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Blood pressure lowering effects of *Ranunculus scleratus* Linn. in normal and fructose induced hypertensive rats and estimation of underlying mechanisms

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Abstract: *Ranunculus scleratus Linn.* is used in folk medicine to treat hypertension. This study was aimed at providing validation to its traditional use and to explore underlying mechanisms of action. Effects of hydro-ethanolic crude extract of the plant and its fractions on blood pressure was evaluated using direct surgical method in normotensive and in fructose induced hypertensive rats. Various doses of crude extract, RSC, (5, 10, 20, 30mg/kg) and all fractions (3, 5, 10, 20mg/kg) were studied. Results suggested that aqueous fraction of R. scleratus (RSA) produced most pronounced effects at 10mg/kg in normotensive and at 20mg/kg in hypertensive animals. Underlying mechanisms, using various pharmacological antagonists were also elucidated. Results suggested the involvement of muscarinic receptor, angiotensin converting enzyme (ACE) inhibition, ganglionic block and nitric oxide (NO) release in presenting hypotensive response.

Keywords: Hypertension, *Ranunculus scleratus Linn*, ACE inhibition, adrenergic blockade.

INTRODUCTION

Ranunculus scleratus Linn (Family Ranunculaceae) is a herbaceous plant found in damp places usually along riverside or along small water bodies (Mei *et al*., 2012). The specie is widely distributed in various regions of Asia. This plant is traditionally used to lower blood pressure, against oesophageal or breast cancer (Ikram, Bhatti and Parvaiz 2014).

High fructose consumption causes rise in blood pressure along with establishment of insulin resistance (Elliott *et al*., 2002). Hypertension due to high fructose is basically as a result of several alterations including expansion of fluid volume, dysfunction of endothelium, dyslipidaemia, increase in oxidative stress, specially sodium and electrolyte retention. High fructose also triggers sympathetic responses causing vasoconstriction and hence hypertension (Tran *et al*., 2009). It also causes endothelial dysfunction resulting in serious vascular issues (Vasudevan *et al*., 2006).

Considering the traditional uses of *R. scleratus*, its therapeutic potential against cardiovascular diseases especially hypertension and lack of scientific background to support the traditional use, the current study was aimed to explore hypotensive and antihypertensive potential of the said plant and to investigate the mechanisms involved.

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MATERIALS AND METHODS

Chemicals and drugs

Ethanol, n-hexane, dicloromethane, ethyl acetate, n-butanol, N-ω-Nitro-L-arginine methyl ester (L-NAME; NO synthase inhibitor), atropine (muscarinic receptor antagonist), hexamethonium bromide (A ganglionic blocker), captopril (ACE inhibitor), propranolol (beta-adrenoceptor antagonist) and thiopental sodium were purchased from Sigma–Aldrich Chemical Co. (St- Louis, MO, USA). All the chemicals obtained were of analytical grade.

Animals

Local strain of Sprague-Dawley rats (200-250gm) were used for this study. Animals were housed in a controlled environment (40-60% humidity, 25±1°C temperature, 12 h light/dark cycle) at Animal Resources Center, University of Sargodha, Sargodha, Pakistan. Principles for laboratory animal use and care (NIH publication number # 85-23, revised in 1985) were followed. Prior to the conduct of study, documented approval from the Animal Ethical Committee, University of Sargodha (No. IAEC/UOS/2016/47) was obtained. At the completion of the experiment, animals were euthanized by injecting overdose of thiopental (over 40mg /kg, i.v.).

Extraction and fractionation of the plant

Ranunculus scleratus (whole plant) was collected from Sheikhupura (Pakistan). Plant was identified and authenticated by Prof. Dr. *Zaheerud Din Khan*,

Government College University, Lahore (GCUL), Pakistan. (GC. Herb. Bot. 3476). Crude hydro-ethanolic extract (RSC) was obtained by cold maceration process (31%yield) (Cifuentes *et al*., 2016). Sequential fractions were prepared with solvent in order of increasing polarity using polarity-based fractionation technique. n-hexane (RSH), dichloromethane (RSD), ethyl acetate (RSE), n-butanol (RSB) and aqueous (RSA) fractions were obtained (Khan *et al*., 2017). RSD was obtained in extremely less quantity (almost negligible), therefore, this fraction was not used for further studies.

