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EFFECT OF LIV.100 AGAINST ANTITUBERCULAR DRUGS (ISONIAZID, RIFAMPICIN AND PYRAZINAMIDE) INDUCED HEPATOTOXICITY IN RATS.

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SUMMARY

Objectives: To assess the protective effect of Liv.100 against antitubercular drugs (isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA)) induced hepatotoxicity in rats.

Methods: The simultaneous treatment of Liv.100 (400 mg/kg body weight) on antitubercular drugs (INH, RMP and PZA) induced lipid peroxidation in liver was studied in rats. Levels of marker enzymes such as lactate dehydrogenase, aspartate amino transferase, alanine amino transferase and alkaline phosphatase were assessed in liver and serum. The glutathione content and the activities of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase were estimated in liver. The levels of lipid peroxides and the activities of Na +K+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase were also assayed in the liver of experimental groups.

Results: In antitubercular drugs administered rats, a significant decrease was observed in the levels of marker enzymes in the liver with a corresponding increase in their levels in serum. The levels of lipid peroxidase (in terms of YBA reactants") were increased significantly in their serum and liver. The activities of $Na^{+}K^{+}$ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase were decreased significantly in their liver. The gluta thione content and the activities of antioxidant enzymes decreased significantly in the liver of antitu bercular drugs administered rats when compared to normal control. Simultaneous administration of Liv. 100, showed near normal levels of marker enzymes, and the levels of lipid peroxides glutathione content on comparison with normal control.

Conclusion: Simultaneous treatment with Liv. 100 offers protection against hepatotoxicity induced by antitubercular drugs by reducing lipid peroxidation and restoring the antioxidant defense system.

KEY WORDS Hepatoprotection lipid peroxides antioxidant enzymes catalase glutathione

INTRODUCTION

Isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), used in short-course chemotherapy of tuberculosis are known to be potentially hepatotoxic[1]. Administration of anti-tuberculous drugs (INH+ RMP + PZA) to white rats results in cytolytic liver injury.This manifests by hyperamino trans ferasemia, initiation of lipid peroxidation and suppression of the antioxidant system[2]. It has been established in experimental animals that anti-tuberculous drugs administered in toxic doses affect the liver, its membranes and organelles. This is supported by release of aspartate and alanine aminotransferases and alkaline phosphatase in serum and by a decrease of the activity of $Na^{+}K^{+}$ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in liver[3].

Liv.100 (Himalaya Drug Co. Pvt. Ltd.,) is a modified formulation of Liv.52, a popular hepatic stimulant[4]. Liv.100 is a scavenger of free radicals and it exhibits dose and time dependant protective response against hydrogen peroxide induced lipid peroxidation[5].

Since, free radical induced lipid peroxidation has been reported to be associated with various deleterious effects including tissue damage and necrosis, Liv.100 being a good scavenger of free radicals, has been chosen to study its effect on lipid peroxidation and activites of antioxidant enzymes during anti-tubercular drugs induced hepatotoxicity.

MATERIALS AND METHODS

Liv.100 an improvised and indigenous preparation of Liv.52 contains *Cichorium intybus, Solanum*

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Table 1. Serum and hepatic levels of aspartate amino transferase (AST), alanine amino transferase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) in experimental groups.

		Treatment (Group)			
		Normal control (I)	INH+RMP+PZA (II)	Liv.100 (III)	Liv.100 +INH+RMP+PZA (IV)
LDH	A	1.35 ± 0.11	2.73 ± 0.19"'	1.31 ± 0.06	1.47 ± 0.05'
	B	1.55 ± 0.04	0.8 ± 0.2"'	1.53 ± 0.04	1.48 ± 0.05'
AST	A	0.38 ± 0.04	0.76 ± 0.04"'	0.35 ± 0.04	0.44 ± 0.03
	B	0.68 ± 0.05	0.49 ± 0.03"'	0.71 ± 0.07	0.64 ± 0.05
ALT	A	0.53 ± 0.04	0.89 ± 0.04***	0.54 ± 0.04	0.58 ± 0.04
	B	0.93 f 0.03	0.05 ± 0.02"'	0.94 ± 0.03	0.91 ± 0.03
ALP	A	0.69 ± 0.03	1.37 ± 0.04"'	0.67 ± 0.02	0.71 ± 0.03
	B	1.10 ± 0.03	0.81 ± 0.02"'	1.07 ± 0.03	1.08 ± 0.04

A: Serum B: Liver; INH: Isoniazid RMP: Rifampicin PZA: Pyrazinamide.

Values are expressed as mean ± S.D. for 6 animals in each group.

Students 't' test : Vs Gp I : ***P<0.001, *P<0.05.

The levels of LDH, AST, ALT and ALP in Serum are expressed as µkat/litre.

