Short communication

The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study

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Abstract

Increasing the ectopic uterine motility is the major reason for primary dysmenorrhea. This motility is the basis for several symptoms including pain is the main complaint of patients with primary dysmenorrhea. There are several mechanisms, which initiate dysmenorrhea. Therefore, different compounds can be employed to control its symptoms. In long-term therapy, combination of oestrogens and progestins may be useful. In short-term therapy, dysmenorrhea sometimes non-steroidal anti-inflammatory drugs (NSAIDs) are used. Most of NSAIDs in long-term therapy show severe adverse effects. In an attempt to find agents with less adverse effect the fennel essential oil (FEO) was chosen for this investigation. In this article, effects of FEO on the uterine contraction and estimation of LD50 in rat were described. For assessment of pharmacological effects on the isolated rat uterus, oxytocin (0.1, 1 and 10 μg/ml) and prostaglandin E2 (PGE2) (5 × 10−5 M) were employed to induce muscle contraction. Administration of different doses of FEO reduced the intensity of oxytocin and PGE2 induced contractions significantly (25 and 50 μg/ml for oxytocin and 10 and 20 μg/ml PGE2, respectively). FEO also reduced the frequency of contractions induced by PGE2 but not with oxytocin. LD50 of FEO was obtained in the female rats by using moving average method. The estimated LD50 was 1326 mg/kg. No obvious damage was observed in the vital organs of the dead animals. © 2001 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Dysmenorrhea; Fennel essential oil; Pain

1. Introduction

Menstrual pain that occur in the absence of visible organic pelvic origin is nominated primary dysmenorrhea. More than half of all postmenarcheal women complain of dysmenorrhea at least once during their life. About 10% of these women cannot have normal life for 1–3 days in each month (Dawood, 1990). It is shown that the pain associated with this disorder is caused by hypercontractility of uterine muscle, subsequent reduction in blood flow and concomitant uterine ischemia (Akerlund, 1979). Several factors may involve in the uterine hypercontractility including: ovarian steroids, cervical obstruction, pituitary hormones and most notably prostaglandins. It has been demonstrated that prostaglandin production in the uterine lining is up to seven times greater in women with clinically diagnosed dysmenorrhea as compared with controls. For this reason, successful treatment of this disorder has been achieved with drugs that inhibit prostaglandin synthesis and reduce uterine hypercontractility (Dawood, 1990). Tocolytic agents such as β2 stimulator or calcium channel blockers are employed to inhibit muscular contraction and increase uterine blood flow. In
spite of their effectiveness, side effects of these drugs in long-term therapy limit its clinical uses (Hansen and Secher, 1975; Anderson et al., 1978). Fennel (foeniculum vulgare mill) is a well-known umbelliferous plant. The seeds of this plant have been known to be able to promote of menstruation, alleviate the symptoms of female climacteric, and increase libido (Albert-Puleo, 1980). FEO also possesses emmenagogue and galactagogue properties (Hare et al., 1916). It has been previously reported that FEO used in the pediatric colic and some respiratory disorders due to its anti-spasmodic effects (Reynolds, 1982). Seeds of fennel are used in folk remedies for treatment of dysmenorrhea. This traditional usage might be related to anti-spasmodic effects of FEO. The present study was designed to examine the scientific basis of traditional use of FEO by routine pharmacological and toxicological studies.

2. Methods and materials

2.1. Evaluation of pharmacological activity

Uterine strips were obtained from virgin Wistar rats (180–250 g) kept in a controlled temperature (25 ± 1 °C) and illumination (12 h on, 12 h off) room. The animals were treated with oestradiol valerate (5 mg/kg, s.c.) 24 h before the experiments. Animals were then be scarified by cervical dislocation and the uterine horns were removed and placed in dejonal solution (NaCl, 154 mmol/l; KCl, 5.6 mmol/l; CaCl2, 0.3 mmol/l; MgCl2, 1.4 mmol/l; NaHCO3, 1.7 mmol/l and glucose, 5.5 mmol/l). Uterine strips (15-mm long) free from adhering tissues were mounted in 50 ml jacketed organ bath containing dejonal solution, maintained at 31 °C and continuously aerated. One end of tissue was tied to perspex holder and the other end of the tissue was tied under 1 g resting tension to grass f-50 isometric transducer. Tension was recorded on a physiograph recorder as described by Calixto and Yunes (1991).

