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Studying the Effect of Imipramine on Morphometric and Histopathological Changes of Ovary in Adult Female Rat

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Purpose and background: the aim of this study was investigating intraperitoneal injection of different doses of Imipramine on the number of follicles in the adult female rat. Infertility that occurs as a result of taking drugs is an important clinical issue. Rate of infertility that has been reported as a result of taking antidepressant drugs is 60%, in spite of its popularity a slight amount of studies has been formed to clarify the mechanism of action on these drugs, this study has written about Imipramine. forty female wistar 180 ± 20 g, selected and distributed them into groups experimental 1, 2, and 3. And they injected 6.25, 12.5, and 25 mg/kg bodyweight Imipramine respectively for 4 weeks, control group receives nothing, and sham group receives distilled water, at the end the samples of ovary tissue were taken to the laboratory for slice preparation, and slides of different parts of ovary made us available to count total number of follicles in each ovary. Results showed that this drug has no effect on bodyweight, ovary and primitive, secondary, graffian and atretic follicles. Different doses of imipramine, number of primary follicles, also 25 mg/kg dose of this drug has caused Corpusluteum to decrease.

Key words: Imipramine, ovary, female rats.

Tricyclic antidepressants have been inclinical use for almost four decades. An important example of this group is Imipramine, with complete name of Imipramine hydrochloride and chemical formula of $C_{19}H_{24}N_2$ and amitriptyline[1].

It seems that antidepressant effect of Imipramine is the result of inhibiting reuptake of serotonin and norepinephrine in presynaptic nerve terminals so it increases activity and density of neurotransmitters in synaptic vesicles. Studies has showed that tricyclic antidepressants trough change in the balance of catecholaminergic inhibition and stimulation of catecholaminergics affect secretion of prolactin, and increase in prolactin secretion can cause infertility[2] also this drug slightly affects reuptake of dopamine[3]. It seems lenitive effects of tricyclic antidepressants decrease synchronous discharge of GnRH[1] and libido reduction, impotence, ejaculation delay or failure, through increase of prolactin. Due to the fact that control of sexual behavior happens

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through the cortex, this will be understandable[4-5]. Studies on the Amitriptyline drug showed that, this drug by affecting on the serotonin or norepinephrine neurotransmitters increase the gonadotropin secretion[6]. Research on the tricyclic antidepressants has showed that these drugs will increase ovarian and breast cancer[7]. Studies has showed that tricyclic antidepressants trough change in the balance of catecholaminergic inhibition and stimulation of catecholaminergics effect secretion of prolactin, and increase in prolactin secretion can cause infertility[2]. It has also reported that imipramine has not changed sexual hormones[8].it has also reported that imipramine has not caused drastic changes in the level of prolactin in serum[9].

So, with the effect of this drug on the sexual hormones, extensive research on its effects is required. With the same reason,the purpose of this study is investigating the effect of intraperitoneal injection of imipramine drug on rat follicles.

MATERIALS AND METHODS

This is an experimental study in which all ethics of working with the laboratory animals has honored.in this study. We used 40 adult female Wistar rat with approximate weight of 180±20 g obtained from a center of laboratory animals in Azad University, Jahrom center and they kept in condition of 12 hours light and 12 hours of darkness and pellet used to fed rats. Animals were divided into 5 groups of 8. The control group received nothing. Sham group received distilled water, the groups experimental groups 1,2 and 3 respectively received 6.25, 12.5 and 25 mg/ kg body weight Imipramine. Disposable insulin syringe used for intraperitoneally injections for four weeks.. With respect to this that, a lethal dose of Imipramine, determined to be (LD50) 50mg/kg, so the sublethal doses of 25, 12.5 and 6.25 mg/kg were used. Imipramine obtained from Sobhan Pharmaceutical Company. At the end of experiments animals weighted by a Japan Made digital scale and with the accuracy of .01 and then anesthetized with ether. The abdominal area dissected and ovary separated from surrounded fat tissue and Fallopian tubeby scalpel and forceps. Ovaries weighted by digital scale and washed out by physiological saline and entered into a 3% formalin solution (fixation) and sent to histology Lab for slide preparation. In each slide primitive follicles, antral follicles, graffian follicles, atretic follicles and corpus luteum respectivelycounted, with the magnification of 4, 10 and 40 percentage of each follicles in each ovary estimated. Results with software (SPSS version 15), and one-way analysis of variance (ANOVA) and Duncan test were analyzed. Mean and standard deviation calculated and $p<0.05$ was considered as statistically significant.

