The effect of pomegranate extract against endothelin-1 expression, inducible nitric synthase, dan thickness of smooth muscles in pulmonary artery media tunics

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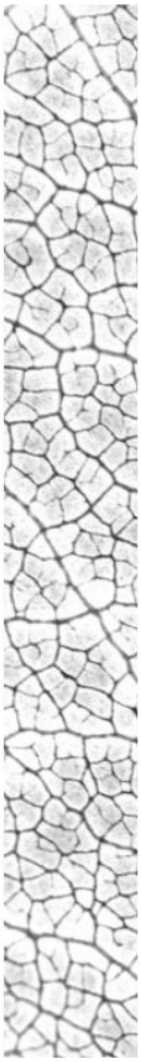

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The effect of pomegranate extract against Endothelin-1 expression, inducible nitric oxide synthase, Dan thickness of smooth muscles in pulmonary artery media tunics

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Abstract

Background: Congenital Heart Disease which if not corrected on time can result in increased pulmonary artery pressure or what is referred to as Pulmonary Artery Hypertension (HAP). The pathogenesis of HAP involves remodeling of the pulmonary artery Pomegranate extract (EBD) can be developed as a therapy in cardiovascular disease as anti-proliferation and anti-inflammatory.
Purpose: Analyzing the effect of pomegranate extract administration on the expression of Endothelin-1 (ET-1), Inducible Nitric Oxide Synthase (iNOS), and thickness of the tunica smooth muscle media of pulmonary arteries in rats modeled by pulmonary arterial hypertension.
Method: This study was an observational analytic retrospective cohort study design. Analysis of the data used is a descriptive analysis of the number of cells expressing ET-1 and iNOS as well as the thickness of the tunica smooth muscle media of the pulmonary arteries for each group.
Result: In the HAP model mouse group that was given EBD, the average number of cells expressing ET-1 decreased sharply from observation after 2 weeks to observation after 4 weeks. The mean number of cells expressing iNOS and the mean thickness of smooth muscle of the pulmonary artery also experienced a sharp decline from observation after 2 weeks to observation after 4 weeks.
Conclusion: The administration of EBD can reduce the expression of ET-1, iNOS, and smooth muscle thickness in tunica pulmonary artery media in hypertensive mice.

Keywords: congenital heart disease, pulmonary artery hypertension, pomegranate, endothelin, nitric oxide synthase

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INTRODUCTION

One of the most high urgency-level diseases is heart disease (Widiyanti, et al. 2016), Congenital heart disease (CHD) is one of the most commonly found congenital abnormalities of heart disease, with an incidence of 8-10 life per 1000 babies born (Wilkins, 2012). The most common risk factors for heart disease occur are (Maharani, et al. 2019) hypertension. Likewise, congenital heart disease that if not corrected on time can lead to increased pulmonary arterial pressure or as pulmonary arterial hypertension (HAP) (Haarman, et al. 2020). Estimated over worldwide there are 600,000 babies born each year with (CHD), 50% or more will die due to infections and heart failure.

The pathogenesis of HAP involves the *remodeling* of pulmonary arteries (Geiger, Sharma, Mooi, & Berger, 2009). In the *remodeling* process, endothelial cells experience dysfunction as a response to increased pressure in the form of proliferation, apoptosis, and changes in homeostasis (Humbert, et al. 2004). Endothelin-1 which is produced by the endothelial cells has the mycogenic and inflammatory properties by working through the mediation of Endothelin (et_A) AND endotheline B (et_b) receptors in the smooth muscle cells of the pulmonalis artery. The presence of shear stress and inflammatory process, due to interaction NO with $O2^-$ become Peroksinitrat ($ONOO^-$), resulting in an increase of iNOS and decreased production of nitric oxide (NO) which gives rise to contraction, the proliferation of smooth muscles, the thickening of tunics media, and the stiffness of the artery wall pulmonalis. Endothelial cell changes in Tunika media relate to the narrowing of the lumen of the blood vessels and the

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progressive decrease of ability respond from vascular to vasodilatory stimulation. So it can be conclude that endothelial cells and smooth muscle cells experienced changes in signaling extra and intercellular pathways, proliferation, and protein synthesis of extracellular matrices in response to increased pressure and hemodynamic changes. As a result of such complex changes, occurred the pathological structural *remodeling* of pulmonary arteries that led to advanced HAP stage which often did not respond to therapy and eventually led to right heart failure and death.

