Original Article

Nephrotoxicity after Sub Chronic Misuse of Gabapentin and the Protective Role of Alpha-Tocopherol, an Experimental Study

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ABSTRACT:

Background: In Egypt, gabapentin (GBP) misuse and abuse have been increased in the last decade after scheduling of its analogue pregabalin in 2012. Although many studies confirmed the deleterious effects of pregabalin, those of GBP are minimal. **The aim of this research** is to study and evaluate the nephrotoxic effects of sub chronic high dose administration of GBP and the protective effect of alpha-tocopherol. **Methods:** Thirty five (35) healthy male albino rats were included. They were randomly divided into; four groups: group I (15 rats) which were subdivided into group Ia (5 rats) negative control (normal diet), group Ib (5 rats) positve control (normal saline), group Ic (5 rats) positve control (Corn Oil), group II which were further subdivided into group IIa (5 rats) (GBP misuse), group IIb (5 rats) (GBP withdrawal), group III (5 rats) (alpha-tocopherol) and group IV (5 rats) (GBP + alpha-tocopherol). All rats received the commenced drugs for 40 days. Serum levels of urea, creatinine and uric acid were measured. Kidney tissues were taken for histopathology. **Results:** GBP increased renal biomarkers levels, disrupted renal tissues and increased the number of degenerated cells. Alpha tocopherol treatment significantly attenuated the deleterious effects induced by GBP. **Conclusions:** High dose sub chronic administration of GBP was associated with nephrotoxic effects in rats. The histopathological effects were less appeared during withdrawal period. Alpha tocopherol protects against GBP induced impairment of kidney functions.

Keywords: Gabapentin, Misuse, Nephrotoxicity, alpha-tocopherol, Rats.

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I. Introduction

Gabapentin is an analog of gamma amino butyric acid (GABA) *(Maneuf et al., 2003)*. It does not bind to GABA A, GABA B, benzodiazepine, opioid or cannabinoid receptors, but it can increase GABA and decrease glutamate concentrations *(Smith et al., 2016)*. Its mechanisms of analgesic and anti-epileptic actions are unknown, although some have speculated that GBP may reduce the release of pain-related peptides and may decrease opioid-induced hyperalgesia *(Compton et al., 2010)*.

The US Food and Drug Administration (FDA) approved GBP initially in 1993 only as an adjunct to anti-convulsant therapy for treatment of epilepsy, but later in 2004, it was also approved for post-herpetic neuralgia as a sole analgesic *(Smith et al., 2016)*. Gabapentin was approved by The European Medicines Agency (EMA) in 2006 for certain types of neuropathic pain and epilepsy *(EMA, 2006)*. It was also recommended by The UK National Institute for Clinical Excellence (NICE) for all neuropathic pain as a first-line therapy *(NICE, 2013)*.

Gabapentin is used widely off-label to treat many disorders, including neuropathic pain conditions, insomnia, anxiety, alcohol and drug addiction, borderline personality disorder, bipolar disorder, menopausal conditions, migraines, vertigo and pruritic disorders. The mechanisms of action of GBP are unclear so it is assumed to have no abusive potential. In fact, the worldwide GBP off-label usage are reported to range from 83 to 95% *(Mersfelder and Nichols, 2016)*.

Many small sample studies reported that addicts used GBP as a catalyst to get and achieve the opioids euphoria and 'high' effect. Gabapentin abusers commonly tend to combine it with many drugs of abuse, such as cocaine, opioids, alcohol and even clozapine *(Evoy et al., 2017)*. Also it is widley used for the concept that it can decrease or supress the opioid withdrawal manifestation especialy somaic pain and distress *(Baird et al., 2014)*.

Both pregabalin and gabapentin are centrally acting γ-amino butyric acid (GABA) analogs, hence their name gabapentinoid drugs. Although they are structurally related to the inhibitory neurotransmitter GABA, they don't affect GABA receptors. Both have wide-ranging therapeutic actions with some differences. Gabapentin, pregabalin and GABA can all modulate voltage-activated Ca^{2+} channels. Pregabaline has the lowest abuse liability among scheduled drugs. Although pregabalin was classified as a controlled substance (Schedule V) by US Drug Enforcement Administration (DEA), uptill now GBP is not a DEA-scheduled drug *(Peckham et al., 2018)*.

There are many reasons behined misuse/abuse of gabapentinoid drugs most of them are not understood. Tolerance and craving vary greatly among abusers, they may abuse these drugs to achieve euphoria or altered mental status or misuse them to self-treat a variety of untreated or nondiagnosed medical problems *(Evoy et al., 2017)*.

Furthermore, after the Egyptian health authorities had moved pregabalin from the third schedule to the first one with the highly addictive substances list in 2012, the misuse

and abuse of GBP had been increased. Although GBP is not considered a controlled substance by the federal government, as of September 2022, GBP was classified as a controlled substance in few countries Alabama, Kentucky, Michigan, North Dakota, Tennessee, Virginia, and West Virginia *(Bassiony et al. 2016).*

Although GBP has potential of abuse, it has been described as a relatively safe drug even with high ingested amount up to 50,000 mg which is greatly exceeded the maximum daily dose of 3600 mg that recommended by the FDA *(Baird et al., 2014).* Higher doses used during substance abuse may be associated with tolerance, addiction and greater withdrawal symptoms. Also people experiencing withdrawal symptoms after stopping higher off-label doses of GBP *(Peckham et al., 2017).*

In a survey, documented cases of withdrawal symptoms were reported in people who took an average daily dose of 3000 mg/day, ranging from 600 mg to 8,000 mg/day for at least 3 weeks. It was found that reported withdrawal occurred within 12 hours to 7 days of discontinuation of the medication *(Mersfelder and Nichols, 2016).*

The most reported overdose clinical manifestations were minimal and include somnolence, mild sedation, dizziness, nausea and loose stooling. Only mild supportive and symptomatic management were required. Furthermore, a three poison control centers study in USA examined GBP toxicities between (1998 – 2000) reported only twenty cases of GBP overdose wherein the sole drug involved was GBP *(Klein-Schwartz et al., 2003).*

There is no approved medication to treat GBP withdrawal. Slow discontinuation can help to relieve symptoms. Behavioral and supportive medical care, such as treatments for nausea, anxiety or insomnia can help to support the withdrawal. Treatment for any other substance use disorder should be initiated (for example, opioid or alcohol use) if this accompanies GBP misuse *(Peckham et al., 2018).*

Vitamin E (Alpha-tocopherol, "Vit E") is known to decrease lipid peroxidation, toxic effects and oxidative damage caused by reactive oxygen species (ROS). Alpha-tocopherol is the most active form of vitamin E and considered as a strong antioxidant that acts by many mechanisms for example; it acts as a scavenger for free radicals, inhibiting peroxidation of lipids and suppressing apoptosis caused by ROS *(Rezq, 2014).*

In addition, Alpha-tocopherol acts like many important factors in peroxidase-dependent antioxidant defense complex system, such as glutathione peroxidase (GPx) and catalase enzymes. Furthermore, Alpha-tocopherol, such as a powerful biological antioxidant, acts by synergistic mechanisms with other antioxidants to decrease free radicals *(Duan et al., 2018).*

In Egypt, both GBP misuse and abuse have been increased in the last decade. Although several pregabalin deleterious effects have been reported in many studies, those of GBP are understood as well as understudied. Few studies in the literatures have discussed the nephrotoxic effects of GBP and the protective effect of Alpha-tocopherol in

GBP induced nephrotoxicity *(Evoy et al., 2021).*

Both GBP and vitamin E are lipophilic and fat soluble drugs. However, this combination hasn't been previously studied in literatures. The present work is the first, to our knowledge, to study the nephrotoxic effects of sub chronic misuse of high dose-gabapentin in an addiction model in albino rats to simulate what occur in human addicts and to study the protective role of Alpha-tocopherol. So, it can be considered as an adjuvant therapy for gabapentin dependence or for patients on prolonged GBP treatment as those complaining of chronic neuropathic conditions.

