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ANTIHYPERTENSIVE EFFECT OF THE ALCOHOLIC EXTRACT OF SEEDS OF *PHASEOLUS VULGARIS* LINN. (FABACEAE) ON HIGH SALT DIET INDUCED HYPERTENSION IN MALE RATS

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Keywords:

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ABSTRACT: Aim: To evaluate the antihypertensive effect of ethanolic extract of seeds of *Phaseolus vulgaris* Linn. (EEPV) on high salt diet induced hypertension in male rats. **Methods:** Male wistar rats (180-200g) received high salt diet (8% NaCl) and 1% saline water for 6 weeks, then cannulated for dosing of Enalapril, angiotensin converting enzyme inhibitor (0.5 mg/kg, i.v.) in standard group and EEPV (50 and 100 mg/kg, i.v.) in test group and measured mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment. **Results:** Elevated blood pressure of the animals was significantly ($P < 0.05$) decreased by EEPV (100 mg/kg, i.v). Enalapril, at the dose of 0.5 mg/kg, i.v. showed significant ($P < 0.05$) reduction in the elevated blood pressure. The antihypertensive activity of ethanolic extract of seeds of *Phaseolus vulgaris* Linn. may be due to the action on rennin-angiotensin system. The antihypertensive activity of EEPV might also be explained by the presence of phytochemical constituents like flavanoids and saponins in the plant. **Conclusion:** The work demonstrated that the concomitant administration of high-salt and EEPV elicits prevention of high salt-induced hypertension in rats. Overall, results justify and support the use of *Phaseolus vulgaris* Linn. as antihypertensive medicine.

INTRODUCTION: Hypertension or high blood pressure is a chronic medical condition resulting to elevated blood pressure in the arteries assessed by systolic and diastolic measurements ¹. The European society of Cardiology has defined hypertension as systolic values above 140 mmHg and diastolic values above 90 mmHg.

However, these values are affected by different factors like age, sex and size ². Hypertension is referred to as a silent killer because in most cases, it does not manifest any symptoms or signs until complications develop in vital organs ³. A direct progressive correlation between blood pressure and cardiovascular risk has long been acknowledged ^{4, 5}.

Only about one-third of patients attain optimal blood pressure control using drug therapy ⁵. Because a reduction of 5 mm Hg in systolic blood pressure has been associated with a 7% reduction in all-cause mortality, it is important to study other involvements that reduce blood pressure ^{4, 6}.

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In spite of progress in prevention, detection, treatment, and control of high blood pressure, hypertension remains an important public health challenge^{4, 5}. Some researches have shown that complementary and alternative medicine has potential in the treatment of hypertension^{7, 9}. The approval of complementary and alternative medicine has grown over the past two decades among physicians and patients alike^{9, 10}.

Herbal medication is an increasingly common form of alternative medicine globally. Herbal products have over the ages created indispensable tools for research and development of new drugs^{11, 12}, and in spite of that there are still many plants whose therapeutic values have not been exploited, it is rational to describe the plant kingdom as a sleeping giant for potential drug development¹³.

Phaseolus vulgaris Linn. (Family- Fabaceae), commonly known as common bean or black bean or kidney bean, is the largest cultivated and consumed pulse legume in the world. It is a slender-stemmed, annual climber growing up to 4 m (12 ft). It has curly tendrils, pointed oval leaflets, clusters of white or lilac flowers and a bean pod containing kidney-shaped seeds. Beans are thought to have originated from South America. But today, varieties are cultivated throughout the globe. The ripe beans gathered in summer. It contains lectin, anthraquinone glycosides, polyphenols, saponins, flavonoids, allantoin, aminoacids, sugar, steroids, terpenoids and tannins¹⁴.

Pharmacologically this plant has been investigated for its antidiabetic activity¹⁵, ACE inhibitory activity¹⁶, antioxidant activity¹⁷ and iron reductase activity¹⁸.

In order to further explore the potential activity of *Phaseolus vulgaris* Linn., the antihypertensive effect of ethanolic extract of seeds of *Phaseolus vulgaris* Linn. (EEPV) were investigated against high salt diet induced hypertension mode.

MATERIAL AND METHODS:

Plant material: The seeds of *Phaseolus vulgaris* Linn. were collected from local market of Lucknow and its identification and authentication were done from CSIR-National Botanical Research Institute, Lucknow, Uttar Pradesh, India (Ref. No.: NBRI/CIF/276/2012). The seeds were break into

small pieces and shade dried. The dried material was then pulverized separately into coarse powder by a mechanical grinder and then passed through Sieve No. 40 to get uniform powder and stored in an air tight container for further use.

