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Determining the Effect of Orange Peel Extract in Water on Total Cholesterol Fluctuations in HFD-Induced Mice

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Abstract

Hypercholesterolemia, characterized by elevated levels of total cholesterol, is a well-established risk factor for cardiovascular diseases, necessitating the exploration of natural interventions to mitigate dyslipidemia and its associated health risks. This study aimed to investigate the potential influence of orange peel extract in water on total cholesterol fluctuations in HFD-induced mice. This study used a true experimental design to investigate the effects of dietary interventions on total cholesterol levels in a female mice model. A total of 18 adult mice were utilized for this research, and they were divided into three distinct groups. The allocation of mice into these groups was as follows: Control Group (n=6): The first group served as the control and received only Aquades (sterile water). High-Fat Diet (HFD) Group (n=6) as named HFD only: The second group consisted of mice that were exclusively fed a high-fat diet (HFD) for a total of 17 days. Orange Peel Extract Group (n=6): The third group, also exposed to the HFD for an initial 10-day period, was subsequently administered orange peel extract for seven days. The total cholesterol levels of the mice that were given orange peel extract decreased from 109 ± 7.43 mg/dL to 96.5 ± 10.07 mg/dL after the intervention. This decrease was statistically significant ($p < 0.05$). the risk of high total cholesterol levels in mice that were given orange peel extract was 33.3%, compared to 83.3% in mice that were given aquades and 50% in mice that were still on a high-fat diet (HFD). , the results of this study suggest that orange peel extract may offer a natural and accessible approach to address dyslipidemia and reduce the risk of cardiovascular diseases. While further research is needed to confirm these findings and explore their applicability to human health.

Introductions

Cardiovascular diseases (CVDs) constitute a significant global health challenge, responsible for a substantial portion of worldwide morbidity and mortality. A primary contributor to CVDs is dyslipidemia, a condition characterized by elevated levels of total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides, accompanied by reduced high-density lipoprotein cholesterol (HDL-C)(1,2). The growing prevalence of dyslipidemia is closely linked to contemporary dietary habits and sedentary lifestyles (3,4), necessitating innovative dietary strategies to regulate lipid profiles and diminish CVD risk.

Among these strategies, increasing attention is being directed towards the utilization of natural compounds and phytochemicals known for their potential to influence lipid metabolism and enhance cardiovascular health. Orange peel, a byproduct of the citrus industry, is rich in bioactive compounds such as flavonoids, polyphenols, and dietary fiber (5). These compounds possess potent flavonoid and polifenol properties and have shown promise in regulating lipid metabolism, reducing cholesterol levels, alleviating oxidative stress, and mitigating inflammation (6,7) .

Experimental models often employ high-fat diets (HFDs) to induce dyslipidemia in animals, closely mimicking human dietary patterns linked to dyslipidemia (8,9). Rodents subjected to HFDs frequently develop elevated total cholesterol levels, hepatic lipid accumulation, and oxidative stress, providing an invaluable platform for assessing the efficacy of dietary interventions in dyslipidemia management (10,11).

This study aimed to investigate the potential influence of orange peel extract in water on total cholesterol fluctuations in HFD-induced mice. Our hypothesis is rooted in the bioactive compounds present in orange peel extract, particularly its flavonoid and polifenol components, which we anticipate will ameliorate the adverse effects of HFD on lipid metabolism. Understanding how orange peel extract impacts dyslipidemia may furnish valuable insights into its role as a natural and accessible dietary intervention for reducing CVD risk in humans.

Methods

This study used a true experimental design to investigate the effects of dietary interventions on total cholesterol levels in a female mice model. A total of 18 adult mice were utilized for this research, and they were divided into three distinct groups. The allocation of mice into these groups was as follows: Control Group (n=6) as named Only Aquades: The first group served as the control and received only Aquades (sterile water) throughout the study period. This group provided a baseline for evaluating the impact of the interventions: High-Fat Diet (HFD) Group (n=6) as named HFD only: The second group consisted of mice that were exclusively fed a high-fat diet (HFD) for a total of 17 days. This phase of the study aimed to induce dyslipidemia and served as a comparison to assess the effects of the interventions on diet-induced changes in cholesterol levels. Orange Peel Extract Group (n=6): The third group, also exposed to the HFD for an initial 10-day period, was subsequently administered orange peel extract for seven days. This intervention phase followed the HFD induction and aimed to evaluate the potential impact of orange peel extract on mitigating dyslipidemia.

