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**Hypotensive and diuretic activities
of aqueous-ethanol extract of
*Asphodelus tenuifolius***

Hypotensive and diuretic activities of aqueous-ethanol extract of *Asphodelus tenuifolius*

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Abstract

In order to rationalize the traditional uses of *Asphodelus tenuifolius* in cardiovascular complaints, aqueous-ethanol extract of the plant was investigated for hypotensive and diuretic activities using *in vivo* and *in vitro* models. Intravenous administration of the extract in anesthetized rats produced 14.5 (95% CI; 13.3–15.6), 24.5 (95% CI; 21.3–27.9) and 35.3% (95% CI; 32.0–42.5) fall in mean arterial blood pressure at the doses of 3, 10 and 30 mg/kg, respectively. The extract increased the urine volume and electrolytes excretion significantly at the doses of 300 and 500 mg/kg in rats. In rabbit's isolated aorta preparations, the extract, like verapamil, relaxed K⁺ (80 mM)-induced contractions more potently than phenylephrine (1 μM)-induced contractions, indicating Ca²⁺ antagonistic activity. The extract produced dose-dependent stimulant followed by depressant effects in spontaneously contracting rabbit's paired atria preparations. The results suggest that the extract of *A. tenuifolius* has hypotensive and diuretic effects in animals.

Introduction

Asphodelus tenuifolius Cav. (Asphodelaceae) is an annual herb and weed, native to Mediterranean region, Asia and Mascarene Islands. The plant is 15-50 cm tall having erect hollow cylindrical leaves and white flowers with pinkish central stripe. The plant is known by vernacular name of Piazi (Urdu), Asphodel and Onion weed (English). It is used as vegetable (IUCN, 2005). Seeds, roots and whole plant are also used for medicinal purposes as diuretic, anti-inflammatory, insecticide, treatment for skin diseases, constipation (Quattrocchi, 2012), diarrhea, epilepsy, diabetes (Ahmed et al., 2014a), paralysis (Abu-Rabia, 2012), narcotic, sedative, sexual problems (Ahmed et al., 2015), cold, cough, fever (Ahmed et al., 2014), piles (Qureshi and Raza, 2008), swellings, hypertension (Mahmood et al., 2011; Ouhaddou et al., 2015) and jaundice (Yaseen et al., 2015).

Phytochemical investigation revealed that the plant

contains alkaloids, anthraquinones, phenols, flavonoids, tannins and steroids (Eddine et al., 2015). Polyphenols including trans-N-feruloyltyramine, luteolin, luteolin-7-O-β-D-glycopyranoside, apigenin, chrysoeriol (Khaled et al., 2014), vanillin, rutin and caffeic acid (Eddine et al., 2015) have been identified in various extracts of the plant. Anthraquinones including chrysophanol, aloe-emodin, anhydrorugulosin; three other bianthraquinones and chrysophanol-8-mono-β-D-glucoside have been isolated from the plant (Basaif and Abdel-Mogib, 2002; Hammouda et al., 1974). Seeds contain considerable amount of fixed oils consisting of glycerides of myristic, palmitic, stearic, oleic and linoleic acids along with a non saponifiable component, fucosterol (Khaq et al., 1961). Asphorodin, a triterpene diglycoside having lipoxygenase inhibitory activity, has been isolated from ethyl acetate soluble fraction of the plant (Safder et al., 2009).

Pharmacological investigations revealed that the plant possesses anti-oxidant and antibacterial activities

(Eddine et al., 2015; Kalim et al., 2010; Panghal et al., 2011). Although the plant is used for treatment of various ailments by traditional healers, but pharmacological reports to justify its traditional uses are scarce.

The present study was undertaken to study the cardiovascular and diuretic effects of the title plant in order to rationalize its traditional uses in hypertension and as diuretic.

Materials and Methods

Plant material

Complete plants of *A. tenuifolius* were collected in the month of April 2015 from Cholistan Deseret, District Bahawalpur, Pakistan. The plant material was identified by Dr. Zafar Ullah, Department of Botany, Bahauddin Zakariya University, Multan (voucher number Fl-PK-50-3). The plants material was rendered free from extraneous matter, chopped into small pieces and dried in shade for two weeks.

