Available online at **www.ijmrhs.com**

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2017, 6(9): 114-120

The Effectiveness of Ambon Banana Peel Extract (*Musa sapientum*) as Atherosclerosis Prevention through Inhibition of NF-κβ and Increased eNOS Expression in Atherogenic Rat Model

Arlinda Silva Prameswari*, Kartika Puji Rahayu, Eria Nahrani Pranadita, Dyah Puspa Ardani and Wafiyah Hasana

Muhammadiyah University of Malang, Malang, Indonesia

**Corresponding e-mail: arlindaofficial@gmail.com*

ABSTRACT

Atherosclerosis is a disease in which plaque builds up inside the arteries. During the early phase, endothelial nitric oxide nitrate (eNOS) decrease and nuclear factor kappa beta (NF-κβ) increase. Ambon banana peel contains XJP-1 and flavonoid which has been shown to inhibit the translocation of NF-κβ and increase eNOS expression in endothelial cells. This study was designed to investigate the effectiveness of Ambon Banana peel as atherosclerosis preventive agent through the inhibition of NF-κB and increasing eNOS expression in atherogenic rats. This study was post-test only control group design for 14 days using 30 male Wistar rats, divided into 5 groups: negative control with normal diet, positive control with atherogenic diet, and three control groups with atherogenic diet + 200 mg/kg body weight/ day, 400 mg/kg body weight/day, and 800 mg/kg body weight/day extract dosage. On the 15th day, the rats were terminated, taken its abdominal aortic and analyzed using immunohistochemical method. Statistical analysis showed that the extract significantly decreases NF-κβ (p=0.00) and increase eNOS (p=0.00). Pearson correlation proves that higher dose can lower NF-κ NF-κβ activity and increase eNOS expression (p<0.05). Linear regression shows that the extract can 82.1% lower NF-κβ activity and increase eNOS expression for 95.2%. In conclusion, the extract of Ambon banana peel has been proven to be effective in preventing atherosclerosis.

Keywords: Ambon banana, Atherosclerosis, eNOS, NF-κβ, XJP-1

INTRODUCTION

Atherosclerosis is a global number one cause of death including Indonesia [1]. According to Ministry of Health, Republic of Indonesia [2], 50% of death in Indonesia is caused by atherosclerosis and in 2030 the estimated number of death by atherosclerosis is around 23.6 million [3]. Atherosclerosis is a popular disease but the prevention is hard to find because most of the cause is asymptomatic. The process of atherosclerosis starts during childhood and since that the development of fatty streak keeps going on [4].

Atherosclerosis is an inflammatory process as a response from lipid accumulation in arterial walls [5]. Oxidation of low density lipoprotein (LDL) cholesterol holds an important role in the process of atherogenesis. Oxidized LDL (oxLDL) phagocytosed by macrophages that expressed by nuclear factor kappa beta (*NF-κβ*) in blood vessels' sub-endothelial cells and become foam cells that trigger the secretion of pro-inflammatory cytokines and it can also induce cell apoptosis [6]. C-reactive proteins, inflammatory mediators, is increasing during the first base of atherogenesis which lowering the level of endothelial nitric oxide synthase (eNOS) that increasing monocyte adhesion as a first phase of foam cell formation [7].

Until today, the therapy for atherosclerosis is still in the form of anti-inflammation and anti-hyperlipidemia which only inhibits the progression of the already-exists atherosclerial plaque, not preventing its formation [8]. The high price of medicine is also another problem for the patients and another preventive action other than healthy lifestyle are needed.

In the other side, Indonesia is a country that rich in natural diversity. Pisang Ambon (*Musa sapientum*) that its peel is mostly throwed away surprisingly contains XJP-1 and flavonoid that lowers the excess regulation and expression

of ICAM-1 and VCAM-1 in a dose dependent manner in human umbilical venous cells (HUVECs), lowering the regulation of lipoprotein receptor low-density (LOX)-1 as a result of oxLDL induction, lowering the activity of ROS, inhibits the activity of *NF-κβ* translocation [9], and increasing the activity of eNOS in endothelial cells [10].

