[8,9] The existence of insulin resistance is thought to have a significant factor on the onset of NAFLD. In the insulin resistance, there will be an increase in the synthesis and transport of triglycerides to the liver and an increase in lipolysis, especially in adipose in the central part of the body with the main product of free fatty acids (FFA). The FFA resulting from lipolysis are then transported through the portal vein to the liver for further processing, causing high FFA levels in the liver.[6,10] Lipogenesis and excess triglyceride synthesis in the liver will trigger an inflammatory reaction lead to hepatic steatosis due to oxidative stress.[11] Hepatic steatosis, accompanied by chronic inflammation, triggers the liver tissue's fibrogenesis process, and decreases liver function.[5]

Antioxidant compounds such as vitamin C and flavonoids from medicinal plants have been proven inhibit NAFLD occurrence in rats induced by high-fat diet. These compounds are believed can prevent oxidative stress and chronic inflammatory reactions.[12] Gotu kola or *Centella asiatica* (L.) Urban is a medicinal plant that contains lots of flavonoids.[13] Flavonoids have been shown to protect the fatty liver in rats fed a high-fat diet.[14] Gotu kola extract has been shown to have antioxidant and hepatoprotective activity.[13,15,16] A chewable tablet preparation containing gotu kola extract has been developed as a hepatoprotector.[17] This study was conducted to evaluate the effect of chewable tablet of *C. asiatica* (L.) Urb extract (CTCE) in Wistar rats induced by high fat diets.

MATERIALS AND METHODS

Materials

The equipment used in this study were: microhematocrit, Eppendorf, micropipette, animal scale, refrigerator, centrifuge, microcentrifuge tubes, glassware, spectrophotometer, syringe, and capillary tube. Whereas, the materials used in this study were the CTCE prepared by the Phytochemical and Pharmaceutical Team of Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta, simvastatin tablets obtained from UAD Pharmacy Yogyakarta, and pork oil with giving orally mixed with PKN feed. 124/BR2/VIII/2004 (1: 9), TRIS pH 7.15, L-alanine, 2-oksoglutarate, NADH, L-aspartate, LDH, MDH.

Animal dan study design

Twenty-one male Wistar rats (*Rattur norvegicus* L.) aged from 8 to 12 weeks with body weight ranging from 100 to 150 g were used in this study. Rats were obtained from the Intergrated Research and Testing Laboratory, Universitas Gadjah Mada, Yogyakarta. Rats were adapted for one week before experiment in individual cages with standard pellet food and tap water ad libitum. Rats

were then randomly divided into seven groups i.e. Group 1 as a normal control, rats were given standard pellet food and drink, Group 2 as negative control, rats were induced by HFD, Group 3 as positive control, rats were induced by HFD and given simvastatin at dose of 0.9 mg/kg BW, Group 4 as control media, rats were induced by HFD and given placebo, Group 5, 6 and 7 as treatment groups, rats were induced by HFD and given CTCE at doses of 100, 200 and 300 mg/kg BW, repectively.

The HFD was conducted based on the previous study. The high fat food was prepared by mixing until homogenous between standard BR II rat feed and pork oil in ratio of 90:10. The high fat food was adiministered daily for five weeks. The rats body weight were measured twice a week. At the end of HFD inducation, blood triglycerides levels of rats were measured. The chewable tablet of *C. asiatica* (L.) Urb extract (CTCE) was given orally once a day daily for one weeks started at fourth week after HFD induction. The research protocol was approved by the Research Ethics Committee, Faculty of Pharmacy, Universitas Ahmad Dahlan, Yogyakarta.

Measurement of blood triglyceride levels

At the end of the experiment, 1.5 mL of blood samples were taken from orbital sinus of the rats and collected into an Eppendorf. The blood samples were left to stand for 15 min and then centrifuged for 10 min at 4000 rpm. The blood serum were taken for analysis. Thetriglyceride levels were measured by GPO-PAP method, an using enzymatic colorimetric assay. Ten µL serum sample was mixws with 500 µL of reagent mix in a wells and incubated for 10 min. The absorbance of the mixture was measured at λ 520 nm within 60 min.

