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Aqueous extracts of castor seed (*Ricinus communis*) increase the contractile activities of mouse uterine tissues *in vivo*

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Abstract

Administration of castor oil from seeds of *Ricinus communis* is a commonly cited herbal preparation used to induce labor. The ricinoleic acid found in castor oil is more polar than most fats and activates uterine EP₃ prostanoid receptors. The purpose of this study was to determine if an aqueous extract from the castor seed would also demonstrate uterotonic behaviors, implicating ricinoleic acid's solubility in water. Aqueous extracts of castor seed were tested on isolated mouse uterine tissues in an organ bath. Increases in uterine contractile forces were observed, and at higher concentrations were greater than those produced by acetylcholine and similar to those from oxytocin. Results suggest that ricinoleic acid is present in aqueous extracts. Attempts to characterize uterine contractile responses were done with the understanding that the extract may contain a number of active constituents whose contributions may be synergistic, additive, or antagonistic to the overall tissue contractile response.

Keywords: *Ricinus communis*, uterine contractions, ricinoleic acid, mouse, labor induction

1. Introduction

Castor oil is a commonly cited herbal preparation used to stimulate labor [1, 2, 3]. Support for the use of castor oil has been garnered through several clinical investigations [4, 5, 6]. Others have concluded that, although no harmful effects were observed in the administration of castor oil to induce labor, the herbal treatment did not produce a significant change in induction time when compared to no treatment [7]. Furthermore, it was considered that castor oil is a poor choice given the availability of modern-day pharmacological induction using the synthetic forms of the human hormones oxytocin and prostaglandin E₂ [8].

In the hospital setting, the most common labor induction method is intravenous pitocin. However, this method may be associated with a number of potential complications, and often requires several additional interventions, including intravenous needle placement, continuous internal/external fetal monitoring, internal uterine pressure catheters, epidural anesthesia, and Foley catheter placement. These interventions often increase further risks to the health of both mother and fetus [9]. For that reason, many women are electing to use natural herbal methods to stimulate labor.

Castor oil is a triglyceride and can be produced by the extraction of oil from the seeds of the castor plant *Ricinus communis* [10]. Castor oil is digested by pancreatic lipases into glycerol and ricinoleic acid, a monounsaturated 18-carbon fatty acid, of which considerable amounts are absorbed in the intestine [11] and induce a strong laxative effect [12]. It was formerly postulated that the increased intestinal motility accompanying the resulting diarrhea was mechanically irritating the uterus and initiating uterine contractions [13, 14].

Tunaru *et al.* [15] however, has recently shown that ricinoleic acid activates EP₃ prostanoid receptors on intestinal and uterine smooth muscle cells, mediating these motility effects. Since EP₃ prostaglandin receptors have been localized to uterine smooth muscle cells and their role in the contractility of the myometrium is well documented [16, 17, 18] these findings identify that ricinoleic acid in castor oil may be an active component inducing labor [15].

Ricinoleic acid may account for 87.4% to 90.4% of the fatty acid content in castor oil [19]. It is an unusual fatty acid in that it has a hydroxyl functional group on the 12th carbon, making it more polar than most fats [20]. The purpose of our study was to determine if an aqueous extract from *Ricinus communis* would also demonstrate uterotonic behaviors and implicate ricinoleic acid's solubility in water. Our specific project objectives were to collect baseline

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concentration-contractile response data, compare the contractile behaviors to other known uterotonics, namely oxytocin, acetylcholine, and blue cohosh, and use receptor antagonism to investigate other possible extract interactions with selected tissue receptors.

2. Materials and methods

2.1. Castor seed extract preparation

Castor seeds from *Ricinus communis* were obtained from Mountain Rose Herbs, Eugene, Oregon, U.S.A. Herbarium samples preserved for future reference are housed at Bethel University. An aqueous extract of *Ricinus communis* seed was prepared based on the methods published by Lis-Balchin *et al.* [21] and Kaingu *et al.* [22]. Seeds were ground to a powder using a mortar and pestle. Subsequently, the necessary mass of ground seeds to produce a given concentration was mixed with 100 mL of deionized water. The mixture was then boiled for 10 min. at 100 °C, allowed to cool, and then vacuum filtered through filter paper to remove castor seed particulates. This aqueous extract was miscible with the polar DeJalons solution in the muscle baths and was freshly prepared before each experiment.

