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Effect of *Gundelia Tournefortii* leaves extract on in immature mouse oocytes

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ABSTRACT

Background & Aim: Considering that antioxidants are known as effective free radicals scavenger, it may be able to improve the in vitro oocyte maturation and the fetal quality. This study was designed to determine the effect of *Gundelia Tournefortii* leaves (GTE) hydro alcoholic extract as a source of antioxidant on in vitro oocyte maturation.

Experimental: Germinal vasicle (GV) were recovered from 6-8 weeks old NMRI ovaries. GV were cultured for 24 hours in maturation medium in MEMα supplemented with 7.5IU/ml hCG, 100mIU/ml rhFSH, 5% FCS (control group) and adding different doses of GTE extract (10µg/ml: group 1, 20 µg/ml: group 2, 40 µg/ml: group 3) and then in vitro maturation stages and resumption of meiotic in all groups was recorded by inverted microscope.

Results: In group 1, maturation rates were improved compared to the control group. But this difference was not significant. In group 2, maturation rates showed a significant increase compared to the control group ($p<0/05$). In group 3, also maturation rates showed a significant increase compared to the control group ($p<0/05$).

Recommended applications/industries: The results of this study showed that the *Gundelia Tournefortii* leaves extract, has a positive effect on oocyte maturation that is doses dependent. So with increasing concentration of Gundelia Tournefortii extract, the rate of maturation immature oocytes is increased. Generally, we conclude that addition of appropriate amounts of natural extracts such as Gundelia Tournefortii to maturation medium improves oocytes maturation.

1. Introduction

In vitro growth of follicles and in vitro maturation (IVM) of oocytes are novel approaches to obtain mature oocytes. Complete maturation of oocytes needs both nuclear and cytoplasmic maturation. Therefore, the lack of complete cytoplasmic maturation can lower the ability to form a male pronucleus and decrease in developmental competence of oocytes (Ka *et al*., 1997; Marchal *et al*., 2003, Marchal *et al*., 2001). The culture conditions of oocytes during in vitro maturation play a critical role in the rate of embryo production and quality including temperature, gas tension composition of media, etc (Wongsrikeao *et al*., 2006). Therefore, many researchers are investigating ways to optimize

the condition of IVM of oocytes. It is well known that one of the problems that impair in vitro production of embryo is the oxidative stress, that is mainly caused by reactive oxygen species (ROS) generation such as hydrogen peroxide (H2O2), hydroxyl radicals (•OH), superoxide anions (O2•–) and nitric oxide (NO), the highly reactive molecules formed by oxygen metabolism (Goto *et al*., 1993). This is damage the cell by breaking the DNA and RNA or inducing lipid peroxidation (Halliwell *et al*., 1991). Additionally, unfavorable media conditions in mammals leads to declines in developmental competence. Oocyte viability is reduced by the effects of heat stress, oxygen concentration and glucose content (Banwell *et al*., 2007). In vitro culture conditions have higher concentrations of O2 than in vivo conditions. It has been found that higher levels of reactive oxygen species (ROS) produced through in vitro cultures affect and impress fertilization rate, subsequent embryo development and clinical pregnancy rates (Tatemoto *et al*., 2004). Anti-oxidative substances prevent detrimental actions of free radicals (Aruoma *et al*., 2003). Previous investigations have indicated that the addition of anti-oxidants such as ethylene diamine tetra-acetic acid (EDTA) (Nasr-Esfahani *et al*., 1992), diethylene triamine penta-acetic acid (DTPA) (Nasr-Esfahani *et al*., 1990), vitamin C and vitamin E (Vermeiden *et al*., 1995), ascorbic acid (Kere *et al*., 2013), during in vitro culture increased embryonic developmental competency. Numerous studies have shown that plant extracts have several in vitro anti-oxidative properties (Alpinar *et al*., 2009; El *et al*., 2004). The anti-oxidative properties Gundelia tournefortii have been demonstrated in previous studies (Azeez *et al*., 2012). In this study the effect of *Gundelia tournefortii* leaves extract on in vitro maturation was evaluated.

2. Materials and Methods

2.1. Animals

Female Naval Medical Research Institute (NMRI) mice (6–8 weeks old) were used (purchased from Pasteur Institute, Tehran, Iran) for the experiment. Animals were kept on 12 h light: 12 h dark photoperiod and controlled temperature with ad libitum access to water and food.

