



Study of *Foeniculum vulgare* (Fennel) Seed Extract Effects on Serum Level of Oxidative Stress

Nahid Sadeghpour¹, Azadeh Montaseri², Alireza Najafpour³, Hodayun Dolatkhan⁴, Asghar Rajabzadeh¹, Amir Afshin Khaki^{5*}

Abstract

Objective: The *Foeniculum vulgare* (FVE), known as fennel, has a long history of herbal uses as both food and medicine. The seed of this plant has been used to promote menstruation, alleviate the symptoms of female climacteric, and increase the number of ovarian follicles. The aim of this study was to evaluate the fennel extract effects on serum level of oxidative stress in female mice.

Materials and Methods: Totally, 28 virgin female albino mice were divided into four groups (n = 7). Groups 1 and 2 (experimental groups) were administered FVE at 100 and at a concentration of 100 and 200 mg/kg for 5 days, interaperitoneally. Group 3 (negative control) received ethanol and Group 4 (positive control) received normal saline. Animals were scarified at 6th day, sera were collected and the level of oxidative stress was determination of using total antioxidant status kit.

Results: Data analysis revealed that there is a significant difference in the mean level of serum oxidative stress between four different groups. P value in experimental groups compared to the control group was (P < 0.0001).

Conclusion: Fennel extract can decrease the serum level of oxidative factors in female mice; it can be introduced as a novel medicine for treatment of infertility

Keywords: *Foeniculum vulgare*, Infertility, Mice, Oxidative Stress

Introduction

Infertility is a multifaceted issue with increasing prevalence in both developed and developing countries. Due to its importance, many researchers try to assist this matter and promote the health status of families and society (1,2). 15% of young couples in different societies suffer from infertility (3) inappropriate diet, obesity, smoking, psychological stress, and genital tract infections are some important factors which can result in infertility (1,2). Current treatment for infertility includes ovulation-inducing medicines and assisted reproductive technology which have own side effects and higher cost, so most infertile couples cannot

afford for these treatments (4). Female factors are responsible for about 40% of infertility, among different factors, ovulatory dysfunction seems to be one of the most important one, which will be identified in about 15% of infertile couples (5). Traditional medicine has a long history for the treatment of female and male infertility for more than thousands of years (5). Oxidative stress (OS) is a recognized factor with the ability to affect human fertility. Free radicals are continuously produced as byproducts under aerobic cellular metabolism and play a key role in physiological and pathological processes. Antioxidant defense system assists the organism to respond and fight against the excess

Received: 9 Dec 2014, Revised: 25 Jan 2014, Accepted: 14 Feb 2015, Available online: 15 Apr 2015

¹ Department of Anatomical Sciences, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

² Stem Cell Research Center AND Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

³ Department of Clinical Sciences, Urmia Branch, Islamic Azad University, Urmia, Iran

⁴ Department of Clinical Biochemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran

⁵ Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

*Corresponding Author: Amir Afshin Khaki, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Tel: +98 9144157161, Email: dr.aakhaki@gmail.com

amount of free radicals (6). Overproduction of free radicals such as reactive oxygen species (ROS) or failure in antioxidant defense system results in oxidative stress (5), which is involved in several pathological conditions. The role of OS in decreasing the egg quality, fertilization, and pregnancy rates in mouse and human has been previously reported (1). On the other hand, it is reported that antioxidant depletion leads to infertility and use of antioxidants can be helpful in the treatment of infertility (1).

Foeniculum vulgare (FVE), known as Fennel, a plant belonging to the family apiaceae, has a long history of herbal uses and widely cultivated, both in the native habitat, India and Egypt, and elsewhere, for its edible strongly flavored leaves and seeds (7). The FVE fruit has a long history of use as both food and medicine. Traditionally, it is believed that the plant acts as a carminative (assists with flatulence control) and increase breast milk production (8). It has been reported that this plant can also enhance libido, facilitate birth, alleviate the symptoms of the male climacteric, promote menstrual flow, and soothe indigestion and cough (9,10).

