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Effect of Pomegranate Extract (*PunicaGranatum L*) on Pulmonary Arterial Pressure in Sprague Dawley Rat with Pulmonary Arterial Hypertension

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Abstract— Congenital heart disease causes pulmonary arterial hypertension, heart failure, and early death. Pulmonary arterial hypertension causes pulmonary arterial wall remodeling, inflammation, deposition of extracellular matrix protein, collagen synthesis, and fibrosis. This study aims to analyze the effect of Pomegranate extract on pulmonary arterial pressure in Sprague Dawley rats with pulmonary arterial hypertension. Twenty-fourSprague Dawleyrat age 3-month bodyweight 250-350 gram allocated randomly into Pomegranate extract and control group, 6 rats in each group. Pulmonary arterial hypertension.was induced with monocrotaline 60 mg/kg body weight injected subcutaneously. Pulmonary arterial pressure both groups were measured at 2 weeks and 4 weeks of observation. Rats were euthanized after 2 and 4 weeks of observation. Mean pulmonary arterial pressure the Pomegranate extract group was significantly lower compared to the control group in two weeks and four weeks of observation, 27,57±9,17 mmHg vs 47,48±5,58 mmHg (p=0.001) and 32,43±9,64 mmHg vs 46,53±5,53 mmHg (p=0.001) respectively. Pomegranate extract can decrease pulmonary arterial pressure in Sprague Dawleyrats with pulmonary arterial hypertension induced by monocrotaline. Pomegranate extracts effective preventing the progressivity of pulmonary arterial hypertension in the rat.

Keywords— Pomegranate extract, pulmonary arterial hypertension

1. Introduction

The incidence of congenital heart disease (CHD) is 2-9.4 per 1000 live births.[1] Non-cyanotic CHD if not corrected on time will result in pulmonary arterial hypertension (PAH). The pathogenesis of PAH is characterized by vasoconstriction, micro thrombosis, and pulmonary arteries remodeling. The triggers of vasoconstriction are hypoxia and hypertension of left atrium, causing calcium influx and calcium release from intracellular, and contraction of smooth muscle cells. Deficiency of protein C and S, and increase activity of von Willebrand factor causing micro thrombotic events. Arterial wall remodeling including increases apoptosis, endothelial cell loss, increased proliferative pathway activity, thickening of smooth muscle cells, and resistance to programmed cell death.[2] Collagen accumulation causes fibrosis, a stiff and narrow lumen of blood vessel walls which leads to an increase in pulmonary artery pressure and ultimately causes impaired right ventricular function.[3]A hallmark of pulmonary hypertension is an increased pulmonary vascular resistance which leads to progressive elevations in pulmonary artery pressure, resulting in compensatory right ventricular hypertrophy and, ultimately, heart failure.[4]Specific medications currently approved for therapy of PAH target three major pathways consist of nitric oxide, endothelin, prostacyclin pathway. Some PH centers use off-label drugs for PAH therapy.[5] PAH existing therapies are nothing can cure, so the search for new therapeutic strategies continues.[6]

Studies in PAH treatment with drugs derived from natural plants have demonstrated unique advantages and broad application prospects. That natural plant products including *Ginkgo biloba leaf, Green tea, Salvia miltiorrhiza Bunge, Uncariarhynchophylla, Astragalus membranaceus, Panax notoginseng, and Anemarrhenaasphodeloides*. Active substances in that plant are flavonoids, glycosides, diterpenoids, pyranocoumarins, and stilbenes.[7]Pomegranates use as a natural remedy due to their capability against pathogens. Every part of the pomegranate, the fruit juice, peel, arils, flowers, and bark have wide ranges of phytochemical propertiestherapeutic activity including anticancer, anti-inflammatory, anti-atherogenic, anti-diabetes, hepatoprotective, and antioxidant activity. Ellagic acid andhydrolyzable tannins, such as punicalagin, have the most activities.[8]Pomegranate juice effects in reduce blood pressure, improve endothelial function, reduce stiffness, and slow or reverse the progression of atherosclerosis.[9] Until now, there have not been many studies that prove the effect of PGE on the improvement of pulmonary arterial

pressure on PAH. PGE is expected to be used in clinical research as an adjunct drug for therapy and inhibits the progression of PAH. The study aimed to prove the effect of pomegranate extract on pulmonary artery pressure reduction in Sprague Dawley rats with pulmonary artery hypertension.

