Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(1): 15-19
Received: 22-10-2022
Accepted: 12-12-2022

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Trigonella foenum-graecum seed extract produces contractions in mouse uterine horns *in vitro*

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DOI: https://doi.org/10.22271/phyto.2023.v12.i1a.14602

Abstract
Many of the most commonly used labor interventions come with risks to both mother and fetus. For that reason, some women use herbal methods to promote labor. Previous research suggests that fenugreek, *Trigonella foenum-graecum*, may serve to favor disease prevention. Fewer inquiries have targeted the role that fenugreek may have on uterine smooth muscle contractility and its potential to influence labor and delivery. This investigation used an organ bath system for assessing changes in contractile tension following the application of a prepared seed extract from fenugreek. Fenugreek concentrations (0.15 - 0.74 mg/20 mL) significantly increased contractile forces as compared to the baseline of the tissues endogenous motility ($p < 0.0001$). These outcomes, even though collected from isolated tissues, potentially provide support for any oral traditions claiming that fenugreek consumption may augment or aid in the promotion of labor.

Keywords: Fenugreek, uterus, *in vitro*, saponins, labor

Introduction
More and more frequently, pregnant women are considering the use of herbs as a means to promote a safe and effective delivery of the fetus. The use of medicinal herbs has been reported to bring about labor [1, 2, 3]. For example, a survey sent out to certified nurse-midwives over 20 years ago [1] reported using herbals such as blue cohosh (*Caulophyllum thalictroides*), black cohosh (*Actaea racemosa*), red raspberry leaf (*Rubus idaeus*), castor oil (*Ricinus communis*), and evening primrose oil (*Oenothera biennis*) to intiate labor. A common reason given by these midwives for not using medicinal herbs was that there was simply too little research documenting that the herbal options were effective and safe [1].
Fenugreek (*Trigonella foenum-graecum)* can be found in the Mediterranean region, southern Europe, and western Asia, and is used in cooking and as a medicine [4]. It is proposed that fenugreek might induce, facilitate, and/or augment labor. An early study using an aqueous extract from fenugreek seeds demonstrated that they contained very high levels of saponins [5]. More recently, these concentrations were shown to be 1.34 grams/100 grams of dry weight [6], placing fenugreek among the top known plants containing saponins [7]. When saponins (30-500 micrograms/mL; Sigma-Aldrich St. Louis, Missouri, USA) were applied to isolated rat uterine strips collected on day 20 of pregnancy, the amplitude of spontaneous contractions increased [8]. It would thus seem reasonable that fenugreek seed extract itself would produce contractions in isolated uterine smooth muscle.
Thus the research objectives herein were as follows: 1) to investigate if variable concentrations of fenugreek seed extract had the potential to stimulate smooth muscle contractions in isolated mouse uterine tissue; and if so, 2) determine if the contractile responses were concentration-dependent. Failure to reject the null hypothesis that fenugreek will have no contractile response from uterine smooth muscle, would remove it as a potential herbal candidate involved in the processes of labor and delivery.

Materials and Methods
Several of the procedures detailed below are based on previous work done by Quam et al. [9] with modifications.

Preparing the aqueous extract
Fenugreek seeds were obtained from Raja Foods, India. The procedures for making the seed extract were based on those reported by Kaingu et al. [10]. Whole fenugreek seeds were ground to a powder, and then a stock solution was made by dissolving 1.8 grams in 100 mL of boiling deionized water prior to the testing of the uterine tissue. The solution was then vacuum filtered several times with the final resulting filtrate ready for use.

Any non-filtered material from the fenugreek original stock solution was subject to evaporation at room temperature for 24 hours. The remaining mass was when subtracted from the original 1.8 grams and used to calculate the actual concentrations of fenugreek treatments (mg) applied per 20 mL organ bath.

Specimens

Non-pregnant female mice, outbred ICR (CD-1® (n = 18) were provided by Envigo (Indianapolis, Indiana, USA). The mice had free access to mouse chow and water. All animal husbandry and experimental protocols were approved by the institution's animal care and use committee.

The reproductive cycle for the mice used (*Mus musculus*) is 4 - 5 days long [11]. Diethylstilbesterol, a synthetic estrogen agonist, was administered intraperitoneally into the mice 24 hours prior to an experiment. This action brings the mouse into the estrus stage of its estrous cycle [12] and also promotes the addition of gap junctions [13] between the uterine smooth muscle cells which would further enable the uterine myocytes to contract as a single unit.

Preparation of the uterine horns

On the day of an experiment, the mice were sacrificed by a controlled administrated overdose of CO_2. The two uterine horns were dissected out of the abdominopelvic cavity and deposited into an iced DeJalons solution containing (grams/5 L): 45 g NaCl, 2.1 g KCl, 0.4 g $CaCl_2$, 2.5 g D-glucose, and 2.5 g $NaHCO_3$ [14]. This salt solution served to favor the distribution of electrolytes for membrane excitability and muscle contraction.

