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Study of Acute Pregabalin Intoxication in Patients Admitted to Poison Control Center Ain Shams University Hospitals and its Pathological Effect on Rat Brain: Clinical and Experimental Study

Dalia Hamdy El-Said Lasheen,[1] Nahla Hassan Tolba Sherif,[1] Enas Abo Elwafa El-Taftazani,[1] Suzi Sobhy Atalla[2], Salma I. Abdellkader[1]

[1] Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
[2] Histology and cell biology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

Abstract

Background: Pregabalin (PGB) has been an anti-epileptic and an effective treatment of neuropathic pain; it acts by inhibiting certain presynaptic calcium channels and decreased excitatory neurotransmitters. There are reported serious effects following PGB exposure beside its addictive effect. **Aim of the work:** Study the pattern of acute PGB toxicity in patients admitted to Poison Control Center-Ain Shams University Hospitals (PCCASUH) and its histopathological effects after acute toxic dose in adult albino rat brain.
Methods: The study was comprised of two parts. The clinical part: on 31 patients admitted to PCCASUH with a history of acute PGB toxicity. The experimental part: on thirty adult albino rats, divided into two groups (acute toxicity group received acute single dose of PGB5000 mg/kg and the control group received normal saline). **Results:** Acute PGB exposure leads to mild toxicity and most of symptoms related to CNS included DCL, with a significant relation between the prolonged period of hospital admission and the DCL. The experimental results revealed that the cerebral cortex (CC) and cerebellum showed degeneration of nerve cells, pyknosis with karyolysis of nuclei, and a marked increase in the glial cells with positive glial fibrillary acidic protein (GFAP) cytoplasmic granules in the CC (toxic group). **Conclusion:** The course of PGB intoxication is mostly mild self-limiting and most of patients discharged on the next day of admission. The experimental study concluded that PGB has degenerative effects on brain nerve cells. **Recommendations:** further studies with larger sample size to evaluate the acute and chronic toxicity of PGB.

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Key words Acute Pregabalin toxicity, Abuse, histopathology

Introduction

Pregabalin (PGB) is a gabapentinoid agent. It acts mainly by binding at the alpha-2-delta site that reduce depolarization-induced calcium influx at nerve terminals, with a subsequent reduction in the release of several excitatory neurotransmitters, including glutamate, noradrenaline and substance P (Fink et al., 2002; Calandre et al., 2016).
Pregabalin has analgesic, anticonvulsant and anxiolytic properties, so it was approved for the treatment of different diseases including treatment of partial seizures, post-herpetic neuralgia, diabetic neuropathy, spinal cord injury, neuropathic pain, and generalized anxiety disorder (GAD)(Daniel et al., 2007 and Roth et al., 2014).

There are reported serious side effects following PGB administration e.g., atrio-ventricular block and hepatotoxicity (Sendra et al., 2011; Aksakal et al., 2012). In addition to the reports of abuse and misuse of PGB which have been raised in recent years. Also, acute intoxications from an accidental overdose and attempted suicide with fatal consequences have been reported (Cantrell et al., 2015; Evoy et al., 2017).
According to the US (United states) National Poison Data System, the incidence of PGB abuse cases increase 4.3-fold in the period from 2006 to 2014, with clinical outcomes ranging from clinical effects to death (Dart et., al 2017).

In August 2019, it has been declared by the Egyptian Ministry of Health that PGB is illegal and added to the schedule list (III) of illicit drugs without prescription because of its potential abuse. This could affect the number and pattern of PGB intoxicated cases (Ministerial Decision number (475), 2019).

So, the present study was conducted aiming at determining the pattern of acute PGB toxicity among patients admitted to Poison Control Center Ain Shams University Hospitals (PCCASUH) in a period from the first of March 2019 to the end of February 2020; beside the histopathological study of the effect of acute PGB

toxic dose on adult albino rat brain try to find a primary suggestion to explain the clinical manifestations.

Methodology

The current study was comprised of two parts clinical and experimental.

The clinical part

Study design: Cross sectional observational study carried out during the period from the first of March 2019 to the end of February 2020.

Study population: All symptomatic patients admitted to (PCCASUH) secondary to acute PGB toxicity of different age groups and both sex. The diagnosis of acute PGB toxicity was established by detailed history obtained from patients or guardians. Full clinical examination of patients was performed to evaluate the patients' condition. The following patients were excluded from the study: patients with history of concomitant overdose of any agent with Central Nervous System (CNS) effects like; benzodiazepines, barbiturates, alcohol and opiates after confirmation by clinical and laboratory tests; to avoid their CNS effects which may affect the results of the study *(Druid, 2001; Kraut and Kurtz, 2008).*Past medical history of epilepsy as convulsions could be a symptom of PGB toxicity*(Reedy and Schwartz, 2010).*Chronic renal failure and chronic cardiac disease to avoid their effect on the obtained results *(Asconapé, 2014).*

Study variables:

- Socio-demographic data (age, gender and residence).
- Manner of poisoning (suicidal, accidental or addiction with overdose), amount and delay time.
- Clinical data included general examination (vital data, skin and pupil examination), and systemic examination of various body systems. Reed's classification used to assess the conscious level.
- Laboratory investigations: included ABG and routine laboratory tests (kidney functions, glucose level and serum(s) electrolytes (sodium, potassium) on admission.
- Electrocardiography (ECG) at the time of admission. Interpretation of ECG was done, with focusing on the heart rate and rhythm, PR interval, QRS width, and QTc interval (corrected by using the Bazett's formula).
- The treatment given to each patient which included decontamination, supportive and symptomatic treatment according to each case, site and period of hospital admission and patients' outcome either survival or death.
- The severity grade of patients assessed at the time of peak manifestations according to Poisoning Severity Score (PSS). Poisoning Severity Score (PSS) (0) included asymptomatic patients, PSS (1) (patients with minor presentations), PSS (2) (patients with moderate presentations), PSS (3) (patients with severe manifestations), and PSS (4) (dead patients) *(Persson et al., 1998).*

The experimental part was carried out in the Medical Research Center, Ain Shams University on groups of adult albino rats.

The animals: Thirty adult albino rats of both sex and average weight of 200gm were chosen. They were housed in cages under controlled conditions of temperature and humidity. At the start, all rats were left in their housing for 2 weeks, for adaptation to the environmental condition and to displace the diseased one. All animals received balanced diet containing essential elements and vitamins. Clean containers were supplied with tap water throughout the experiment.

Drug and preparation: Commercially available Lyrica tablets (Pfizer). Each tablet contains 300 mg PGB were reconstituted with normal saline (0.9%) 3 ml.

Time and route of administration: prepared drug was administered once for each animal in the group of acute toxicity. It was administered orally by a curved needle like oral tube that was introduced directly into stomach (a gavage process).

Dose: The dose was calculated according to 5000 mg/kg (the highest toxic oral dose in rats) *(Pfizer, 2017).* Pregabalin was calculated for the rats according to their body weights and the toxic dose for each rat was calculated to be 1000 mg then was reconstituted in normal saline (0.9%) 3 ml.

