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The influence of common cuisine spices such as ajwain, cumin, dill, fenugreek, and papaya on the contractile behaviors of isolated strips of mouse uterine tissue

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Abstract
Pregnant women, as they prepare for labor, often seek herbal treatments as a more natural and holistic approach to medicine. This study used isolated uterine tissues from mice to investigate smooth muscle contractility in response to Indian Ayurveda herbals. The herbals chosen are typically used in the Indian cuisine, and are also used to stimulate the gastrointestinal tract. The extracts were ajwain, cumin, dill, fenugreek, and papaya. All produced contractile forces that were greater than the tissues own spontaneous motility ($p < 0.001$). At similar concentrations, fenugreek produced the strongest contractile forces and cumin the weakest (90.4% and 58.5% of individual oxytocin control responses, respectively). These results provide support for the rural and traditional use of these herbals in facilitating childbirth. These results also create an awareness of the uterotonic properties of common spices, information which might be applicable to the treatment plans of midwives and other medical practitioners.

Keywords: Herbals extracts, uterine contractions, mice, *in vitro*

1. Introduction

1.1 Background

Inducing and augmenting labor is potentially challenging when delivering a baby. In developing countries, over 80% of women rely on natural and herbal medicine to aid them in their labor [1]. In a study [2] using an *in vitro* human uterine collagen model, nine different herbal extracts from Nigerian plants (*Calotropis procera*, *Commelina africana*, *Duranta repens*, *Hyptis suaveolens*, *Ocimum gratissimum*, *Saba comorensis*, *Sclerocarya birrea*, *Sida corymbosa* and *Vernonia amygdalina*) were analyzed and the investigators found that each of these extracts did produce an increase in contractility in smooth muscle tissues. Such results contribute support that folklore remedies and medicines are effective.

The women in these and several other countries rely on the information that has been passed down traditionally by word of mouth to inform them about the labor experience. In India, this information can come from a Dai, a woman who has chosen a career in assisting in the delivery process. Due to the uncleanliness associated with childbirth and the afterbirth's bodily fluids, there is no formal training for the Dais who are in rural villages [3]. Knowledge is gained through apprenticeship and is passed down from one Dai to another. This means that if there is false information, it may be passed down somewhat blindly since there is a lack of formal input from external empirically derived results. Similarly, any new information has little hope of being passed down because there is limited input of new knowledge being shared with the general public.

The growing demand for natural childbirth methods is fueled by the perception that they are less expensive and safer [4, 5]. Some of the more common and expensive labor aids used in hospital settings, such as misoprostol and pitocin, have common side effects such as hypertension, bradycardia, headache, nausea, vomiting, and rupture of the uterus [6]. Misoprostol is a synthetic prostaglandin E1 analogue that is used to induce labor in pregnant women [7]. Pitocin is a synthetic form of oxytocin that works to stimulate and augment labor [8]. Oxytocin is the main endogenous hormone that stimulates the beginning of labor contractility in the myometrium's smooth muscle, and is responsible for creating the forceful uterine contractions that can evacuate the contents of the uterus [9]. The side effects of misoprostol and pitocin, combined with high costs, have led to an increased interest in lower cost holistic approaches to facilitating labor, and a movement away from approaches used in hospital-centered medicine [1].

1.2 Objectives
In this research project, the efficacy of four spices and one fruit in inducing uterine contractions were tested in an animal model system. It was anticipated that *Trachyspermum ammi* (ajwain), *Cuminum cyminum* (cumin), *Anethum graveolens* (dill), *Trigonella foenumgraecum* (fenugreek), and *Carica papaya* (papaya) would augment or initiate contractions in isolated uterine tissue extracted from mice. Since mouse uterine contractions work similarly to those of human uterine contractions, a mouse model system has served as a proxy for evaluating the effectiveness of other proposed herbal uterotonics in humans [10-12].

2. Methods
2.1 Herbal Preparation
Bagged seeds of ajwain, cumin, dill, and fenugreek were supplied from SWAD distributing company, India. The papaya fruit was purchased from a local Asian fruit market near Minneapolis, Minnesota.

To create a seed extract, 1.8 grams of the seed of interest was ground in a coffee bean grinder and mixed for 30 minutes with 100 mL of deionized water that had been boiled. The mixture was then vacuum filtered through Whatman filter paper into a Buchner funnel to remove the seed particles from the liquid [13]. For the papaya, the fruit pulp was pulverized in a blender and strained through cheesecloth. All treatments were prepared fresh for use within 24 hours of the experiment.