Preparation of extract

The dried plant material was converted into coarse powder with help of an electric grinder and soaked in aqueous-ethanol (30:70 v/v) at room temperature with occasional stirring for three days followed by filtration using muslin cloth and then through Whatman grade 1 filter paper. The procedure of soaking and filtration was repeated with the residue using the fresh solvent for two more times. All three filtrates were combined and evaporated using rotary evaporator (Rotavapor, BUCHI Labortechnik AG, Switzerland) under reduced pressure at temperature not exceeding 45°C to yield a thick semisolid mass of greenish brown color; i.e. the crude extract of *A. tenuifolius*. Percentage yield of crude extract was found 9.2% and stored at -4°C until used. The crude extract was suspended in normal saline for oral administration or dissolved in normal saline containing 5% DMSO for intravenous administration to animals. For *in vitro* studies, the crude extract was dissolved in distilled water containing 10% DMSO to form stock solution of 300 mg/mL, which was further diluted with distilled water. Solutions were prepared fresh on every day of experiment.

Animals

Sprague-Dawley rats of either sex (200-250 g) and locally available breed of albino rabbits of either sex (1-1.5 kg) were used in experiments. Animals were exposed to 12 hours light-dark cycles at 25 ± 3°C temperature and housed at animal house of the Department of Pharmacy, Bahauddin Zakariya University Multan. The animals had free access to water and standard diet.

Chemicals and reagents

All chemicals used in the study were of the analytical grade. Atropine sulfate, acetylcholine chloride, verapamil hydrochloride, furosemide hydrochloride, potassium chloride and magnesium chloride were purchased from Sigma Chemicals Co., USA. Potassium-di-hydrogen phosphate, glucose, calcium chloride, magnesium sulfate, sodium bicarbonate, sodium-di-hydrogen phosphate, ethanol and DMSO were purchased from Merck, Darmstadt, Germany. Sodium chloride was purchased from BDH Laboratory Supplies, England. Ketamine injection (Ketalar®, Akahai Pharmaceuticals, Karachi), diazepam injection (Valium®, Roche Pharmaceuticals, Karachi), adrenaline injection (PDH Pharmaceuticals, Lahore) and heparin injection (Heparol®-5000, China) were purchased from the local medicine stores. All chemicals were dissolved in distilled water.

Hypotensive activity in anesthetized rats (Video Clip)

Blood pressure of anesthetized rats was recorded by slight modification of already described method (Jabeen et al., 2009). Briefly, the animals were anesthetized with i.p. injections of diazepam (5 mg/kg) and ketamine (50-80 mg/kg). Each animal was fixed in supine position on dissecting table and body temperature was maintained with help of an overhead lamp. A small mid tracheal incision was made to expose trachea, left jugular vein and right carotid artery. Trachea was catheterized with polyethylene tube to facilitate spontaneous respiration. Drugs were administered intravenously in 0.1 mL volumes followed by 0.1 mL saline flush through jugular vein catheterized with polyethylene tubing (PE-50). Blood pressure was monitored through carotid artery catheterization with polyethylene tubing (PE-50) connected to a pressure transducer (MLT0699 disposable BP transducer, ADInstruments, Australia) filled with heparinized saline (60 i.u./mL) and connected to PoweLab data acquisition system (ADInstruments, Australia). Blood pressure was allowed to return to basal values between drug administrations. Change in blood pressure was calculated as percent difference from basal value immediately before dose to lowest reading after dose. Control responses of acetylcholine (1 µg/kg) and adrenaline (1 µg/kg) were obtained before injections of extract. Mean arterial blood pressure was calculated by adding one-third of pulse pressure to diastolic blood pressure.

