College of Pharmaceutical Sciences and Drug Manufacturing
A college accredited by "NAQAAE"

Journal of Pharmaceutical Sciences and Drug Manufacturing
ISSN: 3009-6553 (Print)
ISSN: 3009-6677 (Online)

JPSDM

Section D: Pharmacognosy and Phytochemistry.

Research Article

Pharmacological activity of *Pomegranate* peels ethyl acetate extract: antihypertensive potential

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Received: 22 January 2024
Accepted: 19 May 2024
Published: 2 June 2024

Editors
Marwa Mohanad
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Keywords
Punica granatum.
Peels.
Phenolics.
Hypertension.
Medicinal plants.
Editable plants.

Abstract

Numerous biological effects of pomegranate fruit (Punica granatum Linn.) have been found. This fruit's juice has been shown to be a significant source of high concentrations of phyto-constituents. It is rich in antioxidants and polyphenolic compounds, including punicalagins, anthocyanins, and hydrolyzable tannins, which have numerous nutritional and health benefits. This study examined the effects of 400 mg/kg/day of pomegranate peel ethyl acetate extract (PGE) on heart rate and blood pressure in Wister rats with fludrocortisone-induced hypertension. Vascular reactivity studies were conducted in segments of the aorta from normal rats, hypertensive control rats, and hypertensive rats treated with PGE. PGE treatment was found to significantly lower blood pressure in hypertensive rats treated with fludrocortisone salt; however, it had no effect on the mean blood pressure or heart rate in normotensive rats. PGE therapy considerably significantly decreased the hypertensive rats' isolated aortic strip's contractile responses to noradrenalin. This study will continue to separate the bioactive compounds and to design natural active formula.

Introduction

Chronic increases in peripheral vascular resistance are attributed to abnormalities in the peripheral vascular system, which are primarily responsible for the alteration of essential hypertension in humans. These abnormalities can be structural (increased arteriolar wall-to-lumen ratio) or functional (changes in vascular reactivity) [1-2]. Numerous experimental and clinical findings support the central role of the sympathetic nervous system in regulating vascular tone, vascular opposition, and the progression of hypertension. Increased levels of pro-inflammatory cytokines in the bloodstream are also linked to elevated blood pressure [3–4]. This pathology may modify the regulation of vascular tone and the vascular expression of enzymes such as inducible nitric oxide synthase (iNOS). Indeed, increased vascular iNOS activity and/or protein expression have been related to hypertension [5]. Previous studies [6–8] by others have investigated the role of iNOS-derived NO in endothelium-dependent vasodilator and vasoconstrictor responses in arteries stimulated by lipopolysaccharide or interleukin-1b. Different mechanisms can be used by oxidative stress to influence vascular reactivity. Reactive oxygen species act as second messengers by inducing a range of signaling molecules, which has a substantial effect on vascular physiopathology [9–10]. The presence of superoxide anion (O2-) sources inside of vessels has been reported multiple times. Among them, xanthine oxidase, uncoupled NOS, and COX can all produce O2 in different conditions. However, it is well known that nicotinamide adenine dinucleotide (phosphate) (NADPH) oxidase, which is present in all three vessel layers [11–13], is the main source of O2- at the vascular level. Numerous characteristics of the animal model of hypertension used in this study are also present in the human model. Many of these models were developed using the etiological factors thought to be responsible for human hypertension, including excessive consumption of salt, renin-angiotensin-aldosterone system (RAAS) hyperactivity, [14]. The regulation of blood pressure (BP) is complex, so the effectiveness of an antihypertensive medication in one model does not always indicate that the pathophysiology of high blood pressure in that specific model is linked to the drug's mechanism of action. Fludrocortisones increased blood volume and blood pressure by causing salt and water to be reabsorbed. Vasopressin secretion is elevated in addition to changed RAAS activity, which raises sympathetic activity [15]. The goal of the current study was to use a model of hypertensive rats to examine any potential antihypertensive activity and changes in vascular reactivity caused by PGE. Apart from creating antihypertensive medication to stop and/or reverse structural and functional alterations in the microcirculation.

