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Brittany Kothari
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

Teresa DeGolier
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

Corresponding Author:
Teresa DeGolier
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, MN 55112, USA

The contractile effects of *Matricaria chamomilla* on *Mus musculus* isolated uterine tissue

Brittany Kothari and Teresa DeGolier

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Abstract
Modern medical practices for labor induction demonstrate success yet sometimes cause unwanted side effects for mothers and infants. Some individuals have turned to herbal remedies to induce labor, replacing current medical practices, such as Pitocin administration. One remedy, German chamomile, *Matricaria chamomilla,* is noted to induce contractions as an oral capsule *in vivo* and hydro-alcoholic extract *in vitro*. However, one of its constituents, α-bisabolol, has been associated with relaxation of smooth muscle tissue *in vitro*. The primary goal of this project was to determine whether or not *M. chamomilla* would contract the isolated uterine horns of mice, and if the contractions were concentration-dependent. Results showed that concentrations of *M. chamomilla* (0.07-1.16 mg/mL) produced contractile forces equivalent to 75% of the tissue's contractile response to the positive control (oxytocin 10^{-5} M). Additionally, *M. chamomilla* produced contractile forces almost 2.5 times greater than the tissues' own spontaneous motility ($p<0.0001$). These outcomes show *M. chamomilla* may augment labor, however, further research is required before it can be utilized with confidence in labor.

Keywords: Uterus, smooth muscle, chamomile, labor, *in vitro*

Introduction
In the United States, from 2000 to 2014 there was an increase in estimated maternal mortality rate from 18.8% to 26.6% in 48 states [1]. However, this same observational study also noted that the World Health Organization reported that from 2000 and 2013, 157 of 183 international countries experienced decreases in maternal mortality rates. Many have tried to explain this increased maternal mortality in the United States as a function of mental health, disease, drugs, obesity, substance abuse, and even race [2], but many are also concerned with the usage of medical drugs during labor.

One way that women have induced labor during pregnancy is by the exogenous use of oxytocin. Oxytocin was used to "speed up" labor from 2012 to 2013 by 31% of women giving birth. Historically, oxytocin has only been used since the early 1900s compared to herbal remedies which date back to the ancient Greeks. Oxytocin is involved to some extent in mood or pain alteration, and to a larger extent, activities such as stress reactivity and maternal attachment behaviors. Although there are strong responses from oxytocin for contracting or augmenting labor, malpractice has caused pharmacotherapy to frequently associate oxytocin with adverse perinatal outcomes [3].

Matricaria chamomilla (German Chamomile)
Many mothers who are concerned about these potential adverse perinatal outcomes have converted to the use of herbal remedies. Many of these herbs are effective in the induction of labor, however there is still a very limited amount of research done, which may lend to a number of safety warnings [4]. One such herb, *Matricaria chamomilla*, is used therapeutically as an antidepressant, antioxidant, antimicrobial, and anti-inflammatory [5]. *M. chamomilla*'s known constituents include numerous phenolic compounds, mainly the flavonoids patuletin, luteolin, quercetin, apigenin, and their glucosides [6]. The main constituent is a terpenoid, α-bisabolol, which has been found to have spasmolytic [7] and contractile properties [8]. These findings have led to a growing interest in *M. chamomilla*'s effects on smooth muscle contractility, and more specifically its potential use as a substitute for synthetic oxytocin during labor.

Previous Work
Numerous studies have been performed *in vivo* and *in vitro* to test *M. chamomilla*'s effects on smooth muscle. Gholami [9] found that a hydroalcoholic extract of *M. chamomilla* primed with estrogen stimulated contractions in post-term pregnant women during labor.

This further supports an earlier study which found that *M. chamomilla* tested *in vitro* had a tonus-raising effect on isolated rabbit and guinea pig uteri [10]. In contrast, *M. chamomilla* was reported to produce a dose-dependent spasmolytic effect on isolated guinea pig ileum [11].

