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Research Article

KUMIS KUCING (*ORTHOSIPHON STAMINEUS* [BENTH.]) LEAVES ETHANOL EXTRACT AS ANTI-OBESITY AGENT IN HIGH-FAT DIET-INDUCED OBESE MICE

ARI YUNIARTO[1]*, HENI PURWANI[1], DADANG JUANDA[2], FINNA SETIAWAN[3], IKA KURNIA[1]

[1]Pharmacology Research Group, Bandung School of Pharmacy, Bandung, Indonesia. [2]Pharmaceutical Biology Research Group, Bandung School of Pharmacy, Bandung, Indonesia. [3]Pharmacology and Clinical Pharmacy Research Group, Bandung Institute of Technology, Bandung, Indonesia. Email: ariyuniarto@yahoo.co.id

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ABSTRACT

Objective: The leaves of kumis kucing (*Orthosiphon stamineus* [Benth.]) are used as folk medicine for lifestyle-related diseases such as hypertension, hyperlipidemia, and diabetes mellitus in Southeast Asia countries, especially Indonesia. Obesity is closely associated with lifestyle-related disease. The objective of this research was to evaluate the effect of kumis kucing leaves ethanol extract in high-fat diet-induced obese mice, and its ability to inhibit pancreatic lipase enzyme activity *in vitro*.

Methods: Obese mice groups were fed a high-fat diet for 30 days and treated with kumis kucing leaves ethanol extract for 2 weeks. The measurement parameter includes body weight, organ weight, and visceral fat mass. Experiments were also carried out to clarify the effect of kumis kucing leaves ethanol extract inhibited pancreatic lipase enzyme activity *in vitro*.

Results: Kumis kucing-treated groups showed a significant decrease in body weight, organ weight, and visceral fat mass after 2 weeks treatment compared with untreated groups. Furthermore, kumis kucing leaves ethanol extract showed potency as the pancreatic lipase activity inhibitor *in vitro*.

Conclusion: Kumis kucing leaves ethanol extract can inhibit the development of obesity in high-fat diet-induced obese mice. The effect appears mediated by inhibiting the pancreatic lipase activity. The results from this study suggested that the kumis kucing leaves may have a considerable impact as potential anti-obesity agents.

Keywords: Kumis kucing, Extract, Obesity, High-fat diet.

INTRODUCTION

Recently, obesity has become an epidemic on a global scale and one of the world's most serious public health problems [1]. Obesity condition increases the risk of morbidity and mortality. World Health Organization (WHO) defines obesity as abnormal or excessive fat accumulation which influences body health. In 2005, WHO gave estimation that obese adults in the world average 400 million and predicted that in 2015 it will increase to 700 million adults with obesity [2]. Obesity results from disequilibrium between energy intake and energy expenditure [3]. Therefore, inhibition of digestion and absorption of dietary fat can be useful in treatment of obesity. Furthermore, obesity is with various diseases, including Type 2 diabetes mellitus (DM), hypertension, dyslipidemia, cardiovascular diseases, atherosclerosis, and cancer [4,5].

Diet modification and activity as obesity treatment have low effectiveness in long-term weight loss and maintenance. Several studies suggest that adjunctive therapy in combination with exercise and nutrition therapy increase efficacy. However, some of these medications have been correlated with serious adverse effects [6].

Orlistat, is one of the two clinically approved drugs for obesity treatment, has been shown to act by inhibiting pancreatic lipase enzyme. The long-term effects of Orlistat administration are oily stools, oily spotting, and flatulence, among others. So, the potential of natural products for the treatment of obesity might be an excellent alternative strategy for the development of safe and effective anti-obesity drugs [7].

Kumis kucing or java tea classified under the family Lamiaceae or Labiatae. Kumis kucing has some synonyms, such as *Orthosiphon aristatus* (Blume) Miq, *Orthosiphon longiflorum* Ham., *Orthosiphon grandiflorum et aristatum* Bl., *Orthosiphon grandiflorus* Bold., and *Orthosiphon spiralis* Merr. This plant can be found in Southeast Asia (Indonesia, Malaysia, Myanmar, and Vietnam) and Australia. Kumis kucing is one of the popular traditional plants in Southeast Asia, especially in Indonesia which used to treat several diseases such as arthritis, DM, hypertension, kidney disorder, inflammation, and menstrual disorders [8].

