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ORIGINAL RESEARCH ARTICLE

Hepatoprotective Effect of Rimonabant Against Isoniazid Induced Liver Damage In Albino Wistar Rats

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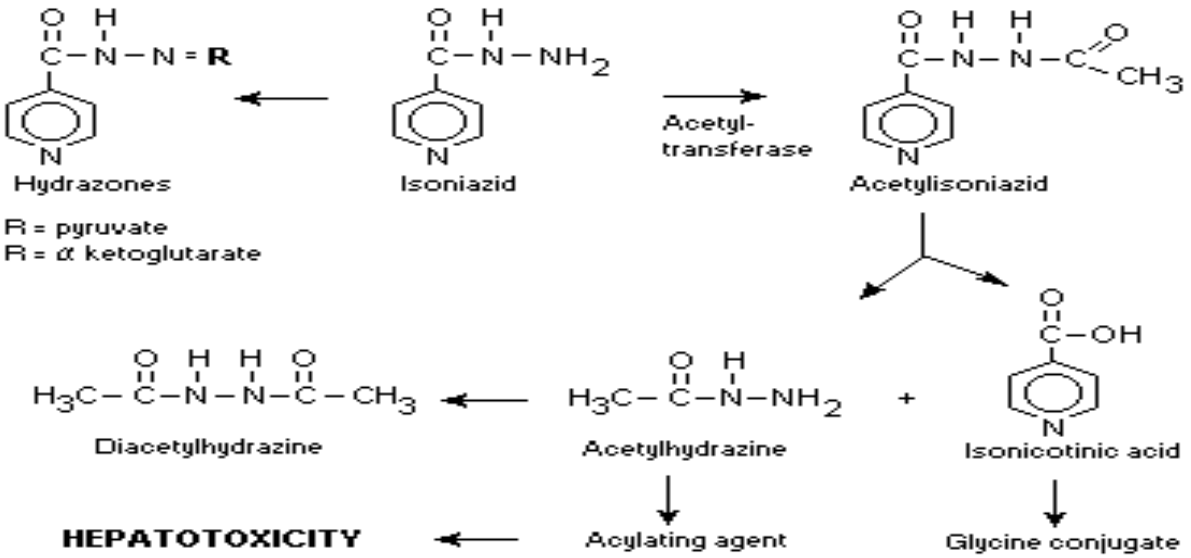
ABSTRACT

The present study was conducted to evaluate the hepatoprotective activity of the rimonabant against isoniazid-induced liver damage in albino wistar rats. Hepatotoxicity was induced by daily dose of isoniazid (250mg/kg p.o.) for 14 days as manifested by statistically significant increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and bilirubin level. Pretreatment of rats with the rimonabant at doses of 2.5, 5 and 10 mg/kg prior to isoniazid dosing at 250mg/kg p.o statistically lowered the serum liver enzyme activities. The activity of the rimonabant was comparable to the standard drug, silymarin (50mg/kg, p.o.). Results obtained from histopathological studies also supported hepatoprotective activity against ethanol-induced hepatotoxicity. Thus the study demonstrates that rimonabant possess antihepatotoxic effect against isoniazid.

Key words: rimonabant, isoniazid, hepatoprotective activity and biochemical parameters

INTRODUCTION

Isoniazid (INH) is used as a first-line drug in the treatment and chemoprophylaxis of tuberculosis [1]. It inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. At therapeutic levels INH is bacteriocidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms. INH undergoes extensive *N*-acetyltransferase - catalyzed acetylation to acetylisoniazid, which is then hydroxylated by cytochrome P450 enzymes to the hepatotoxic intermediate acetylhydrazine, a metabolite capable of forming covalent cellular adducts. The risk of liver toxicity is higher in slow acetylators, in the elderly, and in association with concomitant use of cytochrome P450 inducers such as alcohol [2].



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Presently there are only few hepatoprotective drugs and not a single effective allopathic medication, are available for the treatment of liver disorders [3].

