

Ovarian protection in cyclophosphamide-treated mice by fennel

Hassanpour Azam^a, Yousefian Shirin^b, Askaripour Majid^c, Shariffar Fariba^d,
Ezzatabadipour Massood^{e,*}

^a Department of Anatomical Sciences, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

^b Department of Agricultural Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

^c Physiology Research Center and Department of Physiology, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

^d Herbal and Traditional Medicines research Center, Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran

^e Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

ARTICLE INFO

Article history:

Received 21 December 2016

Received in revised form 10 February 2017

Accepted 7 March 2017

Available online 14 March 2017

Keywords:

Cyclophosphamide

Fennel

Mice

Ovary

ABSTRACT

Evaluation of protective effect of fennel on mouse ovary against the destructive effects of cyclophosphamide (CP) was the aim of this study. Adult female NMARI mice were randomly divided into six groups ($n = 8$): (A) negative control, (B) CP200 mg/kg, (C) fennel 400 mg/kg/day, (E, F, and D) that received fennel 200, 400 and 100 mg/kg/day respectively + CP200 mg/kg. Their ovary weight, volume, and diameter (WVD) were measured. Five micron sections were stained using the H&E method. The serum levels of oestrogen and progesterone were measured using ELISA kit. The results showed that WVD significantly reduced in the CP-treated groups in comparison with the A and C, but WVD increased after treatment of the mice with fennel extract, in comparison with B group. A significant decrease of serum in terms of oestrogen and progesterone levels among CP-treated groups in comparison with the A group was observed. In the CP-treated groups a reduction in the number of different ovarian follicles in comparison with the A and C groups was observed. However, in the treated animals with fennel extract, these parameters significantly increased in comparison with the B group. Finally, it is concluded that fennel can protect ovary from cyclophosphamide side effects.

© 2017 The Author(s). Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Usage of antineoplastic drugs is sometimes accompanied by side effects that need to be countered. One of the most successful and widely used antineoplastic drugs is cyclophosphamide (CP). However, it can cause reproductive dysfunction. Because of the immunosuppressive effect of this drug, some researchers advise it for the treatment of some autoimmune diseases [1]. In addition to destructive morphological changes to the ovaries, uterus, and testis, the most significant side effects of CP are decreasing gonadal function, amenorrhea, azoospermia, and oligospermia [2–4]. Moreover, lowering of the blood testosterone level and impaired secretion of gonadotropins were observed on exposure to CP [5]. Premature ovarian failure (POF) and infertility are the common result of CP treatment. Dysfunction of the ovary in rats which are treated with CP is related to destruction of the granulosa cells and cause infer-

tility [6]. Survivors of breast cancer treated with CP suffer from chemotherapy-related amenorrhea (CRA) because of ovarian damage and this may lead to early menopause [7]. Ovarian toxicity of CP therapy may be prevented with the use of gonadotrophins, thereby preserving ovary function [8]. Some researchers propose consumption of antioxidant as adjuvant antineoplastic drugs to reduce their side effects [9].

Fennel (*Foeniculum vulgare* L.), a well-known Mediterranean aromatic herb from the family of Apiaceae, is rich in phytoestrogens such as lignans and has wide spectrum usage in herbal medicine. This medicinal plant has been used to reduce pain in menstruation (dysmenorrhea), to regulate menstruation, to reduce menopausal aches and symptoms, to reduce gastrointestinal pains [10–12], to treat osteoporosis [13] and to treat hirsutism [14]. Fennel compounds have been analysed and their effective fractions defined [12] but there is the possibility of different percentage composition of their fractions in different regions. To aid local exploitation of herbal medicine, the aim of the present study was to assay the

* Corresponding author.

E-mail address: ezzatabadipm@gmail.com (M. Ezzatabadipour).

protective effect of wild indigenous fennel on mouse ovary against the destructive effects of cyclophosphamide.

