Vol 5|Issue 1| 2015 | 24-30.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

ANTIHYPERTENSIVE POTENTIAL OF *BOERHAAVIA DIFFUSA* L. IN ADRENALINE-INDUCED HYPERTENSIVE MODEL

Vandana. S. Nade*, laxman. A. Kawale, Anjali. B. Tajanpure

Department of Pharmacology, M.V.P. Samaj's College of Pharmacy, Gangapur Road, Nashik, Maharashtra-422002, India.

ABSTRACT

The objective of the present study was to investigate antihypertensive potential of *Boerhaavia diffusa* roots in adrenaline-induced hypertension in rats. Hypertension was induced by administration of adrenaline (0.5 mg/kg, i.p.) for 10 days consequently. Methanolic extract of *Boerhaavia diffusa* (MEBD) roots was administered at doses of 100, 200 and 300 mg/kg, p.o for 20 days in hypertensive rats. Propranolol (10 mg/kg, i.p.) was used as a standard drug. The effect of MEBD was determined on serum glucose, cholesterol and triglyceride levels and cardiovascular parameter such as blood pressure (BP), heart rate (HR), mean arterial pressure (MAP) and pulse pressure (PP). Blood pressure was determined weekly by non invasive tail cuff method. At the end of the study, blood pressure was measured by invasive method and vascular reactivity was tested with adrenaline, noradrenaline and phenylephrine. The effect of MEBD was studied on isolated rat aortas by acetylcholine-induced vasorelaxation. Chronic treatment with MEBD significantly decreased weight gain, serum glucose level; normalize the heart rate and BP in adrenaline treated rats. All the treatments significantly reduced the pressor response to catecholamines. The significant improvement in the relaxant response to acetylcholine was obtained on isolated aorta. The results suggested that the MEBD possesses significant antihypertensive activity through decrease in sympathetic activity by inhibition of adrenoreceptors or release of nitric oxide. Further detail investigation can explore the mechanism of action of plant to establish the plant as an antihypertensive agent.

Keywords: Adrenaline, *Boerhaavia diffusa*, Blood pressure, Heart rate.

INTRODUCTION

Hypertension is the most common cardiovascular illness and is a major public health issue in economically developing as well as developed countries [1]. Hypertension is a lifestyle- related disease. The prevalence of sustained hypertension is on the rise in younger age groups. This is possibly related to sedentary life style, altered eating habits, increased fat content of diet and decreased physical activity [2]. The significant numbers of individuals with hypertension are unaware of their condition and among those with diagnosed hypertension treatment if frequently inadequate. Recent studies shows that a high prevalence of hypertension in Indian population. As hypertension is an important public health challenge worldwide, its prevention, detection, treatment and control of this condition should received high priority [3].

Boerhaavia diffusa L. (known as Punarnava means which rejuvenates or renews the body) is one of the most famous medicinal plants in the treatment of a large number of human ailments. Boerhaavia species have been in phytochemical and pharmacological research due to their excellent medicinal values. They are rich sources of alkaloids, steroids and flavones. *Boerhaavia diffusa* has attracted a lot of attention due to its prevalent uses in Ayurvedic system of medicine. It is widely used in jaundice, hepatitis, oedema, oligurea, anemia, inflammation, eye diseases, hypertension etc. The plant has been mentioned as cardioprotective and also lowers blood sugar level [4]. Pharmacologists and clinicians have

Corresponding Author

Vandana S. Nade
Email id: kawalevl@rediffmail.com

investigated *B. diffusa* for various activities. The plant is known to improve and protect eye sight, has diuretic and antibacterial activities [5, 6]. The leaves of *B. diffusa* have shown antioxidant and hepatoprotective activities in various pharmacological models [7]. An alkaloid isolated from *B. diffusa* punarnavin has shown anticancer, antiestrogenic, immunomodulatory and antiamoebic activities [8, 9, 10]. *B. diffusa* is clinically proved as a useful and safe drug in patients of nephrotic syndrome and causes regeneration of kidneys [11, 12].

