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Research Article

EVALUATION OF ANTIHYPERTENSIVE ACTIVITY OF *EVOLVULUS ALSINOIDES* IN ADRENALINE INDUCED HYPERTENSIVE RATS

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ABSTRACT

Objective: To evaluate antihypertensive effects of methanolic extract of *Evolvulus alsinoides* herb (MEEA) in adrenaline induced hypertensive rats.

Methods: MEEA of authentified sample was prepared using soxhlet extraction using methanol as an extracting solvent. Antihypertensive effect of MEEA was investigated using adrenaline induced hypertension in wistar rats. Hypertension like condition i.e. raised in systolic and diastolic blood pressure was induced in rats by i.p. administration of adrenaline (0.5 mg/kg/100 µL). % inhibition in systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP) and pulse pressure (PP) were measured in MEEA (600 mg/kg, i.p.); propranolol, nifedipine and enalapril (standards) using tail-cuff apparatus with BIOPAC power lab to evaluate antihypertensive effect.

Results: Induction in SBP, DBP, MABP and PP were significantly decreased in MEEA treated rats as compared to disease control group *(p<0.001/ p<0.01/ p<0.05)*. % inhibition in SBP, DBP, MABP and PP were significantly increased in MEEA, propranolol, nifedipine and enalapril treated rats as compared to disease control group *(p<0.001/ p<0.01/ p<0.05)*.

Conclusion: MEEA possess significant antihypertensive activity in adrenaline induced hypertensive rats.

Keywords: MEEA, Antihypertensive effect, Adrenaline, BIOPAC power lab.

INTRODUCTION

Hypertension is the most common cardiovascular illness and is a major public health issue in developed as well as in developing countries. Hypertension (HTN) or high blood pressure, sometimes called arterial hypertension, is a chronic medical condition in which the blood pressure in the arteries is elevated. Normal blood pressure at rest is within the range of 100-140mmHg systolic and 60-90mmHg diastolic. High blood pressure is said to be present if it is persistently at or above 140/90 mmHg. [1]

World Health Organization (WHO) has carried out epidemiological studies in India in between 1995 to 2006. According to this, prevalence of hypertension is 29.3% in men and 25.2% women has been found at the end of 2006. In India cardiovascular diseases cause 1.5 million deaths annually. Deaths due to hypertension arise from cerebrovascular and cardiovascular complications such as stroke, end-stage renal disease, congestive heart failure, myocardial infarction and cardiac arrest. Hypertension is directly responsible for 57% of all stroke deaths and 24% of all coronary heart disease deaths. This fact is important because hypertension is a controllable disease and a population wide 2 mmHg decrease in BP can prevent 151,000 stroke and 153,000 coronary heart disease deaths. [2]

Hypertension involves mainly two types, essential and secondary. The pathogenesis of essential hypertension is multi factorial and highly complex which will be caused by increase in sympathetic nervous system activity, increase in production of sodium-retaining hormones and vasoconstrictors, deficiencies of vasodilators such as prostacycline and nitric oxide, inappropriate or increased renin secretion and genetic predisposition while pathogenesis of secondary hypertension will be caused by chronic kidney disease, renovascular disease, Cushing's syndrome, pheochromocytoma, drugs such as non-steroidal anti-inflammatory drugs and oral contraceptives. [1]

Symptoms associated with high blood pressure can include, shortness of breath (dyspnea), fatigue, dizziness or fainting spells (syncope), chest pressure or pain, bluish color to lips and skin (cyanosis), racing pulse or heart palpitations, headache and nosebleeds. [1]

Normally, hypertension is diagnosed by physical history, laboratory tests, sphygmomanometer and digital blood pressure monitor. [1]

Although, many antihypertensive allopathic remedies are readily available to prevent and manage the hypertension, but many of this drugs cause serious and life threatening adverse effects. Synthetic antihypertensive like diuretics cause muscle cramps, dehydration, extreme tiredness, skin rash, blurred vision and abnormal heart rate, ACE inhibitors cause cough, kidney failure, skin rash and fever, calcium channel blockers cause fatigue, skin rash, constipation and edema, β-blockers cause bronchospasm, Reynaud's syndrome, heart failure and postural hypotension, as β-blockers cause bronchospasm so contraindicated in asthma, others like centrally acting drugs cause sexual dysfunction. In addition, all antihypertensive drugs are contraindicated during pregnancy accept methyldopa. Other major drawback of synthetic anti-hypertensive is that most of these drugs are very costly. [2]

