Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 1252-1258
Received: 19-07-2018
Accepted: 21-08-2018

Brian Bristol
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, USA

Teresa Degolier
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, USA

Correspondence
Teresa Degolier
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, USA

Quillaja saponins are a potent contractor of uterine smooth muscle tissue *in vitro*

Brian Bristol and Teresa Degolier

Abstract
Caulophyllum thalictroides, commonly known as blue cohosh, is a plant indigenous to the northeastern region of North America. The roots and rhizomes have been used in herbal medicine for centuries as they are thought to stimulate uterine contractions during labor; they have been shown to contract mouse uterine tissues *in vitro*. The active constituents thought to be responsible for these actions are the alkaloids and saponins. The primary purpose of this research was to expose isolated mouse uterine tissues to *Quillaja saponaria*, which contains saponins similar to those isolated from blue cohosh, and observe any contractile responses. Tissues collected from mice were suspended individually in organ baths and saponin solutions (0.1-20%) were added. Contractile responses were analyzed as a percent of the tissues contractile response to 10^{-5} M oxytocin. Saponins were found to increase force (P<0.0001) as well as frequency (P<0.0001) of contractions when compared to 0% treatment (the tissues endogenous spontaneous motility). The saponin responses, however were not statistically different from each other. Saponins are considered to be permeating substances that create pores in the plasma membrane, allowing for the influx of extracellular Ca^{2+} ions and initiating the smooth muscle contractile cascade. Since *Quillaja* saponins do contract uterine smooth muscle, it is likely that the saponins found in the roots and rhizomes of blue cohosh would also contribute to the overall contractile response as seen from extracts of the parent plant.

Keywords: Saponins, uterine smooth muscle, contractions, blue cohosh

1. Introduction

1.1 Blue cohosh as an oxytocic agent

Caulophyllum thalictroides (L.) Michx, family Berberidaceae, also known as blue cohosh, is a plant native to northeastern North America and is commercially available as a dietary supplement [1].The roots and rhizomes of blue cohosh have been used in traditional Native American practices for the relief of numerous conditions including pain from childbirth, colic, cramps, hysteria, epilepsy, inflammation of the uterus, and rheumatism [2, 3, 4].

Numerous Native Americans have used blue cohosh as a gynecological aid [5], it was considered to induce labor as its consumption was correlated with increased uterine contractions during delivery. John King [6], one of the first physicians to document the use of an herbal preparation of blue cohosh, administered it during the birthing process so as to provide tone and give activity to a uterus with abnormal function. He considered blue cohosh to be a gentler and safer alternative to ergot, a fungus of the genus *Claviceps* [7], which at that time was the primary herbal treatment for promoting labor. Since then, several practitioners have used and promoted the use of blue cohosh as a labor-inducing agent in obstetrics [8]. According to a national survey of certified nurse-midwives in [9], 64% of midwives were still using blue cohosh to assist in inducing labor.

Aqueous extracts of the roots and rhizomes of blue cohosh tested on isolated uterine tissues from mice were observed to produce significant increases in contractile forces [10]. There is, however, little documentation on which constituents of blue cohosh (the saponins or the alkaloids) may cause these oxytocic effects.

1.2 Saponin active constituents

Saponins are glycosides that consist of a sugar moiety glycosidically linked to a hydrophobic aglycone (sapogenin), which may be a triterpenoid or steroid in nature [11]. They are common in a large number of plants and plant products that are important in human and animal nutrition. Several biological effects have been ascribed to saponins, including anti-inflammatory [12], immunostimulant [13], hypocholesterolaemic [14], and membrane-permeabilising [15].

Several saponins have been found in preparations of blue cohosh [1, 16, 17]. A crystalline glycoside isolated by Ferguson and Edwards [18] was demonstrated to have definite oxytocic action on isolated rat, rabbit and guinea pig uterus by acting as a smooth muscle stimulant.

1.3 Alkaloid active constituents

The isolated alkaloids include *N*-methylcytisine, baptifoline, anagyrine, and magnoflorine [19, 20, 21]. Power and Salway [19] were the first to publish the discovery of *N*-methylcytisine in blue cohosh. It is abundant in the roots and rhizomes with its highest content reported to be 4.58 ± 0.9 mg/g dry weight [1]. The structure of *N*-methylcytisine resembles the structure of nicotine and stimulates responses similar to nicotine, but to a weaker effect [22, 23, 24]. Using receptor-rich membranes from porcine brains, *N*-methylcytisine, when compared to anagyrine (when both isolated from *Anagyris foetida)*, was found to have the highest functional binding strength to nicotinic acetylcholine receptors. Weaker affinities were observed at the muscarinic acetylcholine receptors [24].

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 [25, 26, 27]. 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 [28].

When these alkaloids were tested for teratogenic activities in rat embryo cultures, Kennelly *et al*. [29] found *N*-methylcytisine to be the most active, resulting in varying levels open anterior neural tube defects, poor or absent eye development, and twisted tail.

Magnoflorine is an aporphine alkaloid [20] in contrast to the other three mentioned alkaloid constituents which are quinolizidine [19]. It makes up approximately 65% of the total alkaloids in the roots and rhizomes of blue cohosh [30]. Magnoflorine isolated from the seeds of *Arisolochai bracteata*, was shown to induce uterine contractions in isolated pregnant rat uterus [31].

