

The effects of pomegranate juice on monocrotaline-induced hypertensive pulmonary vascular changes and right ventricular hypertrophy in rats

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ABSTRACT

Pulmonary hypertension is associated with structural pulmonary vascular changes. Due to the increase in pulmonary vascular resistance, right ventricular hypertrophy and right heart failure develop. Previous studies have shown that pomegranate juice has anti-inflammatory and anti-proliferative effects. The purpose of this study was to determine whether pomegranate juice intake could prevent or reverse changes in pulmonary circulation in a monocrotaline-induced pulmonary hypertension rat model. Pulmonary hypertension module was established in Westar rats by a single subcutaneous injection of monocrotaline (60mg/kg). Experimental groups were as follows: control, monocrotaline for 3 weeks, monocrotaline +Pomegranate juice for 3 weeks, and monocrotaline for 3 weeks followed by Pomegranate juice for 3 weeks (n=10 per group). The pathohistological changes of pulmonary arteries, right ventricular hypertrophy caused by monocrotaline, and pomegranate juice were analyzed. The thickness of the pulmonary arterioles and right ventricular are reduced significantly by the intake of pomegranate juice. Giving it with monocrotaline at day zero resulted in less thickening of pulmonary arterioles, alveolar septa, and right ventricle wall. We conclude that pomegranate juice intake ameliorates the pulmonary vascular remodeling developed in monocrotaline-induced pulmonary hypertension.

Keywords: Monocrotaline; Pomegranate juice; Right ventricular hypertrophy; Pulmonary blood vessels.

INTRODUCTION

Pulmonary hypertension (PH) is associated with a variety of adult and pediatric diseases, however, it is characterized by common features, such as sustained pulmonary vasoconstriction due to increased vascular tone and progressive structural remodeling of pulmonary arteries (1). Patients with severe PH have combinations of small pulmonary arteries adventitial and medial thickening, occlusive intimal lesions, and obliterating thrombotic and plexiform lesions (2). Pulmonary hypertension follows a progressive and fatal course due to

the development of right ventricular (RV) dysfunction and failure (3). Several models have been developed and extensively studied over the years, yet the most commonly used animal models are rodents exposed to either hypoxia or monocrotaline (4). Monocrotaline (MCT), an organic alkaloid extract of the plant *Crotalaria Spectabilis*, induces mild-moderate increases in pulmonary arterial pressure (30-40 mmHg), most prominently 3-4 weeks after injection (5). Pulmonary vascular remodeling is more severe than that found in chronic hypoxia-exposed rats, however the more complex occlusive concentric laminar and plexiform lesions are lacking (6).

The high mortality in PH patients necessitates an in-depth evaluation of the available treatments. In 2007,

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Macchia and colleagues performed an extensive meta-analysis of clinical trials on PH, which revealed a statistically insignificant 30% reduction in mortality in patients receiving experimental treatments (7). The same group showed in 2010 that the “pooled” effect of all treatments had a significant 39% reduction in “all-cause” mortality, although no class of drugs, per se, produced a statistically significant reduction (8). These disappointing results reveal the inadequacy of the current treatments of PH, which, in turn, mandates more effort in finding new, more effective drugs and a wider range of therapeutic options.

Although there is general agreement on at least three components of PH pathogenesis: sustained pulmonary vasoconstriction, progressive structural remodeling and occlusion of small pulmonary arteries and arterioles, and in-situ thrombosis, the relative contribution, chronology, and triggers of these processes have not yet been elucidated (9). Patients with severe PH have combinations of small pulmonary artery medial wall thickening, and occlusive concentric neointimal and plexiform lesions (10). Such complex pathology probably reflects the multifactorial nature of PH, which means several signaling pathways are aberrant in the process. At present time and despite the development of many drugs, there is no single fully effective therapy (11). Given the history of poor patient compliance for using multiple drugs for the same problem, it is logical to start evaluating multifunctional compounds that can, in theory, achieve the same results as a combination of drugs.

Pomegranate fruit has been used as a medicinal substance by ancient civilizations such as Greeks and Egyptians (12). Pomegranate juice (POM) is rich in polyphenols like punicalagin which is the main phytochemical ingredient of POM, which has many pharmacological properties due to its high antioxidant punicalagin (13). The antioxidant activities of POM are three times more than those found in red wine and green tea (14). The hemodynamic indicators, like PH and right

ventricular hypertrophy, in hypoxia-induced PH in rats were improved by treatment with punicalagin (15). Other polyphenols like tannins and flavonoids are also found in POM, both of which have high antioxidant and anti-inflammatory activities. The oxidative stress was attenuated in mice and humans by intake of POM (16). POM juice supplementation reduces the size of atherosclerotic lesion by 44% and decreases foam cells number in apolipoprotein E- deficient mice. Also, Pomegranate juice has anti-proliferative effects (17) which could be attributed to its free radical scavenging properties. The anti-inflammatory properties of POM are through its inhibitory effect on some enzymes with relevant pharmacological properties like cyclooxygenase 2 which is needed for the synthesis of prostaglandin and leukotrienes (18). The anti-proliferative (17) and vasodilatory effects (19) of POM are also documented. Such a multifactorial nature of pomegranate makes it an attractive therapeutic target for disease states characterized by heterogeneous and complex pathogenic mechanisms like PH.

