EFFECTS OF Punica granatum JUICE ON CONTRACTILITY OF ISOLATED AORTA IN FEMALE ALBINO RATS

Lina N. Adam
Biology Department, Faculty of Science, University of Zakho, Iraq.
Omer Habib
College of Science, International University of Erbil.

Abstract

The present study focused on the mechanisms of smooth muscle relaxation induced by Punica granatum juice (PJ) in the rat thoracic aorta. The thoracic aorta was removed from female adult albino rat and placed in an organ bath containing Kreb's solution and the contractions were recorded isometrically. The results demonstrated that the P. granatum juice (1.5 to 5 mg/ml) significantly reduces the contractions induced by phenylephrine (PE) (0.01 mM), with IC50 ± SEM of 2.859 ± 0.101 mg/ml, and percentage of relaxation for PE-induced contraction was 81.8 ± 0.358. Also, the vasodilatory activity of PJ was not modified by endothelial denuded aorta with IC50 ± SEM 3.389 ± 0.084 mg/ml and the percentage of relaxation was 79.77 ±0.824 %. Pomegranate juice induced relaxation in the PE-induced contraction in intact aorta was unaffected by nitric oxide synthase inhibitor (L-NAME) (3*10-4), gaunyl cyclase inhibitor (methylene blue) (1*10-5) and PGI2 inhibitor (Indomethacin) (3*10-5) with IC50 ± SEM 3.424 ± 0.065, 3.286 ± 0.168 and 3.490 ± 0.054, respectively. The percentage of relaxation was 75.23 ± 0.661, 119.61 ± 4.128 and 83.87 ± 0.715%, respectively. In addition, Pre-incubation of aortic rings with TEA (1mM), GLIB (10-5), BaCl2 (1mM) and 4-AP (1mM) with IC50 ± SEM 3.099 ± 0.049mg/ml, 2.759 ± 0.087mg/ml, 2.889 ± 0.054mg/ml and 2.436±0.049 mg/ml, and the percentages of relaxation were 101.38 ± 0.151%, 88.55±0.58%, 90.25±0.168%, and 104.72±0.939%, respectively. Finally, PJ significantly increased dose-response relaxation after incubation of thoracic aortic rings with Nifedipine (10-6 M) with IC50 ± SEM, 3.495 ± 0.1012 and the percentage of relaxation was 100.276 ± 3.378%.

Keywords: Punica granatum juice, K+-channels blockers, Ca++-channels blocker, L-NAME, COX., Endothelium and hyperpolarizing factors.

INTRODUCTION

The pomegranate belongs to the family Punicaceae and it is a native plant distributed from the area of Iran to the Himalayas in northern India and has been cultivated and naturalized over the whole Mediterranean area since antiquated times (Viuda-Martos et al., 2010). In the archaic Ayurveda system of medicine, the pomegranate has been extensively utilized as a source of traditional remedies for thousands of years. The seeds and juice are considered tonic for the heart, throat, and eyes and used for different purposes, such as stopping nose and gum bleeding, taning skin, firming-up sagging breasts and treating haemorrhoids (Herlekar, 2014). Awareness of various preventative measures against diseases such as cancers, obesity, type II diabetes and osteoporosis has influenced the society to adopt a healthier lifestyle. Through the course of history, health practitioners have evolved in their way of treating diseases from a natural herbal approach by using medicinal synthetic drugs, and now modern science has returned to study natural products again (Watson and Preedy, 2009). Pomegranate, as a functional food has increased consumer interest due to the presence of bioactive compounds in the different parts of the plant (Viuda-Martos et al., 2011). The phenolic compounds that are distributed in different parts of the pomegranate plant contribute to the total antioxidant activity and may play a role in cancer prevention and therapy (Kim et al., 2002). Some bioactive components reported in pomegranate arils in variable proportions are anthocyanins, ascorbic acid and β-carotene (Tzulker et al., 2007). Anthocyanins are responsible for the attractive colour of pomegranate arils and some of the fruit's antioxidant activities (Borochov-Ne-

