

Histopathological Study about the Effects of Chronic Concomitant Administration of both Pregabalin and Ibuprofen on the Skeletal, Cardiac and Smooth Muscles of Male Albino Rats

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ABSTRACT

Background: there is not enough information available to assess the relationship between pregabalin use and muscular damage. Also, the effect of pregabalin ibuprofen combination on musculature not well assessed till now.

Aim of the study: The study aimed to evaluate the possible chronic concomitant effect of both pregabalin and ibuprofen on skeletal, cardiac and smooth muscles in male albino rats.

Patients and Methods: One hundred adult healthy male albino rats weighting 200 – 250 gm were obtained from the animal house, Faculty of Medicine, Assiut University, Egypt. The animals were classified into five groups: Group one (Negative control):10 male albino rats, received normal feeding, group two (Positive control): 15 male albino rats given 1 ml of distilled water, group three: 25 male albino rats were given pregabalin at dose 250 mg/kg/day orally (1/20 LD50), group four: 25 male albino rats were given ibuprofen at dose of 80 mg/kg/day orally (1/20 LD50), group five: 25 male albino rats were given ibuprofen at dose of 80 mg/kg/day and pregabalin at of dose 250 mg/kg/day. All animals were treated for three months.

Results: as regard weight of animals, there were statistically significant difference between positive control group versus pregabalin, and pregabalin+ ibuprofen groups. Also histopathological examination showed that chronic use of pregabalin was associated with atrophy, inflammatory cells and cell degeneration of skeletal muscle.

Conclusion: The current study demonstrated that the chronic use of pregabalin was associated with atrophy, inflammatory cells and cell degeneration of skeletal muscle of male albino rats.

Keywords: Histopathological; Pregabalin; Ibuprofen; Musculature; Albino Rats.

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INTRODUCTION

Pregabalin (PGB)[S-(+)-3-isobutyl-GABA] is derived from gamma aminobutyric acid (GABA) that exerts its anticonvulsant activity through binding to alpha 2 delta 1 auxiliary subunit of the voltage-gated calcium channel, although it was originally designed to target the GABA system.¹

Pregabalin has a chemical structure similar to gabapentin but PGB is absorbed faster and has pharmacokinetic advantages. PGB has received FDA approval for patients who suffer from central neuropathic pain, partial seizures, generalized anxiety disorder, fibromyalgia and sleep disorders.²

Ibuprofen is a nonsteroidal anti-inflammatory drug (NSAID) of the propionic acid chemical class. Ibuprofen is a racemic mixture of 2 isomers; however, only the l-isomer of ibuprofen has been shown to have clinical activity. Although d-isomer is considered inactive, it is slowly and incompletely converted to the l-isomer in adults and probably children and may serve as a circulating reservoir for the active drug.³

The study aimed to evaluate the possible chronic concomitant effect of both pregabalin and ibuprofen on the different body musculature in male albino rats.

MATERIALS AND METHODS

Material:

Drugs: pregabalin capsule (each contain pregabalin 150mg) and ibuprofen tablet (each contain ibuprofen 400mg).

Chemicals: Distilled water a vehicle, absolute ethyl alcohol (100%), diethyl ether for anesthesia, formalin 10%, paraffin wax and hematoxyline and eosine stain.

Instruments: Plastic container for storage of slides, glass container for storage of samples, gavage tube and cages for housing of animals.

Experimental animals: One hundred (100) adult healthy male albino rats weighting 200 – 250 gm were obtained from the animal house, Faculty of Medicine, Assiut University, Egypt.

Methods:

The animals were housed in a clean capacious macro-lane cages (5 per cage) under standard laboratory conditions including good aerated room with suitable temperature, relative humidity, maintained at good light with alternating 12 hours light/dark cycles. Standard food and water ad libitum.

The animals were classified into five groups:

Group one (Negative control group): Ten (10) male albino rats, which is the blank group in which animals were allowed to receive nothing except normal feeding.

Group two (Positive control group or vehicle control): Fifteen (15) male albino rats. Each animal were given 1 ml of the vehicle which is distilled water orally by gavage for three months.