Studies on isolated aorta and atria

In our laboratory, rabbits are commonly used for *in vitro* studies, thus using multiple tissues from the same animals to maximize ethical and economical utilization. Direct cardiac and vascular effects were studied using isolated atria and aorta preparations from rabbits. Rabbits were killed by cervical dislocation. Rabbit thoracic aorta was removed, cleaned off from

connective tissue and cut into 2-3 mm wide rings. Aortic rings were individually mounted in 15 mL organ bath (Radnoti tissue organ bath system, ADInstruments, Australia) filled with Krebs-Henseleit solution having composition in mmol/L; NaCl 118.2, NaHCO₃ 25.0, CaCl₂ 2.5, KCl 4.7, KH₂PO₄ 1.3, MgSO₄ 1.2, and glucose 11.7 (pH 7.4). The solution was maintained at 37°C with thermocirculator and continuously gassed with O₂ containing 5% CO₂ (carbogen). A preload tension of 2.0 g was applied to aortic rings and change in the tension was recorded with isometric transducer connected to PowerLab data acquisition system (AD Instruments, Australia). An equilibration period of 1 hour was allowed and preparations were stabilized by repeated treatments with submaximal doses of phenylephrine (1 µM) to obtain two reproducible contractile responses (usually 3-4 treatments). Vasodilator effects of the test substance were then assessed on aortic rings previously contracted with either phenylephrine (1 µM) or K⁺ (80 mM) by adding the extract or standard drug in increasing doses in cumulative fashion.

Paired atria were carefully and rapidly isolated by cutting out ventricles of spontaneously beating isolated heart of rabbit. The paired atria were set up in 15 mL organ bath (Radnoti tissue organ bath system, AD Instruments, Australia) containing Krebs-Henseleit solution maintained at 34°C and continuously gassed with carbogen. The force and rate of spontaneous contractions of atria were recorded under 1.0 g tension by isometric transducer connected to PowerLab data acquisition system (ADInstruments, Australia). An equilibration period of 30 min was allowed with fluid changes at 10 min intervals before addition of any drug. Percentage changes in rate and force of baseline contractions were calculated after addition of test substance in cumulative doses (Janbaz et al., 2011).

Diuretic assay

The diuretic assay was conducted with slight modification of already described method (Chen et al., 2014). Sprague-Dawley rats of either sex were deprived

of food but not water 12 hours before the test. Each animal was then administered normal saline at a dose of 20 mL/kg to impose saline load and hydration. 30 min later, animals were randomly divided into five groups of six animals each. The control group received normal saline (10 mL/kg, orally). Another group of animals was given furosemide (20 mg/10 mL/kg, orally) as standard diuretic. The treatment groups of animals received different doses of extract orally. Immediately after dosing, animals were individually placed in diuretic cages (Techniplast, Italy) and the urine was collected for 6 hours. Total volume of urine was determined. Na⁺ and K⁺ concentrations were measured by clinical flame photometer (Model 410C, Sherwood Scientific Ltd., UK) and Cl⁻ concentration was measured by using commercial kit (Chloride FS, DiaSys Diagnostic Systems GmbH, Germany).

Statistical analysis

The data is expressed as mean ± standard error of mean (SEM) and/or median effective concentrations (EC₅₀) with 95% confidence interval (CI). GraphPad Prism Software (GraphPad, San Diego, CA, USA) was used to analyze data and construct graphs. One-way analysis of variance followed by Dunnett's test was used to compare experimental groups with control group and values of p<0.05 were regarded as statistically significant.

Results

Intravenous administration of the crude extract of *A. tenuifolius* decreased the mean arterial blood pressure of normotensive anesthetized rats in dose-dependent fashion. Figure 1 represents a typical tracing of hypotensive effects of acetylcholine and crude extract of *A. tenuifolius* in one animal and combined results obtained from various experiments are plotted in Figure 2. The extract caused 14.5 (95% CI; 13.3–15.6, n=5), 24.5 (95% CI; 21.3–27.9, n=5) and 35.3% (95% CI; 32.0–42.5, n=5) fall in mean arterial blood pressure at respective doses of 3, 10 and 30 mg/kg. Intravenous

