Conference Paper

Low Serum Cholesterol in Mice Pre-treated with *Imperata cylindrica L.* after Acute Olive Oil Gavage

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Abstract

Imperata cylindrica L. extract was studied for its effect on cholesterol absorption rate in mice (*Mus muscularis*). Male mice (20-25g) were divided control and pre-treated group. Pretreated group was further divided into two groups that received different dose of *Imperata cylindrica L.* extract gavage (90mg/KgBW and 115mg/KgBW) for 2 weeks. Mice were fasted 12 hour before acutely given olive oil gavage with dosage of 10 µl/g, and blood was collected at 0, 2, and 6 hour-time points on the indicated day. Blood was collected from the retroorbital plexus at indicated time points. Total cholesterol level was significantly ($p<0.05$) decreased in both pretreated groups at 6 hours after acute olive oil gavage. In conclusion, there is a significant effect of *Imperata cylindrica L.* extract to reduce cholesterol level in serum, suggesting its potential effect as an antihypercholesterolemia therapy.

Keywords: *Imperata cylindrical L.*; cholesterol absorption; antihypercholesterolemia; lipid absorption; herbal medicine.

1. Introduction

People with high total cholesterol in blood has a higher risk of heart attack and death in the future. Association of high blood cholesterol with the occurane of atherosclerosis and coronary heart disease (CHD) has been demonstrated [1, 2]. Atherosclerosis and CHD are the most serious cardiovascular disease that influenced by several factors such as obesity, diet, heredity, stress and aging that result in serious vascular imbalances.

Many clinical trials have established a relationship between hypercholesterolemia and atherosclerosis, coronary endothelial function will increase and the risk of CHD will be reduced by reducing concentration of total and LDL-cholesterol in the blood [3–5].

Cholesterol in the body is derived from dietary intake and biosynthesized de novo. 70% of daily total cholesterol requirement is synthesized in liver, the rest comes from dietary intake [6]. Under certain conditions, cholesterol obtained from diet together with synthesized cholesterol might exceed body requirement for producing cellular membranes, steroids or biles. This excessive cholesterol accumulates in blood vessels and results in plaque formation that contributes to the development of various cardiovascular diseases. 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase is rate limiting enzyme that controls cholesterol synthetized. Excessive cholesterol formation controlled by feedback mechanism. Levels of HMG-CoA reductase reduces by decreasing the gene transcription of this enzyme when cholesterol concentrations increased in blood vessels [7, 8].

Recently, herbal medicine used for treatment of many diseases has attracted more attention. The useful and well-known plant is *Imperata cylindrica L.* which might be exhibit hypocholesterolemic effect [10]. The scaly rhizomes of *Imperata cylindrica L* spread and invade any disturbed ecosystem, including cultivated field [11]. It is commonly used as traditional medicine for its empirically-proven abilities to cure fever, muscle ache, asphyxia, and nosebleed. It also has ability to lower fat and glucose concentrations in blood [12–14]. *Imperata cylindrica L.* contains tannin, saponin, flavonoid, alkaloid, and terpenoid [15, 16]. Flavonoid significantly reduces glucose concentration, plasma cholesterol, and triacylglycerol [17–19].

Drugs for hypercolesteremia have side effects such as myositis, gallstones, myopathy, myalgia, and liver damage [9]. Overwhelmed by many side effects of several conservative drugs, scientists find herbal medicine to be an alternative option for treating various diseases. As *Imperata cylindrica L.* shows a potential effect to treat hypercholesterolemia, we conduct this study to investigate the hypocholesterolemic effects of *Imperata cylindrica L.* in mice.

2. Methods

2.1. Preparation of the extract

Imperata cylindrica L. was collected from the local regions of Java and was authenticated by School of Natural Science, Bandung Institute of Technology, Indonesia. Roots were separated and washed thoroughly with water. It was dried under shade for 2

weeks and processed into powder using electrical blender. This powder was successively extracted with ethanol 96% (Merck, U.S.A) by maceration process for 72 hours. The macerated pulp was filtered through a coarse sieve and the filtrate was concentrated in rotary vacuum evaporaror. Imperata cylindrica extract was diluted with Carboxyl methyl cellulose (CMC) 0,5% (Merck, U.S.A) to get concentrations of 90 mg/Kg Bw and 115 mg/KgBW.

