Acalypha Indica and Gemfibrozil Lowering Cholesterol and Triglyceride Levels in High Fructose-Cholesterol Diet Induced Rats

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Abstract

High fructose and cholesterol diets (HFCDs) cause hypercholesterolemia, hypertriglyceridemia, and many acute and chronic serious diseases. Current established treatments, such as simvastatin (SIM) and gemfibrozil (GEM), have been successful in lowering cholesterol and triglyceride levels, but their long-term use poses a risk for organ dysfunction. Herbal medicine addition to this treatment can improve patient outcomes. This study examined the effects of *Acalypha indica* L. (AI) root extract in improving the efficacy of SIM and GEM treatment and attempted to reduce their side effects. Five of the seven male Sprague–Dawley rat groups were maintained daily on HFCD for 4 weeks while being treated with either SIM, GEM, AI, SIM+AI, or GEM+AI. The remaining two groups were given only HFCD and normal diet, respectively. Liver HMG-CoA reductase and PPAR-α, blood total cholesterol and triglyceride levels, and liver histopathology were measured after a 1-month therapy. The SIM+AI group had low HMG-CoA reductase levels, whereas the GEM+AI group had high PPAR-α levels. The GEM+AI group showed normal liver histopathology, whereas the SIM+AI and HFCD-only groups showed similar features. Adding AI to SIM and GEM lowered triglyceride levels. GEM+AI significantly lowered cholesterol levels, indicating that AI functions synergistically as a PPAR-α agonist.

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Introduction

A high fructose and cholesterol diet (HFCD) is becoming increasingly common in many communities, thus contributing to the increase in hypercholesterolemia and hypertriglyceridemia cases. Fructose and cholesterol play important roles in acute and chronic fatal diseases. Agents for lowering cholesterol and triglyceride, such as statins and fibrates, have been proved effective but can have systemic side effects such as liver toxicity, muscle toxicity, cardiac hypertrophy, and renal insufficiency.[1–3] The prolonged use of these medicines can harm the liver.[1] To enhance the efficacy of these treatments in lowering cholesterol and lipids and to minimize the negative side effects, the incorporation of herbal medicine into these treatments may be a promising option.

Our focus here is the herb *Acalypha indica* L. (AI), which is known for its antihypercholesterolemia and antihyperlipidemia effects. However, the mechanism by which AI lowers total cholesterol and triglyceride is still unclear. AI contains flavonoid and polyphenol, which have shown the ability to suppress apolipoprotein B, thus leading to a decrease in triglyceride in serum and in the liver.[4] Tannins, glucoside, acalyphamide, and sucinimide are also present in AI andhave been shown to modulate peroxisome proliferator–activated receptor-α (PPAR-α), which is a target of triglyceride-lowering therapy.[5] Our prior study showed that AI and a combination of AI and gemfibrozil (GEM) significantly and consistently reduced total cholesterol, triglyceride, atherogenic index, and lipid deposition in fatty liver tissue in HFCD-fed rats.[6] We continued the study to learn more about AI mechanism in

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decreasing cholesterol and triglyceride levels. We measured the PPAR-αlevel as the GEM target and the HMG-CoA reductase as the SIM target in the liver of an HFCD-fed rat.

Materials and methods

This preclinical experimental study was approved by the Ethics Committee of the Faculty of Medicine at Universitas Indonesia (509/UN2.F1/ETIK/2015). A total of 42 male Sprague–Dawley rats(8–12 weeks old each) were divided into seven groups (Table 1).Experimental groups were fed with an HFCD comprising 2 mL 55% fructose and 10% cholesterol in rat's chow. Groups 1–6 were given HFCD food twice daily for four weeks to achieve twice the normal cholesterol level or more than 140 mg/mL triglyceride. For another four weeks after this initial period, the treatments were as follows: group 1 (HFCD) as negative control received CMC 1%, group 2 (SIM) received SIM10 mg/kgBW daily, group 3(GEM) received GEM31 mg/kgBW daily, group 4(AI) received AI 250 mg/kgBW daily, groups 5(SIM+AI)and 6(GEM+AI)received combinations of these in the same amounts, and group 7 was given a normal diet without any treatment.

