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ORIGINAL ARTICLE

Effect of sunkist orange peel extract nanoparticle granules on the lipid profile levels in diabetic wistar rat

Maya Sari Mutia[1], Safriani Harahap[2], Fahira Zahra Nurhady[2], Robby Gunawan[3], Yunita Dewani[3], Muhammad Chairul[4], Qori Fadillah[5]*

Abstract

The research was conducted to determine the effect of Sunkist orange (*Citrus sinensis (L.) Osbeck*) peel extract nanoparticles in granular form on lipid profile levels in alloxan-induced Wistar rats (*Rattus norvegicus*). The study employed an experimental method with a posttest-only control group design. The population for this research was Wistar rats. The study used five treatment groups, resulting in a total of 25 rats, with five rats in each treatment group, as determined using the Federer formula. After the administration of sunkist orange peel extract nanoparticles at different doses for 14 days, the results showed a decrease in total cholesterol, LDL, triglycerides, and HDL levels. The research findings demonstrated the influence of reducing lipid profile levels in diabetic rats. The effective dose for reducing the lipid profile in this study was found to be 100 mg for total cholesterol, 70 mg for triglyceride, and 70 mg for both HDL and LDL when using the ethanol extract of sunkist orange peel.

Keywords: granule, nanoparticle, lipid profile

Introduction

Diabetes mellitus (DM) is a global issue.[1] According to data from the International Diabetes Federation (IDF), Indonesia ranks fifth among countries or regions with the highest number of adults (aged 20-79) with DM in 2021, with a total of 19.5 million people, and is estimated to increase to 28.6 million by 2045.[2] The prevalence of diabetes in the age group of 55-64 years was 4.8% in 2013, and increased to 6.29% in 2018, according to data from Riskesdas (Baseline Health Research).[3] The increasing number of patients is a problem for all of us, and it is important for patients with diabetes to control their blood sugar levels to prevent unwanted complications. Education about diabetes is needed for the community, as many people are unaware that they have type 2 diabetes, and delayed diagnosis can lead to complications such as heart disease, stroke, and vision problems.[2,4] Proper management is necessary for DM patients with uncontrolled blood sugar levels, with the aim of avoiding complications.[1]

Citrus sinensis is a type of orange that is widely cultivated in the world, commonly grown in semi-tropical and tropical regions.[5] The Sunkist orange or *Citrus Sinensis (L.) Osbeck* is very rich in vitamin C and can support the immune system due to its natural antioxidant content.[6] The ethanol extract of Sunkist orange peel contains flavonoids, saponins, tannins, terpenoids, alkaloids, and glycosides.[7] Sunkist oranges can combat the inflammation process, reduce the risk of metabolic disorders and chronic diseases, due to their rich content of bioactive compounds such as polyphenols, anthocyanins, and flavonoids.[8] Natural ingredients can be a good management for diabetes, and ethanol extract of Sunkist orange peel, which

Affiliation
[1]Departement of Histology, Universitas Prima Indonesia
[2]Undergraduate Programme in Medical Sciences, Universitas Prima Indonesia
[3]Department of Physiology, Universitas Prima Indonesia
[4]Department of Neurology, Universitas Prima Indonesia
[5]Department of Microbiology, Universitas Prima Indonesia

Correspondence
qorifadillah@gmail.com

contains tannins, phenolics, polyphenols, flavonoids, saponins, alkaloids, and terpenoids, has been proven to lower blood sugar levels.[9] The Sunkist orange peel is rich in phenolic compounds, which have benefits such as anti-diabetic, anti-gastrointestinal disease, antioxidant, and free radical-fighting properties.[10] Nanoparticles have a size ranging from 1 to 1000 nm. Nanoparticles have shown their ability in good drug delivery and can also protect drugs and other molecules.[11]

Due to the high incidence of diabetes, which can lead to numerous complications, there is a growing interest in the potential anti-diabetic properties of sunkist orange peel compounds. Therefore, researchers are motivated to further investigate the effects of sunkist orange peel extract on alloxan-induced diabetic rats. Given that nanoparticles can serve as effective drug carriers, this study aimed to evaluate the impact of nanoparticle-encapsulated sunkist orange peel extract on the lipid profile of alloxan-induced Wistar rats.

Method

This research used an experimental method with a post-test control group design. This study was conducted from February to September 2023 at the Integrated Research Laboratory of the Faculty of Medicine, Universitas Prima Indonesia, Focus Lab, and UPTD Laboratorium Kesehatan. The tools used were a fan scale, oven, analytical balance, autochek glucometer, glucose strips, blender, petri dish, sample bottle, microscope, incubator, Pyrex measuring glass, Pyrex beaker glass, gloves, mask, cage, and food and drink containers for 25 male Wistar rats. The research materials used were sunkist orange peel extract, aquades, 96% ethanol, aloxan, metformin, phosphate-buffered saline (PBS) solution, 70% alcohol, Wistar male white rat pellet food, and filter paper.