Use of appropriate herbals causes least or no side effects with multiple actions and cost is also relatively low. Scientific studies suggest different lifestyle changes and use of appropriate herbal medicine use in the management of hypertension. *Rauwolfia serpentine*, *Ginkgo Biloba*, *Allium sativum* and *Crocus sativus* are the most popular herbs are used for management of hypertension today. The way they work is that they improved blood circulation by dilating the arteries. [3]

Modern science has already, accepted the potential of the herbs as a source of new bio-active constituents. There are numerous plants-derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine, including Ayurveda, Homeopathy and Unani system of medicine.

The recent development of science of phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines in various diseases. [4]

Looking at the dire need of a new, safe and economical antihypertensive molecule, we resolved to investigate beneficial effects of Evolvulus alsinoides commonly known as shankhpushpi belongs to family Convolvulaceae responsible for its antihypertensive activity.

In Ayurvedic literature, entire plant of *Evolvulus alsinoides* is reported for its beneficial effects in hypertension. [5, 6] These drugs also act as Ayurvedic rasayana because these contain many of therapeutic phyto-chemical constituents (i.e. glycosides, alkaloids, flavanoids, tannin, resins etc.) with no adverse effects. [7] Whole herb

of *Evolvulus alsinoides* also possesses brain-tonic (memory stimulant) [8], immunomodulatory, antioxidant activity, diuretic, gastro protective, antibacterial [9], anthelmintic, adaptogenic (anti-stress) and anti-amnesic activities without any side effects [9].

Evolvulus alsinoides have no significant adverse effects as compare to other antihypertensive herbals, they do not interfere with synthetic drugs during hypertension treatment and any other disease treatment, they also prevent cardiovascular complications which accelerate hypertension additionally both drugs are also available at low costs [7].

MATERIALS AND METHODS

Collection and authentification of plants

Evolvulus alsinoides herb was collected from the medicinal garden of R. K. College of Pharmacy, Rajkot. Then was authentified by Department of Botany, Christ College, Rajkot.

Preparation of plant extracts

The collected whole plant of *Evolvulus alsinoides* was subjected to dry to brittle material at 60°C in hot air oven to remove moisture. This dried herb was subjected for size reduction using mixer grinder and comminuted to very fine powder. Methanolic extracts of *Evolvulus alsinoides* were prepared using methanol as a solvent in soxhlet apparatus. [10]

Selection of animals

Either sex Wistar albino rats (n=6) of weighing 220-300 g were used for the present study. The animals were procured from animal house, Department of Pharmacology, R. K. College of Pharmacy, Rajkot, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±20˚C and relative humidity of 30 – 70 %. A light and dark cycle was followed. All animals were fed on standard balance diet and provided with water *ad libitum*.

All the experimental procedures and protocols used in study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of R. K. College of Pharmacy and care of laboratory animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

In vivo anti-hypertensive study using adrenaline induced hypertensive rats (Non-invasive Method)

Method to induce hypertension by adrenaline

Rats were anaesthetized with diethyl ether and 0.1 ml of adrenaline was injected into rats by intraperitoneal (I.P) injection using a 1 ml disposable syringe for 10 consecutive days to induce hypertension. To confirm the induction of hypertension, different hemodynamic parameters were measured by using Non-invasive tail cuff method with BIOPAC power lab. [11]

Trial design (Groups and receivers)

Control: Dimethyl sulfoxide (DMSO) (1% v/v) (as a vehicle) [10]

Disease control: Adrenaline (0.5 mg/kg/100 µL, i.p) [12]

Standard:

1. Propranolol inj. (10 mg/kg, i.p.)[11]
2. Nifedipine tab. (6 mg/kg, i.p.; with DMSO) [13]
3. Enalapril inj. (48 mg/kg, i.p.) [14]

Test: Methanolic extract of *E. alsinoides* (600 mg/kg i.p) [15]

