

Conference Paper

Hispathology of Coronary artery of male rat (*Ratus Norvegicus*) with high fat diet after being given ethanol extract of Indian acalypha (*Acalipha indica. L*)

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Abstract

This research aims to know the effect of ethanol extract of Indian acalypha (*Acalipha indica L*) to histopathology of coronary artery of male rat (*Ratus norvegicus*) with high fat diet. Twenty male rats (*Rattus Norvegicus*) males aged 3-5 months with mean weight 200 grams were divided into 5 groups and 4 replications. Group 1 (negative control) was only given standard feed and distilled on ad libidum. Four groups (Positive control, Treatment 1, 2 and 3) were made hypercholesterolaemia by administering 1 mL peroral pork oil for 56 days. On day 14, the cholesterol levels were checked. After hypercholesterolaemia, from day 15 to day 56, each group was given ethanol extract of Indian acalypha peroral with a dose of 200 g / kg BW (P₁), 400 g / kg BW (P₂) and 800 g / kgBW (P₃), whereas the positive control groups were not given ethanol extract of Indian acalypha. On day 57, the dying rat was dissected for the removal of the cardiac organ and the making of coronary artery histopathological preparation. Data on histopathologic observation of coronary artery were analyzed by the *Kruskal Wallis* test, and if there was a significant difference between treatment groups ($p < 0.05$), then it continued with the Mann-Whitney test. The results showed that in infiltration of fat cells and endothelial cells that experienced damage treatment P₁, P₂, and P₃ which were given extracts of Indian acalypha experienced decrease compared to positive control treatment groups. The result of statistical test of fat cells and endothelial cells in the coronary artery that experienced damage showed significant differences between treatment groups ($p < 0.05$), whereas in the statistical test of coronary artery foam cells showed no significant differences between treatment groups ($P > 0, 05$). From the research, it can be concluded that ethanol extract of Indian acalypha (*Acalipha indica L*) can improve histopathology coronary artery of rat fed high fat diet.

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1. Introduction

Cardiovascular disease is still the leading cause of death in the world. The WHO data suggest that hypercholesterolemia contributes to 56% of coronary heart disease cases worldwide and causes 4.4 million deaths annually. The WHO also predicts that by 2020 heart disease and stroke will be the leading cause of death worldwide with a projected number of more than 20 million per year and more than 24 million per year by 2030 (WHO, 2004). One of the causes of cardiovascular disease is the increased levels of low density lipoprotein (LDL) and the decreased levels of High Density Lipoprotein (HDL). Hypercholesterolaemia is also triggered by hepatic disorders, such as diabetes mellitus, nephrotic syndrome, and saturated fat diet habits (Bahri, 2004). All of these things can synergistically cause early atherosclerosis, as well as at risk of heart disease and blood vessels.

The treatment of hypercholesterolemia in general use synthetic drugs; the types of drugs that are often used are including statins and fibrates (Helen, 2005). The use of hypercholesterolemia drugs in animals has been successful in lowering cholesterol levels in blood, but the long-term use of hypercholesterolic drugs will have side effects (Sherwood, 2010). The side effects of the long-term statin use are elevated levels of liver enzymes, muscle and joint pain in patients with myopathy, whereas the frequent side effects of fibrate therapy include gastrointestinal disorders and elevated liver enzymes (Helen, 2005). The alternative use of traditional medicine is needed to minimize the side effects of synthetic drugs. Traditional medicine is very important for the community because it is more easily obtained without a prescription (Suarsa, 2005). One of the herbal remedies that can be used to overcome hypercholesterolemia is Indian acalypha (*Acalypha indica* L.).

Indian acalypha is known to have many health benefits; it has effects as anti-inflammatory, diuretic, laxative, and hemostaksis (Grace, 2009). It is also known to contain tannin that has alkaline content which can prevent the occurrence of cholesterol attachment in the lumen of blood vessels (Grace, 2009). The attachment of cholesterol to the lumen of the blood vessels causes atherosclerosis.