Following an equilibration period of 30–40 min, cumulative curves (Von Rossum, 1963) for oxytocin (obtained from Pars Minoo Co., Ltd., Iran) were constructed. After the concentration–response curves became constant, different concentration of FEO (25, 50 and 100 µg/ml final concentration in the organ bath, obtained from Barij Essence Co. Ltd., Iran) were added to the bath and left in contact with the tissue for 10–20 min. Different concentrations of FEO (10, 20 and 40 µg/ml) were employed to inhibit response of uterine when was treated by 5 × 10−5 M of prostaglandin E2 (PGE2) (Prostin E2, Upjohn, USA) final concentration in organ bath. Oxytocin and PGE2 were added to separate strips and control experiments were performed with the vehicle (90% ethyl alcohol added to the organ bath).

2.2. Acute toxicity assay

The routine acute toxicity assay was performed using female Sprague–Dawley rats, which were bred on house and acclimated 2 weeks before experiment (Ecobichon, 1997). Due to possible oral use of FEO for dysmenorrhea, the oral LD50 protocol was performed for it. FEO was dissolved in minimum amount of 90% ethyl alcohol and used throughout experiments. Due to small amount of FEO, it was used with normal saline. Vehicle group was treated in the same manner without using FEO. To obtain toxic dose level and reducing the number of animals, which should be used, five rats were chosen randomly with average weight of 183 ± 34 g. Four doses of 50, 500, 1000 and 2000 mg/kg of FEO and vehicle were administered by gavages to each rat. Animals were starved for 16 h before each experiment. Furthermore, the volume of gavages for each experiment was constant by using different concentration of FEO in 90% ethyl alcohol. Maximum amount of alcohol, which was used in the test group, was chosen for vehicle control. Animals were evaluated for any possible toxic effects. Dosages for final experiment was chosen according to the results obtained from observed our experiment.

2.2.1. LD50 assay

LD50 assay was studied in eight groups of rats. Each group contained five rats with average weight of 180 ± 25 g. These groups were divided into control, vehicle, 1000, 1125, 1250, 1375 and 1500 mg/kg of FEO, respectively. After administration of FEO animals were observed closely during first 48 h and then every 12 h. Lung, liver, kidney, stomach and intestine of dead animals were examined for any pathological sign by using histological methods. Alive animals were observed for any behavioural, respiratory and cutaneous changes.

2.3. Statistical analysis

Statistical analysis of the pharmacological data was performed with an analysis of variance followed by the Newman–Keuls test. Differences with P < 0.05 and 0.01 were considered statistically significant. The moving average method was employed for determination of LD50.

3. Results

3.1. Pharmacological study

Figs. 1 and 2 show the inhibitory effect of fennel essential oil (FEO) on the response of uterine when treated by oxytocin and PGE2, respectively. As it is
shown in Fig. 1, 25 μg/ml of FEO caused no remarkable effect in uterine contraction. FEO at 50 μg/ml caused a parallel and concentration dependent rightward displacement of oxytocin (0.1–10 μU/ml) dose–response curve (P < 0.01). Fig. 2 shows the significant inhibitory effect of FEO on the response caused by 5 × 10⁻⁵ M PGE₂ (P < 0.01). In the case of PGE₂, both the frequency and intensity of uterine contraction decreased during experiment but in the case of oxytocin only the intensity decreased by increasing the dose of FEO.

