Research Article

Toxicological Effects of Long Term Pregabalin Abuse on Liver of Adult Albino Rats

Sherien S. Ghaleb[a], Amir Eid [b], Olivia G. Michel Zaki[c] and Amr R. Zaki [d]

[a] Professor of Forensic Medicine and Clinical Toxicology Department, Faculty of medicine, Cairo University,

[b] Lecturer of Forensic Medicine and Clinical Toxicology Department, Faculty of medicine, Beni-Suef University,

[c] Demonstrator of Forensic Medicine and Clinical Toxicology Department, Faculty of medicine, Beni-Suef University,

[d] Assistant Professor of Forensic Medicine and Clinical Toxicology Department, Faculty of medicine, Beni-Suef University

Abstract

Background: Pregabalin (trade name 'Lyrica') is one of the most recent antiepileptic drugs. It is also used in several medical therapies such as neuropathic pain and anxiety disorders. Recently, pregabalin abuse potential has increased among vulnerable population with doses that exceed the therapeutic ones which was the leading cause of the focusing of the new scientific studies on its susceptible toxicological effects. Previous studies discussed many case reports concerning the toxic effect of pregabalin on liver in addition to limited in vitro studies that showed its serious effect on the histopathological level. **Objective**: The aim of our study is to assess toxicological effects of pregabalin abuse for a long period on different biochemical and histopathological parameters of liver of adult Albino Rats. **Materials and methods:** our study was carried out on eighty rats which are randomly allocated into 2 groups. Group (I) included 40 rats which received distilled water orally for 8 weeks and considered as control group. Group (II) included 40 rats which received pregabalin at a dose of 300 mg/kg once daily, orally for 8 weeks and considered as pregabalin group. Then all animals were scarified. Blood samples were collected for biochemical analysis. Histopathological examination of liver was conducted through H & E staining. **Results**: Our data showed that pregabalin at this high dose harmed the liver on both biochemical and histopathological levels. It elevated AST, ALT, T.B, alkaline phosphatase and glucose values. Microscopically, pregabalin produced severe degenerative changes and necrosis of hepatocytes, congestion of blood vessels and lymphocytic infiltration with proliferation of van kupffer cells. **Conclusion:** Pregabalin abuse can markedly affect the liver on both levels, biochemically and histopathologically

Keywords: Pregabalin, Liver, Van kupffer cells.

Introduction

Pregabalin is drug with a structure resembling gamma aminobutyric acid (GABA) which is an inhibitory neurotransmitter. Pregabalin is approved pharmaco-therapy for the treatment of some pain and epileptic disorders, and also for generalized anxiety disorder (GAD)[1].

Within the last decade pregabalin has become blockbuster prescription drug with large number of prescriptions around the world. Pregabalin has been assumed to produce considerable abuse liability as it became easily obtainable over the internet and also were sold on black markets[2].

At least six cases of acute liver injury resulting from the use of pregabalin have been reported universaly. After 8 days of pregabalin use, the patients developed jaundice with elevation of the hepatic enzymes ALT (alanine transaminase) 43-fold increase and AST (aspartate transaminase) over 4-fold increase. Pregabalin-associated acute liver injury may be classified as an idiosyncratic hepatocyte injury[3].

Aim of the work

The aim of this study is to assess toxicological effects of Pregabalin abuse for a long period on different biochemical and histopathological parameters of liver of adult albino Rats.

Materials and Methods

The trade name of the used drug is Lyrica (Pfizer Pharmaceutical Industries, Cairo, Egypt) 300 mg which is presented in hard capsules containing 300 mg of pregabalin. A solution was prepared by dissolving capsules' contents of pregabalin 300mg -white to off - white crystalline powder- in distilled water and administered orally for eight successive weeks at a dose of 300 mg/Kg/day, 1/15 LD50[4]. The dose schedule was so adjusted that the amount of pregabalin administration per animal was as per their respective weight[5]. These concentrations are approximately equivalent to 3000 mg in humans according to the conversion equation[6]:

Human equivalent dose (mg/kg) = animal dose (mg/kg) × (animal Km / human Km).

Km is the average body weight (kg) of species divided by its body surface area (m2).

The dose of 3000 mg/day pregabalin is the dose that produces the euphoric and dissociative effects desired by addicts for recreational purposes[7].

