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The contractile responses of blue cohosh, *Caulophyllum thalictroides*, and some its constituents on isolated longitudinal strips of mouse gastric tissues

Andrew Kremer and Teresa De Golier

Abstract
Blue cohosh (*Caulophyllum thalictroides*) is an herbal supplement shown to evoke contractile responses from uterine smooth muscle. It has been reported to induce labor among Native American and midwife patients. Since blue cohosh is ingested, the question of what effect it may have on other tissues is important. This study considers the contractile response from *Mus musculus* stomach fundus tissue subjected to blue cohosh and two of its constituents, *N*-methylcytisine and saponins. These compounds were administered *in vitro* to isolated stomach tissues in an organ bath. The overall effect of the treatments showed an increase in contractile forces when compared to the "0" treatment ($p<0.0001$). These responses however, were extremely small and are much smaller when compared to uterine contractile responses under similar conditions. The results herein would imply that taking blue cohosh as an aid for inducing labor should not result in unintended dramatic consequences from gastric motility.

Keywords: Stomach muscle, blue cohosh, N-methycytisine, saponins

1. Introduction

1.1 Background

Blue cohosh (*Caulophyllum thalictroides*) is commonly found in the northeastern United States and has been used medicinally over many centuries [1]. A common historical application was the use of blue cohosh by Native Americans in preparation for childbirth [1, 2]. The roots and rhizomes of blue cohosh are still occasionally used to stimulate the uterus of pregnant women in an effort to induce labor [3, 4]. Aqueous extracts from the roots and rhizomes of blue cohosh do indeed produce strong contractile forces in a concentration dependent manner when tested on isolated mouse uterine tissues suspended in an organ bath [5].

The roots and rhizomes of blue cohosh possess many alkaloids and glycosides [1, 6, 7]. *N*-methylcytisine is one of the alkaloids [6, 8, 9] and a variety of saponins make up the glycoside group [10, 11]. The application of *N*-methylcytisine to isolated mouse uterine tissues was recently shown not to produce any significant changes in contractile activity when compared to the tissues own spontaneous motility patterns [12]. This same study, however, showed that the application of saponins (isolated from *Quillaja saponaria)*, resulted in the production of intense uterine contractile activity [12], and is likely be the greatest contributor to the overall contractile response as seen from the parent plant.

Since blue cohosh is sold as a dietary supplement and is orally consumed, it is important to question if there are other systemic effects from blue cohosh following its assumed absorption and distribution in the body. It is important to note that the safety and use of blue cohosh for labor induction has been an area of much debate due to the potential toxicity of the alkaloid constituent to the mother and fetus, as observed in neonatal congestive heart failure and myocardial infarction, and stroke [13-15]. The bradycardia observed in rat hearts *in vitro* when exposed to aqueous extracts of blue cohosh is also considered to be a function of the alkaloid constituent [16].

As the aforementioned references show, the constituents of blue cohosh can interact with smooth muscle and with cardiac muscle. Since both of these tissues types do respond to intrinsic input from the autonomic nervous system, it was reasonable to investigate whether blue cohosh and two of its constituents (*N*-methylcytisine and *Quillaja* saponins) would affect the motility of smooth muscle associated with the gastrointestinal tract. It was recently shown that these same agonists did produce a positive contractile response from isolated distal colon tissues from mice, but the responses were very small, and many of the increases in colon muscle tension were statistically insignificant when compared to the tissues own spontaneous

motility patterns [17]. Therefore, the goal of the investigation, using similar methods as employed by the isolated mouse colon (hindgut) study, was to determine what affect blue cohosh and the two constituents *N*-methylcytisine and *Quillaja* saponins would have on the motility of the mouse foregut, namely the smooth muscle of the gastric fundus. Previous pilot studies using rat tissues did show that extracts from the parent plant, blue cohosh, did increase contractile forces in isolated segments from both the distal rat colon [18] and the fundus of the stomach [19]. These results did show however, that the gastric smooth muscle was much less responsive and produced smaller contractions than those evoked from the colon.

1.2 Project objectives
Thus, the primary objectives of the experiment herein were to individually examine and quantify the contractile responses produced by blue cohosh and two of its constituents, *N*-methylcytisine and *Quillaja* saponins, on the fundus of the mouse stomach *in vitro*. The resulting changes in contractile activities were then compared to the tissues own spontaneous motility as well as the different treatment responses to each other. Furthermore, the results were also compared to those previously reported in the literature from isolated mouse colon and uterine tissues using very similar experimental conditions.

