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Authors: Berger, Jennifer, and DeGolier, Teresa

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Pharmacological effects of the aqueous extract of *Caulophyllum thalictroides* (blue cohosh) on isolated *Mus musculus* uteri

Jennifer Berger and Teresa DeGolier

*Department of Biological Sciences, Bethel University, St. Paul, Minnesota 55112*

**Abstract.** The roots and rhizomes of *Caulophyllum thalictroides* (blue cohosh), traditionally used as an aid for childbirth, contain several active alkaloids and saponins, which act directly on uterine smooth muscle resulting in an oxytocic response. The historical use of this herbal supplement has been well documented, but there are few clinical studies addressing its efficacy and potential side effects. This research investigated the physiological and pharmacological responses of blue cohosh on isolated strips of murine uterine tissue. Uterine horns from mice were suspended in a smooth muscle bath and exposed to the aqueous extract of blue cohosh (doses ranging from 0.037–23.8 mg). All tissues showed an increase in the strength of contractile force, the frequency of the contraction, and basal tonus. Contractile forces were significantly greater with higher doses (P = 0.0027). The stages of estrous were determined by vaginal smears and dose-dependency was consistent in all stages of estrous observed (diestrus, estrus, metestrus). Blocking experiments with d-tubocuranine, a nicotinic receptor antagonist, were inconclusive as decreases in contractile responses were not statistically different from the observed fatigue following control cumulative dosing.

**Introduction**

Labor is a natural process in which a fetus moves from the uterus through the birth canal and into the world. Towards the end of a woman’s pregnancy, collagen fibers in the cervix of the uterus break down, as relaxin, a hormone secreted by the placenta and corpus luteum, causes the cervix to soften. In the last trimester of pregnancy, a woman often experiences weak, irregular contractions known as Braxton-Hicks contractions. During the last few weeks of pregnancy, these contractions become stronger and more frequent, and within a few hours of labor the contractions become regular, occurring every 15–30 minutes (Spence and Mason, 1992). It is not known exactly how labor is initiated; however, it is believed that both oxytocin and prostaglandins play an important role in this process. Oxytocin is a peptide hormone that is synthesized in the neurohypophysis, and stimulates smooth muscle of the uterus to contract late in pregnancy. Prostaglandins are hormone-like substances synthesized by the placenta, and are also known to contract the uterus. One theory explaining the initiation of labor is that the concen-
tration of oxytocin receptors in the uterus and the placenta increases throughout the course of a woman’s pregnancy due to increasing levels of estrogen and uterine expansion. Although the concentration of oxytocin circulating in a woman’s blood does not significantly elevate at the onset of labor, the increased number of receptors increases sensitivity to oxytocin resulting in strong uterine contractions. Oxytocin also binds to placental receptors stimulating prostaglandin synthesis, which then diffuses into the uterus and works agonistically with oxytocin to produce even stronger uterine contractions beneficial to successful labor (Spence and Mason, 1992).

Most of the time, a woman’s body naturally goes into labor. However, a recent survey reported that in 9.5 to 33.7% of pregnancies, labor needed to be induced for the welfare of the mother or the baby (Tenore, 2003). Induction is the process of stimulating uterine contractions using pharmaceutical or surgical techniques, until spontaneous labor results. It is also common for doctors to prescribe estrogenic hormones, unsaturated fatty acids, vitamins, trace elements, and calcium preparations to help manage labor for women with a high risk of complications, such as uterine contractile dysfunction (Ventoskovskiy, 1990). In contrast, Abramchenko and Bogdachkin (1988) believe that estrogenic drugs have teratogenic action; furthermore, some women may experience side effects like nausea, diarrhea, or worsening of chronic cholecystitis from consuming drugs containing unsaturated fatty acids. Thus, patients that experience such complications with contemporary obstetric medicine often turn to homeopathic techniques as an option to help induce labor.

In the United States 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 (Steinberg and Beal, 2003). In a large group of people surveyed reporting the use of such alternative medicine, 49% were women in their reproductive age (Eisenberg et al., 1998), and interestingly, labor stimulation is one of the most cited uses for herbal treatments (Allaire, 2001). According to a survey of North Carolina midwives (Allaire et al., 2000), 93.9% recommended homeopathic remedies to their pregnant patients and the most often prescribed remedy was herbal therapy (73.2%). Furthermore, a national survey of certified nurse-midwives stated that 64% used the perennial herb blue cohosh (Caulophyllum thalictroides), to help induce labor in pregnant women (Tyler, 1993; McFarlin et al., 1999).

