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ScienceDirect
Journal of Ethnopharmacology 117 (2008) 102–107
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effects of essential oil from *Croton tiglium* L. on intestinal transit in mice

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Received 2 May 2007; received in revised form 18 October 2007; accepted 23 January 2008
Available online 3 February 2008

Abstract

Aim of the Study: Croton tiglium (*Croton tiglium* L., Euphorbiaceae) is widely used as a herb for treatment of gastrointestinal disturbances. Previous studies established its purgative and inflammational properties. The present study aimed to investigate the effects of *Croton tiglium* oil (CO) on intestinal transit in mice.

Materials and Methods: Gastrointestinal transit in mice and contractile characteristics of isolated intestinal strips from mice were evaluated. Intestinal inflammation was confirmed by histological examination.

Results: Low dose of CO increased the gastrointestinal transit of charcoal and barium meal as well as the production of fecal pellets in mice. In contrast, high dose exerted inhibitory effects. For normal colonic circular strips, both high and low dose of CO inhibited the contractile frequency. Low doses (0–20 μg/ml) of CO enhanced the phasic contractions, while high doses (>40 μg/ml) reduced them. Colonic longitudinal strips in CO-treated mice were less sensitive to electrical field stimulation than those in control mice. The contraction of colonic longitudinal, colonic and jejunal circular strips in CO-treated mice was more sensitive to atropine than that in control mice.

Conclusions: CO might modulate gastrointestinal motility and induce intestinal inflammation related to immunological milieu and motor activity. Our findings may highlight the ethno-medical uses of *Croton tiglium* on intestinal disorders.

Keywords: *Croton tiglium* L.; Euphorbiaceae; Traditional medicine; Gastrointestinal motility; Muscarinic receptor; Inflammation

1. Introduction

Croton tiglium (*Croton tiglium* L., family Euphorbiaceae) is a plant grown in tropical and subtropical zone. The seed of *Croton tiglium* is well known as Ba-Dou (or Badou) in China. The Chinese had written records in the second century B.C. for using Ba-Dou to treat gastrointestinal disorders, intestinal inflammation, rheumatism, headache, peptic ulcer and visceral pain (Qiu, 1996; Wang et al., 2002a; Morimura, 2003; Tsai et al., 2004). The fruits, leaves, barks and roots of *Croton tiglium* contain lipid-soluble contents of terpenes and hydrocarbons. The main components, the sesquiterpenes and monoterpenes, comprise the great parts of the extracted essential oil from seed, bark and leaves of *Croton tiglium* and it is widely used in medicine (Qiu, 1996). In addition to its popular use for the treatment of gastrointestinal disturbances in China, Brazil and other countries (Giday et al., 2007), essential oil of *Croton tiglium* or other species of Croton family, for instance, *Croton nepetaefolius*, had also been used as agents for generating animal models of diarrhea and intestinal inflammation (Pol et al., 1994; Pascual et al., 2002; Tsai et al., 2004; Santos et al., 2005; Jimenez et al., 2006). However, the mechanisms as how essential oil of Croton can modulate gastrointestinal motility remain unclear.

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doi:10.1016/j.jep.2008.01.023

Previous reports showed that the essential oil of *Croton tiglium* had purgative, analgesic, antimicrobial, and inflammatory properties (Qiu, 1996; Tsai et al., 2004). We have also reported the direct effect of essential oil of *Croton tiglium* on human intestinal epithelial cell and guinea pig colonic smooth muscle cells *in vitro* (Wang et al., 2002a). Keeping in view the traditional use of *Croton tiglium* in gastrointestinal disturbances and our previous findings, the present study investigated the effects of *Croton tiglium* oil (CO) on intestinal motility in mice and explored the possible mechanisms.

2. Materials and methods

2.1. Animals and plant materials

Male and female Balb/C mice (20–25 g) were from the experimental animal centre of Fourth Military Medical University. Animals were sacrificed by cervical dislocation after 20% urethane (0.1 ml/10 g, i.p.). Animals' experiments were performed with the approval of our Institute Committee on the Care and Use of Animals for experimentation in accordance with the guidelines of the International Association for the Study of Pain. Extracting and characterizing CO from seed of *Croton tiglium* were described before (Wang et al., 2002a). Animals received CO diluted with edible oil 400 μl or control agent (edible oil 400 μl without CO) by i.g.