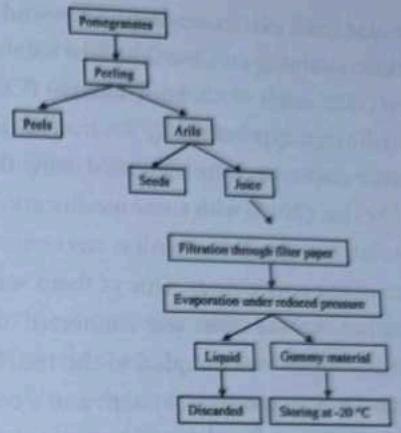

Figure 1. The general scheme for preparations of crude pomegranate juice.

Animals Breeding and Housing

Adult female albino rats (Rattus norvegicus) weighing 200-250 grams bred in the animal house of Department of Biology, Faculty of Science, University of Zakho, were used during the current study. Animals were housed under controlled environmental conditions at 20-24°C, relative humidity between 30–70%, and a photoperiod of 12 hours' light-dark cycle. The rats had free access to rodent food pellets and supplied with dechlorinated tap water ad libitum. The animals were reared in rat cages (460 x 30 x 20 cm) bedded with shredded recycled wood ships at a density of 6 individuals/cages. The rats were reared under hygienic conditions with a daily cleanliness of the housing environment.

Aorta Preparation

The animals were injected intraperitoneal (IP) with heparin (1500 units/ kg body weight) and left for about 30 min to prevent blood coagulation and the possibility of damaging of the aorta endothelial layer (Fulton et al., 1996). Animals were then anaesthetized with intraperitoneal injection of Ketamine (40 mg /kg) and Xylazine (10 mg/Kg). The descending thoracic aorta was rapidly

ori et al., 2009). Since no data are available on the effect of P. granatum juice on contractility of the isolated aorta; the present study aimed to investigate the role of potassium (K+) calcium (Ca++) channel subtypes, cyclooxygenase (COX) and endothelium hyperpolarizing factors in the vascular relaxation induced by PJ.

Materials and Methods

Plant Materials

The fresh sour pomegranate fruits (Punica granatum), collected from Armishte Agricultural field/Zakho during November 2014 were used in the current study. Pomegranates were washed and manually peeled; the arils were carefully separated and washed with excess water for the removal of sugars and adhering materials. The crude juice was obtained by using a commercial blender (BOMNANN, Germany). The juice was filtered through Whatman's No.1 filter paper, concentrated using a thin film rotary evaporator (BÜCHI) and the obtained gummy material was kept in dark bottles stored at -20°C until use.

Juice preparation

The Pomegranate Juice was performed in the Physiology Research Lab / Department of Biology, University of Zakho, Faculty of Science, Kurdistan Region – Iraq. Pomegranate juice was prepared by using a commercial professional automatic juicer (BOMANN, Germany) that separates the juice from seeds. The obtained juice was filtrated through a muslin cloth and stored at 4°C overnight for the settling of suspended particles. After decantation, the supernatant was filtered through Whatman No. 1 filters paper. The filtrate was completely evaporated under reduced pressure at 40°C using thin film rotary evaporator (BÜCHI). The evaporated fluid was discarded and the gummy material was stored in glass vials at -20 °C (figure 1). The stock solution was prepared by dissolving the desired quantity of the material in 0.9% NaCl and DMSO (Shekha, 2010).

intimal surface of the aorta with toothpick stick. The presence of functional endothelium was assessed in all preparations by determining the ability of acetylcholine (ACh, 10–5 M) to induce more than 50% relaxation in aortic rings precontracted with PE (10–5 M). Vessels were considered to be denuded of functional endothelium when there was no relaxation response to ACh (Nakamura et al., 2002).

