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EFFECT OF PROPRANOLOL;
RIFAMPICIN INDUCED HEPATOTOXICITY IN RABBIT'S LIVER

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ABSTRACT: Tuberculosis is the disease required long term treatment. Rifampicin is the major element of antiTB therapy if resistance is not documented. Potential of antitubercular dugs to produced hepatoxicity is very high and among all antiTB agents rifampicin (RIF) induced hepatoxicity stands on top. Rifampicin is the major element of antiTB therapy if resistance is not documented. But its hepatotoxic effects are the main hurdle to continue with this therapy. In this study RIF were administered to the rabbit alone or in combination of propranolol to evaluate the hepatotoxic effects of RIF and reduction of hepatotoxicity by propranolol. Histological evaluation of liver tissue on higher magnification, its micrometric analysis and SEM (scanning electron microscopy) of liver were used to estimate the effects of this combination. Micrometry revealed that number of viable hepatocytes, their diameter and nuclear diameter were altered. SEM micrograph showed distorted and swollen hepatic cords. All of these changes successfully turned to normal by combined administration of propranolol. Propranolol successfully improves the hepatic architecture proved by both qualitative and quantitative microscopy.

Key words: Hepatotoxicity, antitubercular therapy, micrometric analysis, scanning electron microscopy, hepatic cords, hepatocytes.

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INTRODUCTION

Rifampicin is the bactericidal antimicrobial of rifamycin group. RIF is the major part of antitubercular combination therapy. RIF produce bactericidal activity by inhibiting DNA dependent RNA polymerase. Antitubercular drug induced hepatotoxicity is the major problem associated with the therapy and it is the main reason to decline the therapy and non compliance. Rifampin may cause cholestatic jaundice and strongly induce cytochrome P450 which increases the elimination of several other drugs.[1] In USA 14% liver plantation is due to drug induced hepatic damage among them 0.2% is due to antitubercular drugs. A 10 years old child developed anti-TB induced hepatotoxicity after 40 days of therapy and he required hepatic transplantation.[2] Chronic liver disease, alcoholism and old age appeared to increase the incidence of severe hepatic problem when rifampicin is given alone or concurrently with isoniazid.[3] The pathogenesis ranges from hepatic adaptive changes to hepatocellular damage.[4] Several researches have been conducted to evaluate the hepatotoxicity and its treatment via plant extrats and various medications.[5-7]

Propranolol is effective in the prevention of esophageal variceal bleeding.[8] This effect of propranolol is due to reduction in portal blood flow.[9-11] As propranolol reduces the portal blood flow, it is our scientific belief that propranolol might be effective in reducing the hepatotoxicity of RIF. Therefore, present study was designed to focus the effect of reduced hepatic blood flow induced by propranolol in reduction of hepatoxicity of RIF.

MATERIALS AND METHODS

Animals

In the present study rabbits were selected as experimental animals due to the similarity in hematological biochemistry to human beings.[12] Thirty six healthy male rabbit of weight 1200 to 1400 grams 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 hour light and dark cycle. All the animals had full access of water and food adlibitum.

Experimental design

All the animals were randomly divided into three groups and each group comprised of 12 animals. Drugs were administered orally as following schedule.

Group A: Control group received distilled water orally for 8 day.

Group B: the animals of group B received rifanpicin 100mg/kg for 28 days as single daily dose in oral solution.[13]

Group C: the animals of group C received RIF 100mg/kg and propranolol 30mg/kg for 28 days as single daily dose in oral solution through gastric tube.[14]

Preparation of liver tissue for histological examination

The liver of the animals were also collected and flushed with saline and put into 10 % normal buffered formalin for histopatholgical evaluation. After 24 hours, 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.[15] 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.

Scanning electron microscopy

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.[16]

Statistical analysis of data

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.

RESULTS

Microscopy

At 1000X, swollen hepatocytes were displayed with almost normal granular cytoplasm and dilated sinusoidal spaces. Large numbers of binucleated hepatocytes were also noted (Figure-a). Hexagonal shaped hepatocytes restored with normal granular cytoplasm. Hepatocytes were lined by normal kupffers cells and edothilial cells (Figure-b).

