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Effects of Pregabalin Drug on Level of Reproductive Hormones, Sexual Activity, Number and Weight of Offspring in Female Albino Rats

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Abstract: ***This study was aimed to investigate the effect of two doses of pregabalin (PGB) on hormonal level and sexual activity in female albino rats. Ninety female rats with age (9-10 weeks) and weight (200±20 g) were divided into three major groups of thirty rats. First group was considered as control G1, the second G2 and third G3 groups were exposed to PGB into two doses 150, and 300 mg/kg body weight per day respectively. Each major group was divided into three subgroups (subgroup A, B, and C of each has ten rats), the treatments last for one month for subgroup A, two months for subgroup B, and three months for subgroup C. Five rats from each subgroup were placed separately into two breeding cages with two isolated males and waiting the pregnancy and then delivery. Blood samples were collected from other five rats in each subgroup by heart puncture technique for hormonal assessment, The results showed a significant (P≤0.05) decrease in the levels of luteinizing hormone (LH), follicular stimulating hormone (FSH), and prolactin hormone (PRL) in treated groups (G2 and G3), when compared to the control group . A significant (P≤0.05) increase in the levels of testosterone, estradiol, and progesterone was observed in the serum of treated groups (G2 and G3), when compared to control rats (G1).The results showed a significant (P≤0.05) delay in the delivery of the offspring in (G2 and G3) groups as compared with control (G1). There was a significant (P≤0.05) decrease in the number and weights of offspring in the treated rats (G2 and G3), as compared to the number and weights of offspring of the control groups (G1).***

Keywords: Pregabalin, female rat, sex hormones, Offspring Weight and Number

1. Introduction

Process of reproduction in female of mammals includes different stages like ovarian growth, follicular development, fertilization, implantation, gestation and lactation. Different phases of the reproductive cycle reflect the different stages of ovarian development [1]. Rodents are classified as incomplete cycles, as the corpus luteum of previous cycle remains during the next cycle [2]. Pregabalin (PGB) is a newer generation gabapentinoid or antiepileptic drugs (AEDs), which followed the use of gabapentin (GBP). Pregabalin was approved for neuropathic pain (NeP) management in 2004 within the USA and Europe [3]. Pregabalin, like gabapentin, however, receptor affinity of pregabalin is six times higher than gabapentin, pregabalin is a specific ligand of the α2δ type 1 and 2subunits of voltage-gated calcium channels, which attenuates depolarization-induced calcium influx at nerve terminals [4].

Pregabalin decreases calcium inward currents, and reduces glutamate, norepinephrine in the brain [5]. Reproductive endocrine disorders are more common among patients with epilepsy than in the general population; these endocrine disorders are not only attributed to the epilepsy itself, but also to the use of antiepileptic drugs [6]. Antiepileptic drugs may interfere with the hypothalamic pituitary axis; in females treated with carbamazepine is associated with decrease levels of (LH and FSH), and increase in level of prolactin in addition to lower levels of progesterone and estradiol, and no change with testosterone level, while in male treated patients, the levels of LH and FSH were increased and total testosterone level not altered [7].

In healthy animals studies; many of studies proved clearly that antiepileptic drugs affects on gonadal morphology, endocrinology and fertility. Animal studies support a drug-specific effect of VPA on ovarian morphology and endocrinology, alteration on number of corpora lutea and follicular cysts in healthy female rats [8], and alteration on endocrine function in both sexes [9]. Valproic acid (VPA) and oxcarbazepine also affects on uterus and ovary cells of rats, they trigger apoptotic and deterioration of folliculogenesis in rat ovarian follicles and degenerative effects on rat uterine and ovarian cells, prevents implantation of embryo to the uterus and causes abortion via endometrial eosinophilic infiltration [10].

