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Raw ginger root juice (*Zingiber officinale*) produces a biphasic contractile response in isolated mouse uterine tissue under resting baseline tension

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Abstract
Ginger root (*Zingiber officinale*) is an herbal supplement used to control severe nausea and vomiting during pregnancy. It is also prepared as a tincture to revitalize the uterus during labor. The purpose of this study was to determine if revitalizing the uterus involved changes in uterine contractile activity. Volumes of ginger root juice (20-1000 µL) were applied directly to isolated mouse uterine tissues in an organ bath under baseline conditions. At each volume, increases in contractile force occurred within five minutes ($p < 0.001$). Increases in contractile frequency were less remarkable ($p = 0.5343$). After five minutes, the greater volumes of ginger root juice inhibited all contractile activity. These results indicate that raw ginger root juice evokes a biphasic response as a function of time and treatment volume. This data would advocate for the revitalizing use of ginger root for calming uterine spasms rather than inducing labor.

Keywords: *Zingiber officinale*, ginger, uterine contractions, mice, *in vitro*

1. Introduction
Ginger (*Zingiber officinale*) is a flowering plant whose rhizome (herein root) has been widely used as a food additive in cooking [1]. Native to southeast Asia, ginger is now cultivated in a number of tropical countries including India, China, Nepal, Indonesia, and Thailand [2].
The therapeutic use of ginger root has been historically employed as a strong natural treatment for a number of health problems [3]. Today ginger root is highly valued for its anti-inflammatory properties [4] and its effectiveness in treating and reducing motion sickness, postoperative vomiting, and pregnancy-induced nausea and vomiting [5, 6, 7].
Some of the phytochemical constituents found in ginger root, namely the gingersols and shogoals, are considered to be the agents that reduce stomach upset and inhibit vomiting [3]. Others may work as cholinergic agents, promoting the movement of food through the intestinal tract *in vivo*, or as calcium channel antagonists, promoting the relaxation of K^+ -induced contracted intestinal tissues *in vitro* [8].
Ghayur and Gilani [9] demonstrated that ginger root relaxed K^+ -induced contracted rat uterine tissues *in vitro*, yielding support for claims that ginger root reduces uterine spasms when in a pre-contracted state. With respect to its effect during parturition, ginger root has been called a uterine revitalizer [5]. The results from Ghayur and Gilani [9] may indicate that this revitalization is due to a soothing relaxation of smooth muscle, mediated by an agent in ginger root that acts as a Ca^{2+} channel antagonist.
The purpose of the study herein was to apply raw ginger root juice to isolated mouse uterine tissues to determine if revitalizing the uterus might also involve changes in uterine contractile activity in tissues that were under resting baseline tensions, in contrast to tissues that were in a pre-contracted state [9]. The specific objectives were to 1) demonstrate if raw ginger root juice would contract uterine smooth muscle suspended under resting baseline tension; 2) if so, determine whether the responses were volume dependent for force and frequency of contractions; and 3) to determine if the contractile responses were biphasic as a function of the amount of time the tissues were exposed to the treatment as well as the volume of treatment given.

2. Materials and Methods
2.1 Ginger root
Fresh ginger root (*Zingiber officinale*) was purchased at a local grocery store (St. Paul, MN). The entire root, unpeeled, was diced into small pieces and pulsed in a blender. The pulp was then collected in a mortar and pestle, and further ground into a paste.

The paste was then squeezed through cheesecloth to filter out the fibrous components and to accumulate the raw juice from the ginger root. Excess pulp was disposed of and the juice was kept on ice for the remainder of the experiment and thoroughly mixed prior to each application.

2.2 Specimens

Eight virgin female mice, *Mus musculus* (outbred ICR CD-1), each weighing 25-30 g, were obtained from Envigo, Inc. (Indianapolis, IN). The mice were housed in cages in the Department of Biological Sciences of Bethel University (St. Paul, MN) and had access to water and standard mice chow *ad libitum*. All procedures were completed in accordance with the Institutional Animal Care and Use Committee of Bethel University.

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 mice into the estrus stage of their estrous cycle [10], thereby increasing the responsiveness of the smooth muscle [11]. An 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 [12].

