Research Article

Antidiabetic and antihypercholesterolemic activities of *Citrus sinensis* peel: in vivo study

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Abstract

Background: Fruit peels are still regarded as useless materials and wastes; however, the chemical content in these fruit peels have pharmacological activities. **Aims and Objective:** To determine the *in vivo* antidiabetic and antihypercholesterolemic activities of sweet orange fruit peels extract. **Materials and Methods**: The study was conducted based on pre- and posttesting groups design with control. Twenty-five rats were divided into five groups—group I, negative control (0.5% CMC-Na); group II, positive control (glibenclamide and simvastatin); groups III, IV, and V were given sweet orange peel extract doses of 125, 250, and 500 mg/kg body weight (BW), respectively. For the antidiabetic study, the rats were induced by alloxan monohydrate (150 mg/kg BW intraperitoneally), and the blood glucose levels 4 days later were ± 200 mg/dL, which is a diabetic condition. Meanwhile, a hypercholesterolemic study was carried out by providing by a high-fat feed and feed high-fat diet to achieve blood cholesterol levels at values > 130 mg/dL. **Result:** The study showed that the sweet orange peel extract of these doses could reduce blood glucose levels with decreasing values of 39.24% ± 4.96%, 46.18% ± 6.60%, and 61.36% ± 5.57% in groups III–V, respectively. The most interesting feature was the activity of extracts in lowering blood cholesterol levels, which was almost similar, with the value around 55%, and this activity was higher than cholesteramine (800 mg/kg BW), for which the value was 34.20% ± 10.48%. **Conclusion:** The extract of *Citrus sinensis* peels with dose of 500 mg/kg BW showed the highest antidiabetic and antihypercholesterolemic activities in rats models.

KEY WORDS: *Citrus sinensis*; antidiabetic; antihypercholesterolemic; in vivo

Introduction

Fruit peels are still regarded as useless materials and pollute the environment; however, the chemical content in these fruit peels have pharmacological activities. For example, the mangosteen peel extract has been reported to present antioxidant and antimicrobial,[1] anti-inflammatory,[2] anticancer, antiallergy, and antiviral activities.[3,4]

The peels of citrus also had been studied and reported to have several antibacterial,[5] antioxidant,[6] larvicidal, pupicidal, repellent, and adulticidal pharmacological activities.[7] Gil-Izquierdo et al.[8] suggested that the flavonone and hesperidin in the sweet citrus can be used as an anti-inflammatory and antihypertensive and prevent cardiovascular disease.

This article describes the findings of research that had been conducted on antidiabetic and antihypercholesterolemic activities of sweet orange (*Citrus sinensis*) peels in rats. The results of this study are expected to contribute both scientifically by obtaining scientific data of antihypercholesterolemic and antidiabetic activities and solutions to the problems of waste fruit peels and the utilization in traditional medicine in the community.

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Materials and Methods

Sweet orange fruit peels were obtained from Pasar Gede, Surakarta. Herbarium voucher specimen was prepared and deposited in the Herbarium of Pharmacy Biology at Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Indonesia. The peels were collected, then cleaned, chopped into small pieces, dried, and powdered with a blender. Dried powder was then weighed and ready to be extracted.

Animals used for testing were male Wistar rats, which were obtained from the Laboratory of Pharmacology of Universitas Muhammadiyah Surakarta. The age of Wistar rats was approximately 2–3 months and weighed 175–225 g. This study was approved by Health Search Ethics Committee of Faculty of Medicine, Universitas Muhammadiyah Surakarta.

Extraction of Orange Fruit Peels

The extract of sweet orange peels was prepared by maceration using solvents system of 96% ethanol and acetone with ratio 4:1. A total of 2.5 kg of sweet citrus fruit peels powder was soaked in 10 L of ethanol 96% and 2.5 L of acetone, which was kept away from sunlight and stirred for 3 days. The maceration was then filtered with a Buchner funnel. The remaining pulps were subjected to remaceration for another two times. The filtration of extracts was combined and concentrated using an evaporator to obtain a dried extract.

Testing of Preclinical Antidiabetic Condition

Blood sampling. Blood sampling was collected through the lateral vein in rats, which contained as much as 0.5 mL in the Eppendorf tubes, and, then, centrifuged using mini spin for 20 min at 12,000 rpm to obtain the serum. Furthermore, the supernatant was transfered as much as possible into a 10 uL cuvette using micropipette; then, 1,000 mL GOD-PAP reagent mixture was added and incubated for 10 min at 37°C. Then, the absorbance of the blank, standard, and samples were determined using λ 500 nm visible spectrophotometer.