2. Materials and methods

2.1. Plant material and extraction

Seeds of fennel were collected from local areas of Bardsir, Kerman and authenticated by a botanist. A voucher specimen was deposited in the Herbarium Center at the Department of Pharmacognosy, Kerman University of Medical Sciences and Kerman, Iran. An ethanolic extract of fennel seeds was prepared by ultrasonication assisted extraction (UAE). One hundred grams of seeds were dried, powdered and added to 500 ml of 80% ethanol (v/v) and sonicated three times. Under ultrasonic action the solid and liquid phases were vibrated and the solute quickly diffused out from the solid phase into the solvent [15]. After each sonication (30 min), filtration was performed. The obtained extract was concentrated under vacuum to dryness (40–50 °C). The dried extract was stored at –20 °C until its use in experimental work.

2.2. Animals

Forty-eight mature female NMARI mice (25–30 g each) were procured from the institutional animal house of Kerman Neuroscience Research Center (Kerman, Iran). The animals were acclimatised for one week under standard husbandry conditions (room temperature of 21.2 ± °C and a 12-hour light-dark photoperiod schedule) of animal house. The animals had free access to water and a standard mouse pellets diet during the experiment. Ethics approval was obtained from the ethics committee at Kerman University of Medical Sciences (approval number K/92/621).

2.3. Experimental design

The healthy mice were selected and divided randomly into six groups ($n=8$). The group distribution was as follows: A, as negative control (water); B, received CP200 mg/kg; C, received fennel 400 mg/kg/day; D, received fennel 100 mg/kg/day + CP200 mg/kg; E, received fennel 200 mg/kg/day + CP200 mg/kg, and F, received fennel 400 mg/kg/day + CP200 mg/kg.

Cyclophosphamide was diluted in water (200 mg/kg) and was administered orally to animals of groups E, F, D, and B for three days at seven-day intervals. It is noteworthy that in the groups treated with CP, the first treatment was on the seventh day of the test. In the groups treated with fennel extract (C, D, E, and F), the treatment was initiated one week before the first CP treatment and continued until the twenty-first day.

It should be noted that the doses of fennel were selected based on effective doses in pregnancy, lactation, and for their effects on ovarian tissue. The most important criterion for the dose consideration is the level of oestrogenic compounds in plant [16–19]. The dose of CP (purchased from Helal Ahmar pharmacy, Kerman, Iran) was selected based on an effective dose causing ovarian damage in rats and mice [18–20]. The experimental duration and the administration time of CP were based on the sexual cycle in female mice, the half-life of the drug, and its toxic effects on the ovary.

2.4. Measurement of sexual hormones

Twenty-four hours after the last day of administration, the animals in all groups were anaesthetised, blood samples from the heart were centrifuged, and their serum was extracted. Serum samples were kept at –70 °C, and the sexual hormones (oestrogen and progesterone) were measured using appropriate laboratory kits (ELISA

Kit, Diaplus, USA, Diagnostic System Laboratories Inc., STAT FAX, USA).

2.5. Study of ovarian histology

Animals in all groups were sacrificed. The ovaries were dissected out and after cleaning of fat, their weight was measured on a sensitive scale. They were fixed in 10% buffered formaldehyde and then embedded in paraffin. Sections of 5 μm thickness were cut using a microtome and after tissue processing, were stained using the H&E method. To estimate each parameter, 10–14 microscopic fields were examined for each ovary.

2.6. Statistical analysis

For statistical analysis, one-way ANOVA was used with LSD post hoc tests. P values equal to or less than 0.05 were considered as statistically significant. Statistical analysis was performed using SPSS 20.

3. Results

3.1. Ovary weight, volume and diameter

The ovary weight, volume, and diameter of E (fennel 200 mg/kg/day + CP), F (fennel 400 mg/kg/day + CP), D (fennel 100 mg/kg/day + CP), and B (CP 200 mg/kg) groups in comparison with the negative control A (water) and C (fennel 400 mg/kg/day) groups significantly reduced ($P<0.05$), but the ovary weight, volume, and diameter increased after treatment of the animals with fennel extract, in comparison with experimental group B ($P<0.05$).

