APPLE CIDER VINEGAR INDUCED WEIGHT LOSS IN OBESE RATS

Fitriyani¹, Kurniasari²*

ABSTRACT

BACKGROUND
Obesity has become a global problem in the last ten years. The increasing incidence of obesity caused an increase in deaths from various obesity-related diseases. Pharmacotherapy for obesity that exists today is limited and has a variety of side effects. Apple cider vinegar is one type of natural ingredient used by the general public to lose weight. Acetic acid is an active component of apple cider vinegar that plays an important role in weight loss.

METHODS
This study is an experimental study using experimental animals. Thirty-six Sprague Dawley rats aged 1-2 months were given high-fat diet for 5 weeks. Rats with obesity were divided into 3 groups, the control group, the P1 group were given apple cider vinegar doses of 0.096mL/100g twice daily and the P2 group were given apple cider vinegar doses of 0.192mL/100g twice daily. Treatment is given for 4 weeks. Weight and leftovers food are weighed during treatment period. The final weight was analyzed with the Kruskal-Wallis test and the weight difference was analyzed with the one-way ANOVA test followed by the Turkey post hoc test.

RESULTS
There was no difference in final weight between the 3 groups (p=0.55), but there was a significant difference in weight before and after treatment between the control group and the P1 group (p=0.001) and the control group with the P2 group (p=0.000).

CONCLUSION
Apple vinegar has the potential to be used as an anti-obesity treatment in obese subjects.

KEYWORDS: Obesity, Apple Cider Vinegar, Weight Loss

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Cuka Apel Menurunkan Berat Badan pada Tikus dengan Obesitas

LATAR BELAKANG
Obesitas telah menjadi masalah global dalam sepuluh tahun terakhir. Angka kejadian obesitas yang terus bertambah menyebabkan meningkatnya jumlah kematian akibat berbagai penyakit terkait obesitas. Farmakoterapi obesitas yang ada saat ini terbatas dan memiliki berbagai efek samping. Cuka apel merupakan salah satu jenis bahan alamiah yang digunakan oleh masyarakat umum untuk menurunkan berat badan. Asam asetat merupakan komponen aktif cuka apel yang berperan dalam penurunan berat badan.

METODE
Penelitian ini merupakan penelitian eksperimental dengan menggunakan hewan coba. Sebanyak 36 ekor tikus Spraque Dawley berusia 1-2 bulan diberikan diet tinggi lemak selama 5 minggu. Tikus dengan obesitas dibagi dalam 3 kelompok, yaitu kelompok kontrol, kelompok P1 yang diberi cuka apel dosis 0.096ml/100g/kali dan kelompok P2 yang diberi cuka apel dosis 0.192ml/100g/kali, masing-masing 2 kali sehari. Perlakuan diberikan selama 4 minggu. Berat badan dan sisa makanan ditimbang selama perlakuan. Data berat badan akhir dianalisa dengan uji Kruskal-Wallis dan selisih berat badan dianalisa dengan uji one-way ANOVA yang dilanjutkan dengan uji post hoc Turkey.

HASIL
Tidak ada perbedaan yang bermakna berat badan akhir antara 3 kelompok (p=0.55), namun terdapat perbedaan selisih berat badan bermakna antara kelompok kontrol dengan kelompok P1 (p=0.001) dan kelompok kontrol dengan kelompok P2 (p=0.000).

KESIMPULAN
Cuka apel memiliki potensi untuk digunakan sebagai terapi anti obesitas pada subjek dengan obesitas.