Preparation of extract: The ethanolic extract of seeds of *Phaseolus vulgaris* Linn. (EEPV) was prepared by soxhletion. The powdered dried seeds of *Phaseolus vulgaris* Linn. (250g) were extracted with 50% ethanol (EtOH). The extract was filtered, dried and concentrated in rotary evaporator. The resultant extract was used for further studies.

Preliminary phytochemical screening: An attempt was made to observe the presence and absence of diverse phytochemical constituents in the EEPV, viz., alkaloids (Wagner's test), flavonoids (Shinoda test), tannins (Ferric chloride test), steroids and triterpenes (Lieberman-Burchard's test), terpenoids (Salkowski test), glycoside (Baljet's test) and saponins (Foam test) according to standard methods¹⁹.

Animals: The experiments were carried out with male Albino wistar rats weighing 180-200 g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in agreement with the internationally accepted principles for laboratory animal use and care (1087/07/ CPCSEA). They were kept in polypropylene cages (22.5 cm²x 37.5 cm) and were kept under standard housing conditions (room temperature, 24-27°C and humidity, 60-65 %) with a 12-h light and dark cycle.

They were allowed free access to standard pellet diet and water, *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures. [Hygia/M.Pharm/02/2011-12].

Chemicals and Instruments: Enalapril and Sodium chloride were purchased from SD Fine

Chem Limited (Mumbai, India). Rest of the chemicals was also purchased from SD Fine Chem Limited (Mumbai, India). Power Lab 6 Pro (AD Instruments, Australia).

Acute Toxicity Study: Acute oral toxicity studies were performed as per OECD guidelines. The EEPV was administered orally in doses of 50, 100, 250, 500, 1000 and 2000 mg/kg to different groups of rats. The mortality rate was observed and recorded for a 24-h period.

Induction of high salt-induced hypertension: Each rat in Group II - V received high salt diet (8% NaCl) and 1% saline water *ad libitum* for six weeks. After 6 weeks each rat was anaesthetized with Urethane 1.5 mg/kg, i.p. The jugular vein and carotid artery were cannulated for drug administration and blood pressure measurements with Power Lab 6 Pro. Enalapril (0.5 mg/kg, i.v.) was administered in standard group III and EEPV (50 and 100 mg/kg, i.v.) were administered in tested groups IV and V and then mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate was measured after 60 minutes of treatment.

Experimental Design:

Evaluation of antihypertensive activity: The animals were divided into five groups and each group consisted of six rats ($n=6$).

Group I: Normal control

Received normal diet and drinking water for 6 weeks, then cannulated for measuring mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment.

Group II: Disease control

Received high salt diet (8%NaCl) and 1% saline water for 6 weeks, then cannulated for measuring mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment

Group III: High salt diet-induced hypertensive rats treated with Enalapril

Received high salt diet (8%NaCl) and 1%saline water for 6 weeks, then cannulated for dosing of Enalapril (0.5mg/kg, i.v.) and measured mean

blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment

Group IV: High salt diet-induced hypertensive rats treated with EEPV (50 mg/kg, i.v.)

Received high salt diet (8% NaCl) and 1% saline water for 6 weeks, then cannulated for dosing of EEPV (50 mg/kg, i.v.) and measured mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment

Group V: High salt diet-induced hypertensive rats treated with EEPV (100 mg/kg, i.v.)

Received high salt diet (8% NaCl) and 1% saline water for 6 weeks, then cannulated for dosing of EEPV (100 mg/kg, i.v.) and measured mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate with the help of Power Lab 6 Pro after 60 minutes of treatment.

Statistical analysis: Results are expressed as mean \pm S.E.M. The statistical significance of differences between the groups was determined by one-way ANOVA, followed by Dunett's multiple comparison tests using the software Graph Pad Prism 5 (San Diego, CA, USA).

RESULTS:

Phytochemical screening: The result of phytochemical investigation of ethanolic extract of *Phaseolus vulgaris* Linn. seeds has indicated the presence of flavonoids, tannins, steroids and triterpenes, saponins and glycosides.

Acute Toxicity Studies: Oral administration of ethanolic extract of *Phaseolus vulgaris* Linn. up to 2000 mg/kg did not produce any toxic effects. No mortality was observed and *Phaseolus vulgaris* Linn. was found to be safe at the given doses.

Effects of EEPV on Mean blood pressure, Systolic blood pressure, Diastolic blood pressure and Heart rate in high salt-induced hypertension after 60 minutes of treatment:

High salt treatment in disease control group showed significant ($P < 0.05$) increase in mean blood pressure, systolic blood pressure, diastolic blood pressure and heart rate as compared to

control group. The heart rate was significantly normalized by EEPV at 50 and 100 mg/kg. EEPV produced a significant decrease in mean blood pressure, systolic blood pressure and diastolic blood pressure of hypertensive rats at the dose of 50 and 100 mg/kg, i.v. in a dose dependent manner.

