Arch. Pharm. Res. Vol. 17, No. 3, pp. 145-149, 1994

Hypotensive and Spasmolytic Activities of Crude Extract of *Cyperus scariosus*

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(Received December 15, 1993)

Intravenous administration of hydro-methanolic extract of *Cyperus scariosus* (3-10 mg/kg) produced hypotensive and bradycardiac effects. These effects remained unaltered in atropinized animals indicating that cardiovascular effects of the plant extract are not mediated through activation of muscarinic receptors. In the *in vitro* studies, it suppressed the spontaneous contractions of guinea-pig paired atria, rat uterus and rabbit jejunum in a concentration-dependent (0.1-1 mg/ml) manner. It also inhibited histamine or acetylcholine-induced contractions of guinea-pig ileum indicating non-specific spasmolytic action. In rabbit aorta, it inhibited norepinephrine (10 μM) as well as K^+ (80 mM)-induced contractions at similar concentrations (0.1-1 mg/ml). These data indicate that *Cyperus scariosus* contains Ca^{2+} channel blocker-like constituent(s) which may explain hypotensive effect observed *in vivo* and the general spasmolytic activity of plant may explain its folkloric use in diarrhoea.

Key words: *Cyperus scariosus*, Hypotensive, Spasmolytic, *In vivo*, *In vitro*

INTRODUCTION

Cyperus scariosus, Br. (Syn: *C. pertenuis*, Roxb.; family: Cyperaceae) is a selender, delicate grass, growing luxuriously in damp places of eastern and southern parts of the Indo-Pak-Bangla Desh subcontinent (Watt, 1972). Plant roots has a folkloric reputation as a cordial, tonic desicant, emmenagogue, diaphoretic and diuretic (Kirtikar and Basu, 1918; Watt, 1972; Said, 1982). It remained to be an important ingredient of several prescriptions used in indigenous system of medicine to treat a variety of diseases including diarrhoea, epilepsy, fever, gonorrhoea, syphilis and liver damage (Kiritkar and Basu, 1918; Said, 1982). The essential oil obtained on steam distillation of rhizomes and roots of the plant has its value in perfumary (Kahol *et al.*, 1987), and is also known to possess antibacterial (Laharia and Rao, 1979), antifungal (Desmukh and Jain, 1985) as well as plant growth-regulating (Kalsi *et al.*, 1980) properties. Phytochemical studies revealed the presence of sesquinterpenes (Nerali and Chakravarti, 1969; Nerali *et al.*, 1970), steroidal saponins (Bhatt *et al.*, 1982), aurone (Bhatt *et al.*, 1984) and substituted hydrocarbons (Nevelle *et al.*, 1968; Uppal *et al.*, 1984).

In this investigation, we describe hypotensive action in normotensive anesthetized rats and spasmolytic action in different isolated tissue preparations.

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MATERIAL AND METHODS

Plant Material

Dried tubers of *Cyperus scariosus* plants were collected from a local herbal store in Karachi and authenticated with the help of a botanist at The University of Karachi. The plant material was shade dried, powdered and macerated in 80% aqueous-methanol (BDH Ltd. Poole, England) for one week with occasional shaking. The extract was filtered and concentrated to thick reddish brown residue under reduced pressure on a rotary evaporator. The approximate yield was 10%. The plant extract was dissived in saline for pharmacological testing.

Drugs and Animals

The following reference materials were obtained from the sources specified: acetylcholine chloride, atropine sulphate, norepinephrine hydrochloride, potassium chloride (Sigma Chem. Co. St. Louis, MO, USA),

Calcium chloride (E. Merck, Darmstadt, F.R. Germany) and pentothal sodium (Abbott Laboratories, Pakistan). All drugs were dissolved in distilled water and dilutions were made fresh in normal saline (0.9% NaCl) on the day of experiment.

Animals used in this study were housed at the Animal House of The Aga Khan University at 23-25°C and were given a standard diet and tape water *ad libitum*.

