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Evaluation of the Prophylactic Effect of Fennel Essential Oil on Experimental Osteoporosis Model in Rats

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Abstract: The water-distilled essential oil of Iranian fennel seeds (*Foeniculum vulgare* Mill.) was investigated for its composition and anti-osteoporotic activities in ovariectomized rat osteoporosis model. After oil analysis by GC/MS, 15 components were identified in the oil. Among them, trans-anethole (81.1%) and fenchone (9.2%) were the major components. Healthy female albino rats were divided into five groups of six animals each including sham operated (control), ovariectomized-vehicle and ovariectomized treated animals receiving fennel essential oil (FEO 500, 750, 1000 mg kg^{-1}) or estradiol valerate (5 mg kg^{-1}) for 30 days. The findings assessed on the basis of bone mineral density and uterine weight showed that the fennel essential oil has a preventive effect on development of osteoporosis in ovariectomized rats. This protective effect of FEO on early post-ovariectomy bone loss was dose dependent and at the dose of 1000 mg kg^{-1} it was even more than estradiol (BMD of 0.082±0.008 g cm^{-2}, $p<0.05$) but more toxic than other doses. *Trans-anethole,* probably has an important role in these pharmacological effects.

Key words: Fennel, anethole, osteoporosis, bone mineral density

INTRODUCTION

Osteoporosis is the most common metabolic bone disease (Cheung *et al*., 2004). WHO defines osteoporosis as a systematic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures (Consensus Development Conference, 1993). The most common type of osteoporosis is the bone loss associated with menopausal ovarian hormone deficiency. Convention therapies for treating osteoporosis in women include hormone replacement therapy, either estrogen alone or combination of estrogen and progesterone, calcitonin, raloxifen and bisphosphonates (North American Menopause Society; 2002, Larsen *et al*., 2003).

The Women's Health Initiative initial study results demonstrated a statistically significant reduction in fractures, including hip fractures, in a large group of otherwise healthy women using hormone therapy. However, it also indicated a significantly increased risk of cardiovascular events and breast cancer for women taking combined estrogen and progestin therapy (Rossouw *et al*., 2002). Thus, active research is still vigorously pursued to identify new agents for the treatment of postmenopausal osteoporosis with minimal side effects, especially in herbal medicine area.

Phytoestrogens have attracted new attention as a potential agent to prevent and treat postmenopausal osteoporosis in women. The evidence from both animal and human studies demonstrates that soy isoflavones favorably impact bone health (Brynin, 2002).

Although the potential beneficial effects of a high phytoestrogen diet have been reported in some articles (Whitten and Patisaul, 2001; Mazur and Adlercreutz, 2000; Adlercreutz and Mazur, 1997), only few articles are available on the potential impact of these compounds on osteoporosis treatment and/or prevention.

The preventive effect of soybean protein on ovariectomized (OVX) induced bone loss (Arjmandi *et al*., 1996) has been attributed to its high content of the isoflavones genistein and daidzein phytoestrogens which possess a range of biological effects including estrogenic, antiestrogenic, antiviral and antiproliferative actions (Adlercreutz, 1990). Zhang *et al*. (2004) has reported increased BMD level, serum estradiol and inorganic phosphorus content and uterine index, lower tartrate-resistant acid phosphatase (TRAP), alkaline phosphatase (AKP) and interleukin-6 levels after oral administration of phytoestrogens for 90 days to OVX rats.

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They have concluded that phytoestrogen is effective in preventing and treating osteoporosis in ovariectomized rats.

The ovariectomized rat is the most appropriate model for studying the mechanism as well as potential treatments of postmenopausal osteoporosis in humans. It is also a good model to study the efficacy of various candidates for their prevention and/or reversal of bone loss (Miller, 1997; Wronski and Yen, 1991).

The consumption of phytoestrogens is becoming increasingly popular as food supplements because of their associated myriad of health benefits. They are frequently advertised as a natural alternative to Estrogen Replacement Therapy (ERT).

Fennel (*Foeniculum vulgare* Mill) is an Umbelliferous plant. Fennel oil is used as a carminative and flavoring agent. It is used in herbal remedies for respiratory tract disorders, indigestion and is also used to increase milk flow in nursing mothers. Fennel seeds have been reputed to increase milk secretion, promote menstruation, facilitate birth and alleviate the symptoms of the female climacteric, increase libido and alleviation of the dysmenorrheal symptoms (Ibn Sina, A., 1998; Ostad *et al*., 2001; Namavar Jahromi *et al*., 2003). Avicenna, one of the famous Iranian practioners was also familiar with this plant and has mentioned its medicinal properties in his books (Ibn Sina, 1998).

