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SHORT COMMUNICATION

Relaxant and Antispasmodic Activities of Aqueous Extract from *Thymus algeriensis* Boiss. and Reut.

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Abstract

Thymus algeriensis have been vastly utilized for intestinal disorders. The purpose of this investigation was to scrutinize the probable mechanism for its utilization in the spasm disorder. Aqueous extract of this medicinal plant (AqTA) was tested *in vitro* on rat and rabbit jejunum. The extract produced relaxation of rabbit jejunum. This relaxation does not depend on the adrenergic pathway, the AqTA induces inhibition irrespective of the presence or absence of adrenergic inhibitors. AqTA engendered a concentration-dependent (0.1-5 mg/ml) relaxation of carbamylcholine chloride (CCh) and K^+ provoked tones in rat intestine with IC_{50} values of 2.06 ± 0.26 and 3.55 ± 0.48 (mg/ml) respectively. This extract likewise induced a dose-dependent (0.1-3 mg/ml) rightward shift in the CCh and Ca^{++} dose-response curves. The AqTA alone has decreased more significantly the percentage of contraction of rat jejunum than the AqTA pre-incubated with atropine or hexamethonium then contracted with KCl; but there is no significant difference by those pre-incubated with methylene blue or L-NAME. When the intestine was pretreated with nifedipine and contracted by CCh, the antispasmodic effect provoked by AqTA with and without pre-incubation with nifedipine is statistically not significant. In conclusion AqTA acts possibly on the voltage dependent Ca^{++} channel and cholinergic receptors but did not act on adrenergic receptors, NO and guanylatecyclase pathway. This investigation may explicate some of its traditional utilization in gut illnesses.

Keywords: Antispasmodic, Jejunum, Relaxant, Smooth muscle, *Thymus algeriensis*

1. Introduction

Thymus algeriensis Boiss. and Reut. is an endemic species found in African countries along the Mediterranean Sea. It belongs to the Thymus genus. Numerous Thymus species are greatly employed in culinary and medicine. In Morocco *Thymus algeriensis*, known locally under the common name "Zaatar", is found in the Atlas and Rif mountains and in the BéniSnassen Forest. It is a perennial plant with 4 to 7 mm long internodes emerging as a tuft from the short, woody stump[1]. Its active principles are gallic acid, catechin, epicatechin, coumaric acid, apigenin, naringenin, rutin, quercetin, kaempferol, tannins, rutin[2], flavonol, flavone glycoside derivatives and phenolic acids, specially rosmarinic acid and kaempferol-O-glucuronide[3]. The volatile components varied among populations; at least five chemotypes according to main compounds have been found: -pinene/ 1,8-cineole / -pinene, 1,8-cineole/ caryophyllene oxide/ camphor, linalool and thymol chemotypes[4]. In North Africa traditionally it is used to cure the problems of respiration, digestion and against abortion[5,6]. Previous studies have shown that it had antioxidant, antimicrobial, healing gastric ulcers[7,8] and antitumor[9] activities.

In a preceding work, we obtained an antidiarrheal action of the aqueous extract of the *T. algeriensis*[10]. The purpose of this report is to continue this work on the digestive tract problems and to attempt to elucidate how

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this extract intervenes on the relaxation pathways of the muscle fibers of rabbit and rat jejunums.

2. Materials and Methods

2.1 Solutions and Drugs

For twenty years we have always used the same solutions for antispasmodic tests. See Normal Krebs-Henseleit Buffer (KHB), Calcium-free high K^+, High K^+ KHB (75 mM) and Calcium-free KHB solutions composed of (in mM) in the reference[11]. The drugs employed in spasmolytic tests were L-NAME from Calbiochem, methylene blue from Sigma-Aldrich, carbamylcholine chloride (CCh), prazosin, nifedipine, yohimbine, propranolol were purchased from Sigma chemical co, hexamethonium from Across organics, atropine from Fluka.

2.2 HPLC Analysis of Phenolic Compounds

HPLC analysis was performed to analyze the phenolic compounds present in the plant[12].

2.3 Plant Material

T.algeriens was harvested from Oujda province in the spring of 2014, was authenticated by Prof. Benyounes Haloui and a voucher specimen was deposited in the Herbarium of the Sciences Faculty, University Mohammed the First, Oujda, Morocco under the reference number (HUMPOM 425).

2.4 Preparation of the Extract

Inspired by the Moroccan tradition, 50g of the aerial part of *T. algeriensis* (AqTA) was boiled in 1l of water for 15 min and dehydrated to dryness to give a crude residue (yields: 18%).

