



## Effect of *Citrus amblycarpa* Hassk Extract Against *Rattus norvegicus* Body Weight with High-Fat Diet

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### Abstract

**Background:** Obesity is a complex disease generally accompanied by insulin resistance, increased oxidative stress, and the expression of inflammatory markers that contribute to the accumulation of body fat mass. Citrus family contain high levels of flavonoids and essential oils, so they have the potential to control appetite and play a role in fat breakdown.

**Objective:** to determine the effect of *Citrus amblycarpa* Hassk extract on weight loss of rats fed a high-fat diet.

**Methods:** *Rattus norvegicus* Wistar strain aged 2-3 months and randomly divided into six groups. The dried *Citrus amblycarpa* Hassk peel was extracted by maceration with 70% ethanol as solvent. Variations in the dose of *Citrus amblycarpa* Hassk peel extract given were 200, 300, and 400 mg/kg BW. There were six groups of treated rats, with 30 total rats sample. The weight of the rats from each treatment group was measured for six weeks, and then the average body weight of each Group was analyzed using Kruskal Wallis with the Mann-Whitney further test.

**Results:** The data showed that the administration of *Citrus amblycarpa* Hassk peel extract significantly affected the weight loss of Wistar rats fed a high-fat diet with  $p=0.00$  ( $p < 0.05$ ). The average body weight of the 300 mg/kg BW and 400 mg/kg BW extract-treated rats was significantly different from the control group that was not given the *C. amblycarpa* Hassk peel extract, but the average body weight in the 200 mg/kg BW dose group did not show a significant difference.

**Conclusion:** There is an effect of *C. amblycarpa* Hassk peel extract in reducing the body weight of Wistar rats fed a high-fat diet significantly at doses of 300 mg/kg BW and 400 mg/kg BW.

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## INTRODUCTION

Obesity is a disease caused by the entry of excessive caloric intake into the body and is not balanced with physical activity.<sup>1</sup> In addition, the diet of modern society is shifting towards high-calorie and high-fat foods, which is the leading cause of the rising obesity rate.<sup>2</sup> Obesity can occur if a person consumes foods and drinks high in fat. A high-fat diet applies a low-carbohydrate and high-fat diet that can lead to obesity through an increase in the proportion of body fat so that it is associated with high cholesterol levels and is considered bad for health.<sup>3</sup> Metabolic disorders such as obesity, dyslipidemia, hypertension, heart disease, and inflammatory disease pathologies can cause obesity. This pathology occurs due to an increase in blood cholesterol levels. Obesity is usually accompanied by insulin resistance and increased oxidative stress.<sup>4</sup>

A survey by the World Health Organization (WHO) in 2016 stated that more than 340 million children and adolescents aged 5-19 years were overweight or obese, and the value always increases yearly.<sup>5</sup> Based on the Indonesian Basic Health Research in 2018, there has been an increase in the proportion of obesity in adults aged >18 years since 2007 (10.5%), 2013 (14.8%), and 2018 (21.8%) in Indonesia.<sup>6</sup> The incidence of obesity according to Indonesian Basic Health Research South Kalimantan in 2018 19.52% of adults aged >18 years.<sup>7</sup>

The condition of excess fat in the body causes abnormalities in lipids. One of the typical South Kalimantan herbal plants, a flavoring and kitchen spice in Banjarese cuisine, is *Citrus amblycarpa* Hassk. One of the centers for producing *C. amblycarpa* Hassk is in Sungai Tuan

Village, Astambul District. Research by Irwan *et al.* suggests that limes contain active ingredients of secondary metabolites in alkaloids, saponins, triterpenoids, tannins, and flavonoids. The main flavonoids of Citrus include naringin, narirutin, and hesperidin. In Devy *et al.* study revealed multiple components in orange juice affected inhibiting the formation of fat accumulation. Flavonoids act as lipase inhibitors that prevent obesity.<sup>8,9</sup>

## METHODS

This research is an experimental study with a randomized control group design with pretest and post-test design with the research location in the Experimental Animal Laboratory at the Veterinary Research Institute, Banjarbaru-South Kalimantan. This research has passed the ethics test by the Health Research Ethics Commission-Faculty of Medicine, University of Lambung Mangkurat, with No. 10/KEPK-FKULM/EC/II/2022.

