Antidiabetic and Antihypercholesterolemic Activities of *Citrus Sinensis* Peel in Rats

Haryoto\textsuperscript{a}, Muhtadi\textsuperscript{a}, Tanti Azizah\textsuperscript{a}, Andi Suhendi\textsuperscript{a}

Abstract

Fruit peel of sweet oranges (*Citrus sinensis*) has been reported to contain flavonoid compounds and to have antioxidant and hypoglycemic effects. This study aims to determine the antidiabetic and antihypercholesterolemic activities of sweet orange fruit peel extract were tested against the white male rats in vivo. Each of these preclinical testing used the approach pre and post testing with control group design. Twenty-five rats were divided into 5 groups, namely group I (negative control) were given 0.5% CMC-Na, group II (positive control), group III, IV, V given sweet orange peel extract with successive doses 125, 250 and 500 mg/kgBW. Antidiabetic testing, rats induced by alloxan monohydrate dose of 150 mg/kgBW intraperitoneally, 4 days later the mice blood glucose levels have reached a hyperglycemic condition, ± 200 mg/dL. While the induction of hypercholesterolemia testing is done by providing a high-fat feed and feed high-fat diet, to achieve conditions in rat blood cholesterol levels peak at values > 130 mg/dL. The resulted of the study in diabetic rats for 10 days with 4 times the blood sampling days 0, 4, 11 and 15, showed that the sweet orange peel extract dose of 125, 250 and 500 mg/kg had reduced activity blood glucose levels with the percentage decreasing, 39.24 ± 4.96%; 46.18 ± 6.60% and 61.36 ± 5.57%, respectively. The resulted of research on antihypercholesterolemia testing measured at days 0, 14, 28, showed that the ethanolic extract of sweet orange peel with a dose of 500, 250 and 125 mg/kgBW had reduced blood cholesterol levels up to 57.61 ± 7.23%, 57.06 ± 6.47% and 54.77 ± 2.10%, stronger than the cholesteramine 800 mg/kgBW was a positive control, which only reduces blood cholesterol levels by 34.20 ± 10.48%.

**Keywords**: *Citrus sinensis*, blood glucose level, total cholesterol level, the white male rat

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Introduction

The garbage including fruit peel wastes are still regarded as useless material and pollute the environment. Though the wastefruit peels are a chemical that would have biological and pharmacological activity. As mangosteen peel extract that has been reported to have many pharmacological activities, such as antioxidant and antimicrobial (Palakawong et al., 2010), Anti-inflammatory (Nakataniet al., 2002), anti-tumoral, anti-allergy, and anti-viral activities (Suksamrarn et al., 2006; Pedraza-Chaverri et al., 2008).

The peel of citrus fruits has also been studied and reported to have several pharmacological activities, such as antibacterial (Wijiaustiti, 2010), antioxidant (Hegazy and Ibrahim, 2012), larvicidal, pupicidal, repellent and adulticidal activity (Murugan et al., 2012). Gil-Izquierdo et al. (2001) suggested that the flavonoid content of sweet citrus namely hesperidin (flavones) can be used as anti-inflammatory, anti-hypertensive, and prevent cardiovascular disease.

This paper describes the results of research that has been conducted on antidiabetic and antihypercholesterolemic activities of sweet orange (*Citrus sinensis*) peel extract in rats. The selection of antidiabetic and antihypercholesterolemic activities testing, because the incidence of diabetes and hypercholesterolemia from year to year has increased. The results of this study are expected to contribute both scientifically by obtaining scientific data of antihypercholesterolemic and antidiabetic activity and solutions to the problems of waste fruit peel and the utilization of traditional medicine in the community.
Methodology

Preparation of Materials

Sweet orange fruit peels obtained from Pasar Gede, Surakarta. The peels were collected, then cleaned, chopped into small pieces, dried, and then powdered with a blender. Rind powder is then weighed and ready to be extracted.

Extraction of Orange Fruit Peels

The extract of sweet orange fruit peels prepared by maceration method using 96% ethanol: acetone (4:1). A total of 2.5 kg of sweet citrus fruit peels were soaked in 10 L of ethanol 96% and 2.5 L of acetone protected from sunlight and stirred every day for 3 days. Results maceration then filtered with Buchner funnel. The remaining pulp was dried and stored at room temperature for 3 times. Liquid extract obtained was concentrated via an evaporator and then evaporated with an air bath until dried extract.

