Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2023; 11(1): 180-183

Received: 08-10-2022
Accepted: 16-12-2022

Benjamin Stephens
Department of Biological Sciences, 3900 Bethel Drive, Bethel University, St. Paul, Minnesota, 55112, USA

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

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

The effects of ajwain, *Trachyspermum ammi*, on the contractile behavior in isolated uterine smooth muscle tissue from *Mus musculus*

Benjamin Stephens and Teresa DeGolier

Abstract
Trachyspermum ammi, commonly known as ajwain, is a commonly consumed medicinal herb used to treat a variety of ailments. Oral tradition suggest that it is unsafe for pregnant women to consume as it may cause early contractions and miscarriages. To test this claim, isolated uterine horns from laboratory mice that had been brought to the same physiological state as a pregnant mouse, were suspended in a physiological organ bath. Oxytocin was administered as a viability check prior to treating with ajwain concentrations of 0.41, 0.82, and1.64 mg/20 mL DeJalons buffer. ANOVA results indicated significant increases in both uterine contractile force (p<0.0001) and frequency (p<0.0001) following ajwain applications. While further testing is needed to see if this effect would translate to humans *in vivo*, these results do provide enough evidence to warrant cautiousness about consumption of ajwain while pregnant.

Keywords: Ajwain, smooth muscle, contractions, *in vitro*, uterus, mice

Introduction
Trachyspermum ammi, also known as ajwain, is an annual herb that is primarily grown and cultivated in Egypt, Iraq, Iran, Afghanistan, Pakistan, and India [1, 2]. *T. ammi* is from the family *Umbelliferae* and goes by many different names, including ajowan, caraway, ajave seeds, ajvain, ajwan, Ethiopian cumin, omam, and omum [3]. It has historically been used to treat various ailments including diarrhea, abdominal pains, hyper/hypotensive disorders, and bacterial and microbial infections [4, 5]. Some of these treatments are largely anecdotal, and have been passed down through word of mouth from generation to generation.

Ajwain contains approximately 40% carbohydrates, 11% fiber, 15% of proteins, and 15% fat [6]. It has also shown to contain an abundance (3%) of saponins [7]. This is of particular interest as saponins have been shown to have a notable role in uterine muscle contractions [8].

A recent pilot study investigated uterine smooth muscle contractility in response to a number of Indian Ayurveda herbals including ajwain [9]. The researchers found that a large bolus of ajwain, at a concentration of 0.64 mg/mL, produced a significant increase in contractile force in isolated mouse uterine tissues when compared to the tissue's own spontaneous motility. With that study in mind, the goals of this investigation were to (1) further explore if additional concentrations of ajwain would produced contractile responses in a concentration dependent manner, and (2) to investigate if ajwain also affected the contractile frequency of the spontaneous motility that is endogenous to uterine smooth muscle under tension. If so, this additional information would be useful, as it validates the anecdotal stories passed down passed down via oral tradition which claim that ajwain may promote and/or augment labor.

Methods and Materials
The methods used in this investigation were modified from previous work done by Bristol and DeGolier [8], and were also approved by the Institutional Animal Care and Use Committee at Bethel University, St. Paul, Minnesota, USA.

Preparation
Eighteen virgin female mice (*Mus musculus*) were purchased from Envigo Inc. (Indianapolis, Indiana, USA) and were housed and cared for in Bethel's animal care facility. Each mouse was injected with 0.1 mg of diethylstilbesterol (Sigma Aldrich, St. Louis, Missouri, USA) 24 hours prior to each experiment. This was done to bring the reproductive hormone levels in the uterine horns to the same levels as that of a pregnant mouse.

A stock solution of ajwain was prepared fresh before each experiment. This was done by grinding ajwain seeds (Deed Foods Inc., Union, New Jersey, USA) into a powder and mixing 1.8g with boiled distilled water. The solution was then filtered to remove any undissolved solid material. This undissolved material was then dried and the resulting mass was subtracted from the initial 1.8g ajwain in order to calculate the amount of ajwain (g/ml) that was administered to an organ bath. A DeJalons buffer was also prepared with the following composition (g/ L): 9 NaCl, 0.42 KCl, 0.5 $NaHCO_3$, 0.5 Glucose, and 0.08 $CaCl_2$ [10].