2. Materials and Methods
2.1 Animal specimens
Twelve virgin female mice, *Mus musculus* (outbred ICR CD-1), each weighing 25-30 g, were obtained from Envigo (Indianapolis, Indianapolis, USA). They were housed in cages in the Department of Biological Sciences at Bethel University (St. Paul, Minnesota, USA) and had access to water and standard mice chow *ad libitum*. All procedures were completed in compliance with the Institutional Animal Care and Use Committee of Bethel University.

2.2 Preparation of gastric fundus smooth muscle
On the day of an experiment, fresh Krebs buffer solution (g/5 L: 34.5g NaCl, 10.5g $NaHCO_3$, 10g D-Glucose, 0.8g KH_2PO_4, 1.8g KCl, 1.45g $MgSO_4*7H_2O$, and 1.85g $CaCl_2*2H_2O$) was made to simulate extracellular fluid conditions. Mice were euthanized via carbon dioxide asphyxiation, and afterwards pinned supine, and the stomach excised. Two pieces of stomach fundus (including some of the body from the greater curvature of the stomach) were prepared in a longitudinal orientation and a surgical suture was tied on each end of a sample tissue; one suture was attached to a stationary rod for eventually placement into a 20 mL organ bath, and the other for eventual attachment to a force transducer.

At the start of each experiment, the organ baths were flushed multiple times with the Krebs solution warmed to 30° C, and continually aerated (~2 psi) with 95% O_2/5% CO_2. Then a given isolated strip of stomach tissue was lowered into the organ bath, the stationary rod was anchored into the bath, and the other suture was attached to an isometric force transducer (MLT500, ADInstruments, Colorado Springs, Colorado, USA), and placed under 1.0 g of tension [20]. The force transducer was connected to an amplifier and a PowerLab data acquisition system (ADInstruments, Colorado Springs, Colorado, USA) that collected data from the suspended tissue and translated the tissue's contractile activities into visual waveforms.

The tissue samples were then equilibrated for one hour with flushes (tissue washouts) every 15 minutes, replenishing the system with fresh Krebs solution each time. These methods were based on previously published studies conducted by Bristol and DeGolier [12] and Cermin and DeGolier [17]. During this time, tissues demonstrated some spontaneous motility which was usually measurable but very small.

2.3 Experimental protocol
An initial experiment was completed using the cholinergic agonist acetylcholine (10^{-5} M) to validate that the stomach fundus tissue was viable for experimentation. Since the stomach tissues did show repeatable fatigue after two treatments, the remaining experimental tissues were only exposed to the blue cohosh, *N*-methylcytisine, and saponin treatments.

All treatment applications were made after the completion of any discernable spontaneous motility cycles and under baseline tension. Data collection began with application of one of the following treatments into the organ bath: 0.5 mg/ml blue cohosh, 10^{-5} M *N*-methylcytisine, or 10% saponin solution. Individual treatments were left in the tissue bath for 20-30 minutes. Following a tissue washout, tissues were allowed to re-equilibrate for an additional 30 minutes before a second occasional dosing of a different treatment was applied.

2.4 Chemicals
The powdered root and rhizomes of blue cohosh were purchased from Mountain Rose Herbs (Eugene, Oregon, USA) and was mixed with 100 mL of boiling deionized water, allowed to cool, and then vacuum filtered through Whatman filter papers via Buchner funnel to separate the aqueous extract from unnecessary plant particles.

The *N*-methylcytisine and the sapogenin glycoside (from the bark of the South American soap tree, *Quillaja saponaria)* were both purchased from Sigma-Aldrich (St. Louis, Missouri, USA). *N*-methylcytisine was dissolved in DMSO and the saponin was dissolved in Krebs solution and pipetted directly into the organ bath.

2.5 Measurements
The resulting waveform data was used to measure changes in contractile force from the baseline tension of the waveform to the maximal force produced within the treatment exposure. To control for the possible force contribution that the tissue's own spontaneous motility might have on the treatments, the contractile amplitude of those forces were also measured in a similar manner before the treatment applications, and were considered as the control, or the "0" treatment.

