

Fennel fortified diet: New perspective with regard to fertility and sex hormones

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Abstract: The objective of this study was to evaluate the effect of *Foeniculum vulgare* (FV) on fertility of mice and on serum sex hormone values of rats and rabbits. We used powdered form of *Foeniculum vulgare* seed and added it to food of mice, rats and rabbits in 2% and 4% ratio which was provided ad libitum throughout study period. Fertility study on mice was conducted for a period of nine months and 3 generations of mice fed on study drug were observed. Rats and rabbits were given *Foeniculum vulgare* seed powder containing diet for two month and then serum levels of FSH, LH, estrogen, progesterone and testosterone were checked. The number of pups increased in each succeeding generation and no gross abnormality was observed in any of the pups of all 3 generations. The sex hormone levels increased in both sexes of rats and rabbits with exception of FSH and LH levels which were less than 0.1mIU/mL in all study animals. Data was analysed on SPSS. Fertility and sex hormones increased in both male and female when given special diet containing 2% and 4% *Foeniculum vulgare*.

Keywords: Estrogen, *Foeniculum vulgare*, progesterone, sex hormones, testosterone.

INTRODUCTION

Unable to produce offspring is a major problem encountered by young couples these days. It could be defined as a couple's inability to achieve pregnancy after a year of unprotected, regular intercourses (Rad *et al.*, 2013). Common reasons of infertility are considered to be consuming unhealthy diet, being overweight or obese, cigarette smoking, stress and acute or chronic infections of genital tract (Petraglia *et al.*, 2013). In almost 40% of cases female partner is responsible for infertility, in which, ovulatory dysfunction is documented as one of the most important and commonest as seen in 15% of infertile couples (Tan *et al.*, 2012). Customarily, traditional therapies are tried initially before seeking medical advice because of cost and social issues. Since traditional medicines have a prime role in health care at individual and community level, usage of herbal therapies is popular in many regions around the globe (Khazaei *et al.*, 2011). Health of 80% people residing in developing countries is managed by utilization of therapeutic effects of many medicinal herbs.

Foeniculum vulgare belong to the group of medicinal herbs which are used for the treating various ailments (Jamwal *et al.*, 2013). *Foeniculum vulgare* is a biennial aromatic and medicinal member of Apiaceae (Umbelliferae) family. It is a sturdy plant having amber flowers with feathered leaves (Rather *et al.*, 2016). Its strong flavour and aroma has popularized its culinary and medicinal usage (Kaur *et al.*, 2013). Fennel is the ripe fruit or seed, of *Foeniculum vulgare*. Its odour is compelling and taste is strongly bitter sweet (Amin and Nagy, 2009). Essential oils are mostly concentrated in the

mericarps and are accountable for the unique aroma and taste (Sadrefozalayi and Farokhi, 2014). In fact, it has a number of therapeutic properties related to digestive, endocrine, reproductive and respiratory systems (Badgujar *et al.*, 2014). The osteoprotective effects of a *Foeniculum vulgare* ethanol extract were also reported (Pacifico *et al.*, 2015).

Foeniculum vulgare has diuretic, analgesic, antipyretic and antioxidant activities. It is also known to promote menstruation, being useful in child birth, attenuate the symptoms of climacteric in both sexes and enhance sex drive. The essential oil has emmenagogue and galactagogue properties. In folk medicine, *Foeniculum vulgare* is utilized to stimulate gastrointestinal motility, lessen intestinal gas build up, enhance eyesight, reduce productive coughs, relieve spasm, boost courage and mental strength, decrease stress and anxiety and have calming and soothing effect (Abbas *et al.*, 2019). Pharmacological studies demonstrated antibacterial, antithrombotic, hepatoprotective, and anti-inflammatory activities in many *in vitro* and *in vivo* settings. Antifungal, antiviral and antiprotozoal effects have also been documented (Perveen *et al.*, 2017). One of the prime components of *Foeniculum vulgare* oil is anolor dimethylated anethole which has estrogenic potential. It causes the proliferation of lobule alveolar tissue of the mammary glands in young female rabbits. It also generates oestrus in mice (Mallni *et al.*, 1985).

The objective of this trial was to evaluate the fertility enhancing potential of *Foeniculum vulgare* fortified diet (containing seeds of *Foeniculum vulgare*) in successive generations of mice and its effect on sex hormones in rats and rabbits of both sexes.

