Research Article

Investigation of Antihypertensive activity of Leaves of *Barleria Prionitis* in Doca Salt Induced Hypertensive Rats

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ABSTRACT

The present study was designed to determine the antihypertensive activity of methanolic extracts of leaves of *barleria prionitis* linn using DOCA salt induced antihypertensive model. The plant material was extracted using solvent methanol. *Barleria prionitis* L. (Family Acanthaceae; commonly known as Vajradanti), has a great potential against various disorders. For evaluation of this activity male albino wistar rats were uninephrectomized and randomly divided into five groups. Hypertension was induced by injecting DOCA-salt, 25 mg/kg BW subcutaneously, twice a week for six weeks, with NaCl 1% instead of tap water for drinking throughout the study. Systolic and diastolic Blood pressure was measured every week. *B.prionitis* showed significant anti hypertensive effect in DOCA salt induced hypertensive rats in dose of 200mg/b.w and 400mg/b.w.

Keywords: Antihypertensive effects, Uninephroctomy, DOCA salt, *B.prionitis*

INTRODUCTION

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. This requires the heart to work harder than normal to circulate blood through the blood vessels. Hypertension is one of the risk factors for cardiovascular diseases (CVD), the leading cause of death in developed countries[1]. This study was specifically carried out to evaluate the effect of crude ethanolic extract of *barleria prionitis*.

Barleria prionitis L. (Family Acanthaceae; commonly known as Vajradanti), is an annual shrub, found throughout tropical Asia and in South Africa[2]. Leaves and roots of *B.Prionitis* are used for a variety of purposes in traditional Indian medicine. The aerial parts (stem, leaves & flower) are used in catarrhal affections of children, glandular swellings, boils, fever, toothache, inflammation & gastrointestinal disorders[3-4], bark in whooping cough as an expectorant; the whole plant and especially the roots are used as tonic[5-6]. Leaves, stem and root of *B. prionitis* possess antibacterial and anti-inflammatory activities. Iridoid enriched fraction of aerial parts (leaves and stems) was showed hepatoprotective activity in various acute and chronic animal models [7]. Juice of the leaves is used in ulcer and fever. Plant is also used in stiffness of limbs, enlargement of scrotum and sciatica[8-9]. Balarenone, prioniside A, prioniside B and prioniside C has been isolated from the ethanolic extract of B. prionitis[10]. 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester, barlerin, acetylbarlerin, 7-methoxydiderroside, lupulinoside has been also isolated from the aerial parts[11-12].

Barleria prionitis has no significant adverse effects as compare to other antihypertensive herbals and also available at low costs. The present study is aimed at determining the antihypertensive effect of *B.Prionitis* in hypertensive animal models to substantiate the clinical findings.

MATERIALS AND METHODS

Plant Collection and authentication

Fresh leaves are collected during the flowering and fruiting stage of *B.Prionitis* from the Surendranagar district, Gujarat. The collection was made by picking out leaves. The plant was authenticated by Dr. H. B. Singh, Scientist and Head, Raw Materials Herbarium and

Museum, National Institute of Science and Communication and Information Resources, New Delhi (NISCAIR) deposited this plant at NISCAIR, New delhi.

Preparation of plant extract

The collected leaves of *B.Prionitis* were subjected to dry to brittle material at 60°C in hot air oven to remove moisture. These dried roots were subjected for size reduction using mixer grinder and comminuted to very fine powder. Methanolic extracts of *B.Prionitis* was prepared using methanol as a solvent in soxhlet apparatus.

Selection of animals

Either sex Wistar albino rats (n=6) of weighing 220-300 g were used for the present study. 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 C. U. Shah. 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).

Drug and chemicals

DOCA and dimethyl formamide were purchased from Sigma-Aldrich Chemical Company, St. Louis, Missouri, USA. All other chemicals used in this study were of highest analytical grade obtained from Sisco Research Laboratories (SRL) or Himedia, Mumbai, India.

***In vivo* anti-hypertensive study using DOCA salt induced hypertensive rats (Non-invasive method)**

Method of uninephrectomy

Left uninephrectomy was performed on all rats. Rats were anaesthetized with intraperitonial injection of ketamine (75 mg/kg bw), kidney was visualized two 1-cm incisions were made at midscapular region, one on the skin and other on the body cavity, respectively. The kidney was freed from the surrounding tissues and pulled out gently. The adrenal glands, which is attached loosely to the anterior pole of the kidney by connective tissue and fat, was gently freed by tearing the attachments, and was put back into the abdominal cavity. The renal artery and ureter were tied by silk thread, and then the kidney was removed. The muscle and skin layers were closed separately by using achromic sterile absorbable suture[13].