Therefore, in this paper we would like to discuss about the results of *Musa sapientum* peel extract effectivity as a prevention of atherosclerosis through the inhibition of *NF-κβ* and the increase of eNOS expression.

The problems of the background above is how is the effectivity of Musa sapientum peel extract as atherosclerosis prevention through the inhibition of *NF-κβ* and the increase of eNOS expression in the atherogenic rats?

The purpose of this research is to understand the effectivity of *Musa sapientum* peels as atherosclerosis preventive thru the inhibition of *NF-κβ* and increasing the expression of eNOS in atherogenic rat.

The outcome of this research is to get the effective dose of *Musa sapientum* peel extract as the prevention of atherosclerosis and to publish scientific articles in accredited journals.

The advantage of this research is increasing the knowledge about *Musa sapientum* as a prevention of atherosclerosis with minimal side effects, economic, and easy to get. This research can also be used as a base of further research about *Musa sapientum*.

MATERIALS AND METHODS

Study design

This study is true experimental using the post-test only control group design. The population used in this study are *Rattus norvegicus* strain Wistar with inclusion criteria: age of 2-3 months, weight of 150-200 g, male, strain Wistar, and health (active in movement, white, and clear in eyes) and exclusion criteria: ill, stress, and death.

Rats were divided in 5 groups as follows:

- **Group 1:** Negative control (normal diet)
- **Group 2:** Positive control (only atherogenic diet)
- **Group 3:** Control 1 (Atherogenic diet and extract doses 200 mg/kg body weight/day)
- **Group 4:** Control 2 (Atherogenic diet and extract doses 400 mg/kg body weight/day)
- **Group 5:** Control 3 (Atherogenic diet and extract doses 600 mg/kg body weight/day)

Each group consists of 5 rats. The extracts were administrated per oral using modification gastric spuit in 14 days. Rats were euthanized at Day 15 and their aortic endothelial tissue were taken out.

Material and reagents

The banana's peel (80% ethanol) extract was purchased from UPT. Matria Medica Batu (Malang, Indonesia). Primary antibody, antibodies against eNOS and *NF-κβ*, SC654 and SC101752, Mayer's hematoxylin, PBS pH 7.4, H_2O_2, serum FBS, SA-H, and DAB were provided by Biomedical Laboratory, Medical Faculty, University of Brawijaya (Malang, Indonesia).

Atherogenic diet

Wistar rats were injected with bitratras adrenalin (i.v) 0.006 mg/200 g, then orally fed with intermittent yolk egg 3% to 4% weight using modificated gastric spuit in 14 days (Constatinindes method).

Data preparation and analysis

Data were collected through observation using microscope with 1000x magnification in 10 areas. Homogeneity test and normality data test were used. Furthermore, comparative test with one-way analysis of variance (ANOVA) to test the average equality hypothesis between groups then continued with post hoc test. Pearson correlation test was performed to find a significant relationship between dose of banana extract with *NF-κβ* activity and eNOS expression and continued with simple linear regression test to analyze how strong the effect between dosage of banana extract with activation of *NF-κβ* and expression of eNOS in cell rats aortic endothelial tissue.

RESULTS

Musa sapientum peel's extract inhibit the *NF-κβ* activation

The *NF-κβ* activity is indicated by the appearance of a brown cell nucleus. The calculation uses a light microscope at 1000x magnification at 10 field of view (Figure 1).