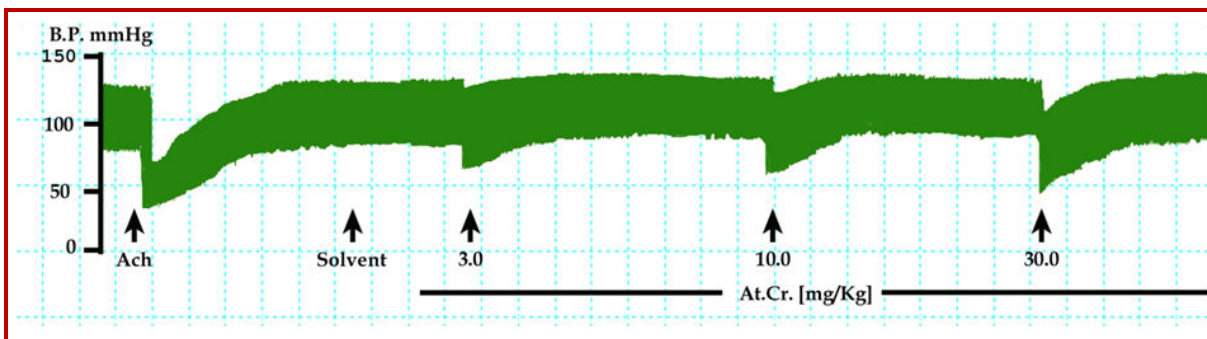


Figure 1: A typical tracing showing effects of intravenous administrations of acetylcholine (Ach, 1 µg/kg) and different doses of aqueous-ethanol extract of *Asphodelus tenuifolius* (At.Cr.) on blood pressure in anesthetized rat. Solvent used has no effect on blood pressure

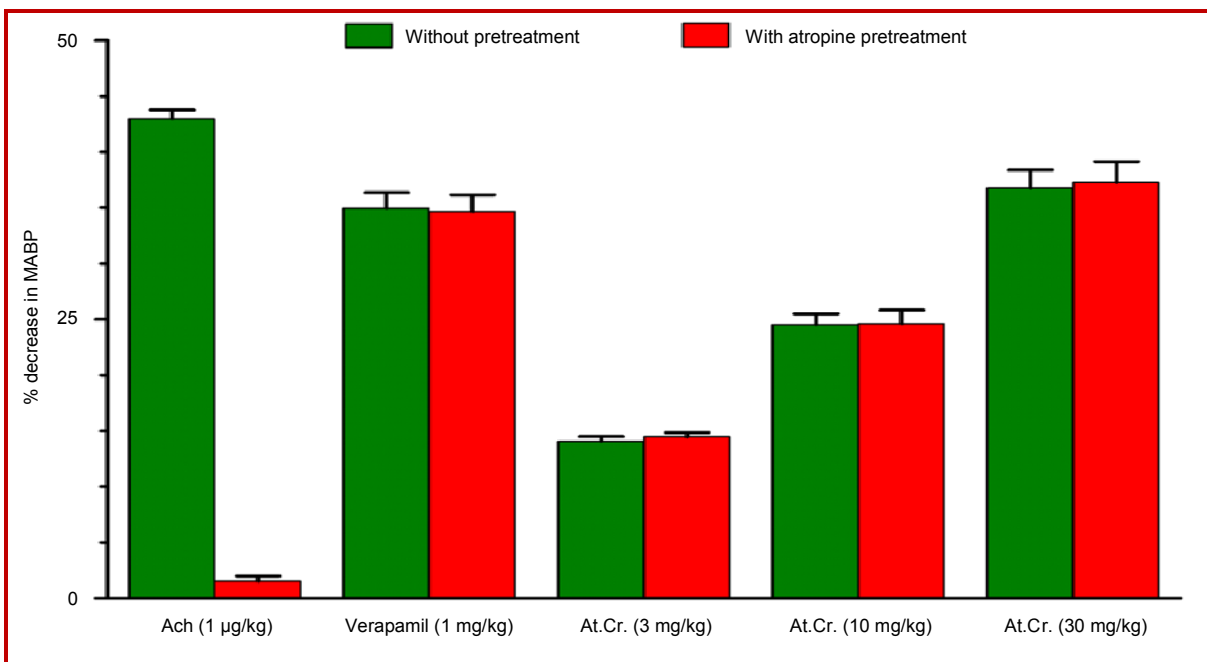


Figure 2: Bar diagram showing effects of acetylcholine (Ach), verapamil and different doses of crude extract of *Asphodelus tenuifolius* (At.Cr.) on mean arterial blood pressure (MABP) in anesthetized rats, with no pretreatment (green bars) and with atropine pretreatment (red bars). The hypotensive effect of Ach is blocked by pretreatment of animals with atropine (1 mg/kg) but remain unaffected for verapamil or At.Cr. Values are mean \pm SEM of 4-5 measurements

