ORIGINAL ARTICLE

Drug Induced Liver Injury with Diclofenac and Febuxostat Combination in Mice at Histopathology

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ABSTRACT

Febuxostat is a novel drug administered for the treatment of hyperurecemic state; usually it leads to gout flare during first few days of treatment and joint pain aggravates. In such situations patients are advised to take Diclofenac to alleviate pain. A study was planned to explore the hepatotoxic effects of this combination. Six groups were arranged having 10 mice in each. Group I was given normal saline which served as control. Group II administered Diclofenac (100 mg/kg). Group III treated with Febuxostat (50 mg/kg). Group IV, Group V and Group VI were treated with drug combinations. In these three groups dose of Diclofenac was same (100 mg/kg) and dose of febuxostat gradually increased as for Group IV 5 mg/kg, Group V 10 mg/kg and Group VI 50 mg/kg. After 7 days of treatment, liver tissue preserved for histopathological assessment from each animal. Liver injury was evaluated by examining liver macroscopically as well as histologically.

Keywords: Diclofenac, Febuxostat, combination, hepatotoxicity, histopathology.

INTRODUCTION

Drug induced hepatic injury affects 10 to 15 per 10,000 people annually worldwide (Sgro et al., 2002) The list of drugs is continuously growing which are being withdrawn from market due to DILI (Regav., 2013). Febuxostat was approved by FDA in 2009. Abnormal liver function tests have been reported in 2% to 13% patients receiving febuxostat. The severity, nature and timing of these abnormalities have not been described; however liver enzymes elevations were the major reason for febuxostat discontinuation during these clinical trials (U.S NLM., 2014).

MATERIAL AND METHODS

It was an experimental study. Sample size was 10 ; it was calculated by using formula with 90% power of study and 5% level of significance (Aydin et al., 2002). Adult healthy male BALB-*c* mice, weighing 25-30gm, were kept for 1 week to acclimatize to the environment. Studies were performed in accordance with standard animal care and use committee guidelines . Healthy adult male 60 BALB-*c* mice were randomly divided into 6 groups, having 10 animals each.

Group I served as control and each subject was given 0.3ml normal saline (0.9%) once daily by gavage for 7 days. In group II mice had diclofenac 100 mg/kg once daily for 7 days by gavage (EMEA., 2003). Group III he animals treated with febuxostat 50 mg/kg by gavage once daily for 7 days (Patel et al., 2010)**.** Group IV Subjects were administered diclofenac at a dose of 100mg/kgand after 2 hrs febuxostat 5 mg/kg was given by gavage once daily for 7 days (Xinxu et al*.,* 2008). Group V Experimental animal had diclofenac at a dose of 100mg/kg and after 2 hrs febuxostat10mg/kg by gavage once daily for 7 days (Patel et al., 2010). Group VI mice had diclofenac at a dose of 100mg/kg and after 2 hrs febuxostat 50mg/kg by gavage once daily for 7 days.

All animals were sacrificed. Liver tissue of each experimental animal was dissected out and stored in 10 % formalin. Hepatic tissue fixed in 10% formaldehyde buffer and processed in automated histology tissue processing machine for dehydration, clearing, impregnation and embedding in paraffin. Paraffin embedded tissue blocks were prepared. Sections of the liver tissue were cut at a thickness of 4-6µm by rotary microtome. From each block at least two tissue sections were cut. Then samples were stained with heamotoxylin and eosin (Skip et al., 2012). Slides were observed under light microscope using different magnifications for histological changes (Marques et al., 2015).

RESULTS

Liver histopathology

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Fig 1:Control group showing normal liver parenchyma (20X) with Hematoxylin & Eosin staining.Hepatocytes appear normal in shape and size. Stroma and peri portal area showed no histopathological changes.