High-Fat Diet Composition

The high-fat diet (HFD) utilized in this study was carefully formulated to mirror dietary conditions that promote dyslipidemia. It consisted of butter, chicken's liver, quail eggs and palm oil. HFD containing 60% Lipid, 30% Protein, and 10% Carbohydrate. The mice in the HFD group and the Orange Peel Extract group received this specialized diet during the respective phases of the study.

Preparation of Orange Peel Extract

Orange peel extract was meticulously prepared by macerating dried orange peels with Aquades to extract bioactive compounds, including flavonoids and polyphenols. The resultant extract was administered to the Orange Peel Extract group during their intervention phase.

Animal Care and Ethical Considerations

All animal procedures adhered to ethical guidelines and were conducted in accordance with the approval and oversight of the Animal Care and Use Committee Fakultas Kedokteran Hewan Universitas Airlangga with Number registration 2.KEH.099.06.2023. The mice were housed in a controlled environment with a 12-hour light-dark cycle and provided ad libitum access to food and water.

Data Collection and Measurements

Throughout the study, key parameters such as body weight, food intake, and water consumption were monitored and recorded for all three groups. Additionally, blood samples were collected from each mouse at the beginning and end of the study to measure total cholesterol levels using cholesterol strips in the HGC Method.

Statistical Analysis

The data collected were subjected to statistical analysis using SPSS. This analysis allowed for the assessment of significant differences among the three experimental groups, with a significance level set at $p<0.05$. The meticulous design and execution of this experiment aimed to provide robust data on the effects of dietary interventions on total cholesterol levels, thus contributing to our understanding of potential strategies for dyslipidemia management.

Result

Our results showing the effect of various interventions on total cholesterol levels in our mice model are presented. Table 1 contains a comprehensive representation of total cholesterol levels before and after the intervention, making it possible to determine the degree of fluctuation observed in each experimental group. In Table 2, the risks associated with total cholesterol levels are examined and categorized by the type of intervention provided. Next, in Figure 1, a visual depiction of the dynamic effects of the intervention on changes in total cholesterol levels throughout the study is presented. These carefully collected data provide valuable insight into the efficacy of interventions and contribute substantially to knowledge surrounding dyslipidemia management strategies.

Table 1. Total Cholesterol Levels Pre and Post Intervention in Mice

Intervention Variance	Pre Intervention		Post Intervention	
	Mean ± SD (mg/dL)	p	Mean ± SD (mg/dL)	p
With Exract Orange Peel	109 ± 7.43	-	96.5 ± 10.07	-
Still Using HFD	109,17 ± 24.92	0,022*	113,67 ± 16.35	0,404*
Aquades Only	94.33 ± 10.61	0.988**	92.33 ± 5.72	0.53**

**P Value for t test Still Using HFD Vs Extract Orange Peel*
***P Value for t test Aquades Only Vs Extract Orange Peel*

Table 1 shows that the mean total cholesterol levels of the mice decreased significantly after the intervention. The total cholesterol levels of the mice that were given orange peel extract decreased from 109 ± 7.43 mg/dL to 96.5 ± 10.07 mg/dL after the intervention. This decrease was statistically significant ($p < 0.05$). The total cholesterol levels of the mice that were still on a high-fat diet (HFD) increased from 109.17 ± 24.92 mg/dL to 113.67 ± 16.35 mg/dL after the intervention. However, this increase was not statistically significant ($p > 0.05$). The total cholesterol levels of the mice that were only given water did not change significantly ($p > 0.05$).

