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Comparative Study of the Hepatotoxic and Nephrotoxic Effects of Gabapentin versus Clonazepam in Rats

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Abstract

Background: Gabapentin is an anticonvulsant adjunct and a neuropathic pain analgesic that has become widely abused in Egypt in the last decade after scheduling its analogue pregabalin in 2012. Clonazepam is an antiepileptic drug approved for treatment of various types of seizures. Clonazepam has a high-recognized potential for abuse, as well as tolerance, physical dependence, and ultimately addiction.
The aim of this research is to study and evaluate the hepatotoxic and nephrotoxic effects of sub chronic misuse of gabapentin in relation to a well-known highly addictive drug like clonazepam.
Methods: Using a previously validated animal model, 30 healthy adult male albino rats were included, divided into three equal randomization groups: group I (normal saline), group II (clonazepam misuse), and group III (gabapentin misuse). Rats in each group received the respective drugs for 50 days. After this time, liver enzymes (AST, ALT and ALP) and renal biomarkers (urea, creatinine and uric acid) were measured and hepatic and renal histopathology was evaluated.
Results: Both gabapentin and clonazepam were associated with numerous biochemical and histopathological alterations relative to controls. Clonazepam was associated with higher elevation of (AST and ALT) as well as more histopathological changes to a greater degree than gabapentin.
Conclusions: The study underscores the importance of careful monitoring of protein markers of hepatocyte injury as well as renal biomarkers in patients receiving gabapentin for long-term duration, either as a misuse or in addict patients during withdrawal.

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Keyword: Gabapentin, Clonazepam, Hepatotoxicity, nephrotoxicity, histopathology.

Introduction

Gabapentin is considered a gamma amino butyric acid (GABA) analog [1], but it is not a GABA agonist. It does not bind to GABA A, GABA B, benzodiazepine, opioid or cannabinoid receptors, but it can increase GABA concentration and decrease excitatory neurotransmitters glutamate and noradrenaline concentrations by binding to and blocking the alpha-2-delta-1 subunit of pre-synaptic, voltage-gated calcium channels. This leads to the inhibitory effects in the central and peripheral nervous system, which explains its anticonvulsant effects [2]. Its mechanisms of analgesic actions and neuropathic pain relieving effects are not fully understood, although some have speculated that gabapentin may reduce the release of pain-related peptides (substance P) and may decrease opioid-induced hyperalgesia [3].

Gabapentin was initially approved by the United States Food and Drug Administration (FDA) in 1993 for the treatment of epilepsy as an adjunct to anti-convulsant therapy. In 2004, it was also approved as an analgesic for post-herpetic neuralgia [2]. The European Medicines Agency (EMA) for neuropathic pain and epilepsy [4] approved Gabapentin in 2006. Furthermore, the UK National Institute recommended gabapentin as a first-line treatment for all neuropathic pain for Clinical Excellence (NICE) [5].

It is used widely off-label to treat several disorders, including neuropathic pain conditions, insomnia, anxiety, bipolar and borderline personality disorder, drug and alcohol addiction, migraine headaches, vertigo, pruritic disorders and menopausal conditions. It is estimated that the overwhelming majority of gabapentin use is for off-label conditions, with estimates ranging from 83 to 95% of all users [6]. Gabapentin is used concomitantly with cocaine, opioids, benzodiazepines, alcohol, and even antipsychotics, to achieve a heightened euphoria among drug abusers [7, 8, 9].

Clonazepam is a high-potency; long-acting anticonvulsant and anxiolytic benzodiazepine approved in 1976 by the FDA for treatment of seizure, panic disorders and non-convulsive status epilepticus. It also has many off-label indications for its use, such as restless leg syndrome, acute mania, insomnia, sleep behavior disorder and tardive dyskinesia [10, 11]. Clonazepam is an agonist at GABA-A receptors. Its GABAnergic action occurs by increasing chloride channel opening frequency, resulting in neuronal hyperpolarization. Decreased neuronal firing leads to calming and relaxation, and in some patients, euphoria [12]. The development of euphoria, and dysphoria when it is discontinued, has been suggested as reinforcing factors for its misuse [13].

Although clonazepam is primarily metabolized in the liver, gabapentin does not appreciably metabolized in humans and is 100 % eliminated as unchanged drug. Both clonazepam and gabapentin are renally excreted. Monitoring the dosage and clinical effect is required in patients with renal or hepatic impairment. Diminished metabolism or elimination may result in accumulation of the drugs and their toxic metabolites, especially in patients at extremes of age such as infants or the elderly. Clonazepam, and to lesser extent gabapentin, are relatively contraindicated in patients with severe hepatic or renal dysfunction [14].

The clinical manifestations of most overdoses of either clonazepam or gabapentin are typically minimal and self-limited, commonly causing somnolence, mild sedation, lethargy, dizziness, slurred speech, nystagmus and ataxia without respiratory depression. Thus, treatment usually requires only minimal supportive and symptomatic care [15]. Patients have tolerated Gabapentin ingestion of large doses, such as 50,000 mg and up to 90,000 mg without resulting in major adverse effects [16]. These doses far exceed 3600 mg/day, which is the FDA-recommended maximum daily dose [2, 7, 17].

A less common complication with clonazepam is exacerbation of dyslipidemia, development of fatty liver, and clonazepam-induced steatosis or steatohepatitis, in patients with preexistent dyslipidemia, and liver disease, respectfully [18].