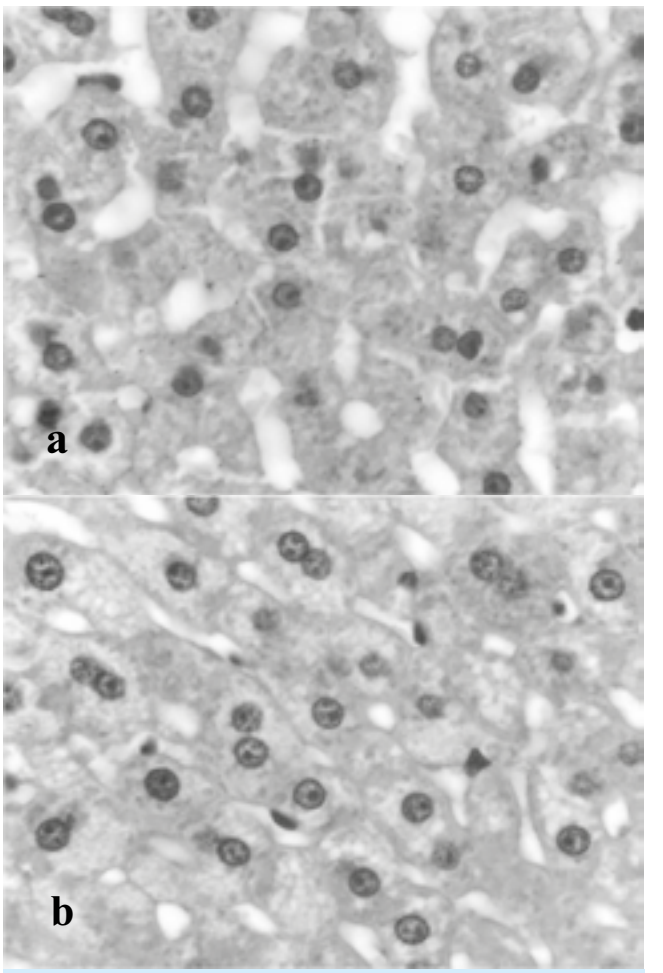

Figure a; Photomicrograph of 5 micron thick H & E stained paraffin section from liver of rabbit of group D (RIF treated group) showing swollen hepatocytes with almost normal darkly stained nucleus, nucleolus and granular cytoplasm and dilated sinusoidal spaces (1000X).
Figure b; Photomicrograph of 5 micron thick H & E stained paraffin section from liver of rabbit of group E (RIF and propranolol treated group) illustrating normal size polygonal hepatocytes with cytoplasm and nucleus (1000X).

MICROMETRIC ANALYSIS IN GROUP A, B AND C			
Parameters	**GROUP A**	**GROUP B**	**GROUP C**
Hepatocyte count (cell/reticule)	21.70± 1.13	14.1±2.15	18.78±2.01
Hepatocyte diameter (μm)	13.90± 0.33	13.92±1.95	14.16±1.14
Nuclear diameter (μm)	5.89± 0.07	5.16±0.62	5.85±0.60
Data expressed as Mean±SEM			

Micrometric Analysis and Their Comparison in Different Group (A, B and C)

The mean values of viable hepatocyte count per field in control, RIF treated (B) and RIF plus propranolol treated (C) animals are 21.70 cell/reticule, 14.10 cell/reticule and 18.78 cell/reticule, respectively. It was indicated that number of hepatocytes were significantly reduced in group B as compared to group A. Number of viable hepatocytes was also reduced in group C but not as significant as in group D. The hepatocyte diameter in group A, B and C are 13.90μm, 13.92μm and 14.16μm, respectively. These results showed that there were not significant ($P>0.05$) alterations in diameter of hepatocytes seen in group B and C. The nuclear diameter in group A, B and C were 5.85μm, 5.16μm and 5.82μm respectively. These results showed that nuclear diameters were significantly ($p<0.05$) reduced in group B as compared to group A and C while this nuclear diameter reduction is insignificant in RIF and propranolol treated group as compared to control.

SCANNING ELECTRON MICROSCOPY OF LIVER SECTION

At 2000X and 4300X, SEM micrograph of surface structure of section of liver of control rabbits showed normal hepatic cords and regular polyhyderal structure of hepatocytes (Figure-c). Hepatocytes are separated by normal sinusoidal spaces (Figure-d).

At 2000X magnification, scanning electron microscopy of rifampicin treated liver section showed deformd hepatic architecture. Hepatocytes were separated with dilated sinusoidal spaces. Polygonal shapes of hepatocytes were also lost due to membrane swelling (Figure-e).