The dose was tested first in a pilot study. There were no deaths in the toxic group with presences of histopathological changes so, the dose used in the study.

At the end of the experiment, animals of both groups were euthanized; 24 hours after the dose. The brain rapidly dissected and excised, rinsed in saline solution, and cut into 1-2 cm^3 pieces which were fixed in neutral buffered formaldehyde solution (10%) for a minimum of 48 hours before being embedded in paraffin wax. Sections of 5 microns thickness were mounted on glass slides and stained with hematoxylin and eosin (Hx& E) according to *Drury & Wallington, (1980)* and examined by light microscope for histopathological investigation. Two slides of each group were stained by glial fibrillary protein (GFP) (immune staining), *and* examined by light microscope.

Ethical consideration: The study was approved by the ethical committee of Ain Shams University: FMASU M S 142/2019. Regards the clinical part an informed written consent was obtained from each patient or from his/her guardians for inclusion in the study, and the confidentiality of the patients and their data were maintained. The study was conducted in accordance with the guideline of the animal use and care of Ain Shams ethical committee.

Statistical Analysis: Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version23. The method of data presentation depends on the type of data. The comparison between groups carried out by using *Mann-Whitney and Kruskall-Wallis statistical analysis. Spearman coefficient* was used as a test of correlation. P-value> 0.05: Non significant (NS), P-value < 0.05: Significant (S) and P-value < 0.01: Highly significant (HS).

Results

I- **The clinical part:**

The study included thirty-one acutely PGB intoxicated patients. They were selected according to predetermined inclusion criteria.

- Socio-demographic characteristics: (54.8%) of the included patients were males and 45.2% were females with a wide range of age between 6 months to 50 years and most of patients were children and adolescents (64.5%). The majority of the patients (83.9%) were from urban areas (table 1).
- Manner of poisoning, amount of drug and delay time: The suicide was the most common (48.4%). The amount of ingested PGB ranged from 2 to 12 tablets. The delay time between the exposure and presentation to PCCASUH ranged between 2 to 12 hours (table 1). There is no significant correlation between age, the ingested amount and delay time and the severity grade of the patients (table 2)
- Clinical manifestations: Tachycardia observed in 22.6% of patients, 6.4% of patients presented with hypotension. Thirty-two percentages of patients presented with tachypnea (table 3) with positive correlation between the respiratory rate and increased PSS (table4) and (figure 1a). All included patients presented with disturbed conscious level (DCL) but in different grades. About 39% of patients were drowsy and 29% were in coma grade (I). Other neurological manifestations included convulsions (9.7%), and agitation (12.9%). About 13% of patients presented with chest wheezes and crepitation, 3% developed cyanosis (table 5). There was significant relation between long hospital stay and the patients' presentation with DCL, convulsion, chest manifestations, cyanosis and the need for MV as shown in table (6)
- Laboratory investigations: the included patients had normal s. electrolytes, s. urea and creatinine, and 12.9% of patients developed hypoglycemia (table 7) with non-significant relation with hypoglycemia and the severity (PSS) (table 9). Regards ABG; 29% of patients had respiratory acidosis, and 12.9% had metabolic acidosis with significant relation between the long hospital stay and the presence of acidosis (low pH) and decrease O_2 saturation as shown in table (8) and figure 1b but both respiratory and metabolic acidosis didn't significantly relate to the degree of the severity (PSS) (table 9).
- As regards ECG changes; sinus tachycardia was observed in 25.8% of cases and 3.2% of patients had wide QRS complex, with non-significant correlation between the ECG findings and the severity of patients (PSS) (table 10).
- All patients in the present study received supportive and symptomatic treatment in the form of intravenous (IV) fluids (100%) beside observation of the vital signs, 9.68% of cases needed anticonvulsant medications, 3.2% required antibiotic for treatment of chest infection, and 9.68% required MV (figure Ic).
- Intensive care admission was required for 45.1% of patients. The duration of hospitalization ranged between 1 – 8 days. Thirty cases of studied patients were completely recovered, and only 1 case died this patient presented with coma grade IV, respiratory distress, cyanosis, and mechanically ventilated.
- Poison severity score (1) recorded in the majority of patients (45.1%), PSS (2) in (29.1%), 16 % of patients were PSS (0). The death occurred in only one patient with PSS (4) (figure Id).

II- **The experimental part**

- cerebral cortex (CC):

In acute toxicity group sections stained by Hx&E showed focal areas of hemorrhage and congestion, most of the pyramidal cells are degenerated, pyknotic with karyolysis of their nuclei (figure 2), areas of fibrous tissue deposition (figure 3, 5) with areas of multiple focal loss of brain tissue (figure 4), and presence of remnants of karyolytic cells and cell lysis (figure 6,7). This pattern was the same among male and female groups compared to the same study in the control group showed normal cc structure (figure 1).

Sections of CC stained by GFAP in toxic group (figure 8) showed marked increase in the glial cells with positive GFAP cytoplasmic granules than control group (figure 9).

- Cerebellum:

In acute toxicity group sections showed maintained architecture of cerebellar cortex layers with lysis of purkinje cells, and increased pyknotic cells, areas of hemorrhage and congestion of the blood vessels and focal loss areas of cerebellar tissue (figures 11, 12, 13, and 14). Both molecular and granular layers were preserved normal architecture, this pattern was the same among both sex groups compared to control group (figure 10).

Table (1): Distribution of sociodemographic variables (Sex, age and residence), delay time, and manner of poisoning among studied patients.

Socio-demographic data		Total number (n=31)
Sex	Female	14(45.2%)
	Male	17(54.8%)
Age (years)	Range	0.5-50
	Median (IQR)	18(11-28)
	Children and adolescents (<18 years)	20 (64.5%)
	Adults (>= 18 years)	11 (35.5%)
Residence	Urban	26 (83.9%)
	Rural	5 (16.1%)
Delay time (hours)	Range	2 – 12
	Mean ± SD	4.90 ± 3.27
Amount of drug (tablets) Concentration 150-300 mg	Range	2–12
	Mean±SD	7.81±2.33
Manner of intoxication	Suicidal	15 (48.4%)
	Addiction (acute on top of chronic)	7 (22.6%)
	Accidental	9 (29.0%)

N: number, IQR: interquartile range, SD: standard deviation

Table (2) Spearman correlation coefficient between age, delay time and amount of drug and the PSS in acute PGB intoxicated patients.

Total number (31 patients)	PSS	
	r	P-value
Age	0.217	0.241
Delay time (hours)	-0.141	0.45
Amount (tablets)	0.087	0.642

P-value > 0.05: Non significant, PSS: poison severity score, r: Spearman correlation coefficient

Table (3): Distribution of vital signs at presentation time of studied patients.