The individual uterine horns were then further dissected to free them of any excessive non-uterine tissue. A suture was placed on each free end of a uterine horn allowing for attachment to an anchored force transducer (AD Instruments, Colorado Springs, Colorado, USA) as well as a fixed hook located in the bottom of a 20 mL Panlab organ bath (AD Instruments, Colorado Springs, Colorado, USA). The tissue preparation was then placed in 20 mL organ bath, filled with fresh DeJalons solution and suspended at 0.8 g of tension [14].

Testing protocol

After one hour of tissue equilibration to the organ bath, which included a chamber washout with fresh DeJalons solution at 15 minute intervals, individual tissues were treated with 10^{-5} M oxytocin to ensure that the tissues within the isolated uterine horns were still viable and responsive to chemical stimuli. Since oxytocin is an endogenous uterotonic [15, 16], a positive control response (*i.e.*, a contraction) would affirm that the tissue remained viable. Prior to any fenugreek treatments, each uterine horn was flushed with DeJalons solution twice more (at ten minute intervals), so as to remove any further potential of newly induced contractile responses from oxytocin.

Then a single uterine horn was given only one specific concentration of fenugreek and any changes in contractile activity were recorded using a Power Lab 4/SP Data Acquisition system (AD Instruments, Colorado Springs, Colorado, USA). The concentrations tested were 0.15, 0.30, 0.44, 0.59, 0.74, and 1.18 mg fenugreek /20 mL organ bath. Sample sizes per treatment concentration ranged from 3 to 6 tissues.

Measurements and statistical analysis

Fenugreek or oxytocin applications were given in between observed smooth muscle spontaneous contractile activities. The changes in the actual contractile force produced (in mN) were measured as the distance from the muscle baseline tension to the highest tension (or contractile amplitude) produced following treatment applications. The amplitude of the uterine spontaneous motility was also measured in a similar fashion [17] and was considered as the "0" treatment.

These same uterine horn contractile forces (in mN) measured as a function of fenugreek or its own spontaneous motility were further calculated as a percent of their own control responses to oxytocin (% OXY). This was done to potentially correct for any disparity in the amount active contractile smooth muscle under tension between the two uterine horn sutures [18].

Measurements for raw contractile forces (mN) for oxytocin, spontaneous motility, and fenugreek concentrations were summarized as means (± SE). The same data were further calculated to present as means (± SE) contractile force for spontaneous motility (% OXY) and in response to the various fenugreek concentrations (% OXY). Individual data were subjected to a One-Way ANOVA in order to compare the differences between the means of more than two different concentrations of fenugreek. Resulting *P* values that were less than or equal to 0.05 indicated that the contractile responses based on the treatments given, were significantly different from each other.

Results

Isolated uterine horn contractile activity

Spontaneous motility was observed from all uterine horns before any treatment, confirming tissue viability and proper functioning of the organ bath system. Each tissue produced a strong contractile response to the oxytocin treatment (Fig. 1). Contractile responses to oxytocin were induced very quickly and typically plateaued for 6 - 8 minutes before gradually losing tension (Fig. 1, letter A). The contractile responses of the highest fenugreek concentration used in this investigation (1.18 mg/20 mL), did not reach the full contractile force as produced by oxytocin. Contractile forces in response to fenugreek also peaked very quickly, but in contrast to oxytocin, tapered off more slowly (Fig. 1, letter B).

Isolated uterine horn contractile responses to fenugreek

For contractile data expressed as % OXY (Fig. 2), the mean (± SE) spontaneous motility contractile force was 11.46 ± 1.69% OXY (n=29). Mean (± SE) contractile forces produced in response to increasing concentrations of fenugreek (mg/20 mL) were: for 0.15, 28.84 ± 18.29% OXY (n=3); for 0.30, 35.31 ± 12.84% OXY (n=4); for 0.44, 36.36 ± 12.12% OXY (n=6); for 0.59, 44.27 ± 11.27% OXY (n=6); for 0.74, 79.92 ± 9.09% OXY (n=4), and for 1.18, 51.71 ± 1.31% OXY (n=6).

Increasing fenugreek concentrations (0.15 - 0.74 mg/20 mL) elicited concentration-dependent contractile responses (Fig. 2). While all fenugreek concentrations used produced an increase in the amplitude of spontaneous motility (*i.e.* 0.00 mg/20 mL), the concentrations of 0.59, 0.74, and 1.18 mg/20 mL were significantly more forceful ($p < 0.001$). When the fenugreek induced contractile responses (% OXY) were compared to each other, the 0.74 mg/20 mL concentration evoked a greater increase in muscle tension that did the other concentrations ($p < 0.0001$, Fig. 2).