In vivo Experiments

The effect of drugs on blood pressure was studied as reported previously (Gilani, 1991). Wistar rats of either sex (200-250 g) were anesthetized with thiopental sodium (50 mg/kg, i.p.). The trachea was exposed, and cannulated to facilitate spontaneous respiration. The arterial blood pressure was measured from the carotid artery via an arterial cannula connected to a pressure transducer (Washington PT-400) coupled with FC-137 (pressure) and FC-128 (heart rate) couplers using MD_4 Bioscience recorder. Drugs were injected in the form of bolus injection (0.1 ml) via a cannula inserted into the external jugular vein followed by a saline flush (0.2 ml). The temperature of the animal was maintained at 37°C by use of a heated table and over head lamp. Animals were allowed to equilibrate for at least 20 min before administration of any drug. Mean blood pressure (MBP) was determined from the sum of diastolic BP plus one-third pulse width. Changes in blood pressure were expressed as percentages of control values obtained immediately before the administration of test substance(s).

In vitro Experiments

These experiments were carried out by the method previously used in our laboratory (Gilani and Cobbin, 1986; Gilani, 1991).

Guinea-pig Atria

Guinea-pigs of either sex (400-600 g) were killed by cervical dislocation. Paired atria were removed carefully and mounted in a 20 ml tissue bath filled with Krebs-Henseleit solution maintained at 32°C and aerated with 5% carbon dioxide in oxygen. The composition of physiological salt solution was (mM): NaCl, 118.2; KCl, 4.7; $MgSO_4$, 1.2; KH_2PO_4, 1.2; D-glucose, 11.7; $NaHCO_3$, 25.0 and $CaCl_2$, 2.5 (pH 7.4). The spontaneous atrial contractions were recorded via a force displacement transducer (FT-03) using a Grass model 79 polygraph. Each preparation was allowed to equilibrate under 1 g resting tension for at least 30 min before administration of any drug.

Rabbit Aorta

New Zealand white rabbits of either sex weighing 2-3 kg were killed by a blow to the back of the head. The descending thoracic aorta was quickly removed and cut into rings of 2-3 mm width which were opened by cutting perpendicular to the axis of symmetry of the cylindrical vessel to make strips. Each strip preparation was mounted in a 20 ml tissue bath containing Krebs-Henseleit solution, maintained at 37°C and continuously bubbled with a mixture of 95% oxygen and 5% carbon dioxide. A resting tension of 2 g was applied to each tissue and an equilibrium period of one hour was allowed before drug-induced changes in isometric tension of the strips were measured as described for atria.

Guinea-pig Ileum

Segments of ileum about 2 cm length obtained from guinea-pigs were supended in a 10 ml tissue bath, filled with Tyrode's solution, maintained at 37°C and aerated with 5% carbon dioxide in oxygen. The composition of the Tyrode's solution was (mM): NaCl, 136.9; KCl, 2.7; $MgCl_2$, 1.1; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9; D-glucose, 5.6; $CaCl_2$, 1.8 (pH 7.4).

An initial loading of 0.7 g was applied to the tissue and isotonic contractions to acetylcholine (ACh) were recorded with a Bioscience transducer (T_3) coupled to a Bioscience (PR 200) chart recorder.

The tissue was exposed for upto 20 seconds, to a constant concentration of ACh which produced a sub-maximal response, then washed by over flow and the cycle repeated at 3 min intervals until constant responses were recorded (usually 10-15 contractions).

Rat Uterus

Young female rats (170-200 g) were killed by a blow on the head. the middle 2 cm of the uterine horns were cut longitudinally and each strip was mounted in a 10 ml tissue bath containing Tyrode's solution maintained at 37°C and gassed with a mixture of 5% of CO_2 in oxygen. The tissue was allowed to equilibrate under 0.5 g basal tension for 20 min before isotonic contractions were recorded as described for ileum.