Vital signs	Total No. = 31	
Pulse (heart rate) (Beat / minute)	Normal	24(77.4%)
	Tachycardia	7(22.6%)
Blood pressure (mmHg)	Normal	28(90.3%)
	Hypotension	2(6.4%)
	Hypertension	1(3.1%)
Respiratory rate (Breath / minute)	Normal	21(67.7%)
	Tachypnea	10(32.3%)
Temperature	Normal	31(100%)

No: number

Table (4): Spearman correlation coefficient between vital signs and PSS in acute PGB intoxicated patients.

Total number (31 patients)	PSS	
	r	P-value
Pulse (beat /minute)	0.094	0.616
Systolic BP (mmHg)	-0.047	0.803
Diastolic BP (mmHg)	-0.173	0.353
Temperature (^{0}C)	-0.157	0.398
Respiratory rate (breath/minute)	**0.570**	**0.031***

P-value > 0.05: Non significant, P-value < 0.05: Significant*, P-value < 0.01: Highly significant, PSS: poison severity score, r: Spearman correlation coefficient

Table (5): Distribution of clinical findings among studied acute PGB intoxicated patients.

Neurological examinations	Total no. = 31	
Conscious level	Drowsy	12 (38.7%)
	Coma I	9 (29.0%)
	Coma II	8 (25.8%)
	Coma IV	2 (6.5%)
Agitation	Negative	27 (87.1%)
	Positive	4 (12.9%)
Convulsions	Negative	28 (90.3%)
	Positive	3 (9.7%)
Pupil	Normal reactive	23 (74.2%)
	Constricted reactive	8 (25.8%)
Cyanosis	Normal	30 (96.8%)
	Cyanosis	1 (3.2%)
Chest manifestations	normal	27 (87.1%)
	Wheezes and Crepitation	4 (12.9%)

No: number

Table (6): Mann-Whitney and Kruskall-Wallis statistical analysis between both neurological and chest examinations and the period of hospital stay in studied acute pregabalin intoxicated patients

		Period of hospital stay (days)		Test value	P-value	Sig.
		Median (IQR)	Range			
Conscious level	Coma 0	1 (1 – 1)	1 – 2	10.735##	0.013	S
	Coma I	1 (1 – 1)	1 – 2			
	Coma II	1 (1 – 1.5)	1 – 3			
	Coma IV	5.5 (3 – 8)	3 – 8			
Agitation	Negative	1 (1 – 1)	1 – 8	-1.371≠	0.17	NS
	Agitation	1.5 (1 – 2.5)	1 – 3			
Convulsions	No	1 (1 – 1)	1 – 8	-2.149≠	0.032	S
	Convulsions	3 (1 – 3)	1 – 3			
Wheezes	Negative	1 (1 – 1)	1 – 2	-3.840≠	0	HS
	Positive	3 (3 – 8)	3 – 8			
Crepitations	Negative	1 (1 – 1)	1 – 2	-3.104≠	0.002	HS
	Positive	3 (2 – 5.5)	1 – 8			
Cyanosis	Normal	1 (1 – 1)	1 – 8	-2.065≠	0.039	S
	Cyanosis	3 (3 – 3)	3 – 3			
Mechanical Ventilation	No	1 (1 – 1)	1 – 2	-3.840≠	0	HS
	Yes	3 (3 – 8)	3 – 8			

P-value > 0.05: Non significant (NS), P-value < 0.05: Significant (S), P-value < 0.01: Highly, significant (HS), IQR: interquartile range, ≠: Mann-Whitney test, ##: Kruskal-Wallis test

Table (7): Laboratory investigation (glucose, Na, K, kidney functions) in studied patients

Laboratory results	Total number (31 patients)	
Blood glucose level (mg/dL)	**Normal**	27 (87.1%)
	Hypoglycemia	4 (12.9%)
	Range (Mean ± SD)	50 – 136 (94.32 ± 19.5)
Na (mEq/L)	**Range (Mean ± SD)**	130 – 145 (138.35 ± 3.15)
K (mEq/L)	**Range (Mean ± SD)**	2.7 – 4.5 (3.52 ± 0.35)
Urea (mg/dL)	**Range (Mean ± SD)**	5 – 33 (16.61 ± 5.7)
Creatinine (mg/dL)	**Range (Mean ± SD)**	0.6 – 1.2 (0.88 ± 0.13)

SD: standard deviation

Table (8): Spearman correlation coefficient between ABG findings and period of hospital stay in studied acute pregabalin intoxicated patients

	Period of hospital stay (days)		Sig.
	r	P-value	
pH	**-0.385**	**0.032**	**S**
PO_2 (mmHg)	-0.28	0.127	NS
PCO_2 (mmHg)	0.207	0.263	NS
SO_2 (%)	**-0.394**	**0.028**	**S**
HCO_3 (mEq/L)	-0.076	0.686	NS

P-*value > 0.05: Non significant (NS), P-value < 0.05: Significant (S), P-value < 0.01: Highly significant (HS), r: Spearman correlation*

Table (9) Mann-Whitney and Kruskall-Wallis statistical analysis of glucose, ABG and PSS in studied acute PGB intoxicated patients.

		PSS		Test value	P-value	Sig.
		Median (IQR)	Range			
Glucose	Normal	1 (1 – 2)	0 – 4	-1.288≠	0.198	NS
	Hypoglycemia	2 (1.5 – 2)	1 – 2			
ABG (pH)	Normal 18 (58.1%)	1 (0 – 2)	0 – 3	4.753##	0.093	NS
	R. acidosis 9 (29%)	1 (1 – 2)	1 – 4			
	M. acidosis 4 (12.9%)	2 (1.5 – 2.5)	1 – 3			

P-value > 0.05: Non significant (NS), R.: respiratory, M.: metabolic, IQR: interquartile range, PSS: poison severity score, ≠: Mann-Whitney test, ##: Kruskal-Wallis test

Table (10): Spearman correlation coefficient between ECG findings and PSS among studied patients.

	PSS	
	r	P-value
ECG rate (beat/min)	0.042	0.822
PR interval (ms)	-0.28	0.127
QT interval (ms)	0.144	0.439
Duration of QRS (ms)	0.1	0.592

P-value > 0.05: Non significant, P-value < 0.05: Significant, P-value < 0.01: Highly significant, r: Spearman correlation coefficient, PSS: poison severity score

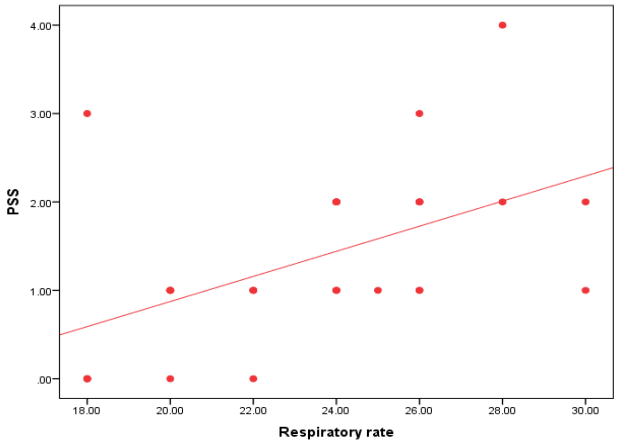

Figure (1a): Positive correlation between respiratory rate (breath/minute) and PSS in studied patients.

