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The chronotropic effects of blue cohosh, *Caulophyllum thalictroides*, on frog hearts *in situ* and rat hearts *in vitro*

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Abstract

Blue cohosh (*Caulophyllum thalictroides*) is a North American herb traditionally used for inducing labor. Case studies report the effectiveness of blue cohosh in inducing uterine contractions, but have also reported fetal cardiac complications due to overdose. Little research investigating such cardiac responses at an organ is available. In this study, blue cohosh treatments (30 mg) were administered to frog hearts *in situ* and rat hearts *in vitro*. The consequent changes in heart rate were compared to control treatments. In both models, the administration of blue cohosh resulted in a negative chronotropic effect when compared to the controls ($P < 0.0001$). Bradycardia was observed in both cardiac preparations exposed to the same concentration that has been shown to effectively contract isolated uterine tissues from mice. These results further stimulate the need to understand how blue cohosh can be safely and holistically used without side effects in pregnant women.

Keywords: *Caulophyllum thalictroides*, hearts, bradycardia, frogs, rats

1. Introduction

Blue cohosh, *Caulophyllum thalictroides* (Berberidaceae), is a perennial herb native to northeastern North America and eastern Asia [1]. It stands from 0.3-.75 meters tall and has a smooth stem that grows one to three feet in height and terminates in yellowish-green flowers. It bears dark blue berries from which the name "blue" cohosh is derived [2, 3]. Blue cohosh is also known by several other common names such as blue ginseng, papoose root, squawroot, or yellow ginseng [4]. It is harvested in the fall and the dried roots and rhizomes that contain the active ingredients [3, 5], can be ground up and are commonly available.

Blue cohosh is one of the oldest indigenous American plant drugs and was introduced to the medical field in 1813 by Peter Smith, an Indian herb doctor [6]. It was officially listed in American Pharmacopeia from 1882 to 1905 as a labor inducer, and 19th century physicians regularly recorded its use as a uterine stimulant. More recently, blue cohosh has been reported to treat amenorrhea, dysmenorrhea, contraction-like spasms, rheumatic symptoms, and symptoms resulting from uterus atonia [3, 7].

Blue cohosh in homeopathy is considered very useful in establishing labor [8] as well as preventing poor contraction patterns [9]. However, two studies involving 133 women compared a homeopathic *Caulophyllum* experimental treatment to a placebo. No differences were found in the onset of uterine contractions [10] or in the length or difficulty of the labor [11]. It is possible that the experimental design utilized in those studies [10, 11] may not reflect routine homeopathic practice [12].

The use of homeopathic techniques has dramatically increased in the last decade. Homeopathic therapeutics are often very dilute and are less likely to result in undesirable and potentially harmful side effects compared to those of conventional pharmaceuticals [13]. A survey of midwives reported by Allaire *et al.* [14] found that over 90% recommended homeopathic remedies to their pregnant patients, with the most commonly prescribed remedy being herbal therapy. Furthermore, a national survey of certified nurse-midwives stated that 64% used blue cohosh to help induce labor in pregnant women [15].

Even though the historical and contemporary use of blue cohosh in pregnant woman has been well documented [16], there are only a few empirical studies addressing its efficacy at the reduced level of individual uterine tissues. Aqueous extracts of the roots and rhizomes of blue cohosh have been shown to increase the force of uterine contractions when sampled in isolated strips of mouse uterine smooth muscle [17]. These results are consistent with much earlier investigations demonstrating that blue cohosh has a definite oxytocic action in uterine tissue [18, 19].

While these *in vitro* results help provide credibility for the use of blue cohosh in augmenting or inducing labor, they also stimulate the need to understand how blue cohosh can be used safely and holistically, and without side effects in pregnant women. In 1997, a case study followed an expectant mother who was ingesting blue cohosh under the supervision of her midwife for three weeks prior to her due date. The mother opted to take a triple dose from what was recommended by the midwife, and reported a reduction in fetal activity during this time. Upon delivery, the infant suffered from congestive heart failure and severe myocardial infarction. Two years after recovery, cardiomegaly and reduced left ventricular contractions persisted in the child. With other variables ruled out, blue cohosh was identified as the source of the complications^[20].