Several studies identified that kumis kucing contains important compounds including polyphenols, flavonoids, terpenoids, sterols, orthosiphols, saponins, caffeic acid, and oleanolic acid. The compounds were known to be associated with anti-obesity [9]. Therefore, the aim of the present study is to evaluate the effect of kumis kucing leaves ethanol extract as anti-obesity agents in high-fat diet-induced obese mice model.

METHODS

Reagents

All reagents in this study were of analytical grade. Porcine pancreatic lipase, oleic acid, and bovine serum albumin were obtained by Sigma-Aldrich. Orlistat (Xenical)® was purchased from Kimia Farma Pharmacy.

Plant material and identification

Kumis kucing leaves were obtained from The Centre for Research and Development of Medicinal Plants and Traditional Medicine, Tawangmangu, Central Java, Indonesia. Plant identification was done in Laboratory of Taxonomy, Faculty of Mathematics and Natural Sciences, Padjajaran University, Bandung, Indonesia.

Preparation of extract

The leaves of kumis kucing were dried and then powdered. The powder of kumis kucing was extracted with 96% ethanol by maceration method and evaporated by *Rotary Evaporator*. This kumis kucing leaves ethanol extract was used for the *in vivo* and *in vitro* assays.

Phytochemical screening of the kumis kucing extract
Phytochemical screening was performed to monitor the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

Pancreatic lipase enzyme *in vitro* assay
Pancreatic lipase enzyme activity was determined by measuring the release rate of oleic acid from sesame oil. This method is based on Han *et al*. [10] but with a slight modification. The substrate (5 ml in a 10 ml tube) was prepared through sonication process for 5 minutes. The substrate contained 15 mmol/l sesame oil, 1 mmol/l NaCl, 1 mmol/l $CaCl_2$, 10 mg of bovine serum albumin/ml, and phosphate buffer solution with pH 8.0. After sonication, the substrate was incubated with 50 µl of *porcine* pancreatic lipase enzyme and kumis kucing extract for 30 minutes at 37°C with various concentrations. 30 minutes after incubation at 37°C, the substrate was added to 3 ml of a 1:1 (v/v) mixture of chloroform and n-heptane, extracted by shaking on the tube for 10 minutes in a shaker. The mixture was centrifuged at 2000 rpm for 10 minutes. The upper aqueous phase was removed, and the lower phase was added with a copper reagent with volume 0.5 ml. The tube was shaken again for 10 minutes, was centrifuged at 2000 rpm, and 0.5 ml of the upper phase (organic phase) was added with 0.5 ml diethyldithiocarbamate-sodium solution. The absorbance was then measured at 480 nm.

The inhibitory activities of kumis kucing leaves ethanol extract and orlistat, a positive control of pancreatic lipase were measured at concentrations of 1000, 100, 10, 1, and 0.1 µg/ml. The measurements were performed in triplicate, and the IC_{50} value, the concentration of the extract that results in 50% inhibition of maximal activity was determined.

Anti-obesity activity *in vivo* assay
The anti-obesity activity of kumis kucing leaves ethanol extract has been examined in male *Swiss-Webster* mice, and all animal experiments were conducted under institutional ethical guidelines. Animal study was conducted in the Laboratory of Pharmacology, Bandung School of Pharmacy, Indonesia. 25 *Swiss-Webster* mice, of 2-3 months, weighed about 25 and 30 g, were adapted under room temperature. The animals were fed with standard diet and drink *ad libitum*.

Following adaptation period, the healthy mice were used in the experiments. The mice were randomly divided into five groups of five mice each. The experimental group consisted of the following groups control group, high-fat diet group, orlistat 15.6 mg/kg, kumis kucing leaves extract 100 mg/kg, and kumis kucing leaves extract 200 mg/kg. The control group was fed standard animal laboratory diet and other groups were fed high-fat diet for 30 days.

