Comparison of The Effectiveness of Lime [Citrus aurantifolia (Christm.) Swingle] and Lemon [Citrus limon (L.) Burm.f.] Juice to Prevent Lipid Profile Aberration

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ABSTRACT

Hyperlipidemia is a cause of various degenerative diseases. In recent years, there is a trend to consume lemon juice to improve blood lipid profiles. In this study, the effectiveness of lemon compared to lime was studied. The aim of this study was to determine differences in the lipid profile of total cholesterol (TC), low density lipid (LDL), very low density lipoprotein (VLDL), and triglycerides (TG) in rats given High Fat Diet (HFD), lemon and lime juices. This study used 32 males of white Rattus norvegicus Wistar which were assigned into 8 treatment groups with various doses of lemon and lime juice. The animals model that received the treatment of lime had mean lipid levels which were not significantly different from the lemon treatment group (LDL (P<0.151; p<0.05), VLDL (P<0.187; p<0.05), TG (P<0.216; p<0.05)), even the total cholesterol level in the lime treatment group showed lower levels than both the control and lemon treatment groups. The consumption of lime seems to be an alternative to lemon to prevent an aberration in the lipid profile. Lime juice treatment has an impact on the lipid profile similar to lemon juice, even more, effective in the case of total cholesterol.

Key words: lemon, lime, TC, LDL, VLDL, TG

ABSTRAK

Hiperlipidemia merupakan penyebab dari berbagai penyakit degeneratif. Mengonsumsi air perasan lemon menjadi tren di Indonesia beberapa tahun ini. Pada penelitian ini, dilakukan perbandingan efektivitas air perasan lemon dan jeruk nipis. Tujuan dari penelitian ini adalah untuk menganalisis perbedaan profil lemak darah: kolesterol total, low density lipid (LDL), very low density lipoprotein (VLDL), dan trigliserida (TG) pada tikus yang diberi diet tinggi lemak, air perasan lemon dan jeruk nipis. Penelitian ini menggunakan model tikus Wistar berwarna putih 32 ekor yang diberi makanan tinggi lemak (HFD) dengan variasi dosis air perasan jeruk nipis dan lemon. Dari hasil penelitian diketahui bahwa model tikus yang diberi bahan pangan berbentuk cairan dari perasan jeruk nipis tidak menunjukkan perbedaan signifikan dengan model tikus yang diberi air perasan lemon (LDL (P=0.151; p<0.05), VLDL (P=0.187; p<0.05), TG (P=0.216; p<0.05)), bahkan kadar kolesterol total pada model tikus yang diberi air perasan jeruk nipis menunjukkan tingkat lebih rendah dibandingkan dengan model tikus yang diberi makanan tinggi lemak tanpa perasan dan model tikus yang diberi air perasan lemon. Kebijaksanaan konsumsi air perasan jeruk nipis tampaknya bisa menjadi pilihan alternatif untuk mencegah ketergantungan dalam profil lemak darah. Penambahan air perasan jeruk nipis ke dalam makanan tinggi lemak memiliki dampak yang sama dengan air perasan lemon, bahkan lebih efektif dalam hal kolesterol total.
INTRODUCTION

Recently there is a trend in the people's everyday practices in consuming warm lemon juice every morning (lemon shots) or with infused water (water that has been soaked all night with lemon slices or other fruit). Unfortunately, lemon is hard to find in some areas in Indonesia, and the price is quite expensive. People in Indonesia sometimes replace it with lime (1).

Lemon [Citrus limon (L.) Burm.f.] is a citrus fruit that contains high flavonoids, such as rutin, hesperidin, quercetin, eriocitrin, narirutin, didymin and naringenin, vitamin C and carotenoids which function as antioxidants that can prevent atherosclerosis. In previous studies conducted on mice, it was shown that lemon can reduce the risk of obesity and improve lipid profile (2).

