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Antispasmodic Effects of Aqueous extract of *Anthemis mauritiana* Maire & Sennen Flowers

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ABSTRACT

The effects of aqueous extract of *Anthemis mauritiana* Maire & Sennen flowers (AM) on rabbit and rat jejunum were studied. The AM (0.1-3 mg/mL) showed reversibly relaxation of spontaneous contractions on isolated rabbit jejunal smooth muscle The spasmolytic effect was dose-dependent with IC_{50} value of 1,48 ± 0,02 mg/ml. Similarly this extract inhibited the contractions of rat jejunum induced by KCl (75mM) and Carbachol (CCh, 10^{-6}M) with IC_{50} values of 0,48 ± 0,09 mg/ml and 1,53 ± 0,03 mg/ml respectively. Furthermore, AM exhibited an inhibitory effect on the dose-response curves induced by CCh and $CaCl_2$ on rat jejunum. These results clearly demonstrated the antispasmodic effect of AM which was strongly suggested to be mainly due to the inhibitory effect on Ca^{++} influx through membrane of jejunal smooth muscle.

INTRODUCTION

The use of medicinal herbs among the general population gives rise to the possibility of therapeutic or toxic effects in patients seeking conventional medical assistance. Aerial parts from different species of the genus Anthemis have been used in traditional medicine as anti-inflammatory, antioxidant, antibacterial, and antispasmodic agents (Papaioannou *et al*., 2007, El Hanbali *et al*., 2007, Maschi *et al*., 2008). *Anthemis mauritiana* Maire & Sennen is an endemic specie distributed in Morocco and Algeria. In our previous investigations we have revealed that the essential oil of this plant had antioxidant and antispasmodic activities (Karim *et al*., 2010 and Karim *et al*. 2011). In the present study, we evaluated the ability of aqueous extract from the flowers of this plant to relax jejunum smooth muscle of rat and rabbit.

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MATERIAL AND METHODS

Solutions and Drugs

Normal Krebs-Henseleit Buffer (KHB) solution composed of (mM) NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and glucose 10. High K^+ KHB (75mM); NaCl, 48; KCl, 75; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and glucose 10. Calcium-free high K^+; KHB (75mM); NaCl, 48; KCl, 75; $CaCl_2$, 0.0; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and glucose 10. Calcium-free KHB; NaCl, 121.7; KCl, 4.7; $CaCl_2$, 0.0; $MgSO_4$, 1.2; $NaHCO_3$, 25; KH_2PO_4, 1.2 and glucose 10, made up in distilled water, the pH was adjusted to 7.4.

The following drugs were used for the experiments: Carbamylcholine chloride (Carbachol, CCh), papaverines, verapamil, were purchased from Sigma. Dimethyl sulfoxide (DMSO) was purchased from Prolabo.

Plant material

The fresh plant was collected locally during the flowering time (in May) from North eastern area of Morocco; the

botanical identification was done by Professor B. Haloui at the department of Biology, Faculty of Science University Mohammed I Oujda, Morocco. A voucher specimen (N° 64666) was previously deposited in Scientific Institute of Rabat.

Aqueous extract of *Anthemis mauritiana* Maire & Sennen (AM)

Air-dried flowers (100g) of *Anthemis mauritiana* were boiled in 1 l and evaporated to dryness gave a crude residue (yield: 18%).

Animals

New-Zeeland rabbits (1.5 - 2 kg) and Wistar rats (200–250 g) bred in the animal house of the department of Biology (Faculty of Sciences, Oujda, Morocco) were housed in a controlled room with a 12 h light-dark cycle, at room temperature of 22±02 ◦C, and kept on standard pellet diet (Société SONABETAIL, Oujda, Morocco). Animal maintenance and handling were in accordance to internationally accepted standard guidelines for use of laboratory animals.

Spasmolytic study

The spasmolytic activity of the essential oil was studied using isolated Wistar rat and New Zealand rabbit jejunum preparations. Animals were fasted overnight before the beginning of the experiment. A portion of jejunum (2 cm) was removed and mounted in 10 ml organ baths containing Krebs-Henseleit buffer (KHB). The bath solution was maintained at 37°C, pH7.4 and gassed continuously with air bubbling. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. AM was added to the organ bath. Each concentration of the AM was at least 7-10 min in contact with the tissue before its effect was evaluated.

Effect of AM on spontaneous contractions of rabbit jejunum

After stabilization of smooth muscle spontaneous contractions of rabbit jejunum, the cumulative doses of AM were added (10-100 µg/ml).

Relaxant effect on K^+ and CCh induced contractions

The jejunum was contracted with K^+ (KCl, 75 mM) or Carbachol (CCh, 10^{-6}M) to a maintained tone. At this point the AM was added to the bath.

Inhibition of dose-response to Carbachol

Cumulative dose-response curves for Carbachol (CCh) were obtained for the tissues according to the method of Van Rossum (1963). After a stabilization period of 60 min, CCh (10^{-8}-10^{-5} M) was added to the organ bath, and different doses of the AM were added to the bath 5 min before commencing the dose-response curve of the agonist.

