Objective: To evaluate the hepatoprotective potential of cimetidine in hepatotoxicity induced by isoniazid-rifampicin combination in albino rabbits. Methods: Six groups of six rabbits each were studied. Three groups received saline (control), isoniazid (50 mg/Kg/d) alone or isoniazid with rifampicin (100 mg/Kg/d) daily orally for 7 days. Other groups received intraperitoneal cimetidine (50 mg/Kg/d) alone, or cimetidine (50 or 120 mg/Kg/d) along with isoniazid-rifampicin combination. Serum levels of liver enzymes were measured at baseline and on day 8, and liver histology was studied on day 8. Results: Rabbits receiving isoniazid alone for 7 days showed no increase in serum ALT and AST levels, whereas those receiving isoniazid-rifampicin combination had a 3-4-fold increase in these levels (p=0.02). Animals receiving cimetidine pre-treatment did not show a significant increase in ALT and AST levels. Histological changes in the liver were more common with isoniazid-rifampicin combination than with isoniazid only. These changes were reduced in animals receiving low-dose cimetidine and prevented in those receiving high-dose cimetidine. Conclusion: Cimetidine in high dose can prevent hepatotoxicity induced by isoniazid-rifampicin combination. [Indian J Gastroenterol 2007;26:18-21]

The use of isoniazid and rifampicin in the treatment of tuberculosis is limited by their potential for hepatotoxicity. The incidence of hepatotoxicity is higher with isoniazid and rifampicin combination than with isoniazid alone.1

The exact mechanism responsible for liver injury caused by these drugs is not clear. Isoniazid is acetylated and then hydrolyzed, resulting in isonicotinic acid and monoacetylhydrazine; the latter compound can be activated to a toxic species by cytochrome P-450.2 In vitro studies indicate that metabolic oxidation of acetylhydrazine leads to a reactive acylating species that binds covalently to microsomal protein. It is postulated that acetylhydrazine and hydrazine act as acetylating agents by binding covalently with liver cell macromolecules, causing hepatocyte injury.3

Rifampicin, a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of isoniazid by enhancing the production of toxic metabolites from acetylhydrazine.4 Cimetidine, a microsomal P-450 enzyme-system inhibitor, has been shown to reduce the hepatotoxicity of several drugs in rats by inhibiting oxidative drug metabolism.5,6,7 Rabbits show a similar genetically determined acetyltransferase activity as in humans and are more sensitive to isoniazid-induced hepatotoxicity due to a high amidase activity, which results in release of large amount of acetylhydrazine, which induces hepatotoxicity.8 We therefore used a rabbit model to study whether cimetidine could ameliorate the hepatotoxicity of isoniazid and rifampicin.

Methods

This study was approved by the institutional animal ethical committee and Committee for Prevention of Cruelty and Supervision of Experiment on Animals. Albino rabbits of either sex, weighing 2-3 Kg, were procured from the central animal house of the institute and housed in individual cages in air-conditioned environment. They were allowed to acclimatize for 7-10 days before start of study, and were provided normal diet and free access to water throughout the study.

Rabbits were divided into 6 groups of six animals each. Animals in Group 1 (control) received normal saline orally. Animals in Group 2 received isoniazid (50 mg/Kg/d), and those in Group 3 received a combination of isoniazid (50 mg/Kg/d) and rifampicin (100 mg/Kg/d) orally for 7 days. Group 4 received only cimetidine (120 mg/Kg/d) intraperitoneally for 7 days. Animals in Group 5 and Group 6 received isoniazid-rifampicin combination with intraperitoneal cimetidine in doses of 50 or 120 mg/Kg/d, respectively, for 7 days. Cimetidine was given 30 minutes before the isoniazid-rifampicin combination.

A baseline blood specimen (2 mL) was drawn from the lateral ear vein of each rabbit, after mop-
Kalra, Aggarwal, Khurana, Gupta

Cimetidine salvage of isoniazid-rifampicin hepatotoxicity

The ear was xylanized on day 1 (before administration of any drug). On day 8 (the day after last day of drug administration), the animals were sacrificed by cervical dislocation followed by exsanguination. Each animal’s blood specimen was collected through cardiac puncture and liver tissue was collected. Blood was centrifuged at 2500 rpm for 10 min and the extracted serum was stored at -20°C till estimation of liver enzymes. The liver tissue was examined with naked eye and preserved in 10% buffered formalin for histological examination. Two wedge sections were made from each liver, one each from the center and the periphery. Tissue was processed by cycling it through different chambers of a histokinette (Leica Corp, Germany) over 15 hours followed by preparation of paraffin blocks. Sections from the blocks were stained with hematoxylin-eosin and examined under a light microscope for portal inflammation, ballooning degeneration, necrosis and fatty changes.

Statistical analysis was done using Wilcoxon’s signed rank test; p values <0.05 were taken as significant.

Results

Food intake and weight of animals

Animals receiving isoniazid alone and in combination with rifampicin had decreased food intake (Table 1). Animals in the isoniazid-rifampicin combination group were also quieter, had less motor activity, and roughening of hair coat. Rabbits in Groups 2, 3 and 5 showed reduction in mean body weight (Table 1).

Liver enzymes (Table 2)

Animals who received isoniazid alone had no significant increase in ALT and AST levels. However, rabbits receiving isoniazid-rifampicin combination showed a 3-4-fold increase in ALT and AST levels (p=0.02). Concomitant administration of cimetidine at the higher dose prevented this increase, but that of low-dose cimetidine did not.