administration of acetylcholine (1 µg/kg) or verapamil (1 mg/kg) also decreased the mean arterial blood pressure in anesthetized rats, whereas the vehicle did not affect the mean blood pressure. Pretreatment of animals with atropine (1 mg/kg) abolished the hypotensive effect of acetylcholine, but did not affect the hypotensive effect of verapamil or extract of *A. tenuifolius*.

Isolated rabbit aortic ring preparations were used for study of direct vasodilator activity and assessment of possible mode of action(s). The extract caused concentration-dependent (0.03–10.0 mg/mL) relaxation of phenylephrine and K⁺-induced contractions with respective EC₅₀ values of 5.5 mg/mL (95% CI; 2.6–11.8, n=5) and 0.8 mg/mL (95% CI; 0.7–0.8, n=5). Verapamil, a standard Ca²⁺ channel blocker and vasodilator also relaxed phenylephrine and K⁺-induced contractions with respective EC₅₀ values of 1.0 µM (95% CI; 0.9–1.1, n=5) and 0.3 µM (95% CI; 0.2–0.3, n=5) (Figure 3).

When tested on spontaneously contracting paired atria of rabbit, the crude extract of *A. tenuifolius* increased force of contractions at tissue bath concentrations ranging from 0.01 to 1.0 mg/mL. With further increase in concentration (3–10 mg/mL), the extract produced inhibition of contractility. The extract produced little or no increase in heart rate at concentration range of 0.01–1.0 mg/mL, followed by decrease in heart rate at higher concentrations (Figure 4).

The results of diuretic assay are summarized in Table I.

Urine volume and urinary electrolytes (Na⁺, K⁺ and Cl⁻) excreted by rats per 100 g of the body weight in 6 hours for control group treated with normal saline (10 mL/kg) were compared with other groups. The increase in volume was not significant (p>0.05) at dose of 100 mg/kg extract, but was significant (p<0.001) at doses of 300 and 500 mg/kg extract. Furosemide increased the urine volume and urinary electrolytes excretion significantly (p<0.001), when compared to the vehicle-treated control group.

Discussion

Results of experiments show that the plant extract cause dose-dependant blood pressure lowering effect in anesthetized normotensive rats, which is in agreement with ethnic uses of the plant as antihypertensive (Mahmood et al., 2011). Blood pressure is the product of cardiac output and peripheral vascular resistance (Benowitz, 2009). The blood pressure lowering effect of the extract may involve either or both components. Some herbs decrease blood pressure by stimulation of muscarinic receptors (Jabeen et al., 2009). *In vivo* administration of muscarinic agonist drugs decrease blood pressure by endothelium-nitric oxide-dependant vasodilatory and cardiodepressant actions (van Zwieten and Doods, 1995). In the present study, pretreatment of the animal with atropine, a nonspecific muscarinic receptor antagonist, blocked the