The measurement parameter included body weight, organ weight, and visceral fat mass. Body weight was measured daily. At the end of the experiment, the mice were sacrificed, and the organs collected. Organs collected included liver, spleen, testicle, kidney, and visceral fat mass such as perirenal fat, perianal fat, retroperitoneal fat, and epididymal fat.

Statistical analysis
Statistical comparisons of the results of the anti-obesity assay were performed using the one-way Analysis of Variance method, coupled with the *post-hoc* least significant difference test. A value of $p<0.05$ was used to denote statistical significance. All data were expressed as mean ± standard deviation of the mean (SD) for each group.

RESULTS AND DISCUSSION

Composition of experimental diet
The composition of the experimental diet is shown in Table 1 and its was composition based on Adnyana *et al.* [11]. By the end of 30 days acclimatization period, the body weight of the obese mice group (including high-fat diet group, orlistat group, and kumis kucing extract group) was approximately 20% higher than the negative control group.

Result of phytochemical screening kumis kucing leaves ethanol extract
The percentage of yield of kumis kucing leaves ethanol extract was found to be 6.11%. Result of phytochemical screening as shown in Table 2. Phytochemical screening of kumis kucing leaves ethanol extract showed the presence of flavonoids, saponins, tannins, quinones, and steroid/triterpenoids. Iswantini *et al.* [12] showed that phytoconstituents, such as flavonoids, saponins, alkaloids, tannins, and steroids/triterpenoids, have effect on obesity and contribute to inhibit pancreatic lipase enzyme activity. The phytoconstituents which were derived from plants such as flavonoids, terpenoids, phenolic acids, and other groups exhibit potential effect as anti-obesity agents.

Effect of kumis kucing leaves ethanol extract on pancreatic lipase activity
The inhibitory activities of kumis kucing leaves ethanol extract against porcine pancreatic lipase are shown in Fig. 1. The kumis kucing leaves ethanol extract inhibited pancreatic lipase activity by 38.55%, 40%, 44.26%, 57.5%, and 63.92% at concentrations 0.1, 1, 10, 100, and 1000 µg/ml *in vitro*, respectively. Orlistat, a pancreatic lipase inhibitor used as anti-obesity agent, inhibited the enzyme activity at the concentrations 0.1, 1, 10, and 100 µg/ml, respectively. The kumis kucing leaves ethanol extract and Orlistat had IC_{50} as 53.68 µg/ml and < 0.1 µg/ml, respectively.

Effect of kumis kucing leaves ethanol extract on body weight
As shown in Fig. 2, the body weight gain was reduced by administration of kumis kucing leaves ethanol extract at the dose 100 mg/kg and 200 mg/kg compared with high-fat diet group. Subsequently, the mean body weight and the body weight gain in these groups were significantly lower than high-fat diet group. Body weight of the kumis kucing leaves ethanol extract group at the dose 100 mg/kg was significantly different to the control group but comparable with high-fat diet group.

Table 1: Composition of the experimental diet

Composition	Standard diet (g/kg)	High-fat diet (g/kg)
Corn starch	250	250
Fish flour	160	160
Bean flour	140	140
Wheat flour	410	130
Fat	-	320
Vegetable oil	40	-

Table 2: Result of phytochemical screening of kumis kucing leaves ethanol extract

Group	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Quinones	+
Steroid/Triterpenoids	+

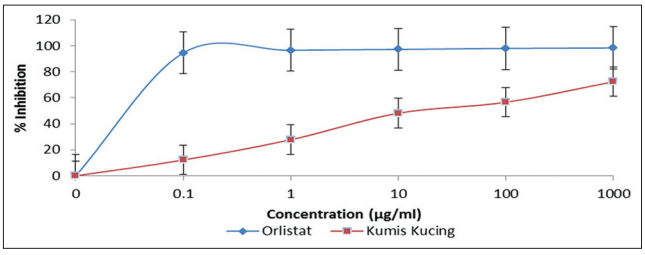

Fig. 1: Inhibition of pancreatic lipase activity by kumis kucing extract and orlistat