Measurementof blood SGPT and SGOT activities

The blood SGPT and SGOT were measured using transaminase kits with colorimetric assay. The SGPT activity was measured using reagent I consisting TRIS, L-alanin, LDH and reagent II consisting 2-oxoglutarate, NADH. The SGOT activity was measured using reagent I consisting TRIS, L-aspartic, MDH, LDH and reagent II consisting 2-oxoglutarate, NADH. The reagent was used as a mixture of reagent I and reagent II in ratio of 4:1. Fifty µLof serum was mixed with500 µL of reagent kit of the SGPT/SGOT, and thenincubated at 37 °C for 1 min. Absorbance of the mixture wasthen measured using spectophotometer at λ 340 nm at 37 °C. The mixture was then brought back to room temperature and incubated at 37 °C for 1 min. The absorbance was then measured at min 1, 2 and 3.

Statisticalanalysis

Data of serum triglyceride levels, SGOT and SGPT activities were presented as mean ± standard deviation (SD) and analyzed using on way analysis of variance (ANOVA). A p value <0.05 was considered significant.

RESULTS

Serumtriglyceride levels

Serum triglyceride levels of rats in all groups are presented in TABLE 1. The results showed that the HFD induction increased the serum triglyceride levels. The serum triglyceride levels of Group 2or negative control were significantly higher than Group 1 or normal control ($p<0.05$). Furthermore, the simvastatin and CTCE administration reduced the serum triglyceride levels. The serum triglyceride levels of Group 3, 5, 6 and 7 were significantly lower than Group 2 and 4 ($p<0.05$).

TABLE 1. Serum triglyceride levels of male Wistar rats (*R. norvegicus* L.) after induced by HFD and given CTCE.

Group	Intervention	Triglyceride level (mean±SD) (n=3)
1. Normal control	Standard pellet	99.33±27.86
2. Negative control	HFD group	134.37±8.91*
3. Positive control	HFD+ simvastatin group	83.80±17.10a,b
4. Placebo control	HFD+ plasebo group	107.60±14.19
5. Treatment I	HFD +CTCE dose of 100 mg	76.83±66.38a,b
6. Treatment II	HFD+CTCE dose of 200 mg	53.90±21.11*a,b
7. Treatment III	HFD+CTCE dose of 300 mg	49.43±17.43*a,b

Note: CTCE = chewable tablet of *C. Asiatica* extract; HFD = high-fat diet; *=significantly different compared tothe normal control ($p<0.05$); a= significantly different compared to the negative control; b=significantly different compared to placebo control

Serum SGOT and SGPT activities

The serum SGOT and SGPT activities of rats in all groups are presented in TABLE 2. No significantly different in SGOT in all groups was observed. It was indicated that the HFD induction and the CTCE administration did not influence the serum SGOT activity ($p>0.05$). However, the HFD induction increased the serum SGPT activity. The serum SGPT activity of Group 2 or negative control were significantly higher than Group 1 or normal control ($p<0.05$). In addition, the simvastatin and CTCE administration reduced the serum SGPT activity. The serum SGPT activity of Group 3, 5, 6 and 7 were significantly lower than Group 2 and 4 ($p<0.05$). No significantly different in the serum SGPT activity between the simvastatin administration and the CTCE administration ($p> 0.05$). It was indicated that the CTCE had similar activity to the simvastatin.

