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The *Citrus sinensis* peel extract's increase HDL and reduce LDL levels in cigarette smoke-induced Rats

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ABSTRACT

Oxidative substances that contain in cigarettes could cause oxidative stress and change the lipid profiles in High-Density Lipoprotein (HDL) and Low-Density Lipoprotein (LDL). However, oxidative stress could be inhibited by antioxidants from the extract of sweet orange peel. The effect of Citrus sinensis peel extract on HDL and LDL levels is investigated in this study. Wistar rats were employed as subjects in this study. The research subjects were divided into five groups, namely, normal control, given cigarette smoke without *Citrus sinensis*, and given *Citrus sinensis* peel extract. The results showed that the administration of *Citrus sinensis* peel extract could inhibit HDL reduction and increase LDL levels. The highest score of HDL levels is shown in the negative control group (37.5 mg/dL). On the other hand, the lowest shows in the normal control group (2.66 mg/dL) with a significant $p < .001$. The lowest post-test HDL levels were found in the negative control group. The highest post-test HDL levels were in group V. The highest difference in LDL levels was in the negative control group (53.57 mg/dL) and the lowest in the normal control group (3.26 mg/dL). The highest post-test LDL levels were found in group V. The lowest post-test LDL levels were in group V.

Keywords: Cigarette Smoke, *Citrus sinensis*, HDL, LDL

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INTRODUCTION

Cardiovascular disease (CVD) is well acknowledged as a major cause of morbidity and mortality worldwide. According to the data presented, it is evident that over 17 million individuals succumbed to cardiovascular disease (CVD), with a significant proportion of over 6 million experiencing early mortality, defined as death occurring before the age of 70. Nevertheless, it is widely acknowledged that most cardiovascular diseases (CVD) can be averted, as stated by the World Health Organization (Mendis et al., 2011).

Hence, dyslipidemia represents a contributing factor to the growth of cardiovascular disease. Dyslipidemia can be effectively managed with dietary modifications, such as reducing the consumption of saturated fat and cholesterol while concurrently increasing the intake of hypolipidemic foods. Several studies have provided evidence supporting the antiatherogenic properties of a diet that is abundant in *polyphenols* and *antioxidants*. *Citrus sinensis* peel contains antioxidants that can prevent escalate lipid peroxidation due to the output of reactive oxygen species (ROS) (Muhtadi et al., 2014). It contains antioxidants, namely phenolic compounds, flavonoids, carotenoids, and anthocyanins. The Fruit peels, as a source of antioxidant compounds, has better biological activity than other parts (Hussain et al., 2023).

It is said that *Citrus sinensis* has antioxidant, anti-inflammatory, and hypolipidemic properties (Mallick & Khan, 2016). Phenols and flavonoids found in different citrus species possess beneficial properties that have attracted scientific attention in the field of human health. These chemicals have been identified for their antibacterial, anti-inflammatory, anticancer, and cardio protecting actions (Saleem et al., 2023). The essential oils contained in orange juice (*Citrus sinensis*) contain a variety of components, including monoterpenes and sesquiterpenes, with d-limonene being a notable component. In contrast, it has been documented that orange juice or its flavonoid constituents have demonstrated the ability to induce reductions in cholesterol levels in both rabbits and people (Mallick & Khan, 2016).

The prevalence of tobacco use in Indonesia is significantly elevated. According to the World Health Organization (World Health Organization, 2021), the number of active smokers among adult males exceeds 60 million, while the corresponding figure for adult females stands at 3.7 million. Additionally, there has been a consistent upward trend in tobacco consumption among young individuals in recent times. According to Tan & Dorotheo (2018), Indonesia held the highest position in terms of smoking rates among Southeast Asian countries in 2016.

Smokers' increased lipid peroxidation and antioxidant deficiency may lead to biomolecular vascular endothelial damage (Kamceva et al., 2016). Smoking leads to decreased concentrations of apolipoproteins AI and AII, mirroring a drop in HDL cholesterol. It, on the other hand, produces an increase in apolipoprotein B and the apolipoprotein B/AI ratio. This emphasizes the negative consequences of smoking on fat metabolism, which increases the risk of heart disease (Kauss et al., 2022).

