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Article

Reproductive and Hormonal Alterations Induced by Single and Combined Doses of Pregabalin and Baclofen Male Albino Rats: A Mechanistic Evaluation

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Abstract: Background and Objective: The prescription of pregabalin and baclofen is prevalent as drugs that act on the central nervous system. Excessive usage or off-label usage of these drugs is of great concern because of their potential effects on reproductive toxicity. This study was conducted to assess and compare the reproductive effects, hormonal effects, oxidative stress, and inflammatory response of pregabalin and baclofen in both single and combination treatments in male albino rats. **Methods:** Rats were subjected to different protocols involving the use of pregabalin and baclofen singly and in combination in standard and double dosages. Measurements included sex hormone profile (testosterone, SSH [FSH], and ICSH [LH]) levels, antioxidant enzymes (superoxide dismutase [SOD], reduced glutathione [GSH], and catalase [CAT]), lipid peroxidation end-products (MDA and IsoPs), and inflammatory markers (TNF-α and NF-κB). **Findings:** The results revealed a dose-dependent disturbance in the HPG axis, which was reflected in the reduction in the concentrations of serum testosterone and gonadotropins. In addition, the medications caused acute oxidative stress, as reflected in the increase in the levels of MDA and IsoPs, together with the reduction in antioxidant capacity (SOD, GSH, and CAT). On the other hand, a significant increase in the levels of pro-inflammatory mediators (TNF-α and NF-κB) was observed, specifically in the groups exposed to double dose and combination therapy. **Conclusions**: Administration of high-dose pregabalin and baclofen, either alone or in combination, causes significant gonadotoxicity and endocrine disturbances in male rats. The mechanism of toxicity appears to be mainly through oxidative stress, inflammation, and HPG axis central suppression.

Keywords: Pregabalin, Baclofen, Reproductive toxicity, Oxidative stress, Inflammatory pathways, Hormonal alterations.

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1. Introduction

Neurological and psychiatric diseases are health problems of great importance worldwide that require the application of CNS drugs with broad-spectrum effects in their treatment. Prominent among these drugs are pregabalin, which is extensively prescribed for epilepsy, neuropathic pain, and generalized anxiety disorders, and baclofen, a selective gamma-aminobutyric acid type B (GABA-B) receptor agonist primarily utilized to alleviate spinal-origin spasticity [1- 2]. In recent years, however, rates of off-label misuse and high-dose administration of these medications have escalated significantly[3].

Despite the substantial clinical utility of these pharmacological agents, emerging preclinical and clinical research has begun to highlight their undesirable adverse effects,

particularly their adverse toxic impacts on the male reproductive system and endocrine function[4-5] . The delicate balance of spermatogenesis and steroidogenesis is tightly orchestrated by the hypothalamic-pituitary-gonadal (HPG) axis, which is highly susceptible to alterations in central neurotransmitter dynamics [6].

Pregabalin acts by binding to voltage-gated calcium channels, thereby reducing neurotransmitter release; this pharmacological action can suppress testosterone synthesis and disrupt pituitary gonadotropin secretion [7] . On the other hand, chronic or high-dose administration of baclofen interferes with central and peripheral neuro-signaling cascades, provoking severe oxidative stress, triggering pro-inflammatory pathways, and inducing cellular apoptosis within testicular tissues [8].

Given that clinical scenarios often involve concurrent co-administration or excessive dosing of these agents, a rigorous mechanistic understanding of their single and combined physiological, endocrine, and testicular impacts is imperatively needed. Therefore, the current study was conducted to evaluate the reproductive and hormonal alterations induced by single and combined doses of pregabalin and baclofen in male albino rats, while providing a detailed mechanistic insight into the underlying oxidative stress and inflammatory signaling pathways.

2. Materials and Methods

2.1. Experimental Animals and Treatment Protocols

This experiment was carried out on 35 adult male albino rats weighing 180-200 grams. The animals were kept in standard plastic cages (5 animals per cage) and maintained under controlled environment (temperature 23 ± 1 °C and 12 hours light/dark cycle). Laboratory animal diet and water were supplied ad libitum during the entire duration of the experiment [9].