Based on the aforementioned background, we propose to test the benefits of POM by virtue of the antioxidant, anti-inflammatory, and vasorelaxation activities of its components in improving or preventing some features of PH and RV dysfunction/failure that are induced experimentally in a rat model of pulmonary hypertension and right ventricular hypertrophy induced by MCT. The results of this work will hopefully broaden our understanding of the PH through studying the effects of pomegranate extract on the animal model of PH. Moreover, confirmation of our working hypothesis may provide a novel mechanistic foundation for an effective, economical, and safe oral therapeutic approach for PH.

Materials and Methods:

Experimental procedures

All animal experimental protocols were reviewed and approved by the Jordan University of Science and Technology animal care and use committee (ACUC,

approval no. 2016/0315). Animals were treated in accordance with guidelines from the Care and Use of Laboratory Animals (8th edition, National Academies Press) and all procedures were confirmed to adhere to the mentioned guidelines by the ACUC. The experiments were performed on 40 male Sprague–Dawley rats (200–250 g) obtained from the university animal house. The rats were randomly divided into four groups (no=10 in each group) (Fig 1): the control group received a subcutaneous (SC) injection of normal saline, the Monocrotaline group (MCT) received a single SC injection of 60 mg/Kg to induce PH as published elsewhere (20), the prevention group (MCT + POM) received single SC injection of 60 mg/Kg on day one and pomegranate juice for 3 weeks, and finally the reversibility group (MCT/POM) received a single SC injection of 60 mg/Kg on day one and received pomegranate juice on day 21 to day 42 after injection of MCT. Since monocrotaline is metabolized in the liver and in higher doses more than 60 mg/kg could result in significant liver failure, we chose this dose to induce pulmonary vascular disease with little non-pulmonary damage over 3 weeks.

Lung and Heart Tissue Preparation

Rat lungs and heart were resected via an open-chest procedure and washed in phosphate-buffered saline to remove blood. For each harvested heart, the RV free wall was quickly and carefully separated from the LV and intra-ventricular septum, and both parts were weighed. Fulton's index was calculated by dividing the RV weight by LV weight plus the septum weight (RV/LV+S) for the evaluation of RV hypertrophy. The right hilum was ligated, and the left lung was fixed for histology by tracheal instillation of a mixture of 1% formalin and 0.5% agarose under constant pressure (20 cm H₂O). The inflated lung was immersed in 10% formalin. Paraffin sections (5- μ m thick) were obtained and stained with hematoxylin and eosin or anti- α -smooth muscle actin antibodies for immunohistochemical study.

Histomorphometric Analysis of Pulmonary Vascular Remodeling

Slides were analyzed using a light microscope by one of the authors in a blinded manner (OR). The microscopic images of intra-alveolar arteries with a diameter of 100–200 μ m were analyzed. The luminal and total cross-sectional area of each examined vessel was obtained using a computerized morphometric program. From the previously mentioned values, the wall thickness was extrapolated. Wall thickness ratio (WT%) of pulmonary arterioles was determined from each animal (average of 15 vessels). WT% was calculated as the area occupied by the vessel wall divided by the total cross-sectional area of the arteriole. The tunica media (smooth muscle layer) of the blood vessel was visualized in anti- α -smooth muscle actin-stained slides, as it is an authentic marker for the medial layer.

Statistical Analyses

All values are expressed as mean \pm standard deviation from the mean (SD). One-way analysis of variance (ANOVA) was used to determine the significance of differences between different groups and the significance of interactions between groups was determined by Tukey's post-hoc tests. P-values less than 0.05 were considered significant. Analyses were performed using Graph Pad Prism, version 8.

Results

Pulmonary Arterial Wall Thickness

Pulmonary arterial wall thickness % was significantly increased in the MCT group to 0.44 ± 0.03 % versus 0.22 ± 0.09 % in control group ($P < 0.05$) (Fig.2). With POM administration on the same day of MCT injection, the arterial wall thickness% had decreased to 0.35 ± 0.07 % ($P < 0.05$ vs MCT group). Giving POM 3 weeks after MCT injection did not prevent the increase in the thickness of the pulmonary arteriolar wall induced by MCT, the arterial wall thickness % was 0.42 ± 0.09 % which is not significantly different from the MCT group (Fig.2).