2.2 Specimens

Twenty-four virgin, female, ICR CD-1 outbred mice (*Mus musculus*) weighing 25-30 g were obtained from Harlan Laboratories, Inc. All mice had *ad libitum* access to water and standard mice chow. All procedures were completed in accordance with the Institutional Animal Care and Use Committee of Bethel University.

Since the estrous cycle of mice is four to five days, injections of diethylstilbestrol (DES), a synthetic non-steroidal estrogen agonist, were given 24 h prior to each experiment to forward each mouse into the estrus stage of their cycle [23]. DES also facilitates the formation of gap junctions within the endometrial cells of the uterus, thus promoting the thickening of the endometrial layer of the uterus and allowing the organ to work as single-unit smooth muscle [24].

2.3 Tissue preparation

On the day of the experiment, the mice were sacrificed via CO₂ asphyxiation. The uterine horns were extracted from the abdominal cavity and immediately placed in chilled DeJalons solution (g/5 L): 45 g NaCl, 2.1 g KCL, 2.5 g NaHCO₃, 2.5 g D-glucose, 0.4 g CaCl₂. The uterine horns were dissected, cleansed of excess connective tissue, and ligated on either end. One end was attached to the distal portion of a fixed stationary hook to be placed inside a 15 mL organ bath, and the other attached to an isometric force transducer (MLT500, AD Instruments, Colorado Springs, Colorado, U.S.A.) coupled to Power Lab 4/SP data acquisition system with Chart 5.2 software (AD Instruments). The uterine tissue was then placed into the bath containing DeJalons solution, suspended at 0.8 g of tension, and allowed to equilibrate for 1 h with the flushing of fresh DeJalons solution every 15 min. A mixture of 95% O₂ and 5% CO₂ was bubbled into the baths throughout the duration of the experiment. The baths were maintained at 30 °C so as to dampen the tissue's normal endogenous contractile patterns, herein called spontaneous motility, and enable contractions produced by castor seed extract treatment to be more easily distinguished [25].

2.4 Isolated uterine tissue testing

All tissues were first given 10⁻⁵ M acetylcholine (ACh) to produce a positive contractile response and affirm tissue

viability [25]. Tissues were then rinsed with fresh DeJalons solution, allowed to equilibrate for an additional 10 min and return to baseline motility patterns. Each tissue was then given the desired treatment and the resulting contractile forces were recorded and measured. Each uterine horn was treated as a unique sample. The final castor seed extract concentrations used were 2.5, 5, 10, 20, and 30 mg /15 mL bath. A sample size of four or five uterine horns was used for each concentration.

A sub-set of these uterine horns were also given 10⁻⁵ M oxytocin in order to compare contractile responses with those evoked from 10⁻⁵ M ACh, and from 10 mg and 30 mg castor seed extract.

2.5 Blocking experiments

To determine if receptors other than EP₃ might be interacting with the aqueous castor seed extract, experiments using selective antagonists were completed. After a uterine horn was allowed to re-equilibrate following a positive contractile response from 10⁻⁵ M ACh, it was given an application of 10⁻⁵ M of a receptor antagonist, followed by a 5 to 10 min incubation period, followed by a single application of 30 mg of aqueous castor seed extract.

2.6 Measurements and statistical analyses

All treatment applications were made after the full completion of a spontaneous motility cycle and under basal tension. Changes in contractile force were measured from baseline tension to the maximal force produced within the first 10 min of treatment exposure. The mean values for change in maximal contractile force relative to 10⁻⁵ M ACh at each castor seed extract concentration were determined. The percent change in uterine contractile frequency for each specimen was determined by dividing the number of contractions produced for 10 min following the application of castor seed extract by the rate of spontaneous motility exhibited 10 min prior to extract application.