Treatment of Diabetic-Induced Rats. The animals used for testing were randomly divided into five groups, each consisting of five rats. Each rat was fasted for 12–15 h before the blood sample was drawn, and the blood glucose levels at baseline were measured. Furthermore, all the groups were induced by alloxan monohydrate intraperitoneally (150 mg/kg).[9] After 4 days, the blood glucose levels of alloxan-induced rats were measured again; an increase, if any, in the blood glucose levels of the rats up to ± 200 mg/dL was considered as diabetic condition. Then, each group was treated as follows:

Group I: negative control was given 0.5% CMC-Na.
Group II: positive control was given glibenclamide dose of 0.45 mg/kg body weight (BW).
Group III: Orange fruit peel extract dose of 125 mg/kg BW daily.
Group IV: Orange fruit peel extract dose of 250 mg/kg BW daily.
Group V: Orange fruit peel extract dose of 500 mg/kg BW daily.

The treatment of the extract was conducted for 15 days.

Antihypercholesterolemic Condition Testing

Preparation of diet and high-fat feeding. The rats used for testing were fed with high-fat diet for 28 days. High-fat diet was made to consist of 50 mL cooking oil, 10 g quail egg yolk, 0.1% propylthiouracil (PTU), and water to 100.0 mL; other high-fat feed consisted of 150 g standard feed (pellets), 20 g quail egg yolk, and 50 g margarine. Drinking water with 0.1% PTU at a dose of 2 mL/200 g BW was administered and always made new.

Treatment of Test Animals. The test animals used were white rats. They were divided into five groups. Each group consisted of five rats. All the test animals first adapted to the standard and distilled water were fed ad libitum for 7 days. Before giving the diet and a high-cholesterol feeding, the total cholesterol level of the test animals was measured. Then, they were given a high-fat diet for 4 weeks and treated with the extract for 2 weeks. The rats' blood samples of 1.5 mL were taken from the tail vein. High-fat feed were given as much as 30 g daily for five rats and high-fat diet with a dose of 2 mL/200g BW, while the treatment of the extract was conducted for 2 weeks (after hypercholesterolemia with total cholesterol levels $>$ 150 mg/dL) in all groups, as discussed further:

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.

Statistical Analysis

All data, blood glucose levels (mg/dL) and cholesterol levels (mg/dL) were expressed as mean ± SD (n = 5). The data in post-treatment of extract were assessed by Shapiro–Wilk test to obtain the prevalence of normal distribution. Statistical differences between the groups (treated groups and control) were identified by using one-way ANOVA; degree of freedom was $p < 0.05$. If the test was significant, it was then followed by LSD post hoc test.

Result

The orange fruit peels were extracted using cold maceration with a mixture solvents of ethanol:acetone (4:1). Sanjaya[10] stated that the solvent mixture of ethanol and acetone can provide a good extract, because it was more selective, nontoxic, neutral, hot to less concentration, and ethanol can be mixed with acetone in all comparisons. The percentage yield for the extraction of orange fruit peel was 20.35%.

Induction of diabetes in rats, in this study, was conducted based on the method of destruction of the pancreas by giving diabetonic alloxan. Dose of 150 mg/kg alloxan monohydrate was given by intraperitoneal route, which was able to induce

Table 1: The mean of blood glucose levels of rats, baseline, and pre- and post-treatment

Treatment	Glucose levels (mg/dL)			
	Baseline	Postalloxan	Pos treatment of extract	Percentage of decreasing
Negative control (CMC-Na 0.5%)	81.60 ± 20.16	217.80 ± 15.27	227.80 ± 21.58	—
Positive control (Glibenclamide 0.45 mg/kg BW)	66.60 ± 6.88	213.60 ± 13.94	130.40 ± 28.43*	42.76 ± 12.48
Orange fruit peel extract dose of 125 mg/kg BW	81.60 ± 16.29	214.00 ± 14.02	138.40 ± 11.30*	39.24 ± 4.96
Orange fruit peel extract dose of 250 mg/kg BW	92.00 ± 13.78	218.60 ± 7.70	122.60 ± 15.04*	46.18 ± 6.60
Orange fruit peel extract dose of 500 mg/kg BW	88.60 ± 14.96	219.20 ± 17.88	88.00 ± 12.69*(**)	61.36 ± 5.57

Data are expressed as mean ± SD (n = 5).
*Significant at $p < 0.05$ with negative control (CMC-Na).
**Significant at $p < 0.05$ with positive control (Glibenclamide)

the diabetic condition in rats.[9] Induction of alloxan caused an increment in blood glucose levels to ± 200 mg/dL, which was considered as diabetic rats. The negative control rats were treated with CMC-Na administration of 0.5%, while the rats administered with glibenclamide dose of 0.45 mg/kg were taken as postive control. All the treatments were given the orange peel extract with dosage of 125 mg/kg, 250 mg/kg, and 500 mg/kg for 10 days. The average measurements of rat blood glucose levels after the test are summarized in Table 1.

