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Coronary Artery Wall Thickness and Lumen Diameter of Nicotine-Induced White Rats (*Rattus norvegicus*)

Nur Anisah¹, Maya Nurwantanti Yunita^{2*}, Hani Plumeriastuti³, Bodhi Agustono¹, and Reina Puspita Rahmaniar⁴

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Abstract

Nicotine can cause hemodynamic disorders for good health. This study aimed to observe the histopathology of lumen diameter and coronary artery wall thickness in white rats exposed to nicotine. Rats were divided into five treatment groups, C (without nicotine exposure), T1, T2, T3, and T4 (nicotine concentration 0,005mg/ml; 0,05mg/ml; 0,2mg /ml, and 0.65 mg/ml). The results showed a significant difference in coronary artery wall thickness between the control and treatment groups. The diameter of the coronary artery lumen was narrowed. In conclusion, a higher concentration of nicotine can increase the thickness of the coronary artery wall and decrease the lumen diameter.

Key words : coronary artery, good health, histopathology, nicotine, white rats

Nicotine can stimulate the nervous system, increase heart rate, and increase blood pressure. Chronic effects of nicotine exposure are disorders of blood vessels, such as narrowing or thickening of the blood (Isdadiyanto, 2019). Nicotine can cause endothelial cell dysfunction. Endothelial cell dysfunction will affect cardiac vasoconstriction resulting in ischemia and infarction in the myocardium (Benowitz *et al*, 2017).

The blood vessels of the heart consist of the coronary arteries and cardiac veins, which supply most of the blood to and from the myocar-

dium (Moore *et al*, 2017). The main cause of most cardiovascular diseases is atherosclerosis. One of the predisposing factors of atherosclerosis comes from the toxins produced by nicotine from cigarettes. Nicotine is associated with the presence of intravascular inflammation that contributes to the development of atherosclerosis (Yankelevitz *et al*, 2013).

Atherosclerosis is characterized by the thickening of the arterial wall from the accumulation of fat cells, macrophages, fibrous tissue, and calcium (Ackermann *et al*, 2017). Thickening of the arterial wall can cause narrowing of the arterial lumen which disrupts the blood flow (Montecucco, *et al*. 2009). This flow disturbance can lead to ischemia and tissue death in the heart area. The wall thickness artery coronary atherosclerosis can also cause coronary heart disease (Lee *et al*, 2007).

Based on the phenomenon and background, research is needed on the histopathology of Lumen Diameter and Wall Thickness Coronary Artery Of White Rats (*Rattus norvegicus*) induced by nicotine.

Materials and Methods

The sample size used in this study adjusted to the Completely Randomized Design (CRD) formula, namely: $t(n-1) \geq 15$, so the number of samples needed was 20 white rats. The experimental animals used in this study were Sprague Dawley (*Rattusnorvegicus*) white rats from UD Tiput Abadi Jaya, Yogyakarta. The rat used was male sex four months old and weight 130-150 grams.

The white rat was adapted for seven days, and given food and water ad libitum. All the experimental animals were treated with: C (without exposure to electric vapor), T1

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Table I. Mean and standard deviation on coronary artery wall thickness.

Treatment	Mean \pm Standard deviation
C	9,002 ^a \pm 0,649
T1	10,860 ^{ab} \pm 1,857
T2	13,372 ^{bc} \pm 1,263
T3	14,277 ^c \pm 1,237
T4	15,875 ^c \pm 3,199

^{a,b,c}The different superscripts on the bar chart show significant differences ($p < 0,05$). C (without nicotine exposure), T1, T2, T3, and T4 (nicotine concentration 0,005mg/ml; 0,05mg/ml; 0,2mg /ml, and 0.65 mg/ml).

Table II. Mean and standard deviation of luminal diameter coronary artery

Treatment	Mean \pm Standard deviation
C	42,217 ^a \pm 9,423
T1	41,805 ^a \pm 11,421
T2	40,072 ^a \pm 5,351
T3	38,205 ^a \pm 5,302
T4	32,685 ^a \pm 7,305

^{a,b,c}The different superscripts on the bar chart show significant differences ($p < 0,05$). C (without nicotine exposure), T1, T2, T3, and T4 (nicotine concentration 0,005mg/ml; 0,05mg/ml; 0,2mg /ml, and 0.65 mg/ml).