II. Material and Methods

Study locality:

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

Animals

Thirty five (35) healthy male albino rats, weighing (200-250) gram were included in the current research. They were put in plastic cages under a standard laboratory condition of 12-h dark and 12-h light cycle at a constant temperature of 25°C. The standard laboratory diet (libitum and pellet) and water were used for feeding. Animals will be randomly divided into four groups (5 rats each).

Drugs and chemicals

- Gabapentin (Neurontin® cap 300 mg gabapentin powder, Neurontin® Pfizer, Cairo, Egypt under license of Pfizer Inc., USA).
- Vitamin E (Vitamin E cap 1000 mg from Pharco Pharmaceuticals Cairo, Egypt).
- Corn oil (Pharco Pharmaceuticals Cairo, Egypt).

Study design:

Group I: control groups

- **Group Ia negative control:** each rat received normal diet (libitum, pellet and water) oral by gavage method for 40 days.
- **Group Ib positive control:** each rat received one ml 0.9% normal saline / day oral by gavage method for 40 days.
- **Group Ic positive control:** each rat received one ml corn oil oral by gavage method for 40 days.

Group II (Gabapentin treated groups):

- **Group IIa (GBP misuse):** Each rat received a starting dose of 32.4 mg/kg/day of GBP dissolved in normal saline 0.9% orally by gavage for three days. The dose was gradually increased by adding the starting dose every three days until they reached the dependent dose (64.8 mg/day) at the end of the 30 days. Furthermore, this last dependent dose (64.8 mg/day) was given every day for the next 10 days. After that, rats were sacrificed for evaluation of both

biochemical and histopathological renal changes *(Mersfelder and Nichols, 2016; Bonnet and Scherbaum, 2017).*

- **Group IIb (GBP withdrawal):** the same as group IIa but after fourty days GBP was suddenly stopped and rats were kept for another 10 days to evaluate GBP induced histopathological changes during withdrawal period after sudden stoppage of GBP *(Mersfelder and Nichols, 2016; Bonnet and Scherbaum, 2017).*

Gabapentin starting dose was equal to the daily therapeutic dose of 360 mg/day while the dependent dose is equal to the dose which leads to the dissociative effects and desired euphoria in human addicts 3600 mg/day with conversion to the rat dose according to Paget equation. The equivalent dose for a rat weighing 200 gm is = 18/1000 x average adult human therapeutic daily dose *(Paget and Barnes, 1964; Nair and Jacob, 2016; Badawy et al., 2019).*

Group III (Vit E): Each rat received alpha tocopherol (100 mg/kg/day) or (20 mg/day) dissolved in corn oil orally by gavage for 40 days. The dose of vitamin E (100 mg/kg/day) was equal to the upper tolerable intake doses of vitamin E in human adult according to Paget equation *(Paget and Barnes, 1964; Taha et al., 2020).*

Group IV (GBP + Vit E): Each rat received GBP in normal saline 0.9% (32.4 mg/kg/day) plus alpha tocopherol in corn oil (100 mg/kg/day) orally by gavage for three days. After that each rat received the same daily dose of alpha tocopherol in corn oil (100 mg/kg/day) + the GBP dose were increased by adding the starting dose every three days till the end of the 30 days. Further, the dependent dose of GBP (64.8 mg/day) + alpha tocopherol (100 mg/kg/day) were given daily for another 10 days *(Bonnet and Scherbaum, 2017; Taha et al., 2020).*

Sampling:

Cervical decapitation was used as a method of euthanasia of the animals for avoiding any contamination or chemical injury to rat tissues *(Nakai et al., 2005; Underwood and Anthony, 2020).* At end of the study, the weight of the rat of all groups was measured and compared with their weight at the start of the study *(Huang et al. 2018).* Samples of the blood were taken from abdominal aorta. Centrifugation at 5000 rpm for 15 min was performed to obtain the serum and then was kept frozen for biochemical testing at - 80 ◦C. The kidney tissues were excised and then were held and washed using cold saline solution then were divided and prepared for histopathological examination.

- **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:**

Paraffin blocks were made by putting liver and kidney specimens in 10% formol-saline then sectioned at 5-µm-thickness and stained with hematoxylin and eosin (H and E) *(Carleton and Drury, 1980).*

- **Histopathological score:**

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) *(Carleton and Drury, 1980).*

III. Data management and statistical analysis:-

The data collected were coded, processed, and analyzed with Statistical Package for the Social Sciences (SPSS) version 27 for Windows® (SPSS Inc., Chicago, IL, USA). The normality of distribution for the analyzed variables was tested using Kolmogorov-Smirnov test assuming normality at $P > 0.05$. The collected data were summarized in terms of median and Inter Quartile Range (IQR) as appropriate for nonparametric data and as number and percentage for qualitative data. Friedman test was used to compare non parametric quantitative data for repeated measures followed by post hoc multiple comparisons using Bonferroni adjusted tests to detect the significant pairs. The one-way analysis of the variance (one-way ANOVA) was used to compare normally distributed quantitative variables. Intergroup significance was tested by (Tukey-HSD) post hoc test to indicate which significant difference between pairs of groups. Krauskal Wallis test was used as test of significance to compare independent non-parametric quantitative data. Significant Krauskal Wallis was followed by adjusted Bonferroni test to detect significant pairs. All tests were two sided. The accepted level of significance in this work was ($p < 0.05$), $p \leq 0.001$ was considered highly statistically Significant (HS), and $p > 0.05$ was considered Non statistically Significant (NS).

IV. Results

There were no statisitically significant results according to one-way ANOVA test between control groups (Ia, Ib, Ic) as regard to body weight, kidney weight, biochemical parameters and histopathological changes, so we choose group Ia for comparison with other groups of the study. By the same way, there were no significant results as regard body weight and kidney weight between group IIa and IIb so we choose group IIa for comparison with other groups.

1) Effect of long term gabapentin administration on weight gain:

Initial body weight was statistically comparable between the four groups. Table 1 shows initial and final body weights as well as weight gain percentage of all groups. There was significant increased body weight in GBP misuse group (G IIa) and Group IV (GBP + Vit E) ($p < 0.001$) at the end of the study. The body weight change percent was significantly high in groups (G IIa, IV) as compared to other groups (G I, III) (table 1).

Table (1): Comparison of body weight among the studied groups.

Groups / Parameters	Group Ia Negative Control (n = 5)	Group IIa GBP misuse (n = 5)	Group III Vit E (n = 5)	Group IV GBP + Vit E (n = 5)	Significance test
Initial body weight (g) Mean ± SD	195.50 ± 9.26	193 ± 10.59	194 ± 10.22	196 ± 9.07	F= 0.197 P= 0.898
Final body weight (g) Mean ± SD	252.80 ± 8.34	268 ± 11.35	251.50 ± 8.18	265 ± 9.72	F= 7.829 P < 0.001*
P1		0.005*	0.990	0.033*	
P2			0.002*	0.894	
P3				0.015*	
Percent of change (%) in body weight Mean ± SD	29.41 ± 3.16	38.96 ± 2.55	29.77 ± 3.02	35.27 ± 2.25	F= 27.605 P < 0.001*
P1		< 0.001*	0.991	< 0.001*	
P2			< 0.001*	0.025*	
P3				< 0.001*	

N: number of rats; F: One-Way ANOVA; P: Tukey-HSD post hoc test; *: Statistically significant ($p \leq 0.05$); P1: Significance in relation to G I; P2: Significance in relation to G II; P3: Significance in relation to G III; GBP: gabapentin; Vit E: vitamin E.

