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Ameliorating Effect of a Beta-Blocker, Propranolol on Carbamazepine-Induced Hepatotoxicity in Rabbits

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ABSTRACT

Propranolol is non cardioselective beta blocker used to treat various cardiac and non-cardiac diseases including arrhythmia, hypertension, portal hypertension and oesophageal varices. The study was undertaken in rabbits to investigate the effect of propranolol to reduce hepatoxicity of carbamazepine (CBZ). Animals were divided into three groups; control, CBZ administered group (200 mg/Kg for 10 days) and CBZ plus propranolol (30 mg/Kg for 10 days) treated group. Liver function test and histological evaluation by H and E staining and scanning electron microscopy (SEM) was carried at the end of dosing by using standard procedures. Serum level of ALT, ALP, γGT and bilirubin was significantly ($p<0.05$) increased in CBZ treated group as compared to control, whereas the hepatic parameters were significantly reduced in CBZ plus propranolol group. The histopathological examination reveals various features of hepatic architecture damage in CBZ treated group but the hepatic damage induced by CBZ was successfully ameliorated by propranolol. To conclude propranolol is effective in reducing the hepatoxic effects of CBZ probably by affecting hepatic blood flow.

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HA, MRA, ABA and HY conceived and designed the study, collected the data and wrote the manuscript. SNHN supervised the research.

Key words
Hepatoxicity, Portal hypertension, Oesophageal varices carbamazepine, Propranolol.

INTRODUCTION

Drug induced liver diseases are the commonest problem seen with majority of the pharmaceutical products. Drugs produce wide range of hepatic disorder ranging from asymptomatic jaundice to liver cirrhosis leading to life threatening hepatic failure (Chen *et al*., 2015). It is estimated that 1 in 100 patients have faced the problem of drug induced liver injury during hospitalization (Tarantino *et al*., 2009). In USA it is reported that 50% cases of hepatic failures are drug induced (Chalasani *et al*., 2008; Zheng and Navarro, 2016). The susceptibility of drug induced liver damage depends on several risk factors like age, sex, poly pharmacy, concomitant disease state and genetic polymorphism of metabolic pathways *etc*. (Chalasani and Bjornsson, 2010).

Different classes of drugs are well reported to cause hepatic injury including antimicrobial, antineoplastic, NSAIDs, antidiabetics, antitubercular drugs, anticonvulsants, antibiotics and antihyperlipidimics (Chalasani *et al*., 2015). Carbamazepine (CBZ) is one of the anticonvulsant drugs that is widely used to treat partial tonic clonic seizure in children and adults. Besides of its extensive use, many side effects has been reported including hepatotoxicity (George *et al*., 2016). CBZ related hepatotoxicity is due to metabolic and immunologic factors (Higuchi *et al*., 2012). Oxidative stress involvement is the basic mechanism of CBZ induced hepatotoxicity (Santos *et al*., 2008; Elliott *et al*., 2012). Arene oxide is the toxic metabolite of CBZ which is also reported to cause hepatotoxicity (Pandit *et al*., 2012). Numerous studies have been conducted to test various herbal formulation and vitamins to prevent the hepatotoxicity of CBZ (Santhrani *et al*., 2013; Maheswari *et al*., 2014; Shi *et al*., 2014).

Propranolol is effective in the prevention of esophageal variceal bleeding (Tursi, 2010). This effect of propranolol is due to reduction in portal blood flow (Bosch *et al*., 1984; Ohnishi *et al*., 1985; Pizcueta *et al*., 1989). As propranolol reduces the portal blood flow, it is our scientific belief that propranolol might be effective in reducing the hepatotoxicity of CBZ. Therefore, present study was designed to focus the effect of reduced hepatic blood flow induced by propranolol in reduction of hepatoxicity of CBZ.

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MATERIALS AND METHODS

Thirty six healthy male rabbit of weight 1.2 to 1.4 Kg were recruited from the animal house of Baqai Medical University, Karachi, Pakistan. All the animals were acclimatized for housing condition before starting the experiment. Each animal was kept in separate cage under controlled climatic condition during entire study in an alternating 12 h light and dark cycle. All animals had full access to water and standard laboratory food *ad libitum*.

All the animals were randomly divided into three groups, each comprised of 12 animals. Drugs were administered orally as follows: One group received distilled water orally for 10 days (control). The second group received CBZ 200mg/kg dissolved in distilled water orally for 10 days (Mesdjian *et al*., 1996). The third group received CBZ 200mg/kg and propranolol 30mg/kg orally for 10 days (Huang *et al*., 1998). After 24 h of last dose, the thoracic cage was exposed, approximately 5ml of blood was collected from each rabbit by cardiac puncture technique (Parasuraman *et al*., 2010). Blood sample were then transferred into gel tube and sent to the laboratory, where serum was separated by centrifugation at 4000rpm for 8 min. Alkaline phosphatase (ALP), alanine transaminase (ALT/SGPT) and γ-glutamyl transaminase (γ GT) and total bilirubin were estimated within 2 h of serum separation on automatic analyzer using standard kits purchased from Merck.