2.6 Statistical analysis
The data was summarized as means ± SE for contractile force for each treatment. One-sample T-tests were run to determine whether treatment contractile responses were significantly different from their respective spontaneous motilities. Individual data were further analyzed using ANOVA for multiple comparisons among the treatment means. Resulting *p* values ≤ 0.05 were subjected to the Tukey-Kramer post hoc test (JMP 4.0, SAS Institute, Cary, North Carolina, USA) which indicated which treatment means were considered to be statistically different from each other.

3. Results

3.1 Contractile waveform responses

Treatment contractile responses were significantly increased from their respective spontaneous motilities: blue cohosh p=0.0002; *N*-methylcytisine p=0.01; and saponin p <0.0001. While individual responses were variable, they were consistently observed as very small increases in tension. A typical gastric fundus smooth muscle waveform following an application of 0.5 mg/ml blue cohosh treatment is shown in Figure. 1. The waveform responses of all the other treatments were similar in shape, but on average, lesser in magnitude.

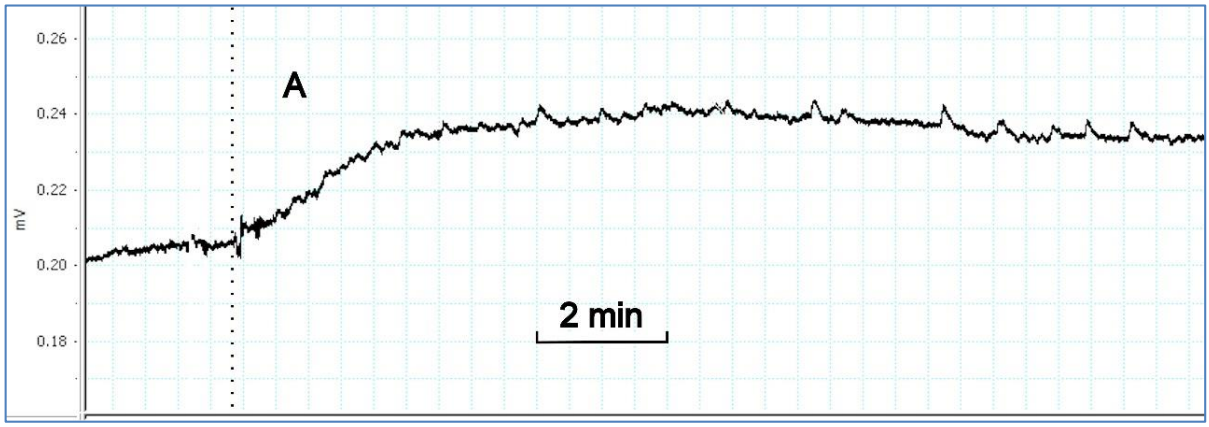

Fig 1: Typical gastric fundus smooth muscle waveform before and after an application of 0.5 mg/ml blue cohosh herbal treatment (letter **A**). This responses was typically observed as a slow increase in baseline tension that eventually plateaued over 10 min of exposure, frequently resulting in a more discernable motility pattern. Maximal contractile force observed in this sample was 1.9 mN. The vertical axis represents contractile force in mV, later converted to mN.

3.2 Contractile forces

The average contractile responses among the different treatments are compared to each other in Figure 2. The overall effect of the treatments as a group did show an increase in the force of tissue contractile responses when compared to the average "0" treatment (p<0.0001). Significant increases in contractile forces were found between "0" treatment (0.15 ± 0.07 mN, n=29) and both blue cohosh (1.46 ± 0.25 mN, n=14) and the saponins (1.52 ± 0.05 mN, n=6). The saponin and blue cohosh treatments were not statistically different from each other.