The increase in pulmonary arterial wall thickness (**Fig.3**) observed in the MCT group represents arterial tunica medial hypertrophy in these rats (**Fig.4**), which was prevented largely by the administration of POM at day zero.

Pulmonary alveolar septum thickness:

Pulmonary alveolar septum thickness was increased in the MCT group compared with the control group (**Fig.5**). With POM administration concurrently with MCT or after 3 weeks following administration of MCT, the alveolar septum thickness was decreased compared with the MCT group.

Ventricular hypertrophy assessment

RV Hypertrophy was estimated by changes in RV weight divided by LV+S weight ratio (Fulton index) (**Fig.6**) and examination of the ventricular wall thicknesses using H&E stained slides of the cross-sections of the heart taken 2 mm below the atrioventricular junction (**Fig.7**). The ratio was almost doubled by MCT treatment compared with the control group (0.22 ± 0.026 % versus 0.44 ± 0.09 % in the MCT group) and this was obvious in the cross-section of the heart. Administration of POM on the first day of MCT treatment succeeded in bringing down the ratio significantly (0.35 ± 0.07 % versus 0.44 ± 0.09 % in the MCT group). Administration of POM after three weeks of MCT administration failed to reduce the ratio significantly (0.42 ± 0.09 % in MCT and then POM group versus 0.44 ± 0.09 % in the MCT group). Body weight was significantly decreased in MCT group at 3 weeks compared to before administration of monocrotaline ($190\text{g} \pm 11.5$ vs $231\text{g} \pm 13.3$, $P < 0.05$). Also, it was significantly reduced in MCT/POM group at six weeks compared to before administration of the monocrotaline ($183\text{g} \pm 11.5$ vs $229\text{g} \pm 13.3$, $P < 0.05$).

Discussion:

Pulmonary hypertension is a fatal disease affecting mostly young people. It is associated with structural vascular changes in the pulmonary vessels. Concomitantly

and due to the increase in the pulmonary vascular resistance, right ventricular hypertrophy and right heart failure develop. The burden of this disease is increasing worldwide with the lack of sufficient treatments and pharmacological options to be considered (7,8). Fortunately, animal models of pulmonary hypertension can be induced by different methods and the changes shown in these models are comparable to those changes seen in humans (4, 5, 6). In this study, we investigated whether the structural changes in pulmonary arteries and arterioles and right ventricle hypertrophy in the rat monocrotaline model can be prevented or reversed by POM. Previous studies have shown pulmonary vessel remodeling in MCT-treated rats and ends in the development of PH (21). Severe PH developed in MCT-injected rats within 3 weeks where the right ventricle systolic pressure is almost doubled and the RV/LV+S ratio increases by 70% compared with control animals (22). Thus, we used this rat MCT-PH model to study the changes seen in the lungs and the heart.

In the rat MCT-PH model that we used, we did not notice any plexiform-like lesion or concentric neointimal lesion that is usually seen in patients with severe PH or the SUGEN/hypoxia-induced experimental severe HP (23). Although this model does not exactly mimic the pathophysiology and pulmonary arteriopathy of human PH, many hallmarks of this disease can be seen in this model like the thickening of blood vessels wall and right ventricular hypertrophy. Moreover, the severity of arterioles remodeling in this model is higher than the other models and can serve as a good tool in screening and investigating the pharmacological effects of potential drugs on the PH (6). Rat model of PH induced by monocrotaline is superior to mice model since it showed significant increase in pulmonary pressure, right ventricular hypertrophy and remodeling of pulmonary arteries (24). Nevertheless, Rat MCT model of PH has some limitation like the lack of certain vascular pathological lesion seen in human (23). Compared with

other model of PH used in research like chronic hypoxia and hypoxia combined with Sugren-5416, MCT rat model of PH is reproducible and inexpensive, and does not require meticulous technical skills (25).

In agreement with that, our study focused on the effects of POM intake on the parameters we studied. We have shown that giving POM from day 1 after the MCT injection and for 3 weeks significantly prevented the pulmonary vascular remodeling caused by MCT and improved the pathohistological picture of the lungs. The thickening of the blood vessels walls and the widening of the tunica media of pulmonary arteries were decreased significantly by the intake of the POM juice. As well, the thickening of the alveolar walls was improved. This later improvement in the lungs histopathology was reflected on the right ventricle where the Fulton index, a parameter for the degree of right ventricle hypertrophy, was reduced significantly.