Boerhaavia diffusa plant contains a large number of compounds such as flavonoids, alkaloids, steroids, triterpenoids, lipids, lignins, carbohydrates, proteins, and glycoproteins. The plant is reported to contain the phytoconstituents like punarnavin, boeravinone, punarnavoside, lirodendrin, b-sitosterol, a-2-sitosterol, palmitic acid, ester of b-sitosterol, tetracosanoic, hexacosonoic, stearic, arachidic acid, urosilic acid, Hentriacontane, b-Ecdysone, triacontanol etc. as the main active principles. Many rotenoids have been isolated from the roots of the *Boerhaavia diffusa*. Boeravinones *viz.* boeravinone A, boeravinone B, boeravinone C, boeravinone D, boeravinone E and boeravinone F and Punarnavoside (a phenolic glycoside), are present in roots [13] . Although several medicinal uses have been reported for *B. diffusa*, but no investigative report pertaining to it's an anti-hypertensive and cardioprotective potential exists. Hence, an attempt has been made to evaluate the effect of *B. diffusa* on *hypertension and its related complications.*

MATERIALS AND METHODS

Plant material

The roots of *B. diffusa* were collected in the month of August from local area of Nashik and authenticated at the Botanical Survey of India, Pune, where a voucher specimen was submitted (Voucher no. MAKBOR2).

Extract preparation

The roots were shade dried and reduced to coarse powder. The powdered roots of *B. diffusa* were defatted with Petroleum ether (60 – 80°C) under Soxhlet extraction. The defatted marc was air-dried and put for exclusive extraction under Soxhlet using methanol. The extract was then filtered and evaporated to dryness under reduced pressure (yield 6.3 w/w). Methanolic extract of *B.diffusa* (MEBD) suspended in distilled water using 0.5% carboxy methyl cellulose (CMC) as suspending agent. The extract was administered in doses of 100, 200 and 300 mg/kg per orally (p.o.) for 20 days daily after induction of hypertension. Control group was given only vehicle (0.5% CMC) in volume equivalent to that of the plant extract.

Experimental Animals

Wistar rats (230 - 250 g) of either sex were obtained from National Institute of Toxicology Pune, India and used for the experiment. Animals were housed in polypropylene cages and maintained under the standard laboratory environmental conditions; temperature 25± 2ºC, 12: 12 h L: D cycle and 50 ± 5% RH with free access to food and water *ad libitum*. Animals were acclimatized to laboratory conditions before the test. Each group consisted of six (n = 6) animals. All the experiments were carried out during the light period (08:00-16:00 h). The studies were carried out in accordance with the guidelines given by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi (India). The Institutional Animal Ethical Committee of approved the protocol of the study (IAEC/ 2013/ 03).

Drugs and chemicals

Adrenaline (Neon Laboratories, Thane, India), Noradrenaline (Samarth Life Sciences, Mumbai, India), Phenylephrine (Neon Laboratories, Thane, India) were used for study. Biochemical kits for glucose, triglyceride and cholesterol (Auto Span, Surat, India) were used. All the chemicals used were of analytical grade and purchased from standard manufacturers.

Phytochemical investigation

Phytochemical investigation of the extract for the presence of alkaloids, saponins, flavonoids, tannins, phenolic compounds, triterpenes and sterol was carried out [14].

Acute Toxicity Study

Acute oral toxicity studies were performed as per OECD guidelines. The extract was administered orally in doses of 100, 200, 300, 500, 1000, and 2000 mg/kg to different groups of mice. The mortality rate was observed and recorded for a 24-h period.

Induction of Hypertension

Adrenaline (0.5 mg/kg, i.p.) was administered to rats for ten consecutive days to induce hypertension [15].

Experimental design

After induction of hypertension, animals were divided into 5 groups (n = 6). Group I: Vehicle (0.5% CMC in distilled water, p.o.), Group II: Adrenaline (0.5 mg/kg, i.p.) for 10 days. Group III: Adrenaline + MEBD (100 mg/kg, p.o) 20 days. Group IV: Adrenaline + MEBD (200 mg/kg, p.o) 20 days. Group V: Adrenaline + MEBD (300 mg/kg, p.o) 20 days. Group VI: Adrenaline + Propranolol (10 mg/kg, i.p.) for 20 days.

MEBD and propranolol were administered for 20 days after induction of hypertension. The body weight, systolic pressure, heart rate, serum glucose, serum cholesterol and serum triglyceride were measured before and after treatment and thereafter at weekly interval. Using tail cuff method systolic BP and pulse rate were recorded on Power Lab Data acquisition system (AD Instrument, Australia). Blood samples were collected through retro

orbital plexus under ether anesthesia weekly for determination of serum glucose, cholesterol and triglyceride.