2.5 Animals

Female and male New Zealand rabbits (1.-2 Kg) and Wistar rats (200-300kg) were provided from the animal house of the Sciences Faculty in Oujda (Morocco). Animals had free access to water but food was retiring 18h prior to experimentation. All animals were treated for in conformity with the internationally accepted Guide for the care and use of laboratory animals, published by US National Institutes of Health (NIH Publication N°. 85-23, revised in 1985).

2.6 Spasmolytic Study

The spasmolytic activity of the jejunums was tested exactly like the one we studied on *Origanum majorana*[11].

2.7 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.

3. Results

3.1 HPLC

HPLC analysis of the phenolic composites showed that the AqTA contains syringic acid, rutin, coumaric acid, luteolin, cinnamic acid, apigenin, and quercetin (Table 1, Figure 1).

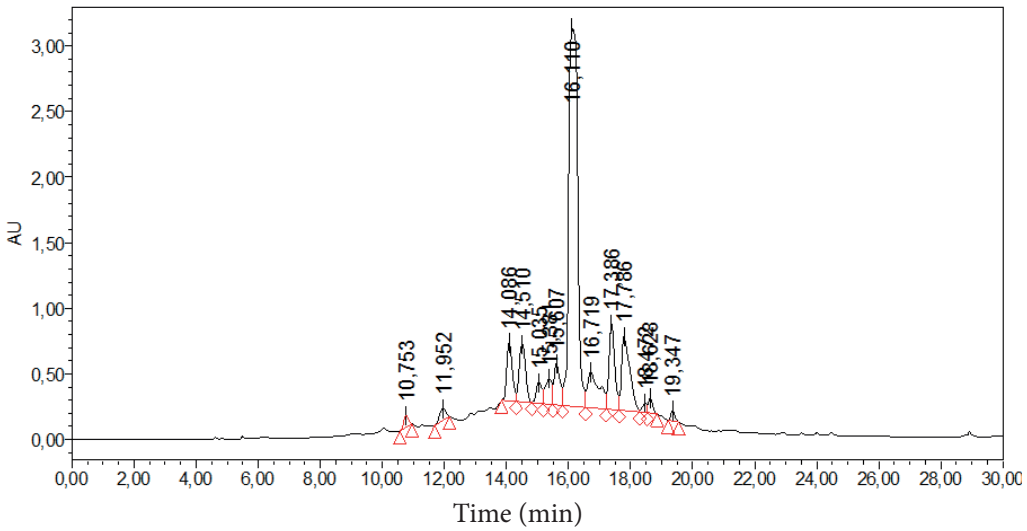

Figure 1. HPLC chromatogram of aqueous extract from *Thymus algeriensis*.

3.2 Effects of AqTA on Rabbit Intestine

Contact of the intestines from 0 to 5 mg/ml of the AqTA decreased the mean magnitude of the spontaneous tonus to almost total inhibition of control with an IC_{50} value of 1.88 ± 0.07 mg/ml (Figure 2). This action was reversible as the intestine recovered its basal activity after rinsing twice with KHB. Adrenaline at 10^{-6} M inhibited rabbit jejunum contractions. The addition to the KHB medium of β (Propranolol), α2 (Yohimbine) and α1 (Prazosin) adrenergic inhibitors with AqTA provoked inhibition of the spontaneous contractions of the rabbit jejunum.

Table 1. Results of HPLC analysis Aqueous extract from *Thymus algeriensis* compared to Retention time of 18 standard phenol acids and flavonoids

Standards	RT(min)	AqTA
Chlorogenic acid	9.23	-
Catechin	9.33	-
3,4-dihydropyran	9.60	-
4-hydro phenyl	10.27	-
Vanillic acid	10.50	-
4-hydroxybenzoic acid	10.62	-
Syringic acid	10.76	+
Caffeic acid	11.11	-
Vanillin	11.35	-
p-coumaric acid	12.73	-
trans-ferulic acid	13.35	-
Rutin	15.18	+
Coumaric acid	15.47	+
Luteolin	16.14	+
Cinnamic acid	16.72	+
Apigenin	17.46	+
Quercetin	17.72	+
trans-chalcone	20.65	-

"+" sign indicates the presence of phenolic compounds;
"–" sign indicates the absence of phenolic compounds.

3.3 Inhibition of Dose-response to Carbachol and $CaCl_2$

AqTA caused a dose-dependent (0.1-5mg/ml) inhibition CCh and K^+ induced tones of rat jejunum preparations with IC_{50} values of 2.06 ± 0.26 and 3.55 ± 0.48(mg/ml) respectively (Figures 3 and 4). Our extract likewise caused a dose-dependent (0.1-3 mg/ml) rightward shift in the CCh (Figure 5) and Ca^{++} dose-response curves (Figure 6).

