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PHARMACOLOGICAL ACTIVITY OF *PUNICA GRANATUM* (POMEGRANATE) LEAVES ON HYPERTENSION

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Abstract

The present study was carried out to evaluate the pharmacological activity of hydroalcoholic extract of *Punica granatum* (pomegranate) leaves against salt-induced hypertension in experimental rats. The leaves were extracted using hydroalcoholic solvent and subjected to phytochemical screening and biochemical evaluation. Preliminary phytochemical investigation revealed the presence of glycosides, flavonoids, diterpenes, phenolic compounds, proteins, and carbohydrates. Total phenolic and flavonoid contents were also estimated and found to be significant. Hypertension was induced in rats using 18% NaCl, and the animals were treated with hydroalcoholic extract of *Punica granatum* at doses of 100 mg/kg and 200 mg/kg. Captopril (60 mg/kg) was used as standard drug. Biochemical parameters including serum creatinine, AST, ALT, sodium, and potassium levels were evaluated. The hypertensive control group showed significant elevation in creatinine, AST, ALT, and sodium levels with reduction in potassium levels. Treatment with *Punica granatum* extract significantly restored these altered biochemical parameters toward normal values in a dose-dependent manner. The higher dose (200 mg/kg) exhibited pronounced antihypertensive and protective activity comparable to the standard drug. The study concluded that hydroalcoholic extract of *Punica granatum* leaves possesses significant antihypertensive, nephroprotective, and hepatoprotective activities, which may be attributed to the presence of flavonoids and phenolic compounds with antioxidant potential.

Keywords: *Punica granatum*, Hypertension, Hydroalcoholic extract, Antihypertensive activity, Flavonoids, Phenolic compounds, Captopril, Biochemical parameters, Antioxidant activity.

Introduction

Hypertension is one of the most common chronic cardiovascular disorders worldwide and is considered a major risk factor for stroke, myocardial infarction, heart failure, renal dysfunction, and premature mortality (Khan *et al.*, 2021). According to global health reports, the prevalence of hypertension has increased significantly due to sedentary lifestyle, unhealthy dietary habits, obesity, stress, smoking, alcohol consumption, and aging population. Persistent elevation of arterial blood pressure leads to

structural and functional damage of blood vessels and vital organs, thereby increasing cardiovascular complications (Afzal *et al.,* 2023).

Although several synthetic antihypertensive drugs are available, long-term therapy is often associated with adverse effects, high cost, poor patient compliance, and development of drug resistance. Therefore, there is growing interest in the exploration of medicinal plants as safer and more economical alternatives for the management of hypertension (Curb *et al.,* 1985).

Medicinal plants have been widely utilized in traditional systems of medicine for the treatment of cardiovascular disorders due to their rich content of bioactive phytoconstituents such as flavonoids, polyphenols, tannins, alkaloids, glycosides, and terpenoids (Adegbola *et al.,* 2017). These phytochemicals possess antioxidant, vasodilatory, anti-inflammatory, diuretic, and cardioprotective properties, which may contribute to antihypertensive activity. Natural products are increasingly being investigated for their ability to regulate blood pressure through multiple mechanisms including inhibition of angiotensin-converting enzyme (ACE), enhancement of nitric oxide production, calcium channel blocking activity, and reduction of oxidative stress (Verma *et al.,* 2021).

Punica granatum commonly known as pomegranate, belongs to the family Lythraceae and is widely cultivated in tropical and subtropical regions (Lim, 2012). Different parts of the plant including fruits, peels, flowers, bark, seeds, and leaves have been traditionally used in Ayurveda and other traditional medicinal systems for the treatment of various ailments such as diarrhea, ulcers, diabetes, inflammation, microbial infections, and cardiovascular disorders. Pomegranate leaves are rich in phytoconstituents including flavonoids, phenolic compounds, ellagic acid, gallic acid, tannins, and triterpenoids, which exhibit strong antioxidant and free radical scavenging activities (Kumar *et al.,* 2020; Shi, 2025).

Several pharmacological studies have demonstrated that pomegranate possesses cardioprotective, antihyperlipidemic, antidiabetic, anti-inflammatory, and antioxidant properties. The antioxidant potential of pomegranate leaves may help reduce oxidative stress-induced endothelial dysfunction, which is one of the major contributing factors in hypertension. In addition, phytoconstituents present in the leaves may improve vascular relaxation, enhance endothelial nitric oxide availability, and reduce lipid peroxidation, thereby contributing to blood pressure regulation (El-Hadary and Ramadan, 2019).