The study used five treatment groups, with five rats per treatment group, for a total of 25 rats. Twenty-five white rats, weighing approximately 200 g and aged 2-3 months were used. The rats were acclimatized to the laboratory environment for one week. There were five groups of rats, each consisting of five control rats (-) administered aquadest and 125 mg/kg BW alloxan, five control(+) rats administered 125 mg/kg BW alloxan + 150 mg/KgBW metformin, treatment 1 administered 125 mg/kg BW alloxan + 50 mg granular nanoparticle extract of sunkist orange peel, treatment 2 administered 125 mg/kg BW alloxan + 70 mg granular nanoparticle extract of sunkist orange peel, and treatment 3 administered 125 mg/kg BW alloxan + 100 mg granular nanoparticle extract of sunkist orange peel. The data were analyzed using one-way analysis of variance (ANOVA) for normally distributed data and Kruskal–Wallis test for non-normally distributed data. The results of the statistical test, which showed differences in the means ($p<0.05$), were followed by a post-hoc test.

Results

In this study, 25 Wistar rats were divided into five groups to investigate the effect of extracellular nanoparticles of sunkist orange peel in the form of granules on the lipid profile of Wistar rats. The study was conducted for 28 days, with seven days of adaptation, followed by intraperitoneal induction of rats, and blood glucose levels exceeding 200 mg/dl on the sixth day. The rats were then divided into groups according to treatment, and after 14 days of treatment, blood samples were collected for lipid profile analysis. The results of the normality test showed that the total cholesterol and triglyceride data were normally distributed, while the HDL and LDL data were not normally distributed. The total cholesterol and triglyceride data were analyzed using the One-Way ANOVA test, and the comparison of average LDL and HDL cholesterol levels between groups was performed using the Kruskal-Wallis test.

Table 1. Cholesterol levels of rats

Treatment	Total (Mean±SD)	p
Group 1 Control (+)	104.00 ± 9.54	
Group 2 Control (-)	90.67 ± 11.02	
Group 3 (dose 50 mg/kg BW)	85.00 ± 4.00	< 0.001
Group 4 (dose 70 mg/kg BW)	61.67 ± 8.20	
Group 5 (dose 100 mg/kg BW)	69.33 ± 1.53	

Table 2. LDL levels of rats

Treatment	Total (Mean±SD)	p
Group 1 Control (+)	3,00 ± 1,00	
Group 2 Control (-)	2.33 ± 0.58	
Group 3 (dose 50 mg/kg BW)	2,00 ± 1,00	0.672
Group 4 (dose 70 mg/kg BW)	2.33 ± 0.58	
Group 5 (dose 100 mg/kg BW)	2,00 ± 1,00	

Table 1 shows the potential of extracting granule nanoparticles from jackfruit peels to reduce total cholesterol. Based on the statistical results, a significant difference in total cholesterol level was observed

among the groups (p<0.001). The Post Hoc Games-Howell test showed a significant difference in the mean values between the control group (+) and group 4 (p=0.021), control group (+) and group 5 (p=0.003), and groups 3 and 5 (p=0.044). The results of this study indicate that 1000 mg/kg BW is the optimal dose, showing an effective reduction in cholesterol levels. Table 2 reveals that the obtained p-value of 0.672 (>0.05) indicates that there was no significant difference in LDL cholesterol levels among the groups.

As shown in Table 3, HDL levels among the treatment groups were significantly different (p< 0.05). The Mann–Whitney U test indicated that there was no significant difference in the mean values among these groups (p>0.05).

Table 3. HDL levels of rats

Treatment	Total (Mean±SD)	p
Group 1 Control (+)	62.33 ± 5.86	
Group 2 Control (-)	55.33 ± 5.86	
Group 3 (dose 50 mg/kg BW)	49.33 ± 0.58	0.028
Group 4 (dose 70 mg/kg BW)	45.33 ± 0.58	
Group 5 (dose 100 mg/kg BW)	42.67 ± 7.37	

Table 4. LDL levels of rats

Treatment	Total (Mean±SD)	p
Group 1 Control (+)	193.33 ± 14.74	
Group 2 Control (-)	178.67 ± 31.79	
Group 3 (dose 50 mg/kg BW)	167.67 ± 20.01	0.002
Group 4 (dose 70 mg/kg BW)	142,00 ± 13.53	
Group 5 (dose 100 mg/kg BW)	71.33 ± 43.50	

In Table 4, it can be seen that the triglyceride levels among the treatment groups have a significant result, with a p-value of 0.002 (<0.05), indicating that the granular extract of sunkist orange peel has a significant effect on triglyceride levels. The Post Hoc Bonferroni test showed a significant difference in the mean values between the control group (+) and group 4 (p=0.003), the control group (-) and group 4 (p=0.007), and groups 3 and 4 (p=0.015). The results of this study indicate that 70 mg/kg BW is the optimal dose, showing an effective reduction in triglyceride levels in aloxan-induced diabetic rats.