Material & methods

Animals

We used male albino Wistar rats weighing 100–150 grams. When conducting research, the National Institutes of Health's guidelines for the use and care of laboratory animals were adhered to. We were allowed to conduct experiments on active animals because they were housed at room temperature and on a 12-hour light/dark cycle. To be used in laboratory settings, rats were domesticated. The rats were fed a regular diet and water. All aspects of the research and animal care were carried out with ethical approval from the local regulatory body.

Plant material

For a week, one kilogram of Punica granatum peels were macerated in 70% ethanol. Following evaporation, the residual residue was fractionated using ethyl acetate, and the resulting ethyl acetate fraction was evaporated to produce 200 gm of PGE powder. The PGE powder was given orally to the experimental animals in a solution of deionized water.

Drugs and chemicals

All chemicals used in the research were of analytical grade. Noradrenalin and Fludrocortisone acetate were purchased by Sigma-Aldrich, USA.

Mineralocorticoid induced hypertension

Groups of rats were given a diet high in sodium chloride; however, these rats were given 2% sodium chloride solution to drink in place of water. They were given fludrocortisones dissolved in sesame oil at a dose of 10 mg/kg once daily for three weeks after they reached a weight of roughly 250 gm [16].

Antihypertensive activity in normotensive rats

There were three groups of five normal rats used. PGE was administered orally to Groups 2 and 3, at a dose of 200 and 400 mg/kg, respectively, with Group 1 acting as a typical control. Basal blood pressure and heart rates were measured at 0, 2, 4, and 6 hours using a non-invasive blood pressure recorder device (Ugo Basile Instruments, Varese, ITALY). After each rat was placed in the controller, it was heated to a temperature of approximately 33 to 35 °C and given an assumed cuff with a sensor attached to its tail. Heart rate, systolic and diastolic blood pressure, and pulse rate were measured directly using the tail cuff and pulse sensor after it was inflated to a pressure higher than 200 mmHg [17].

Antihypertensive activity in hypertensive rats

Over a period of three weeks, oral treatments with deionized water, 10 ml/kg (control), 200 mg/kg and 400 mg/kg of PGE, and three groups (n=5) of hypertensive rats (250 ±10 gm) were administered once daily. A non-invasive blood pressure recorder device was used to measure the heart rate and systolic blood pressure both before and after therapy. Rats were first trained for blood pressure measurement at least three times, which established a baseline blood pressure.

Vascular reactivity experiments

The study of vascular reactivity was conducted using isometric pressure on aortic segments [18]. Two stainless-steel pins were placed within the fragments' lumen; one was fastened to the tissue bath's wall, and the other to a force transducer (Ugo Basile, Italy) that was subsequently connected to an amplifier. The pieces were kept at 37 ± 0.5 °C in an organ bath with 25 ml of Krebs–Henseleit solution, constantly effervescent with a mixture of 95% O2 and 5% CO2 (pH 7.4). Before any drugs were added, the ideal resting tension for each aortic segment was set at 1.5 g [19]. During the equilibration phase, this tension was adjusted every fifteen minutes. The vessel rings were subjected to two grams of tension. To reach the maximum KCL-induced contractile plateau, 60 mM of KCl was utilized. The collective dose-response curve for noradrenaline (10-10-10-5M) could be obtained. As a percentage of the maximum contraction induced by KCl, the amounts of contraction induced by NA were reported. The tissue was cleaned every fifteen minutes for two hours. The study looked at the nor-adrenalin dose response

Table 3: Effect of Punica granatum extract on systolic BP of hypertensive rats on continuous therapy for 21 days.

