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ENDOCRINE REPRODUCTIVE EFFECTS OF PREGABALIN DRUG IN FEMALE ALBINO RATS

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ABSTRACT

The aim of present study was to investigate the effect of Pregabalin on hormonal profile of pituitary and ovarian hormones in female albino rats. Three groups of healthy adult female albino rats having fifteen rats in each group were selected for present study. The rats of groups G2 and G3 were administered with Pregabalin with two doses 150 mg and 300mg/kg b.wt/day respectively by orally route daily for 1month, 2 months, and 3 months. Animals of group G1 (Control) were given saline alone. After the experimental periods, the rats were sacrificed and the study of Hormonal profile was carried out by collecting blood samples from the heart puncture method, centrifuged sera and analyzing concentrations of estrogen, progesterone and gonadotropins for hormonal profile via RIA method. The results were shown significant decrease in the body weight among various groups. Also ovarian weight and ovarian diameter in the experimental groups G2 and G3 were decreased significantly ($p \leq 0.05$). Significant decrease in the levels of FSH, LH and PRL hormones were observed in the low dose and high dose. In contrast to the levels of progesterone, estradiol, and testosterone hormones were showed a significant increase in all experimental groups compared with the control group. Final remarks of our investigation are that, the oral administration 150 mg and 300mg/kg b.wt/day of Pregabalin can produce adverse effects on hormonal profile.

KEYWORDS: Pregabalin, LH, FSH, PRL, Progesterone, Estrogen, Testosterone, Ovary.

INTRODUCTION

Pregabalin (PGB) has demonstrated analgesic, and anxiolytic properties in preclinical models.[1] It is the latest compound that joins the list of approved "new" antiepileptic drug.[2]

In addition to epilepsy, it has demonstrated efficacy for the treatment of neuropathic pain[3] and generalized anxiety disorder.[4] Pregabalin (Lyrica, Pfizer) is the pharmacologically active S-enantiomer of racemic 3-isobutyl GABA.[5]

Reproduction is a wonderful phenomenon and very important for all living organisms in order to the continuity of their species. It is controlled by the function of hypothalamo-hypophyseal gonadal axis.[6] The hypothalamus and pituitary forms a functional unit that secretes the gonadotrophins in pulses. The hypothalamus regulates the rhythmic release of gonadotrophins i.e., FSH and LH through neural stimulus to gonadotrophins releasing hormone-GnRH.[7] Antiepileptic drugs known to interfere with the functions of central nervous system including hypothalamus there by modify the activity of hypophysis and gonads.[8] The secretion and release of pituitary follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL) are directly dependent on hypothalamic releasing hormone – GnRH.[9] As a result, the function of the gonads may also be modified. Therefore, there is probably that CNS influencing drugs can modify the reproductive and endocrine activities.[10] Therefore, in the present work, effects of pregabalin drug on female endocrine activities were undertaken and we hereby report an investigation of pregabalin-induced changes on ovarian and hormonal profile of healthy albino 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 of the High Institute of fertility Diagnosis and Assistant Reproductive Technology/Al-Nahrin University. 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 and tap water *ad libitum*. They were maintained as per the principles of laboratory animal's care.[11] The animals were divided into three main groups (group-1, 2 and 3), each consisting of fifteen rats in each group and treated , then each group was divided into A, B, and C, which represents the periods as 1, 2, and 3 months of administration respectively) as follows: The group1 was given (0.5ml saline) and served as control. Group 2 and 3 were given pregabalin at dose 150 and 300mg/ kg of body weight/day dissolved in 0.5ml saline in each dose. The treatment was given for 1, 2, and 3 months once orally between 9:00-10:00AM.

After 24h from last treatment, all the animals from each group were weighed and anesthetized with ether, blood samples were collected by cardiac puncture, from control and pregabalin treated rats. Blood samples were centrifuged at the rate of 2800 RPM for 10 min for hormone assay. The pituitary gonadotrophins and ovarian steroid hormones were assayed by RIA, serum FSH/ LH were assayed using the double antibody RIA techniques of Peterson and Swerdloff,[12] based on the principle of competitive binding. The estradiol was assayed by procedure as described by Xing et al.,.[13] Progesterone was assayed by solid phase RIA as described by Kubasik et al.,[14] After blood samples were collected, animals were sacrificed, the ovary were dissected out, freed from extra depositions of adherent tissue and weighed to the nearest mg on an electronic balance. 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 rat body weight

Body weight of rats at low (150 mg/Kg b.wt./day) and high (300mg/Kg b.wt./day) dose level of pregabalin treated rats after 1month (Table 1), 2months (Table 2), and 3months (Table3) were reduced respectively, when compared to control group at each same period of treatment. The reduction in the body weight was significant ($P<0.05$) in all the periods of treatment. The reduction in the body weight was increased with the high dose of PGB and with long lasting of exposure to PGB, as shown in tables 1, 2, and 3.