2) Effect of long term gabapentin administration on kidney weight:

No significant results were detected as regarding kidney weight changes in GBP misuse group (G IIa) as compared to other groups (G Ia, III, IV) (table 2).

Table (2): Comparison of kidney weights among the studied groups.

Groups / Parameter	Group Ia Negative Control (n = 5)	Group IIa GBP misuse (n = 5)	Group III Vit E (n = 5)	Group IV GBP + Vit E (n = 5)	Kruskal Wallis test	P value
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
Kidney weight (g)	1.3 (1.4)	1.6 (0.7)	1.3 (1.4)	1.4 (0.5)	7	0.07

N: number of rats; P value: intergroup significance was tested by Bonferroni post hoc test; GBP: gabapentin; Vit E: vitamin E.

3) Evaluation of biochemical markers:

Gabapentin misuse group (G IIa) and Group IV (GBP + Vit E) showed increased renal function tests (urea, creatinine and uric acid levels) as compared to the other groups (G I, III) (table 3).

Table 3 shows that urea level is high among groups (G IIa, IV) (median=33 and 26) respectively compared to other groups (G I, III) and this difference is of high statistically importance (P value 0.007*). Similarly, both creatinine (median=0.86 and 0.67) (P value= 0.006*) and uric acid levels (median=5.8 and 3.9) (P value=0.001*) respectively showed similar highly statistically significant results. Co-administration of vitamin E with GBP (G IV) showed significant decreased serum levels of urea, creatinine and uric acid as compared to GBP misuse group (G IIa). This indicates its renal ameliorative role (table 3, Fig 1, 2).

Table (3): Comparison of biochemical markers of the Kidney in different groups of the study.

Groups / Parameters	Group Ia Negative Control (n = 5)	Group IIa GBP misuse (n = 5)	Group III Vit E (n = 5)	Group IV GBP + Vit E (n = 5)	Kruskal Wallis test	P value
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)		
Urea (mg/dl)	17 (24.2) #	33 (4)	17 (24.2) #	26 (12) ¥	12	0.007*
Creatinine (mg/dl)	0.6 (0.6) #	0.86 (0.4)	0.6 (0.6) #	0.67 (0.6) ¥	12.4	0.006*
Uric acid (mg/dl)	2.8 (3.2) #	5.8 (2.3)	2.8 (3.2) #	3.9 (1.5) ¥	15.5	0.001*

N: number of rats; *Significance P value < 0.05 (intergroup significance was tested by Bonferroni post hoc test); # Significant difference with G II and G IV; ¥ Significant difference with G II; GBP: gabapentin; Vit E: vitamin E.

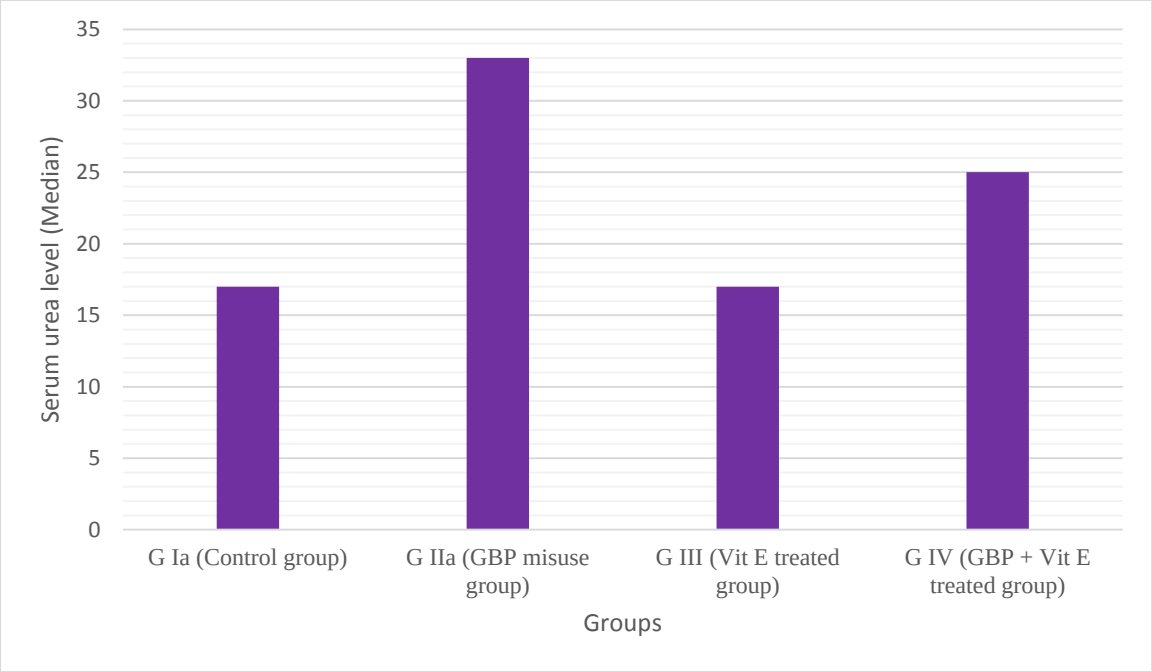

Figure (1): Serum urea levels in different groups of the study.

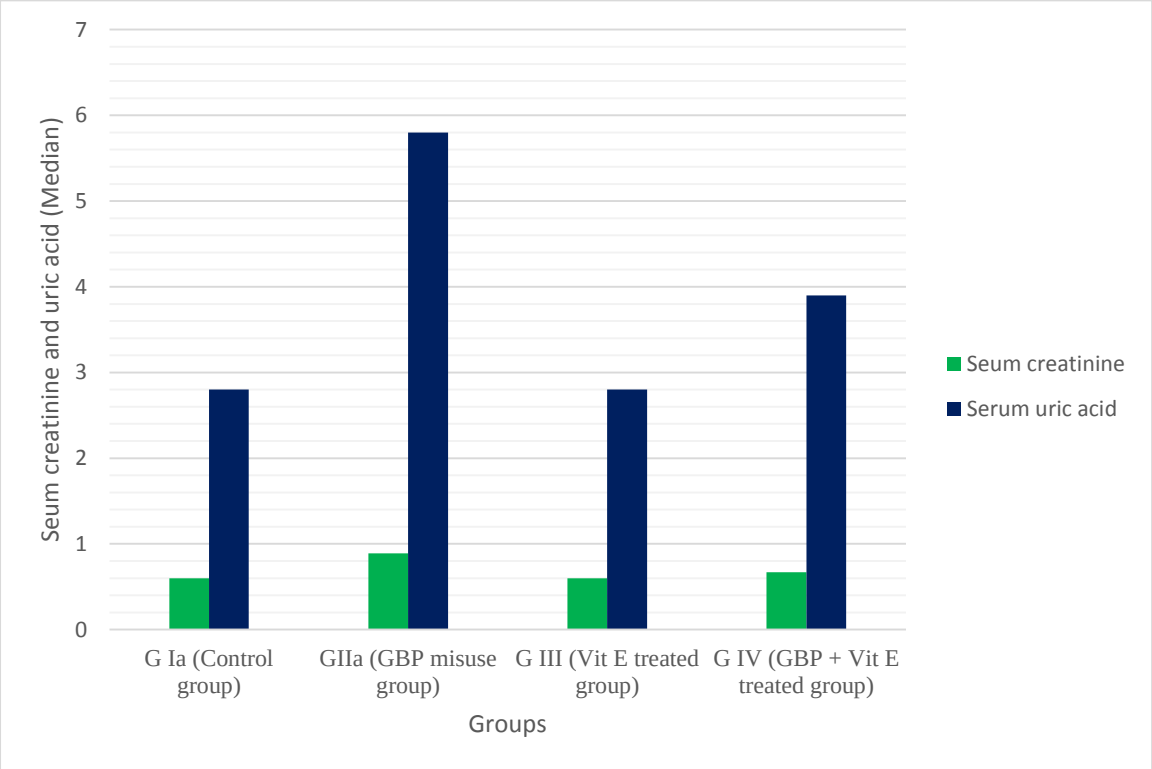

Figure (2): Serum creatinine and uric acid levels in different groups of the study.