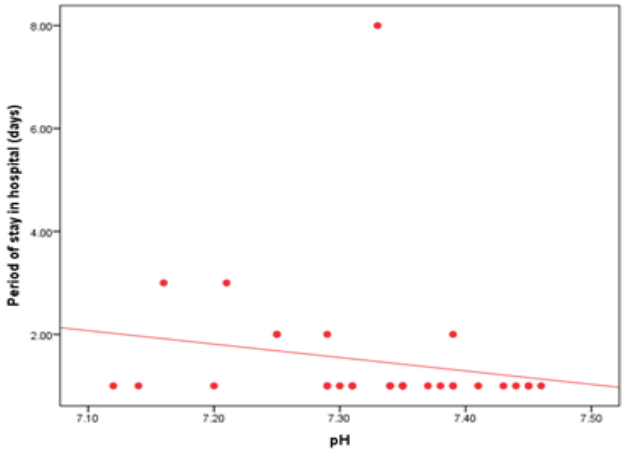

Figure (1b) Negative correlation between pH and the period of hospital stay in studied patients.

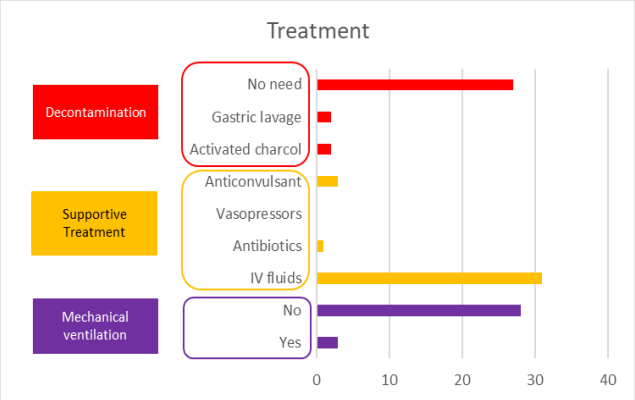

Figure (1c) shows decontamination and supportive treatment among studied patients

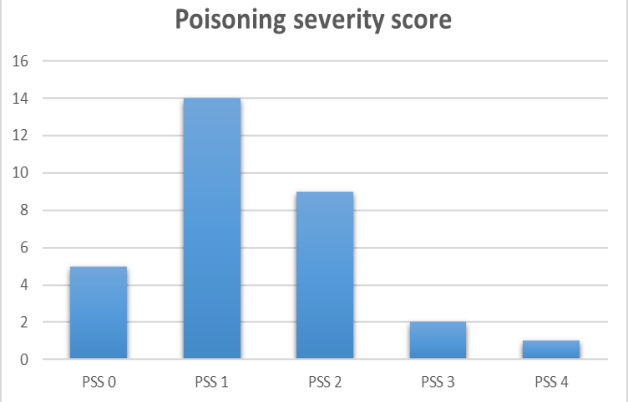

Figure (Id) Bar chart shows Poisoning severity score (PSS) in studied patients.

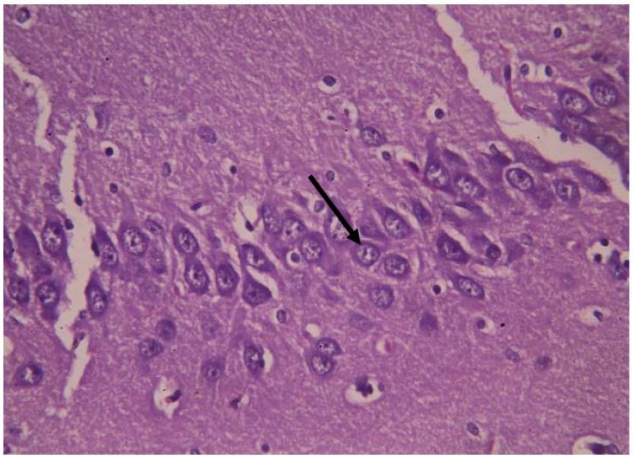

Figure (1): A photomicrograph of rat cerebral cortex showing pyramidal cells with centrally located vesicular nuclei (Control group H&E x640).

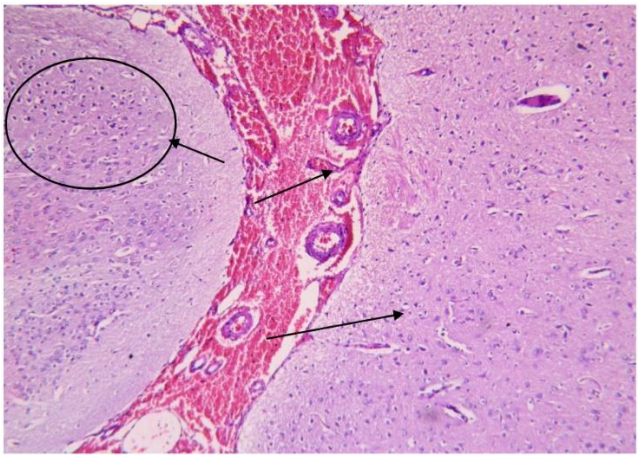

Figure (2): A photomicrograph of cerebral cortex showing areas of cerebral hemorrhage and congestion and multiple pyknotic pyramidal cells (Acutely toxic group H&E x250).

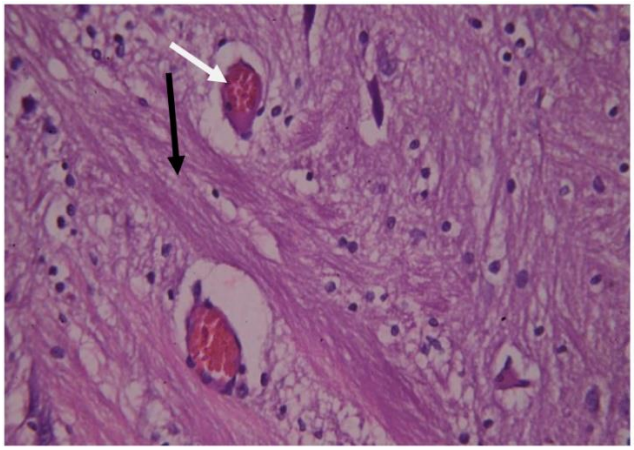

Figure (3): A photomicrograph of cerebral cortex showing areas of cerebral haemorrhage (white arrow) note the presence of fibers more than cells (black arrow) (Acutely toxic group H&E x640).

