ORIGINAL RESEARCH

Effect of Heated Canola Oil on Aorta Wall Thickness in Rats

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ABSTRACT

Background: Structural alteration of blood vessels such as formation of atheroma or changes in the thickness of vessel walls, are heavily involved in pathogenesis of cardiovascular disease. Several studies have shown that canola oil has a positive effect on such diseases by reducing LDL and cholesterol levels. However, there may be several negative impacts on reheating canola oil upon administration, similar to other oils. Although canola oil can improve lipid profiles, studies related to how canola oil alters the structure of blood vessels are limited. **Objective:** The aim of this study was to investigate the effect of heated canola oil on intimal-to-medial thickness (IMT) of Thoracic aorta in high-fat diet rats. **Material and Method:** A total of 27 rats were divided into 3 groups, the K+, P1, and P2. Rats among the three groups were given a high-fat diet for 14 days, accompanied by consumption of canola oil without heating in the P1, and with repeated heating in the P2. Thoracic aorta was taken on the 15th day and then processed into histological preparations. IMT was measured using CellSens software on a microscope with a magnification of 400. The difference between groups was tested using the one-way ANOVA test on SPSS. **Result:** The mean and standard deviations of each group in a row were K+ **(**134.96 and 21.27) P1(132.04 and 27.30) and P2 (152.05 and 31.75). There was no significant difference in IMT between groups ($p > 0.05$). However, the P2 group showed the highest mean of IMT. **Conclusion:** The consumption of canola oil with or without repeated heating did not result in the changes of the IMT in rats fed with a high-fat diet.

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BACKGROUND

Cardiovascular disease (CVD) was ranked first in leading causes of death worldwide in 2012 (WHO, 2014). Even in 2016, cardiovascular diseases accounted for 31% of global mortality, which was an increase from the 14.5% in 2006. The main cause of cardiovascular disease is atherosclerosis (Borén, et al., 2020). Atherosclerotic cardiovascular disease (ACD) includes two major conditions: ischemic heart disease (IHD) and cerebrovascular disease (mainly ischemic stroke). IHD and stroke are the world's first and third causes of death (Barquera, et al., 2015). Atherosclerotic cardiovascular disease (ACD) is the leading cause of mortality worldwide (Barquera, et al., 2015). It is caused by the infiltration of low-density lipoprotein (LDL) into the vascular epithelium, which damages the blood vessel walls, some of which are trapped and partly oxidized to LDL-ox. (Borén, et al., 2020). The oxidized LDL attracts many monocyte cells, T lymphocytes, and endothelial cells to become activated (Chen & Khismatullin, 2015; Parung, et al., 2016). Endothelial cell activation results in adhesion of molecules, changes in permeability, and triggers infiltration of macrophages and T cells, along with the reduction of NO production and an increase in arterial vascular tone (Platt et al., 2007; Förstermann, et al., 2017). This inflammatory process involves several types of cells which include, foam cells, endothelium and smooth muscle that form atherosclerotic plaques. This process causes an increase in the thickness of the vessel wall and a decrease in the diameter of the blood vessel (Parung, et al., 2016). In addition, the uneven constriction of blood vessels affects blood flow velocity (Zhuge, et al., 2020). Moreover, the existence of foam cells in atherosclerotic plaques produces proteases that cause the plaque to become unstable, causing thrombus (Badimon & Vilahur, 2014). The thrombus makes the diameter of arteria smaller and becomes detachable, resulting in formation of an embolus. The presence of an embolus that joins the bloodstream can eventually occlude completely a smaller blood vessel than the embolus along the course of the embolus (Seidman, et al., 2020). Subclinical atherosclerosis may predict future CVD events. Therefore, the identification of subclinical atherosclerosis will provide benefits to prevent future CVD events (Mawarti, et al., 2020). In subclinical vascular disease, an increase in the intimal medial thickness (IMT) is an important disease marker. This is not limited to atherosclerosis but also in aging and hypertension (Winston, et al., 2013; Lalenoh & Aminuddin, 2019).

Canola oil is one of the oils with high levels of unsaturated fats (Adeyemi, et al., 2016). It is obtained from canola flower extract (*Brassica napus* L.) (Yu, et al., 2012). It has also been shown to reduce LDL fat levels in plasma (Lin, et al., 2013). In the study by (Gulesserian & Widhalm, 2002), it was found that there was a significant decrease in total serum cholesterol (TC, LDL-C, and TAG) in children and adults with familial hypercholesterolemia after receiving dietary counselling by replacing the fat source with canola oil for 5 months.