RESULTS

We can conclude from changes in body weight, and ovary that there is no significant difference between experimental groups, sham group and control group. (Table 1)

After counting follicles we noticed that there is no significant decrease between promaty follicles, secondary follicles, graffian follicles and atretic follicles in experimental groups in comparison with, sham group and control group.

The primitive follicles in three experimental groups compared with the control and sham groups showed a significant decrease. The amount of 25 mg per kg of the drug was a significant decrease in corpus luteum compared with control and sham groups.

Table 1. Comparisons between different groups. (mean ± SD, n=8)

(mg/kg25)	(mg/kg12.5)	(mg/kg6.25)	sham	control	
0.51a±2.6	0.78a±4.5	1.2a±3.4	0.47a±2.7	0.24a±2.6	primitive follicles (percent)
1.7a±4.6	1.3a±5	1.2a±5.3	1.5b±9.5	0.58b±9.2	Primary follicles (percent)
0.36a±1.4	0.49a0±1.6	0.52a±1.7	0.65a±2.1	0.51a±2.8	Secondary follicles(percent)
0.96a±7	0.98a±8.1	1.4a±8	0.79a±6.8	0.78a±6.4	graffian follicles (percent)
1.6a±3	1.6b±5.2	0.64b±6	1.35b±7.3	1.02b±7.4	The corpus luteum (percent)
0.42a±0.71	0.33a±0.66	0.14a±0.14	00a±00	00a±00	Atretic Follicle (percent)

DISCUSSION

The results obtained in this study shows the primitive and secondary follicles have no significant difference in the experimental group compared to the control group. But the primary follicles in this study, in the experimental groups, showed significant differences compared with the control group. The results of this study showed that Imipramine reduced primary follicles significantly.

Follicle and Lutenizing stimulating hormones both stimulate their own ovarian target cells to attach to highly specific FSH and LH receptors, in the ovarian target cell membranes. Then activated receptors speed up secretion from the cells and also increase growth and proliferation of these cells. Almost all of these stimulatory effects produce by cyclic adenosine monophosphate second messenger system activation in the cytoplasm of the cells. The first stage of follicular development is mean enlargement of the egg, which it's diameter increases, two to three times. After this growth some of additional layers of granulosa cells grow in some follicles and the follicles are called primary follicles.Gonadotrophin-Releasing Hormones especially Follicle stimulating hormone speed up follicles growth after the proliferative phase of follicular growth that lasts several days granulosa cells secrete a fluid containing large concentrations of estrogen[10].

The number of primary follicles according to Table (1) showed a significant decrease in the experimental groups,compared with the control and sham groups. These follicles grow under the influence of follicle-stimulating and Lutenizing hormones especially FSH hormone. Also, a significant reduction in the primary follicles could also be due to increased prolactin[11-12]. This study showed that imipramine has no significant effect on the number of graffianfollicles. In this study corpus luteum

According to Table (1) no significant difference seen between theleast amount and middle amount in the experimental group compared to sham and control groups. But in comparison between control and Experimental in max amount significant difference observed. About 16 hours before ovulation, LH secretion by the pituitary gland were significantly increased up to 6 to 10 times, after ovulation, granulosa cells and remaining interior single cells quickly become effected by LH and convert Lutenizing cells. Diameter of these cells grows up to two times, the initial cell, and fills with Lipid inclusions that give them a yellow appearance. This process is known as Lutenizing and the total mass of the cells is called the corpus luteum[10].

In this study, the corpus luteum generally decreased, but decrease in groups with minimum and middle amounts has been very small and do not show a significant difference, but in the group with maximum amount we see a significant decrease. According to Table (1) the mean number of atretic follicles in the experimental groups compared to control group and the sham group showed no significant change. Successful differentiation of follicles is dependent on the presence of steroids and growth factors that stimulate follicular differentiation and protects cells against cell death. Growth and differentiation of ovarian follicles happens by proliferation and differentiation process of granulosa cells. Estrogenic hormones have the most important role in the regulation of growth, development, and homeostasis and programmed cell death in ovary[10] in these study estrogenic hormones in the experimental groups showed a significant decrease so we can estimate an increase in atretic follicles, but the reason for this meaningless increase might be injection time and by increasing the injection time we can be expect that a significant increase in the number of atretic follicles happen.

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