Atherosclerosis in large and small arteries is characterized by the accumulation of fat deposits, thrombocytes, neutrophils, monocytes, and macrophages throughout the depth of the intima tunica (endothelial cell layer) and finally to the media tunica (the smooth muscle layer) (Corwin, 2009). Coronary vessels at the cross section will be seen 3 layers: the tunica intima (inner layer), tunika media (middle layer), and tunica adventitia (outer layer). The surface of the inner blood vessels is coated with a layer

of cells called endothelium. The thin layer of endothelial cells is a layer that provides a slippery surface between the blood and the artery walls as well as the subendothelium layer. These endothelial cells produce substances such as prostaglandins, heparin, and plasminogen activators that help prevent platelet aggregation and vasoconstriction. In addition, endothelial also has a rapid regeneration power to maintain the anti thrombogenic power of the arteries (Kusuma and Hanafi., 2003, Suharto 2004).

The artery coronary histopathology image can be used to assess the function of coronary blood vessels. Thus, this research is significant in order to prove the potential of Indian acalypha (*Acalypha indica L.*) ethanol extract terhadap gambaran histopatologis arteri koronaria tikus putih jantan (*Rattus Norvegicus*) yang diberi pakan tinggi lemak.

The histopathological features of the coronary arteries can be used to assess the function of coronary blood vessels. Therefore, this research is needed to prove the potency of ethanol extract of Indian acalypha (*Acalypha indica L.*) to histopathologic image of coronary artery of male white rat (*Rattus Norvegicus*) fed with high fat diet.

2. Materials and Research Methods

2.1. Material Research

2.1.1. Experimental Animal

The experimental animal in this study was white male rats (*Rattus Norvegicus*) aged 3-5 months with an average weight of 200 grams obtained from the Department of Pharmacology Faculty of Medicine, Universitas Airlangga Surabaya.

Material Research

The research materials used were Indian acalypha (*Acalypha indica L.*), 96% ethanol, sterile distilled water, pellet 511 (Charoen Pokphan), pork, formalin 10%, 70%, 80%, 90%, 95%, absolute alcohol, xylene, paraffin liquid, dye hematoxylin eosin, and oil emery.

3. Research Tools

The tools needed for experimental animals during the research were rat cages and feeding places, drinking places and cage mats, 5 cc skates, sonde, disposable hand gloves, pots, weights ohaus, blenders, mesh sizes 100, beaker glass, measuring cups,

glass jar, glass funnel, stirrer bar, rotavapor, erlenmeyer flask, porcelain cup, vacuum pump, Buchner filter, dissecting set, and Olympus® CX-41 microscope.

3.1. Research Method

The Production of Indian Copperleaf (*Acalipha indica L.*) Extract. The production of Indian acalypha (*Acalipha indica L.*) extract used maseration method. The Indian acalypha were dried at room temperature and made powder. Indian acalypha powder was macerated with 96% ethanol solvent done repeatedly until the resultant liquid had been clear. The maseration was done for 3 × 24 hours, then filtered. The obtained maserate was evaporated using a rotary evaporator at a temperature of 50-55 °C at a speed of 40 rpm to obtain a viscous extract.

3.2. Research Procedures

A total of 20 rats were grouped randomly into 5 groups. Each group consisted of 4 rats and had been adapted. The division of research groups:

K(-): Control group (-) without the provision of high-fat feeding diets and without the provision of Indian acalypha extract.

K(+): The control group (+) was fed high-fat diet without the ethanol extract of Indian acalypha.

P (1): Group P (1) was fed high-fat diet and extracts of Indian acalypha extract dose 200mg / kg bw / day.

P (2): Group P (2) was fed high-fat diet and given the ethanol extract of Indian acalypha dose 400mg / kg bw / day.

P (3): Group P (3) was given high-fat diet and given the ethanol extract of Indian acalypha dose 800mg / kkg bw / day.

The provision of high-fat diet was by giving peroral pork oil dose 1 ml / day (Merisa, 2011) from day 1 to day 56. Giving ethanol extract of Indian acalypha (*Acalipha indica L.*) was done for 42 days (day 15 to day 56) as much as 1 mL orally. On the day 57, it was done surgery to take the heart organ, then fixed with 10% formalin to produce histopahtological preparation of coronary arteries.