Despite the opportunistic use of blue cohosh, Romm and Upton^[16] have clearly summarized that the medicinal capacity of blue cohosh as a homeopathic remedy is still questionable. Other problematic neonatal occurrences such as multi-organ hypoxic injury^[21] myocardial infarction and ischemic stroke^[22] and dangerous fetal cardiac arrhythmias^[23-25] have been reported.

The goal of the investigation herein was to determine what effect blue cohosh, as prepared as an aqueous extract from the pulverized roots and rhizomes, would have directly on heart rate when using isolated hearts. Preliminary results were first recorded from frog amphibian hearts *in situ* as this endothermic model is very robust. Then further data was collected from a more challenging set-up using isolated rat hearts, representing an endothermic mammalian model. We chose to collect baseline cardiac contractile responses to blue cohosh using the same concentration as what has been shown to successfully contract isolated mouse uterine tissues^[17].

2. Materials and Methods

2.1 Blue cohosh roots and rhizomes extract preparation

Blue cohosh was purchased from Viable Herbal Solutions, Inc. (Bensalem, Pennsylvania, USA). Each 600 mg capsule contained 100% powdered roots and rhizomes of blue cohosh with no filler. The extract was prepared by dissolving the capsule contents into an appropriate amount of deionized water to create the desired concentration of extract per 15 mL organ bath. Once thoroughly mixed into solution (~ ten min), the extract was vacuum filtered through Whatman filter papers (#4, 1, 5) using a *Büchner funnel* under vacuum to remove any non-dissolved plant particles. The resulting filtrate was stored on ice in a closed container for the duration of each experiment, and warmed to the temperature of the tissue bath prior to application. The extract was made fresh for each experiment.

2.2 Specimens

Six adult female frogs *Rana pipiens* (large grass frogs, 6.4-7.6 cm) were purchased from Wards Biological Supply (Rochester, New York, USA). They were housed in chlorine-free fresh water (2.5 cm) and refrigerated at 3 °C. On the day of the experiment, they were allowed to equilibrate to room temperature (20 °C) for one hour.

Six, fully mature female Hsd: Sprague Dawley rats (*Rattus norvegicus*) weighing 200-250 g, were obtained from Wards Biological Supply. All specimens had *ad libitum* access to water and standard chow. All procedures were completed in accordance with the Institutional Animal Care and Use Committee of Bethel University.

2.3.1 Preparing the frog heart specimens *in situ* and testing the tissues

A frog was doubly pithed and placed in a dissection tray ventral side up. A midsagittal incision was made through the skin and muscle tissue of the torso. The sternum was cut in order to open up the chest cavity and pericardium. Chilled Ringer's solution (g/L: 6.5g NaCl, 0.14g KCl, 0.12g CaCl₂, 0.20g NaHCO₃) was used to wash out the chest cavity. A small fishhook was inserted through the tip of the ventricle of the heart. One end of a string was tied to the fishhook, and the other end was tied to a FT-102 Force Transducer (iWorx, Dover, New Hampshire, USA) plugged into an ETH 200 Series Transducer Amplifier (iWorx) plugged into the PowerLab 4/25T box (ADInstruments, Colorado Springs, Colorado, USA) connected to a computer. The string was tightened so that the heart was lifted up and inverted. The tension was set to not place too much stress on the heart, but yet give enough tension so that heart rate could be clearly visible and monitored on the computer. The amphibian pulmonary and systemic blood vessels remained intact. Once a steady heart rate was achieved, a 5 min baseline contractile rhythm was established before adding any blue cohosh (treatment) or Krebs buffer (control). Then either the treatment (n=3) or the control was applied (n=3). The treatment group received a 30 mg concentration contained in 2.5 ml deionized water that was slowly pipetted down the underside of the heart in order to bring the drug into contact with the sinus venosus. The control frogs received an equal volume of the Krebs buffer applied in a similar manner. The heart rate was then recorded for an additional ten minutes.

2.3.2 Preparing the rat cardiac specimens *in vivo* and testing the tissues

A small 15 mL organ bath was used as a controlled system for the isolated rat heart in order to mimic the biological environment of the organism. The bath was kept at 32 °C in an effort to preserve tissue viability. A Krebs solution (g/2L) was composed of 13.8 NaCl, 4.2 NaHCO₃, 4.0 D-Glucose, 0.32 KH₂PO₄, 0.72 KCl, 0.58 MgSO₄•7H₂O, 0.74 CaCl₂•2H₂O and was constantly bubbled with 95% O₂ and 5% CO₂ aeration^[26].