Current HAP therapies is the inhibitors of Endothelin-1 (ET-1) receptor activity, the inhibitor of phospholidiasterase-5, and the Prostacyclin (PGI_2) (Abman *ET al*., 2015). HAP Therapies used today are vasodilators, while the inflammatory process and fibrosis on HAP have never been touched, so the remodeling process is still ongoing. More and More researchers are looking for the best handling of HAP, such as anti-proliferative therapy, genetic variation, *stem cell*use, and other strategies that can be used in the clinic practice to prevent early progresifitas (Dewachter, et al. 2010).

Pomegranate fruit is commonly used as an herbal medication. Extracts from all parts of this fruit have a protective effect against damage in the body, including a decrease in the setting of apoptosis mechanisms due to the Ellagic acid (Anggraini, & Hendarto, (2018) compounds. Extract Pomegranate fruit is suspected to have some potential, one of which is anti-fibrotic, anti-proliferative, anti-inflammatory, and antioxidant effects that can be developed as a therapy to cure Kardiovascular disease (Jurenka, 2008). Content in EBD can also serve as an anti-proliferation and anti-inflammatory in cardiovascular disease in mice model (Seeram, et al. 2005, Putri, 2019).

However, until now there has been no research about alternative HAP therapy that proves *remodeling* mechanism of the effects of pomegranate extract to mechanisms that related to vasoactivity, through nitric oxide pathways, and endothelin so that it can Manipulating the process of angiogenesis in pulmonary arterial hypertension due to increased blood flow to the lungs. Based on the above data, further research is expected to be used as an additional therapy in pulmonary arterial hypertension to reduce the long-term mortality rate.

METHOD

This study is an analytical observational Study of retrospective cohort design. In this study researcher search for the influence of treatment carried out against the paraffin block, namely the smooth muscle cell lung tissue pulmonary artery tunics media

Population and Sample

The paraffin block of lung tissue from HAP rat model derived from the white rat (*rattus norvegicus*) strain

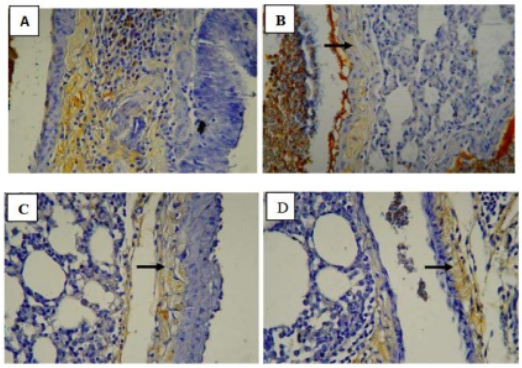

Fig. 1. Expression ET-1 tunika arterial media pulmonalis rat Model Control (a) and the given an EBD (B) observation 2 weeks control (C), and given an EBD (D) observation after 4 weeks. 100x magnification. The black arrow indicates the cell that expresses the ET-1 in the pulmonary artery.

Table 1. Number of cells that Mexpressed ET-1

Group	n	ET-1 (cell/ field of view)		Oneway Anova p
		Mean	SD	
I	6	10[c]	2,09	p <0,001*
II	6	12[d]	0,89	
III	6	7,16[b]	1,47	
IV	6	3,50[a]	1,37	

[abcd] *superscript* shows there is no difference between groups (based on multiple *comparison* LSD).