Group	One-month Treatment
1	HFCD diet
2	HFCD diet + SIM
3	HFCD diet + GEM
4	HFCD diet + AI
5	HFCD diet + SIM + AI
6	HFCD diet + GEM + AI
7	Normal diet

Table 1. Treatment groups. HFCD: high fructose and cholesterol diet; SIM: simvastatin; GEM: gemfibrozil; AI: *Acalypha indica.*

The preparation of the ethanolic extract of AI roots, as well as blood sample collection, lipid profile measurement, and liver histopathology assessment, was performed as described in our previous study.[6] Part of the liver tissue was also stored at −80°C and measured for HMG-CoA reductase, PPAR-α, and protein. HMG-CoA reductase and PPAR-α levels were measured using ELISA kit (Elabscience Catalog No. E-EL-R0515 and E-EL-0725). Both levels were normalized to liver protein levels.

All statistical analyses were performed using GraphPad Prism. The Kruskal–Wallis test was used to analyze the differences between the groups. The correlation between liver histopathology score and HMG-CoA reductase or PPAR-α levels was analyzed using Spearman's test.

Results

Figure 1 and Figure 2 show the HMG-CoA reductase and PPAR-α levels among groups. Data were not normally distributed and there were no significant differences between groups (p>0.05). As expected, the SIM group had low HMG-CoA reductase levels (2.779, 1.182–3.208 ng/mg protein), and these were slightly higher in the SIM+AI group (3.315, 1.253–5.174). The GEM + AI group had high PPAR-α levels (17.01, 15.84–25.38 ng/mg protein). The PPAR-α level in the GEM-only group (14.92, 13.10–15.75) was less than that in the GEM+AI group.

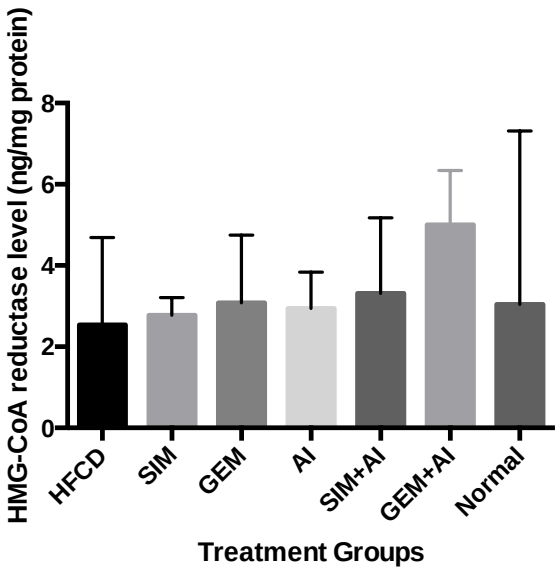

Figure 1. HMG-CoA reductase levels in rat livers (n=4). Values are represented as median (range). HFCD: high fructose and cholesterol diet; SIM: simvastatin; GEM: gemfibrozil; AI: *acalypha indica*.

The serum lipid profiles and liver histopathology for all groups other than SIM and SIM+AI has been published before.[6–8] The AI group showed lower total cholesterol and triglyceride levels (121.2; 111.98–155.45 and

137.18±38.22) than the SIM group (141.81; 113.54–148.2 and 145.83±28.73). However, this difference was not significant.[7]The addition of AI to SIM did not have an effect on total cholesterol or triglyceride levels. The liver histopathology study showed that the SIM group had the same lipid deposition features as the HFCD group, which did not improve with AI addition (Figure 3). There was a perfect correlation between the SIM group liver histopathology score and the HMG-CoA reductase level (R = 1), but it was not significant (p = 0.333).We could not analyze the correlation of GEM or GEM+AI liver histopathology scores with the PPAR-αlevels because the rats in both groups had normal liver features (zero score).[6,8]

