Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/repbio

Original research article

The effect of cinnamon extract on isolated rat uterine strips

Mohammed Alotaibi *

Department of Physiology, College of Medicine, King Saud University and King Khalid University Hospital, P.O Box 2925, Riyadh 11461, Saudi Arabia

ARTICLE INFO

Article history:
Received 28 July 2015
Received in revised form
24 November 2015
Accepted 5 December 2015
Available online 17 December 2015

Keywords:
Cinnamon
Uterus
Contraction
Smooth muscle
L-type Ca^{2+} channel

ABSTRACT

Cinnamon is a spice used by some populations as a traditional remedy to control blood pressure and thus hypertension. Cinnamon extract decreases contractility in some smooth muscles, but its effect on uterine smooth muscle is unknown. The aim of this study was to determine the physiological and pharmacological effects of cinnamon extract (CE) on the contractions of isolated rat uterine strips and to investigate its possible mechanism of action. Isolated longitudinal uterine strips were dissected from non-pregnant rats, mounted vertically in an organ bath chamber, and exposed to different concentrations of CE (10–20 mg/mL). The effect of CE was investigated in the presence of each of the following solutions: 60 mM KCl, 5 nM oxytocin, and 1 μM Bay K8644. CE significantly decreased the force of uterine contraction in a concentration-dependent manner and significantly attenuated the uterine contractions elicited by KCl and oxytocin. In addition, CE significantly decreased the contractile force elicited when L-type Ca^{2+} channels were activated by Bay K8644. CE's major mechanism may be inhibition of L-type Ca^{2+} channels, which limits calcium influx. These data demonstrate that CE can be a potent tocolytic that can decrease uterine activity regardless of how the force was produced, even when the uterus was stimulated by agonists. As a result, cinnamon may be used to alleviate menstrual pain associated with dysmenorrhoea or prevent unwanted uterine activity in early pregnancy.

1. Introduction

Primary dysmenorrhea is one of the most common gynaecological disorders [1]. More than 80% of the populations of developing countries rely on traditional medicine to treat their health conditions (see [2] for review). Different alternative medicines have been studied to test their effectiveness in alleviating menstrual pain [3–5]; one of these is cinnamon [6], an ancient herbal medicine [7] cited by the Chinese naturopathic medicine literature 4000 years ago [8].

Cinnamon is a common name for the tropical evergreen trees that belong to the genus "Cinnamomum" of the *Lauraceae* family. There are two main types of cinnamon:

* Tel.: +966 11 4672629; fax: +966 11 4672567.
E-mail address: mfalotaibi@ksu.edu.sa

http://dx.doi.org/10.1016/j.repbio.2015.12.001

one of them is Ceylon Cinnamon (*true Cinnamon*), which is scientifically known as *Cinnamomum zeylanicum* or *Cinnamomum verum* and originally cultivated and grown in Sri Lanka (the most common type). The other type of cinnamon is Cassia Cinnamon or Chinese Cinnamon, which is scientifically known as *Cinnamomum Cassia* or *Cinnamomum aromaticum* and cultivated and grown in southern China. Chemical analysis of cinnamon extracts (CEs) revealed the presence of essential oils, tannins, cinnamaldehyde (CA), eugenol, cinnamophilin, coumarins, cinnzeylanine, cinnzeylanol, arabinoxylan, 2′-hydroxycinnamaldehyde, and 2′-benzoloxycinnamaldehyde [7,9–13]; CA constitutes the major component of cinnamon bark (45–65%) [14,15] and the spicy taste of cinnamon is due to this component [16]. CA has been used in biomedical research worldwide for its known biological effects [17–20]. Cinnamon is widely used as an essential herbal medicine to treat many pathophysiological disorders [21]; it is effective as an anticancer [22], antimicrobial, anti-inflammatory, and antioxidant compound [23,24], and was more recently used in the management of menstrual disorders [6,25]. Cinnamon possesses anti-diabetic properties in rats and humans [26], and CA increases the activity of antioxidant enzymes in kidneys [27]. In the vascular system, CA relaxes the contraction of rat aortic smooth muscles induced by different agonists in a dose-dependent manner [20,28]. Furthermore, CA lowered blood pressure and left ventricular systolic pressure in anesthetised rats [29] and protected rats from hypertension associated with diabetes [18].