Rimonabant appears to be a promising drug in an entirely new class called selective cannabinoid (CB1) receptor antagonists. Recent studies have demonstrated the beneficial effects of rimonabant in tackling obesity, smoking cessation and metabolic syndrome. The drug may be approved for treatment of obesity and smoking cessation [4]. Ongoing studies may provide information on its other clinical uses.

Rimonabant which is selective endocannabinoid CB1 receptor antagonist that inhibits the pharmacological effects of cannabinoids agonists in vitro and vivo. The chemically described as N-peperidino-5(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide. Rimonabant is reported to increase HDL-C and decrease atherogenic LDL-C levels [5]. The unique property of this drug may, in turn, improve cardiovascular risk factors and metabolic syndrome. In addition to weight loss [6], rimonabant is reported to produce improvement in HbA_{1C} levels and may be helpful in diabetes. It also prevents weight gain in persons who are quitting Smoking [7].

MATERIALS AND METHOD

Experimental animals

Three months old Wistar albino rats of either sex weighing 150- 250g were used for the study. The animals were procured from National Toxicology Center, Pune. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2 ^{0}C and relative humidity of 30-70%. A 12/12 h light and dark cycle was followed. All animals were fed on standard balanced diet and provided with water ad libitum**.**

All the experimental procedures and protocols used in the study were reviewed and approved by the (IAEC) Institutional Animal Ethical Committee of R.C.Patel IPER, Shirpur and were in accordance with the guidelines of the Committee for the purpose of Control and Supervision of Experiments on Animals(CPCSEA). Registration No.RCPCOP/ IAEC/2008-2009/30

Toxicity Study [8]: Acute oral toxicity was conducted for rimonabant on albino mice according to OECD 425 and median effective dose (ED50) of rimonabant was selected based on LD50 obtained from acute toxicity studies.

INH induced liver damage [9]: Albino wistar rats of either sex (150-250g) were used. All the animals were divided into the six groups each group consists of 6 animals and they received the treatment as follows.

Group I: Normal (1% CMC.p.o.) for 14 days
Group II: INH (250mg/kg p.o.) for 14 days
Group III: Standard drug (Silymarin 50mg/kg p.o) + INH (250mg/kg p.o.) for 14 days
Group IV: Rimonabant (2.5mg/kg p.o) + INH (250mg/kg p.o.) for 14 days
Group V: Rimonabant (5mg/kg p.o) + INH (250mg/kg p.o.) for 14 days
Group VI: Rimonabant (10mg/kg p.o) + INH (250mg/kg p.o.) for 14 days

All the test drugs were administered orally by suspending in 1% CMC solution. Twenty-four hours after last dose of INH, blood was obtained from all groups of rats by puncturing retro-orbital plexus. The blood samples were allowed to coagulate for 45 min at room temperature. Serum was separated by centrifugation at 3000 rpm at room temperature for 20 min and used for the biochemical estimation.

Biochemical estimation: The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) [10], alkaline phosphatase (ALP) [11], lactate dehydrogenase (LDH), total bilirubin (TB) and direct bilirubin (DB) were measured according to the reported methods.

Histopathological examination: The livers of all animals were removed and determine its liver weight and liver volume. A thin slice of livers preserved in 10% buffered formalin solution for histopathalogical investigations.

Statistical Analysis: All the data expressed as Mean ± S.E.M and analyzed statistically using ANOVA followed by Dunnett test and compare with respective control group. A value was of $P<0.05$ was considered significant.

RESULTS

Administration of INH at a dose of 250 mg/kg (p.o.) caused a significant increase in liver weight and volume but no significant change in body weight was observe (Table 1), and showed significant rise ($P<0.01$) in level of serum marker enzymes such as AST, ALT, ALP, LDH, TB, and DB. Silymarin significantly ($P<0.01$) reduced these levels near to normal. A significant ($P<0.01$) decrease was observed in the AST, ALT, ALP, LDH, TB and DB (Table 2) in the animals treated with different doses (2.5mg/kg, 5mg/kg and 10 mg/kg) of rimonabant and showed dose dependant activity. At the dose of 10mg/kg rimonabnt showed comparable activity with the standard drug silymarin.