(exposure to electric vapor with nicotine concentration 0,005mg/ml), T2 (exposure to electric vapors with nicotine concentration 0.05mg/ml), T3 (exposure to electric cigarette vapor with nicotine concentration 0.2mg/ml), and T4 (exposure to vapor from electric cigarettes with 0.65mg/ml nicotine concentration) after adaptation. This concentration is the nicotine dose that has been labeled on the e-liquid packaging. All treatments were carried out twice a day for 14 days. Each rat will get nicotine exposure vapor with an electric cigarette tube that has been paired in the treated cage. Electric cigarettes that have been dripped with liquid are turned on and automatically stop within 10 seconds of suction.

Euthanasia of white rats used xylazine and ketamine injection on day 15. After anesthesia, albino rats are terminated by cervical dislocation (Underwood, *et al.*, 2015). Histopathological preparations used Haematoxylin - Eosin (HE) staining. In this study, an observation of wall thickness and coronary artery lumen diameter using a trinocular microscope (Benowitz, *et al.*, 2017). Data were collected and analyzed using Program SPSS Version 25.0 software. The data obtained were tested by Analysis of Variant

(Anova) with confidence (5%), and if there was a significant difference, then proceed with the distance test by Duncan Test.

Results and Discussion

Based on the results of Duncan's post hoc test which can be seen in the superscript in Table I, the T2, T3, and T4 group with nicotine exposure (0,05mg/ml; 0,2mg /ml, and 0.65 mg/ml) had significantly different of coronary artery wall thickness measurement results compared to the C groups (without nicotine exposure). Increased wall thickness at T1, T2, T3, and T4 is caused because the nicotine in the liquid of electric cigarettes was inhaled and entered the white rats's lungs. Nicotine in the lungs undergoes diffusion and enters the bloodstream through pulmonary circulation and towards the heart. This is in line with the opinion of Platt, *et al.*, (1994) that nicotine receptors that influence cells in the walls of blood vessels.

Nicotine can cause the proliferation and migration of vascular smooth muscle cells by stimulating $\alpha 1$ type nicotinic cholinergic receptor (nAChRs) (Ackermann, *et al.*, 2017; Montecucco, *et al.*, 2009). Then AChRs (nicotinic acetylcholine receptors) which are nicotinic

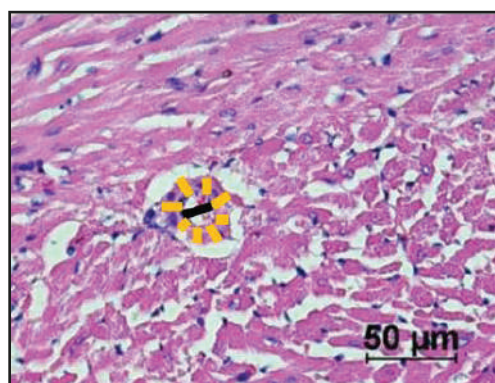


Fig 1. The coronary artery without an electric cigarette (C) was seen as normal, the mean wall thickness is 8.12 μ m with a lumen diameter of 42.69 μ m, black line (—) coronary artery lumen, yellow line (—) coronary artery wall (HE, 40x10 microscope Trinocular).

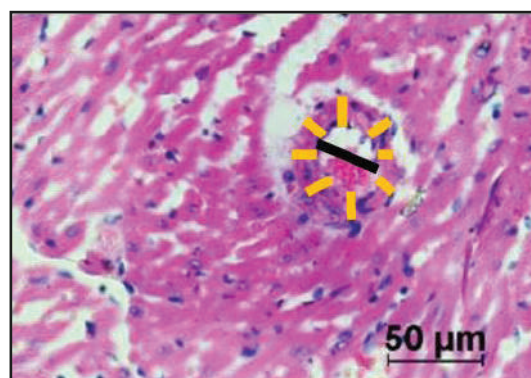


Fig 2. The coronary artery with 0.005 mg/ml nicotine exposure (T1) was seen as normal, the mean wall thickness is 11.27 μ m with a lumen diameter of 41.71 μ m, black line (—) coronary artery lumen, yellow line (—) coronary artery wall (HE, 40x10 Trinocular microscope).