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MATERIALS AND METHODS

Study design

Laboratory-based randomized control trial

It was a laboratory-based randomized controlled study conducted at the animal house of the Pharmacology Department in University of Karachi. The study design was based on the guidelines for the care and use of laboratory animals (Council, 2010) and the experimental design and protocol was approved by Board of Advanced Studies and Research (BASR), University of Karachi in October 2016 Resol. No. 10(P)14.

Plant Material

Foeniculum vulgare seeds (dry) were purchased from a general store and were identified from Department of Pharmacognosy, University of Karachi, assigned voucher no. FVF-02-15/17 and submitted in the herbarium of Pharmacognosy. Seeds were coarsely powdered, mixed with standard diet of mice, rats and rabbits in ratio of 2% and 4% and food pellets were prepared. These pellets were stored separately under hygienic condition in animal food store of animal house (Singh and Kale, 2008; Abbas^a *et al.*, 2020) of Faculty of Pharmacy, University of Karachi. Standard rodent diet contained fish meal (11.1%), corn gluten (11.1%), wheat flour (44.4%), gram flour (11.1%), barley flour (22.25) and milk powder (1%) (Abbas^b *et al.*, 2020).

Animals

Adult albino, healthy mice of both sexes, having weight range of 20-25grams and Wistar rats weighing 200-240grams were taken from animal house of Pharmacology Department of University of Karachi. Albino adult rabbits weighing 1500-2000grams were taken from the supplier of lab animals. All the animals were fed on standard food and prepared pellets according to groups plus water ad libitum throughout the experiment. Mice, rats and rabbits were kept in transparent cages with saw dust covered floor separately. Mice were paired, each pair consisting of a male and a female mouse. Rats and rabbits were caged according to gender. All animals were kept at a standard temperature (22-25°C), humidity (50-60%) and 12 hour light and dark cycle (Fatima *et al.*, 2018).

Fertility Study

This study was conducted for total duration of nine months. Thirty pairs of nulliparous mice were divided in three groups for F₀ generation and kept in separate cages in animal house.

Group 1: Control group, these pairs were fed on standard diet and water ad libitum

Group 2: 2% group was given standard diet containing 2grams of powdered *Foeniculum vulgare* seeds per 100grams of diet.

Group 3: 4% group was given standard diet containing 4grams of powdered *Foeniculum vulgare* seeds per 100grams of diet.

Pairs of F₀ generation were given prepared food pellets starting from pairing, during mating, pregnancy, birth of pups (labelled as F₁ generation) till the weaning of F₁ generation. Pairs from F₁ generation were taken, grouped as parent generation and were fed on prepared diet until F₂ generation pups were weaned. This protocol was continued till birth of F₃ generation. Offspring of each generation were counted and observed for any abnormality on Day 0 and Day 5 (Riaz *et al.*, 2010).

Hormonal assessment

The duration of this study was two months. Twelve pairs each of nulliparous adult rats and rabbits were divided in three groups each group containing equal number of males and females. Males and females rats and rabbits were housed separately for a period of 2 months.

Group 1: Control group was given standard rodent diet.

Group 2: 2% *Foeniculum vulgare* group was given standard food containing 2grams of powdered *Foeniculum vulgare* seeds per 100grams of diet.

Group 3: 4% *Foeniculum vulgare* group was given standard food containing 4grams of powdered *Foeniculum vulgare* seeds per 100grams diet.

Blood samples were collected under aseptic conditions and kept in B-Ject gel clot activator vacuum tube from all study animals after 2 month dosing and hormonal assay (progesterone, estrogen and testosterone) was carried out in PCMD diagnostic, Dr. Panjwani Center for Molecular Medicine and Drug Research, University of Karachi and Dow Diagnostic Reference & Research Laboratory, Dow University of Health Sciences, respectively, using standard kit method. Samples were centrifuged in HUMA X 14-K at 2000 rotations per minute and serum was stored at temperature of 2-8°C. Hormonal levels were assessed within 3 hours of collection time (Riaz *et al.*, 2018).

STATISTICAL ANALYSIS

All experimental values were shown as mean ± SD. SPSS 17 was utilized for the statistical analysis. Statistical analysis was carried out by Students t-test and one-way ANOVA followed by tukey hsd. P<0.05 was considered statistically significant.