Histopathological studies, showed ethanol to produce extensive vascular degenerative changes and centrilobular necrosis in hepatocytes. Treatment with silymarin and rimonabant produced mild degenerative changes and absence of centrilobular necrosis when compared with control (fig. 1). All these results indicate a hepatoprotective potential of rimonabant. Rimonabant showed a dose dependent activity which was confirmed by Histopathological examination.

Table 1: Effect of Rimonabant, Silymarin and Isoniazid on liver weight and liver volume.

	Body weight (gm)	Liver weight (gm)	Liver volume (ml)
Normal	245	7	8
INH(250 mg/kg) Treated	259	13	11
STD drug (Silymarin) + INH(250mg/kg)	225	9	10
Rimonabant(2.5mg/kg) + INH(250mg/kg)	235	13	13
Rimonabant (5mg/kg) + INH(250mg/kg)	243	11	9
Rimonabant(10mg/kg) + INH(250mg/kg)	215	8.5	8

Table 2: Effect of Rimonabant, Silymarin and Isoniazid on different biochemical parameters.

Group	AST (I.U./L)	ALT (I.U./L)	ALP (I.U./L)	LDH (I.U./L)	T.B. (%mg)	D.B. (%mg)
Normal	64.19 ± 1.2*	47.09 ± 0.6*	185.1 ± 1.7*	146.3 ± 1.3*	0.18 ± 0.006	0.17 ± 0.004**
INH(250mg/kg) Treated	172.9 ± 1.3**	125.8 ±3.2	352.5 ± 3.2*	273.5 ± 1.4*	1.08 ± 0.1*	0.6 ± 0.016
Silymarin(50mg/kg) + INH	95.91 ±1.1	71.03 ±0.8	210.3 ± 0.9*	177.3 ± 3.5*	0.35 ± 0.01*	0.24 ± 0.004
Rimonabant (2.5mg/kg)+ INH	135.5 ±1*	107.1 ±0.6	305.3 ± 1.2*	230.8 ± 1.6*	0.6 ± 0.03**	0.50 ± 0.01*
Rimonabant (5mg/kg) + INH	114.9 ±0.9	96.86 ±0.7**	281.9 ± 2.4*	215.7 ± 1.8*	0.5 ±0.02	0.42 ± 0.01*
Rimonabant (10mg/kg)+ INH	103.8 ±1	85.88 ±0.3	240.7 ±2.6*	196.8 ± 0.4*	0.45 ±0.01	0.32 ± 0.01*

Values are expressed as mean ± S.E.M. (n=6)

* $P<0.05$, when compared with the INH treated groups (one-way ANOVA followed by Dunnetts test)

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Histopahological Changes:

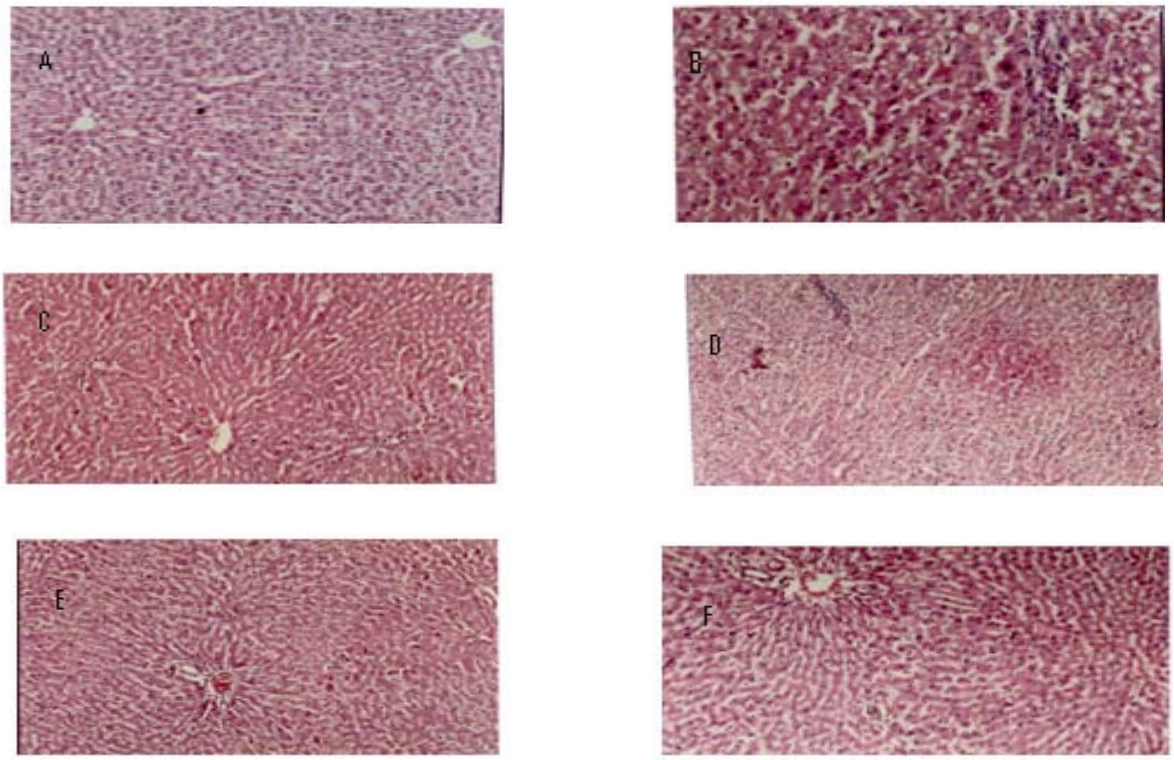

Figure 1: Effect of rimonabant on isoniazid induced liver damage in rats (X 100)

A) Normal liver Section showing prominent central vein & normal hepatocytes & sinusoids of liver; B) INH treated liver showing focal areas of liver cell necrosis and degeneration and fatty changes ; C) Silymarin treated liver showing normal hepatocytes and few areas of necrosis ; D) Rimonabant (2.5mg/kg) treated liver showing focal hepatocytes necrosis & inflammation ; E) Rimonabant (5mg/kg) treated liver showing few areas of liver cell necrosis & degeneration; F) Rimonabant (10mg/kg) treated liver showing normal central vein and hepatocytes are seen few area of fatty change .

DISCUSSION

During metabolism of INH, hydrazine is produced directly (from INH) or indirectly (from acetylhydrazine). It is evident that hydrazine plays a role in INH-induced liver damage. It can cause moderate abnormalities in serum transaminases leading to hepatotoxicity; hence the measurement of serum transaminases is often advocated during INH administration, to assess the extent of INH-induced hepatotoxicity [12].

Administration of INH significantly elevated levels of AST, ALT, ALP, LDH and bilirubin, due to damaged structural integrity of the liver because these are cytoplasm in location and are released into circulation after cellular damage [13]. Riomonabant treatment with INH prevented the INH-induced perturbations in the activities of AST, ALT, ALP, LDH, TB, DB, Sodium and potassium ions in both the serum and liver tissue. It is likely that the INH-derived hepatotoxic metabolites, namely acetyl.isoniazid and acetyl hydrazine would have been trapped by rimonabant [14]

The results in this study were cofirmed by histopathological observation. In contrast to the control group, INH-intoxicant rat showed mild inflammatory cell infiltration and fatty changes, but Rimonabant administration for 14 days attenuated this histopathological changes.

CONCLUSION

In conclusion, rimonabant has hepatoprotective activity against hepatotoxicant like isoniazid and its activity is comparable with the standard drug silymarin. Drug has shown the ability to maintain

the normal functional statues of the liver. From the above preliminary study, we conclude that the rimonabant is proved to be one of the allopathic medication for liver ailment.

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