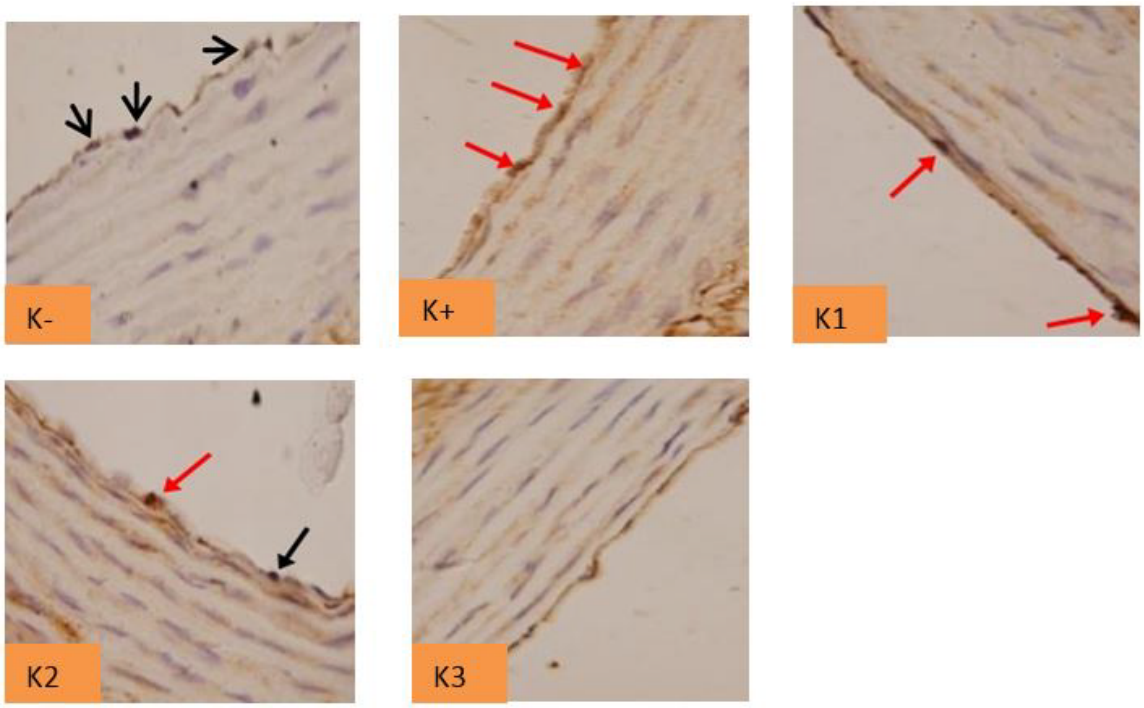

Figure 1 Histopathology of aortic endothelial tissue with *NF-κβ* Immunohistochemical assay in 1000x magnification. Description: The red arrow indicates the activation of *NF-κβ* at the endothelial cell nucleus, the black arrow indicates an inactive *NF-κβ* at the endothelial cell nucleus

Table 1 *NF-κβ* Immunohistochemical assay histopathological observation data

Groups	Repetition					Total	Mean
	1	2	3	4	5		
K −	2	1	0	0	1	4	1
K +	73	79	72	75	75	374	75
K 1	60	62	62	63	59	306	61
K 2	63	60	62	65	59	309	62
K 3	41	30	35	37	38	181	36

Based on data obtained from observations, Table 1 indicates that increased doses result in *NF-κβ* inhibition. ANOVA assay of *NF-κβ* activation in rat aortic endothelial tissue showed $p=0.000$ ($p<0.05$). This shows that there are at least 2 groups that have significant differences. Therefore, it is necessary to proceed with Bonferroni post hoc test to know the differences between groups. Post hoc test results showed that there was a significant difference between positive control group and all treatment groups (K1 ($p=0.000$), K2 ($p=0.000$), K3 ($p=0.000$)). The correlation test $p=-0.906$ ($p<0.05$) showed that there was a significant relationship between each group. This suggests that an increase in dose of the extract of the Ambon banana causes a decrease in the activity of *NF-κβ*.