The experiment carried out on 80 adult albino rats weighing about (150-200)g after a stabilization period in the animal house, for 8 weeks. The study performed in accordance with the guidelines for the care and use of laboratory animals approved by Research Ethical Committee (Beni Suef University, Egypt), approval number 019-84. All animals housed in plastic cages in a room with a controlled temperature (22 ± 2°C) and humidity level (50 ± 5%). The animals had a 12-hour light and dark cycle. Animals allowed free access to diet and water in good air conditioned room for two weeks before starting the experiment. One group served as control. Animals were weighed and randomly allocated into two equal groups as following:

Group (I): Control group (40 rats treated with distilled water orally for 8 weeks).

Group (II): Pregabalin (40 rats received pregabalin at a dose of 300 mg/kg once daily, orally for 8 weeks).

At the end of the experiment, blood samples obtained all rats was sacrificed *via* decapitation under light ether anesthesia and the following investigations were carried out:

Histopathological study:

Autopsy samples were taken from the rats in the two previously mentioned experimental groups. Then, liver samples were fixed in 10% formal saline solution for twenty-four hours. All samples were washed in tap water then diluted serially with absolute ethyl alcohol for dehydration. Specimens were cleared in xylene and submerged in paraffin at 56°C in a hot air oven for twenty-four hours. Blocks of paraffin beeswax tissue were prepared for sectioning at four microns thickness sections by sledge microtome. The obtained sections were fixed on glass slides, cleared from paraffin and stained with H & E stain for histopathological examination under the light microscope[8].

Biochemical analysis

Biochemical essay included assessment of liver functions (AST, ALT and total bilirubin), alkaline phosphatase and glucose. The serum AST and ALT activities were estimated photometrically using AST and ALT tests kits supplied by *DIAMOND Diagnostics*, Egypt according to the method described by[9]. The blood serum total bilirubin was estimated photometrically by using total bilirubin test kits supplied by SPINREACT according to the method described by[10]. The blood serum alkaline phosphatase and glucose were estimated photometrically by using alkaline phosphatase and glucose tests kits supplied by BioSystems reagents & instruments according to the method described by[11, 12].

Statistical analysis

Analysis of data was performed using SPSS v. 25 (Statistical Package for Social science) for Windows. Mean values were considered significantly different at $P < 0.05$. Data are expressed as (mean values ± SD).

Results

The measured AST, ALT, total bilirubin, alkaline phosphatase and glucose were found to be significantly ($p < 0.05$) higher in pregabalin rats when compared with normal control animals (Table 1, Figs.1, 2, 3, 4 and 5).

Histopathological findings

Table (1) : Comparison between control and pregabalin groups regarding tested liver functions, alkaline phosphatase and glucose levels in serum.

	AST (U/L)		ALT (U/L)		T.B (mg/dl)		Alkaline Phosphatase (U/L)		Glucose (mg/dl)	
	Control group No=40	Pregabalin group No=40	Control group No=40	Pregabalin group No=40	Control group No=40	Pregabalin group No=40	Control group No=40	Pregabalin group No=40	Control group No=40	Pregabalin group No=40
Mean±SD	28.9±4.4	48.3±3.9	23.5±4.2	43.4±4.5	0.39±0.12	1.1±0.2	168.8±8.6	230.8±25.3	110.3±7.8	159.1±8.4
Range (min-max)	24.8-33.6	43.6-52.5	18.9-26.9	38.3-48.1	0.27-0.51	0.88-1.4	162.1-178.5	198.3-268.9	103.1-118.6	148.5-171.2
Median	28.4	48.7	24.62	43.9	0.38	1.1	165.73	230.68	109.2	158.91
P-value	0.001**		0.001**		0.002*		0.007*		<0.001**	

*P-value is significant at ≤0.05
**P-value is highly significant at ≤0.001

Histopathological examination of liver sections of the control group showed normal histological structure which consists mainly of central vein and hepatic cords radiating from the central vein towards the periphery and alternating with narrow blood sinusoids, which lined with single layer of Van Kupffer cells and normal histological structure of portal areas including blood vessels and bile ducts (Figs. 6a-b).