Phenobarbitone (PB) has caused adverse effect on ovarian components, biochemical studies, estrous cycle and hormonal profile in health albino rats [11]. Svalheim *et al.* [12] confirmed that LEV has caused a drug-specific effect on reproductive endocrine function and ovarian morphology in female wistar rats at clinically relevant doses following long-term drug treatment. While Tauboll *et al.* [13] have shown for the first time that the effect of LEV on endocrine function in human ovarian cells differs from that of VPA at therapeutically relevant drug concentrations, in addition to alteration of endocrine function LEV act as apoptotic agents.

2. Materials and Methods

Ninety healthy, sexually matured, regularly cycling, colonies bred virgin female albino rats (aged 2.5- 3) months and weighing (200±20) gm. was used in this study. The rats were housed in special cages, under restricted conditions (temperature: 22-25 °C, photoperiod: 12 hours natural light

and 12 hours' natural dark). The rats were fed with normal rodents pellets and received tap water *ad libitum*. They were maintained as per the principles of laboratory animal's care [14].

The female rats were divided into three main groups (group-1, 2 and 3), with thirty rats in each group, then each main group was divided according to period of administration into A, B, and C, which represents 1, 2, and 3 months respectively: Group1 was given (0.5ml saline) and served as control. Group 2 was given pregabalin at dose 150 mg/ kg of body weight/day and group 3 was given pregabalin at dose 300mg/ kg of body weight/day dissolved in 0.5ml saline in each dose. The treatment was given for 30, 60, and 90 days once orally. Blood samples were collected after 24h from last treatment, by heart puncture for hormones analysis. The 2nd half of each group, female rats were placed separately into two breeding cages with 2 isolated males and waiting the pregnancy, then allowed for normal vaginal delivery and measuring Alived birth Index, the weight, and the number of alived and died offspring. Radio immuno assay was used for analysis of kits of all hormones (IRMA kits) (Beckman coulter/France).

3. Results

1. General behavior

It was observed that the groups of rats which treated with PGB particularly the higher dose and for long-term exposure were sedation (movements or running, climbing), and dizziness for 3-4 hours after administration of PGB in compared with control (normal) group.

2. Measurements of FSH, LH, and PRL hormones levels

Results in table (1) represent the effect of two doses of PGB after one, two, and three months treatment on serum FSH, LH, and PRL levels. A significant decrease ($P<0.05$) in the FSH level in (G2) and (G3) as compared to control group (G1) in all periods of treatment, the decrement in FSH was dose dependent and long lasting exposure to PGB. Also there was a significant decrease ($P<0.05$) in FSH level between treated groups (G2 and G3) in each period of treatment.

The level of LH, showed significant decrease ($P<0.05$) in treated groups (G2 and G3) when compared to G1 in all periods of treatment, the decrement in LH was dose dependent and long lasting exposure to PGB. Also there was a significant decrease ($P<0.05$) in LH level between treated groups (G2 and G3) in each period of treatment.

Finally PRL hormone showed a significant decrease ($P<0.05$) in its level in (G2) and (G3) as compared to control group (G1) in all periods of treatment, the decrement in PRL hormone was dose dependent and long lasting exposure to PGB. Also there was a significant decrease ($P<0.05$) in its level between treated groups (G2 and G3) in each period of treatment.

Table 1: Effect of two doses of PGB after 1, 2, and 3months treatment on the levels of FSH, LH, and PRL hormones (mean ± SE)

