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Effects of Raloxifeneon on Ovarian Tissue of Mature Female Rats

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Raloxifene as a selective non-steroidal regulator has profound estrogen-like effects on bone but anti-estrogen effects on endometrium and breast tissue. The aim of this study is to evaluate the effect of Intra-peritoneal injection of Raloxifene in different doses on ovarian tissue of mature female rats. Forty female Wistar rats weighing 180 20 g range were selected and divided into control, sham and experimental (1,2,3) groups. Raloxifene dissolved in distilled water with dosage of 30, 60 and 120 mg/kg BW were within four weeks injected to experimental groups1, 2 and 3 respectively. Control group did not receive any drugs while sham group received distilled water. All the injections were conducted intraperitoneally in patological studies. The parameters were the counts of initial, first, secondary, graph, uterus follicles and yellow body. The results of initial and uterus follicles counts in comparison with the control group show no significant difference (P<0.05). Secondary and graph follicles in experimental groups 2 and 3 showed no significant decrease compared to the control group (P<0.05) and number of initial follicles and yellow body in experimental group 3 showed a significant decrease compared to the control group (P<0.05). Results revealed that Raloxifene may cause a slight change in the number of ovarian follicles.

Key words: Raloxifene, ovary, female rats.

Evista, a drug with the generic name "Raloxifene hydrochloride",is used for the treatment of osteoporosis. Raloxifene hydrochloride and its hydrochloride salt have recently beenrecognized as keoxifene which has been invented two decades ago[1]. Raloxifene hydrochloride has the same structure as benzothiophene. Since this drug onlyaffects female sex hormones, itis merely prescribed for women.It is also used in order to prevent vertebral fractures[2]. Raloxifene as a non-steroidal selective estrogen regulatorfunctions as estrogen imitator in some tissues and has non-estrogenic activities in other ones. In fact Raloxifene is an alternative meansof estrogen treatment for those who are inrisk of osteoporosis whereasit has an anti estrogenic property[3]. The general chemical formula of Raloxifene is $C_{28}H_{27}NO_4S.HCl$ which corresponds to a net molecular weight of 510.05. Raloxifene belongs to a class of drugs known as SERMs (Selective Estrogen Response Modifiers)[4].

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Raloxifene has anti-estrogenic effects on breast cancer. This drug can decrease breast cancer hormones by preventingthe secretion of these hormones[5].This drug can favorably change the biochemical signs of vascular disease by reducing LDL-cholesterol, fibrinogen and lipoprotein and also by increasingLDL2-cholesterol without increasing triglyceride.Scientists are investigating the question that whether these favorable chemical effects can cause protection against vascular disease[5]. The main concern in using Raloxifene is the high risk of blood clottingin veins. Therefore blood clotting in veins prevents the use of Raloxifene[6].

In a study on rats in which each rat,before and during intercross, hadreceived 5 mg/kgof Raloxifene on a daily basis,no pregnancy was observed in the end. As a result the sexual cycle in female rats was disrupted and ovulation process was interrupted. Implantation of the embryo was also delayed and failed. The size of newborn rats became smallby using of this drug during pregnancy. The reported growth effectsincludea decrease in the newbornweight, delay in skeleton growth, delay in growth, incomplete or abnormal formation of heart, edema and Hydrocephalus in children[7].In a studydone by Patrick *et al*. (1997), onthe effects ofRaloxifene on ovarian cancer in postmenopausal women, it was shown that Raloxifeneshould not be used on women suffering from ovarian cancer. Furthermore, a treatment with Raloxifene in postmenopausal womendoes not cause any increasein ovary size[8].