In contrast, the liver of Pregabalin group showed marked pathological changes in the form of congestion of portal area blood vessels, central veins and hepatic sinusoids and hemorrhages. Severe degenerative changes including vacuolar degeneration and severe necrotic changes which characterized by nuclear pyknosis and homogenous acidophilic cytoplasm of hepatocytes (Figs. 6c-d) could be found. Focal lymphocytic infiltration within hepatic parenchyma and portal area was found associated with marked proliferation of Van Kupffer cells (Figs. 6e-f).

Figures:-

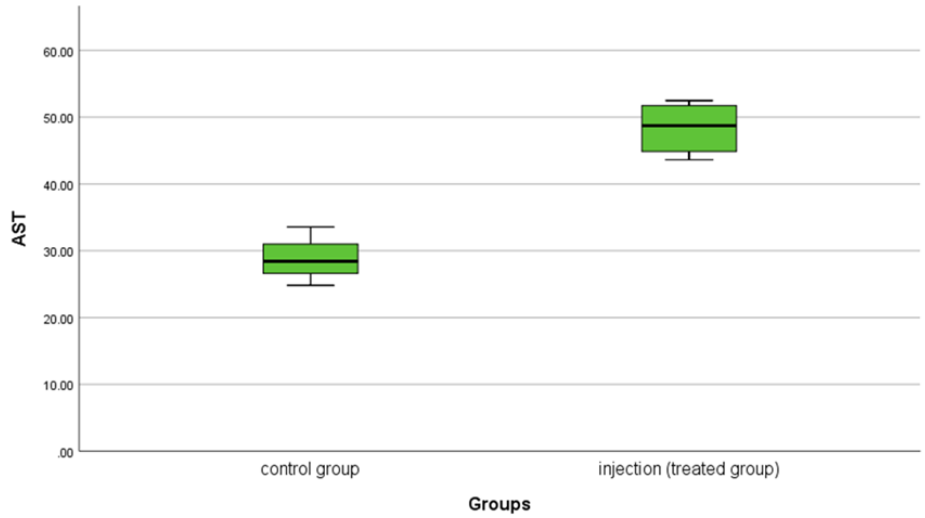

Figure (1): Distribution of AST in both groups.

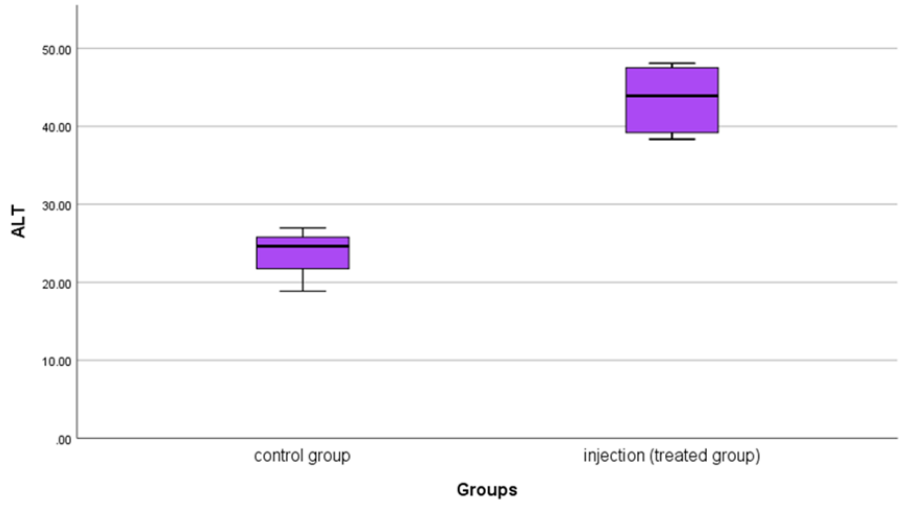

Figure (2): Distribution of ALT in both groups.