KATA KUNCI: Obesitas, Cuka Apel, Penurunan Berat Badan

INTRODUCTION
Obesity has become a global problem in the last ten years. Changes in community trends that lead to unhealthy lifestyles, such as increasing consumption of high-fat foods, have led to an increasing number of individuals suffering from obesity.\(^{(1,2)}\) The prevalence of obesity in the world in 2025 is estimated to reach 18% for men and 21% for women. These data were obtained from a study of 1698 populations with 19.2 million individuals in 200 countries from 1975 to 2014.\(^{(3)}\) In Indonesia, based on the 2016 National Health Indicators Survey (SIKERNAS), the prevalence of obesity in individuals aged >18 years with a body mass index (BMI) of 27 kg/m\(^2\) increased from 15.4% to 20.7%. The obesity rate at age >18 years with a BMI of 25 kg/m\(^2\) also increased from 28.7% to 33.5%.\(^{(4)}\)

The high rate of obesity causes an increased risk of death caused by various diseases related to obesity, including type 2 diabetes mellitus (type 2 DM), cardiovascular disease, malignancy, and chronic respiratory disease.\(^{(5)}\) Death from various diseases in obese individuals was lowest at BMI 20 kg/m\(^2\) <25 kg/m\(^2\) and increased at BMI 25 kg/m\(^2\).\(^{(6)}\) Therefore, obesity management is needed to achieve the ideal BMI.

Management of obesity includes diet modification, increased physical activity, pharmacotherapy, and surgical therapy. There are not many obesity pharmacotherapies currently used, namely orlistat, phentermine-topiramate, bupropion-naltrexone, and liraglutide.\(^{(7)}\) These anti-obesity drugs have side effects of oily stools, urinary incontinence, vitamin A D E K deficiency, constipation, diarrhoea, nausea, vomiting, dyspepsia, increased blood pressure, paresthesias, and insomnia, anxiety, depression, and seizures.\(^{(8)}\) The side effects of available anti-obesity drugs have led many researchers to research safer alternative drugs, such as vinegar. This is based on using vinegar since the 18th century to treat obesity.\(^{(9)}\)

Vinegar is a natural product resulting from the fermentation of glucose found in various fruits and various types of foods rich in carbohydrates.\(^{(9)}\) Currently, apple cider vinegar is a popular type used by the general public because it is claimed to...
have various health benefits.\(^{(10)}\) One of the benefits of consuming apple cider vinegar is weight loss.\(^{(10-12)}\) Apple cider vinegar contains organic acids,\(^{(13)}\) flavonoids,\(^{(13,14)}\) and vitamins.\(^{(15)}\) The biggest content in apple cider vinegar is acid. Acetate. The acetic acid in apple cider vinegar (6-9\%) has the effect of losing weight\(^{(13)}\) by increasing satiety.\(^{(16)}\) The mechanism of acetic acid in increasing satiety, among others, is by slowing gastric emptying,\(^{(17)}\) affecting palatability,\(^{(18)}\) and increased pro-opiomelanocortin mRNA expression in the hypothalamus.\(^{(19)}\) Other compounds in apple cider vinegar that can affect body weight are gallic acid and caffeic acid.\(^{(14)}\) Gallic acid increases insulin sensitivity and energy expenditure without affecting food intake,\(^{(20)}\) whereas caffeic acid suppresses lipogenic enzymes and hepatic fat accumulation.\(^{(21)}\)

Research on the effect of apple cider vinegar on body weight in experimental animals has been carried out. One is a study conducted by Halima BH et al.,\(^{(22)}\) who found a significant difference in body weight in a group of rats fed a high-fat diet and apple cider vinegar with a group of rats that were only given a high-fat diet for 6 and 9 weeks. In the group of rats that received apple cider vinegar, it was found that body weight was lower than the group of rats that did not get apple cider vinegar. This result differs from the study conducted by Ousaaid D et al.,\(^{(23)}\) who examined the effect of apple cider vinegar on body weight in rats fed a high-caloric diet with 10\% D-glucose. In this study, it was found that there was no significant difference in body weight in the group of rats that received apple cider vinegar compared to the group of rats that did not receive apple cider vinegar.

Based on the explanation above, there are differences in the results of previous studies regarding the effect of apple cider vinegar on the body weight of experimental animals. This study was conducted to analyze the effect of giving apple cider vinegar in different doses on the body weight of obese experimental animals given a high-fat diet. In addition, this study also analyzes the possibility of increasing satiety as a cause of weight loss in experimental animals given apple cider vinegar.