Treatment	FSH (IU/L)	LH (IU/L)	Prolactin (ng/ml)
Control for 1month (G1A)	0.632 ±0.01[a]	0.350 ±0.00[a]	0.490 ±0.004 [a]
150 mg/kg b.wt/day (G2A)	0.404 ± 0.002[b]	0.238 ± 0.005[b]	0.470 ± 0.008 [b]
300 mg/kg b.wt/day (G3A)	0.310 ± 0.005[c]	0.196 ± 0.002[c]	0.402 ± 0.002 [c]
LSD value	0.0195 *	0.0113 *	0.0235 *
Control for 2months (G1B)	0.626 ±0.009[a]	0.334 ±0.009[a]	0.506 ± 0.006 [a]
150 mg/kg b.wt/day (G2B)	0.304 ± 0.002[b]	0.156 ± 0.002[b]	0.436 ±0.004 [b]
300 mg/kg b.wt/day (G3B)	0.206 ±0.002[c]	0.124 ± 0.002[c]	0.424 ±0.010 [c]
LSD value	0.0185 *	0.0185 *	0.0224 *
Control for 3 months (G1C)	0.654 ±0.002[a]	0.324 ±0.011[a]	0.540 ±0.018 [a]
150 mg/kg b.wt/day (G2C)	0.204± 0.002[b]	0.104 ±0.002[b]	0.394 ±0.002 [b]
300 mg/kg b.wt/day (G3C)	0.108 ±0 .004[c]	0.100 ±0.00[b]	0.358 ±0.008 [b]
LSD value	0.0107 *	0.0196 *	0.0365 *
* ($P\leq0.05$)			

Each group n = 5 female rats, the different letters mean significant ($P<0.05$) between treated groups in same period

4. Measurements of Some Ovarian Hormones Levels

Table (2) represents the results in the levels of Testosterone, progesterone, and Estradiol (E_2) hormones in treated groups with their controls after treated with PGB. Table (2) showed that there was a significant ($P<0.05$) increase in levels of steroids hormones in each period as compared with their control, and the increment was dependent on dose and long lasting exposure to PGB. Testosterone levels exhibits that there was a manifold increase in a significant ($P\leq0.05$) difference in G2 and G3 as compared with G1 especially in the 3rd period of treatment. Progesterone level showed multiplier increase with a significant ($P\leq0.05$) difference in its levels with ascending concurrently of doses of PGB (G2) and (G3) as compared to the control (G1). Estradiol levels showed a multiplier increase in a significant ($P\leq0.05$) difference in treated groups (G2) and (G3) when compared with the control (G1).

Table 2: Effect of two doses of PGB after 1, 2, and 3months treatment on the levels of testosterone, progesterone, and estradiol hormones (mean ± SE)

Treatment	Testosterone (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control for 1month (G1A)	0.190 ± 0.004 [a]	28.00 ± 0.00 [a]	90.80 ± 1.80 [a]
150 mg/kg b.wt/day (G2A)	0.386 ± 0.009 [b]	45.40 ± 0.24 [b]	202.00 ± 3.74 [b]
300 mg/kg b.wt/day (G3A)	0.466 ± 0.002 [c]	72.00 ± 1.22 [c]	242.00 ± 3.39 [c]
LSD value	0.0188 *	2.222 *	9.537 *

Control for 2months (G1B)	0.196 ± 0.004 [a]	30.40 ± 0.24 [a]	90.20 ± 0.73 [a]
150mg/kg b.wt/day (G2B)	0.498 ± 0.002 [b]	65.40 ± 0.24 [b]	271.80 ± 1.31 [b]
300 g/kg b.wt/day (G3B)	0.568 ± 0.002 [c]	93.20 ± 0.91 [c]	327.00 ± 5.38 [c]
LSD value	0.0087 *	1.743 *	9.949 *
Control for 3 months (G1C)	0.194 ± 0.002 [a]	30.80 ± 0.49 [a]	99.60 ± 0.24 [a]
150 mg/kg b.wt/day (G2C)	0.550 ± 0.00 [b]	90.60 ± 0.60 [b]	374.80 ± 1.28 [b]
300 mg/kg b .wt/day (G3C)	0.664 ± 0.01 [c]	123.20± 0.91 [c]	420.20 ± 5.29 [c]
LSD value	0.0196 *	2.134 *	9.701 *
* (P≤0.05)			

►Each group n=5 female rats, the different letters mean significant (P<0.05) increase between treated groups in the same hormone (same column).