Method to measure hemodynamic blood pressure parameters using BIOPAC Power lab with tail cuff apparatus

After administration of dose to animals, blood pressure was measured by Non-invasive Tail cuff method using pressure meter (NIBP250 and NIBP200) [16] (Fig. 3 and Fig. 4). The rat was kept in strainer and the tail cuff was applied on the tail of rat for determination of blood pressure. Normal blood pressures of all the rats were recorded as baseline blood pressure. After that the animals were treated with respective treatment and again blood pressure was recorded as after drug treatment. The blood pressure as SBP (systolic blood pressure), DBP (Diastolic blood pressure), were displayed on monitor were recorded and other hemodynamic parameters like MABP (Mean Arterial Blood Pressure) and PP (Pulse pressure) were calculated using equations [(SBP-DBP)/3] + DBP [17] and SBP-DBP [17] respectively. To evaluate anti-hypertensive effect of drugs, adrenaline was injected after 5 minutes. Again the blood pressure was recorded and the difference between baseline blood pressure and blood pressure after adrenaline treatment were calculated and compared. [18, 19]

Statistical analysis

To check the significance of data, following statistical tests were performed:

ANOVA: to see the variability within all the groups.

INSTAT software: to derive all the statistical terms like Standard Error of Mean (SEM), ANOVA, *p* – value, Degree of freedom, Standard deviation, etc.

RESULTS

Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) were measured in normal rats with respective before and after drug treatment using tail-cuff apparatus with BIOPAC. Induction of SBP and DBP as well as % inhibition in SBP and DBP were measured which indicated antihypertensive action of methanolic extract of *Evolvulus alsinoids* herb which was compared with disease control group and with propranolol, nifedipine and enalapril. (Table 1 and Table 2)

Table 1: Systolic blood pressure in adrenaline induced hypertensive rats.

Groups	Before treatment SBP (mmHg)	After Adrenaline SBP (mmHg)	Induction of SBP (mmHg)	% inhibition in SBP
Normal Control	134	-	0	-
Disease Control	113.67	162	48.33	0
Propranolol	137	150.83	13.83	70.57
Nifedipine	153	175.67	22.67	53.10
Enalapril	157.17	184	26.83	44.40
Evolvulus alsinoides	136.5	162.33	26.33	45.50

Table 2: Diastolic blood pressure in adrenaline induced hypertensive rats.

Groups	Before treatment SBP (mmHg)	After Adrenaline SBP (mmHg)	Induction of DBP (mmHg)	% inhibition in DBP
Normal Control	95	-	0	-
Disease Control	102.67	130	27.33	0
Propranolol	109.33	123	13.67	49.57
Nifedipine	115.83	135.67	19.83	26.87
Enalapril	116.5	139.5	23	15.71
Evolvulus alsinoides	113	134.83	21.83	19.73

From previous observation, induction of SBP and DBP in normal control, disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were measured, in which induction of SBP and DBP in standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were significantly decreased as compared to disease control group which indicate that all three standard drugs and test drugs decrease in systolic blood pressure. (Table 3)

Table 3: Induction of systolic blood pressure and diastolic blood pressure in adrenaline induced hypertensive rats.

Groups	Induction of Systolic Blood Pressure (mmHg)	Induction of Diastolic Blood Pressure (mmHg)
Normal Control	0	0
Disease Control	48.33 ± 0.84#	27.33 ± 0.71#
Propranolol	13.83 ± 0.83***	13.67 ± 1.05***
Nifedipine	22.67 ± 0.61***	19.83 ± 1.08***
Enalapril	26.83± 1.74***	23 ± 1.21*
Evolvulus alsinoides	26.33 ± 0.88***	21.83± 0.65***
F	***256.84***	***117.21***
df	***41 (6, 35)***	***41 (6, 35)***
p	***< 0.001***	***< 0.05***

n = 6 and results were shown as mean ± SEM

indicate significant difference in the data compared to control group and the level of significance was $p<0.05$ ≈ significant

*** indicate significant difference in the data disease compared to control group and the level of significance was $p<0.001$ ≈ highly significant

* indicate significant difference in the data compared to disease control group and the level of significance was $p<0.05$ ≈ significant

Induction of Mean Arterial Blood Pressure (MABP) and Pulse Pressure (PP) in normal control, disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were calculated by [(SBP-DBP)/3] + DBP and SBP-DBP equations respectively, in which induction of MABP and PP in standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) in significantly decreased as compared to disease control which indicate that all three standards an test drug decrease mean arterial blood pressure as well as pulse pressure. (Table 4)

Table 4: Induction of mean arterial blood pressure and pulse pressure in adrenaline induced hypertensive rats.