The cholesterol-lowering effect test, which is used to determine the effect of citrus fruit peel extract in lowering cholesterol levels in the blood, was conducted in dosages with three different ratings. The positive control was treated with cholesteramine form of 0.8 g/kg BW daily. Sujono and Sutrisna[9] stated that cholesteramine, which was given 800 mg/kg BW, reduced cholesterol by 52.97% ± 1.12% when administered for 30 days. In this study, cholesteramine given for 14 days (2 weeks) reduced the blood cholesterol by 34.20% ± 10.42%. The CMC-Na 0.5% was used as a negative control, and it does not affect the blood cholesterol levels.

Discussion

The result revealed that a higher dosage of orange fruit peel extracts exhibited a greater reduction in blood levels [Table 1]. For example, the highest dosage of orange fruit peel extract (500 mg/kg BW) showed a decrease of 61.36% ± 5.57% in blood glucose level when compared with the lowest dosage (125 mg/kg BW) that showed a decrease of 39.24% ± 4.96%, which was the lowest reduction in blood glucose level. The antidiabetic activity was also reported by Parmar and Kar[11] with the percentage of lowering glucose levels to be 19.30%.

Orange is known as a rich source of vitamin C, flavonoids, phenolic compounds, and pectin. The main flavonoids found in citrus species are hesperidin, narirutin, naringin, and eriocitrin.[11,12] Antidiabetic activity of orange is owing to flavonoids such as hesperidin and naringin in citrus fruit peels. It decreases the activities of glucose-6-phospate and phosphoenol pyruvate. The antidiabetic potential of orange peel appears to be mediated via antiperoxidation, inhibition of α-amylase enzyme activity that is responsible for the conversion of complex carbohydrates to glucose, increased hepatic glycogen content, stimulation of insulin secretion, and repair of secretory defects of pancreatic β-cells.[13]

Furthermore, both the antidiabetic and antihypercholesterolemic activities were owing to the active compounds such as flavonoids and phenolic contents. Orange fruits are well known as a source of vitamin C, flavonoids, phenolic compounds, and pectin. The main flavonoids found in citrus species are hesperidin, narirutin, naringin, and eriocitrin.[14,15] Antidiabetic activity of orange is owing to flavonoids such as hesperidin and naringin in citrus fruit peels. The mechanism of the activity is by enhanced insulin levels in blood, as direct or indirect effect on the improvement of beta cells of pancreas as reported for some

Table 2: The mean cholesterol levels decrease after being given the extract

Treatment	Cholesterol levels (mg/dL)			
	Baseline	Induction for 4 weeks	After giving of extract	Decreasing levels (%)
Positive control (cholesteramine)	58.60 ± 4.16	116.80 ± 10.23	77.20 ± 16.02*	34.20 ± 10.48
Negative control (CMC-Na)	61.00 ± 11.87	145.80 ± 23.40	126.60 ± 32.17*	13.44 ± 15.45
Orange fruit peel extract dose of 500 mg/kg BW	85.80 ± 4.21	188.60 ± 38.73	78.20 ± 8.20*	57.61 ± 7.23
Orange fruit peel extract dose of 250 mg/kg BW	64.60 ± 12.99	196.60 ± 37.48	82.60 ± 5.27*	57.06 ± 6.47
Orange fruit peel extract dose of 125 mg/kg BW	80.00 ± 16.23	166.80 ± 10.99	72.80 ± 7.12*	54.77 ± 2.10

Data are expressed as mean ± SD (n = 5)
*Significant at $p < 0.05$ with negative control (CMC-Na).

others medicinal plants extracts.[16] Lallan and Shyam.[17] have reported that pectin in orange fruit peel can reduce the cholesterol levels in the serum and blood glucose levels.

The active compounds that corresponded to the antidiabetic and antihypercholestermia activities of sample were hesperidin and naringin. Bok et al.[18] reported that naringin and hesperidin reduced the cholesterol and triglyceride levels significantly by inhibiting HMG-CoA reductase and acetyl-coenzyme A acetyl-transferase.[19] According to the recent WHO report, citrus fruits offer protection against cardiovascular diseases by reducing homocysteine level. Orange fruit contains vitamin C, carotenoids, and flavonoids, which possess cardiovascular-protective effects. Cholesterol-lowering effect of orange is produced by limonene. Polymethoxylated flavones are present in citrus fruit peel, which can lower cholesterol more effectively than some prescription drugs, without showing any side effects.[20]

Conclusion

Sweet orange (*C. sinensis*) peels extract have antidiabetic and antihypercholestermic activities at doses from 125 to 500 mg/kg BW and showed significant activities when comparable with the positive control.