3.3. Preparation Assessment and Coronary Artery Histopathology Scoring

The observed histopathologic examination was the infiltration of fat cells, foam cells, and endothelial cells that were damaged using a microscope with 400 × magnification. The degree of damage to the arteries was assessed by the Brunt et al. (1999) scoring method based on the percentage of damage. The observation of arterial blood vessel damage was seen from the number of foam cells and infiltration of fat cells in the artery, then scored 1-4 (score 1 = <25%), 2 = 25-50%, 3 = 50-75% and 4 => 75 %) (Utami et al., 2016).

Assessment of the rate of arterial damage was done by observing the damage to endothelial cells by scoring: score 1 for minor severity (endothelial cells slightly damaged, but still regular), score 2 for moderate severity (endothelial cells damaged, Accumulation of fat), score 3 for the severity of large (endothelial cells damaged, irregular shape and lots of fat accumulation) (Rahimatul et al., 2016).

3.4. Data Analysis

The data obtained were processed by using the Kruskal Wallis test. If there was a significant difference between treatment groups ($p < 0.05$), then Mann Whitney test was followed.

4. Result and Discussion

The observation of histopathologic preparations used Hematoxilin Eosin (HE) staining. It was made by calculating the total number of arteries in all the field of view with magnification 100x, then enlarged 400x. Histopathologic changes in the coronary arteries observed were fat cell infiltration, foam cells and damaged endothelial cells. The results can be seen in Figure 1.

Giving the pork oil continuously can cause hypercholesterolemia because pork fat contains saturated fatty acids. Saturated fatty acids result in increased levels of triglycerides and decreased High Density Lipoprotein (HDL) so the risk of atherosclerosis. Consuming excessive fat cause hypercholesterolemia. It is characterized by the increasing level of LDL cholesterol (Reinaldo dkk., 2014). hypercholesterolemia can interfere with the function of endothelial through an increase in the formation of oxygen free radicals which disables the nitric oxide. The fatty chemical changes which are triggered by free radicals which is generated in macrophages or endothelial cells