Direct blood pressure measurement in anesthetized animals

Direct surgical method was followed to estimate BP and heart rate. Method proposed by Khan and Kumar, 2017 was followed with some alterations. Briefly, animals were anesthetized with thiopental sodium (i.p; 80mg/kg). First, trachea was cannulated to facilitate respiration throughout experiment. Then, jugular vein was cannulated for drug injection and carotid artery for recording BP using PE-50 catheter. Catheter injected into carotid artery was attached to power-lab data acquisition system and an application program (Chart,v 4.1; ADI Instruments, Australia). After surgery, animals were stabilized for 30-45 minutes prior to drug administration (Tolouei *et al*., 2019).

Hypotensive dose response relationship of R. scleratus

Normotensive animals were divided into seven groups (n=6) that received iv injections of crude extract/fractions in following order: Group I=0.9% NaCl; Group II = captopril (2.5mg/kg); Group III = RSC (5, 10, 20, 30mg/kg); Group IV = RSH (3, 5, 10, 20mg/kg); Group V = RSE (3, 5, 10, 20mg/kg), Group VI = RSB (3, 5, 10, 20mg/kg), Group VII = RSA (3, 5, 10, 20mg/kg). Test agents were freshly prepared before use and administered at 1 ml/kg body weight. Before administration, all test agents were sterilized using syringe filter (0.22 µm pore size) hydrophilic Polyethersulfone (PES) membrane (Merck Millipore, Burlington, Massachusetts, EUA).

On the basis of results, RSA was found to be the most active fraction as it produced most pronounced effect. So, this fraction (RSA) at 10mg/kg was selected for further study. In a separate set of experiment, delayed response of RSA was observed for a period of 45 min.

Anti-hypertensive dose response relationship of R. scleratus

Fructose induced hypertensive rats were divided into seven groups (n=6) with the arrangement as described in the previous section and antihypertensive effect was evaluated.

Evaluation of mechanisms underlying the hypotensive effect of R. scleratus in normotensive rats

In this experiment, normotensive rats were anesthetized and prepared for BP recording. Various pharmacological antagonists were injected 10 min before RSA (10mg/kg) administration. Changes in blood pressure were recorded for 45 min after treatments (Shih *et al*., 2008). Animals were divided into the following groups: Group I = NaCl 0.9%; Group II=RSA (10mg/kg); Group III= hexamethonium bromide (30mg/kg) + RSA (10mg/kg); Group IV = atropine (1mg/kg) + RSA (10mg/kg); Group V = captopril (2.5mg/kg) + RSA (10mg/kg); Group VI = L-NAME (20mg/kg) + RSA (10mg/kg); Group VII = propranolol (100mg/kg) + RSA (10mg/kg).

STATISTICAL ANALYSIS

Results were expressed as mean ± S.E.M. Statistically, two-way ANOVA was applied using Graph pad prism 5.0. P values ≤ 0.05 were considered as significant.

RESULTS

R. scleratus produced acute hypotensive effect

Compared to vehicle control, treatment with RSC and RSA reduced blood pressure significantly in a dose dependent fashion in normotensive animals (fig 1 A, B). Average baseline reading of SBP, DBP, MAP and HR were 125±5.30, 115±5.18, 118±5.13 mm-Hg, and 265±7.10 bpm respectively. Treatment with RSC decreased SBP, DBP and MAP ($p<0.05$ $p<0.001$). however, RSA produced reduction with $p<0.001$. Maximum effect with RSA was observed at 10mg/kg declining SBP (58.98±5.38mm-Hg), DBP (49.03± 5.31mm-Hg) and MAP (52.45±5.38mm-Hg).

RSH produced a significant decline at higher doses (data not shown), however, RSE and RSB did not produce any significant changes (data not shown). No significant changes were observed in heartrate at all tested doses. As most potent hypotensive effect was observed with RSA, it selected for further experimentation.

R. scleratus produced acute anti-hypertensive effect

Treatment with RSC and RSA reduced SBP, DBP and MAP significantly in a dose dependent fashion in fructose induced hypertensive animals (fig. 2 A, B). Compared to control, RSC produced anti-hypertensive effect at 5mg/kg with $p<0.01$and at 10, 20 and 30 mg/kg with $p<0.001$. while RSA produced a significant decline in SBP, DBP and MAP with $p<0.001$. Maximum reduction with RSA was produced at 20mg/kg for SBP (32.95±12.32 mmHg), DBP (22.09±12.18mm-Hg) and MAP (26.08±12.09 mm-Hg).

R. scleratus produced persistent hypotensive effect

Moreover, RSA produced persistent hypotensive effect in anesthetised normotensive rats for 45 min (fig. 3).

RSA produced hypotensive effect mediated by muscarinic receptors, NO/cGMP pathway, ganglionic receptor and RAAS

Hypotension produced by RSA was significantly inhibited in the presence of atropine, L-NAME and hexamethonium while potentiated in the presence of captopril. Propranolol did not alter its effect (fig. 4).