Goal of Study

With these studies in mind, the goal of this project was to determine whether or not *M. chamomilla* would contract the smooth muscle as found in isolated uterine horns of mice, and if so, were the contractions concentration-dependent. It was hypothesized that *M. chamomilla* would cause contractions in isolated uterine horns of mice and that these contractions would be concentration-dependent.

Materials and Methods

This protocol was developed based on that published by Bristol and DeGolier [12].

Mus musculus

For this study a total of twelve virgin female *Mus musculus* 9-10 weeks old were purchased from Envigo, (Indianapolis, Indiana, United States). All the mice were housed in cages in the animal room in the Department of Biological Sciences at Bethel University. Food and water were provided *ad libitum*. This research was completed in accordance with Bethel University's Institutional Animal Care and Use Committee.

***Matricaria chamomilla* Preparations**

Flowers of *M. chamomilla* were purchased from Richters Herbs (Goodwood, Ontario, Canada). Once obtained, *M. chamomilla* was ground in a coffee grinder. Next, 1.8g was weighed out and mixed into 100mL of deionized, boiling water at 190 RPM for 8 minutes. Once mixed, the solution was vacuum filtered through Whatman filter paper via a Buchner funnel for 10 minutes, and the liquid was collected, measured, and cooled to room temperature.

Tissue Preparations

A DeJalons Ringer's solution containing 36g NaCl, 1.68g KCl, 2g $NaHCO_3$, 2g D-glucose, 0.32g $CaCl_2$ in 4 liters of deionized water was prepared before extraction of the uterine horns from the mice [13]. The solution was warmed to 32°C in 20 mL organ baths which were continuously gassed with 95% O_2/5% CO_2 at approximately 2 psi. Diethylstilbestrol (DES) 0.2 mg was injected into each mouse 24 hours prior to CO_2 asphyxiation. DES is a synthetic estrogen agonist which increases the number of gap junctions in uterine smooth muscle, thus increasing its responsiveness and allowing the uterus to contract as a single-unit [14].

Immediately after euthanasia, a 3cm incision was made to the ventral abdomen and the uterine horns were removed. The horns were temporarily placed in cold DeJalons solution while they received two sutures: one attaching a uterine horn to a stationary rod in the bath, and the other to a force transducer. Each uterine horn was then submerged into an isolated organ bath.

Tissue Bath Procedure

A Power Lab Data Acquisition System, which gathered analog data and digitized them into visible waveforms, was attached to a 50g force transducer (MLTF050/ST). All data collection tools used were products of AD instruments (Colorado Springs, Colorado, United States). All uterine horns were suspended in the organ baths at 0.8 grams of tension [13], and left for an hour to equilibrate. During this time, fresh DeJalons was flushed over the tissues every 15 minutes. Data was collected and recorded during this equilibration period.

After one hour, 10^{-5} M oxytocin, an endogenous hormone produced by the posterior pituitary that promotes uterine contractions during childbirth [15], was applied to each tissue. For this experiment, oxytocin was used as a positive contractile control and the resulting contractions were observed and recorded for ten minutes. After the tissues were washed over with fresh DeJalons followed by another ten-minute equilibration. Then, a single aqueous *M. chamomilla* extract was added to the tissues at a concentration of 0.07 mg/mL, 0.14 mg/mL, 0.28 mg/mL, 0.58 mg/mL, or 1.17 mg/mL. The *M. chamomilla* extract was left in the organ bath for 10 minutes and any contractile activity was observed and recorded.

All chemicals used in the procedure above were purchased from Sigma-Aldrich (St. Louis, Missouri, United States).

Statistical Analyses

Uterine contractile forces were measured for a given waveform response starting at the baseline value (zero tension) to the highest amplitude of the force produced within five minutes following the application of oxytocin or chamomile treatment. Similar measurements were also made for the tissue's spontaneous motility. Contractile forces were expressed both in mN and as a percent of their initial oxytocin control responses. Average contractile forces per *M. chamomilla* concentrations were summarized as means ± standard error (SE) for both contractile forces (mN) and as a percent response of their oxytocin control (% OXY).