Discussion

In this study, it was found that the administration of the nanoparticle extract of sunkist orange peel in granule form resulted in a statistically significant change in the lipid profile levels of male Wistar rats induced by alloxan. The control group (-), which was not administered alloxan, did not experience an increase in blood sugar. This group was used for comparison with the other groups. The average total cholesterol level in the control group (-) was 90.67 ± 9.54, indicating normal cholesterol levels. The control group (+) had an average total cholesterol level of 104 ± 9.54, which was higher than that of the control group (−). Treatment 1 (dose of 50 mg/kgBW) showed a cholesterol level of 85 ± 4.00, which was significantly different from the other groups. Treatment 2 (dose of 70 mg/kgBW) showed a cholesterol level of 61.67 ± 8.2, and treatment 3 (100 mg/kg BW) showed a cholesterol level of 69.33 ± 1.53. These results indicate that the cholesterol level in rats treated with sunkist orange peel extract nanoparticles was better at reducing total cholesterol levels.

The average HDL level in the control group (-) was 55.33 ± 5.86, while that in the control group (+) was 62.33 ± 5.86. The group that received treatment 1 (dose of 50 mg/kgBW) showed an HDL level of 49.33 ± 0.58, treatment 2 (dose of 70 mg/kgBW) was 45.33 ± 0.58, and treatment 3 (dose of 100 mg/kgBW) was 42.67 ± 7.37. From these data, it can be seen that the control (+) group administered metformin 150 mg/kg BW was better than the other treatment groups. The average LDL level in the control group (-) was 3 ± 1, the control group (+) was 2.33 ± 0.58, treatment 1 was 2 ± 1, treatment 2 was 2.33 ± 0.58, and treatment 3 was 2 ± 1. From these data, it can be seen that treatments 1 and 3 were better at reducing the LDL levels. The average triglyceride level in the control group (-) was 178.67 ± 31.79, the control group (+) was 193.33 ± 14.74, treatment 1 was 167.67 ± 20.01, treatment 2 was 142 ± 13.53, and treatment 3 was 71.33 ± 43.50. From these data, treatment 3 (dose of 100 mg/kg BW) was the best in reducing triglyceride levels compared to the other groups and treatments.

The toxic action of alloxan on pancreatic β cells is initiated by free radicals (ROS) formed by redox reactions.[12] In patients with diabetes mellitus, leading to an increase in blood sugar levels beyond the normal limit. The increase in blood glucose is proportional to the increase in free radicals (ROS) in the body, triggering various complications, one of which is dyslipidemia.[13] Dyslipidemia is characterized by an increase in total cholesterol levels, an increase in the bad cholesterol LDL (Low-Density Lipoprotein), an increase in triglyceride levels (TG), and a decrease in the good cholesterol HDL (High-Density Lipoprotein) in the blood.[14]

The orange peel extract contains flavonoids, which have excellent effects on the human body, and polyphenols, which also have anti-inflammatory and antioxidant potential. Synthetic antioxidants can help scavenge free radicals in the human body.[15] This is in line with the research conducted by Hammad et al.[16] who conducted a study on rats fed a diet containing orange peel at varying levels of 5%, 7.5%, and 10% for 28 days. Diabetes was induced in male rats by intraperitoneal injection of 150 mg/kg alloxan. Sunkist orange peel extract can be beneficial to patients with diabetes and help reduce excessive sugar levels. This is because of the natural pectin present in oranges, which helps reduce blood sugar levels. The data obtained in this study showed that the highest reduction in blood sugar levels was achieved in diabetic rats fed a 10% orange peel diet. Orange peel also contains phenolics, which, when consumed in high amounts, can increase HDL levels and protect LDL from oxidation. Research conducted by Rajiv et al.[17] supports this finding, showing that the administration of orange peel extract (50 and 100 mg/kg) along with metformin can reduce total cholesterol, triglyceride, and LDL levels compared to the control group. Polyphenols in orange peel, which also have antioxidant properties, are believed to function better within the body.

Conclusion

Administration of the nanoparticle extract of sunkist orange peel to diabetic male Wistar rats demonstrated a significant effect on lipid profile reduction. The effective dosages for total cholesterol (100 mg), triglyceride (70 mg), HDL (70 mg), and LDL (70 mg) were determined. Sunkist orange peel extract significantly reduced the lipid profile of diabetic Wistar rats. The extract of sunkist orange peel reduced the lipid profile in diabetic Wistar rats.

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