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Research Article

Preventive Effect of Jasmine Flower Ethanol Extract on MSG-High Fat Diet Induced in Male Wistar Rats

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ABSTRACT

Objectives: The study was conducted to determine the activity of jasmine flowers ethanol extract (JFEE) in preventing obesity and obtain its mechanism on obesity model in male Wistar rats.

Methods: This study was conducted by administering 2 mg/kg bw of MSG subcutaneously. It was combined with high-fat diet in obtaining obesity model. There were five groups including normal, control, JFEE with a dose of 100 mg/kg bw, JFEE with a dose of 200 mg/kg bw, and Orlistat with a dose of 21.67 mg/kg bw.

Results: Administration of JFEE 200 mg/kg bw was able to decrease significantly on parameters: gaining body weight onset, urine index, feces index, food index, serum level of triglycerides (TG), and total fat index compared to the control group.

Conclusion: JFEE with a dose of 200 mg/kg bw through this study was possibly effective in preventing weight gain and fat accumulation by preventing lipid absorption, besides diuretic effect and suppressing appetite.

Keywords: Jasmine flowers, obesity, high-fat diet, MSG.

INTRODUCTION

Obesity is a high risk factor for many diseases worldwide. Approximately 3.4 billion people in the world suffer from diseases caused by obesity, such as diabetes, cardiovascular disease, and cancer every year. According to data from WHO in 2008, around 1.4 billion adults (over 20 years) are overweight, more than 200 million men and 500 million women have obesity problem. Overall, more than 10% of the population are obese. It seems that currently, obesity becomes major health problems as either adults or children suffer from such a disorder. The incidence of obesity in childhood has reached epidemic levels in developed countries[1]. According to data of Health Research of Republic of Indonesia in 2013, the highest prevalence of obesity reached 15.4%, in which of 13.3% was classified as overweight[2]. Natural medicines are widely used in Indonesia, both empirically and as registered medicines. Many herbs have been developed to treat abnormalities of human body, such as obesity. One potential herb that may be lowering body weight is jasmine. Jasmine (*Jasminum sambac* (L.) Ait) is one of decorative plants that is commonly used in traditional wedding ceremonies in Indonesia. Several studies reported that jasmine flower possessed anti-lipidemic activity, anti-peptic ulcer, anti-acne, and antimicrobial effect[3]. The ability of jasmine flower to reduce lipid seems considerable to be observed further for preventing obesity pre-clinically. Therefore, this study was conducted to determine jasmine flower, *Jasminum sambac* (L.) Ait.) in preventing weight gain as well as other possible mechanisms.

MATERIALS AND METHODS

Chemicals

All chemicals reagents used in this study are pharmaceutical grade. Orlistat (Xenical®) was purchased from PT. Kimia Farma Pharmacy. Meanwhile, MSG was purchased from Ajinomoto.

Plant collection and identification

The fresh jasmine flower was collected from the Manoko garden in Lembang, Bandung, West Java. The flower was determined in School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia.

Preparation of extract

Flowers were collected, pulverized, and extracted by ethanol 96% through maceration for 24 hours. The procedure was repeated three times consecutively. Afterwards, obtained extracts were separated by using filter paper. The filtrate was then concentrated using rotary evaporator. The percentage of yield was determined by comparing weight of extract over weight of crude herbs. Before analysis, it was kept in refrigerator 4°C. For pharmacological studies, the extract was dispersed in 0.3 % CMC Na.

Phytochemical screening

The jasmine flowers ethanol extract (JFEE) was subjected to preliminary phytochemical screening for plant constituents. Secondary metabolites that were observed were alkaloid, flavonoid, quinone, tannin, and

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Table 1: Composition of experimental diets.

Component	Normal (g/kg)	High fat-diet (g/kg)
Rice flour	-	300
Corn flour	250	200
Wheat flour	340	150
Fish meal	160	100
Mung bean flour	140	100
Beef tallow	70	200

Table 2: Characterization result from herb and extract of jasmine flower.