The experimental rats were divided into 7 groups (n=5 per group) and subjected to oral administration of the drug through gavage for 60 days as follows:

- **Group 1 (Control):** Received standard chow, drinking water, and oral administration of distilled water.
- **Group 2 (Pregabalin Standard Dose):** Received pregabalin at a standard therapeutic dose of 150 mg/kg body weight.
- **Group 3 (Pregabalin Double Dose):** Received pregabalin at a double dose of 300 mg/kg body weight.
- **Group 4 (Baclofen Standard Dose):** Received baclofen at a standard therapeutic dose of 14.5 mg/kg body weight.
- **Group 5 (Baclofen Double Dose):** Received baclofen at a double dose of 29 mg/kg body weight.
- **Group 6 (Pregabalin + Baclofen Standard Doses):** Received a combination of pregabalin (150 mg/kg) and baclofen (14.5 mg/kg).
- **Group 7 (Pregabalin + Baclofen Double Doses):** Received a combination of pregabalin (300 mg/kg) and baclofen (29 mg/kg).

2.2. Sample Collection and Biochemical Assays

On completion of the 60 days of administration, overnight fasting was done before anesthesia and then collection of blood through cardiac puncture in test tubes without any anticoagulants. The serum was obtained by centrifuging the blood at 3,000 rpm for 15 minutes, then stored in -20 °C until further analysis.

Levels of serum sex hormones such as testosterone, follicle-stimulating hormone (SSH/FSH), and interstitial cell-stimulating hormone (ICSH/LH) were analyzed using enzyme-linked immunosorbent assay (ELISA) kits. Levels of enzymatic and non-enzymatic antioxidants such as superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) along with lipid peroxidation such as malondialdehyde (MDA) and isoprostanes (IsoPs) were determined. Furthermore, levels of inflammatory mediators like TNF-α and NF-κB were also detected.

2.3. Statistical Analysis

Data analysis was carried out using the SPSS statistical package (Version 16.0). One-way Analysis of Variance (ANOVA) was used to compare the results obtained from various experimental groups, with the results expressed as Mean ± SD. Post-hoc analysis was conducted to compare means between groups using Duncan's multiple range test at $p \leq 0.05$.

3. Results

3.1. Sex Hormone Profile

The effects of single and combined doses of pregabalin and baclofen on serum sex hormones in male albino rats are detailed in Table 1. Administration of both drugs, either individually or in combination, resulted in a statistically significant decrease ($p \leq 0.05$) in serum testosterone, SSH (FSH), and ICSH (LH) concentrations compared to the control group. The most profound suppression of the pituitary-gonadal axis was observed in the combination high-dose group (Pregabalin + Baclofen Double Dose), which recorded the lowest overall levels across all three parameters.

Table 1. Effect of pregabalin and baclofen administration (standard and double doses) on serum sex hormone concentrations in male albino rats.

Group	Testosterone (ng/mL)	SSH / FSH (mIU/mL)	ICSH / LH (mIU/mL)
Control	13.555 ± 1.171 a	56.720 ± 2.402 b	44.601 ± 1.207 a
Pregabalin (Standard)	5.105 ± 1.099 e	32.022 ± 1.250 e	23.312 ± 1.406 c
Pregabalin (Double)	7.806 ± 1.323 c	41.790 ± 2.371 c	20.961 ± 0.849 d
Baclofen (Standard)	5.178 ± 0.149 e	59.515 ± 1.556 a	36.203 ± 0.809 b
Baclofen (Double)	9.374 ± 1.724 b	33.363 ± 1.114 e	21.971 ± 1.631 d
Pregabalin + Baclofen (Standard)	6.266 ± 0.470 d	35.279 ± 1.232 d	23.919 ± 1.077 c
Pregabalin + Baclofen (Double)	5.377 ± 0.819 e	26.581 ± 1.217 f	14.560 ± 2.531 e

The values have been presented as Mean ± SD. The means with different superscripts in the same column are significantly different at $p \leq 0.05$ by Duncan's multiple range test.