4) Evaluation of histopathological changes:

Gabapentin-induced renal damage:

Light microscopy analysis of H and E-stained sections from group (Ia, III) control and vitamin E groups revealed the renal cortex's typical histological architecture. Renal corpuscles, glomerular capillaries, Bowman's capsules, and urine space filled the renal cortex. The proximal convoluted tubules make up the majority of the renal cortex. The distal convoluted tubules had a broad lumen and were bordered with short cuboidal cells (Fig. 3).

While sections from group IIa (GBP misuse) showed injury with widened tubular lumens. Some tubules showed luminal casts. Most tubular cells had vesicles and vacuolated cytoplasm. Cells without nuclei and high capillary congestion and mild lymphocytic infiltration had occurred. Cell nuclei were damaged in proximal and distal convoluted tubules, implying disintegration. Hydropic degeneration clouded the convoluted tubule endothelial lining cells. Most glomeruli were swollen inside Bowman's capsule without capsular space. Few segmented and congested glomeruli were seen (Fig. 4).

After sudden stoppage of GBP in group IIb (GBP withdrawal group), most renal glomeruli and tubular structure were restored. Bowman's capsule contained normal-sized glomeruli. Some glomeruli have dilated Bowman space. Some areas of renal cortex showed tubules with degenerated features and degenerated cells. Few tubules revealed widen lumen and other with luminal cast (Fig. 5).

Treatment of rats with both gabapentin and vitamin E (GBP + VE) group IV showed substantial protection against the development of kidney injuries that present in group II. Renal convoluted tubule epithelial cells showed mild vacuolation and degeneration. Bowman's capsules contain typical glomeruli and normal sized capsular space. Congestion was mild (Fig. 6).

Gabapentin withdrawal (G IIb) and G IV (GBP + VE) groups revealed signs of improvement of renal tissue organization as compared with GBP misuse group (G IIa) with more better results in group IV (table. 4).

Table 4: Comparison of histopathological changes amonng the four groups.

Groups / Parameters	Group Ia Negative Control (n = 5)	Group IIa GBP misuse (n = 5)	Group IIb GBP withdrawal (n = 5)	Group III Vit E (n = 5)	Group IV GBP + Vit E (n = 5)
Congestion	-	+++	-	-	+
Lymphocytic infiltration	-	+	-	-	-
Atrophied glomeruli	-	-	-	-	-
Tubular hydropic degeneration	-	++	+	-	+
Swollen glomeruli	-	+++	+	-	-

N: number of rats; GBP: gabapentin; Vit E: vitamin E.

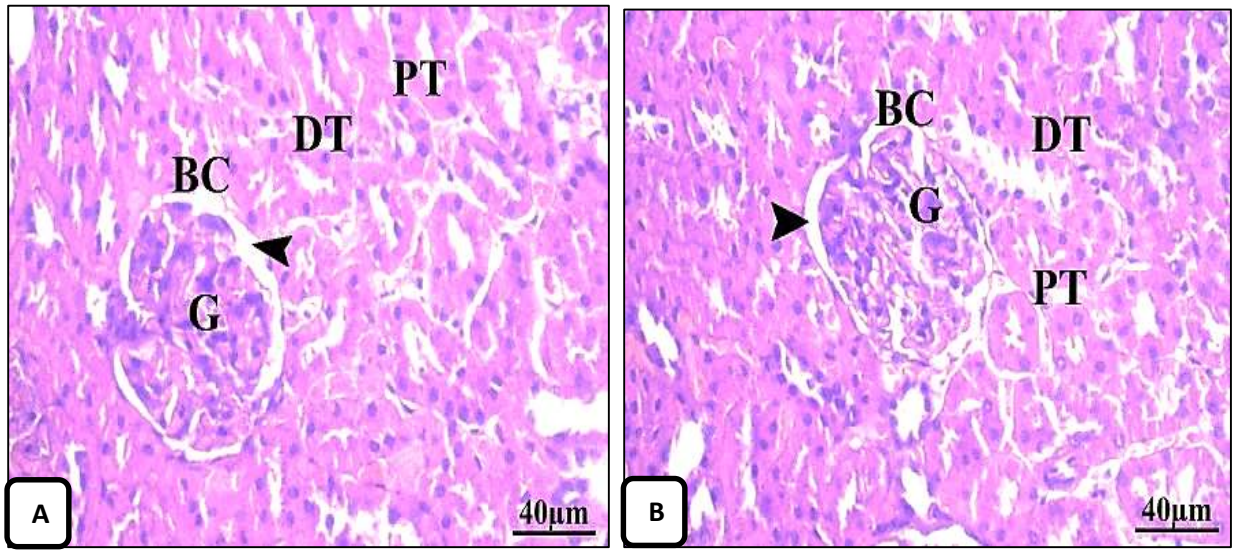

Figure 3: H and E-stained sections from control (G Ia) (A) and vitamin E group (G III) (B) revealed the renal cortex's typical histological architecture. Renal corpuscles, glomerular capillaries (G), Bowman's capsules (BC), and urine space (arrowhead) filled the renal cortex. The proximal convoluted 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.

