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ASCORBIC ACID;

ROLE IN PROTECTION AGAINST FOCAL NECROSIS INDUCED BY RIFAMPICIN

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ABSTRACT… Objective: Rifampicin continues to be an effective drug for treatment of tuberculosis. A variety of drug reactions have been reported of which hepatotoxicity is well known. This study was conducted to investigate the effects of ascorbic acid as dietary supplementation in case of Rifampicin induced hepatotoxicity. **Data source:** Animal house NIH (National Institute of Health). **Study design:** Randomized control trial. **Materials and Methods:** Thirty adult BALB/c mice weighing 30-60 grams were taken. They were kept under standard laboratory conditions. Mice were randomized and divided into three groups A, B and C each containing 10 mice. Group A was given Rifampicin 100 mg/kg body weight, group B was administered Rifampicin 100 mg/kg body weight along with ascorbic acid 500 mg/kg body weight orally and group C was given regular NIH lab diet for six weeks. **Result:** Liver specimens of animals given rifampicin showed formation of necrotic foci. Simultaneous administration of ascorbic acid significantly reduced histological changes induced by Rifampicin. **Conclusions:** Ascorbic acid has protective role against hepatotoxic effect of Rifampicin used in chemotherapy of tuberculosis in animal models.

Key words: Rifampicin, Ascorbic acid, Focal necrosis

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INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis. Morbidity and mortality from TB and related disorders remain a major health issue in under developed and developing world[1]. TB is major cause of morbidity among infectious diseases worldwide[2]. 95 percent of cases of this disease occur in third world countries[3]. The AIDS epidemic has led to a resurgence of the disease in the west as a part of AIDS related complex.

Tuberculosis remains the leading cause of death worldwide among infectious diseases. It is the second leading cause of death in undernourished adult population. According to a report published by World Health Organization (WHO) in March 2012 the incidence of TB per 100,000 people in Pakistan is 181, case notification per 100,000 per year is 150 while the treatment success rate is 85%. Pakistan ranks fifth amongst TB high-burden countries worldwide. It accounts for 61% of the TB burden in the WHO Eastern Mediterranean Region. In 2012, an estimated 8.6 million people developed TB and 1.3 million died from the disease, including 320,000 deaths among HIV-positive people, according to WHO report[4].

MATERIALS AND METHODS

The study was carried out in the department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad. The study was carried out with permission of "Ethical committee on experimental animals" of Army Medical College Rawalpindi.

Thirty adult male BALB/c mice having weight of 35-50 g and age between 10 -12 weeks were obtained

from animal house of NIH, Islamabad. Mice were kept under standard laboratory conditions. These mice were maintained on pelleted diet which was prepared at animal house. They were kept at 12 hours light and dark cycle in a room at 22-24 °C and were given food and water ad libitum.

Study Design
Study design was experimental randomized control trial.

Grouping of Animals
Animals were divided into 3 groups each containing 10 mice.

I. Group A
Mice were administered Rifampicin orally in a dose of 100mg/kg of body weight for 6 consecutive weeks.

II. Group B
Mice were administered rifampicin orally in a dose of 100mg/kg of body weight with concurrent administration of ascorbic acid in a dose of 500mg/ kg body weight for 6 consecutive weeks.

III. Group C
This group received regular laboratory diet for 6 consecutive weeks. This was the control group.

For focal (spotty) necrosis criteria modified by Ishak et al was used.5 Slides were stained with H& E and examined under 10X objective. Scoring was done as 0 if no focus was present, '1' if one focus was found 2' if there were one to four foci, 3 if five to ten foci were present and 4 if more than ten foci were counted. For photography X5 model of General Electric digital camera was used through ocular lens of microscope. Pictures were edited using microsoft image analyzer. Data was entered in a data base using Statistical Package for Social Sciences (SPSS) windows version 15. The results were expressed as the mean and standard error (S.E). Comparison of focal necrosis between different groups was done by chi square test and p- value less than 0.05 was considered significant.