On the other hand, giving POM after the establishment of the PH (3 weeks after the MCT injection) for the same period of 21 days (from day 21 to day 42) was not accompanied by a significant improvement in the lungs' histology or the right ventricular hypertrophy. This is possibly due to the establishment of the structural changes (pulmonary vascular remodeling) that have been already formed and reached an irreversible stage (fixed components of PH), and therefore, POM was not able to affect this remodeling process. However, giving POM to those animals with irreversible remodeling was able to improve their survival rate where all rats in this group survived. In agreement with that, a previous study using the same model of HP showed that all rats without treatment have died by day 30, due to right ventricular failure, after 60 mg/kg MCT injection (26). It seems that POM administration halts or slows the progress of PH caused by the MCT (although may not reach a statistically significant difference) or POM may possess or enhance some other cardiac protective properties that are not necessarily directly related to the PH pathology (27).

Moreover, this protective effects of POM could be due to the improvement in some of the hemodynamic indicators of the pulmonary circulation by punicalagin, the main phytochemicals found in POM, which causes vasodilatation through enhancement of NO-cGMP pathway in the pulmonary vascular tree (15). In addition, many pieces of evidence from human studies showed the advantageous effects of POM on the inflammation, blood circulation, muscular damage, reducing exhaustion, vasoprotective mechanisms, and the circulation levels of active blood components (28).

As previously mentioned, accumulating data shows that pomegranate has many beneficial effects on human health, and the interest in pomegranate and its health properties have been greatly increased. Pomegranate juice is rich in polyphenols like tannins and flavonoids where both have high antioxidant and anti-inflammatory activities (14, 29). Moreover, several researchers have shown that pomegranate has the highest antioxidant activities compared with other juices (14). Also, in vitro studies using cultured vascular endothelial cells found that POM has many effects on nitric oxide (NO) levels which acts as a potent vasodilator. POM causes more expression of endothelial nitric oxide synthase (30), protection the generated NO against oxidative destruction by superoxide anions (31) and reverts down-regulation of the expression of eNOS (32). All these effects of POM may lead to increased bioavailability of NO in lung tissues. One of the main factors implicated in the pathophysiology of PH is the imbalance between vasoconstriction and vasodilatation of the pulmonary vascular tree, and therefore the benefit of POM was possibly restoration of this balance by increasing bioavailability of NO. The improvement in the lung histology by POM could also be attributed to the inhibitory effect of NO on vascular smooth muscle cell proliferation (31). This was documented in animal studies where POM intake decelerated the progression of atherosclerosis in mice (33) and pigs, and it could reduce coronary endothelial dysfunction induced by

hyperlipidemia (29). In humans, it attenuates the platelet aggregation by reduction of thromboxane A2 production and hydrogen peroxide production (34). All these mechanisms may contribute to the direct or indirect effect of POM in reducing vascular and right ventricular remodeling and in prolonging the survival rates of treated animals.

The results of this work will hopefully broaden our understanding of the pathophysiology of pulmonary hypertension (PH) disease through the segregated analysis of the effects of pomegranate extract on a rat model of monocrotaline-induced PH. As well, it may pave the way for more useful utilization of the pomegranate extracts in

the treatment of PH. Confirmation of our working hypothesis may provide a novel mechanistic foundation for an effective, economical, and safe oral therapeutic approach for PH.

Conflict of interest:

The authors declare that they have no competing interests.

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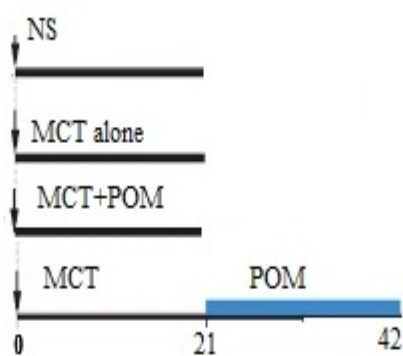


Figure 1: A diagram for the experimental protocol: male rats were injected (SC) with monocrotaline (MCT) or normal saline (PBS) on Day 0. The thick horizontal lines represent the study period for each experimental group. On Day 21, animals were either killed (control, MCT, and MCT+POM), or the MCT/POM group received POM only from Day 21 to Day 42.

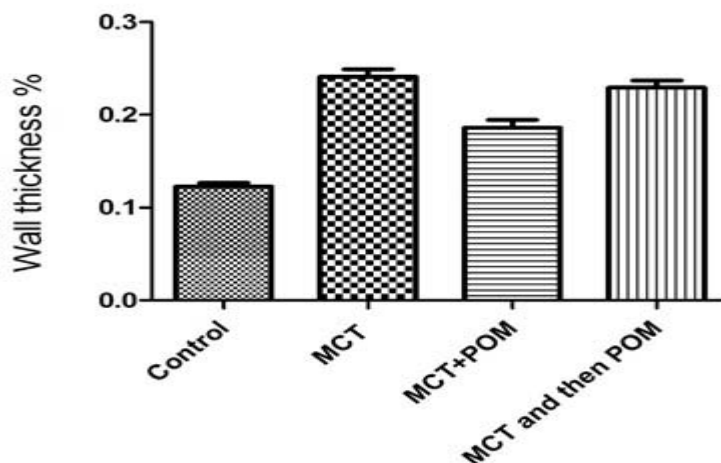


Figure 2: Pulmonary artery thickness% was increased significantly ($P < 0.05$) by all treated groups (MCT, MCT+POM, and MCT then POM). MCT+POM decreased significantly ($P < 0.05$) the thickness compared with MCT groups but MCT then POM treatment did not decrease the thickness compared with the MCT group.