2.2. Plant material and extract preparation method

About 200 g of dried Gundelia tournefortii leaves were added to ethanol 70% and macerated at 45-50◦C for 48 h. Thereafter, the extract was filtered and concentrated using a rotary evaporator apparatus. The final weight of the crude extract was 20 g. The GTE was maintained at 4°C throughout the experiments. Before adding GTE to maturation medium, it was filtered by 0.22 µm filters.

2.3. Antioxidant Activity by DPPH Assay

The percentage of antioxidant activity (AA%) of GTE was assessed by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical assay. The measurement of the DPPH radical scavenging activity was performed according to methodology described by Brand-Williams *et al*. (Brand-Williams *et al*., 1995). When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in color (from deep violet to light yellow) were read [Absorbance (Abs)] at 517 nm after 100 min of reaction using a UVVIS spectrophotometer.

2.4. In vitro maturation

Female mice were sacrificed by cervical dislocation. The ovaries were excised and placed in a medium that contained minimum essential medium alpha (MEMa) supplemented with 5% fetal bovine serum (FBS), 100 IU penicillin and 100 IU streptomycin (Daman *et al*., 2008). Antral ovarian follicles were punctured using 26-gauge needles. Cumulus oocyte complexes (COCs) at the germinal vesicle (GV) stage were collected and washed three times in maturation medium droplets including MEMa supplemented with 100 IU streptomycin, 100 IU penicillin, 5% FBS, 7.5 IU/ml recombinant human follicular stimulating hormone (rhFSH) (Organon, Holland), and 100 IU/ml human chorionic gonadotrophin (HCG) (Organon, Holland). Different concentrations of GTE (0, 10, 20 and 40 µg/ml) were added to the maturation medium. The 10-15 COCs were transferred to 25 µl drops that were covered with mineral oil and cultured for 24 hours at 37°C and 5% CO2. At various intervals from the onset of incubation, oocytes were observed by invert microscope (Nikon,Japan) and observation of nucleus morphological changes [GV and germinal vesicle break down (GVBD)] or the extrusion of first polar body (Metaphase II: MII) were used as the criterion for nuclear maturation of GV-stage oocytes.

2.5. Statistical analysis

ANOVA and Duncan's protected least significant test, using SAS program (Statistical Analysis System version 1.9) was used for all statistical analyses. All percentages of values were subjected to arcsine transformation prior to analysis. All data was expressed based on mean ± SEM. A probability of $p<0.05$ was considered statistically significant.

3. Results and discussion

3.1. DPPH Assay

The results are presented in Figure 1. All concentrations exhibited antioxidant activity. However, the values of antioxidant activity were always higher for high concentrations (Fig 1). Earlier studies have shown *Gundelia tournefortii* contain phenol compound such as Quercetin, this substance has strong antioxidant effects (Apak *et al.*, 2007; Filipe *et al.*, 2004). Antioxidant components protect oocytes from free radicals and improve quality of oocyte. Antioxidant therapy protects against oxidative stress and improves fertility parameters.

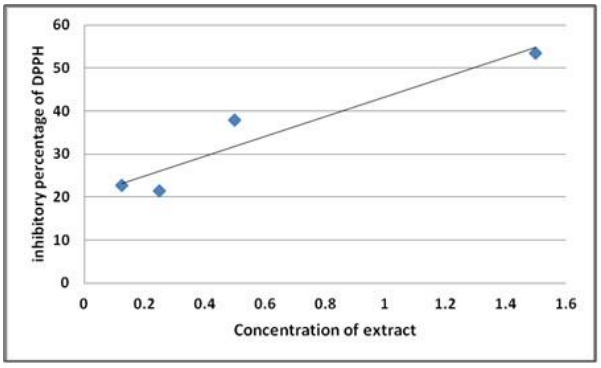

Fig 1. Inhibitory percentage of DPPH by GTE different concentrations.

3.2. In vitro maturation

In figure 2 has shown oocyte maturation different stages. In this study, oocytes were cultured for 24 hours in IVM medium supplemented with various concentrations of GTE. As shown in table 1, the percentage of metaphase oocytes significantly increased in all experimental groups (exceptionally, 10 µg/ml concentration) compared to the control group ($p<0.05$) (Fig 2).

