Pakistan J. Zool., pp 1-9, 2023. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20220219060234

Effect of Propranolol in Hepatic Blood Flow: A Way to Encounter Phenytoin Induced Hepatotoxicity in Albino Rabbits

Hina Abrar[1]*, Hina Yasin[1], Hira Naeem[2], Rabia Bushra[1], Shaukat Mahmood[2] and Kaneez Fatima[3]

[1]Dow College of Pharmacy, Dow University of Health Sciences, Ojha Campus, Gulzar E Hijri, Scheme-33, Karachi, Pakistan
[2]Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Super Highway, Gadap Road, Karachi, Pakistan
[3]Institute of Pharmaceutical Sciences, Jinnah Sindh Medical University, Rafiqui H.J Shaheed Road, Karachi, Pakistan

Article Information
Received 19 February 2022
Revised 23 January 2023
Accepted 15 February 2023
Available online 18 May 2023
(early access)

Authors' Contribution
HA conceptualization and execution of project. HY and HN conduction of experiments. HN and RB result analysis and manuscript drafting. SM and KF result analysis.

Key words
Phenytoin, Propranolol, Hepatotoxicity, Micrometry, Scanning electron microscopy, Hepatoprotective

ABSTRACT

Phenytoin has proved to be the most effective anti-seizure drug to treat the partial and tonic clonic seizure. However; hepatotoxicity associated with the Phenytoin (PHY) is of great concern in clinical settings. Contrarywise, Propranolol (PRL) is reported to possess the significant hepatoprotective effects owing to the reduced blood flow. Therefore, the present study was aimed to evaluate the hepatotoxicity of PHY alone and in combination with PRL in healthy male rabbits. Post treatment level of various liver enzymes including alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ-GT) and bilirubin (BRB) were measured and compared with the normal values. Additionally, histological examination was done through micrometry and scanning electron microscopy (SEM) to estimate the intensity of hepatotoxicity induced by PHY and PHY with PRL. Animals received PHY alone treatment showed a significant elevation of liver enzymes than control and combination. Conversely, the lower level of serum ALT, ALP, γGT and BRB were found when administered PRL with PHY ($p<0.005$). Prominent change in hepatic cells structure and diameter of nucleus were observed ($p<0.05$). Severe hepatic damage reflected by necrosis, hemorrhage and dilation of sinusoids, inflammation with dilation and congestion of portal vein were obvious in PHY treated group. Combination treatment of PHY with PRL showed normal liver function and identical tissue pattern as control group exhibited. It is concluded that the PRL actually reduces the blood flow to the liver consequently, supply of noxious substance is blocked. Henceforth, the PRL would provide the beneficial effects when co-administered with the hepato-toxic drugs.

INTRODUCTION

Epilepsy is a neurologic disorder affected millions of people worldwide and considerable cause of morbidity and mortality. The prevalence of epilepsy is relatively higher in developing countries than the developed countries. The predicted efficacy of antiepileptic drugs has to be weighed against potential adverse effects, while at the same time considering the risks associated with withholding of treatment (Schmidt and Schachter, 2014). Phenytoin, carbamazepine and valproate are utilized to be the first line antiepileptic drugs. It is extensively documented that Phenytoin (PHY) has been found to be highly effective in management of partial and tonic clonic seizure. It is also prescribed in neuropathic pains and cardiac arrhythmias especially in digitalis induced arrhythmias. Beyond these treatment benefits unfortunately such aromatic antiepileptic drugs are prone to cause hepatic injuries (Vidaurre *et al*., 2017).

PHY is almost 95% metabolized in the liver while only 5% is excreted via kidney. It belongs to the aromatic antiepileptic category, often associated with hepatotoxicity usually manifested as idiosyncratic reaction. Symptoms of hepatotoxicity are usually appeared within first six weeks of the therapy (Curry *et al*., 2018; Patocka *et al*., 2020). Common warning signs are often include pyrexia,

* Corresponding author: hina.abrar@duh.edu.pk
0030-9923/2023/0001-0001 $ 9.00/0

skin irritation, lymphadenopathy, hepatomegaly, anorexia and myalgias or joint pain. Hospitalized patients may develop jaundice, periorbital and facial edema. Alteration in liver function parameters related with the PHY hepatotoxicity are elevation in serum aminotransferase, lactic dehydrogenase, alkaline phosphatase, bilurubin and prothombin time (Smythe and Umstead, 1989; Misra *et al*., 2013). Although the exact mechanism of hepatoxicity is still unknown but probably the immune related hypersensitivity reaction would lead to an oxidative stress, resulting in liver damage (Santos *et al*., 2008; Kamp *et al*., 2012). PHY induced hepatotoxicity was manifested as cholestasis with hepatitis, granulamatous hepatitis and acute hepatitis (Cadman and Featherstone, 2003; Diwan *et al*., 1993; Mahendra and Hendra, 2011).

Drug induced hepatotoxicity is now become a global burning issue especially when drugs are to be administered for long duration (Yoon *et al*., 2016; Kaplowitz, 2004). Various herbal formulation and vitamins are studied to evaluate their effects in PHY induced hepatotoxic animal models (Sasaki *et al*., 2013; Owoeye *et al*., 2015; Saraswathy *et al*., 2015b).