There were no significant differences in ovary weight, volume, or diameter among the E, F, and D experimental groups (Graph 1).

3.2. Hormone assays

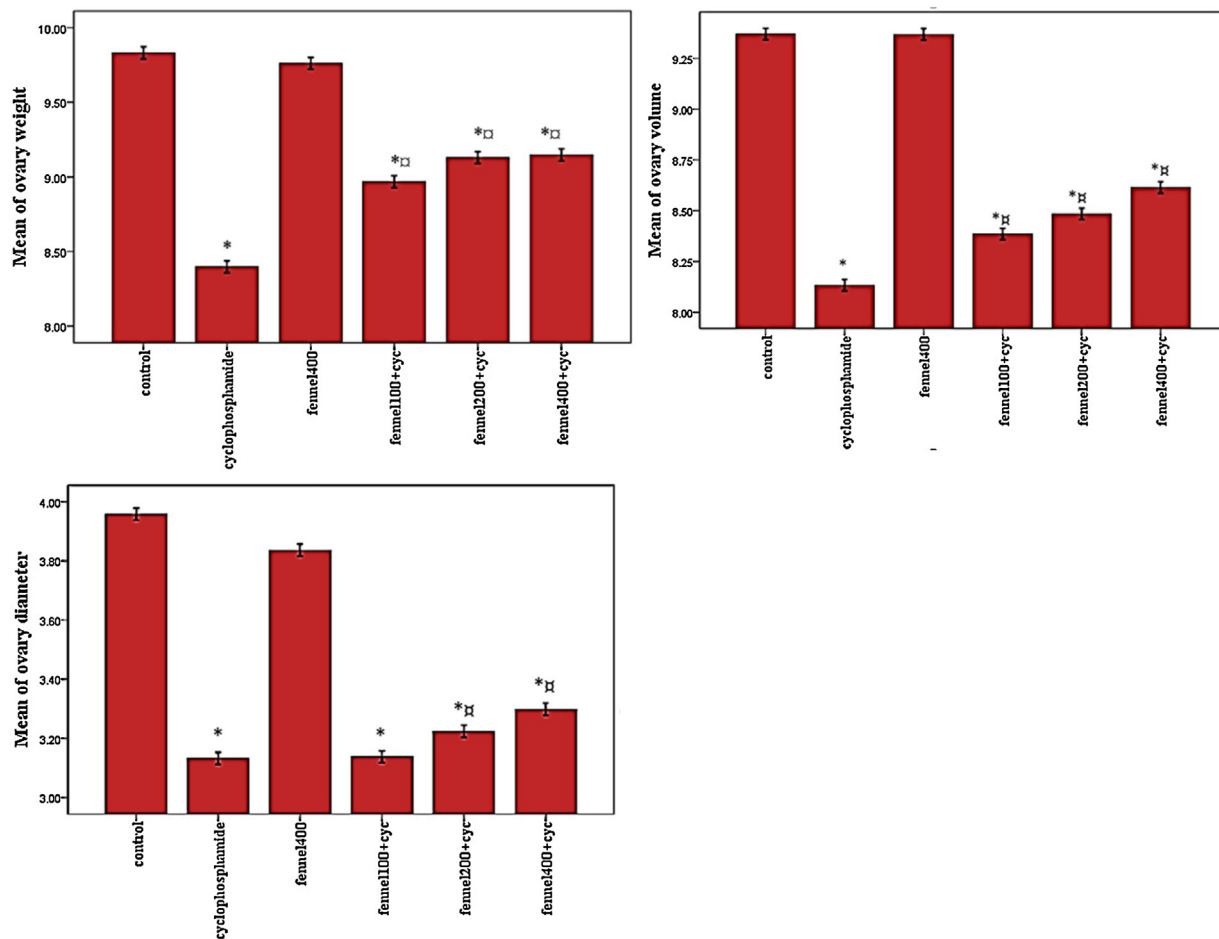
Graph 2 shows the results of female hormone profiling in the A (negative control) and C (fennel 400) groups in comparison with the E (fennel 200 mg/kg/day + CP), F (fennel 400 mg/kg/day + CP), D (fennel 100 mg/kg/day + CP), and B (CP-treated) groups. The serum levels of oestrogen and progesterone decreased significantly in the E, F, D, and B groups in comparison with the A group (negative control) ($P<0.05$).