5. Sexual Activity of Treated Female Rats

The observations about sexual behavior in female rats of treated groups with PGB (G2 and G3) for three periods (1, 2, and 3 months), not receptive the males, and nervous behavior when trying males to approach the females for the purpose of mating when compared to the G1, which are normal in their behavior towards males, and receptive the males for mating. Table (3) showed that the mother rats treated with PGB (G2 and G3) after 1,2, and 3 months have developed their offspring after male presence, and there is a significant (P<0.05) increase in the number of days of delay in mating as compared to control group (G1). The results also showed a significant (P<0.05) increase in the number of days in developing their offspring between G3 and G2 in all periods of treatment.

Table 3: Effect of two doses of PGB after 1, 2, and 3 months treatment on developing female rats of their offspring after male presence in days (mean ± SE)

Treatment	Delay in mating and developing female rats of their offspring (in days) after		
	1month (A)	2 months (B)	3 months (C)
Control (G1)	23.00 ± 0.002 [a]	25.00 ± 0.00 [a]	24.60 ± 0.40 [a]
150mg/kgb.wt/day (G2)	26.00 ± 0.32 [b]	32.80 ± 0.20 [b]	39.80 ± 0.20 [b]
300mg/kgb.wt/day (G3)	30.00 ± 0.00 [c]	38.00 ± 0.31 [c]	44.40 ± 0.40 [c]
LSD value	0.562 *	0.665 *	1.067 *
* (P≤0.05)			

Each group n= 5 female rats, the different letters mean significant (P<0.05) difference between treated groups

6. Number of Offspring

Table (4) showed a significant (P < 0.05) decrease in the alived birth index for offspring after 1,2, and 3 months of treatment belong to mother rats treated in (G2) and (G3) in compared to mother rats in the control group (G1). Also there were significant (P < 0.05) differences between G3 and G2 in alived birth index. In the number of alived litters results showed a significant (P < 0.05) decrease was found from mother rats treated with PGB in (G2) and (G3) as compared to healthy mother rats in (G1). Significant (P < 0.05) differences were observed between G3 and G2 in the number of alived litters. Also there was a significant (P < 0.05) increase in the number of died neonates from treated mother rats in (G2) and (G3) as compared to the control (G1). Also there was significant (P < 0.05) increase in died neonates between G3 and G2.

Table 4: Effect of two doses of PGB after 1, 2, and 3 months of treatment on lived birth index, the number of alive and died Litters (mean ± SE)

Treatment	Alived Birth Index %	No. of alive litters	No. of died litters
Control (G1A)	100.00% [a]	9.40 ± 0.24 [a]	0.00 ± 0.00 [a]
150 mg/kg b. wt/day G2A)	44.00 % [b]	2.20 ± 0.20 [b]	2.80 ± 0.20 [b]
300 mg/kg b. wt/day(G3A)	43.48 % [b]	2.00 ± 0.00 [b]	2.60 ± 0.24 [b]
LSD value	12.539 *	0.562 *	0.562 *
Control (G1B)	100.00% [a]	8.40 ± 0.24 [a]	0.400 ± 0.20 [a]
150 mg/kg b.wt/day (G2B)	36.36% [b]	1.60 ± 0.24 [b]	2.80 ± 0.20b [b]
300 mg/kg b.wt/day (G3B)	25.00% [c]	1.00 ± 0.00 [b]	3.00 ± 0.00 [b]
LSD value	9.117 *	0.616 *	0.795 *
Control (G1C)	100.00% [a]	8.40 ± 0.24 a	0.00 ± 0.00 a
150 mg/kg b.wt/day (G2C)	27.78 % [b]	1.00 ± 0.00 [b]	2.60 ± 0.24 [b]
300 mg/kg b.wt/day (G3C)	0.00 % [c]	0.00 ± 0.00 [c]	3.60 ± 0.24 [c]
LSD value	12.829 *	0.435 *	0.616 *
* (P≤0.05)			