Vascular reactivity to catecholamines

After the completion of treatment schedule, rats from each group were anesthetized with ketamine and xylaxine (75 mg/kg and 15 mg/kg i.p. respectively). Right jugular vein was cannulated with fine polyethylene catheter for administration of drugs. BP was recorded left common carotid artery using pressure transducer by direct method on Power Lab Data acquisition system (AD Instruments, Australia). Heparinized saline (100 IU/ml) was filled in transducer and the fine catheter cannulated to the carotid artery to prevent clotting. After 30 minutes of stabilization mean change in BP in response to NA (1µg/kg), Adr (1µg/kg) and PE (1µg/kg) was recorded [16].

***In Vitro* study**

Acetylcholine-induced vasorelaxation on isolated rat aorta

Immediately after completion of vascular reactivity studies, rats were sacrificed by cervical dislocation. Midline abdominal incision was made and entire descending thoracic aorta from arch down to the diaphragm was isolated and placed in krebs solution at a temperature of 37 ^{0}c and aeration with aerator. Connective tissue and adhering fat was removed from aorta. Rings of 3 mm length were prepared and mounted in an organ bath containing 15 ml krebs solution. Contractions were recorded by suspending the rings between two stainless steel hooks, one of which was attached to the end of a bathing tube and other to the force transducer (PowerLab, AD Instruments). Care was taken to ensure that endothelial layer was not damaged during preparation of aortic rings. The resting tension of 1g was applied to the preparation and equilibrated in a 15 ml bathing solution for 90 to 120 min before the experiment with change of solution every 15 min. After equilibration, the rings were exposed to 10^{-6} M PE. When the contractile response to PE was plateaued, acetylcholine was added in a cumulative fashion and vasorelaxation was recorded [17].

Statistical Analysis

Results are expressed as mean ± SEM, and the statistical analysis of data was done using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

RESULT

Phytochemical investigation

Phytochemical screening of *B. diffusa* reveals the presence of alkaloids, flavonoids, and saponins and triterpenoids.

Acute Toxicity Studies

Oral administration of methanolic extract of *B. diffusa* up to 2000 mg/kg did not produce any toxic effects. No mortality was observed and *B. diffusa* was found to be safe at the given doses.

Effect of methanolic extract of *B. diffusa* on serum glucose, cholesterol and triglyceride levels

Administration of adrenaline for 10 days significantly ($p < 0.01$) increased serum glucose as compared to vehicle group. Treatment with MEBD (200 and 300 mg/kg) and propranolol showed significant ($p < 0.01$) decrease in serum glucose level as compared to adrenaline treated group. MEBD (100 mg/kg) showed slight decrease in serum glucose level ($p < 0.05$).

MEBD only at 300 mg/kg showed significant reduction in cholesterol level ($p < 0.05$). MEBD has no major effect on triglyceride level.

Effect of methanolic extract of *B. diffusa* on blood pressure by non-invasive and invasive method in adrenaline-induce hypertension

The systolic blood pressure was significantly ($p < 0.001$) increased after administration of adrenaline for 10 days in rats as compared to vehicle group. Treatment with MEBD (100, 200 and 300 mg/kg) and propranolol showed significant ($p < 0.01$) decrease in systolic blood pressure measured by both non-invasive and invasive methods.

Effect of MEBD on heart rate, mean arterial pressure and pulse pressure in adrenaline- induce hypertension

Adrenaline treatment group showed significant ($p < 0.01$) increase in heart rate, mean arterial pressure and pulse pressure as compared to vehicle treated group. The heart rate was significantly normalized by MEBD at 200 and 300 mg/kg. The mean arterial pressure was also significantly decreased by MEBD (100, 200 and 300 mg/kg). MEBD (200 and 300 mg/kg) showed slight ($p < 0.05$) decrease, propranolol group showed moderate decrease ($p < 0.01$) and MEBD (100 mg/kg) showed no significant change in pulse pressure as compared to adrenaline group.

Vascular reactivity

The vehicle group showed a normal vascular reactivity to catecholamines [Adr (1µg/kg), NA (1 µg/kg) and PE (1µg/kg)], whereas adrenaline treated group showed a significant ($p < 0.01$) exaggeration in mean change in BP to catecholamines as compared to vehicle group. Treatment with MEBD (200 and 300 mg/kg) and propranolol showed a significant ($p < 0.01$) fall in mean change in BP to catecholamines as compared to adrenaline treated group.

