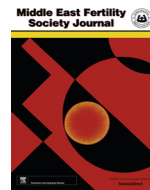




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Original Article

The effect of Melatonin on histological changes of ovary in induced polycystic ovary syndrome model in mice

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ABSTRACT

Background: Antioxidants can be used as adjuvant treatment of polycystic ovary syndrome (PCOS). Melatonin (MT) is one of the antioxidant that is used nowadays.

Objective: In this study, the effect of MT on the histological changes of ovary in the experimental model of polycystic ovary syndrome is investigated.

Methods: In this study 30 immature female NMRI mice were divided into 5 groups including: (1) control group received distilled water, (2) received a dose of 10 mg/kg MT for 5 days, (3) received Dehydroepiandrosterone (DHEA) at a dose of 6 mg/kg for 20 days to induce PCOS, (4) after induction of PCOS received MT at a dose of 10 mg/kg for 5 days, and (5) received 6 mg/kg DHEA and 10 mg/kg MT for 20 days simultaneously.

Results: The evaluation of ovarian tissue characteristics such as the granulosa layer, theca, number and diameter of cysts and follicles was performed. PCOS caused a significant reduction in the number of antral follicles and corpus luteum and an increase in the number of primordial, primary, pre-antral and cystic follicles in comparison with the control group ($P < 0.05$). Moreover, MT resulted in a significant increase in the granulosa layer thickness in group 4 ($P < 0.001$), and group 5 ($P = 0.001$) and a significant reduction in the thickness of theca layer between groups 4 and 5 compared with group 3 ($P < 0.001$).

Conclusion: These findings indicate that MT have a protective effects on polycystic ovary damages induced by DHEA, although the mechanism is unclear. It is likely that this is happening by reducing oxidative damage.

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1. Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of infertility (20% of infertile couples) and 6–8% of women suffer from this endocrine disorder in their reproductive age; this disorder was discovered by Stein and Leventhal for the first time in 1935 [1,2]. Several studies have shown that this syndrome can cause histological abnormalities such as bilateral ovarian enlargement of more than 10 mm, presence of more than 12 follicles of less than 10 mm size in the central dense stroma, increasing the thickness of the follicular sheath and ovarian stroma due to increase in angiogenesis, vasculogenesis, ovarian blood flow and, consequently, reduction or chronic anovulation and infertility [3,4].

Women with polycystic ovary syndrome have a wide range of clinical symptoms, but usually they seek medical advice due to three disorders that include irregular menstruation, infertility and symptoms associated with an increase in androgens such as hirsutism and acne [5]. Various treatment methods have been proposed for polycystic ovary syndrome, such as changing lifestyle, surgery and taking medication. Now the most recognized way of treatment is using medications such as clomiphene citrate, metformin, Letrozole and Tamoxifen [6]. Considering the side effects of these medications, identification and preparation of alternative medicine is important. However, extensive research to find new treatments have shown that oxidative stress, inflammation and activation of endothelial cells in the ovary plays an important role in the pathogenesis of PCOS and it leads to the development of atherosclerotic lesions in the ovaries. Therefore, a positive correlation between the reduction of oxidative stress and increased oocyte maturation in women with PCOS and infertility has been found. It seems that antioxidants reduce oxidative stress in different ways and as a

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result they can improve the prognosis of PCOS [7]. Melatonin (MT) is one of the antioxidants that are in focal attention nowadays. MT and its metabolites are potent antioxidants that remove free radicals and increase the expression of several antioxidant enzymes [8]. Consuming MT in laboratory mice protects cells against oxidative damage. In fact, it reduces oxidative stress in various ways [9]. MT, N-acetyl-5-methoxy-tryptamine which is a lipophilic Indolamine is synthesized mainly by the pineal gland. MT, in a circadian rhythm, is secreted during the night with high levels in all species. In mammals, including humans, MT rhythm is produced by an endogenous circadian clock in the suprachiasmatic nucleus and it impacts the hypothalamus-pituitary-gonad axis [10]. MT receptors have been identified in the nerve cells of the hypothalamus, which governs the release of gonadotropins pituitary gland, in the pituitary gland, in both male and female gonads and other reproductive organs [11–13]. In mammals, MT can affect reproductive function through the activation of the receptors in hypothalamic hypophyseal gonadal axis [14]. MT is also found in the fluid of the ovarian follicles [15]. The increase of MT in follicular fluid leads to a lower incidence of oxidative damage inside the follicle. Reducing oxidative stress increases the fertilization and pregnancy in women who could not be pregnant due to poor quality of oocytes [11]. MT receptor in rat, mouse and pig ovary cells has been found which suggests that it's has a direct effect on oocyte maturation [11,13]. Recently, *in vitro* studies have shown that MT plays an important role in optimal egg development and ovulation, and has a positive impact on early embryonic development [16]. Interestingly, MT concentration in large follicles is more than small follicles. High levels of MT in large follicles are likely to protect the granulosa cells of radicals that are induced during ovulation [17]. Considering the aforementioned issues, the inhibitory effect of MT on pituitary gonadotropin secretion and inhibiting intra follicular oxidative stress, and considering the fact that no report was found up to conducting this study, the effect of MT on the histological changes in induced polycystic ovary syndrome was analyzed.