Experimental induction of hypertension

Animals were given, weekly twice, subcutaneous injections of DOCA (25 mg/kg BW) in dimethyl formamide (vehicle) solution, and salt was administered by substitution of 1% NaCl solution for drinking water *ad libitum* throughout the experimental period.

DOCA-salt induced hypertensive rats

The rats were randomly divided into six groups of six rats each. Group one served as normal control and group two served as diseased control treated with DOCA-salt hypertension control. Group III as Enalapril inj. (48 mg/kg, i.p.), groups IV and V were hypertensive rats which received different doses of B.prionitis, 200 and 400 mg/kg BW and group VI received nifedipine 20 mg/kg BW. Test drugs or nifedipine were administered orally once daily for 6 weeks.

Group I (Control): Water (as a vehicle)

Group II (Disease control): DOCA salt (25 mg/kg, s.c. twice weekly for 43 days)

Group III (Standard): Enalapril inj. (48 mg/kg, i.p.)

Group IV (Test) : Methanolic extract of *B.Prionitis* (200 mg/kg i.p)

Group V (Test) : Methanolic extract of *B.Prionitis* (400 mg/kg i.p)

At the end of 6th week, all the rats were anesthetized with intramuscular injection of ketamine and sacrificed by cervical dislocation. Blood was collected in two different tubes, i.e., one with anticoagulant for the separation of plasma and another without anticoagulant for the serum. Plasma and serum were separated by centrifugation [14-15].

Blood pressure measurements

Systolic and diastolic blood pressure was determined by the tail-cuff method (IITC, model 31, Woodland Hills, CA, USA) from 0th day to 6 weeks. The tail cuff approach to determine arterial blood pressure requires certain precautions such as reduction of stress of the animals, appropriate training of rats over multiple days and adequate prewarming to dilate the tail artery. The animals were placed in a heated chamber at an ambient temperature of 30–34°C for 15 min, and from each animal, 1–9 blood pressure values were recorded. The lowest three readings averaged to obtain a mean blood pressure[16].

Statistical analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The significance level was set at $p \leq 0.05$

RESULTS AND DISCUSSION

25 mg/kg s.c. Deoxycorticosterone acetate (DOCA) was injected into nephrectomised rats produced moderate hypertension. When all rats were put in tail-cuff apparatus with AD instrument, they were displayed Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) according to respective treatment in normal control, disease control, standard (enalapril) and test groups (*B.Prionitis*) respectively, which were observed (Table 1). SBP in normal control, disease control, standard (enalapril) and test groups were measured. There were significant reduction in SBP were found in standard (enalapril) and test groups (*B.Prionitis*) as compared to diseases control group. The systolic and diastolic blood pressure were considerably ($P<0.05$) increased in DOCA-salt hypertensive rats. Oral administration of test groups for a period of six consecutive weeks considerably ($P<0.05$) decreased systolic and diastolic blood pressure in DOCA-salt treated rats (groups 3, 4 and 5). The effect exerted by 400 mg/kg bw of *B.Prionitis* was better than the 200mg dose of *B.Prionitis*.

Table 1: Antihypertensive effect of methanolic extracts of *B.Prionitis leaves* in rats.

	Systolic blood pressure (mm Hg)		Diastolic blood pressure(mm Hg)	
Group	**0 week**	**6th week**	**0 week**	**6th week**
Normal Control	128.5±0.90	135.5±2.05	83.5±1.30	99.5±1.45
Disease Control	178±2.22	231.5±4.89	104.5±2.94	123.5±3.57
Enalapril	131.5±1.99**	136.5±2.51**	87.5±1.88**	103±2.54**
Barleria prionitis	132±3.57*	146±2.21**	92.5±2.26*	100.5±2.74*
Bareleria prionitis	128±1.42**	143±3.11**	89±1.57**	105.5±2.35**

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, considered for significance.

CONCLUSION

DOCA salt induces reabsorption of salt and water leading to increased blood volume and hence increased blood pressure. SBP and DBP were increased persistently in DOCA salt treated nephrectomised rats as compared to normal rats and *B.Prionitis* extracts. *B.Prionitis* revealed the presence of alkaloids, flavonoids, steroids, saponins, tannin & phenolic compounds, because of these compounds the plants shows significant antihypertensive activity. The present study revealed that methanolic extracts of *B.Prionitis* possessed profound antihypertensive activity. Further research work is required for biofragmentation and isolation of constituents which is responsible for its antihypertensive activity.

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