Table (1): Effect of two doses of PGB after 1 month treatment on the rat body weight (gm)

Treatment	Mean ± SE	
	Initial weight (gm)	Weight after 1 month (gm)
Control (G1A)	198.30 ± 2.72	212.60 ± 2.03
150 mg/kg b.wt/day (G2A)	200.10 ± 2.53	193.70 ± 2.01 *
300 mg/kg b.wt/day (G3A)	203.10 ± 1.51	190.90 ± 1.73 *
LSD value	---	5.599 *
* ($P<0.05$)		

►Each group n=5 female rats, ***** = $P<0.05$ in compared with control group

Table (2): Effect of two doses of PGB after 2 months treatment on the rat body weight (gm)

	Mean ± SE

Treatment	Initial weight (gm)	Weight after 1 month (gm)	Weight after 2 months (gm)
Control (G1B)	200.80 ±2.38	207.80 ±1.63	222.80 ± 1.45
150mg/kg b.wt/day (G2B)	202.20 ± 1.37	191.70 ± 1.54*	185.50 ± 1.36*
300mg/kg b.wt/day (G3B)	203.00 ± 1.48	196.00 ± 1.25*	183.20 ± 1.45*
LSD value	-----	4.317 *	4.132 *
* (P<0.05)			

►Each group n=5 female rats, *= P<0.05 in compared with control group

Table (3): Effect of two doses of PGB after 3 months treatment on rat body weight (gm)

Treatment	Mean ± SE			
	Initial weight (gm)	Weight after 1 month (gm)	Weight after 2 months (gm)	Weight after 3 months (gm)
Control (G1C)	201.10 ± 2.85	206.70 ± 2.32	218.00 ± 2.35	243.30 ± 2.64 *
150mg/kg b.wt/day (G2C)	204.60 ± 1.86	194.30 ± 1.80 *	186.90 ± 0.99 *	177.40 ± 1.03 *
300mg/kg b.wt/day (G3C)	206.50 ± 2.28	198.10 ± 1.31 *	185.80 ± 1.74 *	173.40 ± 1.00 *
LSD value	--	5.394 *	5.180 *	5.047 *
* (P<0.05)				

►Each group n=5 female rats, ***=** P<0.05 in compared with control group

Changes in the ovarian weight of rats

As shown in Table 4, PGB induced significant (P<0.05) decline in the ovarian weights, this decrease was with low and high dose of PGB administration and dependent with long lasting exposure when compared to control group.

Table (4): Effect of two doses of PGB on rat ovarian weight (mg) after 1, 2, and 3 months treatment

	Mean ± SE		
Treatment	Wt. after 1 month(mg) (A)	Wt. after 2 months(mg) (B)	Wt. after 3 months(mg) (C)
Control (G1)	65.76 ± 0.36	74.78 ±0.30	85.90 ±0.26
150mg/kg b.wt/day (G2)	62.38 ±0.34 *†	61.64 ±0.51 *†	60.04 ±0.15 *†
300mg/kg b.wt/day (G3)	58.22 ±0.26 *†	52.82 ±0.54 *†	43.14 ±0.85 *†
LSD value	1.005 *	1.431 *	1.617 *
* (P<0.05)			

►Each of group n=5 female rats, ***=** P<0.05 in compared with control group,

† =P<0.05 between (G2)and(G3) in same period (same column).

Changes in diameter of ovaries

Ovarian diameter was reduced in the experimental groups. This reduction in the diameter of ovary was significant (P<0.05) with low and high dose of PGB, and dependent to the dose and long lasting of exposure to the drug (Table 5).

Table (5): Effect of two doses of PGB after 1, 2, and 3 months treatment on diameter of rat ovary (µm)

Treatment	Mean ± SE		
	Diameter after1month treatment (µm)(A)	Diameter after2months treatment(µm)(B)	Diameter after 3months treatment (µm)(C)
Control (G1)	5942.40 ± 314.94	6284.0 ±132.12	6724.60 ±214.19
150mg/kg b.wt/day (G2)	4790.80 ± 152.41 *	4496.00 ±92.82 *	4185.0 ±101.29 *
300mg/kg b.wt/day (G3)	4153.40 ± 149.84 *	4140.20 ±147 *	3918.00 ±61.18 *
LSD value	677.12 *	389.62 *	435.34 *
* (P<0.05)			

►Each of group n=5 female rats, ***=** P<0.05 in compared with control group

Effect of two doses of PGB on pituitary gonadotropins (FSH, LH, and PRL hormone)

The pituitary gonadotropins, FSH, LH, and PRL in the blood were reduced with both the low and high doses of PGB. This reduction in the levels of hormones was significant (P<0.05) with the two doses and dependent on long lasting of exposure to the PGB drug, as shown in Tables 6, 7, and 8.

