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Research Article

THE ANTIHYPERTENSIVE EFFECT OF METHANOLIC EXTRACT OF *HYGROPHILA SPINOSA* IN RATS

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Abstract: Objective: To evaluate the effect of methanolic extract of Hygrophilaspinosa (Acanthaceae) in acute renal hypertension in rats. Materials and methods: Animals were divided into five groups of eighteen animals in each group.Methanolic extract of Hygrophilaspinosa (HSME) (250 and 500 mg/kg body weight), were administered orally to male wistar albino rats. At the end of treatment, animals were undergone left renal artery occlusion for 4 hrs on 7th, 14th and 21st day. The mean arterial blood pressure was measured using BIOPAC student lab data acquisition system. Results: Occlusion of left renal artery of rats induced acute renal hypertension. Mean arterial B.P. of rats of 2K1C group was found to be significantly ($P<0.01$) increased as compared to control group. Pretreatment with HSME 250 and HSME 500 mg/kg significantly ($P<0.05$, $P<0.01$) prevented this increase in comparison with 2K1C group. Conclusion: The results indicate that the aerial parts of Hygrophilaspinosa are endowed with antihypertesiveactivity, thereby justifying its traditional claim and augmenting it into the present day systems of medicine.
Key words: *Hygrophilaspinosa*, Acuterenal hypertension, Biopac

INTRODUCTION

Hypertension is one of the leading causes of disability, mortality and morbiditythroughout population. It is the most common chronicillness among the world faces. Hypertension is the most common cardiovascular diseases and constituents a major factor for several cardiovascular pathologies including atherosclerosis, coronary artery disease, myocardium infract, heart failure, renal insufficiency, stroke and dissecting aneurysm of aorta. An elevated arterial pressure is an important public health issue in developed countries. Although it is common, asymptomatic and readily detectable but it can often lead to lethal complication, if left untreated. The method in practice to control high BP is 'long-term' drug therapy. Drugs have side effects that can create more clinical problems than are solved. That is why medical professionals worldwide are seeking non-drug treatment and preventative strategies. Recently attention has been focused towards herbal preparations which traditionally used as potential therapeutic agents in the prevention and management of cardiovascular diseases[1].The present study is an attempt to screen the methanol extract of *H. spinosa*for its antihypertensive activity. *H. spinosa*(K. Schum) Heine (syn.) *Asteracanthalongifolia* Nees, Acanthaceae are described in ayurvedicliterature as Ikshura, Ikshugandha and Kokilasha "having eyeslike the Kokila or Indian Cuckoo". The plant is widely distributedthroughout India, Srilanka, Burma, Malaysia and Nepal. Theleaves,roots, seeds and ashes of the plant are extensively used in traditionalsystem of medicine for various ailments like jaundice,hepatic obstruction, rheumatism, inflammation, pain, urinaryinfections, urinary calculi, edema and gout. It is classified in ayurvedic systemas seethaveeryam, mathuravipaka and used for the treatment of premeham (diabetes), athisaram (dysentery) [2, 3]. A literature survey revealed that *H. spinosa*is endowed with various chemical components such as flavonoids, steroids, polysaccharides, triterpenoids such as lupeol, saponins, etc. which possibly contribute to its diverse uses in folklore medicine. The plant is known to possess antitumor, hypoglycaemic, free radical scavenging and lipid peroxidation[4]. Hepatoprotective activity of seeds of *H. auriculata*in thioacetamide, paracetamol-induced liver damage in rats[5],ethylene glycol induced nephrolithiasis in rat[6]. The literature survey revealed that there are no scientific studies carried out regarding *H. spinosa*in antihypertensive activity. Hence the present study is focused to evaluate the antihypertensive activity in rats.

Animals

Male wistar rats, procured from commercial breeder, were kept for a week for acclimatization under environmentally controlled conditions with free access to standard food (Amrut Feed, Sangali, India) and water. Rats weighing 150-250 g were used for the experiments. All animal experiments were carried out according to the guidelines and approval of institutional animal ethic committee (IAEC).

Plant material

The whole plant *H. spinosa* syn. *Asteracanthalongifolia, H. schulli* (Acanthaceae) was collected in month of September from region of Indore, Madhya Pradesh, India and authenticated at Botanical Survey of India (BSI), Government of India, Ministry of Environment and Forests, Pune, India by Dr. P. G. Diwakar. A voucher specimen of the plant was deposited in the BSI herbarium under the number BSI/WC/Tech/2010/851. The

whole plant material was dried in shade and ground to get a coarse powder.

