



E-ISSN: 2278-4136  
 P-ISSN: 2349-8234  
 JPP 2017; 6(3): 346-350  
 Received: 23-03-2017  
 Accepted: 24-04-2017

**Zeinab Mohamed Ahmed**  
 Department of Pharmacognosy,  
 Faculty of Pharmacy, University  
 of Medical Sciences and  
 Technology, Khartoum, Sudan

**Ikram Mohamed Eltayeb**  
 Department of Pharmacognosy,  
 Faculty of Pharmacy, University  
 of Medical Sciences and  
 Technology, Khartoum, Sudan

**Amna El Hassan Hamad**  
 Department of Pharmacology,  
 Medicinal and Aromatic Plants  
 Research Institute, Khartoum,  
 Sudan

## Phytochemical Screening and contractile activity of *Foeniculum vulgare* seeds on rat intestinal and uterine muscles

**Zeinab Mohamed Ahmed, Ikram Mohamed Eltayeb and Amna El Hassan Hamad**

### Abstract

*Foeniculum vulgare*, commonly known as fennel is one of the commonest herbs used in traditional medicine. It is used to treat various ailments such as intestinal and uterine pain. There is no study had been done on the contractile activity of *F. vulgare*. This study was carried out to attempt the contractile activity of *F. vulgare* seeds extract on the intestinal and uterine muscles. The ethanolic extract of *F. vulgare* seeds were tested *in vitro* using the isolated tissue technique. The results showed that, the small doses of the of *F. vulgare* seeds extract caused an increase in the uterus muscle contractility of the wistar rat, while the higher doses caused a relaxant effect. No significant effect had been seen on the ileal muscle contractility.

**Keywords:** Phytochemical, Screening, Contractile, Activity, *Foeniculum, vulgare*, Seeds, Rat Intestinal, Uterine, Muscles

### Introduction

Uterine contractions are the contractions of uterine smooth muscles which play an important role in many reproductive functions such as the transport of sperms and embryo, menstruation, pregnancy and parturition (childbirth). Irregular uterine activity can cause various disorders such as infertility, improper implantation, dysmenorrhea, preterm labor (abortion), and weak uterine contraction during labor [1]. There are a number of intestinal motility disorders includes abnormal intestinal contractions, such as spasms and intestinal paralysis in which the gut loses its ability to coordinate muscular activity due to the endogenous or exogenous causes [2-4]. These disorders may be primary or secondary and may occur in a variety of ways such as abdominal distention, constipation, irritable bowel syndrome, and fecal incontinence.

*Foeniculum vulgare*, is an ancient, perennial herb of the Apiaceae family. It is well known to the ancient Egyptians, Romans, Indians and Chinese. It is originated in the southern Mediterranean region and grows through naturalization and cultivation in vegetable and herb gardens for its anise -flavored foliage and seeds due to their use in cooking [5].

The plant has been used in many traditional medicine systems such as the Indian, Unani, and Iranian systems [6]. The different parts of the plant, stem, fruit, seeds and whole plant are used to treat simple and complicated diseases and also has veterinary uses [7]. It is one of the most common medicinal plants traditionally used to treat the uterine and intestinal problems. It used for the treatment of various diseases such as abdominal pains [8], colic in children, diarrhea, fever, flatulence, gastralgia, gastritis constipation, and irritable colon. It is also used for arthritis, cancer [9], for conjunctivitis, insomnia [10], kidney ailments, laxative [11], leucorrhoea, liver pain, mouth ulcer [12]. The plant was reported to contain many secondary metabolites such as volatile oils, flavonoids and many phenolic compounds [13].

This study was carried out to investigate the contractile activity of *F. vulgare* seeds extract on the intestinal and uterine muscles of wistar rats.

### Materials and Methods

#### Plant Material Collection and Preparation

*F. vulgare* seeds were collected from Omdurman local market, and identified by taxonomist at the National Centre for Research, Medicinal and Aromatic Plants Research Institute (MAPRI), Khartoum, Sudan. The voucher specimen was deposited at Pharmacognosy Department, Faculty of pharmacy, University of Medical Science and Technology (UMST).The plant materials were dried and crushed by using mortar and pestle.

**Correspondence**  
**Ikram Mohamed Eltayeb**  
 IDepartment of Pharmacognosy,  
 Faculty of Pharmacy, University  
 of Medical Sciences and  
 Technology, Khartoum, Sudan

### **Phytochemical Screening Test**

The phytochemical constituents of the plant were detected using standard procedures as described by Trease and Evans (2002) [13] and Sofowora, (1993) [14]. The extract was prepared by reflux of 20 grams of powdered plant material with 250 ml 70% ethanol for two hours, the extract was then filtered and used for phytochemical screening.