Group three: Twenty five (25) male albino rats. Each animal were given pregabalin capsule dissolved in distilled water at a dose 250 mg/kg/day orally by gavage (which represent 1/20 LD50) for 3 months (90 days). The oral LD50 of pregabalin in rats is estimated to be about greater than 5000 mg/kg body weight.⁴

Group four: Twenty five (25) male albino rats. Each animal were given ibuprofen tablet dissolved in distilled water at a dose 80 mg/kg/day orally by gavage (which represent 1/20 LD50) for 3 months (90 days).

Group five: Twenty five (25) male albino rats. Each animal were given both pregabalin capsule dissolved in distilled water at a dose of 250mg/kg/day orally by gavage (which represent 1/20 LD50) for 3 months (90 days) and ibuprofen tablet dissolved in distilled water at a dose 80 mg/kg/day orally by gavage (which represent 1/20 LD50) for 3 months (90 days).

Histopathological examination: At the end of 3 months and under diethyl ether anesthesia all animals were sacrificed after 24 hours of the last dose.

The skeletal muscle of the hind leg, cardiac muscle and smooth muscle of intestine of all rat groups were fixed in 10% formalin. After fixation, the specimens were dehydrated through ascending grades of alcohol, cleared and then embedded in paraffin. Paraffin sections of 5 μ m thickness were prepared and stained with Hematoxylin and Eosin. The stained sections were examined using light microscope according to study of Bancroft and Gamble.⁵

Computer Assisted digital image analysis (Digital morphometric study): Slides were photographed using Olympus® digital camera installed on Olympus® microscope with 1/2 X photo adaptor, using 4 \times X objective. The result images were analyzed on Intel® Core I7® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for area, % area, measurement, object counting and contact_Angle.

Statistical analysis: The collected data were revised, organized, tabulated and statistically analyzed using statistical package for social sciences (SPSS) version 23.0 for windows. Data are presented as the Mean \pm standard deviation (SD), frequency, and percentage. Continuous variables were compared by the Student t test (two-tailed) and one – way ANOVA test for parametric data with Bonferroni post hoc test to detect differences between subgroups. The level of significance was accepted if the P value < 0.05.

RESULTS

Animals body weight: There were no statistically significant difference between positive control group versus negative control, and ibuprofen groups, $P > 0.05$, whereas there were statistically significant difference between positive control group versus pregabalin, and pregabalin+ ibuprofen groups (Table 1).

| Studied groups | Weight (gm) Mean \pm SD | P-Value* | |
|----------------------------|------------------------------|------------------|-------------------|
| | | Positive control | VS studied groups |
| Positive control, N=15 | 243.2 \pm 2.3 | | |
| Negative control, N=10 | 244.2 \pm 1.36 | p>0.05 | |
| Pregabalin, N=25 | 232.3 \pm 1.03 | P<0.05 | |
| Ibuprofen, N=25 | 241.32 \pm 1.8 | p>0.05 | |
| pregabalin+ibuprofen, N=25 | 235.3 \pm 3.1 | P<0.05 | |
| P-Value** | P<0.05 | | |
| Among all groups | | | |

*Independent sample t-test, **F-test (Anova), \rightarrow S: Statistically significant difference ($p \leq 0.05$)

Table 1: comparison of animals weight after 3 month between positive control group versus negative control and pregabalin, ibuprofen, and pregabalin+ ibuprofen groups.

Digital image analysis results:

As regard inflammatory cells percentage in skeletal muscles of all study groups: There were no statistically significant difference between positive control group versus negative control, and ibuprofen groups, whereas there were statistically significant difference between positive control group versus pregabalin, and pregabalin+ ibuprofen groups (Table 2).

| Studied groups | N (%) |
|----------------------------|-----------|
| Positive control, N=15 | 0 (0%) |
| Negative control, N=10 | 0 (0%) |
| Pregabalin, N=25 | 0 (0%) |
| Ibuprofen, N=25 | 25 (100%) |
| pregabalin+ibuprofen, N=25 | 25 (100%) |

Table 2: Illustrating percentage of inflammatory cells of positive control group versus negative control, pregabalin, ibuprofen, and pregabalin+ ibuprofen groups.