At 2000X magnification, the SEM of liver section of group E (rifampicin and propranolol treated) rabbits showed partial restoration of hepatic architecture. The polygonal shape of hepatocytes also preserved (Figure-f).

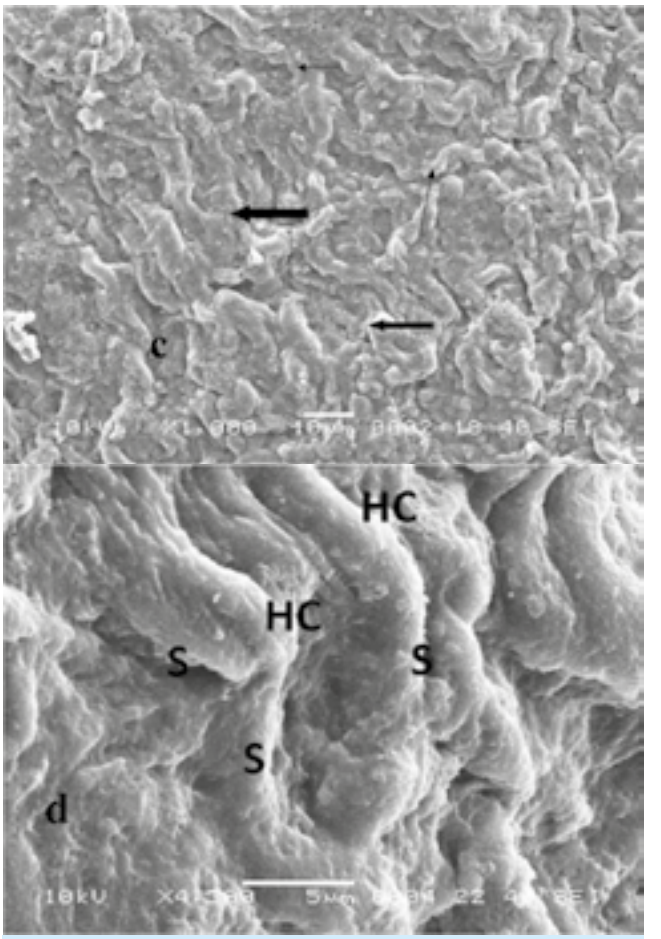

Figure c; Scanning electron micrograph of a liver section of a rabbit in the control group (A) showing hepatic cord of normal polyhederal hepatocytes (thick arrow) separated by normal sinusoidal spaces (thin arrow). Figure d; Scanning electron micrograph of a liver section of a rabbit in the control group (A) showing normal hepatic cords (HC) and sinusoids (S) (4300X).

DISCUSSION

Drug induced liver injury is the major cause of morbidity and mortality in wide range of population who received various medication for long duration.