### **Detection of Flavonoids**

Two ml of the extract were mixed with diluted NaOH to produce yellow coloration. Disappearance of the color upon addition of dil. HCl indicates the presence of flavonoids.

### **Detection of Sterols**

Two ml of the extract were evaporated to dryness, the residue was then dissolved in two ml of chloroform and transferred to clean dry test tube, two ml of acetic anhydride were added followed by addition of conc. H<sub>2</sub>SO<sub>4</sub> carefully to the wall of the tube. Color development from violet to blue or green indicates presence of a steroidal moiety.

### **Detection of Terpenoids (Salkowski Test)**

Two ml of the extract were mixed with two ml of chloroform. Three ml of conc. H<sub>2</sub>SO<sub>4</sub> were added carefully to form a layer, formation of a reddish brown color at the interface indicates the presence of terpenoids.

### **Detection of Alkaloids**

Two ml of the extract were acidified with 1% HCl, few drops of Mayer's reagent were added, appearance of turbidity indicates the presence of alkaloids.

### **Detection of Saponins**

#### **Frothing Test**

One gram of the powdered drug was extracted by boiling with ten ml of distilled water for ten minutes and was filtered. The filtrate was shaken vigorously in a test tube for thirty seconds and was allowed to stand for thirty minutes. Result was observed.

### **Ether Test**

One gram of the powdered drug was extracted by boiling with twenty ml. of methanol, under reflux, for 10-15 minutes and was filtered. The filtrate was cooled and 5-10 ml of ether was added. Result was observed.

### **Detection of Tannins**

One gram of the powdered drug was extracted by boiling with 20ml. of distilled water for 10 minutes and was filtered.

### **Ferric Chloride Test**

One drop of ferric chloride was added to two ml of the extract, in a test tube. Result was observed.

### **Gelatin Test**

Few drops of 1% gelatin solution containing NaCl were added. Result was observed.

### **Detection of Anthraquinones (Borntrager's Test)**

One gram of the powdered drug was extracted by boiling with 20 ml HCl, filtered and cooled. The extract was shaken with 5 ml chloroform. The organic layer was separated and shaken with dil. ammonia. Result was observed.

### **Detection of Cardiac Glycosides**

Two grams of the powdered drug was extracted with 25 ml

70% ethanol on water bath for 15-30minutes, and then filtered. The filtrate was diluted with the same volume of distilled H<sub>2</sub>O; 1-2 ml of strong lead acetate was added to precipitate resins, tannins and pigments. The filtrate was extracted with 15 ml (5 X 3) chloroform. The extract was divided into two portions (I and II),

### **Extract I (Keller Killiani test)**

- Extract I was evaporated to dryness (rotary evaporator)
- Extract I was dissolved in 2 ml of 3.5% FeCl<sub>3</sub> in glacial acetic acid, transferred to a clean dry test tube.
- 2 ml conc. H<sub>2</sub>SO<sub>4</sub> was poured on the wall of the test tube. Then the result was observed.

### **Extract II (Kedd's test)**

Extract II was evaporated to dryness, then dissolved in few drops of alcohol, 3,5dinitrobenzoic acid (Kedd's A) was added followed by NaOH (Kedd's B), then the color was noticed.

### **Detection for Bitter Principles (Furanochromone): Khellin**

0.5 g of the p was extracted with 10 ml 70% alcohol on water bath for five minutes and filtered. Few KOH pellets were added to the filtrate. The color was then observed.

### **Detection of Carbohydrates**

The alcoholic extract was dissolved in five ml distilled water and filtered. The filtrate was treated with two drops of alcoholic -naphthol. The formation of a violet ring at the junction was observed.

### **Detection of Reducing Sugars**

Two ml of the extract was heated with equal volumes of Fehling solution A and B. Appearance precipitate indicates the presence of reducing sugars.

### **Detection of Compound Reducing Sugars**

Two ml of the extract was hydrolyzed by boiling with five ml diluted HCL; the resulting solution was neutralized with NaOH solution. A few drops of Fehling solution were added, and then it was heated on a water bath for two minutes. Appearance of a reddish-brown was observed.

