American Journal of Pharmacology and Toxicology 2 (3): 130-134, 2007
ISSN 1557-4962

Cardiovascular Effect of *Capapris spinosa* Aqueous Extract in Rats. Part II: Furosemide-like Effect of *Capparis spinosa* Aqueous Extract in Normal Rats

[1]N.A. Zeggwagh, [2]J.B.Michel, [3]T. Sulpice and [1]M. Eddouks
[1]UFR PNPE, BP.21, Errachidia, 52000, Morocco
[2]U460 INSERM, Faculty of Medicine Xavier Bichat, Paris, France
[3]Physiogenex, Toulouse, France

Abstract: The purpose of this study was to examine the diuretic effect of the aqueous extract of *Capparis spinosa* (Cs) at a dose of 500 mg kg^{-1} h^{-1} in normal rats. The aqueous extract was administred intravenously and the diuresis was followed within four hours after starting the treatment. Intravenous administration of aqueous Cs extract produced a significant increase on diuresis ($p<0.001$) and glomerular filtration rate from the second hour of treatment to the fourth hour ($p<0.001$). In addition, urinary Na+, Cl- and K + were significantly increased after two hours of Cs perfusion ($p<0.01$). Furosemide at a dose of 0.1 mg kg^{-1} h^{-1} had similar effect when compared to Cs administration. We conclude that the water extract of Cs fruit exhibited a significant diuretic effect accompanied by in crease in Na+, K+ and Cl- urinary excretion in normal rat. This effect may be mediated via a Furosemide like effect.

Key words: Diuretic effect, ethnophamacology, aqueous extract

INTRODUCTION

Increasing diuresis phenomena might be a useful tool in the treatment of hypertension[1]. Medicinal plants was commonly used for traditional treatment of some renal diseases[2]. Many investigations have demonstrated the hypotensive effect of several plants with a diuretic activity[3-11]. Thus the use of diuretic plants in the treatment of hypertension may be beneficial in the reduction of blood pressure and kidney damage associated with hypertension.

Capparis spinosa (Cs) is a plant belonging to Capparidaceae family wildly grown in the mediterranean basin[12]. From ancient time, Cs fruit is used in the folk medicine for its hypoglycaemic[13,14] diuretic, antihypertensive and tonic properties[15].
This study aims to evaluate diuretic effect of continuous intravenous perfusion of aqueous Cs extract in anaesthetized rats.

MATERIALS AND METHODS

Plant material: Specimens of Cs (Capparidaceae) were collected from the Tafilalet region (semi-arid area) of Morocco in May-June 2003 and air-dried at 40°C. The plant was previously identified and authenticated by Pr. M. Rejdali (Agronomy and Veterinary Institute, Rabat) and a voucher specimen (ME 60) was deposited at the herbarium of the Faculty of Sciences and Techniques Errachidia.

Preparation of the aqueous extract: About 1 g of powdered fruits mixed with 100 ml distilled water were boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St Quentin en Yvelines, France) to remove particulate matter. The filtrate was then freeze-dried and the desired dose (mg of lyophilized aqueous extract of Cs fruits per kg body weight) was then prepared and reconstituted in physiologic saline solution just before administration. The extract was green coloured with a percent yield of 14%, its average osmolarity was 32 mOsm, pH 6.5 and with a very low viscosity.

Animals: The hypotensive effect of Cs was studied in adult male wistar rats weighing (250-300 g). Animals were housed under standard environmental conditions (23±1°C, 55±5% humidity and a 12 h light/dark cycle) and had free access to water and *ad libitum* standard laboratory diet.

Surgery: The rats were anaesthetized by an intraperitoneal injection of Inactin at a dose of 50 mg kg^{-1} of body weight. They were then placed on a

Corresponding Author: Mohamed Eddouks, BP 21, Errachidia, 52000, Morocco Tel: +212 55 57 44 97 Fax: +212 55 57 44 85

thermostated table to keep them at a constant temperature. Two catheters PE50, one filled with physiological saline solution (NaCl), the other filled with heparinized physiological saline solution were introduced respectively to the right jugular vein of the cardiac side and to the left jugular vein of the encephalic side. The first serves to perfuse the test solutions and the second for blood sampling. The bladder was also catheterized in order to collect urine for determination of different parameters. At the end of the experiment, the animals were sacrificed by cutting their carotids, urine and plasma were conserved 2 and 4 h of treatment. All experiments were performed in fasted rats.

Parameters: Blood samples from experimental rats were collected from the jugular vein. Urinary samples were collected from the catheterized bladder. Creatinine and plasma urea levels were evaluated by colorimetric methods, according to the manufacturer's protocol (Boerhinger, Germany) using a spectrophotometer (HITACHI Model U-2001).

Sodium, potassium and chloride levels were determined in urine samples using an auto-analyser (HITACHI 911-Boehringer).

Glomerular filtration rate was evaluated by the clearance of creatinine.

Statistical analysis: Results were expressed as mean ± S.E.M of six observations. ANOVA was used to calculate the levels of significance for comparison made within group and between groups using Graphpad prism 4.0 software.