Although clonazepam misuse is a long-standing health problem, gabapentinoid misuse/abuse are still developing and not fully understood, but are clearly related to the desirable sedation and/or euphoria users seek. This is not only for abuse, but also for self-medication to treat a variety of undiagnosed or undertreated medical conditions [8, 19]. In 2012, after Egyptian health authorities designated pregabalin from schedule three "drugs with a moderate to low potential for physical and psychological dependence" to schedule one "highly restricted drugs with a high potential for abuse", gabapentin misuse and abuse has been increased [20].

The deleterious effects of clonazepam are well understood, and many studies have evaluated the pharmacological and toxicological profile of clonazepam. The toxicological effects of gabapentin are less studied, and are still being discovered and described. A limited number of publications [21, 22, 23] and case reports [24, 25 26, 27, 28] have mentioned and discussed the hepatotoxic effects of both clonazepam and gabapentin.

The study presented here directly compared the hepatotoxic and nephrotoxic effects of high dose, sub chronic gabapentin exposure relative to that of a well-known highly addictive drug like clonazepam in adult male albino rats, to simulate exposure in human addicts. The specific aims are to compare protein markers of hepatocyte injury (AST, ALT, ALP), renal biomarkers (urea, creatinine and uric acid) and hepatic and renal histopathological changes among the study groups.

Methods

Study locality and ethical approval:

This prospective experimental randomized controlled study was conducted at **Animal House of Research Institute of Ophthalmology (RIO)** – in collaboration with the institutional Animal Care and Use Committee, Faculty of Medicine, Cairo University, Egypt (CU-IACUC). Approval of (CU-IACUC) was obtained **(code number is CU-III-F-78-22).** The National Research Council's Guide was followed as regard the use and care of laboratory animals.

Animals
Thirty healthy adult male wistar albino rats (Rattus norvegicus), each weighing 200-250 grams were used. They were held in plastic cages at a constant temperature of 25°C under standard laboratory conditions of 12-h dark and 12-h light cycles. The standard laboratory diet that used for feeding was "ad libitum", pellets and water. Animals were randomly assigned to either control, clonazepam, or gabapentin each with 10 rats.

Drugs and chemicals
- Clonazepam (Rivotril® 2 MG 30 tablets F.HOFFMAN LA ROCHE, Egyptian Pharmaceutical Trading Company).
- Gabapentin (Neurontin® cap 300 mg gabapentin powder, Neurontin® Pfizer Company, Cairo, Egypt under license of Pfizer Inc., USA).

The rationale for the doses
The initial clonazepam dose was equal to the daily therapeutic dose of 2 mg/day, while the dependent dose is (20 mg/day), with conversion to the rat dose using Paget calculation [29, 30, 31]. The initial gabapentin dose was equal to the daily therapeutic dose of 360 mg/day, while the dependent dose is 3600 mg/day, with conversion to the rat dose according to Paget equation [2, 7, 17, 32]. **Paget equation:** The equivalent dose for a rat weighing 200 gm is = 18/1000 x average adult human therapeutic daily dose [33, 34].

Study design
Group I (control): Each rat received one mL 0.9% normal saline/day orally by gavage for 50 days and used as negative control group.

Group IIa (clonazepam misuse): Each rat received an initial dose of 0.036 mg/day (0.18 mg/kg/day) of clonazepam dissolved in normal saline 0.9% orally by gavage for three days. The dose was progressively increased by adding the starting dose of 0.036 mg to the total dose every three days for 30 days. At that time, the maximum therapeutic or dependent dose (0.36 mg/day) was reached which was reported and usually produces the dissociative effects and desired euphoria in human addict**.** This dependent dose (0.36 mg/day) was given daily for an additional twenty days [29, 30, 31].

Group IIIa (gabapentin misuse)
Each rat received an initial dose of 6.48 mg/day (32.4 mg/kg/day) of gabapentin dissolved in normal saline 0.9% orally by gavage for three days. The dose was gradually increased by adding the starting dose of 6.48 mg/day every three days for 30 days. At that time, the maximum therapeutic or dependent dose (64.8 mg/kg/day) was reached which was reported and usually produces the dissociative effects and desired euphoria in human addict. This dependent dose was given daily for an additional twenty days [2, 7, 17, 32].

Sampling
At the experiment conclusion, to avoid any chemical contamination of the tissues, cervical decapitation was used to euthanize the rats [35, 36, 37]. Blood samples were obtained from the abdominal aorta; sera were separated by centrifuging at 5000 rpm for 15 min and were frozen at - 80°C for biochemical analysis. The liver and kidneys were excised from each rat; then washed with cold saline, sectioned, and prepared for histopathological examination.

Methods

Evaluation of protein markers of hepatocyte injury:
Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured by colorimetric kinetic assays using commercially available kits (Diamond Diagnostic, Egypt) following the manufacturer's instructions.

Evaluation of kidney functions
Serum Creatinine was measured by standard Jaffe method (colorimetric kinetic method) using commercial kits (Diamond diagnostic, Egypt). Serum urea was measured by Brethelot enzymatic colorimetric assay (Diamond diagnostic, Egypt). Serum Uric acid was measured by uricase method, colorimetric assay (Diamond diagnostic, Egypt). The concentration was measured against known standard concentrations according to the manufacturer.

Histopathological study
Specimens of the livers and the kidneys were put in 10% formol-saline; paraffin blocks were made, sectioned at 5-µm-thickness, and stained with hematoxylin and eosin (H & E) [38].