As can be seen in Graph 2, treatment with fennel extract at the three concentrations caused a significant increase in the serum levels of oestrogen and progesterone compared with those of the B group. No significant differences were found in the female hormonal profile results among the three doses of fennel extract.

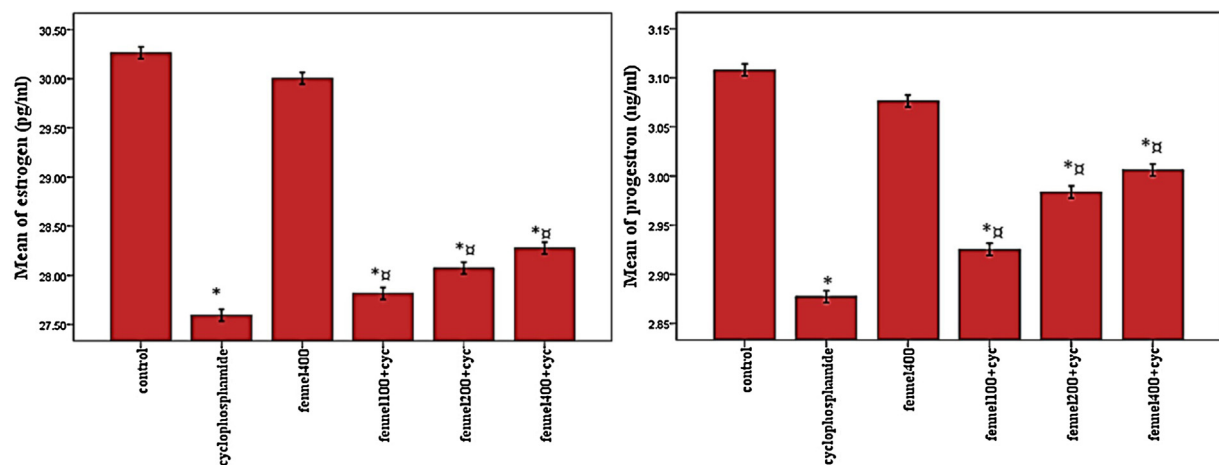
3.3. The number of different follicles in the ovarian parenchyma

The normal structural compartments of the ovary in the A (negative control) and C (fennel 400 mg/kg/day) groups are shown in Fig. 1 (a, b respectively). The histological changes in the ovary caused by fennel and CP in the E, F, D, and B groups are shown in Fig. 1 (c, d, e, and f respectively) and Graph 3.

In the E, F, D, and B groups, treatment with CP caused a reduction in the number of primary, secondary, antral, and atretic follicles in the ovarian parenchyma in comparison with the A and C groups ($P<0.05$). However, in the animals treated with fennel extract (E, F, and D groups), these parameters increased significantly in comparison with the B (CP200 mg/kg) group (Graph 3).



Graph 1. Comparison of the ovary weight, diameter, and volume among different groups.
*Significant difference with control and fennel 400 ($P < 0.05$); □Significant difference with CP ($P < 0.05$).



Graph 2. Serum levels of female sexual hormones in the E, F, D, B, A, and C groups.
*Significant difference with control and fennel 400 ($P < 0.05$); □Significant difference with CP ($P < 0.05$).

4. Discussion

Although the impact of traditional medicine is controversial, there are hopes for its effectiveness. Much research has been performed on fennel as a Mediterranean herb that also has a history of usage in Asia. The present study aimed to survey the protective effect of fennel against the destructive effect of cyclophosphamide

(CP) on ovarian tissue. The effect of concurrent administration of CP and fennel on ovaries was studied in three fields: morphology, hormone production, and follicle condition. The present findings indicated a protective effect of fennel on ovary tissue.