This study used one of the commercial apple cider vinegar, which people in Indonesia quite often use. The method in this study is different from previous studies, namely from the dose and duration of administration of apple cider vinegar to experimental animals.

**METHODS**

This research is a pure experimental study with Post-test-Only Control Group Design, using 31 male rats (Rattus norvegicus) Sprague Dawley strain 1-2 months old with initial body weight of 90 – 105 grams which are included in the juvenile age category. A total of 4-5 rats were placed in a cage measuring 47cm x 33 cm x 15cm. Before starting the study, acclimatization was carried out for 7 days. Standard pellet feed 551 containing 13\% water, 18.5-20.5\% protein, 4\% fat, 6\% fiber, 8\% ash, 0.90\% calcium, and 0.70\% phosphorus was given from the beginning to the end of the treatment. Before the study, body weight was checked after administration high-fat diet for 5 weeks to determine the mice that met the obesity criteria. Rats are said to be obese when they gain 20\% of their initial body weight.\(^{(25)}\)

Experimental animals that met the criteria for obesity were divided into three groups by randomizing 32 obese rats. The research group consisted of a control group given 0.7 mL of distilled water; treatment group 1 (P1) was given 0.096 mL/100gBW/time apple cider vinegar; treatment group 2 (P2) was given 0.192 mL/100gBW/time apple cider vinegar. All interventions orally using a gastric probe were given two times a day for four weeks.

This study used commercial apple cider vinegar produced by a producer in Indonesia with a total acid of 18.746 g (6.25\%).\(^{(26)}\) The dose of apple cider vinegar used in this study was determined based on the dose of apple cider vinegar which has a weight loss effect in humans. 1–2 tablespoons (15–30 mL) per day.\(^{(27)}\) The dose was converted to a rat experimental dose using the Laurence table.

Rats were weighed using a digital weight scale once a week from the end of the 6th week to the 10th week. The rest of the feed in each group
was weighed every day from the beginning of the 7th week to the 10th week. High-fat diet continues to be given from week 6 to week 10.

The data is processed using the Statistical Program for Social Science (SPSS) program. Data analysis was carried out using one-way variance analysis (one-way ANOVA). The test for the normality of the data distribution was carried out using the Shapiro Wilk test, and the homogeneity test was carried out using the Levene test. Data with non-normal and/or inhomogeneous distribution were tested by the Kruskal-Wallis test.

This research passed the ethical review from the Faculty of Medicine, Universitas Trisakti, on 27 July 2018 with the ethical review number: 83/KER-FK/VIII/2018.

RESULTS

The sample of experimental animals, which initially consisted of 36 animals, was reduced to 32 because four experimental animals died during the acclimatization process. As a result, the average body weight of rats after acclimatization was 97.31±3.632 grams. The experimental animals were weighed at the end of the 6th week, and all experimental animals gained weight >20% from the initial body weight (27.45% - 91.40%). At the end of the 7th week, one rat from the P1 group died, leaving a total of 31 experimental animals.

The mean weight of the control group tends to increase every week compared to the P1 and P2 groups. The most significant weight loss occurred in experimental animals in groups P1 and P2 in the first week of administration of apple cider vinegar (Figure 2).
Statistical analysis using the Kruskal-Wallis test on the body weight of rats at week 10 showed no difference between the three groups (p=0.55). The average difference in body weight of experimental animals, which showed a decrease in body weight during the administration of apple cider vinegar, was mainly observed in the P2 group. In contrast, in the control group, body weight increased (Table 1).

The results of the One-Way ANOVA test for the difference in body weight of rats at the 6th and 10th weeks showed a significant difference between the groups (p = 0.000). Therefore, the Turkey test carried out further analysis to determine which groups had significant differences. Statistical analysis showed a significant difference in the control group with P1 (p=0.001) and the control group with P2 (p=0.000), while between groups P1 and P2, there was no difference (p=0.255) (Figure 3).

This study also observed differences in the remaining feed in each group from the start of giving apple cider vinegar to groups P1 and P2. The Kruskal-Wallis test showed no difference in feed residue between the three study groups (p=0.445).