Blue cohosh is a perennial herb native to North America and grows across the continental United States in a humus-rich soil within deciduous forest with at least 75% shade (Adam, 2004). Blue cohosh has a smooth stem that grows one to three feet in height and terminates in yellowish-green flowers. When the plant is young it is purplish in color, but when it matures, it is bluish-green in color and bears dark blue berries from which the name “blue” cohosh is derived (Herbs2000.com, 2006). Blue cohosh is also known by several other common names such as beechdrops, blue ginseng, papoose root, squaw root, or yellow ginseng. The rhizomes and roots of the blue cohosh plant contain the active ingredients (Kennelly et al., 1999; Ganzaera et al., 2003), and are harvested in late fall and ground into a powder which can then be consumed (Herbs2000.com, 2006).

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, (Tyler, 1993). It was officially listed in American Pharmacopoeia from 1882 to 1905 as a labor inducer, and 19th century physicians regularly recorded its use as a uterine stimulant.

Blue cohosh was first chemically examined by Mayer in 1863 (as cited by Power and Salway, 1913) who claimed it contained a saponin element and a colorless alkaloid. The saponin element was eventually deduced to contain the substances caulosaponin, $C_{51}H_{88}O_{17}, 4H_2O$ and caulolphyllosaponin, $C_{66}H_{104}O_{17}$ (Power and Salway, 1913). In 1887, the alkaloid component was demonstrated by Lloyd (Power and Salway, 1913) to have a significant presence in the roots and rhizomes. Then, in 1913 Power and Salway isolated the alkaloid and identified it as $N$-methylcytisine, $C_{11}H_{13}ON_2(CH_3)$.

In 1916, Pilcher et al., tested blue cohosh and
several other herbs (e.g. colicroot *Aletris farinosa*, wind flower *Pulsatilla pratensis*, fragrant valarian *Valerian officinalis*) which claimed to affect the contractile activity of isolated guinea-pig uteri. They concluded that blue cohosh was the only drug that increased the rate and amplitude of uterine muscle contraction. This was further substantiated when Ferguson and Edwards (1954) injected anesthetized albino rats with varying amounts of the isolated crystalline glycoside and demonstrated increase in uterine tone, contraction rate, and duration of contraction. Today, *N*-methylcytisine is understood to be the agent responsible for the oxytocic effect, increasing the regularity and strength of uterine contractions by directly binding to nicotinic cholinergic receptors on the smooth muscle (Schmeller et al., 1994; Perri, 2002).

Despite blue cohosh’s long reported history of being used to stimulate uterine contractions in humans, there is really little empirical evidence supporting or disputing this claim. In 1987, a French clinical study (Smith, 2006) compared the effects of five herbal supplements, one being blue cohosh, to a placebo in 93 women who were 36 weeks into their pregnancy. No significant difference in uterine contractile activity was reported between women given a placebo and those given blue cohosh. Furthermore, in a later 1999 German clinical study, 40 women who were 38–42 weeks into their pregnancy were given either blue cohosh or a placebo. They showed no significant difference in the length of their labor, their oxytocin requirements, or the method of delivery (Beer and Heiliger, 1999). The quality of these clinical studies however, are under question because of lack of detail in the research reported and the small sample sizes used.

It seems then that the variability of effect of this herbal supplement on the whole organism as well as on isolated uterine tissue warrants further research. The primary aim of the *in vitro* experiment herein was to study a selected aspect of uterine tissue, namely muscle contractility, under conditions when the influence of external factors (i.e., central nervous system, circulating hormones) are removed, but the uterus itself performs in a manner analogous to its *in vivo* capacity (Percy, 1996). The specific project objectives using the aqueous extract of blue cohosh, *Caulophyllum thalictroides*, as it is available to the consumer, were to (1) observe and quantify the contractile action on the uterine tissue, (2) investigate whether the responses were dose-dependent, (3) determine whether the contractile effect is influenced by the frequent reproductive cycling of the mouse, and (4) attempt to further characterize the mechanism underlying the contractile responses.