Mimica Dukic *et al*. (2003) have reported the antifungal activity of the Fennel Essential Oil (FEO). Significant reduction in the intensity of oxytocin and prostaglandin E induced uterine contractions by different doses of FEO (25 and 50 μg mL^{-1} for oxytocin and 10 and 20 μg mL^{-1} for PGE and its efficacy for the treatment of primary dysmenorrheal (with lower potency than mefenamic acid) has been reported as well (Ostad *et al*., 2001; Namavar Jahromi *et al*., 2003). Also, fennel seed extract has been shown to have estrogenic activity (Malini *et al*., 1985). Antioxidant effect (Oktay *et al*., 2003) and antihirsutism activity (Javidnia *et al*., 2003) of fennel extract has been reported. Therefore, based on its estrogenic activity, the present study was carried out in an attempt to evaluate the potential activity of Fennel Essential Oil (FEO) in rats using OVX model of osteoporosis.

Since there are some reports of compounds with estrogenic like activities in FEO, we also analyzed FEO constituents using GC/MS.

MATERIALS AND METHODS

Plant collection: Ripe fruits of *F. vulgare* were collected from cultivated plants at northern parts of Isfahan province, Iran in summer 2003. The plant identity was confirmed by the Herbarium Department of Shiraz School of Pharmacy, Shiraz, Iran. A voucher specimen of the plant was deposited in the Herbarium of our School.

Essential oil preparation: The freshly dried fruits of the plant were segmented and the volatile fraction was isolated by a water-distillation method for 2 h according to the method recommended in British Pharmacopoeia (1998). The oil obtained in ca. 3.0% (v/w) yield was dried over anhydrous sodium sulfate and refrigerated at 4°C.

Essential oil analysis: The oil was analyzed by GC/MS using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 GC according to a standard method (Ghannadi *et al*., 2004).

Identification of components in the oil was based on retention indices relative to n-alkanes and computer matching with the WILEY 275. L library, as well as by comparison of the fragmentation patterns of the mass spectra with those reported in the literature (Adams, 1995; Sandra and Bicchi, 1987).

Study protocol: Ninety-day-old Sprague-Dawley nulliparous female rats, with mean weight of 200 g, were selected and housed at general food and environmental conditions. Access to food and water was *ad libitum*.

Thirty six rats were randomized into six groups of 6 animals. Group 1 was normal rats (sham operated), five other groups (group 2-6) were OVX.

The day before surgery, three OVX groups of rats were started on daily intrapritoneal injections of 500, 750 or 1000 mg kg^{-1} of body weight of FEO, up to 30 days. One other OVX group (group 2, OVX-Veh) and sham operated animals (group 1, Sham-Veh) received intraperitoneal injection of vehicle (normal saline) for the same period. In remained OVX group, estradiol valerate was injected subcutaneously at a dose of 5 mg kg^{-1}. Selection of the doses of FEO was based on the reported estimate of LD_{50} of FEO (1326 mg kg^{-1}) by Ostad *et al*. (2001).

The animals were killed 30 days after surgery, the right leg of each rat was dissected and the tibiae were harvested for densitometric analysis. The uterus of each rat was also dissected out for immediate determination of weight. Bone densitometry of the whole tibiae was performed with Dual-Energy X-Ray Absorption (DEXA) scanner (NORLAND, USA) equipped with a high resolution for the evaluation of bone mineral in small subjects. Both Bone Mineral Content (BMC) and Bone Mineral Density (BMD) was determined.

Statistical analysis: Data were obtained from 2-3 measurements for each animal and were expressed as the mean±SD. Statistical comparisons were made by ANOVA with Tukey posthoc test using SPSS version 10 software. Differences with $p<0.05$ were considered significant.

Table 1: List of the components of *F. vulgare* fruits oil

Compound	%	RI
alpha-pinene	0.5	938
camphene	0.2	953
beta-pinene	0.2	978
beta-myrcene	0.1	990
para-cymene	0.1	1024
limonene	5.0	1031
cis-beta-ocimene	0.3	1037
gamma-terpinene	0.1	1053
fenchone	9.2	1076
trans-limonene oxide	0.1	1119
camphor	0.1	1129
estragole	0.3	1185
trans-anethole	81.1	1272
alpha-copaene	0.1	1369
germacrene-D	0.3	1475

Table 2: Bone density and uterine weight in different study groups (control, OVX and FEO-treated rats) 30 days after ovariectomy

Groups	Bone density (g cm^{-2}) Mean±SD	Uterine weight (g) Mean±SD
Control (Sham-veh),	0.075±0.007	0.444±0.128***
Ovarectomy (OVX-Veh)	0.0633±0.004*	0.410±0.080***
FEO (500 mg kg^{-1}) 30 days treatment	0.0710±0.006	0.521±0.053***
FEO (750 mg kg^{-1}) 30 days treatment	0.076±0.005**	0.805±0.340**,***
FEO (1000 mg kg^{-1}) 30 days treatment	0.082±0.008**	0.925±0.192*,**,***
Estradiol valerate (5 mg kg^{-1}) 30 days treatment	0.074±0.008	1.335±0.222*,**,***