PRL is a non-selective beta adrenergic receptors antagonist, excellently reduce the portal hypertension and bleeding from esophageal varices. The effectiveness of drug in such disorders is due to the decrease hepatic blood flow (Tursi, 2010). It is therefore, hypothesized that the reduction in blood flow to hepatic portal system by PRL would be responsible to subside the toxic effect of PHY to liver.

Based on the above mentioned facts, the current study is design to evaluate the extent of liver damage produced by the PHY. Additionally, the role of PRL in PHY induced injuries will be explored with the concurrent administration of PHY and PRL. The liver strength will be assessed by various enzymatic and non-enzymatic parameters of liver function test including alanine transaminase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (γ-GT) and bilirubin (BRB). The histo-pathological studies of liver will be performed using scanning electron microscopy of hepatic tissue to estimate the hepatic toxicity induced by the phenytoin and the repair of the hepatic injuries produced by PRL. Moreover, the micrometric analysis of liver by means of viable hepatocyte count and diameter and nucleus were also analyzed.

MATERIALS AND METHODS

PHY and PRL were obtained from Lisko Pakistan (Pvt) Ltd. Heamotoxylin and eosin (H and E) staining solution kit ALP, γ-GT, ALT and BRB standard kits and poly-1-lysine coated glass slides were purchased from Sigma– Aldrich, St. Louis, MD, USA. Hard paraffin wax and formalin were purchased from Merck.

Animal model

Healthy male rabbits were selected for the study due to identical human blood parameters (Feroz and Khan, 2013). Thirty six rabbits 1000-1200 g were purchased from the animal house of Baqai Medical University, Karachi, Pakistan. All rabbits were adapted from housing condition one week before experiments. Animals were kept in animal house of Baqai Medical University according to the standard protocol (Albus, 2012). All procedures of animal handling complied with international ARRIVE guidelines. Temperature was maintained to 25 ± 2 °C for 12 h light/dark cycle. Each rabbit was placed in a separate cage during the study period. Animal fed with laboratory standard balance diet and water adlibitum.

Experimental design

Total 36 animals were included in the study. Rabbits were divided into three groups normal saline, PHY and PRL having 12 animals in each one. Control group received normal saline orally only. In PHY treated group rabbits were treated with PHY 300mg/kg for 14 days as a single daily dose in form of oral solution through gastric gavage (Huang *et al*., 2004). In PHY+PRL treated group rabbits were received the PHY 300mg/kg and PRL 30mg/kg simultaneously once daily for 14 days in the form of oral solution via gastric tube (Huang *et al*., 1997; D'Amico *et al*., 2012).

Blood samples (5ml) were collected from the animals after 24 h of last administered dose. Serum was isolated after centrifugation at 4000 rpm for 8min (Parasuraman *et al*., 2010) and used for estimation of liver enzymes ALT, ALP and γ–GT and BRB by Automatic Hematology Analyzer by DRAWELL using standard kits within 2 h of serum separation.

Histological studies

Animals were sacrificed after 24 h of last administerd dose and livers were delicately separated. Livers were flushed with sterile normal saline and finally fixed in 10% buffered formalin. The fixed tissues were processed embedding for paraffin following the standard procedures (Alturkistani *et al*., 2016). Thick sections (5µm) of portions were cut and stained with haemotoxylin (H) and eosin (E) (Sharma and Janmeda, 2013). The micrometric analysis of hepatic tissues was done by measuring the number of viable hepatocyte with their diameter and diameter of

nucleus using Olympus BX40 bright field microscope camera (AmScope) and Toup View software (v3.7.7934).

Scanning electron microscopy (SEM)

The sectioned hepatic tissue were dehydrated and mounted on specimen stub with the help of electro-conductive double sided adhesive tape. The sample was coated with gold to observe under electron microscope (Murtey and Ramasamy, 2016).

Statistical analysis

The quantitative results were statically evaluated by statistical package for social sciences SPSS software (version 21). Findings of PHY treated and PHY+PRL treated group treated group were compared with control group by taking mean and standard errors using one way analysis of variation ANOVA, considering the $p<0.05$ is significant for any response.

RESULTS

Gross toxicity

The animals of PHY treated group were unhealthy and less reactive to external stimuli as compared to control group, where all individuals were found to be alive. There were no major architectural abnormality observed in the liver of PHY treated group animals rather only a slight increase in liver size. The color of livers was observed to be dull brown and dark brown respectively for treated and control group without the adhesion of fatty tissues and abnormal contracture. Upon post treatment analysis, the animals of phenytoin + propranolol treated group were active, healthy and well-responsive to external stimuli. All the animals of this set were alive but less active than control group. The liver of PHY+PRL treated group animals had regular architecture having smooth surface and normal reddish brown color without any adhesion and contracture.

Hepatic biomarkers

The mean serum levels of ALT, ALP and γ–GT and BRB are given in Table I. The statistical analysis showed that there was significant increase in the levels of all hepatic biomarkers in PHY treated group when compared to control. These biomarkers were also found to be increased in PHY and PRL treated group than control group. However; the level of ALT, ALP and γ–GT and BRB were significantly reduced in PHY+PRL treated group in comparison to the means of PHY treated group.

Histological analysis

H and E stained sections of liver of control group animals showed normal hepatic lobules with radial arrangement of hepatocytes and central vein. Portal triad was appeared to be normal with portal vein, hepatic artery and one or two bile ducts. The sinusoids were seemed to be lined by Kupffer cells and endothelial cells (Fig. 1A, B, C).

