

Research article

Effect of pregabalin on ovary tissue of albino female rats

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ABSTRACT

The present study aimed to investigate effect of Pregabalin (PGB) on ovary tissue and number of follicles in female albino rats. Three groups of healthy adult female albino rats, fifteen rats in each group were used in current study. The rats of groups, G2 and G3 were administered orally with two doses 150 mg and 300mg/kg b.wt/day of pregabalin, respectively. The doses were given daily for 1 month, 2 months, and 3 months. Animals of group G1 (Control) were given saline alone. After the experimental periods, the rats were sacrificed and the isolated ovaries were histologically examined. The results of histological analysis of the ovaries in treated rats (G2, and G3) showed a significant ($P \leq 0.05$) decrease in the number of preantral, antral, corpora lutea, and total number of follicles when compared to the control group (G1). While the number of atretic follicles was showed a significant ($P \leq 0.05$) increase in the treated groups (G2 and G3) when compared with control group. The difference was dependent on the dose and period. Final remarks of present study are oral administration 150 mg and 300 mg/kg b.wt/day of pregabalin produce adverse effects on the ovary tissue and affects on the fertility in female rats.

Keywords: Antral, atretic, corpus luteum, female, follicle, preantral, pregabalin.

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INTRODUCTION

Reproduction is a phenomenon for the living organisms; its important came from the ability to continue their species. It is controlled by the function of hypothalamo hypophyseal gonadal axis [1]. The hypothalamus and pituitary forms a functional unit that secretes GnRH, and then gonadotrophins, which affects on the gonads [2]. In females, the different stages of ovarian development are reflected through the different phases of reproductive cycle. The rat estrus cycle is short, lasting 4 to 5 days with 4 stages [3], and for this reason female rats are ideal for most of fertility studies [4]. Antiepileptic drugs known to interfere with the functions of central nervous system including hypothalamus thereby modify the activity of hypophysis [5]. As a result, the function of the gonads may also be modified [6]. Antiepileptic and other drugs that affect on CNS can modify the

reproductive and endocrine activities [7]. Pregabalin (PGB) recently was proved its affects on endocrine hormones in healthy female rats especially for fertility [8]. Therefore, in the present work, the effects of pregabalin drug were undertaken to discover if pregabalin-induced changes on ovarian tissue of healthy albino female rats after 1, 2, and 3 months of orally administration.

MATERIALS and METHODS

Forty five healthy, sexually matured, regularly cycling, colonies bred virgin female rats of Wistar strain (*Rattus norvegicus* L.) aged 2.5- 3 months and weighing 200 ± 20 gm was purchased from animal house unit, High Institute of Fertility Diagnosis and



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Assistant Reproductive Technology, Al-Nahrin University, Baghdad, Iraq. The rats were housed in polypropylene cages, under well ventilated animal house conditions (temperature: 22-25 °C, photoperiod: 12 hours natural light and 12 hours' natural dark). The rats were fed with balanced pellets (tap water and *ad libitum* diet). They were maintained as per the principles of laboratory animal's care [9]. The animals were divided into three main groups (G1, G2 and G3), fifteen rats in each group, the animals were treated and then each group was divided into subgroups; A, B, and C, according to periods post administration; 1, 2, and 3 months, respectively) as follows: G1 was given 0.5 ml saline and served as control. Group 2 and 3 were given pregabalin at dose 150 and 300mg/kg of body weight/day, respectively. Pregabalin was dissolved in 0.5 ml saline in each dose. The treatment was given for 1, 2, and 3 months once orally between 9:00-10:00AM. After 24 h from last treatment, all the animals from each group anesthetized with ether, animals were sacrificed, the ovary were dissected out, freed from extra depositions of adherent tissue. Ovaries from each animal were fixed in formalin (10%) [10] for histological examinations [11]. The ovarian components like preantral follicles, antral follicles, atretic follicle and corpus luteum, and total number of follicles were calculated from randomly chosen 20 sections from each group using ocular lens [12, 13].

Statistical Analysis

All the values were statistically analyzed by ANOVA –Least significant difference test. Data are expressed as the Mean + S.E. Statistical significance was set at $p < 0.05$.

