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The contractile effects of purple holy basil (*Ocimum Tenuiflorum*) on isolated mouse uterine muscles

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Abstract
For centuries, herbal agents have been used in the practice of midwifery to holistically induce labor and reduce the stress of post-term labor. This research specifically evaluated purple holy basil (*Ocimum tenuiflorum*), a species of basil found primarily in tea and prepared with other varieties of basil collectively referred to as Tulsi. Several concentrations of this herb were prepared as aqueous extracts and applied to isolated mouse uterine smooth muscle tissues and tested in an organ bath. Following analysis, it was found that the contractile forces produced by the herb were significantly greater when compared to the tissues own spontaneous motility ($p<0.0001$). Higher concentrations of *O. tenuiflorum* (0.274-1.121 mg/mL) were equal to or greater than contractile forces produced by the positive control, oxytocin (10^{-5} M). These results support that purple holy basil does have a contractile effect on isolated strips of uterine smooth muscle.

Keywords: Purple holy basil (*Ocimum tenuiflorum, Ocimum sanctum*), uterus, smooth muscle, *in vitro*, labor

Introduction
Human female anatomy is a highly organized and meticulous system, specifically in its supportive function of the physiological regulation of uterine smooth muscle contractility. Smooth muscle plays a vital role in regulating homeostasis, as it is able to sustain contractions much longer as necessary in the instance of labor [1]. This process, however, can become complicated when the contractile forces are not strong enough to favor reaching the threshold needed for full cervical dilation and/or uterine pushing action. These instances of dysfunctional labor can cause tremendous stress on both the mother and her offspring and are the leading cause of emergency Cesarean sections [2]. In order to increase uterine contractile forces, doctors often treat their patients with small and steady concentrations of oxytocin, the same hormone naturally produced during childbirth to initiate or elevate uterine contractions [3]. The use of herbal remedies is not novel in the world of midwifery, as they have been used in varying capacities to treat an array of labor symptoms and pains [4]. In addition to the versatile nature of herbs in their availability, they are also often much more affordable in comparison with other methods including the costs of hospitalization and delivery drugs. While these plant derivatives can be cheaper and widely available, there is also a lack of data and empirical evidence to draw conclusions regarding their effectiveness or potential safety hazards associated with treatments [5]. Certain studies do warn of falling for the implicit belief that the organic nature of herbal remedies automatically correlates to increased safety. Forward progress is needed to gather a better understanding of the complex pathophysiology as well as pharmacology involved in the use of herbal treatments during labor [6].
In spite of the lack of causal evidence, the exploration of herbal medicines in relation to inducing labor continues to persist and they are considered safe in the terms of the recommended methods in which they are applied [4]. However, safety allegations are often directed towards Pitocin, a synthetic form of oxytocin that works as an agent to stimulate and augment labor. This is often at a cost to the health of the mother and her child [7], and can include nausea, vomiting, abdominal pain, memory problems, convulsions, and in serious cases excessive bleeding post childbirth [8].
An example of a more holistic approach by midwives is to induce labor by using herbal treatments. One such herbal is purple holy basil, *Ocimum tenuiflorum*, also referred to as *Ocimum sanctum*. It is a common garden herb and ingredient native to India that is used frequently in the preparation of teas, meals, or other substances of nutritive value [9]. While most associate this edible herb and many others, such as parsley, oregano, and culinary varieties of basil, as simple common seasonings for the kitchen, there are some traditions and

undocumented sources stemming from the Middle Ages and onward that suggests these plant varieties have also been used as cheap and widely available abortifacients [10]. Other studies have shown that purple holy basil might serve multiple purposes, one of which is for antifertility treatments [11].

In order to evaluate the efficacy of herbal remedies in humans, studies utilizing a mouse model system have been shown to undergo smooth muscle contractile pathways very similar to mechanisms as understood in human models [12]. The aims of this study were to support or refute the claims of uterine contractile properties as observed in the common garden herb purple holy basil, based on the appearance and strength of contractile forces within mouse uterine muscle tissues. In doing so, it is imperative that the study be able to either provide some validity or at the very least, more support towards these suggestions of herbal remedies in relation to uterine contractile effects.

Materials and Methods

The procedures followed in this research project reported herein are modeled from those published by Quam [13] with a few modifications.