2.2 Tissue Preparation
Twenty virgin female mice (*Mus musculus*) were supplied from Envigo, Inc (Indianapolis, Indiana, USA). The mice were housed as pairs and cared for at Bethel University's animal facilities, and received standard care, food, and water based on the guidelines approved by their Institutional Animal Care and Use Committee.

Twenty-four hours before the experiment, each experimental mouse was given an injection of diethylstilbestrol (DES). DES brings the uterine tissue to hormonal levels that would be similar to that of a pregnant mouse. It results in more visible vascularization of the uterine horns, which allows for a faster identification and removal from the pelvic cavity [14]. On the day of the experiment, each mouse underwent CO_2 asphyxiation and the uterine horns were surgically removed and put into a petri dish that contained cold De Jalon's solution. Fresh De Jalon's solution was made for each experiment and was composed as follows (g/4L): 36 NaCl, 1.68 KCl, 2 $NaHCO_3$, 2 D-glucose, and 0.32 $CaCl_2$ [15].

Each uterine horn was then tied with a suture at each end and hung in an organ bath filled with 20 mL of De Jalons solution. One end was tied to a stationary metal rod and the other end was tied to a PowerLab force transducer (AD Instruments, Colorado Springs, Colorado, USA). Each uterine horn was equilibrated at 0.8g tension for one hour with fresh De Jalon's solution washes every fifteen minutes. A 95% O_2/ 5% CO_2 supply aerated the organ bath during the entire experiment [15]. A PowerLab data acquisition system (AD Instruments, Colorado Springs, Colorado, USA) recorded the changes in contractile force throughout the experiment.

2.3 Application of the Treatments
In order to test for the viability of the uterine tissues, and to normalize contractions based on the size of the available uterine tissues, oxytocin (10^{-5} M) was applied as a positive contractile control and established a baseline for what a normal contractile waveform would look like. The effects of oxytocin were recorded for approximately 10 minutes, followed by multiple washes of De Jalons in an effort to remove all traces of oxytocin from the organ bath, and to bring the contractile forces back down to the initial baseline prior to the application of the herbal treatment.

Any herbal treatment was pipetted directly onto the tissue and contained either a low extract concentration in 0.833 mL, or a high extract concentration in 1.6 mL of the stock volume. Final concentrations of the different herbals in the bath were variable due to constituent solubilities unique to each of the seed species. For example, a volume of 0.833 mL ajwain contained 0.64 mg/mL, cumin contained 0.31 mg/mL, dill contained 0.29 mg/mL, and fenugreek contained 0.59 mg/mL. The concentrations were considered doubled for 1.6 mL volumes.

Contractile data was recorded for approximately thirty minutes after the herbal was administered. Upon occasion, when two treatments were administered, a De Jalons wash was done after the first treatment and the tissue was equilibrated for fifteen minutes prior to the second treatment application.

2.4 Statistical Analyses
Contractile forces were measured and presented as herbal treatment means ± SE. Data was analyzed for differences among the treatment means using an ANOVA, and differences were considered significant at $p \leq 0.05$. Data was also analyzed using t-tests to compare contractile responses from each herbal extract to its own spontaneous motility.

The contractile forces that were produced during spontaneous motility or from the oxytocin and herbal treatments were measured from the baseline of the resulting waveform to the highest force produced within five minutes of treatment application. Data was recorded as mN of force for the oxytocin, herbal treatments, and the spontaneous motility. Both the spontaneous motility and the herbal treatments were further analyzed as a percent of each tissue's initial oxytocin response.

3. Results
3.1 Smooth muscle waveform responses: spontaneous motility, oxytocin, herbal treatments
All of the individual herbal treatments tested did produce contractile forces that were significantly greater than those exhibited by the tissue's own spontaneous motility ($p < 0.05$). Fig. 1 illustrates a typical contractile waveform produced by fenugreek. All herbal treatments contracted the tissues quickly, produced a strong contractile force usually within five minutes, and then decreased the contractile force back to its pre-treatment baseline.

At the concentrations tested, all of the herbal treatments produced tissue contractile forces less than their oxytocin controls. These contractile peaks were short lived, and unlike contractile responses evoked from oxytocin, did not maintain a contractile plateau (Fig. 1).