RESULTS

Changes in number of different ovarian follicles after 1 month of treated female rats

Table 1 shows that PGB induced non significant ($P \geq 0.05$) changes in the number of preantral follicles in both doses (150 and 300mg/kg b.wt/day) in treated female rats (G2A)(G3A) respectively when compared with control group(G1A). Also the table showed a significant ($P < 0.05$) decrease in the number of antral follicles in G2A and G3A in compared to the G1A. A significant ($P < 0.05$) increase in the number of atretic follicles was showed in G2A and G3A in the same table in compared to the control group (G1A). The number of corpora lutea was affected by treatment with PGB (in two doses) and decreased significantly ($P < 0.05$) in G2A and G3A when compared with G1A, finally a non significant difference ($P \geq 0.05$) was noticed in the total number of follicles in G2A and G3A as compared to the control group (G1A).

Table 1. Effect of two doses of PGB after 1 month treatment on different types of ovarian follicles and corpora lutea in treated female rats. The data represented by mean \pm SD. Each group contains 5 female rats; *, $P < 0.05$ as compared from control group; NS, Non-significant.

Treatment	Pre- antral follicles	Antral follicles	Atretic follicles	Corpus luteum	Total no. of follicles
Control (G1A)	9.60 \pm 0.24	6.00 \pm 0.45	5.00 \pm 0.32	3.60 \pm 0.24	20.60 \pm 0.68
G2A	9.60 \pm 0.51 NS	4.00 \pm 0.44*	8.00 \pm 0.32*	1.60 \pm 0.24*	21.60 \pm 0.87 NS
G3A	9.60 \pm 0.51 NS	3.60 \pm 0.40*	9.20 \pm 0.48*	1.40 \pm 0.24*	22.00 \pm 0.54 NS
LSD value	1.354 NS	1.331 *	1.180 *	0.754 *	2.193 NS

Changes in number of different ovarian follicles after 2 months of treated female rats

Table 2 shows that there was significant decrease ($P < 0.05$) in the number of pre-antral and antral follicles in treated females (G2B) and (G3B) with PGB when compared to their numbers in the control group (G1B). Also significant increase in number

of atretic follicles was showed in G2B and G3B as compared to the control G1B. A significant ($P < 0.05$) decrease was shown in the number of corpora lutea in treated groups (G2B and G3B) when compared with control (G1B). And in total number of follicles no significant difference ($P \geq 0.05$) was showed in treated groups (G2B and G3B) in compared to the control (G1B).

Table 2. Effect of two doses of PGB after 2 months of treatment on different types of ovarian follicles and corpora lutea in treated female rats. The data represented by mean \pm SD. Each group contains 5 female rats; *, $P < 0.05$ comparing to control group; NS, Non-significant.

Treatment	Pre- antral follicles	Antral Follicles	Atretic follicles	Corpus luteum	Total no. of follicles
Control G1B)	10.00 \pm 0.00	5.80 \pm 0.48	6.00 \pm 0.32	4.00 \pm 0.00	22.60 \pm 0.24
G2B)	8.00 \pm 0.31*	3.20 \pm 0.20*	9.40 \pm 0.50*	1.20 \pm 0.20*	22.80 \pm 1.62 NS
(G3B)	8.00 \pm 0.22*	2.20 \pm 0.58*	10.00 \pm 0.00*	0.80 \pm 0.20*	20.20 \pm 0.96 NS
LSD value	1.378 *	1.401 *	1.067 *	0.503 *	3.394 NS

Changes in number of different ovarian follicles after 3 months of treated female rats

Table 3, fig.1, fig.2, fig.3, show PGB decreased significantly ($P < 0.05$) the number of preantral and antral follicles in treated groups (G2C) and (G3C) after 3 months of treatment as comp-

ared to control group (G1C). Also the table shows a significant ($P < 0.05$) increase in the number of atretic follicles in G2C and G3C as compared with control (G1C). Finally, a significant ($P < 0.05$) decrease in the number of corpora lutea and the total number of follicles in treated groups (G2C) (G3C) as compared with control group (G1C).

Table 3. Effect of two doses of PGB after 3 months of treatment on different types of ovarian follicles and corpora lutea in treated female rats. The data represented by mean \pm SD. Each group contains 5 female rats; *, $P < 0.05$ as compared from control group; NS, Non-significant.