On the day of an experiment, a rat was sacrificed by cervical dislocation. It was then pinned to a dissection tray ventral side up and a longitudinal incision was made through the thoracic cavity in order to expose the heart. After the heart was extracted from the body, it was placed in an iced Krebs solution until transferred to the organ bath. Non-cardiac tissue was trimmed off and the heart was mechanically massaged while still in the iced Krebs solution in order to empty as much blood as possible from the cardiac chambers. Two small fishhooks attached to fishing line were pierced through the cardiac tissue: one through the right atrium and the other through the apex. The hook associated with the cardiac apex was attached to a brass bar that served as an anchoring apparatus for the heart lowered into the 15 mL organ bath (Fig. 1). The hook associated with the right atrium was attached to an MLT500 force transducer that was linked to computer via ML301 Bridge Pod and PowerLab hardware. Data were collected via Chart 5.2 software on a computer. All instruments were obtained from AD Instruments (Colorado Springs, Colorado, USA).



Fig 1: A rat heart anchored to a force transducer in an organ bath. The top hook is attached through an atrium, and tied to a force transducer (not pictured). The bottom hook is attached to the ventricular apex and secured to a brass bar. The bubbles at the top of the organ bath are an indication of the saponin constituent found in blue cohosh. (Photo courtesy of Joshua Hulst)

After the heart was lowered into the organ bath, the tension of the fishing line was adjusted to ~1 gram so as to establish a clear, visible baseline heart rate on the computer. The heart was allowed to equilibrate in the bath for one hour with a tissue washout every 20 min. During equilibration, the ventricles usually stopped contracting. This was not a concern since the right atrium contains the cardiac pacemaker and the atrial baseline rhythm did produce a very clear measurement of heart rate. If a heart became unresponsive during the

equilibration period, it was mechanically stimulated by cardiac massage with sterile swabs.

After an hour of equilibration, a baseline contractile heart rate was established for ten minutes. Then either a single 30mg concentration of blue cohosh (treatment; n=3) or an equal volume of the Krebs buffer (control; n=3) was applied directly over the suspended tissue, targeting the atria, and observed for ten minutes. The 30 mg concentration was chosen because it was considered to be roughly equivalent to a triple dose in humans (by a mass ratio of rats to humans). This same concentration has also been shown to effectively evoke a contractile response from isolated mouse uterine tissues^[17].

2.4 Response measurements and analyses for the hearts

Both the rat and the frog heart rates were measured in 15-second intervals throughout the duration of an experiment. For each heart, the experimental rate was expressed as percent change from the five min baseline rate observed before administration of the treatment or control. Mean (\pm S.E.M.) heart rates were calculated at each time interval for both groups. Differences among the resulting heart rates were analyzed using ANOVA for multiple comparisons among the means. Following the rejection of the null hypothesis that blue cohosh would have no chronotropic effect on frog hearts *in situ* or rat hearts *in vitro*, the Tukey-Kramer post hoc test was used to indicate at which time intervals the differences were significantly different from each other. Data were considered to be statistically different at $P \leq 0.05$ (JMP 4.0, SAS Institute, Cary, NC).

3. Results

3.1 The effect of blue cohosh on frog hearts

A typical frog cardiac contractile waveform response following the administration of blue cohosh is shown in Fig. 2. The atrial and ventricular contractions of each cardiac cycle as seen on the waveforms were visually matched with the physical contractions of the heart. An initial spike in heart rate occurred in every frog following either the administration of blue cohosh or the control buffer. It represents a mechanical artifact of the treatment fluid being pipetted onto the heart.