Cigarette smoke contains oxidants that increase lipid peroxidation (oxidative damage). The increasing of lipid peroxidation to lipid biomolecules in the reactivity of ROS compounds. Lipid peroxidation causes changes in lipid profiles, specifically, lower levels of HDL and higher levels of LDL (Venkatesan et al., 2006).

This study focuses on finding out the effect of *citrus sinensis* on HDL and LDL levels through rat cigarette smoke induction.

MATERIALS AND METHOD

Materials

Plant material

Citrus sinensis was procured at a nearby grocery store by carefully considering its qualities as described in relevant literature and scientific sources. *Citrus sinensis* is a flowering tree that is evergreen. Its trees are typically 9–10 m tall and have huge spines on its branches. The leaves are arranged in pairs and have thinly winged petioles that are 3-5 mm in width and 6.5-15 cm in length. The blades themselves can be elliptical, oval, or oblong in shape, and have blunt teeth. Because they contain a lot of oil, the leaves have a distinct citrus scent (Steduto et al., 2012). Flowers are axillary and are borne individually or in whorls of six (5 cm wide), with 20–25 golden stamens and five white petals. The fruit ripens to an orange or golden color and can be globose to oval (6.5 to 9.5 cm broad). The fruit is divided anatomically into two parts: the endocarp, or pulp, which has juice sac glands, and the pericarp, commonly known as the peel, skin, or rind (Han, 2008; Orwa et al., 2009). Water is utilized to cleanse *Citrus sinensis*. Following the washing process, the object in question is subjected to peeling, whereby the outer layer is carefully removed. Subsequently, the peeled material is subjected to a drying technique carried out at a temperature of 40 degrees Celsius in order to facilitate the removal of moisture. The peel of *Citrus cinensis* is subsequently dried and processed into a powdered form, known as simplicia. A quantity of Simplisia weighing up to 100 grams is combined with a solvent consisting of 70% ethanol, with a volume of up to 800 mL. The mixture is subjected to maceration for a duration of 48 hours, with intermittent stirring. Subsequently, a filtration process is employed until two distinct outcomes are achieved, namely pulp and mascerat. The outcomes of maceration are introduced into an evaporator that rotates, resulting in the formation of a dense extract.

Animals

The current study used a sample of 25 male white rats (*Rattus norvegicus*) aged 2 to 3 months, with an approximate body weight of ± 200 g and overall good physical condition. The rats underwent a three-day period of acclimation, during which they were provided with sufficient nourishment in the form of a standard pellet rat diet, access to drinking water, and exposure to ambient lighting conditions. This acclimatization was done at the Laboratory of the Centre for Food and Nutrition Studies at Universitas Gadjah Mada, which is housed in the Pusat Antar Universitas Building. On July 18, 2017, the Research Ethics Committee of the Faculty of Medicine and Health Sciences at Universitas Muhammadiyah Yogyakarta granted ethical permission for the study under reference number 394/EP-FKIK-UMY/VII/2017.

Methods

Induction of cigarette smoke

When taken at a dosage of two cigarettes per day, kretek cigarettes without side flow filters exhaled cigarette smoke. Every group was comprised of five mice that were housed together in the same enclosure. Located at the lowermost section of the enclosure, there exists an aperture designated for the disposal of extinguished tobacco products. Conversely, the uppermost part of the enclosure is sealed, although it possesses an opening to facilitate the egress of smoke. The process of fumigation is typically conducted until the consumption of two cigarettes has been completed.

Experimental Design

The intervention was administered via oral administration, utilizing a singular dosage over a period of 14 consecutive days. The rats were categorized into five distinct groups (n = 5). Group I represented the normal control, where the animals were provided with food and drinking water without the extract and subjected to the cigarette smoke test. Group II, on the other hand, served as the negative control and was exposed to cigarette smoke. *Citrus sinensis* peel extract was administered to groups III, IV, and V at doses of 37.5, 75, and 112.5 mg/kg body weight (BW). The peel extract of *Citrus sinensis* was administered through sonde to the entire group once daily at a consistent time.

In the present investigation, blood samples were permitted to undergo coagulation for an approximate duration of 60 minutes at ambient temperature. Furthermore, the blood was centrifuged for 10 minutes at 3000 rpm to get the serum. Biochemical factors, such as the levels of HDL and LDL, are subject to estimation. The biochemical parameters were assessed by employing a cholesterol kit (DiaSys Diagnostic System GmBH & Co) that was acquired from Sigma Chemical Co.