2.2. Animal

Eight to ten weeks old of male mice (*Mus muscularis*) were collected from Animal Laboratorium, Department of Phamarcology and Therapy, Faculty of Medicine, Universitas Padjadjaran, Bandung, Indonesia. The ethical implications of experimental animals has been approved by The Institutional Animal Care and Use Committee (Faculty of medicine, Universitas Padjajaran). Mice were housed in a temperature-controlled room in a 12-hour light 12-hour dark cycle and has unrestricted access to water and chow. Mice were given *Imperata cylindrica* extract intragastrically with dose 90 mg/Kg (dose 1) and 115 mg/KgBW (dose 2) for two weeks.

2.3. Acute Olive Oil Gavage

Control and treated mice were given olive oil (10 µl/ g) intragastrically after fasting overnight. Blood was collected from the retro-orbital plexus with indicating time point.

2.4. Measurement of Total Cholesterol

Blood samples were centrifuged for 15 minutes at 1,200 × g to separate the serum. Total cholesterol (AIIMS, Indonesia) were measured according to the manufacturer's protocols.

3. Discussion

3.1. Statistical Analysis

Statistical analysis was performed using one way ANOVA for 3 samples at the same group of indicated time points. Bonferroni's post hoc multiple comparison tests were performed to evaluate differences between control and treated groups. P-value of 0.05 was considered statistically significant.

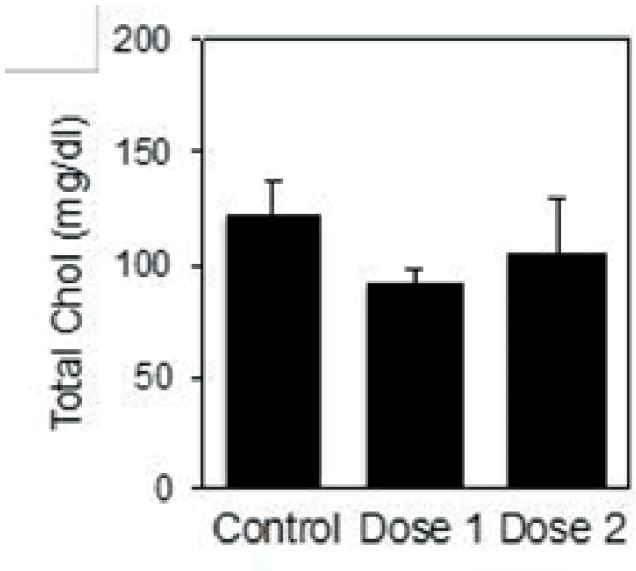

Figure 1: **Total cholesterol in serum.** Serum was collected from retroorbital blood after fasting overnight and total cholesterol was measured. Chol = cholesterol.

4. Results

After two weeks of *Imperata cylindrica L.* extract gavage, blood from retro-orbital was collected and measurement of total cholesterol was performed. Total cholesterol in serum was not different among groups (Figure 1). We further gave olive oil gavage to see cholesterol serum level after acute lipid gavage. As shown in Figure 2, at two hours following olive oil gavage, cholesterol serum level in dose 2 group reached the same level as control group, while the dose 1 group the level did not change. After 6 hours of olive oil gavage, cholesterol serum in both treated mice were lower than control group. Our results showed *Imperata cylindrica L.* reduces cholesterol metabolism after acute olive oil gavage.

The purpose of this study is to examine the effect of *Imperata cylindrica L.* toward cholesterol absorption rate. Controlled group showed an increase in total cholesterol level consequently at 2 and 6 hours after acute olive oil gavage. Both of treated mice groups (90mg/KgBW and 115mg/KgBW) showed a reduction in cholesterol serum level at 6 hours folowing acute olive oil gavage.

There was no significant difference between total cholesterol levels in 90mg/KgBW group compared to control group at 2 hour after acute olive oil gavage. In other hand, group of 115mg/KgBW treatment showed lower level of total cholesterol that was 27% less compared to control group. At 6 hours after acute olive oil gavage there was a significant change in both treated group which was a decrease in total cholesterol.