Concurrent use of antioxidant agents and CP ameliorates its side effect on the liver and kidney [21]. The inhibitory effect of CP on rat ovarian steroidogenic activity can also be avoided by using ascor-

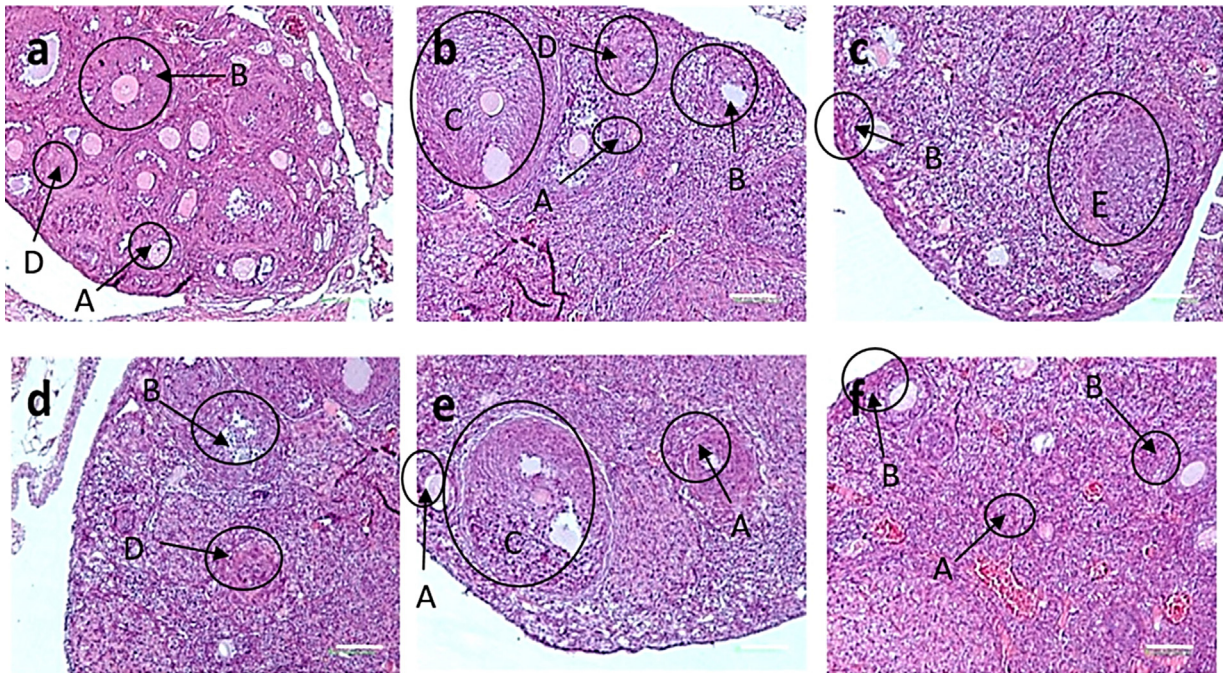
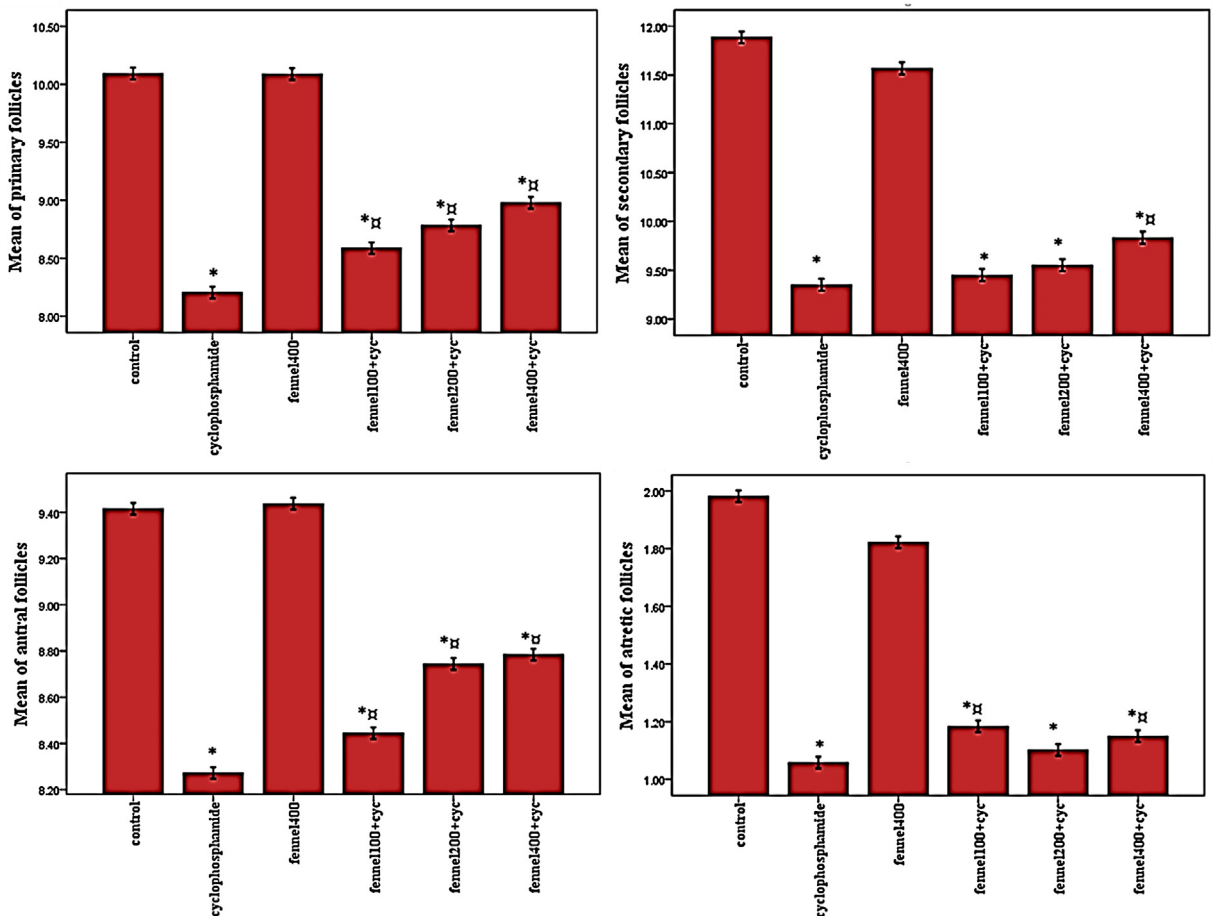