Statistical analysis

The present study employed statistical analysis utilizing the Paired t test to compare different therapies. The analysis was conducted using Jamovi software, specifically Version 2.3.16. At a significance threshold of p 0.05, statistical differences were found to be significant. Consequently, the mean values of each sample are presented along with their corresponding standard deviations (SD).

RESULT AND DISCUSSION

Citrus sinensis contains a variety of chemical compounds that can be found in many sections of the plant, including the fruit, peels, leaves, extract, and roots. Flavonoids, steroids, hydroxides, alkanes, fatty acids, coumarins, peptides, carbohydrates, carbamates, alkylamines, carotenoids, volatile chemicals, potassium, magnesium, calcium, and salt are all present. According to (Favela-Hernández et al., 2016). *Citrus sinensis* is a botanical species that serves as a reservoir of vitamin C, possessing inherent antioxidant properties that contribute to the enhancement of the immune system (Etebu & Nwauzoma, 2014).

The antioxidant activity of *Citrus sinensis* seed extract was assessed in the test. The study conducted by (Dongre et al., 2023) employed power reduction and radical scavenging tests to evaluate the efficacy of *Citrus sinensis* extract. Gallic acid, with an IC50 value of 29.5 µM, was utilized as the control standard in the experiment (Atrooz, 2009).

The results of this investigation are presented in Table 1 and 2. Table 1 illustrates a statistically significant disparity in the average HDL levels before and after the test across the normal control, negative control, groups III, IV, and V. This study aims to examine the disparities in the average high-density lipoprotein (HDL) values between the groups through the utilization of the Wilcoxon test. The positive control group exhibited the lowest post-test HDL levels, whereas group V demonstrated the highest values.

Table 1. The HDL level in white rats (Rattus norvegicus) on average induction of cigarette smoke and *Citrus sinensis* peel extract in pre- and post-test

Groups	Average of HDL levels (mg/dL)		P value (Paired t test)
	Pre test	Post test	
normal control	72.78 ± 2.64	70.12 ± 2.52	< .001
negative control	69.07 ± 4.22	31.52 ± 17.85	< .001
37.5 mg/kg BW	72.20 ± 3.58	44.10 ± 17.93	< .001
75 mg/kg BW	68.29 ± 4.63	52.878 ± 6.60	0.002
112.5 mg/kg BW	67.51 ± 3.61	62.47 ± 5.52	0.015

The data was presented as mean SD (Standard Deviation). If the p value was 0.05, the data was statistically different

The observed disparity lies in the significant reduction of average HDL values across several groups, particularly in the positive control group where participants were exposed to cigarette induction without the administration of *Citrus sinensis*. The treatment group that had the smallest average decrease difference was group V, which was exposed to induced cigarette smoke and *Citrus sinensis* peel extract 112.5 mg/kg BW).

Table 2 shows a significant difference between LDL levels of white rats (*Rattus norvegicus*). The difference in the highest increase in average LDL levels was in the positive group (induction of cigarette without treatment). The lowest average increase in the treatment group shown in group V (induced cigarette smoke and treatment 112.5 mg/kg BW).

Citrus sinensis is useful in cases of high cholesterol. For 15 days, rats (200-250 g) were given lyophilized *Citrus sinensis* juice at a dose of 5 g/kg in a dilute container of 0.5 mL / 100 g body weight so it reduced plasma cholesterol levels (31%), LDL (44%) and triglycerides (33%) (Trovato et al., 1996). Insoluble microfiber from *Citrus sinensis* fruit decreased serum triglyceride concentrations (15.6% -

17.8%) and serum total cholesterol concentrations (15.7% -17.0%) by increasing cholesterol excretion (123% -126%) and bile acids (129% - 133%) in feces (Wu et al., 2009).

Through direct scavenging of free radicals, flavonoids can prevent cell damage and thus prevent their damaging effects. Oxidize flavonoids by free radicals become more stable, even though free radicals become less reactive. Flavonoids can inhibit the oxidation of LDL by scavenging radicals. It is due to preventive measures against atherosclerosis (Panche et al., 2016).

