Potential Role of *Centella asiatica* and *Sauropus androgynus* in High-Fat and High-Fructose Diet-Induced-Obesity Animal Model

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**ABSTRACT**

Obesity is a condition characterized by excessive fat accumulation, leading to metabolic syndrome diseases including hyperglycemia, hyperlipidemia, hyperuricemia, and hypertension. Efforts to reduce obesity can minimize the risk of the disease. Therefore, this study aimed to determine the antiobesity activity of *Sauropus androgynus* L. Merr (SA) and *Centella asiatica* L.Urban (CA) combination in Swiss Webster obese mice model. A total of 36 mice were randomly grouped into six groups, including the control (receiving drug carriers), positive (receiving drug carriers), and standard (receiving orlistat 15.6 mg/kg BW). A total of three groups received a combination of SA & CA with a dosage ratio of SA:CA namely 25:25 mg/kg BW, 25:50 mg/kg BW, and 50:25 mg/kg. All groups except the normal were induced with obesity using a high-fat and high-fructose (HFHF) diet for 28 days. Subsequently, body weight, feed, feces, organ, fat index, as well as serum triglyceride levels, and percent inhibition of lipid peroxidation using malondialdehyde (MDA) absorbance were measured. The results showed that there were significant differences in parameters of body weight, feed, feces, organ, fat index, serum triglyceride level, lipid-peroxidation inhibition, and histology of adipocytes between groups of animals treated with the combination extract compared to the positive control (p<0.05). Therefore, it was concluded that the combination of SA and CA had antiobesity activity.

**Keywords:** *Centella asiatica*, Obesity, *Sauropus androgynus*, Triglycerides

**INTRODUCTION**

Obesity is considered a noncommunicable disease, serving as the initial indicator for the onset of numerous non-infectious diseases prevalent in developing and developed countries. This condition is also defined as a long-term imbalance between calories taken and those burned, leading to the formation of a negative habit pattern. Poor diet, an unhealthy lifestyle, a lack of exercise, and an excessive intake of calories are the leading causes of obesity (Ministry of Health of the Republic of Indonesia, 2018). High-calorie intake is typically caused by eating foods high in fat and sugar, while low-calorie burn results from habitual inactivity (Romieu et al., 2017). In Indonesia, obesity is a problem for all wealthy quintiles, affecting both children and adults (Mahendradhata et al., 2017).

The proportion of epidemics on a global scale suggests that obesity is a significant health issue requiring serious attention. Therefore, studies on this topic are commonly carried out to prevent the emergence of numerous illnesses that lead to metabolic syndrome. Globally, approximately 1 billion adults suffer from obesity. Among this group, people with clinical obesity are estimated to reach 300 million, playing an active role as the main factor in chronic diseases and disabilities (World Health Organization, 2021). Obesity can trigger metabolic syndrome or conditions related to dyslipidemia, hypertension, and insulin resistance resulting from low-grade chronic inflammation. Type 2 diabetes mellitus, heart or cardiovascular disease, osteoarthritis, and cancer development are all effects of metabolic syndrome. In addition, physical health issues occur, and metabolic syndrome can also result in psychological disorders such as depression (Łopuszańska et al., 2014).

Based on the results of Basic Health Research (*Riset Kesehatan Dasar*) in 2018, the number of obese people in adults increased significantly, namely 21.8%, compared to data in 2013, at 14.8%. The prevalence rate of obese...
people increased as well, rising from 11.5% in 2013 to 13.6% in 2018 (Ministry of Health of the Republic of Indonesia, 2018). These data show the widespread prevalence of obesity in Indonesian society. Due to a rise in body weight above 20% of the usual, obesity can result in abnormal serum lipoprotein levels in the blood and triglycerides have an essential role as the primary fat store in adipose tissue. People with obesity typically have higher triglyceride levels than those with average weight. In these people, excess fat builds up, increasing the amount of free fatty acids (FFAs), which are subsequently digested by LPL (Lipoprotein Lipase) in endothelial tissue (Sakers et al., 2022). Obesity can also cause imbalances in redox homeostasis, causing oxidative stress. Consumption of foods high in fat leads to lipid peroxidation, free radicals, and reactive oxygen species. Free radicals are created by lipid peroxidation, typically occurring in every cell membrane structure. Studies using the obese mice model have shown that obesity increases plasma lipid peroxidation (Agrawal & Singh, 2017).