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 0.32g $CaCl_2$) 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 through a 4 cm abdominal incision made cranially from the vaginal orifice. The two uterine horns were isolated from each mouse and a suture was tied on each end of a horn: one attached to a stationary rod for eventual placement into the organ bath, and the other for eventual attachment to a force transducer.

2.3 Smooth muscle bath

At the start of each experiment the organ baths were flushed multiple times with the DeJalons solution warmed to 32° C, and continually aerated with 95% O_2/5% CO_2 throughout the investigation. A prepared uterine horn was then 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, ADInstruments, Colorado Springs, CO), and placed under 0.8 g of tension [13]. The force transducer was connected to an amplifier and a PowerLab data acquisition system (ADInstruments, Colorado Springs, CO) that collected data from the tissue and translated the tissue's contractile responses into visual waveforms.

The tissue samples were equilibrated in individual baths for one hour with tissue washouts (or flushes) every 15 min, replenishing the system with fresh DeJalons. During this time, the tissues demonstrated spontaneous motility representative of healthy uterine smooth muscle under tension. Oxytocin at 10^{-5} M was then added to elicit a control contractile response, as it is an endogenous hormone known to contract smooth muscle of the uterus [14]. Following a 10 min exposure to oxytocin, tissues were flushed again and allowed to return to their normal baseline spontaneous contractile rhythm before any further treatment.

Each tissue was then given only one of the desired treatments (*i.e.* volumes of ginger root juice: 20, 100, 500, and 1000 µL) and left in the organ bath for approximately 25-30 minutes. Changes in uterine contractile force frequency were observed and recorded.

2.4 Measurements

All treatment applications were made after the completion of a full spontaneous motility cycle and under resting baseline tension (*i.e.* in a non pre-contracted state). Changes in contractile force were measured from the baseline to the maximal force produced within the first 5 min of treatment exposure. To control for the possible contribution that the tissue's spontaneous motility might have on treatment responses, the contractile amplitude of these endogenous forces were considered as the control, or the "0" treatment. These contractile forces were also measured 5 min prior to the application of the ginger root juice. Furthermore, to normalize for the slight variation in the uterine tissue masses, each tissue's maximal contractile response to any given applied volume of raw ginger juice 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 5 min prior to the application of the treatment and 5 min after.

2.5 Statistical analyses

The data was summarized as means ± SE for each treatment for both contractile forces (% Oxy) and contractile frequencies. 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, NC) which indicated which means were considered to be significantly different from each other.

3. Results

3.1 Uterine contractile responses to oxytocin and ginger root

All uterine tissues reported herein exhibited spontaneous motility before the application of either oxytocin or raw ginger root juice (Fig 1). Oxytocin evoked a persistent positive contractile response from uterine tissues which was typically maintained at ~70% of its maximal plateau profile until flushed with DeJalons solution approximately 10 min later. After the tissues regained their spontaneous motility, they did respond to given volumes of raw ginger juice as evidenced by changes in contractile force and frequency.

In this 46 min selection, the addition of oxytocin (letter **A**) resulted in a strong contractile force of 37.73 mN as compared to the spontaneous motility force just prior of 4.92 mN. The dotted vertical line (letter **B**) indicates a tissue washout with fresh DeJalons. The addition of 20 µL raw ginger root juice (letter **C**) resulted in an increased contractile force greater than that observed in the spontaneous motility (from 18.77 mN to 42.32 mN). For analysis, the default y-axis was converted to mN based upon calibration of the force transducer

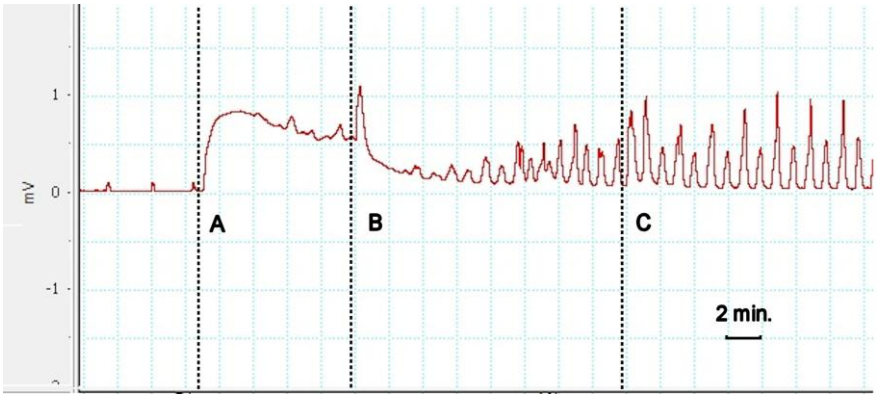

Fig 1: Typical contractile waveform responses following by the application of 10^{-5} M oxytocin and 20 µL raw ginger root juice as recorded from a single isolated mouse uterine horn.