Experimental Protocols

In this study, cumulative dose-response relationships for the effects of PJ (1.5 to 5 mg/ml) were established for aortic rings. For all experiment, the relaxant effects of PJ were studied in aortic rings precontracted with PE (1X10-5 M). In the experiment for evaluating the role of endothelial cells, the denuded aortic rings were prepared as previously described and preincubated with L-NAME (3X10-4 M), a nitric oxide synthesis inhibitor, methylene blue (1X10-5 M) and indomethacin (3X10-5 M) for 30 min before PE pre-contraction to evaluate the mechanism of the vasorelaxant response. For examining the role of K+ channels, the aortic rings were pre-incubated for 20 minutes with the following K+ channel inhibitors, (TEA, 1 mM), (GLIB, 1X10-5 M), (BaCl2, 1mM) and (4-AP, 1 mM). The blockers of KCa channel, KATP channel, KIR channel and KV channels blockers, respectively. Finally, to clarify the functional role of Ca++ channel in the relaxation of aortic rings with intact endothelium, the rings were incubated with Nifedipine (3X10-5), an L-type Ca2+ channel blocker for 20 min prior to contraction with PE.

Statistical Analysis

The statistical analysis was performed using two-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out a pairwise comparison between the same dose of different groups using Graph pad prism program. Analysis of variance for repeated measurements was applied to data consisting of repeated observations at successive time points. P-values less than 0.05 ($P<0.05$) were considered as statistically significant. In all figures, the symbols (*, ** and ***) represent mean differences are significant at the 0.05, 0.01 and 0.001 levels respectively.

removed and cleaned from extraneous connective and fatty tissues after transferring to a beaker containing an ice-cold Krebs solution aerated with carbogen [95 % oxygen (O2) and 5 % Carbone dioxide (CO2)]. Then, the isolated aorta cuts into small rings approximately 2-4 mm long.

Isometric contractile responses were identified using the procedure described by Al-Habib and Shekha, (2010) with some modifications to study the vascular reactivity in the isolated aorta. Two stainless steel wires were carefully passed through the lumen of the aortic rings. One of them was anchored to the base of glass organ chamber and the other was connected to force transducer (AD instrument Australia) which was coupled to the trans bridge amplifier connected to Power Lab Data Acquisition System and a computer running chart software (Version 7) used for isometric tension measurement. Maximum care was taken during the assembly of aorta rings to avoid the damaging of the endothelium. The extents of contraction and relaxation were measured by the tension developed by the transducer and recorded by the system.

Prior to the experiment, the organ bath system set at 37°C was operated for at least two hours to allow the water in an organ to reach the thermal stability. Later, 10 ml of Krebs solution was placed inside the tissue glass chamber and continuously aerated with carbogen passed through the inlet at the base of the bath. Aortic rings were connected to the base of the chamber from one end and mounted to the transducer from the other end. Tissues were maintained under an initial tension of 2 g and allowed to equilibrate for 1 h during which bath solution was replaced every 15 min. The aortic segments were initially exposed to 10μM (1X10-5 M) phenylephrine (PE) to test their functional integrity and 10μM (1X10-5 M) acetylcholine (Ach) to test endothelium integrity. This was followed by changing the bath medium several times until a stable resting tone was recorded, and then the experiments were started (Shekha, 2010).

After each experiment, bath solution was replaced every 15 min several times (3 to 4) with Krebs solution to allow the aortic rings to restore their initial tension. Then the relaxant effects of eight pomegranate concentrations of (1.5 to 5 mg/ml) on the contractility of PE-precontracted aortic rings were studied. In denuded experiments, endothelium was removed by gently rubbing the

Results and Discussion

Effect of Pomegranate Juice

A typical trace from a representative experiment on relaxing effects of PJ on rat's PE-precontracted aorta is shown in Figure (2-A). Dose-response curve of the effect of PJ against PE-induced contractions are shown in figure (2-B). Phenylephrine, a selective α1-adrenergic receptor agonist class phenethylamine (Horak et al., 2009) is primarily used in the current study. It stimulates Ca+2 release from intracellular stores through a mechanism of ryanodine-sensitive which is not associated with increased production of inositol 1, 4, 5-trisphosphate (IP3), (Jorgensen et al., 1996).