The levels of LDH, AST and ALT in liver are expressed as µ moles of pyruvate liberated/sec/g of protein. The level of ALP in liver is expressed as µ moles of phenol liberated/sec/g protein.

nigrum, Phyllanthus amarus, Piccorhiza kurroa and Embelica oficinalis (5:3.75:2.62:2:1) and it was gifted by Himalaya Drug Company. Anti-tubercular drugs [viz, isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA)], epinephrine, NADPH, glutathione (reduced) and bovine serum albumin were obtained from Sigma Chemical Company (St. Louis, MO, USA). All the other chemicals used were of analytical grade.

Adult male albino rats of Wistar strain weighing 120-l 50g were fed with commercial pelleted rat chow (M/s Hindustan Lever Limited, Bombay) and water *ad libitum.* They were maintained in clean, sterile, polypropylene cages. The rats were divided into 4 groups of 6 animals each.

Group I served as normal control. Group II rats were administered a combination of three anti-tubercular drugs (INH-7.5 mg/kg bodywt., RMP-10 mg/kg body wt. and PZA-35 mg/kg body wt. for 45 days by intragastric administration) in sterile saline. Group III rats were given Liv.100 (400 mg/kg body wt., daily for a period of 45 days, orally). Group IV rats were administered both Liv.100 and the combination of three antitubercular drugs (INH+RMP+PZA) at the abovementioned dosage for 45 days.

After the experimental period, the rats were sacrificed by cervical decapitation. Blood was collected and the serum was used for the assay of marker enzymes, (lactate dehydrogenase[6] (LDH), aspartate amino transferase[6] (AST), alanine amino transferase[6] (ALT) and alkaline phosphatase[6] (ALP)). Immediately after the sacrifice, the liver was dissected out, washed in ice-cold saline, and a homogenate was prepared in 0.1 M Tris- HC1 buffer (pH7.4). The homogenate was centrifuged and the supernatant was used for the assay of marker enzymes, glutathione (GSH)[7], superoxide dismutase (SOD)[8], catalase (CAT)[9], glutathione peroxidase (GPX)[10], glutathione-S-transferase (GST)[11], glutathione reductase (GRD)[12], Na+K+ ATPase[13], Ca^{2+} ATPase[13] and Mg^{2+} ATPase[13]. Lipid peroxide levels were estimated in serum and liver in terms of TBA reactants using 1,

Table 2. The levels of lipid peroxides and the activities of Na +K+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in the liver of experimental groups.

	Treatment (Group)			
	Normal Control (I)	INH+RMP+PZA (II)	Liv. 100 (III)	Liv. 100+INH+RMP+PZA (IV)
Lipid peroxides				
Liver	3.69 ± 0.14	6.41 ± 0.10"'	3.60 ± 0.16	3.55 ± 0.06
Serum	2.37 ± 0.12	5.67 ± 0.14"	2.26 ± 0.04	2.30 ± 0.14
Na+K+ ATPase				
Liver	0.94 ± 0.04	0.53 ± 0.04'"'	0.97 ± 0.03	1.04 ±0.06
Ca^{2+} ATPase				
Liver	0.06 ± 0.04	0.34 ± 0.04***	0.64 9 . 0 3	0.57 ±0.04
Mg^{2+} ATPase				
Liver	0.73 ± 0.05	0.39 ± 0.03'"'	0.69 ± 0.01	0.70 ± 0.04

INH: Isoniazid; RMP: Rifampicin; PZA: Pyrazinamide. Values are expressed as mean ± S.D. for 6 animals in each group.

Students 't' test : Vs Gp I: ***P<0.001, **P<0.01, *P<0.05.

The level of lipid peroxide in liver is expressed as pmoles of TBA reactants/g protein whereas that in serum are expressed as pmoles of TBA reactants/litre.

The activities of Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase are expressed as µmoles of phosphorus liberated/sec/g protein.

1; 3,3' tetramethoxy propane as the standard[14]. Protein was determined by the method of Lowry et *al*[15].

Student 't' test was used for statistical analysis of data.

RESULTS

The rats which received anti-tubercular drugs showed a significant decrease in the levels of marker enzymes such as ALT, AST, LDH and ALP in liver accompanied by a corresponding significant increase in their levels in serum (Table 1). Rats administered with Liv.100 maintained the levels of marker enzymes in serum and liver. Group IV rats restored the levels of marker enzymes in the liver thereby preventing their increase in the serum.

The levels of lipid peroxides (as TBA reactive substances) and the activities of membrane bound Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase in liver are presented in Table 2. Rats administered anti-tubercular drugs (INH+RMP+PZA) alone, showed a significant increase in lipid peroxide in serum ($p<0.01$) and liver ($p<0.001$) when compared to normal control.The rats from same group showed a significant decrease in the activities of Na^+K^+ ATPase Ca^{2+} ATPase and Mg^{2+} ATPase. Group IV rats (Liv.100+INH+RMP+PZA) retained near normal lipid peroxide levels in serum and liver, and the activities of membrane bound enzymes in liver.