The prevalence of mortality due to the changing lifestyle of modern people. The Global Burden of Disease in 2010 estimates that 29.6% of worldwide deaths are caused by cardiovascular diseases (3). Cardiovascular diseases (CVDs) are the most prominent cause of mortality in Europe and throughout the world (4). In Indonesia, CVDs is one of the highest causes of mortality (5). The main causes of CVDs are hyperlipidemia (4). Hyperlipidemia is a medical condition characterized by an increase in some or all of the lipids and lipoprotein profiles in the blood (6). During the 2003-2006 period the prevalence of hyperlipidemia in American adults who has total cholesterol high (> 240 mg / dL) of 16.3% (7), whereas in Indonesia the prevalence of hyperlipidemia in the elderly in Jakarta (58.4%), Padang (56%), Bandung (52.2%) and in Jogjakarta (27.7%) (8).

Lime [Citrus aurantifolia (Christm.) Swingle] is a polyembryonic plant that has been cultivated in many countries, especially in subtropical and tropical regions. Just like lemon, lime is also rich in flavonoids and vitamins such as naringenin, vitamin C, vitamin B1, and other flavonoids such as poncirin, hesperidin, and rhoifolin (9). Based on the United States Department of Agriculture (2006) on the content of flavonoids in food, lemon juice contained naringenin of 1.43 mg/100 grams, and in whole lemon (raw) containing naringenin 0.55 mg/100 grams while 100 grams of lime juice containing naringenin is slightly lower at 0.38 mg (10). In 100 grams of lime contains 63 mg of vitamin C and 15.64 mg of hesperidin. Vitamin C and hesperidin in lime is more than lemon and sweet oranges (11). Based on the Food Science Laboratory Central America analysis report, the content of niacin in 100 grams of the edible portion of lime is 0.14-0.25 mg (12); while the content of niacin in 100 grams of the portion of lemon edible is 0.1 mg (13) (14). It can be concluded that with almost the same type and quantity of antioxidant content,
lemons and lime can replace each other as a hypocholesterolemic agent.

The trend of people in Indonesia, especially in households level who live in urban areas, have low education levels, private workers, have high economic status, tend to prefer Traditional Chinese Medicine (TCM). The primary reason for the rising number of traditional medicine usage nowadays is that patients take a more proactive approach to their health and look for various forms of care (15). Lime and lemon are one example of any types of citrus fruits that have been used as traditional medicinal herbs to cure diseases in several Asian countries (16). If it is found that there is no difference in the effect of lime juice compared to lemon juice, the market value of lime in Indonesia will increase as one of the agricultural commodities that has a hypocholesterolemic effect such as lemon (17).

Based on this background it is necessary to examine the differences in the effectiveness of giving lemon and lime juice to changes in lipid profile (total cholesterol, VLDL, LDL, and triglycerides) male white rats (Rattus norvegicus) Wistar strain which are given high fat diet (HFD). The choice of HFD as a one of diet during treatment because the administration of HFD has been shown to induce hyperlipidemia (18).

MATERIALS AND METHODS

Research Design

This study uses true experimental laboratory design with Post Test Only that included Controlled Group Design and grouping of experimental animals into the treatment group by using the Simple Random Sampling method. Figure 1 displays the general procedure of treatments.

Sample/Subjects

The age of rats chosen was 8-10 weeks with consideration because at that age it was classified as adults age (19). Selection of rats classified as adults was adjusted to the age of adults who according to many data were at risk hyperlipidemia, as evidenced by American Heart Association data that at the age of> 20 years, a person has cholesterol levels> 240 mg / dL and HDL levels <40 mg / dL (10).

Animal Handling

Animal handling and measurement of lipid profile levels were performed in the Laboratory of Parasitology and Clinical Pathology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java - Indonesia.

The materials used included normal diet, high-fat diet, lemon, lime, and Simvastatin. Inhalation anesthetic was practiced using diethyl ether and laboratory examination materials for lipid levels and direct enzymatic colorimetric test (Diasys) reagents with colorimetric spectrophotometric method (20)(21). The tools used included analytical scales CAMRY brand (EK3650/EK3651) and spectrophotometer with a wavelength of 500 ± 20 nm.

The Treatments and Measurements

Male white rats through an adaptation phase for 1 week with a normal diet. The subjects were then divided into eight research groups (P0-P7). In the next 5 weeks during the treatment, the rats were treated with lemon and lime juice by oral gavage every day at 9:00 a.m., then the HFD was given 40 grams ad libitum. Rats were weighed every 3 days, and at the end of the treatment phase, calculation of lipid profile levels was performed.