All the animals were sacrificed and livers were collected, flushed with saline in 10% normal buffered formalin for histopatholgical evaluation. After 24 h, liver tissues were embedded in paraffin wax as standard protocol. Five micrometer thick section were carried out from these block and put into poly-1-lysine coated glass slide and stained with haemotoxylin and eosin as standard procedure (Piao *et al*., 2016). The slides were observed under light microscope for histological changes induced by CBZ alone and in combination with propranolol. In micrometric studies number of intact hepatocytes, diameter of hepatocyte and diameter of nucleus were analyzed.

The formalin fixed tissues of liver were dehydrated by standard procedure. The samples were mounted on specimen stub using electrically conductive double sided adhesive tape and sputter coated with gold before examination in electron microscope (Echlin, 2011).

All the quantitative results were analyzed statistically using SPSS Software (Version 21). All the values were compared with control by taking mean and standard errors of mean (SEM) using ANOVA, considered $p<0.05$ was significant.

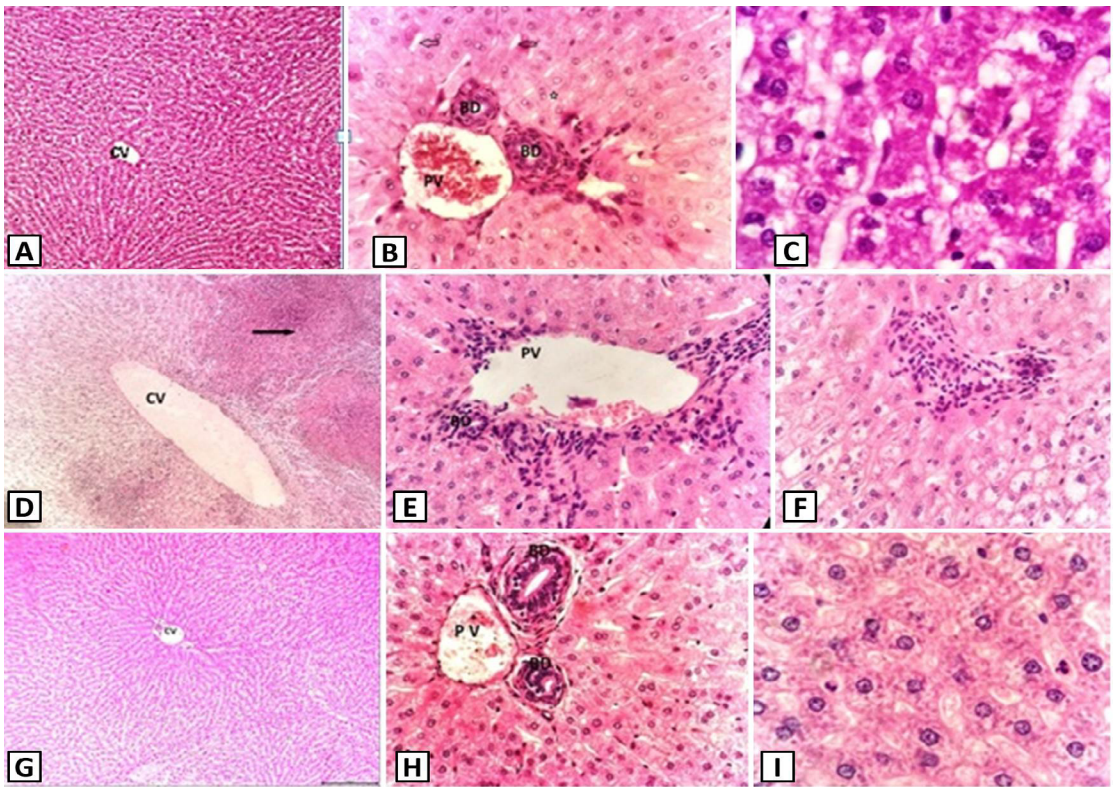

Fig. 1. Histological structure of rabbit: Control (A, B and C), CBZ treated rabbits (D, E and F) and CBZ and propranolol treated rabbits (G, H and I). Stain: H&E. Magnification: A, D and G, 100X; B, E and H, 400X; C, F and I, 100X.