2.2. Gastrointestinal motility essay by differential dosages of CO treatments

After fasting (12 h), each mouse in 25 mg/10 g body weight ($n=5$), 15 mg/10 g ($n=5$), 2.5 mg/10 g ($n=10$), 0.5 mg/10 g ($n=10$) and control group ($n=10$) was given CO or edible oil once and fed in single cage rebased with white filter paper. The survival number and production of fecal pallets within 24 h after the administration in groups without death were counted.

2.3. Gastrointestinal transit (GIT) of charcoal

GIT of charcoal were measured in the control group ($n=10$), low dose (0.5 mg/10 g, $n=10$) and high dose CO-treated mice (2.5 mg/10 g, $n=10$). After fasting (24 h), each received charcoal (0.1 ml/10 g weight; 10% charcoal suspension in 5% gum Arabic, i.g.) to assess GIT as described (Pol et al., 1994) and was sacrificed in 20 min. GIT was a percentage of the traveled distance of the charcoal front in the total length of the intestine from pylorus to caecum.

2.4. Barium meal X-ray examination

Fifteen mice (both sexes) were randomly divided into low dose (0.5 mg/10 g, $n=5$), high dose (2.5 mg/10 g, $n=5$) and control group ($n=5$). 20 min after administration of CO or edible oil, 0.8 ml 160% barium sulphate suspension was given by i.g. The transit of barium was measured by X-ray contrast examination (Wang et al., 2002b). When the barium out of the body at 10th, 40th and 90th min was recorded, the transit grade was noted as 4, 3 and 2, respectively. At the 180th min, if most of the barium entered large intestine, the grade was 1, and if most entered small intestine or even stayed in gastric, the grade was 0. Then, 0–4 was transferred as index 0, 25, 50, 75 and 100% for further analysis, respectively.

2.5. Histological examination

Four strips from each 1 cm colon or jejunum segment in CO-treated and control mice were available for histological examination. Colon (middle) and jejunum (middle) were fixed by 4% formalin and prepared for HE staining and electron microscopy.

2.6. Contractile activity of intestinal smooth muscle strips

CO 0.5 mg/10 g was given for 8 days (one time a day), and edible oil 400 μl without CO was for the control mice each time. Mucosa-intact strips (2× 10 mm) were cut approximately parallel to the longitudinal smooth muscle strips of the middle isolated colon and jejunum colon by a tool (specially made by us) with two parallel operating knife blades. Circular smooth muscle strips of colon and jejunum were cut into 2 mm in length (without open) (Boddy and Daniel, 2005). Strips was immediately placed into tissue bath with Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 25 mM $NaHCO_3$, 2.5 mM $CaCl_2$, 10 mM glucose, pH 7.4, 37 ± 0.5 °C) which had bubbled with carbogen (95% O_2–5% CO_2) continuously.

Longitudinal strips were placed in 5 ml tissue bath containing Krebs solution, between two platinum concentric circular electrodes. One end was tied to a hook with 3/0 silk suture thread, the other was tied with thread and attached to a strain gauge (SEN-3301, Japan). According to preliminary experiment (data not shown), the square wave pulses of electrical field stimulation (EFS) (0.5 ms duration, 10 Hz, train duration of 60 s and 15 V) was used to elicit neural response.

Circular strips were placed in 10 ml tissue bath, and the open side of a thin metal triangle was slid through the lumen of the ring (Boddy and Daniel, 2005). A stainless steel rod attached to the bottom of the holder was inserted into the lumen of the ring under the metal triangle to secure the triangle and provide slight tension. A long vertical fine stainless steel hook attached at the apex of the triangle opposite to the tissue, was hold to the strain gauge.

Segments were equilibrated in Krebs solution for 60 min. According to the contractile response of strips to KCL (EC_{50} was 60 mM), the optimal tension for longitudinal and circular strips is 1.0 and 2.0 g, respectively. Contractile activity of strips was recorded on a Powerlab system (Australia). The recording system was different for longitudinal strips and circular strips. RM6280 and RM6240 software (China) were used for data acquisition. Atropine was used, and EC_{50} 1.4×10^{-5} M was determined by preliminary experiments (data not shown). The contractions of 10 min before and after atropine treatment were counted and the mean frequencies (min^{-1}) were computed.