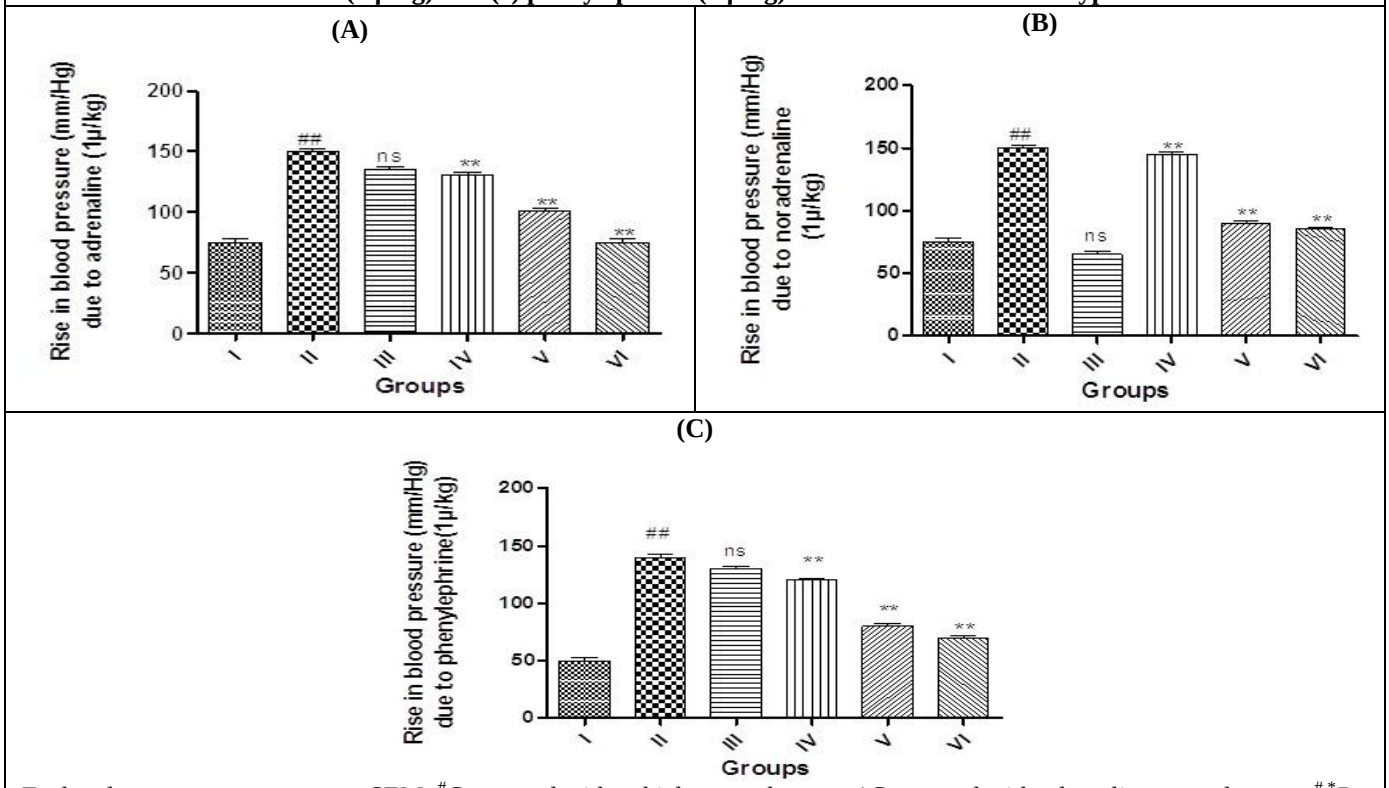
(a)I: Vehicle
II: Adrenaline
III: Adr + MEBD (100 mg/kg)
IV: Adr +MEBD (200 mg/kg)
V: Adr + MEBD (300 mg/kg)

VI: Ad r + Propranolol`

Acetylcholine-induced relaxation of rat aorta pre-contracted with phenylephrine (1×10^{-6}M)

The aorta from vehicle group showed normal relaxant response to cumulative doses of acetylcholine (10^{-7}M to10^{-3}M). This signifies normal endothelial function. Adrenaline treated group showed a significant ($p < 0.001$) impairment of relaxation. Aortas from MEBD (200 and 300 mg/kg) and propranolol showed significant improvement in relaxant response ($p < 0.05$) and ($p < 0.01$) respectively as compared to adrenaline group. However, MEBD (100 mg/kg) group showed no significant change in relaxant response as compared to adrenaline group.