Tissue dissection and equilibration

Twenty-four hours after injection, the mice were euthanized via CO_2 asphyxiation. Two uterine horns were dissected from each mouse and placed into chilled DeJalons buffer to help delay decomposition of the tissue. Each horn was then sutured on each end: one end was suspended on an internal standard anchored into a 20 mL organ bath filled with the DeJalons buffer, and the other end was tied to an AD Instruments 50g force transducer (Colorado Springs, Colorado, USA). Once fully suspended, the uterine horns were subjected to a tension of 0.8 g and allowed to equilibrate for a period of one hour [10]. During this time, the organ baths were flushed with DeJalons buffer every 15 minutes.

Oxytocin and ajwain treatments

Once the equilibration period passed, each uterine horn was administered 10^{-5} M oxytocin (Sigma-Aldrich, St. Louis, Missouri, USA). This was done to confirm tissue viability and to serve as a positive contractile control as this endogenous hormone known to contract uterine tissue [11]. The oxytocin solution was left on each tissue sample for ten minutes, and then flushed with buffer at ten-minute intervals until the spontaneous motility of the uterine horns returned back to their basal levels of activity.

Then, each uterine sample was administered a single concentration of the ajwain solution (g/20 ml organ bath) and allowed to interact with the tissue for 30 minutes. These applications were made after the completion of a full spontaneous motility cycle and under baseline tension. The resulting waveforms were collected and visualized using an AD Instruments PowerLab/4SP and AD Instruments LabChart 7 software (v7.3.8, Colorado Springs, Colorado, USA).

Measurements and statistical analysis

Changes in contractile force were measured from the resulting waveform trough to the peak force produced within the first five minutes of treatment exposure. To control for the possible force contribution that spontaneous motility might have had on the treatments, these were also measured in a similar manner and were considered as the "0" treatment.

To normalize for the slight variation in the harvested uterine tissue masses, each uterine horn's maximal contractile response to a given treatment was expressed as a percent of its initial contractile response to 10^{-5} M oxytocin (% Oxytocin).

To determine if ajwain evoked any changes in contractile frequency, the number of spontaneous motility waveforms produced five minutes before an application of the ajwain treatment were counted and compared the number of waveforms produced in the first five minutes after the ajwain treatment.

The data were summarized as means ± (SE) for each treatment for both contractile force and contractile frequency. Individual data were further analyzed using ANOVA for multiple comparisons among the means. Resulting P values $\leq$ 0.05 were subjected to the Tukey-Kramer post-hoc test (JMP 4.0, SAS Institute, Cary, North Carolina, USA) which indicated which means were considered to be significantly different from each other.

Results

Figure 1 shows a resulting series of waveform patterns typical of the data collection. Spontaneous motility at baseline levels is observed prior to letter A. At letter A, oxytocin was applied and produced a rapid increase in contractile tension which slowly declined until the tissue was subjected to a washout. The contractile force following ajwain application (letter B) was also rapid, but in contrast to oxytocin, declined more rapidly and exhibited less of a plateau pattern. During this time (after letter B), it was observed that ajwain clearly increased the both the amplitude and frequency of the spontaneous motility.