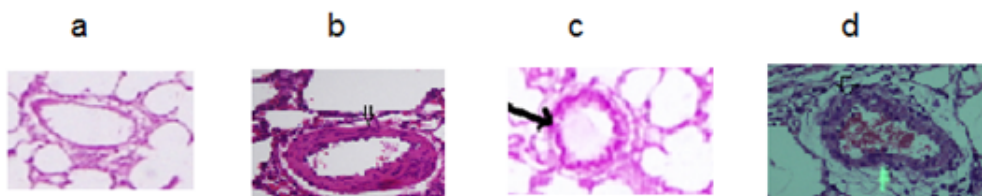


Figure 3: Representative images of H&E staining of the pulmonary arteries. The original magnification of the images was $\times 100$ **a.** control, **b.** 21 Days after challenge in MCT, **c.** 21 Days after challenge in MCT and POM, **d.** 21 Days after challenge in MCT and then another 21 days with POM. Monocrotaline-treated animals (**b**) exhibit pulmonary vascular media hypertrophy and mononuclear cell infiltration compared with control animals (**a**).

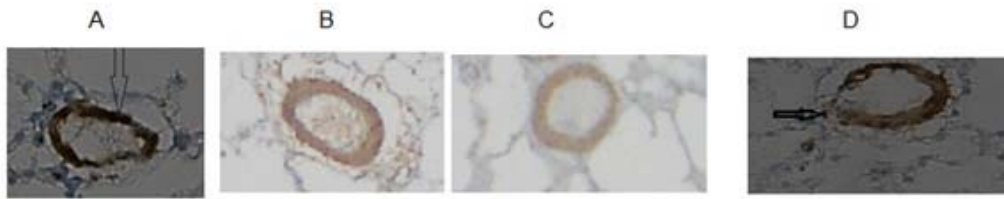


Figure 4: Immunoperoxidase labeling of pulmonary arterioles stained with anti- α smooth muscle actin antibody (brown). **A-D** representative images of paraffin sections of pulmonary arterioles stained with anti- α smooth muscle actin antibody. Control (**A**), MCT (**B**), MCT+POM (**C**) and MCT then POM (**D**).

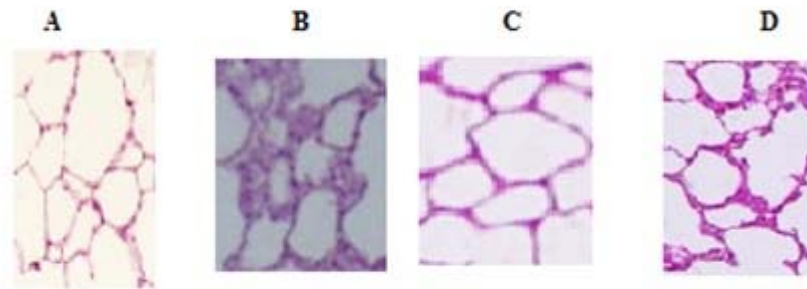


Figure 5: Pulmonary alveolar septum thickness: H&E staining of lung sections in Control (**A**), MCT alone (**B**), MCT+POM (**C**), and MCT then POM (**D**). The alveolar wall is thickened in the MCT group and this thickening was ameliorated partly in the MCT+POM group.