Other studies have shown that canola oil consumption can reduce serum cholesterol levels (Gillingham, et al., 2011; Lin, et al., 2013). Food processing using deep-fried techniques, namely food processing techniques by frying at a temperature between 163 ^{o}C to 196 ^{o}C can reduce the quality of the oil (Zeb, 2019). Frying with oil at high temperatures converts oil from cis fat into saturated fat bonds or trans fats (Song, et al., 2015). High levels of trans fats will increase plasma LDL levels as well as saturated fatty acids (Islam, et al., 2019). However, consumption of trans fats also reduces blood HDL levels, which will increase the risk of cardiovascular disease (de Souza, et al., 2015). We hypothesized that consumption of canola oil prevented the thickening of IMT, but overcooked canola oil also increased the IMT of thoracic aorta of rats.

OBJECTIVE

This study aimed to investigate the effect of heated canola oil on intimal-to-medial thickness (IMT) of thoracic aorta in high-fat diet rats.

MATERIAL AND METHOD

Research design

The research was true experimental study with a post-test only control group designed. Experimental animals consisted of 27 male wistar rats. These rats were divided equally into 3 groups (K+, P1, and P2) through simple random sampling technique.

Animal care and diet intervention

Each group of rats was put in two cages of 30 x 45 x 20 cm. The rats were then adapted to the cage for 7 days. On the 8th day, the rats received an additional diet according to the group for 14 days. The positive control group (K+) was the group of rats that receiving a high-fat diet of egg yolks as much as 1 ml/day through a gastric tube. Treatment group 1 (P1) was a group of rats fed with a high-fat diet of 1 ml/day with added canola oil of 1 ml/day. Treatment group 2 (P2) was a group of rats that consumed a high-fat diet along with an additional diet of canola oil which had been heated repeatedly. The heating of the canola oil at high temperatures was done 5 times for 15 minutes with 10 minutes between each heating.

Specimen preparation

On the 15th day, the rats were killed through ether anesthesia and proceeded to be dissected through abdominal surgery and severing the ribs. The heart and aorta were collected and placed into a 10% formalin buffer solution for fixation. Preparation utilizing hematoxylin eosin staining was done after sample collection.

IMT measurement

The slices of aorta were observed using a microscope, whereas the measurement was conducted using the Cellsens software. Measurement of IMT started from the border between media and adventitia layers perpendicular to the inner surface of the aorta. Each section was randomly measured in 15 different random sites at a magnification of 400. The results were averaged to acquire the average thickness of the individual IMT of the aorta.

Data Analyse

All data were analyzed by SPSS 18 with the average aortic IMT of individual rats and was carried out using descriptive statistics in the form of average and standard deviation. Kruskal Wallis test was utilized for normality tests. If the distribution were proven to be normal, the mean difference between those groups was tested using an independent t-test with 95% confidence interval (CI).

RESULT

The results can be seen in Table 1. All data of the IMT were normally distributed.

Table 1. The average of IMT

Group	Mean ± SD (μm)	P1	P2
K+	134.96 ± 21.27	p = 0.803	p = 0.644
P1	132.04 ± 27.30		p = 0.991
P2	152.05 ± 31.75		

At a glance, the results of the descriptive statistics showed that the P2 was the thickest IMT, while the thinnest was in P1 group. There was an increasing trend in the dispersion ratio from K+ to P2. Therefore, the data from P2 was proven to be more diverse than the data from K+ or P1. The independent t-test showed no significant differences between the control and treatment groups. Therefore, a conclusion drawn from this experiment was that there was no significant difference between groups. Hence, all aforementioned hypotheses were rejected.

Measurement of IMT in the three groups can be seen in Figure 1.

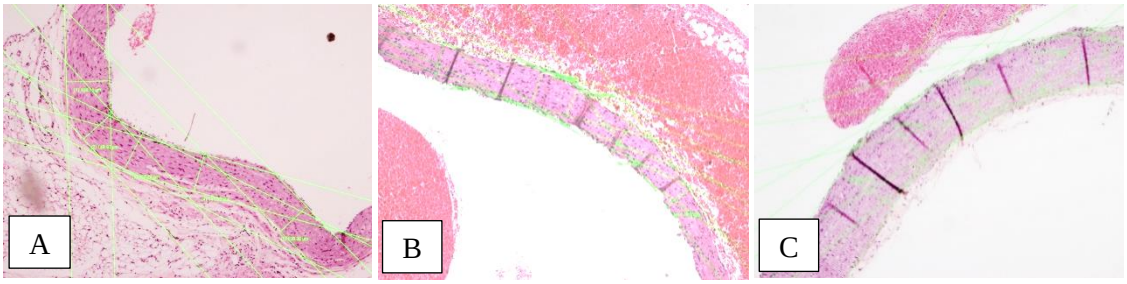

Figure 1. Measurement of intimal to a medial thickness of the aorta in different sites using CellSens software. a). control group, b). P1 group, and c). P2 group. HE staining, x400.