RESULTS
The histological specimens of control group C showed normal architecture and there was no evidence of necrotic lesions (Fig.1). Necrotic foci were present in all members of experimental group A (Fig 2). Members of experimental group B also revealed necrotic foci but percentage of affected animals was reduced (Fig 3). The experimental group A revealed necrotic injury in 100% of animals with grade 3 injury in 80% of animals and grade 2 injury in 20% of animals. The experimental group B showed grade 1 injury in 20% of animals, grade 2 in 30% of animals, grade 3 in 40% of animals while 10% of animals remained free of any necrotic lesion (Fig. 4). The difference was statistically significant when compared by applying Chi square test (Table-I).

DISCUSSION
Tuberculosis is a chronic infectious disease caused by Mycobacterium tuberculosis. Currently TB is the leading cause of mortality among infectious diseases worldwide. Ninety five percent of TB cases and ninety eight percent of deaths due to TB occur in developing countries[6].

Groups	0	1	2	3	4	p value between groups
Experimental Group A				8	2	0.00*
Experimental Group B	1	2	3	4		
Control Group C	10					

Table-I. Comparison of Grading of Focal Necrosis between Control and Experimental groups
Statistical difference by applying Chi square test in grading of Necrotic Foci between:

Group A and B < 0.05 Group A and C < 0.05* Group B and C < 0.05**
**Statistical difference is significant*

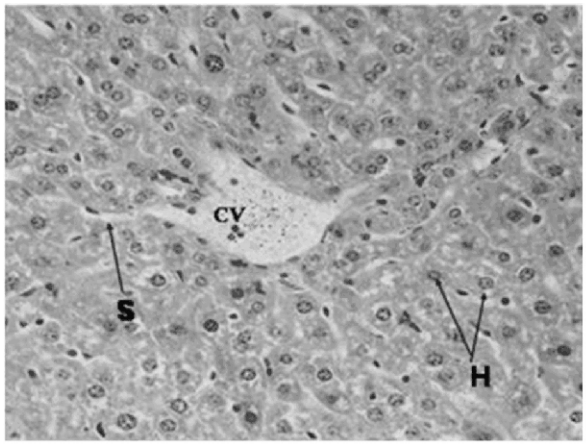

Fig. 1.Photomicrograph of section of liver of control group C showing hepatic parenchyma with Central Vein (CV) Sinusoids(S) and Hepatocytes (H).

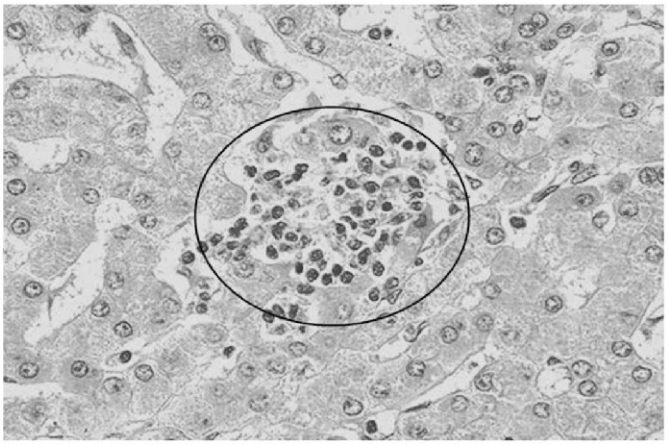

Fig.2.Photomicrograph of section of liver of experimental group A showing hepatic parenchyma with inflammatory focus in a circle (H&E stain).

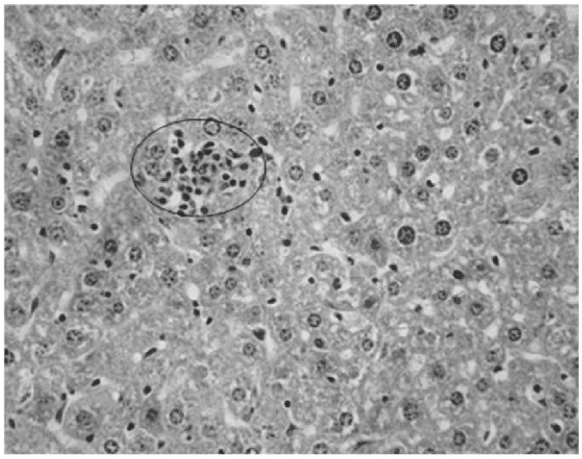

Fig.3. Photomicrograph of section of liver of experimental group B showing hepatic parenchyma with inflammatory focus in a circle (H&E stain).