Inhibition of dose-response to $CaCl_2$

After an initial incubation period of 60 min in normal KHB's solution, the nutrient solution was replaced by calcium-free KHB during 15 min, then replaced by calcium-free hyperpotassic medium (K^+ 75 mM). Cumulative dose-response curves to $CaCl_2$ (0.1, 0.3, 1, 3, 10 mM) were obtained in the presence of different doses of AM (Farre, Columbo, Fort & Gutierrez, 1991).

Statistics

The results are expressed as means ± S.E.M. The statistical significance of data was analyzed using Student's t–test, $P<0.05$ was considered as significant. The 50% inhibitory concentration (IC_{50}) was determined by linear regression method.

RESULTS

When tested on a spontaneously contracting rabbit jejunum, AM exhibited a spasmolytic effect in a dose-dependent manner ranging from 0.1 to 3 mg/ml (Fig. 1). The IC_{50} of the spasmolytic effect was 1.48 ± 0.02 mg/ml. and showed a total reversible inhibition at 3 mg/ml of rabbit jejunal muscle till the rinsing.

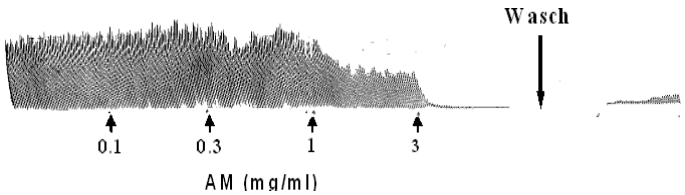

Fig. 1: Tracing showing the spasmolytic effect of *Anthemis mauritiana* aqueous extract (AM) (mg/ml) on spontaneously contracting isolated rabbit jejunum preparation.

Carbachol analogue of acetylcholine yielded a concentration-dependent contraction of tissue; the AM in a concentration-dependent manner inhibited the jejunum contraction induced by CCh (10^{-6} M) with an IC_{50} value of 1.53 ± 0.03 mg/ml (Fig. 2).

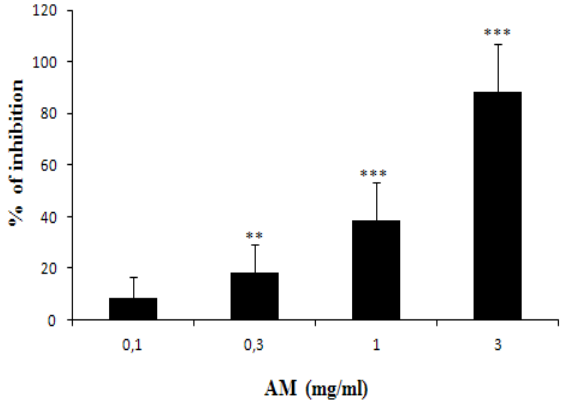

Fig. 2: Relaxant effects of different doses of AM (mg/ml) on CCh (10^{-6} M)-induced contractions. * P<0.05, ** P<0.01 and *** P<0.001 were statistically significant difference from control, (±S.E.M, Student's t-test; n = 6).

Moreover, to assess whether the spasmolytic activity of the tested AM was through calcium channel blockade pathway, K^+ was used to depolarize the preparations as described previously. KCl (75 mM) was added to the tissue bath, in order to produce a sustained contraction; the AM significantly reduced the maximal response to the KCl in a concentration-dependent manner with an IC_{50} value of 0.48 ± 0.1,09 mg/ml (Fig. 3).

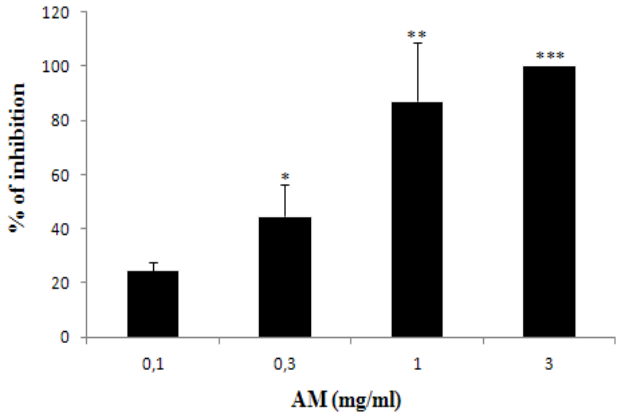

Fig. 3: Relaxant effects of different doses of AM (mg/ml) on K^+ (75 mM)-induced contractions. * P<0.05, ** P<0.01 and *** P<0.001 were statistically significant difference from control, (±S.E.M, Student's t-test; n = 6).

To confirm the calcium channel blocking activity of the test substances, dose-response curves of $CaCl_2$ were constructed in the absence and presence of AM. Pretreatment of the tissue with the AM (30, 50 and 100 µg/ml), shifted the $CaCl_2$ curves to the right (Fig. 4). The AM had also a significant inhibitory effect on CCh concentration–response curve by reducing the maximum induced contraction (fig. 5).