DISCUSSION

According to the results of the study, there was no effect of administering canola oil on the thickness of the IMT of male *Rattus norvegicus* strain wistar rats. However, there was a decrease in the mean thickness of IMT in treatment group 1 (P1) against the positive control group (K+). This may be due to the lack of duration of the study in comparison to other previous studies. In the study of (Maramis, et al., 2014), it was found that giving a high-fat diet in the form of pork oil for 14 days increased the thickness of the aortic IMT and the formation of foam cells in the aorta of male *Rattus norvegicus* strain wistar rats. However, in this study, the administration of different high-fat diets might have contibuted to different results. The absence of a negative control group in this study caused the researchers unable to evaluate the positive control group, whether the positive control group had a different IMT thickness compared to normal rats. The smallest average thickness value of the aortic IMT was identified in P1 group. As for P2 group, aortic wall thickening was seen in 7 subjects with a thickness of more than 150 μm.

Such results might have been caused by the richness of canola oil and its containing poly unsaturated fatty acid (Kostik, et al., 2013). Oil with a high amount of unsaturated and polyunsaturated fatty acids such as linoleic acid is more prone to oxidation (Roiaini, et al., 2015). Furthermore, reheated oil will increase trans fat in its composition (Wang, et al., 2016). A study has prove that the consumption of oil with repeated heating can increase the risk of various chronic diseases (Stott-Miller, et al., 2013). However, there was a decrease in glutathione levels and liver enzyme activity. Glutathione levels indicate the body's reaction to free radical compounds in the blood vessels. These free radicals can cause adhesion of molecules to blood vessel walls and cause inflammation. Decreased levels of glutathione can be a risk factor for cardiovascular disease (Buijsse, et al., 2012). In a study by (Putra & Selviana 2017), it was found that the administration of used cooking oil increased the thickness of the aortic wall in rats. This follows of studies that the consumption of oil that has been heated repeatedly can increase the risk of various chronic diseases such as coronary heart disease (Stott-Miller, et al., 2013; Lippi, et al., 2015). However, the second treatment group had a higher standard deviation and dispersion. A higher dispersion number indicates a higher level of variation from the data. This could be caused by the composition of the heated canola oil which has yet to be measured.

The mean of P1 was lower than other groups. This result was in accordance to a study which found that the canola oil contained rich omega 3 fat. Omega-3 has many health benefits, such as preventing heart disease, improving blood coagulation function and controlling inflammation in the body (Gammone et al., 2018). Canola oil can reduce total LDL cholesterol and especially apoprotein B, along with a decrease in atherogenic indicators such as LDL/ HDL, TC/ HDL and apo B/ apo A-1 (Jones, et al., 2014; Ghobadi, et al., 2019; Amiri, et al., 2020) but the serum level of TG and HDL did not statistically change following canola oil intervention (Ghobadi, et al., 2019). Canola oil has also been shown to improve cardiac diastolic function in obese rats compared to feeding them with lard (Thandapilly, et al., 2017).

The mechanisms of canola oil on serum lipids are not well understood, but it is probably related to its FA composition, which is high in MUFAs and PUFAs, especially alphalinolenic acid (ALA) (Ghobadi, et al., 2019). Ramprasath, et al., (2012) reported that a high-PUFA diet decreased LDL and TC. However, the TG and HDL levels were not affected.

Although experiments on experimental animals can be a basis for studies on treatment effects and are useful to study human health conditions (Frumkin, et al., 2017), the differences in biological conditions between types of experimental animals, along with potential bias when designing the procedures with certain clinical conditions can cause differences in results between animals trials and human subjects (Akhtar, 2015).

The results in this study were not significant. Several factors that might cause the results to not be projected as desired included an unrepresented negative control group. This led to an impossible determination of whether the thickness of IMT in the positive control group was different from normal rats. In comparison to previous research, this research was done in an overly short duration of time. In addition, there was only one variable studied, the thickness of the IMT. Therefore the discussion was less comprehensive and sufficient to describe the effects of canola oil intake on the cardiovascular system, along with observation of immeasurable effects from the administration of canola oil and repeatedly heated canola oil to the experimental subjects.

CONCLUSION

Consumption of canola oil without heating or with repeated heating in a short period did not contribute to a significant difference in the results of the thickness of the aortic IMT in rats.

Acknowledgment
None declared.

Conflict of Interest
All authors have no conflict of interest.

Ethics Consideration
The research protocol approved by the Committee of Medical Research Ethics in Faculty of Medicine Universitas Airlangga (Number 253/EC/KEPK/FKUA/2021).

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Author Contribution
All authors have contributed to all process in this research, including preparation, data gathering and analysis, drafting and approval for publication of this manuscript.

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