Original Article

Evaluation of the Effects of Mentha Spicata Extract on In-Vitro Maturation of Mouse Oocytes

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Abstract

In traditional medicine, Mentha spicata is widely used for many diseases, especially in digestive system. It has anti-androgenic effects thus can be used by women experiencing polycystic ovary syndrome (PCOS). Also, it has shown to possess antioxidant properties. Although beneficial, studies have shown its detrimental effect on some tissues. The present study was carried out to determine the effect of Mentha spicata extract on oocyte maturation. To this aim, germinal vesicles (GV) were obtained from ovaries of 6-8 week old female C57 mice and cultured for 24 hours in maturation media containing different concentrations of Mentha spicata extract (0, 10, 20, 40 µg/ml). Another maturation medium containing dimethyl sulfoxide (DMSO) was determined as a Mentha spicata extract solvent. After 24 hours, number of degenerated, germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) oocytes were determined in each group using an inverted microscope. According to our results, the percentage of degenerated oocytes was higher in experimental groups compared to the control group. This has however not been statistically significant (*P*=0.473). Additionally, the percentage of GV oocytes was not different in control and experimental groups (*P*=0.774). The percentage of MI and MII oocytes was lower in experimental as compared to control groups, however statistically insignificant (*P*=0.410 and 0.855, respectively). In conclusion, Mentha spicata extract has mild detrimental effects on oocyte in vitro maturation. However, with increased concentration, its detrimental effects decrease which may be due to higher level of its antioxidants contents. Therefore addition of appropriate amounts of Mentha spicata as a natural extract in maturation medium may improves oocytes' maturation rate.

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Introduction

The use of medicinal plants for treatment of a wide variety of diseases has long been experienced in Iran. However, the exact effects of these herbal plants have not been determined thoroughly (1). Mentha spicata, known as spearmint, is a flowering plant which is widely used in complementary medicine for a variety of purposes, including treatment of respiratory and digestive system disorders, hypertension, anxiety and even for relieving menstrual pain (2-4). It is mainly recommended for its antispasmodic effects, which is related to its carvone content. Studies have demonstrated that Mentha spicata has antioxidant, anti-fungal, anti-microbial, anti-inflammatory, anti-tumor activity (5-9). Also, since it has antiandrogenic effects, it has been suggested for treatment of hirsutism and PCOS (10,11). Despite its beneficial effects, some studies have

demonstrated the harmful effects of this herbal plant on tissues such as kidney, uterus, liver and testis (12-15). This study was an attempt to evaluated the possible beneficial or detrimental effects of Mentha spicata extract on in vitro maturation of mouse oocytes.

Materials and Methods

Plant material and extract preparation method

Dried Mentha spicata leaves were purchased form PurSina Pharmaceutical Company, Tehran, Iran. For preparation of Menthe spicata extract, 100 gr of dried leaves was added to 500 ml 70% ethanol and macerated for 72 hours. Thereafter, the mixture was filtered through a 0.45 µm filter and put on rotary to dry at 40°C. After all, solvent evaporation was performed by vacuum desiccator for 24 h. The final weight of the extract was 13 g. The extract was maintained at 4°C throughout the experiments.

Animals

Mature female C57 mice (6-8 weeks-old) were bred in the animal house of Shiraz University of Medical Sciences. Animals were kept on 12 h light: 12 h dark period and controlled temperature condition with free access to water and food. The animal experiments were performed according to the principles of the care and use of laboratory animals established by the National Institutes of Health, Bethesda, MD, USA.

Preparation of media

Dissection medium was prepared using minimum essential medium (MEM-a), supplemented with 10% fetal bovine serum (FBS), 100 IU penicillin and 100 IU streptomycin. Drops of dissection medium were placed in a Petri dish, covered with mineral oil and preincubated three hours before cumulus-oocyte complexes (COC) collections.

In vitro maturation (IVM) medium was prepared using αMEM, 100 IU streptomycin, 100 IU penicillin, 10% FBS supplemented with 0.075 IU/mL FSH and 0.075 IU/mL LH (Menopur; Ferring Pharmaceuticals, Kiel, Germany). Four maturation media with different Menthe spicata extract (MSE) concentrations (0, 10, 20 and 40 µg/ml) were prepared. For this purpose, MSE was first dissolved in dimethyl sulfoxide (DMSO) and then the above-mentioned IVM medium was prepared. In addition, one IVM medium was determined with DMSO as a solvent. The final concentration of DMSO in each medium was 0.1%.