Statistical analyses were completed only on tissues that demonstrated spontaneous motility, responded to oxytocin, and collectively had a sample size greater ≥ 3 per chamomile concentration. An ANOVA was used to determine if the contractile forces generated by spontaneous motility were statistically different from those produced by the various chamomile concentrations. A p-value ≤ 0.05 was considered to be statistically significant. The Tukey-Kramer *post hoc* test was utilized in order to identify which concentration-induced contractile responses were statistically different from each other.

Results

Waveform Data

Figure 1 represents a typical example of raw waveform data collected throughout a single experiment. Spontaneous motility was recorded for each tissue at the beginning of the experiment. Once oxytocin was added, a positive contractile response was observed for several minutes which can be seen at point A (Fig. 1). After 10 minutes, the organ bath was flushed twice with DeJalons solution over a 20-minute period with the intention of removing any remaining oxytocin and allowing the contractile baseline to return to pre-oxytocin levels. When spontaneous motility was re-established, *M. chamomilla* was added (point B) and produced a waveform spike greater than that of the previous spontaneous motility, indicating contractile activity as produced by the chamomile extract.

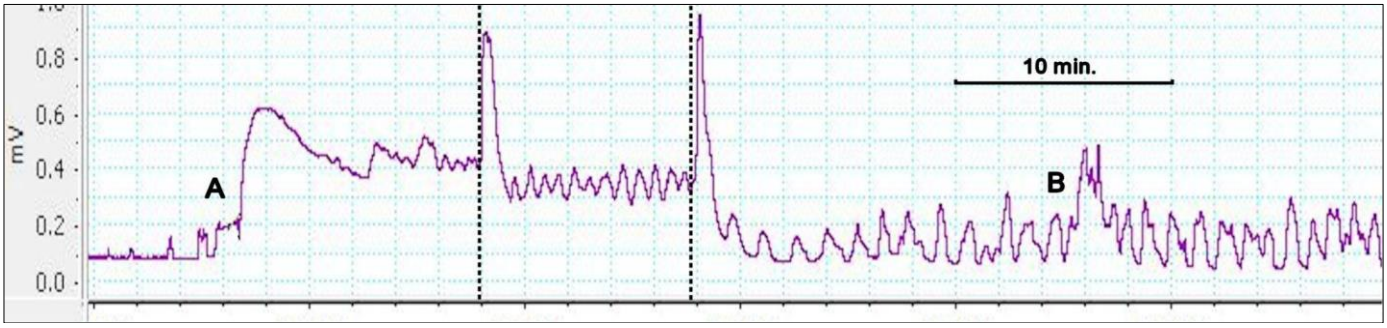

Fig 1: A typical example of collected waveform data. Oxytocin (10^{-5}M) application at point A shows an immediate increase of 23.35 mN of contractile force. The tissue bath was then flushed twice creating two artifact spikes in the waveform (dotted lines). Once the tissue returned to spontaneous motility, *M. chamomilla* (0.28 mg/mL) was applied to the bath at point B and created a brief contractile force of 18.10 mN. The default y-axis (mV) was converted later to mN force.

***Matricaria chamomilla* Concentration-Dependent Contractions**

Different concentrations of *M. chamomilla* were tested to determine if contractile forces were dependent on the concentration extract. Multiple concentrations were used, and the resulting contractile forces were measured and recorded. For the raw data represented in mN, the spontaneous motility, or "0" concentration, had an average force of 9.05±1.36 mN (n=27). Spontaneous motility contractile forces were significantly lower than the 0.14 mg/mL, 0.28 mg/mL, 0.58 mg/mL as well as the oxytocin control. The oxytocin contractile response was significantly greater than the contractile forces produced by *M. chamomilla* concentrations of 0.07 mg/mL, 0.58 mg/mL, and 1.17 mg/mL. The average contractile force of the 0.14 mg/mL chamomile concentration was 24.76±2.21 mN (n = 6) and 0.28 mg/mL was 24.48 ± 3.02 mN (n=7). Finally, the 0.58 mg/mL concentration had an average contractile force of 20.63±2.86 mN (n=5) and the 1.17 mg/mL at 17.97±2.64 mN (n=4). Based on the data reported herein, it can be observed that the contractile forces produced by *M. chamomilla* increased with higher concentrations, plateaued, and then began to decrease ($p < 0.0001$, Fig. 2).