Hypertensive rats (SBP ± SEM

Treatment	Days			
	0	7	14	21
Normal control	110.50±3.64	110.83±3.38	90.50±0.81	112.30±1.89
Hypertensive control	145.16±0.79*	144.83±1.47*	155.40±1.41*	140.11±1.63*
PGE 200mg/kg	143.50±1.31	152.40±1.89	131.20±0.89**	123.90±1.29**
PGE 400mg/kg	146.10±1.38	143.20±0.83	128.40±1.12**	119.26±0.89**

SBP: Systolic Blood Pressure, All values are mean of 5 observations + SE, *P<0.05 compared to Normal control group, **P<0.05 compared to Hypertensive control group.

curve in rats with hypertension and VAD treatment. It ranged from 8.47 x 10-7 to 1.73 x 10-4. To record the contractile response to the cumulative addition of agonist, an Ugo basile transducer was employed. Whole collective dose-response curves to NE were reliably acquired at 30-minute intervals with and without the studied chemical added five minutes before the second curve. The graphic valuation of the concentration of NE responsible for the half-maximal contraction was made possible by plotting increased pressure against the log of NE concentration [20].

Data analysis and statistics

The results were presented as the mean ± standard error of the number of rats shown, and any discrepancies were evaluated using Student's t-test. A P<0.05 threshold indicated statistical significance. Vasoconstrictor responses elicited by noradrenalin were expressed as a proportion of tone produced by 75mM KCl. Using linear regression, log dosage response curves—or straight lines—were created.

Results

Hypotensive activity in normotensive rats

In normotensive rats, baseline values for systolic blood pressure and HR were (118 ± 1) mmHg and (376 ± 10) bpm, respectively. None of the PGE given at 200 mg/kg and 400 mg/kg showed effect on HR or caused any noticeable hypotension in normotensive rats when compared to rats given the vehicle alone (Table 1, Table 2).

Table 1: Effect of Punica granatum extract on the systolic Blood pressure of normotensive rats.

NORMOTENSIVE RATS (SBP MMHG ± SEM)

	TREATMENT	
Time	200 mg/kg	400 mg/kg
0 hr	118±1.08	123±1.02
2 hr	115±1.10	115±1.18
4 hr	121±0.99	117±0.77
6 hr	112±0.87	122±0.81

SBP: Systolic Blood Pressure, all values are mean of 5 observations + SE.

Table 2: Effect of Punica granatum extract on the heart rate of normotensive rats.

Normotensive rats (Heart beats/min ± SEM)

	Treatment	
Time	200 mg/kg	400 mg/kg
0 hr	385±2.99	372±3.22
2 hr	371±3.01	420±3.84
4 hr	451±3.21	397±3.44
6 hr	402±3.88	375±3.72

All values are mean of 5 observations + SE.

Antihypertensive activity

The oral administration of fludrocortisones once daily caused a significant rise in blood pressure following three weeks of treatment. However, the daily oral administration of various PGE doses led to varying reductions in blood pressure. Table 3 reveals that PGE at 200 mg/kg and 400 mg/kg had a significant antihypertensive effect following three weeks of treatment. Furthermore, at a dosage of 200 mg/kg, PGE demonstrated a significant decrease in heart rate in the second and third week. Similarly, PGE at 400 mg/kg showed, by the end of the third week, a significant decrease in heart rate in hypertensive rats relative to the hypertensive control group, as shown in (Table 4).

Contractile response of vascular ring to NA

Vasoconstriction is elevated in vascular dysfunction, and diastolic function is decreased. Thus, our primary goal is to assess any alterations in vascular function by determining the aortic rings' vascular reactivity to the physiological modulator noradrenalin (NA). The concentration inside isolated aortic rings affected the contractile responses that came from adding NA at escalating concentrations (10-10-10-5M). Our findings indicate that the group treated with DOCA exhibited a significantly higher vasoconstrictive response to NA (P<0.01). On the other hand, the PGE-treated group showed a lower vasoconstrictive effect than the DOCA-treated group (P<0.01) (Figure 1). Additionally, the PGE treated group demonstrated a significantly reduced contractile response to NA (P<0.01) in comparison to the control group (Figure 1). The dose-response curve of noradrenalin in the isolated aortic strip shifted significantly (P < 0.001) to the right when PGE (400 mg/kg/day) was given orally to the rats treated with

Table 4: Effect of Punica granatum extract on heart rate of hypertensive rats on continuous therapy for 21 days.