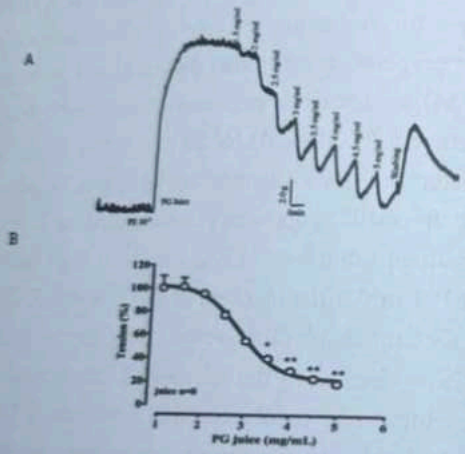

Figure 2. (A) A typical chart view trace showing the relaxant effects of different concentrations of Pomegranate juice on rat aortic rings, precontracted with 10–5 M PE. (B) A cumulative dose-response curve for the effects of P. granatum juice on PE (10–5 M) induced contraction in rat's aorta.

Pomegranate juice at concentrations from 1.5-5 mg/ml caused significant relaxant effects in PE (10-5) precontracted rat's aortic ring. The IC50 ± SEM, (IC50 of CI 95%) and percentage of relaxation are shown in the table (4.1). The Juice produced an inhibitory effect on PE-induced contractions, with IC50 ± SEM of 2.859 ± 0.101 mg/ml (with IC50 of CI 95% between 2.657 to 3.061).

The percentage of relaxation for PE-induced contraction was 81.8 ± 0.358. Pomegranate contains a lot of antioxidants that are, in fact, mediated by NO and, thereby, intensify NO actions (De Nigris et al., 2007). Pomegranate juice is acquired from arils which are considered rich sources of a bioactive compound like phenolic and flavonoids (Anahita et al., 2015). It contains antioxidants such as soluble polyphenols, tannins, and anthocyanins that may reduce the carotid artery intima-media thickness in humans (Sumner et al., 2005). In the current study, it was observed that pomegranate juice significantly inhibits the transient contractions induced by PE.

Role of Endothelium in Pomegranate Juice Induced relaxation in Rat Aorta

Lab chart traces from representative experiments and dose-response curves on the relaxant effect of juice on endothelium-intact and denuded aortic rings are displayed in Figure (3). From the results, it is obvious that cumulative addition of juice at the plateau phase of the contraction induced by PE (10-5 M) in rat's thoracic aortic rings caused a concentration-dependent inhibition in PE-induced contraction in both endothelium-intact and denuded preparations.

Endothelium denudation also induced significant relaxation ($P > 0.05$) at doses (1.5 - 5 mg/ml) for juice with IC50 ± SEM 2.935 ± 0.112 mg/ml (with IC50 of CI 95% 2.710 to 3.161). The percentage of relaxation for endothelium intact and denuded rings were 82.15 ±0.183 % and 79.77 ±0.824 %, respectively.

It was well-known that NO is a major EDRF that induces relaxation in vascular smooth muscle cells (Vanhoutte, 2009). Endothelial nitric oxide synthase (eNOS) is the major enzyme responsible for NO production in the blood vessels. Nitric oxide synthesis increases when the level and activity of eNOS in the endothelial cells is increased (Ugusman et al., 2014). The vascular endothelium plays an important role in controlling vascular tone via the release of relaxant and contractile factors. The relaxant effects of several groups of flavonoids have been demonstrated to be endothelium-dependent or independent (Ajay et al., 2003).