The antioxidant activity of water and ethanol extracts of fennel seeds was evaluated by various antioxidant methods, including total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities, and reducing power (1). Those various antioxidant activities were compared to standard antioxidants such as (BHA), (BHT), and alpha-tocopherol. The water and ethanol extracts of fennel seeds showed strong antioxidant activity (1). Our previous study reported that fennel seed extract at concentration of 100 and 200 mg/kg can increase the number of different ovarian follicles and serum level of sexual hormones such as estrogen, progesterone and also prolactin, dramatically, but we did not measure the level of oxidative stress which probably are responsible for this ovulation-induction effect of the plant in our last research. Hence, the aim the present study was to determine whether the administration of fennel seed extract can alter the serum.

Materials and Methods

Fennel seeds were purchased from local markets authenticated by a botanist (School of Pharmacy, Tabriz University of Medical Sciences). The extract was prepared according to World of Health Organization (WHO) protocol for preparation of an alcoholic extract (11). Briefly, 100 g of fruit was shed-dried, powdered and added to 1000 ml of 70% ethanol (v/v) and left to macerate at room temperature for 20 h. The basin was slowly rotated during this time. After filtration, ethanol was evaporated at low pressure at 30°C.

The Ethical Committee of Tabriz University of Medical Sciences approved all procedures used in this study. A total of 28 virgin female albino mice with the weight range of 25-30 g were used. The

animals were fed with standard laboratory chow and water during the experiment. We used the Whitten effect for co-cycling animals and controlled vaginal changes for determining estrous cycle (12).

Pro-estrus mice were divided into four groups (n = 7). Animals in experimental groups (1 and 2) were received fennel extract at 100 mg/kg (Group 1) and 200 mg/kg (Group 2) for 5 days, interaperitoneally (11). Group 3 (negative control) received ethanol as the extract solvent. Group 4 received normal saline and was considered the normal group. Ethanol was administered in the same volume as groups 1 and 2. Animals in all groups were sacrificed on the sixth day of the study.

A simple method for determining estrus cycle stage is known as a vaginal lavage or vaginal smear. The 4 stages can be distinguished by observation of cell type characteristic and differences in cell density. A small amount of a physiological saline solution is inserted into the mice vagina with a disposable pipette, removed, placed on a slide and examined under a microscope.

After determining the estrogenic effect of FVE extract, we examined for oxidative stress with measurement of superoxide dismutase (SOD) enzyme activity, glutathione peroxidase enzyme activity, and total antioxidant capacity. Whole blood samples were slipping by hemolysis solution, and we measured the enzyme activity in red blood cells. SOD activity in whole blood samples was determined using RANSOD kit (Randox Labs, crumlin, UK) (14). GPX enzyme activity in wool blood samples was determined using RANSEL kit (Randox Labs, crumlin, UK) (13). Whole blood hemoglobin was measurement by SYSMEX KX-21 Automated Hematology Analyzer up to the enzyme activity according to IU/mgHb reported. Enzyme activity according to the reportTAC in serum samples measurement by Randox Total Antioxidant Status kit (Randox Labs, crumlin, UK) (14).

Statistical analysis

Statistical analyses of data were performed using a one-way analysis of variance and Tukey's post-hoc test. A value of $P < 0.050$ was considered statistically significant.

Results

The effects of fennel seed extract on Blood SOD enzyme Activity

As shown in Figure 1 significant differences were observed in the mean levels activity of blood SOD enzyme in the four groups. The mean \pm standard error (SE) in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 6.36 ± 0.33 , 8.78 ± 0.52 , 3.94 ± 0.25 , and 3.97 ± 0.25 IU/mgHb, respectively, and p values in experimental groups compared to the control groups showed a significant increase ($P = 0.0012$).

The effects of F. vulgare seed extract on blood glutathione peroxidase (GPX) enzyme activity

As shown in Figure 2, significant differences were

observed in the mean activity of blood GPX enzyme in the four groups. The mean \pm SE in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 0.85 ± 0.024 , 0.97 ± 0.022 , 0.31 ± 0.020 and 0.35 ± 0.033 IU/mgHb, respectively, and P values in experimental groups compared to the control groups showed a significant increase ($P = 0.0042$).