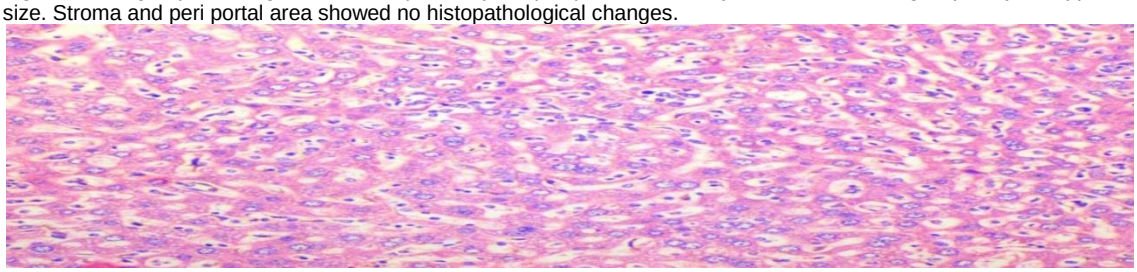

Fig 2: Group II treated with DF (100 mg/kg) showing normal liver parenchyma (20X) .Hepatocytes appear normal in shape and size. Stroma and peri portal area has no signs of inflammation.

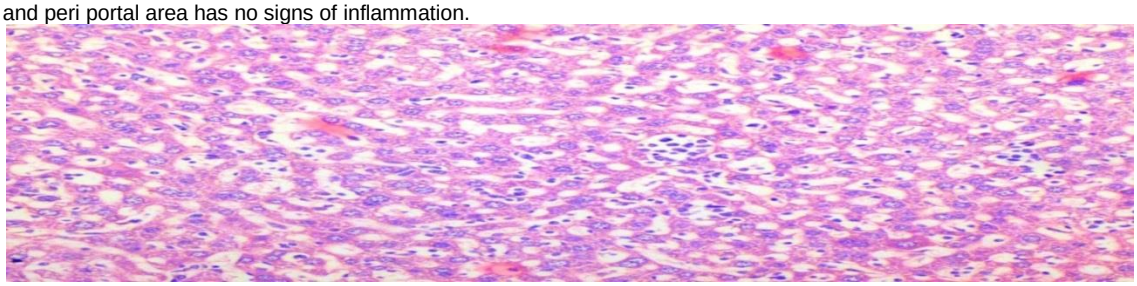

Fig 3: Group III treated with FX (50 mg/kg) showing normal liver parenchyma (20X) with Hematoxylin & Eosin staining.Hepatocytes appear normal in shape and size. No pathological changes in stroma and peri portal area were found.

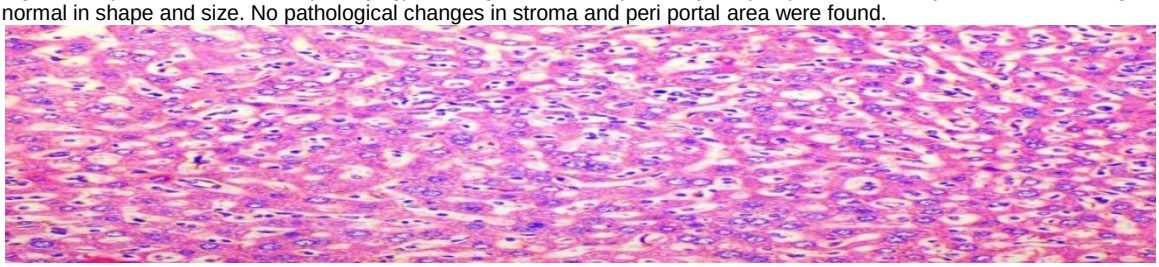

Fig 4: Group IV treated with DF (100mg/kg) and FX (5 mg/kg) combination showing focal parenchymal inflammation involving the major part of the parenchyma (100X) with Hematoxylin & Eosin staining. Hepatocytes showed damaged cell outline, apoptosis and cellular degeneration. Kupffer cells were also observed.