Sprague Dawley A male aged 3 months with a bodyweight of 250-500 grams. There are 4 treatment groups with each group containing 6 living mice. In this study Parafin block were used in all the group.

Data Analysis

Research Data results are presented in tables form, graphs and text/writings. Data Analysis used is analytic descriptive to the number of cells expressing the ET-1 and INOS as well as the thickness of the smooth muscles of the pulmonary artery media tunic for each group. Data is analyzed with a trust level of 95% (α = 0.05).

RESULT

An expression of ET-1 in the control group as well as a group of EBD treatment in intima tunics and media apulmonarypulmonary tissue preparation of the HAP rat model is shown by **Figure 1**.

Figure 1(A) Black Arrow shows an expression of ET-1 in a medium pulmonary artery tunic Rat Model control group 2 weeks. It appears the number of cells that express the ET-1 more than in the **Figure 1(B)** groups that get the EBD of 2 weeks, fewer cells THAT express the ET-1 shown with the black arrows. **Figure 1(C)** of the black arrow shows the expression ET-1 in the media tunic artery rat model control 4 weeks. Visible cells that express the ET-1 much more than in the **Figure 1(D)**

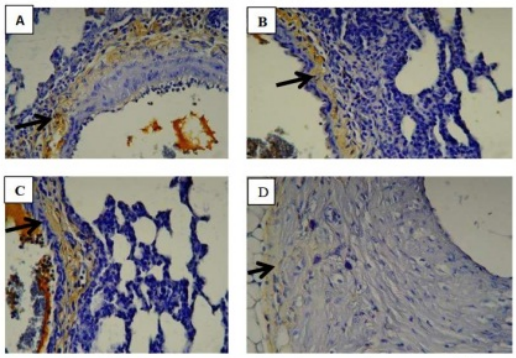

Fig. 2. The iNOS expression (black arrow) on a media tunica artery rat Model Control (a), which given an EBD (B) on 2-week observation, Control (C), and that given an EBD (D) on a 4-week observation. 100x Per magnitude.

Table 2. Average test number of cells that Express INOS based on control group and treatment and difference of observation time 2 weeks as well as 4 weeks.

Group	n	iNOS (cell/field of view)		Oneway Anova p
		Mean	SD	
I	6	12^{c}	1,78	p <0,001*
II	6	12,50^{c}	1,76	
III	6	8,17^{b}	0,98	
IV	6	2,33^{a}	1,03	

* p value mean if < 0.05
^{abc} *superscript* ABC shows no differences between groups (based on multiple *comparison* LSD)

group of Rats given the EBD 4 weeks, the cells that express the ET-1 fewer are shown the black arrows.

Table 1 shows that average cell that expresses the ET-1 in group control group rat model continues to increase sharply from observation after 2 weeks until observation after 4 weeks. In contrast to the rat group HAP models which given EBD, the average number of cells expressing ET-1 suffered a sharp decline from observation after 2 weeks until observation after 4 weeks.

An iNOS expressions on the control group as well as the group of EBD Administration treatment on intima tunics and pulmonary ARTERIAL media. The lung tissue preparations of HAP models were shown by **Figure 2**. **Figure 2(A)** Black arrow shows iNOS expression in the tunic of pulmonary artery Media Model 2-week control group. The number of cells that express iNOS appears slightly. In **Figure 2(B)**, the number of cells expressing iNOS in a group given 2 weeks also seemed a bit like the designated black arrows. **Figure 2(C)** Arrow Black shows iNOS expression in tunic medium pulmonary artery control Model 4 weeks. It appears that the cell is slightly compressed, compared to the **Figure 2(D)** group that is given a 4-week EBD, only a few number of cells that express iNOS, shown black arrows. The number of cells that Express iNOS on a group that gets a 2-week EBD is higher than the number of cells that Express iNOS in a 4-WEEK EBD group.