Liver morphology

Mean liver weights were similar in all the groups. Livers from the control group did not show portal inflammation, necrosis, fatty change or ballooning degeneration (Table 3). Animals receiving isoniazid or isoniazid-rifampicin combination showed portal inflammation, fatty changes (Fig 1) and liver cell necrosis (Fig 2); the changes were more

Table 1: Body weight, liver weight, liver morphology and food intake in different treatment groups (n = 6 each)

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (Kg)</th>
<th>Liver weight (g)</th>
<th>Animals with changes in liver (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 8</td>
<td>Baseline</td>
</tr>
<tr>
<td>Group 1</td>
<td>2.4 (0.4)</td>
<td>2.3 (0.4)</td>
<td>90 (5.5)</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.4 (0.5)</td>
<td>1.7 (0.3)*</td>
<td>70 (2.0)*</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.6 (1.7)</td>
<td>1.8 (0.3)*</td>
<td>65 (6.0)*</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.5 (0.4)</td>
<td>2.2 (0.3)</td>
<td>80 (2.5)</td>
</tr>
<tr>
<td>Group 5</td>
<td>2.8 (0.3)</td>
<td>2.1 (0.4)*</td>
<td>75 (6.5)*</td>
</tr>
<tr>
<td>Group 6</td>
<td>2.4 (0.3)</td>
<td>2.0 (0.3)</td>
<td>75 (5.0)*</td>
</tr>
</tbody>
</table>

Data as mean (SD). Group 1: control; Group 2: isoniazid; Group 3: isoniazid + rifampicin; Group 4: cimetidine (120 mg/Kg/d); Group 5: isoniazid + rifampicin + cimetidine (50 mg/Kg/d); Group 6: isoniazid + rifampicin + cimetidine (120 mg/Kg/d)

*p=0.02 as compared to baseline values. *p=0.009, *p=0.004, *p=0.01, *p=0.02 as compared to Group 1

Table 2: Effect of different treatments on AST and ALT serum levels (n = 6 each)

<table>
<thead>
<tr>
<th>Groups</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Day 8</td>
</tr>
<tr>
<td>1</td>
<td>27 (13-58)</td>
<td>28 (13-43)</td>
</tr>
<tr>
<td>2</td>
<td>34 (13-109)</td>
<td>83 (13-200)</td>
</tr>
<tr>
<td>3</td>
<td>24 (13-47)</td>
<td>124 (27-200)*</td>
</tr>
<tr>
<td>4</td>
<td>20 (13-47)</td>
<td>20 (13-47)</td>
</tr>
<tr>
<td>5</td>
<td>27 (13-57)</td>
<td>74.5 (20-181)</td>
</tr>
<tr>
<td>6</td>
<td>20 (13-57)</td>
<td>42 (20-200)</td>
</tr>
</tbody>
</table>

Data as median (range); *p<0.02, *p<0.04 as compared to baseline

Group 1: control; Group 2: isoniazid; Group 3: isoniazid + rifampicin; Group 4: cimetidine (120 mg/Kg/d); Group 5: isoniazid + rifampicin + cimetidine (50 mg/Kg/d); Group 6: isoniazid + rifampicin + cimetidine (120 mg/Kg/d)

Fig 1: Portal inflammation (grade 1). Mild portal inflammation seen in livers of animals treated with isoniazid-rifampicin combination (Group 3) (H&E, 100X)
marked in the latter group. Animals receiving only cimetidine did not show any necrotic or fatty changes in their livers. Cimetidine pre-treatment was associated with reduction in changes induced by isoniazid-rifampicin combination; this effect was more marked in animals receiving a higher dose of cimetidine.

**Discussion**

In a study of 427 hospital employees receiving isoniazid chemoprophylaxis for tuberculosis, abnormal liver function test results correlated well with histological evidence of hepatocellular damage. Further, several studies suggest that hepatitis is more frequent and more severe in patients receiving isoniazid-rifampicin combination treatment than in those receiving isoniazid alone. Our study confirms these findings using an animal model.

Isoniazid-induced hepatitis is associated with ballooning degeneration, focal hepatocyte necrosis, with minimal cholestasis. Another study reported diffuse microvesicular fatty infiltration with mild portal triaditis. Similar changes were seen in our study, confirming the validity of our animal model.

Cimetidine has been reported to have a hepatoprotective effect in animals receiving toxic doses of paracetamol. These studies showed a marked reduction in serum liver enzyme levels; however, histology was not studied. To the best of our knowledge, the effect of the microsomal-enzyme inhibitor cimetidine on isoniazid- and rifampicin-induced hepatotoxicity has not been reported in the literature. In the current study, we found that cimetidine at the higher dose level prevented an increase in AST and ALT levels as well as the histological changes associated with isoniazid-rifampicin combination. In addition, it also prevented the reduction in body weight caused by isoniazid-rifampicin combination. This may be due to inhibition of enhanced production of toxic reactive metabolites.

In conclusion, our data show that, in a rabbit model, rifampicin potentiates the hepatotoxicity caused by isoniazid, and that cimetidine administration in a high dose ameliorates biochemical and histological changes associated with administration of isoniazid-rifampicin combination.

**References**


**References**