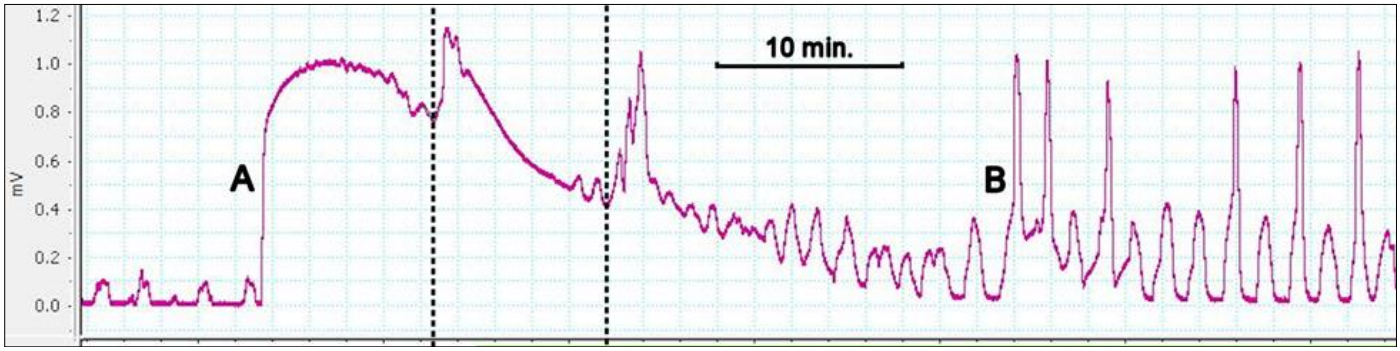

Fig 1: A typical uterine smooth muscle recording from a single uterine horn, illustrating spontaneous motility (before letter A, 2.42 mN; 0.08% OXY), a response to oxytocin (at letter A, 31.68 mN), and a response to 0.74 mg/20 mL fenugreek (letter B, 25.97 mN; 81.98% OXY). The dotted lines prior to the artifact spikes represent when the tissue bath was flushed with fresh DeJalons solution. Once the tissue contractile forces returned to baseline tensions similar to those prior to oxytocin treatment, the fenugreek treatment was applied. The y-axis (mV default) seen here was calibrated to grams and then converted to mN force.

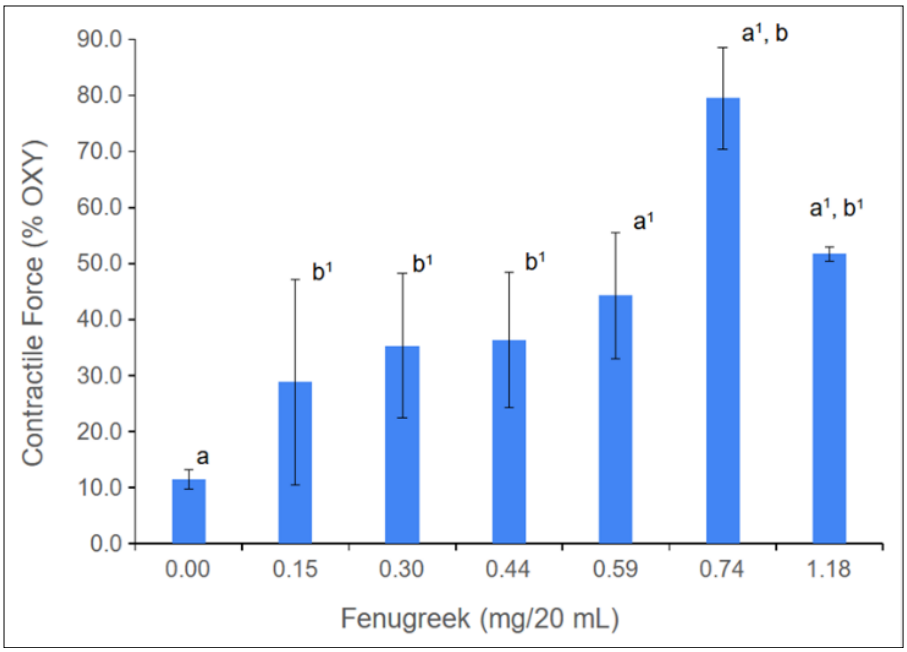

Fig 2: Means (± SE) uterine horn contractile responses (% OXY) as a function of increasing fenugreek concentrations. All treatments increased contractile responses when compared to baseline spontaneous motility (0.00 mg/20 mL). The fenugreek concentrations of 0.59 - 1.18 mg/20 mL (a^1) were significantly greater than spontaneous motility (a) ($p<0.0001$). The peak fenugreek response was produced at a concentration of 0.74 mg/20mL (b, $p < 0.001$).