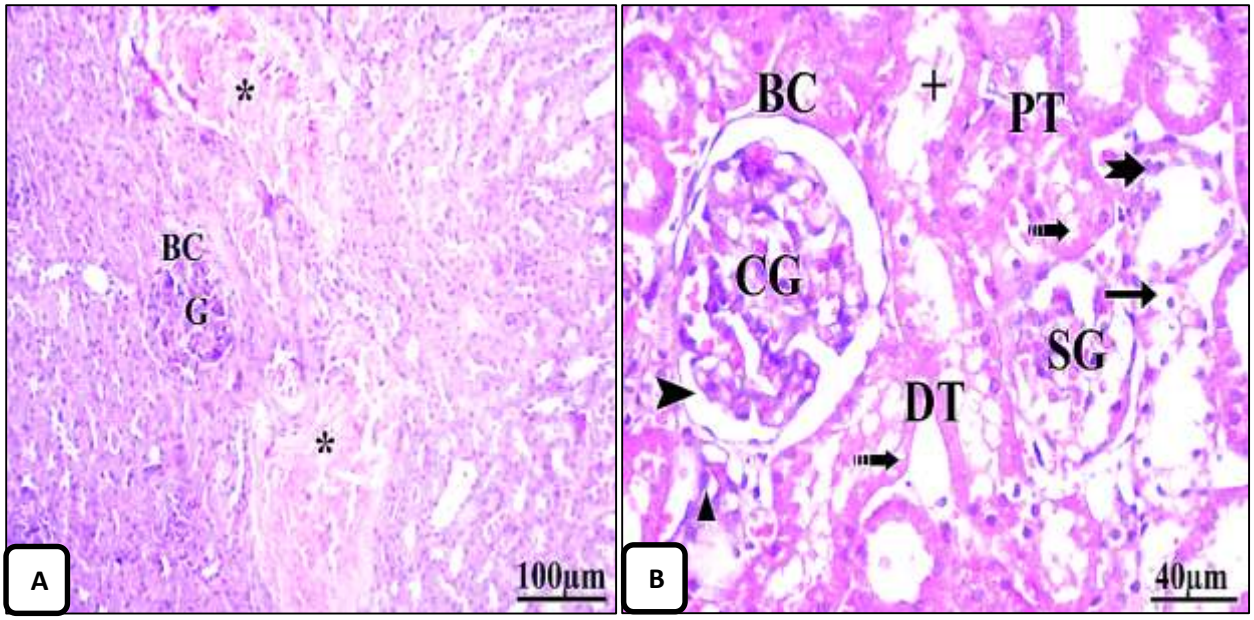

Figure 4: H and E-stained renal cortical sections from group (G IIa) (GBP misuse): (A) showed injury under light microscopy. Most glomeruli (G) were swollen inside Bowman's capsule (BC) without capsular space. Cells without nuclei and high capillary congestion (*). (B) showed Widened tubular lumens (notch arrow). Some tubules showed luminal casts (+). Most tubular cells had vesicles and vacuolated cytoplasm. and mild lymphocytic infiltration (triangle). Cell nuclei were damaged in proximal (PT) and distal convoluted tubules (DT), implying disintegration (dotted arrow). Hydropic degeneration (arrow) clouded the convoluted tubule endothelial lining cells. Few segmented (SG) and congested glomeruli (CG).

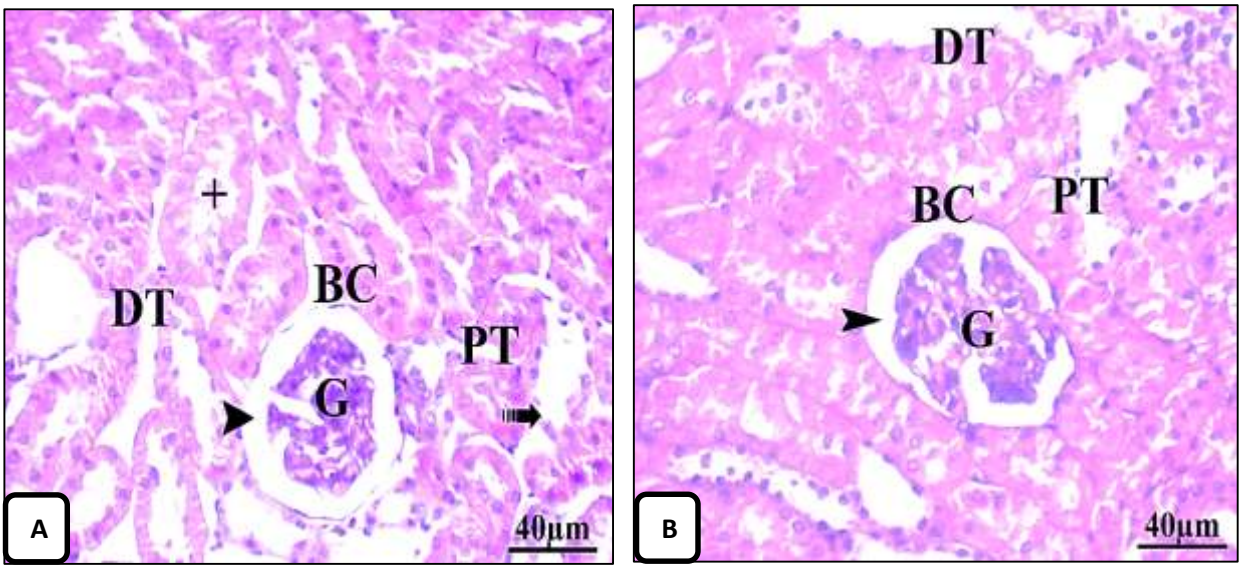

Figure 5: H and E-stained renal cortical sections from group IIb (GBP withdrawal group). (A) showed that Some areas of renal cortex showed tubules with degenerated features and degenerated cells (dotted arrow). Few tubules revealed widen lumen (notch arrow) and other with luminal cast (+). (B) showed that most renal glomeruli (G) and tubular structure were restored. Bowman's capsule (BC) contained normal-sized glomeruli. some glomeruli (G) have dilated Bowman space (arrow head).

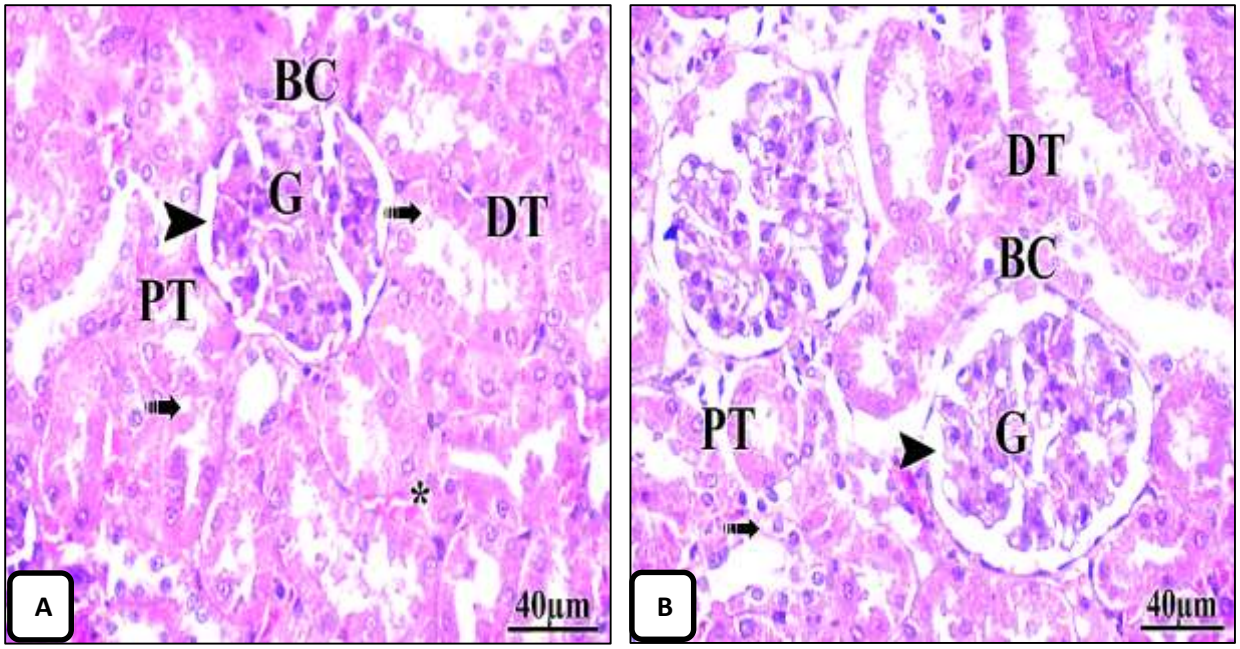

Figure 6: H and E-stained renal cortical sections from group (G IV) (GBP + vit E). (A) revealed that renal convoluted tubule epithelial cells showed mild vacuolation and degeneration (dotted arrow). Congestion was mild (*). (B) showed Bowman's capsules (BC) contain typical glomeruli (G) and normal sized capsular space (arrow head).

V. Discussion

Addiction is considered a prevalent and lethal disease all over the world that leads to many economic and social impacts. Many evidences demonstrated increasing both misuse and abuse of gabapentinoid drugs among the adolescents that forces some authorities including Egypt to include pregabalin in the addicting drugs schedule *(Al-Husseini et al., 2018).*

After scheduling pregabalin, the problem of abusing GBP had become devastating especially it is purchased without prescription. The development of the euphoria as well as mood changes as adverse effects of GBP had been suggested as the enforcing factors to take more frequent doses *(Schifano et al., 2011; Schjerning et al., 2016).*

The current experiment studied the nephrotoxic potential of GBP sub chronic high dose exposure in adult male albino rats and the protective role of alpha-tocopherol against GBP induced renal toxicity.

