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Editorial

Artigos Originais

Artigo Original

An experimental investigation on effect of *Foeniculum vulgare* Mill. on gestation

Investigação experimental sobre o efeito de *Foeniculum vulgare* Mill. na gestação

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Abstract

Foeniculum vulgare (fennel), common in culinary and herbal Medicine, is also been used to induce menstruation and abortion. Therefore, its abortive and teratogenic potential were evaluated in this study. Seed infusion, inflorescence and leaf hydroalcoholic extracts were orally administered to mice from the first to the third gestational day (pre-implantation period), from the fourth to the sixth gestational day (implantation period) or from the seventh to the ninth gestational day (early organogenesis). On 18th gestational day, the number of *corpora lutea*, implantation sites, embryonic resorptions and fetuses were recorded. Fetuses were examined for malformations and skeletal anomalies. Seed infusion caused neither pre-implantation loss, nor embryonic or fetal death, but skeletal variations were observed. When administered from the fourth to the sixth gestational day, inflorescence and leaf extracts reduced the implantation success and consequently the birth rate. Malformations were more frequent in these treated groups. The results obtained in this study demonstrated that seed infusion did not exhibit adverse effect on reproductive parameters and rates. Otherwise, leaf and inflorescence hydroalcoholic extracts reduced the implantation and consequently the birth rate when administered on implantation period. Therefore, the seed infusion taken popularly might not affect the pregnancy, but the fennel has an anti-implantation potential, and the concentration or isolation of active substances is necessary to this result. Skeletal anomalies findings in seed infusion-treated groups and the significant incidence of external and visceral malformations in leaf and inflorescence extracts-treated groups from the fourth to the sixth gestational day suggest teratogenic potential by fennel.

Uniterms: *Foeniculum vulgare*; Medicinal plants; Abortifacient agents; Pregnancy; Mice.

Resumo

Foeniculum vulgare (funcho), comum na culinária e na medicina popular, é também usada para induzir menstruação e aborto. Assim, seu potencial abortivo e teratogênico foi avaliado neste estudo. A infusão das sementes e os extratos hidroalcoólicos das inflorescências e das folhas foram administrados a camundongas do primeiro ao terceiro dia de gestação (período pré-implantação), do quarto ao sexto dia de gestação (período da implantação) ou do sétimo ao nono dia de gestação (início da organogênese). No 18º dia de gestação, o número de corpos lúteos, sítios de implantação, reabsorções embrionárias e fetos foi registrado. Fetos foram examinados para malformações e anomalias esqueléticas. A infusão das sementes não causou perda pré-implantação, nem morte embrionária ou fetal, mas variações esqueléticas foram observadas. Quando administrados do quarto ao sexto dia de gestação, os extratos das inflorescências e das folhas reduziram o sucesso de implantação e consequentemente o índice de natalidade. Malformações foram mais frequentes nesses grupos tratados. Os resultados obtidos neste estudo demonstraram que a infusão das sementes não exibiu efeito adverso sobre os parâmetros e índices reprodutivos. Em contrapartida, os extratos hidroalcoólicos das inflorescências e

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das folhas diminuíram o sucesso de implantação e consequentemente o índice de natalidade quando administrados no período da implantação. Portanto, a infusão das sementes tomada popularmente pode não afetar a gestação, mas o funcho tem potencial anti-implantação, e a concentração ou o isolamento de substâncias ativas é importante para esse resultado. Os achados de anomalias esqueléticas nos grupos tratados com infusão das sementes e a incidência significativa de malformações externas e viscerais nos grupos tratados com extratos das inflorescências e das folhas do quarto ao sexto dia de gestação sugerem potencial teratogênico por funcho.

Palavras-chave: *Foeniculum vulgare*; Plantas medicinais; Abortivos; Gestação; Camundongos.

Introduction

Foeniculum vulgare Mill. (Apiaceae), known popularly as fennel, is commonly used in herbal Medicine and in culinary. Seed and leaf infusions are used for its antispasmodic and carminative effects[1], and seeds or its essential oils are used as flavoring agents in food.

Fennel has been reported to contain estrogenic substances and to increase milk secretion, promote menstruation and alleviate symptoms of climacteric[2]. The juice of fresh leaves is taken for fertility controlling purposes in Ethiopia[3]. In addition, the use of *F. vulgare* extracts as remedy for control of primary dysmenorrhea increases concern about its teratogenic potential due to its estrogen-like activity[4]. Considering the related toxicity of fennel extracts, the use during gestation can be harmful since the embryo, during its phase of organogenesis, may be sensitive to toxic insult from certain drugs and other xenobiotics[5].