Fig 1. Effect of methanolic extract of *Boerhaavia diffusa* on vascular reactivity to (a) adrenaline (1 µ/kg) (b) noradrenaline (1 µ/kg) and (c) phenylephrine (1 µ/kg) in adrenaline - induced hypertension



Each column represents mean ± SEM. #Compared with vehicle treated group. *Compared with adrenaline treated group. $^{\#,*}P<0.05$, $^{\#\#,**}P<0.01$, $^{\#\#\#,***}P<0.001$. (One-way ANOVA followed by Dunnett's test).

Fig 2. Effect of methanolic extract of *Boerhaavia diffusa* on acetylcholine–induced relaxation of aorta pre-coated with phenylephrine (1×10^{-6} M) in rats with adrenaline- induced hypertension

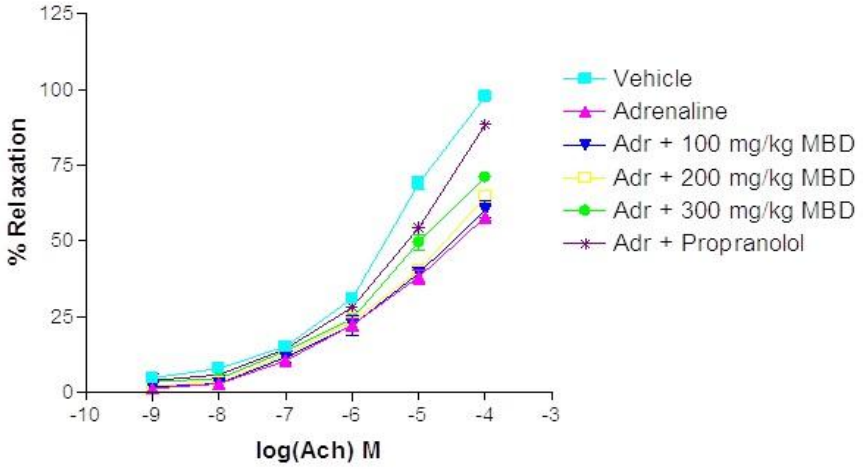


Table 1. Effect of methanolic extract of *B. diffusa* on serum glucose, cholesterol and triglyceride levels

Treatments	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)
Vehicle	91.24 ± 1.9	103.6 ± 7.8	110.8 ± 9.3
Adrenaline (0.5 mg/kg, i.p.)	**140.4**±3.0***	138.4 ± 7.2^{ns}	159 ± 5.2^{ns}
Adrenaline + MEBD (100 mg/kg p.o.)	120.3±2.4**	129.1 ± 4.4 ^{ns}	157.8 ± 3.5^{ns}
Adrenaline + MEBD (200 mg/kg p.o.)	97.44 ± 2.6**	125.4 ± 2.7^{ns}	156.3 ± 5.7 ^{ns}
Adrenaline + MEBD (300 mg/kg p.o)	90.38 ± 1.9**	119.4 ± 1.98*	141.4 ± 4.8 ^{ns}
Adrenaline + Propranolol (10 mg/kg, i.p.)	88.4 ± 1.8**	112.2 ± 3.2**	116.3 ± 3.2*

All values are expressed as mean ± SEM. $^{\#}$Compared with vehicle treated group. *Compared with adrenaline treated group. ^{ns} non significant. $^{\#,*}P< 0.05$, $^{\#\#,**}P< 0.01$ (One-way ANOVA followed by Dunnett's test).