Test	Result (%w/w)	
	Herbs	Extract
Water content (%v/w)	-	12.5
Volatile matter content	23.4	-
Water soluble content	-	23.24
Ethanol soluble content	-	60.48
Insoluble ash content	0.34	0.42
Specific gravity (g/ml)	-	1.16
Yield	-	16.33

- = parameters were not quantified

Table 3: Serum levels of triglyceride.

Group	Serum TG levels (mg/dl)
Normal	168.33 ± 27.97
Control	280.16 ± 30.16$^{\#}$
JFEE 100 mg/kg b.w	203.80 ± 22.45$^{*\#}$
JFEE 200 mg/kg b.w	127.50 ± 28.52$^{*\#}$
Orlistat 21.67 mg/kg b.w	191.80 ± 25.41*

*: Significantly difference compared to the control group at $p<0.05$; (n=5)
#: Significantly difference compared to the normal group at $p<0.05$; (n=5)

Table 4: Food Index Determination.

Groups	Food Index (%) w/w
Normal	19.08 ± 1.93
Control	30.08 ± 5.16
JFEE 100 mg/kg b.w	19.67 ± 2.38*
JFEE 200 mg/kg b.w	15.00 ± 1.92$^{*\alpha}$
Orlistat 21.67 mg/kg b.w	22.50 ± 3.66*

*: Significantly different compare to the control group at $p<0.05$;
α: Significantly difference compare to the orlistat group at $p<0.05$. (n=5)

steroid/triterpenoid.

Experimental Design

The pharmacological studies was conducted at Laboratory of Experimental Animals, School of Pharmacy, Bandung Institute of Technology, Indonesia. All method has been approved by Ethical Comission, School of Pharmacy, Bandung Institute of Technology, approval document No.10/KEPHP-ITB/03-2015. Experiment was conducted according to Deeman's protocol[4]. Twentyfive male Wistar rats (4 week, 90 to 110 gram in weight) were used in this study. They were kept under standard environment for laboratory animals. Before the study, they were acclimatized for seven days. Afterwards, rats were divided randomly into five groups in which a group consisted of five rats. Groups that were observed include normal, control, JFEE 100 mg/kg, JFEE 200 mg/kg, and Orlistat 21.67 mg/kg. All groups, except the normal received MSG 2 mg/kg for the first 5 days through subcutaneous injection along with high fat diet. They were given high fat food for 42 days consecutively. However, the normal was fed by standard commercial chow. All rats had free access to water during the experiment. The composition of both normal and high fat chow was presented on table 1. Starting from day 22, either JFEE or orlistat was given to rats according to their group, meanwhile control group received CMC Na suspension only. Twenty-four hour after the last day of experiment, all rats were sacrificed by using carbon-dioxide. After the euthanasia procedure, liver, perirenal and perianal fat were immediately isolated and were stored in freezer -20°C until analyzing time.

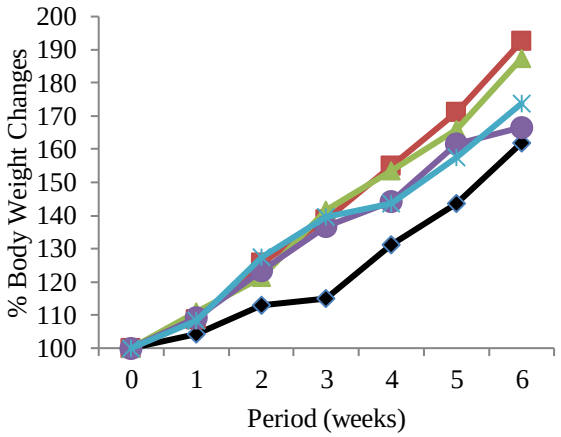

Figure 1: Effect of the jasmine flowers extract ethanol administration on preventing weight gain. (⬥) normal, (■) control, (▲) JFEE 100 mg/kg , (●) JFEE 200 mg/kg, (✳) Orlistat 21.67 mg/kg.

