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In Vitro Bronchorelaxant Effects of *Capparis Spinosa* Aqueous Extracts on Rat Trachea

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ABSTRACT

Capparis spinosa (Capparidaceae) dicotyledons from the class of spermaphytes, is a shurb, enduring and woody plant, typically Mediterranean, largely used in folk medicine in the Mediterranean countries including Algeria. The aim of the present research is to assess the in vitro effects of aqueous extract of different parts of *Capparis spinosa* (leaves, fruits and seeds) on rat trachea in order to establish them as a real source for the isolation of bioactive compounds with potential use as anti-obstructive or anti-allergic agents. Rings of windpipes of rat Wistar were isolated, streamlined cut and suspended in organ bath containing 10 ml of Krebs physiological solution. The addition of *Capparis spinosa* extracts (0.1, 1 and 10 mg/ml) during the step of contraction by acetylcholine showed various effects on trachea. Incubation of the windpipe for 30 mn with extracts proves to be so efficient. The dose of 10 mg/ml showed a significant relaxant effect for fruits and seeds, and constrictor effect for the leaves. The results showed a potent relaxant effect of the fruit aqueous extract of *Capparis spinosa*, on rat trachea, with a dose dependant manner. However, the leaf aqueous extract has a contractive effect. A muscarinic receptor blockade/stimulation was suggested for caper/leaf extracts.

INTRODUCTION

Capparis spinosa L. common perennate shrub with medicinal and aromatic properties, growing widely in the Mediterranean basin including Algeria. The floral buttons of *Capparis spinosa*, were used in traditional medicine as a poultice and for their diuretic, antihypertensive and tonic properties (Baytop, 1984; Çalis *et al*., 1999). Methanol extract of *C. spinosa* buds, rich in flavonoids, including several quercetin and kaempferol glycosides, has strong antioxidant/free radical scavenging effects in different *in vitro* tests. Lemhadri *et al*. (2007) have shown a potent antihyperglycemic and anti-obesity actions. The study of Eddouks *et al*. (2004, 2005) showed that the aqueous extract has potent activity on the reduction of cholesterol, triglycerides and glucose in normal and severe hyperglycemia. Panico *et al*. (2005) and Feng *et al*. (2011) proposed the caper extract to be tried in clinical tests for its anti-arthritic effect. A preliminary experiment indicated that the seed extract had antiproliferative activity toward tumor cells (Lam and Ng, 2009). The methanolic extract of *C. spinosa* caper possessed a marked inhibitory effect (46.07%) against histamine-induced skin erythema (Trombetta *et al*., 2005). Caper aqueous extract effectively inhibited the carrageenan induced paw edema in mice (Zhou *et al*., 2010). Other activities included antiviral, immunomodulatory (Arena *et al*., 2008), chondrocyte protective (Panico *et al*., 2005), antifungal (Ali-Shtayeh & Abu Ghdeib, 1999), anti-Leishmania (Jacobson and Schlein, 1999) and antimicrobial (Mahasneh, 2002) activities, as well as inhibitory effect on fibroblast proliferation and type I collagen production in progressive systemic sclerosis (Cao *et al*., 2008). Recently, we have shown that all parts of *C. spinosa* possess antioxidant effects with certain correlation with their polyphenols and flavonoids contents (Arrar *et al*., 2013). Acetylcholine is the primary parasympathetic neurotransmitter in the airways, and is traditionally associated with inducing airway smooth muscle contraction and mucus secretion (Gosens *et al*., 2006). Parasympathetic activity is increased in airway inflammation, which is the basis for the use of anticholinergic therapy in asthma and chronic obstructive pulmonary disease (COPD) (Gross & Skorodin, 1984). Anticholinergics are widely used for the treatment of COPD, and to a lesser extent for asthma. Primarily used as bronchodilators, they reverse the action of vagally derived acetylcholine on airway smooth muscle contraction.

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We aim here to study the effect of the aqueous extract of different parts of *Capparis spinosa* used in traditional medicine on rat trachea contracted with acetylcholine in order to confirm the use of this plant in allergic conditions.