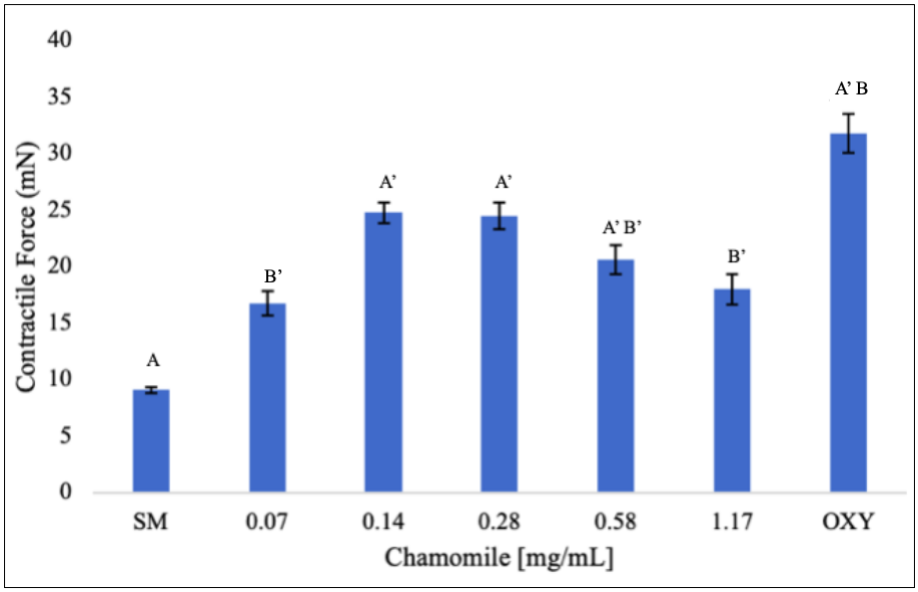

Fig 2: Means (± SE) of uterine tissue contractions (mN) as produced by increasing concentrations of *M. chamomilla*. Spontaneous motility (SM) was significantly lower than chamomile concentrations of 0.14 mg/mL, 0.28 mg/mL, 0.58 mg/mL, and OXY ($p<0.0001$). Significant changes in contractile forces are indicated as differences between letters and their primes.

For contractile data expressed as % OXY, the spontaneous motility had an average force of 27.40±17.53% OXY (n=27). Spontaneous motility was not significantly lower than the contractile forces produced by the 0.07 mg/mL concentration of *M. chamomilla* which had an average force of 43.18±14.95% OXY (n=5). It was however significantly lower than the contractile forces following application of 0.14 mg/mL, 0.28 mg/mL 0.58 mg/mL, and 1.17 mg/mL chamomile. The average contractile force of the 0.14 mg/mL concentration was 81.97±19.27% OXY (n=6), 0.28 mg/mL was 70.61±14.53% OXY (n=7), 0.58 mg/mL was 83.22±28.17% OXY (n=5), and 1.17 mg/mL was 71.77±16.94% OXY (n=4). The 0.07 mg/mL concentration produced a contractile force significantly lower than the 0.14 mg/mL (82% OXY) and 0.58 mg/mL (83% OXY) concentrations. Similar to that observed in Fig. 2, the resulting uterine contractile forces increased with increasing concentrations of chamomile, but the 0.14-1.17 mg/mL concentrations were not significantly different from each other ($p< 0.0001$, Fig. 3).