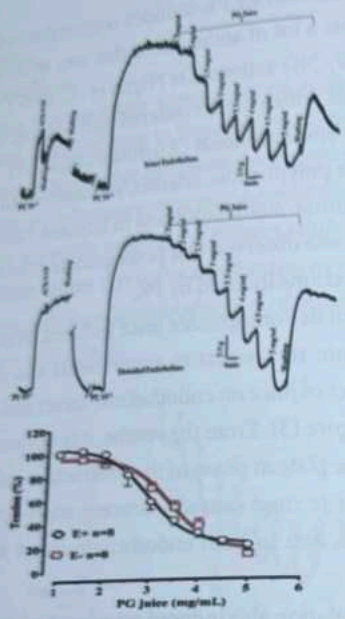

Figure 3. A typical chart view traces showing the relaxant effects of different concentrations of P. granatum juice on, endothelium intact (A) and denuded (B) rat's aortic rings, precontracted with PE (10–5M). (C) Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and endothelium-denuded rat's aortic rings, precontracted with 10–5 M PE.

These novel results indicate that the endothelium played no role in the vasorelaxation induced by juice. This was clearly indicated when treating the denuded aortic rings with juice, which showed a non-significant difference in the rate of relaxation when compared with the control intact endothelium. This implies that pomegranate causes vascular relaxation by two mechanisms: a direct effect on the vascular smooth muscle that is independent of the endothelium and a mechanism that is dependent on the presence of a functional endothelium at high concentration.

The Role of Endogenous NO, cGMP and PGI2 on Relaxation Action Induced by the Effect of juice in Rat's Aorta.

Figures (4, 5 and 6) show the effect of L-NAME, methylene blue and Indomethacin on the juice-induced relaxation of aortic rings contracted with PE and the dose-response curves of the effect of juice against PE-induced contractions. From the result, it can be concluded that the suitable concentrations of L-NAME (3X10-4), NOS inhibitor, Indomethacin (3X10-5), PGI2 inhibitor, and methylene blue (1*10-5), which is a soluble guanylate cyclase inhibitor did not produce any inhibitory effects on the vasodilator responses induced by increasing the concentration of juice in PE-precontracted rat's aortic rings. The results clearly indicate that the above inhibitors produced no blocking effect on the vasodilator response induced by P. granatum juice.

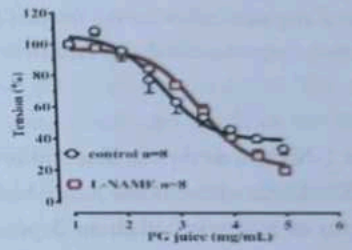

Figure 4. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with L-NAME (3X10-4 M), precontracted with 10–5 M PE.

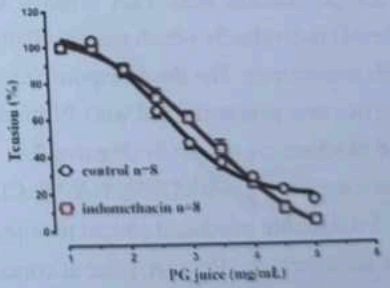

Figure 5. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with Indomethacin (3X10-5M), precontracted with 10–5 M PE.

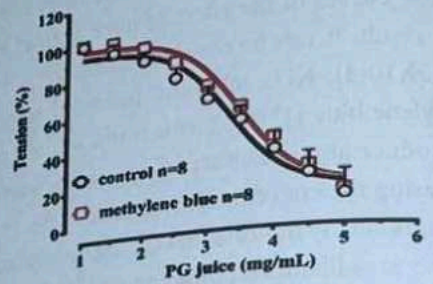

Figure 6. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with methylene blue (3mM), precontracted with 10–5 M PE.

The results showed that L-NAME, methylene blue and Indomethacin did not modify the relaxant effect of juice with endothelium, which demonstrates that NO, cGMP and PGI2 play no role in endothelium-dependent relaxations induced by juice.

The Role of Potassium Channels in Vasorelaxant Action Produced by P. granatum Juice.

To investigate the role of K+ channels in juice induced relaxation, aortic rings were preincubated for 20 minutes with TEA (1mM), GLIB (10-5), BaCl2 (1mM) and 4-AP (1mM) individually which preferentially block KCa, KATP, KIR and KV channels respectively. The dose-response curves for the relaxing effects of juice on aortic rings precontracted with PE that preincubated with the above K+ channel blockers are shown in (Figures 7, 8, 9 and 10).