2. Method

This study was the laboratory experimental research conducted on 24 Sprague Dawley rats. PAH was induced by subcutaneous monocrotaline injection at a dose of 60 mg/kg body weight. The rats meet the study requirements: 3 months old, body weight 250-350 grams, and healthy. Rats were grouped randomly into 2 study groups and 2 control groups, six rats in each group. The study group was treated with pomegranate extract, extracted from all parts of pomegranate fruits in powder form and has been standardized to contain 40% ellagic acid produced by Xi'an Biof Bio-Technology Co., Ltd. (Room 1-1111, High-tech Venture Park, No. 69 Jinye Road, Gaoxin District of Xi'an, People Republic of China). The PGE 150 mg / kg body weight suspended in a 0.3% CMC solution of 0.3% 2 ml. The control group was given a 0.3% sodium carboxymethylcellulose solution 2 ml. PGE and CMC were given once daily, start after 3 days of monocrotaline injection. Evaluation of pulmonary arterial pressure was done after 2 weeks and 4 weeks of observation. Pulmonary arterial pressure measurement was made after the following steps: rats were anesthetized, intubated via tracheostomy using a 16G IV catheter, connected with an oxygen tube through a three way with an oxygen flow of 0.5 L / minute. A left lateral thoracotomy was performed at 3-4th intercostal space, widened with a spreader, and the heart exposed. The 20G IV catheter was inserted into the main pulmonary artery through the right ventricular outlet under the pulmonary artery valve, and connected to the pressure gauge monitor hose. On the monitor screen, pulmonary arterial pressure waves appear in the systolic and diastolic phases. Pulmonary artery pressure was recorded at least 3 times. The best pressure trace was chosen and the mean pressure was calculated. The rats were euthanized by exsanguination after the measurement of pulmonary arterial pressure.[10]

Statistic analysis of MANOVA and Least Significant Difference (LSD) were used and the level of significance of < 0.05 using SPSS. This study was approved by Animal Care and Use Committee Veterinary Faculty of UniversitasAirlangga (No. 400-KE December 23rd, 2014).

3. Result

Two rats died on the 7th and 11th day of observation, both included in the control group 2 weeks and 4-weeks observation. The pulmonary arterial pressure was as shown in table 1.

Table 1. Pulmonary arterial systolic and diastolic pressures

Group	Systolic BP(mmHg)	Diastolic BP(mmHg)
	Mean ± SD (Min-Maks)	**Mean ±SD (Min-Max)**
Control 2 weeks	66.64 ± 11.07[a] (45.9-79.1)	37.90 ± 4.26[a] (30.9-42.8)
PGE 2 weeks	41.47 ± 13.47[b] (20.5-57.4)	20.62 ± 7.25[b] (10.7-32.6)
Control 4 weeks	65.03 ± 5.50[a] (59.5-71.4)	37.28 ± 7.99[a] (26.9-44.1)
PGE 4 weeks	44.73 ± 12.66[b] (26.0-60.6)	26.28 ± 9.15[b] (13.7-38.2)
p	0.002*	0.002*

Note: * significant at α = 0.05. The same a,b, superscript shows no differences between groups (based on multiple LSD comparisons)

The mean systolic and diastolic pulmonary arterial pressure in the PGE group was significantly lower than in the control group 2 weeks (p = 0.001 and p = 0.001) and 4 weeks (p = 0.014 and p = 0.032) observations. The mean systolic and diastolic pulmonary arterial pressure in the PGE group 2 weeks and 4 weeks observation was not significantly different (p = 0.631 and p = 0.196). Meanwhile, the mean systolic and diastolic pulmonary arterial pressure in the control group 2 weeks and 4 weeks observation was not significantly different (p=0.831 and p=0.222) (Figure 1)