Current obesity treatment options aim to restore energy balance primarily by suppressing appetite or interfering with lipid absorption in the small intestine. Due to the rapid rise in obesity prevalence worldwide and the ineffectiveness of current medical therapies, efforts to develop new pharmacological therapies for obesity have been stepped up (Buhman et al., 2002). The use of natural resources for prevention or treatment also contributes to developing obesity management therapies, including Sauropus androgynus (L.) Merr (SA) and Centella asiatica (L.) Urban (CA). SA and CA are both commonly found in Indonesia and are generally used for treating diseases by the local people. According to several studies, SA contains tannins and saponins, which have antiobesity properties. Consuming SA juices or beverages may help people lose weight, as well as manage hypertension, and hyperlipidemia (Yu et al., 2006). Moreover, SA has been reported as an antiobesity agent at a dosage of 200-400 mg/kg BW, comparable to Orlistat (Patonah et al., 2018). CA extract also showed antiobesity in histopathological studies at dosages of 100, 200, and 400 mg/kg BW, as evidenced by mild cytoplasmic fat infiltration and granular degeneration compared to the normal and control groups (Begum & Swamy, 2019). Therefore, this study aimed to examine the potential of combining the dosages of two different plants to provide better efficacy with a lower amount than the single extract.

**MATERIALS AND METHODS**

**Plants material authentication**

The leaves of Sauropus androgynus (SA) and Centella asiatica (CA) were obtained from the Research Center for Spice and Medicinal Plants (BALITTO), Bogor, West Java. Plant determination was carried out at the Taxonomy Laboratory of Padjadjaran University and the presence of active compounds contained in the samples was tested qualitatively.

**Extracts preparation**

The dried SA leaves and CA herbs weighing 200 grams were macerated using 96% ethanol for three days, with solvent replacement carried out every 1 x 24 hours. Due to the low water content, 96% ethanol was used to ease the process of separating solvents following extraction. The macerate was thickened using a rotary evaporator at 50°C and 40 rpm until a viscous extract was obtained. The thick extract was then collected and its weight was measured gravimetrically.

The dosages of SA and CA extracts used in this study were I (CA-SA 25:25 mg/kg), II (CA-SA 50:25 mg/kg), and III (CA-SA 25:50 mg/kg). These dosages were based on an earlier study conducted by Patonah et al., (2018) showing considerable antiobesity activity with single-extract SA. Each extract was weighed on an analytical balance according to a predetermined dosage ratio. The weighted extract was dissolved in 4 mL of a 0.5% CMC-Na solution, then using a funnel, the mixture was transferred to a 10-mL volumetric flask. Distilled water was further added to the volumetric flask up to the limit, and the mixture was shaken until homogenous.

**Preparation of Normal and High-fat and high-fructose diet (HFHF)**

The standard feed contained 25% corn starch, 16% fish flour, 14% mung bean starch, 41% flour, and 4% vegetable oil. In contrast, the HFHF feed contained 25% corn starch, 16% fish flour, 14% mung bean starch, 13% flour, and 32% beef fat. The ingredients were mixed and kneaded into an even dough, then formed lengthwise and dried under a 40-watt incandescent lamp for three days. The dried feed was stored in an airtight container (Patonah et al., 2018), while fructose of 20 grams was dissolved in 100 mL of aquadest, and the solution was used as the drinking water.
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Animals protocol
The animal model used was the Swiss Webster strain of mice, approved by the Health Research Ethics Commission of the Faculty of Medicine, Universitas Padjadjaran, with No. 491/UN6.KEP/EC/2022. A week before the test, the mice were acclimatized under an experimental setting by being given standard feed and drink. To maintain the cleanliness of the cage, the husks were also changed every three days.