3.2 Change in uterine contractile force in response to raw ginger root juice.

The control contractile amplitude (~ spontaneous motility) occurred with an average force of 21.97 ± 2.88 mN (n=14). There was an in increase in contractile force following the administration of 20 µL (35.06 ± 2.73 mN; n = 4), 100 µL (48.75 ± 5.60 mN; n = 4), 500 µL (61.68 ± 4.11 mN; n = 3), and 1000 µL (52.21 ± 3.33 mN; n = 3) raw ginger root juice. Fig 2 presents the mean ± SE contractile force response (% Oxy) for each volume of raw ginger root applied. All treatment responses were statistically greater from that of the control treatment ($p < 0.001$). The treatment responses themselves, however, were not volume dependent within the 5 min assigned measurement parameters.

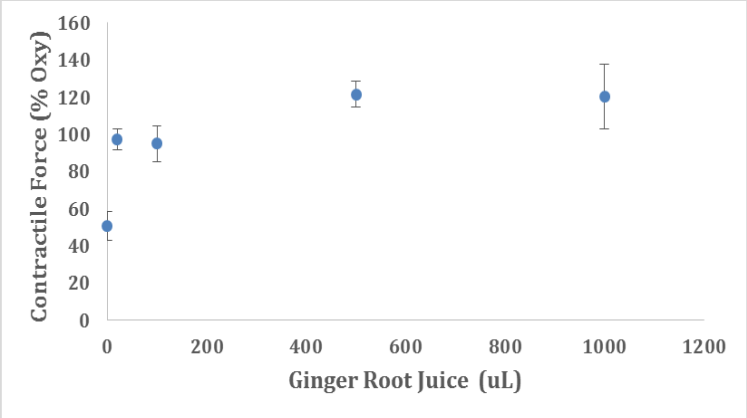

Fig 2: Means ± SE uterine contractile force (% Oxy) in response to increasing volumes of ginger root juice. Each increase in volume resulted in a forceful contraction, statistically greater from "0" treatment ($p < 0.001$). The increases in contractile forces however, were not volume dependent within the 5 min assigned measurement parameters.

3.3 Change in uterine contractile frequency in response to raw ginger root juice

Fig 3 presents the mean ± SE contractile frequency responses to each volume of raw ginger root juice applied. After the application of 20, 100, and 500 µL, there was a noticeable increase in contractile frequency when compared to that of the control (4.82 ± 0.26 waveforms/five min). It would appear that the 1000 µL volume was a threshold resulting in a decrease in contractile frequency. Overall, there was no statistical change in frequency in response to raw ginger root juice within the 5 min assigned measurement parameters ($p = 0.5343$).

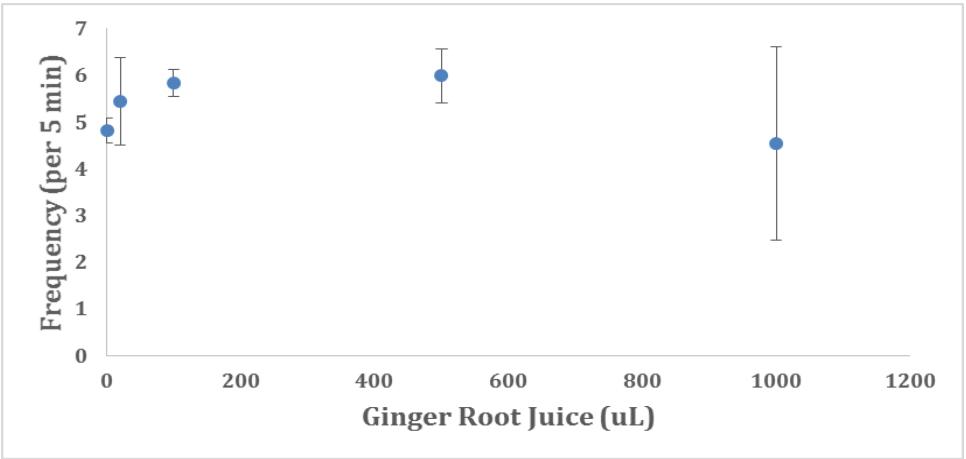

Fig 3: Means ± SE changes in uterine contractile frequency in response to increasing volumes of applied raw ginger root juice. Although there is

a slight increase in frequency at the 20, 100, 500 µL applications, the differences are not statistically greater than the control treatment (p = 0.5343, n = 14).