The morphological examination of the H and E stained liver sections of PHY treated rabbits' liver showed moderately disturbed but intact hepatic cord with dilated sinusoids especially in periportal area. Necrotic patches and hemorrhagic sinusoids were also found in various fields. The central vein was seem to be congested and markedly dilated. Moderate to severe mononuclear cell infiltrations were also observed in portal tract with dilated and congested portal vein. Neutophils and plasma cells were noticed within the inflammatory area (Fig. 1D, E, F, G).

Table I. Effect of propranolol on liver function and structure parameters of albino rabbits with phenytoin induced hepatotoxicity.

Parameters (Units)	Control (n=12)	Phenytoin (n=12)	Phenytoin+Propranolol (n=12)
ALT(IU/L)	40.80 ±1.14	128±3.07*	6.75±1.55**
ALP(IU/L)	42.60±2.79	114.8±3.61*	55.1±1.34**
γ GT (IU/L)	8.00 ± 0.93	24.2±1.29*	14.60±1.08**
Total BRB (µmol/L)	8.52± 1.03	35.98±3.32*	21.51±2.82**
Hepatocyte count (cell/reticule)	21.70±1.13	15.27±2.02*	20.00±1.10
Hepatocyte diameter (µm)	13.90± 0.33	11.98±1.86*	13.24±1.14**
Nuclear diameter (µm)	5.89± 0.07	4.27±0.52*	5.65±0.35**

Results values are expressed in Mean±SEM. ALT, alanine transaminase; ALP, alkaline phosphatase; γ-GT, gamma-glutamyltransferase; BRB, bilirubin. *Significant when Phenytoin compard with control. **Significant when Phenytoin+propranolol compared with control.