Histopathological score
Hepatic histopathological score was determined in 10 randomly chosen, non-overlapping fields. The tissue was evaluated for histopathological changes including: venous congestion, necrosis, hydropic degeneration of hepatocytes and lymphatic infiltration. The score grades were - (no lesion), + (mild damage), ++ (moderate damage), +++ (severe damage) [38].
Renal histopathological score was determined in 10 randomly chosen non-overlapping fields. This score involved some histopathological changes in the renal corpuscle, in renal tubules degeneration, and in the interstitial mononuclear cell infiltration and hemorrhage. The score grades were - (no lesion), + (mild damage), ++ (moderate damage), +++ (high damage) [38].

Statistical Analysis
The collected data were processed, coded, and analyzed using SPSS version 27 for Windows ® (IBM SPSS Inc, Chicago, IL, USA). The normality of distribution for the analyzed variables was tested using Kolmogorov-Smirnov test assuming normality at $p > 0.05$. Parametric data were expressed as mean ± standard deviation, while the non-parametric data were expressed as median (range). The one-way analysis of the variance (one-way

ANOVA), was used to compare the normally distributed quantitative variables and Krauskal Wallis test was used as test of significance comparing independent, non-parametric quantitative data. The highly statistically significant (HS) value was ($p \leq 0.001$), while the accepted significance level was ($p \leq 0.05$) and non-statistically significant (NS) was ($p > 0.05$).

Results

1) Evaluation of biochemical markers

Both clonazepam and gabapentin treatment **(G II, III)** had elevated serum ALT, AST and ALP relative to the control group **(G I)** ($p < 0.001$).

Both AST and ALT elevation in the clonazepam group **(G II)** had highly significant elevation relative to the gabapentin group (G III) (p 0.001 & 0.005) respectively **(table 1).**

Similarly, urea, creatinine and uric acid serum levels had been increased in treated groups **(G II, III)** as compared to the control group **(G I)** ($p < 0.001$) but there were no significant results in between clonazepam and gabapentin groups **(table 2).**

Table 1: Hepatic enzymes within the study groups

Variables Mean ± SD	Group I Control (n= 10)	Group II Clonazepam misuse (n= 10)	Group III Gabapentin misuse (n= 10)	Significance test
AST (IU/L)	**21.10 ± 2.51**	**53 ± 5.35**	**44.90 ± 4.38**	**F= 152.183 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.001***	
ALT (IU/L)	**19.20 ± 2.04**	**48.1 ± 2.45**	**41.50 ± 2.95**	**F= 362.164 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.005***	
ALP (IU/L)	**532.70 ± 18.59**	**670 ± 58.64**	**661.50 ± 40.35**	**F= 32.805 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.896**	

F: One-Way ANOVA
***:** Statistically significant ($p \leq 0.05$)
P1: Significance in relation to G I
P2: Significance in relation to G II
N: number of rats

Table 2: Renal biomarkers within the study groups

Variables Mean ± SD	Group I Control (n= 10)	Group II Clonazepam misuse (n= 10)	Group III Gabapentin misuse (n= 10)	Significance test
Urea	**21.80 ± 2.10**	**34.20 ± 3.26**	**32.50 ± 3.34**	**F= 51.766 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.415**	
Creatinine	**0.60 ± 0.04**	**0.80 ± 0.05**	**0.80 ± 0.06**	**F= 51.589 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.996**	
Uric acid	**3.18 ± 0.15**	**5.65 ± 0.41**	**5.30 ± 0.37**	**F= 150.672 P < 0.001***
P1		**< 0.001***	**< 0.001***	
P2			**0.077**	

F: One-Way ANOVA
***:** Statistically significant ($p \leq 0.05$)
P1: Significance in relation to G I
P2: Significance in relation to G II
N: number of rats

2) Evaluation of hepatic histopathological changes:

Liver sections obtained from the Control group (I) had normal histopathologic appearance for all variables. Hepatocytes were grouped in cords emanating from central veins, demonstrating the classical hepatic architecture. Hepatocytes were polyhedral with acidophilic granular cytoplasm and central vesicular spherical nuclei. Hepatic sinusoids appeared as narrow gaps between hepatic cords lined by flat endothelial cells and a few numbers of Kupffer cells. At the periphery of the hepatic lobules, portal tracts consisted of a branch of the portal vein and a bile duct (Fig.1).

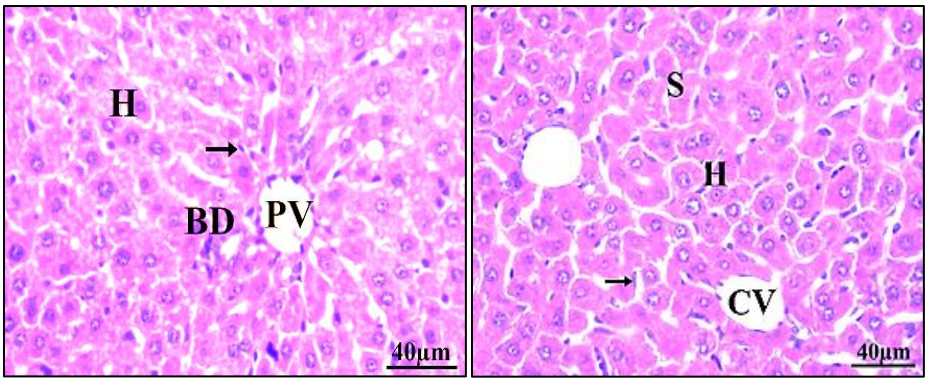

Figure 1: H&E-stained hepatic sections: Control group (I) revealed that the hepatocytes were grouped in cords emanating from the central veins (CV), demonstrating the classical hepatic architecture. Hepatocytes (H) were polyhedral with acidophilic granular cytoplasm and central vesicular spherical nuclei. Hepatic sinusoids (S) were lined by flat endothelial cells and few numbers of bulging Kupffer cells (Arrow). At the periphery of the hepatic lobules, portal tracts consist of a branch of the portal vein (PV) and a bile duct (BD).

