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Original Article

Activity of *Corylus avellana* seed oil in letrozole-induced polycystic ovary syndrome model in rats

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ABSTRACT

The aim of the present study was to assess the activity of the hazelnut oil in the treatment of polycystic ovary syndrome in rats. Serum follicle-stimulating hormone, luteinizing hormone, estradiol, progesterone, testosterone, serum lipid parameters, leptin and glucose levels were evaluated. Moreover, antioxidant activity was tested using superoxide dismutase, malondialdehyde, catalase, glutathione peroxidase levels. The phytosterol content of the oil was determined by HPLC. The plasma high density lipoprotein-cholesterol level was found to be significantly high and leptin and glucose concentrations were found to be significantly low in the treatment group. According to the phytochemical analysis, the main components of the oil were detected as α-tocopherol, γ-tocopherol, squalene, β-sitosterol, campesterol and stigmasterol. *Corylus avellana* oil was found to be effective in the treatment of polycystic ovary syndrome *via* regulating gonadotropins, steroids and serum lipid parameters and possesses antioxidant activity.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder which causes anovulation in animal and women of reproductive age (Dehghan et al., 2012; Zhai et al., 2012; Arikawe et al., 2013). Its hyperandrogenic manifestations include menstrual irregularity, acne, hirsutism and oligo-ovulation/anovulation. Metabolic abnormalities such as dyslipidemia, insulin resistance, therefore, diseases including diabetes, obesity, cancer and infertility as well as coronary heart diseases could be seen along with PCOS (Maharjan et al., 2010; Zhai et al., 2012; Ghasemzadeh et al., 2013).

PCOS is characterized by small arrested antral follicle formation in the development process. In PCOS estrogen level decreases, however, the progesterone level increases and LH/FSH ratio becomes three times of the normal level. Androgens are synthesized by the theca cells (Walters et al., 2012).

Corylus avellana L., Betulaceae, is growing wild in Europa, Western Asia and Northern Africa as large shrubs or small trees about 3.5–4.5 m high. The leaves are deciduous, rounded, 6–12 cm long, softly hairy on both surfaces, with a double-serrate margin. The flowers are monoecious, with single-sex catkins, the male pale yellow and 5–12 cm long. The edible part of the hazelnut is the roughly spherical seed, which is covered by a dark brown perisperm and protected by a hard, woody shell. The ripening nut is enclosed in a green fringed tube (Könemann, 1999; Contini et al., 2011). This plant was reported to contain vitamins including vitamin E, B6 and B9, unsaturated fatty acids, plant sterols and polyphenols (Jakopic et al., 2011). One of the most important features of *C. avellana* is to have the highest ratio of unsaturated/saturated fatty acids. Moreover, *C. avellana* was shown to reduce plasma total and LDL cholesterol concentrations, by its polyunsaturated fatty acid, phytosterol and soluble dietary fiber content (Weststrate and Meijer, 1998; Brown et al., 1999; Feldman, 2002). A high fiber diet can be beneficial for the health of heart and digestive system and can help to regulate the blood glucose level (Anderson et al., 2009). *C. avellana* is a poor source of isoflavones, indeed, seed oil contains only trace amounts of phytoestrogens (Mazur, 2000).

According to the data reported in ethnobotanical studies, *C. avellana* has been used for the treatment of varicose veins, hemorrhoids, diabetes mellitus and gynecological disorders (Sezik et al., 1997; Ramalhosai et al., 2011; Abeer and Amr, 2013). The aim of the present study was to evaluate the activity potential of *C. avellana* oil in the treatment of PCOS and analyze the phytochemical constituents by using chromatographic methods in order to find out the compounds responsible from the efficacy.

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Materials and methods

Plant material

Seeds of *Corylus avellana* L., Betulaceae, were collected from Trabzon, Akçabat village, Turkey in August 2013. A voucher specimen was deposited in the Herbarium of Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Preparation of the oil

The plant material (50 g) was air dried and grinded. Ground hazelnut was weighed (10 g) and the oil was extracted with hexane in a Soxhlet apparatus during 6 h (James, 1995). The extract was evaporated at 40 °C. The oil content of the hazelnut was determined to be 51.23%. The oil was kept in a dark bottle in a freezer at −25 °C until the time of analysis.

Saponification of sterol, tocopherol and squalene for HPLC analysis

The oil (30 mg) was mixed thoroughly with 50% KOH (w/v) (200 μl) and 1% ethanolic pyrogallol (w/v) (1.5 ml). The tubes were kept at 70 °C in a water bath for 40 min. Water (0.5 ml) and hexane (3 ml) were added to the tubes after cooling on ice. The tubes were shaken and centrifuged at 400 × *g* for 10 min. The hexane phase was removed and the extraction repeated with hexane (1 ml). The combined hexane extracts were dried and was redissolved in ethanol (150 μl) and transferred into an HPLC vial.

