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Inhibitory Effects of *Cistus salvifolius* on Contractile Responses in the Isolated Rabbit and Rat Jejunum.

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ABSTRACT

The effects of the aqueous extract of *Cistus salvifolius* aerial parts were studied on the rodent isolated jejunum. The extract produced a reversible concentration dose-dependent (0.1–3 mg/ml) inhibition of the spontaneous motility of the rabbit jejunum and carbachol-induced contractions of the rat jejunum. The inhibitory effects of the extract were not affected by pretreatment with the inhibitors of the α and β adrenergic receptors yohimbine, prazosin or propranolol. The ability of the *Cistus* extract to inhibit both carbamylcholine chloride (Cch) (1.5×10^{-7} , ..., 1.5×10^{-5} M) and CaCl_2 (0.1, 0.3, 1, 3, 10 mM) -induced contractions and the relaxing effect on K^+ (75 mM) -induced contractions may indicate a non-specific receptor antagonist. However, this action may be related to the influx of extracellular Ca^{2+} . These antispasmodic effects confirm the traditional use of *Cistus salvifolius* in treating intestinal ache.

Keywords: *Cistus salvifolius*; Jejunum; Antispasmodic effect; Cistaceae.

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INTRODUCTION

The Genus of *Cistus* is widely distributed in Morocco and used by traditional medicine for their therapeutic effects [1]. Aqueous extracts obtained from some of them have been described as hypotensive [2], antiulcerogenic and cicatrizant agents [3]. Anti-inflammatory [4, 5], and antispasmodic [6, 7].

The species *C. Savifolius* L. is a shrub belonging to the family Cistaceae which grows in the perimeter of Mediterranean Sea. It is a medium sized tree locally known as “Rbibi” in North-East of Morocco [1]. It is present in the mountainous regions of the West Rif, central zone, and the Beni Znassen of Morocco. This plant is widely used in herbal medicine by the local population of the North-east of Morocco as anti-diarrhoeal, antacid and antispasmodic agent. However, the therapeutic effects have not been scientifically evaluated.

The aim of this paper was to study the effect of the aqueous extract of *Cistus salvifolius* aerial part on isolated rabbit and rat jejunum and to try to evaluate a possible mechanism of action of this extract by comparing its effect with some well-known smooth muscle relaxants.

MATERIALS AND METHODS

Plant material and extraction procedure

The *Cistus salvifolius* aerial parts were collected in Taza a town of the North-east of Morocco at flowering time (May) as identified by B. Haloui from the Biology Department of Oujda Sciences Faculty (Morocco). A voucher specimen (N° of classification 461/N° of herbarium 76303) was previously deposited in Scientific Institute of Rabat.

The leaves and stems (50 g) were boiled in 1 l distilled water and evaporated to dryness gave a crude residue (yield: 20%)

Smooth muscle preparation

Male Wistar rats (body weight 250 ± 10 g) and New Zealand male rabbits (1.5–2 kg) from an animal room maintained in standard environmental conditions of temperature, humidity and light. The animals were subjected to a 12 h fast and had access to water ad libitum prior to experimentation and killed by decapitation and the jejunum was removed. A 2cm long segment of the jejunum was placed in a 10 ml organ bath containing oxygenated Tyrode's solution of the following composition (mM): NaCl 136.89, KCl 2.68, CaCl_2 1.80, MgCl_2 1.05, NaHCO_3 11.90, NaHPO_4 0.42, and glucose 5.55 at 37°C under a resting tension of 1 g. The suspended jejunum was allowed to equilibrate for 60 min.

Four kinds of experiments were carried out

Spontaneous contractions effect of extract of rabbit jejunum

After stabilization of smooth muscle spontaneous contractions of rabbit jejunum, the effect of the extract against noradrenaline (NA) at 10^{-6} M and the inhibitors of the α and β adrenergic receptors yohimbine, prazosin, propranolol at 5×10^{-5} M was evaluated.

Relaxant effect on K^+ or carbachol induced contractions

The jejunum was contracted with K^+ (75 mM) or carbamylcholine chloride (carbachol, Cch) to a maintained tone, at this point the aqueous extract were added to the bath.

Inhibition of dose_response curves to Carbachol

After a stabilization period of 60 min Cch (1.5×10^{-1} , 5×10^{-5} M) was added to the organ bath in a geometric progression doubling the amount each time. The different doses of the *Cistus* aqueous extract were added to the bath 5 min before commencing the dose-response curve of the agonist.