1.4 Project objectives

The purpose of this research was to expose isolated mouse uterine tissues to commercially available saponins, and to the alkaloids *N*-methylcytisine, and magnoflorine. Anagyrine and baptifoline were not available at the time of this investigation. Our specific objectives were to 1) collect baseline data on the contractile activity of each of these three treatments on isolated mouse uterine tissues, 2) analyze if the resulting contractile responses were concentration-dependent, and 3) review their contractile contributions to the overall contractility of aqueous extracts from the roots and rhizomes of the blue cohosh plant itself.

2.0 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 uterine tissues

Twenty-four hours prior to uterine horn extraction, mice were given an injection of diethylstilbestrol (DES). DES is a synthetic non-steroidal estrogen agonist used to promote the mouse into the estrus stage of their estrous cycle [32] thereby increasing the responsiveness of the smooth muscle [33]. The epigenetic change induced by DES stimulates the formation of gap junctions and allows the uterus to function as a single-unit of smooth muscle through endometrial thickening within the uterine wall [34].

On the day of the experiment, fresh DeJalons Ringer's solution (g/4 L: 36g NaCl, 1.68g KCl, 2g $NaHCO_3$, 2g D-glucose, and .32g $CaCl_2$) was made to simulate extracellular fluid conditions. Mice were then euthanized via CO_2 asphyxiation, placed on a dissection board, and the uterine horns were removed by means of a 4 cm abdominal incision made cranially from the vaginal orifice. The two uterine horns were individually isolated from each mouse and a suture was tied on each end of a horn; 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 De Jalons warmed to 32° C, and continually aerated (~2 psi) with 95% O_2/5% CO_2. A prepared uterine horn was lowered into the organ bath; the stationary rod was anchored into the bath and the other sutured uterine horn was attached to an isometric force transducer (MLT500, AD Instruments, Colorado Springs, CO), and placed under 0.8 g of tension [35]. The force transducer was connected to an amplifier and a Power Lab data acquisition system (AD Instruments, Colorado Springs, Colorado, USA) that collected data from the tissue and translated the tissue's contractile responses into visual waveforms.

The tissue samples were equilibrated for one hour with flushes every 15 minutes, replenishing the system with fresh DeJalons. During this time, tissues demonstrated spontaneous motility representative of healthy uterine smooth muscle under tension.

2.3 Experimental protocol

After equilibration, 10^{-5} M oxytocin, an endogenous hormone known to evoke contractions of uterine smooth muscle via oxytocin receptors [36] was applied to each tissue. Oxytocin served as a positive contractile control and the resulting contractions were observed and recorded for ten minutes. The tissues were then flushed and allowed to return to their normal spontaneous rhythm before any of the three treatments were applied.

The saponin treatment was dissolved in DeJalons solution and pipetted into the organ baths at percent concentrations of 0.1, 0.2, 0.5, 1, 2, 5, 10, and 20. The *N*-methylcytisine and magnoflorine treatments (10^{-5} M) were dissolved in DMSO. Blue cohosh was dissolved in deionized water to give the desired concentration of 30mg/20 mL organ bath. Once fully dissolved, the solution was vacuum filtered through Whatman filter papers via Buchner funnel to separate the extract from unnecessary plant particles.

All treatments were left on the tissues for 10 minutes and the results were observed and recorded. After 10 min, the tissues were flushed with fresh DeJalons solution and allowed to re-equilibrate. Saponin tissues that returned to normal spontaneous motility were used again with a different saponin concentration.

2.4 Chemicals
The powdered root and rhizomes of blue cohosh were purchased from Mountain Rose Herbs (Eugene, Oregon, USA). All the other chemicals were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). The sapogenin glycoside was obtained from the bark of the South American soap tree, *Quillaja saponaria*. In water, the *Quillaja* saponin yields micelles with an average MW of 56,000 (Sigma Prod. No. S4521). Individual saponin mass measurements obtained from blue cohosh itself range from 1074.6-1236.6 MW [1].
The magnoflorine was obtained from the dried root and rhizome of *Coptidis rhizome* (Sigma Prod. No. SMB00377) and has the same molecular weight (342) at that isolated directly from blue cohosh [1]. *N*-methylcytisine (Sigma Prod. No. SMB00353) also had the same molecular weight (204) as that isolated directly from blue cohosh [1].

2.5 Measurements
All treatment applications were made after the completion of a full spontaneous motility cycle and under baseline tension. Changes in contractile force were measured from the baseline tension of the waveform to the maximal force produced within the first five minutes of treatment exposure. To control for the possible force contribution that the tissue's spontaneous motility might have on the treatments, the amplitude of these forces were also measured in a similar manner five minutes before the application the treatments and are considered as the control, or the "0" treatment.
To normalize for the slight variation in the uterine tissue masses, each tissue's maximal contractile response to any given treatment was expressed as a percent of its initial contractile response to 10^{-5} M oxytocin.
To determine changes in contractile frequency, the waveforms produced were counted five minutes prior to the application of the treatment and five minutes after.

