Pathological Effects of Pregabalin Toxicity in Rats

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Abstract

Background: Pregabalin (PGB) is one kind of gabapentinoid. The main mechanism of action is binding at the alpha-2-delta site, which inhibits calcium influx in response to depolarization at nerve terminals and, in turn, suppresses the release of glutamate, noradrenaline, and substance P. **Objectives:** This study aimed to study the pathological effects of PGB toxicity on brain and liver of rats. **Materials and Methods:** We chose 20 mature albino rats, both sexes, averaging 220 g in weight; these were divided into two groups (10 rats per group): the toxic group and the control group. Tablets of Lyrica (Pfizer) may be purchased over the counter. The 300 mg of PGB in each tablet was dissolved in 3 mL of 0.9% normal saline. The dosage was determined using the maximum lethal oral dose in rats (5000 mg/kg) (Pfizer, 2017). Based on the rats' weights, a toxic dosage of 1000 mg of PGB was determined and reconstituted in normal saline (0.9% in 3 mL). Each animal in the acute toxicity group received a single dose of the produced medication orally. After 24 h, all of the animals in both groups were euthanized. The brain and liver were quickly dissected and removed, washed in saline solution, and then processed for histopathological study. **Results:** Focal regions of hemorrhage and congestion were seen in H&E-stained sections from the acute toxicity group, and most pyramidal cells were degraded, pyknotic, and exhibited karyolysis. Cerebellar cortical layers were preserved; however, Purkinje cells were destroyed in the acute toxicity group, which also exhibited an increase in pyknotic cells, hemorrhage, vascular congestion, and localized loss of tissue. Hemorrhages, congestion of portal region blood vessels, and central veins and hepatic sinusoids were some of the most notable pathological abnormalities seen in the livers of those using PGB. Hepatocytes show nuclear pyknosis and a homogeneously acidophilic cytoplasm as a result of severe degenerative alterations, such as vacuolar degeneration and severe necrotic changes. **Conclusion:** PGB can cause pathological lesions in the brain and liver at a single toxic dose.

Keywords: Brain, histopathology, liver, pregabalin, rats

Introduction

Pregabalin (PGB) is one kind of gabapentinoid. The main mechanism of action is binding at the alpha-2-delta site, which inhibits calcium influx in response to depolarization at nerve terminals and, in turn, suppresses the release of glutamate, noradrenaline, and substance P.[1,2]

Because of its anticonvulsant, analgesic, and anxiolytic properties, PGB has been approved for the treatment of a wide range of medical conditions, including partial seizures, diabetic neuropathy, postherpetic neuralgia, neuropathic pain, spinal cord injury, as well as generalized anxiety disorder.[3,4]

Atrio-ventricular block and hepatotoxicity are only two of the significant adverse events linked to PGB use.[5] In addition to concerns about PGB's possible misuse and abuse that have been voiced in recent years. There have also been reports of people becoming very intoxicated due to an accidental overdose or attempting suicide, both of which ended in death.[6,7]

Cases of PGB misuse increased by a factor of 4.3 between 2006 and 2014, as reported by the US National Poison Data System. Clinical outcomes ranged from clinical effects to death.[8]

Because of its potential for misuse, in August 2019, the Egyptian Ministry of Health decided that PGB

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was unlawful and put it on schedule III of illicit pharmaceuticals without prescription. The frequency and distribution of PGB intoxication cases may change as a result of this.[9]

There have been at least six confirmed incidences of acute liver damage due to PGB usage. Patients who took PGB for 8 days experienced jaundice, with hepatic enzyme elevations of 43-fold for alanine transaminase and nearly 4-fold for aspartate transaminase. Hepatocyte damage caused by PGB may be considered an isolated event.[10]

This study aimed to study the pathological effects of PGB toxicity on the brain and liver of rats.

Materials and Methods

We chose 20 mature albino rats, both sexes, averaging 220 g in weight; these were divided into two groups (10 rats per group): the toxic group and the control group. They were housed in cages under regulated conditions of temperature and humidity. For the first 2 weeks, every rat was left in its cage so that it could become used to its new surroundings and rid itself of the infected rats. All the animals were fed a healthy, nutritious diet that included all the nutrients they needed. Throughout the experiment, fresh containers were refilled with tap water.

Tablets of Lyrica (Pfizer) may be purchased over the counter. The 300 mg of PGB in each tablet was dissolved in 3 mL of 0.9% normal saline.

The dosage was determined using the maximum lethal oral dose in rats (5000 mg/kg).[11] Based on the rats' weights, a toxic dosage of 1000 mg of PGB was determined and reconstituted in normal saline (0.9% in 3 mL).

