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Spasmolytic activity of *Rosmarinus officinalis* L. involves calcium channels in the guinea pig ileum

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ABSTRACT

Ethnopharmacological relevance: Rosmarinus officinalis L. is a plant used around the world for its properties to cure pain in several conditions, such as arthritic and abdominal pain or as an antispasmodic; however, there are no scientific studies demonstrating its spasmolytic activity. Therefore, the aim of the present study was to investigate the effect of an ethanol extract from *Rosmarinus officinalis* aerial parts and the possible mechanism involved by using rings from the isolated guinea pig ileum (IGPI).
Materials and methods: The IGPI rings were pre-contracted with potassium chloride (KCl; 60 mM), acetylcholine (ACh; 1×10^{-9} to 1×10^{-5} M) or electrical field stimulation (EFS; 0.3 Hz of frequency, 3.0 ms of duration and 14 V intensity) and tested in the presence of the *Rosmarinus officinalis* ethanol extract (150, 300, 600 and 1 200 μg/mL) or a referenced smooth muscle relaxant (papaverine, 30 μM). In addition, the possible mechanism of action was analyzed in the presence of hexametonium (a ganglionic blocker), indomethacine (an inhibitor of prostaglandins), L-NAME (a selective inhibitor of the nitric oxide synthase) and nifedipine (a calcium channel blocker).
Results: Rosmarinus officinalis ethanol extract exhibited a significant and concentration-dependent spasmolytic activity on the contractions induced by KCl ($CI_{50} = 661.06 \pm 155.91$ μg/mL); ACh ($CI_{50} = 464.05 \pm 16.85$ μg/mL) and EFS ($CI_{50} = 513.72 \pm 34.13$ μg/mL). Spasmolytic response of *Rosmarinus officinalis* (600 μg/mL) was reverted in the presence of nifedipine 1 μM, but not in the presence of hexamethonium 0.5 mM, indomethacine 1 μM or L-NAME 100 μM.
Conclusion: The present results reinforce the use of *Rosmarinus officinalis* as antispasmodic in folk medicine. Moreover, it is demonstrated the involvement of calcium channels in this activity, but not the participation of nicotinic receptors, prostaglandins or nitric oxide.

1. Introduction

Rosmarinus officinalis L. is a species of Mediterranean origin. It is well known around the world as a common spice for culinary purposes (Polunin and Smythies, 1973; Davis, 1982). In folk medicine, its aerial parts are used orally to relief pain in renal colic and dysmenorrhoea, and as antispasmodic (Al-Sereiti et al., 1999). A tea made of the boiled leaves is also used to improve digestion and to alleviate stomachache (Romo de Vivar, 1985; Martínez, 1989; Argueta et al., 1994). In Mexico, it is prepared also as maceration in ethanol and used topically to relief rheumatic pain. Some studies have reported its effects as diuretic (Haloui et al., 2000), antipyretic (Martínez et al., 2004) and as a mood stabilizer (Moss et al., 2003). The powerful antioxidant activity of its constituents supports protection against damage induced by free radicals (Ramírez et al., 2004; Peng et al., 2005). Furthermore, it has been demonstrated that *Rosmarinus officinalis* produces antinociceptive effect in experimental models of pain like acid acetic and formalin, but also in the pain-induced functional impairment model in the rat (González-Trujano et al., 2007). Despite the widespread use of this plant in popular medicine and the pharmacological studies that demonstrate their antinociceptive and/or anti-inflammatory activity, there is a lack of information to support its antispasmodic activity. On the other hand, diarrheal diseases are a major problem in Third World countries and are responsible for the death of millions of people each year (Celia and Mediadora, 1990). For this reason, international organizations including the World Health Organization have encouraged studies pertaining to the treatment and prevention of diarrheal diseases using traditional medical practices, in which medicinal plants like *Rosmarinus officinalis* are a

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promising source of antidiarrheal drugs (Alanís et al., 2005). Therefore, the present study was performed to evaluate the effects of an ethanol extract of *Rosmarinus officinalis* aerial parts on the contractile response induced by several spasmogens in the isolated guinea pig ileum.

2. Materials and methods

2.1. Animals

Adult male guinea pigs weighing 300–400 g and obtained from our breeding facilities were used. Animals were housed one per cage in a temperature-controlled room (22 ± 2 °C) with an automatically timed cycle of 12 h light/dark (lights on 08:00–20:00). Food (Purina Chow, St. Louis, MO, USA) and water were available *ad libitum*. Twenty-four hours before experiments, food was withheld and free access to water was maintained. Experiments were performed between 08:00 and 14:00 h. The experimental protocol was carried out under the provisions of the Declaration of Helsinki, and adhered to the National Health Ministry guidelines for the use of laboratory animals.

2.2. Plant material

Aerial parts of *Rosmarinus officinalis* L. (Lamiaceae) were collected in June 2004 in the State of Morelos, Mexico. MSc Abigail Aguilar identified the specimen and a voucher specimen (IMSSM-15005) was deposited in the Herbarium of the Instituto Mexicano del Seguro Social in Mexico City for future reference.

