IOSR Journal Of Pharmacy
(e)-ISSN: 2250-3013, (p)-ISSN: 2319-4219
www.iosrphr.org Volume 5, Issue 7 (July 2015), PP. 44-47

Effect of *Piper crocatum* Extract Against Weight Loss and Liver Enzyme Levels in High Fat Diet Induced Obese Rats

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Abstract: *Piper crocatum is one of Indonesian medicinal plant that contain flavonoids, tannins, alkaloids, and saponins. Aims of this study were to evaluate the effect of Piper crocatum aqueous extract against a decrease in body weight (BW) and the activity of enzymes involved in lipid metabolism (AMPK, ACC, FAS) in liver obese rats. This study used four groups of Sprague dawley rat (n = 6), including normal group (N), obese controls (OC), Piper crocatum extract dose 1260 mg/kgBW (PcA), and Piper crocatum extract dose of 1890 mg/kgBW (PcB). Measurement of metabolic liver enzyme levels (AMPK, ACC, FAS) are using ELISA kit (Cusabio™). Results of this study showed that the PcA group produce the highest reduction in body weight (4.52%), and the lowest levels of ACC (9.13 ng/g) and FAS (360.68 ng/g) which was significantly different from obese control group (95% CI). Piper crocatum extract can't activate AMPK. The highest levels in rat liver AMPK is in N group with 8.42 ng/g, but this value is not significantly different from other groups.*

Keywords - *ACC, AMPK, FAS, Obese, Piper crocatum*

I. INTRODUCTION

Obesity is a problem that gets serious attention in the medical world today, because the condition of obesity can be a precipitating factor other diseases that can endanger lives. Many studies have shown that obesity can lead to metabolic syndrome conditions and become intermediate precursor to heart disease, type 2 diabetes mellitus, stroke, and cancer [1-3]. Obesity occurs due to the accumulation of body fat or adipose tissue excess or abnormal. Fat accumulation is generally caused by out fewer calories than the calorie intake.

The incidence of obesity is increasing rapidly due to inactive lifestyles, as well as genetic factors. Energy of physical activity everyday use decreases as globalization and technological progress. Such as motorized transport facilities, elevators, lifts, air conditioning, and heating causes the energy used to move a little more. Minimal physical activity in leisure time as watching television and playing video games on children increases the incidence of obesity. An imbalance of energy intake from food with the energy released by the body, if it occurs in a long time (chronic) can lead to obesity [4].

WHO [5] report that 1.6 billion adults have excess body weight (overweight) and 400 million of them are obese or overweight. The prevalence of obesity in Indonesia based on data from the Health Research (Riskesdas) in 2007 showed that obesity is common in people aged ≥ 15 years were 10.3% [6].

Several studies that examine the antiobesity effects of medicinal plants have been carried out [7,8]. Red betel (*Piper crocatum*) is one of Indonesia's traditional crops that have been widely studied and used as a medicinal plant. Water extract of *Piper crocatum* leaves contain flavonoids, tannins, and alkaloids. The content of tannins and alkaloids from aqueous extract of *Piper crocatum* leaf is greater than 30% ethanol extract [9]. According to George & Nimmi [7], medicinal plants which have anti-obesity effects mostly contain flavonoids, tannins, alkaloids, and saponins.

Aqueous extract of *Piper crocatum* has antihyperglycemic effect at optimum dose of 1260 mg/kgBW which is characterized by a significantly decrease in blood glucose levels in Sprague Dawley rats were given streptozotosin diabetes, also can prevent the increase in blood lipid levels, as well as non-toxic in vivo [10]. Based on these effects, the extract of *Piper crocatum* believed to have the ability to improve the condition of obesity and the incidence of metabolic syndrome. Hutchinson *et al.* [11] found that drugs used clinically for the treatment of type 2 diabetes (such as metformin and a glitazone), capable of lowering blood sugar levels through the mechanism of activation of the enzyme AMP-activated protein kinase (AMPK). This mechanism does not depend on insulin signaling pathway, making AMPK as a promising therapeutic target in the treatment of diabetes and obesity.

Activation of AMPK phosphorylated enzyme would lead to inhibition of acetyl co-enzyme A carboxylase (ACC), the first enzyme involved in fatty acid biosynthesis [12]. AMPK activation also indirectly result in inhibition of fatty acid synthase (FAS), which plays a role in lipogenesis [13].