Although GBP has been associated with both misuse and abuse potential, the duration of intake differentiates between them. If any person use GBP in higher doses for more than 12 months for un prescribed purposes and to get high, he is considered as a drug abuser or addict according to DSM-5. However, any period less than 12 months is diagnosed as a drug misuse *(Buttram and Kurtz, 2021).* In this research, we tried to simulate what occur with human addicts as they usually misuse gabapentin during withdrawal period from opioid drugs so we are concerning with misuse terminology in human addicts.

The authors intended to produce an addiction model of GBP as regards the mode of administration as well as the dose and duration. So GBP in (G II) was given orally with a starting dose which was equivalent to the daily therapeutic dose in human (360 mg/day), then the dose was gradually increased to reach the supratherapeutic doses (equivalent to 3600 mg/day) which was reported to produce the desired euphoria and dissociative effects in human addicts at the end of the 30 days. Furthermore; continuous intake of the last dose was given for the next 10 days and another 10 days were included for withdrawal effects after sudden stoppage of GBP. This goes in the same way with many researches that use this addiction module *(Taha et al., 2020; Elgazzar et al., 2021; Elsukary et al., 2022)* and case reports *(Yargic and Ozdemiroglu, 2011; Carrus and Schifano, 2012; Ashwini et al., 2015).*

In the current research, the misuse of high doses of GBP was associated with significant weight gain, Gabapentin treatment induced weight gain at the end of the experiment. Animals in groups (G II, IV) showed significant weight gain compared to other groups (G I, III). This might occurred because GBP may increase the appetite and causes water retention. *DeToledo et al. (1997*) reviewed changes in body weight in 44 patients treated with GBP for a period of 12 or more months. Twenty-eight patients were receiving GBP dosages of > 3000 mg/day. Observed changes in body weight were as follows: 10 patients gained more than 10% of their baseline weight, 15 patients gained 5% to 10% of baseline, 16 patients had no change, and 3 patients lost 5% to 10% of their initial weight.

Based on the mechanism of action of GBP that it is supposed to modulate neuro-signaling through attaching to the voltage gated calcium channels specific subunit, *Tuluc et al. (2021*) suggested that prolonged GBP use may be associated with weight gain because it may affect voltage gated calcium channels in pancreatic β-Cell that modulate insulin release.

Recently, *Buraniqi et al. (2022)* systematically reviewed studies and stated that Anti-seizure drugs have several side effects, and many of them may affect appetite, thus impacting weight gain and normal growth. In this research, GBP wasn't included among the drugs which decrease the appetite, however; the author concluded that gabapentinoid drugs may also lead to increased appetite or moderate weight gain in children.

Unfortunately, the result of this study goes in opposite way with a previous study on tramadol and pregabalin *(Elsukary et al., 2022),* similar studies *(Shokry et al. 2020; Elgazzar et al., 2021)* and GBP misuse study *(Welson et al., 2021)* that were associated with loss of weight. This may be due to many factors such as; long duration of their studies that leads to prolonged depletion and exhaustion of the rats, relatively short duration of our study, tramadol use usually associated with gastritis and increased acid secretion that leads to loss of appetite and finally; the authors of these researches explained the loss of weight in rats to its lack of interest in their food intake.

In the current research, the sub chronic misuse of high doses of GBP had elicited

several renal deleterious effects as indicated by both biochemical and histopathological derangements in comparison to control groups. Furthermore, GBP withdrawal for 10 days provides some improvements in histopathological appearance. Also the use of alpha-tocopherol with GBP has inhibited the much more histopathological damage that occured with GBP alone. The biochemical markers levels also weren't highly elevated in vitamin E treated groups as in groups treated with GBP only. This emphasizes the protective role of vitamin E.

In the current work, GBP treated rats showed elevated levels of urea, creatinine and uric acid that indicate renal dysfunction. This is going in the same way with many previous studies and case reports that demonstrated high doses of GBP was associated with biochemical renal dysfunction *(Torregrosa-de Juan et al., 2012; Cruz‐Álvarez et al. 2018).*

Renal function tests had been elevated after sub chronic administration of GBP. Previous studies supported our finding as they stated that long term use of GBP can induce renal failure although was very rare adverse effect. They also advised prolonged follow-up for serum creatinine, urea, and uric acid results during GBP prolonged therapy *(Yılmaz et al., 2017).*

The present histopathological findings supported the latter biochemical results. Rats treated with GBP demonstrated renal tissues structural changes similar to previous studies that showed hydropic degeneration, nuclear disintegration, high fatty degeneration and necrosis. Lymphocytic infiltrations were also reported together with glomerular atrophy, renal tubular epithelial degeneration, vacuolated cytoplasm, luminal casts and hemorrhage *(Hauben, 2002; Bureau, et al., 2003; Contreras-Mota and Rosales-Cortés, 2021).*

Degenerative lesions and disintegrated nuclei in renal cortex together with hydropic degeneration, tubular cast, and glomerular atrophy as well as abnormal renal function tests had been detected by previous researches that administer GBP for subchronic period *(Torregrosa-de Juan, et al., 2012; Badawy et al., 2019).*

Hung et al. (2008) stated that GBP is widely used in the management of pain. It is eliminated solely by renal excretion. GBP toxicity should be considered as one of the differential diagnoses of altered consciousness in patients with compromised renal function even after a single dose. Dosing adjustment is required according to patient's renal function.

Miller and Price, (2009); Guddati et al. (2012) and Kaufman et al. (2013) described case reports of significant deterioration in conscious level and myoclonus due to high doses GBP in chronic kidney disease patients. Furthermore, *Zand et al. (2010)* awarenessed health care professionals that these patients often receive inappropriately high GBP dosage for their kidney function. Occasioning overt toxicity; advanced age and comorbidity predispose these patients for toxicity. Heightened awareness of such preventable risk, amid the chronic kidney disease epidemic, would be cost-effective and improve healthcare quality.

When vitamin E was used in parallel with GBP there was some decrease in serum levels of renal functions (serum urea, creatinine and uric acid) and reduction in the histopathological abnormalities in comparison to GBP group this indicates its protective role. These findings are in agreement with previous reports about vitamin E protective role as antioxidant against oxidative cell injury induced by different xenobiotics *(Santos, et al., 2016; Welson et al., 2021).*

The proposed protective mechanisms of alpha tocopherol had been postulated in many previous researches. These include that it acts as scavenger for reactive oxygen harmful radicals, enhancing glutathione formation and inhibits oxidative lipid peroxidation. This highlights its important role in ameliorating GBP induced renal cell injuries *(Cruz‐Álvarez et al. 2018; Welson et al., 2021).*

There are some limitations of this research as the small sample size included, the relatively short period of the study (40 days) to indicate chronicity, so we are concerning of sub chronic terms (less than 90 days) not chronic. Also, the need for reevaluation of renal biochemical markers after withdrawal period. Even though it was present in the original study design but unfortunately it wasn't performed by the operating technician during the experiment. So we couldn't include their results in the research. Therefore, this theory may be evaluated with larger-sample studies and complete biochemical analysis in the future.

VI. Conclusion

In this study the current findings showed that high dose sub chronic administration of GBP was associated with nephrotoxic effects in male albino rats as demonstrated by both biochemical and histopathological changes. The histopathological deleterious effects were less appeared during withdrawal period after sudden stoppage of GBP. Alpha tocopherol protects against GBP induced impairment of kidney functions mainly through improving renal functions tests and enhancing histopathological regeneration that emphasizing its protective role.