Treatment	Pre- antral follicles	Antral follicles	Atretic follicles	Corpus luteum	Total no. of follicles
Control (G1C)	10.80 \pm 0.48	8.00 \pm 0.54	7.20 \pm 0.37	4.40 \pm 0.24	26.00 \pm 0.94
G2C	8.40 \pm 0.40*	1.60 \pm 0.50*	10.00 \pm 0.00*	0.80 \pm 0.20*	20.00 \pm 0.83*
G3C	8.20 \pm 0.20*	0.80 \pm 0.58*	12.20 \pm 0.20*	0.60 \pm 0.24*	21.40 \pm 0.51*
LSD value	1.180 *	1.687 *	0.754 *	0.711 *	2.426 *

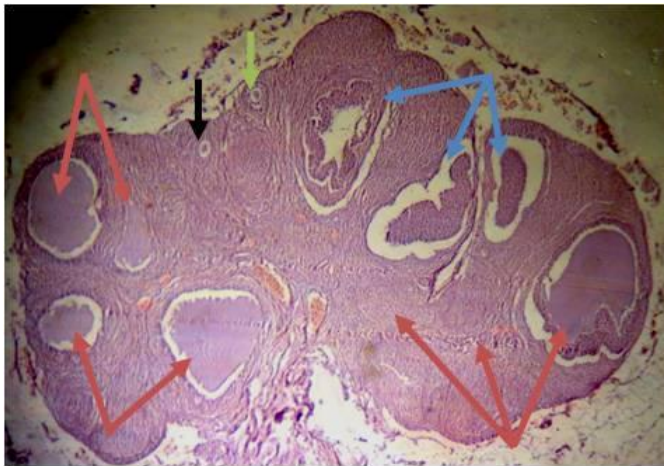


Fig 1. Section in ovary of control rat after 3 months, showing normal ovarian components preantral (primary) follicle \blackrightarrow , antral follicle \blackrightarrow , corpus luteum \blackrightarrow , and variable stages of atretic follicles \blackrightarrow (H and E stain 4X).

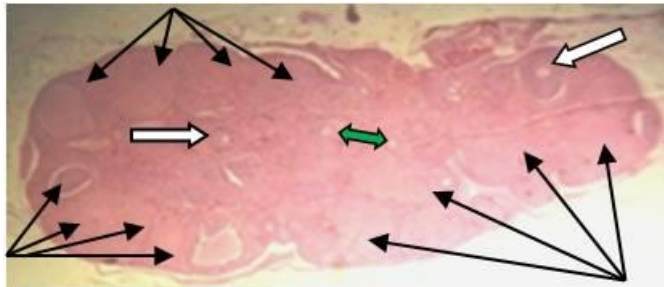


Fig 2. Section in ovary of treated rat with PGB 150 mg/kg b.wt/ day after 3 months, showing diffuse atretic follicles changes \blackrightarrow with residual preantral (primary) follicles $\blackleftarrow\blackrightarrow$ and antral follicle \blackrightarrow (H and E stain 4X).

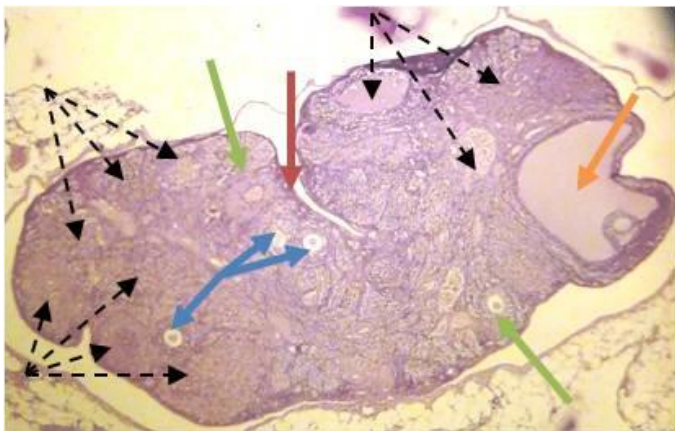


Fig 3. Section in ovary of treated rat with PGB (300mg/kg b.wt/ day) after 3 months showing a large developed antral follicle with large single antrum filled with follicular fluid \blackrightarrow , preantral follicles (several primordial follicles \blackrightarrow , unilaminar primary follicles \blackrightarrow , two secondary follicles \blackrightarrow , and more numbers of variable stages of atretic follicles \blackrightarrow with congested vessels (H and E 4X).

DISCUSSION

The results of the present study exhibits that ovary affected by treating with PGB, and ovarian follicles were affected by