Table (6): Ef11fect of two doses of PGB after 1 month treatment on the levels of LH, FSH and PRL hormone

Treatment	Mean ± SE		
	FSH (IU/L)	LH (IU/L)	Prolactin (ng/ml)
Control (G1A)	0.632 ±0.01	0.350 ±0.00	0.490 ± 0.004
150 mg/kg b.wt/day (G2A)	0.404 ± 0.002*†	0.238 ± 0.005*†	0.470 ± 0.008*†
300 mg/kg b.wt/day (G3A)	0.310 ± 0.005*†	0.196 ± 0.002*†	0.402 ± 0.002*†
LSD value	0.0195 *	0.0113 *	0.0235 *
* (P≤0.05)			

►Each group n = 5 female rats, *= P≤0.05 in compared with control group, †= P≤0.05 between (G3A) and (G2A)in the same hormone (same column).

Table (7): Effect of two doses of PGB after 2 months treatment on the levels of LH, FSH and PRL hormone

Treatment	Mean ± SE		
	FSH (IU/L)	LH (IU/L)	Prolactin (ng/ml)
Control (G1B)	0.626 ±0.009	0.334 ±0.009	0.506 ± 0.006
150 mg/kg b.wt/day (G2B)	0.304 ± 0.002*†	0.156 ± 0.002*†	0.436 ±0.004*
300 mg/kg b.wt/day (G3B)	0.206 ± 0.002*†	0.124 ± 0.002*†	0.424 ±0.010*
LSD value	0.0185 *	0.0185 *	0.0224 *
* (P≤0.05)			

► Each group n = 5 female rats, * = P≤0.05 in compared with control group, †= P≤0.05 between (G3B) and (G2B) in the same hormone (same column).

Table (8): Effect of two doses of PGB after 3 months treatment on the levels of LH, FSH and PRL hormone

Treatment	Mean ± SE		
	FSH (IU/L)	LH (IU/L)	Prolactin (ng/ml)
Control (G1C)	0.654 ± 0.002	0.324 ±0.011	0.540 ±0.018
150 mg/kg b.wt/day (G2C)	0.204±0.002*†	0.104 ±0.002*	0.394 ±0.002*
300 mg/kg b.wt/day (G3C)	0.108 ±0 .004*†	0.100 ±0.00*	0.358 ±0.008*
LSD value	0.0107 *	0.0196 *	0.0365 *
* (P≤0.05)			

►Each group n = 5 female rats, *= P≤0.05 in compared with control group, †= P≤0.05 between (G3C) and (G2C) in the FSH hormone (same column).

Effect of two doses of PGB on the levels of ovarian hormones (Testosterone, Progesterone, and Estradiol (E_2) hormones

Table 9, Table 10, and Table 11 shown the effect of low and high dose of PGB for 30, 60, and 90 days respectively, and PGB induced significant (P≤0.05) increase on the levels of steroids hormones. This increasing in the levels of all steroid hormones was dose and periods dependent to PGB drug.

Table (9): Effect of two doses of PGB after 1 month treatment on the levels of Testosterone, Progesterone, and E_2 hormones

Treatment after 1 month	Mean ± SE		
	Testosterone (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control (G1A)	0.190 ± 0.004	28.00 ± 0.00	90.80 ± 1.80
150 mg/kg b.wt/day (G2A)	0.386 ± 0.009*†	45.40 ± 0.24*†	202.00 ± 3.74*†
300 mg/kg b.wt/day (G3A)	0.466 ± 0.002*†	72.00 ± 1.22*†	242.00 ± 3.39*†
LSD value	0.0188 *	2.222 *	9.537 *
* (P≤0.05)			

►Each group n=5 female rats, *= P≤0.05 in compared with control group, †= P≤0.05 between (G3A) and (G2A) in the same hormone (same column).

Table (10): Effect of two doses of PGB after 2 months treatment on the levels of Testosterone, Progesterone, and E_2 hormones

Treatment	Mean ± SE		
	Testosterone (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control (G1B)	0.196 ± 0.004	30.40 ± 0.24	90.20 ± 0.73
150mg/kg b.wt/day (G2B)	0.498 ± 0.002*†	65.40 ± 0.24*†	271.80 ± 1.31*†
300 g/kg b.wt/day (G3B)	0.568 ± 0.002*†	93.20 ± 0.91*†	327.00 ± 5.38*†
LSD value	0.0087 *	1.743 *	9.949 *
* (P≤0.05)			

►Each group n=5 female rats, *= P≤0.05 in compared with control group, †= P≤0.05 between (G3B) and (G2B) in the same hormone (same column).