### Material and Methods

#### Specimens

In preparation for uterus isolation, fifty virgin female mice *Mus musculus* ranging in age from 5 weeks to 4 months old (Harlan) were injected with 0.2 mg diethylstilbestrol (DES) (Sigma-Aldrich) intramuscularly 24 hours prior to the experiment. DES is a synthetic estrogenic drug that is relatively more potent than natural estrogen and will promote thickening of the uterine endometrial layer (Burger et al., 2001) allowing for the extraction of intact uterine horns from the pelvic cavity without undo stress. On the day of the experiment the mice were sacrificed by cervical dislocation. The horns of the uterus were dissected out, cleansed of excess fat and connective tissue, and cut into longitudinal strips. Ligatures were tied around both ends of the tissues with one end attached to the distal end of a stationary bar and the other end connected to an isometric force transducer (AD Instruments Inc.) coupled to an amplifier and computer data acquisition software (PowerLab, AD Instruments Inc.).

In similar descriptive studies, uterine horns as opposed to the isolated myometrium have been used to successfully demonstrate contractile responses to pharmacological agonists (Ferguson and Edwards, 1954; Eno and Itam, 1998; Mohamed et al., 1999; Amos, et al., 2002; Kantas et al., 2002). Since uterine tissue is very sensitive to stretch (Kitchen, 1984), the use of full thickness strips minimizes the amount of handling. Furthermore, it is unknown whether isolating the myometrium would eliminate access to the nicotinic receptor. Thus, the strips of uterine horn
were used as the target tissue for this investigation.

**Vaginal smears**
A vaginal smear was taken from all the mice before the uterine horns were dissected out. Using methods described by Allen (1922), a small amount of saline was pipetted directly into the vagina, withdrawn, and then placed on a slide. The slide was allowed to dry before it was stained with 8.3% light green and 0.17% eosin y for 2 minutes, then rinsed with tap water, and allowed to air dry. The distribution of leukocytes, cornified epithelial cells, and nucleated epithelial cells observed on the slide was used to determine the mouse’s stage of estrous (Thung et al., 1956).

**Smooth muscle bath**
The isolated uterine tissues were suspended longitudinally in 20 ml organ baths filled with De Jalons solution (g/5 L): 2.1g KCl, 45g NaCl, 2.5g NaHCO₃, 2.5g D-glucose, and 0.4g CaCl₂. The bath was maintained at 30°C and a mixture of 95% O₂/5% CO₂ gas was bubbled throughout the duration of the experiment. The use of a bath solution with a low CaCl₂ dose, such as De Jalons (0.5mM), coupled with the maintenance of the bath temperature at 30°C helped reduce the amount of spontaneous motility and allowed more accurate measurement of the contractile changes due to drug application (Kitchen, 1984). Regular spontaneous motility was induced by suspending the tissue at 0.8g of tension. Tissue viability was verified by spontaneous motility and an initial contractile response to 20µL 10⁻⁵M acetylcholine (ACh); any tissues not demonstrating these responses were not included in the data set. To test for contractile responses to blue cohosh, the tissues were flushed with fresh De Jalons solution and allowed to equilibrate for 10 min. Blue cohosh extract (single doses ranging from 0.037 to 23.8mg/20 mL buffer solution) was then applied directly to the top of the suspended uterine tissue and left on until a maximal response was observed.

**Blue cohosh extract**
The aqueous extract of blue cohosh was prepared by breaking open blue cohosh capsules, each containing 595 mg of rhizome and root components with no filler (Viable Herbal Solutions, Inc.) and dissolving them in distilled water. The solution was then filtered through increasing filter gradients (size 4, 1, 5) to remove the undissolved plant particles. As purchased, it is not known how much N-methylcytisine is contained in the 595 mg of rhizome and root. Since all capsules used in this experiment were from the same lot (June 6, 2006) the investigation proceeded with the assumption that all capsules were uniform in composition. The goal of this project was not to uniquely test for N-methylcytisine effect, but to simply use the herbal supplement as it is available to the consumer.

**Prostaglandin removal**
Since prostaglandins are known to contract the uterus and are also believed to be present in plants to influence flowering (Salisbury and Ross, 1992), pilot studies were run with the prostaglandins separated out from the aqueous blue cohosh extract. This was done by placing the blue cohosh extract into a separatory funnel with either petroleum ether or methylene chloride. The solutions were thoroughly mixed so as to bind the non-polar prostaglandins and separate them out of the blue cohosh solution. The extract in which the prostaglandins had been removed was then applied directly to the uterine tissue.