2.6 Statistical analysis
The data was summarized as means ± SE for each treatment for both contractile force and frequency. Each set of means included data 1) with a sample size greater than three, 2) which had experienced spontaneous motility prior its respective positive contractile control, and 3) responded to its respective positive contractile control. Individual data were further analyzed using ANOVA for multiple comparisons among the 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 means were considered to be significantly different from each other.

3. Results
3.1 Uterine smooth muscle responses to oxytocin
Uterine horn viability was affirmed by the presence of spontaneous motility observed at the beginning of each experiment, as well as a contractile response after the addition of oxytocin (Figure 1). On average, oxytocin evoked a force of 44.23 ± 3.1 mN, (n=18) within 5 min of application. The typical oxytocin response shows an immediate increase in contractile force followed by a sustained plateau response that gradually declined over time.

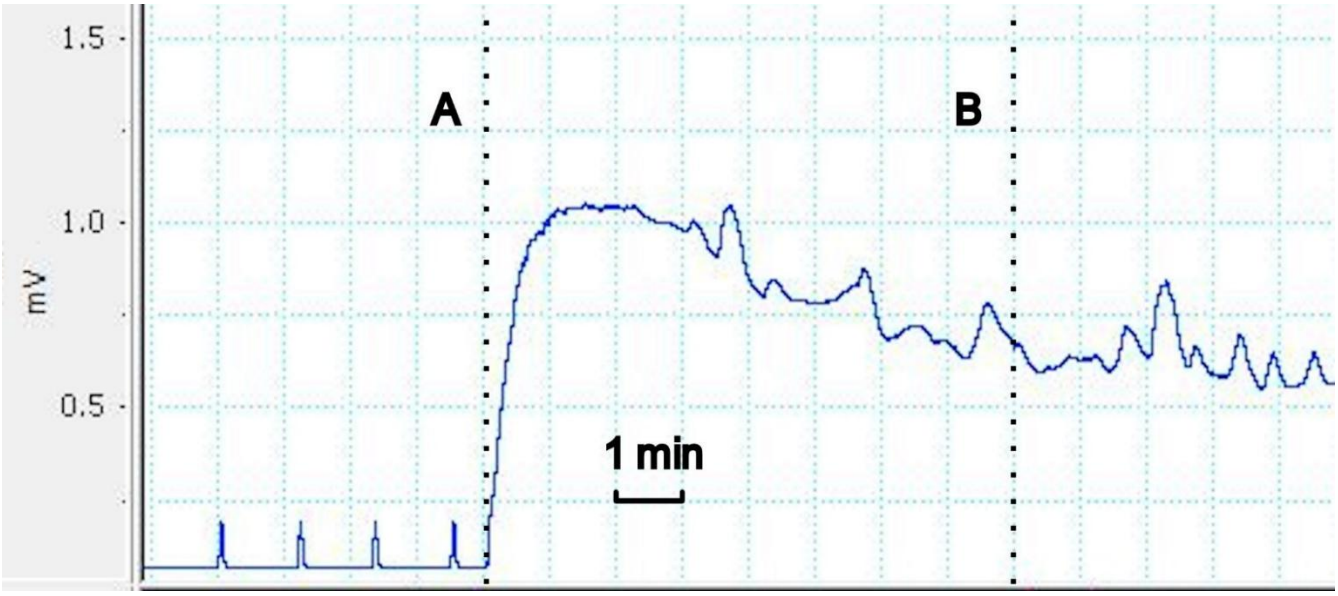

Fig 1: Typical uterine smooth muscle waveform response for 10^{-5}M oxytocin. In this sample, the spontaneous motility amplitude prior to letter **A** was 9.28 mN. Oxytocin was applied at letter **A** and produced a maximal contractile force of 42.96 mN. The tissue was flushed at letter **B** and the basal tone eventually returned to baseline. The y-axis (in mV) was later converted to mN of force.

3.2 Uterine smooth muscle responses to *Quillaja* saponin
3.2.1. Waveform response
A typical waveform response following the addition of saponins after spontaneous motility is shown in Figure 2. In contrast to oxytocin, the contractile response to *Quillaja* saponin did not appear as a plateau, but did produce an increase in both the force and frequency of contractions.