RESULTS

Liver function

The serum analysis of bilirubin and liver enzymes SGPT (ALT), ALT, GGT between control and treated groups were used for assessment of hepatic injury. Statistical analysis showed the significant increase in all above parameters in CBZ treated group as compared to control and CBZ plus propranolol treated group ($p<0.05$). All these biochemical parameters were significantly reduced in CBZ plus propranolol treated group although, the values were more than the control.

Histological structure of liver

Control group specimens showed normal architecture of hepatic tissue with hepatocytes radiating from a central vein separated by normal sinusoidal spaces. The hepatocytes were polygonal with round nucleus with surrounding eosinophilic cytoplasm (Fig. 1A, B, C).

The morphological examination of the H & E stained liver sections of CBZ-treated animals showed distorted hepatic cord with marked mononuclear cell infiltrations in the periportal and pericentral area (Fig. 1D). The portal and central veins were also dilated and congested (Fig. 1E). Minimal swelling and marked congestions of sinusoids were also observed. At 400X the pyknotic necrosis of hepatocytes with cytoplasmic vacuolization were also noted. At 1000X inflammatory patches were observed and almost completely lost hepatic cell were seen. Hemorrhagic necrosis was also observed (Fig. 1F).

Liver sections of carbamazepine and propranolol treated liver showed preservation of hepatic architecture with minimal inflammatory cell infiltration. The central vein demonstrated mild dilation but the sinusoids appeared nearly normal. The pericentral area showed mild inflammatory cells infiltration. At 400X Slight mononuclear cell infiltrations were observed in portal tract with moderate portal vein dilation. At 1000X the polyhedral structure of hepatocytes were restored with normal nucleus and nucleolus (Fig. 1G, H, I).

The surface structure of section of liver of control rabbits showed normal hepatocyte cords and regular polyhyderal structure of hepatocyt (Fig. 2A). SEM of Carbamazepine treated liver sample showed marked changes in the surface of hepatocytes. There is multiple bleb formation in the cell membrane of the hepatocytes and swelling and rounding of hepatic cells are observed in group B (Fig. 2B). While SEM micrograph of CBZ plus propranolol treated group showed restoration of normal hepatocytes surface. The blebs have disappeared and polyhederal shape of hepatocytes was preserved (Fig. 2C).

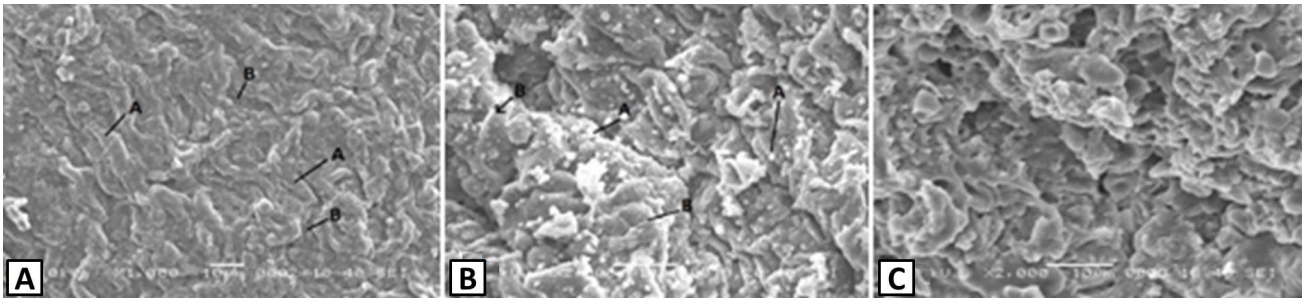

Fig. 2. A Scanning electron micrograph of a liver section of a rabbit in the control group (A) CBZ treated group (B) CBZ and propranolol treated group (C).

Table I.- Effect of propranolol on liver of carbamazepine (CBZ) treated rabbits.

Parameters	Control (n=12)	CBZ treated (n=12)	CBZ + propranolol treated (n=12)
Liver function test			
ALT(IU/L)	40.80 ±1.14	155.60±3.94*	118.40 ± 4.21**
ALP(IU/L)	42.60±2.79	71.20±4.01*	52.80±4.93**
γ GT (IU/L)	8.00 ± 0.93	17.00 ±1.50*	10.80 ±1.82**
Total bilirubin (m.mol/L)	0.50 ± 0.06	3.16 ± 0.39*	1.06 ± 0.12**
Micrometric parameters of liver			
Hepatocyte count (cell/reticule)	21.70± 1.13	14.30± 0.91*	16.70±1.01**
Hepatocyte diameter (µm)	13.90± 0.33	18.70± 0.54*	15.18± 0.58**
Nuclear diameter (µm)	5.89± 0.07	4.46± 0.12*	5.24± 0.14**

Data are expressed as Mean+SEM (standard error of mean). *Significant when compare with control. **Significant when compare with CBZ treated group. ALT, alanine transaminase; ALP, alkaline phosphatase; γ GT, γ-glutamyl transaminase.