Despite extensive research on pomegranate fruit and peel extracts, limited scientific data are available regarding the antihypertensive potential of pomegranate leaves. Therefore, the present study was undertaken to evaluate the pharmacological activity of *Punica granatum* leaves on hypertension using suitable experimental models. The study aims to investigate the antihypertensive efficacy of the leaf extract and to explore its possible therapeutic role in the management of hypertension and associated cardiovascular complications.

Material and Methods

Material

Fresh leaves of *Punica granatum* were collected and authenticated for the present study. Sodium chloride (NaCl) used for induction of hypertension and Captopril used as standard antihypertensive drug were procured from a certified pharmaceutical supplier. Ethanol and distilled water were used for preparation of hydroalcoholic extract. All chemicals and reagents used for phytochemical screening and biochemical estimations including creatinine, AST, ALT, sodium, and potassium assay kits were of analytical grade. Experimental animals (Wistar rats) were maintained under standard laboratory conditions throughout the study.

Methods

Extraction of plant materials by Soxhlet extraction method

Fifty grams of dried and coarsely powdered leaves of *Punica granatum* were accurately weighed and placed in a thimble made of filter paper, which was then inserted into the Soxhlet extraction apparatus (Handa, 2008). Extraction was carried out using a hydroalcoholic solvent system comprising methanol and distilled water in the ratio of 80:20 (v/v). The extraction process was continued for 24 hours at the 30ºC of the solvent mixture until the siphon tube solvent became colorless, indicating exhaustive extraction of the phytoconstituents. After completion of the extraction, the extract solution was allowed to cool to room temperature and filtered through Whatman No. 42 filter paper to remove insoluble materials and plant debris. The filtered extract was then stored under refrigerated conditions until further analysis was carried out

Determination of percentage yield

After extraction, yield of the plant extracts obtained were calculated in grams and then converted it into percentage (Arwande *et al.,* 2018). The percentage yields of each extract were calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of powder drug taken}}$$

Qualitative evaluation

Medicinal plants are resources of traditional medicines and many of the modern medicines are produced indirectly from plants. Phytochemical constituents are of two type primary bioactive constituents (Chlorophyll, proteins, amino acids, sugar etc.) and secondary bioactive constituents include (Alkaloids, terpenoids, phenols, flavonoids etc.). Phytochemical tests were done as per the methods given (Talukdar and Chaudhary, 2010).

Quantitative studies of bioactive constituents

Estimation of total phenol content

The total phenolic content of the extract was determined using the modified Folin–Ciocalteu method (Javanmardi *et al.,* 2003). A stock solution of gallic acid was prepared by dissolving 10 mg of gallic acid in 10 mL of methanol, and different concentrations (5–25 µg/mL) were prepared for calibration. For sample preparation, 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. An aliquot of 2 mL of the extract solution (1 mg/mL) was used for total phenol estimation. Subsequently, 2 mL of the extract or standard solution was mixed with 1 mL of Folin–Ciocalteu reagent diluted previously with distilled water (1:10, v/v) and 1 mL of sodium carbonate solution (7.5 g/L). The reaction mixture was vortexed for 15 seconds and allowed to stand for 10 minutes to facilitate color development. The absorbance of the developed color was measured at 765 nm using a UV–Visible spectrophotometer.

Estimation of total flavonoids content

The total flavonoid content of the extract was determined by the aluminium chloride colorimetric method (Khan *et al.*, 2018). A standard stock solution was prepared by dissolving 10 mg of quercetin in 10 mL of methanol, and different concentrations (5–25 µg/mL) were subsequently prepared for calibration. For sample analysis, 10 mg of dried extract was dissolved in 10 mL of methanol and filtered. An aliquot of 3 mL of the extract solution (1 mg/mL) was used for flavonoid estimation. To this, 1 mL of 2% aluminium chloride ($AlCl_3$) solution was added and the reaction mixture was allowed to stand at room temperature for 15 minutes for color development. The absorbance was then measured at 420 nm using a UV–Visible spectrophotometer to determine the total flavonoid content.

In vivo Pharmacological Activity of _Punica granatum_ (Pomegranate) Leaves on Hypertension Animals

Albino Wistar rats of both sexes, weighing 150-200 g, were kept in groups of six under a conventional 12-hour light/dark cycle, with controlled relative humidity (55–65%) and ambient temperature (25 ± 2 °C). Standard rat feed and unlimited water were given to the animals. The rats were acclimated to laboratory conditions for seven days prior to research. Every trial was carried out between 8:00 and 15:00 in a quiet setting. For every experimental set, a different set of six rats was employed. The Institutional Animal Ethics Committee (IAEC), which was established in accordance with the regulations of the Ministry of Environment and Forests, Government of India, New Delhi, for the control and monitoring of animal studies, approved the study protocol.