Hypertensive rats (Heart beats/min ± SEM)

Treatment	Days			
	0	7	14	21
Normal control	411.33±3.64	387.3±3.45	378.7±3.64	418.14±3.89
Hypertensive control	445.16±2.79	444.73±4.47*	455.10±4.41*	440.11±3.63
PGE 200mg/kg	443.50±3.31	452.30±3.89	431.20±3.89	423.90±3.29
PGE 400mg/kg	446.40±3.38	443.40±3.83	435.40±1.12	376.25±2.89**

***P<0.05 compared to Normal control group, **P<0.05 compared to Hypertensive control group, all values are mean of 5 observations + SE.**

DOCA salt for three weeks, in contrast to the animals treated with DOCA salt who had hypertension.

Discussion

According to the current study, PGE significantly lowers high blood pressure in experimental models of hypertension. The hypertension brought on by DOCA is brought on by the retention of water and sodium. Additionally, the study shows that vasoconstriction, which raises arterial blood pressure, is caused by the atypical cation turnover in the hypertensive models given DOCA salt. Furthermore, in hypertensive rats given DOCA salt, there is an increased mobilization of calcium ions into the smooth muscle of blood vessels, which accounts for the increased sensitivity to noradrenaline [21]. It is plausible that modifications in calcium permeability or voltage-operated calcium channels are necessary to maintain hypertension in the DOCA salt-treated hypertensive model [22–23]. Recent studies conducted both in vitro and in vivo have shown that the vascular reactivity to noradrenaline and adrenaline is increased in hypertensive models treated with DOCA salt. This vasomotion may be related to variations in K+ absorption. The effects of phenylephrine (PE) and acetylcholine (ACh) on vascular reactivity in rat aortic rings were measured in previous studies. When 10(-7) M PE was incubated with aortic rings, the most noticeable rhythmic contractions were observed, with a maximum amplitude of 210 ± 28 mg, and 10(-6) M ACh produced a maximum amplitude of 177 ± 6 mg. Notably, in contrast to the control (121% for PE and 117% for ACh), 10 (-7) M PE and 10 (-6) M ACh both markedly increased K (+) uptake in endothelium-intact aortas [24]. Following VAD administration, hypertensive rats treated with DOCA salt showed a decrease in vascular reactivity, which suggests a change in the adrenoceptors' sensitivity to noradrenaline and adrenaline. PGE may have antihypertensive effects due to its influence on cation transport across the cell membrane, according to the mechanism of hypertension in hypertensive models treated with DOCA salt.

Conflicts of Interest

The authors clarified that there is no conflict of interest in this study.

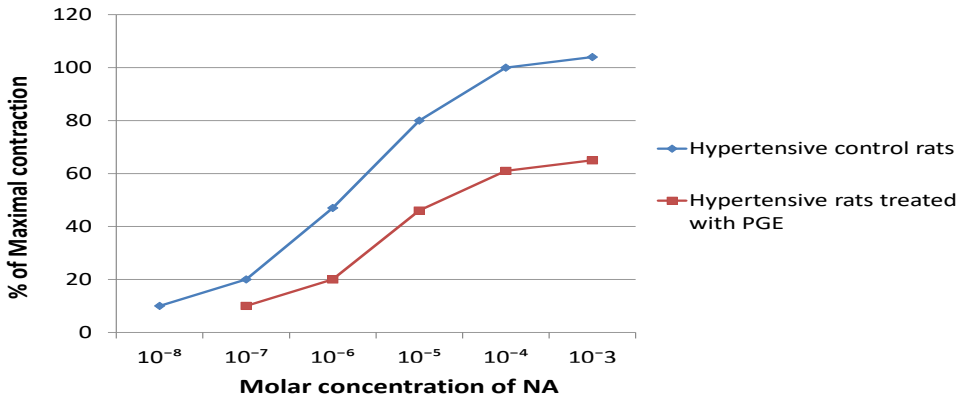

Figure 1: Cumulative dose-response curve to noradrenaline in aortic strips of rats made hypertensive by DOCA (hypertensive control rats) and hypertensive rats treated with PGE (400 mg/kg). P < 0.05 compared to hypertensive control group

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