changing on their number. The number of preantral follicles (primordial, primary and early secondary follicles) after one month of treatment with PGB did not change. This observation is agreement with Sneha *et al.* [14] they reported that number of growing follicles in female rats is not changed after one month phenobarbital (PB) treatment. According to McGee and Hsueh [15] who reported that in the fewer or absence of gonadotrophin stimulation follicles can progress to the small antral stage but gonadotrophin stimulation accelerates cell division and differentiation. In our prior study in Tawfeeq *et al.* [8], we found PGB affects on endocrine hormones leading to decrease the levels of FSH, LH, and Prolactin after 1,2, and 3 months treatment. Hence, the development of follicles prior to the antral stage may not be gonadotrophin-dependent, but is gonadotrophin-responsive, and the present results may agree with this opinion. Preantral follicles number after 2, and 3 months of treatment with PGB in current study reduced dependent with long-lost of exposure and dose of PGB. In the present results the decrease was noted with secondary follicles number (data not shown) and that is expected because they are prior to the antral stage and they are in this condition are responsive [16] to FSH level, which decreased from affects of PGB directly on pituitary gland or on hypothalamus [8]. Antral follicles numbers in present study are decreased as dependent of both dose and long-lasting exposure to PGB. These observations agree with the results of Sneha *et al.* [14], decreasing in numbers of antral and Graafian follicles in female rats after one month of PB treatment. The antral follicles in general are under the effect of FSH (this phase of growth the follicle is dependent on FSH level) [17], since levels of FSH are decreased in treated female rats as dependent of dosage and periods of administrations of PGB in previous study [8], so the results are consistent with the prevailing information that the existence of gonadotrophic hormones at appropriate levels especially FSH is supporting the growth and maturing of ovarian follicles [18].

In each estrous cycle, only a limited number of follicles mature and ovulate. The vast majorities of follicles stops developing after the formation of an antrum and then become atretic in a process characteristic of programmed cell death (Apoptosis) [19]. Few were selected to become ovulatory follicles are transformed into corpora lutea following ovulation [20]. While the numbers of preantral and antral follicles are decreased, the numbers of atretic follicles are increased in the present study. In spite of atretic follicles is a normal condition [21], apoptosis of follicles represents > 99.9% of all follicles over the life in females undergo degeneration and only 0.1% of the rest of follicles will survive and undergo ovulation after puberty [22], but in current study the atretic follicle numbers were increased and this increment is harmonized with the decrease of levels of gonadotrophic hormones FSH, and LH [8].

Because of physiological levels of FSH and LH are stimulated the follicles to enhance steroidogenesis and avoiding apoptosis in the follicles [23], and expression of enzyme caspase-3 in granulosa cells of atretic follicles, that is responsible for apoptosis is depended on and regulated by the levels of gonadotropins because it is absent in the healthy follicles [24]. From our previous study [8] it may expect that the decrease of levels of FSH and LH is the reason of increase of number of atretic follicles in the treated ovaries in this study. The observation about increase of numbers of atretic follicles after PGB treatment is agreement with another study about effects of Valproate and oxcarbazepine on folliculogenesis in female rats that there were decrease in the total number of follicles, in numbers of atretic follicles and impairment of folliculogenesis in ovary of rats [25]. Also this result is agreement with the results

of Sneha *et al.* [14] reported that there was increase of atretic follicles number after 1 month of treatment with PB.

From the results of present study, a decrease in the number of corpora lutea was dependent on PGB doses and period. The increase of the number of atretic follicles in this study may reflect that all or most of growing follicles undergo atresia or degeneration (apoptosis either in normal conditions or induced from direct effects of PGB on these follicles), this is logic in current result because when a large number of follicles undergo atresia, also may be from the decrease of the levels of FSH and LH [8] and this led to a little or no occurrence of ovulation and thus led to low number or lack of corpus luteum. The observation in current study agrees with the results of Sneha *et al.* (14) which showed decrease in number of corpora lutea after 1 month of treatment with PB.

The observation in the present study about the decrease of total number of follicles after 3 months treatment with PGB may be reflected the effect of PGB on GnRH / and or FSH and LH inadequate of their levels [8], which are responsible for stimulating and fasten the preantral follicles to transition to antral follicles; antral follicles which are responsive to the level of FSH [26], these two stages are affected from effect of PGB and number of follicles was decreased, then subsequently the total number of follicles is decreased because of inadequate growth support [17,27] or may be PGB affects on the growth of follicles in the ovary via Ca^{2+} channels as in its mechanism of its action, which Ca^{2+} ions are important as one of factors required in addition to FSH and growth factors {e.g. epidermal growth factor (EGF) and insulin-like growth factor1 (IGF-1)} for complete growth and differentiation of follicles [28]. From current study it can be concluded that pregabalin has effect dependent on quantity of dose and periods on ovary tissue by decreasing the number of preantral and antral follicles, also PGB affects on atretic follicles by increasing their number, and PGB affects by decrease the number of corpora lutea leading to decrease in the total number of follicles in the ovary of treated female rats.

Conflict of interest

The authors declare that they have no conflict of interests.

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