**DISCUSSION**

All experimental animals were given a high-fat diet to induce obesity in this study. Fat contains more calories in 1 gram (9 kcal/g) than other macronutrients. High fat consumption causes relatively rapid weight gain because fat is metabolized into free fatty acids, which are the building blocks for triglyceride synthesis. Excess triglycerides are stored by the body in the liver and adipose tissue. Pork oil has high saturated fat and cholesterol content. The amount of saturated fat in a food ingredient affects the amount of weight gain. Pork oil has been shown to increase body weight when consumed in large quantities significantly. In this study, all experimental animals given a high-fat diet were obese.

The effect of apple cider vinegar on changes in body weight of obese rats in the P1 and P2 groups on control could be observed from the 6th to the 10th week of the study (Figure 2). The most significant weight loss seen at week seven may be due to a decrease in appetite and an increase in satiety which is influenced by the palatability of experimental animals to apple cider vinegar. Poor palatability may result from the strong sour taste and the pungent odour of apple cider vinegar. Daily exposure to apple cider vinegar can increase the palatability of apple cider vinegar. This may be the cause of the increase in body weight again at week 8. This finding is in line with the observation that food waste from groups P1 and P2 was more abundant at week seven than the following week. The Kruskal-Wallis test showed no difference in food waste in the control group, group P1 and group P2 (p=0.445). This study's results differ from the research conducted by Halima BH et al. They found a significant decrease in food intake in the rats that received apple cider vinegar at the 6th and 9th weeks of administration of apple cider vinegar. Khezri SS et al. also found a decrease in food intake in a

### Table 1. Rat Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>BW 1st Week</th>
<th>BW 6th Week</th>
<th>BW 10th Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>97.55 ± 4.180</td>
<td>144.36 ± 13.99</td>
<td>164.36 ± 26.73</td>
</tr>
<tr>
<td>P1</td>
<td>10</td>
<td>97.00 ± 2.683</td>
<td>154.80 ± 11.03</td>
<td>147.60 ± 17.85</td>
</tr>
<tr>
<td>P2</td>
<td>10</td>
<td>97.40 ± 4.22</td>
<td>164.20 ± 9.47</td>
<td>154.29 ± 20.65</td>
</tr>
</tbody>
</table>

Note: data on body weight (BW) are presented in mean ± SD
group of obese subjects who received apple cider vinegar for 12 weeks. The longer duration of apple cider vinegar administration in both studies may be the reason for the decreased food intake observed in the group receiving apple cider vinegar.

The average body weight between the study groups from the end of the sixth week to the tenth week of the study showed that the P1 and P2 groups had a lower mean final weight than the control group (Table 1). Statistical analysis using the Kruskal Wallis test to compare the final body weight of rats in the three groups showed no difference between groups (p=0.055). The results obtained in this study are different from previous studies. Halima BH et al. (22) investigated the effect of giving apple cider vinegar for 6 and 9 weeks on changes in body weight of male Wistar rats fed a high-fat diet containing 22% fat. Significant differences could be observed in the rats that received apple cider vinegar for 6 and 9 weeks compared to the rats that only received a high-fat diet. These changes were in line with lower blood glucose levels, total cholesterol levels, and triglyceride levels in the group of rats that received apple cider vinegar. The difference in results with this study may be due to apple cider vinegar being administered for a longer time. Another study by Urtasun R et al. (33) used low doses of raw organic apple cider vinegar. This study compared the effects of apple cider vinegar, probiotic Bacillus coagulant, and a combination of apple cider vinegar and probiotic Bacillus coagulants for five weeks on C57B/6 mice fed a high-fat diet. The results showed that the most significant weight loss effect was found in the group of mice that received the combination treatment, followed by the group of mice that received apple cider vinegar and the group of mice that received probiotics. The type of organic apple cider vinegar in Urtasun R et al. (33) differs from apple cider vinegar in this study. This may be why the results in this study did not show a difference in final body weight between groups. Organic apple cider vinegar is produced through a more stringent method and has more heterogeneous microbes, so it has better quality than conventional apple cider vinegar. (34) In addition, previous studies have shown a significant difference in the final body weight of the study in the group that received apple cider vinegar and those who did not get apple cider vinegar may be because the mean weight before being given treatment in the control group is the lowest compared to the average body weight in P1 and P2. This can be seen in a study conducted by Dios Lozano J et al. (35) They examined the effect of vinegar fermented by golden apple, pineapple, honey, and sugarcane on rat body weight. The study compared the initial and final body weight in 4 groups, namely a group of healthy and obese rats that were not given and given apple cider vinegar. The average weight gain of the group of obese rats not given apple cider vinegar was greater than those given apple cider vinegar. Still, statistical analysis showed no difference in the final body weight of the four study groups (p>0.05). In this study, it was observed that the average initial body weight of healthy rats (320 grams) before being given vinegar was lower than obese rats (425 grams).