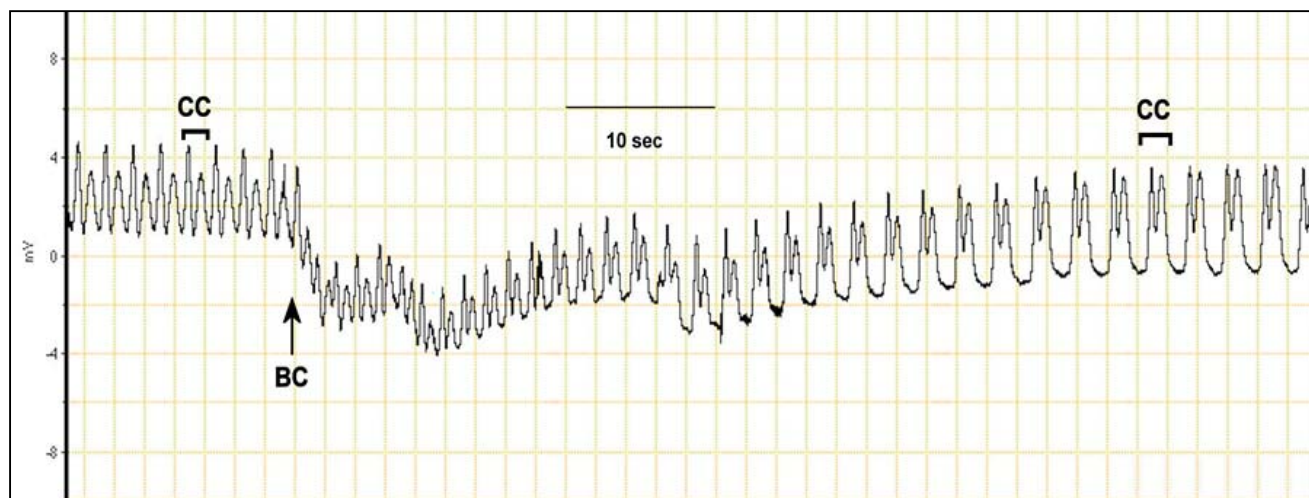


Fig 2: A typical frog cardiac contractile waveform response following the application of blue cohosh (BC). The baseline cardiac rhythmicity prior to BC treatment can be observed as 9 heart beats/15 sec, or 45 beats/min. A slight artifact-induced tachycardia can be observed within the first 20 sec following BC treatment, but after 60 sec the heart rate had been reduced to 5.5 beats/15 sec or 22 beats/min. Each heartbeat, or one cardiac cycle (CC), included both the ventricular and atrial contraction.

Fig. 3 illustrates the mean response of heart rate to blue cohosh in comparison to the control. The statistical analyses were only completed on data recorded after 3 min of time, when the frog hearts had returned to a more consistent baseline following the initial artifact induced bradycardia. After 10 min, the control hearts showed no significant change in heart rate ($P = 0.980$). The hearts receiving the blue cohosh

treatment also did not demonstrate a significant decrease in heart rate over time ($P = 0.439$). However, when comparing the heart rates evoked from blue cohosh to those evoked from the controls, blue cohosh did produce a negative chronotropic effect on the frogs when compared to the controls ($P < 0.0001$).