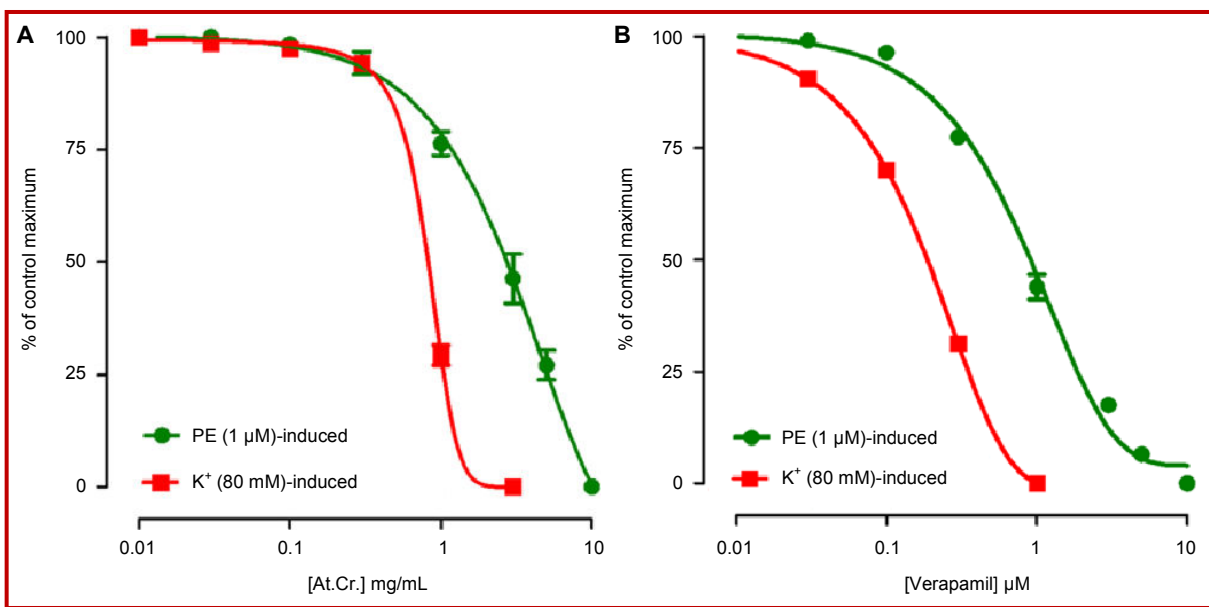


Figure 3: Effects of (A) the crude extract of *Asphodelus tenuifolius* (At.Cr.) and (B) verapamil on phenylephrine (PE, 1 μ M)-induced and K^+ (80 mM)-induced contractions in isolated aorta preparations. Vales are mean \pm SEM of 4-5 determinations

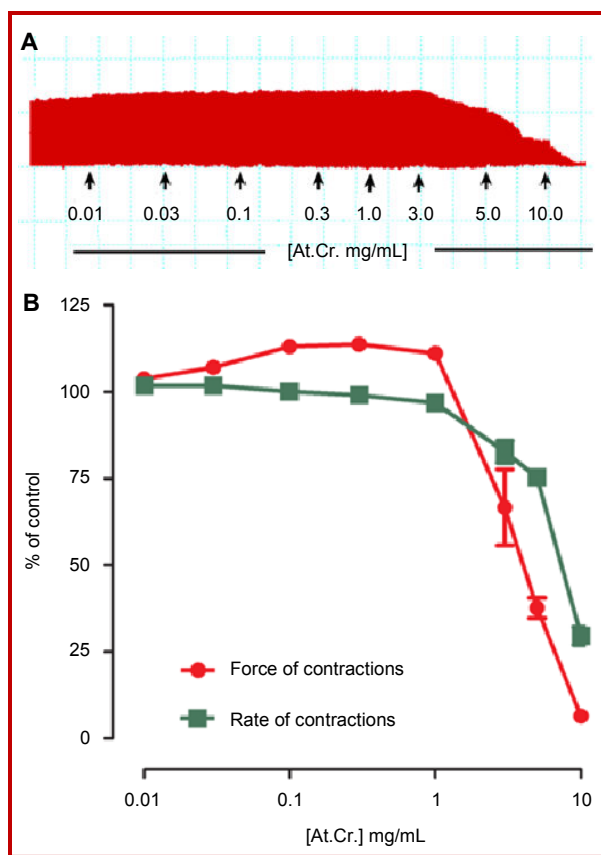


Figure 4: The upper panel (A) represents a typical tracing showing effect of cumulative addition of crude extract of *Asphodelus tenuifolius* (At.Cr.) on rabbit's paired atria preparation, and the lower panel, (B) a graph showing effects of the At.Cr. on force and rate of contractions of rabbit's paired atria preparations. Vales are mean \pm SEM of 4 determinations

hypotensive effect of acetylcholine but did not affect the hypotensive effect of the extract or verapamil, suggesting that muscarinic receptors are not involved in the hypotensive effect of the extract or calcium channel blockers.