3.4 Inhibition of uterine contractile activities in response to volume and time

All tissue samples receiving the 1000 µL application of raw ginger root juice experienced a suppression of further motility after 5 min. In two of three sampled tissues, the relaxation of the tissue with little to no spontaneous motility began as early as 5 min (Fig 4). Tissues receiving 500 µL were also affected, but the earliest indication of relaxation did not occur until at least 20 min after treatment. Some of these same tissues were flushed at the end of the experiment in an attempt to washout the effect of the ginger root juice. These tissues did eventually return to their normal spontaneous motility patterns, with the 500 µL treatment recovering sooner than the 1000 µL treatment. There were no contractile relaxation of any type observed in tissues that received the smallest treatment volumes of 20 and 100 µL (see Fig 1).

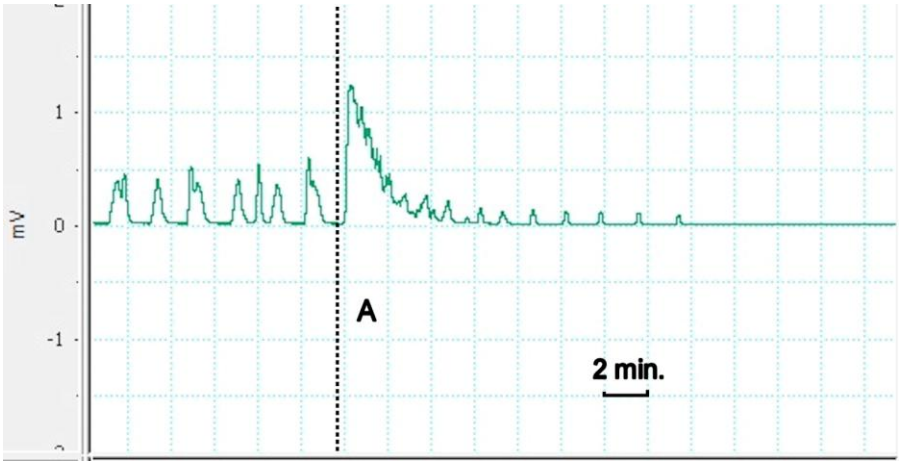

Fig 4: A sample contractile waveform response as recorded from a single isolated mouse uterine horn, following the application of 1000 µL of raw ginger root juice. In this 35 min selection, the addition of the ginger juice resulted in an immediate strong contractile force of 53.61 mN compared to the contractile force of the spontaneous motility of 16.96 mN prior to treatment. However, 5 min after application, the magnitude of the spontaneous motility had decreased to 5.46 mN and disappeared altogether after 16 min. For analysis, the default y-axis was converted to mN based upon calibration of the force transducer

4. Discussion

4.1 A biphasic uterine contractile response from raw ginger root juice

The application of raw ginger root juice (20-1000 µL) to isolated uterine tissues under resting baseline conditions resulted in an immediate increase in contractile forces that were nearly twice as great as the tissues endogenous spontaneous motility patterns. Increases in contractile frequencies were less remarkable. After 5 min, however, the 500 and 1000 µL volumes of ginger root juice led to a complete inhibition of contractile activity. These observations would indicate that raw ginger root juice evoked a biphasic contractile response as a function of the amount of time the tissues are exposed to the treatment, as well as the volume of treatment given. The post 5 min inhibitory effects were successfully reversed after a tissue washout.

Of unique interest to this project is the fact that Ghayur and Gilani [9] did not demonstrate any tissue contractile response following the application of ginger extract (up to 10 mg/mL) to rat uterine tissues under resting baseline conditions. At first glance, our study seemed to be in contrast with this research as we showed significant increases in the force of contractions at resting baseline conditions. Although not analyzed, it is possible that the mg/mL concentrations found in the volumes of raw ginger root juice used herein was greater than 10 mg/mL. We do concur that our observed inhibition of contractile activity induced 5 min after the application of the raw ginger root juice in a mouse model, is likely due to any one or more of the receptor interactions reported and summarized as follows.