A total of 36 mice were randomly divided into six groups, including 1 (negative control given 0.5% CMC-Na), 2 (positive control received 0.5% CMC-Na), 3 (comparator treated with 15.6 mg/kg Orlistat), 4 (dosage I), 5 (dosage II), and 6 (dosage III). Drugs and extracts were administered orally every day. All groups, except from 1, were given an HFHF diet for 28 days, to induce obesity, and mice with a weight greater than 20% of the initial weight (T0) were obtained. Feed and leftovers were weighed daily, body weight was measured three times a week, and the collected feces were weighed twice a week. On the last day, euthanasia was performed using CO₂ and the mice were dissected with surgical scissors to isolate the liver, kidney, and heart for organ index analysis. Fats attached to the retroperitoneal and epididymal parts were carefully separated for fat index analysis, while the blood was taken for triglyceride level analysis.

During the triglyceride test preparation step, 10 μL of aquadest was prepared, and 1000 μL of Proline Triglycerides FS 10’ reagent was added for blank measurement. About 10 microliters of serum were added to a 1000 microliter reagent, then the mixture was incubated for 20 minutes at 20-25°C. After setting the solution, the triglyceride levels were measured for 60 minutes using a Microlab 300 at a wavelength of 500 nm.

The malondialdehyde (MDA) levels were measured from the liver organs of mice using the previous methods. The measurement of lipid peroxidation in 20% liver homogenate was carried out with three repetitions using a 15 mL falcon tube. A total of 2.2 mL Tris HCl pH buffer 7.4, 0.2 mL of FeSO₄.7H₂O, and 0.6 mL of organ homogenate were incubated in a mechanical shaker incubator at a temperature of 37°C for 60 min. Furthermore, 0.5 mL of 40% trichloro-acetic acid, 0.25 mL of HCl SN, and 0.5 mL of 2% thiobarbituric acid were added. The mixture was incubated in a water bath at a temperature of 100°C for 10 minutes. The tube was cooled, and then 3 mL of chloroform was added and homogenized. All the falcon tubes were centrifuged at 2500 rpm for 10 minutes. The organic layer (bottom part) was separated, and the supernatant absorbance was measured at 532nm. The final test entailed applying Hematoxylin-Eosin staining and histological methods to check the size and number of fatty cells under the skin.

Analytical Data
The normality (Shapiro Wilk for <100 data samples) and the homogeneity test (Levene’s test) were the first statistical steps in data analysis. The data was then analyzed using the One-Way ANOVA to determine group differences, augmented by the Post Hoc test.

RESULTS AND DISCUSSION
The initial step in this study entailed phytochemical screening of Sauropus androgynus (SA) and Centella asiatica (CA) extracts to identify the compounds after the maceration process. The presence of the biochemical compound was tested qualitatively by reacting the extracts with specific reagents. The test results showed that the extract of SA contained saponins, tannins, flavonoids, alkaloids, and triterpenoids. This was consistent with a previous study in which these substances were found to be bioactive chemicals in SA during phytochemical screening (Mustarichie et al., 2019). The flavonoids in SA reportedly have antioxidant effects (Hikmawanti et al., 2021), while obesity is associated with oxidative stress and susceptible to various degenerative diseases. Therefore, the antioxidant activity of SA extract is closely related to antiobesity (Fernández-Sánchez et al., 2011).

The CA extract was found to contain saponins, tannins, flavonoids, and triterpenoids, consistent with previous studies stating that these compounds were identified during phytochemical screening (Chandrika & Prasad Kumara, 2015). Furthermore, previous studies reported that the active compounds functioning as antiobesity in the extract of CA were Asiatic acids and flavonoids. Asiatic and madecassic acids are triterpenoid groups studied in vivo and in vitro as antioxidants. These compounds can regulate lipid metabolism by increasing LCAT (lecithin-cholesterol acyltransferase) and SR-BI (scavenger receptor class B type 1), which are enzymes implicated in the biochemical pathway of cholesterol. Therefore, CA has the potential to be used as an oxidative stressor or lipid regulatory (Zhao et al., 2014).
Body weight

An animal model of obesity can be obtained by feeding a high-fat and fructose diet for 28 days. The positive control group experienced a significant increase in body weight, reaching 24.02%, after 28 days of treatments. The treatment groups differed significantly from the positive control on day 28. This suggested that all dosage variations of SA and CA in combination and the Orlistat (15.6 mg/kg) successfully reduced body weight. A previous study discovered that both SA and CA at single dosages reduced body weight in an animal model (Begum & Swamy, 2019; Patonah et al., 2018). This study showed that the group administrated SA and CA in all variations of the combination dosage was effective in reducing body weight and comparable to Orlistat.