VII. Author Contribution

This work was carried out in collaboration with all authors. The authors Mohammad El-Kattan and Ahmed Elshatory designed the study and wrote the protocol, the authors Mahmoud Ahmed Khattab, Fatma Abdel Wahab Abdel Maksoud, Maha Emad Eldien, Nada Elsayed Abdel-Roaf, managed the literature research and the authors Mohammad El-Kattan wrote and revised the final manuscript. All authors read and approved the final manuscript.

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X. Declarations

Competing Interests: The authors declare no competing interests.

XI. References:

Al-Husseini, A., Van Hout, M. C. and Wazaify, M. (2018): Pregabalin misuse and abuse: a scoping review of extant literature. Journal of Drug Issues, 48(3), 356-376.

Ashwini, S., Amit, D. R., Ivan, N. S., and Alka, P. V. (2015): Pregabalin dependence with pregabalin induced intentional self-harm behavior: A case report. Indian journal of psychiatry, 57(1), 110-111.

Badawy, G. M. (2019): Ginger ameliorates the nephrotoxicity induced by gabapentin in rat fetuses (Doctoral dissertation, Menoufiya University, Egypt).

Baird, C. R., Fox, P. and Colvin, L. A. (2014): Gabapentinoid abuse in order to potentiate the effect of methadone: a survey among substance misusers. European addiction research, 20(3), 115-118.

Bassiony, M. M., Youssif, U. M., Hussein, R. A. and Saeed, M. (2016): Psychiatric comorbidity among Egyptian patients with opioid use disorders attributed to tramadol. Journal of Addiction Medicine, 10(4), 262-268.

Bonnet, U. and Scherbaum, N. (2017): How addictive are gabapentin and pregabalin? A systematic review. European neuropsychopharmacology, 27(12), 1185-1215.

Buraniqi, E., Dabaja, H., and Wirrell, E. C. (2022): Impact of antiseizure medications on appetite and weight in children. Pediatric Drugs, 24(4), 335-363.

Bureau, C., Poirson, H., Péron, JM. and Vinel, JP. (2003): Gabapentin-induced acute hepatitis. Gastroenterol Clin Biol. 2003 Dec;27(12):1169-70. French. PMID: 14770125.

Buttram ME. and Kurtz SP. (2021): Descriptions of Gabapentin Misuse and Associated Behaviors among a Sample of Opioid (Mis)users in South Florida. J Psychoactive Drugs. Jan-Mar;53(1):47-54. doi: 10.1080/02791072.2020.1802087. Epub 2020 Aug 4. PMID: 32748701; PMCID: PMC7858688.

Carleton, HM, Drury, RAB: (1980). Carleton's Histological Technique. 5th ed, Wallington, Description,New York, Oxford. pp 1–200.

Carrus, D., and Schifano, F. (2012): Pregabalin misuse–related issues; intake of large dosages, drug-smoking allegations, and possible association with myositis: Two case reports. Journal of Clinical Psychopharmacology, 32(6), 839-840.

Compton, P., Kehoe, P., Sinha, K., Torrington, M. A. and Ling, W. (2010): Gabapentin improves cold-pressor pain responses in methadone-maintained patients. Drug and alcohol dependence, 109(1-3), 213-219.

Contreras-Mota, M. and Rosales-Cortés, V. (2021): Hepatotoxicidad probablemente asociada a gabapentina. Revista Médica del

Instituto Mexicano del Seguro Social, 59(2), 157-162.

Cruz- Álvarez, L. E. D. L., Zúñiga-Romero, Á., Huerta- Cruz, J. C., Flores-Murrieta, F. J., Reyes- García, J. G., Araiza- Saldaña, C. I. and Rocha-González, H. I. (2018): Antiallodynic interaction and motor performance of the pregabalin/thioctic acid and pregabalin/α-tocopherol combinations in neonatal streptozotocin- induced diabetic rats. Drug Development Research, 79(7), 362-369.

DeToledo, J. C., Toledo, C., DeCerce, J. and Ramsay, R. E. (1997): Changes in body weight with chronic, high-dose gabapentin therapy. Therapeutic drug monitoring, 19(4), 394-396.

Duan, Y., Duan, J., Feng, Y., Huang, X., Fan, W., Wang, K. and Yang, S. (2018): Hepatoprotective activity of vitamin E and metallothionein in cadmium-induced liver injury in Ctenopharyngodon idellus. Oxidative medicine and cellular longevity, 2018.

Elgazzar, F. M., Elseady, W. S. and Hafez, A. S. (2021): Neurotoxic effects of pregabalin dependence on the brain frontal cortex in adult male albino rats. Neurotoxicology, 83, 146-155.

Elsukary, A. E., Helaly, A. M., El Bakary, A. A., Moustafa, M. E. and El-Kattan, M. A. (2022): Comparative study of the Neurotoxic Effects of Pregabalin versus Tramadol in Rats. Neurotoxicity Research, 40(5), 1427-1439.

EMA. (2006): ummary information on referral opinion pursuant to Article 30 of Council Directive 2001/83/EC for Neurontin and associated names (see Annex I) International Non-Proprietary Name (INN): Gabapentin: Background information. London, UK: EMA.

Evoy, K. E., Morrison, M. D. and Saklad, S. R. (2017): Abuse and misuse of pregabalin and gabapentin. Drugs, 77, 403-426.

Evoy, K. E., Sadrameli, S., Contreras, J., Covvey, J. R. and Peckham, A. M. (2021): Authors' Reply to Bonnet et al."Abuse and Misuse of Pregabalin and Gabapentin: A Systematic Review Update". Drugs, 81(5), 615-617.

Guddati, A. K., Zafar, Z., Cheng, J. T. and Mohan, S. (2012): Treatment of gabapentin-induced myoclonus with continuous renal replacement therapy. Indian Journal of Nephrology, 22(1), 59.

Hauben, M. (2002): Re: Lasso-De-La-Vegaet al. Gabapentin As A Probable Cause of Hepatotoxicity and Eosinophilia. Official journal of the American College of Gastroenterology| ACG, 97(8), 2156-2157.

Huang, C. T., Chen, S. H., Lin, S. C., Chen, W. T., Lue, J. H., and Tsai, Y. J. (2018): Erythropoietin reduces nerve demyelination, neuropathic pain behavior and microglial MAPK s activation through erythropoietin receptors on S chwann cells in a rat model of peripheral neuropathy. Glia, 66(11), 2299-2315.

Hung, T. Y., Seow, V. K., Chong, C. F., Wang, T. L., and Chen, C. C. (2008): Gabapentin toxicity: an important cause of

altered consciousness in patients with uraemia. Emergency Medicine Journal, 25(3), 178-179.

Klein-Schwartz, W., Shepherd, J. G., Gorman, S., and Dahl, B. (2003): Characterization of gabapentin overdose using a poison center case series. Journal of Toxicology: Clinical Toxicology, 41(1), 11-15.

Kaufman, K. R., Parikh, A., Chan, L., Bridgeman, M., and Shah, M. (2014): Myoclonus in renal failure: two cases of gabapentin toxicity. Epilepsy and Behavior Case Reports, 2, 8-10.

Maneuf, Y. P., Gonzalez, M. I., Sutton, K. S., Chung, F. Z., Pinnock, R. D., and Lee, K. (2003): Cellular and molecular action of the putative GABA-mimetic, gabapentin. Cellular and Molecular Life Sciences CMLS, 60, 742-750.

Mersfelder TL, Nichols WH. (2016): Gabapentin: Abuse, Dependence, and Withdrawal. Ann Pharmacother. Mar;50(3):229-33. doi: 10.1177/1060028015620800

Miller, A., and Price, G. (2009): Gabapentin toxicity in renal failure: the importance of dose adjustment. Pain medicine, 10(1), 190-192.