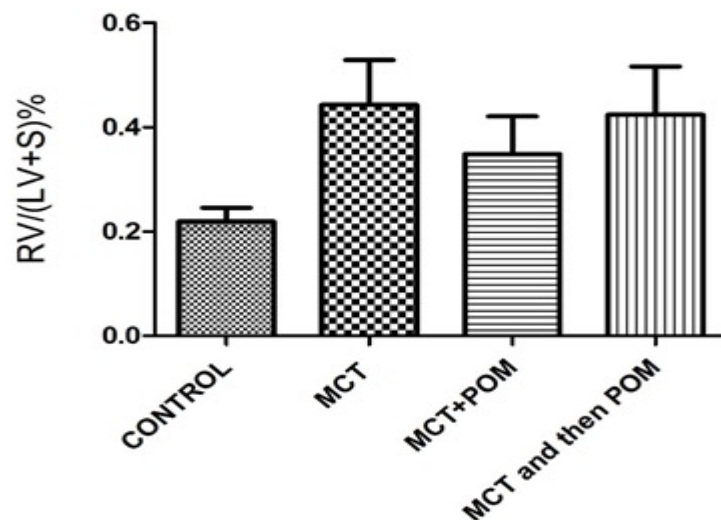


Figure 6: Measurement of the right ventricular hypertrophy: RV hypertrophy, which was measured by Fulton index [RV/ (LV + S)] of control, MCT 60 mg/kg, MCT + POM and MCT for three weeks then POM for another three weeks. All treated groups (MCT, MCT+POM and MCT then POM) significantly ($P < 0.05$) increased RV/ (LV + S) compared control group. RV/ (LV + S) is decreased significantly ($P < 0.05$) by MCT+POM treatment compared with the MCT group while MCT then POM is not significantly different from the MCT group.

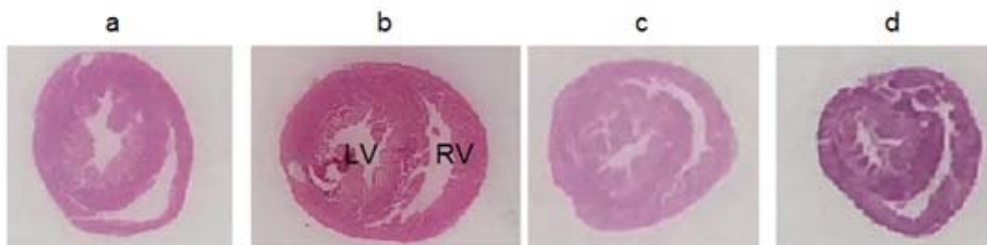


Figure 7: Hematoxylin-eosin staining for heart cross-sections in male rats. Control (a), MCT (b), MCT plus POM (c) MCT and then POM (d).

REFERENCES

- (1) Simonneau G, Robbins IM, Beghetti M, Channick RN, Delcroix M, Denton CP, et al. Updated clinical classification of pulmonary hypertension. *Journal of the American College of Cardiology*. 2009;54(1 Suppl):S43-54.
- (2) Alzoubi A, Almalouf P, Toba M, O'Neill K, Qian X, Francis M, et al. TRPC4 inactivation confers a survival benefit in severe pulmonary arterial hypertension. *The American journal of pathology*. 2013;183(6):1779-88. animal model. *Front. Pharmacol.* 7:397. doi: 10.3389/fphar.2016.00397
- (3) Badesch DB, Raskob GE, Elliott CG, Krichman AM, Farber HW, Frost AE, et al. Pulmonary arterial hypertension: baseline characteristics from the REVEAL Registry. *Chest*. 2010;137(2):376-87.
- (4) Stenmark KR, Meyrick B, Galie N, Mooi WJ, McMurtry IF. Animal models of pulmonary arterial hypertension: the hope for etiological discovery and pharmacological cure. *Am J Physiol Lung Cell Mol Physiol* 2009;297:L1013-L1032.
- (5) Kay JM, Harris P, Heath D. Pulmonary hypertension produced in rats by ingestion of *Crotalaria spectabilis* seeds. *Thorax*. 1967;22(2):176-9.
- (6) Meyrick B, Gamble W, Reid L. Development of *Crotalaria* pulmonary hypertension: hemodynamic and structural study. *The American journal of physiology*. 1980;239(5):H692-702.
- (7) Macchia A, Marchioli R, Marfisi R, Scarano M, Levantesi G, Tavazzi L, et al. A meta-analysis of trials of pulmonary hypertension: a clinical condition looking for drugs and research methodology. *American heart journal*. 2007;153(6):1037-47.
- (8) Macchia A, Marchioli R, Tognoni G, Scarano M, Marfisi R, Tavazzi L, et al. Systematic review of trials using vasodilators in pulmonary arterial hypertension: why a new approach is needed. *American heart journal*. 2010;159(2):245-57.
- (9) Chan SY, Loscalzo J. Pathogenic mechanisms of pulmonary arterial hypertension. *Journal of molecular and cellular cardiology*. 2008;44(1):14-30.
- (10) Tuder RM. Pathology of pulmonary arterial hypertension. *Seminars in respiratory and critical care medicine*. 2009;30(4):376-85.
- (11) Spaczyńska M, Rocha SF, Oliver E. Pharmacology of Pulmonary Arterial Hypertension: An Overview of Current and Emerging Therapies. *ACS Pharmacol Transl Sci*. 2020;3:598-612. doi: 10.1021/acscptsci.0c00048. PMID: 32832865; PMCID: PMC7432664.
- (12) El-Soud NHA. Herbal medicine in ancient egypt-review article. *J. Med. Plant Res*. 2010;4:82-86.
- (13) Xu X., Li H., Hou X., Li D., He S., Wan C., et al. Punicalagin induces Nrf2/HO-1 expression *via* upregulation of PI3K/AKT pathway and inhibits LPS-induced oxidative stress in RAW264. 7 macrophages. *Mediators of Inflammation*. 2015;Article ID 380218 705-715. <https://doi.org/10.1155/2015/380218>.
- (14) Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of agricultural and food chemistry*. 2000; 48(10):4581-9.
- (15) Shao J., Wang P., Liu A., Du X., Bai J., Chen M. Punicalagin prevents hypoxic pulmonary hypertension *via* anti-oxidant effects in rats. *Am. J. Chin. Med.* 2016;44 (04):785-801. 10.1142/S0192415X16500439
- (16) Aviram, M., Dornfeld, L., Rosenblat, M., Volkova, N., Kaplan, M., Coleman, R., et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. *Am. J. Clin. Nutr.* 2000;71:1062-1076. doi: 10.1093/ajcn/71.5.1062
- (16) Budi, HS, Juliastuti, WS, Sulistyowati, YP. The bleeding and clotting time analysis of the stem extract of *Musa paradisiaca* var. *sapientum* (L.) Kunze on