Inhibition of dose_response curves to $CaCl_2$

After an initial incubation period of 60 min in Tyrode's solution, the nutrient solution was replaced by a calcium-free hyperpotassic medium (K^+ 75mM). Cumulative dose-response curves to $CaCl_2$ (0.1, 0.3, 1, 3, 10 mM) were obtained in the absence and presence different doses of the aqueous extract.

Statistical analysis

Student's t-test was used for statistical analysis and $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

The jejunum of rabbit was used to study the spontaneous basal contractions because their amplitude was easier to evaluate than the rat jejunum. The aqueous extract of *Cistus salvifolius* has exhibited a reversible inhibitory effect on the spontaneous motility of the rabbit jejunum in a dose-dependent fashion (Fig1). The extract also inhibited carbachol-induced contractions of jejunum of the rats (Fig3). This effect tends to suggest some anticholinergic activity likely mediated through cholinergic receptors. As envisaged we found that the blocking of all adrenergic receptors, α_1 by the prazosin and α_2 by the yohimbine and β by the propranolol, shows that noradrenaline does not have any effect on the contractions (Fig. 2A), while the aqueous extract at the concentration of 3 mg/ml, the dose previously shown to produce a clear antispasmodic effect, causes the inhibition of these contractions (Fig. 2B). These results suggest that these effects were not mediated through the adrenergic receptors. The contractions of smooth muscle preparations

including rabbit jejunum are dependent upon an increase in the cytoplasmic free $[Ca^{2+}]$ [8], which activates the contractile elements [9]. The increase in intracellular Ca^{2+} is due to either influx via voltage dependent Ca^{2+} channels (VDCs) or to release from intra cellular stores in the sarcoplasmic reticulum. The Periodic depolarization and repolarization regulate the spontaneous movements of the intestine and at the height of depolarization the action potential appears as a rapid influx of Ca^{2+} via VDCs [10]. SC aqueous extract (SCAE) inhibits the spontaneous movements of rabbit jejunum, and this may be due to interference either with the Ca^{2+} release or with the Ca^{2+} influx through VDCs. In order to test, whether the spasmolytic effect is mediated through the blockade of Ca^{2+} influx, a high dose of K^+ (75 mM) was used to depolarize the tissue. Addition of SCAE caused a dose-dependent inhibition of the K^+ precontracted jejunum (Fig4). The contractions induced by high K^+ are dependent upon entry of Ca^{2+} into the cells through VDCs [11], and a substance which inhibits K^+ -induced contractions is, therefore, considered to be a calcium channel blocker [12]. Thus, inhibition of high K^+ (75 mM)-induced contraction of rat jejunum by the plant extract may reflect restricted Ca^{2+} entry via VDCs. These assumptions were further confirmed when pretreatment of the tissue with SCAE caused a dose-dependent rightward shift of the dose-response curves to Ca^{2+} ($CaCl_2$) (Fig5) and carbachol (Cch) (Fig.6), this extract significantly reduced the maximal response of $CaCl_2$ and Cch in a concentration dependent manner.

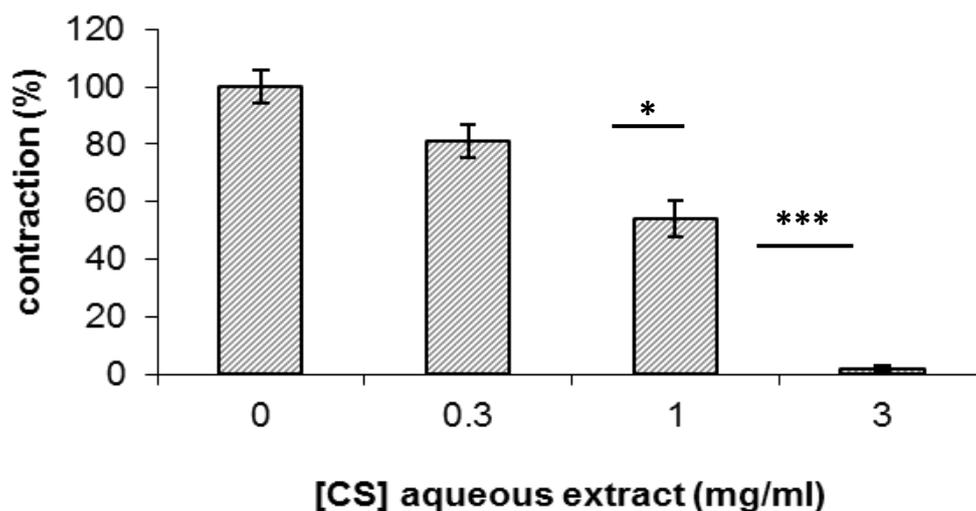


Figure 1 : Effects of the Cistus Salvifolius (CS) aqueous extract on spontaneous contractions of isolated rabbit jejunum. * P < 0.05, *** P < 0.001 statistically significant difference from control (n=6).