Feed Index

Based on the results, the positive control group showed no leftover feed, while the treatment showed a higher leftover feed index. The group treated with the extract combination showed an appetite inhibition comparable to that of Orlistat. This might be due to the central appetite inhibition mechanism by lowering neuropeptide-Y (NPY), which is in the central nervous system (Onakpoya et al., 2011).

Similar results were also reported by Yu et al., (2006), stating that the flavonoid contained in SA could prevent weight gain. By supplying SA extract to obese mice, food intake significantly decreased by 15%, and serum-free triglyceride levels were lowered (Yu et al., 2006). CA has also been reported to reduce appetite, leading to a decrease in food intake. Catechins, a type of flavonoid found in CA, are known to promote the catabolism of fat in the body (Hussin et al., 2007). Consequently, both SA and CA can treat obesity by synergistically reducing food intake.

Feces index

The action of Orlistat reduces body weight by preventing fat buildup in adipose tissue. This leads to a reduction in fat content and excretion along with feces, resulting in a decline in body weight. A previous study reported that Orlistat showed a significant increase in the weight of feces. The mechanism of action is inhibiting the lipase enzyme in the gastrointestinal tract, resulting in reduced fat absorption and excretion through feces (Ahnen et al., 2007).

The positive control group showed the lowest feces index compared to others (Figure 1), demonstrating that the HFHF diet reduced the feces index. The groups administered dosages I and II had the most significant feces index accumulation. This result proved that the combination of SA and CA effectively increased the feces index comparable to Orlistat.

Organ Index

Compared to other groups, the negative and positive controls had the highest average organ index (Figure 2). There were no significant differences between the negative and positive control groups, indicating that the HFHF diet for 28 days did not affect the organ index. Several antiobesity studies require up to 45 days of treatment time in modeling obesity test animals (Qowiyyah et al., 2020).

The group that received the combination extract showed significant potential to reduce the organ index. Specifically, the group treated with dosage I (CA-SA combined dosage of 25: 25 mg/kg) had the lowest average organ index, including liver, heart, and kidney (Figure 2) but there were no statistical differences. These results underscore that although the induction did not sufficiently affect the organ index in the animal model, the combination of SA and CA extracts can potentially reduce the organ index (Noeman et al., 2011).

Fat Index

The measurement of the fat index is closely related to obesity, specifically in long-term metabolism factors at risk of fat accumulation in several cavities or organs (Gruzdeva et al., 2018). Numerous studies reviewing the parameters of retroperitoneal fat cells aimed to assess the risk of central obesity impacting metabolic and cardiovascular syndrome diseases (Han & Lean, 2016). The calculation of fat weights was carried out by weighing the retroperitoneal and epididymal fat tissue around the abdomen to the pelvic fascia, divided by the body weight of the test animal.

The positive control group showed the highest average fat index, including retroperitoneal (1.78 ± 0.53) and epididymal fat (2.56 ± 0.23) (Figure 3). However, the negative control was comparable to the positive, and there was no significant difference statistically. This indicated that feeding the HFHF diet for 28 days did not affect the weight of fat.
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The retroperitoneal fat buildup is a significant contributor to the occurrence of obesity. In the male group, excessive accumulation of white fat tissue occurs mainly in the abdominal area, while in females, it occurs primarily in the buttocks area. Both sexes have varied fat deposition patterns in the omentum, mesentery, and retroperitoneal regions. Fat in this area is the first to be released when the body requires energy. Central obesity refers to fat accumulation in the omentum and retroperitoneum (Park et al., 2014).

Figure 1. Accumulation of faeces index after 28 day treatment in all groups. The negative control group (0.5% CMC-Na with standard diet); positive control/induced group (0.5% CMC-Na with HFHF); Dosage I (CA-SA combined dosage of 25:25 mg/kg), dosage II (CA-SA combined dosage of 50:25 mg/kg); dosage III (CA-SA combined dosage of 25:50 mg/kg); CA (extract of Centella asiatica), SA (extract of Sauropus androgynus). Data are presented as the mean ± standard deviation of the 4 data sets.