3.2. Oxidative Stress Markers

Table 2 presents the changes in serum lipid peroxidation products (MDA and IsoPs). Single standard doses caused modest increases in oxidative damage, whereas double doses of individual treatments produced substantial increases in both MDA and IsoPs. The combination of pregabalin and baclofen caused a dramatic, synergistic rise in serum IsoPs and MDA levels, reaching peak values in both the standard and double combination groups compared to control animals ($p \leq 0.05$).

Table 2. Effect of pregabalin and baclofen administration (standard and double doses) on serum oxidative stress markers (MDA and IsoPs) in male albino rats.

Group	MDA (µM/L)	IsoPs (pg/mL)
Control	174.9 ± 18.25 e	72.53 ± 3.93 d
Pregabalin (Standard)	284.4 ± 28.00 c	89.81 ± 2.79 c
Pregabalin (Double)	344.1 ± 29.51 b	104.15 ± 11.98 b
Baclofen (Standard)	185.5 ± 13.61 f	76.04 ± 3.08 cd
Baclofen (Double)	239.7 ± 16.33 d	85.12 ± 3.10 d
Pregabalin + Baclofen (Standard)	466.4 ± 30.40 a	242.90 ± 21.72 a
Pregabalin + Baclofen (Double)	454.9 ± 27.12 a	255.92 ± 26.41 a

Values are presented as mean ± SD. Different superscript letters in the same column indicate significant differences between the means ($p \leq 0.05$), based on Duncan's multiple range test.

3.3. Antioxidant Defense System

The results presented in Table 3 revealed that the pharmacologic intervention greatly inhibited the antioxidant activity in male rats. The levels of SOD, GSH, and CAT in the serum were considerably reduced ($p \leq 0.05$) in all groups compared to the control group. The inhibition was concentration dependent and achieved maximum suppression in the double combination group (Pregabalin + Baclofen Double Dose).

Table 3. Effects of Pregabalin and Baclofen treatment (normal and double dose) on serum antioxidant levels (SOD, GSH, and CAT) in male albino rats.

Group	SOD (U/mL)	GSH (μM)	CAT (U/mL)
Control	0.8005 ± 0.0540 a	1743.9 ± 45.3 a	249.30 ± 8.22 a
Pregabalin (Standard)	0.6465 ± 0.0516 b	805.3 ± 45.9 c	172.96 ± 6.95 c
Pregabalin (Double)	0.4005 ± 0.0335 d	968.0 ± 27.0 b	98.52 ± 2.96 d
Baclofen (Standard)	0.4815 ± 0.0934 c	804.9 ± 34.9 c	191.05 ± 2.93 b
Baclofen (Double)	0.4507 ± 0.1026 cd	989.9 ± 47.9 b	171.91 ± 3.61 c
Pregabalin + Baclofen (Standard)	0.3247 ± 0.0376 e	571.8 ± 35.3 d	93.07 ± 4.96 d
Pregabalin + Baclofen (Double)	0.2630 ± 0.0344 f	308.7 ± 35.5 e	69.51 ± 4.37 e

Values are presented as mean ± SD. Means with different superscripts in the same column are significantly different ($p \leq 0.05$), based on the Duncan's Multiple Range Test.

3.4. Inflammatory Markers (TNF-α and NF-κB)

Serum levels of pro-inflammatory transcription factor NF-κB and cytokine TNF-α showed pronounced, statistically significant elevations ($p \leq 0.05$) in all treated groups compared to control rats (Table 4). Double-dose combination treatment provoked the highest upregulation of NF-κB (3718.7 ± 62.2 pg/mL) and TNF-α (329.49 ± 8.17 pg/mL), highlighting a strong pro-inflammatory cascade driven by high-dose combination regimens.

Table 4. Effect of pregabalin and baclofen administration (standard and double doses) on serum inflammatory markers (NF-κB and TNF-α) in male albino rats.