Table 2. LDL cholesterol levels in White Rats (*Rattus norvegicus*) on average induction of cigarette smoke and *citrus sinensis* peel extract in pre- and post-test

Groups	Average of LDL levels (mg/dL)		P value (Paired t-test)
	Pre test	Post test	
normal control	23.67 ± 2.65	26.89 ± 3.04	0.003
negative control	24.63 ± 2.22	78.20 ± 1.89	< .001
37.5 mg/kg BW	27.48 ± 1.24	62.30 ± 2.78	< .001
75 mg/kg BW	25.99 ± 1.89	37.54 ± 1.57	0.001
112.5 mg/kg BW	25.31 ± 3.02	30.82 ± 1.70	0.022

The data was presented as mean SD (Standard Deviation). If the p value was 0.05, the data was statistically different

Citrus flavonoids increase lipid metabolism and mediate the effect of antioxidant capacity (Jung et al., 2006; Assini et al., 2013). Rutin is an effective radical scavenger, which may be owing to its inhibitory effects on the enzyme xanthine oxidase (XO). Naringenin's antioxidant action is mostly attributable to reduced ROS and improved antioxidant defenses in chronic illnesses, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) (Mahmoud et al., 2019).

In this study, the pre-test and post-test HDL and LDL levels in the negative control group with cigarette smoke induction showed a significant difference (p = 0.043). Post-test HDL levels decreased by a difference of 37.55 mg / dL, while for post-test LDL levels increased with differentiation of 53.57 mg / dL. So, that shows that *Citrus sinensis* inhibits the decrease in HDL levels and increases LDL levels. It is due to the presence of antioxidants that can inhibit fat oxidation (Arifin & Ibrahim, 2018). A similar study from (Nakamura et al., 2021) showed current and previous cigarette smoking has been linked to dyslipidemia, characterized by increased TG, LDL-C, and lower HDL-C.

(George et al., 2014) stated that a exsiccate of HDL and an escalate of LDL due to cigarette exposure can cause oxidative stress in the body, thereby increasing lipid peroxidation. A study conducted by (Kumar et al., 2015) found a significant decrease in HDL and an increase in LDL compared to non-smokers. The strong association between smoking and elevated serum lipids. Chronic smokers are at high risk of increasing LDL and lowering HDL (Singh, 2016).

Ashraf et al. (2017) showed that the *Citrus sinensis* peel extracts effectively reduce LDL and total cholesterol levels due to the content of flavonoids and hesperidin. The review by (Favela-Hernández et al., 2016) showed that Citrus sinensis possesses anti-cholesterol effects, which is a health concern. In Wistar male adult rats (200-250 g), administration of Citrus sinensis extract at 5 g/kg dose with a volume dilution of 0.5 mL/100 g BW for 15 days can reduce plasma cholesterol levels (31%), LDL (44%), and triglycerides (33%). Administration of *Citrus sinensis peel* extract's raises HDL levels to near normal control values in the 8 mL/kg/day dose group. *Citrus sinensis* contains flavonoids which is one of the antioxidants that can increase HDL concentrations and reduce LDL and VLDL levels in hypercholesterolemia rats. Therefore, the flavonoids and polyphenols found in *Citrus sinensis* are considered promising in increasing HDL and reducing LDL and VLDL (Mallick & Khan, 2016).

Flavonoid compounds have a mechanism to increase HDL levels by increasing cholesterol release from macrophages and increasing the expression of ATP-binding cassette (ABC) A-1 (Millar et al., 2017). Another study mentioned that the mechanism of flavonoids to increase HDL can also be increased by the production of apoprotein A-1 which is a constituent of HDL so that HDL in the blood can increase (Vijayakumar and Nalini, 2006; Baba et al., 2007; Rahmayanti et al., 2021).

Flavonoids also play a role in inhibiting cholesterol absorption, increasing bile excretion and excretion of LDL receptors, an increase in LDL receptors can indicate a decrease in LDL levels (Zeka et al., 2017). The inhibition of cholesterol absorption will cause the formation of chylomicrons and decreased VLDL resulting in serum LDL levels also decreasing (Benito-Vicente et al., 2018). Besides the lipophilic nature of flavonoids that caused flavonoids can bind LDL (Amrizal et al., 2017).

CONCLUSION

Citrus sinensis peel extract administration at 37.5 mg/kg BW/day, 75 mg/kg BW/day, and 112.5 mg/kg *BW/day* inhibit decreasing HDL and increasing LDL levels in white rats (*Rattus norvegicus*) through inducing cigarette smoke.

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