RESULTS

Diuretic activity: Intravenous injection of aqueous Cs extract at a rate of 50 mg kg^{-1} min^{-1} provoked an increase of urinary outflow (Fig. 1). This effect was observed form the second hour ($p<0.001$) of Cs perfusion to the fourth hour ($p<0.001$). Furosemide perfusion (0.1 mg kg^{-1} h^{-1}) induced a similar and cumulative effect; the urine flow was significantly elevated from the first hour ($p<0.001$) to the end of treatment ($p<0.001$).

Effect on Glomerular Filtration Rats (GFR): Perfusion of aqueous Cs extract during two hours caused a significant increase in Glomerular Filtration Rate (GFR), as estimated by creatinine clearance ($p<0.01$), this effect was maintained at the fourth hour of treatment ($p<0.01$) (Fig. 2). On the other hand, Furosemide did not change the GFR.

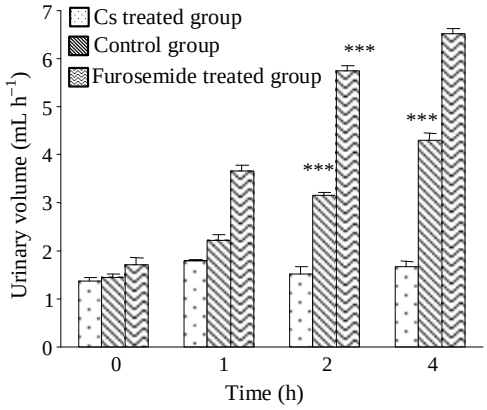

Fig. 1: Effect of intravenous administration of aqueous Cs extract (500 mg kg^{-1} h^{-1}) on urinary excretion of water (mL h^{-1}). Data are expressed as means±SEM, n = 6 rats per group. **p<0.01, p<0.001 when compared to the respective control values

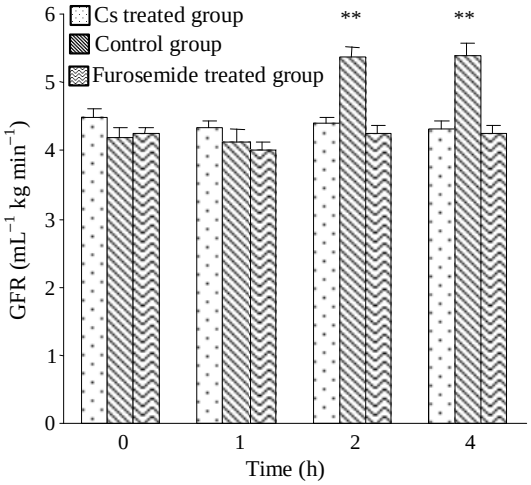

Fig. 2: Effect of intravenous administration of aqueous Cs extract (500 mg kg^{-1} h^{-1}) on glomerular Filtration Rate (GFR) (mL kg^{-1} h^{-1}). Data are expressed as means±SEM, n = 6 rats per group. **p<0.01, p<0.001 when compared to the respective control values

Effect on electrolytes excretion: Figure 3 shows the natriuretic effect of aqueous Cs extract perfusion. Cs perfusion induced an increase in urinary excretion of sodium in the second hour of perfusion ($p<0.01$), this effect was maintained until the end of treatment ($p<0.01$). Earlier increase in the urinary sodium levels was observed in Furosemide-treated group from the first hour of treatement ($p<0.05$) and further increase was observed in the second and the fourth hour of treatment ($p<0.001$).

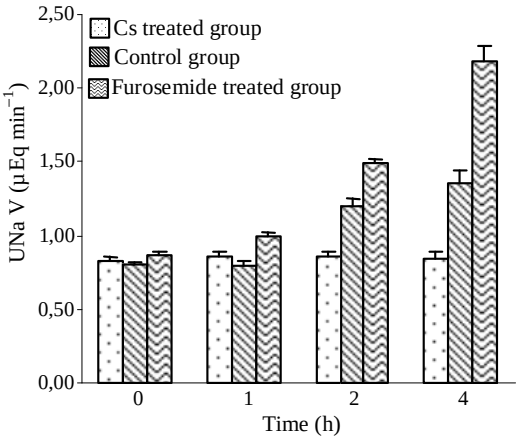

Fig. 3: Effect of intravenous administration of aqueous Cs extract (500 mg kg^{-1} h^{-1}) on urinary excretion of sodium (UNa+V) (µEq min^{-1}). Data are expressed as means±SEM, n = 6 rats per group. **p<0.01, p<0.001 when compared to the respective control values