RESULTS

Three generations of swiss albino mice were studied, fed on standard diet (Group 1), 2% *Foeniculum vulgare* diet (Group 2) and 4% *Foeniculum vulgare* diet (Group3). Ten pairs of mice were kept in each groups of all generations observed. Pups were counted in each generation and observed for congenital abnormalities on day 1 and day 5. No congenital abnormalities were noted in any of the pups of all the groups of three generations. Number of

Table 1: Number of pups in three generations

Groups	F0 generation (No. of pups)	F1 generation (No. of pups)	F2 generation (No. of pups)
Control	62	71	72
2% <i>Foeniculum vulgare</i>	66	77 ^{***}	78 ^{***}
4% <i>Foeniculum vulgare</i>	68	79 ^{***}	80 ^{***}

n=10 pairs

Values are mean \pm SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)** $p < 0.01$ is considered moderately significant as compared to control*** $p < 0.001$ is considered highly significant as compared to control^{sss} $p < 0.001$ is considered highly significant when compared within a group**Table 2:** Hormonal Assay of Male Rats

Hormones	Group 1 (Control)	Group 2 (2% FV)	Group 3 (4% FV)
Testosterone (ng/ml)	1.94 \pm 0.30	5.71 \pm 2.7 ^{**}	1.28 \pm 0.61 ^{!!!}
Estrogen (pg/ml)	24.21 \pm 2.8	12.81 \pm 2.7 ^{***}	9.98 \pm 2.1 ^{***!}
Progesterone (ng/ml)	1.83 \pm 1.07	1.08 \pm 1.13	1.31 \pm 1.0

n=10 pairs

Values are mean \pm SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)** $p < 0.01$ is considered moderately significant as compared to control*** $p < 0.001$ is considered highly significant as compared to control! $p < 0.05$ is considered significant when both study groups are compared among each other!!! $p < 0.001$ is considered highly significant when both study groups are compared among each other**Table 3:** Hormonal Assay of Female Rats

Hormones	Group 1 (Control)	Group 2 (2% FV)	Group 3 (4% FV)
Estrogen (pg/ml)	18.16 \pm 1.66	18.53 \pm 1.88	19.84 \pm 2.03
Progesterone (ng/mL)	2.86 \pm 1.88	9.06 \pm 2.08 ^{***}	6.12 \pm 1.92 ^{***!!}
Testosterone (ng/mL)	0.49 \pm 1.03	1.12 \pm 1.04 ^{**}	0.81 \pm 1.0

n=6 Values are mean \pm SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)** $p < 0.05$ is considered very significant as compared to control*** $p < 0.001$ is considered highly significant as compared to control.!! $p < 0.05$ is considered very significant when both study groups are compared among each other**Table 4:** Hormonal Assay of Male Rabbits

Hormones	Group 1 (Control)	Group 2 (2% FV)	Group 3 (4% FV)
Testosterone (nmol/L)	7.12 \pm 1.48	25.31 \pm 2.24 ^{***}	18.75 \pm 1.07 ^{***!!!}

n=6 Values are mean \pm SD, data analysed by one way ANOVA followed by multiple comparison (post hoc Tukey's test)*** $p < 0.01$ is considered highly significant as compared to control!!! $p < 0.001$ is considered highly significant when both study groups are compared among each other**Table 5:** Hormonal Assay of Female Rabbits

Hormones	Group 1 (Control)	Group 2 (2% FV)	Group 3 (4% FV)
Estrogen (pg/ml)	<5.00	<5.00	<5.00
Progesterone (ng/ml)	0.57 \pm 1.15	0.66 \pm 1.0	1.61 \pm 1.03

n=6 Values are mean \pm SD, data analysed by Students t-test.

pups increased in second and third generations of both *Foeniculum vulgare* fed groups (table 1).

Hormonal analysis in male rats showed highly significant decline in serum estrogen levels in groups of rats fed on 2% and 4% FV diet when compared to control. Similarly,

comparison among group 2 and group 3 revealed significant difference in serum estrogen levels. By analysis of serum progesterone in male rats, it was noted that p-value was insignificant when *Foeniculum vulgare* fed groups were compared to control group. Similarly, there was no significant difference found between both

group 2 and group 3. Regarding serum testosterone, elevation in testosterone was noted in group 2 as compared to control group, whereas, no statistically significant difference was noted in group 3 as compared to control group. Highly significant difference was noted among group 2 and group 3 (table 2).