This study wanted to see the antiobesity effects of *Piper crocatum* aqueous extract through a reduction in body weight and respond to the enzymes involved in lipid metabolism through activation of AMP-activated protein kinase (AMPK), also the inhibition of acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) in the liver of obese rats. Results of this research is expected to contribute knowledge about the mechanisms of biomolecular antiobesity effects of *Piper crocatum* plant, as well as utilization can be applied by the public as a traditional medicinal plant which can reduce body weight.

II. MATERIAL AND METHODE

This research was conducted from May 2014 until February 2015. Animal maintenance were performed at the Animal Hospital Bogor Agricultural University (BAU), while the sample testing carried out in the Laboratory of Biochemistry Department of the University.

Manufacture of aqueous extract of *Piper crocatum* leaf is based on the use of traditional medicine Indonesian society that has been modified according to the rules of Indonesian Health Ministry [14]. *Piper crocatum* leaf dry were milled to obtain a powder with a size of 40 mesh, then the water content simplicia determined using a modified ISO 01-2891 -1992 [9]. Dry powder of *Piper crocatum* leaves as much as 100 g was then extracted with 1 L of water (1:10) for 2 hours at 100 °C as reflux, then filtered with filter paper. Extraction is repeated three times. The extract is dried using a rotary evaporator temperature of 50 °C to obtain crude extract of *Piper crocatum* leaf.

Animals male Sprague Dawley rats (SD) age 5 weeks were divided into four groups (6 each): normal (N), obese control (OC), *Piper crocatum* dose 1260 mg/kgBW (PcA), and *Piper crocatum* dose 1890 mg/kgBW (PcB). Rats in the normal group (N) only fed the standard until the end of the treatment, while the remaining (18 rats) were fed a high-fat diet (HFD) then the obesity level were examined after 12 weeks. Obese rats assessed based on *Lee obesity index* (values > 0.3) [15] or statistical analysis showed a significant difference with normal group.

$$Lee\ Obesity\ Index\ = \frac{\sqrt{Body\ weight\ (gram)\ x\ 10}}{Nasoanal\ length\ (mm)}$$

Before the treatment begins, the obese rats in group OC, PcA, and PcB replaced feed into standard feed. This period is referred to "Washing Out" (WO) period and changes in body weight was observed for 4 weeks. The period of treatment is restricted for 2 weeks, that is until the body weight (BW) of rats treatment groups was not significantly different from normal. The extract of *Piper crocatum* and distilled water (for normal and control group) administered per oral every day by using the sonde, according rat body weight and not more than 2 ml/100 g BW [16].

At the end of treatment, the rats were terminated with *Ketamine* 80 mg/kgBW and *xylazine* 10 mg/kgBW. Blood were taken by cardiac puncture (5 ml). The abdomen was opened, then the livers were removed and cleaned. Liver tissue samples were fixed in NaCl 0,9 %. Liver samples then homogenized with homogenizer Ultraturax and centrifuged to obtain a supernatant for testing the metabolic liver enzyme levels (AMPK, ACC, FAS). Measurement of the enzyme AMPK, ACC, and FAS is done by using the ELISA kit from Cusabio, namely Rat Acetyl-CoA Carboxylase 1 (CSB-EL001119RA), Rat Fatty Acid Synthase (CSB-E16440r), and Rat phosphorylated Adenosine Monophosphate activated Protein Kinase (CSB-E11337r). The color that appears is measured by ELISA Reader BioRad absorbance at a wavelength 450 nm.

Data were statistically analyzed according to the method completely randomized design (CRD). Effect of treatment differences between groups were analyzed using one-way ANOVA test with significance level (Level of Significancy) $\alpha = 0.05$, and further test with Tukey to see the significance of the differences of each treatment group.

III. RESULTS

III.1. Changes of Rats Body Weight

Rats that were fed with HFD for 12 weeks showed an increase in body weight higher than normal group that given only the standard feed (Table 1). Differences in body weight is starting to be seen after 8 weeks of high fat feeding. Lee obesity index shows the value < 0.3 in group N rats while obese rats group (OC, PcA, and PcB) managed to reach Lee index > 0.3. Assessment showed statistically significant differences between groups N to obese rats group ($p < 0.001$).

Wash out (WO) periode which lasted for 4 weeks, aimed to see the effect of feed changing without additional treatment. There are weight loss in obese groups (OC, PcA, PcB) but this change does not take effect statistically, because the differences of body weight between normal and obese groups is still significant ($p < 0.05$) (Table 1).