Pretreatment of thoracic aortic rings with GLIB, 4-AP, BaCl2 and TEA at a concentration between 1.5-3.0 mg/ml produced almost no effect on the vasorelaxant effect produced by juice extract. However, juice at concentrations between 3.0 - 5.0 mg/ml, GLIB and 4-AP mildly and non-significantly enhanced the vasorelaxation effects produced by P. granatum juice with IC50 ± SEM 2.759 ± 0.087mg/ml (IC50 of CI 95% 2.585 to 2.934) and 2.436±0.049 mg/ml (IC50 of CI 95% 2.338 to 2.534). The percentages of relaxation were 88.55±0.58% and 104.72±0.939% respectively. While BaCl2 and TEA produced a significant (P<0.05) and (P<0.01) relaxant effect at juice concentrations 4.0-5.0 mg/ml with IC50 ± SEM 3.099 ± 0.049mg/ml (IC50 of CI 95% 2.838 to 3.219) and 2.889 ± 0.054mg/ml (IC50 of CI 95% 2.781 to 2.997) and the percentages of relaxation were increased to 101.38±0.151% and 90.25±0.168% respectively, (Figures 7, 8, 9 and 10).

A possible mechanism that mediates relaxation of vascular smooth muscle is the opening of K+ channels. The opening of membrane K+ channels, specially Kir in vascular smooth muscle increases K+ efflux which leads to membrane hyperpolarization, the closing of voltage-dependent Ca+2 channels, and subsequent relaxation (Yılmaz & Usta, 2013). However, the results of the current study showed that at least the studied K+ channels play no role in membrane hyperpolarization and ultimately vasorelaxation. This may indicate the presence of another K+ channel subtypes which are not blocked by the conventional of K+ channel blockers used.

These findings clearly suggest that K+ channels (KATP and Kv,) may play no role in the vasorelaxant effect of juice in rat aortic rings. On the other hand, KCa and Kir channel may be attributed to the inhibitory effects on hyperpolarizing factors released from endothelium. Also, indicate that these novel results can't be compared since no data are available on this subject.

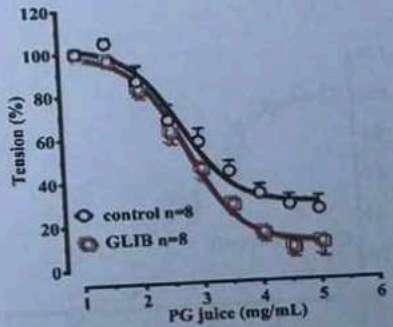

Figure 7. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with GLIB (10-5 M, precontracted with 10–5 M PE.

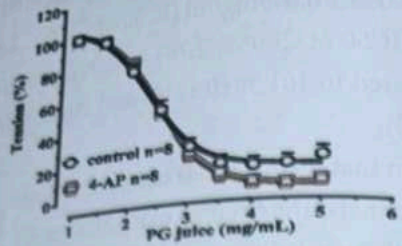

Figure 8. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with 4-AP (1mM), precontracted with 10–5 M PE.

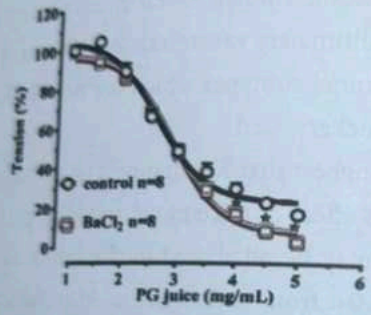

Figure 9. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with BaCl2 (1mM), precontracted with 10–5 M PE.

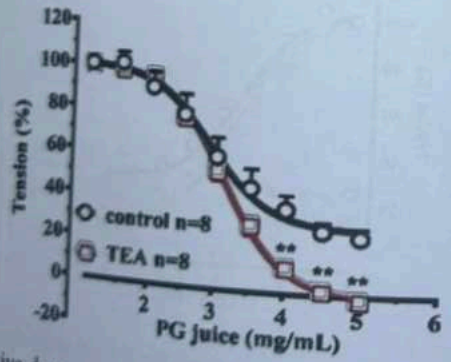

Figure 10. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with TEA (1mM), precontracted with 10–5 M PE.