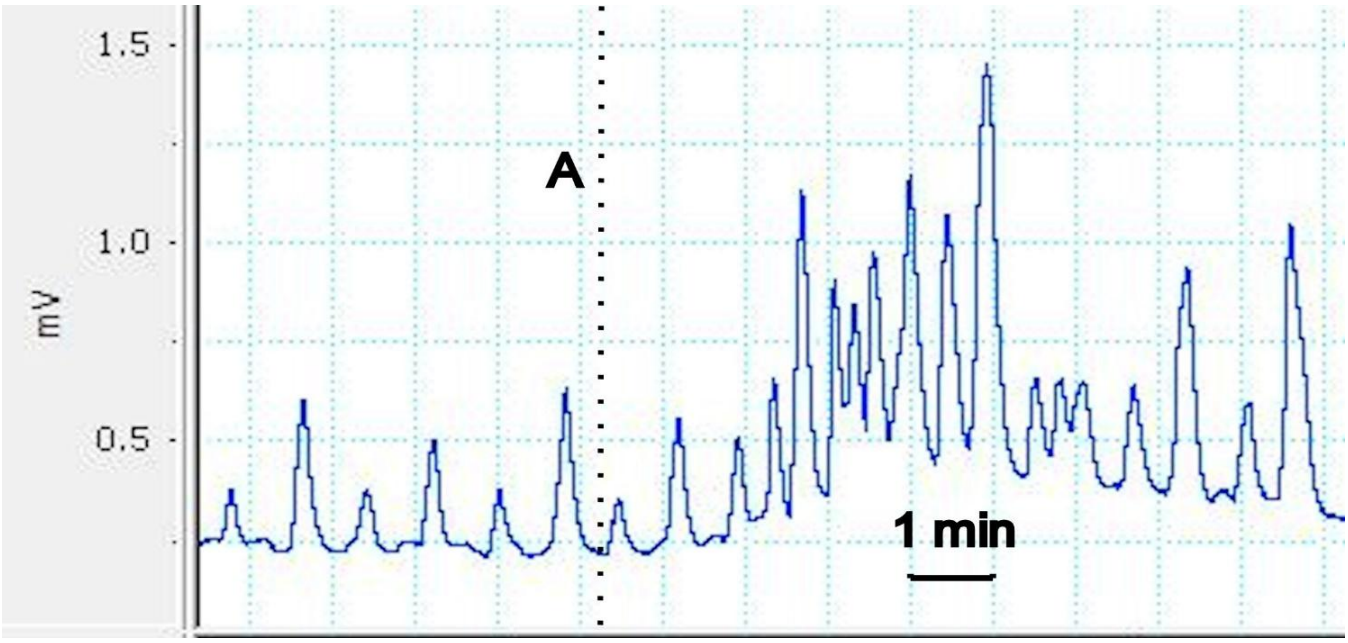

Fig 2: Typical uterine smooth muscle waveform response for *Quillaja* saponin. This 1% saponin solution (applied at letter **A**) produced a gradual contractile response of 50.71 mN response (or 118.02% of its oxytocin control) within 5 min of application. Most saponin treatments produced a small increase in basal tone that eventually returned to baseline after tissue washout. The y-axis (in mV) was later converted to mN of force.

3.2.2. Contractile force

Saponins increased contractile forces following the applications of 0.1% (51.94 ± 13.01 mN; n=8), 0.2% (27.78 ± 1.81 mN; n=5), 0.5% (104.18 ± 7.20 mN; n=4), 1% (74.94 ± 13.52 mN; n=9), 2% (71.13 ± 11.31 mN; n=7), 5% (107.01 ± 8.55 mN; n=8), 10% (84.01 ± 12.85 mN; n=10), and 20% (83.12 ± 17.11 mM; n=7). Spontaneous motility prior to saponin treatments had an average contractile force of 36.22 ± 3.20 mN; n=58).

These increases in uterine contractile forces in response to saponins (presented as a percent of their initial oxytocin) are summarized in Figure 3. *Quillaja* saponin evoked significant increases in uterine contractile forces at concentrations of 0.5%, 1%, 5%, 10%, and 20% when compared to 0% saponin concentration (P<0.0001). The observed threshold concentration was about 0.5%. Overall, most concentrations of saponin were near 100% of their respective 10^{-5}M oxytocin response. Even though the saponin treatments did produce strong uterine tissue contractions, the responses were not concentration-dependent.