Liver sections from the Group II (clonazepam misuse) demonstrated hydropic degenerated cells, altered lobular shape, nuclear degradation in certain areas, disruption of normal hepatic cells, and moderate fatty degeneration and necrosis. There were enlargements of the hepatic central vein with a disconnected wall. Lymphocytic infiltration was observed in the portal region with congested portal vein. Some cells were enlarged and swollen and their typical shape was lost, as ghost nuclei were observed (Fig. 2).

Similarly, liver sections from Group III (gabapentin misuse) demonstrated hydropic deteriorated cells, modified lobular structure and nuclear disintegration in some locations, disarray of regular hepatic cells, high fatty degeneration, and necrosis. Hepatic central vein congestion and disconnected wall were found. Lymphocytes were seen in the portal area with a thick wall around the portal vein and normal bile duct appearance (Fig. 3).

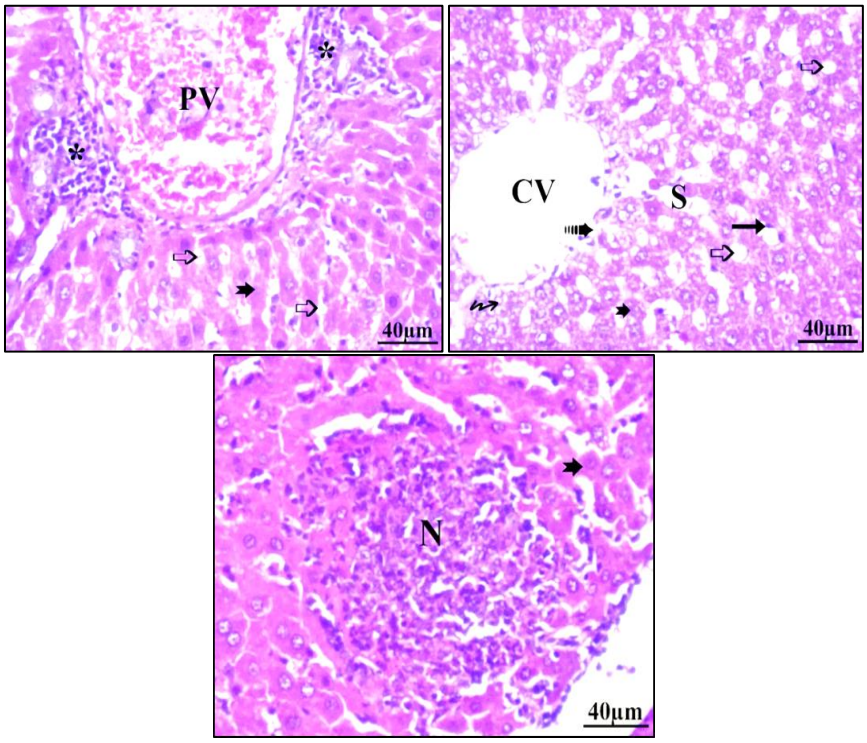

Figure 2: H&E-stained hepatic sections: from group II (clonazepam misuse) hydropic degenerated cells, altered lobular shape, nuclear degradation (notch arrow) in certain areas, disruption of normal hepatic cells, necrosis (N), and moderate fatty degeneration (hollow arrow). There were enlargements of the hepatic central vein (CV) with a disconnected wall (pointed arrow). Lymphocytic infiltration (*) was observed in the portal region with congested portal vein (PV). Some cells were enlarged and swollen (wavy arrow) and their typical shape were lost, as ghost nuclei were observed (notch arrow).