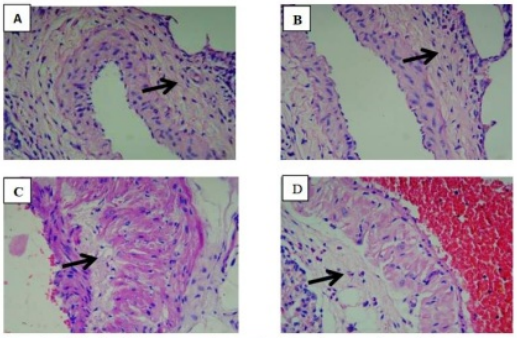

Fig. 3. Thick plain muscle wall (black arrow) tunic of pulmonary artery medium of the HAP rat model. Control (a) and that is in a 2-week observation, control (C) and that is in a 4-week observation (D). Painting Hematooxylin eosin, with P 100 x. Arrows showing thick smooth muscles of the tunic of the pulmonary artery media.

Table 3. Thick smooth muscle Tunika media Apulmonary artery

Group	n	Thick tunika media A.pulmonalis (mm)		Oneway Anova p
		Mean	SD	
I	6	7,33^{c}	1,21	P <0,001*
II	6	4,33^{b}	1,63	
III	6	9,33^{d}	1,86	
IV	6	2,33^{a}	1,03	

* p value mean if < 0.05
^{abcd} *superscript* shows no difference between groups (based on *multiple comparison* LSD)

Table 2 shows average cell expresses iNOS in the Rat group's control model continues to increase sharply from observations after 2 weeks until 4 weeks observation. In contrast to the of the HAP rat group models given an EBD, the average number of cells that express iNOS is experiencing a sharp decline from observation after 2 weeks until observation after 4 weeks. As the time goes there is a decreases number of cells that express iNOS.

Histology of smooth muscle pulmonary tissue tunics of the pulmonary artery media with the coloration of *Hematoxylin Eosin* (HE) in the rat model of THE control group HAP and the group of EBD is shown by **Figure 3**.

Mouse Model group control. Thick smooth muscles on the control group seem thicker than the thick smooth muscles of the group that get an EBD in 2 weeks, Black arrow **Figure 3(B)**. **Figure 3(C)** Arrow Black show thick smooth muscles tunic artery medium artery rat model Observation control in 4 weeks. Tunika smooth muscles pulmonary artery media is thicker than in the **Figure 3(D)** Rat group of HAP models that get EBD 4 weeks indicated Black arrow.

Table 3 shows the smooth muscles of the pulmonary artery plain in the Rat Group control model continue to increase sharply from observation after 2 weeks until observation after 4 weeks. In contrast to the rat group of the HAP models that were given EBD, the average of

the pulmonary artery smooth muscles suffered a sharp decline from observation after 2 weeks until observation after 4 weeks. The passage of time further lowers the thickness of the smooth muscles of the pulmonary artery and can inhibit the proliferation of smooth muscles of pulmonary artery media tunics.

DISCUSSION

This research proves that pomegranate extract inhibits the increase in the number of cells that express ET-1 on 2-week observation as well as 4-week observation. This is evident from the absence of increased expression ET-1 and its lower expression in the mice model group that were given EBD during the study. The opposite state in the rat group of model control of the ET-1 expression increased meaningless and a higher expression during the study took place. With the presence of barriers to THE expression ET-1 BY EBD, the proliferation of smooth muscles of pulmonary artery can be inhibited so that HAP progression can also be inhibited.