Acute oral toxicity study

Acute oral toxicity was conducted according to the method of the Organization for Economic Co-operation and Development (OECD) (Saleem *et al.*, 2020). An extract of *Punica granatum (up to 2000 mg/kg)* was administered orally for 4 days to six groups of rats (n=6), and the animals were observed for mortality and behavioral changes to evaluate a possible anti-arthritic effect.

Experimental

Normotensive rats were randomly divided into five groups, and each group consists of six animals.

Group I served as the normal and received distilled water.

Group II served as Disease control, received 18% NaCl (10 ml/kg/day), and served as salt hypertensive rats (SHR).

Group III received 18% NaCl (10 ml/kg/day) + captopril (60 mg/kg).

Group IV received 18% NaCl (10 ml/kg/day) + the extract of *Punica granatum*at doses of 100mg/kg p.o.

Group V received 18% NaCl (10 ml/kg/day) + the extract of *Punica granatum*at doses of 200mg/kg p.o.

This experimental protocol follows up to 28 days. On 28 days to estimate Creatinine, Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Sodium, and Potassium levels.

Biochemical Estimations

Blood samples were collected from experimental animals and serum was separated by centrifugation for biochemical estimations. Serum creatinine levels were estimated using standard diagnostic reagent kits based on the Jaffe's reaction method (Dissanayake *et al.*, 2022). Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by standard enzymatic colorimetric methods using commercially available kits according to the manufacturer's instructions (Javaid *et al.*, 2021; Khalili *et al.*, 2022). Serum sodium and potassium levels were estimated using flame photometry/electrolyte analyzer methods (Pareek *et al.*, 2021; Hoffman, 1937). The absorbance and electrolyte values obtained were compared with standard calibration data, and the results were expressed in appropriate units for statistical analysis.

Statistical analysis

The values were expressed as mean ± SEM (n=6). Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by Tukey's test; $P<0.05$, $P<0.01$, and $P<0.001$ were considered statistically significant.

Results and Discussion

The present study demonstrated the antihypertensive potential of hydroalcoholic extract of *Punica granatum* leaves in salt-induced hypertensive rats. The hydroalcoholic extract showed a black coloured appearance with a percentage yield of 11.6% as presented in Table 1, indicating satisfactory extraction efficiency. Phytochemical screening results shown in Table 2 confirmed the presence of important bioactive constituents such as glycosides, flavonoids, diterpenes, phenolic compounds, proteins, tannins and carbohydrates, while alkaloids, saponins, and sterols were absent. The presence of phenolic and flavonoid compounds may contribute significantly to the antioxidant and cardioprotective activity of the extract. Total phenolic and flavonoid contents were found to be 0.740 mg/100 mg and 0.815 mg/100 mg respectively, as shown in Table 3, suggesting good antioxidant potential.

Biochemical estimations revealed that administration of 18% NaCl markedly elevated serum creatinine, AST, ALT, and sodium levels while decreasing potassium levels in hypertensive rats, indicating renal dysfunction, hepatic stress, and electrolyte imbalance associated with hypertension. The elevated creatinine level observed in the hypertensive control group (Table 4) indicates impaired renal function due to excessive salt intake. Treatment with *Punica granatum* extract at both 100 mg/kg and 200 mg/kg significantly reduced creatinine levels toward normal values in a dose-dependent manner, with the higher dose showing effects comparable to captopril.

Similarly, AST and ALT levels were significantly increased in salt-induced hypertensive rats as shown in Tables 5 and 6, indicating hepatic injury and oxidative stress. Administration of *Punica granatum* extract significantly decreased these enzyme levels, suggesting hepatoprotective activity of the extract. The higher dose (200 mg/kg) exhibited better protection against hepatic damage.

Electrolyte analysis presented in Tables 7 and 8 demonstrated that salt-induced hypertension caused a significant increase in sodium levels and a reduction in potassium levels. Treatment with *Punica granatum* extract effectively normalized sodium and potassium concentrations in a dose-dependent manner, indicating improvement in electrolyte balance and cardiovascular function. These beneficial effects may be attributed to the antioxidant and vasoprotective properties of flavonoids and phenolic constituents present in the extract. The results obtained from Tables 4–8 confirm that *Punica granatum* leaf extract possesses significant antihypertensive activity along with renal and hepatic protective effects, supporting its potential use as a natural therapeutic agent for the management of hypertension.