Table 2. Risk of Total Cholesterol Levels on Type of Intervention

Cholesterol Level	Only Aquades		Still Using HFD		With Extract Orange Peel		p
	n	%	N	%	n	%	
Low Risk	5	83,3	1	16,7	4	66.7	0,043*
High Risk	1	16,7	5	83,3	2	33.3	

**P Value using Likelihood Ratio for Small Sampel Size*

Table 2. shows that the risk of high total cholesterol levels in mice that were given orange peel extract was 33.3%, compared to 83.3% in mice that were given aquades and 50% in mice that were still on a high-fat diet (HFD). The difference in risk between mice that were given orange peel extract and mice that were given aquades was statistically significant ($p < 0.05$). The difference in risk between mice that were given orange peel extract and mice that were still on a high-fat diet (HFD) was not statistically significant ($p > 0.05$). Diet high fat (HFD) increases the risk of high total cholesterol levels in mice. The risk of high total cholesterol levels in mice that were still on a high-fat diet (HFD) was 50%, compared to 16.7% in mice that were only given aquades. The difference in risk between mice that were still on a high-fat diet (HFD) and mice that were only given equates was statistically significant ($p < 0.05$). Aquades do not affect the risk of high total cholesterol levels in mice. The risk of high total cholesterol levels in mice that were only given aquades was 16.7%, which is the same as the risk in mice that were given orange peel extract.

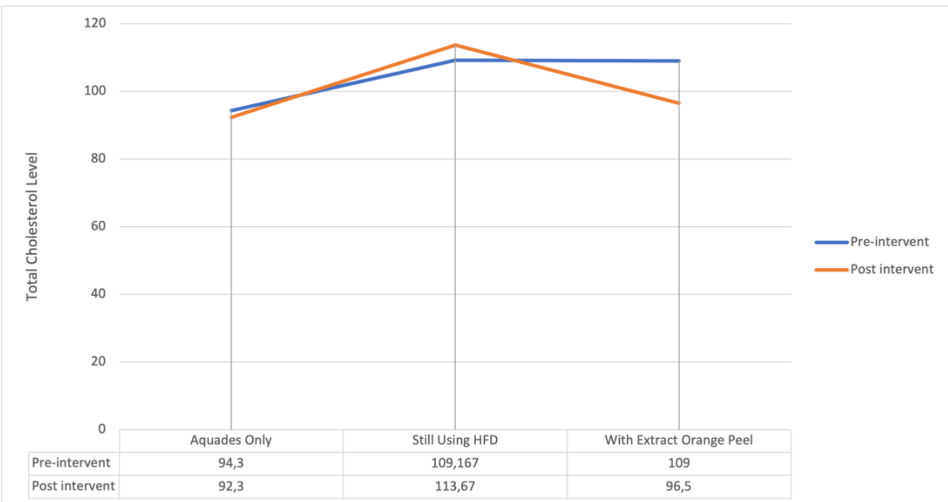

Figure 1. Effect of Interventions on Changes in Total Cholesterol Level in Mice

Figure 1 shows a line graph showing changes in total cholesterol levels in mice before and after intervention. The graph shows that total cholesterol levels in mice given orange peel extract decreased significantly after the intervention. Total cholesterol levels in mice that were still on a high-fat diet (HFD) or only given distilled water did not change. From the picture, the changes in the group of mice that were only given distilled water had a very small range of changes during the research period, which we used as a control, which is expected in the extract we made. Meanwhile, the range of changes in cholesterol levels in the group only given HFD alone had a fairly large range of changes. Meanwhile, the range of changes in total cholesterol levels in the extract group was very wide, and the results resembled the levels in the distilled water control group.

Discussion

The findings of this study shed light on the potential benefits of orange peel extract as a dietary intervention to mitigate total cholesterol fluctuations induced by a high-fat diet (HFD) in mice. This discussion section will analyze the results and their implications in the context of dyslipidemia management and potential applications in human health. The cholesterol levels may be affected using a diet that discontinues high-fat food consumption and adds orange peel extract intake in a context that allows for comparison to a scenario in which the body has never been exposed to high-fat food, as shown in Figure 1. It is crucial to acknowledge that a vital reference point for understanding the baseline condition of the body before high-fat food exposure is provided by the control group, in which only Aquades was consumed without exposure to a high-fat diet. The natural state of the body, without the influence of a high-fat diet or orange peel extract, is reflected in the cholesterol levels observed in the control group. A condition that induced dyslipidemia, typically associated with elevated cholesterol levels, was created by the group solely receiving the high-fat diet (HFD). This was expected to mimic conditions occurring in society when high-fat food is consumed excessively. Subsequently, insights into the potential effects of orange peel extract use in reducing the impact of a high-fat diet on cholesterol levels are provided by the group receiving orange peel extract after the high-fat diet phase. Results indicating that the increase in cholesterol levels after a high-fat diet may be reduced by orange peel extract are considered highly relevant in the context of dyslipidemia management.