4.2 The influence of ginger root on a uterine relaxation phase

Ghayur and Gilani [9] determined that 0.03-0.3 mg/ml of ginger root extract evoked a dose-dependent relaxation of pre-contracted (80 mM K^+ induced) uterine muscle from rat tissues *in vitro*. Similar investigations using guinea pig tracheal preparations [9] as well as rat, mouse, and guinea pig ileum [8] led them to conclude that a calcium antagonist present in the ginger root constituent is responsible for the relaxant effects observed in pre-contracted tissues. Work by Borrelli *et al.* [15] indicated the inhibitory effect might also also be mediated antagonistically on cholinergic and vanilloid receptors on smooth muscle.

Other studies have demonstrated that some of the pungent constituents found in ginger root such gingerol, shogaol, and galanolactone, act as antagonists of the 5-HT receptor, blocking the action of serotonin on smooth muscle [16, 17, 18], further advocating for ginger root's relaxant effect. Since serotonin is an inducer of uterine contractions and operates through the 5-HT receptor [19] it would seem likely that these ginger constituents would act in a similar manner on uterine smooth muscle.

Other pungent compounds isolated from ginger ([6]- and [10]-dehydrogingerdione and [6] - and [10] - gingerdione) have been show to be potent inhibitors of prostaglandin biosynthesis [20, 21] who play an essential mechanistic role in the contractions of uterine smooth muscle.

4.3 The influence of ginger root on a uterine contractile phase

Ghayur and Gilani [8] demonstrated that ginger root enhanced intestinal propulsion in a manner similar to that observed with the parasympathetic agonist carbachol. Using a phytochemical

analysis, they found alkaloids, saponins, and flavonoids all to be contained in their ginger root extract. Both alkaloids and saponins have been shown to evoke contractile activities on smooth muscle. For example, Akah *et al*. [22] has showed that some alkaloids and the saponins from *Carica papaya* root are capable of contracting isolated guinea pig ileum. A cholinergic-mediated system for the alkaloid contraction is indicated since these contractile responses were inhibited by atropine.

Recently, it has been demonstrated that saponins isolated from the soap tree *Quillaja saponin* contract uterine tissues [23]. This is likely due to the hydrophobic aglycone moieties of the saponin molecules forming insoluble complexes with membrane cholesterol leading to saponin-cholesterol micelles that disrupt the lipid bilayer [24]. These disruptions result in invaginations and subsequent pore formation [25] likely allowing for the influx of Ca^{2+} ions as found in the DeJalons solution bathing the isolated tissues.

The formation of soap bubbles is typically observed in aerated organ bath preparations that are receiving saponin-containing compounds. This was observed in similar experiments using *Quillaja saponin* [23] and the roots and rhizomes as found in blue cohosh *Caulophyllum thalictroides* [26] both evoking contractions in isolated uterine tissues from mice. The lack of bubble formation in our ginger root investigation may indicate that the saponin content may be lower in *Zingiber officinale.*

It remains to be determined what affect isolated flavonoids have on isolated uterine smooth muscle, but they have been shown to inhibit both intestinal motility in mice [27] and contractility in guinea pig ileum [28].

4.4 Variations in ginger root extract preparation.

The preparation of the ginger root extract within these various studies may lead to the unique isolation or inclusion of different constituents. Ghayur and Gilani [9] soaked their samples in methanol or distilled water, and then concentrated them with a rotary evaporator to obtain separate methanolic and aqueous crude extracts of ginger (mg/mL). Both extracts did induce relaxation responses on the uterine tissues. Further separation of the aqueous extract, however, indicated that it was the organic fraction (from petroleum ether) that was responsible for the relaxation activity.

The study herein simply used raw ginger root juice, filtered through cheesecloth, directly available from the root. It may be possible that raw ginger root juice, containing as much of the skin and pulp constituents in solution as possible, may have either 1) contained a greater proportion of contractile agents, or 2) exhibited a contractile synergy which was eventually overcome as the relaxation threshold for the tissues was met as a function of the time the juice was interacting with the tissue or as the volume of the juice constituents was increased.