Group	NF-κB (pg/mL)	TNF-α (pg/mL)
Control	2146.5 ± 72.6 g	150.12 ± 9.60 f
Pregabalin (Standard)	2557.4 ± 53.2 d	180.78 ± 4.53 d
Pregabalin (Double)	2764.3 ± 87.2 c	268.37 ± 4.39 c
Baclofen (Standard)	2331.3 ± 63.8 f	165.33 ± 4.86 e
Baclofen (Double)	2402.1 ± 82.2 e	174.68 ± 3.59 de
Pregabalin + Baclofen (Standard)	3003.6 ± 89.1 b	357.50 ± 9.61 a
Pregabalin + Baclofen (Double)	3718.7 ± 62.2 a	329.49 ± 8.17 b

Values are presented as Mean ± SD. Means with different superscripts within the same column differ significantly ($p \leq 0.05$) by Duncan's multiple range test.

4. Discussion

Despite the extensive therapeutic usage of pregabalin and baclofen that has led to higher prescription rates globally, it is still unclear how these two drugs affect the physiology of the reproductive system in males. Taking into account the lack of comparable research, the aim of the current study was to determine the endocrine, oxidative, and inflammatory pathways that lead to the induction of reproductive disorders in male albino rats by pregabalin and baclofen in single and combined doses.

Hormonal analysis demonstrated serious disturbance of HPG axis associated with the significant decrease in the concentrations of testosterone, SSH, and ICSH; specifically, the lowest level of ICSH was observed in rats receiving double doses of both drugs (14.56 ±

2.53 mIU/mL). This endocrine failure is due to the combination of neuroendocrine mechanisms. Pregabalin decreases central dopaminergic and noradrenergic activities causing hyperprolactinemia, which leads to the suppression of conversion of cholesterol to pregnenolone induced by nitric oxide in Leydig cells [10-11]. Meanwhile, activation of central GABA-B receptors by baclofen leads to the suppression of the pulsatility of GnRH in the hypothalamus. This central suppression, together with oxidative and inflammatory damage to Leydig cells, blocks the pituitary activity [12].

MDA and F2-isoprostanes are very important indicators of the processes of lipid peroxidation and damage to cell membranes. An abrupt increase in MDA and IsoPs in serum in combination groups is evidence of the potent synergism of oxidative stress due to concurrent administration of pregabalin and baclofen. Lipid peroxidation of polyunsaturated fatty acids in plasma membranes is linked to sperm dysmorphism, degeneration of tubules, and lack of germ cell viability. This finding agrees well with previous observations on formation of radicals caused by xenobiotics and membrane instability [13-12].

The analysis of the level of the antioxidants SOD, CAT, and GSH showed their pronounced depletion that was dose-dependent and increased progressively according to different combination schemes. This depletion reflects an insufficient protective ability of the testis against excessive production of reactive oxygen species [14]. Due to high levels of polyunsaturated fatty acids in membranes of testicular germ cells, depletion of activity of catalase (CAT) and levels of glutathione (GSH) deprives tissues of important intrinsic protection.ROS accumulation causes fragmentation of genomic DNA and damage to Leydig and Sertoli cells [15,16].

NF-κB acts as one of the major transcription factors that regulates the activation of inflammation and pro-inflammatory cytokines. Chronic administration or exposure to CNS drugs such as baclofen and pregabalin leads to cellular stress responses, which results in increased production of TNF-α in the testis and in immune cells [17-18]. Acute inflammation in the spaces and seminiferous tubules is directly linked to microdamage in the tissues, sloughing of the germinal epithelium, and Sertoli cell apoptosis [12-19].

5. Conclusions

The current study reveals that chronic administration of pregabalin and baclofen, especially at double doses or through combined therapies, causes highly negative consequences to the reproductive system of male albino rats. These medications cause severe disruptions in the function of hypothalamus-pituitary-gonads axis, which is reflected in a highly decreased concentration of serum levels of testosterone, follicle-stimulating hormone (SSH), and luteinizing hormone (ICSH). At a molecular level, these agents induce highly oxidative stress, which is reflected in the increased levels of MDA and F2-isoprostanes together with a highly decreased level of endogenous antioxidant defense mechanisms such as SOD, GSH, and CAT. In addition, the treatment caused an increase in inflammatory processes that is shown by the increase in NF-κB and TNF-α levels. Overall, the above data demonstrates that an excessive or combined clinical administration of pregabalin and baclofen leads to severe gonadotoxicity and endocrine dysfunction.

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