Preparing Aqueous Extracts of *Ocimum tenuiflorum*

Purple holy basil (*O. tenuiflorum*) was obtained from Richters Herbs (Ontario, Canada). The dried leaves of this herb were blended into a fine powder, and from this powder, 1.8 g was dissolved into 100 mL of boiling deionized H_2O, stirred and heated on low heat for 10 minutes. Undissolved particles were filtered out using vacuum filtration and left to dry for 24 hours in order to determine their dry mass. The remaining filtrate was then transferred to a graduated cylinder where its final volume was recorded before applying the solution to tissues. For each experiment a volume of known concentration was pipetted directly onto isolated uterine tissue.

Specimens and Tissue Preparation

Sixteen virgin female laboratory mice, *Mus musculus* were obtained from Envigo (Indianapolis, Indiana, USA) and kept in cages located in the rodent room within the Bethel University Department of Biological Science (St. Paul, Minnesota, USA). All mice were handled in an ethical manner, given proper care (food, water, and shelter) following the guidelines established by Bethel University's Institutional Animal Care and Use Committee. The females used in these experiments were between four to six months of age.

Twenty-four hours before an experiment, mice were given an injection of 0.2 mg of diethylstilbestrol, a synthetic estrogen agonist used to bring the mice into the estrus stage of their estrous cycle [14]. This ideally increases the responsiveness of the smooth muscle in the uterine tissues by increasing the number of gap junctions [15].

On the day of an experiment, two mice were euthanized utilizing carbon dioxide asphyxiation and dissected via a ventral abdominal incision to allow for the removal of the two uterine horns. These tissues were then placed in a 20 mL organ bath containing freshly made DeJalons Ringer's solution (in 6L of deionized H_2O: 54 g of NaCl, 2.52 g KCl, 3 g $NaHCO_3$, 3 g D-glucose, and 0.48 g $CaCl_2$) which served to mimic the extracellular fluid conditions that these tissues would be exposed to *in vivo* [14].

Tissue Bath Testing

In order to collect uterine contractile forces and convert them into visible and measurable waveforms, a 50 g force transducer instrument was connected to a PowerLab Data Acquisition System (AD Instruments, Colorado Springs, Colorado, USA). One end of each uterine horn was sutured and tethered to the force transducer, and the other end was tied with a suture to a stationary rod. The contractile force produced between the knots on either end of the suspended uterine horn was held at 0.8g of tension [14] in the organ bath, and allowed to equilibrate for up to one hour, during which time they were flushed with fresh DeJalons solution every 10-15 minutes. After this, 10^{-5} M of oxytocin was applied to the tissues to act as the positive contractile control. The resulting contractions were recorded for ten minutes and this ensured proper viability of the tissue. The tissues were then washed out (flushed) with fresh DeJalons solution (1-2x) to allow for any remaining oxytocin to be removed from the organ bath. Then a ten-minute re-equilibration period followed and various single concentrations of *O. tenuiflorum* were added and their responses were observed for an average of 15-20 minutes.

Statistical Analysis

Contractile waveforms were generated during periods of spontaneous motility, following the addition of oxytocin, and following the addition of an aqueous extract of purple holy basil at a given concentration. Resulting waveforms were measured from their baseline prior to treatments to the peak contractile force produced during their treatments. The recorded values were divided by a calibration constant (g), then multiplied by gravitational force (9.81 m/s) to derive the contractile forces (mN). For each herbal concentration applied (mg/mL), the mean (±SE) of the maximal contractile responses were calculated for both the raw contractile force (mN) as well as a percent of their controlled oxytocin response. ANOVA tests were run for both sets of data (mN, % Oxy). Following each ANOVA, a Tukey Kramer test was used to evaluate any significant differences among the contractile forces as a function of the herbal concentrations used. Differences were considered to be significant at $p \leq 0.05$.

Results

Smooth Muscle Activity

Each tissue included in these results showed spontaneous motility before any treatment, and also demonstrated a standard contractile response to treatment with oxytocin (Figure 1). A 0.561 (mg/mL) of *O. tenuiflorum*, (administered at letter B) surpassed the full contractile force of oxytocin (administered at letter A), but was observed to quickly lose contractile strength and within ten minutes returned to spontaneous motility with a basal tonus similar to pre-herbal treatment.