Evaluation

Body weight from each group was monitored every week throughout periods. Other parameters which were also determined include food index, urine index, feces index, and serum level of triglycerides. Obtained feces during the study was observed further to examine the presence of oil and/or fat by exposing them to the filter paper.

Statistical Analysis

Results were analyzed using the Statictical Package for the Social Sciences (SPSS) version 16 by applying Analysis of Variance (ANOVA) with the post-hoc LSD. A value below 0.05 was considered as statistically significant. The results were expressed as mean ± standard deviation.

RESULTS

Phytochemical screening of flowers extract

With regards to the herbs determination in Herbarium Bandungense, School of Natural Science and Technology ITB, the tested herb is *Jasminum sambac* (L.) Ait. The results of characterization was shown in the table 2. The yield value of the extract was 16.33%. Secondary

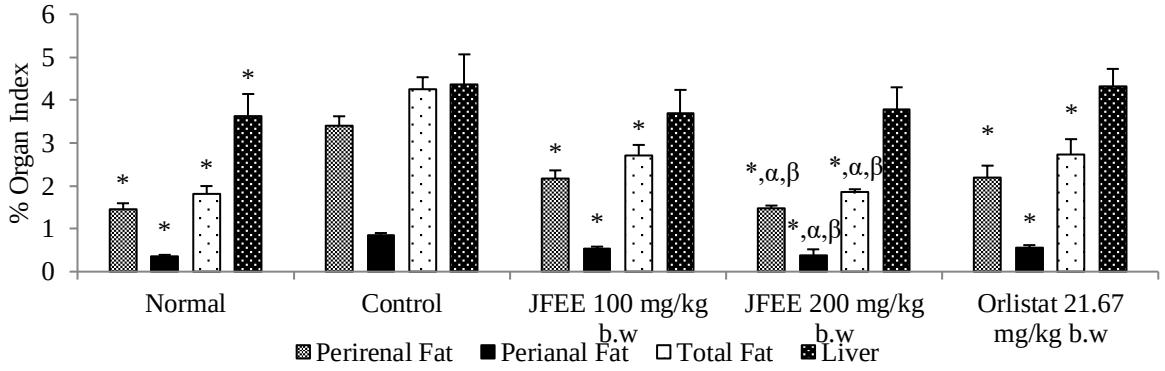

Figure 2: Effect of the jasmine flowers extract ethanol administration on fat index and liver index at the end of the experiment. The sign *: Significantly difference compare to the control group at $p<0.05$; α: Significantly difference compare to the orlistat group at $p<0.05$; β: Significantly difference compare to the JFEE 100 mg/kg b.w group at $p<0.05$. (n=5)

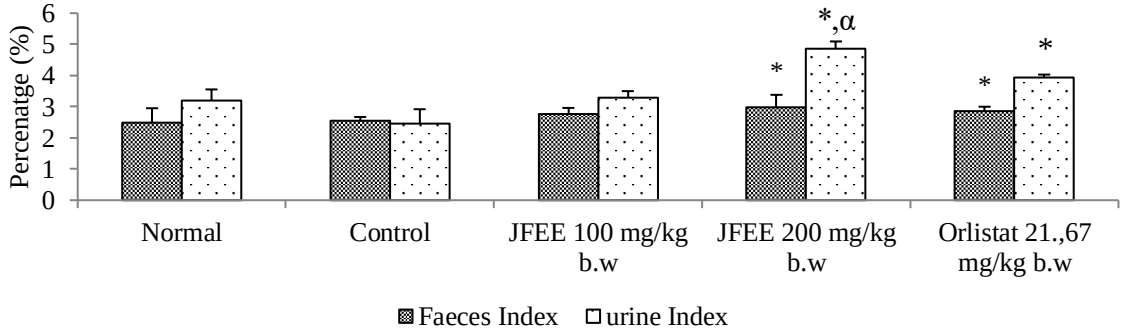

Figure 3: Effect of administration tested substances on feces and urine index at the end of the experiment. *: significantly difference from the control group ($p<0.05$); α: Significantly difference compare to the orlistat group ($p<0.05$). (n=5)