4.5 Variations in the ginger root itself

The characteristic constituents of ginger root (% fatty oils, proteins, carbohydrates, raw fiber, ash, water, volatile oils, pungent substances) can vary based on their place of origin [29, 30, 6] as well as their maturity at harvest, ranging from 6-20 months [6].

In an effort to use as much of the root constituents as possible, we did not peel or scrape the skin. Oil content and pungent flavor are greatest in roots that are not scraped. The secretory cells that produce these pharmacologic compounds are located in the cortex, often right under the cork covering, and can be lost in post harvesting if scraping occurs [6].

5. Conclusion

This discussion is timely for pregnant women who seek natural therapies during the labor process as opposed to the standard intravenous methods performed in hospitals that can lead to possible complications [31]. Midwifery techniques and herbal treatments such as blue cohosh *(Caulophyllum thalactroides),* black cohosh *(Cimifuga racemosa),* red raspberry leaf *(Rubis idaeus)* and castor bean *(Ricinus communis)* can provide alternative therapies for inducing or preparing the uterus for labor [5, 32]. All of these herbal supplements have been shown to contract the uterus *in vitro* [26, 33, 34, 35].

A suggested labor tincture from herbalist Susun S. Weed [5] includes ginger along with black cohosh, blue cohosh, and birthroot (*Trillium* spp.) to "initiate labor, strengthen contractions, unstall and stimulate labor, deal with exhaustion during labor, expel the placenta, and help control postpartum hemorrhage." Ginger's implied role is to increase energy available to the uterus [5]. With this in mind, we would recommend the revitalizing use of ginger root for calming uterine spasms rather than inducing labor. Its ability to be used as a relaxant [36] as well as possessing no central nervous system adverse effects when orally ingested supports its reliability for use in labor [37, 38].