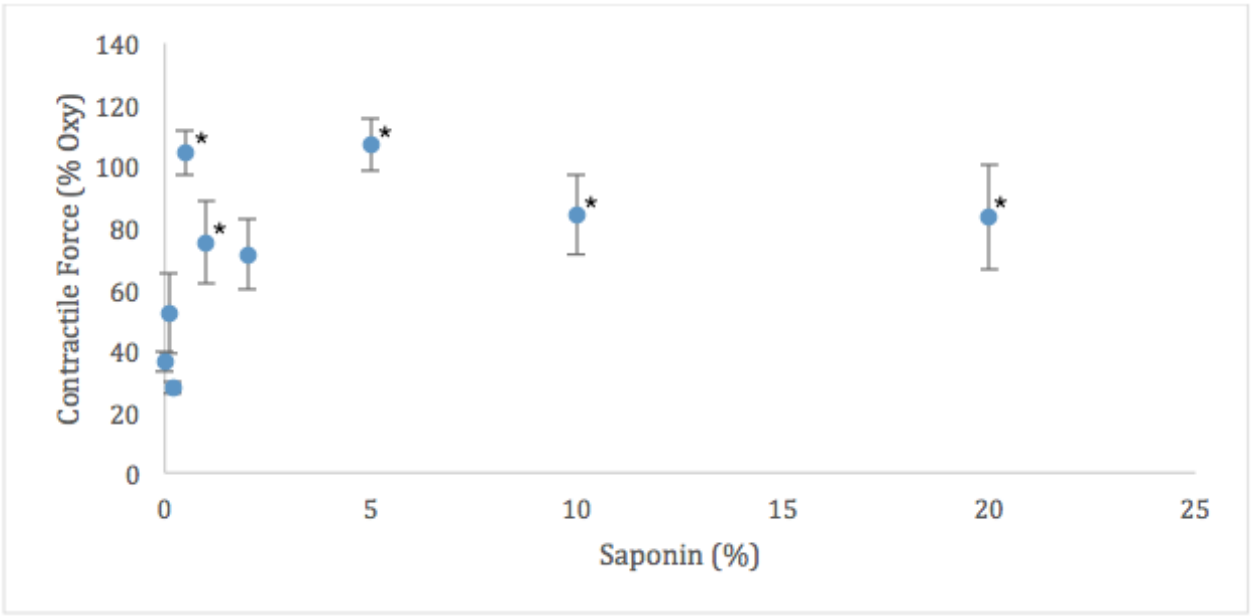

Fig 3: Each percent *Quillaja* saponin contractile force is presented as percent of their oxytocin contractile force (means ± SE). Spontaneous motility is represented as 0% saponin concentration. *Quillaja* saponin does contract the uterine tissue (P<0.0001). Significant increases in contractile force relative to spontaneous motility are noted with *.

3.2.3. Contractile frequency

The frequency of spontaneous motility prior to saponin treatment was 3.9 ± 3.10 contractions/5 min (or 0.78 contractions/min). Contractile frequencies increased following saponin applications of 0.1% (12.63 ± 3.05), 0.2% (7.7 ± 2.31), 0.5% (13.63 ± 4.03), 1% (17.61 ± 3.97), 2%

(8.14 ± 2.01), 5% (10.13 ± 1.36), 10% (8.00 ± 1.30), and at 20% (12.36 ± 2.48) per 5 min.

The increase in uterine contractile frequency in response to saponins are presented in Figure 4. *Quillaja* saponin evoked a significant increase in contractile frequency at concentrations of 0.1%, 0.5%, 1%, 5%, and 20% when compared to 0% saponin (P<0.0001). The results tended to show a greater contractile rate at some of the lower concentrations of saponin (0.1, 0.5, and 1%). Concentrations ≥ 2% essentially doubled the "0" treatment baseline values. Even though the saponin treatments did produce increases in contractile rate, the responses were not concentration-dependent.

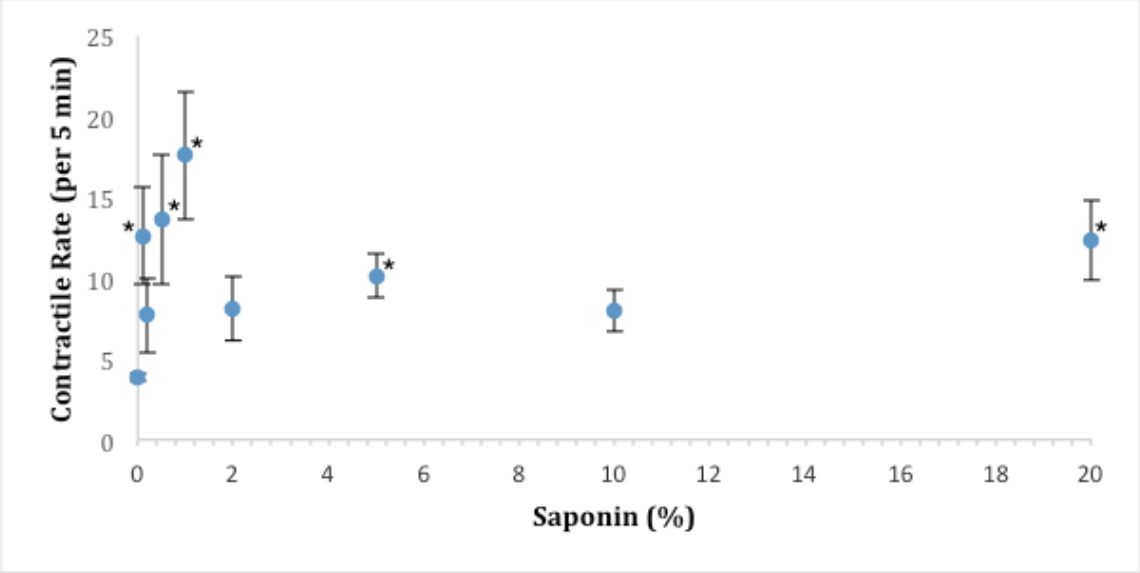

Fig 4: Mean ± SE contractile rate for each percent concentration of saponin after 5 minutes of exposure. *Quillaja* saponin does increase the frequency of contractions relative to the 0% saponin (P<0.0001); * indicates which means are statistically greater than 0% treatment.

3.3 Uterine smooth muscle responses to *N*-methylcytisine and magnoflorine

Tissues treated with either *N*-methylcytisine (n=4) or magnoflorine (n=4) did not produce any significant increases in uterine contractile forces when compared to the tissues spontaneous motility (P=.9258, P=.9763, respectively) and no further analysis was considered for this investigation.

3.4 Uterine smooth muscle responses to blue cohosh

A typical contractile response of uterine tissue before and after an application of blue cohosh is illustrated in Figure 5. The tissues immediate response showed a rapid increase in contractile force and frequency. The basal tone was elevated and remained at about 50% of its maximal increase until the bath was flushed 10 min later.