- hemostatic response. *Jordan Journal of Pharmaceutical Sciences*. 2021; 14: 1-8
- (17) Balasundram N SK, Sammar S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chem*. 2006;68:191-203.
- (18) Schubert SY, Lansky EP and Neeman I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J. Ethnopharmacol*. 1999;66:11-17.
- (19) de Nigris F, Balestrieri ML, Williams-Ignarro S, D'Armiento FP, Fiorito C, Ignarro LJ, et al. The influence of pomegranate fruit extract in comparison to regular pomegranate juice and seed oil on nitric oxide and arterial function in obese Zucker rats. *Nitric oxide: biology and chemistry / official journal of the Nitric Oxide Society*. 2007a;17(1):50-4.
- (20) Alencar AK, Pereira SL, da Silva FE, Mendes LV, do Cunha VM, Lima LM, et al. N-acylhydrazone derivative ameliorates monocrotaline-induced pulmonary hypertension through the modulation of adenosine AA2R activity. *Int J Cardiol*. 2014;173:154–162. [PubMed: 24630383]
- (21) Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF et al. The monocrotaline model of pulmonary hypertension in perspective. *Am J Physiol Lung Cell Mol Physiol*. 2012; 302: L363-L369. doi: 10.1152/ajplung.00212.2011.
- (22) Kuang T, Wang J, Pang B, Huang X, Burg ED, Yuan JX et al. Combination of sildenafil and simvastatin ameliorates monocrotaline-induced pulmonary hypertension in rats. *Pulm Pharmacol Ther*. 2010; 23(5):456-64. doi: 10.1016/j.pupt.2010.02.003.
- (23) Abe K, Toba M, Alzoubi A, Ito M, Fagan KA, Cool CD, et al. Formation of plexiform lesions in experimental severe pulmonary arterial hypertension. *Circulation*. 2010;121(25):2747-54. <https://doi.org/10.1161/CIRCULATIONAHA.109.927681>
- (24) Ryan J.J., Marsboom G., Archer S.L. Rodent Models of Group 1 Pulmonary Hypertension. In: Humbert M., Evgenov O., Stasch JP. (eds) *Pharmacotherapy of Pulmonary Hypertension. Handbook of Experimental Pharmacology*. 2013 vol 218. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-38664-0_5
- (25) Gomez-Arroyo JG, Farkas L, Alhussaini AA, Farkas D, Kraskauskas D, Voelkel NF, Bogaard HJ. The monocrotaline model of pulmonary hypertension in perspective. *Am J Physiol Lung Cell Mol Physiol* 2012;302:L363–L369.
- (26) Umar, S., Iorga, A., Matori, H., Nadadur, R. D., Li, J., Maltese, F., et al. 2011. Estrogen rescues preexisting severe pulmonary hypertension in rats. *Am. J. Respir. Crit. Care Med*. 2011;184(6),715–723. doi: 10.1164/rccm.201101-0078OC)
- (27) Al Hariri, M., Zibara, K., Farhat, W., Hashem, Y., Soudani, N., Al Ibrahim, F., et al. Cigarette smoking-induced cardiac hypertrophy, vascular inflammation and injury are attenuated by antioxidant supplementation in an animal model. *Front. Pharmacol*. 2016;7:397. doi: 10.3389/fphar.2016.00397
- (28) Wang D, Özen C, Abu-Reidah IM., Chigurupati S, Patra JK, Horbanczuk JO, et al. Vasculoprotective Effects of Pomegranate (*Punica granatum L.*) *Frontiers in Pharmacology* 2018; 9 article 544 doi.org/10.3389/fphar.2018.00544
- (29) Vilahur, G., Padró, T., Casaní, L., Mendieta, G., López, J. A., Streitenberger, S., et al. 2015. Polyphenol-enriched diet prevents coronary endothelial dysfunction by activating the Akt/eNOS pathway. *Rev. Esp. Cardiol. (Engl. Ed)*. 2015;68,216–225. doi: 10.1016/j.recesp.2014.03.023
- (30) Bajes, HR and Al-Dujaili, EAS. Polyphenolic-rich fruits and supplements enhance exercise performance; general review. *Jordan Journal of Pharmaceutical Sciences*. 2017; 10: 135-151.
- (30) de Nigris, F., Williams-Ignarro, S., Lerman, L. O., Crimi, E., Botti, C.,

