# Association Between Carbamazepine Toxicity, Liver Bile Duct Injury, Granuloma and Inflammatory Cells Infiltration in Female Mice

**A.T. Nawar R. Jaber[1], Nahla A. Al-Bakri[2], A.T. Ola Al-Bhadly[3]**

*[1]Assistant Lecture / Dept. of Medical Laboratory Science / Dijlah University College/Iraq, [2]Professor / Dept. of Biology / College of Education for Pure Science (Ibn- Al-Haitham) / University of Baghdad/Iraq, [3]Assistant lecture / Dept. of Biology / College of Education for Pure Science (Ibn- Al-Haitham) / University of Baghdad/Iraq.*

## Abstract

The liver is an important organ in the body that can be affected by many drugs and toxins. The hepatotoxins can cause oxidant stress that lead to activation of inflammatory cells and cause liver damage. Drug induced bile duct injuries are related to drug toxicity, multiple drugs have been known to cause the development of liver granulomas. Carbamazepine (CBZ) among other antiepileptic drugs is believed to cause hepatic injury. In this study we investigated the effect of (CBZ) 20mg/kg/day on female mice liver after 14 and 30 days of treatment. The histological findings showed that (CBZ) can cause histological alterations in the liver components such as bile duct proliferation, biliary hypertrophy, ductopenia, inflammatory cells infiltration and granulomas.

***Key words**: Carbamazepine, liver injury, bile duct injury, infiltration, granuloma.*

## Introduction

The liver is an important organ in the body that can be affected by many drugs and toxins, some drugs can cause death or even liver transplant [(1)]. The liver has different cells that help the organ maintain immune homeostasis, drug induced liver injury can cause inflammatory cells infiltration, those hepatotoxins can cause oxidative stress that lead to activation of these cells [(2)]. The responded of these cells can cause liver damage, innate immune cells take a part in liver inflammation and are the first line of defense. As well as they play a role in tissue repair and treat of the inflammation [(3)] infiltration of these cells can cause hepatocytes damage especially lymphocytes that can be seen in chronic liver diseases [(4)].

Drug induced bile duct injuries are represented in many pathological forms in the portal areas of the liver. The destruction of bile ducts epithelium after drug treatment is the main character to be seen in response to drug toxicity and a very common side effect of some therapies [(5,6)].

Multiple drugs have been known to development the liver granulomas [(7)]. Granulomas are the aggregation of multiple immune cells such as lymphocyte and macrophages, due to endogenous or exogenous stimuli [(8,9)], which can be seen in chronic inflammation [(10)]. There are different type's granulomas such as foreign body, necrotizing, non-necrotizing, diffuse and suppurative granulomas [(11)], they rarely cause damage to liver structure [(12)].

Antiepileptic drugs are known to cause acute and chronic toxicity, beside adverse effects like neurotoxicity [(13,14)] which are common with these treatments.

Carbamazepine (CBZ) among other antiepileptic drugs is connected to hepatic granuloma, this drug works by blocking $NA^+$ channels in the brain [(15)], it metabolizes in the liver by Cytochrome P450 [(16)], it`s metabolites work as neoantigenes and can lead to immune reactions [(17)]. Other organs suffer from CBZ toxicity such as kidneys [(18)] and the brain. Therefore,

**Corresponding author:**
**A.T. Nawar R. Jaber**
Rukia_1986@yahoo.com

the study aims to investigate the negative pathological effects of carbamazepine treatment on liver components of mice.

## Materials and Methods

### Chemicals

Carbamazepine (CBZ) oral suspension used in this study was purchased from Novartis pharma AG basal Switzerland.

### Animal of study

A total of40 adult female mice were used in this experiment, average weight 22-29 gm, they were brought to the animal house in Department of Biology / College of Education for Pure Science- Ibn- Al-Haitham and all under the same conditions, they were separated in to 4 groups with 10 mice each. The first group was administered with tap water and named as a control group. The second group was orally administered (CBZ) 20 mg/kg/day for 14 days. The third was also a control group administered with tap water and the fourth group was administered (CBZ) 20 mg/kg/day orally for 30 days. The first two groups were sacrificed after 14 days of treatment and the second two groups were sacrificed after 30 days of treatment.

### Histological sections preparation

The livers of scarified mice were collected and immersed in 10% formalin and tissue samples were prepared for histological study by using paraffin method then sections were made and stained with conventional histological stains hematoxylin and eosin [19].