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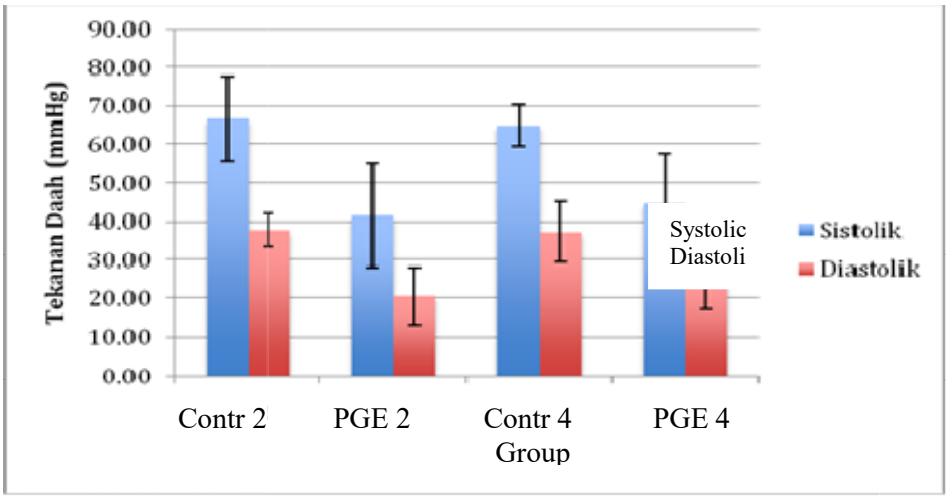

Figure 1. Average pulmonary arterial systolic and diastolic pressure
Notes: Blue bar chart = mean pulmonary arterial systolic pressure.
Red bar chart = mean pulmonary arterial diastolic pressure.

The mean pulmonary arterial pressure in the PGE group and the control group was as shown in table 2.

Table 2. Mean pulmonary arterial pressure

Group	**n**	**Mean pulmonary arterial pressure (mmHg)**				**OnewayAnova p**
		Mean	**SD**	**Minimum**	**Maximum**	
Control 2 weeks	5	47.48[b]	5.58	39.03	53.63	0.001*
PGE 2 weeks	6	27.57[a]	9.17	13.97	40.87	
Control 4 weeks	5	46.53[b]	5.53	40.50	53.20	
PGE 4 weeks	6	32.43[a]	9.64	20.47	41.67	

Note: * significant at α = 0.05. The same superscript shows no differences between groups (based on multiple LSD comparisons)

The mean pulmonary arterial pressure in the PGE group was significantly lower than the control group 2 weeks and 4 weeks of observations (p=0.000 and p=0.013). The mean pulmonary arterial pressure in the PGE group 2 and 4 weeks observation was not significantly different (p=0.302). The mean pulmonary arterial pressure in the control group 2 and 4 weeks observation was not significantly different (p=0.854).