Due to common use of *F. vulgare* in herbal Medicine, including by pregnant women, this research was performed to investigate its effects on the gestation. Seed aqueous extract and inflorescence and leaf hydroalcoholic extracts were administered to female mice on the pre-implantation, implantation or post-implantation period, and reproductive parameters were assessed. Fetuses were examined for anomalies and malformations, because if pregnancy is not interrupted, teratogenicity might be produced.

Material and Methods

Vegetal material

F. vulgare was collected from a garden at Caxias do Sul (RS), Brazil. The aerial parts were dried in a ventilated place at room temperature for 15 days.

The infusion was prepared with 9 g of seeds in 350 mL of hot distilled water for 12 minutes. The extract was filtered and stored in aliquots in a freezer until use. To establish the solid content, a sample was lyophilized: 10 mL of aqueous extract had 10.3 mg of dry matter. Therefore, the yield was 4% in relation to seed mass.

Inflorescence (seeds and stems) and leaf were ground separately in an electric grinder. Inflorescence (366.3 g) and leaf (385.5 g) powder were extracted by maceration with 4 L of 70% ethanol under stirring for 96 hours. Extracts were filtered, concentrated in a rotary evaporator at 50ºC and lyophilized. Concentration and lyophilization were made at Laboratory of Phytochemistry, Faculty of Pharmacy at *Universidade Federal do Rio Grande do Sul* (UFRGS). The yields of the inflorescence and leaf extracts in relation to the dried vegetal material were 14 and 15.8%, respectively. The extracts were stored in a freezer until use.

Animals

This evaluation was carried out in Laboratory of Reproduction Biology, Department of Morphological Sciences at UFRGS. All procedures were performed in accordance with the ethical principles for animal research adopted by the Brazilian College of Animal Experimentation and approved by Research Committee of the Institute of Health Basic Sciences at UFRGS.

CF1 mice acquired from the State Foundation of Production and Research in Health (Porto Alegre, RS, Brazil) were used. They were maintained under temperatures from 22 to 26°C and 12-hour light/dark cycle. Animals were fed with Nuvilab Cr 1® rat food (Nuvital, Colombo, PR, Brazil) and water ad libitum.

Evaluation of abortive activity

Nuliparous female mice (2- to 3-month-old) were mated with male mice in a 2:1 ratio and examined for a vaginal plug on the following morning. The day on which the vaginal plug was observed was considered as first gestational day (GD).

Females received 1,000 mg/kg/day of inflorescence or leaf extract (suspended in distilled water in a proportion of 1,000 mg to 4 mL) or 4 mL/kg/day of seed infusion or distilled water (control group). The dose of 1,000 mg/kg corresponds to that of the limit test in the guideline for reproduction and teratogenicity tests[6]. The administration was oral, using a curved feeding needle and a tuberculin syringe. Treatments were restricted to first half of gestation, segmented in the pre-implantation (first to third GD), implantation (fourth to sixth GD) and post-implantation (seventh

to ninth GD) periods. According to recommendations for reproductive toxicity using rodents, each group contained 20 animals[6-8]. Animals were weighed on the first GD, on the first day of administration, on the day after the last dose, and on the 18th GD, when they were euthanized by cervical dislocation and laparotomized. Ovaries were collected and placed in a Petri dish with saline for counting their *corpora lutea* (or albicans) using a stereomicroscope. Uterus was opened for counting of live and dead fetuses, degenerated embryos and late resorptions, and incubated for 10 minutes in 10% ammonium sulfide for counting of implantation sites and early resorptions[9]. Placentae and live fetuses were weighed. Fetuses were examined for external malformations and fixed either in Bouin's fluid for posterior analysis of internal malformations[10] or in 95% ethanol for staining with alizarin red S[11] and identification of skeletal anomalies, observation of skull plates and counting of metacarpals, metatarsals, sternebrae and xiphisternum, ribs, lumbar vertebrae, and sacral and caudal vertebrae.

Statistical analysis

The weight of the body, placenta, fetus and uterus, and the number of *corpora lutea*, implantation sites and live fetuses were expressed as mean ± standard deviation and analyzed by one-way ANOVA, post hoc multiple comparisons Dunnet's *t*-test.