**Blocking experiments**
To further investigate and characterize the proposed mechanism for the contractile effects of blue cohosh, the receptor selective antagonists hexamethonium (alpha-Bungarotoxin insensitive) and d-tubocurarine (alpha-Bungarotoxin sensitive) were used to block nicotinic cholinergic receptors (Watling, 2006).
For all blocking experiments, each uterine horn of each uteri received 4.0 mg blue cohosh applied directly to provide a controlled contractile response. The tissues were then flushed with fresh DeJalons solution and allowed to equilibrate for ten minutes. Then one of the two uterine horns was incubated for two minutes with either 10⁻⁵M hexamethonium or 10⁻⁵M d-tubocura-
nine, followed by the same initial dose of blue cohosh. The response of the uterine tissue to blue cohosh before and after antagonist application could then be directly compared.

The other uterine horn received an equal volume and incubation of DeJalons solution instead of the receptor antagonist between the first and second application of blue cohosh. This was done to help determine to what extent possible contractile fatigue may have had on tissue responsiveness to blue cohosh, as opposed to receptor antagonism.

**Statistical analyses**

Changes in frequency, contractile force, and overall muscle tone were observed. Contractile responses (force) were determined from the baseline to the top of the blue cohosh-induced response. Since tissues exhibited spontaneous motility, the midpoints of these motility patterns were considered as the baseline for all measurements (Kitchen, 1984). Contractile responses were measured and means ± S.E.M were calculated for each dose. Differences in responses among varying doses were statistically analyzed using ANOVA for multiple comparisons among means. Following the rejection of the null hypothesis that there would be no statistical difference in the strength of uterine contractions between the experimental group and the control group, the Tukey-Kramer Honestly Significant Difference test (JMP, 2001) was used to determine which of the dose responses was significantly different from each other. The significance of receptor antagonism was also compared to possible fatigue due to cumulative dosing of blue cohosh using a student’s t-test. Differences were considered significant at $P \leq 0.05$.

**Results**

A typical contractile response of uterine tissue before and after an application of blue cohosh is illustrated in Figure 1. All tissues responded immediately to an application of blue cohosh and demonstrated an observable increase in the initial force of contraction, frequency of contractions, and basal tonus. The force of contraction

![Figure 1](https://bioone.org/journals/BIOS/2008/79/3/107/article-fig.png)

*Figure 1.* Typical uterine tissue response to an application of blue cohosh. This illustration represents 36 minutes of recorded time (1 square = 100 sec). The dotted line represents when blue cohosh (5.95 mg/20 mL buffer solution) was applied to the tissue. The default axis is in mV, and when calibrated $158 \text{ mV} = 1 \text{ gram of tension}$. In this sample, spontaneous motility prior to the application of blue cohosh had a maximal contractile force of 1.45 grams of tension. The maximal contractile response evoked by blue cohosh was 3.53 grams of tension. For this study, the midpoint of the spontaneous motility patterns (e.g. 1.45 grams of tension/2) was used as the baseline for measuring agonist evoked contractile responses. Thus, in the example provided, the contractile response from blue cohosh is 2.805 grams of tension.
was the most consistent and repeatable measured response observed, and thus is the focus of quantified measurements presented herein.

Results from pilot studies showed that when prostaglandins were removed from the blue cohosh aqueous extract prior to application to the uterine tissues, typical and consistent contractile responses were still observed. This then confirmed the presence of other active ingredients in the blue cohosh extract, presumably N-methylcytisine.

Individual contractile force responses are shown in Figure 2 for all uterine tissues exposed to blue cohosh, doses ranging from 0.037 to 23.8 mg/20mL buffer solution. Each data point represents a single uterine horn responding to the first dose applied that elucidated a response. As the doses increased from 0.037 to 5.0 mg, there was a general increase in contractile strength from 0.0903 to 3.8271 grams of tension. Doses greater than 5.0 mg appear to plateau at about 2.5 grams of tension.

Figure 3 presents the mean ± S.E.M. uterine contractile response for each blue cohosh dose ranging from 0.35 to 23.8 mg/20mL buffer solution, in which at least three to six uterine tissues were given each dose. Blue cohosh demonstrated a somewhat high-low dose-dependency (P = 0.0027). Contractile responses to 5.0 mg blue cohosh were significantly greater than those given 1.49 mg. Additionally, contractile responses at 3, 5, 11.9 and 23.8 mg blue cohosh were also significantly greater than those of 0.35 mg.