### ***In vitro* Contractility Test of Uterus and Intestine Muscles**

#### **Preparation of the Isolated Tissues**

#### **Preparation of the Rat Uterus Muscles**

A young female Wister rat, weighing 100 gm was used. The rat was brought into estrus by subcutaneous administration of estradiol valerate (2 mg/kg) 24 hrs prior to the experiment. The rat was killed by a blow on the head and bleeding. The abdomen was opened and the two uterine horns were exposed by pulling aside the intestine. Each horn was freed carefully from surrounding fat and mesenteric attachments, cut out separately and transferred to a petri-dish containing Ringer's solution. Each horn was cut and opened longitudinally to form a sheet of muscle instead of a narrow tube. A thread was attached at each end of piece and the preparation was mounted in a 25-ml organ bath containing aerated Ringer's solution maintained at 37° C with one attached to a fixed pin and the other to an isometric transducer (Harvard) connected to Harvard oscillographic recorder. The preparation was allowed to equilibrate for 45 min, under 0.5 tensions before addition of the plant extract and the reference drugs.

#### **Preparation of the Rat Ileum Muscles**

A Wister rat was killed by a blow on the head and bleeding.

The abdomen was opened and the ileum (last part of the small intestine) was taken; a portion of about 4-5 cm was cut out. The ileum was then transferred to a Petri dish containing Tyrode's solution where it was completely cleaned from fecal contents. A thread was attached at each end and the preparation was mounted in a 25ml organ bath full with aerated Tyrode's solution at 37 °C. One end was attached to a fixed pin and the other was connected to an isotonic transducer (Harvard) which was connected to Harvard Oscillograph recorder. The preparation was allowed to equilibrate for 45 min under 0.5g tension prior to drugs and plant extract administration.

### The Procedure of *in vitro* Contractility Effect

#### Addition of *F. vulgare* Seeds Extract and Drugs to the Rat Uterus

1 g of the extract, 0.01 g of isoprenaline and 0.01 g of 5-hydroxytryptamine were dissolved in water separately. First the normal contraction of the uterus muscle was observed and recorded, then doses of 1,2,4,8 mg of the extract were added and results were observed and recorded. Then doses of 10, 20,40 ng of isoprenaline and 80,160 ng of 5-hydroxytryptamine were added and results were recorded. All doses had washing periods between them, and contact time between the doses given and the uterus and we waited for 30 seconds after applying the drug or extract before recording of the response.

#### Addition of *F. vulgare* Seeds Extract and Drugs to the Rat Ileum

0.5 g of the extract, 0.01 g of acetylcholine, and 0.01 g of atropine were dissolved in water separately. First the normal contraction of the ileum muscle was observed and recorded. Then doses of 1, 2,4,8,16,32 ng of acetylcholine were added to increase the ileum contractility. Then doses of 1, 0.1, 0.01 mg of fennel ethanolic extract were added and results were observed and recorded. Finally doses of 1,2,4,8,16,36,64,128 ng of atropine were added and results were recorded. All doses had washing periods between them, and contact time between the doses given and the ileum and

we waited for 30 seconds after applying the drug or extract before recording of the response.

### Results and Discussion

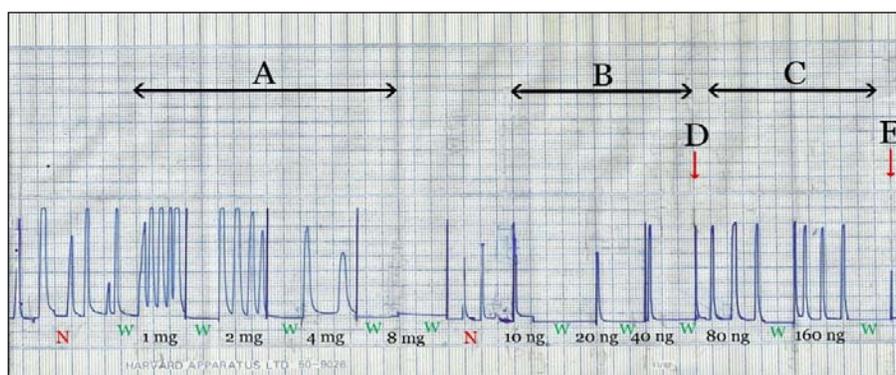
The results of phytochemical screening of *F. vulgare* seeds are shown in table 1. The main main secondary metabolites are found to be flavonoids, sterols, terpenoids, tannins, and bitter principles in addition to the compound reducing sugars. The results confirm the previous report of the presence of flavonoids essential oil and terpenoids<sup>[15-19]</sup>