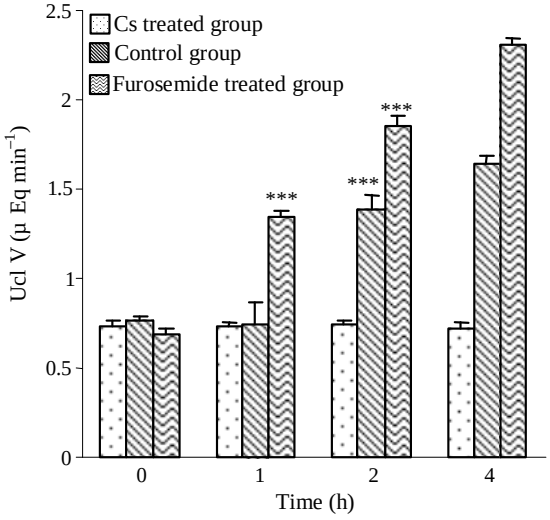

Fig. 4: Effect of intravenous administration of aqueous Cs extract (500 mg kg^{-1} h^{-1}) on urinary excretion of chlorure (Ucl-V) (µEq min^{-1}). Data are expressed as means ± S.E.M, n = 6 rats per group. **p<0.01, p<0.001 when compared to the respective control values

Change in urinary chloride levels is shown in Fig. 4. Significant increase in urinary chloride levels was observed in the second hour in Cs-treated group (p<0.01). However, Furosemide treatment produced a significant increase in urinary chloride levels (p<0.001) from the first hour to the end of treatment (p<0.001).

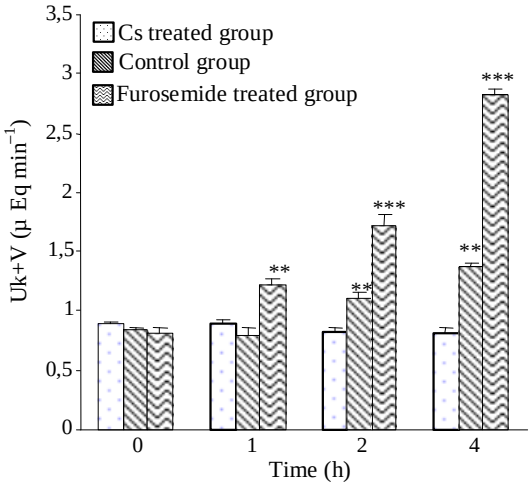

Fig. 5: Effect of intravenous administration of aqueous Cs extract (500 mg kg^{-1} h^{-1}) on urinary excretion of potassium (Uk^{+}V) (µEq min^{-1}). Data are expressed as means±SEM, n = 6 rats per group. **p<0.01, p<0.001 when compared to the respective control values

As it is shown in Fig. 5, Cs perfusion induced a significant increase in urinary potassium levels form the second hour (p<0.001) to the end of treatment (p<0.001). Significant increase in urinary potassium levels was observed in furosemide-treated group after one hour of treatment, additional increase in the urinary potassium levels was observed at the end of treatment.

DISCUSSION

Cs fruits are used in the traditional health care in Tafilalet region (South of Morocco) in the treatment of diabetes and cardiovascular disorders[16]. Our previous studies demonstrated that several plants used in this region have renal effect leading to increase diuresis and inhibition of glucose renal absorption.

In this study, the diuretic effect of intravenous perfusion of aqueous Cs extract at a rate of 500 mg kg^{-1} min^{-1} was evaluated in anaesthetized rats. The effect on electrolyte balance and creatinine clearance was also determined. Cs was administered intravenously because catheterization of the bladder needs surgery and the monitoring of urinary output required a continuous perfusion of the test solution. Furosemide, a high ceiling loop diuretic was used as reference drug[17,18]. Increasing urine output and solute urine levels are the two components of diuresis[17]. Furosemide increases urine output and urinary excretion of sodium by inhibition of Na+/K+/2Cl- symporter in the loop of henle[18].

Cs perfusion caused a diuretic response accompanied with an increase in GFR. This effect may be due to the direct effect on the arterial blood pressure producing vasodilatation by decreasing renal perfusion pressure[19]. Our recent work demonstrated that Cs perfusion reduced mean arterial blood pressure in anaesthetized normal rats (data not shown), this effect may be partially be due to the observed diuretic effect. However, the increase in the creatinine levels must be taken with care since it may be due the a probable renal toxicity[20].

In addition, Cs perfusion incresaed urinary sodium, chloride and potassium levels suggesting a probable inhibition of Na+/K+/2Cl- commonly named as Furosemide like effect. Previous studies have been reported similar diuretic mechanism of plants extract[2,20-22]. We have previously demonstrated that the increase of diuresis was accompanied by a similar qualitative increase of electrolytes after *Spergularia purpurea* and *Retama reatam* water extract treatment[9,23]. The possibility of direct action of potassium content of Cs extract on diuretic effect was ruled out since the K+ content of this plant seems to be lower to affect diuresis[11].

A number of compounds have been identified in Cs including flavonoids, saponins and tannins[15]. The compounds which are responsible for the observed renal effect of Cs aqueous extract are not determined.

Based on the above results, we can conclude that Cs treatment produced a marked diuresis when rats were acutely treated. In our study, no lethality was observed at least for the dose and duration used. However, advanced toxicological studies remain to be performed in mice and rats. The precise site(s) and the molecular and cellular mechanism(s) of Cs extract action remain to be elucidated in further studies.

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