Control and treated sections of the liver were examined under the light microscope for bile duct injury, infiltration and granulomas at 10x, 40x and 100x magnifications power.

## Results

In the present investigation, the liver of the control group showed normal bile duct structure with normal liver parenchyma and cells distribution (fig.1).

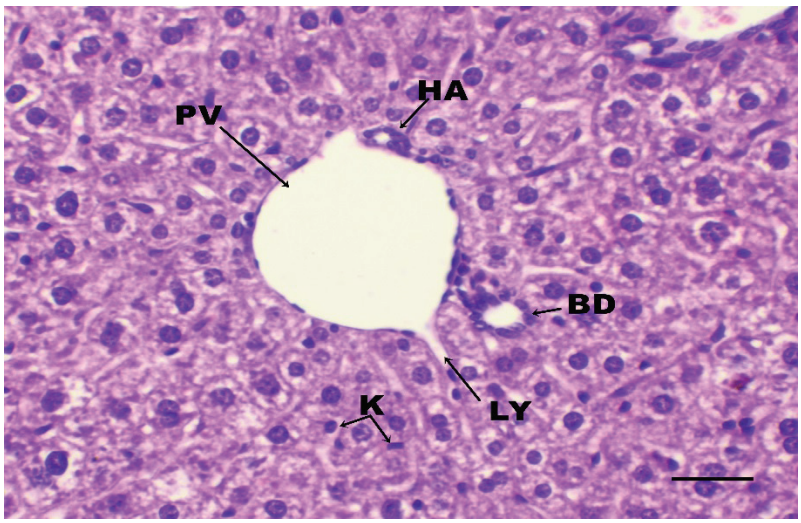


**Figure (1): Cross section through female mice liver of the control group showing normal bile duct (BD), portal vein (PV), lymphatic vessels (LY), hepatic artery (HA) and Kupffer cells (K), H&E ,40x, scale bar 8.81µm.**

The toxic effects of (CBZ) on the liver of female mice after 14 and 30 days of treatment were evaluated, it was found that drug toxicity induced bile duct injury in the 14 days' group, biliary proliferation and biliary hypertrophy was seen (fig.2), as for the 30 days' group, ductopenia was also noticed beside the injuries that were seen in the 14 days' group (fig .3).

Infiltration of inflammatory cells at the portal areas was noticed in the treated groups, consisted of lymphocytes, neutrophils, plasma cells and eosinophils (figs. 4, 2).

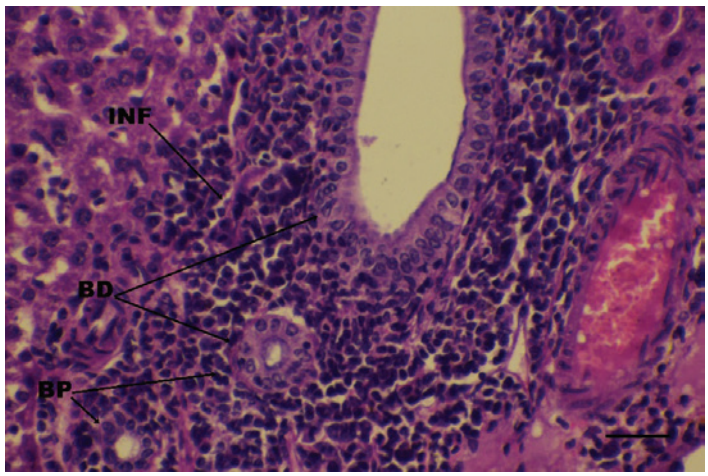


**Figure (2): Cross section through female mice liver treated with CBZ 20mg/kg/day for 14 days demonstrating bile duct (BD) hypertrophy and proliferation (BP), H&E ,40x, scale bar 8.81µm.**