- 31) Mansueto, G., et al. Beneficial effects of pomegranate juice on oxidation-sensitive genes and endothelial nitric oxide synthase activity at sites of perturbed shear stress. *Proc. Natl. Acad. Sci. U.S.A.* 2005;102:4896–4901. doi: 10.1073/pnas.0500998102
- 32) Ignarro, L. J., Byrns, R. E., Sumi, D., De Nigris, F., and Napoli, C. Pomegranate juice protects nitric oxide against oxidative destruction and enhances the biological actions of nitric oxide. *Nitric Oxide* 2006;15:93–102. doi: 10.1016/j.niox.2006.03.001
- (33) de Nigris, F., Williams-Ignarro, S., Botti, C., Sica, V., Ignarro, L. J., and Napoli, C. Pomegranate juice reduces oxidized low-density lipoprotein downregulation of endothelial nitric oxide synthase in human coronary endothelial cells. *Nitric Oxide* 2006; 15, 259–263. doi: 10.1016/j.niox.2005.12.004
- (34) de Nigris, F., Williams-Ignarro, S., Sica, V., Lerman, L. O., D'armiento, F.P., Byrns, R. E., et al. Effects of a pomegranate fruit extract rich in punicalagin on oxidation-sensitive genes and eNOS activity at sites of perturbed shear stress and atherogenesis. *Cardiovasc. Res.* 2007b; 73, 414–423. doi: 10.1016/j.cardiores.2006.08.021
- (35) Mattiello, T., Trifirò, E., Jotti, G. S., and Pulcinelli, F. M. Effects of pomegranate juice and extract polyphenols on platelet function. *J. Med. Food* 2009; 12, 334–339. doi: 10.1089/jmf.2007.0640

تأثير عصير الرمان على التغيرات في الاوعية الدموية الرئوية و تضخم البطين الايمن المصاحبة لارتفاع ضغط الدم الرئوي الناتج من المونوكروتالين -دراسة مورفولوجية

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ملخص

يرتبط ارتفاع ضغط الدم الرئوي بتغيرات هيكلية في الأوعية الدموية الرئوية. وبسبب زيادة مقاومة الأوعية الدموية الرئوية ، ينتج تضخم البطين الأيمن وقشر القلب الأيمن. أظهرت الدراسات السابقة أن عصير الرمان له تأثيرات مضادة للالتهابات وزيادة انقسامات الخلية. كان الغرض من هذه الدراسة هو تحديد ما إذا كان تناول عصير الرمان يمكن أن يمنع أو يعكس التغيرات في الدورة الدموية الرئوية في نموذج ارتفاع ضغط الدم الرئوي في الجرذان الناجم عن المونوكروتالين. تم عمل ارتفاع ضغط الدم الرئوي في فئران ويسترن عن طريق حقنة واحدة تحت الجلد من مونوكروتالين (60 مجم / كجم). كانت المجموعات التجريبية على النحو التالي: المجموعة الضابطة ، المونوكروتالين لمدة 3 أسابيع ، المونوكروتالين + عصير الرمان لمدة 3 أسابيع ، والمونوكروتالين لمدة 3 أسابيع يليها عصير الرمان لمدة 3 أسابيع (العدد = 10 لكل مجموعة). تم تحليل التغيرات النسيجية المرضية للشرابين الرئوية وتضخم البطين الأيمن الناجم عن المونوكروتالين وتأثير عصير الرمان على هذه التغيرات. لقد وجد أن تناول عصير الرمان يؤدي الى تقليل سمك الشرايين الرئوية والبطين الأيمن بشكل كبير. كذلك أدى إعطاء عصير الرمان مع المونوكروتالين في اليوم صفر إلى تقليل سماكة الشرايين الرئوية والحاجز السنخي وجدار البطين الأيمن. نستنتج أن تناول عصير الرمان يحسن إعادة تشكيل الأوعية الدموية الرئوية التي تم تغييرها بسبب ارتفاع ضغط الدم الرئوي الناجم عن المونوكروتالين.

الكلمات الدالة: مونوكروتالين ,عصير الرمان, تضخم البطين الأيمن, الاوعية الدموية الرئوية.

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