Vasorelaxant drugs are commonly used in the treatment of hypertension (Benowitz, 2009). Vasorelaxant effects of the extract were tested in phenylephrine and high K^+ contracted rabbit aorta. It is well-known that K^+ (80 mM) depolarizes the smooth muscle cell membrane, resulting in activation of voltage-dependent Ca^{2+} channels that lead to an influx of extracellular Ca^{2+} and increase in cytosolic free Ca^{2+} causing sustained contractions. The ability of the test substance to relax K^+ (80 mM)-induced contractions suggests the possibility of a voltage-dependent Ca^{2+} channel blocking effect. Whereas, phenylephrine stimulates α_1 -adrenergic receptors leading to the conversion of phosphatidylinositol to inositol-1,4,5-trisphosphate, which in turn increases free cytosolic Ca^{2+} by release from intracellular stores, followed by influx of Ca^{2+} from the L-type voltage-dependent Ca^{2+} channels and the receptor-operated cation channel resulting in contraction of the aorta. Relaxation of the phenylephrine-induced contractions indicates blockade of Ca^{2+} influx through receptor-operated Ca^{2+} channels or Ca^{2+} release from the internal stores (Karaki et al., 1997). Verapamil, a standard voltage dependent Ca^{2+} channel blocker was found more potent in relaxing K^+ -induced contraction compared to phenylephrine-induced contractions. The extract produced vasodilatation in K^+ contracted aorta at much lower dose than required for vasodilatation of phenylephrine-contracted aorta preparation, indicating that the extract may be acting

| Table I | | | | | | | |
|--|------------------------------------|----------------|--------------------------|-------------------------|---------------------------|---------------------------------|--------------|
| Effects of the crude extract of <i>Asphodelus tenuifolius</i> (At.Cr.), normal saline and furosemide on urine volume and urinary electrolytes in sprague-dawley rats | | | | | | | |
| Treatment group (dose) | Volume of urine (mL/100 g/6 hours) | Diuretic index | Na ⁺ | K ⁺ | Cl ⁻ | Na ⁺ /K ⁺ | Ion quotient |
| | | | (μmol/100 g/6 hours) | | | | |
| Normal saline (10 mL/kg) | 1.02 ± 0.1 | - | 121.0 ± 4.0 | 7.8 ± 0.2 | 127.5 ± 2.0 | 15.6 ± 0.8 | 1.0 ± 0.1 |
| Furosemide (20 mg/kg) | 4.1 ± 0.1 ^c | 4.1 ± 0.3 | 379.2 ± 5.6 ^c | 31.4 ± 0.6 ^c | 516.5 ± 9.3 ^c | 12.1 ± 0.4 ^c | 1.2 ± 0.1 |
| At.Cr. (100 mg/kg) | 1.2 ± 0.2 ^{ns} | 1.2 ± 0.1 | 140.3 ± 7.6 ^b | 9.5 ± 0.4 ^b | 140.0 ± 2.8 ^b | 13.9 ± 0.8 ^{ns} | 0.9 ± 0.0 |
| At.Cr. (300 mg/kg) | 1.6 ± 0.1 ^c | 1.6 ± 0.1 | 189.2 ± 6.9 ^b | 13.9 ± 0.3 ^c | 223.3 ± 7.2 ^c | 13.6 ± 0.5 ^{ns} | 1.1 ± 0.1 |
| At.Cr. (500 mg/Kg) | 2.3 ± 0.1 ^c | 2.3 ± 0.1 | 313.0 ± 8.1 ^c | 24.1 ± 0.7 ^c | 364.2 ± 10.6 ^c | 13.0 ± 0.4 ^b | 1.1 ± 0.1 |

Diuretic index = volume of urine in treatment group/volume of urine in saline group, Ion quotient = Cl⁻/(Na⁺ +K⁺)

The values are mean ± SEM of six determinations. P values are compared with normal saline treated group as ^{ns}p>0.05, ^ap<0.05, ^bp<0.01, ^cp<0.001

through voltage-dependent Ca²⁺ channels. Ca²⁺ channel blockers constitute an important class of drugs used clinically in the management of hypertension and angina due to direct vasodilator and cardio depressant actions (Eisenberg et al., 2004).