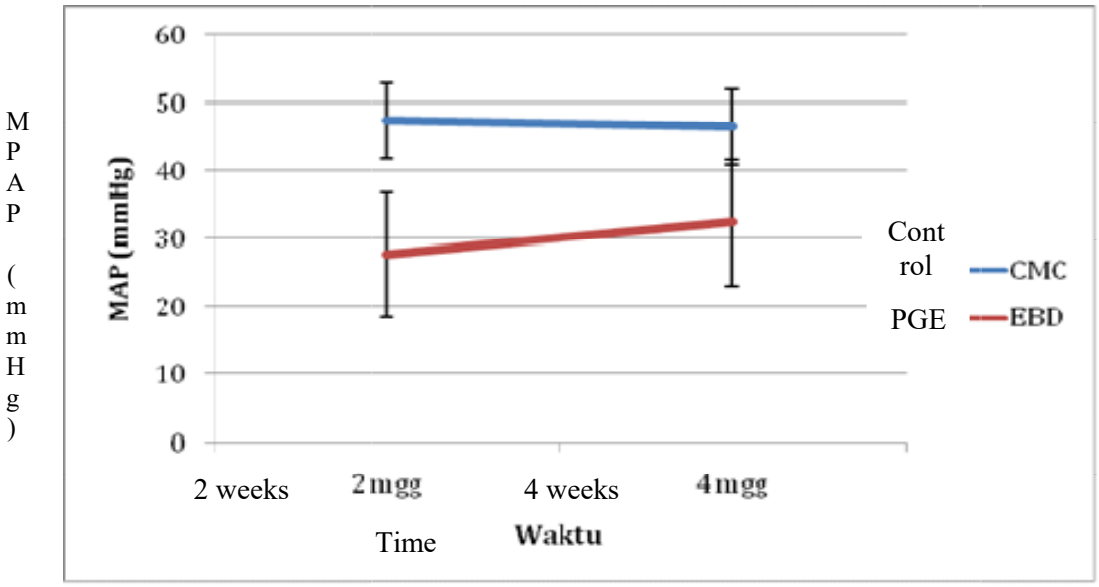

Figure 2 . Mean pulmonary arterial pressure (MPAP). Note Blue line = mean pulmonary arterial pressure control group. Redline = mean pulmonary arterial pressure PGE group.

Figure 2 showed that mean pulmonary arterial pressure in the PGE group maintained lower in 2 weeks period of observation compared with the control group.

4. Discussion

Our study investigated the effect of pomegranate extract for the treatment of pulmonary arterial hypertension in Sprague Dawley rat induced with monocrotaline. The results showed that pomegranate extract effectively reduced/suppressed the pulmonary arterial pressure and slowing its progressivity. The mean pulmonary arterial pressure was significantly lower compared to the control after two weeks of treatment. Increasing mean pulmonary arterial pressure from two weeks until four weeks of observation was not significant. These results revealed that Pomegranate extract can reduce and prevent the progressivity of pulmonary arterial hypertension.

Pulmonary arterial hypertension is a chronic and progressive disease characterized by a persistent increase in pulmonary artery pressure accompanied by right ventricular hypertrophy. The definition of PH in children has been the same as in adults, as mPAP>20 mmHg and PVR ⩾3 WU.[11]Pulmonary arterial hypertension is still an important cause of morbidity and mortality in children. The survival of children with idiopathic PAH remains poor. The treatment of PAH has changed, improved, and led to the approval of 10 drugs from three main pharmacological pathways in the last two decades. Therapy with modern drug improves the symptoms of PAH and slows down the clinical deterioration. However, therapeutic strategies in children have not been sufficiently studied. PAH therapy in pediatric is vastly based on experience and trial data from adults.[5,12]

The use of natural plant products has confirmed in the clinical efficacy and showed unique advantages for PAH treatment. *Ginkgo biloba leaf, Green tea, Salvia miltiorrhiza Bunge, Uncariarhynchophylla, Astragalus membranaceus, Panax notoginseng, and Anemarrhenaasphodeloides*, are the natural plant products that have been used in the treatment of PAH.[7]The effects of herbs and phytochemicals used to treat PH through six mechanisms, including vasodilatory, antiproliferative, anti-vascular remodeling, antioxidant, apoptosis-inducing actions, and anti-inflammatory. Many of these medicinal plants and phytochemicals can have more therapeutic effects than chemical drugs if used appropriately.[12]Pomegranate has been used for thousands of years. It is described as a treatment for tapeworm and other parasites in records dating from around 1500 BCE. Pomegranate has been used for high blood pressure, athletic performance, heart disease, diabetes, and many other conditions.[13]Pomegranates have a wide range of capabilities and use as a natural remedy to chemical treatment. Every part of the pomegranate has