MATERIALS AND METHODS

In this experimental study all code of conducts for research work with laboratory animals which is approved by the Americanorganization called SPCA (Society for the Prevention of Cruelty Animals) in 2006areobeyed.In this research, forty, mature female Wistar rat weighing 180± 20 gwere used whichwas supplied bythe center ofbreeding and keeping of laboratory animals in Islamic Azad universityof Jahrom.They were kept in light condition of 12h light and 12h darkness. Standard compressedanimal food(Pellet) was used in order to feed therats.They were divided into 5 eight-membergroups. The first group was control rats, second group sham rats which received distilled water, third group was the rats which received30 mg/kgRaloxifene, forth group was the rats which wereinjected60 mg/kg Raloxifene and fifth group was the rats which received 120 mg/kg Raloxifene. All injections were doneintraperitoneally during 4 weekswith disposable insulin syringes. In this study the lethal dose of Raloxifene (LD_{50}) was determined 240 mg/kg. Therefore sub-lethal doses (30, 60, 120 mg/kg)were used. Raloxifene was supplied by Osveh Pharmacy Company.At the end of test period,all animals were anesthetized with ether and wereweighted by AND (Japanese model digital scale)in 0.01 accuracy.Then theovaries were removed by surgery and adipose tissues around them were removed carefully. Each animal'sright and left ovaries were separately weighted and until the preparation of tissue slices they were kept in glasses containingformalin fixation. Then tissues were sent to histology lab for the preparation of slides.The results were analyzed by SPSS software (version 20), ANOVA analysis test and DUNCAN statistical test.Average and standard deviation were estimated. $P \leq 0.05$ was taken as statistical significant level.

RESULTS

Due to the body-weight results, there is nosignificant difference between experimental, sham and control groups (table 1).

According to the resultsobtained from measuring the weight of rightovaryin all groups, experimental groups shownosignificant difference compared tocontrol group (table 1).

According to the results obtained from measuring the weight of left ovary in all groups, no group showsa significant difference compared to control group (table1).

Results obtained from counting the number of initial follicles related to ovaries between different groups show that the average of these follicles in non group shows meaningful difference than control groups (table 1).

Results obtained from counting the number of the initial follicles related to ovaries between experimental groups 1, 2 shownosignificant difference compared to control group, but the experimental group 3 shows asignificant decrease compared to control group and experimental group 1 (table 1).

Results obtained from counting the number of secondary follicles related to ovaries between differentgroups show that none of the experimental groups had a meaningful change compared to the control group, but experimental group 3 showsa significantdecreasecompared to the experimental group 1 (table1).

Results obtained from counting the number of the graph follicles related to ovaries between different groups showthat there is no meaningful difference between experimental groups 1, 2, 3compared to control group. But the experimental groups2, 3 showsignificant decrease compared toexperimental group 1 (table 1).

Results obtained from counting the number of the yellow body related to ovaries between different groups show that there is nosignificant difference between experimental groups 1,2 in compared to control group, but the experimental group3 shows a significant decrease compared to experimental group 1 (table 1).

Results obtained from counting the number of the uterus follicles related to ovaries between different groups show that there is nosignificant difference between experimental groups compared to control group (table 1).

Table 1. Average comparisonin different groups

Experimental 3	Experimental 2	Experimental 1	Blank	Control	Parameters
15.6±9.8a	15.510.2a	16.0111.1a	15.210.3a	15.810.6a	Weight of Body
0.0520.02a	0.0410.02a	0.0380.006a	0.0490.002a	0.0470.003a	Weight of Right Ovary
0.0450.008a	0.0380.009a	0.0330.003a	0.0440.002a	0.0480.002a	Weight of Left Ovary
1.70.35a	2.80.45a	3.20.8a	2.10.47a	2.60.24a	Initialfollicles
6.51.7a	71.06ab	10.50.35b	9.51.5b	9.20.58b	First follicles
1.10.64a	2.50.48ab	3.20.86b	2.10.65ab	2.80.58ab	Secondary follicles
5.011.7a	5.81.6a	9.71.5b	6.80.79ab	6.40.87ab	Graph follicles

DISCUSSION

Raloxifene is a drug which is discovered in the past two decades. Due to the selective nature of Raloxifene it is called the Selective Estrogen Response Modifiers (SERMs). Estrogenic and anti-estrogenic effects of Raloxifenein organizationand regulationof nerve system in controlling reproduction performance is quite clear. In fact, there are paradoxical results about estrogenic or anti-estrogenic behaviors of Raloxifene onhypothalamus–hipofisisaxis.