Testing of Preclinical Antidiabetic

Blood Sampling

Blood sampling was done through the lateral vein in rats contained as much as 0.5 mL Eppendorf tubes and stored at 4°C. Then centrifuged using minispin for 20 minutes at 1,200 rpm to obtain the serum. Furthermore, the supernatant was taken using a micropipette inserted into as many as 10 uL cuvettes then added 1000 mL GOD-PAP reagent mixture and incubated for 10 min at 37°C. Then blank, standard and sample absorbance is read using λ 500 nm visible spectrophotometer.

Treatment of Diabetic Induced Rats

Test animals were randomly divided into 5 groups, each consisting of 5 rats. Each rat was fasted blood drawn previously used for 12-15 hours and measured blood glucose levels. Furthermore, all groups were induced by alloxan monohydrate intraperitoneally 150 mg/kg (Sujono and Sutrisna, 2010). Four days after alloxan induced, mice blood glucose levels were measured again, if there is an increase in blood glucose levels of miceto ≥200 mg/dL is considered diabetic rats then. These animals were treated as follows:

Group 1: negative control, were given 0.5% CMC-Na.
Group II: positive control, given Glibenclamide dose of 0.45 mg/kg b.w.
Group III: Orange fruit peel extract dose of 125 mg/kg b.w. daily.
Group IV: Orange fruit peel extract dose of 250 mg/kg b.w. daily.
Group V: Orange fruit peel extract dose of 500 mg/Kg b.w. daily.

Anti Hypercholesterolemic Testing

Preparation of Diet and High Fat Feeding

Feeding and high-fat diet for 28 days was done. High-fat diet consisted of 50 mL of cooking oil, 10 g quail egg yolk, 0.1% PTU and water to 100.0 mL. How to make it is to mix the entire oil and quail egg yolk and then added to 100 mL of water. Mixed 0.1% PTU in drinking water (drinking bottle). Administered at a dose of 2 mL/200 g b.w. and always made new. Additionally fed a high-fat, consisting of 150 g standard feed (pellets), quail egg yolk 20 g, and 50 g margarine. How to make margarine is heated to melting then mixed all the ingredients and stirred until blended. Given high-fat feed as much as 30 g daily.

Treatment of Test Animals

Test animals used were white rats were divided into 25 groups of 5. Each group consisted of 5 rats were divided at random. All test animals first adapted to the standard and distilled water were fed ad libitum for 7 days. Before giving diet and a high-cholesterol feeding, the test animals were measured of cholesterol total levels. Then, after 4 weeks had given a high-fat diet and 2 weeks treated with the extract. Mouse blood samples of 1.5 mL were taken from the tail vein. High-fat feed as much as 30 g daily for 5 rats and high-fat diet with a dose of 2 mL/200 g b.w. While the treatment of the extract conducted for 2 weeks (after hypercholesterolemia with total cholesterol levels > 150 mg/dL) in all groups:

Group 1: 0.5% CMC-Na (negative control).
Group 2: Cholestyramine 0.8 g/kg (positive control).
Group 3: The ethanol extract of orange fruit peels dose of 500 mg/kg daily.
Group 4: The ethanol extract of orange fruit peels dose of 250 mg/kg daily.
Group 5: The ethanol extract of orange fruit peels dose of 125 mg/kg daily.

Results and Discussion

Extraction of orange fruit peels was using techniques maceration with ethanol: acetone (4:1). Sanjaya (2012) stated that the solvent mixture of ethanol and acetone can provide a good extracts because it was more selective, non-toxic, neutral, hot to less concentration and ethanol can be mixed with acetone in all comparisons. Yield obtained from orange fruit peel skin was 20.35%.

Induction of diabetes in rats this research using themethod of destruction of the pancreas by giving diabetogenic alloxan. Dose of alloxanmonohydrate150mg/kg given intraperitoneal alloxan causeable tomatoes condition of diabetes in rats (Sujono and Sutrisna, 2010). Induction of alloxan can cause an increasing blood glucose levelsto ≥200 mg/dL was considered diabetic rats. Then the rats
were treated negative controls (CMC-Na administration of 0.5%), positive control (administration of glibenclamide dose 0.45 mg/kg), orange peel extract dose: 125 mg/kg, 250 mg/kg and 500 mg/kg, for 10 days. The average yield measurement of rat blood glucose levels can be seen in Table 1.

The calculation of the percentage decrease in blood glucose levels showed that the higher dose of orange fruit peel extract greater reduction in blood glucose levels. The percentage decrease in blood glucose levels indicated in the treatment of the most high with orange fruit peel extract dose of 500 mg/kg which is equal to 61.36±5.57%.