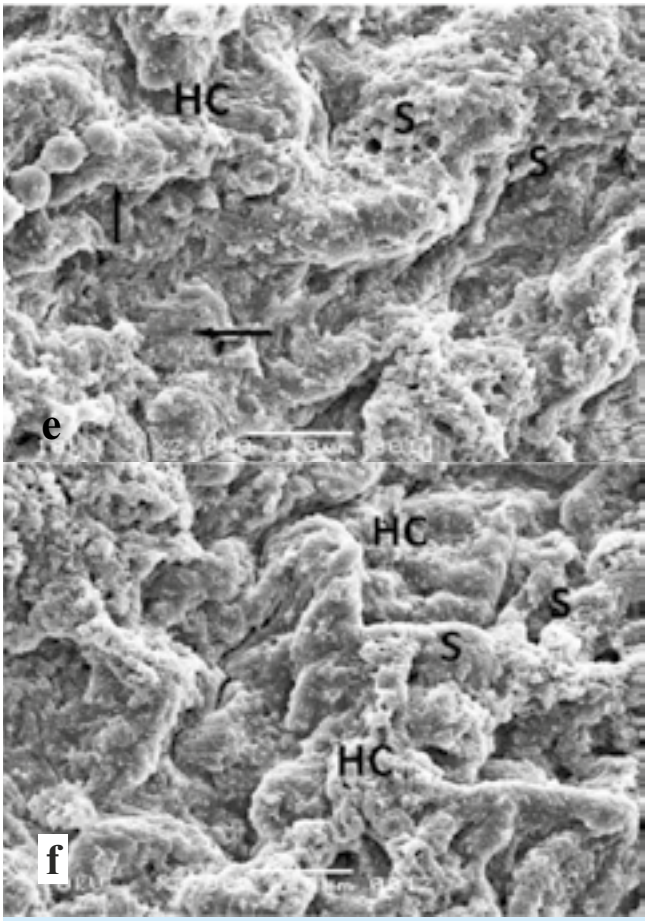

Figure e; Scanning electron micrograph of liver section of rabbits of group B (RIF treated group) showing dilated sinusoids (S) and deformed hepatic cords (HC). Swellings of hepatocytes are also seen (2000X). Figure f; Scanning electron micrograph of liver section of rabbits of group C (RIF and propranolol treated group) showing slightly disturbed hepatic cords (HC). Bleb formations are comparatively reduced (2000X).

DILI is also the leading cause of drug withdrawal from market during and after phase IV clinical trials and post marketing surveillance. Large body of literature discussed the hepatotoxicity of rifampicin in combination with other antitubercular drugs and alone. Histological evaluations of liver tissues were done by almost every scientist to explore the response of toxic compound on liver. Rifampicin and INH cause portal triditis, necrosis specially piecemeal necrosis and it was also concluded that 50 mg/Kg of both drugs are enough to produce hepatotoxic model.[17] It has been reported that esinophilic infiltrations in portal tracts, lobular inflammation, Kupffer cells hyperplasia apoptotic hepatocytes, sinusoidal dilations and central vein damage produced by combination of INH and rifampicin.[18,19] In present study rifampicin alone also produced these kinds of hepatic changes especially sinusoidal dilation, portal triditis and apoptotic hepatocytes. In group C the rabbit liver showed mild degree of inflammation and slight sinusoidal dilation. The portal tract was also mildly congested with dilated portal vein. The comparison of group B and C disclosed that rifampicin induced toxicity was reduced by propranolol. The hepatotoxicity of rifampicin was reduced by cimitidine as it is the potent inhibitor of cytochrome P 450.[19] Hepatotoxic effects of rifampicin and INH was also reduced by vitamin E and theses effects are highly comparable with cimitedine.[7] In this study similar results were observed in group C rabbits that rifampicin and propranolol was coadministered and hepatotoxicity produced by rifampicin was clearly reduced.

These results are further confirmed by micrometric estimation of hepatocytes count, their diameter and nuclear diameter. Micrometric estimation is the definite tool that confirm the hepatotoxicity.[20] In the best of our knowledge the quantitative histology of RIF induced damage was first time evaluated and the results showed that the numbers of viable hepatocytes were significantly reduced in RIF treated group. The values of hepatocyte diameters were not altered significantly. The nuclear diameter is distinctly reduced in group B which showed the reduction in cell viability. It was reported that ciprofloxacin and triterpene reduced the mean viable hepatocyte count and nuclear diameter.[21,22] On the other hand the hepatocyte count and nuclear diameter were significantly restored by propranolol when coadminitered with RIF. These findings are correlated other with the hepatotoxicity of rifampicin was also reduced garlic and other herbal preparations.[23,24]

Scanning electron microscopy is another modified method that reconfirms the changes induced by RIF in rabbit's liver. The SEM micrograph showed that the in group B the hepatic cords are not intact and hepatocytes changed their shape

as compared to control. While in group C the micrograph is not exactly similar as control but hepatic architecture was comparatively intact.

CONCLUSION

On the basis of above discussion it was explored that propranolol may be able to protect the liver from RIF induced damage. Micrometric analysis and SEM of liver tissues are the more advanced and precise techniques that absolutely describes the changes produced by RIF and protection offered by propranolol. For further confirmation of the study should be conducted on large scale and clinical cases should be closely observed who use this combination for another diseases.

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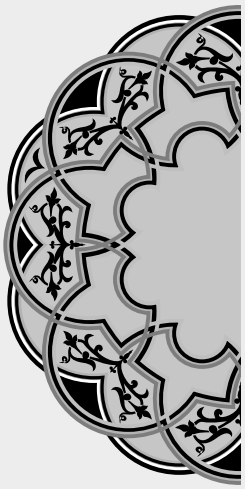

"Keep your friends close,
But your enemies closer."

Al Pacino

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