Number of embryonic resorptions, degenerated embryos and dead fetuses, and reproductive indices were expressed as median and quartiles range and analyzed by Kruskal-Wallis test when compared all groups or by Mann-Whitney U test when compared a treated group to the control group. These tests were used also to skeletal and teratological data[8,12,13].

A probability level of less than 5% was considered significant.

Results

There was significant weight gain in all experimental groups from abortive activity evaluation, even during the administration period.

Data on reproductive parameters and rates are showed in Tables 1 e 2.

Seed infusion caused neither pre-implantation loss, nor embryonic or fetal death. However, misaligned sternebrae were frequent in fetuses from seed infusion-treated groups. The presence of skeletal anomalies in fetuses from group treated with infusion from the first to the third GD was statistically significant (Table 3). When administered from the fourth to the sixth GD, inflorescence and leaf extracts reduced the implantation and consequently the birth rate (Table 2). By analysis of the fetuses fixed in Bouin's fluid, a significant incidence of malformations, particularly cleft palate and renal hypoplasia, was observed in these groups (Table 3).

Placenta and fetus weight is showed in the Table 4. Placenta weight from inflorescence group treated with extract from the seventh to the ninth GD was significantly lesser than other groups.

Discussion

F. vulgare is commonly used in Brazilian herbal Medicine. Estrogenic, antispasmodic, antimicrobial and antioxidant activities were demonstrated by fennel[1,2,14-16]. It is also taken to promote menstruation and fertility controlling[2,3]. Therefore, this study was conducted to evaluate its effect on gestation. To this aim dried seed aqueous extract and inflorescence (seeds and stems) and leaf hydroalcoholic extracts were prepared, resulting in a yield of 4, 14 and 15.8%, respectively. So, a minimum dose and a maximum dose were administered of seed extract: 4 mL (4.12 mg)/kg/day of aqueous extract and

Table 1 – Effect of *Foeniculum vulgare* on reproductive parameters

Group	Corpora lutea	Implantation sites	Embryonic reabsorptions	Degenerated embryos and dead fetuses	Live fetuses
First to third GD					
Control	15.05±1.23	12.85±3.75	1 [0–2]	0 [0–0]	11.10±3.89
Seed infusion	15.25±1.74	13.40±4.10	1 [1–2.5]	0 [0–0.5]	11.05±3.80
Inflorescence extract	15.85±1.79	11.05±5.17	1 [0–2]	0 [0–0]	9.85±4.89
Leaf extract	15.75±1.71	11.35±6.18	1.5 [0–4]	0 [0–0]	8.75±5.20
Fourth to sixth GD					
Control	15.25±2.05	12.50±4.63	2 [0–2.5]	0 [0–0]	10.50±4.27
Seed infusion	16.30±1.84	12.40±5.74	1.5 [0–2.5]	0 [0–0]	10.80±5.21
Inflorescence extract	17.0±1.90	11.90±5.19	0 [1–2]	0 [0–0]	9.95±5.36
Leaf extract	16.90±2.38	10.60±6.39	1.5 [0–4]	0 [0–0]	7.95±5.16
Seventh to ninth GD					
Control	17.15±1.57	13.25±5.29	1.5 [1–2.5]	0 [0–0]	11.10±4.69
Seed infusion	17.55±2.63	13.35±6.22	1 [0–2]	0 [0.0–0.5]	11.40±5.52
Inflorescence extract	17.90±2.59	14.80±5.38	1 [0.5–3]	0 [0–1]	12.50±4.63
Leaf extract	16.35±2.06	11.85±6.23	1 [0–2]	0 [0–0]	8.80±6.05

GD: gestational day.

1,000 mg/kg/day of hydroalcoholic extract. A maximum dose of leaf extract was also administered: 1,000 mg/kg/day of hydroalcoholic extract. Seed infusion and inflorescence and leaf hydroalcoholic extracts were administered to pregnant mice on pre-implantation period, during the implantation or when organogenesis and placentation start.

The results obtained in this study demonstrated that seed infusion did not exhibit adverse effect on reproductive parameters and rates. Otherwise, leaf and inflorescence hydroalcoholic extracts reduced the implantation success and consequently the birth rate when administered on implantation period (fourth to sixth GD). Therefore, the seed infusion taken popularly might not affect the pregnancy, but the fennel has an anti-implantation potential, and the concentration or isolation of active substances is necessary to this result.