Means ± S.E.M. were calculated at each concentration and differences among the contractile responses were analyzed using ANOVA for multiple comparisons among the means. Following the rejection of the null hypothesis that castor bean aqueous extract would have no contractile effect on isolated uterine horn tissues, the Tukey-Kramer post hoc test was used to indicate which extract concentrations were significantly different from each other. The results of the receptor blocking experiments were analyzed using the Mann-Whitney U-Test. For all analyses, data were considered to be statistically different at $P \leq 0.05$ (JMP 4.0, SAS Institute, Cary, NC).

2.7 Chemicals

All drugs were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Acetylcholine, atropine, bradykinin B₂, B-9430 trifluoroacetate salt, and oxytocin were dissolved in deionized water. Indomethacin was solubilized in methanol and nifedipine was solubilized in dimethylsulfoxide.

3. Results

3.1 Concentration-contractile responses to aqueous extracts of castor seed

Overall spontaneous motility prior to the application of castor seed extracts occurred with a consistent force of ~ 9.12 mN and a frequency of 0.53 contractions/min. A representative uterine tissue contractile response to both 5 mg and 30 mg castor seed extract is illustrated in Fig 1.

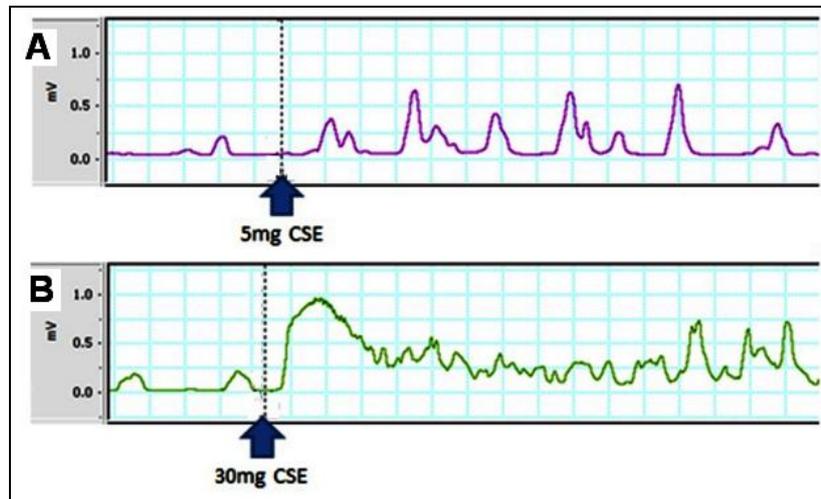


Fig 1: Typical uterine contractile responses showing an increase in both contractile force and frequency following the addition of castor seed extract (CSE)/15 mL organ bath. Each panel represents a single tissue over the course of 10 min. The vertical axis represents uterine contractile force in mV, later converted to mN. In panel A, the force generated from spontaneous motility was 7.09 mN, and the resulting force produced by 5 mg castor seed extract was 29.734 mN. The contractile frequency increased 57%. In panel B, the force generated from spontaneous motility was 7.03 mN, and resulting force produced by 30 mg castor seed extract was 42.69 mN. The contractile frequency increased 110%.

The addition of the aqueous castor seed extract increased the raw force of uterine contractions at all concentrations tested ($P < 0.0001$) and the responses ranged from 19.73-71.4 mN. After the contractile forces were normalized to their respective 10^{-5} M ACh responses, contractile forces following the application of 10, 20, and 30 mg castor seed extract were statistically greater from that of “0” treatment ($P = 0.0057$;

Fig. 2a). Furthermore, at the same extract concentrations, contractile forces were greater than those evoked from 10^{-5} M ACh.

All castor seed extract concentrations yielded increases in uterine contractile frequency; concentrations at 10, 20, and 30 mg yielded significant increases in frequency ($P = 0.0009$; Fig. 2b).