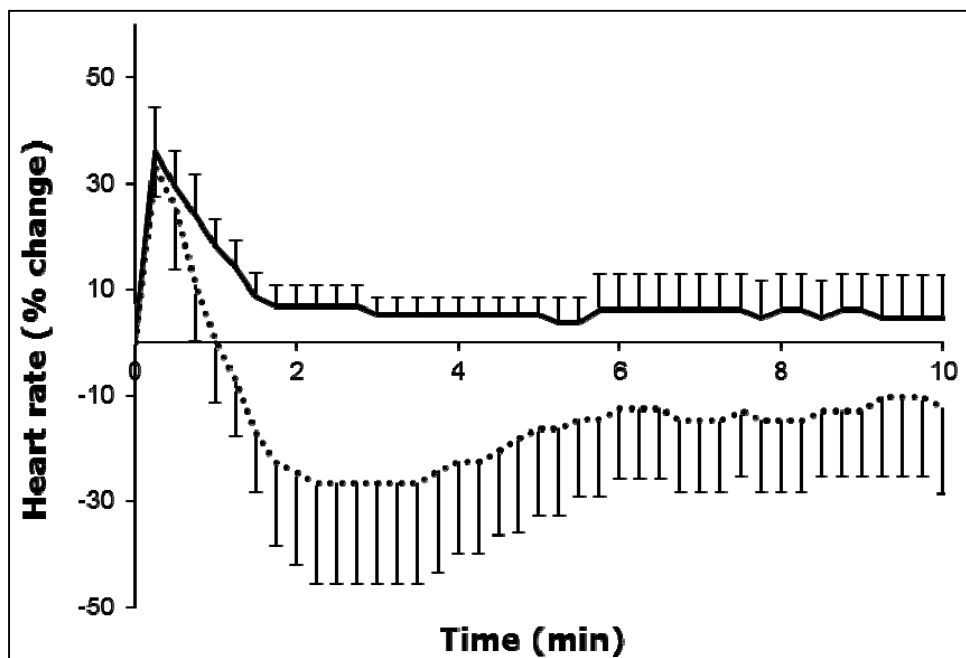


Fig 3: Means \pm S.E.M percent change in frog heart rates following the application of the control solution (solids lines, $n=3$) and 30 mg of the blue cohosh extract (dotted lines, $n=3$). The initial spike is not a response to a treatment but is a mechanical artifact that occurred on all the frog hearts when the fluids were pipetted on the heart. After 3 min, the frog hearts receiving the blue cohosh treatment did demonstrate a significant reduction in heart rate when compared to the control treatments ($P < 0.0001$).

3.2 The effect of blue cohosh on isolated rat hearts

The average baseline heart rate for the control rats was 31.8 ± 5.4 beats/15 sec, or about 127 beats/min. The average baseline heart rate for the blue cohosh rats was slightly higher at 39.7 ± 4.9 beats/15 sec, or about 158 beats/min. Rat hearts receiving the blue cohosh treatment did demonstrate a significant

reduction in heart rate ($P < 0.0001$) as compared to the controls, at the onset of 3.5 min. After ten min of exposure, the blue cohosh heart rate had decreased 52.5% (± 13.5) and the control hearts actually demonstrated a slight increase of 9.6% (± 2.2) in heart rate (Fig. 4).

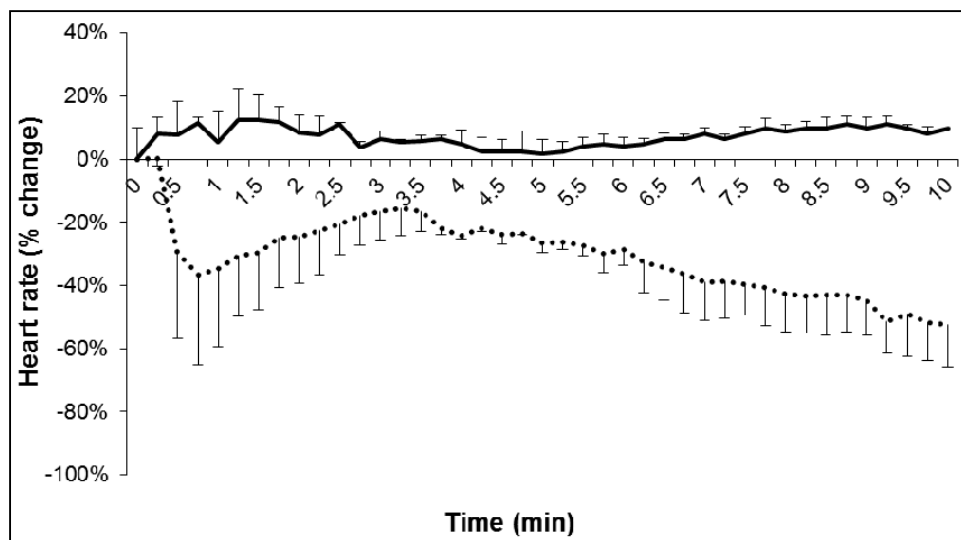


Fig 4: Means \pm S.E.M percent change in the rat heart rates following the application of the control solution (solids lines, $n=3$) and 30 mg of the blue cohosh extract (dotted lines, $n=3$). At the onset of 3.5 min, the rat hearts receiving the blue cohosh treatment did demonstrate a significant reduction in heart rate ($P < 0.0001$) as compared to the controls.

Two additional rat hearts were also treated with blue cohosh, but their baseline heart rates were only ~7 beats/15 seconds, or 28 beats/min. Since this was remarkably slower than the other three treatment hearts, and since both of them completely stopped beating after 7.5 min, they were left out of the data analysis even though their response to blue cohosh is indicative of bradycardia.