Rabbit Jejunum

New Zealand white rabbits (2-3 kg) of either sex starved for 24 hours were killed by cervical dislocation and exsanguination. Segments of jejunum about 2 cm length were mounted in 20 ml tissue bath containing Krebs-Henseleit solution, maintained at 37°C and bubbled with a gas mixture of 95% O_2 and 5% CO_2. A preload of 1.0 g was applied and spontaneous contractions were recorded isotonically via T-3 isotonic transducer on Bioscience MD_4 recorder. The tissue were allowed to equilibrate for one hour before addition

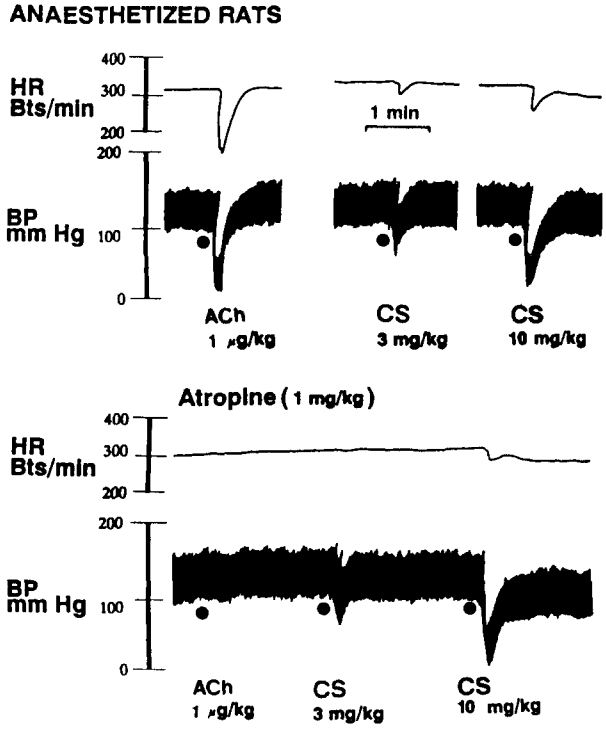

Fig. 1. A representative tracting (n=4) showing comparison of *Cyperus scariosus* (CS) and acetylcholine (ACh) for their effects on blood pressure (BP) and heart rate (HR) in the absence and presence of atropine in anesthetized rats. Atropine was administered 5 min before the redetermination of ACh or CS responses.

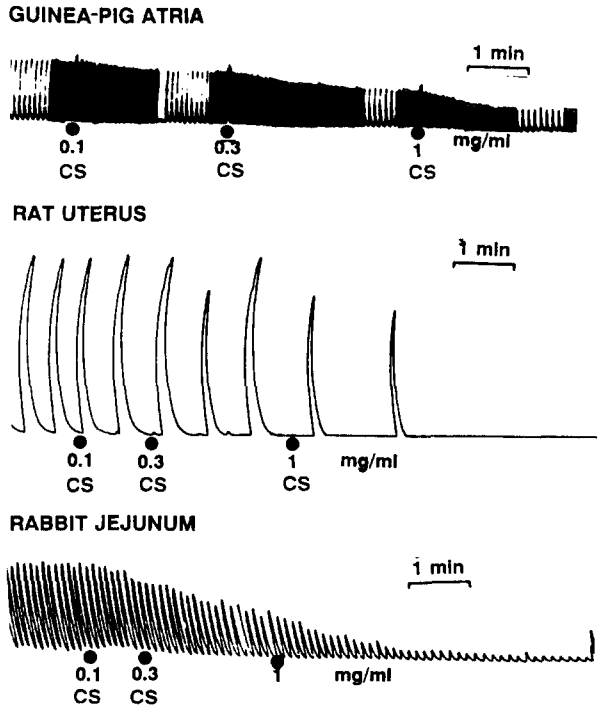

Fig. 2. A representative tracings (n=5) showing inhibitory effects of *Cyperus scariosus* on spontaneous movements of isolated tissue preparations.

of any drug.

The concentrations mentioned in the text or in the figures represent the final bath concentrations.