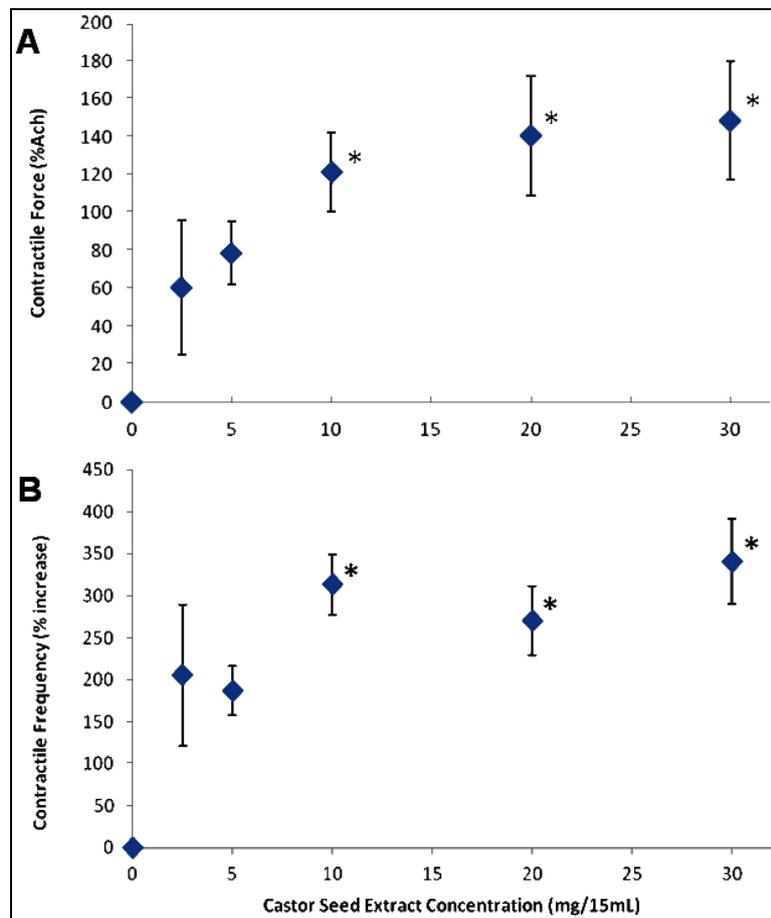


Fig 2: (A) Means \pm S.E.M uterine contractile force (%ACh) produced by castor seed extract. Concentrations marked with an asterisk (*) produced forceful contractions statistically greater from “0” treatment ($P = 0.0057$; $n = 22$). (B) Means \pm S.E.M percent increases in uterine contractile frequency in response to various concentrations of castor seed extract. Concentrations marked with * produced contractile rates that were significantly greater than “0” treatment ($P = 0.0009$, $n = 21$).

3.2 Comparison of castor seed extract with responses from ACh and oxytocin

A representative contractile response evoked from both ACh and oxytocin at 10^{-5} M is shown Fig. 3a. While both agonists produced very strong contractions, the oxytocin response was typically greater (ACh/oxytocin ratio = 0.841 ± 0.146 ; $n = 4$). An oxytocin contractile plateau was sustained and its return to basal tonus was 25% higher until washout (20 min later). Acetylcholine typically returned to its original baseline tension within 5 min following application. Increases in contractile frequencies from pre-treatment spontaneous motility were very similar between ACh and oxytocin.