According to table 1, there was nosignificant difference seen in the weights ofovaries in experimental groups 1, 2, 3 compared tothe control group. TenaSempere, *et al*. (2004) after studying Raloxifene effects on female mice stated that this drug causes a decrease inovary weight[9].

Pinla, *et al* (2002) also claimed that this drug causes a decrease inovary weight[10] which does not match up withthe results of this study. Previous studies expressed that this drug can cause fragmentation in action of hypothalamus – hipofisis-gonad axis and can cause a decrease in hipofisisvolume. According to someresearches carried out in the past, this drug causesa decrease in hipofise weight which can cause ovaryweight loss.In this study, however,this result isnot obtained though.

Results obtained from counting the number of the ovarian follicles showed that the number of initial, secondary and graph follicles did not havea significant difference in any of the experimental groupscompared tothe control group.But the first follicles in experimental group 3 showed asignificant decrease compared to experimental group 1.

According to the research about effects of this drug on female mice whichis carried out by Tena-Sempere, *et al*.(2004) and Gerald (2001) (9,11) ovulation rate and level of progesteronewas reduced. Gerald, on the other hand, stated that this drug causesa swelling in follicles and hyperplasia in granulosa cells, a change in ovarian morphology anddecrease inovulation rate. In another study by

Pinela(2001)on"Raloxifene effects on operation of male mouse reproduction" expressed that this drug cause testis atrophy but it has less effects on reproduction system of male mouse[12]. Furthermore, by injecting this drug to female mice, abundant horny cells in vaginal smear were found. Pinela also stated that this drug increase prolactin level. After maturation alarge amount of FSH and LH hormones are released from anterior hipofisis that can cause a growth in ovaries and some follicles. Gonadotropin releasing hormone (GnRH)releases from hypothalamus and cause secretion of LH (producer of yellow body) and FSH (follicle-stimulating hormone) from anterior hipofisisandthese two hormones cause secretion of ovarian hormones called estrogen and progesterone from ovary. Low concentration of estrogen also inhibits gonadotropins releasing. Increase in prolactin reduced the secretion of gonadotropinsbyaffectingthe pulsatile release of GnRH. Therefore increase in prolactin causes FSH reduction.Reduction of follicle-stimulating hormone, FSH, can affect the growth and reduction offolliclesprocess[13].

According to the table 1,no significant difference wasseen in uterus follicles compared to control group.

Alisiya (2010) observed uterus follicles in ovarian tissue when he injected this drug to female mice[14].

Successful distinction of follicles depends on the presence of steroid and growth factors which stimulates follicledistinction and protectsthe cells against cellular death.The growth and distinction of ovarian follicles are done by proliferation and distinctionof granulosa cells. Estrogenic hormones in ovary have the main role in control of growth, evolution, homeostasis and planned death. In this study the amount of uterus follicles is not significant. Indeedby increasing the time of using of this drug, increasing in uterus follicles may happen.

According to table 1 experimental group 3 showedsignificant decreasecompared toaverage number of yellow body.

In a research conducted by Gerald (2001), it is stated that this drug can causean extremedecrease in yellow body as well asreducing progesterone.Another research claimed that the number of yellow body was reduced which matches upwith the changes ofyellow body in this study[15, 11]. Transformation of granulose cells and inner theca into yellow body is essentially dependent on the secretion of LH hormone from anterior hipofisis. Moreover, yellow body cells,influenced by LH hormone,replace their enzyme group and start to discharge progesterone and estrogen.Yellow body is the main source of progesterone[13]. A decrease in Yellow bodyin this studycan be the causedbythe reduction of the LH hormone.

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