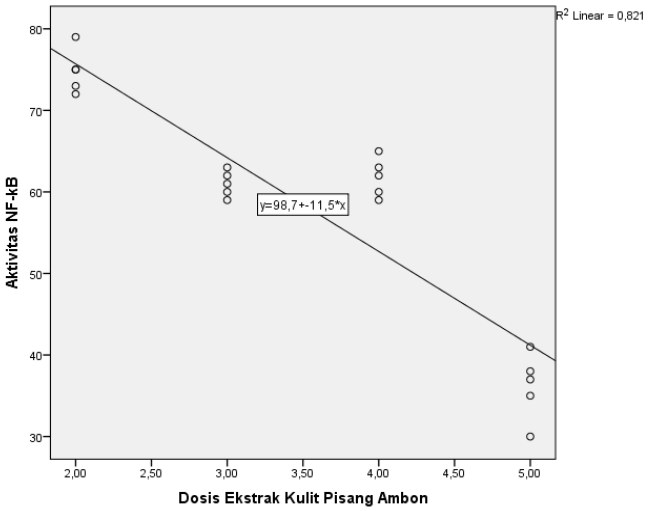

Figure 2 Regression test

Regression test showed R2=82.1% (Figure 2). This means that the dosage of Ambon banana peel extract can decrease the activity of *NF-κ*β by 82.1%, while the rest is explained by another factor which is not investigated by the researcher.

***Musa sapientum* extract increase the expression of eNOS**

The expression of eNOS is indicated by the appearance of the aortic endothelial cell cytoplasmic brown color. The calculation uses a light microscope at 1000x magnification at 10 field of view (Figure 3).

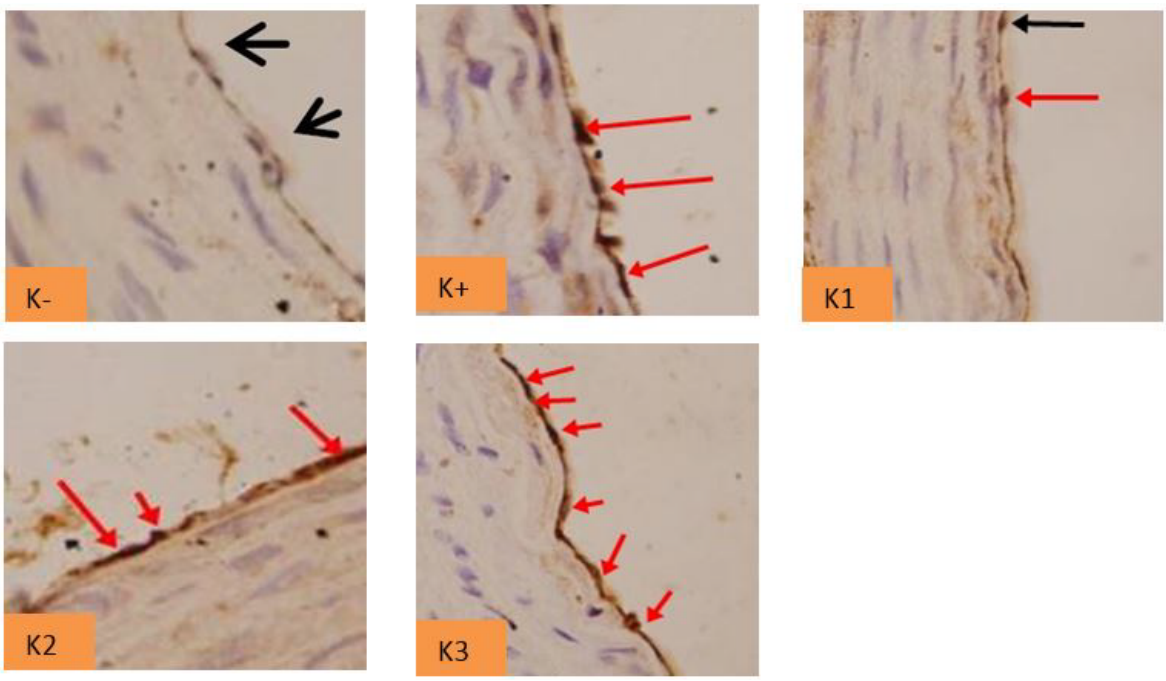

Figure 3 Histopathology of aortic endothelial tissue with eNOS Immuno-histochemical assay in 1000x magnification. Description: The red arrow indicates the activation of eNOS in the cytoplasm of endothelial cells, the black arrow shows eNOS that is not activated in endothelial cell cytoplasm