The role of L-type Ca^{2+} in the mechanism of the relaxant effect induced by pomegranate juice

Dose-response curves for on the relaxant effect of juice on thoracic rat's aortic rings preincubated with Nifedipine (a typical L-type calcium channel blocker) against PE-induced contractions are shown in figure (11).

The relaxant effect juice on aortic rings preincubated with the Nifedipine 10-5 and then precontracted with PE 10-5 was enhanced at highly significant levels ($P<0.001$). This indicates that these results explain the role of PJ in the blocking of Ca+2 channel which indirectly participates and supports the vasorelaxation induced by P. granatum. The IC50 ± SEM of control rings for juice was 4.180 ± 0.278 mg/ml (IC50 of CI 95% between 4.180 ± 0.278mg/ml) whereas that of the treated rings was 3.495 ± 0.1012 mg/ml (IC50 of CI 95% between 3.293 to 3.697), and the percentages of relaxation was 100.276 ± 3.378%.

Calcium, which is an essential ion for smooth muscle contraction, can be derived from the intracellular stores and/or extracellular fluid. Extracellular Ca+2 enters the cell via the voltage-gated dihydropyridine channels at the myocyte plasma membrane. Following the opening of this channel, Ca+2 enters down its concentration gradient. This will then trigger the release of more Ca+2 from the intracellular stores (Salleh and Ahmad, 2013).

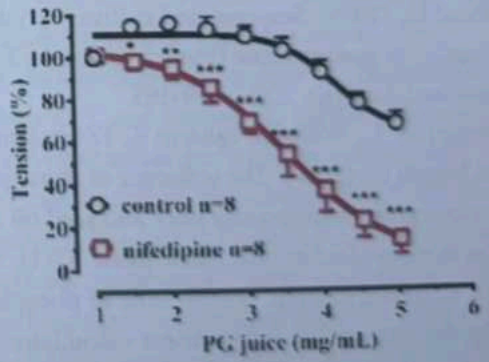

Figure 11. Cumulative dose-response curves for the relaxant effects of P. granatum juice on control and aortic rings preincubated with Nifedipine (3X10-5 M), precontracted with 10–5 M PE.

Conclusion

The results of present research work indicate that Punica granatum juice has relaxant effects on rat aortic rings and induced endothelium-dependent and endothelium-independent relaxation in aortic smooth muscle. Moreover, the endothelium-dependent relaxant effects of the juice are not mediated through NO, PGI2, cGMP. Furthermore, activation of KATP and KV channels didn't alter the relaxant effect of juice, but there was an enhancement in the relaxation mediated by KCa and KIR channel blocker. Lastly, the L-type Ca+2 channel blocker enhanced the relaxation caused by juice.

References

Ajay, M., Gilani A. H. and Mustafa M. R., (2003). Effects of flavonoids on the vascular smooth muscle of the isolated rat thoracic aorta. Life Sciences. 74(5): 603-612.

AL-Habib, O.A.M. and Shekha M.S., (2010). Vasorelaxant Effect of Aqueous Extract of Crataegus azarolus aronia and Quercetin on Isolated Albino Rat's Thoracic Aorta. J. Duhok Univ. 13 (1): 7-13.

Anahita, A., Asmah R. and Fauziah O., (2015). Evaluation of total phenolic content, total antioxidant activity, and antioxidant vitamin composition of pomegranate seed and juice. General Medicine: Open Access, 2015.

Borochov-Neori, H., Judeinstein S., Tripler E., Harari M., Greenberg A., Shomer I. and Holland D., (2009). Seasonal and cultivar variations in antioxidant and sensory quality of pomegranate (Punica granatum L.) fruit. Journal of Food Composition and Analysis. 22(3): 189-195.

de Nigris, F., Balestrieri M. L., Williams-Ignarro S., D'Armiento F. P., Fiorito C., Ignarro L. J. and Napoli C., (2007). 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. 17(1): 50-54.