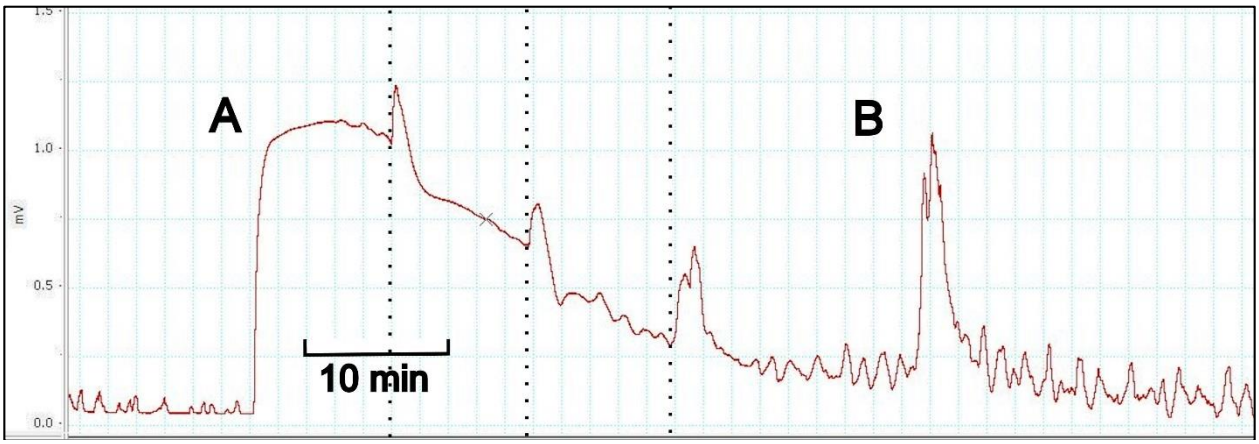

Fig 1: This figure illustrates a typical contractile waveform produced by fenugreek. Baseline spontaneous motility (5.9 mN) can be observed prior the tissue's response to 10^{-5} M oxytocin (letter "A"; 46.3 mN). The three dotted lines represent tissue washouts. The contractile peak observed following letter "B" (37.5 mN) was produced after applying 1.18mg/ml fenugreek onto the uterine tissue. The force produced by fenugreek at this concentration was 80.9% of the peak oxytocin response for this tissue. The vertical axis represents contractile force in mV, later converted to mN.

3.2 Change in uterine contractile activity in response to herbal treatments

All herbal treatments (both high and low concentrations) increased the contractile forces relative to spontaneous motility (p<0.001). While the higher concentration of each herbal did produce a greater contractile force than the lower concentration, the differences were not significant except for fenugreek applications (p = 0.035). This result may indicate that the ajwain, cumin, and dill concentrations may have already reached their maximal contractile response. Since the 1.18 mg/ml fenugreek concentration yielded a statistically greater contractile force then the 0.59 mg/ml concentration (90.4 ± 8.4% OXY, n = 4; 58.1 ± 7.9% OXY, n = 4, respectively), additional trials pursuing a maximal concentration-response curve warrants further research. To employ a larger sample size and to stay within the scope of the project reported herein, contractile responses yielded from both high and low concentrations within each herbal were pooled together.

Contractile forces as a measure of spontaneous motility were 23.7 ± 3.7% OXY (n = 31). Contractile forces produced from ajwain concentrations (0.64, 1.28 mg/mL) were 68.3% ± 8.8 OXY (n = 8), from cumin (0.31, 0.62 mg/ml) were 49.3 ± 9.4% OXY (n = 6), from dill (0.29, 0.58 mg/mL) were (58.1 ± 8.9% OXY (n = 9), and from fenugreek (0.59, 1.18 mg/mL) were 74.2 ± 8.2% OXY (n = 8). Among the herbal treatments themselves, only fenugreek produced contractile forces statistically greater than those produced by cumin (Fig. 2).

