ORIGINAL ARTICLE

The hepatoprotective role of Silymarin in isoniazid induced liver damage of rabbits

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Abstract

Objective: To evaluate the hepatoprotective role of Silymarin against isonicotinylhydrazine-induced hepatotoxicity in rabbit model.

Methods: The experimental animal study was held at Jinnah Postgraduate Medical Centre, Karachi, from April to September 2013 and comprised rabbits weighing 1-1.5kgof either gender. The animals were divided randomly into equal groups: group I underwent liver function test without any drug; in group II effects of Silymarin (50mg/kg/day orally) was observed; in group III isoniazid (50mg/kg/dayorally) was administered; and in group IV combined effects of isoniazid and silymarin were observed. Liver function tests were performed at day0 and after the treatment at day19. SPSS 16 was used for statistical analysis.

Results: The 28 rabbits in the study were divided in four groups of 7(25%) each. No mortality was recorded in any group. In group III, bilirubin level was increased and alanine transaminase was decreased significantly ($p<0.05$ each). In group IV, there was significant improvement in serum billirubin and serum alanine transaminase ($p<0.05$ each).

Conclusion: Isonicotinylhydrazine-induced hepatotoxicity was well treated by concurrent administration of Silymarin.

Keywords: Hepatotoxicity, INH-induced hepatotoxicity, Silymarin, Liver function test. (JPMA 65: 620; 2015)

Introduction

Drug-induced liver injury has become the leading cause of acute liver failure and transplantation in Western countries.[1,2] It has been observed that when experimental animals are treated with anti-tuberculosis drugs, a correlation exists between hepatic injury and oxidative stress (OS).[3,4] Levels of alanine transaminase (ALT) and bilirubin are helpful in the diagnosis of hepatic diseases.[3] Isoniazid has an inhibiting effect on cytochrome P450 enzyme activity which was suggested to be involved in hydrazine detoxification as its active metabolite.[5] Silymarin, commercially available as Milk Thistle, is an extract from the seeds of Silybium Marianum. The protective effect of Silymarin was attributed to its antioxidant and free radical scavenging properties.[6] Silymarin has been shown to be safe in animal models, and no significant adverse reactions are reported inhuman studies.[7] The current study was planned to observe the hepatoprotective effects of Silymarin in rabbits treated with isoniazid.

Material and Methods

The experimental animal study was held at Jinnah Postgraduate Medical Centre, Karachi, from April to September 2013 and comprised rabbits weighing 1-1.5kg of either gender. The animals were divided randomly into equal groups: group I underwent liver function test (LFT) without any drug; in group II effects of Silymarin (50mg/kg/day orally) was observed; in group III isonicotinylhydrazine (INH) (50mg/kg/day orally) was administered; and in group IV combined effects of isoniazid and silymarin were observed. Adequate amount of water and food was supplied. Before starting the experiment, all groups were supplied with normal diet and water for acclimatisation. LFT was assessed by serum level of bilirubin and ALT before and after the treatment. All parameters were recorded by auto-analyser. Blood 4ml was drawn from marginal ear vein of the rabbit with the help of a fine 22G needle. Xylene was applied on target surface to dilate the vein. The blood samples obtained were centrifuged and serum was stored at -20°C till analysed. Normal saline 1000ml intravenous (IV) sol, lignocain injection vials, kits for serum bilirubin and ALT were used. Automated clinical chemical analyser, disposable syringes 5cc 23G, cotton bags, butterfly needles (22G), scissors, measuring cylinder 100ml, glass pipette, glass rod were also used.

The sample size was calculated on the basis of literature[8] by using OpenEpi version 16.

Data was analysed using SPSS 16. The results were presented as mean ± standard deviation (SD). Differences

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among the groups were assessed by paired sample t-Test followed by multiple mean comparisons using independent Sample t-Test and chi-squared test. Wilcoxon Signed-Rank Test was used as an alternative to the independent Sample t-Test while Fisher's exact test was used as an alternative to the chi-squared test to complement the results obtained from parametric tests because the sample size of the study was small and the normality assumption might have been violated. In all statistical analysis, only $p<0.05$ was considered significant.

Results

The 28 rabbits in the study were divided in four groups of 7(25%) each. No mortality was recorded in any group.

In group1, which acted as a control group, mean bilirubin and ALT values were observed at day 0 and at the end of study which was not statistically significant ($p>0.05$).