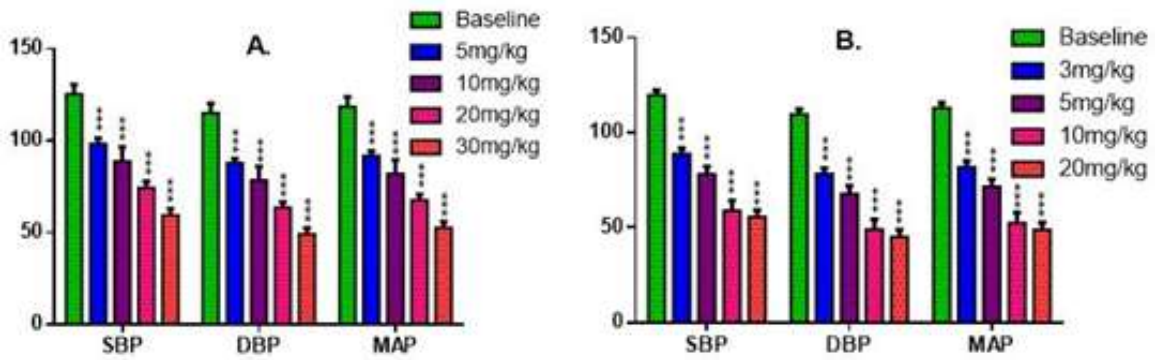

Fig. 1: Effect of various doses of *R. scleratus* A) crude extract (RSC) B) aqueous fraction (RSA) in anesthetized normotensive rats on SBP, DBP and MAP. Results are expressed as mean ± S.E.M, whereas, ***=p<0.001, compared to baseline (vehicle control) using 2-way ANOVA.

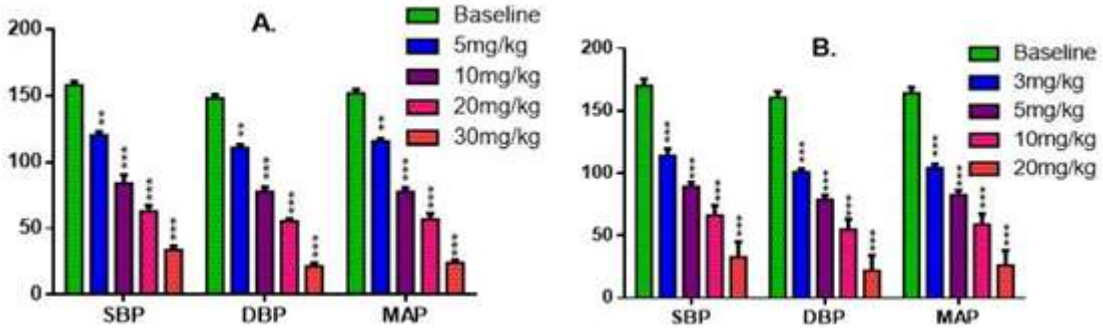

Fig. 2: Effect of various doses of A) RSC B) RSA in anesthetized hypertensive rats on SBP, DBP and MAP. Results are expressed as mean ± S.E.M, **=p<0.01 and *** =p<0.001 compared to baseline (normal saline treated group) using 2-way ANOVA.

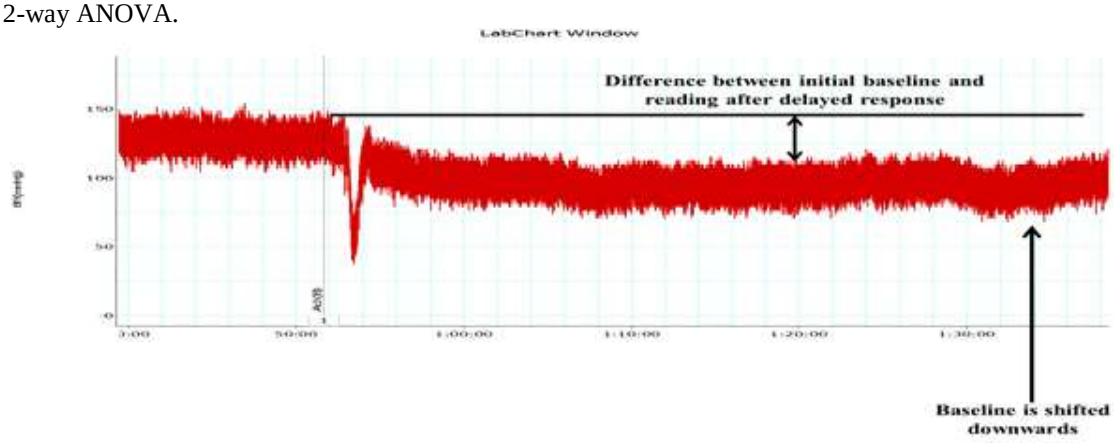

Fig. 3: Original tracing of persistent response of RSA at 10mg/kg over 45 minutes.