Rats that have undergone treatment were sedated using diethyl ether. The collection of rat blood samples
was taken through the heart (intracardial) as much as 2 mL. Blood that has been taken is stored in a syringe into an Eppendorf tube containing (EDTA).

The high-fat diet with Comfeed PARS composition 50%, flour 25%, duck egg yolks 5%, goat fat 10%, coconut oil 1%, pork oil 8.9% and cholate acid 0.1% as much as 40 grams per rat per day (22)]. Normal diet ingredients consist of Comfeed PARS 66.67% (with 12% water content, 11% protein, 4% fat, 7% fiber, 8% ash, 1.1% Ca, 0.9% phosphorus, 53% coccidiostat antibiotics) and 33.33% water (14)].

Determination of dosage of lemon and lime juice in rats based on Purnamasari (2014) was practiced. In this study, a volume of lime administration of 2 ml/head/day was used as a standard because it can give a decrease in cholesterol levels by 28.93% (11)]. However, a lower and higher doses (1 and 3 ml/head/day were tested).

Simvastatin powder used dose was 0.18 mg/day/200g body weight of rats dissolved in 2 ml of distilled water (23). Statins used as a pharmacological treatment of hyperlipidemia for the P1 group. Statins remain the first choice therapy as they have been shown to reduce the annual risk of major coronary events by 24% through lowering of LDL-C by 40 mg/dl (24).

**Data Analysis**

The results obtained were analyzed using SPSS statistical program. Repeated the homogeneity was determined using the homogeneity of variances test. Different lipid profile tests using the One Way ANOVA or Kruskal-Wallis statistical test (if the data is not normally distributed). Posthoc Tukey test was used to determine significantly different treatment group pairs.

**RESULTS**

Table 1 displays the characteristics of the test animal and the body weight (BW) measurements. The most important parameter to mention is the weight gain (WG). That is, the highest WG was at P5 treatment and the lowest was that of P2 treatment. But no statistically significant differences were found in these weight gain among the groups (P>0.05). Table 2 show the readings of the different treatments in lipid levels of the test animals.

Figure 2 and Table 2 column 5 show the average LDL level is 15.44 mg/dl. The highest average LDL level occurred in the P1 group (18.5 mg/dl), while the lowest LDL level occurred in the P6 group (11.75 mg/dl). It was found that there were no differences in LDL levels among the groups (P>0.05).

Figure 2 and Table 2 column 4 show the average VLDL level is 14.29 mg/dl. The highest average VLDL level occurred in the P0 group (19.55 mg/dl), while the lowest LDL level occurred in the P4 group (9.8 mg/dl). It was found that there were no differences in VLDL levels among the groups (P>0.05).

Figure 2 and Table 2 column 2 show the average TC level is 45.41 mg/dl. The highest average TC level occurred in the P0 group (54.0 mg/dl), while the lowest TC level occurred in the P5 and P6 group (34.4 mg/dl). Different from the others, it was found that there were differences in TC levels among the groups (P<0.05). Based on these results, posthoc Tukey test was performed and found that significant differences between groups occurred in the P0-P5 group (P<0.05), P0-P6 (P<0.05), P3-P5 (P<0.05), and P3-P6 (P<0.05).

Figure 2 and Table 2 column 3 show the average TG level is 71.47 mg/dl. The highest average TG level occurred in the P0 group (97.75 mg/dl), while the lowest TG level occurred in the P6 group.
(54.25 mg/dl). It was found that there were no differences in LDL levels among the groups (P0.216; p < 0.05).