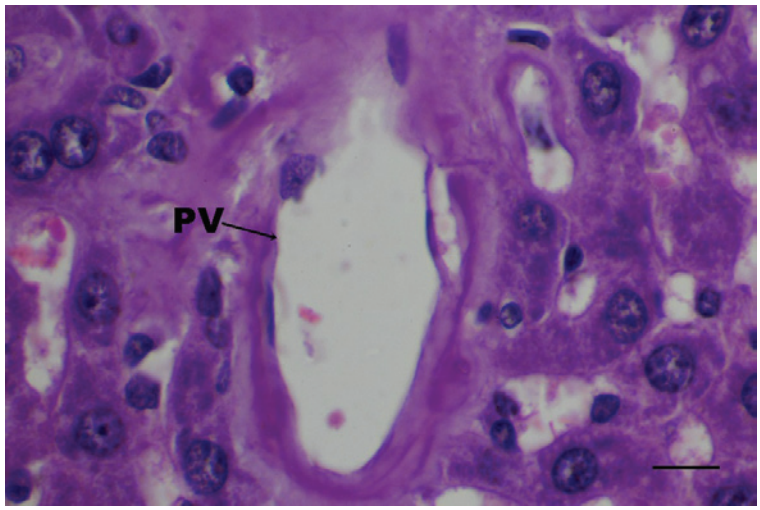


**Figure (3): Cross section through female mice liver treated with CBZ20mg/kg/day for 30 days demonstrating ductopenia, H&E,100x, scale bar 22.03µm.**

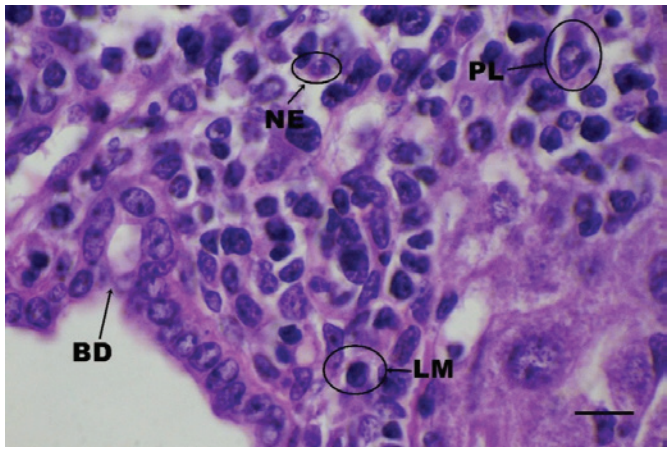


**Figure (4): Cross section through female mice liver treated with CBZ 20mg/kg/day for 30 days demonstrating bile duct (BD) hypertrophy, infiltration of neutrophils (NE), lymphocytes(LM) and plasma cells(PL), H&E, 100x, scale bar 22.03µm.**

Loose granuloma and micro granulomas were detected in the 14 days' group (fig.5), while well-formed granulomas were detected in the 30 days' group (fig.6), the granulomas were consisted of lymphocytes, macrophages and neutrophils(fig.6).

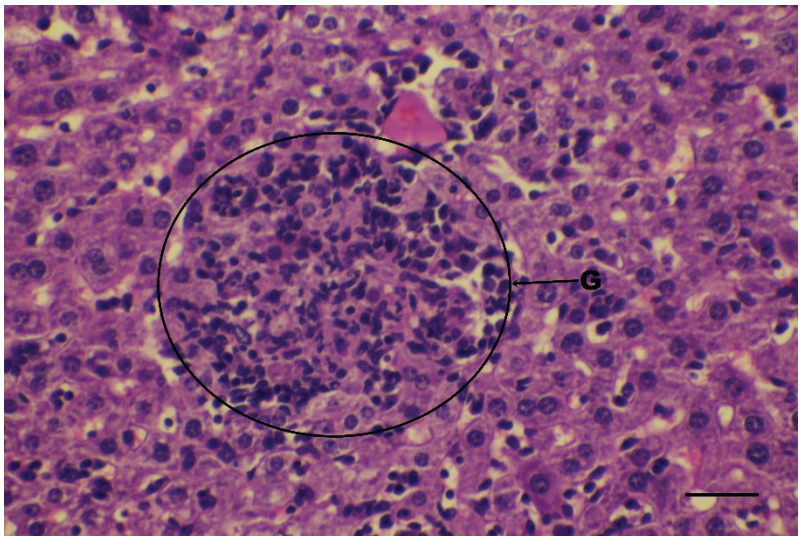


**Figure (5): Cross section through female mice liver treated with CBZ 20mg/kg/day for 14 days demonstrating granuloma (G), H&E ,40x, scale bar 8.81µm.**