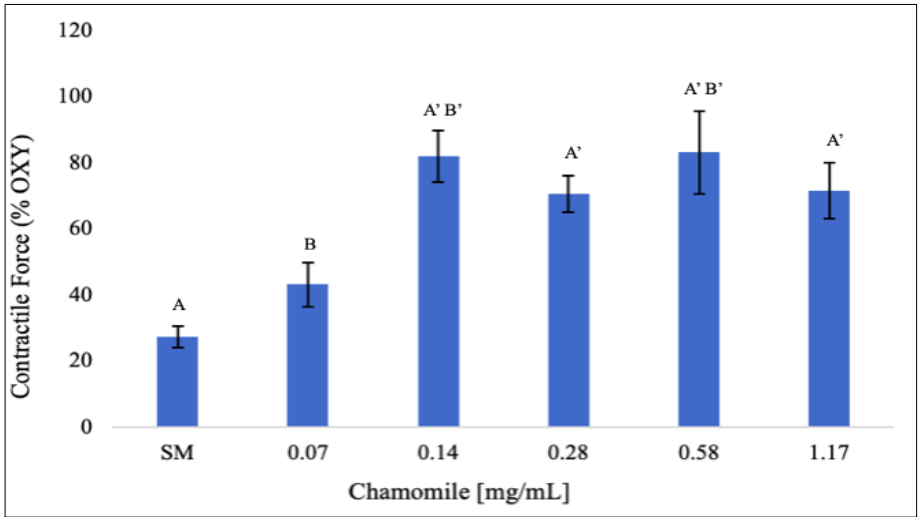

Fig 3: Means (± SE) contractile forces of uterine tissue, expressed as a percent of their oxytocin control, following application of increasing concentrations of *M. chamomilla.* Chamomile concentrations of 0.14 mg/mL, 0.28 mg/mL, 0.58 mg/mL, and 1.17 mg/mL all produced contractile forces significantly greater than the tissues of spontaneous motility (SM) (p<0.0001). These and other significant changes in contractile forces are indicated as differences between letters and their primes.

Discussion

Research Findings and Applications

The application of *M. chamomilla* on isolated *M. musculus* uterine tissues produced small contractile forces. *M. chamomilla* exhibited the strongest contractile responses (mN) at 0.14 mg/mL and 0.28 mg/mL, yet were all lower than the oxytocin control (82% and 70% OXY, respectively). The 0.14 mg/mL and 0.58 mg/mL concentrations produced the greatest contractile responses when the data is presented as % OXY (82% and 83% OXY, respectively). Both expressions of contractile force (mN, % OXY) indicated that *M. chamomilla* demonstrated some concentration-dependency. Further data collection utilizing a greater range of chamomile extract concentrations would favor a more detailed interpretation of concentration-dependent results.

Previous studies completed *in vivo* and *in vitro* have also reported *M. chamomilla*'s ability to induce small contractile responses. A double-blind *in vivo* clinical study with 80 post-term pregnant women [9] found that orally encapsulated *M. chamomilla* stimulated labor in post-term pregnancies. Each participant took 2 capsules (500mg) every 8 hours and reported their condition via a phone call every 24 hours. Results showed labor began during week one of starting the capsules in 92.5% of the chamomile group and 62.5% of the placebo group (p=0.003). It was considered that the chamomile-induced labor was due to its ability to reduce levels of adrenalin, which is an anti-oxytocic in women stressed by post-term pregnancy. Additionally, an *in vitro* study [16] completed using isolated rat uterine tissues also confirmed the contractile activities of a hydroalcoholic extract of *M. chamomilla*. Furthermore, Shipochliev [10] demonstrated a tonus-raising effect on isolated rabbit and guinea pig uteri.

In contrast, Achterrath-Tuckermann [11] found *M. chamomilla* to have a concentration-dependent spasmolytic effect on guinea pig ileum.