Enalapril (0.5 mg/kg, i.v.) showed significant decrease in mean blood pressure, systolic blood pressure and diastolic blood pressure of hypertensive rats as compared to disease control group (**Table 1**).

TABLE 1: MEASUREMENTS OF MEAN BLOOD PRESSURE, SYSTOLIC BLOOD PRESSURE, DIASTOLIC BLOOD PRESSURE AND HEART RATE AFTER 60 MINUTES OF TREATMENT

Treatment	Mean blood pressure (mmHg)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Heart rate (beats/min)
Group I (Normal control)	110.53 ± 01.54	125.65 ± 1.93	83.85 ± 1.62	311.77 ± 2.94
Group II (Disease control)	142.75 ± 2.99*	160.61 ± 1.42*	114.43 ± 2.97*	348.82 ± 1.62*
Group III (Enalapril 0.5 mg/kg)	108.58 ± 1.35*	121.85 ± 1.32	77.24 ± 1.34*	310.84 ± 1.21*
Group IV (EEPV 50 mg/kg)	122.34 ± 1.91*	137.45 ± 1.28	87.59 ± 1.99*	328.84 ± 1.21*
Group V (EEPV 100 mg/kg)	112.47 ± 1.41*	126.49 ± 1.97	80.56 ± 0.96*	317.16 ± 1.76*

Results were expressed as mean ± S.E.M. ($n = 6$). Data are analyzed by One-way ANOVA followed by Dunett's multiple comparison tests. * $P < 0.05$ (significantly different from the control).

DISCUSSION: Hypertension or high blood pressure is a chronic medical condition in which the blood pressure in the arteries is elevated. It is classified as either primary (essential) or secondary. About 90 to 95% of cases are primary hypertension, in which no medical cause can be found²⁰. The remaining 5 to 10% of the cases are secondary hypertension, which are caused by other conditions that affect the arteries, kidneys, heart or endocrine system²¹. An excess of dietary salt is the most common environmental factor that contributes to the pathogenesis of hypertension²²⁻²⁶. Much evidence from epidemiological and genetic studies suggest that a high salt intake is almost uniformly associated with an increase in blood pressure in normotensive and hypertensive humans²⁷⁻³¹. A long-term high salt intake also increased blood pressure in animals³²⁻³³.

The mechanisms of dietary salt-induced hypertension is still not clear, though accumulating evidence has shown a variety of genetic mutations and polymorphisms of sodium channels and related proteins in the kidney result in the dysregulation of sodium metabolism and/or salt-sensitive hypertension. These include mutations affecting synthesis and circulating levels of mineral corticoids³⁴, the role of renal inflammation, oxidative stress, and intra-renal angiotension activity³⁵⁻³⁷. Dietary salt-induced hypertension is also associated with the down-regulation of the renal VEGF expression³⁸.

Conventional antihypertensive drugs are generally coupled with many side effects. About 75 to 80% of the world population use herbal medications, mainly in developing countries, for primary health care because of their better tolerability and lesser side effects. In the last three decades, a lot of rigorous efforts have been done to find out the local medicinal plants with hypotensive and antihypertensive therapeutic potentials³⁹.

Preliminary phytochemical investigation revealed that EEPV showed positive response to flavonoids, tannins, saponins, glycosides, steroids and triterpenes.

This study investigated the antihypertensive effect of the ethanolic extract of seeds of *Phaseolus vulgaris* Linn. in high salt diet-induced hypertensive rats. Administration of EEPV produced a significant decrease in mean blood pressure, systolic blood pressure and diastolic blood pressure of hypertensive rats at the dose of 50 and 100 mg/kg, i.v. in a dose dependent manner.

The heart rate was significantly normalized by EEPV at 50 and 100 mg/kg. Enalapril (0.5 mg/kg, i.v.) showed significant decrease in mean blood pressure, systolic blood pressure and diastolic blood pressure of hypertensive rats as compared to disease control group.

The pharmacological activities of EEPV might be explained by the presence of phytochemical

constituents like flavanoids and saponins in the plant. These bioactive phytoconstituents are known to possess vasorelaxant, antihypertensive and antihyperlipidemic effects⁴⁰⁻⁴².

CONCLUSION: The data showed the efficacy of ethanolic extract of seeds of *Phaseolus vulgaris* Linn. against high salt-induced hypertension in rats. Overall, results justify and support the use of *Phaseolus vulgaris* Linn. as antihypertensive medicine.

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