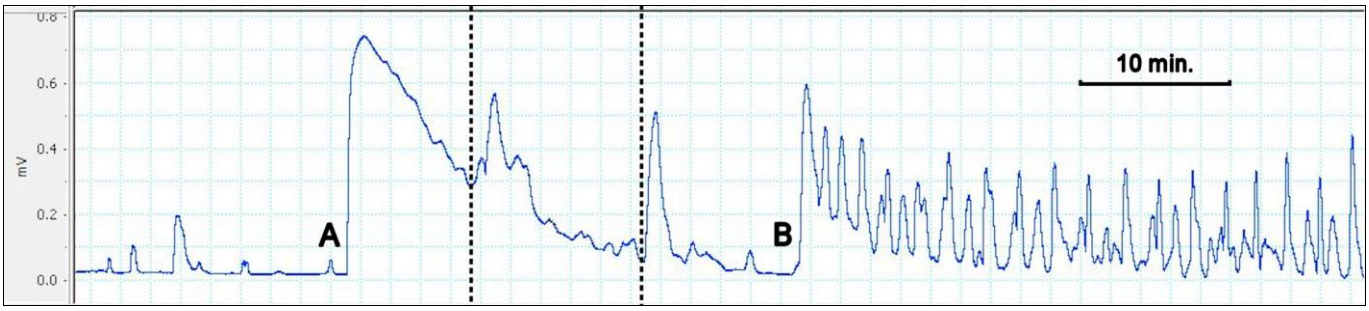

Fig 1: A) typical waveform obtained from an experimental trial. In this figure, the far-left portion of the waveform shows typical spontaneous motility (6.73 mN, 11.76% Oxytocin) from the uterine horn sample (prior to letter A). At letter A, oxytocin was applied and, in this sample, produced a contractile force of 31.7 mN. The dotted lines represent when the tissue bath was flushed with fresh DeJalons solution, creating two artifact spikes in the waveform. Once the tissue tension returned to spontaneous motility at levels similar to those prior to oxytocin treatment, the ajwain treatment (Letter B, 0.82 mg/20 mL) was applied and produced a contractile force of 18.05 mN (56.94% Oxytocin). The default y-axis (mV) was calibrated to grams and later converted to mN force.

The means ± (SE) contractile forces and contractile frequencies for each ajwain treatment and the tissues spontaneous motility are presented in Figs. 2 and 3, respectively. Analyses were only completed only on tissues that demonstrated spontaneous motility (n=31), responded to oxytocin (n=31), and collectively had a sample size greater $\geq$ 3 per ajwain concentration. This included the concentrations of 0.41 mg/20 mL (n=9), 0.82 mg/20 mL (n =14), and 1.64 mg/20 mL (n=8).

All ajwain treatments increased contractile force when compared to spontaneous motility (Fig. 2), with the ajwain concentrations of 0.82 and 1.64 mg/20 mL producing forces

which were significantly greater. The peak ajwain response was produced by the 0.82 mg/20mL concentration which was also significantly greater than those produced by the 0.41 mg/20 mL (Fig. 2, $p<0.0001$).

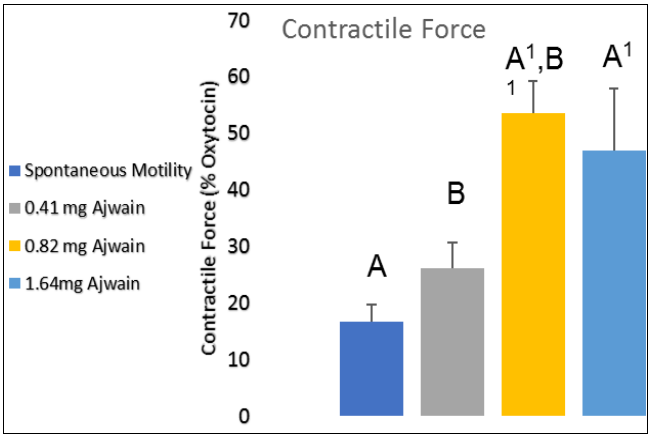

Fig 2: Means (± SE) of uterine tissue contractile forces (% OXY) in response to increasing concentrations of ajwain (mg/20 mL organ bath). All ajwain treatments increased contractile force when compared to spontaneous motility. Statistical differences ($p<0.0001$) between columns can be denoted by a capital letter and a matching primed letter

All ajwain treatments significantly increased contractile frequency when compared to spontaneous motility (Fig. 3, $p<0.0001$). The peak ajwain response was produced with the 1.64 mg/20mL concentration.