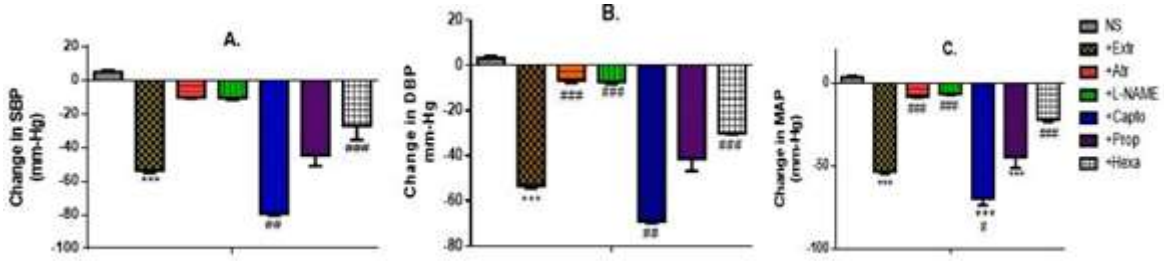

Fig. 4: Effect of RSA at 10 mg/kg on SBP, DBP and MAP in anesthetized rats pre-treated with different antagonists. Results are expressed as mean ± S.E.M. ***= p<0.001 compared to normal saline treated group, while #=p<0.05, ###= p<0.001 compared to RSA alone following 2-way ANOVA.

DISCUSSION

Plants are considered important tool for the development of effective medicinal agents. Several studies have shown that medicinal plants have been scientifically assessed based upon their traditional uses (Sharif *et al*., 2018). Many currently used drugs are synthesized from phytoconstituents isolated form medicinal plants (Al Disi *et al*., 2016).

High fructose consumption causes rise in blood pressure (Elliott *et al*., 2002) basically due to several alterations including expansion of fluid volume, dysfunction of endothelium, dyslipidaemia, increase in oxidative stress, specially sodium and electrolyte retention. High fructose also triggers sympathetic responses causing vasoconstriction and hence hypertension (Tran *et al*., 2009). It also causes endothelial dysfunction resulting in serious vascular issues (Vasudevan *et al*., 2006).

Literature shows that RAAS blockers, β- blockers, ganglionic blockers, muscarinic agonist and NO causes decline in blood pressure. Thus, from the results, it could be concluded that RSA showed its effect possibly through mediation of NO, cholinergic pathway, ganglionic block operating central sympathetic neural responses or RAAS. Acetyl choline increases level of NO, hence it can be said that cholinergic activity might be responsible for rise in NO level in producing hypotension (Gil-Longo and González-Vazquez, 2010). Muscarinic receptor M-2 causes contraction of smooth muscles in bladder by inhibiting adenylyl cyclase while M-3 receptors causes contraction of detrusor muscles increasing urination. Therefore, agonist of M-2 and M-3 receptors causes a drop in blood pressure (Hegde and Eglen, 1999). As atropine caused reduction in effect of RSA, it can be presumed that hypotensive response of RSA could be due to mediation of M2/M3 induced diuresis due to contraction of detrusor muscles which produce reduction in blood pressure. Wang *et al*. 2009 documented indirect hypotensive effect of another species of Ranunculus, *R. japonicus* without effecting heartrate. Results of our study are in line with Wang *et al*. (2009).

Literature confirms the presence of several flavonoids in Ranunculus scleratus namely 5-hydroxy tryptamine, apigenin, apigenin 4′-O-α-rhamnopyranoside, apigenin 7-O-β-glucopyranosyl-4′-O-αrhamnopyranoside, tricin 7-O-β-glucopyranoside (Hussain *et al*., 2009; Aslam *et al*., 2012). Flanonoids is an important class of phyto-constituents that possesses many pharmacological activities. Majewska-wiezbicka and Czeczot, 2012 documented effects imposed by flavonoids in preventing rise in blood pressure.

CONCLUSION

Results of this study given scientific validation to the hypotensive and antihypertensive effect of *Ranunculus scleratus Linn*. Through several mechanisms mediated by mediated by muscarinic receptors, NO/cGMP pathway, ganglionic receptor and RAAS.

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