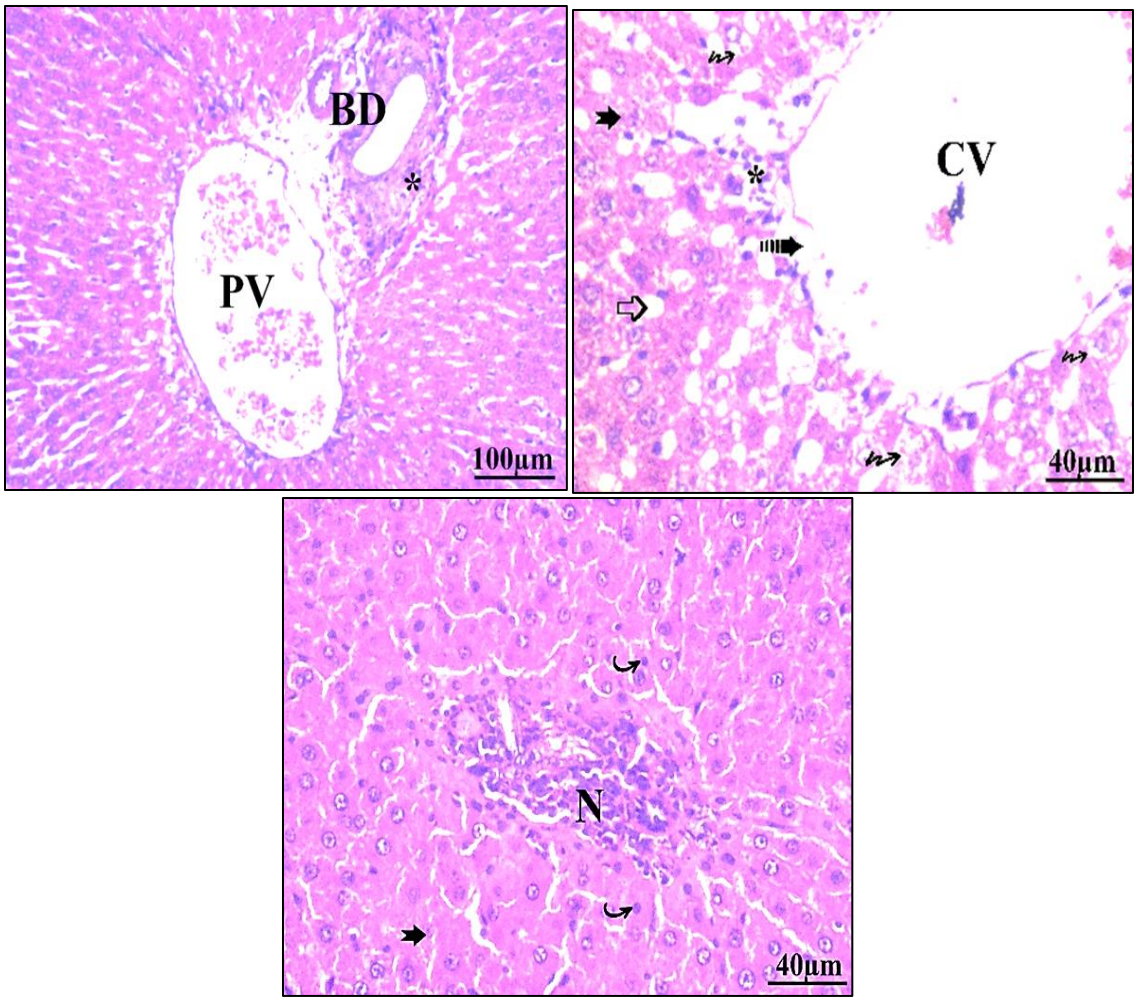

Figure 3: H&E-stained hepatic sections: from group III (gabapentin misuse) hydropic deteriorated cells (Wavy arrow), modified lobular structure and nuclear disintegration (notched arrow) in some locations, disarray of regular hepatic cells, high fatty degeneration (hollow arrow) and necrosis (N) were evident. Hepatic central vein (CV) congestion and disconnected wall (dotted arrow) were found. Lymphocytes (*) were seen in the portal area with a thick wall around the portal vein (PV) and normal bile duct (BD) appearance.

Each drug resulted in numerous histopathological alterations in comparison to the control. Each drug triggered disturbance of hepatic tissue, severe congestion, moderate lymphocytic infiltration. Clonazepam prompted lymphocytic infiltration, dilated portal vein, dilated sinusoid and necrosis to a greater degree than gabapentin (table 3).

Table 3: Comparison of hepatic histopathological score between study groups

	Group I Control	Group II Clonazepam misuse	Group III Gabapentin misuse
Congestion blood vessel	-	+++	+++
Lymphatic infiltration	-	++	+
Dilated portal vein	-	++	+
Dilated sinusoid	-	++	+
Necrosis	-	++	+
Fatty degeneration	-	++	+++
Hepatocyte degeneration	-	++	++

3) Evaluation of renal histopathological changes

Light microscopy analysis of H&E-stained sections from group (I) control revealed that renal cortex had typical histological architecture. Renal corpuscles, glomerular capillaries, Bowman's capsules, and urine space filled the renal cortex. The proximal convoluted tubules were bordered with cuboidal epithelial cells and had limited lumens. The distal convoluted tubules were bordered with short cuboidal cells with an acidophilic cytoplasm that was less granular with rounded nuclei (Fig. 4).