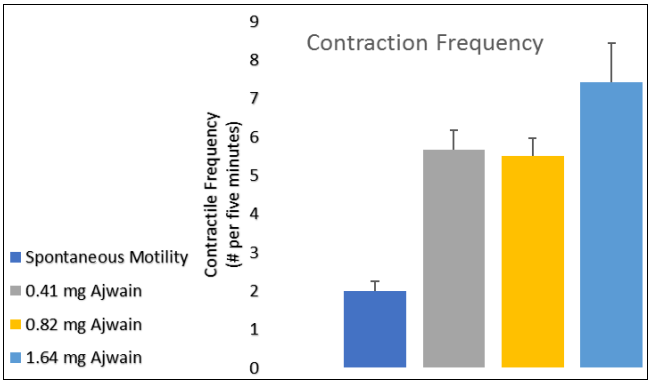

Fig 3: Means (± SE) of uterine tissue contractile frequencies in response to increasing concentrations of ajwain. All ajwain treatments (0.41 – 1.64 mg/20 mL organ bath) significantly increased contractile frequency when compared to the tissues spontaneous motility ($p<0.0001$)

Discussion

The results from this investigation support that ajwain increases both the force and frequency of smooth muscle contractions in isolated mouse uterine horns. This was logically expected as previous research has showed that ajwain contains a high portion of saponins [7], and that saponins have been shown to increase the contractile activity of isolated mouse uterine tissue [8].

The scientific literature documents that smooth muscle contractile responses to ajwain are quite variable. This likely depends upon (1) what organs the tissues were harvested from, (2) whether the experimental framework was completed *in vitro* or using a whole animal model, and (3) which biologically active constituents interacted with variable receptor signaling pathways.

For example, smooth muscle samples that were isolated from the ileum [12, 13], the trachea, and the bronchial tree [12] and treated with ajwain, all showed significant increases in contractile force. In contrast, Gilani *et al*. [14] demonstrated that ajwain had an antispasmodic effect on isolated jejunum preparations from rabbits. Similarly, Hejazian *et al*. [15] found that ajwain had an antispasmodic effect on isolated rat ileum. Such antispasmotic tissue behaviors could support claims from oral tradition that ajwain is effective in treating indigestion and diarrhea [2, 16].

Ajwain administration also affects muscle tone from tissues within the cardiovascular system. Ajwain is reported to reduce heart rate in anaesthetized rats [17], relax isolated rabbit aorta preparations [14] as well as to reduce blood pressure in anaesthetized rats [14, 17, 18].

Of the known active constituents in ajwain, saponins likely have a role in increasing contractile activity. Saponins may increase cell membrane permeability by creating large pores in muscle cells which would potentially allow calcium to enter the muscle cell and initiate contraction [19]. However, over time, a muscle cell in a hypercalcemic state may show signs of weakness and fatigue [20]. It might be possible that if a uterine horn sample had been exposed to very high concentrations of saponins, it may experience weakness and fatigue due to the elevated influx in calcium. Perhaps this is why the results presented in Fig. 2 show that while the ajwain concentration of 0.41 and 0.82 mg/20 ml bath produced concentration-dependent responses, the greatest concentration used, 1.64 mg/20 mL, actually evoked a weaker response than 0.82 mg/20 ml concentration.

It would be worthwhile to determine if any of the other active constituents work synergistically or in tandem with saponins to promote contractile activity. For example, *Trachyspermum ammi* protein (TAP) has been shown to remove excess calcium from epithelial tissues within the kidney [21]. It remains to be determined if TAP could aid in preventing uterine smooth muscle tissues from reaching hypercalcemia once it becomes saturated with saponins.

