Toxicology Reports 4 (2017) 160–164

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Ovarian protection in cyclophosphamide-treated mice by fennel

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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.

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 infertility [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

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http://dx.doi.org/10.1016/j.toxrep.2017.03.002

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 212 ± °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).