Figure 4 shows mice uterine contractile responses to increasing doses of blue cohosh as sorted by tissues whose stages of estrous could be identified (n = 47). Again, as the blue cohosh dose increased, the tissues as a group increased significantly in contractility (P = 0.0023). However, dose patterns were no longer statistically significant when responses were sorted by their stages of estrous: diestrus P = 0.1323, n = 13; estrus P = 0.3004, n = 13; metestrus P = 0.0674, n = 21. No mice in proestrus were observed. Trend lines for diestrus and estrus indicated that the increasing contractile response to blue cohosh doses, from 0.14875 mg and up to 5.95 mg, were linear. In the samples identified, only the metestrus stage was subjected to doses greater than 5.95 mg, clearly indicating a logarithmic dose response to blue cohosh.

![Figure 2](https://bioone.org/journals/BIOS)  
**Figure 2.** Individual uterine horn contractions (n = 56) in response to a single application of blue cohosh, doses ranging from 0.037 to 23.8 mg/20mL buffer solution. Tensions generated ranged from 0.0903 to 3.8271 grams of tension. Doses greater than 5.0 mg appear to plateau at about 2.5 grams of tension.

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Figure 3. Mean ± S.E.M. contractile responses of uterine tissue exposed to increasing doses of blue cohosh ranging from 0.35 to 23.8 mg/20mL buffer solution (n = 40). Samples plotted represent a minimum of three tissues at any one given dose, each tissue receiving only a single application of one dose of blue cohosh. A high-low statistical dose-difference (P = 0.0027) existed between contractile responses to 5.0 mg blue cohosh (a1) and 1.49 mg (a), and in between 3.0, 5.0, 11.9, 23.8 mg (b1) and 0.35 mg (b).

Figure 4. Uterine contractile force in response to increasing doses of blue cohosh as sorted by stages of estrous. For all tissues in estrous, uterine contractile force significantly increased with dose (P = 0.0023; n = 47). However, dose patterns were no longer significant when responses are sorted by stages of estrous (dierstrus n = 13, estrus n = 13; metestrus n = 21). No mice in proestrus were observed. Each data point represents a single piece of tissue given one dose that elicited a response. Trend lines for each of the stages of estrous are included.
In Figures 2, 3, and 4, uterine contractile responses to blue cohosh were reported based upon their initial application only. It was generally observed throughout the entire investigation that subsequent applications of blue cohosh on the same tissue demonstrated fatigue. Thus, any interpretation of receptor antagonism needed to be understood in light of tissue fatigue. Attempts to block the contractile response of blue cohosh by antagonizing the nicotinic cholinergic receptors with $10^{-5}$M hexamethonium were ineffective ($n = 4; P = 0.6805$). D-tubocurarine ($10^{-5}$M) may have reduced some of the induced blue cohosh contraction, but the responses were not different enough when compared to the reduction in contractile force of the fatigue controls ($n = 4; P = 0.2385$). Thus, the effect of receptor blocking was not conclusive.

**Tissue responses to oxytocin**

Because blue cohosh yielded a considerable contractile response on isolated strips of uterine smooth muscle, experiments were also completed using a similar protocol so as to generate an oxytocin dose-response curve and make quantitative comparisons regarding the response to blue cohosh.

Contractile actions of oxytocin ($10^{-11}$M to $10^{-5}$M) on isolated longitudinal strips of uterine smooth muscle are shown in figure 5. Doses at $10^{-5}$M and $10^{-6}$M yielded contractile responses that were significantly greater than those evoked from $10^{-9}$M $-10^{-11}$M and $10^{-8}$M $-10^{-11}$M, respectively ($P = 0.0004$). When comparing the oxytocin dose-response curve (Figure 5) to the blue cohosh dose-response curve (Figure 3), approximations can be made for equivalent responses. For example, oxytocin $10^{-6}$M (or 1 microgram) yielded the greatest mean contractile response at 1.7 grams of tension. This same amount of tension was produced by 1.6 mg of blue cohosh.