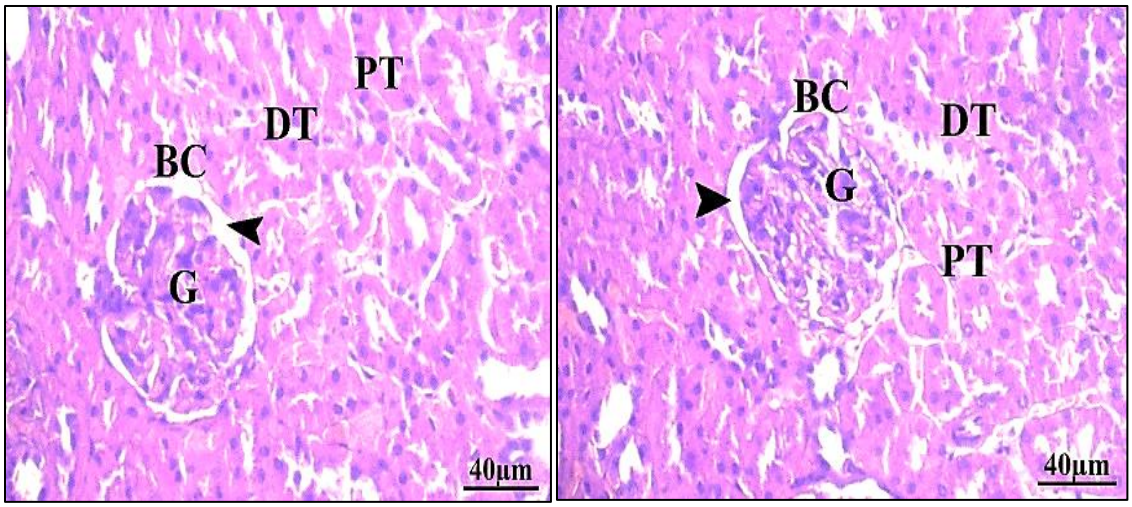

Figure 4: H&E-stained sections: Control group (G1) revealed the renal cortex's typical histological architecture. Renal corpuscles, glomerular capillaries (G), Bowman's capsules (BC), and urine space (arrowhead) filled the renal cortex. The proximal convoluted tubules (PT) make up the majority of the renal cortex and are situated close to the renal corpuscles. They were bordered with cuboidal epithelial cells and had limited lumens. The distal convoluted tubules (DT) had a broad lumen and were bordered with short cuboidal cells with an acidophilic cytoplasm that was less granular with rounded nuclei.

While sections from group II (clonazepam misuse) showed injured irregular congested glomeruli. The majority of tubular cells exhibited vacuolated cytoplasm and nuclei with vesicles. Peritubular space dilation and capillary congestion were observed. In the proximal and distal convoluted tubules, cell nuclei displayed total or partial damage, indicating disintegration. Moreover, the nuclei compacted (pyknosis). Some tubules revealed irregular casts (Fig. 5). On the other hand sections from group III (gabapentin misuse) showed widened tubular lumens with luminal casts. Most tubular cells had vesicles and vacuolated cytoplasm (PT, DT). Disintegrated tubular lining cells were also observed. Most glomeruli were swollen inside Bowman's capsule, congested; others are irregular segmented degenerated (Fig. 6).

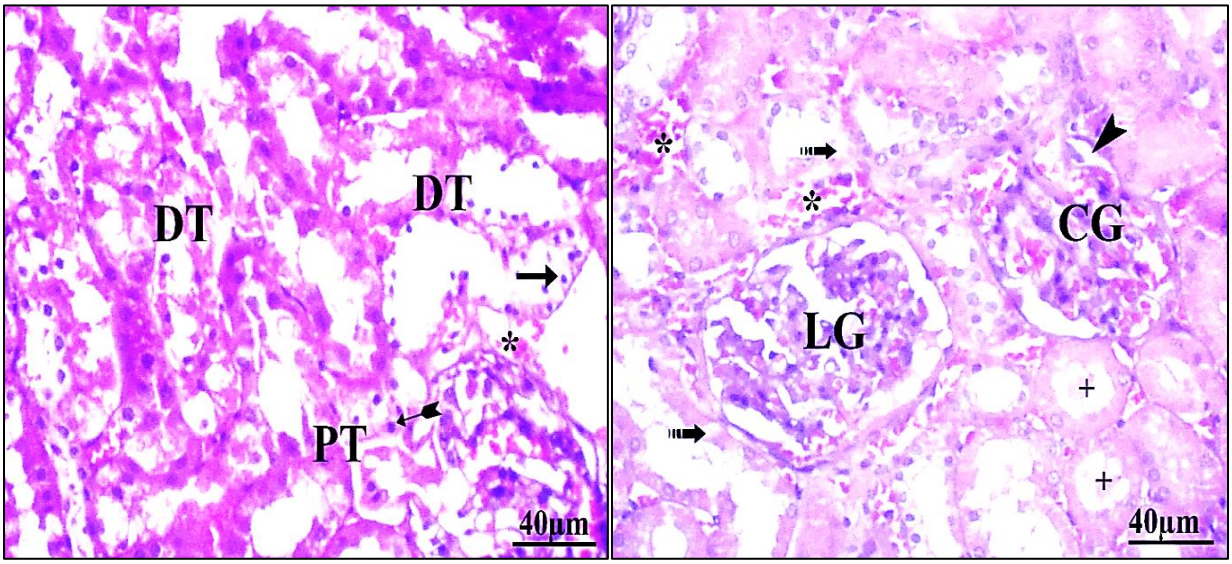

Fig. 5: H&E-stained renal cortical sections from group II (clonazepam misuse) showed injured irregular congested glomeruli (notched arrow). The majority of tubular cells exhibited vacuolated cytoplasm (arrow) and nuclei with vesicles. Peritubular space dilation and capillary congestion (*) were observed. In the proximal and distal convoluted tubules, cell nuclei displayed total or partial damage, indicating disintegration (doted arrow). Moreover, the nuclei compacted (pyknosis) (bifid arrow). Some tubules revealed irregular casts (+).