*The effects of *F. vulgare* seed extract on serum total antioxidant capacity (TAC) level*

As shown in Figure 3, significant differences were observed in the mean levels of serum TAC in the four groups. The mean \pm SE in the experimental groups (1 and 2), Group 3 (negative control) and Group 4 (normal control) were 1.61 ± 0.055 , 1.84 ± 0.027 , 1.19 ± 0.084 and 1.25 ± 0.051 nmol/l, respectively,

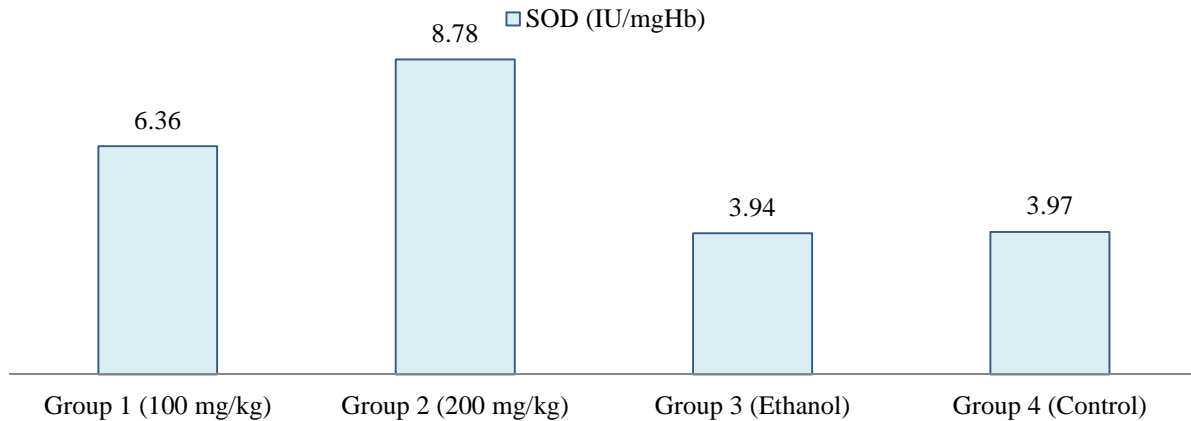


Figure 1. Comparison of the mean activity of blood superoxide dismutase enzyme in the groups of study
SOD: Superoxide dismutase

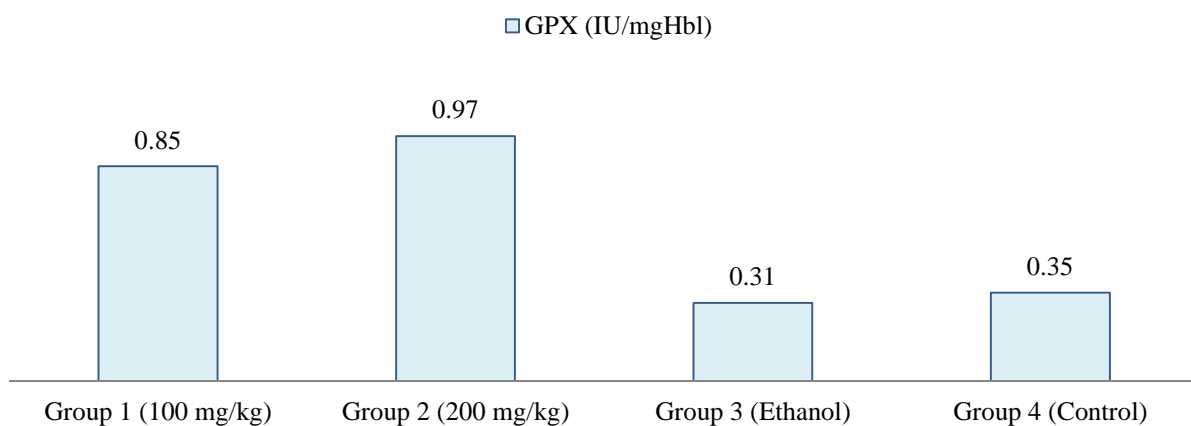


Figure 2. Comparison of the mean activity of blood glutathione peroxidase enzyme in the groups of study
GPX: Glutathione peroxidase