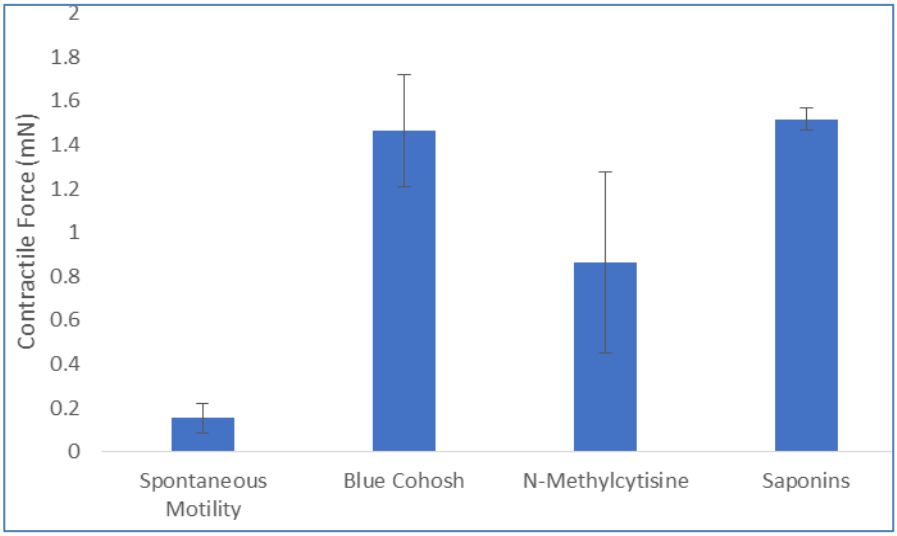

Fig 2: Means ± SE mouse fundus contractile force (mN) in response to various treatments. All treatments produced an increase in contractile force relative to overall spontaneous motility with the blue cohosh and saponin treatment groups producing significant increases (p<0.0001).

4. Discussion

The overall effect of the treatments (blue cohosh, *N*-methylcytisine, and saponins) did produce an increase in the force of tissue contractile responses when compared to the spontaneous motility or "0" treatment (p<0.0001). The contractile activity from the *N*-methylcytisine is likely a result of its high functional binding strength to nicotinic acetylcholine receptors [9, 21, 22]. The contractile activity from the saponins may be characteristic of their interaction with cholesterol within the plasma membrane to create pores, thus allowing Ca^{2+} to diffuse into the cell [23-25].

Spontaneous motility from the stomach fundus was difficult to discern and frequently absent from recorded tissues. Takaki [26] points out that the fundus is not myogenically active even though the antral portion of the stomach is, and suggests that this may be due to a differential distribution of the Interstitial Cells of Cajal within the myenteric plexus and longitudinal/circular muscle layers.

The stomach contractile responses from the treatment were extremely small and were measurably smaller when compared to the contractile responses evoked from isolated colon tissues [17] using a similar protocol with the same species and treatment concentrations (Table 1).

Table 1: Contractile forces (means ± SE) produced by an aqueous extract of blue cohosh (0.5 mg/ml), *N*-methylcytisine (10^{-5} M), and saponin solution (10%) in isolated murine stomach and colon smooth muscle tissues (n=sample size). Treatment "0"=spontaneous motility. *Data source from Cermin and DeGolier [17].

		Contractile force (mN)		
Tissue site	**Treatment "0"**	**Blue cohosh**	***N*-methylcytisine**	**Saponin**
Stomach	0.15 ± 0.07 (29)	1.46 ± 1.33 (14)	0.86 ± 0.41(6)	1.52 ± 0.05 (6)
Colon*	2.44 ± 0.51 (22)	4.63 ± 1.33 (8)	9.68 ± 3.00 (6)	15.81 ± 6.19 (4)

Both of these gastrointestinal tissue locations, however, were much less responsive to both blue cohosh and the saponin treatments when compared to contractile forces produced by uterine tissues under similar conditions. Cermin and DeGolier [17] recorded very forceful uterine contractions in isolated mouse tissues following the application of blue cohosh (57.65 mN ± 5.95, n=4) and from the saponin (84.01mN ± 12.85, n=10).

5. Conclusion

Even though the isolated suspended stomach tissues were slightly shorter in length and were visibly less dense when compared to uterine tissues employed in the Cermin and DeGolier study [17], the resulting stomach contractile forces were only ~3% of the forces produced by uterine tissues. This data further supports the conclusion that taking blue cohosh orally as an aid for inducing labor (at least at the concentrations reported herein) should not result in unintended dramatic consequences from gastrointestinal motility [17].

Blue cohosh is categorized in the United States as a food supplement and does not undergo rigorous evaluation of safety and efficacy before receiving the Food and Drug Administration approval for marketing [27]. While several studies have identified alkaloid and saponin glycoside constituents [1, 6, 7, 9, 10, 11, 21] very little information is available detailing how (and whether or not) blue cohosh (and its constituents) are absorbed, distributed, metabolized, and excreted in a whole animal model. Mechanisms at best have been deduced by reporting *in vivo* side effects and then testing isolated tissues models for positive or negative responses (such as the investigation reported herein) and employing receptor antagonism studies. The applicability of using results obtained from isolated tissue models is best practiced when the bioavailability of blue cohosh and its constituents can be demonstrated.

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