When considering dose approximations for an adult human, the pharmacokinetics of blue cohosh (i.e. how the body will handle the drug) must be considered. The assimilation efficiency of blue cohosh is poorly understood. The powdered root and rhizome recommended intake for blue cohosh to produce a therapeutic effect is $0.3-1.0$ g per day, taken three times a day (Herbs

![Figure 5. Oxytocin dose-response curve demonstrated on isolated longitudinal strips of uterine smooth muscle. Each data point represents the mean contractile amplitude ± S.E.M. in response to oxytocin $10^{-11}$M to $10^{-5}$M ($n = 4$). Doses at $10^{-5}$M and $10^{-6}$M yielded contractile responses that were significantly greater than those evoked from $10^{-9}$M $-10^{-11}$M and $10^{-8}$M $-10^{-11}$M, respectively.](https://bioone.org/journals/BIOS)
2000.com). Adult dosage for inducing labor in humans with oxytocin is 1 milliunit/min IV (Adams et al. 2008) or about 2 micrograms of pure peptide (Cort et al., 1980).

**Discussion**

The data reported herein show that the roots and rhizomes of blue cohosh produce an increase in contractile force in isolated longitudinal strips of murine uterine smooth muscle. These results are consistent with earlier investigations demonstrating that blue cohosh has a definite oxytocic action in uterine tissue (Pilcher et al., 1916; Ferguson and Edwards, 1954). In the present study, tissue contractile responses to lower doses of blue cohosh were statistically less than those to larger, indicating that blue cohosh has some dose-dependency. This relationship is likely due to nicotinic cholinergic receptor binding to \( N \)-methylcytisine (Schemmler et al., 1994) as found in *Caulophyllum thalactroides* (Betz et al., 1998; Ganzera et al., 2003).

Nicotinic cholinergic receptors are a family of ligand-gated channels, permeable to \( \text{Na}^+ \) and \( \text{K}^+ \). They are characterized on the basis of their activation by the plant alkaloid nicotine; acetylcholine (ACh) is the endogenous ligand. There are several nicotinic cholinergic receptor subunits expressed in neuromuscular junctions and as neuronal nicotinic receptors in both the central and autonomic nervous system (Watling, 2006). In autonomic transmission, preganglionic neurons release ACh which rapidly depolarizes nicotinic receptors on postganglionic neurons, creating an excitatory postsynaptic potential in muscarinic receptors on the target tissues.

Papka et al., (1999) using a rat rodent model demonstrated that cholinergic neurons do project some of their axons into the myometrium of the rat. This would be consistent with results reported herein demonstrating that nicotinic receptors may actually be in the mouse myometrium. In some of our initial investigations (\( n = 4 \)), care was taken to deliberately leave 2–3 mm of connective tissue containing fat, blood vessels, and peripheral nerves on the uterine horns, assuming that the postganglionic nicotinic receptor would be located outside of the uterine horns and would need to be intact for the preparation to work. However, it was observed that when as much of this connective tissue was removed macroscopically as possible from the preparation, blue cohosh still exhibited as effective a contractile response. This supports that the nicotinic receptor at the very least must be on the surface of the mouse uterine horn.

Throughout the mouse estrous cycle, estrogen’s primary effects are to increase the vascularity and permeability of the endometrium, and to increase water retention, electrolytes, mitotic activity, and the dry weight of the uterine tissue (Roberts and Szego, 1953). When uterine muscle responses to blue cohosh were interpreted based on the stage of the estrous cycle that the experimental mouse was in, there were no significant differences in contractile responses with respect to the stage of estrous. In an earlier study, Pilcher et al., (1916) also concluded that similar contractile results were seen between various stages of pregnancy and the estrous cycle when using isolated guinea pig uteri. These results would seem to infer that the number of neuronal nicotinic receptors available for blue cohosh binding is not influenced by fluctuating levels of estrogen (or estrogen-like) compounds.

Adham and Schenk (1969), however, using histochemical techniques, observed that the density of cholinergic innervation in the rat uterus is the most prominent during estrus and the least so during diestrus. Since the entire estrous cycle of a mouse can take place in four to five days (Allen, 1922), a possible reason for the different conclusions between Pilcher et al., (1916); Adham and Schenk, (1969), and the results herein may relate to the intraspecific variation occurring within such a continuous cycle as estrous. For example, the administration of DES 24 hours prior to experimentation served as an attempt to standardize the mice, moving them towards estrus. Despite this, vaginal smears indicated that the sacrificed mice were either in metestrus, diestrus, or estrus. One may speculate that additional sampling of mice in estrus and diestrus as well as in the intermediate phases of proestrus and metestrus would further elucidate whether DES and/or fluctuating estrogen levels actually
increase the agonist activity of blue cohosh due to an increase in the number of ganglionic nicotinic receptors.