Table 2 eNOS Immunohistochemical assay histopathological observation data

Groups	Repetition					Total	Mean
	1	2	3	4	5		
K −	56	50	53	57	60	4	1
K +	31	20	25	27	28	131	26
K 1	45	48	42	43	46	224	45
K 2	55	54	53	52	50	264	53
K 3	67	68	69	65	65	334	67

Based on data obtained from observations, Table 2 suggests that increased doses result in increase of eNOS expression. The ANOVA assay of eNOS expression in rat aortic endothelial tissue cells showed a value of p=0.000 ($p<0.05$). This shows that there are at least 2 groups that have significant differences. Therefore, it is necessary to proceed with Bonferroni post hoc test to know the differences between groups. Post hoc test results showed that there was a significant difference between positive control group and all treatment groups (K1 (p=0.000), K2 (p=0.002), K3 (p=0.000)). The correlation test p= −0.975 ($p<0.05$) showed that there was a significant relationship between each group. This suggests that an increase in dosage of the Ambon banana peel extract causes an increase in eNOS expression.

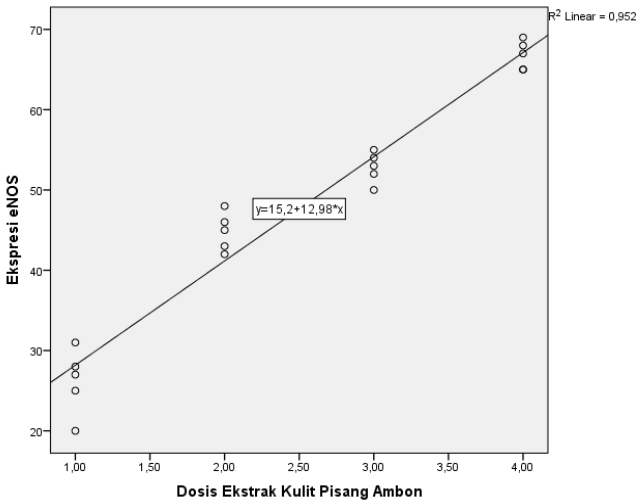

Figure 4 Regression Test

Regression test showed R2=95.2% (Figure 4). This means that doses of Ambon banana peel extract can decrease eNOS activity by 95.2%, while the rest is explained by another factor which is not examined by researcher.

DISCUSSION

Atherogenic diet can increase LDL, then activate *NF-κβ* at proximal aorta and triggers endothelial dysfunction, an early process in atherosclerosis [11]. *NF-κβ* plays an important role in various aspects of pathogenesis in atherosclerosis. *NF-κβ* is a transcription factor that regulates cytokine production, cytokines that play a role in the response Inflammatory agents such as TNF-α, IL-1, IL-6. *NF-κβ* also regulates the expression of the molecule adhesion such as VCAM-1, ICAM-1, P-selectin, and E-selectin thus cause adhesion of monocytes in vascular endothelium and migrate to sub-endothelial vessels, resulted in the formation of foam cells [12].

In this study, the administration of *Musa sapientum* peel's extract can prevent the increase activation of *NF-κβ* significantly ($p<0.05$).

The atherogenic diet also causes the formation of LDL to oxLDL due to oxidative stress so that oxLDL enters the vascular endothelial cells, leading to an early phase of atherogenesis involving multiple expressions of several genes

such as eNOS that function in nitric oxide (NO) synthesis as vasodilators, cyclo-oxygenase (COX), growth factor and monocyte chemoattractant protein-1 (MCP-1).

Upregulation of gene expression in endothelial cells may induce an early process of atherogenesis involving inflammatory cell migration to the activated endothelial cell side [11,13]. In this study, the administration of *Musa sapientum* peel's extract can prevent the decrease of eNOS expression significantly ($p<0.05$). This is caused by the uptake inhibition of oxLDL into endothelial cells and prevent the decrease of intracellular NO levels due to prevention of *NF-κβ* activation so that endothelial cell dysfunction and apoptosis prevented.