It was noted that there was no significant difference in estrogen levels in all study groups of female rats. Whereas, serum progesterone levels markedly increased in 2% FV groups and p-value was found to be highly significant. Same was the case in 4% FV group and increase in serum progesterone levels was significant. Comparison among groups of female rats fed on 2% and 4% *Foeniculum vulgare* diet was also significant in regard to serum progesterone levels. Serum testosterone level estimation and analysis, in female rats, showed an elevation of serum testosterone in both group 2 and 3 as compared to control group, highest value noted in 2% FV group (table 3).

Serum testosterone levels markedly increased in male rabbits fed on 2% *Foeniculum vulgare* and 4% *Foeniculum vulgare* diet, more elevation is noted in 2% *Foeniculum vulgare* group animals (table 4). Analysis of hormonal levels in female rabbits revealed insignificant difference in serum Progesterone levels in both group 2 and group 3. No change was noted in serum values of estrogen, in any of the three groups of female rabbits (table 5).

DISCUSSION

Sedentary ways of life and bad eating habits have negatively afflicted human beings around the globe. Apart from other problems, infertility is an issue of prime importance worldwide. We conducted this trial, to note the effect of powdered seeds of *Foeniculum vulgare* on fertility and serum levels of sex hormones. *Foeniculum vulgare* seeds fortified diet showed a positive effect on litter population. Pups number increased in subsequent generation particularly F₂ generation of 4% group and there were no abnormalities in any of the pups of study groups.

Elevation noted in serum progesterone levels in this study in female rats of both 2% and 4% *Foeniculum vulgare* groups was supported by a study done earlier on albino mice using seed extract of *Foeniculum vulgare* (Sadeghpour *et al.*, 2015). Brožič *et al.*, reported inhibitory activity of phytoestrogens on enzyme (20 α -hydroxysteroid dehydrogenase) responsible for metabolism of progesterone (Brožič *et al.*, 2006). The increase in progesterone levels noted in our study might be due to decreased metabolism of progesterone caused by phytoestrogens found in *Foeniculum vulgare*.

Foeniculum vulgare contain isoflavones which have inhibitory effect on two enzymes; aromatase P450 responsible for converting testosterone to estradiol and 5 α -reductase which stimulate conversion of testosterone to 5 α -dihydrotestosterone (Brooks and Thompson 2005). These compounds (isoflavones) also enhance sex hormone binding globulin (SHBG) synthesis. Elevated SHBG bind more testosterone, thus, decreasing levels of free hormone promoting its synthesis (Celec *et al.*, 2005), as noted in our study. It had been documented in other studies that estrogen levels in females do not change after intake of isoflavones (Lund *et al.* 2001; Lephart *et al.* 2003) as observed in this study. Decreased action of aromatase P450 caused by isoflavones might be the contributing factor to lowering of estrogen levels.

Dianethole and photoanethole found in extract of seeds of *Foeniculum vulgare* relate structurally to stilbene and diethylstilbestrol whereas anethole resemble catecholamines and could affect prolactin production (Garga *et al.*, 2009). Prolactin and steroids play role in folliculogenesis. *Foeniculum vulgare* extract may bring about estrus in rats. A study revealed estrogenic effects of another active compound found in *Foeniculum vulgare*, diosgenin is a steroid sapogenin, which also enhance dehydroepiandrosterone (DHEA) synthesis (Sautour *et al.*, 2004). It had also been investigated and utilized for osteoporosis treatment of ovariectomized adult rats (Higdon *et al.*, 2001).

Increase in weight of sex organs had been noted as the effect *Foeniculum vulgare* extract. Orally given extract led to elevating concentration of total protein in prostate gland and seminal vesicles of male rats and increasing the weight of mammary gland, oviduct and uterus of female rats (Malini *et al.*, 1985). Moreover, another study revealed that *Foeniculum vulgare* seeds alcoholic extract resulted in increased production of antral, graffian and multilaminar primary follicles, enhancing folliculogenesis in mice (Khazaei *et al.*, 2011), thus explaining its traditional use as treatment of female infertility. Folliculogenesis was known to be dependent upon multiple factors including pituitary gonadal hormones such as follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin plus local factors (autocrine and paracrine) (Guraya, 1998). The significant elevation in the number of follicles by *Foeniculum vulgare* seed extract revealed in study done by Khazaei *et al.*, showed the estrogenic activity of this herb.

CONCLUSION

The positive effect of *Foeniculum vulgare* seed fortified diet on number of offspring (study duration nine months) and sex hormones (study duration two months) reveal its potential as treatment of infertility. However, clinical studies should be conducted to further evaluate this beneficial effect.

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