Additional data not reported herein was collected from mice not receiving DES. Uterine tissues from these mice were substantially smaller and were much less responsive to both acetylcholine and blue cohosh. Burghardt et al. (1984) showed that DES works directly on the uterine tissue causing uterine fluid to accumulate, leading to a stretch of the uterine walls, and interestingly, an increase in the number of gap junctions between myometrial and serosal cells. In hypophysectomized female rats that were injected with a single dose of 500µL DES, muscular gap junctions could be detected which had not previously been observed twenty-four hours earlier. In a later study, Burghardt et al. (1987) further found that estrogen stimulates the gap junctions to not only increase in quantity but also in size. The resulting increase in myometrial gap junction activity could then enhance the response of a single fiber depolarization to spread rapidly to an increased number of neighboring muscle fibers, allowing for a strong, coordinated contraction of the uterus, which would be very beneficial in parturition. This single-unit smooth muscle response is a modification of the normal, multi-unit smooth muscle behavior of the uterus that occurs towards the end of pregnancy (Silverthorn, 2004). These explorations may support why the non-DES injected mice had substantially smaller uterine horns, and their contractile activity was much less responsive to both the acetylcholine and blue cohosh.

Blue cohosh is available to the consumer as a dietary supplement and is not approved by the FDA as a drug. Since it has such a strong effect on uterine tissue in vitro, additional in vivo input regarding its safety or toxicity on excitable systemic tissues that have nicotinic receptors would be of practical interest. Scott and Chen (1943) compared N-methylectysine’s effect to nicotine’s effect on various smooth muscle tissues and found that N-methylectysine stimulated, then paralyzed the ganglion cells of the cardiac vagus in dogs, and stimulated motility in isolated small intestine in rabbits. They concluded that the N-methylectysine’s effects were similar in pharmacological actions to nicotine, but less potent.

Studies by Ferguson and Edwards (1954) found that blue cohosh caused spasmodic contractions and decreased intestinal peristalsis in isolated intestine in mammalian models, decreased and then elevated blood pressure in intact blood vessels of rats, and created tachycardia in rats and cardiac irregularities in both turtles and frogs that in some cases even led to systolic arrest in situ. Further more, Jones and Lawson (1998) reported that a newborn, whose mother ingested three times the recommended dose of blue cohosh taken as an aid for labor induction, suffered acute myocardial infarction associated with congestive heart failure.

In a recent in situ study, significant cardiac arrhythmias (P = 0.0239) were observed in amphibian hearts following an application of blue cohosh. A low dose of blue cohosh (0.5 mg) resulted in a 15% drop in heart rate within 1.5 minutes, and higher doses of blue cohosh (2.0 mg, 5.0 mg) exhibited immediate tachycardia followed by bradycardia (30% drop in heart rate). After 3 minutes, all cardiac rhythms had returned to 85% of their original pace (Stanley, 2007). Perhaps, this more prompt rebound to baseline rhythms may indicate that the in situ frog heart is a more appropriate model for elucidating receptor mechanisms.

Other herbal supplements also claim to stimulate uterine contractions. For example black cohosh Cimicifuga racemosa, red raspberry leaf Rubus idaeus, castor oil Ricinus communis, and evening primrose Oenothera biennis are often consumed by women to help induce uterine contractions and stimulate labor (Johns and Sibeko, 2003). Similar to blue cohosh, little to no substantial research has been done on the effectiveness of these herbs on isolated uterine tissue.

In conclusion, this study researched the effects of blue cohosh as it is supplied over the counter, on the contractile responses in isolated mouse uteri. The major findings of this study: 1) demonstrated that blue cohosh produces a significant
increase in contractile force; 2) quantified the force of the contractions and showed them to be generally dose-dependent; 3) showed that blue cohosh responses may not be dependent on the stage of the estrous cycle as all stages sampled in this project responded similarly when similar doses were applied; 4) showed that blue cohosh elicits such a strong response in uterine tissue that fatigue eliminates the possibility of cumulative dosing experiments; and 5) supports that blue cohosh works through the receptors, presumably, within or on the uterine tissue.

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