Figure 2. Average of organ index after 28-day treatment in all groups

The retroperitoneal fat buildup is a significant contributor to the occurrence of obesity. In the male group, excessive accumulation of white fat tissue occurs mainly in the abdominal area, while in females, it occurs primarily in the buttocks area. Both sexes have varied fat deposition patterns in the omentum, mesentery, and retroperitoneal regions. Fat in this area is the first to be released when the body requires energy. Central obesity refers to fat accumulation in the omentum and retroperitoneum (Park et al., 2014).

Data are presented as the mean ± standard deviation of the 4 data sets.
Figure 3. Average of fat index after 28-day treatment in all groups

\( a \) = There was a significant difference compared to the control group (\( p<0.05 \)); * = There was a significant difference compared to the induced group (\( p<0.05 \)); control group (0.5% CMC-Na with standard diet); positive control/induced group (0.5% CMC-Na with HFHF); Dosage I (CA-SA combined dosage of 25 mg/kg), dosage II (CA-SA combined dosage of 50 : 25 mg/kg); dosage III (CA-SA combined dosage of 25 : 50 mg/kg); CA (extract of \textit{Centella asiatica}), SA (extract of \textit{Sauropus androgynus}).

Data are presented as the mean ± standard deviation of the 4 data sets.

Figure 4. Average of triglyceride level after 28-day treatment in all groups

\( a \) = There was a significant difference compared to the control group (\( p<0.05 \)); * = There was a significant difference compared to the induced group (\( p<0.05 \)); control group (0.5% CMC-Na with standard diet); positive control/induced group (0.5% CMC-Na with HFHF); Dosage I (CA-SA combined dosage of 25 mg/kg), dosage II (CA-SA combined dosage of 50 : 25 mg/kg); dosage III (CA-SA combined dosage of 25 : 50 mg/kg); CA (extract of \textit{Centella asiatica}), SA (extract of \textit{Sauropus androgynus}).

Data are presented as the mean ± standard deviation of the 4 data sets.
The group that received Orlistat and a combination of SA and CA extracts had a lower average fat index than the positive control group. Treatment with dosages I and II led to a significant difference in both fat indexes compared to the positive control. However, dosage III differed significantly from the positive control on the epididymal fat index. This result suggests that the combination of SA and CA extracts can potentially reduce the fat index in obese animal models induced by the HFHF diet for 28 days, comparable to Orlistat.

**Triglyceride Levels**

After 28 days of treatment, the next test was the measurement of triglyceride levels. One of the consequences of high-fat and high-carbohydrate feeding is the imbalance of triglyceride levels. In models of obese animals, excessive fructose feeding can cause insulin resistance, hypertension, hypertriglyceridemia, and hyperinsulinemia (Elliot et al., 2002). Fructose is a carbohydrate that acts as the main ingredient for the formation of triglycerides. Obesity, which is strongly associated with a rise in triglyceride levels, can be caused by excessive and long-term carbohydrate intake (Charrez et al., 2015).

Obesity is a balance disorder that occurs when energy intake exceeds output, with most excess calories being converted to triglycerides and stored in adipose tissue. Inhibiting triglyceride synthesis is one potential treatment strategy for obesity (Chen & Farese, 2000). Although triglycerides are essential for normal physiology, excessive accumulation leads to obesity and, more specifically, insulin resistance in non-adipose tissue (Chen & Farese, 2000). The DGAT1 (Diacylglycerol O-acetyltransferase 1) enzyme is predominantly expressed in the small intestine. Inhibiting this enzyme prevents fat absorption in enterocytes after administering a high-fat diet. The inhibition of DGAT1 increases energy expenditure, which improves insulin and leptin sensitivity. Furthermore, the enzyme can inhibit triglyceride synthesis and accumulation in the liver, resulting in its use for treating non-alcoholic hepatic steatosis (Buhman et al., 2002). DGAT1 inhibition can prevent potential body weight gain through the mechanism of reducing adiposity fat mass and tissue triglyceride levels (Smith et al., 2000).