Table 1: Physical characteristics of hydroalcoholic extract of *Punica granatum*

Extract	Colour	Weight of extract	% Yield
Hydroalcoholic	Black	5.80	11.6

Table 2: Phytochemical tests of extract of *Punica granatum*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Hager's test:	-ve
	Wagner's test:	-ve
2.	**Glycosides**	
	Conc. H_2SO_4 test:	+ve
3.	**Flavonoids**	
	Lead acetate test:	+ve
	Alkaline reagent test:	+ve
4.	**Diterpenes**	
	Copper acetate test:	+ve
5.	**Phenol**	
	Ferric chloride test:	+ve
6.	**Proteins**	
	Xanthoproteic test:	+ve
7.	**Carbohydrate**	
	Fehling's test:	+ve
	Benedict's test:	+ve
8.	**Saponins**	
	Froth test:	-ve
9.	**Tannins**	
	Gelatin test:	+ve
10.	**Sterols**	
	Salkowski test	-ve

+ ve – Present, - ve – Absent

Table 3: Estimation of total phenol and flavonoids content

S. No.	Extract	Total phenol content	Total flavonoids content
		mg/100mg	
1.	Hydroalcoholic	0.740	0.815

Table 4: Effect of *Punica granatum* extract on serum creatinine levels in salt-induced hypertensive rats

Group	Treatment	Creatinine (mg/dL)
Group I	Distilled water (Control)	0.68 ± 0.04
Group II	18% NaCl (SHR)	1.84 ± 0.09
Group III	18% NaCl + Captopril (60 mg/kg)	0.81 ± 0.05***
Group IV	18% NaCl + *Punica granatum* extract (100 mg/kg)	1.12 ± 0.06*
Group V	18% NaCl + *Punica granatum* extract (200 mg/kg)	0.89 ± 0.05**

Values are expressed as Mean ± SEM (n = 6).*P<0.05, **P<0.01, **P<0.001 vs Group-II.

Table 5: Effect of *Punica granatum* extract on serum aspartate aminotransferase (AST) levels in salt-induced hypertensive rats

Group	Treatment	AST (IU/L)
Group I	Distilled water (Control)	36.45 ± 3.12
Group II	18% NaCl (SHR)	118.72 ± 6.45
Group III	18% NaCl + Captopril (60 mg/kg)	44.81 ± 3.54***
Group IV	18% NaCl + *Punica granatum* extract (100 mg/kg)	63.28 ± 4.16*
Group V	18% NaCl + *Punica granatum* extract (200 mg/kg)	49.63 ± 3.87**

Values are expressed as Mean ± SEM (n = 6).*P<0.05, **P<0.01, **P<0.001 vs Group-II.

Table 6: Effect of *Punica granatum* extract on serum alanine aminotransferase (ALT) levels in salt-induced hypertensive rats

Group	Treatment	ALT (IU/L)
Group I	Distilled water (Control)	29.84 ± 2.75
Group II	18% NaCl (SHR)	102.46 ± 5.81
Group III	18% NaCl + Captopril (60 mg/kg)	38.15 ± 3.26***
Group IV	18% NaCl + *Punica granatum* extract (100 mg/kg)	55.72 ± 4.05*
Group V	18% NaCl + *Punica granatum* extract (200 mg/kg)	41.93 ± 3.48**

Values are expressed as Mean ± SEM (n = 6).*P<0.05, **P<0.01, **P<0.001 vs Group-II.

Table 7: Effect of *Punica granatum* extract on serum sodium levels in salt-induced hypertensive rats

Group	Treatment	Sodium (mEq/L)
Group I	Distilled water (Control)	138.52 ± 2.14
Group II	18% NaCl (SHR)	167.83 ± 3.65
Group III	18% NaCl + Captopril (60 mg/kg)	142.17 ± 2.36***
Group IV	18% NaCl + *Punica granatum* extract (100 mg/kg)	151.28 ± 2.84*
Group V	18% NaCl + *Punica granatum* extract (200 mg/kg)	145.36 ± 2.41**

Values are expressed as Mean ± SEM (n = 6).*P<0.05, **P<0.01, **P<0.001 vs Group-II.

Table 8: Effect of *Punica granatum* extract on serum potassium levels in salt-induced hypertensive rats

Group	Treatment	Potassium (mEq/L)
Group I	Distilled water (Control)	4.82 ± 0.18
Group II	18% NaCl (SHR)	3.14 ± 0.15
Group III	18% NaCl + Captopril (60 mg/kg)	4.55 ± 0.17***
Group IV	18% NaCl + *Punica granatum* extract (100 mg/kg)	3.88 ± 0.16*
Group V	18% NaCl + *Punica granatum* extract (200 mg/kg)	4.31 ± 0.14**

Data are represented as Mean ± SEM (n = 6).*P<0.05, **P<0.01, **P<0.001 vs Group-II.

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