Group one (Negative control group): showed normal tissue of smooth muscle of intestine, cardiac

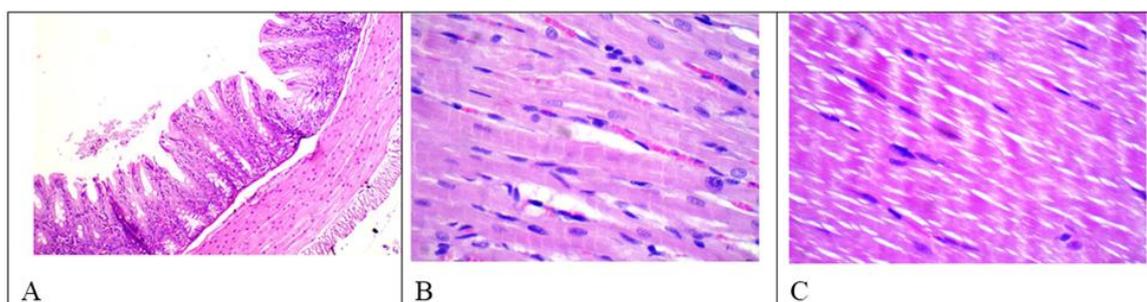


Fig 1: A) Photomicrographs section of intestine showing normal tissue (H&E–10X). B) Photomicrographs section of cardiac muscle showing normal tissue (H&E–40X). C) Photomicrographs section of skeletal muscle showing normal tissue (H&E–10X).

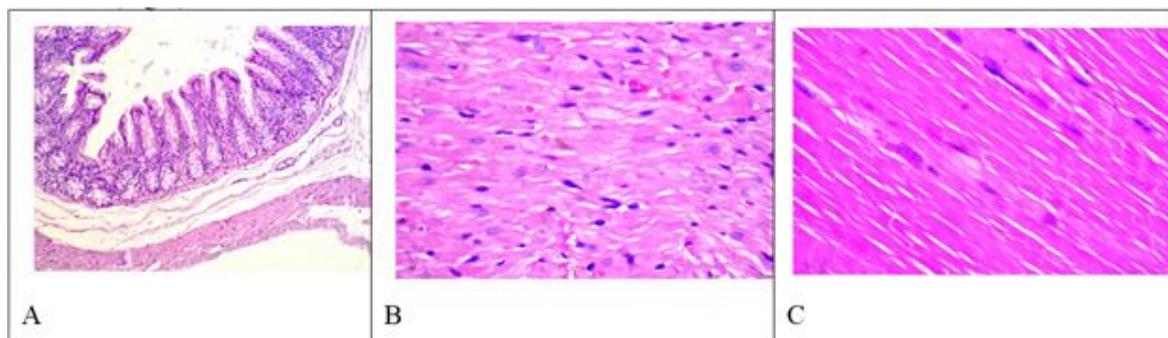


Fig 2: A) Photomicrographs section of intestine showing normal tissue (H&E–10X). B) Photomicrographs section of cardiac muscle showing normal tissue (H&E–40X). C) Photomicrographs section of skeletal muscle showing normal tissue (H&E–40X).

muscle and skeletal muscle of male albino rats (Fig 1).

Group two (Positive control group): showing normal tissue and no effect on smooth muscle of intestine, cardiac muscle and skeletal muscle of male albino rats (Fig 2).

Group three: showing normal tissue and no effect on smooth muscle of intestine and cardiac muscle and there are a cells atrophy, inflammatory cells and cells degeneration of skeletal muscle of male albino rats (Fig 3).

Group four: showing normal tissue and no effect on smooth muscle of intestine, cardiac muscle and skeletal muscle of male albino rats (Fig 4).

Group five: showing normal tissue and no effect on smooth muscle of intestine, cardiac muscle but there are a cells atrophy and cells degeneration of skeletal muscle of male albino rats (Fig 5).