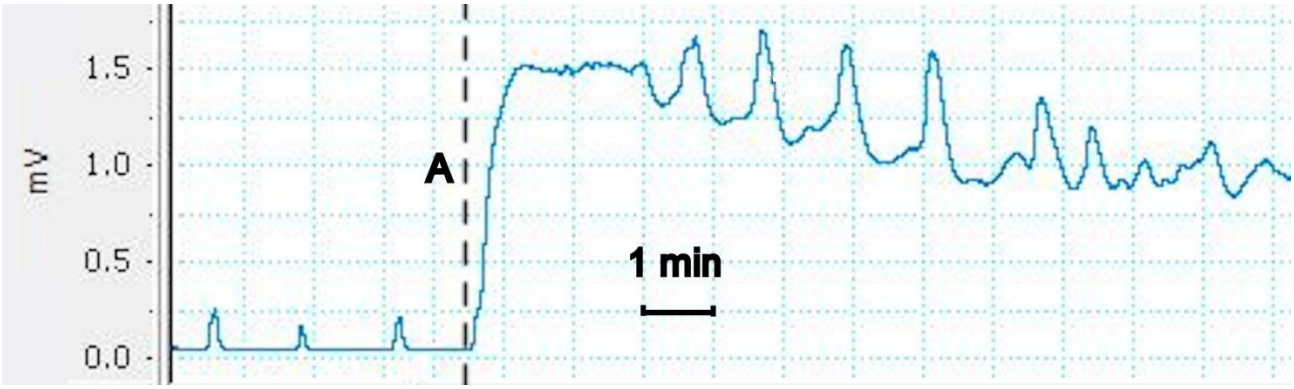

Fig 5: Typical uterine smooth muscle waveform response for blue cohosh (30mg/20 mL bath). The amplitude of the spontaneous motility prior to the administration of blue cohosh was 9.01 mN. Blue cohosh was applied at letter **A** and within 5 min of treatment, contractile forces had increased up to 71.28 mN. The y-axis (in mV) was later converted to mN of force.

4. Discussion

4.1 *Quillaja* saponin response

Quillaja saponaria saponin produced an increase in force and frequency of uterine contractions at almost all concentrations when compared to the tissues endogenous spontaneous motility. These results indicate that indeed saponins are a potent contractor of smooth muscle as observed in the isolated uterine tissue and, are proposed to contribute to the overall contractile response as seen from the parent plant, blue cohosh, *Caulophyllum thalictroides*, as well.

Saponins are cell membrane permeating agents, which work because of their detergent-like properties [11, 13, 15, 37, 38]. It is thought that the hydrophobic aglycone moieties of the saponin molecules form insoluble complexes with plasma membrane cholesterol constituents leading to saponin-cholesterol micelles disrupting the lipid bilayer [39]. These disruptions in the lipid bilayer result in invaginations and subsequent pore formation [11].

These pores likely allow for the influx of Ca^{2+} ions (as found in the DeJalons solution bathing the isolated tissues) to bind to calmodulin and initiate the smooth muscle contraction

cascade. Support for this conclusion was gathered from a small pilot study in which nifedipine, an L-type Ca^{2+} channel blocker, was added to the uterine muscle tissue prior to saponin treatment. This failed to prevent any saponin evoked contractile response, indicating that the saponins were not interacting with the L-type Ca^{2+} channels on the cell membrane. Furthermore, an additional experiment employing a Ca^{2+}-free DeJalons solution was used, and upon treatment with saponins, there was no contractile response. This would indicate that the saponins indeed are interacting with the cell membrane and they themselves are not entering the cell and inducing Ca^{2+} release from the sarcoplasmic reticulum.

The typical waveform response from the isolated saponin constituent of *Quillaja saponaria* (Fig. 2) is comparable to the waveform response from an aqueous extract of blue cohosh (Fig. 5) in that both treatments do evoke increases in uterine contractile force, frequency, and basal tone. However, there are both quantitative and qualitative visual differences between the two waveform patterns. Most obvious is the slower onset of increasing contractile force, the lack of an extended contractile plateau, and a less forceful contraction produced by the saponin constituent. This might reflect the unique concentrations of the two different treatments. The *Quillaja* saponins used in this experiment may also have had a different structure from the saponins found in blue cohosh; the structure of saponins can vary greatly depending on their plant source [11, 30]. Using saponins extracted directly from the roots and rhizomes of blue cohosh would be most informative in determining percent contributions from the extract of the parent plant itself.

Since saponins are found in many plants [30, 40], it is likely that they are an important contributor to the smooth muscle contractions observed from other plant extracts which also demonstrate oxytocic properties *in vitro* (e.g. red raspberry leaves *Rubrus ideas* [41], castor seed *Ricinus communis* [42], calabash fruit *Crescentia cujete* [43], evening primrose *Oenethera biennis*) [44].

4.2 Alkaloid response

It is also possible that the resulting difference in waveform responses between the blue cohosh plant extract and the *Quillaja* saponin constituents indicate that saponins are not the only active ingredient in blue cohosh. In the full plant, it would seem likely that synergistic activity among the saponins and the alkaloids are at work. It seemed reasonable to test *N*-methylcytisine as an individual constituent since it has been demonstrated to be a potent nicotinic acetylcholine receptor agonist [24] and too slightly (one tenth less than nicotine) stimulate motility via intrinsic ganglia using isolated segments of small intestine from rabbits [22].