Collection of immature cumulus-oocyte complexes

Female mice were sacrificed by cervical dislocation and the ovaries were removed, cleaned of fats and transferred into dissecting medium. Cumulus-oocyte complexes were released by puncturing them with a 26-gauge sterile needle under a stereomicroscope, collected by a flame-pulled glass Pasteur pipette and washed in three drops of the same medium by gentle pipetting.

In vitro maturation

All cumulus oocyte complexes were divided and transferred into 20 µl drops of different IVM media, covered with mineral oil and pre-incubated 3 hours before COC collections. Then IVM dishes containing immature oocytes were placed in 5% CO2 incubator at 37°C. After 24 hours, granulose cells were removed by pipetting for precise evaluation of oocytes, and the quality of oocytes was evaluated using an inverted microscope (Nikon, Japan). Oocytes were divided into degenerated, germinal vesicle (GV), metaphase I (MI) and metaphase II (MII) according to their morphology.

Statistical analysis

Statistical analysis was done using the SPSS 16 software (SPSS Inc, Chicago, USA). For data analysis, we used one-way ANOVA. $p<0.05$ was considered statistically significant.

Results

In this study, oocytes were cultured for 24 hours in IVM medium supplemented with various concentrations of MSE. As shown in Table 1, the percentage of degenerated oocytes was higher in the experimental groups than in the control and DMSO group, whereas statistically insignificant (P=0.473). Furthermore, the percentage of GV oocytes was not different in control and experimental groups (P=0.774).

The percentage of MI and MII oocytes was lower in experimental groups in comparison to the control group, while not statistically significant (P=0.410 and 0.855, respectively).

Table1. The number of degenerated, GV, MI and MII oocytes in control, DMSO and three experimental groups (10, 20, 40 µg/ml MSE). Percentage of degenerated, germinal vesicle (GV), metaphase I (MI) and metaphase II oocytes (MII) in different groups.

Groups	Maturation stage of oocytes				
	Total COCs	Number of degenerated oocytes (%)	Number of GV oocytes (%)	Number of MI oocytes (%)	Number of MII oocytes (%)
Control (0 µg/ml MSE)	93	5 (5.38)	5 (5.38)	47 (50.54)	36 (38.71)
DMSO	112	16 (14.29)	5 (4.46)	66 (58.93)	25 (22.32)
MSE (10 µg/ml)	98	23 (23.47)	11 (11.22)	35 (35.71)	29 (29.59)
MES (20 µg/ml)	105	27 (25.71)	8 (7.62)	38 (36.19)	32 (30.48)
MSE (40 µg/ml)	102	18 (17.65)	8 (7.84)	41 (40.20)	35 (34.31)

Discussion

In the current study, the possible beneficial or detrimental effects of Mentha spicata extract, which is widely in use in traditional medicine, was studied on the maturation of mouse oocytes.

Findings of this investigation showed that Mentha spicata slightly increases degenerated and GV oocytes and decreases the number of MI and MII oocytes. Additionally, according to our results, the number of GV oocytes in the 40 µg/ml group was less than the 10 and 20 µg/ml groups, and the number of MI and MII oocytes in the 40 µg/ml group was almost near that of the control group. This may be related to the antioxidant capacity of this plant. It is probable that by increasing the doses of extract. As such its antioxidant capacity seems to dominate its detrimental effects, and using higher doses of this extract may improve IVM success rate. It has been determined that optimizing the culture condition for oocyte in vitro maturation is a critical factor for the success of IVM (16,17). Higher generation of reactive oxygen species in in-vitro cultures is one of the most well-known factors to exert detrimental effects on IVM success through reducing the developmental competence of embryos (18). Therefore, one of the main factors that can improve IVM success is the use of antioxidants (19, 20). some natural extracts have demonstrated improving effects on IVM (21-23).

The antioxidant potential of Mentha spicata has been determined by several studies and are thought to be associated with its carvone content (24-26).

Kamkar et al (27) showed that ethanolic extract of the Iranian Mentha spicata has noticeable antioxidant ability in vitro, and can be used as a natural antioxidant in food supplements or in the pharmaceutical industry. According to the higher percentage of MII oocytes in 40 µg/ml dose rather than in 10 and 20 µg/ml dose and also in DMSO, which is toxic for oocyte maturation (28), and the importance of adding optimum concentration of antioxidants to IVM medium (29), it is probable that by increasing the doses of Mentha spicata extract in IVM medium, oocyte maturation can be improved because of the higher antioxidant activity of this extract. Further studies are therefore warranted to determine precise effects of this extract on oocyte maturation. Finally, if higher doses improve oocyte maturation, the exact dosing level with no or minimal untoward effects should be determined.

Conclusion

Mentha spicata at applied doses does not seem to have any significant detrimental or beneficial effects on oocyte maturation.

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