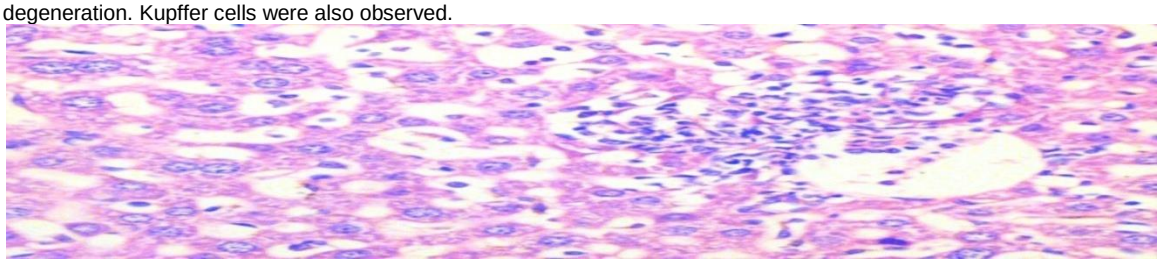

Fig.5: Group V treated with DF (100mg/kg) and FX (10 mg/kg) combination showing parenchymal inflammation and damaged cell outline (100X) with Hematoxylin & Eosin staining. Inflammation of portal tract, vascular congestion, ballooning degeneration and pyknosis was also observed.

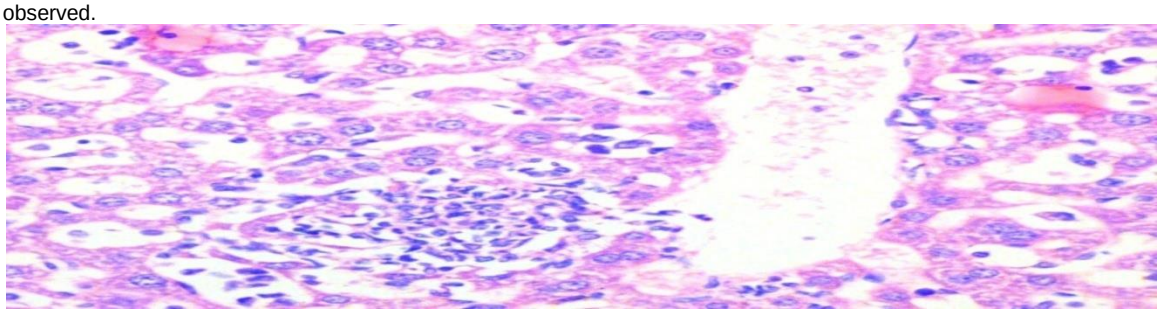

Fig 6: Group VI treated with DF (100mg/kg) and FX (50 mg/kg) combination showing cellular degeneration and severe apoptosis (10X) with Hematoxylin & Eosinstaining**.** Inflammation of hepatocytes with ballooning degeneration, focal necrosis, minimal cholestasis and early fibrosis was also observed. Apoptotic body was visible.

DISCUSSION

On histopathological examination the control group was observed for normal histological findings. Group II (DF 100 mg/kg) showed no microscopic signs of liver damage. It is described in literature that DF raises liver enzymes but does not cause liver histological changes when used for short duration. Group III (FX 50 mg/kg) also exhibited normal liver histology. It indicates that when it is prescribed alone, it may lead to increased enzymes levels but causes no histological changes. In group IV (DF 100 mg/kg + FX 5 mg/kg) focal parenchymal inflammation was found. It is a sign of early damage. In group V (DF 100 mg/kg + FX 10 mg/kg), there is parenchymal inflammation involving the major part of the parenchyma. It was also having damaged cell outline. It is because of apoptosis and cellular degeneration. Inflammation of portal tract, ballooning degeneration and pyknosis was also observed. It is relatively advanced stage of cell injury. In group VI (DF 100 mg/kg + FX 50 mg/kg) there were more adverse changes showing inflammation of hepatocytes with ballooning degeneration, focal necrosis, minimal cholestasis and early fibrosis.

Group II and group III which were treated with DF and FX respectively, did not affect histology of the liver but when drugs were administered in combination, they caused histopathological changes as described above. Such changes increased in intensity with increasing dose of FX.

CONCLUSION

Histological findings in groups which were administered Diclofenac and Febuxostat separately in group II and group III, showed no liver damage. when these were used in combination, histological damage increased with increasing dose of Febuxostat in group IV, V and VI.

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