Nair, A. B., and Jacob, S. (2016): A simple practice guide for dose conversion between animals and human. Journal of basic and clinical pharmacy, 7(2), 27.

Nakai, J. S., Elwin, J., Chu, I., and Marro, L. (2005): Effect of anaesthetics [sol] terminal procedures on neurotransmitters from non-dosed and aroclor 1254- dosed rats. Journal of Applied Toxicology: An International Journal, 25(3), 224-233.

NICE. (2013): Neuropathic Pain in Adults: Pharmacological Management in Non-Specialist Settings. London, UK: NIC.

Paget, G. E., and Barnes, J. M. (1964): Interspecies dosage conversion scheme in evaluation of results and quantitative application in different species. Evaluation of drug activities: pharmacometrics, 1, 160-162.

Peckham, A. M., Fairman, K. A., and Sclar, D. A. (2017): Prevalence of gabapentin abuse: comparison with agents with known abuse potential in a commercially insured US population. Clinical Drug Investigation, 37, 763-773.

Peckham, M., A., Ananickal, M. J., and Sclar, D. A. (2018): Gabapentin use, abuse, and the US opioid epidemic: the case for reclassification as a controlled substance and the need for pharmacovigilance. Risk management and healthcare policy, 11, 109-116. doi:10.2147/RMHP.S168504.

Radley, D. C., Finkelstein, S. N., and Stafford, R. S. (2006): Off-label prescribing among office-based physicians. Archives of internal medicine, 166(9), 1021-1026.

Rezq, A. A. (2014): Effects study of Nigella sativa, its oil and their combination with

vitamin E on oxidative stress in rats. American Journal of Applied Sciences, 11(7), 1079-1086.

Santos, S. A. D., Silva, E. T. D., Caris, A. V., Lira, F. S. D., Tufik, S., and Dos Santos, R. V. T. (2016): Vitamin E supplementation inhibits muscle damage and inflammation after moderate exercise in hypoxia. Journal of Human Nutrition and Dietetics, 29(4), 516-522.

Schifano, F., D'Offizi, S., Piccione, M., Corazza, O., Deluca, P., Davey, Z. and Scherbaum, N. (2011): Is there a recreational misuse potential for pregabalin? Analysis of anecdotal online reports in comparison with related gabapentin and clonazepam data. Psychotherapy and psychosomatics, 80(2), 118-122.

Schjerning, O., Rosenzweig, M., Pottegård, A., Damkier, P., and Nielsen, J. (2016): Abuse potential of pregabalin: a systematic review. CNS drugs, 30, 9-25.

Shokry, D. A., El Nabrawy, N., Yassa, H. D., Gaber, S. S., Batiha, G. E. S., and Welson, N. N. (2020): Pregabalin induced reproductive toxicity and body weight changes by affecting caspase3 and leptin expression: protective role of wheat germ oil. Life Sciences, 260, 118344.

Smith, R. V., Havens, J. R., and Walsh, S. L. (2016): Gabapentin misuse, abuse and diversion: a systematic review. Addiction, 111(7), 1160-1174.

Taha, S. H. N., Zaghloul, H. S., Ali, A. A. E. R., Gaballah, I. F., Rashed, L. A., and Aboulhoda, B. E. (2020): The neurotoxic effect of long-term use of high-dose Pregabalin and the role of alpha tocopherol in amelioration: implication of MAPK signaling with oxidative stress and apoptosis. Naunyn-Schmiedeberg's archives of pharmacology, 393, 1635-1648.

Torregrosa-de Juan, E., Olagüe-Díaz, P., Royo-Maicas, P., Fernández-Nájera, E., and García-Maset, R. (2012): Acute renal failure due to gabapentin. A case report and literature review. Nefrología (English Edition), 32(1), 130-131.

Tuluc, P., Theiner, T., Jacobo-Piqueras, N., and Geisler, S. M. (2021): Role of High Voltage-Gated Ca2+ Channel Subunits in Pancreatic β-Cell Insulin Release. From Structure to Function. Cells, 10(8), 2004.

Underwood, W., and Anthony, R. (2020): AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved on March, 2013(30), 2020-1.

Welson, N. N., Rofaeil, R. R., Ahmed, S. M., Gaber, S. S., Batiha, G. E. S., and Shahataa, M. G. (2021): Vitamin E protects against gabapentin-induced chronic hepatic and renal damage associated with the inhibition of apoptosis and tissue injury in rats. Life sciences, 267, 118940.

Yargic, I., and Ozdemiroglu, F. A. (2011): Pregabalin abuse: a case report. Klinik Psikofarmakoloji Bülteni-Bulletin of Clinical Psychopharmacology, 21(1), 64-66.

Yılmaz, S., Kaya, E., and Comakli, S. (2017): Vitamin E (α tocopherol) attenuates toxicity and oxidative stress induced by aflatoxin in rats. Advances in clinical and experimental medicine: official organ Wroclaw Medical University, 26(6), 907-917.

Zand, L., McKian, K. P., and Qian, Q. (2010): Gabapentin toxicity in patients with chronic kidney disease: a preventable cause of morbidity. The American journal of medicine, 123(4), 367-373.

السمية الكلوية الناتجة عن التعرض شبه المزمن للجابابنتين والدور الوقائي لألفا توكوفيرول، دراسة تجريبية

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الملخص والتوصيات:

الخلفية: ازداد سوء استخدام عقار الجابابنتين و إدمانه في العقد الماضي في مصر، بعد جدولة مثيله البريجابالين في عام 2012. على الرغم من أن العديد من الدراسات أكدت الآثار الضارة للبريجابالين ، إلا أن الدراسات علي عقار الجابابنتين ضئيلة وقليلة. **الهدف من هذا البحث:** هو دراسة وتقييم التأثيرات السمية الكلوية لإعطاء جرعات عالية شبه مزمنة من عقارالجابابنتين والتأثير الوقائي لألفا توكوفيرول"فيتامين هـ". **الطريقة:** تم تضمين خمسة وثلاثين (35) ذكور جرذان ألبينو أصحاء. تم تقسيمهم بشكل عشوائي إلى أربع مجموعات متساوية (5 فئران لكل منهما): المجموعة الأولى أ (طعام عادي) ، المجموعة الأولى ب (محلول ملحي عادي) المجموعة الأولى ث (زيت الذرة) المجموعة الثانية أ (إساءة استخدام الجابابنتين) ، المجموعة الثانية ب (إنسحاب الجابابنتين) والمجموعة الثالثة (ألفا توكوفيرول في زيت الذرة) والمجموعة الرابعة (الجابابنتين + ألفا توكوفيرول). تلقت جميع الفئران الأدوية لمدة 40 يومًا . تم قياس مستويات اليوريا والكرياتينين وحمض البوليك في الدم. و تم أخذ أنسجة الكلى من أجل التشريح النسيجي. **النتائج و الإستنتاج:** يؤدي تناول الجابابنتين بجرعات عالية إلي زيادة مستويات المؤشرات التحليلية للكلي ، واضطراب أنسجة الكلى ويزيد من عدد الخلايا المتدهورة، كما خفف استخدام علاج ألفا توكوفيرول بشكل كبير من الآثار الضارة التي يسببها جابابنتين. **التوصيات:** يوصي بتقييم هذه النظرية من خلال دراسات عينة أكبر وتحليل كيميائي كامل لوظائف الكلي في المستقبل.

الكلمات المفتاحية: الجابابنتين ، سوء الاستخدام ، السمية الكلوية ، ألفا توكوفيرول ، الجرذان.