MATERIALS AND METHODS

Plant material

Capparis spinosa was collected from the region of Beni-Aziz, Wilaya of Setif northeast of Algeria and identified by Pr H. Laouer, Faculty of Nature and Live Sciences, University Setif 1, Algeria. A voucher specimen was preserved in the laboratory. Leaves, seeds and capers (fruits) of *C. spinosa* were dried in shadow and powdered before the extraction.

Preparation of *C. spinosa* extracts

Ten grams of powdered plant parts (leaves, seeds and capers) were mixed with 100 ml distilled water, heated for 15 min and cooled for 15 mn. The aqueous extract was stirred overnight at 4°C (Arrar *et al*., 2013).

Preparation of rat trachea

Healthy adult rats Wistar, 250-300 g were used in this study. They were maintained under standard laboratory conditions with free access to food and water. Trachea ring were used to study the possible bronchoactive effects of extracts. The rats were anesthetized, the trachea were excised, placed in Krebs solution, cleaned of connective tissue and cut into approximately 3 mm wide rings. Each trachea ring was mounted in a 10 ml organ bath containing Krebs buffer solution (pH 7.4), maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. During this period Krebs solution was replaced every 10 min. Changes in force were recorded by isometric force displacement transducer (Narco F-60) and Physiographs (Linseis).

Treatment of trachea with plant extract

Before starting the experimental protocol, contractile responsiveness of the trachea rings, acetylcholine (Sigma Aldrich) at 10^{-5} M was added during the plateau phase to analyze the reactivity of trachea rings. Trachea rings were pre-contracted with acetylcholine 10^{-5} M, once the plateau was reached the bath fluid was renewed several times until the preparations returned passively to initial resting tone. Then, the preparations were incubated 30 min with aqueous extracts of *Capparis spinosa* (0,1 mg/ml 1 mg/ml and 10 mg/ml). Subsequently, increasing concentrations of acetylcholine (10^{-9} to 10^{-4}) were added in the organ bath.

Statistical analysis

All determinations were conducted in triplicate and all the results were calculated as mean ± standard deviation (SD). Statistical analysis was performed using Student's *t* test for significance. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Results of *C. spinosa* effects on dose response curves induced by acetylcholine in rat trachea are presented in table 1. It is clear that even pEC50 were similar, the Emax showed a relaxant effect of caper extract.

Table. 1: pEC50 and Emax values of the leaf (LAE), capper (CAE) and seed (SAE) on the concentration curve obtained with acetylcholine in rat trachea pEC50 and maximum response were calculated by non-linear regression of experimental data.

Extract	control	SAE	LAE	CAE
pEC50	5.8 ± 0.3	5.7 ± 0.4	5.6 ± 0.3	5.6 ± 0.2
Emax	1.9 ± 0.2	1.9 ± 0.0	1.3 ± 0.7	0.9 ± 0.3

At low concentration of the extracts of 0.1 mg/ml (figure 1A), no significant effect was observed at all concentrations of acetylcholine (10^{-9} to 10^{-4}). When extracts were used at 1 mg/ml (figure 1B) a relaxant effect but only at high concentrations of acetylcholine (10^{-6} to 10^{-4} M) was shown by caper extract which gave the same effect at 10 mg/ml. In contrast, leaf extract showed a contractile role. However, at the concentration of 10 mg/ml (figure 1C), leaf extract give a contractile effect at all doses of acetylcholine (synergic effect). After pre-contraction of trachea preparations with acetylcholine (10^{-5} M) and passively return to initial resting tone, inhibition of the contraction using 30 min incubation of SAE, LAE and CAE at dose of 0.1, 1 and 10 mg/ml was followed on contraction behavior at 10^{-4} M of acetylcholine. Aqueous extract of *Capparis spinosa* caper possessed a bronchodilation on acetylcholine pre-contracted trachea as shown in figure 2. This effect could not be attributed to the quantity of polyphenols and flavonoids but to their quality. We have previously found (Arrar *et al*., 2013) that the caper contained 7.2 mg GAc-Eq/g dry extract and only 1.1 mg R–Eq/g dry extract. However, leaf and seed extract contained more polyphenols (57.0 and 35.8 GAc-Eq/g dry extract, respectively) and flavonoids (11.2 and 2.4 mg R–Eq/g dry extract, respectively). The muscarinc receptors blockade was suggested for the extract effect since these receptors are responsible for bronchial and tracheal smooth muscle contraction and this is evident from the functional affinities of a variety of selective antagonists in airway tissues from diverse species, including humans (van Nieuwstadt *et al*., 1997; Roffel *et al*., 1990). The relaxant effects of the caper extract observed in the present study could be translated to bronchodilation and might have potential benefits in asthmatic patients.