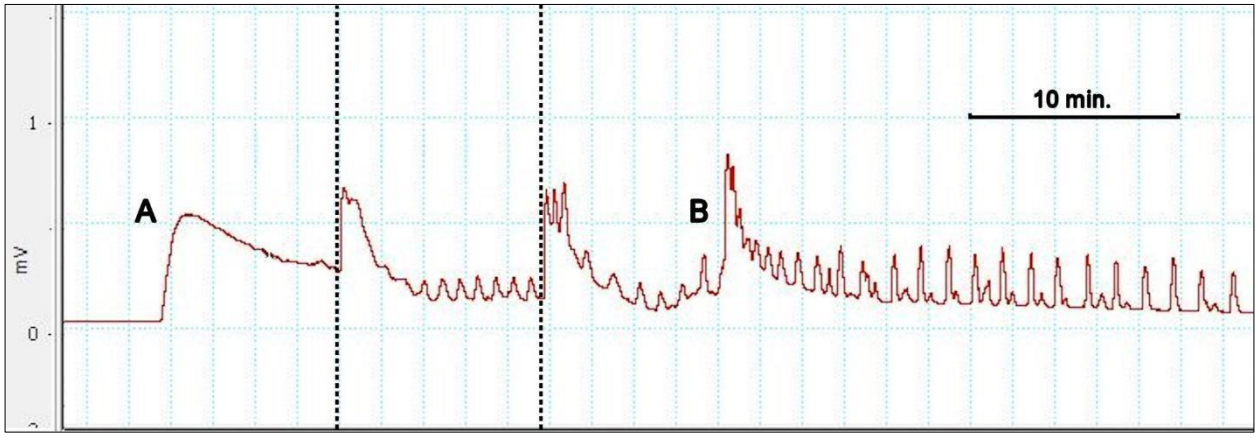

Fig 1: Typical contractile waveform patterns, showing mouse uterine smooth muscle tissue responses to 10^{-5} M oxytocin (A), spontaneous motility, here, appearing after tissue washouts (dotted lines), and a response to 0.561mg/mL aqueous *O. tenuiflorum* (B). For analysis the default y-axis (mV) was later converted to mN based on earlier calibrations with force transducer values (mV). Contractile forces at letter A were 21.8 mN and at letter B, 27.3 mN.

Smooth Muscle Response to *O. tenuiflorum*

As illustrated in Figure 2, treatments with *O. tenuiflorum* were shown to produce variable contractile responses depending on the applied concentration. As concentrations increased, contractile responses also increased but appeared to level off while approaching the final concentration used (1.121 mg/mL). Each treatment did produce an increase in contractile force when compared to the tissues overall spontaneous motility ("0" treatment). When compared among each other, the herbal concentration means that produced the highest contractile responses ranged from 0.274-1.21 mg/mL and were all significantly greater than their own spontaneous motility (p<0.0001). Based on the concentrations used in this investigation, 0.136 mg/mL was observed to be the threshold for a significant increase in contractile force. Interestingly, the highest concentrations used (0.274-1.21 mg/mL) were not significantly different from each other, but they were greater than the force produced by oxytocin, but not statistically. These same higher concentrations also yielded significantly greater contractile responses than the lower herbal concentration used (0.063 and 0.136 mg/mL).