Analyses of phytosterols, squalene and tocopherols by HPLC

HPLC conditions on Varian a ProStar HPLC system include Varian ProStar 240 Pump, Varian ProStar 410 AutoSampler, Varian ProStar 510 Column Oven, Varian ProStar 335 UV/VIS Detector. Sample (10 μl) was injected onto a Phenomenex luna C8 5u Silica 100A (150 mm × 4.6 mm LC Column) for phytosterol analysis. The chromatographic separation of the sample was performed using a mobile phase consisting of 80% acetonitrile and 20% water at a flow rate of 1.5 ml/min. For the analyses of tocopherol and squalene, Supelcosil LC-18 Column, 3 μm, (4.6 mm × 150 mm i.d.; Supelco, Bellefonte, PA, USA) and mobile phase consisting of 99% methanol and 1% water was used at a flow rate of 1.3 ml/min. Peak areas were recorded using Galaxie Chromatography Workstation software version 1.9.3.2 (Varian, Inc. Marketing Department 2700 Mitchell Drive Walnut Creek, CA 94598). Chromatograms were extracted at 205 nm, 292 nm and 215 nm for phytosterol, tocopherol and squalene analysis respectively (Maguire et al., 2004).

Animals

Eleven-week-old female, non-pregnant, eighteen mature Sprague Dawley rats (200–250 g) with 4–5 days regular estrus cycles were used in the study. All rats were purchased from Kobay Experimental Animals Laboratory, Ankara, Turkey and quarantined for at least two weeks. The animals were housed in polysulfone cages at 21–24 °C, 40–45% humidity, and light and dark cycles of 12 h conditions at Laboratory Animals Breeding and Experimental Researches Center, Faculty of Pharmacy, Gazi University, Ankara, Turkey. The experiments were conducted in accordance with the directions of Guide for the Care and Use of Laboratory Animals. The experiment was approved by the Experimental Animal Ethics Committee of Gazi University (G.Ü. E.T-14-022).

Experimental design

Letrozole (Letrasan®, DevaHolding A.S., Tekirdag, Turkey) (concentration of 1 mg/kg) was dissolved in 0.5% carboxymethylcellulose (CMC) was administered to the rats by gavage once daily throughout 21 days. This dose was chosen according to the previous studies in which the cystic follicle formation was induced (Rezvanfar et al., 2012a). During the experiment, the estrus cycle was microscopically evaluated by the analyses of relative proportion of leukocytes, epithelial and cornified cells. Body weight change due to the administration of letrozole was weekly observed. All rats were randomly divided into following three groups consisting of six rats in each group: (i) control group (CMC; 2 ml/rat/day, *p.o.*), (ii) reference group (buserelin acetate; 20 mg/rat/week, *s.c.*) and (iii) treatment group (*C. avellana*; 2 ml/rat/day, *p.o.*). The test materials were administered throughout 45 days.

Termination of the procedure

The animals were euthanized 24 h after the last dose of the treatment. Blood samples were collected by cardiac puncture. Serum was separated and kept in a freezer at −20 °C for determination FSH, LH, estradiol, progesterone, testosterone, TC, HDL-C, LDL-C, TGs, leptin and glucose, SOD, MDA, catalase, GPx levels. Uteri and ovaries were dissected, and weighed for the evaluation of the endocrine function.

Measurement of circulating levels of serum gonadotropins and steroids

Serum gonadotropin levels were determined using radioimmunoassay (RIA). All RIA kits were obtained according to the manufacturer's instruction from Beckman Coulter Co., Marseille, France. Serum FSH was assayed by sandwich RIA using a commercially available RIA kit. Serum LH, estradiol, progesterone, and testosterone were measured by competition radioimmunoassay with commercially available RIA kits.

Evaluation of the blood lipid, leptin and glucose levels

Plasma leptin (Cat. EZRL-83K) concentration was determined by ELISA using a rat kit (Linco Research, Inc., St. Charles, USA). Plasma triacylglyceride, total cholesterol, HDL-C LDL-C values were measured with commercially available assay kits (Human Diagnostica. GmbH, Germany). Blood glucose level was determined using Glucose Roche Diagnostic glucometer strip.

Determination of antioxidant activity

SOD, GPx, MDA, catalase levels were analyzed for the determination of the antioxidant activity potential. SOD and GPx activities were measured as described in Rezvanfar et al. (2012b). SOD assay was evaluated by the rate of increase at 560 nm. GPx activity was read by the determination of the decrease in absorbance at 365 nm. MDA level and catalase activity were studied biochemically in erythrocyte lysate samples. Catalase activity was measured and calculated according to Aebi's method by recording the hydrogen peroxide degradation spectrophotometrically at 240 nm (Aebi, 1984). MDA was measured by the modified Yagi (1984).

Histology

The specimens were fixed in 10% buffered formalin, processed, and embedded in paraffin and sectioned 5 μm thicknesses. These sections were stained with hematoxylin-eosin (HE). Pathological-physiological structures in ovaries were classified on the HE stained