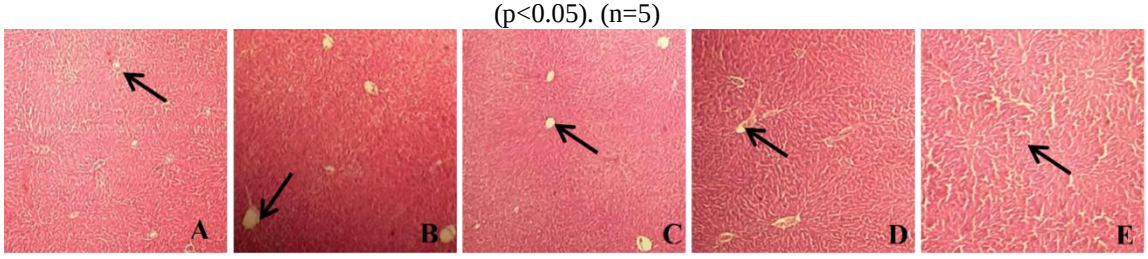

Figure 4: Histology of liver from each group after 21-day treatment on microscope with magnification of 40 times. A: normal group, B: control group, C: JFEE 100 mg/kg b.w; D: JFEE 200 mg/kg b.w; E: Orlistat 21.67 mg/kg b.w. The arrow is pointed at small circle where the fat existed.

metabolites that existed in the extract of jasmine flowers include flavonoid, tannin, and steroid/triterpenoid.

Changes in Body Weight

The body weight change throughout experimental period is represented in fig.1. There were significant differences in body weight between control and normal group ($p<0.05$). Even though the body weight of all groups grew throughout the periods, elevation of that in a group treated with JFEE 200 mg/kg was lower than that of the control group.

Serum levels of triglyceride

Determination of triglyceride serum levels was shown on the table 3. The measurement showed a significant increase of triglyceride serum levels on the control group comparing to normal group. The results proved that administration of high fat diet caused elevation in the body. Group of JFEE 200 mg/kg showed lower triglyceride levels remarkably compared to the control group. *Organ Index*

In terms of perirenal and perianal fat, the group treated with JFEE 100 mg/kg and 200 mg/kg were lower than that of the control group. At a dose of 200 mg/kg, jasmine extract possessed the ability to reduce both perirenal and perianal fat compared to the orlistat treated group. Treatment of JFEE with dose of 100 mg/kg and 200 mg/kg b.w seem ineffective on reducing the liver enlargement, which was induced by administration of high fat food[5].

Urine and Feces Index

According to the fig.3, the group treated with JFEE 200 mg/kg had significant lower urine index compared to the

control group ($p<0.05$). The similar figures were presented in measurement of feces index, in which JFEE 200 mg/kg showed lower value than control ($p<0.05$). Moreover, there were oil spots when rats' feces exposed to the filter paper.

Histopathological Studies

Examination on liver histology displayed that, in the normal group, hepatocytes are structurally organized with a few small circles. As shown in fig.4, there were small circles (pointed by an arrow) distributed in the liver tissues that was seemingly used as fat storage. However, the size of each spots was higher in the control group. It is most likely mean that without any treatment, absorbed fat will be stored particularly in the liver with greater size of vesicles. This histopathological findings was relevant to measurement of liver index which was shown on figure 2. There was a significant increase in the liver index value in all induced group compared to normal group (fig.2, liver index parameter).