While a few studies examined the *in vitro* effect of CE on different types of smooth muscle cells [17,19,28], there appear to be no such studies on uterine contraction *in vitro*. Because there is a clinical need to find promising and cost-effective drugs to help control uterine activity and alleviate menstrual pain [30,31], we hypothesised that CE decreases uterine activity in the animal model *in vitro* and investigated the effects of varying CE concentrations on non-pregnant rat uterine contractility *in vitro*. Specifically, we studied the effect of CE on (i) spontaneous uterine contractions; (ii) uterine strips depolarized by high concentrations of KCl; (iii) uterine contractions induced by oxytocin (OT); and (iv) uterine contractions induced by an agonist of L-type calcium channels (Bay K8644).

2. Materials and methods

2.1. Plant materials

Fresh dried barks of Ceylon Cinnamon (*C. Zeylanicum*) were collected in Sri Lanka in December 2014, purchased from a local herb shop, identified and authenticated by an expert taxonomist, Mr A. Nazer. The identity of the purchased cinnamon was verified and confirmed by Dr O. Basudan (College of Pharmacy, King Saud University, KSA). A voucher specimen was deposited for future use (reference no. 0898).

2.2. Extraction and isolation of cinnamon extract

Cinnamon barks were cleaned, air-dried, and then ground to a very fine powder. Five hundred grams of this ground powder were weighed and then extracted with a 50:50 water–ethanol solution for 24 h at room temperature (26 ± 2 °C). The extraction mixture was filtered using Whatman paper No. 1 to remove residues and the filtrates were evaporated under reduced pressure in a rotary evaporator at 45 °C to eliminate the ethanol and water. This yielded a solid, dried, and dark extract (45 g, 9%), which was further ground and kept in a sterile bottle. The extract was then kept at 4 °C until use.

2.3. Animals and isolation of uterine strips

Six non-pregnant female Wistar rats (200–250 g) were used in this study. The oestrous cycle stages were monitored daily using vaginal smears and the rats were sacrificed only in the metoestrous or dioestrous stages because the uterus produces regular spontaneous contractions in these stages [32]. All rats were maintained in accordance with the guidelines of the Animal Care Centre, College of Medicine, KSU. The study was approved by the experimental animal ethics committee at KSU. The rats were humanely sacrificed by carbon dioxide inhalation and cervical dislocation. The abdomen was opened longitudinally and the uterus was removed and immediately placed in a buffered physiological salt solution (PSS) of the following composition in [mM]: 115 NaCl, 4.7 KCl, 2 $CaCl_2$, 1.16 $MgSO_4$, 1.18 KH_2PO_4, 22 $NaHCO_3$, and 7.88 dextrose, pH 7.40. The uterus was placed in a shallow dish containing PSS and placed under a microscope. Fats and blood clots were removed and a fine longitudinal uterine strip with intact endometrium (2 mm × 0.5 mm × 10 mm) was dissected from the ovary end and used immediately for experimentation. Uterine strips with endometrium were treated with or without 15 mg/mL CE ($n = 3$) in order to check whether the endometrium contributes to the effect of CE on uterine contractility.

2.4. Measurement of tension

The dissected uterine strips were transferred and mounted vertically in a 5 mL tissue organ bath (Panlab, ADInstruments Ltd., Sydney, Australia), which was continuously perfused with PSS at a rate of 4 mL/min and bubbled with 95% O_2/5% CO_2 at 37 °C; the pH was maintained at 7.40 throughout the experiment [33]. The uterine strips were connected to an isometric force transducer (ADInstruments Ltd.) using surgical silk. Changes in isometric force were measured, amplified, recorded, and displayed using LabChart software (ADInstruments). Before experimentation, 1 g of resting tension was applied and the strips were allowed to equilibrate for at least 30 min to obtain stable and regular uterine contractions.

2.5. Drug challenges and chemicals

All chemicals used in this study were of analytical grade and purchased from Sigma (St. Louis, MO, USA). All stock solutions were made and stored in accordance with the manufacturer's guidelines.

Concentrated KCl (60 mM/L) solution was made fresh by isoosmotic replacement of NaCl [34]. Oxytocin was prepared by dissolving it in distilled water and used at a final working concentration of 5 nM [33]. Bay K8644 is a dihydropyridine Ca^{2+} channel agonist that can elevate the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by Ca^{2+} entry through L-type Ca^{2+} channels.