Groups	Induction of Mean Arterial Blood Pressure (mmHg)	Induction of Diastolic Blood Pressure (mmHg)
Normal Control	0	0
Disease Control	29.50 ± 0.54#	29.50 ± 0.54#
Propranolol	13.33 ± 0.74***	13.33 ± 0.74***
Nifedipine	20.72 ± 0.75***	20.72 ± 0.75***
Enalapril	23.89 ± 0.99***	23.89 ± 0.99*
Evolvulus alsinoides	23.33 ± 0.52***	23.33 ± 0.52**
F	***237.85***	***11.643***
df	***41 (6, 35)***	***41 (6, 35)***
p	***< 0.05***	***< 0.05***

n = 6 and results were shown as mean ± SEM

indicate significant difference in the data compared to control group and the level of significance was $p<0.05$ ≈ significant

*** indicate significant difference in the data disease compared to control group and the level of significance was $p<0.001$ ≈ highly significant

** indicate significant difference in the data disease compared to control group and the level of significance was $p<0.01$ ≈ moderately significant.

* indicate significant difference in the data compared to disease control group and the level of significance was $p<0.05$ ≈ significant

Table 5: % inhibition in systolic blood pressure and diastolic blood pressure in adrenaline induced hypertensive rats.

Groups	% inhibition in Systolic Blood Pressure (%)	% inhibition in Diastolic Blood Pressure (%)
Disease Control	0	0
Propranolol	70.57 ± 1.77***	49.57 ± 4.69***
Nifedipine	53.10 ± 1.04***	26.87 ± 5.43**
Enalapril	44.40 ± 3.75***	15.71 ± 4.50*
Evolvulus alsinoides	45.50 ± 1.71***	19.73 ± 3.70*
F	***136.86***	***13.385***
df	***35 (5, 30)***	***35 (5, 30)***
p	***< 0.001***	***< 0.05***

% inhibition in SBP and DBP in disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were measured. % inhibition in SBP and DBP in disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were significantly increase as compared to disease control group which indicate that standard and test drugs decrease in systolic blood pressure. (Table 5)

% inhibition in MABP and PP in disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were calculated by [(SBP-DBP)/3] + DBP and SBP-DBP

equations respectively. % inhibition in MABP and PP in disease control, standard (propranolol, nifedipine, enalapril) and test groups (*Evolvulus alsinoides*) were significantly increased as compared to disease control which indicate that standards and test drugs decrease mean arterial blood pressure as well as pulse pressure. (Table 6)

Table 6: % inhibition in mean arterial blood pressure and pulse pressure in adrenaline induced hypertensive rats.

Group	% inhibition in Mean Arterial Blood Pressure (%)	% inhibition in Pulse Pressure (%)
Disease Control	0	0
Propranolol	56.83 ± 3.17***	30.64 ± 4.70***
Nifedipine	35.28 ± 3.71***	26.72 ± 5.51**
Enalapril	25.27 ± 3.38***	25.99 ± 4.08*
Evolvulus alsinoides	28.31 ± 2.37***	25.77 ± 4.52*
F	***41.551***	***7.641***
df	***35 (5, 30)***	***35 (5, 30)***
p	***< 0.001***	***< 0.05***

n = 6 and results were shown as mean ± SEM

*** indicate significant difference in the data disease compared to control group and the level of significance was $p<0.001$ ≈ highly significant

** indicate significant difference in the data disease compared to control group and the level of significance was $p<0.01$ ≈ moderately significant.