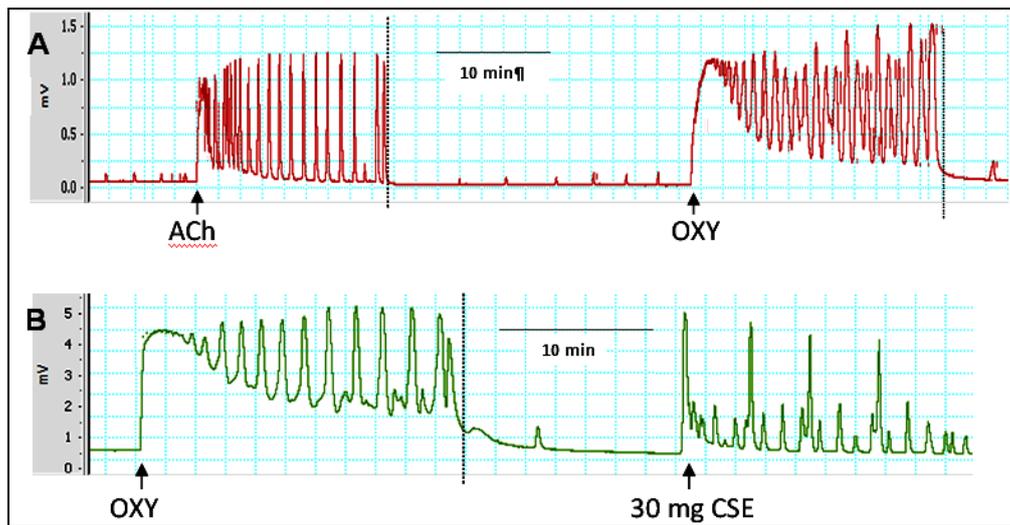


Fig 3: Representative traces comparing contractile responses of (A) 10^{-5} M ACh (52.742 mN) to that produced by 10^{-5} M oxytocin (OXY) (55.192 mN), and (B) 30 mg castor seed extract (CSE) (66.051 mN) to that produced by 10^{-5} M oxytocin (OXY) (71.456 mN). The marked arrows indicate the addition of agonists; all produced a rapid increase in muscle tension. The dotted vertical lines indicate tissue washout with fresh DeJalons. For analysis, the default y-axis was converted to mN based upon calibration of the force transducer.

3.3 Blocking Experiments

Both ACh and bradykinin produced strong uterine contractile responses and their respective antagonists successfully blocked their activities. However, antagonists for these same receptors, atropine and B-9430 trifluoroacetate salt respectively, failed to inhibit any castor seed extract contractile response. Further attempts to block the uterine contractile response were also unsuccessful with indomethacin, a prostaglandin synthase inhibitor. Nifedipine, an L-type calcium channel blocker, however, produced a substantial though incomplete inhibition of contractile action from the castor seed extract ($P = 0.1000$).

4. Discussion

4.1 Aqueous extract of *Ricinus communis* as an uterotonic

The use of *Ricinus communis* as an herbal method for labor induction is an area of both interest and concern within the midwifery community. In a survey of certified nurse-midwives, the most commonly cited reason for the use of any herbal preparation for labor induction was that they are “natural.” However, the most common reason cited for not using them was their lack of documentation regarding their efficacy, safety, and use [1].

Our results provide empirical support for the potential use of *Ricinus communis* as an uterotonic. Recent work by Kaingu *et al.* [22] has also demonstrated that a constituent in an aqueous extract of *Ricinus communis* made from the root bark also produced slight increase in the frequency of uterine contractions in non-pregnant, DES injected rabbit tissues *in*

vitro. It remains to be determined whether the active agent(s) evoking the contractile responses are from sources unique to the plant tissue types (e.g., seeds or roots) or systemically transported within the plant.

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4.2 Applicability of results from isolated tissues to whole animal

It is important to consider the applicability of our *in vitro* results using rodent models, to those resulting following the oral consumption of *Ricinus communis* in humans. Since ricinoleic acid also activates EP₃ prostanoid receptors in intestinal tissues [15], the consequent propulsive effects on gut motility should be an important consideration for health care professionals in deciding whether or not to use *Ricinus communis* to induce labor. The resulting laxative response and concurrent side effects of nausea, vomiting, intestinal colic and diarrhea are common occurrences associated with the consumption of castor oil [23]. It is noteworthy that a recent Cochrane review did not find a difference in meconium stained amniotic fluid between those who consumed castor oil and those with a control or no treatment [20].