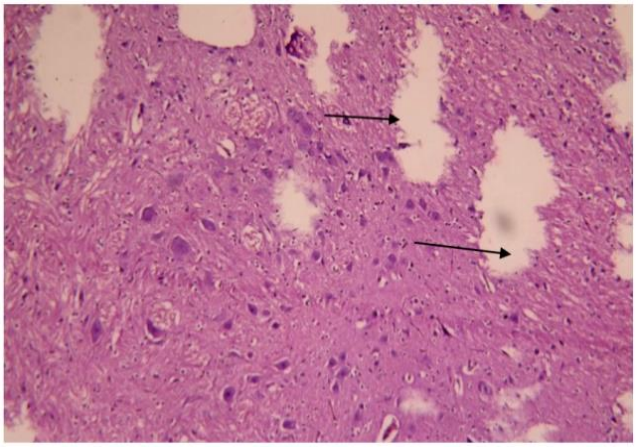

Figure (4): A photomicrograph of cerebral cortex showing multiple areas of focal loss of brain tissues. (Acutely toxic group H&E x 640)

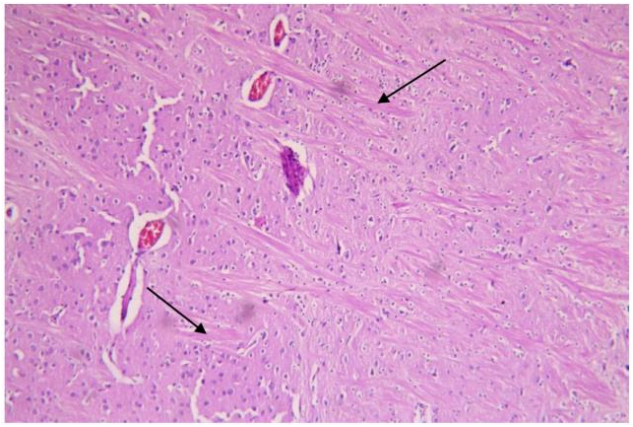

Figure (5): A photomicrograph of cerebral cortex showing areas of cerebral congestion and excessive deposition of acidophilic bundles of fibrous tissue. (Acutely toxic group H&E x250)

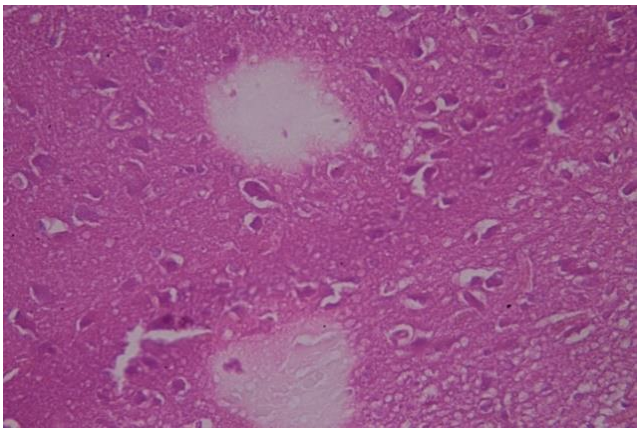

Figure (6): A photomicrograph of cerebral cortex showing remnants of karyolytic cells (ghost cells) within areas of necrotic brain tissues. (Acutely toxic group H&E x 640)

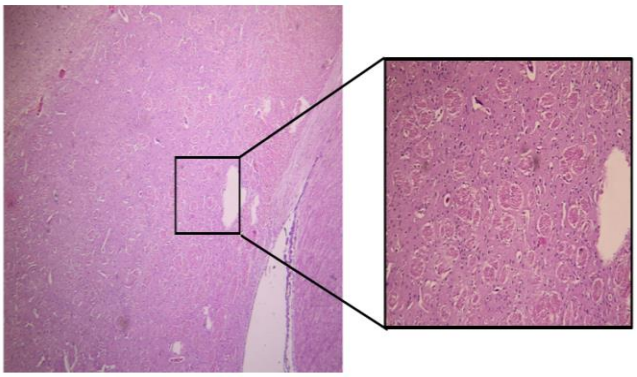

Figure (7): A photomicrograph of cerebral cortex showing loss of architecture and cell lysis. (Acutely toxic group, H&E left x 250, Right x 640)

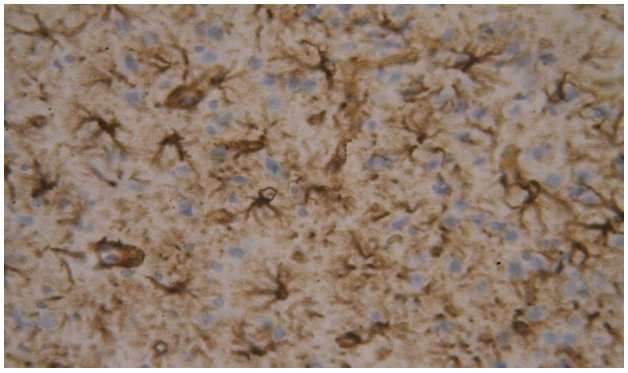

Figure (8): A photomicrograph of cerebral cortex showing multiple glial cells with positive GFAP brown cytoplasmic granules among nerve cells. (Normal control group, GFAP&H. x640)

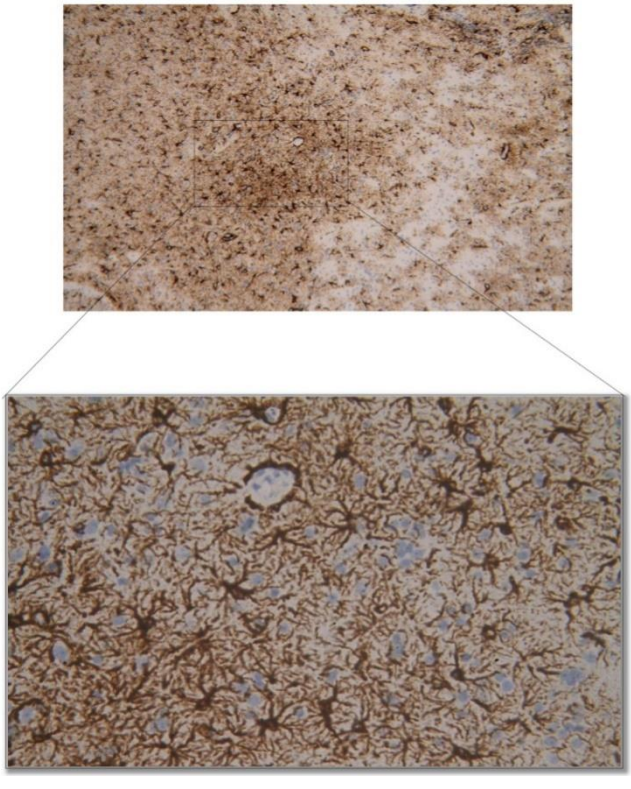

Figure (9): A photomicrograph of cerebral cortex showing marked increase in the glial cells with positive GFAP brown cytoplasmic granules among nerve cells. (Acutely toxic group, GFAP &H. left x 250 Right x 640)