Pomegranate fruit contains polyphenols that serve as anti-inflammatory and antioxidant, including *Ellagic Acid* (EA), flavonoids, tanins, and Anthrocyanins. The anti-inflammatory role of EBD in this case is suppressing the expression of genes that produce cytokines which are inflammatory mediators through inhibitions on *p38 mitogen Activated Protein kinase* (P38MAPK) and NF-kB transcription factor. The role of anti-inflammatory EBD also through inhibitions in the enzyme *cyclooxygenase* (COX) and *lipooxygenase* (LOX) which is an important enzyme in the changes of fig acid kidonat to prostaglandin and Leukotriene. Ekstract Pomegranate has effects as an anti-Inflammation and anti-proliferative by inhibiting the activity of MAPK and Endothelin, so as to lower the levels of ET-1. Pomegranate extract inhibits the path of *P38-mitogen-activated protein kinase* (P38-MAPK) and Transcription factor, NF-KB (*nuclear factor kappa-light-chain-enhancer of activated B cells*). Activation of P38-MAPK and NF-kB is associated with increased expression of the gene TNF-α, IL-1 β, MCP1, iNOS, and COX-2 that is the most important mediator of (Yu et al., 2019) inflammatory inflammation.

This research also proves that pomegranate extract inhibits the increase in the number of cells that express iNOS on 2-week observation as well as 4-week observation. This is evident from the absence of an increased expression of iNOS and their lower expression in the model rat group that was given up during the study. The opposite state in the rat group of models of iNOS expression control is highly increased and a higher expression during the study progresses. With The barriers to the expression of iNOS by EBD, the proliferation of smooth muscles of pulmonary artery can be inhibited so the progression HAP can also be inhibited.

The decrease in the expression of iNOS in pulmonary artery mediums of paraffin block rat pulmonary arterial hypertension model occurs in the administration of pomegranate extract. Extract Pomegranate Fruit can be used as an anti-inflammatory and antioxidant by suppressing free radicals due to the interaction of nitric oxide with the $O2^{-}$ being Peroksinitrat ($ONOO^{-}$) and inhibiting the effect of pro-inflammatory cytokines. The antioxidant and anti-inflammatory abilities and activities of pomegranates are suspected to be attributed to its very high polyphenolite content, such as *ellagic acid* (EA). *Ellagic acid* is one of the active ingredients of *the whole* pomegranate extract is in the *free form* as *Ellagic acid glycosides* or bonded in the form of *ellagitannins*. The activity and concentration of EA in low plasma, due to solubility in low water, besides the EA easily undergo transformation and degradation before it is absorbed. Proinflammatory Cytokine Interleukin-1beta (IL-1 β) is involved in the process of HAP (Jurenka, 2008).

This research also proved that pomegranate extract inhibits the thickness of the smooth muscles of the pulmonary artery media in the observation in 2 weeks as well as observation in 4 weeks. This proved from the absence of the addition of smooth muscles of the tunica of the pulmonary artery media and lower cut in the rat group of models that get EBD during research Last. The opposite state in the Rat Group control model of smooth muscles of the pulmonary artery media tunic increased highly and higher as long as the study progresses. as the time goes, EBD proved to be able to decrease the thickness of the smooth muscles of the pulmonary artery media in the HAP rat model compared to the Rat HAP control model. Occurrence of obstacles to the thickness of the smooth muscles of the pulmonary artery media, the proliferation of smooth muscles of pulmonary artery can also be inhibited so that the progression HAP can be inhibited.

The administration of pomegranate extract can prevent the thickening of the smooth muscles of the monalist artery in the control fin block group, as well as the administration of EBD can inhibit the progression of HAP. The disrupted pulmonary artery flow resulted in an increase in vascular smooth muscle cell count and contractility of the HAP due to a decrease in the expression of prostaglandins and NO with an increase in the number of thromboxines and plasma endothelins. The imbalance of the mediator results in the occurrence of vasoconstriction and proliferation of smooth muscle cells of the pulmonary artery, the migration and Disfung of thecells, and abnormal apoptosis (Barst, et al. 2011. Wardle, 2012).

CONCLUSION

Pomegranate extract can reduce the expression of endothelin-1, inducible nitric Oxide synthase, and thick smooth muscles in pulmonary artery media tunics in hypertensive rats mode.

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