CONCLUSION

The results showed that the aqueous extracts from *Capparis spinosa* leaves can elicit broncho-contractant action. In contrast, *C. spinosa* capers possessed a broncho-relaxant effect or antagonist to the receptors activated by acetylcholine in rat trachea. Therefore, blocking effects upon Ca^{2+} influx through voltage-dependent calcium channels may be implicated. Further experiments could elucidate the active constituent(s) contained in the extracts and possible mechanism of action.

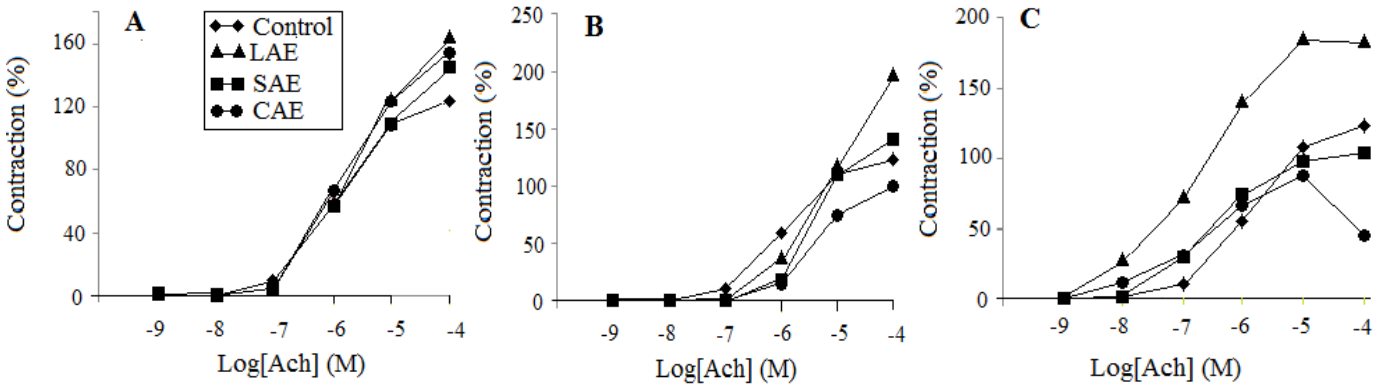

Fig.1: Effect of aqueous extracts: leaf (LAE), capper (CAE) and seed (SAE), at dose of 0.1 mg/ml (A) 1 mg/ml (B) and 10 mg/ml (C) on the contraction curve induced by acetylcholine (Ach) on rat trachea rings.

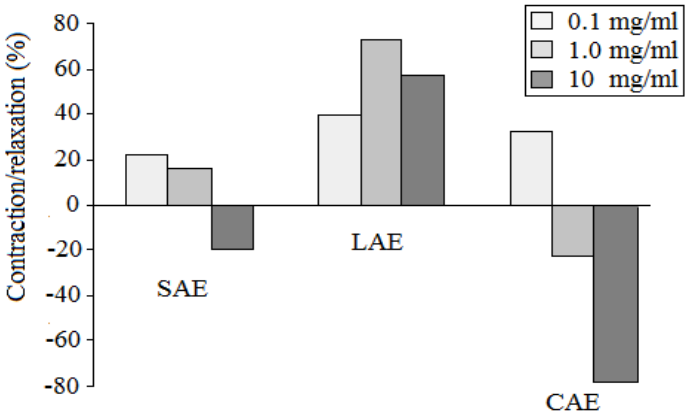

Fig. 2: Effect of SAE, LAE and CAE at dose of 0.1, 1 and 10 mg/ml addition in the plateau contraction induced by Acetylcholine (10^{-4} M) in rat trachea ring. Results are expressed as differences between percentage of maximum response of treated and control trachea ring obtained after 30 mn incubation.

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