4. Discussion

4.1 Summary and relevance.

The purpose of this study was to collect preliminary data to determine if blue cohosh would have a positive or negative chronotropic effect on cardiac tissues. Either blue cohosh or a control treatment was given to frog hearts *in situ* and rat hearts *in vitro*. The consequent changes in heart rates were compared over ten minutes of time, and in both models, the administration of blue cohosh resulted in a negative chronotropic effect. These results were observed using the same concentration shown to effectively contract isolated uterine tissues from mice^[17].

These outcomes further stimulate the need to understand how blue cohosh can be used safely and holistically, without side effects in pregnant women. During such a physically demanding act as childbirth, it is important for the heart to deliver enough blood to the body to carry essentials such as oxygen and nutrients for energy. A decreased heart rate inhibits the heart's ability to do so and in turn places the wellbeing of the mother and child at risk.

4.2 Proposed activation pathways

The alkaloid and saponin compounds found in blue cohosh are considered to be the active physiological agents^[3, 27]. The alkaloid compounds consist of *N*-methylcytisine, baptifoline, anagyrine, and magnoflorine^[28-30]. The current literature proposes that one possible mechanism of the stimulatory action on uterine smooth muscle is due to the activation of nicotinic receptors by *N*-methylcytisine, which is pharmacologically similar to nicotine^[31]. *N*-methylcytisine has been shown to have an affinity to nicotinic receptors, and to a lesser extent, muscarinic receptors^[32, 33].

It is likely that *N*-methylcytisine and perhaps other of the biological constituents of blue cohosh, interact with cholinergic receptors at both the uterine and the cardiac tissue level. Plasma membrane cholinergic receptors are considered to be one of the triggers activating prostaglandin synthetase pathways and ultimately resulting in the contraction of smooth muscle in the uterine myometrium^[34]. Agonist binding to cholinergic receptors on cardiac atrial pacemaker tissues result in the activation of G protein-coupled receptors and second messenger pathways that would ultimately result in the hyperpolarization of autorhythmic cells^[35], and the consequent reduction in heart rate.

If the negative chronotropic effect produced by blue cohosh could be blocked, then perhaps the use of blue cohosh may be a safe alternative to other labor inducing methods, such as the use of oxytocin, prostaglandins, or intracervical Foley bulbs^[36]. This would be beneficial for individuals who may feel more comfortable with a homeopathic treatment or who may experience complications from more conventional methods.

4.3 Challenges in collecting data

The main difficulty of this protocol was keeping the mammalian cardiac tissue viable for experimentation. Although the hearts were removed and transferred to the

organ bath within minutes, there was often trouble keeping the tissue beating. The initial pilot study used hearts from mice, but tissue viability was greatly improved when the model was switched to using the larger heart of a rat.

When the hearts were removed from the rats, they were mechanically pumped in a rhythmic fashion in an attempt to push out as much blood as possible from the coronary vessels and cardiac chambers. However, it proved impossible to mechanically push all of the blood out, and any blood left in the ventricles coagulated once in the Krebs solution, possibly making it more difficult to observe and maintain ventricular contractility^[37].

To improve upon the protocol used for this experiment, a Langendorff apparatus would be better suited to sustain cardiac tissue viability. This technique would ensure continuous perfusion of all blood vessels and chambers of the heart with Krebs solution and would clear out any remaining blood more efficiently, allowing the heart to beat for hours *in vitro*. Additionally, a larger sample size for all treatments and the controls would be beneficial in affirming the trends noted.

In terms of continued investigation, it would be important to determine how the constituent compounds, namely *N*-methylcytisine, baptifoline, anagyrine, and magnoflorine individually contribute to the response. However, there are multiple components to every herbal supplement, and they may be working in a synergistic manner to elicit the chronotropic response.

4.4 Recommendation

The implications of this research demonstrate a possible adverse chronotropic effect if blue cohosh herbal supplement is used to assist in giving birth. *In vivo*, high doses of blue cohosh treatment may result in bradycardia if used to augment labor. McFarlin *et al.*^[15] concluded that blue cohosh should be used with extreme caution during pregnancy and should no longer be offered as an over-the-counter product but instead be used only under medical supervision.

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