Biological Composition of *Matricaria chamomilla*

Interpretation of the results presented herein requires an understanding of the chemical composition of *M. chamomilla* in order to elucidate possible pathways in which *M. chamomilla* may augment labor. A recent study employing GC–MS experimentation determined that *M. chamomilla* has high terpene synthase expression levels and sesquiterpenoid lactones [17]. *Matricaria chamomilla* leaves consist of steroids, tannins, phytosterol, terpenoids, phenolic compounds, flavonoids, and galetin. Galetin, as isolated from *Piptadenia stipulacea*, has been shown to induce spasmolytic behaviors from rat uterine tissues precontracted with carbachol or oxytocin [18]. The research results presented herein did not use leaves, but used chamomile flowers only, which are reported to consist of steroids, tannins, terpenoids, phytosterol, and flavonoids [19]. Thus, it is possible that galetin's relaxing properties were not observed in this smooth muscle preparation.

M. chamomilla contains the terpenoid, α-bisabolol, which has been linked to relaxant activity [7, 8, 20]. α-bisabolol is considered to be one of the principal components of *M. chamomilla* [6]. It is also thought to be a key player in *M. chamomilla's* antimicrobial and disinfectant effects [20].

To test whether or not α-bisabolol had a direct effect on smooth muscle, α-bisabolol has been as applied to multiple sources of isolated smooth muscle. It was concluded that α-bisabolol is biologically active in smooth muscle, but its activity varies depending on the type of smooth muscle organ. For example, α-bisabolol increased the amplitude of spontaneous contractions in the urinary bladder and increased the response of the cholinomimetic drug, carbachol, in tracheal rings *in vitro*, but completely decreased spontaneous contractions in duodenum [21]. Chamomile-derived α-bisabolol was also determined to have only relaxing properties in isolated porcine hearts [22].

Proposed Mechanism

De Sequeira *et al.* [21] has suggested that α-bisabolol may be an inhibitor of voltage-dependent Ca^{2+} channels in tracheal rings which would lead to relaxation of the smooth muscle. Muñoz-Pérez *et al.* [8] also found α-bisabolol was not associated with an increase in cAMP levels which is understood to facilitate relaxation. However, Shanmuganathan *et al.* [23] found α-bisabolol to reduce acetylcholine esterase activity thus increasing acetylcholine concentrations which could possibly increase contractions. Further research antagonizing α-bisabolol activities in a variety of smooth muscle tissue is needed to confirm this terpenoid's mechanism.

Implications for Pregnant Women

Although *M. chamomilla* has been found to induce uterine smooth muscle contractions *in vitro*, its implications for pregnant women require more research. Gholami *et al*. [9] did find that orally encapsulated *M. chamomilla* induced labor in post-term women. However, aromatherapy studies using *M. chamomilla*'s essential oil seem to have mixed results. For example, a randomized clinical trial conducted on 130 pregnant women found *M. chamomilla* aromatherapy to have no effect on the duration and number of contractions [24]. A different randomized, double-blind clinical trial with 128 pregnant women reported that utilizing one drop of a 5% chamomile oil had significantly reduced pain intensity when compared to the placebo group [25]. It may be that *M. chamomilla* reduces pain during labor, but more research is required to determine whether this same outcome allows for a less stressful, more effective, and shortened labor.

Further Research and Considerations

Even though the results presented herein demonstrate that *M. chamomilla* does produce contractions in isolated murine uterine tissue, there should be an awareness of how it responds when paired with other isolated constituents or even with other whole herbs. For example, one such study paired the isolated constituent α-bisabolol from *M. chamomilla* with *Ocimum sanctum*, also known as purple holy basil, and found both agents at high concentrations (0.01-80μM) decreased developmental activity in cardiomyocytes [26].

Future investigation with the goal to further an understanding of *M. chamomilla* behavior in any skeletal, cardiac, and smooth muscle tissue will need to include in its experimental design, the potential for constituent facilitation, synergism, and / or attenuation that is likely to result when using multiple constituents from within the same herbal species or different herbals.

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