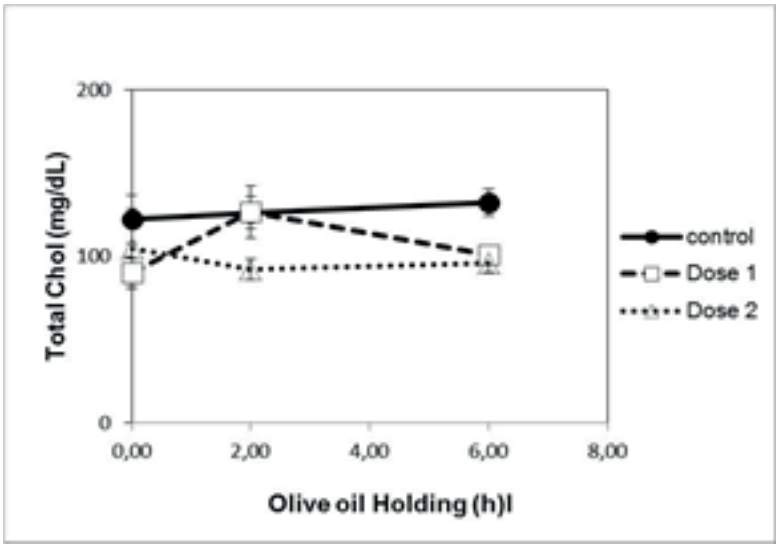

Figure 2: **Acute Olive Oil.** Mice were given olive oil intragastrically and blood was collected from retroorbital before and 2 and 6 hours after olive oil gavage. Serum was collected and cholesterol was measured. Different font indicated significantly different ($p<0.05$). n= 5-6 mice/group. Chol = cholesterol.

Total cholesterol in 90mg/KgBW group was 24% lower and in 115mg/KgBW group was 27% lower compared to control group.

In this study we used acute olive oil gavage to show body's ability to absorb and use lipids. Olive oil is kind of vegetable oils and contains no cholesterol. We suggested that *Imperata cylindrica L.* does not impair cholesterol absorption directly. The treated mice had decrease cholesterol indirectly probably due to decrease cholesterol absorption rate or the use of triglyceride. Our previous study showed that there was disruption of both absorption rate and utilization of triglyceride in mice given *Imperata cylindrica L.* for 2 weeks (data not shown). The pretreated mice had a decrease in cholesterol level that probably due to decrease cholesterol absorption rate or the use of triglyceride. We suggest to conduct further studies using cholesterol substrate to examine cholesterol absorption rate. The study will determine proteins and genes expression associated with cholesterol absorption in small intestine, such as HMG-CoA, sterol regulatory element-binding proteins-1 (SREBP-1), and SREBP-2 [20].

Another possible explanation of reduction in cholesterol serum level is because of the low cholesterol production in liver. Cholesterol synthesized in the body was derived from fatty acids and glucose. Our previous study showed that mice given *Imperata cylindrica L*. extract had lower level of blood glucose (data not shown), that might lead to a reduction in building blocks needed for cholesterol production. Furthermore, the active compound in *Imperata cylindrica L*. might directly regulate the gene related to cholesterol production such as HMG-CoA reductase [21].

Study in China showed that quercentin, a class of flavonoid found in fruits and vegetables, exposes antilipase activity [10]. Some studies have reported that rutin and quercetin decreased total cholesterol in blood, Low Density Lipoprotein Cholesterol (LDL-C, and liver cholesterol levels [22]. Quercetin also reduced 3-HMG-CoA reductase activity [21]. Therefore, it is likely that flavonoid in quercetin may be responsible for the inhibition of HMG-CoA reductase activity. *Imperata cylindrica L*. contain flavonoid, a group of polyphenols known for its lipid-lowering effect [23].

From this study, we conclude that there is a significant effect of *Imperata cylindrica L.* extract to reduce cholesterol level in serum, suggesting its potential effect as an antihypercholesterolemia therapy.

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