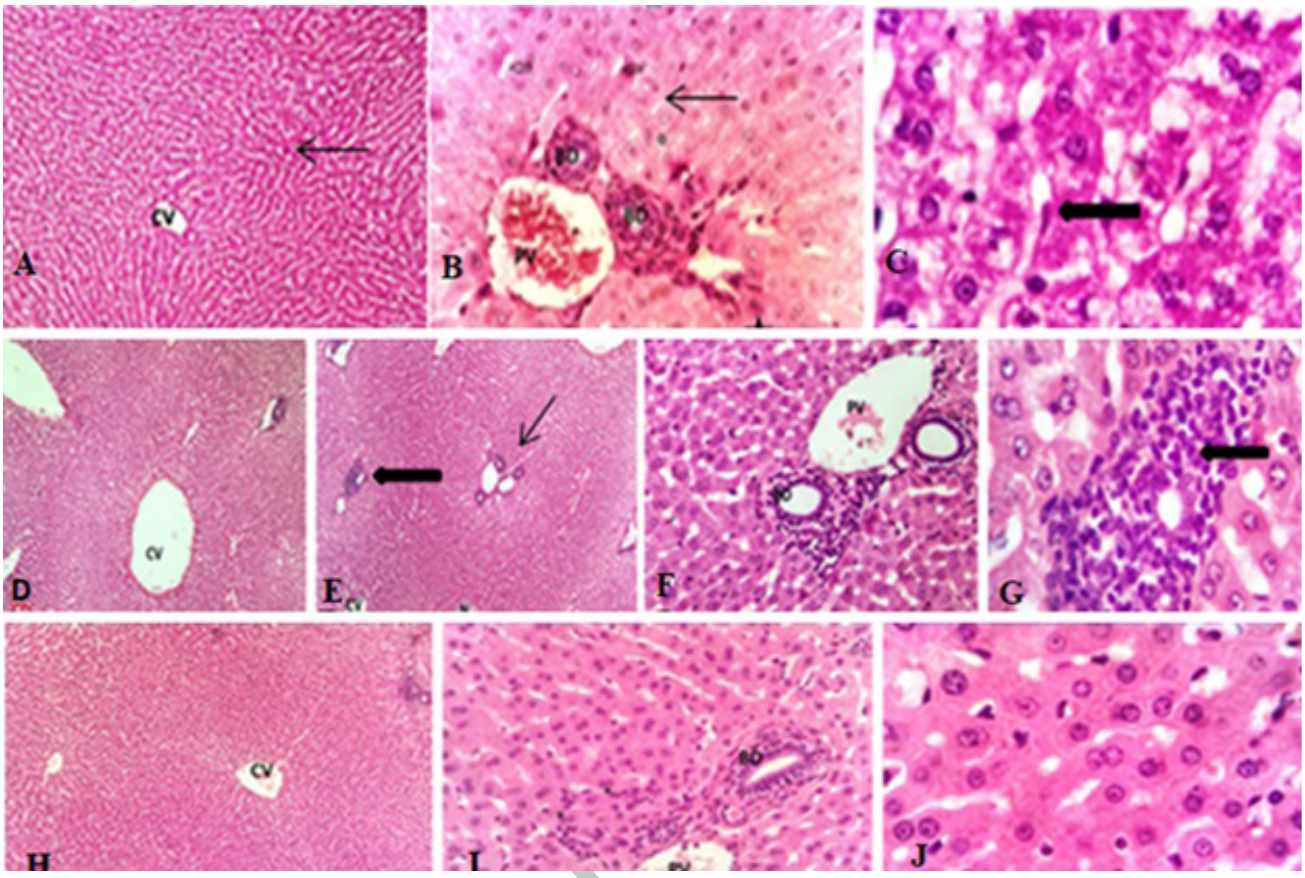

Fig. 1. Effect of propranolol on histological structure of phenytoin-treated liver of albino rabbits.
A, Photomicrograph of 5 micron thick hematoxylin and eosin (H and E) stained paraffin section from liver of rabbits of control group showing normal central vein (CV) and radiating hepatic architecture and normal sinusoidal spaces (arrow)(100X). B, showing normal portal vein (PV) and bile duct (BD), normal sinusoidal spaces (arrow) and binucleated hepaatocytes (star) (400X). C; showing normal hexagonal hepatocytes, kupffers cells (thick arrow) (1000X). D, PHY treated group showing moderately disturbed hepatic cords with dilated sinusoids especially in periportal area. The central vein (CV) was also markedly dilated (100X). E, showing necrotic patches (N), polymorphonuclear cell infiltration (thick arrow) especially within portal tract and hemorrhagic sinusoids (thin arrow) 100X. F, illustrating that the portal tract was severely inflamed with mononuclear cells with dilated and congested portal vein (PV) (400X). G, pointing out the inflammatory patch. Plasma cells and neurophils were observed within the patch (thick arrow) (1000X). H, PHY and PRL treated group showing almost normal hepatic lobules with normal uninflamed central vein (CV) (100X). I, showing small number of inflammatory cells in portal tract. The diameter of portal vein (PV) mildly increased (400X). J, explaining that the hepatic cells were normal with granular cytoplasm and nucleus with nucleolus 1000X.

The H and E stained liver sections of PHY and PRL treated rabbit livers showed mildly dilated heptocytes with normal hepatic cords. The central vein was observed normal and inflammatory cells were almost absent in pericentral area. Few inflammatory cells was observed as well in portal tracts and diameter of portal vein was also slightly increased. The hepatocytes was appeared normal with granular cytoplasm around nucleus. Hepatocytes were present as normal with darkly stained nucleus (Fig. 1H, I, J).

The mean values of viable hepatocyte count, diameter and nuclear diameter were provided in Table I. The results showed that the viable hepatocyte counts were prominently reduced in both PHY groups when compared to other sets of animals (PHY treated group and in PHY+PRL treated group). Likewise, the diameters of hepatocyte were also noticeable decreased in PHY treated group while observed to be almost equal in control group and PHY+PRL treated group. Similar results were observed in case of nuclear diameter. The nuclear diameters were also reduced significantly in PHY treated grouop as compared to control and PHY+PRL treated group.

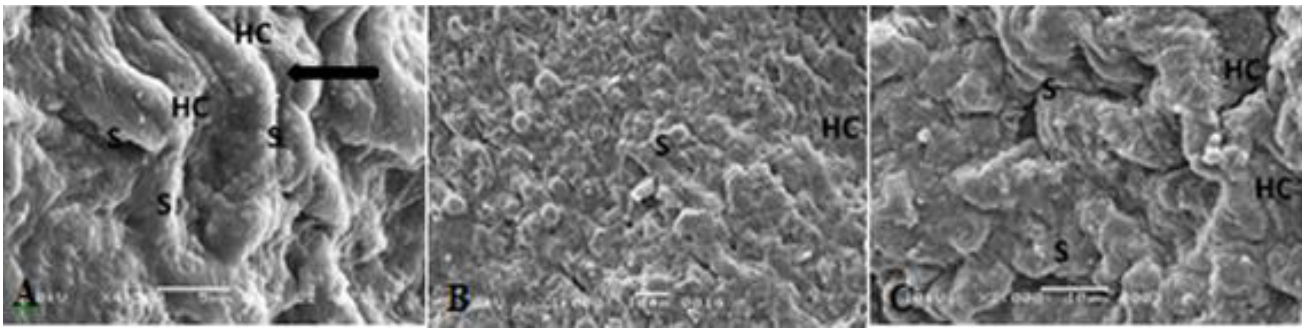

Fig. 2. Effect of propranolol on SEM structure of phenytoin-treated rabbit's liver. A, Scanning electron micrograph of a liver section of a rabbit in the control group shown normal hepatic cords (HC) and sinusoids (S) (4300X). B, PHY treated group showing distinctly distorted hepatic cords (HC) with sinusoidal (S) dilations. Bleb formations on hepatocyt membrane were also observed (thick arrow) (2000X). C, PHY and PRL treated group showing partial restoration of hepatic architecture. Blebs were almost absent (2000X).

Scanning electron microscopy (SEM)

SEM micrograph of surface structure of section of liver of control rabbits showed normal hepatic cords with regular polyhyderal structure of hepatocytes. Hepatocytes were separated by normal sinusoidal spaces as shown in Figure 2A.

SEM of liver section of PHY treated rabbits showed noticeably distorted hepatic cords and sinusoidal dilations. Blebs formations on the membrane of hepatocytes were also observed, with the perforation of liver surfaces as illustrated in Figure 2B.

Scanning electron microscopy of liver section of rabbits of PHY+PRL treated group illustrated partial restoration of hepatic architecture. Blebs formation and round shaped hepatocytes were also reduced. The sinusoids were found to be dilated slightly as shown in Figure 2C.