Perhaps our resulting lack of uterine contractile response from *N*-methylcytisine supports Schmeller's observations that *N*-methylcytisine binds selectively to nicotinic acetylcholine receptors in the central, as opposed to peripheral, nervous system [24]. The lack of uterine contractile response to magnoflorine is in contrast to earlier work reported by El Tahir [31] who was able to show an increase in uterine force and frequency from isolated rat tissues. It remains to be determined if our mice uterine samples provided enough smooth muscle mass to produce a contractile response which is suggested to work by interacting with muscarinic or serotonergic receptors [31].

5. Conclusions

Quillaja saponins do contract isolated uterine smooth muscle. The results herein support the proposal that the saponin constituents of blue cohosh are partially responsible for the uterine contractile responses observed following consumption [8, 45]. This is an important contribution to the current understanding of the pharmacological effects of blue cohosh as most of the previous published work on blue cohosh has focused on the potential toxic effects of the alkaloids.

6. Acknowledgements

This research was partially funded by contributions from the Division of Natural Sciences at Bethel University. The authors would also like to thank Mackenzie Theis for her contributions toward the data collection and Griff DeGolier for his assistance with the graphics.

7. References

1. Satchithanandam S, Roach J, White KD, Mazzola E, Ganzera M, Rader JI. Alkaloids and saponins in dietary supplements of blue cohosh (*Caulophyllum thalictroides*). J AOAC Int. 2008; 91(1):21-32.
2. Smith P. The Indian doctor's dispensatory being Father Smith's advice respecting diseases and their cure. Browne Looker, Cincinnati, 1813, 108p.
3. Felter HW. *Caulophyllum thalitroides*. Med Gleaner 1892; 3:439-434.
4. Ellingwood F. A systematic treatise on material medical and therapeutics. Chicago Medical, Chicago, 1900, 706p.
5. Moerman DE. Native American ethnobotany. Timber, Portland, Oregon, 1998, 927p.
6. King J. American eclectic obstetrics. Moore Wilstach Key, Cincinnati, 1855, 741.
7. Schardl C, Panaccione DG, Tudzynski P. The Alkaloids: Chemistry and Biology. 2006; 63:45-86.
8. Romm A, Upton R, Axentiev P, Swisher D, editors. Blue cohosh root and rhizome. Amer Herb Pharm. California, 2012.
9. McFarlin BL, Gibson MH, Harman P. A national survey of herbal preparation used by nurse-midwives for labor stimulation. J Nurse Midwifery. 1999; 44(3):205-216.
10. Berger J, DeGolier T. Pharmacological effects of the aqueous extract of *Caulophyllum thalictroides* (blue cohosh) on isolated *Mus musculus* uteri. BIOS 2008; 79(3):103-114.
11. Das TK, Banerjee D, Chakraborty D, Pakhira MC, Shrivastava B, Kuhad RC. Saponin: Role in animal system. Veterinary World. 2012; 5(4):248-254.
12. Lee Y, Jung J, Ali Z, Khan IA, Oh S. Anti-inflammatory effect of triterpene saponins isolated from blue cohosh (*Caulophyllum thalictroides*). EVID-BASED COMPL ALT, 2012. http://dx.doi.org/10.1155/2012/798192.
13. Gilabert-Oriol R, Mergel K, Thakur M, Von Mallinckrodt B, Melzig MF, Fuchs H, *et al.* Real-time analysis of membrane permeabilizing effects of oleanane saponins. Bioorgan Med Chem. 2013; 21(8):2387-2395.
14. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: A review. Brit J Nutr. 2002; 88(6):587-605.
15. Berlowska J, Dudkiewicz M, Kregiel D, Czyzowska A, Witonska I. Cell lysis induced by membrane-damaging detergent saponins from *Quillaja saponaria*. Enzyme Microb Technol. 2015; 75-76:44-48.
16. Betz JM, Andrzejewski D, Troy A, Casey RE, Obermeyer WR, Page SW *et al*. Gas chromatographic determination of toxic quinolizidine alkaloids in blue