The ability of the extract to inhibit both Cch and $CaCl_2$ -induced contractions may indicate that the spasmolytic compound included in the aqueous extract is not a specific receptor antagonist. Nevertheless, because this case deals with an extract, we cannot be assured that there is only one spasmolytic compound involved. Furthermore, this antispasmodic effect in particular the dose-dependent relaxant effect of Cistus extract on K^+ (75 mM) induced contraction would also appear to inhibit the calcium influx into the cellular cytoplasm or perhaps interfere in one of the multiple Biochemical processes associated with the influx of calcium into the smooth muscle cells.

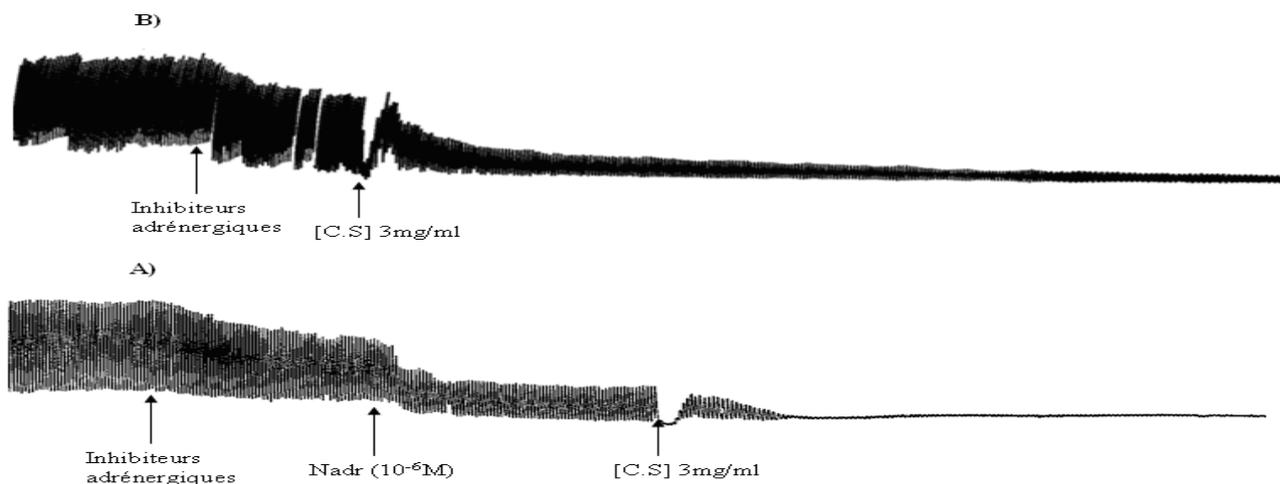


Figure 2 : A: Effect of noradrenaline (Nadr) on rabbit jejunum contractions. B: Effect of the pretreatment with adrenergic receptors antagonists (prazosin, yohimbine and propranolol) on the inhibitory effect of the aqueous extract of Cistus Salvifolius (CS) on rabbit jejunum contractions.

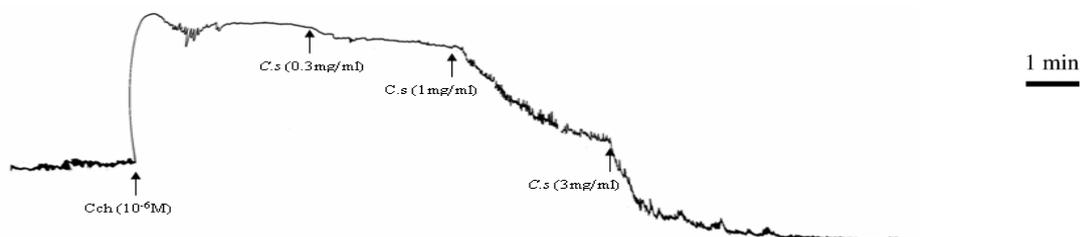


Figure 3. Effect of the C. salvifolius aqueous extract on the carbachol-induced contraction of isolated rat jejunum.