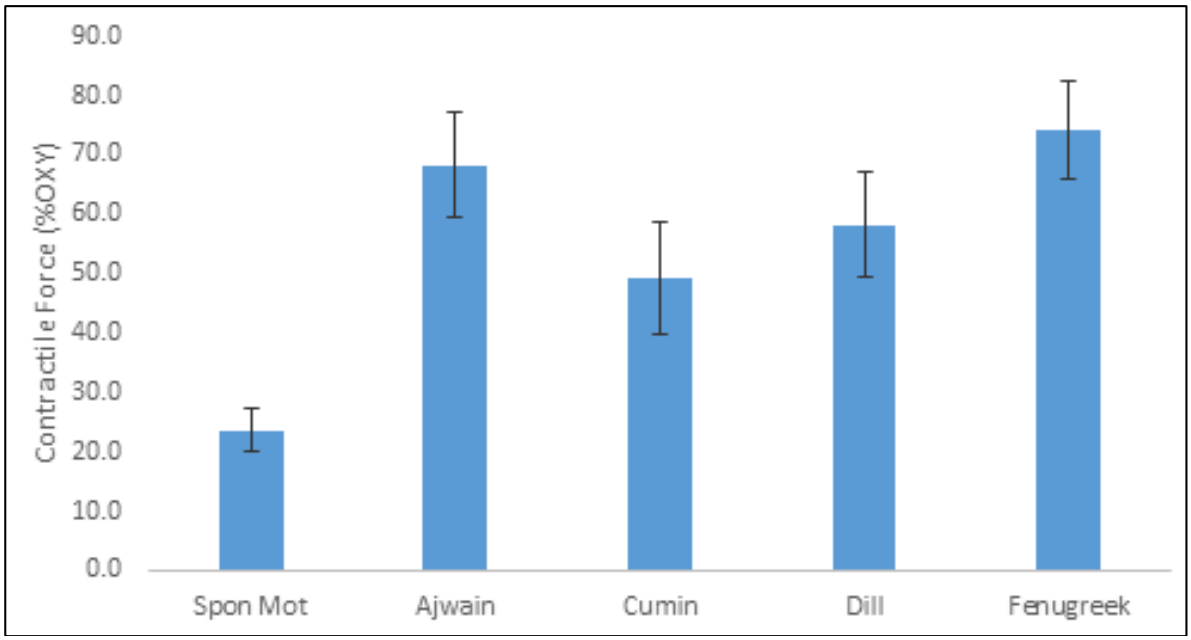

Fig 2: Means ± SE of uterine horn contractile forces presented as a percentage of the tissue's maximal oxytocin response. All treatments increased contractile force relative to spontaneous motility (Spon Mot, p < 0.001). Among the herbal treatments themselves, only fenugreek produced a contractile force statistically greater than those produced by cumin.

3.3 Change in uterine contractile activity in response to papaya fruit pulp

Papaya fruit juice increased contractile forces when compared to those of baseline spontaneous motility (32.7 ± 7.1% OXY; n = 14) following the administration of 100 µL (53.8 ± 20.9% OXY; n = 3), 200 µL (63.4 ± 13.8% OXY; n = 5), and 400 µL (70.7 ± 7.2% OXY; n = 6). Both the 200 µL and 400 µL volumes produced a significant increase in contractile force when compared to overall spontaneous motility (p<0.001; Fig. 3).