**Table 1:** Phytochemical screening results of *F. vulgare* seeds

Screening Test	Observation	Results
Flavonoids	Yellow color	Positive
Sterols	Green color	Positive
Terpenoids <i>Salkowski's test</i>	Red brown ring	Positive
Alkaloids <i>Mayer's reagent</i> <i>Dragendorff's reagent</i> <i>Wagner's reagent</i> <i>Hager's reagent</i>	All no precipitate or change in color	Negative Negative Negative Negative
Saponins <i>Frothing test</i> <i>Ether test</i>	No change No change	Negative Negative
Tannins <i>Ferric chloride test</i> <i>Lead acetate test</i>	Bluish greenish color White precipitate	Positive Positive
Antraquinones <i>Borntrager's test</i>	No change	Negative
Cardiac glycosides <i>Keller Killiani test</i> <i>Kedde's test</i>	All no change	Negative Negative
Bitter principles	Red color	Positive
Carbohydrates	No change	Negative
Reducing sugars	Precipitate	Positive
Compound reducing sugars	No change	Negative

### Contractility Activity Results

A. The Effect of *F. vulgare* Seeds Extract on the Rat Uterus: The contractile responses of the rat uterus to various doses of *F. vulgare* and drugs are shown in figure 1.



**Fig 1:** The effect of *F. vulgare* seed ethanolic extract and drugs on the rat uterus contraction

- A- Different doses of the *F. vulgare* seeds extract.
  - B- Different doses of Isoprenaline (muscle relaxant)
  - C- Different doses of 5- hydroxytryptamine
  - D- The extract + Isoprenaline
  - E- The extract + 5-hydroxytryptamine
- N: normal W: wash

The uterus showed normal spontaneous contractile activity before adding any extract or drug (N).

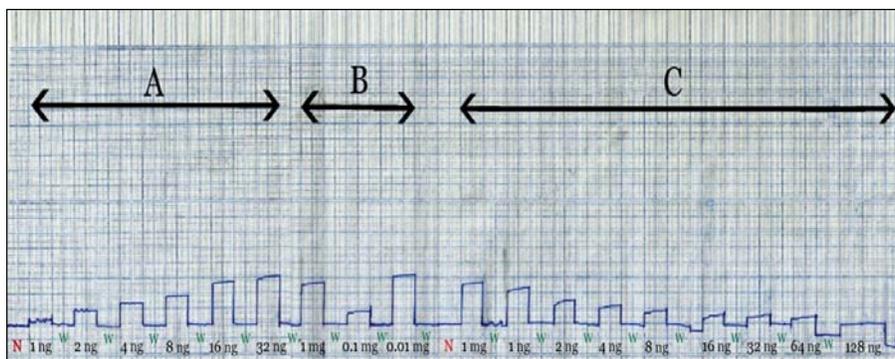
On adding 1mg of *F. vulgare* extract the frequency of these contractions increased. However by adding doses of 2 to 8 mg the frequency and amplitude started to decrease progressively and at a dose of 8 mg there was hardly No any contraction indicating a relaxing effect of this extract on the uterus with higher doses after an initial increase in contractility (A) On adding Isoprenaline in different doses it caused reduction in frequency of uterine contraction, i.e relaxant (B) following washing and adding 40 ng of Isoprenaline with 1mg of the

extract, Isoprenaline reduced the increased frequency of contractions (D).

After washing and adding 5 hydroxytryptamine in various doses it lead to uterine contractions (C). following washing, adding a high dose of the extract (8 mg) to 160 ng of 5 hydroxytryptamine resulted in reduction of the amplitude of

the contractility effect of 5 hydroxytryptamine (E), further confirming the relaxant effect of 8mg of the extract.

**B. The Effect of *F. vulgare* Seeds Extract on the Rat Ileum:**  
The contractile responses of the rat ileum to various doses of *F. vulgare* seed extract and drugs are shown in figure 1



**Fig 2:** The effect of *F. vulgare* seed ethanolic extract and drugs on the rat ileum contraction

- Contraction caused by different doses of acetylcholine
  - Contraction caused by different doses of ethanolic extract of fennel seeds
  - The response of the extract effect to addition of various doses of atropine.
- N: Normal W: wash

As shown in figure 2 the ileum showed contractions that increased progressively with increased doses of acetyl choline (A).

On adding *F. vulgare* extract the ileum showed contractions which were not related to the dose as though the contractility amplitude with a dose of 0.1 mg was less than that which occurred with 1 mg, yet the amplitude on adding 0.01 mg was almost similar to the one with 1 mg. this indicates that with these doses used *F. vulgare* did not show antispasmodic (relaxant) effect (B)

In figure 1(C) the first contraction resulted on adding 1 mg of the extract. After washing and adding another 1 mg of the extract to the ileum it contracted again and these contractility was antagonized by adding progressively increasing doses of atropine after washing and adding 1 mg of the extract each time.