Table (11): Effect of two doses of PGB after 3 months treatment on the levels of Testosterone, Progesterone, and E_2 hormones

Treatment	Mean ± SE		
	Testosterone (ng/ml)	Progesterone (ng/ml)	Estradiol (pg/ml)
Control (G1C)	0.194 ± 0.002	30.80 ± 0.49	99.60 ± 0.24
150 mg/kg b.wt/day (G2C)	0.550 ± 0.00*†	90.60 ± 0.60*†	374.80 ± 1.28*†
300 mg/kg b .wt/day (G3C)	0.664 ± 0.01*†	123.20± 0.91*†	420.20 ± 5.29*†
LSD value	0.0196 *	2.134 *	9.701 *
* (P≤0.05)			

►Each group n=5 female rats, *= P≤0.05 in compared with control group, †= P≤0.05 between (G3C) and (G2C) in the same hormone (same column).

DISCUSSION

The effects of antiepileptic drugs on the reproductive function could be attributed to their central effects on hypothalamic-pituitary-gonad axis.[8] Most of antiepileptic drugs acting as GABA-agonists. The GABA–ergic system is the predominant inhibitory system in the mammalian central nervous system. GABA nerve fibers showed high density in the hypothalamus, median eminence, posterior and intermediate lobes of pituitary.[15] The loss of the body weight in the rats of treated groups may be attributed to the fact that one of uncommon side-effects of this drug is to loss of appetite.[16] The results of this study is disagreement with results of Al-Zubaidi.[17] which reported in his results that the male rats treated with PGB have gained higher weight with higher dose of PGB (600mg/kg B.wt /day) after 35 days of treatment.

The decline in the weight of ovary in the treated groups is gradually with increase dose of PGB, and with increase of periods of exposure of PGB. The results of this study is agreement with results of Sneha *et al*.[18], whom reported in their study that another antiepileptic drug (phenobarbitone) in two doses affects on the weight of ovary of rat (decrease in the weight) after 30 days and the effects are dependent on the doses. Our observation in the present study is agreement with the results of Røste *et al*.[19] in the effect of Valproate on female rats after orally administration for 90-95 days, they reported the weight of ovaries were decreased about 22% from initial their weight and this reduction may imply the beginning of ovarian atrophy.

The decrease of ovarian diameter in treated groups with PGB is gradually with the doses and long lasting exposure to PGB drug, this result is may be similar with a results of a study of Daoud *et al*.[20] effects of some antiepileptic drugs (Gabapentin, Vigabatrin, and Lamotrigine) on male rat reproductive system, and reported that all of these drugs are decreased the diameters both of seminiferous tubules and nuclear of Leydig cells after 60 days of treatments. Bilginer *et al*.[21] were reported that PGB affects on male rats reproductive system by decreasing the diameter of seminiferous tubules after 60 days of treatment with PGB (40mg/kg).

The decline of the levels of FSH, LH, and PRL (pituitary hormones) may be from direct effect of PGB drug on the treated female groups on pituitary gland, or may be the effect is indirect on hypothalamus because GnRH secretion from neurons is dependent on the depolarization-induced entry of extracellular calcium that results from their spontaneous firing of calcium-dependent action potentials[22] and we know that PGB its effects on Ca^{2+}

channels.[23,24] may be by blocking or restricting Ca^{2+} influx on hypothalamus which are sensitive to altering the frequency of the GnRH pulses which leads to decrease of FSH and LH levels in our study.

The levels of ovarian steroids (Progesterone, Estradiol, and Testosterone) increased in the treated groups with PGB with two doses and we expect that PGB affects on either directly from raising the levels of estradiol and progesterone through negative feedback loop inhibits the FSH and LH releasing from pituitary gland , or the low levels of FSH and LH may be due from effect of high level of estrogen, which exerts a negative feedback loop inhibits directly secretion of FSH from pituitary gland (25; 26) or the high level of progesterone may be exerts its major effect at the hypothalamic level and decreases GnRH pulse frequency by inducing the release of beta-endorphin (27).

Our observations about changes on the levels of steroids from effect of antiepileptic drug (PGB in our study) are agreement with the results of other studies with another antiepileptic drugs and all of them alters the steroids hormones (28; 29; 30; 31; 32; 33).

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