* indicate significant difference in the data compared to disease control group and the level of significance was $p<0.05$ ≈ significant

DISCUSSION

Hypertension is common debilitating illness among the people in both developed and developing countries. Community surveys in industrialized countries have shown a prevalence of 15-33 % in people aged 30 years. The disease continues to be a leading cause of morbidity and mortality from coronary artery disease and stroke. [1, 2]

Fortunately, antihypertensive drugs are available to reduce blood pressure to normal level which is necessary to manage cardiovascular disease, coronary heart disease and other cardiovascular related complication. [2]

In this respect, herbal drugs are helpful and render encouraging results in comparison to synthetic drugs due to their fewer or no side effects and easy availability. [3]

The screening of various plants according to their traditional uses and nutritional value based on their therapeutic value leads to discovery of newer and safer alternative for management of hypertension. One of such plant of medicinal value is *Evolvulus alsinoides* herb, belongs to family Convolvulaceae which is commonly known as shankhpushpi. This drug also acts as Ayurvedic rasayana because it contains many of therapeutic phyto-chemical constituents (i.e glycosides, alkaloids, flavanoides, tannin, resins etc.) with no adverse effects. [7]

Pilot *in vivo* study suggested that the antihypertensive activity was highest at dose of 600 mg/kg in methanolic extracts of both the plants.

Antihypertensive effects *Evolvulus alsinoides* herb was evaluated by adrenaline induced hypertensive model. In present study mild to moderate hypertension was induced in rats by 0.1 ml intraperitoneal injection of adrenaline. [11]

Hypertension was induced in rats by adrenaline because of three molecular mechanisms of adrenaline (1) Adrenaline, in blood vessels binding to G-protein coupled (Gs) α1-adrenergic receptors leads to activate phospholipase-c (PL-C) which convert phosphoinositol diphosphate (PIP_2) to inositol triphosphate (ISP_3) and Diacyl glycerol (DAG). This DAG will go into endocrine gland leads to activate protein kinase-C (PK-C) and leads to vasoconstriction similarly ISP_3 increases calcium influx from endoplasmic reticulum leads to vasoconstriction and in heart binding with G-protein coupled (Gs) to β_1 receptors leads to activate adenylate cyclase (AC) which convert ATP to cAMP leads to activate protein kinase-A (PK-A) cause phosphorilation of calcium channel and increase calcium influx from endoplasmic reticulum leads increase in force (positive inotropic effect) and frequency (positive chronotropic effect) by this way adrenaline leads to vasoconstriction and increase in force and frequency to the heart, result in increase in blood pressure. [20, 21] (2) Adrenaline, in heart and blood vessels directly activate 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. [22] (3) Adrenaline binds to β_1 receptors present on juxtaglomerular apparatus (cells) of kidney, lead to activate adenylate cyclase (AC) which convert ATP to cAMP leads to activate protein kinase-A, produces renin release which activate Renin Angiotensin Aldosterone System (RASS) by activating ACE enzyme, convert angiotensin-I to angiotensin-II leads to vasoconstriction result in increase in blood pressure. [23]

SBP and DBP were significantly increased in adrenaline treated rats as compared to normal rats in tail-cuff with BIOPAC instrument; simultaneously derived hemodynamic parameters like mean arterial blood pressure (MABP) and pulse pressure (PP) were also increased in adrenaline treated rats. Due to previous three mechanisms of adrenaline, β-adrenergic blocker-propranolol, calcium channel blocker-nifedipine and angiotensin converting enzyme (ACE) inhibitor-enalapril were used as standards in this study. [11, 13]

Results data suggested that blood pressure (i.e. SBP, DBP, MABP and PP) were significant decreased in different standard groups and in plant extract as compared to disease control group.

CONCLUSIONS

The present study revealed that methanolic extract of *Evolvulus alsinoides* herb possessed profound antihypertensive activity in adrenaline induced hypertensive model.

The intake of *Evolvulus alsinoi*des herb extract as medicine might have potential benefits in management of hypertension. Concominent administration of the extracts might be helpful in better management of hypertension along with available anti-hypertensive without any interference.

Recommendation for further study

Further research is required on isolation, characterization and purification of active constituent which is responsible for antihypertensive activity.

Future aspects of this study include preparation of suitable formulation as well as bulk manufacturing of same at industrial scale.

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