Table 3: Result of kumis kucing leaves ethanol extract on organ weight

Group	Organ weight (g)			
	Liver	Spleen	Kidney	Testicle
Control group	1.47±0.04	0.12±0.01	0.51±0.02	0.14±0.02*
High-fat diet group	1.91±0.26	0.11±0.03	0.49±0.04	0.31±0.03
Orlistat 15.6 mg/kg	1.66±0.50	0.10±0.02	0.52±0.05	0.28±0.02
Kumis kucing extract 100 mg/kg	1.76±0.19	0.07±0.01*	0.51±0.05	0.24±0.05
Kumis kucing extract 200 mg/kg	1.50±0.48	0.04±0.02*	0.36±0.10*	0.21±0.04*

(*) Data represent the mean±SD of observations from 5 mice, significantly different from high-fat diet group at p<0.05, SD: Standard deviation

Table 4: Result of kumis kucing leaves ethanol extract on visceral fat mass

Group	Visceral fat mass (g)			
	Retroperitoneal	Perirenal	Perianal	Epididymal
Control group	1.66±0.17	0.21±0.02	0.29±0.17*	0.40±0.05
High-fat diet group	3.23±0.17	0.41±0.10	1.15±0.03	0.94±0.16
Orlistat 15.6 mg/kg	2.50±0.13	0.41±0.04	0.31±0.03	0.94±0.09
Kumis kucing extract 100 mg/kg	1.33±0.25*	0.14±0.01*	0.08±0.01*	0.42±0.04
Kumis kucing extract 200 mg/kg	0.37±0.22*	0.09±0.05*	0.07±0.01*	0.15±0.01

(*) Data represent the mean±SD of observations from 5 mice, significantly different from high-fat diet group at p<0.05, SD: Standard deviation

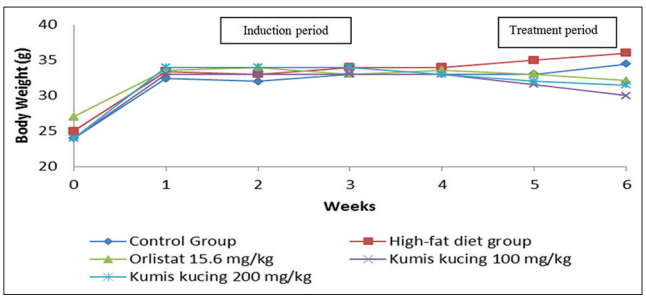

Fig. 2: Effect of the kumis kucing leaves ethanol extract on body weight

Effect of kumis kucing leaves ethanol extract on organ weight

High-fat diet group showed significantly high liver and testicle weight in comparison to the control group, but not spleen and kidney weight (Table 3) (p<0.05). Conversely, groups treated with kumis kucing leaves ethanol extract with dose 100 mg/kg and 200 mg/kg didn't effective to decrease liver weight caused by fat accumulation. Furthermore, kumis kucing extract at the dose of 100 mg/kg and 200 mg/kg showed a significant decrease in spleen weight compared to high-fat diet group. As shown in Table 3, kumis kucing leaves ethanol extract at the dose of 200 mg/kg showed a significantly lower kidney weight and testicle weight compared to the high-fat diet group (p<0.05).

Effect of kumis kucing leaves ethanol extract on visceral fat mass

As shown in Table 4, kumis kucing leaves ethanol extract at 100 mg/kg and 200 mg/kg showed a significantly lower retroperitoneal, perirenal, and perianal weight than that of the high-fat diet group (p<0.05), but less effective to decrease fat accumulation in epididymal. It can be concluded that kumis kucing leaves ethanol extract 100 mg/kg and 200 mg/kg might have anti-obesity effects by preventing visceral fat accumulation.

CONCLUSION

The anti-obesity effect of kumis kucing leaves ethanol extract in high-fat diet-induced obese mice might be mediated by inhibiting pancreatic lipase activity. The present study showed that kumis kucing leaves ethanol extract may have a considerable potential as anti-obesity agents because of its effect on body weight, visceral fat parameters, and organ parameters in obese mice.

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