Further analysis using raw contractile responses (in mN), indicated that the minimal fenugreek concentration of 0.15 mg/20 mL yielded the threshold (or minimal) contractile response of 9.81 mN. The lowest fenugreek concentration to produce the maximal tissue contractile response of 26.29 mN was 0.74 mg/20 mL, yielding an approximate EC_{50} of 0.73 mg/20 mL.

Discussion

Positive uterine contractile response

The applications of aqueous fenugreek seed extract did indeed produce more forceful contractions in the mouse uterine horns in comparison to their own spontaneous activity under baseline tension.

The results presented herein may be of interest to health practitioners involved with pregnancy, labor and delivery [19]. Pregnancies lasting greater than 40 weeks may result in complications involving both the mother and fetus. The uterine smooth muscle contractile activity produced by fenugreek as observed in this study, may help substantiate claims and/or provide credibility that fenugreek can be considered as a medicinal herbal that is effective and perhaps safe since is is already consumed in several dietary cuisines. Fenugreek might also reduce the need for synthesized drugs that may produce desirable outcomes, but at the same time exhibit unintended side effects [20, 21].

An important question to consider is whether results from isolated uterine horns can realistically be expected in a pregnant woman. While *in vitro* techniques have the potential to provide more discernment regarding the mechanisms involved [17], it is important to interpret this data knowing that *in vitro* outcomes may not always be consistent with results from whole animal studies [20]. When an aqueous fenugreek seed extract is consumed, it is possible that the bio-availability is reduced due to breakdown by gastric juices. If any active constituents are successfully absorbed in the intestine, they may be further subjected to enzymatic degradation in the plasma [22]. Nonetheless, the findings herein sampled from isolated mouse uterine tissues can provide some credibility that fenugreek may promote contractile responses in larger mammals, such as humans.

Proposed active constituents

Fenugreek seed extract contains high levels of saponins [5, 7]. Work by Bristol and DeGolier [23] showed that saponin constituents obtained from the *Quillaja saponaria* tree and were applied to mouse uterine horns, produced concentration-dependent contractile responses. Saponins are known to disrupt the lipid bilayer of smooth muscle cells [24], which can result in plasma membrane pore formation [25] and consequent calcium entry. Thus, these research results reported herein do support the hypothesis that the saponins as found in fenugreek are an active constituent that contribute to the increase in contractile force as observed in isolated mouse uterine horns.

Oxytocin has also been found to be a constituent of fenugreek seeds [26]. Since both endogenous or administered oxytocin can contract the uterus *in vivo* [27, 28] as well as *in vitro*, its presence in fenugreek also supports its role as an active constituent promoting uterine smooth muscle contractions. Oxytocin facilitates the liberation of calcium from intracellular storage [29, 30]. It also signals uterine smooth muscle cells to produce prostaglandins [31] which can also play a role in labor induction and delivery [32].

Other fenugreek constituents

A recent review of the primary literature on medicinal plants reports that there are other phytochemical constituents besides saponins and oxytocin, that may be involved in hastening the process of labor and delivery. Some of these same herbals have been documented to have antioxidant properties and/or anti-inflammatory behaviors, and likely may involve some of the same biologically active agents [33].

Isolated constituents from fenugreek that have been reported to have potential to act as antioxidants include vitexin, isovitexin, apigenin, kaempferol and caffeic acid derivatives [34], as well as steroids such as cholesterol, β-sitosterol, diosgenin, and gitogenin, with gitogenin found only in the seeds [35]. It is recommend that these individual constituents be explored for possible uterotonic or oxytocic behaviors.

Future research

Since there are several constituents found within a fenugreek seed extract [3, 5, 7, 34, 35], determining which mechanisms signal activities across the plasma membrane will likely be very involved. Individual constituents could be tested on isolated uterine horns and their contractile responses analyzed and interpreted in light of selective receptor agonists and/or antagonist [36]. The interpretations would be insightful yet challenging as some constituents could act as receptor agonist and potentiate or facilitate smooth muscle contractions, while other constituents could antagonize receptor binding.

Conclusion

Fenugreek (*Trigonella foenum-graecum*) seed extract produced significant increases in smooth muscle tone of isolated mouse uterine horns as suspended in an organ bath. These results may help to justify the use of fenugreek by midwives and other health professionals who might utilize this herb in an attempt to aid in the induction of labor. Additionally, this information should be taken into account by pregnant women who may be exposed to this herb through various cuisines and do not want to go into labor prematurely.

Acknowledgments

The authors would like to thank (1) Beta Beta Beta Research Scholarship Foundation Fund for their financial support in purchasing mice, fenugreek and oxytocin: (2) the Division of Natural Sciences and the Department of Biological Sciences at Bethel University for use of equipment and lab supplies *in kind;* and (3) Griff DeGolier for help in producing the graphics.

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