Table 2. Effect of MEBD on blood pressure by non-invasive and invasive method in adrenaline- induce hypertension

Treatments	BP (mm Hg) by non-invasive method (Tail cuff method)	BP (mm Hg) by Invasive method (Direct)
Vehicle	123.2 ± 1.7	110.2 ± 4.7
Adrenaline (0.5 mg/kg, i.p.)	184.2 ± 2.6$^{\#\#\#}$	160.9 ± 3.9$^{\#\#\#}$
Adrenaline + MEBD (100 mg/kg, p.o.)	154.2 ± 3.5**	148.3 ± 1.8*
Adrenaline + MEBD (200 mg/kg, p.o.)	146.8 ± 3.3**	144.1 ± 2.3*
Adrenaline + MEBD (300 mg/kg, p.o.)	138 ± 2.9**	130.2 ± 1.1**
Adrenaline + Propranolol (10 mg/kg, i.p.)	125.7 ± 2.5**	128.3 ± 5.3 **

All values are expressed as mean ± SEM. $^{\#}$Compared with vehicle treated group. *Compared with adrenaline treated group. $^{\#,*}P< 0.05$, $^{\#\#,**}P< 0.01$, $^{\#\#\#,***}P< 0.001$. (One-way ANOVA followed by Dunnett's test).

Table 3. Effect of MEBD on heart rate, mean arterial pressure and pulse pressure in adrenaline- induce hypertension

Treatment	Heart rate (beats/min)	Mean arterial pressure (mmHg)	Pulse pressure (mmHg)
Vehicle	316 ± 11.8	98.4 ± 0.9	37.2 ± 0.5
Adrenaline (0.5 mg/kg, i.p.)	402.6 ± 7.8$^{\#\#}$	152.7 ± 1.375$^{\#\#}$	47.2 ± 0.2$^{\#\#}$
Adrenaline + MEBD (100 mg/kg, p.o.)	395.6 ± 11.5^{ns}	120.6 ± 3.354**	50.4 ± 1.1^{ns}
Adrenaline + MEBD (200 mg/kg, p.o.)	355.8 ± 15.2*	115.9 ± 1.1**	40.4 ± 2.4*
Adrenaline + MEBD (300 mg/kg p.o.)	274 ± 12.3**	102.2 ± 0.5**	40.2 ± 1.0*
Adrenaline + Propranolol (10 mg/kg, i.p.)	320.2 ± 8.4**	97.0 ± 1.9**	37.1 ± 2.0**

All values are expressed as mean ± SEM. $^{\#}$Compared with vehicle treated group. *Compared with adrenaline treated group. $^{\#,*}P< 0.05$, $^{\#\#,**}P< 0.01$, $^{\#\#\#,***}P< 0.001$. (One-way ANOVA followed by Dunnett's test).

DISCUSSION

Worldwide cardiovascular disease (CVD) is the leading cause of death. Hypertension is the most common cardiovascular disease and a major public health problem in both developed and developing countries [18].

The present study demonstrated the significant antihypertensive effect of *Boerhaavia diffusa* in adrenaline-induced hypertensive model. In this study, hypertension was induced in rats by administration of adrenaline (0.5 mg/kg, i.p.) for 10 consequent days. After induction of hypertension, MEBD was administered at doses of 100, 200 and 300 mg/kg for 20 days. The biochemical parameter like serum glucose, cholesterol and triglyceride were measured. The systolic BP was determined by in non-invasive and invasive methods. Heart rate, mean arterial blood pressure and pulse pressure were also determined.

The hypertension induced by adrenaline is due to activation of α and β- receptors. It is mixed agonist for α and β- receptors. Adrenaline induced hypertension is due to different molecular mechanisms *viz* adrenaline, in blood vessels bind to α_1-adrenergic receptors which are G-protein coupled (Gs) receptors leads to activate phospholipase-c (PL-C) which convert phosphoinositol diphosphate (PIP_2) to inositol triphosphate (IP_3) and Diacyl glycerol (DAG). DAG further activates protein kinase-C (PK-C) and leads to vasoconstriction. Similarly IP_3 increases calcium influx from endoplasmic reticulum leads to vasoconstriction. Vascular smooth muscle contraction caused by increased intracellular Ca^{++} is due to influx of extracellular Ca^{++} and release of Ca^{++} from endoplasmic reticulum by activation of IP_3 and ryanodine receptors.

Adrenaline acts on heart through β_1 receptors, which are G-protein coupled (Gs) receptors. Binding to β_1 receptors leads to activate adenylate cyclase (AC) which converts ATP to cAMP. cAMP activates protein kinase-A (PK-A) which increase calcium influx from endoplasmic reticulum. Released calcium combines with troponin

protein, Ca^{++} - troponin complex phosphorylates myosin light chain kinase (MLCK) enzyme leads to phosphorylation of myosin. Myosin-P combines with actin leading to contraction. Adrenaline increases force of contraction (positive inotropic effect) and frequency (positive chronotropic effect) leading to increase in cardiac output and blood pressure [19]. Adrenaline, in heart and blood vessels also directly activates natriuretic peptide and vasopressin which activate calcium channel in endothelium causes release of calcium and increase calcium influx, lead to vasoconstriction result in increase in blood pressure [19, 20]. Adrenaline binds to β_1 receptors present on juxtaglomerular apparatus (cells) of kidney, release renin which activates renin-angiotensin-aldosterone system (RAS) by activating ACE enzyme, convert angiotensin-I to angiotensin-II leads to vasoconstriction result in increase in blood pressure [21].