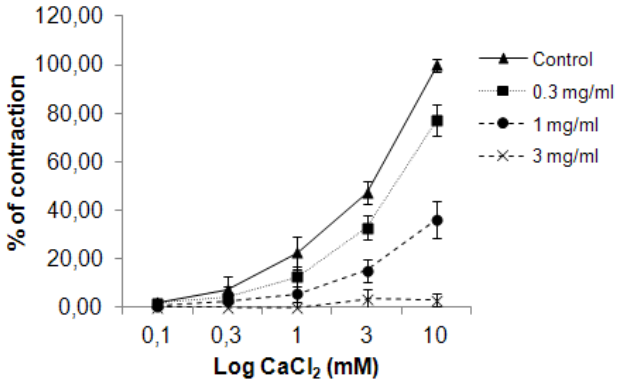

Fig. 4: Cumulative Log concentration-response curves (±S.E.M, Student's t-test; n = 6) for $CaCl_2$ in the presence and absence of AM. The points are means and the vertical bars show the S.E.M. values (n=6).

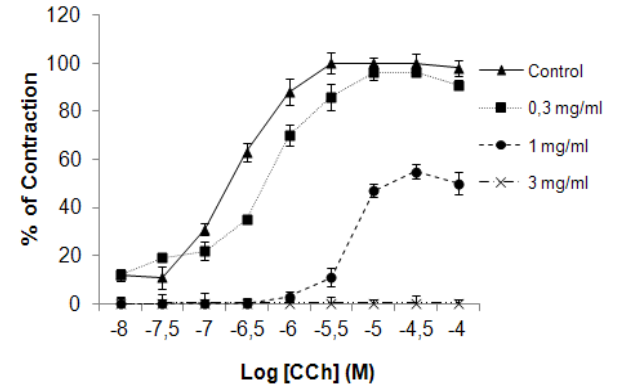

Fig. 5: Cumulative log concentration-response curves (±S.E.M, Student's t-test; n = 6) for CCh in the presence and absence of AM. The points are means and the vertical bars show the S.E.M. values (n=6).

DISCUSSION

In Morocco the herbal medicines are traditionally used for their spasmolytic activity (Bellakhdar 1997). The present data show that the AM exerts concentration dependent reversible inhibitory effects on contractile responses in smooth muscle of isolated rabbit jejunum. This inhibitory effect of the AM on spontaneous movements of jejunum may be due to interference either with the Ca^{++} influx through voltage dependant Ca^{++} channels (VDCs) from the intercellular medium or with the calcium ions release from sarcoplasmic reticulum (Karaki & Weiss, 1988). In order to confirm the interaction of AM with VDCs, the tissue was pretreated with high potassium (75 mM). A depolarization of the membrane occurred, and consequently the VDCs opened and yielded the penetration of Ca^{++} towards cytoplasm (Bolton, 1979). Substances which inhibited contraction induced by KCl is, considered to be à VDCs blocker (Godfraind, Miller & Wibo, 1986). Therefore, inhibition of the contraction of rat jejunum by the AM reflected the limited entry of Ca^{++} through VDCs. This hypothesis was further strengthened when the tissue was pretreated with the AM and caused a concentration-dependent rightward shift in the concentration-response curves of $CaCl_2$ (Rojas, Cruz, Ponce-Monter, & Mata, 1996).

Carbachol, a choline ester, stimulates membrane bound cholinergic receptors, which in subsequent steps leads to increase in intracellular Ca^{++} ion contraction and contraction of the smooth muscle (Goyal, 1988). This effect was mediated by the phospholipase C and the inositol triphosphate (IP3). Competitive antagonists of muscarinic receptors antagonised the response to ACh by antagonising the muscarinic receptors and, therefore, without altering the maximum response they shift ACh concentration–response curve to the right (Hajhashemi, Sadraei, Ghannadi, & Mohseni, 2000). The inhibitory effect of AM on Carbachol concentration–response was like noncompetitive antagonism attenuating the maximum response (Gilani, Shah, Ghayur, & Majeed, 2005; Hajhashemi, Sadraei, Ghannadi, & Mohseni, 2000). We have shown that essential oil of *Anthemis mauritiana* had a relaxing effect on the jejunum of rodents. α-pinene is the major constituent of this oil (Karim & al 2011) and according to Sadraei, Asghari, Hajhashemi, Kolagar, & Ebrahimi (2001) this compound exhibit a clear inhibitory effect on tonic contraction induced by KCl and ACh. It is possible that some compounds of essential oil were mixed with the aqueous extract of Anthemis as this has been the case of other plants (Tschiggerl and Bucar, 2010). Other hydrophilic compounds of the aqueous extract probably play an essential role in the antispasmodic effect observed. Then it is necessary to complete this study in the future.
In conclusion, our results showed that AM exhibits antispasmodic activity which is may be due to the inhibiting of calcium influx into the smooth muscle cells.

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