Fig. 1. Histological sections of ovary: (a) group A (negative control), (b) group C (fennel 400) that show normal structural. In CP treated groups (c, group E, fennel 200 + CP; d, group F, fennel 400 + CP; e, group D, fennel 100 + CP, and f, group B, CP200) reduction in the number of primary, secondary, antral, atretic follicles, and corpora lutea in the ovarian parenchyma were shown. Boundary of Primary, Secondary, Antral, Atretic follicles and Corpora lutea with A, B, C, D, and E respectively have marked (H&E staining; Scale bar, 100 μ m).



Graph 3. Distribution of primary, secondary, antral, and atretic follicles in different groups. *Significant difference with control and fennel 400 ($P < 0.05$); □Significant difference with CP ($P < 0.05$).

bic acid [22]. Several studies on GnRH and HCG as protective factors against the harmful effects of CP have been performed [4,22]. CP is used to treat cancers through chemotherapy as an alkylating agent. This drug is well absorbed through the gastrointestinal tract, widely distributed in tissues and body fluids, and passes through biological barriers [23]. Moreover, lowering the blood level of testosterone and impaired secretion of gonadotropins were observed on exposure to CP [5]. It seems that concurrent administration of fennel and CP could rectify the harmful effects of CP on ovary tissue.

Because of the high costs of treatment and associated social problems, some infertile couples seek traditional methods for their problem. Many herbal and chemical drugs have been proposed for the treatment and modification of infertility and sexual dysfunction. However, due to possible side effects of synthetic drugs, the focus on the use of herbal medicines has increased [24]. Today, the use of herbal products and extracts is common throughout the world, even in industrialised countries that are leaders in terms of technology, science, and possession of synthetic drugs [25].

Fennel is an annual, biennial or perennial aromatic plant belonging to the Apiaceae (Umbelliferae) family. This highly aromatic herb has medicinal uses, in which the most commonly used parts are the fruits and the small seeds [26]. Phenolic and volatile aroma compounds such as trans-anethole, limonene, and fenchone have been reported as the most important constituents of this herb. As mentioned above, treatment effects of fennel on dysmenorrhea, gastrointestinal pain, menstrual regulation, menopausal signs, hirsutism, and osteoporosis may be attributed to its steroidogenic components such as lignans [10–14]. Other notable effects of fennel include enhanced libido, testicular growth, increased mammary glands, induction of folliculogenesis, and increased numbers of growing ovarian follicles [12,27,28]. On the whole, these evidences support the oestrogenic effects of fennel. Studies have shown that fennel seed extract causes increases in sexual organ weight and increased protein concentration in the seminal vesicles and prostate gland of male rats as well as increases in breast, oviduct, endometrium, and myometrium in female rats [27]. It has been reported that the treatment of female rats with fennel essential oil can decrease uterine contractions by reducing the levels of oxytocin and prostaglandin [29]. Moreover, inhibition of the toxic effects of CP on mouse sperm has been evaluated [30]. Iranian traditional medicine claims that fennel improves women's sexual function and infertility [28].

5. Conclusion

Even though it needs further research, based on the achievements of the present study, in instances where using cyclophosphamide as treatment is highly necessary, it is recommended that fennel is prescribed for protecting ovary tissue from side effects.

Transparency document

The [Transparency document](#) associated with this article can be found in the online version.

Acknowledgments

The present study was made possible by the funding provided by Kerman Neuroscience Research Center and Kerman University of Medical Sciences; therefore, authors of this work are deeply grateful. We are also thankful to authors of all articles, journals, and books that are cited and included in references of this manuscript.