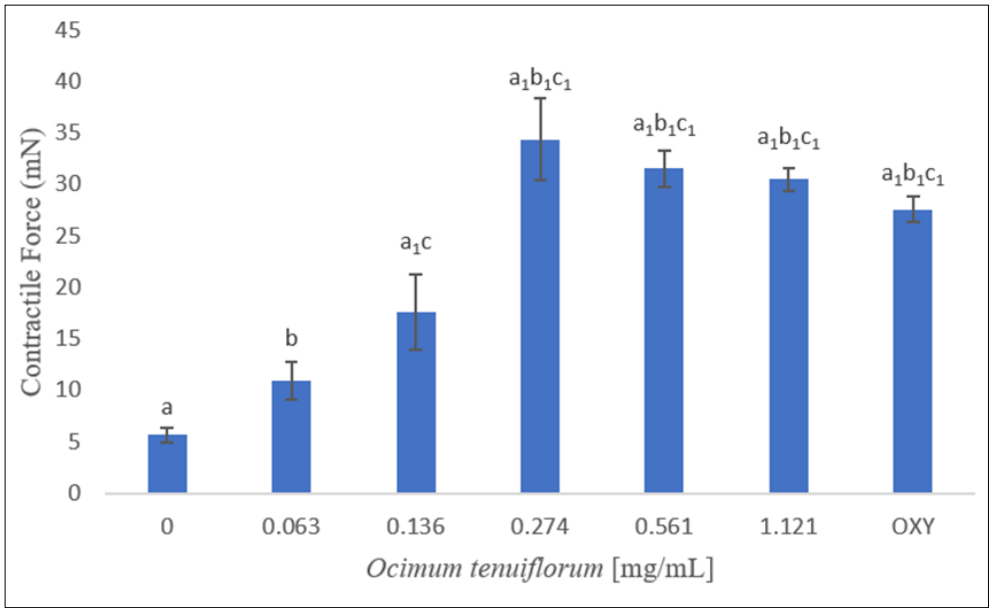

Fig 2: Means (±SE) of uterine contractile forces (mN) in response to increasing concentrations of O. *tenuiflorum* (mg/mL). All treatment concentrations showed an increased response compared to the control tissue, "0" (spontaneous motility). Values with corresponding letters are significantly different from one another (*i.e.* "a" is significantly different from "a_1"; p<0.0001).

Figure 3 illustrates the mean (±SE) contractile forces in response to increasing concentrations of *O. tenuiflorum* (mg/mL) expressed as a percent of their oxytocin control response. The average contractile force for spontaneous motility was 20.08±1.57% OXY (n=27). The contractile responses to the herbal treatments (mg/mL) were as follows: 0.063, 38.85±4.43% OXY (n=4), for 0.136, 61.30±14.45% OXY (n=6), for 0.274, 91.98±8.39% OXY (n=4), for 0.561, 130.13±6.17% OXY (n=8), and for 1.121, 100.52±9.20% OXY (n=5). The ANOVA test indicated that all contractile responses produced by *O. tenuiflorum* concentrations greater than 0.063 mg/mL were significantly greater than the tissues spontaneous motility (p<0.0001), but not from each other. Both purple holy basil concentrations of 0.063 and 0.136 mg/mL produced contractile responses that were significantly lower than the other herbal concentrations of 0.274-1.121 mg/mL.

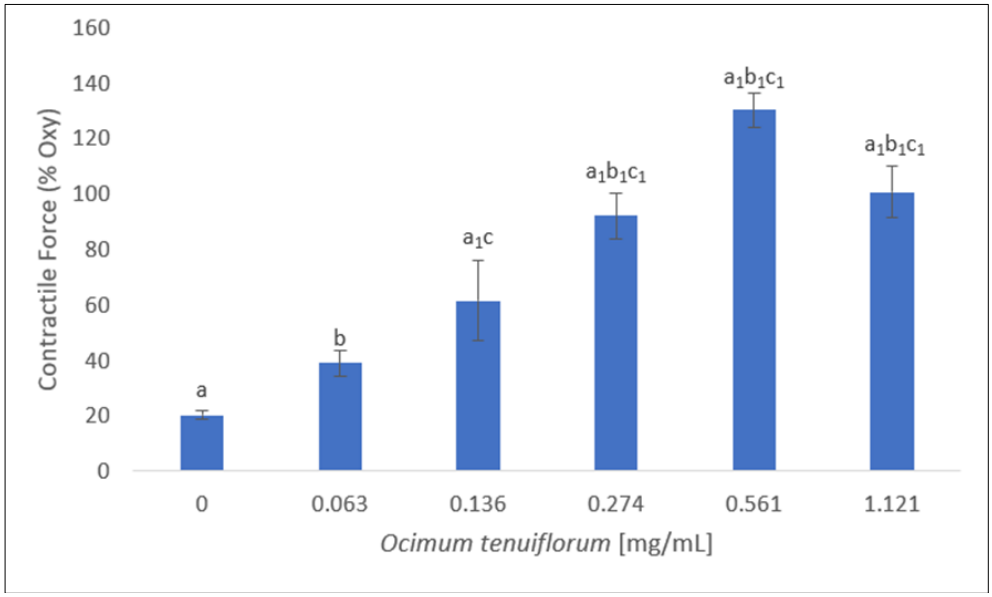

Fig 3: Means (±SE) of uterine contractile forces (% Oxy) in response to increasing concentrations of O. *tenuiflorum* (mg/mL). All concentrations produced an increased contractile response compared to the tissues' spontaneous motility "0" mg/ml. Values with corresponding letters are significantly different from one another (p<0.0001).