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References

1. Palakawong C, Sophanodora P, Pisuchpen S, Phongpaichit S. Antioxidant and antimicrobial activities of crude extracts from mangosteen (*Garcinia mangostana* L.) parts and some essential oils. Inter Food Res J. 2010;17:583–9.
2. Nakatani K, Atsumi M, Arakawa T, Oosawa K, Shimura S, Nakahata N, et al. Inhibitions of histamine release and prostaglandin E2 synthesis by mangosteen, a Thai medicinal plant. Biol Pharm Bull. 2002; 25(9):1137–41.
3. Suksamrarn S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamrarn A. Cytotoxic prenylated xanthones from the young fruit of *Garcinia mangostana*. Chem Pharm Bull (Tokyo). 2006;54(3):301–5.
4. Pedraza-Chaverri J, Cardenas-Rodriguez N, Orozco-Ibarra M, Perez-Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). Food Chem Toxicol. 2008;46(10):3227–39.
5. Wijiastuti L. *Antibacterial Activity of Sweet Orange Fruit Peel (Citrus sinensis (L) Osbeck) Against Staphylococcos aureus, Escherichia coli multiresistant and BSLT (In Bahasa Indonesia)*. *Skripsi*. Fakultas Farmasi, Universitas Muhammadiyah Surakarta, 2011.
6. Hegazy AE, Ibrahium MI. Antioxidant activities of orange peel extracts. World Appl Sci J. 2012;18(5):684–8.
7. Murugan K, Kumar MP, Kovendan K, Amerasan D, Subrmaniam J, Hwang JS. Larvicidal, pupicidal, repellent and adulticidal activity of *Citrus sinensis* orange peel extract against *Anopheles stephensi, Aedes aegypti and Culex quinquefasciatus* (Diptera: Culicidae). Parasitol Res. 2012;111(4):1757–69.
8. Gil-Izquierdo A, Gil MI, Ferreres F, Tomas-Barberan FA. In vitro availability of flavonoids and other phenolics in orange juice. J Agric Food Chem. 2001;49(2):1035–41.
9. Sujono TA, Sutrisna EM. Effect of time pretreatment of rutin against hypoglycemic effects of tolbutamide on male rats alloxan induced (*In Bahasa Indonesia).* Jurnal Penelitian Sains and Teknologi. 2010;11(2):91–9.
10. Sanjaya A. *Powder Production of Food Colouring from Suji Leaves Extract* (Pleomele angsutifolia) *in Soxhlet and Maceration Extraction (In Bahasa Indonesia)*. DIGILIB UNNES (online), 2012. Available at: http://lib.unnes.ac.id/12466/ (last accessed on December 23, 2013).
11. Parmar HS, Kar A. Medicinal values of fruit peels from *Citrus sinensis*, *Punica granatum*, and *Musa paradisiaca* with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin, and thyroid hormones. J Med Food. 2008;11:376–81.
12. Wulandari S, Aji NI, Ratnaningrum H. Agent of anti-cholesterol based on chitosan synthesized from waste shrimp skin: preclinical testing for total cholesterol parameters, triglycerides, LDL-HDL and their influence on the aorta histopathology and blood pressure (*In Bahasa Indonesia*). Khazanah. 2009;2.
13. Abdelbaky MS, Elmehiry HF, Ali NKM. Effect of some citrus peels on hypercholesterolemic rats. Presented at the 1st International and 4th Arab Annual Scientific Conference on: Academic Accreditation for higher specific education Institutions and Programs in Egypt and Arab World "Reality and Expectation"; April 8–9, 2009; Faculty of Specific Education, Mansoura University, Egypt.
14. Guarnieri S, Riso P, Porrini M. Orange juice vs vitamin C: effect on hydrogen peroxide-induced DNA damage in mononuclear blood cells. Br J Nutr. 2007;97(4):639–43.
15. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activiy, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci. 2009;22(3):277–81.
16. Gray AM, Flatt PR. Antihyperglycemic actions of *Eucalyptus globulus* (Eucalyptus) are associated with pancreatic and extra-pancreatic effects in mice. J Nutr. 1998;128(12):2319–23.
17. Lallan R, Shyam S. Medicinal importance of citrus products and by-products—a review. Indian Journals.com. 2006;27(3):170–80.
18. Bok SH, Lee SH, Park YB, Bae KH, Son KH, Jeong TS, et al. Plasma and hepatic cholesterol and hepatic activities of 3-hydroxy-3-methyl-glutaryl-CoA reductase and acyl CoA: cholesterol transferase are lower in rats fed citrus peel extract or a mixture of citrus bioflavonoids. J Nutr. 1999;129(6):1182–5.
19. Wilcox LJ, Borradaile NM, de Dreu LE, Huff MW. Secretion of hepatocyte apoB is inhibited by the flavonoids, naringenin and hesperetin, via reduced activity and expression of ACAT2 and MTP. J Lipid Res. 2001;42(5):725–34.
20. Milind P, Dev C. Orange: range of benefits. Int Res J Pharm. 2012; 3(7):59–63.

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