DISCUSSION

Phenytoin being an aromatic antiepileptic drug (AED) is used to treat variety of seizures and neuropathic pains. It is also prescribed in treatment of arrhythmias especially digoxin induced rhythm failures. Beyond benefits, PHY has potential to induce the hepatic injury owing to hypersensitivity or cholestatic pattern. PHY and other AEDs due to their prospective hepatotoxicity may lead to severe manifestations resulting in liver transplantation or even death. Oxides are formed upon PHY metabolism consequently, leading to the substantial hepatotoxicity either cytotoxic or cholestatic. Till date, no treatment is available, once the PHY hepatotoxicity has been developed (Björnsson, 2008) rather drug withdrawal or treatment discontinuation. Liver dialysis with molecular adsorbent recirculating system device is also often used to treat patients with PHY toxicity (Sen *et al*., 2003).

In this study PHY was administered alone or in combination of PRL to evaluate the role of PRL in PHY induced hepatotoxicity. No kind of macroscopic damage was observed in the rabbits of PHY treated group. Only animals of PHY treated group were found to be lethargic and tired as compared to other group sets (PHY treated group and in PHY+PRL treated group). However; animals who received the combination of both drugs were comparatively active. Their livers were also appeared in normal reddish brown color without any unusual sticking.

The mean serum levels of γ GT, ALT, ALP, and total BRB were determined to assess the extent of liver injuries. Table I showed that the serum levels of mentioned hepatic parameters were prominently higher in PHY treated group animals as compared to control group, reflecting significant PHY induced hepatotoxicity. Sasaki and co-workers (Sasaki *et al*., 2013) had established a mouse model of PHY induced hepatotoxicity and concluded that PHY would surely elevate the serum ALT level by altering an innate immune response. Such elevation of serum levels of all standard hepatic functions (ALT, ALP, γGT and BRB) in PHY treated animals were also reported in past during preclinical and clinical study trials (Altuntas *et al*., 2003; Hussein *et al*., 2013; Saraswathy *et al*., 2015a). Conversely, hepatic enzymes were considerably reduced in animals of PHY+PRL treated group. The comparison among various treatment groups is expressed in terms of mean differences and found a significant variation between PHY treated group and combination of PHY and PRL treated animal groups. On the basis of findings, the role of PRL has proved in reduction of the PHY induced hepatic function elevations. However, PRL being a hepato-protective agent could not be capable to fully reverse the PHY induced values to the normal ranges. In portal biliopathy and biliary obstructive disorders, PRL

is reported to decrease the hepatic biomarkers (Koshy, 2006; Fizanne *et al*., 2008).Utilization of some vitamins including B_{12}, C , folic acid and other hepato-protective herbal drugs had investigated by researchers to assess their function against hepatic parameters when administered concurrently with PHY (Imosemi *et al*., 2010; Ekaidem *et al*., 2007).

PHY is documented to cause variable degree of liver injuries presented as swelling of hepatocyte, dilated central vein, degeneration of hepatocytes, and congestion in periportal area, fatty disintegration and vacuolar degeneration. The present investigation showed the similar histologic patterns as reported in past studies (Saraswathy *et al*., 2010, 2015b). The livers of group B animals displayed dilation of sinusoidal spaces and disrupted hepatic cords especially seen in periportal area. This type of hepatic architectural damage was also endorsed by many researchers (Garzón *et al*., 1989; Singh *et al*., 2000; García-Estrada *et al*., 1990). The PHY treated rabbits' livers also showed necrotic patches, portal vein dilation and congestion. The inflammatory spaces were filled with neutrophil and plasma cells. This was happened probably due to the PHY associated hypersensitivity reactions, hepatitis and cholestasis (Altuntas *et al*., 2003; Bosia *et al*., 1999). The livers of PHY+PRL treated group rabbits showed minor deviations in hepatic architecture when compared with control animals. It was observed that combined administration of PRL and PHY produced lesser degree of damage, when comparing the histology of PHY treated group alone. Henceforth, these outcomes advocate the beneficial effects of PRL in PHY induced liver injury. Non selective beta blocker, PRL is extensively utilized in the treatment of several fatal hepatic disorders including variceal bleeding and portal hypertension in cirrhosis. It has further proved its efficacy not only in animals but bring improvement in gastric and hepatic cirrhotic patients (Dib *et al*., 2006; Pizcueta *et al*., 1989). PRL is actually responsible to reduce the portal pressure and so improve the hepatic histology in animal model of CLD (chronic liver disease) (D'Amico *et al*., 2012). PRL administration has expressed promising results in children who had CLD but the liver biopsy is not allowed due to hyperspleenism. PRL administration is considered to facilitate the treatment of thrombocytopenia and decrease the size of spleen through vasoconstriction in spleenic artery (Poddar *et al*., 2015).

In the current study, the micrometric analysis of H and E stained section of liver provide further authentication for the underlying research hypothesis. The viable hepatocyte count was significantly ($P<0.05$) reduced in PHY treated group rabbits liver having the reduced viability of liver. The shrinkage of the hepatocytes were noted by quantifying the diameter of hepatocytes. The diameter of PHY+PRL treated group is nearly similar to the control, demonstrating the restoration of hepatocytes. Etephon is responsible for the shrinkage of the cell that assessed by decrease in hepatocyte diameter (Bhadoria *et al*., 2015). Mean of nuclear diameter was also reduced in PHY treated group. Such mentioned changes were documented to produce by an ethanol (Rasheed *et al*., 2012). The nuclear diameters were improved in PHY+PRL treated group, indicating the reduction of cellular activity by PHY and improvement was seen by simultaneous administration of PHY and PRL.