An initial dosage was determined during pilot research. The study's dosage was not associated with any fatalities despite the existence of histopathological abnormalities in the toxic group.

Each animal in the acute toxicity group received a single dose of the produced medication. It was taken orally and then gavaged (placed directly into the stomach) using a curved needle-like oral tube.

After 24 h, all of the animals in both groups were euthanized. The brain and liver were quickly dissected and removed, washed in saline solution, and then chopped into 1–2 cm^3 pieces before being preserved in neutral buffered formaldehyde solution (10%) for at least 48 h and then embedded in paraffin wax. For histopathological analysis, 5-µm-thick sections were prepared in accordance with[12] mounted on glass slides and stained with hematoxylin and eosin (H&E).

Ethical consideration

The study was conducted in conformity with the moral guidelines that the Helsinki Declaration served as the foundation for. On July 10, 2023, a local ethics committee examined and approved the research protocol using reference number 4-1 to receive this permission.

Results

Focal regions of hemorrhage and congestion were seen in H&E-stained sections from the acute toxicity group, and most pyramidal cells were degraded, pyknotic, and exhibited karyolysis [Figure 1].

Cerebellar cortical layers were preserved; however, Purkinje cells were destroyed in the acute toxicity group, which also exhibited an increase in pyknotic cells, hemorrhage, vascular congestion, and localized loss of tissue [Figures 2 and 3].

Hemorrhages, congestion of portal region blood vessels, and central veins and hepatic sinusoids were some of the most notable pathological abnormalities seen in the livers of those using PGB. Hepatocytes show nuclear pyknosis and a homogeneously acidophilic cytoplasm as a result of severe degenerative alterations, such as vacuolar degeneration and severe necrotic changes [Figures 4 and 5].

Discussion

Many medical disorders, such as neuropathic pain, partial seizures, migraine, and fibromyalgia, have benefited from the usage of PGB as a medication.[13-15]

Cells with maintained architectural layers in the cerebral cortex and cerebellum were shown to be susceptible to degenerative alterations. The alpha-2-delta 1 (a2d1) subunit of voltage-gated calcium channels is broadly distributed in the peripheral and central nervous systems, suggesting that PGB may be responsible for these alterations.[15]

Significant degenerative alterations in the neurons of the cerebral cortex and cerebellum, as well as increased cellularity

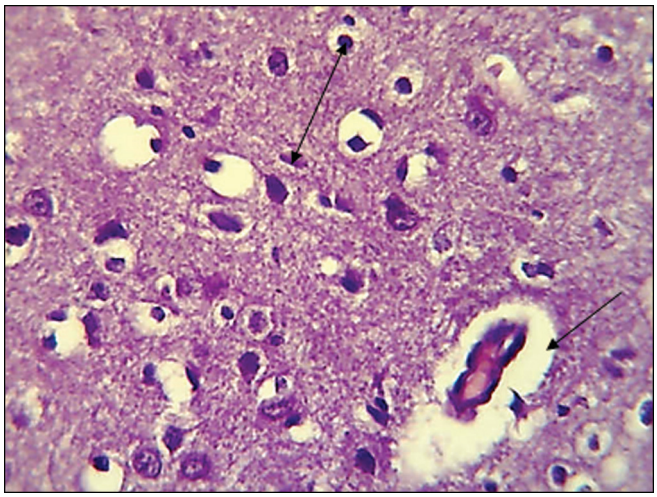

Figure 1: Vascular congestion and perivascular edema were seen in a 400× H&E stained section of the rat brain

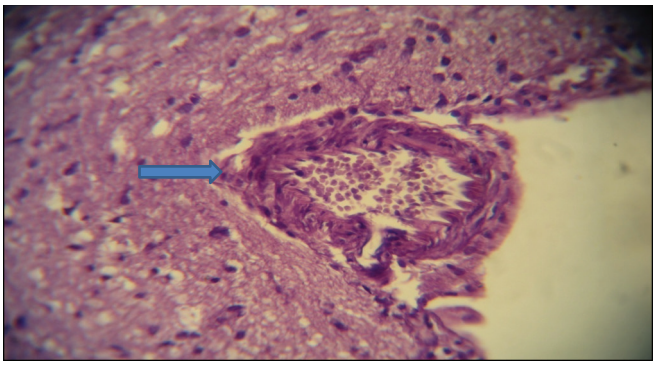

Figure 2: Meningeal blood vessel dilatation and congestion, as shown in a histopathological slice of rat cerebellum after oral administration of a single dosage of pregabalin (H&E stain, 400×)