Adverse effect on implantation might be due to hormonal unbalance. Plant estrogens have the capacity to interrupt early pregnancy in rats and mice through inhibition of estrogen surge necessary for implantation[17]. In mice and humans, estrogens play a pivotal role in implantation because they participate in the estrogen/progesterone balance, thereby, in the uterine receptivity to the embryo[18,19]. Estrogenic activity was verified on cervix and vagina of ovariectomised rats[20]. Anethole, main constituent of fennel essential oil, has been considered to be the active estrogenic agent. Some other studies have suggested that the actual pharmacologically active agents are polymers of anethole, such as dianethole and photonanethole[2].

Effect on uterine motility might also be responsible by lesser implantation rate observed on groups treated with

Table 2 – Effect of *Foeniculum vulgare* on reproductive rates (%)

Group	Implantation rate[a]	Reabsorption rate[b]	Death rate[c]	Birth rate[d]
First to third GD				
Control	93.7 [86.2–100]	6.9 [0–20.8]	0 [0–0]	83.3 [61.3–92.9]
Seed infusion	94.1 [90–100]	7.1 [6.5–18.3]	0 [0–3.1]	80.6 [66.8–89.6]
Inflorescence extract	84.4 [45.8–100]	6.7 [0–16.0]	0 [0–0]	73.3 [39.5–90.2]
Leaf extract	93.3 [58.4–100]	10.7 [0–28.7]	0 [0–0]	61.8 [41.8–82.1]
Fourth to sixth GD				
Control	93.8 [80.6–100]	12.9 [0–18.4]	0 [0–0]	79.9 [62.4–86.2]
Seed infusion	91.4 [73.3–100]	10.4 [0–16.7]	0 [0–0]	73.3 [48.3–89.5]
Inflorescence extract	83.8 [58.8–91.6]*	8.1 [0–15.4]	0 [0–3.1]	67.7 [29.6–84.5]
Leaf extract	77.5 [24.3–94.1]	12.7 [0–26.7]	0 [0–0]	46.9 [7–69.7]**
Seventh to ninth GD				
Control	88.9 [68.3–100]	11.2 [6.1–19.9]	0 [0–2.8]	75.7 [55.3–80]
Seed infusion	93.5 [84.6–100]	6.1 [0.0–14.4]	0 [0–5.4]	73.7 [54.9–90.4]
Inflorescence extract	89.5 [75.6–97.5]	7.4 [1.9–17.7]	0 [0–5.8]	72.6 [64.1–82.8]
Leaf extract	88.2 [54–100]	6.3 [0.0–14.4]	0 [0–0]	70.6 [31.4–83.7]

GD: gestational day; [a]implantation rate: (number of implantation sites/number of corpora lutea) x 100; [b]reabsorption rate: (number of resorptions/number of implantation sites) x 100; [c]death rate: (number of degenerated embryos and dead fetuses/number of implantation sites) x 100; [d]birth rate: (number of live fetuses/number of corpora lutea) x 100. Difference to the control group: *p=0.051, **p=0.016, Mann-Whitney test.

Table 3 – Distribution of malformations and skeletal anomalies

Group	Skeletal anomalies[a]	External and visceral malformations[a]
First to third GD		
Control	52.63% (18/105)	10% (3/119)
Seed infusion	89.5% (34/106)*	31.6% (8/115)
Inflorescence extract	61.1% (17/95)	21% (4/102)
Leaf extract	60% (19/74)	37.5% (8/92)
Fourth to sixth GD		
Control	66.7% (19/100)	0% (0/112)
Seed infusion	87.5% (27/97)	5.9% (1/112)
Inflorescence extract	58.8% (22/95)	52.6% (16/112)**,#
Leaf extract	61.5% (10/64)	23.5% (4/84)***
Seventh to ninth GD		
Control	72.2% (21/106)	16.7% (4/116)
Seed infusion	82.4% (27/107)	5.9% (1/111)
Inflorescence extract	68.8% (22/107)	33.3% (8/129)
Leaf extract	66.7% (20/84)	25% (5/96)

GD: gestational day; [a]percentage of litters with abnormal fetuses; (abnormal fetuses/observed fetuses). Difference relative to the control group: *p=0.012, **p=0.000, ***p=0.032, Mann-Whitney test; difference between the groups: #p=0.000, Kruskal-Wallis test.