3.2. Toxicity study

In present study, during the first 24 h animals in groups of 1500 and 2000 mg/kg FEO died. Based on these results, the final test was performed in the range of 750–1500 mg/kg. Table 1 shows the result of final test. The value of LD₅₀ was 1326 mg/kg that was calculated by probit analysis method. Animals in group 8 showed prostration, sedation, respiratory distress, movement disorder, and unresponsiveness to external stimulation, hind limb weakness, tremor and fasciculation in dorsal muscles during first 24 h after FEO ingestion. The severity of toxicity in groups 7, 6 and 5 decreased respectively and other groups showed no significant toxicity. In groups 4, 5 and 6, the most dominant adverse effect was sedation. In all groups, the amount of 24 h urine increased parallel to the amount of given FEO. Histological observations in different tissues of dead animals did not show any significant tissue damage (Figs. 3–12).

**Table 1**

Results of animal death in the LD₅₀ final measurement

<table>
<thead>
<tr>
<th>Group number</th>
<th>Dose (mg/kg)</th>
<th>Number exposed</th>
<th>Number of death</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>750</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1000</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1125</td>
<td>5</td>
<td>1</td>
<td>20</td>
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<td>6</td>
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<td>5</td>
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<tr>
<td>7</td>
<td>1375</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>1500</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
</tbody>
</table>
4. Discussion

The present results demonstrate that FEO can inhibit contraction of isolated uterus that was induced by oxytocin and PGE\textsubscript{2}. The pattern of response during the administration of oxytocin and PGE\textsubscript{2} showed that the mechanism of FEO inhibition was probably different in these two cases. These effects also confirm the previous
report about uterine relaxation after FEO administration (Saleh et al., 1996). Although all NSAIDs can reduce this contractility by inhibition of arachidonic acid pathway but some NSAIDs such as diclofenac, piroxicam and indomethacin, which have direct muscle relaxation effect, seems to be more efficient. In the case of diclofenac, the contraction of uterine muscle, which is induced by 4 μg/ml oxytocin, was inhibited by 81 mg/ml in vitro (Perez et al., 1990). The optimum dose of FEO in our experiment for inhibition of similar contraction is 100 mg/ml suggesting that the mechanism of action is possibly similar to diclofenac. Although the mechanism of action of fennel oil is unknown but it seems the uterine smooth muscles are directly influenced by FEO as well as its central action in intact animal. Furthermore, the effect can be contributed to oestrogenic activity of FEO. It has been shown that the isometric developed tension of spontaneous contractions in isolated rat uterine strips is clearly influenced by sex hormones (Gimeno et al., 1979; Sterin-Speziale et al., 1980; Chaud et al., 1984). Moreover, uterine preparations isolated from ovariec-tomised rats treated with 17-beta-oestradiol (17-β-E2), exhibited a more diminished spontaneous motility than the same time from 17-β-E2 untreated animals (Sterin-Speziale et al., 1980). The main constituent of fennel oil is anol or dimethylated anethole that may have mild oestrogen-like activity (Albert-Puleo, 1980) and induces oestrus in mice (Dodds and Lawson, 1937) inhibit spasms in smooth muscles (Forster et al., 1980). The results of acute toxicity test showed that the FEO could be classified in the group of slightly toxic substance on the basis for classification of chemical substances. No pathological toxicity was seen in organs of dead animals, which indicate that death may be due to metabolic effects of metabolite imbalance or some kind of nervous toxicity. The emotional and behavioural toxicity in intoxicated animals may confirm this conclusion. Anethole is the major fragrant in the FEO (Duke, 1965). It has been shown that anethole induce ataxia and hypnotic activity in rat (Boissier et al., 1967). Several pathophysiological phenomena can cause dysmenorrhea. Although the reason of dysmenorrhea is not fully understood but it seems irregularity in the peristaltic movement in the uterine muscle should be the major cause of dysmenorrhea. PGs may be one of the groups that cause these pathophysiological disorders and so for several years prostaglandin inhibitors were employed to control symptoms of dysmenorrhea. NSAIDs with muscle relaxant property are more useful
and therefore the major factor for control of symptoms can be muscle relaxation. Increasing muscle relaxation may lead to increase in volume of total bleeding but this increase is not too much problem for normal women (Khorshidi et al., unpublished data). If this negative point outweigh with effect of FEO on reducing dysmenorrhea pain, FEO may be of use for alleviation of dysmenorrhea sequelle.

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References

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