The result of the present study demonstrated that administration of adrenaline (0.5 mg/kg, i.p.) for 10 consequent days significantly ($p < 0.01$) increased serum glucose level, systolic blood pressure, heart rate, mean arterial pressure and pulse pressure, indicating hypertension in rats. MEBD at 200 and 300 mg/kg showed significant ($p < 0.01$) decrease in serum glucose level. The cholesterol level was reduced at 300 mg/kg ($p < 0.05$). No significant change was observed in triglyceride level.

Adrenaline significantly ($p < 0.001$) increased the systolic blood pressure. MEBD at all doses (100, 200 and 300 mg/kg) showed significant ($p < 0.01$) decrease in systolic blood pressure measured by both non-invasive and invasive methods, demonstrating antihypertensive activity of *Boerhaavia diffusa*. Adrenaline significantly ($p < 0.01$) increased the heart rate, mean arterial pressure and pulse pressure. Administration of MEBD normalized the heart rate and significantly decreased mean arterial pressure and pulse pressure, suggesting the effect of *Boerhaavia diffusa* on α and β receptors. The reduction in blood pressure and heart rate may be due to blockage of α and β receptors. Propranolol was used as a standard antihypertensive agent.

Further to clarify the mechanism of action, the effect of *Boerhaavia diffusa* was studies on vascular reactivity to various catecholamines like adrenaline, noradrenaline and phenylephrine. Adrenaline treated group showed a significant ($p < 0.01$) rise in mean change in BP to various catecholamines. The increased vascular sensitivity to these catecholamines was observed, this may be due to altered sympathetic activity. MEBD (200 and 300 mg /kg) significantly normalized the sensitivity to all catecholamines, indicating decreased sympathetic activity mediated through α and β receptors.

The reduction of blood pressure by administration of MEBD for 20 days may be due to the decreased sympathetic activity or increased vasodilating substances, which is further supported by in vitro study.

Acetylcholine-induced vasorelaxation study was done on isolated rat aorta. Aortic ring were prepared without damaging endothelial layer. The aortic rings were exposed to PE. The contractile response to PE was plateaued, and then vasorelaxant response of acetylcholine was recorded in a cumulative fashion [17].

The aorta of vehicle group showed normal relaxant response to cumulative doses of acetylcholine, showing normal endothelial function. Treatment with adrenaline showed significant impairment of relaxation. Administration of MEBD showed significant improvement in relaxant response, suggesting vasorelaxation of aortas. MEBD antagonize the contraction produced by PE. This suggests that the action of MEBD may be directly on vascular smooth muscle cells to induce relaxation or by endothelium derived vasodilator factors such as nitric acid (NO). The results of the present study indicated that *Boerhaavia diffusa* possesses significant antihypertensive activity.

Boerhaavia diffusa contains various chemical constituents like punarnavin, boeravinone, punarnavoside, lirodendrin, b-sitosterol, a-2-sitosterol, Hentriacontane, b-Ecdysone, triacontanol etc. Boeravinones *viz.* boeravinone A, boeravinone B, boeravinone C, boeravinone D, boeravinone E and boeravinone F and Punarnavoside (a phenolic glycoside). The presence of these chemical constituents might be responsible for significant antihypertensive activity of the plant.

CONCLUSION

Boerhaavia diffusa showed antihypertensive potential may be due to decreased sympathetic activity mediated through α and β- receptors or by release of vasodilatory substances such as endothelium dependent relaxing factor (EDRF) which is NO. Thus, this study indicates the beneficial role of *Boerhaavia diffusa* in the treatment and prevention of hypertension.

ACKNOWLEDGEMENT

Authors are grateful to Science and Engineering Research Board (SERB), New Delhi for providing financial assistance.