Another constituent that warrants further research is thymol, which is the most abundant aromatic component found in ajwain's essential oil [6, 22]. Hejazian *et al*. [15] demonstrated that thymol (isolated from ajwain) had an anti-spasmodic effect that was inversely proportional to the contractile activity in rat ileal tissue. This is consistent with other studies which have shown thymol to reduce contractile activity in isolated gastric smooth muscle from guinea pigs [23]. Thymol (isolated from thyme) also reduced contractile activity in tracheal and ileal tissues examined from rats [24].

Ajwain is frequently consumed during lactation as it is high in protein, fiber, calcium, iron, zinc, copper, and phosphorus [25]. It would be beneficial to know what amount of ajwain is considered safe for a woman to consume when pregnant and approaching labor [26]. With the potential for biological activity from several ajwain constituents [13, 28], identifying and understanding their absorption and consequent distribution, as well as their metabolism and excretion would require additional complex investigations beyond the scope of the direct exposure of an aqueous seed extract to isolated uterine smooth muscle as reported herein. Even though ajwain has been the subject of a number of animal studies, there is a lack of clinical human studies which would help to substantiate the medical claims handed down via oral tradition [29].

Conclusion

In conclusion, ajwain was shown to significantly increase

both contractile force and frequency of isolated mouse uterine horns. While further testing is needed to see if the effect would translate to humans *in vivo*, these results do provide enough evidence to warrant cautiousness about consumption of ajwain while pregnant. This information can be considered useful, as it validates the anecdotal stories passed down by way of oral tradition that ajwain may be used to promote and augment labor.