Preparation of extracts

Powder of dried *H. spinosa* was extracted with 70% methanol and concentrated. The concentrated mass was washed with petroleum ether several times to remove the resinous matter. Then the mass was filtered and concentrated under vaccum at temperature 60^0C, dried to get the powdered form of the extract. The methanolic extract of *H. spinosa* was stored in tightly closed glass bottle in refrigerator at 2-8 ^{0}C.

Preliminary phytochemical screening

Preliminary phytochemical screening[8]revealed the presence of phenolic compounds, steroids, alkaloids, flavonoids, and triterpenoids in the extract.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guideline 423 set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One-tenth of the median lethal dose (LD50) was taken as an effective dose[9].

Evaluation of HSMEin acute renal hypertension in rats

Animals were divided into five groups of eighteen animals in each group. The animals of all the groups were again subdivided into three groups on the basis of duration of treatment of 7, 14 and 21days comprising six animals in each group. Group I treated with (vehicle) distilled water was kept as normal. Group II (2K1C) treated with (vehicle) distilled water was kept as untreated group. Group III and IV were treated with methanolic extract of hygrophilaspinosa(HSME) with 250 and 500 mg/kg b. w. p.o. respectively and group V received ramipril 1 mg/kg b.w. i.v. All the treatments were given for 7, 14 and 21 days as per the groups except group V which has given ramiprili.v. after the left renal artery occlusion. At the end of treatment, animals were undergone left renal artery occlusion for 4 hrs on 7^{th}, 14^{th} and 21^{st} day. The mean arterial blood pressure was measured using BIOPAC student lab data acquisition system [1, 10].

a) Induction of acute renal hypertension (2K1C)

The animals were anesthetized by intraperitoneal injection of 1.25gm/kg urethane. Left renal artery was exposed through a retroperitoneal flank incision under antiseptic condition &carefully dissected free from renal vein.A bull dog clip was placed onto the left renal artery (2K1C) and occluded for 4 hours[1, 10].

b) Measurement of blood pressure

The right carotid artery was exposed and cannulated with polyethylene tube filled with small volumes of heparin solution (100 IU/ml, in physiological saline) for the measurement of the mean arterial pressure. The heparin solution was injected in the arterial catheter at 30 min intervals to avoid possible blood coagulation. The catheter (PE-50) was connected to the blood pressure transducerand the transducer was further connected with the Four Channel Data Acquisition System (BIOPAC System, Inc., MP35). After obtaining stable reduced blood pressure values, the renal arterial clip was removed. This led to a rise in blood pressure and within next 15 min a stable hypertension was achieved. The blood pressure was monitored for up to 1hour.

Statistical Analysis

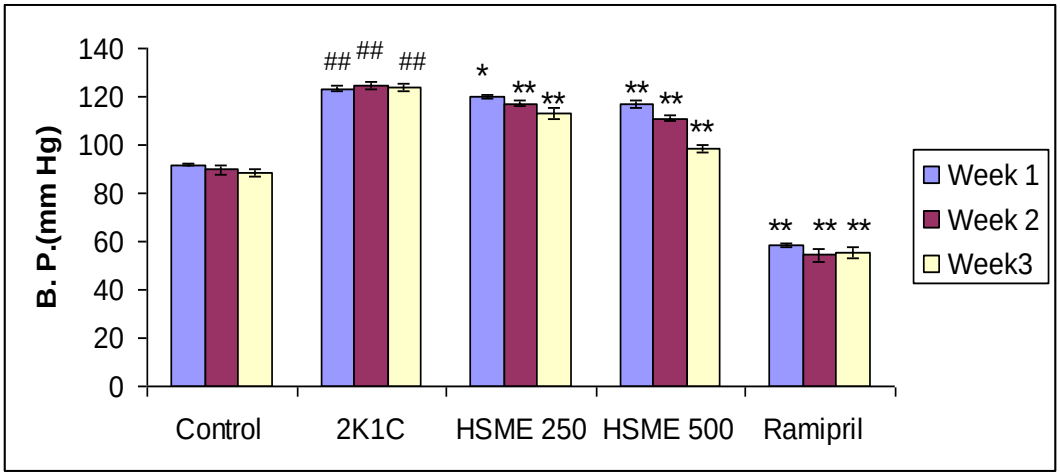
Data expressed as Mean ± S.E.M. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test and $p<0.05$ considered as statistical significant.