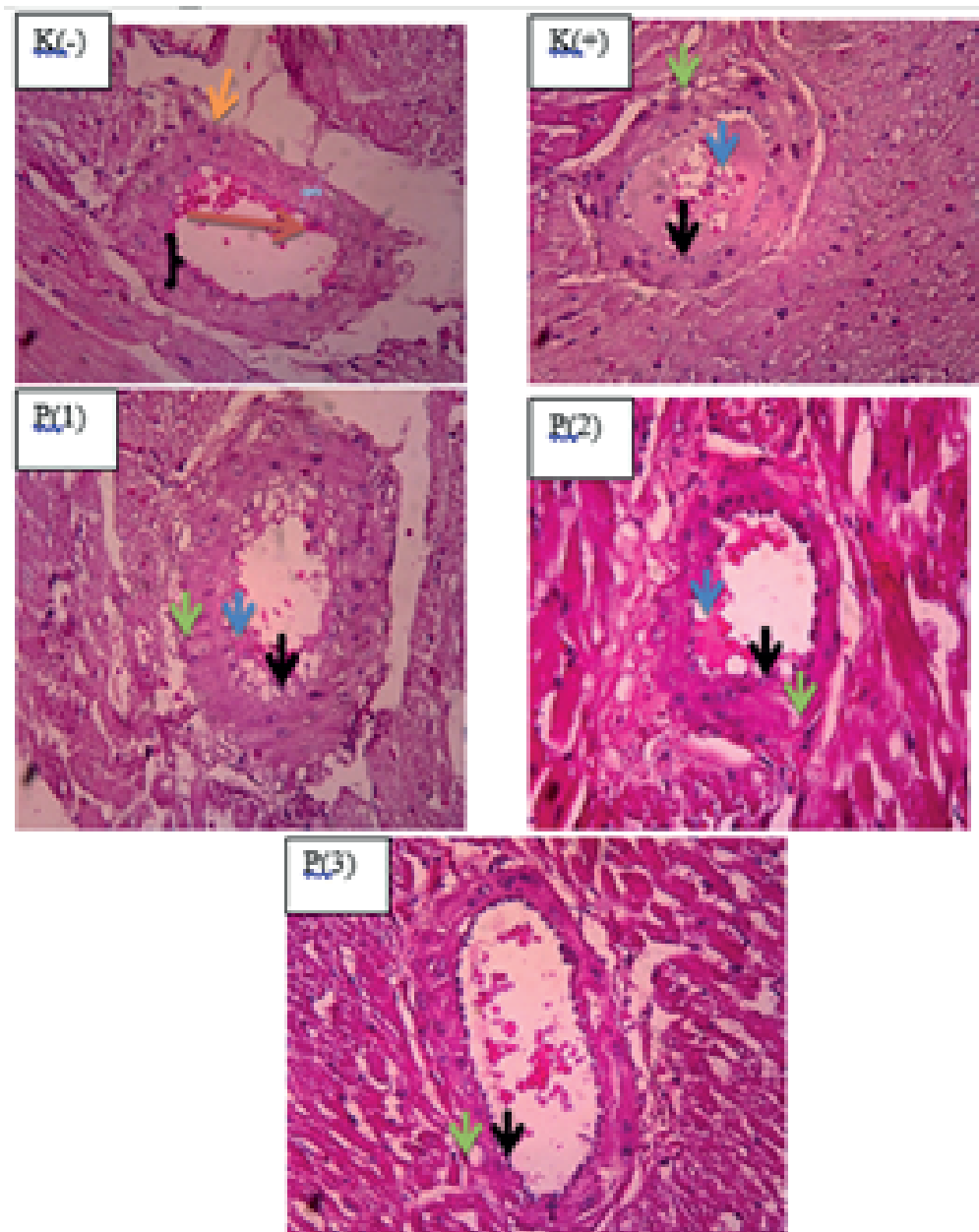


Figure 1: Histopathology of arterial coronary (400x magnification, HE) is examined with a microscope Olympus ® CX-41, K (-) lumen (orange arrow), tunica media (} black), tunica adventitia (yellow arrow). Control (+), P (1), (2) P, P (3), infiltration of fat cells (blue arrow), foam cells infiltration (green arrow), endothelial cells that have been damaged (black arrow).

in artery walls. The process will produce oxidized LDL. Oxidized LDL (LDL-oks) is then continuously captured by macrophages via scavenger receptor so that the macrophage transforms into foam cells and trigger smooth muscle cell migration into tunica intima. This triggered the proliferation of smooth muscle cells on tunica intima, fibroblasts and collagen secretion by fibroblasts. Foam cells then unite to form fatty streak, so, it is considered as a precursor of the atheroma (Mathew and Muawanah., 2008). In this study, the parameter of the examination toward the effect of hypercholesterolemia

which was given ethanol extract of Indian acalypha leaves were the infiltration of fat cells, foam cells, and endothelial cells that have been damaged as a precursor of the atheroma.

The results of the examination of the infiltration of fat cell and cell foam cells as well as damaged endothelial cells can be seen in table 1.

The data of inspection and scoring of each group K (-), K (+), P (1), P (2), and P (3) were analyzed using *Kruskal-Wallis*. *Kruskal-Wallis* test results showed that $p = 0,237$ for infiltration of fat cells, $p = 0,406$ for foam cell infiltration, and $p = 0,015$ to endothelial cells that have been damaged. *Kruskal-Wallis* analysis indicated that there was a significant difference on the damage endothelial cell ($p < 0.05$). It proves that the ethanol extract of Indian acalypha plays a role in fixing the damage of the artery coronaria due to hypercholesterolemia.

The results of the statistical analysis using Mann Whitney on table 1 indicated that the infiltration of fat cells in K (-) differ markedly from K (+), P(1), P(2) and P(3). The treated group, K (+), was not different with P(1) and P(2), real different with P (3). The treatment K (+) showed that fat cells are the most infiltrated compared with the rest of the treatment. It happened because treatment K (+) were not given the therapy but only given high in fat diet (pork oil).

The results of the statistical analysis of the damaged endothelial cells using Mann Whitney test suggested that K (-) differ markedly from K (+), P(1) and P(2) but not different with P (3). Treated group K (+) denoted a noticeable difference with P (1), P (2). The treated group K (+) showed a noticeable difference with P (3) and K (-). Treated group P (1) differ markedly with P(3) but noy different with P (2). It happened because in the other treatment groups i.e. P₁, P₂, P₃ were given the extract of indian acalypha which contains flavonoids, a substance which is able to improve the function of endothelial cells both in the growth, repair, and prevent cholesterol (Harjana et al., 2016).