Each group n= 5 female rats, the different letters mean significant (P<0.05) difference between treated groups in same period

7. Weight of Offspring

In table (5), a significant (P<0.05) decrease in the weight of offspring from mothers treated with two doses of PGB after 1, 2, and 3months in (G2) and (G3) in compared with the offspring from healthy mothers (G1). Also the results showed there were no significant (P>0.05) differences between (G3) and (G2) in the weights of offspring after 1, and 2months of treatment, while there was a significant (P<0.05) difference in the weight of offspring between (G3) and (G2) after 3 months of treatment.

Table 5: Effect of two doses of PGB on the weight (g) of offspring after1, 2, and 3 months treatment of their mothers (mean ± SE)

Treatment	Weight of offspring (g) after		
	1 month A	2 months B	3 months C
Control (G1)	6.11 ± 0.02 [a]	6.14 ± 0.01 [a]	6.11 ± 0.01 [a]
150 mg/kg b.wt/day (G2)	5.78 ± 0.03 [b]	5.52 ± 0.01[b]	5.06 ± 0.01[b]
300 mg/kg b.wt/day (G3)	5.47 ± 0.01[b]	5.06 ± 0.01[b]	3.85 ± 0.05 [c]
LSD value	0.067 *	0.037 *	0.071 *
* (P<0.05)			

In each group n represents all numbers of offspring for the same group, the different letters mean significant ($P<0.05$) difference between treated groups in the same column (same period).

8. Discussion

The results of the study showed all treated female rats with PGB caused sedation for 3 to 4 hours. The extent of sedation was dose dependent. After period of sedation, the animals showed normal activities. The reason may be attributed to the fact that one of very common side-effects of this drug is dizziness, sedation, and slow of movement [15].

The results of this study showed clearly that the pituitary gonadotropins: FSH, LH, and PRL hormone levels in the serum were decreased with two doses of PGB, these observations are disagreement with other studies about effects of other antiepileptic drugs on the LH and FSH in female rats, and the reason as they reported is ADEs may be exert their effect on the gonadal level [16,17], Pregabalin as an antiepileptic drug has known to inhibit the CNS activity , which regulates physiological and behavioral events associated with normal reproductive function in both sexes via hormonal signals [18].

Harden and Pennell [19] pointed out that antiepileptic drugs may interact with gonads. Since PGB drug block voltage dependent calcium channels , one may expect that similar neurochemical mechanisms are engaged in the interaction of these drugs with synthesis of hypothalamic neurohormones such as gonadotropin-releasing hormone (GnRH) , so it may expect that PGB may be indirectly affects on hypothalamus because GnRH secretion from neurons is dependent on the depolarization - induced entry of extracellular calcium from their spontaneous firing of calcium-dependent action potentials , and because PGB exerts its effects on Ca^{2+} channels by blocking or restricting Ca^{2+} influx on hypothalamus which is sensitive to altering the frequency of the GnRH pulses and leads to decrease in LH and FSH levels in the present study[20].

From the present results there were increased in the levels of progesterone, testosterone, and estradiol treated female in two doses of pregabalin. These results are agreement with other antiepileptic drugs that alters the levels of steroid hormones in healthy female rats [16, 17, 9, and 21]. In all of these studies there are no clear explanations for the effect of these antiepileptic drugs and the reason is the lack of full understanding of the mechanical work of each one of them, including the drug used in the current study. The observations about changes in the levels of steroids due to effect of antiepileptic drugs (PGB in this study) are agreement with observations in other studies about the effect of another antiepileptic drugs such as VAP in women complaining from epilepsy [22] and other antiepileptic drugs also alters the levels of steroid hormones in healthy female rats [12,11]. In all of these studies there are no clear explanations for the effect of these antiepileptic drugs and the reason is the lack of full understanding of the mechanical work of each one of them, including the drug used in the current study.