### Preparation of *C. amblycarpa* Hassk Peel extract

Lemon peel (*Citrus amblycarpa* Hassk) which had been dried and cut into pieces as much as 1500 g, was extracted by maceration method using 70% ethanol solvent for five days, and the residue was macerated again for three days. After the extract was collected, it was evaporated using a rotary evaporator to obtain a crude extract.

### Distribution Experimental Animal Group.

Thirty white male rats (*Rattus norvegicus*) Wistar strain aged 2-3 months were randomly divided into six groups, namely the normal diet group (G1), the high-fat diet group + aquadest (G2),

the high-fat diet group + orlistat (G3), and the high-fat diet group + *C. amblycarpa* Hassk peel extract (200 mg/kg BW (G4), 300 mg/kg BW (G5), 400 mg/kg BW (G6)). Before being given a high-fat diet induced, the rats were given seven days acclimatization period. A high-fat diet was given for six weeks until the rat's body weight reached 20% of the normal rat's body weight. They were then treated with *C. amblycarpa* Hassk peel extract for six weeks. Measurements of body weight, abdominal circumference, and body length were measured every week until the end of the treatment period.

### Induction of High Fat Diet (HFD) in Research Rats.

The negative control group rats were fed a normal (standard) rat diet and tap water ad libitum.

Meanwhile, the other Group's rats received a high-fat diet and water ad libitum. A high-fat diet is made with a particular composition that contains high fat consisting of a mixture of milled corn, krosvet, Hi-Provite feed, duck egg yolk, and wheat flour.

### Data Collection Rat Body Weight.

Rats body weight was measured using Krischef brand scales in grams. The weighing was carried out after the acclimatization process was completed and before starting the treatment, namely from day eight to week six in the obesity induction period every two days, then continued until the next six weeks of treatment.

## RESULTS

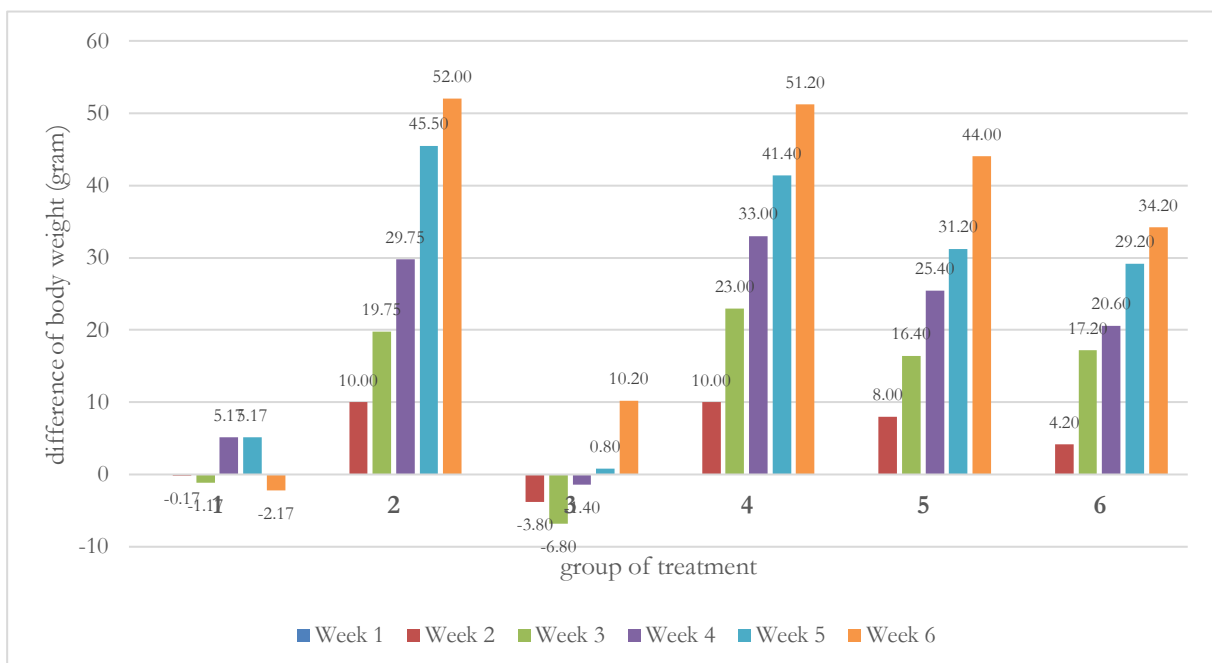


Figure 1. Diagram of the Average Changes in Body Weight of Treated Rats from Week 1 to Week 6 of the treatment period.