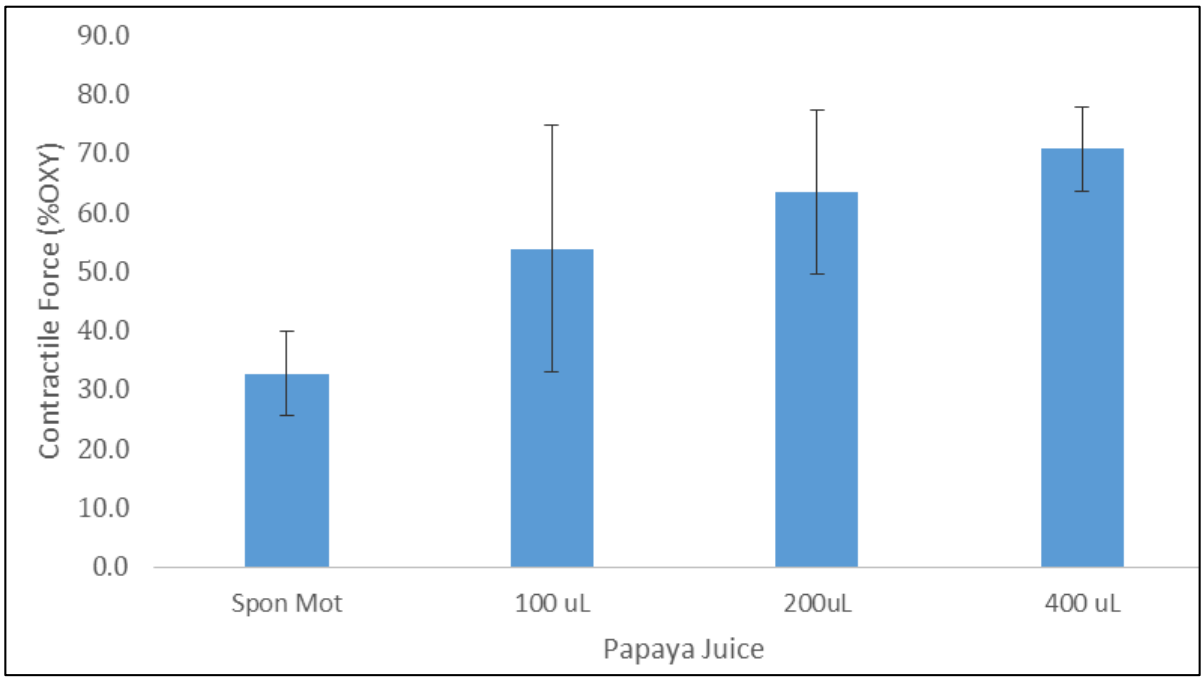

Fig 3: Means ± SE uterine contractile force (% OXY) in response to increasing volumes of papaya juice. Each increase in volume resulted in a more forceful tissue contraction. The 200 µL and 400 µL volumes produced by papaya were statistically greater than those of spontaneous motility (Spon Mot, $p<0.001$).

4. Discussion

4.1 A positive uterine contractile response from ajwain, cumin, dill, fenugreek, and papaya treatments

The results of this research project demonstrated that ajwain, cumin, dill, fenugreek, and papaya treatments all produced significant increases in contractile force in isolated mouse uterine tissues when compared to the tissue's own spontaneous motility. These results do provide support for the rural and traditional uses of these same herbals in facilitating childbirth.

4.2 Biological constituents and interactions with smooth muscle

Most of what is known however, about the biological constituents, their efficacy, and the safety of ajwain, cumin, dill, fenugreek, and papaya, is based upon studies in which these herbals were employed as treatments for other medicinal concerns.

Ajwain seeds have been used to reduce stomach aches and to serve as a mild laxative, yielding small but regular contractions which increases the tone of the muscle [16]. A different study using smooth muscle samples harvested from ileum, tracheal, and bronchial tissues also showed increased contractile force and frequency [17]. Ajwain has been used to reduce menstrual cramps and possibly, to tone the uterus in the months prior to delivery [16]. This may be supported by the awareness that ajwain does contain an abundance of saponins [18] which have been shown to contract isolated uterine smooth muscle [10]. However, there is some evidence which indicates that ajwain may also have the opposite effect on other sources of smooth muscle. For example, ajwain has been shown to reduce contractile forces as observed in vascular smooth muscle cells of mice [19].

In the literature, seed extracts from cumin have primarily been reported to interact with smooth muscle in a relaxant manner as seen in the reduction of colic [20], nausea [21], and the relaxation of guinea pig tracheal tissues [18]. Although several biological constituents have been isolated from cumin [22], receptor antagonism studies have not yet determined which receptor(s) are activated by the cumin constituents. Although the results herein indicate that there is some aqueous constituent of the seed extract that does produce smooth muscle contraction in isolated strips of uterine tissue, the measured responses produced by cumin were the lowest when compared to the other four herbal extracts tested. The result reported in Fig. 2 show that the contractile forces produced were slightly less than 50% of the positive contractile control.

Dill seeds contain tannins and have certain polyphenols that produce contractile behaviors in smooth muscle tissues [6]. Dill seed releases oxytocin [6] which stimulates uterine contractions, increases contractile frequency, and shortens the first stage of labor when the contractions begin, favoring dilation of the cervix [23]. An aqueous extract of dill seed is typically consumed at the very beginning of labor to shorten the duration of the first stage [6]. Dry seeds are added to boiling water and the resulting tea is consumed after a few minutes of steeping [6].