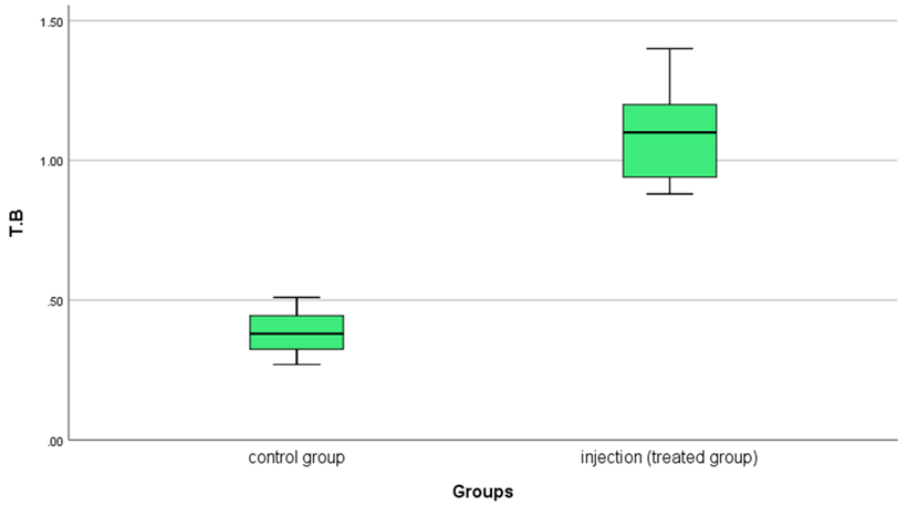

Figure (3): Distribution of T.B in both groups.

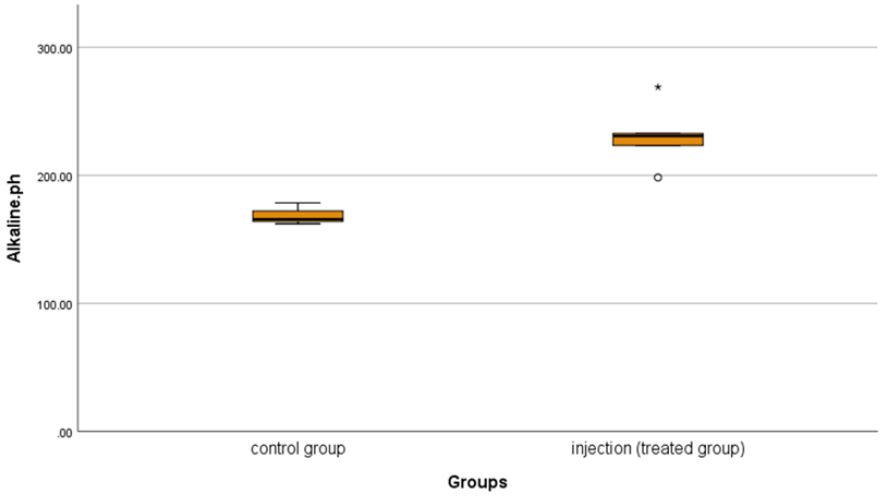

Figure (4): Distribution of alkaline phosphatase in both groups.

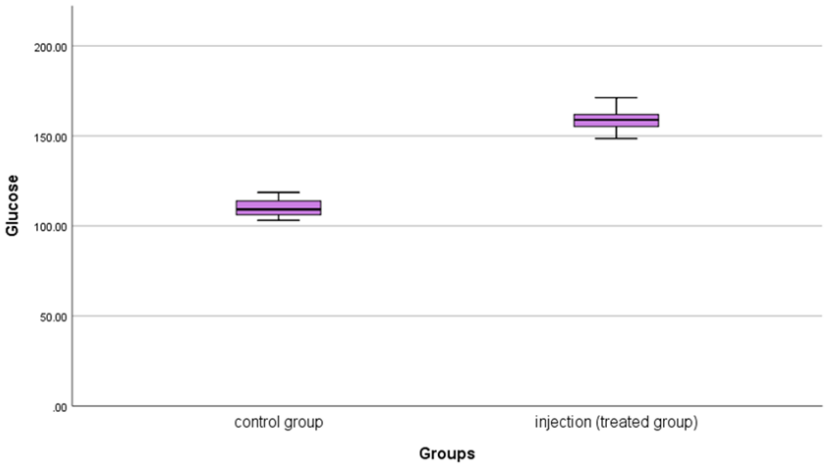

Figure (5): Distribution of glucose in both groups.