Table 1. Significance Values Between Treatment Groups

Treatment Group	Value Significance					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Group 1		0.048*	0.030*	0.017*	0.816	0.520
Group 2			0.000*	0.681	0.027*	0.009*
Group 3				0.000*	0.053	0.128
Group 4					0.009*	0.002*
Group 5						0.681
Group 6						

Information:

Group 1 = Group of rats with BR2 diet and Aquades (normal diet)

Group 2 = Group of rats with a high-fat diet and Aquades

Group 3 = Group of rats with a high-fat diet and orlistat

Group 4 = Group of rats with a high-fat diet and 200 mg/kg BW *C. amblycarpa* Hassk peel extract

Group 5 = Group of rats with a high-fat diet and 300 mg/kg BW *C. amblycarpa* Hassk peel extract

Group 6 = Group of rats with a high-fat diet and 400 mg/kg BW *C. amblycarpa* Hassk peel extract

## DISCUSSION

The results of this study are following several previous studies; Ezeigwe *et al.* revealed that the administration of *Citrus aurantifolia* orange juice for 12 weeks showed a significant difference in body weight with the control group. The concentration of *C. aurantifolia* reduced body weight in mice with a high-fat diet at 400 mg/kg BW. Lime peel is rich in antioxidants, one of which is flavonoids. The most dominating flavonoid group is a flavanone, with the main active compounds being hesperidin and naringin. Both are extensively known to have anti-obesity and anti-diabetic properties<sup>10-12</sup>. Hesperidin can regulate adipokines, cytokines, genes, and other markers in lipid metabolism to reduce fat accumulation and its role as anti-obesity<sup>13</sup>. Food supplementation containing a combination of glucosyl and hesperidin can reduce fat peroxidation and increase total antioxidant capacity in HFD-induced rats<sup>14</sup>.

These compounds are also very effective in increasing lipid metabolism in rats by enhancing triglyceride excretion and inhibiting lipid metabolism enzymes, including glucose-6-phosphate dehydrogenase (G6PDH) and fatty acid synthase<sup>15</sup>.

Naringin can function to lower LDL cholesterol and triglycerides in animals and humans. Naringin plays a role in metabolism, such as regulating fatty acids and cholesterol by utilizing glucose-regulating enzymes. Flavonoid compounds such as naringin and hesperidin can reduce triacylglycerol concentrations by up to 30%. In addition, naringin and hesperidin can increase plasma antioxidant activity and significantly inhibit the increase in plasma lipids.

The anti-obesity effect of naringenin depends on the decline of adipose tissue mass and the blockade of preadipocyte proliferation. This material suppresses preadipocyte proliferation without drawbacks to subsequent adipogenesis. In addition, naringenin stimulates fatty

acid oxidation in hepatocytes by increasing oxidation in peroxisomal in mice<sup>4</sup>. This compound also has the potential to increase the activity of various enzymes related to fatty acid oxidation in hepatocytes, such as acetyl-coenzyme A acetyltransferase (ACAT, also known as thiolase), acyl-coenzyme A oxidase, carnitine O-octanoyl transferase (COT, also known as carnitine medium chain/long chain acyltransferase), and 3-ketoacyl-coenzyme A<sup>16</sup>. In addition, naringenin prevents dyslipidemia and increases glucose metabolism by modulation of fibroblast growth factor 21 (FGR 21) decrease blood glucose and lipids independently<sup>17</sup>.

## CONCLUSION

*Citrus amblycarpa* Hassk peel extract significantly affected weight loss in HFD rats at doses of 300 mg/kg BW and 400mg/kg BW. This effect is due to the high flavonoid antioxidants in the skin of *C. amblycarpa* Hassk, such as hesperidin and naringin. Further in vivo research is needed in molecular biology to determine the mechanism of the effect of *C. amblycarpa* Hassk peel extract with several biological markers such as the hormone leptin, ghrelin, total fat levels, SGOT, and SGPT in rats fed a high-fat diet.

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