TABLE 2. Serum SGOT and SGPT activities of male Wistar rats (*R. norvegicus* L.) after induced by HFD and given CTCE.l

Group.	Treatment	SGOT activity (U/l)	SGPT activity (U/l)
1. Normal control	Standard pellet	111.10 ± 18.80	74.67 ± 22.59
2. Negative control	HFD group	111.23 ± 14.29	130.63 ± 35.07*
3. Positive control	HFD+simvastatin group	97.17 ± 7.10	40.07 ± 3.84a,b
4. Placebo control	HFD+plasebo group	83.50 ± 54.48	107.80 ± 24.12*
5. Treatment I	HFD+CTCE dose of 100 mg	116.50 ± 30.75	64,43 ± 7.43a,b
6. Treatment II	HFD+CTCE dose of 200 mg	100.60 ± 18.47	67.87 ± 13.47a,b
7. Treatment III	HFD+CTCE dose of 300 mg	89.57 ± 30.09	42.60 ± 14.06a,b

Note: CTCE = chewable tablet of *C. asiatica*extract; HFD = high-fat diet;*=significantly different compared to the normal control ($p<0.05$);a= significantly different compared to the negative control;b=significantly different compared to placebo control

DISCUSSION

In this study, induction of HFD caused hypertriglyceridemia as indicated by the increase of the serum triglyceride levels. It also caused the increase the serum SGPT activity which might due to by fatty liver. This results are consistent with the results obtained from the previous study. An HFD in rats also increased triglyceride or cholesterol levels or both.[18] The HFD can cause a nutritional problem that is one of the causes of fatty liver and metabolic syndrome. The excessive fat intake causes an imbalance in the formation and remodeling of triglycerides lead to an insulin resistance which it is bilieved as a significant factor of NAFLD.[8,9]

Non-alcoholic fatty liver disease is a multifactorial disease that difficult to discribethe pathogenesis of the disease in one animal model.[19] However, the majority the *in vivo* NAFLD studies are conducted in animal model. Cholesterol and other lipid components are blood insoluble. To be transported in the bloodstream, cholesterol and other lipids (triglycerides and phospholipids) must bind to proteins to form soluble compounds called lipoproteins. Cholesterol from the liver is transported by LDL to the body cells that need it, including heart muscle cells, brain, and others, to function correctly. However, if the triglyceride or cholesterol levels are too high, it creates the risk of coronary artery blockage, heart disease, metabolism disorders, and hepatocyte damage.[6,20]

The results also showed that the HFD induction successfully incrased the SGPT activity. The results of this study are also reported in the previous studies. In general, an HFD induction in animal model would be followed by an increase in SGPT or SGOT activity or both.[17] Obesity, type 2 diabetes mellitus (non-insulin-dependent), and hyperlipidemia are conditions frequently associated with NAFLD, whereas metabolic syndrome conditions are associated with elevated SGPT or SGOT levels or both levels.[21-23]

This study also showed that the CTCE and simvastatin administrations decreased the serum triglyceride levels and the SGPT activity in rats induced by HFD. Previous studies reported that the gotu kola or *C. asiatica* (L.), Urb. herb's active ingredients can reduce the activity of the SGPT and SGOT activities, hyperlipidemia, and increase the antioxidant activity.[24] Another study reported that asiatic acid can protect against liver cells' damage in mice with fatty liver by affecting fat metabolism.[25] Besides having anti-hepatotoxic activity, flavonoids and asiatic acids from gotu kola herbs have been shown to have beneficial effects on the cardiovascular system,including reducing LDL oxidation, inhibiting platelet aggregation, reducing the body's inflammatory response, healing diabetes wounds, and reducing hyperlipidemia.[26] Gotu kola contains several active compounds belonging terpenoids, flavonoids, and glycosides which have anti-hypertriglyceridemia activity. Flavonoids can reduce the absorption of fatty acids by inhibiting the activity of the lipase enzyme. Flavonoid compounds in plants are known to be antioxidant compounds and can prevent body cells' damage, including liver cells.[13,27]

CONCLUSIONS

One week of CHCT administration could lower triglyceride levels in HFD-induced Wistar rats. In white male rats, Wistar strain, induced by HFD, administration of CHCT could reduce SGPT activity but did not affect SGOT activity. Further research is needed to identify and isolate chemicals containing CHCTs that can help repair liver damage.

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