Table 4 – Effect of *Foeniculum vulgare* in the weight of placenta and fetus (milligram)

Group	Placenta	Fetus
First to third GD		
Control	100.93±9.60	886.77±97.73
Seed infusion	96.45±8.0	923.95±111.88
Inflorescence extract	102.24±22.64	958.44±115.24
Leaf extract	91.52±11.36	966.74±149.52
Fourth to sixth GD		
Control	100.47±7.71	959.86±75.32
Seed infusion	97.10±8.54	895.77±75.41
Inflorescence extract	101.39±22.19	935.8±134.43
Leaf extract	106.30±20.53	900.83±133.83
Seventh to ninth GD		
Control	103.35±9.34	861.23±92.82
Seed infusion	98.73±6.71	894.2±89.83
Inflorescence extract	93.49±6.94*	902.77±66.34
Leaf extract	105.19±15.59	912.94±112.52

GD: gestational day. *significant difference: p=0.005, ANOVA; p=0.013, post-hoc Dunnett's *t*-test.

leaf and inflorescence extracts. The leaf ethanolic extract had uterotonic activity, but aqueous extract had no effect[3]. In a study aiming the use of *F. vulgare* for alleviation of dysmenorrhea pain, the essential oil reduced the intensity of oxytocin and prostaglandin E2 induced contractions and reduced the frequency of contractions induced by prostaglandin E2 on the isolated rat uterus[16].

Other cause to decrease the success of implantation is the embryotoxicity. Fennel might contain cytotoxic substances. Antimicrobial and antioxidant activities were demonstrated[14,15]. In addition, Local Food-Nutraceutical Consortium[21] reported that ethanolic extract of *F. vulgare* contains considerable amounts of polyphenols, important compounds with a huge range of biological activities, including anti-proliferative one.

It is of note that although seed infusion did not affect reproductive parameters, a significant difference was found for skeletal anomalies with administration of 4 mL (4.12 mg)/kg/day from the first to the third GD.

In developmental toxicity studies, skeleton abnormalities found in fetuses at term are classified as variations or malformations. Variations are structural changes that occur within the normal population and are unlikely to affect survival or health, whereas malformations are permanent changes that are likely to adversely affect survival, development or function[22,23].

In our study, frequency of misaligned sternebrae was increased in fennel-treated groups. Misaligned sternebrae are believed to be transient anatomical changes having only minor impact if any on survival and health and therefore they are generally classified as variations[24].

The relevance of skeleton variations for risk assessment is a controversial issue. It has been argued that chemical-produced increases in variations are not to be considered for risk assessment because they are unlikely to adversely affect survival or health. The counter argument is that a treatment-induced increase in the occurrence of variations means that the chemical agent has the potential to perturb skeleton development. According to this view, under a different condition of exposure, or in another species, this perturbation of normal bone formation may give rise to a different and more severe outcome[25].

Evaluating dose-response relationships of skeleton variations and malformations induced by antineoplastic agents 5-fluoro-deoxyuridine, hydroxyurea and 6-mercaptopurine-riboside administered on GD 11, Chahoud and Paumgartten verified incidences of variations started to increase at doses lower than those that elevated the occurrence of malformations. Occurrence of variations, as misaligned sternebrae, enhanced in a dose-dependent manner, supporting that skeleton variations were due to the cytotoxic drugs tested[25]. Significant increase of misaligned sternebrae was observed with other herbal drugs, as *Morinda citrifolia* L. extract, and chemical substances, as valproic acid[26].

Studying fennel essential oil on the rat embryo limb buds culture, there was reduction of cell viability and BMP-4 protein, produced in both prechondrocytes and mature chondrocytes and necessary at multiple stages in limb chondrogenesis. So, fennel essential oil may perturb processes involved in prechondrocyte maturation, and it does not stimulate cells sufficiently to initiate chondrocyte foci formation[5].

Skeletal anomalies findings in seed infusion-treated groups and the significant incidence of external and visceral malformations in leaf and inflorescence extracts-treated groups from the fourth to the sixth GD suggest teratogenic potential by fennel.

Conclusions

F. vulgare seed infusion caused neither pre-implantation loss, nor embryonic or fetal death, but leaf and inflorescence hydroalcoholic extracts had an adverse effect on implantation of the embryos, reducing the birth rate. Teratogenicity potential by *F. vulgare* must be considered.

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