RESULTS

In vivo Studies

In anesthetized rats, hydro-methanolic extract of *Cyperus scariosus* (3-10 mg/kg) caused a fall in systolic, diastolic and mean arterial blood pressure in a dose-dependent manner (Fig. 1). There was slight decrease in heart rate but this effect was not reproducible. These cardiovascular effects were brief, returning to normal within two minutes. The drop in mean arterial blood pressure at the dose of 3 mg/kg was 18.4± 4.5% (mean± S.E.M.; n=4) while 53.55± 2.47% reduction was observed at the next higher dose (10 mg/kg). Fig. 1, shows a representative tracing of comparison of the plant extract and acetylcholine for their hypotensive and bradycardiac effects before and after treatment with atropine (1 mg/kg). Acetylcholine (1 µg/kg) produced inhibitory responses both on blood pressure and heart rate qualitatively similar to those of the plant extract. Pretreatment of animals with atropine did not alter the cardiovascular responses of the plant extract, whereas both vasodilator and bradycardiac effects of acetylcholine were completely abolished. Norepinephrine (1 µg/kg) produced a pronounced increase in arterial blood pressure which was completely blocked by phentolamine (1 mg/kg) but was not affected by pretreatment with the plant extract (data not shown) which rule out the possibility of adrenoceptor blocking action of the extract.

In vitro Studies

Plant extract at the concentration of 0.1-1 mg/ml, caused a concentration-dependent inhibition of spontaneous contractions of guinea-pig paired atria, rat uterus and rabbit jejunum as shown in Fig. 2. This inhibitory effect was reversible as the each tissue regained its spontaneous activity after washing the tissue a couple of times with the fresh bathing fluid.

In guinea-pig ileum, the inhibitory effect of plant extract was studied against agonist-induced contractile responses. Both acetylcholine (ACh) and histamine (His) produced submaximal contractions at 0.1 µM. Pretreatment of tissue with plant extract (0.3 mg/ml) caused approximately 40% inhibition of both acetylcholine and histamine responses (Fig. 3). Next higher concentraction (1 mg/ml) further suppressed the agonist contractile responses to 80-90% inhibition. This inhibitory effect of the plant extract was reversible on

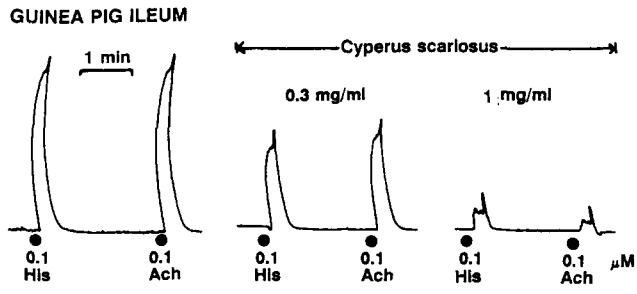

Fig. 3. A reparesentative tracing (n=4) showing inhibitory effect of *Cyperus scariosus* (CS) against acetylcholine (ACh) and histamine (His)-induced contractions of guinea-pig ileum.

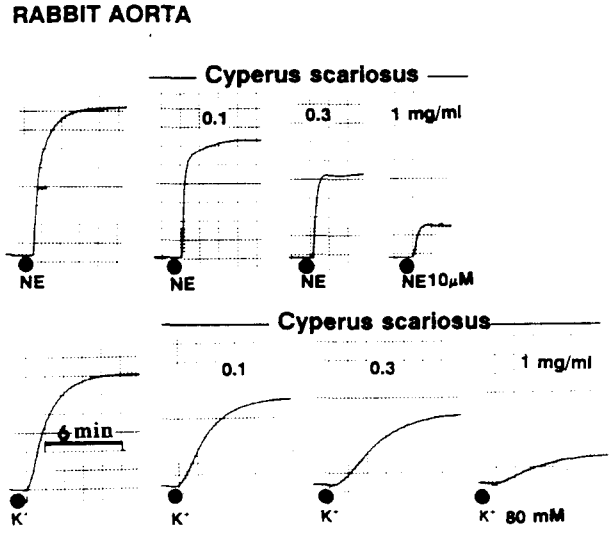

Fig. 4. A representative tracing (n=4) showing inhibitory effect of crude extract of *Cyperus scariosus* against norepinephrine (NE) and K^+-induced contraction in rabbit aorta.

wash out as the tissue regained its initial sensitivity to agonist after 3-5 washings and testing the agonist response between washings.