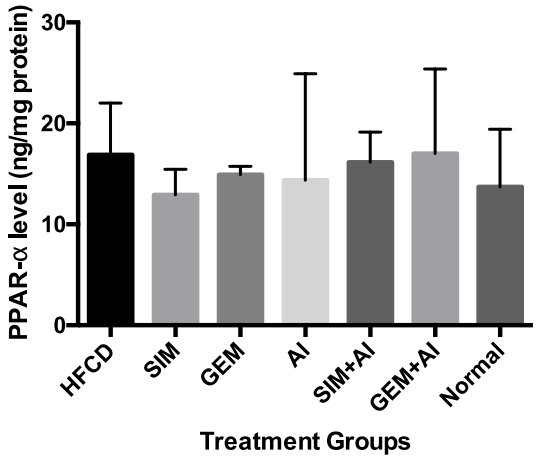

Figure 2. PPAR-α levels in rat livers (n = 4). Values are represented as median (range). HFCD: high fructose and cholesterol diet. SIM: simvastatin; GEM: gemfibrozil; AI:*Acalypha indica*.

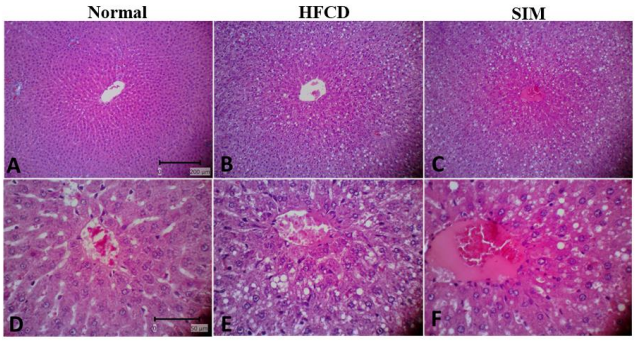

Figure 3. Histopathology of the liver. A-F 10x magnification, D-F 40x magnification. Hematoxylin and Eosin staining. HFCD: high fructose and cholesterol diet.; SIM: simvastatin.

Discussion

We examined the effects of AI root extract as a possible therapeutic agent for lowering cholesterol and triglyceride levels both on its own and in combination with GEM as PPAR-α agonist and SIM as HMG-CoA reductase. Our results show that the addition of AI can improve both total cholesterol and triglyceride levels supported by high PPAR-α level.

We found high levels of PPAR-α in the GEM+AI treatment group. This result is consistent with our previous finding that GEM+AI treatment resulted insignificantly lower triglyceride and cholesterollevels.[6] Therefore, AI appears to increase the positive effects of GEM in improving the blood lipid profile and liver tissue condition. Fawzy et al.[9] also found promising PPAR agonistic effects that are accompanied by anti-inflammatory activities in another *Acalypha* species, namely, *A. fruticosa*. Our results showed that liver histopathology was more normal in the GEM and GEM+AI groups than in the SIM group. GEM, which is a fenofibrate derivative, activates PPAR-α and has pleiotropic effects, such as the reduction of various pro inflammatory markers, thus possibly making it more tolerable than statin.[10] The PPAR-α agonist propertiesof fenofibrate have likewise been shown to attenuate liver disease in mice.[11]

Lipid deposition in the liver after treatment with SIM or SIM+AI may contribute to the risk of liver injury.[12] In this study; the administration of 10 mg/kgBW/day of SIM for four weeks could not protect the liver because the HFCD diet was continuously given during the treatment. Another study by Garip et al[13] using 50 mg/kgBW/day of SIM for 30 days induced hepatic lipid peroxidation and changed the liver structure in healthy rats. This difference could be due to the higher dose of SIM than that used in our study. AI has been shown to be effective at protecting the liver against toxic substances.[14] Our previous study reported that the combination of GEM+AI also had protective effects in other organs, such as the pancreas.[15] All of this might explain the add-on effect of AI on GEM therapy, but the details of this synergy still need further investigations. The many active compounds contained in AI also suggest that the induction of PPAR-α may not be the only mechanism by which AI improves the blood lipid profile.

Conclusions

AI root extract decreases cholesterol and triglyceride levels via its ability to increase PPAR-α without inhibiting HMG-CoA reductase. Together with GEM, AI tends to increase PPAR-α further, thus resulting in a better lipid profile.

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Declaration of Interest

The authors have no conflicts of interest to declare.

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