2. Materials and methods

In this study, thirty NMRI rats which were 14 days old were used. To adapt animals, they were held in their cages for one week in laboratory conditions including 2 ± 21 C, 12 h periods of light, free consumption of food and water and good ventilation. Then, the mice were weighed and randomly divided into 5 groups (each group consisted of 6 mice). Control group: the mice in this group received distilled water intraperitoneally as MT solvent. The treatment control groups: to study the effects of MT on changes in the ovaries and the ovulation power of oocytes under normal circumstances, this group received 10 mg/kg MT intraperitoneally for 5 days. Disease group: In order to induce PCOS, this group received Dehydroepiandrosterone (DHEA) (6 mg/kg) for 20 days intraperitoneally. Treatment group: after inducing polycystic ovarian syndrome on mice in this group, they were treated by MT intraperitoneally for 5 days. The prevention group: in order to study the effects of MT in the prevention of inducing polycystic ovary syndrome, mice in this group received at the same time 6 mg/kg DHEA and 10 mg/kg MT for 20 days. At the end of the experiment mice were weighed and killed by cervical dislocation and their abdomen was opened under sterile conditions and their ovaries were removed for morphology/morphometry study.

2.1. Polycystic ovary syndrome induction method

To induce phenotype of Polycystic ovary syndrome in immature mice (approximately 22–21 days old), they were treated for 20 days with daily injections of DHEA at a dose of 6 mg/kg of body

weight intraperitoneally [18]. In order to confirm the induction of PCOS, three mice were sacrificed and their ovaries were separated from the spiral oviduct tube. Then, excessive fat was carefully dissected without damaging ovarian tissue under loop and ovaries were put inside fixative in order to do histotechnique procedure [19].

2.2. Administration of MT

MT was injected intraperitoneally for 5 days, everyday 10 mg/kg body weight. Duration and dose of MT were decided based on the previous studies conducted on rats and mice [20,21].

2.3. Histological analysis

Both mice ovaries were removed for histological examination. After fixation of tissue samples in Bouin solution for 16–14 h, the samples were dehydrated with alcohol solutions with rising levels 20–100% in each for 45 min to one hour, and then were clarified in xylene alcohol (50:50), xylene (three times) and embedded in paraffin. The samples were cut with a microtome 5 μ m diameter and sections were placed on slides. After Deparaffinization of sections and their yielding with alcohol solutions with lowering levels, they were painted by hematoxylin and they were differentiated with acid-alcohol solution and then they were stained [22]. In the final stage, the tissue sections taken from the ovaries were observed under an optical microscope with a magnification of 40 and different follicular groups were counted. Moreover, the thickness of antral follicles theca layer and granulosa were measured. In order to evaluate changes in ovarian tissue, ovarian structures were categorized into 6 groups based on morphology: Primordial follicles (PRIF), Primary follicles (PF), Preantral follicles (PAF), Antral follicles (AF), Cystic follicles (CF) and Corpus luteum (CL) and their changes in the ovaries were examined [23].

2.4. Statistical analysis

Kolmogorov-Smirnov test, Kruskal Wallis test, ANOVA test, Levene test, Tukey test and SPSS version 20 were used for statistical analysis of the data. P value less than 0.05 was considered significant.

3. Results

By analyzing the histological morphology of ovaries in the control group and the control treatment group different follicles were seen including graafian follicles with a thick layer of granulosa cells and corpus luteum. A number of cystic follicles with a very thin layer of granulosa which is the property of cystic follicles were seen in the ovaries of the disease group. In this group, only a few corpus luteum that indicates ovulation were observed. Therefore, based on the observations of ovarian tissue, injecting DHEA to mice for 20 days resulted in creating cystic follicles and a decrease in the number of antral follicles and corpus luteum and anovulation or very low ovulation (due to the reduction in the number of corpus luteum) in this group. Morphological studies in disease treatment group compared with disease group showed that MT during 5 days had resulted in an increase in the number of follicles, reducing the number of cystic follicles (3 ± 1.41 vs. 33.83 ± 1.60) and increase in the number of CL (7.16 ± 2.31 vs. 0.16 ± 0.40). Also, injecting MT with DHEA at the same time in the prevention group compared with disease group showed a reduction in the number of cystic follicles (5.66 ± 1.63 vs. 33.83 ± 1.60) and an increase in the number of corpus luteum (5 ± 1.41 In contrast, 0.16 ± 0.40) (Fig. 1). 10 mg/kg MT per day did not create a significant change on the

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