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7. References

1. Thirumala-Devi K, Mayo MA, Reddy G, Emmanuel KE. Larondelle Y, Reddy D. Occurrence of ochratoxin A in black pepper, coriander, ginger and turmeric in India. Food Additives and Contaminants. 2001; 18(9):830-835.
2. Ginger production in 2014, Crops/Regions/World/Production Quantity (from pick lists)". Food and Agriculture Organization of the United Nations, Statistics Division. 2014. http://www.fao.org/faostat/en/#search/GingerRetrieved 14 June 2017.
3. Duke, JA. 2000. The Green Pharmacy Herbal Handbook. Rodale Inc, 110-112.
4. Grzanna R, Lindmark L, Frondoza CG. Ginger - an herbal medicinal product with broad anti-inflammatory actions. J Med Food. 2005; 8(2):125-132.
5. Weed SS. 1986. Wise woman herbal for the childbearing year. Ash Tree Publishing, Woodstock, NY, 66-67, 153-154.
6. Langner E, Greifenberg S, Gruenwald, J. Ginger: history and use. Advances in Natural Therapy. 1997; 15(1):25-44.
7. Ding M, Leach M, Bradley H. The effectiveness and safety of ginger for pregnancy-induced nausea and vomiting: a systematic review. Women and Birth. 2013; 26(1): e26-e30.
8. Ghayur MN, Gilani AH. Pharmacological basis for the medicinal use of ginger in gastrointestinal disorders. Dig Dis Sci. 2005; 50(10):1889-1897.
9. Ghayur MN, Gilani AH. Inhibitory activity of ginger rhizome on airway and uterine smooth muscle preparations. Eur Food Res Technol. 2007; 224(4):477-481.
10. Allen E. The oestrus cycle in the mouse. Am J Anat 1992; 30:297-371.
11. 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. Horm Cancer. 2010; 1(3):146-155.
12. Burger H, Healy D, Vollenhover B. The ovary: basic principles and concepts. Endocrinology and Metabolism. McGraw Hill, USA, 2001.
13. Kitchen I. Textbook of *in vitro* practical pharmacology. Blackwell Scientific Publication. London, England, 1984, 32-38.
14. 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.
15. Borrelli F, Capasso R, Pinto A, Izzo A. Inhibitory effect of ginger (*Zingiber officinale*) on rat ileal motility *in vitro*. Life Sciences. 2004; 74(23):2889-2896.
16. Yamahara J, Rong HQ, Iwamoto M, Kobayashi G, Matsuda H, Fujimura, H. Active components of ginger exhibiting anti-serotonergic action. Phytotherapy Research 1989; 3(2):70-71.
17. Huang Q, Iwamoto M, Shunji A, Tanaka N, Tajima K, Yamahar J, *et al*. Anti-5-hydroxytryptamine 3 effect of galanolactone, diterpenoid isolated from ginger. Chem Pharm Bull. 1991; 39(2):397-399.
18. Qian DS, Liu ZS. Pharmacologic studies of antimotion sickness actions of ginger. Chin J Integr Med.1992; 12(2):95-98.
19. Woolley DW. A probable mechanism of action of serotonin. Proc Nat Acad Sci. 1958; 44(2):197-201.
20. Kiuchi F, Shibuya M, Sankawa U. Inhibitors of prostaglandin biosynthesis from ginger. Chem Pharm Bull. 1982; 30:754-757.
21. Kiuchi F, Iwakami S, Shibuya M, Hanaoka F, Sankawa U. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. Chem Pham Bull. 1992; 40:387-391.
22. Akah, PA, Oli AN, Enwerem, NM, Gamaniel K. Preliminary studies on purgative effect of *Carica papaya* root extract. Fitoterapia, 1997; 68(4):327-331.
23. Bristol B. The contractile effects of *Quillaja saponin* on smooth muscle tissue isolated from the uterine horns of *Mus musculus* [B.S. thesis]. 2017. Bethel University, St. Paul, MN.
24. Baumann E, Stoya G, Völkner A, Richter W, Lemke C, Linss W. Hemolysis of human erythrocytes with saponin affects the membrane structure. Acta Histochemica. 2000; 102(1):21-35.
25. Das TK, Banerjee D, Chakraborty D, Pakhira MC, Shrivastava B, Kuhad RC. Saponin: Role in animal system. Veterinary World 2102; 5(4):248-254.
26. 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.
27. DiCarlo G, Autoire G, Izzo AA, Mascolo, Viola P, Diurno V, *et al*. Inhibition of Intestinal Motility Secretion by Flavonoids in Mice and Rats: Structure-activity Relationships. J Pharm Pharmacol. 1993; 45:1054-1059.
28. Fanning M, Macander P, Drzewiecki G, E Middleton. Quercetin inhibits anaphylactic contractions in guinea pig ileum smooth muscle. Int Arch Allergy Immunol. 1983; 71:371-373.
29. Govindarajam, VS. Ginger: chemistry, technology, and quality evaluation; parts 1 and 2. CRC Crit Rev Food Sci Nutr. 1982; 17:189-258.
30. Sakanura F. Changes in volatile constituents of *Zingiber officinale* during storage and cultilvation. Phytochemistry 1987; 26: 2207-2212.
31. Belew C. Herbs and the childbearing woman. J Nurse-Midwifery. 1999; 44(3):231-252.
32. McFarlin BL, Gibson MH, O'Rear J, Harman P. A national survey for herbal preparation use by nurse-midwives for labor stimulation: review of the literature and recommendations for practice. J Nurse Midwifery. 1999; 44(3):205-216.
33. Hachfeld A. The contractile effects of *Actaea racemosa* (black cohosh) on *Mus musculus* uterine tissue *in vitro.* [B.S. thesis]. 2010. Bethel University, St. Paul, MN.
34. Olson AE, DeGolier TF. Contractile activity of *Rubus idaeus* extract on isolated mouse uterine strips. BIOS. 2016; 87(2):39-47.
35. Quam N, Wu K, DeGolier T. Aqueous extracts of castor seed (*Ricinus communis)* increase the contractile activities of mouse uterine tissues *in vivo*. J Pharmacogn Phytochem 2016; 5(4):40-45.
36. Ernst E. Herbal medicinal products during pregnancy: are they safe? Int J Obstet Gyn. 2002; 109(3):227-235.
37. Fischer-Rasmussen W, Kjera SK, Dahl C, Aspring U. Ginger treatment of hyperemesis gravidarum. Eur J Obstet Gynecol Reprod Biol. 1990; 38:19-24.
38. Fulder S, Tenne M. Ginger as an anti-nausea remedy in pregnancy the issue of safety. HerbalGram, USA, 1996.