Discussion

The results of this study support that concentrations of aqueous *O. tenuiflorum* (0.274, 0.561, and 1.121 mg/mL) produced significant increases in contractile forces from isolated mouse uterine tissues when compared to the tissues' own spontaneous motility (p<0.0001). This data thus provides valuable documentation on the overall contractile function of purple holy basil on isolated uterine smooth muscle. It is also important as it adds additional outcomes for further elaboration in conjunction with other studies using different experimental designs that evaluate other medicinal properties of this herb used in different specimens.

Documented sources from the literature identify many of the chemical constituents that make up the leaves, stems, and buds of *O. tenuiflorum*, synonymously called *O. sanctum* [16]. It was determined that among the variety of *O. tenuiflorum* constituents, the two that were recorded to be the most active were eugenol and ursolic acid [17].

Eugenol alone has been shown to relax isolated strips of rat ileum both by reducing the tissue basal tonus as well as reducing preconstructed tissue tension [18]. Eugenol is considered to be an important bioactive constituent in essential oil therapy as it favors a positive response outcome for gastrointestinal concerns [18]. It has been suggested that eugenol might be used to provide energy to sustain smooth muscle contractions, however, this claim was never supported by any data [11].

Methyl-eugenol, an analog of eugenol [11], has also been identified as the predominant aromatic compound in *O. sanctum* seeds [19] and leaves [20, 21]. In like manner to eugenol, methyl-eugenol is also reported to decrease basal tone and relax pre-contracted isolated guinea pig ileum [22]. This supports the more general claims that methyl-eugenol has muscle relaxant properties [23, 24] and hypotensive effects, the latter likely due to vasodilation [25].

Ursolic has also been shown to relax smooth muscle in rat stomachs [26] and produce vasodilation in rabbit aortic rings and in the coronary vessels of the frog heart [27]. Even Tulsi, the mixture of several varieties of basil (whole herbals), is considered as having bronchodilator effects as relaxes smooth muscles in the throat of asthmatic patients [28].

However, in an investigation using a format similar to the experimental design reported herein, Ma [29] was able to show that ursolic acid alone increased both smooth muscle contractile force and frequency in isolated rat uterine tissues. Additional receptor antagonism activities within the same study indicated that the contractile mechanism may involve local prostaglandin synthesis and/or release.

These aforementioned studies indicate that research results may be contingent on the actual location of the smooth muscle (*i.e.* organ specific) as well as whether individual constituents or the whole herb is used. The potential complex outcomes of constituent potentiation, synergism, and attenuation when applied at the same time may not be the same as that of an individual constituent. This may be further complicated by constituent distribution within the whole herbal pending time of harvest or distribution within the plant.

A secondary plant compound present in many plants are saponins, a type of glycoside observed to display a soap-like foam action when mixed rapidly in an aqueous solution [30]. Saponins from the bark of the South American soap tree, *Quillaja saponaria,* have been shown to produce significant increases in contractile force when applied to uterine horns isolated from mice [31]. Saponins are known to interact with smooth muscle cells in a manner disrupting the lipid bilayer [32]. These disruptions result in invaginations and subsequent pore formation [33] allowing for calcium entry. Saponins are present in purple holy basil leaves [24] and therefore likely have a role as a contractile agent in the research results reported herein.

Implications for Pregnant Women

It is important to note that this study examined the application of purple holy basil directly onto isolated strips of uterine smooth muscle tissue, whereas further study might examine its application *in vivo* through vaginal insertion, oral ingestion, or via topical applications. Pregnant women should consider that the consumption of high concentrations purple holy basil should be avoided due to its potential abortifacient responses. The same concern may also apply when/if using isolates from *O. tenuiflorum* as an essential oil which may have antimicrobial properties and may be taken to boost the immune response [34].

It is also important for pregnant women who take purple holy basil to reduce the stress associated with pregnancy [35]. The eugenol constituent has been shown to reduce isolated cardiomyocyte differential activity indicating that eugenol may have teratogenic behaviors which could be detrimental to the health of the developing fetus [36].

Conclusion

This research supports that at specific concentrations, purple holy basil produces a contractile force equal to that of oxytocin (10^{-5} M). Continuing this research at the investigative level of testing additional isolated bioactive constituents from the leaves of *O. tenuiflorum* can then be used to determine how these components might interact with the variety of receptors as found on the uterine smooth muscle cells themselves. Results from such studies would have the potential to determine whether purple holy basil truly helps, harms, or even has any medicinal effect during and after the labor process.

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