A hypercholesterol food can trigger the formation of fatty streak that culminate into atheroma (Mathew, muawanah., 2008). The giving of ethanol extract of Indian acalypha with 800 mg/kg (P₃) indicated infiltration of fat cells and endothelial cells that have been damaged which were different in meaning with K (+). It showed that ethanol extracts of indian acalypha leaves was able to down the infiltration of fat cells and endothelial cells that have been damaged. The potential of ethanol extracts of indian acalypha leaves is possible because the content of the active ingredient in ethanol extracts of Indian acalypha. The research of Wemay and Miranti (2013) explained that the Indian acalypha (*Acalypha indica* L) contains a phytochemical saponin which is

TABLE 1: Infiltration of fat cells, cell foam, and endothelial cells that were experiencing damage to artery coronary on white male rats (*Rattus Norvegicus*).

Treatment	Mean \pm SD		
	Fatty Cell	Infiltration Foam Cell	Infiltration Damaged Endothelial Cell
K(-)	0,75 ^a \pm 0,500	1,00 ^a \pm 0,00	1,00 ^a \pm 0,00
K(+)	1,75 ^c \pm 0,957	1,25 ^a \pm 0,50	2,00 ^c \pm 0,00
P(1)	1,25 ^c \pm 0,500	1,00 ^a \pm 0,00	1,75 ^b \pm 0,500
P(2)	1,25 ^c \pm 0,500	1,00 ^a \pm 0,00	1,50 ^b \pm 0,577
P(3)	1,00 ^b \pm 0,000	1,00 ^a \pm 0,00	1,00 ^a \pm 0,00

^aThe difference of superscript on the same column showed the real difference ($P < 0.05$), whereas the same superscript on the same column showed no real differences ($P 0.05 >$).

present in the extract of the leaves, stems, roots while flavonoids and tannins can be found in the extract of indian acalypha leaves. Maramis et al. (2014) stated that the flavonoids contained in the indian acalypha can provide a protective effect. Saponins are able to eliminate the cholesterol in the colon before absorption into the blood stream, whereas tannins are able to bind and precipitate proteins (Adlhani, 2014). Indian acalypha is known to contain tannins, saponins and flavonoids. Saponins and flavonoids serves as a metabolite of the compound. The metabolites compounds are antioxidant and acts against the mechanisms of lipid profile (Wurdianing et al., 2014).

Flavonoids contained in indian acalypha leaves can reduce blood cholesterol levels in the mice which experienced hyperlipidemia and reduce oxidation of LDL cholesterol that has important role in the process of atherogenesis. Flavonoids reduces cholesterol synthesis by inhibiting the enzyme activity of Acyl-CoA cholesterol acyl transferase (ACAT) in the liver cells. It significantly played a role in the decline of cholesterol esterification in the intestines and liver. It also inhibited the activity of 3-Hydroxy-3-methyl-glutaril-CoA enzyme with bile acids and cholesterol (from food) formed the misel that was also cannot be absorbed by the intestines. The tannin inside the body will bind to the protein of the body and will coat the walls of the blood vessels, so that the absorption of fats will be hampered and atherosclerosis can be inhibitory (Iqbal et al., 2012). Another benefit of tannins is its alkaline, so it can prevent the occurrence of bonding of cholesterol on the vascular lumen (Grace, 2009).

The results of this study are in line with the research done by Rajasekaran et al., (2013) which proved that ethanol extracts of Indian acalypha with doses of 200 and 400 mg/kg is capable of lowering the levels of triglycerides and increase HDL serum levels

of white rat fed a high fat diet with a mixed composition of cholic acid, cholesterol, peanut oil and sucrose.

5. Conclusion

From the results of research that has been done, it can be concluded that the treatment of the ethanol extracts of indian acalypha (*Acalypha indica* L.) was able to improve the male rat (*Rattus Norvegicus*) artery coronary histopathology image which was given a high in fat diet that can be seen from the decrease in the infiltration of fat cells and damaged endothelial cells.

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