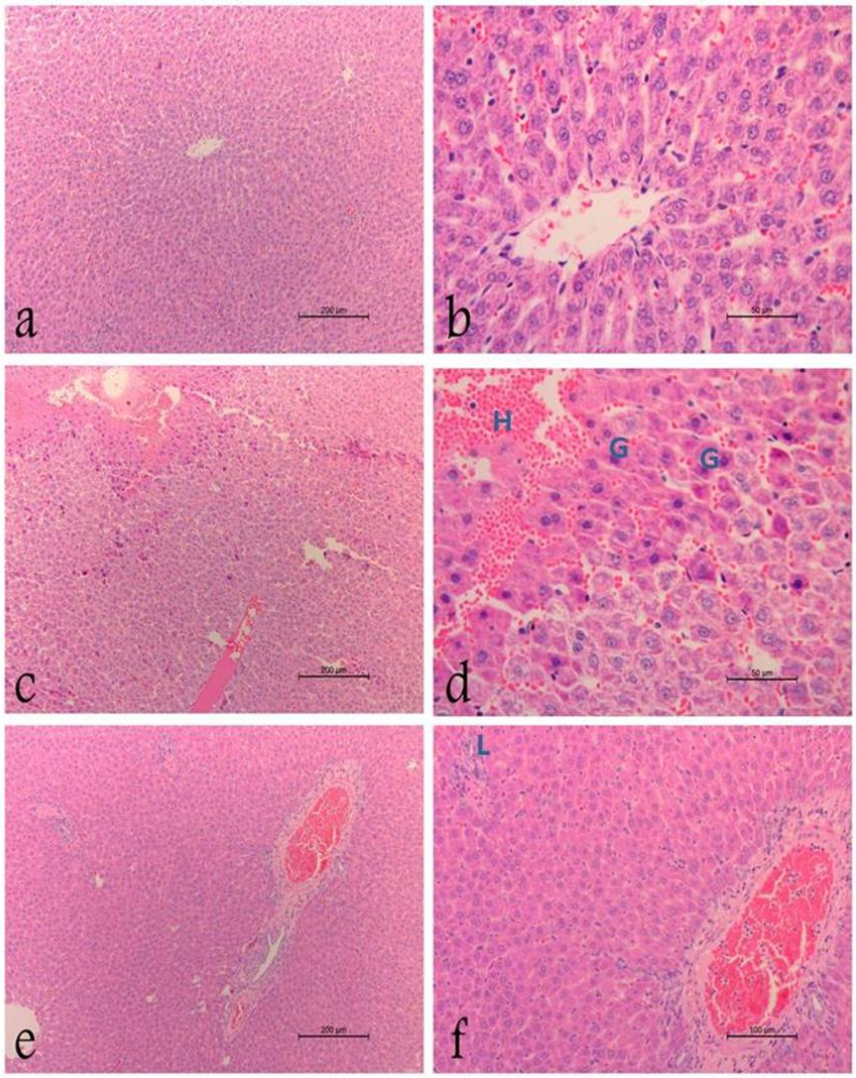

Figure (6): A photomicrograph of liver section of control group rats showed normal histological structures including central veins and hepatocytes (a-b), and pregabalin group showed coagulative necrosis of hepatocytes 'G' and hemorrhages 'H' (c-d) and leuckocytic infiltration in the portal area 'L' (e-f). (HE X100, 400).

Discussion

Recently, pregabalin was added to a European Union list of new recreational psychoactive substances [13], also drug induced liver injury (DILI) is one of the potential side effects of pregabalin[14], our study is focusing on the toxicological effects of pregabalin on liver among abusers due to prolonged pregabalin administration by using doses of 1500 and 3000 mg/day.

Our results demonstrate that pregabalin abuse at those high doses which exceed the therapeutic ones, for a long period of time, could harm liver on biochemical and histopathological levels. Our results show a significant increase ($p \leq 0.001$) in all tested liver functions (AST, ALT and total bilirubin) and alkaline phosphatase in pregabalin group over control one. Many studies agree with our results and discussed different mechanisms of pregabalin induced liver injury.[15] noted pregabalin as a probable cause of hepatotoxicity which was assessed using a specific scale for hepatotoxicity provided by the Council for International Organizations of Medical Sciences.[3] explained liver affection and cholestasis due to pregabalin use as an idiosyncratic hepatotoxicity and also it is previously noted by [16] who recommended more diagnostic measures, especially liver biopsy, to confirm this hypothesis while[17] and[18] added hepatocellular injury as a mechanism of pregabalin-related hepatotoxicity.