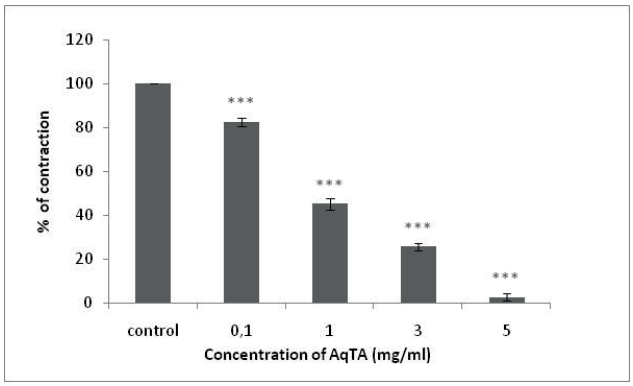

Figure 2. Effect of AqTA on rabbit jejunum preparations. Values shown are mean ± S.E.M. of six experiments (*** P < 0.001).

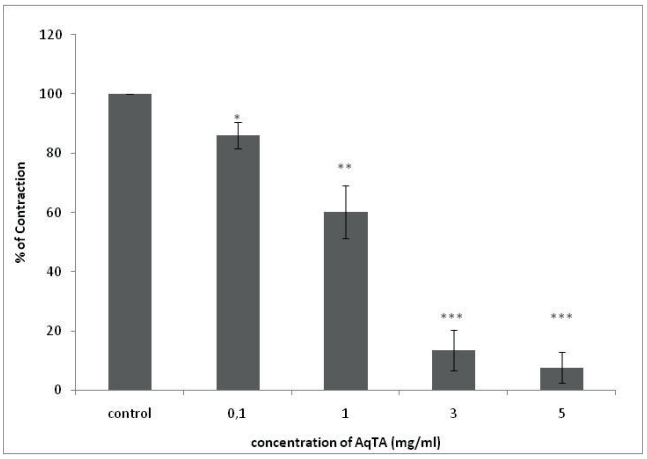

Figure 3. Effect of aqueous extract from *Thymus algeriensis* on Carbachol (10^{-6} M) induced contractions of rat jejunum preparation. Values shown are mean ± S.E.M. of six experiments. *(* P < 0.05; ** P < 0.01; *** P < 0.001).*

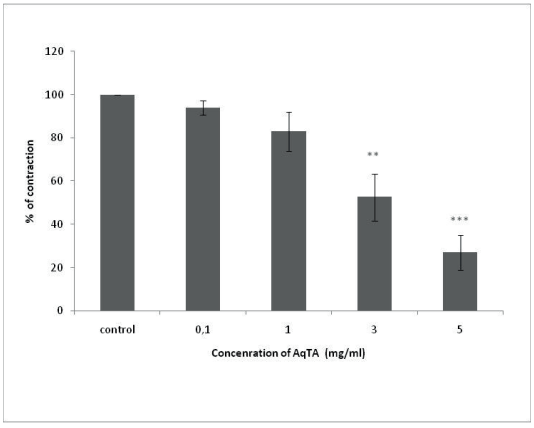

Figure 4. Effect of aqueous extract from *Thymus algeriensis* on KCl (75mM) induced contr actions of rat jejunums. Values shown are mean ± S.E.M. of six experiments. *(** P < 0.01; *** P < 0.001).*

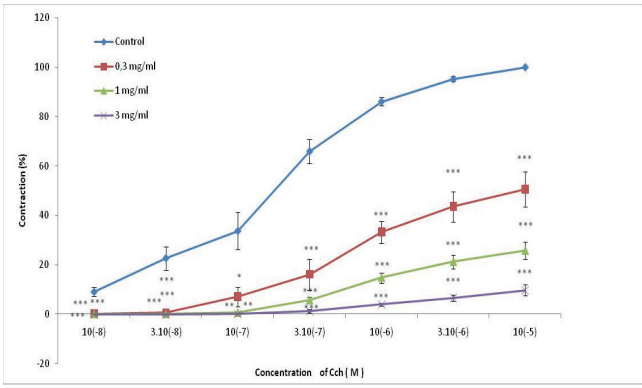

Figure 5. Concentration-response curves of carbachol (CCh) in the presence of different concen trations of AqTA (0; 0.3; and 3 (mg/ml)). Values shown are mean ± S.E.M. of six experiments. *(* P < 0.05; ** P < 0.01; *** P < 0.001).*