### Conclusion

In conclusion *F. vulgare* seed extract with small doses caused increased uterine contractility but higher doses caused relaxation. Therefore the extract in high doses was found to be compatible with the traditional use of *F. vulgare* seed in the treatment of the painful uterine contractions such as dysmenorrhea. However the extract was found to have no effect on the contractility of the ileal muscle contractility, therefore other mechanisms possibly operate or explain the pain-relieving effect of the plant, further studies to explain these mechanisms are needed.

### References

1. Di Lorenzo C, Youssef NN. Diagnosis and management of intestinal motility disorders. *Semin Pediatr Surg.*, 2010; 19(1):50-8.
2. Keller J, Layer P, Intestinal and anorectal motility and functional disorders. *Best Pract Res Clin Gastroenterol.* 2009; 23(3):407-23.

3. Venkatasubramani N, Sood MR. Motility disorders of the gastrointestinal tract. *Indian J Pediatr.* 2006; 73(10):927-30.
4. AlOtaibi M, The physiological mechanism of uterine contraction with emphasis on calcium ion. *Calcium Signaling.* 2014; 1(2):101-119.
5. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *BioMed research international.* 3: 214-216.
6. Rahimi R, Ardekani MR, Medicinal properties of *Foeniculum vulgare* Mill. in traditional Iranian medicine and modern phytotherapy. *Chinese journal of integrative medicine.* 2013; 19(1):73-9.
7. Sharma R, Manhas RK, Magotra R. Ethnoveterinary remedies of diseases among milk yielding animals in Kathua, Jammu and Kashmir. India. *Journal of Ethnopharmacology.* 2012; 141(1):265-272.
8. Savo V, Giulia C, Maria GP, David R, Folk phytotherapy of the amalfi coast (Campania, Southern Italy). *Journal of Ethnopharmacology.* 2011; 35(2):376-392.
9. Tene V, Malagon O, Finzi PV, Vidari G, Armijos C, Zaragoza T, An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchi, Ecuador. *Journal of Ethnopharmacology.* 2007; 111(1):63-81.
10. Oliveira SG, de Moura FR, Demarco FF, da Silva Nascente P, Del Pino FA, Lund RG *et al.* An ethnomedicinal survey on phytotherapy with professionals and patients from Basic Care Units in the Brazilian Unified Health System. *Journal of Ethnopharmacology.* 2012; 140(2):428-37.
11. Mahmood A, Mahmood A, Malik RN, Shinwari ZK, Indigenous knowledge of medicinal plants from Gujranwala district, Pakistan. *Journal of ethnopharmacology.* 2013; 148(2):714-723.
12. Guarrera PM, Forti G, Marignoli S. Ethnobotanical and ethnomedicinal uses of plants in the district of Acquapendente (Latium, Central Italy). *Journal of Ethnopharmacology.* 2005; 96(3):429-444.
13. Akgül A, Bayrak A, Comparative volatile oil composition of various parts from Turkish bitter fennel (*Foeniculum vulgare* var. *vulgare*). *Journal of agricultural and food chemistry.* 1988; 30(4):319-323.
14. Zoubiri S, Baaliouamer A, Seba N, Chamouni N.

- Chemical composition and larvicidal activity of Algerian *Foeniculum vulgare* seed essential oil. *Arabian Journal of Chemistry*, 2014; 7(4):480-485.
15. Faudale M, Viladomat F, Bastida J, Poli F, Codina C. Antioxidant activity and phenolic composition of wild, edible, and medicinal fennel from different Mediterranean countries. *Journal of agricultural and food chemistry*, 2008; 56(6):1912-1920.
  16. Forster HB, Niklas H, Lutz S. Antispasmodic effects of some medicinal plants. *Planta medica*. 1980; 40(4):309-315.
  17. Reiter M, Brandt W. Relaxant effects on tracheal and ileal smooth muscles of the guinea pig. *Arzneimittel-Forschung*, 1984; 35(1):408-414.
  18. Ostad SN, Soodi M, Shariffzadeh M, Khorshidi N, Marzban H. The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study. *Journal of Ethnopharmacology*, 2001; 76(3):299-304.
  19. Khorshidi N, Ostad SN, Mosaddegh M, Soodi M. Clinical effects of fennel essential oil on primary dysmenorrhea. *Iranian journal of pharmaceutical research*. 2010; 20:89-93.