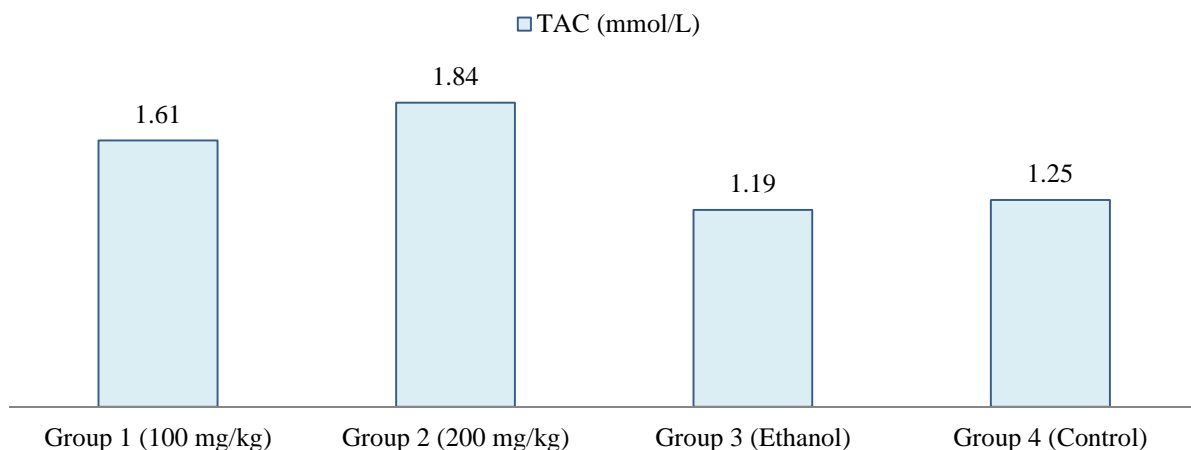


Figure 3. Comparison of the mean levels of serum total antioxidant capacity in the groups of study
TAC: Total antioxidant capacity

and p values in experimental groups compared to the control groups showed a significant increase ($P = 0.0049$).

Discussion

This study revealed that: (1) Fennel extract at concentration of 100 mg/kg and 200 mg/kg can increase the superoxide dismutase serum level, (2) This extract at the above mentioned concentration can increase the amount of glutathione peroxidase level in comparison to control groups and finally (3) Fennel extract at these applied concentrations can increase the TAC serum level compared to controls.

Infertility is defined as the inability to achieve the pregnancy within a year of unprotected intercourse. 15% of young couples in different societies suffer from infertility (15). Infertility is a multifaceted issue with increasing prevalence in both developed and developing countries. Due to its importance, many researchers try to assist this matter and promote the health status of families and society (16,17), and increasing our knowledge about the factors affecting fertilization, would be helpful in solving this problem (18). OS is a recognized factor with the ability to affect human fertility. Free radicals are continuously produced as byproducts under aerobic cellular metabolism and play a key role in physiological and pathological processes. Antioxidants play an important role in preventing the formation of and scavenging of free radicals and other potentially toxic oxidizing species. There are three categories of antioxidant species: enzyme systems (GSH reductase, catalase, peroxidase, etc.), small molecules (ascorbate, uric acid, GSH, vitamin E, etc.) and proteins (albumin, transferrin, etc.). Different antioxidants vary in their reducing power. Trolox is used to standardize antioxidants, with all other antioxidants being measured in Trolox equivalents. Measurement of the combined nonenzymatic antioxidant capacity of biological fluids and other samples provides an indication of the overall capability to counteract ROS, resist oxidative damage and combat oxidative stress-related diseases. Free radicals have been implicated in the progression of numerous conditions including infertility, cancer, diabetes, cardiovascular disease, ageing, and neurological disorders. In some cases, the antioxidant contribution of proteins is desired whereas in other cases only the contribution of the small molecule antioxidants is needed (19).