Table 1 Comparison of Rats Body Weight

Group	Rats Body Weight (g) [a]		
	BW began obese (after 12 weeks feed)	BW after WO periode (4 weeks)	BW before termination (2 weeks treatment)
N	260,36 ± 13,83 a	340,03 ± 37,17 a	385,70 ± 45,15 a
OC	392,65 ± 4,77 b	389,37 ± 9,99 b	418,03 ± 15,65 a
PcA	388,25 ± 11,56 b	380,20 ± 38,88 b	370,70 ± 28,84 a
PcB	391,37 ± 8,33 b	381,37 ± 22,70 b	402,45 ± 21,46 a

[a] The figures in the same column followed by the same letter are not significantly different at the test level 5% (Tukey test)

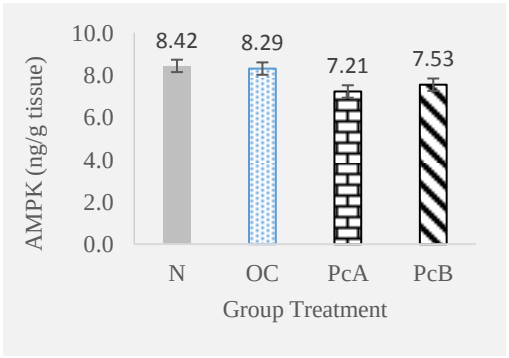
Final measurement of body weight showed that group PcA have the lowest body weight (370.7 g) less than N group (385.7 g) while OC group have a highest body weight (418,03 g). According to the changes in body weight (table 2) it can be seen that weight loss occurs only in the group PcA (-4,52%).

Table 2 Percentage of body weight change from began obese till the end of treatment

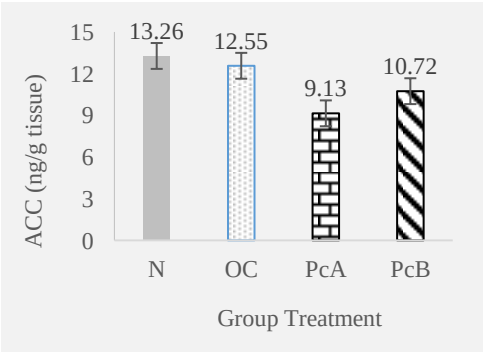
Group	BW changes (%)
N	46,691
OC	6,465
PcA	-4,520
PcB	2,832

III.2. ELISA analysis of rat liver homogenate

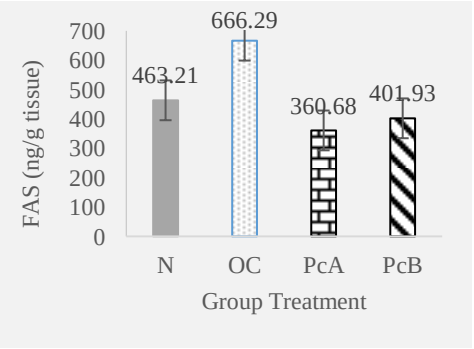
Measurement of the AMPK using ELISA method showed that there are no significant differences between all group ($p > 0.05$), it means the treatments do not lead to AMPK activation. But measurement of the levels of ACC and FAS show that groups of rats which given the extract of *Piper crocatum* has a lower value than N and OC group, which means there is inhibition of these enzymes (Fig.1). PcA group had the lowest levels among all groups. This is in accordance with changes in body weight where the PcA group had the smallest BW.



(a)



(b)



(c)

Fig.1 Concentrations of liver enzymes involved in lipid metabolism: (a) AMPK; (b) ACC; (c) FAS

IV. DISCUSSION

Antiobesity mechanisms of medicinal plants occurred partly by the reduction of fat absorption through lipase inhibition, reducing energy uptake by suppressing the appetite, increase energy expenditure, inhibiting adipocyte differentiation and proliferation, as well as play a role in the regulation of lipid metabolism [17]. This study showed that the aqueous extract of *Piper crocatum* leaf dose of 1260 mg/kgBW can not activate AMPK as previously hypothesized, but capable of inhibiting ACC and FAS.

Acetyl-CoA carboxylase (ACC) is an enzyme that catalyzes the function of acetyl-CoA carboxylation to be malonil-CoA [18], while the fatty acid synthase (FAS) catalyzes malonil-CoA into fatty acids. Inhibition of these enzymes resulted in decreased levels of lipids in the body, which is one of the targets of therapy in the treatment of obesity [19]. This is the mechanism which gives effect to the weight loss in PcA group.

V. CONCLUSION

Aqueous extract of *Piper crocatum* leaf doses of 1260 mg/kgBW has antiobesity effects through inhibition mechanism of ACC and FAS. Aqueous extract of *Piper crocatum* leaf can not activate AMPK.

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