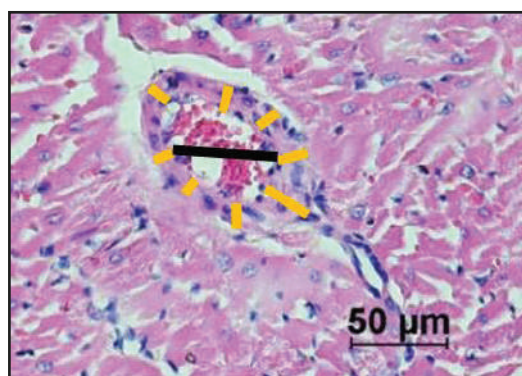


Fig 3. The coronary artery with 0.05 mg/ml nicotine exposure (T2) was seen thickening, the mean wall thickness is 13.34 μ m with a lumen diameter of 37.08 μ m, black line (—) coronary artery lumen, yellow line (—) coronary artery wall (HE, 40x10 microscope Trinocular).

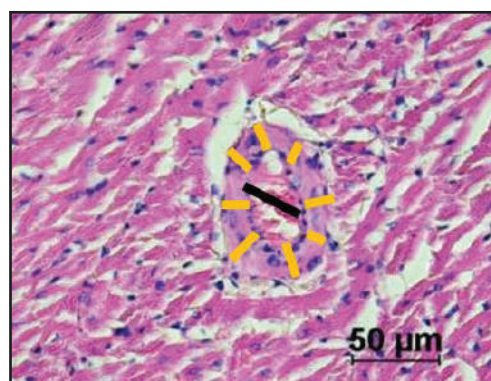


Fig 4. The coronary artery with 0.2 mg/ml nicotine exposure (T3) seen more thickening than T2, the mean wall thickness is 14.45 μ m with a lumen diameter of 36.58 μ m, black line (—) coronary artery lumen, yellow line (—) coronary artery wall (HE, 40x10 microscope Trinocular).

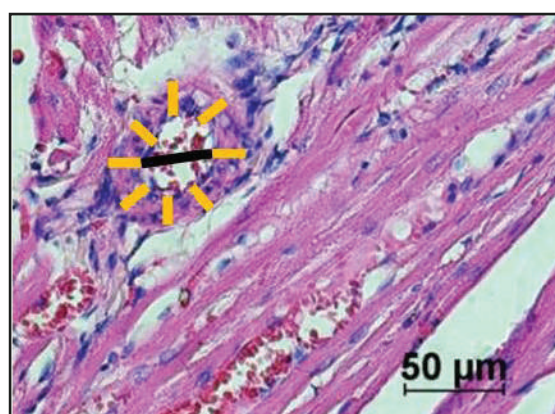


Fig 5. The coronary artery with 0.65 mg/ml nicotine exposure (T4) was seen as the thickest, the mean wall thickness is 15.32 μ m with a lumen diameter of 31.93 μ m, black line (—) coronary artery lumen, yellow line (—) coronary artery wall (HE, 40x10 microscope Trinocular).

cholinergic receptors were first found in easily stimulated cells, but after being identified it was found in other cell types including blood vessels (Lee, *et al*, 2007; Abu-Baker, *et al*, 2010).

The luminal diameter of coronary arteries measurement results in T1, T2, T3, and T4 group had not significantly different compared to the C groups. However the mean of luminal diameter on coronary arteries in this study decreased with the increasing concentrations of nicotine exposure in white rats. Thickening of the wall and narrowing of the luminal diameter of coronary arteries in T1, T2, T3, and T4 are to be one of the risk factors for atherosclerosis. This is in line with the Braunwald, *et al*, (2001) statement that atherosclerosis is characterized by the thickening of the arterial wall due to the proliferation of smooth muscle cells in the media layer, the accumulation of lipids accompanied by fibrous tissue formation. According to Mescher (2016), atherosclerosis is also characterized by a narrowing of the blood vessel lumen. Nicotine has a negative effect on releasing catecholamines, increasing lipolysis, and increasing free fatty acids. Catecholamine secretion will increase free fatty acids which will turn into triacylglycerol and cholesterol esters in the liver into the bloodstream as VLDL which will be converted to LDL in the blood (Sanhia, *et al*, 2015).

The mechanism of atherosclerosis begins with damage to the endothelial cells of blood vessels by oxidative stress, smoking or other factors. Damaged vascular endothelial cells release cytokines (IL-1 β) and chemokines (IL-8). Chemokines released by endothelial cells attract monocytes from the circulating blood to the injured area. Monocytes penetrate the subendothelial space, differentiate and mature into macrophages. Macrophages will take and accumulate LDL cholesterol from the blood and lead to the formation of foam cells and atherogenesis (Ninomiya, *et al*, 2018).

Summary

Based on the results analysis the data obtained showed that the nicotine concentrations were higher, the coronary artery wall was thicker and the lumen diameter was smaller in white rats (*Rattus norvegicus*).

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