Most health care professionals that advocate for the use of castor oil to help initiate labor usually recommend its consumption within an induction regime. Castor oil by itself is considered distasteful, so it is usually ingested along with additional carriers. For example, a common type of cocktail contains two ounces of castor oil coupled with two ounces each of both vodka and orange juice [26]. Thus, the preparation

of an aqueous extract may be of interest if simply to improve palatability.

4.3 Castor seed toxicity concerns

Castor seeds themselves are considered toxic as the phytotoxin ricin is released through chewing or maceration^[27] and may cause cell death by stopping protein synthesis^[28, 29]. Ricin content may vary from 1% to 5%^[30, 31] and clinical symptoms in humans have been documented following the ingestion of 0.5 to 30 castor seeds^[32, 27]. Ricin itself is not found in the castor oil partition following extraction^[33] and can be inactivated if the extraction process is completed under heated conditions^[34, 35]. Despite all the necessary precautions, there is a history of use for the roots, leaves, and seeds of *Ricinus communis* in traditional medicine throughout the world^[36, 20]. Interestingly, Oyewole *et al.*^[37] has shown that the aqueous extracts of *R. communis* prepared from the leaves were effective antimicrobial agents, and did not cause significant alterations in the cellular activities within the host rats.

4.4 Comparison of contractile responses produced by castor seed extract and blue cohosh

The roots and rhizomes of blue cohosh (*Caulophyllum thalictroides*) have also been used to induce labor^[3]. Blue cohosh does create forceful contractions on isolated mouse uterine strips. Using a protocol similar to the preparation of castor seed aqueous extract reported herein, Berger and DeGolier^[38] found that 24 mg blue cohosh (n = 4) evoked a contractile force of 167.772 ± 38.408 (% ACh). This is slightly greater than our 30 mg castor seed (n = 4) response of 148.599 ± 31.394 (% ACh), although the difference is not significant ($P = 0.396$). The literature has reported some problematic neonatal cardiovascular problems with the use of blue cohosh as a labor inducer^[39, 40, 41]. Even though the use of castor oil is generally considered safe; it remains recommended that caution should be exercised^[42].

4.5 Aqueous castor seed extract and uterine tissue receptor activity

Our results indicated that the constituents of the *Ricinus communis* aqueous extract did not mediate their effects via peripheral cholinergic pathways. We were also unable to block contractile responses with a bradykinin receptor antagonist. Calixo *et al.*^[43], when using crude rhizome extracts from a different tropical plant *Mandevilla velutina* (dogbane family), was able to antagonize a bradykinin-induced contractile response in isolated rat uterine tissues. In a study using isolated rings of thoracic blood vessels, Lodge^[44] was able to show that ricinoleic acid alone could induce vasoconstriction and that the tissue responses were insensitive to indomethacin. This is consistent with our results, indicating that the contractile behaviors of our aqueous extract are not directly involved in a prostaglandin synthesis pathway. Nifedipine however, did produce a partial inhibition of the contractile response, indicating that a portion of the activity is mediated by extracellular calcium.

5. Conclusions and recommendations

Aqueous extracts from *Ricinus communis* seeds produced concentration-dependent increases in both contractile force and frequency in isolated mouse uterine tissues. At the higher concentrations, contractile forces were greater than those evoked by the endogenous neurotransmitter acetylcholine, and

similar to those evoked from pituitary oxytocin. Our results indicate that either ricinoleic acid is present and binding to EP₃ prostaglandin receptors on the uterine tissues, or that there are other biologically active constituents within the castor seed extract that are water-soluble. Attempts to characterize the resulting uterine contractile responses herein were done so with the understanding that the extract may contain a number of active constituents whose contributions may be synergistic, additive, or antagonistic to the overall tissue contractile response. Purified ricinoleic acid has yet to be applied to isolated mouse uterine tissues and the results of such a study would create a distinctive link between an active agent within *Ricinus communis* and a physiological response.

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