<table>
<thead>
<tr>
<th>Animal model type</th>
<th>White Rattus norvegicus Wistar strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
</tr>
<tr>
<td>General condition</td>
<td>White, clean, smooth, and shiny fur</td>
</tr>
<tr>
<td>Age</td>
<td>8-10 weeks</td>
</tr>
<tr>
<td>Initial body weight (g) (mean±SD)</td>
<td>177±18.71 190±4.89 181.2±5.38 172.5±21.06 163.5±9.75 183±3.83 166.75±3.63 164.75±13.3</td>
</tr>
<tr>
<td>Final body weight (g) (mean±SD)</td>
<td>229±31.76 239.5±18.79 223.2±14.86 242.2±25.17 220.7±34.82 264.25±35.37 219±21.83 210.5±22.28</td>
</tr>
<tr>
<td>Weight gain (g) (mean±SD)</td>
<td>52±20.03 49.5±15.78 42±15.58 69.75±43.34 57.25±29.55 81.25±38.91 52.25±36.12 45.75±15.59</td>
</tr>
</tbody>
</table>

Table 2. Lipid Profile of Test Animals (mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Triglyceride (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0</td>
<td>56.25±8.96a,b</td>
<td>97.75±10.78</td>
<td>19.5±2.16</td>
<td>18±3.56</td>
</tr>
<tr>
<td>P1</td>
<td>49.75±7.68</td>
<td>65±21.6</td>
<td>13±4.32</td>
<td>18.5±3.31</td>
</tr>
<tr>
<td>P2</td>
<td>41.25±7.71</td>
<td>83±10.23</td>
<td>16.6±2.05</td>
<td>16.5±3.1</td>
</tr>
<tr>
<td>P3</td>
<td>53.5±9.32c,d</td>
<td>88.5±46.60</td>
<td>17.7±9.31</td>
<td>15.75±3.40</td>
</tr>
<tr>
<td>P4</td>
<td>45.75±6.02</td>
<td>54.25±29.33</td>
<td>10.85±5.87</td>
<td>15±2.94</td>
</tr>
<tr>
<td>P5</td>
<td>34±6.05a,b,c,d</td>
<td>67±22.85</td>
<td>13.4±4.57</td>
<td>14.5±2.89</td>
</tr>
<tr>
<td>P6</td>
<td>36.25±3.77</td>
<td>54.5±10.54</td>
<td>10.9±2.1</td>
<td>11.75±2.75</td>
</tr>
<tr>
<td>P7</td>
<td>46.5±10.85</td>
<td>61.75±31.38</td>
<td>12.35±6.28</td>
<td>13.5±5</td>
</tr>
</tbody>
</table>

P0 (High-Fat Diet/HFD), P1 (HFD+Simvastatin), P2 (HFD+lemon juice 1 ml/head/day), P3 (HFD+lemon juice 2 ml/head/day), P4 (HFD+lemon juice 3 ml/head/day), P5 (HFD+lime juice 1 ml/head/day), P6 (HFD+lime juice 2 ml/head/day), and P7 (HFD+lime juice 3 ml/head/day)  

*a,b,c,d statistically significant with p-value<0.05
Figure 1: The General Flow of Treatments

- 32 males of white Rattus norvegicus Wistar strain aged 8-10 weeks
- Adaptation phase (1 week, normal diet)
- HFD (P0)
- HFD + simvastatin (P1)
- HFD + lemon juice 1 ml (P2)
- HFD + lemon juice 2 ml (P3)
- HFD + lemon juice 3 ml (P4)
- HFD + lime juice 1 ml (P5)
- HFD + lime juice 2 ml (P6)
- HFD + lime juice 3 ml (P7)

5 weeks

Rats were weighed every 3 days

Euthanasia of test animal

The laboratorium test of profile lipid

Figure 2. Lipid Profile of Subjects.

P0 (High-Fat Diet/HFD), P1 (HFD+Simvastatin), P2 (HFD+lemon juice 1 ml/head/day), P3 (HFD+lemon juice 2 ml/head/day), P4 (HFD+lemon juice 3 ml/head/day), P5 (HFD+lime juice 1 ml/head/day), P6 (HFD+lime juice 2 ml/head/day), and P7 (HFD+lime juice 3 ml/head/day)

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DISCUSSION

In all treatment groups given HFD experienced an increase in rat body weight during the treatment. This is similar to previous studies that showed an increase in rat body weight when given HFD (25,26). Based on Rabot (2016), rats that were always exposed to HFD would be obese (27)]. This findings was in line with the results of Swithers' study, that animals model fed a high-fat diet triggered positive energy balance which showed an increase in calorie intake, weight gain (WG) and aggravates lipid profile compared to animals model given a low-fat diet (28). By consuming HFD there will be obesity and can cause leptin resistance so that leptin levels and appetite get worse (29)(30).