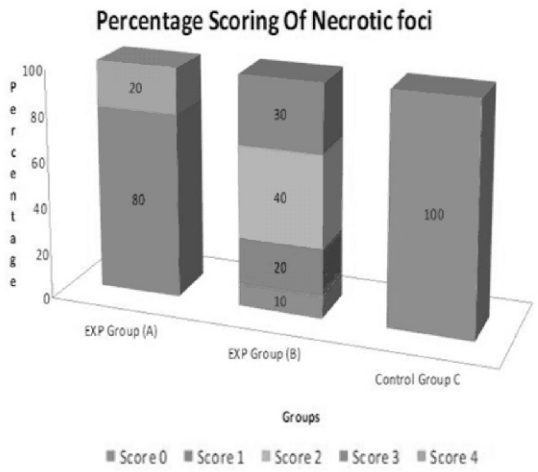

Fig.4. Percentage Grading of necrotic foci in all groups

Rifampicin, isoniazid, pyrazinamide and ethambutol are first line of drugs used for the treatment of tuberculosis[7]. Rifampicin has bactericidal activity against M. tuberculosis by inhibiting bacterial DNA-dependent RNA polymerase[8]. This drug is used in the initial two months of treatment to reduce the duration of therapy[9].

Drugs are not used solely in the treatment of tuberculosis. Instead, the first line drugs are used in combination, or with other medicines. Several adverse reactions of anti-tuberculosis drugs are reported. The best known toxic drug effect is hepatotoxicity as proved previously[10]. The frequency and severity of hepatotoxicity is increased when these drugs are used in combination[11].

Anti-tuberculosis drugs act as inducers of hepatic cytochrome P450 enzymes.The major isoenzyme of cytochrome P450 enzymes in bioactivation is CYP2E1, which is also involved in hepatic toxicity of carbon tetrachloride, ethanol and acetaminophen[12]. Inhibition of this isoenzyme by specific inhibitors or herbal drugs has been shown to be hepatoprotective[13]. Recent studies indicate the existence of a strong correlation between hepatic injury and oxidant stress in experimental animals treated with anti-tuberculosis drugs.

Since all the drugs used in the treatment of tuberculosis are shown to have hepatotoxic effects, studies have been performed to prevent or reduce the toxicity by the use of natural herbal drugs and/or synthetic compounds. It is of importance to note that the inhibition of CYP450 2E1 and antioxidant actions seem to be the common mechanism of action of herbal drugs[14].

The present study was carried to determine the protective effects of ascorbic acid on liver damage induced by rifampicin. Rifampicin is part of antituberculous regime and is potentially hepatotoxic drug. It is metabolised and detoxified in liver making this organ susceptible to injury[15]. Anti tubercular therapy can cause varied degree of hepatotoxicity ranging from asymptomatic rise in transaminases to acute liver failure[16].

The histological examination of liver specimens obtained from animals of experimental group A showed evidence of centrilobular and focal necrosis. Similar or more advanced changes in liver histology were noted by many others[17,18]. There was a significant change in severity of injury between experimental groups A and B proving that Ascorbic acid can scavenge oxygen derived free radicals involved in development and exacerbation of various diseases[19]. This finding suggests protective role of ascorbic acid in drug induced liver injury due to antioxidant properties.

CONCLUSIONS

Drug induced hepatotoxicity remains a challenge to modern hepatology. Because of its significant impact on public health it is essential to understand mechanisms leading to injury and factors responsible for its prevention and treatment.

Current study proved that in a mouse model rifampicin induces hepatotoxicity and that ascorbic acid administration in a high dose ameliorates histological changes associated with rifampicin induced hepatotoxicity.

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