therapeutic activity including antioxidant, anticancer, anti-inflammatory, anti-atherogenic, anti-diabetes, hepatoprotective, and antimicrobial.[8]Pomegranate has several pharmacological actions as an anti-inflammatory, antioxidant, and anticarcinogenic. A growing number of studies of pomegranate apply it to solve medical problems.[14]Punicalagin therapy in rats can protect against endothelial dysfunction and pulmonary hypertension induced with hypoxia. It is induced via anti-oxidant actions, improved nitric oxide (NO)-cGMP signaling, and reduced oxidative stress. Decrease of superoxide generation, phytochemical reduces oxidative stress and raises mRNA expression of MMP-9, HIF-1α, VEGFA, NF-kB, and TNF-α in lung tissue of animals are the indicator of reducing oxidative stress. Punicalagin therapy can be used as an effective treatment for PH.[15]*Punicagranatum* supplementation of chickens fed had the potential to improve growth performance, increasing plasma nitric oxide concentration. and prevent Pulmonary Hypertension Syndrome, significantly suppressed the expression of ET-1 in the liver.[16]

Our study showed pulmonary arterial pressure decreased significantly after PGE administration for two and four weeks. This pressure drop was likely to be through several pathways of inhibition mechanism by PGE. PGE has the ability of anti-oxidant and strong anti-inflammatory properties. The beneficial effects of PGE are related to the active substances; ellagic acid, ellagitannins, punicic acid, and other fatty acids, flavonoids, anthocyanins, anthocyanidins, flavones, and estrogenic flavonols.[17]*Punicagranatum* extract supplementation had the potential to improve growth, prevent pulmonary hypertension syndrome, higher plasma nitric oxide concentration, and suppressed the expression of ET-1 in the liver of chickens fed *Punicagranatum*.[16]Pomegranate-derived compounds exhibit a wide range of vasculoprotective effects through reducing oxidative stress, lipid peroxidation, and generation of foam cells, attenuate platelet aggregation, vasculo-protective effects result in improve vascular function, increased the expression of endothelial nitric oxide synthase (eNOS), protects the generated nitric oxide (NO) against its oxidative destruction, activation of the protein kinase B (Akt) / eNOS pathway and attenuation of vascular inflammation.[18-20]Increase the growth of *Bifidobacterium breve* and *Bifidobacterium infantis* and the production of short-chain fatty acids caused by Pomegranate byproducts and punicalagin have beneficial effects through the activation of peroxisome proliferator-activated receptors (PPARs). PPARs regulate inflammation, metabolism, and immunity.[17]Punicalin, and punicalagin which are constituents of PGE that can increase the production of short-chain fatty acids (SCFAs) by the metabolism of commensal bacteria in the intestine. SCFAs are absorbed and activate peroxisome proliferator-activated receptor γ (PPARγ), which will inhibit the transcription of pro-inflammatory molecules by NF-κB, AP-1, and STAT, and produce anti-inflammatory effects. There are elevated levels of cytokines IL-1β, IL-18, IL-6, IL-13, and TNF-α which contribute to vasoconstriction which causes an increase in pulmonary artery pressure and proliferation of pulmonary artery smooth muscle.[21]The inhibition of the inflammatory process by PGE through inhibition of pro-inflammatory cytokines causes vasodilation and inhibition of pulmonary artery smooth muscle proliferation causing a decrease in pulmonary artery pressure on PAH. In conclusion; Pomegranate extract can decrease pulmonary arterial pressure in Sprague Dawleyrats with pulmonary arterial hypertension induced by monocrotaline.

The limitation of our study was the complexity of the measurement of pulmonary arterial pressure. Our methods used left lateral thoracotomy and direct measurement of pressure through punction of the heart. These methods traumatic enough, sometimes puncture needle stack on the right ventricle wall and can influence the pressure result recorded.

5. Conclusion

This study has demonstrated that Pomegranate extracts effective in decreasing and preventing the progressivity of pulmonary arterial hypertension in the rat. Our results showed the mean pulmonary arterial pressure in the PGE group significantly lower than the control group. The result also showed the increase of mean pulmonary arterial pressure during observation from two weeks to four weeks of treatment was not significantly different. It is important to note that this finding can be an alternative mode of treatment for pulmonary arterial hypertension. Future clinical study effects of PGE can be arranged in children with pulmonary arterial hypertension.

6. References

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