Based on the results (Figure 4), the positive control group had a greater average triglyceride level (98.20 ± 7.89 mg/dL) than the other groups. These results indicate that a diet high in fat and fructose for 28 days can increase triglyceride levels in obese animal models. The group administered dosages I and II were comparable to the negative control in the triglyceride level (p>0.05). Similarly, all groups except for dosage III had significant differences (p<0.05) compared to the positive control. The data showed that the administration of extracts combination at dosages I and II effectively reduced triglyceride levels comparable to Orlistat.

These results are consistent with the action of Orlistat on the digestive system. Orlistat inhibits the lipase enzyme, inhibiting lipid absorption produced by triglyceride hydrolysis. Also, it was reported that administration of Orlistat slightly reduced cholesterol and triglyceride but did not lower lipoprotein(a) levels (Sahebkar et al., 2017). This result confirms previous studies stating that SA and CA play an essential role in regulating lipid synthesis (Begum & Swamy, 2019).

**Lipid Peroxidation Test**

The antioxidant activity of a compound can be determined by inhibiting lipid peroxidation with the TBA test to measure the absorption of malondialdehyde (MDA), one of the products of aldehyde lipid peroxidase. Samples heated with TBA under acidic conditions tend to form a pink product from MDA, which can be measured with a spectrophotometer. Inhibition of lipid peroxidation was shown by the decrease in MDA absorbance in the liver homogenate. The lipid peroxidation test results showed that the group administrated Orlistat 15.6 mg/kg and a combination of extracts at dosage II both obtained the highest percentage of lipid peroxidation inhibition, with the average value of 35.3 ± 2.7% and 34.8 ± 1.2%, respectively (Figure 5). Based on the statistical analysis data, there were no significant differences in the value between the two groups. The lowest percent inhibition of lipid peroxidation was found in the dosage III group, namely 13.20 ± 4.56%, while dosage I had a value of 13.52 ± 4.75%. However, the results showed that each treatment could prevent lipid peroxide in test animals. Treatment with the administration of Orlistat and a combination of SA and CA on the liver homogenate can reduce the absorption of MDA. The presence of flavonoid compounds and Asiatic acid, which presumably act as antioxidants and inhibit the activity of these free radicals, causes a decrease in MDA absorption. Based on the measurement results, the administration of the extract was effective in inhibiting lipid peroxidation and dosage II had an inhibitory ability comparable to Orlistat.
Histology of Adipocytes

The next parameter tested was the size and number of fatty cells in the subcutaneous tissue using the histological method of Hematoxylin-Eosin staining. White fatty tissue is widespread in the subcutaneous tissue, and the primary function is the storage and release of fatty acids (FA) that supply fuel to the organism during the fasting period.
period. These fatty acids are stored in ‘unilocular’ lipid droplets of large size that occupy more than 90% of the cell volume. Adipose white fat has metabolic and endocrine functions, in this context, metabolic functions include lipogenesis, oxidation of fatty acids, and lipolysis, while endocrine functions comprise the production of adipokines. Adipose white fat has a risk of developing obesity-related health problems and branched-chain amino acid metabolism (Torres et al., 2016). In microscopic visualization (Figure 6), the size of fatty cells in the positive control group tended to be larger than in the other groups. In contrast, the size of the fatty cells in the negative control group was smaller than in the positive control. This showed that the HFHF diet affected the size of fatty cells in the subcutaneous tissue.

According to the quantification results, the positive control group had the largest average size with a value of 6.6 ± 3.6 μm and the smallest number of cells, namely 20.2 ± 8.2. Dosage III had a smaller average adipose cell size than the other groups, reaching 3.0 ± 1.5 μm, with a total number of adipose cells at 24.4 ± 4.0. Based on the results, the smaller the adipose cell size, the greater the number of fat cells in one surface area. This study suggested that the extract could potentially reduce the size of fat cells comparable to Orlistat.

**CONCLUSION**

In conclusion, this study found that the combination of *Sauropus androgynus* L. Merr (SA) and *Centella asiatica* L. Urban (CA) extracts had a significant antiobesity effect. The combination with a ratio of dosage 1:1 (25 mg/kg SA and 25 mg/kg CA) showed the best antiobesity effect compared to others.

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**CONFLICT OF INTEREST**

The authors declare that there is no conflict of interest.

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