2.3. Preparation of the extract

The dried mature aerial parts of *Rosmarinus officinalis* were cut into small bits (330 g) and kept in a container; an extraction was carried out by successive maceration at room temperature (22 °C ± 1) for 48 h. A first extraction with hexane (3× 1200 mL) was performed followed by filtration. Residue was extracted with absolute ethanol (3× 1200 mL) and discarded after filtration. Final filtrate was concentrated under vacuum to eliminate ethanol. The final product yielded 111 g (33.6%) of a green solid ethanol extract.

2.4. Drugs

The *Rosmarinus officinalis* ethanol extract was re-suspended and diluted in Krebs solution before adding to the organ bath to get 150, 300, 600 and 1200 μg/mL. Papaverine (as a referenced smooth muscle relaxant), hexamethonium, indomethacin, L-NAME, nifedipine and acetylcholine (ACh) chloride were dissolved in saline solution (0.9%). All these drugs were purchased from Sigma (St. Louis, MO, USA). Potassium chloride (KCl) was bought from J.T. Baker. Drugs were freshly prepared on the day of the experiments.

2.5. Tissue preparation

Guinea pigs were sacrificed by a blow to the base of the skull and cervical dislocation. A selected portion of the ileum (approximately 15 cm), without considering the 10 cm nearest to the ileocecal valve, was removed and placed in a Petri dish with Krebs bicarbonate (K-B) solution maintained at 37 °C and bubbled with 95% O_2 and 5% CO_2. This solution contained (mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25; glucose, 11; and choline chloride, 0.3. Intraluminal contents were flushed out with K-B solution and 2 cm segments of the portion were cut. Each segment was mounted in a 30 mL organ-bath containing K-B solution maintained at 37 °C and constantly bubbled with the mixture of O_2 and CO_2. The resting tension was fixed at 1 g. Preparations were allowed to equilibrate for 60 min under continuous superfusion (10 mL/min) of warm K-B solution, continuously bubbled with 95% O_2 and 5% CO_2 to maintain the pH at 7.4, and then stimulated with ACh (1×10^{-5} M) to ascertain their suitability. The mechanical response of the ileum was recorded with a Grass FT-03C force displacement transducer connected to a Grass 7B polygraph and computerized data acquisition system (PolyView System, version 2.5, Grass instruments).

2.6. Antispasmodic activity

After stabilization for 30 min, the antispasmodic effect of the *Rosmarinus officinalis* ethanol extract was evaluated by the following procedure.

2.6.1. Rings pre-contracted with KCl

For the extract-induced relaxation of preparations pre-contracted with 60 mM of KCl solutions, only those with an at least 5-min lasting plateau contraction were used. The percentage inhibition of contraction induced by KCl in the presence of each concentration of the extract or the reference drug papaverine was calculated for each treatment tested (Nasu et al., 1994).

2.6.2. Rings pre-contracted with ACh

Cumulative concentration–response curves were obtained for ACh (1×10^{-10} to 1×10^{-5} M) in the absence or presence of the *Rosmarinus officinalis* ethanol extract (150, 300 and 600 μg/mL) and papaverine (30 μM), incubated for 20 min, beforehand. Six cumulative concentration–response curves were obtained for each preparation, with a 20 min-rest between each. The maximal response obtained from the first cumulative concentration–response curve (in the absence of compounds) was taken as 100% response value (Teague et al., 2002; Mehmood et al., 2011).

2.6.3. Electrical field stimulation (EFS)

Preparations were electrically stimulated through two nickel electrodes that were positioned parallel to the segments and connected to a square wave stimulator (Grass S88). The anode was placed intraluminally. The following parameters were used in each preparation in order to produce maximal responses: 0.3 Hz of frequency, 3.0 ms of duration and 14 V intensity. Field-stimulated preparations were exposed to graded concentrations of *Rosmarinus officinalis* ethanol extract (150, 300 and 600 μg/mL) or papaverine (30 μM) (Teague et al., 2002).

In all the cases, when a maximal contractile effect was obtained (10 s), the preparation was washed with 30–60 mL of warm K-B solution. Concentrations are expressed as final drug concentrations truly in contact with preparations that cover the full range from no effect to maximal contractile response.

2.7. Mechanism of action analysis

The possible mechanism of action involved in the antispasmodic effect of the *Rosmarinus officinalis* ethanol extract was investigated by exploring the participation of nicotinic receptors, prostaglandins, nitric oxide and calcium channels in independent animal groups (at least 6 rings each) by adding to the chamber: hexamethonium (a ganglionic blocker, 0.5 mM), indomethacin (an inhibitor of prostaglandins, 1 μM), L-NAME (a selective inhibitor of the nitric oxide synthase, 100 μM) or nifedipine (a calcium channel blocker, 1 μM), respectively, when a maximal contractile response was obtained as described in the above subsections (Magalhães et al., 2004).