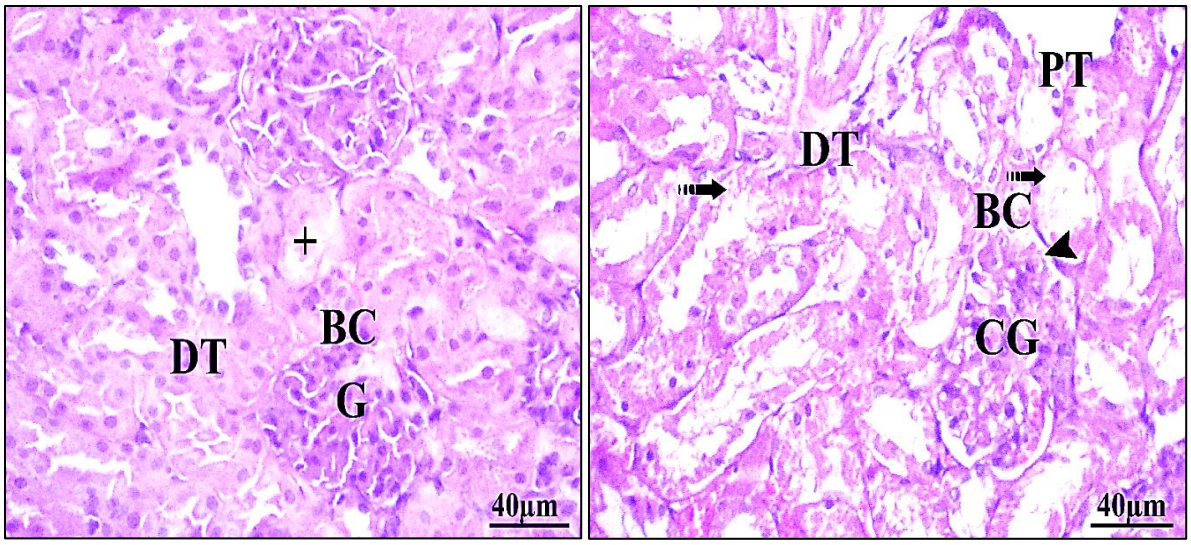

Fig. 6: H&E-stained renal cortical sections from group III (gabapentin misuse) showed widened tubular lumens with luminal casts (+). Most tubular cells had vesicles and vacuolated cytoplasm (PT, DT). Disintegrated tubular lining cells were also observed (doted arrow). Most glomeruli (G) were swollen inside Bowman's capsule (BC), congested (CG), others are irregular degenerated (arrow head). Few segmented (SG) and congested glomeruli (CG) were seen.

The two drugs showed many histopathological changes in comparison to the control. Each drug caused disorder of renal tissue, severe congestion, and mild lymphocytic infiltration. Clonazepam prompted renal tubular hydropic degeneration more than gabapentin (table 4).

Table 4: Comparison of renal histopathological score between clonazepam and gabapentin treated groups

	Group I Control	Group II Clonazepam misuse	Group III Gabapentin misuse
Congestion	-	+++	+++
Lymphatic infiltration	-	+	+
Atrophied glomeruli	-	-	-
Tubular hydropic degeneration	-	+	++
Swollen glomeruli	-	++	+++

Discussion

Substance misuse and addiction is a prevalent disease globally, with many negative health, economic, and social impacts. Misuse and abuse of gabapentinoid drugs, which has become particularly prevalent among the adolescents, have forced Egyptian health authorities to designate pregabalin as an addictive drug [39].

After the scheduling and subsequent restricted access to pregabalin, gabapentin misuse increased in Egypt, particularly since it can be purchased without prescription. Gabapentin misuse results from the use of high doses and frequent dosing to achieve euphorigenic and sedative effects [29, 40].

The presented here evaluated the hepatotoxic and nephrotoxic effects of gabapentin in sub-chronic high dose exposure relative to that of clonazepam in adult male rats. The study design was selected to replicate what may occur in humans misusing these substances. This experimental addiction rat model is consistent with that used frequently in previous research [41, 42, 43] and described in case reports [44, 45, 46].

This study demonstrates deleterious hepatotoxic effects of sub-chronic use of clonazepam, evidenced by both biochemical and histopathological changes. This is consistent with findings in many previous studies [18, 47, 48].

Sabry et al., 2022 [49] proved that animal groups received daily clonazepam for 50 days developed biochemical hepatotoxicity evidenced by elevation of liver transaminases (ALT, AST). Histopathological examination demonstrated cytoplasmic vacuolation and fatty degeneration with distortions in the vasculatures. Kupffer cells proliferation and infiltration of lymphocyte were observed. The authors explained these deleterious effects by increased oxidative stress in the form of increased malondialdehyde (MDA), with decreased total antioxidant activity like glutathione (GSH) contents and glutathione S transferase (GSTs) enzyme.

There are many case reports of clonazepam induced liver injury. Fortunately all these reports have reported complete recovery without evidence of residual or chronic injury after clonazepam induced hepatotoxicity. Neither chronic liver injury nor acute fulminant hepatic failure resulting from clonazepam use has been found [18, 50, 51, 52, 53].

In the study we present, gabapentin also induced hepatotoxicity evidenced by biochemical and histopathological changes. This is similar to previous studies and case reports demonstrating

that high doses of gabapentin were associated with elevated biochemical markers of hepatotoxicity [24, 25] and histological changes [26, 27, 28].

Zimmerman, 1999 [54] stated that the likelihood score of clonazepam induced hepatic injury is (D) (possible but rare cause of clinically apparent liver injury). The cause of clonazepam induced liver injury is probably a rarely produced toxic intermediate metabolite. Hepatic injury with the usual daily doses of clonazepam (0.5 to 2mg) is rare.

Nair et al., 2017 [52] stated that serum ALT elevations are uncommon with clonazepam therapy and clinical evident acute liver injury is extremely rare. However, numerous case reports of clonazepam hepatotoxicity exist [18, 50, 51, 52, 53]. The latency period in acute liver injury has ranged from few weeks to 6 months. The liver enzyme elevations patterns were usually mixed or cholestatic, but hepatocellular injury has also been reported [18; 50], which is usually mild to moderate in severity and self-limited. Autoantibody formation, rash or fever has not been described.