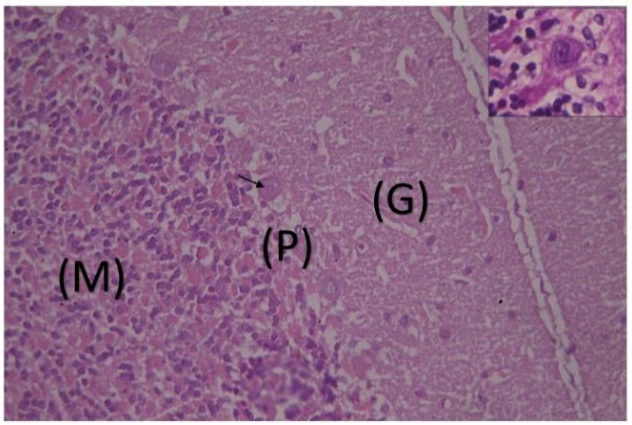

Figure (10): A photomicrograph of cerebellar cortex showing the three layers of cerebellar cortex; molecular cell layer (M), Purkinji cell layer (P) and granular cell layer (G). the incet shows normal purkinji flask shaped cells with vesicular nuclei. (Control group, H&E x 640, Incet H&E x 640)

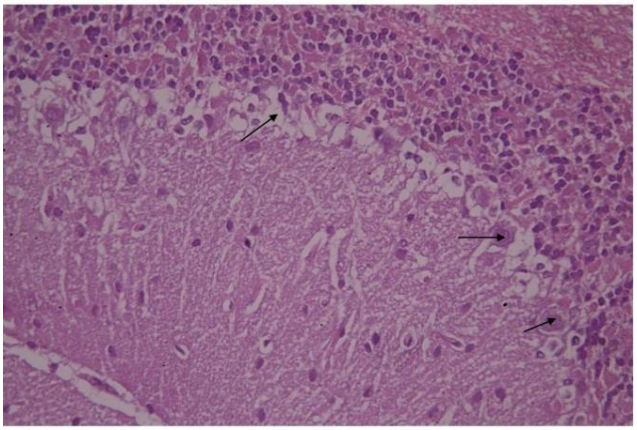

Figure (1): A photomicrograph of cerebellar cortex showing lysis of most of purkinji cells and loss of their uniform distribution and size. (Acutely toxic group, H&E x 640)

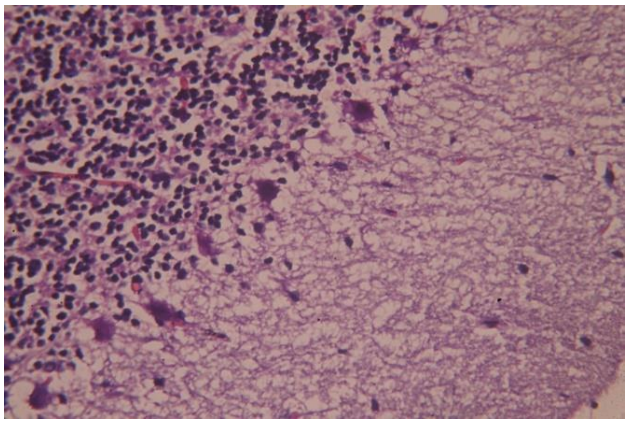

Figure (2): A photomicrograph of cerebellar cortex showing most of purkinji cells with deeply stained pyknotic nuclei (white arrow), note the lysis of some cells (black arrow). (Acutely toxic group, H&E x 640)

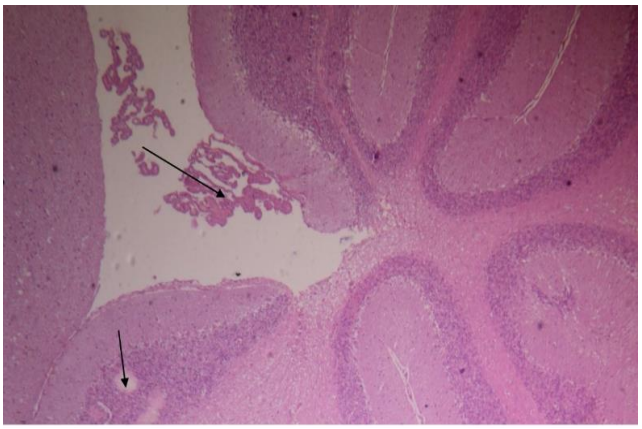

Figure (3): A photomicrograph of cerebellar cortex showing areas of cerebellar hemorrhage and focal loss of cerebellar tissue. (Acutely toxic group, H&E x 100)

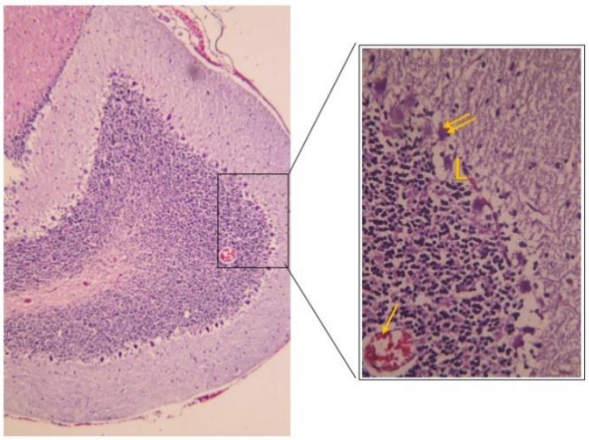

Figure (4): A photomicrograph of cerebellar cortex showing areas of blood vessels congestion (single arrow), lysis of purkinji cells (L) and pyknotic cells (double arrow). (Acutely toxic group, H&E left x 100 Right x 640)

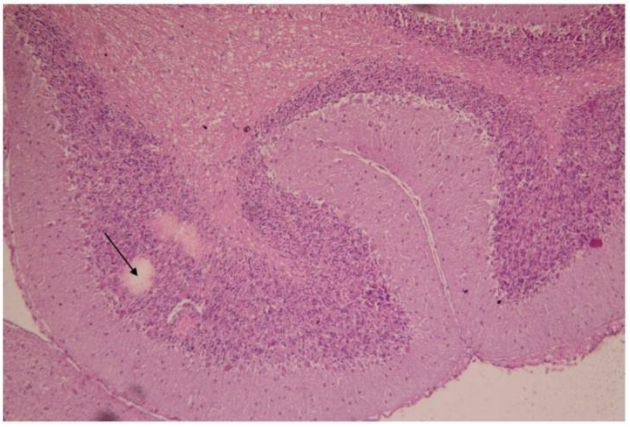

Figure (5): A photomicrograph of cerebellar cortex showing focal loss of cerebellar tissue. (Acutely toxic group, H&E x 100)

Discussion

Pregabalin has been a useful drug in the treatment of many medical conditions including neuropathic pain, partial seizures, migraine, and fibromyalgia (Patel and Dickenson, 2016). In August 2019, it has been declared by the Egyptian Ministry of Health that PGB is illegal and added to the schedule list (III) of illicit drugs without prescription because of its potential abuse. This could affect the number and pattern of PGB intoxicated cases (Ministerial Decision number (475), 2019). The number of patients included in the study fulfilled the inclusion criteria were 31 patients whose median age was 18 years, and most of them were adolescents and children.