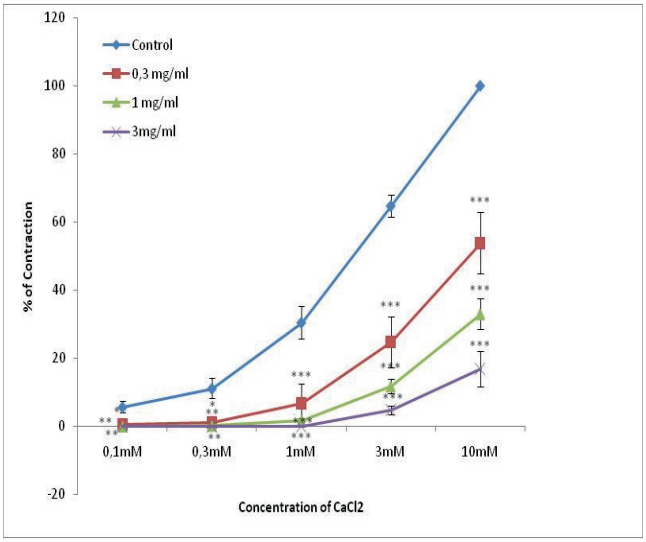

Figure 6. Concentration-response curves of $CaCl_2$ in the presence of different concentrations of AqTA (0; 0.3; 1 and 3 (mg/ml)). Values shown are mean ± S.E.M. of six experiments. *(* P < 0.05; ** P < 0.01; *** P < 0.001).*

3.4 Effect of AqTA with Different Pharmaco logical Blocking Agents on Rat Jejunum Pre-contracted by KCl or CCh

We compared the action of 3 mg/ml of AqTA, concen tration approaching the IC_{50}, with pharmacological blocking agents habitually utilizsed to reduce the contraction provoked by KCl. The AqTA alone has decreased more significantly the percentage of contraction of rat jejunum than the AqTA pre-incubated with atropine or hexamethonium; but there is no significant difference by those pre-incubated with L-NAME or methylene blue (Figure 7A).

When KCl was replaced by Carbachol, the antispasmodic effect provoked by AqTA with and without pre-incubation with nifedipine is statistically not significant (Figure 7B).

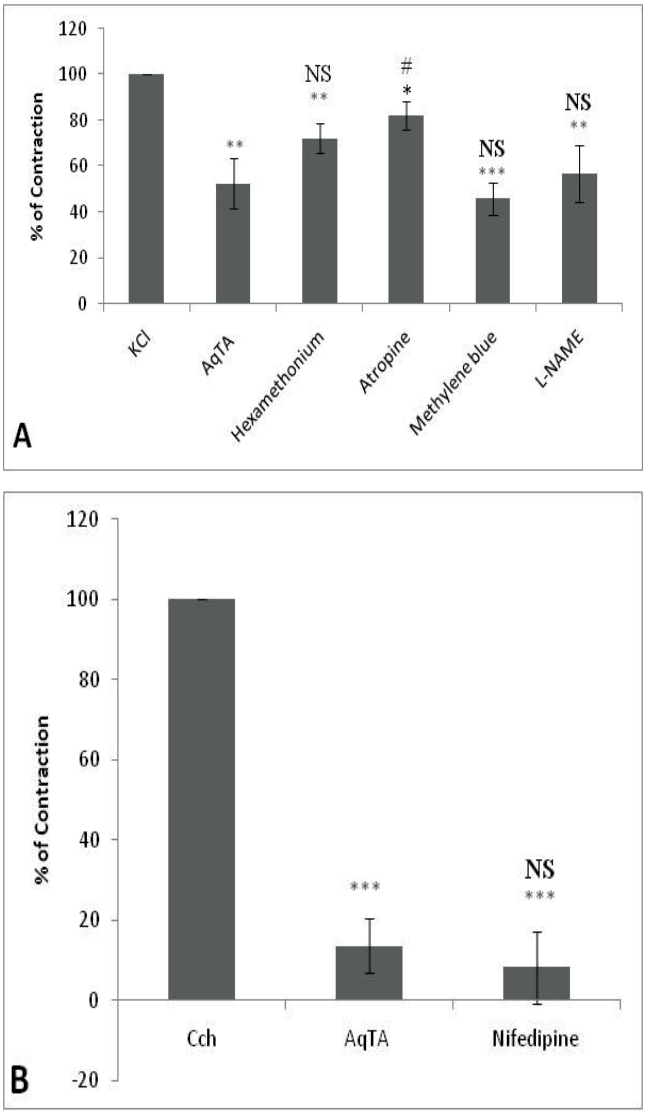

Figure 7. Effect of aqueous extract of *Thymus algeriensis* (3 mg/ml) on contractions of the rat jejunum preincubated with hexamethonium (10^{-4} M), atropine (10^{-6} M), methylene blue (10^{-5} M) and L-NAME (10^{-4} M) for 20 min, and then pre-contracted by 75 mM KCl. (A) or pre-incubated with nifedipine (10^{-6} M) for 20 min, and then pre-contracted by CCh 10^{-6} M (B). Values shown are mean ± S.E.M. of six experiments. *(* P < 0.05; ** P < 0.01; *** P < 0.001).* NS = Not significant. (***compared to the control and # and NS compared to the extract without inhibitors).