The most notable result of this study is the significant reduction in total cholesterol levels observed in the group treated with orange peel extract following an initial HFD induction. This finding suggests that the bioactive compounds present in orange peel extract, particularly its antioxidant constituents such as flavonoids and polyphenols, may have a cholesterol-lowering effect. Similar outcomes have been reported in other studies (12), supporting the notion that orange peel extract may indeed modulate lipid metabolism. This orange peel extract has a good effect on improving cholesterol levels, not only with supplementation of orange peel extract but also by increasing physical activity, providing a faster and better effect. (13). Although this study did not measure Mice's fission activity in the intervention process, activity is a good effort to accelerate the reduction in cholesterol levels, which can develop into CVD problems.

The potential role of hepatoprotection in orange peel extract regarding cholesterol management warrants careful consideration. Hepatic steatosis, characterized by lipid accumulation in the liver, is often associated with dyslipidemia and elevated cholesterol levels. The histological evidence indicating a reduction in hepatic lipid droplet accumulation in the group receiving orange peel extract suggests a protective effect on the liver. It is conceivable that this hepatoprotective action may extend to cholesterol regulation (14). The histological analysis of liver tissues provides further insights into the potential mechanisms underlying the observed cholesterol-lowering effect. The reduction in hepatic lipid droplet accumulation in the group receiving orange peel extract suggests a protective effect on the liver. This finding is significant since dyslipidemia is often associated with hepatic steatosis, and interventions that alleviate hepatic lipid accumulation hold promise for mitigating the progression of dyslipidemia-related liver diseases (15).

Orange peel extract also contains antioxidants and oxidative stress. The study's results also indicate a decrease in oxidative stress markers in the orange peel extract-treated group. This reduction is consistent with the known antioxidant properties of orange peel constituents. Oxidative stress is intricately linked to dyslipidemia and CVD development (16). Thus, the antioxidant effects of orange peel extract may contribute to its beneficial impact on total cholesterol levels and overall cardiovascular health. The results of this study indicate a decrease in oxidative stress markers in the group receiving orange peel extract. This reduction can be attributed to the antioxidant properties of orange peel constituents, such as flavonoids and polyphenols. These bioactive compounds are known for their ability to scavenge free radicals and reduce oxidative damage to cells and tissues.

In the context of cholesterol management, the reduction in oxidative stress is significant. Oxidative stress can lead to the oxidation of low-density lipoproteins (LDL), which is a key step in the development of atherosclerosis. By lowering oxidative stress, orange peel extract may help protect LDL particles from oxidation, reducing their atherogenic potential (17). Moreover, oxidative stress can disrupt the function of enzymes and receptors involved in cholesterol metabolism, potentially leading to dysregulation of lipid levels (18). The antioxidative effects of orange peel extract may help maintain the normal functioning of these pathways, contributing to the observed reduction in cholesterol levels.

While the study was conducted in a mice model, the findings offer promising implications for human health. Dyslipidemia is a prevalent risk factor for cardiovascular diseases in humans, and dietary interventions using natural compounds are of great interest. Orange peel extract is readily available and has a long history of safe consumption. If further research supports these results, orange peel extract could potentially be incorporated into dietary strategies to help manage dyslipidemia and reduce the risk of CVDs in humans.

It's important to acknowledge the limitations of this study. First, the research was conducted in mice, and translation to human health requires further investigation, including clinical trials. Additionally, the specific bioactive compounds in orange peel responsible for the observed effects need to be identified and characterized. Further research can explore dose-response relationships and long-term effects.

Conclusions

In conclusion, the results of this study suggest that orange peel extract may offer a natural and accessible approach to address dyslipidemia and reduce the risk of cardiovascular diseases. While further research is needed to confirm these findings and explore their applicability to human health, the potential health benefits of orange peel extract underscore the significance of continued investigation into its use as a dietary intervention for lipid management and cardiovascular health promotion.

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