cohosh *Caulophyllum thalictroids* (L.) Michx. Phytochem Analysis. 1998; 9:232-236.
17. Ganzera M, Dharmaratne HRW, Nanayakkara NPD, Khan IA. Determination of saponins and alkaloids in *Caulophyllum thalictroides* (Blue Cohosh) by high-performance liquid chromatography and evaporative light scattering detection. Phytochem Analysis 2003**;** 14:1-7.
18. Ferguson H, Edwards L. A pharmacological study of a crystalline glycoside of *Caulophyllum thalictroides*. J Am Pharm Assoc. 1954; 43(1):16-21.
19. Power FB, Salway AH. The constituents of the rhzome and roots of *Caulophyllum thalictroides.* J Chem Soc. 1913; 103:191-209.
20. Flom MS, Doskotch RW, JL Beal. Isolation and characterization of alkaloids from *Caulophyllum thalictroides.* J Pharmac Sci. 1967; 56:1515-1517.
21. Woldemariam TZ, Betz JM, Houghton PJ. Analysis of aporphine and quinolizidine alkaloids from *Caulophyllum thalictroides* by densitometry and HPLC. J Pharmaceut Biomed. 1997; 15:839-843.
22. Scott CC, Chen KK. The paramacological action of *N*-methylcytisine. J Pharamacol Exp Ther. 1943; 79:334-339.
23. Barlow RB, McLeod LJ. Some studies on cytosine and its methylated derivatives. Br J Pharm. 1969; 35:161-174
24. Schemmler T, Saverwein M, Sporer F, Wink M, Mueller WE. Binding of quinolizidine alkaloids to nicotinic and muscarinic acetylcholine receptors. J Nat Prod. 1994; 57:1316-1319.
25. Gunn T, Wright IMR. 1996. The use of black and blue cohosh in labour. New Zealand Med J 1996; 109:410-411.
26. Jones TK, Lawson BM. Profound neonatal congestive heart failure caused by maternal consumption of blue cohosh herbal medication. J Pediat. 1998; 132:550-553.
27. Finkel RS, Zarlengo KM Correspondence: Blue cohosh and perinatal stroke. N Engl J Med. 2004; 351:302-303.
28. DeGolier T, VenOsdel N, Widstrom L, Hulst J. The chronotropic effects of blue cohosh, *Caulophyllum thalictroides*, on frog hearts *in situ* and rat hearts *in vitro*. Journal of Pharmacognosy and Phytochemicals. 2016; 5(5):105-110.
29. Kennelly EJ, Flynn TJ, Mazzola EP, Roach JA, McCloud TG, Danford DE *et al*. Detecting potential teratogenic alkaloids from blue cohosh rhizomes using an *in vitro* rat embryo culture. J Nat Prod. 1999; 62:1385-1389.
30. Rader J, Pawar RS. Primary constituents of blue cohosh: quantification in dietary supplements and potential for toxicity. Anal Bioanal Chem. 2013; 405:4409-4417.
31. El Tahir. Pharmacological actions of magnoflorine and aristolochic acid-1 isolated from the seeds of *Aristolochia bracteata*. Int J Pharmcognosy. 1991; 29(2):101-111.
32. Allen E. The oestrus cycle in the mouse. Am J Anat. 1992; 30:297-371.
33. Doherty LF, Bromer JG, Yuping Z, Tamir AS, Hugh TS. *In utero* exposure to diethylstilbestrol (DES) or bisphenol-A (BPA) increases EZH2 expression in the mammary gland: An epigenetic mechanism linking endocrine disruptors to breast cancer. Hormones and Cancer. 2010; 1(3):146-155.
34. Burger H, Healy D, Vollenhoven B. The Ovary: Basic Principles and Concepts. In Endocrinology and Metabolism, 4th ed. P. Felig, L. Rohman, Eds, McGraw-Hill, Inc. USA, 2011.
35. Kitchen I. Textbook of *in vitro* practical pharmacology. Blackwell Scientific Publication. London, England, 1984, 32-38.
36. Husslein P, Fuchs A, Fuchs F. Oxytocin and the initiation of human parturition: I. Prostaglandin release during induction of labor by oxytocin. Am J Obstet Gynecol. 1981; 141(5):688-693.
37. Hirata M, Kukita M, Sasaguri T, Suematsu E, Hashimoto T, Koga T. Increase in Ca^{2+} permeability of intracellular Ca^{2+} store membrane of saponin-treated guinea pig peritoneal macrophages by inositol 1,4,5-trisphosphate. J Biochem. 1985; 97(6):1575-1582.
38. Kargacin ME, Kargacin GJ. Direct measurement of Ca2+ uptake and release by the sarcoplasmic reticulum of saponin permeabilized isolated smooth muscle cells. J Gen Physiol. 1995; 106(3):467-484.
39. Baumann E, Stoya G, Völkner A, Richter W, Lemke C, Linss W. Hemolysis of human erythrocytes with saponin affects the membrane structure. Acta Histochem. 2000; 102(1):21-35.
40. Lacaille-Dubois MA, Wagner H. Bioactive saponins from plants: an update. Studies in Natural Products Chemistry. 2000; (21 Part B):633-687.
41. Olson A, DeGolier T. Contractile activity of *Rubus idaeus* extract on isolated mouse uterine strips. BIOS. 2016; 87(2):39-47.
42. Quam N, Wu K, DeGolier T. Aqueous extracts of castor seed (*Ricinus communis)* increase the contractile activities of mouse uterine tissues *in vivo*. Journal of Pharmacognosy and Phytochemicals 2016; 5(4):40-45.
43. Theis M, Richard M, Bell K, DeGolier T. *Crescentia cujete* (calabash tree) seed extract and fruit pulp juice contract isolated uterine smooth muscle tissues from *Mus musculus*. Journal of Medicinal Plant Studies. 2017; 5(5):10-15.
44. DeGolier T, Lyle C, Ortmann A. Aqueous extracts from evening primrose seeds (*Oenethera biennis*) contract isolated uterine tissues but have no effect on isolated cervical tissues. International Journal of Herbal Medicine. 2017; 5(3):10-16.
45. Lwung A, Foster S. Encyclopedia of common natural ingredients used in foods, drugs and cosmetics. John Wiley, New York, 1996.