4. Discussion

Due to the traditional reputation as antispasmodic, aqueous extract of *Thymus algeriensis* was tested for its possible spasmolytic effect in spontaneously contracting rabbit jejunum preparations because it's easier to quantify relaxation than in spontaneous contraction of rat jejunum. It is known that intestinal smooth muscle contracts rhythmically in the absence of hormonal and neuronal stimulations; such contractions are referred to as phasic[13] and are commenced by the act of the interstitial cell of Cajal[14]. AqTA inhibited spontaneous contractions in a reversible dose dependent manner. Sympathetic nerves of the autonomic nervous system regulate intestine motility in a round-about way across the enteric nervous system, making an inhibitory action on motility[15]. We wanted to know if our extract acts on the adrenergic pathway. For this, we blocked adrenergic receptors, β by the propranolol, α2 by the yohimbine and α1 by the prazosin (put all together). We remarked that noradrenaline does not have any impact on the tensions; whereas the extract at 5mg/ml, the dose formerly described to provide a manifest relaxant effect, generates the prevention of these contractions. Like extracts from other plants that we have tested in our laboratory, as *Cistus ladaniferus*[16] and *Origanum majorana*[11], AqTA also does not act via adrenergic receptors.

Usually antispasmodic action of medicinal plants is due to Ca^{++} channel blockade[17,18]. To check if the antispasmodic action of the AqTA is as well due via equivalent process, this extract was evaluated on high K^+-induced contraction. This latter provokes a depolarization of the plasma membrane and subsequently the voltage-gated Ca^{++} channels (VGCaCs) open and engendered Ca^{++} get in hyaloplasm[19]. A chemical which can prevent K^+ provoked tone is hence reputed to be a calcium channel blocking[20]. Therefore, antispasmodic effect of rich K^+ medium (75 mM) –induced tone of rat intestine by AqTA may reflect the restrained Ca^{++} entrance through voltage-dependent channels. This supposition was in addition fortified during the pre-treatment of the gut with T. *algeriensis* which produced a concentration-dependent rightward shift in the concentration-response of $CaCl_2$[21]. AqTA apparently does not act on L-type VGCaCs, it could intervene in other types of VGCaCs.

The cholinergic receptor pathway was tested using Carbachol which is a structural analogue of acetylcholine[22,23]. AqTA inhibited by a dose-dependent mode, the tone generated with this analogue. This purpose was reinforced when pre-treatment of the jejunum with thym extract engendered a concentration-dependent rightward shift in the concentration-response of CCh. To know which cholinergic receptors may be involved, we used the muscarinic receptor antagonist atropine[24,25] and the nicotinic receptor antagonist hexamethonium[26,27]. Atropine, but not hexamethonium, have impaired the initial relaxed aftermath of AqTA. Subsequently it can be proposed that our plant intervenes directly on the muscarinic receptors.

Nitric oxide (NO), a transmitter gut nerves in the myenteric plexus nerves in the myenteric plexus throughout the gastrointestinal tract[28], was able to relax intestine[29]. It relaxes smooth muscle fibers via activation of dissolvable guanylate cyclase[30,31] and an increase in intracellular cGMP. We wanted to know if AqTA acts via the NO and/or that of guanylatecyclase. The employment of L-NAME, an inhibitor of NO and blue methylene, a blocker agent of guanylatecyclase pathways[32], showed that AqTA did not act on these two pathways.

The relaxant and antispasmodic effect can be due to Apeginin[33], Luteolin[34–36] and Quercitin[37–39], known by their antispasmodic effect and that are existing in AqTA. These components could act alone or together with other unidentified compounds.

5. Conclusion

These results suggest that the spasmolytic activity of AqTA are mediated possibly through Ca^{++} antagonist mechanism and cholinergic receptors but did not act on adrenergic receptors, NO and guanylate cyclase. This study may explain some of the traditional uses of the plant in gastrointestinal disorders, and with our previous work[10] reinforce positively the practice of Thym in Moroccan folk medicine as a spasmolyticgut agent.

6. Acknowledgement

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