If the inflammatory response can be prevented then the process of atherosclerosis can be inhibited. Thus, administration of the *Musa sapientum* peel extract may have a protective effect on atherosclerosis.

CONCLUSION

In conclusion, we have showed conclusively that *Musa sapientum* peel extract inhibited the inflammatory response by decreasing the activation of *NF-κβ* and improved NO system (eNOS). Collectively, our findings suggested that *Musa sapientum* peel extract is effective in inhibiting the atherosclerotic process by suppressing the expression of chemoattractant molecules and monocyte adhesion. These findings provided evidence that *Musa sapientum* peel extract may serve as a novel therapeutic candidate in atherosclerosis prevention.

DECLARATIONS

Acknowledgment

The authors would like to thank Dr. Dian Yuliartha Lestari, Sp.PA from Department of Pathology Anatomy for the helpful corrections and critically reading the manuscript. This research was supported by grant from Ministry of Research, Technology, and Higher Education Republic of Indonesia for financial assistance.

Conflict of Interest

The authors and planners have disclosed no potential conflicts of interest, financial or otherwise.

REFERENCES

[1] World Health Organization. World Health Day 2015: From farm to plate, make food safe. *World Health Organization, Geneva*, 2 Apr 2015, http://www.who.int/mediacentre/news/releases/2015/food-safety/en/.

[2] Kesehatan, Departemen. "Republik Indonesia." *Laporan Pencapaian Tujuan Pembangunan Millenium Indonesia. Jakarta: DepKes RI,* 2010.

[3] World Health Organization. Cardiovascular diseases (CVDs) Fact sheet, *World Health Organization, Geneva,* May 2017, http://www.who.int/mediacentre/factsheets/fs317/en/index.html.

[4] Libby, Peter. "Inflammation in atherosclerosis." *Arteriosclerosis, Thrombosis, and Vascular Biology* Vol. 32, No. 9, 2012, pp. 2045-51.

[5] Sakakura, Kenichi, et al. "Pathophysiology of atherosclerosis plaque progression." *Heart, Lung and Circulation* Vol. 22, No. 6, 2013, pp. 399-411.

[6] Hansson, Göran K. "Atherosclerosis-an immune disease: the Anitschkov Lecture 2007." *Atherosclerosis* Vol. 202, No. 1, 2009, pp. 2-10.

[7] Osman, Raed, et al. "Critical appraisal of C-reactive protein throughout the spectrum of cardiovascular disease." *Vascular Health and Risk Management* 2.3 (2006): 221-237.

[8] Curtiss, Linda K. "Reversing atherosclerosis." *New England Journal of Medicine* Vol. 360, No. 11, 2009, pp. 1144-46.

[9] Fu, Rong, et al. "Suppression of endothelial cell adhesion by XJP-1, a new phenolic compound derived from banana peel." *Vascular Pharmacology* Vol. 57, No. 2, 2012, pp. 105-12.

[10] Duarte, J., V. Francisco, and F. Perez-Vizcaino. "Modulation of nitric oxide by flavonoids." *Food & Function* Vol. 5, No. 8, 2014, pp. 1653-68.

[11] Hajra, Leena, et al. "The NF-κB signal transduction pathway in aortic endothelial cells is primed for activation in regions predisposed to atherosclerotic lesion formation." *Proceedings of the National Academy of Sciences* Vol. 97, No. 16, 2000, pp. 9052-57.

[12] Gareus, Ralph, et al. "Endothelial cell-specific NF-κB inhibition protects mice from atherosclerosis." *Cell Metabolism* Vol. 8, No. 5, 2008, pp. 372-83.

[13] Tate, Shin-ichi. "Oxidized low-density lipoprotein receptor, LOX-1, on the endothelial cell-the receptor structure and functions of LOX-1 in atherogenesis." *International Journal of Biological Macromolecules* Vol. 7, No. 2, 2007, pp. 11-22.