According to the literature, children younger than 5 years of age have accidents, especially from putting substances to the mouth, and the exposures usually to low PGB doses, caused by exploratory behaviors in children and/or lack of parental supervision (Oliveira and Suchara, 2014; Rietjens et al., 2021).

Regards the group of adolescents, the number of suicide attempts was 6.4 times higher than in the children's group due to a wide range of biological, social and environmental factors, such as cognitive misjudgment, age-related psychological changes (Zakharov et al., 2013).

On the other hand different median age values reported in other studies carried out on PGB intoxicated cases by Häkkinen et al., (2014) and Schwan et al., (2010) who reported median age of 30 and 29 years respectively.

Males were more than females in the studied patients, this agrees with Schwan et al., (2010)and Isoardi et al., (2020) who reported that 56.25% and 57% of cases were males. This could be attributed to the recreational exposures in which PGB was taken to induce its psychoactive effects and males use psychotropic substances especially illicit drugs more than females (Gahr et al., 2013; Isoardi et al., 2020).While this finding is in contrast with Crossin et al., (2019) who reported that females represent most of cases (56.8%).

The majority of the studied patients were from urban areas and this could be related the geographical cause as patients mostly ask medical advice in the nearby hospital. In the current study, the delay time ranged from 2 to 12 hours with mean value 4.90 ± 3.27 hours. These findings are in accordance with Slocum et al., (2018) and Ozturk & Morkavuk, (2019), who mentioned that, delay time in the discussed PGB acute toxicity case report was about three hours. Other case reports by Wood, et al., (2010) mentioned that delay times were less than 30 minutes.

Pregabalin is rapidly and extensively absorbed after oral dosing with maximal plasma concentrations occurring 1.5 hours after single or multiple doses (Ben-Menachem, 2004). This rapid absorption leads to rapid appearance of clinical picture of toxicity.

The suicidal attempt represented the most common manner of intoxication in the present study as most of cases were teenagers followed by accidental manner then acute on top of chronic use. These results agree with Crossin et al., (2019) and, Isoardi et al., (2020) who reported that deliberate self-poisonings accounted for 66% of isolated PGB toxicity.

Lyons, (2018) explained that the misuse of PGB may be achieved by a quicker euphoric response and it can reduces the withdrawal symptoms in those who had history of other drug substance abuse like tramadol and opiate.

Although the severity grade of the patients in the studied patients didn't correlated to the age, delay time or the amount ingested, Rietjens et al., (2021) concluded that higher PGB doses result in more severe poisonings but large inter-individual differences exist in the response to PGB.

In the present study most of cases didn't show changes in the vital signs except for sinus tachycardia observed in 22.6% and tachypnea in 32.3% of cases. This is in agreement with Ozturk & Morkavuk, (2019) who reported that PGB is relatively safe and mostly causing tachycardia and hypotension. Also, there are many reported cases of PGB toxicity with normal vital signs (Wood, et al., 2010; Şengüldür et al., 2018)

Disturbed consciousness was the most common manifestation in the current study and most of comatose patients were in coma grade 0 and 1. Other CNS symptoms included convulsion and agitation also recorded with significant relation between the long hospitalization period and occurrence of DCL and convulsion.

Crossin et al., (2019) noticed that the severity of CNS presentations increased with drug combination with other drugs affect CNS, also Dufayet et al., (2020) reported that PGB toxicity was usually asymptomatic or resulting in non-severe neurological symptoms in all cases.

Although convulsion is uncommon CNS presentation secondary to acute PGB toxicity, it was reported in Slocum et al., (2018) and Ozturk & Morkavuk, (2019) in their studied cases. Like other anticonvulsants such as lamotrigine and carbamazepine, PGB has proconvulsant characteristics in overdose. It can lead to self-limiting seizures especially with large doses (Isoardi et al., 2020), but it potentially important effect as it need meticulous observation for the intoxicated cases and may need longer hospitalization period as observed in the current study.

Cyanosis was a presenting sign in 3.2% of studied patients which significantly increased the period of hospital admission. Gomes et al., (2018) reported that respiratory depression with PGB may be related with several risk factors including compromised respiratory function, respiratory or neurological disease, renal impairment, those using concomitant CNS depressants and people older than 65 years might be at higher risk of experiencing these events.

Hypoglycemia was observed in 13% of studied patients. The mechanism of hypoglycemia is not completely understood but it is thought to be related to the secretion of insulin through the action of PGB on the α2δ subunit of voltage-sensitive Ca2+ channels in the β cells of the pancreas (Yamada et al., 2022).

Respiratory acidosis which observed in 29% of patients was associated with decreased conscious level and respiratory depression as explained by Gomes et al., (2018). Kyung, (2017) also observed development of respiratory acidosis with CO2 retention in the studied patient who received large dose of PGB and the patient required tracheal intubation and MV. After discontinuation of PGB the patient recovered.

Metabolic acidosis also recorded in the current study and it was developed after convulsions or agitation. This is in accordance with reported cases in which patients developed metabolic acidosis and it was accompanied by development of seizures (Tanyildiz et al., 2018; Ocak and Uçar, 2019).

Although the presence of acidosis was significantly associated with longer hospital stay in the current study until treat the cause and normalize the result, both respiratory and metabolic acidosis wasn't significantly associated with increased severity grade of the studied patients.

In our study; ECG abnormalities were in the form of sinus tachycardia (25.8%) and widening of QRS complex duration (3.2%), which resolved spontaneously in the next day and there wasn't significant correlation with the developed ECG finding and the patient severity. This effect on QRS complex could be explained by the effect of PGB on L-type voltage gated Ca++ channels in the heart (Aksakal et al., 2012).

As regards the treatment, generally, PGB doesn't have antidote and the treatment is mainly supportive. Although it can be eliminated by extra-corporeal methods such as hemodialysis and/or hemofiltration, it is treated by supportive measures in most of cases (Wood et al., 2010). In the current study most of patients needed mainly supportive treatment in the form of IV fluids beside observation of the vital signs. Mechanical ventilation indicated for 9.68% of patients to control their conditions where DCL lead to respiratory failure and respiratory acidosis which necessitate MV.

In the current study, 55% of patients needed ICU admission, this is agree with a study done by Browne et al., (2009) in which the clinical effects of acute PGB toxicity were studied, half of the cases were admitted to ICU unit and the other cases were admitted to inpatient. Causes of ICU admission in the current study included coma grad $\geq$ 1, convulsion, agitation, respiratory distress and need of MV.