Fulton, D., McGiff J. C. and Quilley J., (1996). Role of phospholipase C and phospholipase A2 in the nitric oxide-independent vasodilator effect of bradykinin in the rat perfused heart. Journal of Pharmacology and Experimental Therapeutics. 278(2): 518-526.

Herlekar, I., (2014). Fruit power: pomegranate helps fight Hepatitis C infection. CURRENT SCIENCE. 107(7): 1105.

Horak, F., Zieglmayer P., Zieglmayer R., Lemell P., Yao R., Staudinger H. and Danzig M., (2009). A placebo-controlled study of the nasal decongestant effect of phenylephrine and pseudoephedrine in the Vienna Challenge Chamber. Annals of Allergy, Asthma & Immunology. 102(2): 116-120.

Jorgensen, T. D., Dissing S. and Gromada, J., (1996). Cyclic GMP potentiates phenylephrine but not cyclic ADP-ribose-evoked calcium release from rat lacrimal acinar cells. FEBS letters. 391(1-2): 117-120.

Kim, N. D., Mehta R., Yu W., Neeman I., Livney T., Amichay A., Poirier D., Nicholls P., Kirby A. and Jiang W., (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast cancer research and treatment. 71(3): 203-217.

Nakamura, Y., Matsumoto H. and Todoki K., (2002). Endothelium-dependent vasorelaxation induced by black currant concentrate in rat thoracic aorta. The Japanese journal of pharmacology. 89(1): 29-35.

Salleh, N. and Ahmad V. N., (2013). In-Vitro effect of Ficus deltoidea on the contraction of isolated rat's uteri is mediated via multiple receptors binding and is dependent on extracellular calcium. BMC complementary and alternative medicine. 13(1):1.

Shekha, M.S.S., (2010). Physiological Effects of Crataegus aronia Fractions on Isolated Smooth Muscle and Perfused "Langendorff" Heart in Albino Rats. PhD thesis. Duhok University. Kurdistan region-Iraq.

Sumner, M. D., Elliott-Eller M., Weidner G., Daubenmier J. J., Chew M. H., Marlin R., Raisin C. J. and Ornish D., (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart disease. The American journal of cardiology. 96(6): 810-814.

Tzulker, R., Glazer I., Bar-Ilan I., Holland D., Aviram M. and Amir R., (2007). Antioxidant activity, polyphenol content, and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. Journal of Agricultural and Food Chemistry. 55(23): 9559-9570.

Ugusman, A., Zakaria Z., Chua K. H., Megat Mohd Nordin N. A. and Abdullah Mahdy Z., (2014). Role of rutin in nitric oxide synthesis in human umbilical vein endothelial cells. The Scientific World Journal. (2014).

Vanhoutte, P. M., (2009). Endothelial dysfunction the first step toward coro-

nary arteriosclerosis. Circulation Journal. 73(4): 595-601.

Viuda-Martos, M., Fernández-López J. and Pérez-Álvarez J., (2010). Pomegranate and its many functional components as related to human health: a review. Comprehensive Reviews in Food Science and Food Safety. 9(6): 635-654.

Viuda-Martos, M., Ruiz-Navajas Y., Fernández-López J., Sendra E., Sayas-Barberá E. and Pérez-Álvarez J. A., (2011). Antioxidant properties of pomegranate (Punica granatum

L.) bagasses obtained co-product in the juice extraction. Food Research International. 44(5): 1217-1223.

Watson, R. R. and Preedy V. R., (2009). Bioactive foods in promoting health: fruits and vegetables: Academic Press.

Yılmaz, B. and Usta C., (2013). Ellagic Acid-Induced Endothelium-Dependent and Endothelium-Independent Vasorelaxation in Rat Thoracic Aortic Rings and the Underlying Mechanism. Phytotherapy Research. 27(2): 285-289.