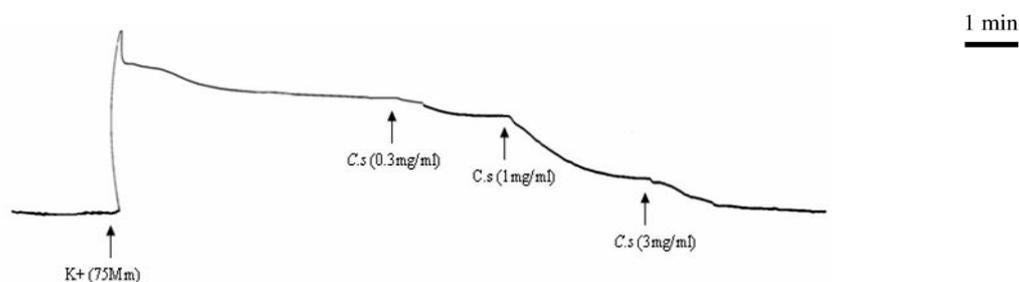


Figure 4 : Effect of the Cistus Salvifolius (CS) aqueous extract on K⁺-induced contraction of isolated rat jejunum.

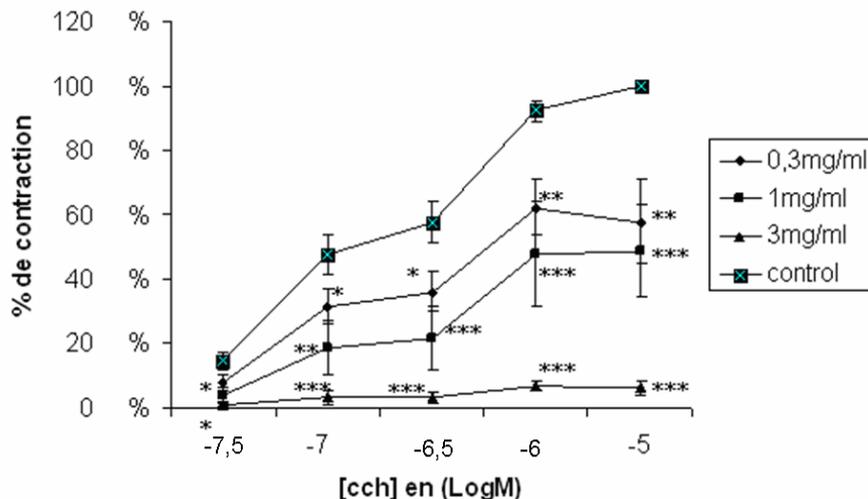


Figure 5 : Cumulative log concentration_response curves \pm S.E.M. for Cch in the presence and absence of *Cistus Salvifolius* aqueous extract. * P < 0.05, **P<0.01, *** P < 0.001 statistically significant difference from control (n=6).

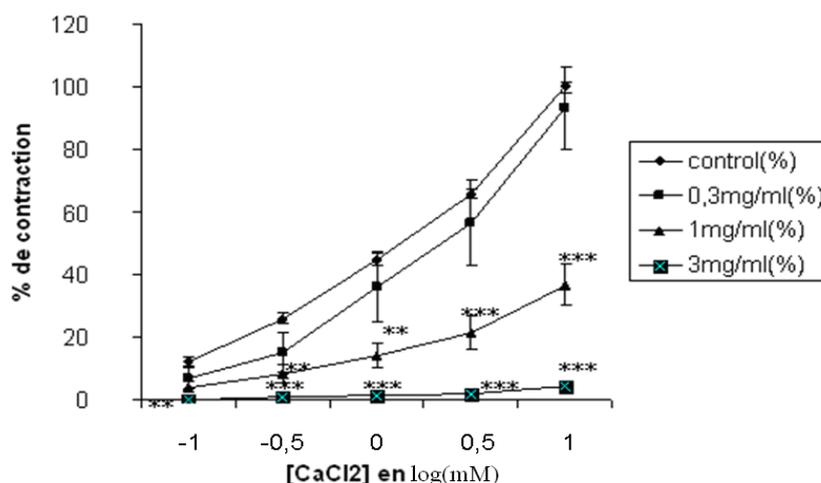


Figure 6 : Cumulative log concentration_response curves \pm S.E.M. for CaCl₂ in the presence and absence of *Cistus Salvifolius* aqueous extract

The presence of constituents with relaxing properties on smooth muscle in *Cistus* aqueous extracts is partly consistent with the use of this plant in folk medicine for certain gastrointestinal disorders. Research for the active constituents is in progress.

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