While the cholesterol lowering effect test was conducted to determine the effect of citrus fruit peel extracts in lowering cholesterol levels in the blood by using three different dose ratings. Positive control treated cholesteramine form of 0.8 g/Kg BW daily. Wulandari (2009) stated that cholesteramine giving 800 mg/kg bw can reduce cholesterol with the value 52.97 ± 1:12% when administered for 30 days. While in this study cholesteramine given for 14 days (2 weeks) and can reduce blood cholesterol of 34.20% ± 10:42. The negative control used was 0.5% CMC-Na as CMC-Na does not affect blood cholesterol levels.

Table 1. The average measurement of blood glucose levels of rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glucose levels early (mg/dL)</th>
<th>Glucose levels of post alloxan (mg/dL)</th>
<th>Final glucose (mg/dL)</th>
<th>Percent increase in blood sugar (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control (CMC-Na 0.5%)</td>
<td>81.60±20.16</td>
<td>217.80±15.27</td>
<td>227.80±21.58</td>
<td>-</td>
</tr>
<tr>
<td>Positive control (Glibenclamide 0.45 mg/kg b.w.)</td>
<td>66.60±6.88</td>
<td>213.60±13.94</td>
<td>130.40±28.43</td>
<td>42.76±12.48</td>
</tr>
<tr>
<td>Orange fruit peel extract dose of 125 mg/kg b.w.</td>
<td>81.60±16.29</td>
<td>214±14.02</td>
<td>138.40±11.30</td>
<td>39.24±4.96</td>
</tr>
<tr>
<td>Orange fruit peel extract dose of 250 mg/kg b.w.</td>
<td>92±13.78</td>
<td>218.60±7.70</td>
<td>122.60±15.04</td>
<td>46.18±6.60</td>
</tr>
<tr>
<td>Orange fruit peel extract dose of 500 mg/kg b.w.</td>
<td>88.60±14.96</td>
<td>219.20±17.88</td>
<td>88±12.69</td>
<td>61.36±5.57</td>
</tr>
</tbody>
</table>

Table 2. Data of cholesterol levels decrease after being given the extract cholesterol levels (mg/dl)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Baseline</th>
<th>Induction for 4 weeks</th>
<th>After giving of extract</th>
<th>Decrease levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control (cholesteramine)</td>
<td>58.6±4.16</td>
<td>116.8±10.23</td>
<td>77.2±16.02</td>
<td>34.20±10.48</td>
</tr>
<tr>
<td>Negative control (CMC-Na)</td>
<td>61±11.87</td>
<td>145.8±23.40</td>
<td>126.6±32.17</td>
<td>13.44±15.45</td>
</tr>
<tr>
<td>Orange peel extract dose of 500 mg/kg b.w.</td>
<td>85.8±4.21</td>
<td>188.6±38.73</td>
<td>78.2±8.20</td>
<td>57.6±7.23</td>
</tr>
<tr>
<td>Orange peel extract dose of 250 mg/kg b.w.</td>
<td>64.6±12.99</td>
<td>196.6±37.48</td>
<td>82.6±5.27</td>
<td>57.06±6.47</td>
</tr>
<tr>
<td>Orange peel extract dose of 250 mg/kg b.w.</td>
<td>80±16.23</td>
<td>166.8±10.99</td>
<td>72.8±7.12</td>
<td>54.77±2.10</td>
</tr>
</tbody>
</table>

Cholesterol decreasing effect by the citrus fruit rind extracts with 3 dose levels did not differ significantly when compared with the positive control can be seen from Table 2. The cholesterol lowering activity may occur because of the ethanol extract of orange fruit peels contain a number of compounds that are responsible for reducing cholesterol levels. Citrus fruit peel extract contains flavonoids including hisperidin which supposedly can decrease cholesterol levels. Hisperidins compound that is responsible for reducing blood cholesterol levels. Ram (2006) also said that there are other contentinorange fruit peel that can reduce cholesterol levels in the serum of pectin which can also lower blood glucose levels.

Conclusion
The resulted of the anti-diabetic activity of orange (Citrus sinensis) peels with a dose of 125, 250 and 500 mg/kg b.w. had reduced activity blood glucose levels with the percentage decreasing, 39.24 ± 4.96%; 46.18 ± 6.60% and 61.36 ± 5.57%, respectively. The resulted of research on antihypercholesterolemia testing, showed that with a dose of 500, 250 and 125 mg/kg b.w. had reduced blood cholesterol levels up to 57.61 ± 7.23%, 57.06 ± 6.47% and 54.77 ± 2.10%, stronger than the cholesteramine 800 mg/kgBWwas a positive control, which only reduces blood cholesterol levels by 34.20 ± 10.48%.

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