The outcome in the current study was favorable where all patients completely recovered except for one case died. The legal restriction which was taken by Egyptian ministry of health lead to decrease number of patients and so affected the total number of deaths.

The majority of patients (45.1%) presented with minor severity (PSS 1). Dufayet et al., (2020) reported that most of the studied cases (77%) remained asymptomatic (PSS0) while 21% and 2% developed minor (PSS1) or moderate (PSS2) neurological symptoms, respectively. No severe complications or fatalities were reported. Generally isolated acute PGB toxicity is largely benign, resulting in mild sedation with low fatality (Isoardi et al., 2020).

The experimental part was designed to study the histopathological effect of acute PGB toxicity on the rat brain. To our knowledge, there are few published articles have addressed the histopathological effects of acute PGB toxicity and most of studies showed the chronic and subchronic effects.

The current study showed degenerative changes affect cells of both cerebral cortex and cerebellum with preserved architecture layers. These changes could be explained by the effect of PGB on alpha 2 delta 1 (a2d-1) subunit of voltage gated calcium channel which are widely distributed in peripheral and central nervous system (Patel and Dickenson, 2016).

Ali and Hassan (2021) also observed significant degenerative changes in the neurons of cerebral cortex and cerebellum with increased cellularity and irregular distribution of the astrocytes, in acute PGB toxicity group.

In the current study there was decrease in gray matter volume and focal area of brain tissue loss and fibrous tissue deposition (cerebral cortex). In a study done by Puiu et al., (2016) altered brain structure and decrease in grey matter volume were noticed after short term administration of PGB in patients with fibromyalgia.

This decrease in gray matter volume may explain the appearance of convulsions as a symptom of acute PGB toxicity as shown in a study done by Huang et al., (2011), on patients with epilepsy as they found that patients showed significant decreases in gray matter volume detected in their MRI in both cerebrum and cerebellum compared to healthy controls.

These results were supported by few case reports that proved that PGB toxicity was the cause of encephalopathy and triphasic waves found on EEG of these cases (Mihir et al., 2017; Anand & Kaplan, 2018).

Pregabalin induced encephalopathy is like any type of acute toxic encephalopathy which is a condition of acute global cerebral dysfunction manifested by disturbed conscious level, behavior changes, and/or seizures in the absence of primary structural brain disease. A common mechanism is interruption of polysynaptic pathways and altered excitatory-inhibitory amino acid balance (Chiriboga et al., 2017).

The pathophysiology of acute toxic encephalopathy accompanied by alterations in central nervous system homeostasis that activate microglial cells and infiltrating myeloid cells, astrocytes, and oligodendrocytes. This process releases cytokines that have independent neurotoxic effects (Chiriboga et al., 2017).

Also, Criswell et al., (2012) explained these histopathological effects on the brain tissue by oxidative stress induced by PGB, Magar et al., (2020) mentioned that hypoxic or direct toxic effect of PGB could lead to this neurotoxicity.

Conclusion

The clinical course of PGB intoxication is mostly mild self-limiting and most of patients were discharged on the next day of admission. Drowsiness then coma grade I were the most common central nervous system (CNS) manifestations of acute PGB toxicity. The experimental study of PGB toxicity on albino rats' brains concluded that PGB has degenerative effects on brain nerve cells in the form of fibrosis, focal areas of hemorrhage and necrosis. The mean optic density in toxic group was decreased in comparison to control group so there was significant relation between mean optic density and acute PGB toxicity.

Recommendations:

Further large-scale surveillance should be done to study the prevalence of PGB abuse in Egypt. More researches should be conducted to study the effect of acute and chronic PGB toxicity on rats' other organs.

Conflict of interest

The authors declare that they have no conflict of interest.

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دراسة لحالات التسمم الحاد من البريجابالين التى تم إدخالها فى مركز علاج التسمم بمستشفيات جامعة عين شمس وتأثيره الباثولوجى على المخ فى فئران التجارب: دراسة إكلينكية وتجريبية

داليا حمدى لاشين[١] و نهلة حسن طلبة شريف[١] و إيناس أبو الوفا التفتزانى[١] و سوزى صبحى عطا الله[٢] و سلمى إبراهيم عبد القادر[١]

الملخص العربى

الخلفية العلمية: يعتبر عقار البريجابالين مضادًا للصرع وعلاجًا فعالًا لآلام الأعصاب. يعمل عن طريق تثبيط بعض قنوات الكالسيوم قبل المشبكي ويقلل من النواقل العصبية المثيرة. وهناك كثير من الآثار الخطيرة التى قد نحدث بعد التعرض للبريجابالين إلى جانب تأثيره الإدماني.

هدف العمل: دراسة نمط السمية الحادة للبريجابالين لدى المرضى الذين تم حجزهم بمركز علاج التسمم - مستشفيات جامعة عين شمس وتأثيراته المرضية النسيجية بعد الجرعة السامة الحادة على دماغ الفئران البيضاء البالغة.

الطريقة: تكونت الدراسة من جزأين. تم إجراء الجزء الاكلينكى على ٣١ مريضًا تم حجزهم بمركز علاج التسمم بجامعة عين شمس ولديهم تاريخ من سمية البريجابالين الحادة. تم إجراء الجزء التجريبي على ثلاثين فأرًا بالغًا من الجرذان البيضاء، مقسمة إلى مجموعتين (تلقت مجموعة السمية الحادة جرعة مفردة حادة من ٥٠٠٠ مجم / كجم من البريجابالين وتلقت المجموعة الضابطة محلول ملحي طبيعي).

النتائج: يؤدي التعرض الحاد للبريجابالين إلى سمية خفيفة ومعظم الأعراض متعلقة بالجهاز العصبي المركزي واكثر الاعراض هى فقدان الوعى، مع وجود علاقة كبيرة بين طول مدة الحجز بالمستشفى وحدوث فقدان الوعى. أظهرت النتائج التجريبية أن القشرة المخية (CC) والمخيخ بها تنكسًا في الخلايا العصبية ، وتضخمًا مع انحلال نوى النواة ، وزيادة ملحوظة في الخلايا الدبقية مع بروتين حمضي ليفي دبقي إيجابي (GFAP) الحبيبات السيتوبلازمية في القشره المخيه للمجموعة السامة.

الخلاصة: إن مسار تسمم البريجابالين هو في الغالب محدود ذاتيًا خفيفًا ومعظم المرضى يخرجون من المستشفى في اليوم التالي من القبول. خلصت الدراسة التجريبية إلى أن البريجابالين له تأثيرات تنكسية على الخلايا العصبية في مخ الفئران.

التوصيات: مزيد من الدراسات مع حجم عينة أكبر لتقييم السمية الحادة والمزمنة للبريجابالين.

١. قسم الطب الشرعى والسموم الاكلينكيه كليه الطب جامعة عين شمس جمهورية مصر العربية

٢. قسم الهستولوجى وبيولوجيا الخلية كلية الطب جامعة عين شمس جمهورية مصر العربية