*p<0.05 vs. Sham-Veh, **p<0.05 vs. Ovx-Veh, ***p<0.05 vs. Estradiol

RESULTS

Analysis of the essential oil: Fruits of *F. vulgare* yielded an essential with a spicy pleasant odor. Fifteen components were characterized, representing 97.7% of the total oil components detected. The chemical composition of the essential oil of *F. vulgare* fruits has been shown in Table 1 with their percentage compositions and retention indices. Many of the unidentified compounds were present in trace amounts. The major constituents of the essential oil were *Trans-anethole* (81.1%), fenchone (9.2%) and limonene (5.0%).

Bone mineral density: BMD of the whole tibiae was significantly lower in the OVX-Veh than in all other groups (0.0633±0.004 g cm^{-2}). Daily injection of OVX rats with 500, 750 and 1000 mg fennel per kilogram body weight reduced the early post-ovariectomy loss of BMD (Table 1, $p<0.05$). This protective effect of FEO on bone loss, at the dose of 1000 mg kg^{-1} (BMD of 0.082±0.008 g cm^{-2}) was significantly($p<0.05$) higher than other doses of fennel and estradiol (0.074±0.008 g cm^{-2}) but this dose was accompanied by higher mortality rate as well (Table 2).

Uterine weight: The uterine weight in OVX rats that were given FEO (especially at higher doses) and estradiol was significantly more than Sham-Veh and OVX-Veh rats, but estradiol had the most prominent effect than FEO (1.335±0.222 g) (Table 2).

DISCUSSION

In this study, intraperitoneal injection of different doses of FEO to short-term OVX rat increased both BMD and uterine weight in a dose dependent trend. It suggests the possible estrogenic activity of FEO and is in accordance with the results of the previous studies (Ostad *et al*., 2001; O'Connel, 1998).

Since FEO do increase the uterine weight as well as the BMD, it is suggested that this agent is possibly function as an estrogen agonist. The effect of the highest administered dose of this study (100 mg kg^{-1}) was even higher than estradiol valerate, but for uterine weight it was less.

To the best of our knowledge, this is the first report of beneficial effect of FEO in prevention of osteoporosis using OVX rat model of osteoporosis. There is not any published study about its effect in other animal osteoporosis models or in human. Its long term preventive effects and its possible effect in the treatment of osteoporosis merits future studies.

Many of the identified oil components were present in the essential oil of fennel fruits reported before (Bilia *et al*., 2002; Yamini *et al*., 2002). More than 80% of the essential oil components of the plant were due to *trans*-anethole. As mentioned previously anethole seemed to have an estrogenic activity (Tabanca *et al*., 2004; Howes *et al*., 2002) and it has been associated with other pharmacological findings of the present study. *trans*-anethole appears to have the potential to interact with estrogen receptors, although its biological significance is uncertain. Therefore, it seems that an important part of the anti-oesteoporotic effects of FEO in rats is due to the presence of *trans*-anethole, although it is not so clear whether this compound is the only contributing component of this plant essential oil or not. Further works are needed to clarify the role of the other constituents of FEO and their exact mechamisms.

One pitfall of our study is using the same diet for both control and FEO treated rats, because standard laboratory chows contain significant amounts of phytoestrogens given their high soy content (Thigpen *et al*., 1999; Boettger-Tong *et al*., 1998). However, despite the above pitfall, the significant difference between FEO treated OVX rat and Sham-Veh and OVX-Veh suggests a potentially more efficacy of FEO in preventing osteoporosis in OVX rats.

It has been reported that both bone resorption and bone formation are promoted by ovarectomy (Wronski and Yen, 1991). Estrogen induces a decrease in bone resorption and may also have some anabolic effect,

particularly in high doses in postmenopausal women at all ages (Prestwood *et al*., 1994; Vedi *et al*., 1999). FEO may act by the same way as estrogen, however its exact mechanism of action still remains to be cleared by further investigation using bone histomorphology methods (such as electron microscopy) and biochemical markers of bone formation and resorption (such as serum osteocalcin, serum alkaline phosphatase, urine hydroxyproline and calcium).

The ovariectomized rat has been widely used as a model of postmenopausal osteoporosis. Although this model is useful for experimental design in studies of postmenopausal bone loss, its relevance in assessing postmenopausal women with osteoporosis remains invalidated (Namkung-Matthai *et al*., 2001). Therefore, well designed clinical trials for assessing this preventive osteoporotic effect of FEO is also warranted.

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