In recent years, application of alternatively medicine, especially herbal medicine, has been considered widely in the treatment of infertility due to its fewer side effects and lower costs (20). Herbs such as *Fructus Lycii*, *Radix Morindae Officinalis*, *Epimedium* and many others have been used for this reason (21).

F. vulgare known as fennel, belonging to the family *Apiaceae*, is being used for its anti-inflammatory, anti-spasmodic, analgesic, and laxative effects in folk medicine (22). Fennel oil contains different ingredients such as anol or dimethylated anethole which may have some estrogenic activity (23).

Another component of this seed oil is flavonoids, a type of phytoestrogen. Some studies revealed the estrogenic actions of phytoestrogens both in *in vivo* and *in vitro* conditions (24). Other studies revealed that fennel seed has various antioxidant activities such as total antioxidant, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities, and reducing power (23).

Previous study of our team showed that Fennel considerably increased the number of graafian, antral and multilaminar follicles at concentration of 100 and 200 mg/kg and also it can increase the serum level of sex hormones such as estrogen and progesterone and prolactin hormone (6). Due to the crucial role of antioxidants in treatment of infertility and based on the previous studies which have been done by other researches about the antioxidant activity of fennel, the present study has been designed to find more details about the serum level of oxidative stress markers in mice treated with fennel at different concentrations.

In this study, we reported that fennel extract at concentration of 100 and 200 mg/kg can significantly increase the serum level of antioxidant markers such as superoxide dismutase, glutathione peroxidase, and total antioxidant capacity in mice. Our data are in accordance with the results described by other researchers. Senatore et al. reported that water and alcoholic fennel seed extracts has strong antioxidant activity, effective reducing power, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, and metal chelating activities (8). Roby et al. reported that fennel leaves have anti-microbial and antioxidant activities (7).

Conclusion

The present study elucidated the fact that fennel extract can increase the serum level of superoxidase dismutase, glutathione peroxidase, and total antioxidant capacity in female mice consistent with its use in folk medicine as a fertility enhancing agent.

Ethical issues

Ethical of this research work was approved by Tabriz University of Medical Sciences, Iran.

Conflict of interests

We declare that we have no conflict of interests.

Acknowledgments

This paper is derived from a MSc thesis of Nahid Sadeghpour (No. 938) in Women's Reproductive Health Research Center, Tabriz University of Medical Sciences. Our sincere appreciation goes to the Women's Reproductive Health Research Center for their financial support.

References

1. Soleimani RS, Abbasalizadeh S, Ghorbani HA, Sadagheyani M, Montaseri A, Soleimani RJ. Serum Levels of Melatonin and Oxidative Stress

