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Hepatoprotective Effect of Liv.52 on Antitubercular Drug-induced Hepatotoxicity in Rats

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SUMMARY

Liv.52, a polyherbal Ayurvedic formulation, exhibited hepatoprotective function when tested against chronic antitubercular drug treated rats. Suppression of GSH and antioxidant enzymes (superoxide dismutase, catalase, GPX and GST) were noticed in the liver of antitubercular drug treated animals, accompanied with an increased production of lipid peroxides. Liv.52 afforded hepatoprotection by inhibiting lipid peroxide production and, as a result, the animals showed improved antioxidant status.

Drug - induced hepatotoxicity is a potentially serious adverse effect of the currently used antitubercular chemotherapeutic regimens containing isoniazed (INH), rifampicin and pyrazinamide[1-3]. Adverse effects of antitubercular therapy are sometimes potentiated by multiple drug regimen. Thus, though INH, rifampicin and pyrazinamide each in itself are potentially hepatotoxic, when given in combination their toxic effect is enhanced. The conversion of monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P_{450} leads to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incident of hepatitis. This has been postulated to be due to rifampicin-induced cytochrome P_{450} enzyme-induction, causing an increased production of toxic metabolites from acetyl hydrazine (AcHz)[4]. Other investigators demonstrated that rifampicin increases the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic[5]. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin and AcHz is quickly converted to its active metabolites by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and rifampicin in combination[6]. Rifampicin induction of the hydrolysis pathway of INH metabolism into the hepatotoxic metabolite hydrazine was reported by Askgaard *et al.*[7] Pharmacokinetic interaction exists between rifampicin and pyrazinamide in tuberculotic patients, when these drugs are administered concomitantly. Pyrazinamide decreases blood levels of rifampicin by decreasing its bioavailability and increasing its clearance[8]. Pyrazinamide, in combination with INH and rifampicin, appears to be associated with an increased incidence of hepatotoxicity[9].

The aim of the present work was to study the effect of Liv.52, an established hepatoprotective polyherbal formulation[10,11], on the hepatoxicity produced by the three antitubercular agents given together.

Liv.52 is an Ayurvedic formulation containing various herbomineral principles designed to combat liver injury and to protect liver against damage. It is available on the market in a tablet form, the composition of which is given in Table 1.

EXPERIMENTAL

Drugs and chemicals – Isoniazid, rifampicin and pyrazinamide, bovine serum albumin and glutathione were obtained from Sigma, USA. Liv.52 was gifted by The Himalaya Drug Company, Bangalore, India.

Table 1: Composition of Liv.52*

Plant	Plant part	Quantity (mg/tablet)
Capparis spinosa	Bark	65
Cichorium intybus	Seeds	65
Solanum nigrum	Whole plant	32
Cassia occidentalis	Seeds	16
Terminalia arjuna	Bark	32
Achillea millefolium	Seeds	16
Tamarix gallica	Whole plant	16

*It also contains 'Mandur bhasma' (33 mg/tablet) which is prepared from ferric oxide, triturated in the juices of many hepatic stimulants and cholagogues.

Animals – Male Wistar rats (150 ± 10g) were maintained in standard environmental conditions. They were fed with commercial pelleted diet obtained from Hindustan Lever Ltd. and water ad libitum. Animals were housed six per cage at 27 ± 2ºC with constant 55% humidity, on a 12-h light/dark cycle.

Antihepatotoxic activity – Liv.52 was suspended in water and administered orally. Isoniazid and pyrazinamide were dissolved in sterile distilled water whereas rifampicin was first dissolved in 0.5 ml of 0.1N HC1 and then made up to the required volume by adding sterile distilled water; all these drugs together were given orally by gastric incubation.

Animals were divided into 4 groups (n=6): Group I – control animals receiving no treatment; Group-II – animals receiving Liv.52 (500 mg/kg, p. o.) for 2 weeks served as drug control; Group-III – animals receiving all the three antitubercular drugs for 2 weeks (isoniazid 7.5 mg/kg, rifampicin 10 mg/kg, pyrazinamide 35 mg/kg, p.o.); Group IV-animals receiving simultaneous treatment of Liv.52 and all the three antitubercular drugs for 2 weeks.

Table 2: Effect of Liv.52 (500 mg/kg, p.o.) on antitubercular drug (isoniazid 7.5 mg/kg + rifampicin 10 mg/kg + pyrazinamide 35 mg/kg, p.o.) - induced hepatotoxicity in rats

Group	Treatment	LPO	SOD	CAT	GSH	GST	GPX
I	Normal Control	0.63 ± 0.08	8.77 ± 0.73	130.72 ± 12.8	8.887 ± 0.78	0.35 ± 0.029	7.225 ± 0.71
II	Liv.52	0.64 ± 0.06	8.53 ± 0.84	128.42 ± 12.3	8.27 ± 0.81	0.348 ± 0.032	7.32 ± 0.69
III	Antitubercular drugs (B)	1.28 ± 0.12***	5.47 ± 0.56***	88.73 ± 8.7***	4.795 ± 0.5***	0.25 ± 0.028***	4.848 ± 0.5***
IV	A – B	0.82 ± 0.09***	7.17 ± 0.74**	116.1 ± 10.9***	6.025 ± 0.54***	0.32 ± 0.029**	6.24 ± 0.61**

LOP=Lipid peroxide content in tissue, expressed as nmoles MDA/mg protein

SOD=Superoxide dismutase activity, expressed as U/mg protein/min (one unit of SOD activity is the amount of protein reviewed to give 50% inhibition of epinephrine autoxidation).

CAT=Catalase activity, expressed as nmoles of H_2O_2 decomposed/min/mg protein.

GSH=Glutathione activity, expressed as nmoles/gm of wet tissue

GST=Glutathione-S-transferase activity, expressed as nmoles of CDNB (1-chloro-2,4-dinitrobenzene) conjugated/min/mg protein.

GPX=Glutathione peroxidase activity, expressed as nmoles of GSH oxidized/min/mg protein.

Values are mean ± SD, n=6; $^{**}p<0.01$, $^{***}p<0.001$, Group III vs Group I, Group IV vs Group III, Student's 't'-test.

At the end of the treatment, the animals were fasted 24 h and sacrificed by decapitation. The liver was dissected out, washed with chilled physiological saline, weighted, homogenized in 0.1M Tris HC1 buffer (pH 7.4) at 4ºC in potter Elvejem homogenizer, and then used for the evaluation: lipid peroxides (LPO)[12] and glutathione (GSH)[13], along with the activities of superoxide dismutase (SOD)[13], catalase (CAT)[14], glutathione peroxidase (GPX)[15] and glutathione S-transferase (GST)[16] were assayed.

RESULTS AND DISCUSSION

Animals treated with the combination of INH, rifampicin and pyrazinamide showed a significant increase in liver peroxide contents, and a significant decrease on SOD, CAT, GPX and GST activities. Also the glutathione level was significantly reduced. Treatment with Liv.52 (500 mg/kg, p.o.) significantly modified the hepatotoxic effect of the antitubercular drugs. As shown in Table 2, Liv.52 afforded protection against lipid peroxidation, the above-mentioned parameters being almost restored to normal values.

Liv.52 affords protection against lipid peroxidation by increasing tocopherol level[17,18]. The extracts of *Cichorium intybus* and *Solanum nigrum* (constitutents of Liv.52) have been reported to contain many polyphenolic compounds, mainly flavonoids. The antioxidant activity of the extract may therefore be due to the presence of polyphenolic constituents[19].

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