SEM micrograph showed bleb formation on the membrane of hepatocytes with distortion of hepatic cords. Livers micrograph have confirmed the PHY induced hepatic damage as well. Contrawise, the micrograph of PHY+PRL treated group has illustrated that the hepatic cords were comparatively intact and blebs were almost absent. The micrograph showed preservation of hepatic architectures and nearly follow the pattern of control group animals.

The hepato-protective effects of PRL in acetaminophene induced hepatic necrosis was explored in an early research conducted 1974 (Gazzard *et al*., 1974). Recently the role of PRL in hepato-toxicity was also established in carbamazepine drug; prone to cause liver impairment in animals. PRL was found to successfully reduced such carbamazepine hepatic damages (Abrar *et al*., 2019). Findings of the current study and the past literature as well are sufficient to justify the role of propranolol in reduction of hapatotoxicity of PHY. Moreover, PHY develops hepato-toxicity possibly due to the reduction of supply of noxious agents to the liver and hepato-protection is achieved due to the antioxidant effect of propranolol. Physicians and clinicians must need to be vigilant during treatment as an early detection of liver injuries may reduce the severity/intensity of hepatic damage and so could safe patients from liver failures.

On the basis of current findings, authors recommended the concurrent administration of PHY and PRL for considerable reduction in PHY induced hepatotoxicity especially for long term treatments clinically. Nevertheless, additional advanced research on molecular and genetic aspects of hepatotoxicity is need to be conducted for clinical implementation of this study.

ACKNOWLEDGEMENT

The principal investigator and the team are highly thankful to BIPS, Baqai Medical University, Karachi, for providing laboratory facilities.

Funding

The authors received no funding from any institute, private or corporate organizations.

IRB approval

The study is approved from the Ehical Committee of Baqai Medical University

Ethical statement

All procedures were approved by the institutional ethics committee for the care and use of animals.

Study approval

The study has been approved by the Board of Advanced Study Research (BASR) of Baqai Medical University, Karachi, Pakistan.

Statement of conflict of interest

There are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

REFERENCES

Abrar, H., Naqvi, S., Ahmed, M.R., Ali, A.B. and Yasin, H., 2019. Ameliorating effect of a beta-blocker, propranolol on carbamazepine-induced hepatotoxicity in rabbits. *Pakistan J. Zool.*, **51**: 341-346. https://doi.org/10.17582/journal.pjz/2019.51.1.341.346

Albus, U., 2012. *Guide for the care and use of laboratory animals (8th edn)*. Sage Publications Sage UK, London, England. https://doi.org/10.1258/la.2012.150312

Altuntas, Y., Ozturk, B., Erdem, L., Gunes, G., Karul, S., Ucak, S. and Sengul, A., 2003. Phenytoin-induced toxic cholestatic hepatitis in a patient with skin lesions: Case report. *South. med. J.,* **96**: 201-204. https://doi.org/10.1097/01.SMJ.0000051269.23361.4A

Alturkistani, H.A., Tashkandi, F.M. and Mohammedsaleh, Z.M., 2016. Histological stains: A literature review and case study. *Glob. J. Hlth. Sci.,* **8**: 72. https://doi.org/10.5539/gjhs.v8n3p72

Bhadoria, P., Nagar, M., Bahrioke, V. and Bhadoria, A.S., 2015. Effect of ethephon on the liver in albino rats: A histomorphometric study. *Biomed. J.,* **38**: 421-421. https://doi.org/10.4103/2319-4170.155589

Björnsson, E., 2008. Hepatotoxicity associated with antiepileptic drugs. *Acta Neurol. Scand.,* **118**: 281-290. https://doi.org/10.1111/j.1600-0404.2008.01009.x

Bosia, J., Borzi, S., Cocozzella, D., Corallini, O., Mayet, M., Malca, M., Padrone, M., Rivero, A., Cecchi, G. and Fraquelli, E., 1999. Acute liver failure by diphenylhydantoin. *Acta gastroenterol. Latinoam.,* **30**: 73-76.

Cadman, B. and Featherstone, B., 2003. Adverse effects of drugs on the liver. In: *Clinical pharmacy and therapeutics.* 3rd ed. Churchill Livingstone, Philadelphia. pp. 843-852.