The effect of apple cider vinegar on body weight in groups P1 and P2 was more clearly seen in rats’ final body weight and initial body weight after being given apple cider vinegar (Figure 3). In the P1 and P2 groups, a decrease in the average weight was observed, while in the control group, there was an increase in body weight. Research by Khezri SS et al. (12) on subjects with obesity showed similar results. In this study, weight loss was observed in the group that received a low-calorie diet and apple cider vinegar 2 X 15mL a day for 12 weeks of administration of apple cider vinegar (p=0.001). Similar results were also observed in the control group who only received a low-calorie diet (p=0.01). The significant difference in weight change for 12 weeks between the two groups (p=0.01) indicated that apple cider vinegar could be used as an adjunct therapy combined with a low-calorie diet for weight loss. In this study, it was also observed that the P2 group had a larger difference in body weight at week six and week ten than the P1 group. This finding may be due to the higher dose used in the P2 group than in the P1 group. Research by Bouhini A et al. (36) analyzed the body weight of rats induced by a high-fat diet and given apple cider vinegar in different doses. The doses of apple cider vinegar used in this study were 3.5 mL/kg/day, 7 mL/kg/day, and 14 mL/kg/day and were administered for 18 weeks. In the three groups of apple cider vinegar doses, it was found that there was a significant difference in
body weight compared to the group of rats that did not receive apple cider vinegar. The average increase in body weight was the smallest in the rats that were given a high-fat diet and received the largest dose of apple cider vinegar (14 mL/kg/day). This shows that the dose of apple cider vinegar given is linear with the reduced weight gain of rats.

Further analysis of the difference in body weight at the 6th and 10th weeks of the three study groups using the one-way ANOVA test followed by the Turkey post hoc test showed a significant difference between the control group and the P1 and P2 groups (Figure 3). In groups P1 and P2, there was no statistical difference between the 6th and 10th-week weight differences. This shows that giving apple cider vinegar in low and high doses does not have a different impact on changes in body weight. This result is different from the study conducted by Kondo et al. (27), who found a significant difference in weight loss in subjects who received high doses of apple cider vinegar (30 mL/day) compared to low amounts of apple cider vinegar (15 mL/day) given for 12 months. Weight loss in the two groups was only seen from the 4th week of apple cider vinegar administration. In contrast, significant weight differences between the low-dose and high-dose groups began in the 8th week of apple cider vinegar administration. The difference between this study and this study may be due to the longer duration of administration of apple cider vinegar, so in this study, a significant difference was not observed at higher doses.