- Markers and Correlation between Them in Infertile Men. *J Caring Sci* 2013; 2: 287-94.
2. Petraglia F, Serour GI, Chapron C. The changing prevalence of infertility. *International Journal of Obstetric and Gynecology* 2013; 123: S4-S8.
 3. Healy DL, Trounson AO, Andersen AN. Female infertility: causes and treatment. *Lancet* 1994; 343: 1539-44.
 4. Khazaei M, Montaseri A, Khazaei MR, Khanahmadi M. Study of *Foeniculum vulgare* Effect on Folliculogenesis in Female Mice. *Int J Fertil Steril* 2011; 5: 122-7.
 5. Baird DT, Collins J, Egozcue J, Evers LH, Gianaroli L, Leridon H, et al. Fertility and ageing. *Hum Reprod Update* 2005; 11: 261-76.
 6. Tan L, Tong Y, Sze SC, Xu M, Shi Y, Song XY, et al. Chinese herbal medicine for infertility with anovulation: a systematic review. *J Altern Complement Med* 2012; 18: 1087-100.
 7. Roby MH, Sarhana MA, Selima KA, Khalela KI. Antioxidant and Antimicrobial Activities of Essential Oil and Extracts of Fennel (*Foeniculum vulgare* L.) and Chamomile (*Matricaria chamomilla* L.). *Ind Crop Prod* 2013; 44: 437-45.
 8. Senatore F, Oliviero F, Scandolera E, Tagliatela-Scafati O, Roscigno G, Zaccardelli M, et al. Chemical composition, antimicrobial and antioxidant activities of anethole-rich oil from leaves of selected varieties of fennel *Foeniculum vulgare* Mill. ssp. *vulgare* var. *azoricum* (Mill.) Thell. *Fitoterapia* 2013; 90: 214-9.
 9. Namavar JB, Tartifizadeh A, Khabnadideh S. Comparison of fennel and mefenamic acid for the treatment of primary dysmenorrhea. *Int J Gynaecol Obstet* 2003; 80: 153-7.
 10. 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. *J Ethnopharmacol* 2001; 76: 299-304.
 11. Albert-Puleo M. Fennel and anise as estrogenic agents. *J Ethnopharmacol* 1980; 2: 337-44.
 12. Birdane FM, Cemek M, Birdane YO, Gulcin I, Buyukokuroglu ME. Beneficial effects of *Foeniculum vulgare* on ethanol-induced acute gastric mucosal injury in rats. *World J Gastroenterol* 2007; 13: 607-11.
 13. Dolatkhah H, Babaee S, Rahbani-Nobar M. Diabetes Mellitus and Helicobacter Pylori: Evaluation of Glycemic Control, Gastric Juice Nitric Oxide and Oxidative Stress in Diabetic Patients Infected by Helicobacter Pylori. Saarbrücken, Germany, LAP LAMBERT Academic Publishing; 2011.
 14. Dolatkhah H, Somi MH, Fattahi E. DNA damage and Cigarette Smoking. Saarbrücken, Germany: Lap Lambert Academic Publishing; 2011.
 15. Kefer JC, Agarwal A, Sabanegh E. Role of antioxidants in the treatment of male infertility. *Int J Urol* 2009; 16: 449-57.
 16. Sharlip ID, Jarow JP, Belker AM, Lipshultz LI, Sigman M, Thomas AJ, et al. Best practice policies for male infertility. *Fertil Steril* 2002; 77: 873-82.
 17. Bonnefont-Rousselot D, Collin F. Melatonin: action as antioxidant and potential applications in human disease and aging. *Toxicology* 2010; 278: 55-67.
 18. Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility - a clinician's perspective. *Reprod Biomed Online* 2005; 11: 641-50.
 19. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 2010; 4: 118-26.
 20. Ried K. Chinese herbal medicine for female infertility: An updated meta-analysis. *Complement Ther Med* 2015; 23: 116-28.
 21. Mosher WD, Pratt WF. Fecundity and infertility in the United States: incidence and trends. *Fertil Steril* 1991; 56: 192-3.
 22. Delaram M, Kheiri S, Hodjati MR. Comparing the Effects of *Echinophora platyloba*, Fennel and Placebo on Pre-menstrual Syndrome. *J Reprod Infertil* 2011; 12: 221-6.
 23. Mohebbi-Kian E, Mohammad-Alizadeh-Charandabi S, Bekhradi R. Efficacy of fennel and combined oral contraceptive on depot medroxyprogesterone acetate-induced amenorrhea: a randomized placebo-controlled trial. *Contraception* 2014; 90: 440-6.
 24. Price KR, Fenwick GR. Naturally occurring oestrogens in foods--a review. *Food Addit Contam* 1985; 2: 73-106.

Citation: Sadeghpour N, Montaseri A, Najafpour A, Dolatkhah H, Rajabzadeh A, Khaki AA. **Study of *Foeniculum Vulgare* (Fennel) Seed Extract Effects on Serum Level of Oxidative Stress.** *Crescent J Med & Biol Sci* 2015; 2(2): 59-63.