In general FSH and LH were responsible for inducing granulosa and theca cells for synthesis steroids hormones in the ovary [23] and in the current study the levels of gonadotropic decreased by pregabalin effects on pituitary gland or on hypothalamus and may be compensate by local secretion of LH and FSH from ovary as pointed [24] that rat ovaries could produce gonadotropic hormones FSH and LH locally in intact follicles, theca cells, corpora lutea, and ovarian content of LH remained unchanged following hypophysectomy. Pregabalin may be affect at subcellular level by induced theca and granulosa cells in the follicles of ovary to synthesize much amount of Testosterone, Progesterone, and estradiol as VAP effects on human theca cells *In Vitro* [25]. Pregabalin maybe have an inhibitory effect on the hepatic P450 enzyme system and, thereby, it may inhibit the metabolism of sex steroids, hence provoking increased steroids levels [26], or may be PGB has direct effects on the ovary and modulates pathway by increasing synthesis of steroids in rat ovary [19].These reasons may explain the increment of levels of steroids hormones in the present study.

Sexual behavior in general reflects complex neural, endocrine, and reproductive organ interactions and is therefore is susceptible to disruption by a variety of toxic drugs, diseases, and pathologic conditions [27,28] and in current results in treated female rats with two doses of PGB (in all periods of treatment) was shown spooky mood behavior towards males, the reason may be attributed to the effects of PGB directly on the developing follicles in the ovary of female rats, which are responsible of estrogen biosynthesis and raised of estradiol level, estradiol is known has proconvulsion effect via increase neuronal excitability and thus mediate proconvulsant effects on female and its either effect directly on the higher centers responsible of behavior in the brain [29,30,31] or PGB may be affects on CNS directly on hypothalamus, the important center for regulation of (Hypothalamus-pituitary-ovary) HPO axis and results on changes in the secretion of estradiol and initiate disruptions in ability of the animal to produce the LH surge required for regulation of the rest of the HPO axis, and dysregulation of any part of this axis will result in a loss of normal reproductive behavior in the animal [28].

Treated females with low dose and with high dose of PGB after 1, 2, and 3 months showed delayed in mating with males and becoming pregnant. The reason for the delayed of pregnancy in PGB treated females is may be due to changes on the hormone level such as decrease of LH, FSH levels and increase of progesterone and estradiol levels, which is mean there were disturbances in estrous cycles be raised from the effects of pregabalin on the treated female rats in the present study, similar observations were reported with PB, which is affects on the of estrous cycles by decreasing their numbers and longer lasting in number of days in treated female rats [17].

The results showed there was a significant decrease on the number of litters synchronized with decrease in the number of live litters accompanied with increase of died litters, and is dependent on both the increase dose of PGB and long-term exposure. This may indicate some possibilities that PGB may affects on the mothers, either failure in

fertilization, reduction in the number of embryos which may even are unable to implant properly, or after implantation there was may be resorption of the embryos or they passed as unnoticed abortion [32]. This observation is agreement with the results of Al-Zubaidi [33], whom reported that there was a reduction in the number of litters from males were treated with PGB for 35 days and mating with healthy mothers. It is known that FSH and LH have their presence in adequate levels may accelerate the growth and maturation of follicles [34].

In the present study FSH and LH levels are low after treatment with PGB and follicles growth is still and slow and may be lead to decrease in the total number of litters. Also the current results showed there was a significant decrease in the weights and the numbers of litters from mothers treated with PGB, this observation is agreement with Al-Zubaidi [33] who showed a reduction in the weight of litters from healthy mothers mated with PGB treated male rats for 35days. Bi *et al*. [35] were reported that when porcine FSH over expression within physiological boundaries in transgenic mouse, it can increase ovulation rate and litter size without causing any reproductive defects *in vivo*. This means that low level FSH in the present study due to PGB treatment may affects on the weights of litters and their sizes.

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