DISCUSSION

Several secondary metabolites that were found in JFEE include flavonoid, tannin, and steroid/triterpenoid. Similar to other herbs, natural medicines contain an abundance of constituents that may have certain pharmacological effects. Jasmine flower extract of *Jasminum sambac* (L/) Ait contains the mixtures of coumarins, cardiac glycosides, essential oils, flavonoids, phenolics, saponins, and steroids[6]. Mittal reported that flavonoid and steroid/triterpenoid may contribute to inhibit lipid absorption[7]. Obesity is considered as a major problem in the world, particularly in developed countries. Researchers are still trying to uncover the mechanism of body-weight regulation and it is still not well understood. Basically, body weight is affected by three components: food intake, nutrient turnover and thermogenesis; and body fat stores[8]. The following feedback mechanism is very complex process including changes on these three components. This study focused on effect of administration of *Jasminum sambac* on modulating food intake and fat storage in obesity-rat-model. To induce obesity, we utilized monosodium glutamate through subcutaneous injection. Monosodium glutamate will broke the arcuate nuclei in the hipothalamus. Arcuate nuclei is a region that is responsible for accepting the signal from leptin inside the adipose tissue, which directly responsive to a pathway of hunger and signals from hormone and metabolites[9]. When there is a damage, there will be loss control of appetite which causes hyperphagia. Recent studies show that with administration of MSG for 5-10 days with the dose of 2 mg/gram bw subcutaneously in an early age of rat, can disrupt the function of arcuate nuclei in hypothalamus that leads to uncontrolled hunger[10]. Along with administration of MSG in the first 5 days of experiment, the rats were fed by high fat diet. The purpose was to induce fat accumulation, so the fat component was doubled compare to normal (table 2)[10]. Recent studies showed that weight gain would be induced within 4 to 6 months[11]. This experiment was conducted by combining MSG method and high fat diet for a 1.5 month. However, we did not successfully induce obesity as the weight gain was no more than 20% compared to the normal group. According to Adnyana, obesity was achieved until the difference of body weight between control and normal group is approximately 20%[5]. On the table 2, there was a noticeable increased in the level of triglyceride serum. Rats which were given high fat diet were fed more energy and gaining weight significantly to rats accessing standard chow. They had more fat intake which lead to higher metabolism of lipid. Lipid that has been absorbed through gastrointestinal tract are disrupted into free fatty acid and triglyceride. Triglyceride will be stored in adipose tissues and in the liver[12]. The administration of JFEE at dose of 200 mg/kg b.w showed a remarkable lower weight gain compared to the control group. This study showed that JFEE at 200 mg/kg bw may reduce body weight gain. Similar results had been reported by Yuniarto[13], whereby JFEE at the dose of 100 mg/kg and 300 mg/kg bw were effective to reduce body weight in high fat induced mice. Observing rat feces, there was a spot of oil in the filter paper in groups treated by JFEE. We assumed that even though rats were fed by high fat chow, it is not absorbed properly and passed through the gastrointestinal. In previous study, in vitro assay of JFEE 100 mg/kg and 300 mg/kg bw were able to inhibit pancreas lipase enzyme which may prevent lipid absorption[13]. In table 3, group treated by JFEE 100 mg/kg and 200 mg/kg presented significant lower food index compared to the control ($p<0.05$). It showed that jasmine flower extract ethanol may suppress the appetite, lowering the weight gain and prevent the obesity with the high-fat diet method to induce obesity, the fat will be stored in many organs, including liver. Using the high fat diet method in long period, for instance two months, may cause hepatic steatosis which is characterized by existence of fat vesicles[11]. The longer induce period, the bigger vesicle would be observed. In this study, the period was relatively short and the vesicles are considerably small in size as in this study we utilized preventive method. However, comparing to the control group, the number of vesicle is much lower than that of the control group.

CONCLUSION

The study has proven that JFEE possess potent activity in preventing weight gain on obese rat model. There are several contributed ways of JFEE as anti-obesity, including: inhibiting lipid absorption, having diuretic properties, and suppressing appetite.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest in this study.

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