At comparable stock solution concentrations, fenugreek aqueous extracts produced the largest uterine contractile forces (see Fig. 2). A study using ultraviolet-visible spectroscopy recently found that oxytocin is produced in fenugreek seeds [24]. It seems likely then, that any consumed seeds should stimulate uterine tissue and help induce childbirth [1]. The medical usage of fenugreek is commonly employed in Egypt, India, and Pakistan, and includes treatments for bronchitis, tuberculosis, asthma, ulcers, constipation, and other digestive problems. In the Middle East, fenugreek is used to treat diarrhea and gastroenteritis [25] as well as aide in the treatment of menstrual cramps [26]. The effectiveness of fenugreek used in this way reinforces its proposed interactions with the smooth muscle in the gastrointestinal, respiratory, or reproductive tracts. This makes sense, as fenugreek has also been shown to contain saponins [25] which have been demonstrated to contract isolated uterine smooth muscle [10].

Papaya fruit is believed to have powerful abortive abilities. It is common in India, especially South India, for women to be discouraged from eating papaya when they are pregnant. The

belief is that the abortive properties come from papain, an enzyme that is within the fruit itself. Papain can be procured from the latex of the fruit and can be used in performing abortions by applying the crude papaya latex intra-vaginally to the opening of the uterus [27]. One study found that crude papaya latex as found in unripe papaya fruit did cause tetanic contractions in pregnant rat specimens, but no significant contractions were observed from ripe papaya [28]. These results suggest that it would be safe for a pregnant woman to eat ripe papaya, but that the danger might lie in the un-ripened fruit. It is important to note that the papaya extracts used in the project herein tested the actual fruit, and not an individual constituent. Furthermore, this papaya sample was from a ripened orange papaya fruit, not un-ripened green papaya fruit.

In a recent study employing a similar experimental design, juice from another tropical ripened fruit Calabash (*Crescentia cujete)* collected from Guatamala, also produced contractions in isolated strips of uterine tissues similar to those produced with the same volume applied herein from papaya [12]. In that project, it was proposed that the role of linoleic acid in calabash seed extract may contribute to the uterine contractile activity. Linoleic acid is a precursor for prostaglandins, so it is reasonable to believe this could be triggering the smooth muscle contraction of the uterus and inducing labor. Interestingly, linoleic acid has also been isolated in papaya seeds [29]. It would seem reasonable that linoleic acid in papaya seed extract may also contribute to the uterine contractile activities as well.

4.3 Future considerations

The herbals of interest in this investigation are commonly used spices that are part of daily Indian and Chinese cuisines. The available chemical constituents of these herbals may already be at work following their ingestion, assuming that they are absorbed and find their way to uterine smooth muscle. Thus, it would be beneficial to transfer the observable effects from isolated tissues to a human whole-body model. Several *in vitro* investigations remain that could build upon the results reported herein, furthering an understanding of the efficacy of these herbal treatments.

Since the null hypothesis can be rejected, the alternative hypothesis can be accepted indicating that these herbals do contract the isolated uterine tissues. A next step is to determine if the uterine contractile responses are dependent on the concentration of the herbals. The application of decreased and increased concentrations of the herbal will help determine (1) the minimal concentration needed to yield the initial (or threshold) response, (2) the minimal concentration needed to yield the maximal response, and (3) the concentration required to contract the tissues at 50% of their maximum response (EC_{50}). It is expected that concentration response curves for these herbal extracts would not be the same, as it has already been stated that each herbal was observed to have different solubilities, and pending what receptors (if any) they interact with may factor when saturation is reached.

A further study could determine which receptors the biological constituents are interacting with. This is challenging when working with an herbal as it may have hundreds of chemicals, some binding to receptors, some creating potential interference, and some even interacting in a synergistic manner. The most obvious strategy here would be to test one isolated proposed biologically active constituent at a time, and test for its contractile effect with and without selective receptor antagonism.

In addition, the bioavailability of the seed constituents *in vivo* cannot be assumed based on results obtained using isolated tissue models alone. When consumed, these constituents may be degraded by gastric juices. If they are absorbed, they might also be rendered useless via enzymatic action in the blood [30]. Protocols that can determine if any of the ingested biological constituents of interest can be found in either the blood or urine would affirm their absorption.

4.4 Conclusions and recommendations

The results of this research project demonstrated that ajwain, cumin, dill, fenugreek, and papaya treatments all produced significant increases in contractile force in isolated mouse uterine tissues, when compared to the tissue's own spontaneous motility. These results provide support for the rural and traditional uses of these herbals in facilitating childbirth, and may help women decide whether to augment their labor by consuming these herbals. Additionally, the awareness of the uterotonic properties of these common ingested spices may be insightful to midwives and other health practitioner's treatment plans.

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