Several mechanisms of the weight loss effect of vinegar have been reported in previous studies. The increase in satiety, thought initially to be a mechanism for the effect of weight loss, has not been proven in this study. The weight loss effect observed at week 10 of the study may occur through other mechanisms, such as increased hepatic fatty acid oxidation (37), increased energy expenditure (38) and increased insulin sensitivity (39). Park JE et al. (37) who examined the effect of pomegranate vinegar on visceral fat in 78 overweight women, found that there was an increase in AMP-activated protein kinase (AMPK) activity in adipose tissue in the group who consumed pomegranate vinegar for eight weeks. Acetic acid is the active component found in all types of vinegar. This acid, with the help of adenosine triphosphate (ATP) is metabolized to acetyl Co-A and causes an increase in the AMP/ATP ratio, which induces AMPK phosphorylation in hepatocytes. (40) This increases fatty acid oxidation. (37,40,41) In 2017, Canfora EE et al. (38) investigated the effect of colonic infusion of a short chain fatty acid mixture on energy metabolism in 12 overweight or obese men. All types of short-chain fatty acid blends, including those high in acetic acid, increase fat oxidation during fasting. Plasma acetic acid levels also positively correlated with increased energy expenditure at rest (r=0.349; p=0.0149). Mitrou P et al. (39) found an increase in insulin sensitivity in 11 subjects with type 2 diabetes mellitus who were given 30 mL of vinegar with 6% acetic acid content and 20 mL of water. Increased insulin sensitivity is characterized by decreased postprandial plasma insulin, glucose and triglyceride levels, and increased glucose uptake in striated muscle. High plasma glucose and triglyceride levels have an impact on increasing the accumulation of triglycerides in adipose tissue. (39) In addition to acetic acid, other studies have shown that gallic acid and caffeic acid are flavonoids in apple cider vinegar. (14) Both of these compounds have a body weight-lowering effect. (20,21) Doan KV et al. (20) found that mice given gallic acid experienced activation of the AMPK/Sirtuin-1/peroxisome-proliferator-activated-receptor-coactivator1 (AMPK/Sirt1/PGC1) pathway which resulted in improved insulin sensitivity. And lipid metabolism, as well as increased levels of uncoupling protein-1 (UCP1) and gene expression (Pgc1a, Ucp3, 3-Adr) in interscapular brown adipose tissue that plays a role in thermogenesis. The anti-obesity effect of caffeic acid was observed in C57BL/6 mice fed a high-fat diet and caffeic acid supplementation (0.02% and 0.08%). Suppression of the sterol regulatory element-binding protein 1c (Srebplc1c) gene and its target enzyme that plays a role in lipogenesis and increased AMPK activation that plays a role in fatty acid oxidation are anti-obesity mechanisms of caffeic acid. (21)

This study used two groups of doses of apple cider vinegar, namely the standard dose (P1) and the high dose (P2). During the administration of apple cider vinegar, in the P2 group, no sick or dead animals were found. However, this study's short duration of apple cider vinegar (4 weeks) could not provide an overview of the long-term
side effects that might occur in the group given high doses of apple cider vinegar (group P2). In group P1, one rat died, but no autopsy was performed on the rat, so the exact cause of death was unknown. In addition, other parameters such as blood glucose levels, lipid profile, and energy expenditure related to apple cider vinegar's mechanism in losing weight have not been measured.

This study showed that apple cider vinegar consumed with a standard and high-fat diet could reduce body weight in experimental animals. However, there was no significant difference in final body weight between groups. This shows that doses of apple cider vinegar equivalent to 2x15 mL and 2x30 mL per day in humans can be used as an anti-obesity therapy. High doses (equivalent to 2x30 mL per day) to be used in humans need to be further investigated for safety and advantages compared to lower doses (equal to 2x15 mL per day). Research using experimental animal subjects to analyze the effects of anti-obesity and side effects of various amounts of apple cider vinegar on long-term consumption also needs to be done. This is related to the findings in this study regarding the weight of rats that tended to continue to lose weight during the administration of apple cider vinegar.

The most appropriate dose and administration of apple cider vinegar to achieve optimal body weight so that there is no excessive weight loss or other side effects need to be determined. Normal control (non-obese) experimental animals can be added as a weight comparison with the experimental group given apple cider vinegar. In addition, the addition of experimental animal groups given conventional anti-obesity drugs can be done as a comparison of the anti-obesity effect with apple cider vinegar.

CONCLUSION
Apple cider vinegar 0.096 mL/100gBW/time and 0.096 mL/100gBW/time given two times a day for four weeks reduced body weight in experimental animals. The addition of apple cider vinegar as much as 2x15 mL and 2x30 mL per day to the diet has the potential to overcome obesity in humans.

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