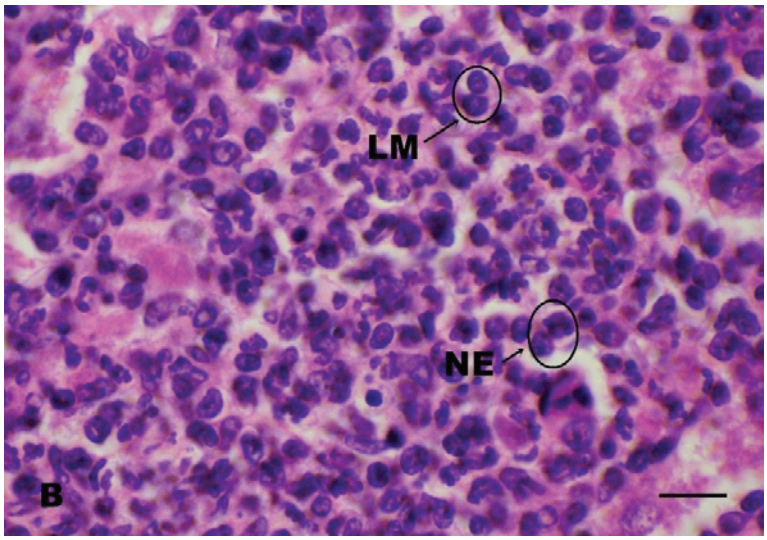


**Figure (6): Cross section through female mice liver treated with CBZ 20mg/kg/day for 30 days demonstrating well-formed granulomas consisting of lymphocytes (LM) and neutrophils (NE), H&E ,100x, scale bar 22.03µm.**

## Discussion

Histological findings in the present study showed groups treated with (CBZ) 20mg/kg/day for 14 and 30 days showed histological alterations in liver compositions. Bile duct proliferation was noticed in both groups, it is thought that toxic effect of the drug can cause bile duct proliferation [20] or this toxicity caused a ductular reaction in this form [21], biliary hypertrophy in the 14 and 30days' group was evident , this indicates that toxic effect of the drug lead to bile duct injury that made bile duct cells go through hypertrophy when hepatic injury occurred [22,23],these cells "the cholangiocytes", are very sensitive to any injury and become activated , also secreting and assisting with the bile duct work [24].

In the 30 days' group, ductopenia was shown in the portal areas as well as fibrosis was induced by the hepatic inflammation and lead to liver injury may have caused it

[25], in addition, the cholangiocytes will start apoptosis at some point and that will lead to this effect [26].

Inflammatory cells infiltration was noticed in both treated groups near portal areas and sometimes seen with or without necrosis it was demonstrated that hypersensitivity reaction to the drug may have caused an inflammation that lead to the infiltration [27] .

Multiple Granulomas of various sizes were seen in liver tissue in both treated groups. The formation of granulomas is linked to the inflammation in the portal areas, and as a result of immune reaction to the toxicity of the drug [28]. Also believed formation of granulomas is due to the failure of the cellular immune response to eliminate the foreign substance [29]. Granulomas can be found anywhere in the tissue and it is connected to hepatitis [30].

## Conclusion

Antiepileptic drug Carbamazepine (CBZ) can cause hepatic injuries, the histological findings showed that (CBZ) causes histological alterations in liver components after short and long period of administration, such injuries include bile duct proliferation, biliary hypertrophy, ductopenia, inflammatory cells infiltration and granulomas.