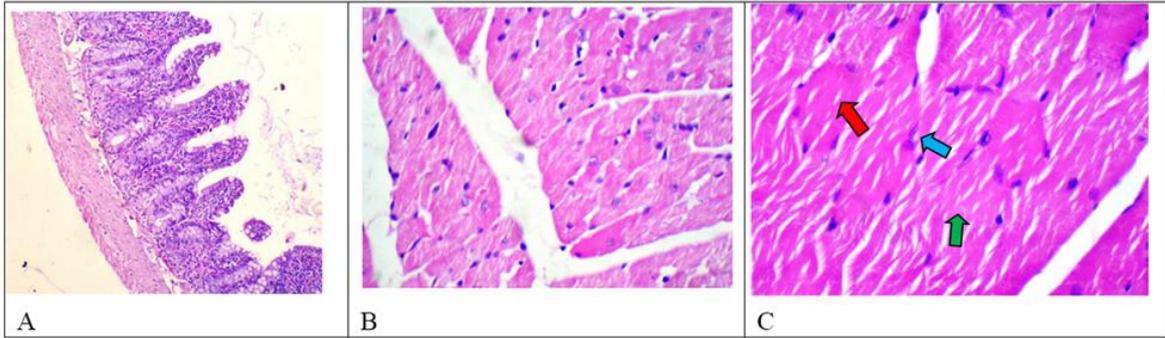


Fig 3: A) Photomicrographs section of intestine showing normal tissue (H&E-10X). B) Photomicrographs section of cardiac muscle showing normal tissue (H&E-40X). C) Photomicrographs section of skeletal muscle showing mild muscle atrophy (red arrow), inflammatory cells (blue arrow) and cell degeneration (green arrow) (H&E-40X).

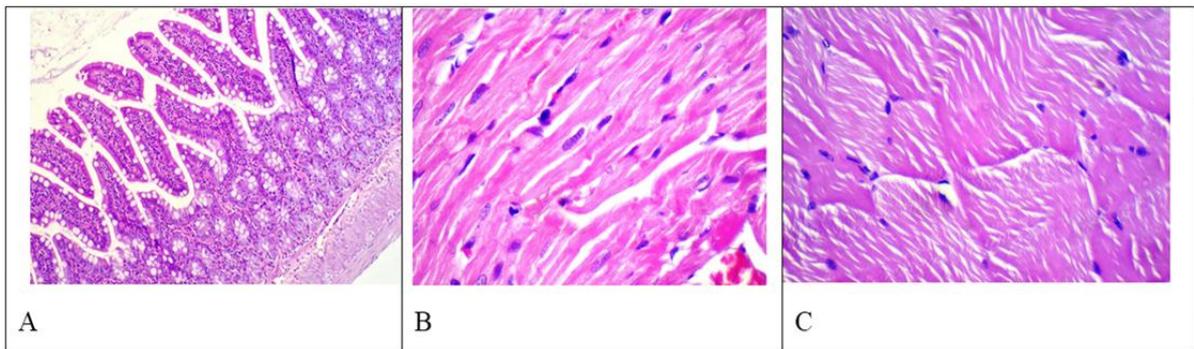


Fig 4: A) Photomicrographs section of intestine showing normal tissue (H&E-40X). B) Photomicrographs section of cardiac muscle showing normal tissue (H&E-40X). C) Photomicrographs section of skeletal muscle showing normal tissue (H&E-40X).