A case report by *Jackson et al.,2018* [25] concluded that gabapentin rarely caused drug induced liver injury, which may be cholestatic, hepatocellular or mixed picture of liver injury. A study by *Abdulhussein et al.,2022* [55] found that gabapentin treatment at recommended doses did not increase AST nor ALT. Gabapentin misuse commonly involves high doses, and thus lack of hepatotoxicity at therapeutic gabapentin dosing has little relevance to situations of misuse and abuse. The serum ALP was significantly increased so they concluded that gabapentin treatment at a dose of 400mg/kg significantly increased ALP; this which is usually associated with cholestatic liver disease, and may be associated with biliary obstruction.

Hepatotoxicity, both biochemically and histopathologically was more severe in our clonazepam rat group relative to the gabapentin group. Our data suggest that, for AST and ALT elevation as well as histopathological variables assessed, clonazepam is more hepatotoxic and still showed more nephrotoxic histopathological changes than gabapentin.

Our study detects nephrotoxicity after sub chronic high doses of clonazepam. Previous studies however were minimal authors stated that although it is a very rare adverse effect as compared to Carbamazepine or Valproic acid, prolonged use of clonazepam can induce acute kidney injury [56].

The study we present showed clonazepam induced nephrotoxicity on both biochemical and histopathological aspects. While evaluating chronic high dose of clonazepam on the kidney *Mahmoud et al.,(2020)* [57] stated that clonazepam may lead to dysuria, enuresis but no evidence of renal impairment, this based on study done to evaluate the incidence of anti-epileptic drugs (AEDs) to induce nephrotoxicity and they concluded that AEDs-induced nephrotoxicity is rare either in post-marketing reports and only reported in less than 1 in 1000 patients in drug product monographs. This discrepancy in results may be related to high doses of clonazepam used in addiction model.

The current study showed Degenerative lesions and disintegrated nuclei in renal cortex together with hydropic degeneration, tubular cast, and glomerular atrophy these changes has also been reported by *Badawy et al.,(2019)* [58] who studied the gabapentin teratogenic effect on maternal rats' pups treated with gabapentin during pregnancy and found kidney affection in the form of dilatation and vacuolar degeneration in the convoluted tubules with hemorrhage between the tubules with edematous glomeruli. At the ultra-structural level, obvious thickening of the glomerular basement membranes with irregular and fused foot processes of the podocytes were observed.

Many case reports recorded the gabapentin-induced nephrotoxicity [59, 60, 61, 62] who described significant deterioration in conscious level due to high doses gabapentin in chronic kidney disease patients. Furthermore, *Zand et al., 2010* [63] awarenessed health care professionals that occasioning overt toxicity; advanced age and comorbidity usually predispose these patients for toxicity. Gabapentin can induce renal failure although was very rare adverse effect. They also advised for prolonged follow-up for serum creatinine, urea, and uric acid results in patients received gabapentin for prolonged period.

Chronic high dose gabapentin exposure, which may occur as a result of therapeutic use as well as misuse, commonly results in hepatotoxicity and nephrotoxicity. Both have been reported by *Welson et al., 2021* [22] with elevation of AST, ALT, ADH, ALP, urea, creatinine as well as p53 gene expression and MDA, with concomitantly reduced GSH. Moreover, Gabapentin administration caused structural changes in the hepatic architecture mediated by apoptosis. This is evident by a positive immunoreaction for BCL2-associated X protein (BAX) and also by glycogen deposition in liver and kidney as evident by a weak Periodic acid-Schiff (PAS) reaction.

Conclusion

This comparative study provides important insights into the hepatotoxicity and nephrotoxicity associated with both drugs. The study's findings demonstrated that sub-chronic misuse of high doses of clonazepam and gabapentin is associated with numerous adverse hepatic and renal effects evidenced by biochemical and histopathological derangements. Both drugs demonstrate toxicity, with clonazepam being clearly more toxic than gabapentin in the model used. The study underscores the importance of careful monitoring of hepatic and renal functions in patients with long-term exposure to gabapentin or clonazepam. This includes both patients taking gabapentin or clonazepam as part of medical therapy, and patients misusing or abusing these medications. Further studies are needed to more fully understand the mechanisms of toxicity induced by these drugs, and to determine the optimal dosages and duration of treatment that minimize the risk of liver and kidney injury. Ultimately, this information can help clinical decision-making and improve patient outcomes.

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate
This study was conducted at Animal House of Research Institute of Ophthalmology (RIO) – in collaboration with the institutional Animal Care and Use Committee, Faculty of Medicine, Cairo University, Egypt (CU-IACUC). Approval of (CU-IACUC) was obtained (code number is CU-III-F-78-22). The National Research Council's Guide was followed as regard the use and care of laboratory animals.

Consent for publication
Not applicable, this study does not include publishing of personal data.

Availability of data and materials
Data supporting our findings can be found with the corresponding author. Data will not be shared. Please contact the author for data requests.

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Author Contribution
This work was carried out in collaboration with all authors. Mohammad El-Kattan and Ahmed Elshatory designed the study and wrote the protocol. Mahmoud Ahmed Khattab, Nada Elsayed Abdel-Roaf and Walaa Awad prepared the drugs and observed the experimental part of the study as regard housing, medication doses and animal behaviour. Fatma Abdel Wahab Abdel Maksoud and Maha Emad Eldien shared in the biochemical and histopathological analysis of the study. Finally, Mohammad El-Kattan and Abdullatif Aloumi managed the literature research, wrote and revised the final manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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Cover letter
- This manuscript is submitted to International Journal of Contemporary Research in Multidisciplinary because the information included is within the scope of the journal of medicine, toxicology.
- We confirm that all authors have agreed to the submission to the journal.
- We also confirm that the manuscript is not currently under submission in any other journal.

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