In rabbit aorta, both norepinephrine (10 μM) and K^+ (80 mM) caused strong reproducible contractions and the tissue attained its resting state 20-30 minutes after wash out. Tissue was pretreated with plant extract (0.1 mg/kg) 30 minutes before redetermination of norepinephrine or K^+ responses and washed following the platea levels of agonist responses. The next higher concentration of plant extract were tested in a similar way. As shown in Figure 4, plant extract inhibited norepinephrine or K^+-induced contractions in a concentration-dependent manner (0.1-1 mg/ml). The inhibitory effect of plant extract was similar against both spasmogens and was reversible after frequent washings and testing agonist responses with regular intervals.

DISCUSSION

The hydro-methanolic extract of *Cyperus scariosus* produced hypotensive and bradycardiac effects in anesthetized rats. Acetylcholine also produced apparently similar responses which were blocked by atropine, a competitive blocker of acetylcholine at muscarinic receptor site (Arunlakshana and Schild, 1959; Gilani and Cobbin, 1987). However, pretreatment with atropine failed to abolish the cardiovascular responses to the extract suggesting that unlike acetylcholine, plant extract mediates its hypotensive action through a mechanism independent of muscarinic receptor activation.

The plant may have direct relaxant action. This speculation was confirmed when plant extract was tested on isolated tissue preparations. The plant extract suppressed the spontaneous movements of guinea-pig paired atria, rabbit jejunum and rat uterus thus, showing its general spasmolytic activity.

The rabbit aorta preparation was used for the study of mechanism of spasmolytic action. Pretreatment of tissue with the plant extract inhibited aorta contractions induced by norepinephrine (10 μM) or K^+ (80 mM). It has been shown that high K^+-concentrations cause marked contractions in blood vessels by depolarizing smooth muscle cells and increasing the influx of calcium through L type voltage operated channels (VOC). In contrast contractions induced by norepinephrine and by other neurotransmitters is due partly to calcium release by intracellular stores and party to influx of extracellular calcium via receptor channels (ROC) (Bolton, 1979; Godfraind *et al.*, 1986; Karaki and Weiss, 1983).

In this study, plant extract inhibited contractions induced by 80 mM K^+. This vasorelaxant effect is not due to opening of K^+ channels because cromokalin and other K^+ channel openers do not inhibit contractions induced by K^+ concentrations greater than about 30 mM (Hamilton *et al.*, 1986; Deitmer *et al.*, 1992). The fact that the inhibitory effect of plant extract against K^+-induced and norepinephrine-induced contractions was observed at similar concentrations, suggests that plant extract may be acting intracellularly and/or on the cell membrane blocking calcium influx through voltage dependent and receptor operated channels.

Thus the results of this study clearly indicate that the hydro-methanolic extract of *Cyperus scariosus* produces direct depressant effects like Ca^{2+} channel blockers and that this action is probably responsible for its hypotensive and bradycardiac effects observed in anesthetized rats.

The plant has been used traditionally for gastro-intestinal disorders such as diarrhoea (Kirtikar and Basu, 1918). Spasmolytics are considered useful in diarrhoea due to the hypermotility of the gastrointestinal tract (Crema and Ponti, 1989) and the general spasmolytic activity of plant extract observed in this study, may provide the pharmacological basis for the usefulness

of the plant in such condition.

ACKNOWLEDEMENTS

This study was supported by the funds made available by The National Scientific Research Development Board and The Aga Khan University Medical College.

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