REFERENCES

1. Rout SK. Antihypertensive therapy: The concepts of management with herbal and synthetic agents for pulmonary hypertension. *International J of Pharmaceutical Science Review and Research*, 3(2), 2012, 13-18.
2. Patrica MK, Megan W, Kristi R, Paul KW, Jiang H. Worldwide prevalence of hypertension: a systematic review. *J of Hypertension*, 22 (1), 2004, 11-19.
3. Kearney PM, Whelton M, Reynolda K, Munter P, Whelton PK, Jiang H. Global burden of hypertension analysis of worldwide data. *The Lancet*, 365-(9455), 2005, 214-223.

4. Irchhaiyar K, Singh M. Morphology, phytochemistry and pharmacological profile of *Boerhaavia diffusa*. *International Journal of current Pharmaceutical Research*, 4(2), 2012, 4-8.
5. Jarald E, Nalwaya N. Sheeja E, Ahmad S, Jamalludin S. Comparative study on diuretic activity of few medicinal plants in individual form and in combination form. *Indian Drugs*, 47 (3), 2010, 20–24.
6. Wagh S, Vidhale N. Antimicrobial efficacy of *Boerhaavia diffusa* against some human pathogenic bacteria and fungi. *Biosciences Biotechnology Research Asia*, 7 (1), 2010, 267–272.
7. Olaleye MT, Akinmoladun AC, Ogunboye AA, Akindahunsi AA. Antioxidant activity and hepatoprotective property of leaf extracts of *Boerhaavia diffusa* L. against acetaminophen-induced liver damage in rats. *Food and Chemical Toxicology*, 48 (8–9), 2010, 2200–2205.
8. Manu KA, Kuttan G. Immunomodulatory activities of Punarnavine, an alkaloid from *Boerhaavia diffusa*. *Immunopharmacology and Immunotoxicology*, 31 (3), 2009, 377–387.
9. Sreeja S. An in vitro study on antiproliferative and antiestrogenic effects of *Boerhaavia diffusa* L. extracts. *Journal of Ethnopharmacology*, 126 (2), 2009, 221–225.
10. Sohni YR, Kaimal P, Bhatt RM. The antiamoebic effect of a crude drug formulation of herbal extracts against *Entamoeba histolytica in vitro* and *in vivo*. *Journal of Ethnopharmacology*, 45 (1), 1995, 43–52.
11. Singh RP, Shukla KP, Pandey BL, Singh RG, Ushana, Singh RH: Recent approaches in clinical and experimental evaluation of diuretic action of *Punarnava* (*B. diffusa*) with special reference to nephrotic syndrome. *Research Education Indian Medicine*, 1, 1992, 26–36.
12. Mishra J, Singh R. The effect of indigenous drug *Boerhaavia diffusa* on kidney regeneration. *Indian Journal of Pharmacology*, 12, 1980, 59–64.
13. Murti K, Mayank A, Lambole V. Pharmacological Properties of *Boerhaavia Diffusa* - A Review. *International Journal of Pharmaceutical Sciences Review and Research*, 5 (2), 2010, 20-24.
14. Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy, Nirali Prakashan, Pune, 31st ed, 2005, 593-597.
15. Marcin A. Reversal of Glomerulosclerosis after high –dose Enalapril treatment in subtotally nephrectomized rats. *Journal of American Society of Nephrology*, 14, 2003, 1833- 1842.
16. Vogel HG: Drug discovery and evaluation: Pharmacological assay. Springerverlag Berlin Heidelberg, New York 2002, 950.
17. Honda H, Ushijima D. A regional variation of Acetylcholine – induced relaxation in different segments of rat aorta. *Physiology and Behavior*, 63, 1998, 55-58.
18. Silagy CA, Neil HA. A meta-analysis of the effect of garlic on blood pressure. *Journal of Hypertension*, 12, 1994, 463- 468.
19. Rang HP, Dale MM, Ritter JM, Flower RJ, Henderson G. Rang and Dale's Pharmacology, 7th ed., Elsevier Chuechill Livingstone, Spain, 2012, 251-259, 266-70.
20. Tripathi KD. Essentials of Medical Pharmacology, 6th ed., Jaypee Brothers Medical Publishers (P) Ltd., New Delhi, 2008, 116-123.

    Barar FSK. Essentials of Pharmacotherapeutics. 3rd ed. Reprint. S. Chand & Company Ltd, New Delhi, 2005, 239-241.