Also, the prospective study by[19], conducted for one year to assess the incidence of drug induced liver injury in patients aged below eighteen years, revealed significant elevations in ALT and AST levels in the most of patients using pregabalin and noted that the predominant type of pregabalin induced liver injury was hepatocellular lesion, followed by cholestatic lesion. In addition, anti-epileptic drugs including pregabalin have been shown causing liver injury by a percentage of 16.7% between other observed drugs and represented the second group of liver affecting drugs after anti-infective drugs.

Our results show significatnt elevations in blood glucose levels, but[20] revealed that pregabalin (20 and 40 mg/kg, intraperitoneal) did not affect the blood glucose levels in mice while[21] showed that pregabalin can cause hypoglycemia. The increase in serum glucose level in our study may be due to liver affection as it is known that the liver plays a central role in the balancing between the storage and uptake of glucose through glycogenesis and the release of glucose through glycogenolysis and gluconeogenesis[22, 23].

Whereas the detection of abnormal liver biochemical test levels usually is the first sign of the evidence of liver disease[24] and upon the recommendations of[16], we are focusing on the histopathological liver affection by pregabalin at abusing doses. Our study demonstrates marked liver cells affection under microscope. Severe necrosis and degenerative changes of hepatocytes are markedly seen and also congestion of portal area blood vessels, central veins and hepatic sinusoids and hemorrhages. In addition to that, focal lymphocytic infiltration within hepatic parenchyma and portal area associated with marked proliferation of Van Kupffer cells could be observed which points to an inflammatory reaction.

Our interesting results are consistent with the hypotheses of[17-19], all of those studies described pregabalin liver affection as an idiosyncratic inflammatory reaction and hepatocellular lesion. Also,[25] revealed that pregabalin increases the incidence of hemangiosarcoma in mice.

Our histopathological features appeared in the liver of pregabalin group could be explained as pregabalin was known by affecting cellular DNA through the binding of pregabalin molecule with the minor groove of the duplex CT-DNA. As DNA minor groove binders represent an important class of derivatives in anti-cancerous therapy[26] and also pregabalin affects tissues by producing tissue hypoxia resulting in endothelial cell proliferation but in mice not in rats[27-29]. To conclude, the absence of pregabalin liver metabolism does not prevent pregabalin induced hepatotoxicity[18].

Conclusion and Recommendations

Pregabalin abuse can markedly affect the the liver on both levels, biochemically and histopathologically.

The study recommends Healthy life style promotion and antagonism of pregabalin abuse

and consumption in non-medical goals, prescribing pregabalin should be with caution to diabetic patients, limiting pregabalin prescribtions for hepatic patients and diagnostic measures, especially liver biopsy, are recommended to confirm liver affection and cholestasis caused due to pregabalin use.

List of Abbreviations

ALT: Serum alanine transaminase.
AST: Serum aspartate transaminase.
DILI: Drug induced liver injury.
GABA: gamma-aminobutyric acid.
SD: Standard deviation.
T.B: Serum total bilirubin.
GAD: Generalized anxiety disorder.
SPSS: Statistical Package for Social science.