Number of hepatocytes, hepatocyte diameter and nuclear diameter were evaluated in all groups. The number of viable hepatocytes were significantly reduced in CBZ treated group when comparing with control. It was also reduced in CBZ plus propranolol treated group but not as significant as in CBZ treated group. CBZ significantly increased the hepatocytes diameter when comparing with control while propranolol reduced the CBZ induced cellular swelling. While the nuclear diameters were significantly reduced in CBZ treated group when comparing with control while this nuclear diameter reduction is insignificant (Table I).

DISCUSSION

Antiepileptic drugs are widely used in the treatment of various psychiatric and neurological disorders. Therapeutic use of AEDs (aromatic anti epileptic drugs) is usually associated with the elevated liver enzymes. These are therapeutically monitored drugs because of high potential of toxicity (Ahmed and Siddiqi, 2006). Involvement of oxidative stress is the major mechanism of CBZ induced hepatotoxicity. Several antioxidant treatments have been reported to reduce the hepatotoxicity of CBZ (Maheswari *et al*., 2015a, b).

Propranolol is a beta blocking agent and binds to the G-protein coupled adrenoceptors, is an effective portal hypotensive agent (Lee *et al*., 2011). Propranolol is commonly used to treat portal hypertension, cirrhosis and oesophageal varices. It is also effective in reducing bacterial translocation which is suggested to prevent hepatocellular carcinoma and in prevention of hepatocellular carcinoma followed by liver cirrhosis (Pérez-Paramo *et al*., 2000). Beside reduction of hepatic blood flow propranolol and its metabolite are accountable as strong antioxidant agent (Adali *et al*., 1999; Mak and Weglicki, 2004). The present study was specially designed to investigate the extent of CBZ toxicity on liver and reduction of the hepatotoxic features of CBZ with the help of propranolol.

Liver function test is the best way to evaluate the status of liver (Kim and Younossi, 2008; Thapa and Walia, 2007). In this study, the levels of serum ALT (SGPT), ALP, and GGT enzymes and bilirubin were measured which are commonly used hepatobiliary biomarkers. Increased SGPT (ALT) activity indicates hepatocellular injury while increased ALP and GGT activities showed obstruction in the bile flow (Cengiz *et al*., 2017). The present study revealed that the levels of ALT, ALP, γGT and bilirubin were considerably increased in CBZ treated group as compared to control and CBZ plus propranolol treated group. The results of biochemical estimation showed that CBZ induced liver injury was indicated by increased hepatic biomarker. These hepatic biomarkers were remarkably reduced by co-administration of propranolol.

Histological examination of liver tissues showed CBZ cause mononuclear cell infiltration in portal tract along with sinusoidal dilatation and central vein congestion, these observations are robustly correlated with the results given by other researchers (Santhrani *et al*., 2013; Maheswari *et al*., 2015a). On the other hand the quantitative microscopic evaluation illustrated that there is reduction in the viable hepatocyte count along with swelling of hepatocytes indicated by increased diameter of hepatocytes. Nuclear diameter was also reduced in CBZ treated group which indicates the reduction in cell viability. The ballooning degeneration of hepatocytes and decreased activity of cells after CBZ administration were also previously reported (Sasaki *et al*., 2016; Eghbal *et al*., 2013). When compared these interpretations of histological examination with CBZ and propranolol treated group it was demonstrated that the combination of propranolol reversed the hepatic damage induced by CBZ. The hepatic architecture was effectively restored when propranolol was co-administered with CBZ.

Thus all the above findings suggested that the hepatotoxicity induced by CBZ was noticeably reduced by concurrent administration of propranolol. Propranolol reduced and reversed the hepatotoxicity and portal hypertension induced by ethanol (Prkacin *et al*., 2001). This effect of propranolol is due to reduced hepatic blood flow by portal vascular contraction. The diameter of central vein was also reduced by propranolol. It was well understood that in heptotoxicity the blood flow of liver was increased as adaptive response to combat hypoxia. Propranolol furthermore possesses an antioxidant effect which was also helpful in reducing the toxicity of CBZ.

CONCLUSION

The present study was carried out to investigate the role of propranolol on various hepatic parameters. Propranolol was co administered with CBZ for specified period of time the results showed that CBZ induced significant hepatoxicity and these observations were confirmed by qualitative and quantitative microscopic examinations and scanning electron microscopy. Propranolol profoundly reduced the hepatotoxicity observed in biochemical and microscopic evaluations. This effect of propranolol is due to reduced hepatic blood flow. However, further advanced studies are required to confirm these results.

Statement of conflict of interest

Authors have declared no conflict of interest.

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