Curry, B., Mican, L. and Smith, T.L., 2018. Phenytoin-induced chronic liver enzyme elevation and hepatic fibrosis: A case report. *Mental Hlth. Clin.,* **8**: 184-187. https://doi.org/10.9740/mhc.2018.07.184

D'amico, M., Mejías, M., García-Pras, E., Abraldes, J.G., Garcia-Pagan, J.C., Fernández., M. and Bosch, J., 2012. Effects of the combined administration of propranolol plus sorafenib on portal hypertension in cirrhotic rats. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **302**: G1191-G1198. https://doi.org/10.1152/ajpgi.00252.2011

Dib, N., Oberti, F. and Calès, P., 2006. Current management of the complications of portal hypertension: Variceal bleeding and ascites. *Can. med. Assoc. J.,* **174**: 1433-1443. https://doi.org/10.1503/cmaj.051700

Diwan, B.A., Henneman, J.R., Nims, R.W. and Rice, J.M., 1993. Tumor promotion by an anticonvulsant agent, phenytoin, in mouse liver: Correlation with CYP2B induction. *Carcinogenesis,* **14**: 2227-2231. https://doi.org/10.1093/carcin/14.11.2227

Ekaidem, I.S., Akpanabiatu, M.I., Uboh, F.E. and Eka, O.U., 2007. Effect of folic acid and vitamin B12 administration on phenytoin induced toxicity in rats. *Indian J. clin. Biochem.,* **22**: 36-40. https://doi.org/10.1007/BF02913310

Feroz, Z. and Khan, R.A., 2013. Hepatoprotective effect of herbal drug on CCl 4 induced liver damage. *Pak. J. Pharma. Sci.,* **26**: 99-103.

Fizanne, L., Régenet, N., Wang, J., Oberti, F., Moal, F., Roux, J., Gallois, Y., Michalak, S. and Calès, P., 2008. Hemodynamic effects of the early and long-term administration of propranolol in rats with intrahepatic portal hypertension. *Hepatol. Int.,* **2**: 457-464. https://doi.org/10.1007/s12072-008-9070-5

García-Estrada, J., Navarro-Ruíz, A., Román-Maldonado, S., Bastidas-Ramírez, B., González-Hita, M. and Navarro-Ruiz, I., 1990. Oral administration of diphenylhydantoin sodium (DFH-Na or phenytoin) predictably affects the liver

and kidney of Sprague Dawley rats. *Arch. Investig. Med.,* **21**: 339-347.

Garzón, D.L.M.P., García-Estrada, J., Navarro-Ruíz, A., Román-Maldonado, S., Bastidas-Ramírez, B., González-Hita, M. and Navarro-Ruiz, I., 1989. Oral administration of diphenylhydantoin sodium (DFH-Na or phenytoin) predictably affects the liver and kidney of Sprague Dawley rats. *Arch. Invest. Med.,* **21**: 339-347.

Gazzard, B., Hughes, R., Portmann, B., Dordoni, B. and Williams, R., 1974. Protection of rats against the hepatotoxic effect of paracetamol. *Br. J. exp. Pathol.,* **55**: 601.

Huang, Q., Jin, X., Gaillard, E.T., Knight, B.L., Pack, F.D., Stoltz, J.H., Jayadev, S. and Blanchard, K.T., 2004. Gene expression profiling reveals multiple toxicity endpoints induced by hepatotoxicants. *Mutat. Res. Fundam. Mol. Mech. Mutagen.,* **549**: 147-167. https://doi.org/10.1016/j.mrfmmm.2003.12.020

Huang, Y-Y., Chung, T-W. and Tzeng, T-W., 1997. Drug release from PLA/PEG microparticulates. *Int. J. Pharma.,* **156**: 9-15. https://doi.org/10.1016/S0378-5173(97)00154-3

Hussein, R.R., Soliman, R.H., Ali, A.M.A., Tawfeik, M.H. and Abdelrahim, M.E., 2013. Effect of antiepileptic drugs on liver enzymes. *Beni-Suef Univ. J. Basic appl. Sci.,* **2**: 14-19. https://doi.org/10.1016/j.bjbas.2013.09.002

Imosemi, I., Osinubi, A., Saalu, L. and Olagunju, J.A., 2010. Phenytoin-induced toxicity in the postnatal cerebellar development in rat: Effect of calotropis procera on selective biochemical and haematological variables. *Int. J. biol. chem. Sci.,* **4**: 2387-2396. https://doi.org/10.4314/ijbcs.v4i6.64974

Kamp, H., Fabian, E., Groeters, S., Herold, M., Krennrich, G., Looser, R., Mattes, W., Mellert, W., Prokoudine, A. and Ruiz-Noppinger, P., 2012. Application of *in vivo* metabolomics to preclinical/toxicological studies: Case study on phenytoin-induced systemic toxicity. *Bioanalysis,* **4**: 2291-2301. https://doi.org/10.4155/bio.12.214

Kaplowitz, N., 2004. Drug-induced liver injury. *Clin. Infect. Dis.,* **38**: S44-S48. https://doi.org/10.1086/381446

Koshy, A., 2006. Medical treatment of portal biliopathy. *J. clin. Gastroenterol.,* **40**: 453-454. https://doi.org/10.1097/00004836-200605000-00021

Mahendra, A. and Hendra, P., 2011. Efek hepatoprotektif infusa daun *Macaranga tanarius* L. pada tikus jantan terinduksi parasetamol. *J. Farmasi Sains dan Komunitas,* **8**: 91-99.