References

1. Silverman, R. B. (2016). Basic science to blockbuster drug: invention of Pregabalin (LYRICA®). Technology & Innovation, 17(4), 153-158.
2. Bonnet, U., & Scherbaum, N. (2017). How addictive are gabapentin and pregabalin? A systematic review European Neuropsychopharmacology, 27(12), 1185-1215.
3. Quintero-Castellanos, M. (2017). Pregabalin-induced liver injury–Case report. Colombian journal of anesthesiology, 45(4), 349-352.
4. Taha, S. H. N., Zaghloul, H. S., Ali, A. A. E. R., Gaballah, I. F., Rashed, L. A., & 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.
5. Tawfeeq, E. N., Fakhrildin, M. B., & Yanzeel, J. H. (2016). Endocrine reproductive effects of Pregabalin drug in female albino rats.
6. Nair, A. B., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. Journal of basic and clinical pharmacy, 7(2), 27.
7. Martinotti, G., Papazisis, G., Santacroce, R., Kouvelas, D., Cinosi, E., Lupi, M., & di Giannantonio, M. (2016). Pregabalin Abuse and Addiction. In Neuropathology of Drug Addictions and Substance Misuse (pp. 945-951). Academic Press.
8. Bancroft, D;Stevens, A;Turner, R (1996): The haematoxylin and eosin. Theory and practice of histological techniques. 4th ed, Ch 6, pp.99–112.
9. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American journal of clinical pathology, 28(1), 56-63.
10. Levitt, D. G., & Levitt, M. D. (2014). Quantitative assessment of the multiple processes responsible for bilirubin homeostasis in health and disease. Clinical and experimental gastroenterology, 7, 307.
11. Ng, V. (2002). Effects of Disease on Clinical Laboratory Tests, Vol. 1 and 2. DS Young and RB Friedman, eds. Washington, DC: AACC Press, 2001, $199.00 ($159.00 AACC members), softcover. ISBN 1-890883-45-X. Clinical Chemistry, 48(4), 682-683.
12. Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry, 6(1), 24-27.
13. Evoy, K. E., Morrison, M. D., & Saklad, S. R. (2017). Abuse and misuse of pregabalin and gabapentin. Drugs, 77(4), 403-426.
14. Vidaurre, J., Gedela, S., & Yarosz, S. (2017). Antiepileptic drugs and liver disease. Pediatric neurology, 77, 23-36.
15. Sendra, J. M., Junyent, T. T., & Pellicer, M. J. R. (2011). Pregabalin-induced hepatotoxicity. Annals of Pharmacotherapy, 45(6), e32-e32.
16. Crespo, P. L., Moreira, V. V., Manzano, F. R., & García, A. X. (2008). Cholestasis associated with pregabalin treatment. Medicina clinica, 130(4), 157.
17. Dogan, S., Ozberk, S., & Yurci, A. (2011). Pregabalin-induced hepatotoxicity. European journal of gastroenterology & hepatology, 23(7), 628.

18. Düzenli, T., Ata, E., Kösem, M., & Bayram Çarli, A. (2017). Pregabaline as a Rare Cause of Hepatotoxicity. Pain Medicine.
19. Cano-Paniagua, A., Amariles, P., Angulo, N., & Restrepo-Garay, M. (2019). Epidemiology of drug-induced liver injury in a University Hospital from Colombia: updated RUCAM being used for prospective causality assessment. Annals of hepatology, 18(3), 501-507.
20. Ikeda, H., Yonemochi, N., Ardianto, C., Yang, L., & Kamei, J. (2018). Pregabalin increases food intake through dopaminergic systems in the hypothalamus. Brain research, 1701, 219-226.
21. Raman, P. G. (2016). Hypoglycemia induced by pregabalin. Journal of The Association of Physicians of India, 64.
22. Nordlie, R. C., Foster, J. D., & Lange, A. J. (1999). Regulation of glucose production by the liver. Annual review of nutrition, 19(1), 379-406.
23. Petersen, M. C., Vatner, D. F., & Shulman, G. I. (2017). Regulation of hepatic glucose metabolism in health and disease. Nature reviews endocrinology, 13(10), 572-587.
24. Martin, P., & Friedman, L. S. (2017). Assessment of liver function and diagnostic studies. In Handbook of liver disease (pp. 1-17). Elsevier.
25. Toth, C. (2012). Drug safety evaluation of pregabalin. Expert opinion on drug safety, 11(3), 487-502.
26. Shahabadi, N., & Amiri, S. (2015). Spectroscopic and computational studies on the interaction of DNA with pregabalin drug. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 138, 840-845.
27. Criswell, K. A., Cook, J. C., Morse, D., Lawton, M., Somps, C., Obert, L., ... & Navetta, K. (2012a). Pregabalin Induces Hepatic Hypoxia and Increases EndothelialCell Proliferation in Mice, a Process Inhibited by DietaryVitamin E Supplementation. Toxicological Sciences, 128(1), 42-56.
28. Criswell, K. A., Cook, J. C., Wojcinski, Z., Pegg, D., Herman, J., Wesche, D., ... & Anderson, T. (2012b). Mode of action associated with development of hemangiosarcoma in mice given pregabalin and assessment of human relevance. Toxicological Sciences, 128(1), 57-71.
29. Criswell, K. A., Wojcinski, Z., Pegg, D., Albassam, M., Duddy, S., Olsen, E., ... & Anderson, T. (2012c). Key components of the mode of action for hemangiosarcoma induction in pregabalin-treated mice: evidence of increased bicarbonate, dysregulated erythropoiesis, macrophage activation, and increased angiogenic growth factors in mice but not in rats. Toxicological Sciences, 128(1), 22-41.