Interestingly, in this study no differences were intended for WG between groups. Although the dosage of lime/lemon is used the same, this result is different from the study of Ajugwo (2012) who found evidence of weight loss in the group treated with lime/lemon/lime and lemon (31). The effects of lime and lemon used traditionally as appetite suppressants have not yet appeared in this study (32). Another thing that might affect it is because in the study of Ajugwo (2012) it was stated that the administration of high cholesterol diets given as a hypercholesterolemic induction only within 7 days did not coincide with the administration of lime/lemon (31). Unfortunately, in our study, no group was solely on lime and lemon treatment so it was not known whether the absence of an appetite suppressant effect was caused by concurrent or not giving HFD.

In this study, the mean of the lipid profile in the HFD+lemon/lime juice treatment group had a lower mean than the lipid profile of the rats in the HFD treatment only group but no significant differences were found. Nevertheless, the average total cholesterol was still within the normal range, according to Smith and Mangkoewidjojo (1988), 10-54 mg/dl (33). Also, levels of triglycerides, VLDL, and LDL were still below the maximum average levels of this lipid profile (in the same age range and gender), 104.15±8.82 mg/dL; 20.83±1.76 mg/dL; 73.93±6.25 mg/dL, respectively (34). In addition, the absence of differences on almost all of the examined lipid profiles (except total cholesterol) between the lemon and lime juice treatment groups showed promising findings for lime consumers in Indonesia. Even total cholesterol was found to be significant differences between the groups given HFD only and the group given HFD+lemon with the HFD+lime treatment group (1 ml/head/day), the mean total cholesterol level in the lime juice treatment group was lower among the other groups. The findings related to total cholesterol levels are in line with a study conducted by Purnamasari and Isnawati (2014) (11). In rabbits which were fed high cholesterol and lime juice for 60 days showed a significant difference in total cholesterol between control and treatment groups. The results of this study are also in accordance with research conducted by Elon and Jacqueline (2015) who found that subjects who were given 1.5 ml/kg of lime juice every morning for 7 days could reduce total cholesterol from 230.30 mm/dl to 205.90 mm/dl (35). Lemon and lime have some of the same substances as naringenin, hesperidin, niacin, and vitamin C. Some of these substances in lemons have higher levels (eg. Naringenin) and some others (eg. hesperidin, niacin, and vitamin C)
found to be higher in lime. Total cholesterol reduction can occur in the presence of hesperidin and vitamin C. In lime, the most and more flavonoid content than lemon, hesperidin, can inhibit the action of HMG-CoA reductase and ACAT so that it can inhibit cholesterol production by the liver and can increase LDL receptor concentration. Inhibition of this enzyme can reduce total cholesterol levels (2). In addition, the content of vitamin C in lime which is higher than lemon can help in cholesterol metabolism by increasing the rate removal of lipids in the form of bile acids and helps the hydroxylation reaction in the forming process of bile acids therefrom increasing cholesterol excretion from the body (11). Niacin which is also found higher in lime can inhibit the process of lipolysis in adipose tissue (36).

**CONCLUSION**

In accordance with the results of this study, the consumption of lime seems to be an alternative to lemon to prevent an aberration in the lipid profile. Lime juice treatment has an impact on the lipid profile similar to lemon juice, even more, effective in the case of total cholesterol.

**ACKNOWLEDGEMENT**

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**REFERENCES**

6. Navar-Boggan AM, Peterson ED, D’Agostino Sr RB, Neely B, Sniderman AD, Pencina MJ. Hyperlipidemia in early adulthood increases long-term

DOI: [http://dx.doi.org/10.21776/ub.ijhn.2020.007.02.7](http://dx.doi.org/10.21776/ub.ijhn.2020.007.02.7)


20. Rahayu T. Kadar Kolesterol

DOI: http://dx.doi.org/10.21776/ub.ijhn.2020.007.02.7


24. Collaboration CTT. Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials.


34. Ihedioha JI, Noel-Uneke OA, Ihedioha TE. Reference values

DOI: http://dx.doi.org/10.21776/ub.ijhn.2020.007.02.7