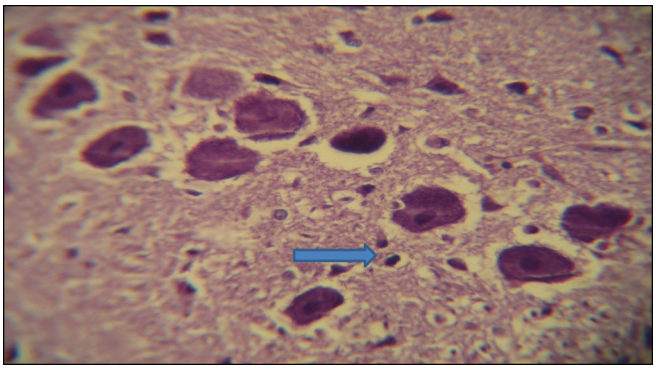

Figure 3: Single-dose pregabalin-induced edema and neuronal shrinkage characterized by pyknotic nuclei and Nissl substance loss in the rat cerebellum (H&E stain, 400×)

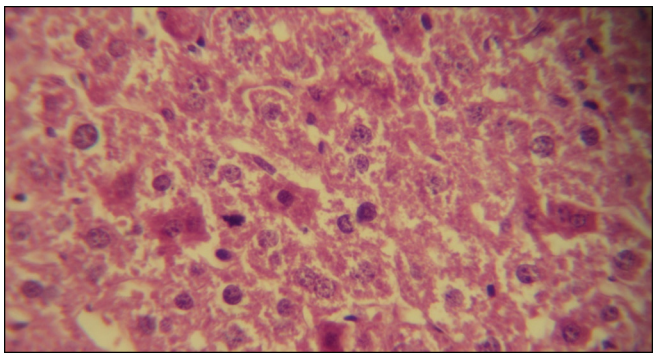

Figure 4: Section of liver stained with H&E at 400× reveals necrosis in a rat that was orally administered pregabalin with congestion of blood vessels

and uneven distribution of the astrocytes, were also identified by Ali and Hassan[16] in the acute PGB poisoning group.

Similar to other forms of acute toxic encephalopathy, PGB-induced encephalopathy is characterized by altered states of consciousness, behavioral abnormalities, and/or seizures in the absence of fundamental structural brain pathology. Polysynaptic disruption and an imbalance between excitatory and inhibitory amino acids are two frequent mechanisms.[17]

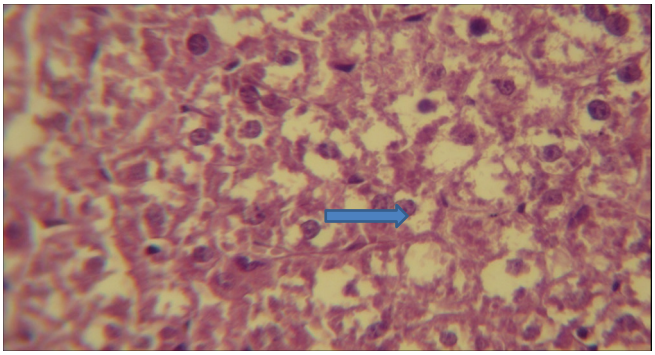

Figure 5: Histopathological analysis of pregabalin-treated rat liver demonstrates vacuolation of hepatocytes and enlarged nuclei with many nucleoli (H&E stain, 400×)

Pathophysiology of acute toxic encephalopathy, including the activation of microglial cells and infiltration of myeloid cells, astrocytes, and oligodendrocytes that disrupt central nervous system homeostasis. This mechanism triggers the production of cytokines, which have been shown to be neurotoxic in and of themselves.[17]

Magar *et al.*[18] indicated that hypoxia or direct toxic action of PGB might contribute to this neurotoxicity, while[19] explained these histopathological effects on brain tissue by oxidative stress generated by PGB.

The histopathological changes seen in the livers of those on PGB are likely attributable to the drug's well-documented ability to disrupt cellular DNA by binding to the minor groove of duplex CT-DNA. PGB affects tissues by causing tissue hypoxia, which results in endothelial cell proliferation, but only in mice and not in rats.[20,21] DNA minor groove binders are a family of significant compounds in anticancer therapy.[22] As a result, it is safe to say that PGB-induced hepatotoxicity occurs even in the absence of PGB hepatic metabolism.[23]

Conclusion

PGB can cause pathological lesions in the brain and liver at a single toxic dose.

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Conflicts of interest

There are no conflicts of interest.

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