**Ethical Clearance**: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest**: Non

**Funding:** Self-funding

## References

1. Kumar V, Abbas A, Fausto N, Aster J. Robbins basic pathology. 9th ed. Philadelphia: WB Saunders Elsevier ;2003.
2. Foureau D, Walling T, Maddukuri V, Anderson W, Culbreath K, Kleiner D et al. Comparative analysis of portal hepatic infiltrating leucocytes in acute drug-induced liver injury, idiopathic autoimmune and viral hepatitis. Clin Exp Immunol. 2015;180(1):40-51.
3. Liaskou E, Wilson D, Oo Y. Innate Immune Cells in Liver Inflammation. Mediat Inflamm. 2012; 2012:1-21.
4. Paronetto F, Colucci G, Colombo M. Lymphocytes in Liver Diseases. Progress in Liver Diseases. 1986; 8:191-208.
5. Zhao Z, Bao L, Yu X, Zhu C, Xu J, Wang Y et al. Acute vanishing bile duct syndrome after therapy with cephalosporin, metronidazole, and clotrimazole. Medicine. 2017;96(36):e8009.
6. Visentin M, Lenggenhager D, Gai Z, Kullak-Ublick G. Drug-induced bile duct injury. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2018;1864(4):1498-1506.
7. Ishak K, Zimmerman H. 11 Drug-induced and toxic granulomatous hepatitis. Baillière's Clinical Gastroenterology. 1988;2(2):463-480.
8. Coash M, Forouhar F, Wu C, Wu G. Granulomatous liver diseases: A review. J Formos Med Assoc. 2012;111(1):3-13.
9. Lagana S, Moreira R, Lefkowitch J. Hepatic Granulomas: Pathogenesis and Differential Diagnosis. Clin Liver Dis. 2010;14(4):605-617.
10. Ferrell L. Hepatic granulomas: A morphologic approach to diagnosis. Surg Pathol. 1990; 3:87-106.
11. Shah K, Pritt B, Alexander M. Histopathologic review of granulomatous inflammation. J Clin Tuberc Other Mycobact Dis. 2017; 7:1-12.
12. Culver E, Watkins J, Westbrook R. Granulomas of the liver. Clin Liver Dis. 2016;7(4):92-96.
13. Camfield P, Camfield C. Acute and Chronic Toxicity of Antiepileptic Medications: a Selective Review. Can J Neurol Sci. 1994;21(S3):S7-S11.
14. St. Louis E. Minimizing AED Adverse Effects: Improving Quality of Life in the Interictal State in Epilepsy Care. Curr Neuropharmacol. 2009;7(2):106-114.
15. Davies J. Mechanisms of action of antiepileptic drugs. Seizure. 1995;4(4):267-271.
16. Waseem M. Carbamazepine Toxicity Clinical Presentation: History, Physical Examination [Internet]. Emedicine.medscape.com. 2020 [cited 27 May 2020]. Available from: https://emedicine.medscape.com/article/813654-clinical.
17. Carey M, Vanpelt F. Immunochemical detection of

flucloxacillin adduct formation in livers of treated rats. Toxicology. 2005;216(1):41-48.

18. Al-Yassiri S, Al-Bakri NA, Al-Kawaz U, Selman M. Histological study on kidney affected by carbamezepine drug in postnatal rat. World J. Pharm. Res.2016;5(11):205-219.
19. Suvarna S, Layton C, Bancroft J. Bancroft's theory and practice of histological techniques. [S.l.]: Churchill Livingstone/Elsevier; 2016.
20. Maronpot R, Yoshizawa K, Nyska A, Harada T, Flake G, Mueller G et al. Hepatic Enzyme Induction. Toxicol Pathol. 2010;38(5):776-795.
21. Alpini G, Lenzi R, Zhai W, Liu M, Slott P, Paronetto F et al. Isolation of a nonparenchymal liver cell fraction enriched in cells with biliary epithelial phenotypes. Gastroenterology. 1989;97(5):1248-1260.
22. Gardner W, Allen E, Smith G. Hyperplasia and Hypertrophy of the Mucosa of Larger Biliary Ducts in Mice Receiving Estrogens. Exp Biol Med. 1941;46(3):511-513.
23. Fausto N. Liver regeneration and repair: Hepatocytes, progenitor cells, and stem cells. Hepatology. 2004;39(6):1477-1487.
24. Maroni L, Haibo B, Ray D, Zhou T, Wan Y, Meng F et al. Functional and Structural Features of Cholangiocytes in Health and Disease. CMGH Cell Mol Gastroenterol. 2015;1(4):368-380.
25. Hübscher S. Histological assessment of the liver. Medicine.2015; 43(10):568-572.
26. Lazaridis K, Strazzabosco M, LaRusso N. The cholangiopathies: Disorders of biliary epithelia. Gastroenterology. 2004;127(5):1565-1577.
27. Rivera L, Rivera I, Toro D, Gutierrez J, Acosta E. Eosinophilic Liver Infiltration. ACG Case Reports Journal. 2015;3(1):63-65.
28. Elshama S, Osman H, El-Kenawy A. Histopathological study of developmental toxicity of Carbamazepine in the mice. Intl Res J Appl Basic Sci. 2013;7(10):616-622.
29. Wahl S. Hepatic granuloma as a model of inflammation and repair: An overview. Methods Enzymol. 1988; 163:605-622.
30. Lamps L. Hepatic Granulomas: A Review with Emphasis on Infectious Causes. Archives of Pathology & Laboratory Medicine. 2015;139(7):867-875.