When tested on spontaneously contracting paired atria of rabbit, the extract produced dual effects on force and rate of atrial contractions. At smaller tissue bath concentrations (0.01-1.0 mg/mL), the extract caused slight positive inotropic action without affecting the rate of atrial contractions. At higher tissue bath concentrations (3-10 mg/mL), the extract was found to possess negative inotropic and chronotropic effects. The negative inotropic and chronotropic effects may be mediated through Ca²⁺ channel blocking activity. The combination of Ca²⁺ antagonist and cardiac stimulant activities in the extract could have side effect neutralizing effects on the heart by balancing extreme cardiac depression associated with pure Ca²⁺ antagonist (Benowitz, 2009), which is especially important in patients with weak hearts. However, further investigations are required to verify mechanism of cardiac effects of the extract.

Medicinal plants such as *Euphorbia granulata* and *Coccinia grandis* have been reported to possess diuretic effect (Saleem et al., 2015; Gupta and Mishra, 2015). Traditional uses of *A. tenuifolius* as diuretic prompted us to perform the diuretic assay. The results of the diuretic study show that the extract increased water and electrolytes (Na⁺, K⁺, Cl⁻) excretion in the given

animal model. In order to investigate the mechanism of diuretic action, urinary electrolytes, and their ratios were determined in all groups. A high urinary Na⁺/K⁺ ratio indicates natriuretic and potassium-sparing effects (Vogel, 2008). Literature also shows that basal urinary electrolyte concentrations and their ratios vary widely; age, strain, dietary intake, housing conditions, method of sample collection and analysis are usually considered as contributing factors of such wide variations (Shevock et al., 1993). Salt loading in the diuretic assay is associated with an increase in urinary Na⁺/K⁺ ratios (Branch et al., 1978). In our study, all the groups were subjected to salt loading before administration of test substance, which possibly contributed in overall high Na⁺/K⁺ ratios in all groups. When compared with saline treated control group, furosemide decreased Na⁺/K⁺ ratio significantly (p<0.01). The extract at all doses decreased Na⁺/K⁺ ratios, but the decrease was only significant (p<0.05) at a dose of 500 mg/kg, when compared to the vehicle-treated control group. Therefore, results excluded potassium sparing effect of the extract. Ion quotient is calculated for estimation of carbonic anhydrase inhibitory activity and its value below 0.8 suggest carbonic anhydrase inhibition (Vogel, 2008). Ionic quotients of all groups were above 0.8; therefore, the possibility of carbonic anhydrase inhibition in diuretic action is not likely to be involved in observed diuresis. Furosemide, a loop diuretic drug,

increase urine output and urinary excretion of electrolytes significantly by inhibiting $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transport in the thick ascending limb of the loop of Henle. While, thiazides, another commonly used group of diuretic drugs, inhibit Na^+/Cl^- co-transport in distal convoluted tubules to bring about the increase in urinary volume and excretion of Na^+ , K^+ and Cl^- (Ives, 2009). Results of the present study suggest that effects of some constituents in the extract on urinary electrolytes and water excretion are furosemide and/or thiazide-like. Diuretics are useful in the treatment of cardiovascular complaints like hypertension and congestive cardiac failure (Ives, 2009). Plants like *A. tenuifolius* containing anti-oxidant phytochemicals (Eddine et al., 2015; Kalim et al., 2010) with hypotensive and diuretic activities are expected to be suitable candidates for the treatment of hypertension associated with renal dysfunction.

Conclusion

The 70% ethanolic extract of *A. tenuifolius* possesses hypotensive, vasorelaxant and diuretic activities in animal models. Hypotensive effect of the extract possibly involves vasorelaxant and Ca^{2+} channel blocking activities. Diuretic effect of the extract involves loop diuretic or thiazide diuretic like actions, while carbonic anhydrase inhibitory and potassium sparing activities were not likely to be present. The study is a step forward towards evidence based phytomedicine.

Ethical Issue

The "Guide for the Care and Use of Laboratory Animals" issued by Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council (1996) was complied with and the study was approved by the ethical committee of the institution.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

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