References

1. Chung I-M, Kahnh TD, Ahmad A. Chemical constituents from ajwain seeds (*Trachyspermum ammi*) and inhibitory activity of thymol, lupeol and fatty acids on barnyard grass and radish seed. Asian J Chem. 2016;19(2):1-11.
2. Anwar S, Ahmed N, Habibatni S, Abusamra Y. Essentials in Food Preservation, Flavor and Safety Ch. 19 - Ajwain (*Trachyspermum ammi* L.) Oils, Editor (s): Victor R. Preedy, Academic Press; c2016. p. 181-192.
3. Sakar P. What Is Ajwain (Carom) Seed? 2022. https://www.thespruceeats.com/carom-seeds-ajwain-1957879, accessed January 16, 2023.
4. Phondani P, Maikhuri R, Kala C. Ethnoveterinary uses of medicinal plants among traditional herbal healers in Alaknanda catchment of Uttarakhand, India. Afr J Tradit Complement Altern Med. 2010;7(3):195-206.
5. Rufino AT, Ribeiro M, Judas F, Salgueiro L, Lopes MC, Cavaleiro C, *et al.* Antiinflammatory and chondroprotective activity of (α)-pinene: structural and enantiomeric selectivity. J Nat Prod. 2014;77(2):264-269.
6. Nagalakshmi S, Naik JP, Shankaracharya NB, Rao LJM. Studies on chemical and technological aspects of ajowan (*Trachyspermum ammi* (L.) syn. *Carum copticum* Hiern) seeds. J Food Sci Technol. 2000;37(3):277-281.
7. Boskabady MH, Alitaneh S, Alavinezhad A. *Carum copticum* (L.): An herbal medicine with various pharmacological effects. BioMed Research International. 2014;4:1-11.
8. Bristol B, DeGolier T. *Quillaja saponins* are a potent contractor of uterine smooth muscle tissue *in vitro*. J Pharmacogn Phytochem. 2108;7(5):1252-1258.
9. Chumber A, DeGolier T. 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. J Pharmacogn Phytochem. 2020;9(2):1145-1150.
10. Kitchen I. Textbook of *in vitro* Practical Pharmacology. Blackwell Scientific Publication. London, England; c1984.
11. Vallera C, Choi LO, Cha CM, Hong RW. Uterotonic medications: oxytocin, methylergonovine, carboprost, misoprostol. Anesthesiol Clin. 2017;35(2):207-219.
12. Dwivedi SN, Mishra RP, Alava S. Phytochemistry, pharmacological studies and traditional benefits of *Trachyspermum ammi* (L.) sprague. International Journal of Pharmacy and Life Sciences. 2012;3(5):1705-1709.
13. Asif HM, Sultana S, Akhtar N. A panoramic view on phytochemical, nutritional, ethanobotanical uses and pharmacological values of *Trachyspermum ammi* (L.). Asian Pac J Trop Biomed. 2014;4:545-553.
14. Gilani A, Jabeen Q, Ghayur M, Janbaz K, Akhtar M. Studies on the antihypertensive, antispasmodic, bronchodilator and hepatoprotective activities of the *Carum copticum* seed extract. J Ethnopharmacol. 2005;98(1-2):127-135.
15. Hejazian S, Bagheri S, Safari F. Spasmolytic and anti-spasmodic action of *Trachyspermum ammi* essence on rat′s ileum contraction. N Am J Med Sci. 2014;6(12):643-647.
16. Singh MB. Maternal beliefs and practices regarding the diet and use of herbal medicines during measles and diarrhea in rural areas. Indian Pediatr. 1994;31(3):340-3.
17. Aftab K, Atta-Ur-Rahman, Usmanghani K. Blood pressure lowering action of active principle from *Trachyspermum ammi* (L.) sprague. Phytomedicine. 1995;2(1):35-40.
18. Zadeh GS, Panahi N. Endothelium-independent vasorelaxant activity of *Trachyspermum ammi* essential oil on rat aorta. Clin Exp Hyperten. 2017;39(2):133-138.
19. Kagacin GJ. Physiological and structural properties of saponin-skinned single smooth muscle cells. J Gen Physiol. 1987;90(1):49-73.
20. Marone C, Beretta-Piccoli C, Weidmann P. Acute hypercalcemic hypertension in man: role of hemodynamics, catecholamines, and renin. Kidney Int. 1981;20(1):92-96.
21. Kaur T, Bijarnia R, Singla S, Tandon C. Purification and Characterization of an anticalcifying protein from the seeds of *Trachyspermum ammi* (L.). Protein and Peptide Letters. 2009;16(2):173-181.
22. Gaba J, Sharma S, Joshi S, Gill P. Gas chromatography-mass spectrometric analysis of essential oil, nutritional and phytochemical composition of ajwain seeds (*Trachyspermum ammi*. L.). JEOBP. 2018;21(4):128-1137.
23. Hisayama T, Takayanagi I. Increased 45Ca-efflux from smooth muscle microsomes by a rise in an extramicrosomal Ca ion concentration, and the effect of thymol. J Pharm Pharmacol. 1983;35(8):532-533.
24. Begrow F, Engelbertz J, Feistel B, Lehnfeld R, Bauer K, Verspohl E. Impact of thymol in thyme extracts on their antispasmodic action and ciliary clearance. Planta Med. 2010;76(4):311-318.
25. Kaushik D, Mathew S. Nutritional composition of traditional supplementary foods consumed by lactating women. Indian J Nutr Diet. 1988;25(10):320-324.
26. Masoodi L, Ahad T, Nisar J, Khurshid S. Ajwain Oleoresin: Characterization and Properties. In Handbook of Oleoresins; c2022. p. 95-100. CRC Press
27. Asif, HM, Hashmi HAS. Bioactive Compounds of Ajwain (*Trachyspermum ammi* [L.] Sprague). In: Murthy, H.N., Paek, K.Y. (eds) Bioactive Compounds in Underutilized Vegetables and Legumes. Reference Series in Phytochemistry; c2020. Springer, Cham. https://doi.org/10.1007/978-3-030-44578-2_16-1.
28. Javed S, Shahid AA, Haider MS, Umeera A, Ahmad R, Mushtaq S. Nutritional, phytochemical potential and pharmacological evaluation of *Nigella sativa* (Kalonji) and *Trachyspermum ammi* (Ajwain). J Med Plants Res. 2012;6(5):768-775.
29. Bhadra P. An overview of ajwain (*Trachyspermum ammi*). IJONS. 2020;10(59):18466-182474.