References

- [1] A. Emadi, R.J. Jones, R.A. Brodsky, Cyclophosphamide and cancer: golden anniversary, *Nat. Rev. Clin. Oncol.* 6 (11) (2009) 638–647.
- [2] F. Farokhi, et al., Morphological and morphometrical study of cyclophosphamide-induced changes in the ovary and uterus in the Syrian mice, *Iran. J. Vet. Res.* 8 (4) (2007) 337–342.
- [3] A. Masala, et al., Use of testosterone to prevent cyclophosphamide-induced azoospermia, *Ann. Intern. Med.* 126 (4) (1997) 292–295.
- [4] D. Meirou, et al., The GnRH antagonist cetrorelix reduces cyclophosphamide-induced ovarian follicular destruction in mice, *Hum. Reprod.* 19 (6) (2004) 1294–1299.
- [5] S.V. Sinhasane, B.N. Joshi, Melatonin and exposure to constant light/darkness affects ovarian follicular kinetics and estrous cycle in Indian desert gerbil *Meriones hurrianae* (Jerdon), *Gen. Comp. Endocrinol.* 108 (3) (1997) 352–357.
- [6] L. Bokser, B. Szende, A. Schally, Protective effects of D-Trp6-luteinising hormone-releasing hormone microcapsules against cyclophosphamide-induced gonadotoxicity in female rats, *Br. J. Cancer* 61 (6) (1990) 861.
- [7] J. Bines, D.M. Oleske, M.A. Cobleigh, Ovarian function in premenopausal women treated with adjuvant chemotherapy for breast cancer, *J. Clin. Oncol.* 14 (5) (1996) 1718–1729.
- [8] K. Manger, et al., Prevention of gonadal toxicity and preservation of gonadal function and fertility in young women with systemic lupus erythematosus treated by cyclophosphamide: the PREGO-Study, *Autoimmun. Rev.* 5 (4) (2006) 269–272.
- [9] K.A. Conlin, Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects, *Nutr. Cancer* 37 (1) (2000) 1–18.
- [10] L.U. Thompson, et al., Mammalian Lignan Production from Various Foods, 1991.
- [11] B.N. Jahromi, A. Tartifzadeh, S. Khabnadideh, Comparison of fennel and mefenamic acid for the treatment of primary dysmenorrhea, *Int. J. Gynecol. Obstet.* 80 (2) (2003) 153–157.
- [12] M. Albert-Puleo, Fennel and anise as estrogenic agents, *J. Ethnopharmacol.* 2 (4) (1980) 337–344.
- [13] F. Jaffary, A. Ghannadi, H. Najafzadeh, Evaluation of the Prophylactic Effect of Fennel Essential Oil on Experimental Osteoporosis Model in Rats, 2006.
- [14] K. Javidnia, et al., Antihirsutism activity of fennel (fruits of *Foeniculum vulgare*) extract – a double-blind placebo controlled study, *Phytomedicine* 10 (6) (2003) 455–458.
- [15] M. Cares, et al., Ultrasonically assisted extraction of bioactive principles from *Quillaja Saponaria* Molina, *Phys. Procedia* 3 (1) (2010) 169–178.
- [16] S.B. Badgajar, V.V. Patel, A.H. Bandivdekar, *Foeniculum vulgare* Mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology, *Biomed. Res. Int.* (2014) 2014.
- [17] N.S. Jamwal, S. Kumar, A. Rana, *Pharma Science Monitor*, 2013.
- [18] J. Jarrell, et al., Ovarian toxicity of cyclophosphamide alone and in combination with ovarian irradiation in the rat, *Cancer Res.* 47 (9) (1987) 2340–2343.
- [19] A. Carmely, et al., Protective effect of the immunomodulator AS101 against cyclophosphamide-induced testicular damage in mice, *Hum. Reprod.* 24 (6) (2009) 1322–1329.
- [20] N.A. Yener, et al., Effects of spirulina on cyclophosphamide-induced ovarian toxicity in rats: biochemical and histomorphometric evaluation of the ovary, *Biochem. Res. Int.* (2013) 2013.
- [21] E.T. Olayinka, et al., Ameliorative effect of gallic acid on cyclophosphamide-induced oxidative injury and hepatic dysfunction in rats, *Med. Sci.* 3 (3) (2015) 78–92.
- [22] S. Ghosh, et al., Effect of ascorbic acid supplementation on liver and kidney toxicity in cyclophosphamide-treated female albino rats, *J. Toxicol. Sci.* 24 (3) (1999) 141–144.
- [23] L. Belfayol, et al., Pharmacokinetics of cyclophosphamide in patients with systemic necrotizing angiitis, *Fundam. Clin. Pharmacol.* 8 (5) (1994) 458–462.
- [24] S. Bent, R. Ko, Commonly used herbal medicines in the United States: a review, *Am. J. Med.* 116 (7) (2004) 478–485.
- [25] J. Barnes, et al., *Herbal Medicines*, Pharmaceutical Press, London, 2007.
- [26] S. De Marino, et al., Phenolic glycosides from *Foeniculum vulgare* fruit and evaluation of antioxidative activity, *Phytochemistry* 68 (13) (2007) 1805–1812.
- [27] T. Malini, et al., Effect of *Foeniculum vulgare* Mill. seed extract on the genital organs of male and female rats, *Indian J. Physiol. Pharmacol.* 29 (1) (1984) 21–26.
- [28] M. Khazaei, et al., Study of *Foeniculum vulgare* effect on folliculogenesis in female mice, *Int. J. Fertil. Steril.* 5 (3) (2011) 122.
- [29] S. Ostad, et al., The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study, *J. Ethnopharmacol.* 76 (3) (2001) 299–304.
- [30] N. Elangovan, et al., Cyclophosphamide treatment causes impairment of sperm and its fertilizing ability in mice, *Toxicology* 222 (1) (2006) 60–70.