RESULTS AND DISCUSSION

Left renal artery of rat was occluded for four hours to induce acute renal hypertension. Mean arterial B.P. of rats of 2K1C group was found to be significantly ($P<0.01$) increased as compared to control group. Pretreatment with HSME 250 and HSME 500 mg/kg significantly ($P<0.05$, $P<0.01$) prevented this increase in comparison with 2K1C group (Table 5.11; Graph 5.20).

Table 1: Effect of HSME on blood pressure in left renal artery occlusion induced acute renal hypertension in rats.

Groups	Control	2K1C	HSME 250	HSME 500	Ramipril 1mg/kg
Week 1	91.817 ± 0.60	123.32 ± 1.17$^{\#\#}$	120.07 ± 0.84*	116.62 ± 1.49**	58.433 ± 0.76**
Week 2	89.817 ± 1.81	124.82 ± 1.43$^{\#\#}$	117.23 ± 1.24**	111.12 ± 1.33**	54.26 ± 2.56**
Week 3	88.483 ± 1.71	123.82 ± 1.20$^{\#\#}$	112.90 ± 2.24**	98.567 ± 1.66**	55.433 ± 2.53**



Graph 1: Effect of HSME on blood pressure in rats in left renal artery occlusion induced acute renal hypertension. The results were expressed as Mean ± SEM (n=6). The data was analyzed using One-way Analysis of Variance (ANOVA) followed by Dunnett's- test. Where;*, #-P<0.05, **, ##-P<0.01, *, ###-P<0.001; #-EG treated group against control; *-HSME treated group against 2K1C group.**

High BP is a reliable indicator of premature death. It is a risk factor for stroke, coronary heart disease and renal vascular disease. The control of BP through diet has been the focal point of public health and mass media attention. The method in practice to control high BP is 'long-term' drug therapy. Drugs have side effects that can create more clinical problems than are solved. That is why medical professionals worldwide are seeking non-drug treatment and preventative strategies. Here, we raise the possibility that HSME can be of significant help in BP management. The occlusion of renal artery upto 4 hour, leads to cause kidney ischemia. Ischemia of the kidneys causes elevation of blood pressure by activation of the renin-angiotensin system. Acute renal hypertension can be induced in rats, by clamping the left renal artery for 4 h. After reopening of the vessel, accumulated renin is released into circulation[1, 10]. Renin acts on angiotensinogen to release the decapeptide angiotensin I. This decapeptide is cleaved by angiotensin converting enzyme to yield the active angiotensin II which is a potent vasoconstrictor leading to hypertension. Angiotensin II undergoes hydrolysis by an aminopeptidase to yield the heptapeptide angiotensin III which is also active. Among other models of experimental hypertension, acute 2K1C, which was used in this study, is a model which development of hypertension mainly occurs via higher activity of ACE and production of angiotensin II [11]. Moreover, it has been shown that other vasoactive agents such as; thromboxane A_2 and prostaglandin F2a are also being involved in producing hypertension in 2K1C [12]. Angiotensin-converting enzyme, a decapeptidyl peptidase, is widely distributed not only in the cardiovascular system, but also in various non-cardiovascular tissues. One of the principal locations of the enzyme is the vasculature, where activity of ACE promotes cellular proliferation. In this study the acute renal hypertension in rats was used to mimic renal artery constriction in humans. It has been suggested that most of the changes in acute renal hypertension (2K1C) model affecting hemodynamic and structural alterations are caused by angiotensin II generation due to an increased ACE activity in the established stages of hypertension[11, 13]. In present study the left renal artery occlusion of rat for 4 hrs increased the blood pressure of rats. The pretreatment of HSME prevented this increase in blood pressure. The effect was increased with duration of treatment. The HSME may inhibit the ACE activity or renin activity. The HSME reported the presence of glycosides, terpenoids and flavonoids which may be responsible for this activity.

The HSME has shown the protective action in above studies so it could be used in renal complications. Further studies are required to reveal the different mechanism of actions by which it prevents renal complications. Further clinical investigation is needed to prove its beneficial effects in humans.

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