Misra, H., Singh, S., Shukla, B. and Rai, Y.K., 2013. A study of effect of phenytoin on liver enzymes in epileptic patients in western Uttar Pradesh. *Int. J. Contemp. Med.,* **1**: 11. https://doi.org/10.5958/j.2321-1032.1.2.026

Murtey, M.D. and Ramasamy, P., 2016. Sample preparations for scanning electron microscopy–life sciences. In: *Modern electron microscopy in physical and life sciences.* Intech Open. https://doi.org/10.5772/61720

Owoeye, O., Adedara, I.A., Adeyemo, O.A., Bakare, O.S., Egun, C. and Farombi, E.O., 2015. Modulatory role of kolaviron in phenytoin-induced hepatic and testicular dysfunctions in Wistar rats. *J. Diet. Suppl.,* **12**: 105-117. https://doi.org/10.3109/19390211.2014.952862

Parasuraman, S., Raveendran, R. and Kesavan, R., 2010. Blood sample collection in small laboratory animals. *J. Pharmacol. Pharmacother.,* **1**: 87-93. https://doi.org/10.4103/0976-500X.72350

Patocka, J., Wu, Q., Nepovimova, E. and Kuca, K., 2020. Phenytoin–An anti-seizure drug: Overview of its chemistry, pharmacology and toxicology. *Fd. Chem. Toxicol.*, pp. 111393. https://doi.org/10.1016/j.fct.2020.111393

Pizcueta, M.P., De Lacy, A.M., Kravetz, D., Bosch, J. and Rodés, J., 1989. Propranolol decreases portal pressure without changing portocollateral resistance in cirrhotic rats. *Hepatology,* **10**: 953-957. https://doi.org/10.1002/hep.1840100610

Poddar, U., Shava, U., Yachha, S.K., Agarwal, J., Kumar, S., Baijal, S.S. and Srivastava, A., 2015. β-Blocker therapy ameliorates hypersplenism due to portal hypertension in children. *Hepatol. Int.,* **9**: 447-453. https://doi.org/10.1007/s12072-014-9575-z

Rasheed, S., Amanullah, A., Rehman, M.H.U. and Javed, M., 2012. Architectural changes of liver in response to alcohol. *Gomal J. med. Sci.,* **9**: 204-207.

Santos, N., Medina, W., Martins, N., Rodrigues, M.C., Curti, C. and Santos, A., 2008. Involvement of oxidative stress in the hepatotoxicity induced by aromatic antiepileptic drugs. *Toxicol. in vitro,* **22**: 1820-1824. https://doi.org/10.1016/j.tiv.2008.08.004

Saraswathy, G., Maheswari, E. and Santhrani, T., 2010. Effect of vitamin C supplementation on phenytoin induced hepatotoxicity. *Glob. J. Pharmacol.,* **4**: 127-135.

Saraswathy, G., Maheswari, E. and Santhrani, T.,

2015a. Protective effect of alpha lipoic acid against phenytoin induced behavioral abnormalities in rats. *J. Mol. Biomark. Diagn.,* **2015**. https://doi.org/10.4172/2155-9929.1000241

Saraswathy, G.R., Maheswari, E., Santhrani, T. and Anbu, J., 2015b. Reversal of phenytoin induced hepatotoxicity by alpha lipoic acid in rats. *Afr. J. Pharm. Pharmacol.,* **9**: 198-204. https://doi.org/10.5897/AJPP2014.4019

Sasaki, E., Matsuo, K., Iida, A., Tsuneyama, K., Fukami, T., Nakajima, M. and Yokoi, T., 2013. A novel mouse model for phenytoin-induced liver injury: Involvement of immune-related factors and P450-mediated metabolism. *Toxicol. Sci.,* **136**: 250-263. https://doi.org/10.1093/toxsci/kft184

Schmidt, D. and Schachter, S.C., 2014. Drug treatment of epilepsy in adults. *Br. med. J.,* **348**: g254. https://doi.org/10.1136/bmj.g254

Sen, S., Ratnaraj, N., Davies, N.A., Mookerjee, R.P., Cooper, C.E., Patsalos, P.N., Williams, R. and Jalan, R., 2003. Treatment of phenytoin toxicity by the molecular adsorbents recirculating system (MARS). *Epilepsia,* **44**: 265-267. https://doi.org/10.1046/j.1528-1157.2003.31402.x

Sharma, V. and Janmeda, P., 2013. Chemopreventive role of *Euphorbia neriifolia* (Linn) and its isolated flavonoid against N-nitrosodiethylamine-induced renal histopathological damage in male mice. *Toxicol. Int.,* **20**: 101. https://doi.org/10.4103/0971-6580.111554

Singh, M., Shah, G. and Singh, K., 2000. Teratogenic effects of dilantin on thoraco-abdominal organs of developing chick embryos. *Indian J. exp. Biol.,* **38**: 1026-1030.

Smythe, M. and Umstead, G., 1989. Phenytoin hepatotoxicity: A review of the literature. *DICP Annls Pharmacother.,* **23**: 13-18. https://doi.org/10.1177/106002808902300102

Tursi, T., 2010. Use of ss-blocker therapy to prevent primary bleeding of esophageal varices. *J. Am. Acad. Nurse Pract.,* **22**: 640-647. https://doi.org/10.1111/j.1745-7599.2010.00567.x

Vidaurre, J., Gedela, S. and Yarosz, S., 2017. Antiepileptic drugs and liver disease. *Pediatr. Neurol.,* **77**: 23-36. https://doi.org/10.1016/j.pediatrneurol.2017.09.013

Yoon, E., Babar, A., Choudhary, M., Kutner, M. and Pyrsopoulos, N., 2016. Acetaminophen-induced hepatotoxicity: A comprehensive update. *J. clin. Transl. Hepatol.,* **4**: 131. https://doi.org/10.14218/JCTH.2015.00052