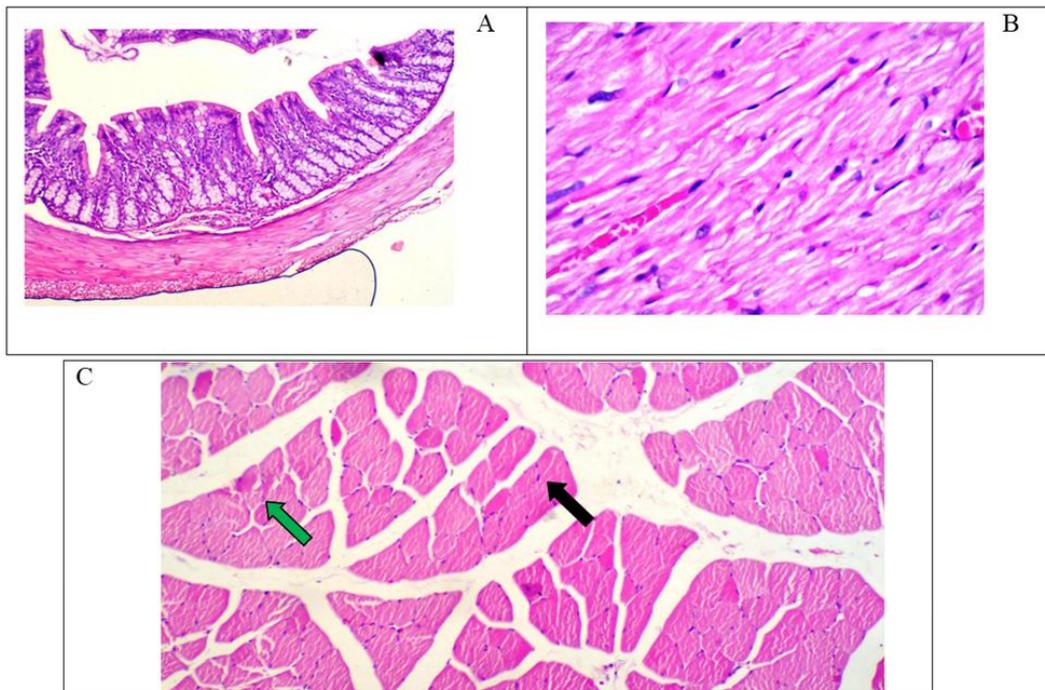


Fig 5: A) Photomicrographs section of intestine showing normal tissue (H&E-10X). B) Photomicrographs section of cardiac muscle showing normal tissue (H&E-40X). C) Photomicrographs section of skeletal muscle showing mild muscle atrophy (black arrow), and cell degeneration (green arrow) (H&E-40X).

DISCUSSION

Pregabalin (PGB) is a new antiepileptic drug that has received FDA approval for patient who suffers from central neuropathic pain, partial seizures, and generalized anxiety disorder, fibromyalgia and sleep disorders.⁶

This is the first study to assess the effect of pregabalin and ibuprofen combination in terms of chronic exposure on male albino rat's musculature. The majority of previous researches were mainly emphasized on the toxic effect of pregabalin alone in terms of central level only.

Our study results showed that, among negative control group there were associated with normal tissue of smooth muscle of intestine, cardiac muscle and skeletal muscle of male albino rats. Also positive control group were associated with normal tissue and no effect on smooth muscle of intestine, cardiac muscle and skeletal muscle of male albino rats.

With regard to pregabalin administrated group, results showing normal tissue and no effect on smooth muscle of intestine and cardiac muscle but there are cell atrophy, presence of inflammatory cells and cells degeneration of skeletal muscle of male albino rats.

This came in accordance with Moshiri et al.,⁶ who reported muscle atrophy, infiltration of inflammatory cells and cell degeneration among pregabalin treated groups. The exact mechanisms behind the muscular damage are unclear and necessitate further investigations.

Bell and Manohar study, demonstrated that pregabalin was associated with the development of focal myositis among pregabalin treated cases.⁷

However, it may be due to the fact that, pregabalin binds with high affinity to the $\alpha 2/\delta 1$ subunit of the voltage dependent calcium channels.⁸ The wide distribution of $\alpha 2/\delta 1$ subunit in the brain and skeletal muscles could explain the mechanism by which pregabalin induce skeletal muscle injury.⁹

Regarding pregabalin ibuprofen combination group, results showed; normal tissue and no effect on smooth muscle of intestine, cardiac muscle but atrophy with inflammatory cells and cell degeneration of skeletal muscle of male albino rats.

This seemed that; ibuprofen plays no role in pregabalin induced skeletal muscle injury.

This was in disagreement with Bondesen et al.¹⁰ who identified that NSAIDs are necessary for muscle regeneration following injury.

Also Soltow et al.,¹¹ study demonstrated that ibuprofen treatment inhibit muscle hypertrophy by approximately 50%.

Another study by Cheung and Tidball, which demonstrated that, administration of ibuprofen prior to increased muscle loading reduces muscle damage.¹²

CONCLUSION

The current study demonstrated that; chronic use of pregabalin was associated with atrophy, inflammatory cells and cell degeneration of skeletal muscle of male albino rats. Moreover, addition of ibuprofen seemed to have no beneficial effect to pregabalin induced muscle injury and pathological changes.

Conflict of interest : none

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