Group II revealed increase in meanbilirubin, but it was not significant ($p>0.05$).

Mean serum ALT decreased but it was not significant ($p>0.05$).

Group III revealed overall significant increase in mean bilirubin ($p<0.05$). Changes in mean ALT value revealed overall decrease and the change was highly significant ($p<0.05$).

In Group IV, changes in mean bilirubin revealed overall increase, but it was not significant ($p>0.05$). Changes in mean ALT revealed overall decrease which was significant ($p<0.05$) (Table).

Table: Changes in mean serum bilirubin and alanine transaminase (ALT) from day 0 to day 19 in different animal groups.

Groups	Serum Bilirubin at day 0 n=07	Serum Bilirubin at day 19 n=07	p-value	Percentage	Serum ALT at Day 0 n=07	Serum ALT at Day 19 n=07	P-value	Percentage
Control	0.23±0.01	0.24±0.01	0.35	-4.35%	47.08±0.62	47.05±0.45	0.48	-0.03
Silymarin	0.23±0.01	0.24±0.01	0.11	-4.35%	47.17±0.50	47.04±0.46	0.7	-0.38
Isoniazid	0.24±0.01	.46±0.02	0.01	-91.66%	47.09±0.35	22.15±0.59	<0.001	-36.02
Silymarin +Isoniazid	0.24±0.01	0.25±0.01	0.2	-4.35%	47.02±0.29	37.03±0.23	<0.001	-10.92

Discussion

INH hepatotoxicity is a common complication of anti-tuberculosis therapy, ranging in severity from asymptomatic elevation of serum transaminases to hepatic failure. It has been reported that during sub-acute or chronic treatment, INH-induced hepatotoxicity results in the rise of serum transaminase levels. But there is also evidence that ALT activities decrease in the serum of rabbits after INH administration. As the extent of INH-induced hepatotoxicity was not clearly observed in the serum, therefore measuring the above-mentioned levels alone does not reflect the actual position of INH-induced liver toxicity during the sub-acute treatment.[8] Rise in the levels of liver enzymes in serum followed by fall in these levels indicates drug and chemical-induced hepatotoxicity. In our study, similar changes were observed, underlining the validity of our animal model. In group II animals treated with Silymarin, increased body-weight of rabbits which may be due to its protein synthesis action as defined by a study.[9] When we compared this group with group III, there was significant increase in body-weight. This suggests that Silymarin was able to arrest the decrease in the weight of rabbits which was observed in isoniazid-treated group. These observations correlate with an earlier study.[10] When we compared this group with the control group, there was no significant difference observed in the levels of liver function enzymes.

These effects of Silymarin indicate the protective effects on liver tissue. In a study,[11] the levels of serum ALT and total bilirubin in Silymarin-treated animals were not raised and remained close to normal, which supported the result of group II (Silymarin) in our study. Biochemical changes seen in group II were also in accordance with a study.[12] In group III, body-weight of rabbits decreased due to less food intake and the animals became lethargic, indicating the toxic effects of isoniazid on liver. On the other hand, serum total bilirubin was significantly increased which showed hepatocellular membrane damage. These changes have also been reported in literature.[13-15] When we compared this group with the control group, there was significant reduction in body-weight, while serum bilirubin was significantly increased, showing the abnormal integrity of hepatocytes. A study[7] also reported similar findings. One study[8] showed the same biochemical changes of INH-induced hepatotoxicity in rabbits, indicating abnormal integrity of hepatocytes, which correlates with our results. In group IV, combination of isoniazid and Silymarin was administered, which was the main objective of our study. Body-weight of animals in this group was slightly increased compared to their weights before starting drug administration at day 0.

When we compared this group with the control group, there was non-significant changes observed in weight and serum bilirubin level and the values were near the control group. There was decrease in serum ALT, which may be due to hepatoprotective role of silymarin. These results correlate with the findings of an earlier study[8] which reported that the effect of combination therapy of isoniazid and Silymarin resulted in decreased incidence and severity of biochemical changes. The result of this group supported the hepatoprotective role of Silymarin. These results correlate with the result of previous studies that proved the protective effect of Silymarin against isoniazid-induced toxicity.[7]

Conclusion

Silymarinacts as a hepatoprotective agent in drug-induced hepatic toxicity. The isoniazid-induced liver biochemical changes are well-treated by concurrent administration of Silymarin.

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