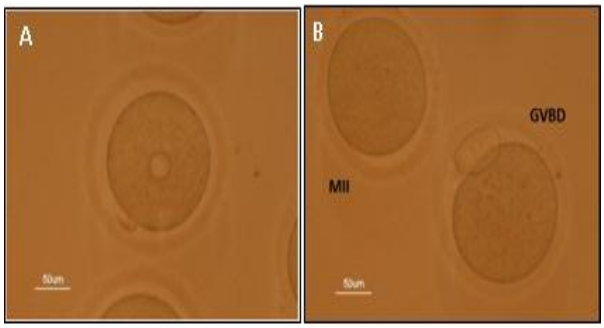

Fig 2. Oocyte maturation different stages. A) GV , B) GVBD & MII.

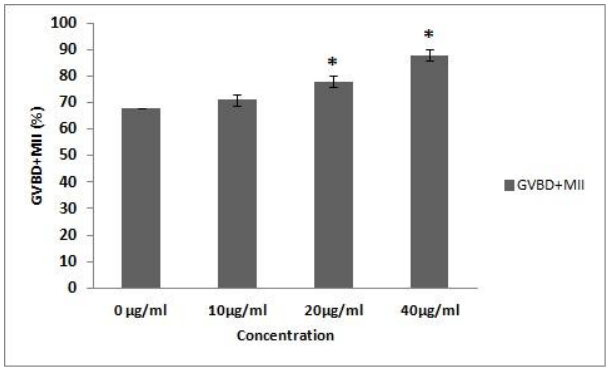

Fig 3. Meiotic resumption and mature of oocytes in different concentration.

Differents Concentrations (µg/ml) of GTE	Total COCs	GV (mean ± SEM)	GVBD (mean ± SEM)	MII (mean ± SEM)
0	92	32±2.2	24±2.1	44±2.6
10	96	29±2.1	21±1.6	50±3.3
20	101	22±1.8	18±1.3	60±3.5
40	98	12±1.1	16±1.3	72±2.8

Table 1. Effect of GTE on IVM rates

This improvement in oocyte maturation might be due to the antioxidant effect of GTE, which scavenge ROS during in vitro embryo culture. The major findings of this research showed that the addition of all experimental amounts of GTE to the maturation medium improved maturation rate. It was demonstrated that the addition of antioxidants such as cysteamine (Eimani *et al.*, 2005), β-mercaptoethanol (Eimani *et al.*, 2006), glutathione (Eimani *et al.*, 2005) and melatonin (Salimi *et al.*, 2014) to maturation medium positively influenced subsequent embryo development of mouse oocytes. In several species, intracellular glutathione

levels in mature oocytes help mediate sperm DNA condensation and male pronucleus formation post-fertilization (Sutovsky *et al*., 1997; Yoshida *et al*., 1993). Therefore, observation of improvement in the rate of oocyte maturation in this research seemed to be related to the effect of *Gundelia tournefortii* on glutathione levels in MII oocytes obtained during IVM. The optimum concentration of antioxidants such as amino acids and vitamins help ROS scavenging (Kitagawa *et al*., 2004). Wang and co-workers have demonstrated that supplementation of appropriate concentrations of green tea polyphenols as antioxidants through maturation of bovine oocytes increased blastocyst formation (Wang *et al*., 2007). Previous study was shown GTE extract contains flavonoids, phenol and Vitamin C (Gulzar Ismeal Ibrahim *et al*., 2013). The antioxidant effects of GTE polyphenols are thought to be associated with their ability to stimulate the antioxidant defense metabolism through redoxregulated transcription factors and mitogen activated protein kinase-dependent cell cycle regulation. It was shown that the optimum concentration of Papaver rhoseas L. extract in maturation medium caused improvement in the rate of oocyte maturation and subsequent embryo development (Golkar-Narenji *et al*., 2010). Similarly, in this research the natural extract of *Gundelia Tournefortii* leaves positively affected IVM, which was concentration dependent. All GTE concentrations increased maturation rates. To our knowledge, the present study is the first to demonstrate the beneficial effect of GTE supplementation of IVM medium on early mouse embryo development. The improving effect of natural extract on IVM has been shown in previous studies (Golkar-Narenji *et al*., 2010; Tavana *et al*., 2012). The results of this research and previous research indicated that the addition of those plant extracts to maturation medium as natural antioxidants was safe and possibly had lower side effects. According to findings of this experiment, *Gundelia Tournefortii* leaves may be regarded as a valuable plant source for use in traditional medicine.

4. Conclusion

Supplementation of IVM media with optimum concentrations of antioxidants such as GTE may help increase the numbers of blastocysts obtained from the IVM procedure. The improved effects might be dependent on the GTE concentrations in maturation medium.

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