Table 3 presents the activities of antioxidant enzymes such as SOD, CAT, GPX, GST, GRD and glutathione in liver of experimental groups. Group II rats showed a significant decrease ($p<0.001$) in the activities of antioxidant enzymes when compared to normal control. Group IV rats restored the activities of antioxidant enzymes at near normal values. Rats administered Liv.100 alone, did not show any significant variations in the above-studied parameters when compared to normal control.

DISCUSSION

Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies[16].The serum levels of a number of hepatic enzymes are used as diagnostic indicators of hepatic injury". Increased levels of LDH, AST, ALT and ALP in serum of the anti-tubercular drugs treated animals certainly indicate liver damage. An increase in the levels of these marker enzymes in serum is due to the leakage of the enzymes from liver as a result of tissue damage. This is consistent

Table 3. The activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione redutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase and glutathione content in liver of experimental groups.

	Treatment (Group)			
	Normal Control(I)	INH+RMP+PZA (II)	Liv. 100 (III)	Liv.100+INH+RMP+PZA (IV)
Superoxide dismutase	61.79 ± 3.01	29.39 ± 0.17"'	63.13 ± 2.51	57.45 ± 1.84"
Catalase	74.33 ± 3.33	56.50 ± 4.17'""	73.50 ± 2.67	71.83 ± 3.0
Glutathione Peroxidase	184.42 ± 6.51	93.29 ± 5.42"'	192.01 ± 10.31	189.30 ± 5.97
Glutathione transferase	160.54 ± 3.54	87.89 ± 2.99***	155.64 ± 4.08	156.46 ± 4.90
Glutathione-S-reducatese	10.38 ± 0.24	5.77 ± 0.25***	10.56 ± 0.31	10.07 ± 0.29
Glutathione	4.58 ± 0.01	2.58 ± 0.05"'	4.59 ± 0.09	4.53 ± 0.08

INH : Isoniazid; RMP : Rifampicin; PZA : Pyrazinamide.Values are expressed as mean ± S.D. for 6 animals in each group.

Students 't' test : Vs Gp I : ***P<0.001, *P<0.05.

Level of superoxide dismutase is expressed in µkat/g protein. Level of catalase is expressed as nmoles of H_2O_2 decomposed/sec/g protein. Activity of glutathione peroxidase is expressed as mmoles of GSH utilised/sec/g protein. Level of glutathione reductase is expressed as mmoles of GSSG utilised/sec/g protein. Activity of glutathione-S-transferase is expressed as µmoles of CDNB conjugated/sec/g protein. Level of glutathione is expressed as nmoles of GSH/g tissues.

with the decrease in the levels of the marker enzymes in liver of anti-tubercular drugs treated animals.

On concurrent treatment with Liv.100, the serum marker enzyme levels in Group IV rats were near normal indicating protection against liver damage.

Lipid peroxidation is a complex and natural deleterious process. The significant increase observed in the levels of lipid peroxides in serum and liver and decrease in activities of Na^+K^+ATP-ase, Ca^{2+} ATP-ase and Mg^{2+} ATPase in liver of rats administered anti-tubercular drugs as compared to normal control are in accordance with the observation of Skakun et al [2,3].

The increase in theTBA reactive substances of liver indicates enhanced lipid peroxidation leading to tissue injury and failure of the antioxidant defense mechanisms to prevent the formation of excess free radicals[18].

Inactivation of Na^+K^+ ATPase and Mg^{2+} ATPase could be due to enhanced lipid peroxidation by free radicals on anti-tubercular drugs treatment since Na^+K^+ ATPase is a 'SH' group containing enzyme and is lipid dependent[19].

The rats given Liv.100 and anti-tubercular drugs concurrently retained the levels of TBA reactive substances to near normal in serum and liver when compared to normal control. This shows the protective action of Liv.100 on cellular membranes by lowering lipid peroxidation[20]. The activities of Na^+K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase were also restored at near normal on administration of Liv.100 concurrently with anti-tubercular drugs. This could be due to the ability of Liv.100 to protect the 'SH' groups from oxidative damage through inhibition of peroxidation of membrane lipids.

GSH together with GSH dependant systems GPX, GST, GRD, and CAT-SOD couple, efficiently scavenge toxic free radicals[21]. Decreased glutathione levels may be due to its increased utilisation in protecting 'SH' containing proteins from lipid

peroxides. The nonavailability of glutathione decreases the activity of glutathione peroxidase and transferase.

Isoniazid is known to induce the cytochrome P450 system resulting in increased metabolism, formation of toxic metabolities, depletion of glutathione stores and subsequent hepatocellular damage**. Inhibition of cellular glutathione biosynthesis by rifampicin in *M. smegmatis* has been reported[23]. Suppression of the antioxidant system in anti-tubercular drugs treated rats has also been reported[2].

The glutathione levels and activities of glutathione dependent enzymes were restored to near normal levels in Group IV rats, which were treated simultaneously with Liv.100 and the combination of anti-tubercular drugs.

GSSG, the oxidised product of GSH has been reported to accumulate due to the inactivation of glutathione reductase. GSSG inactivates many enzymes containing the 'SH' group and inhibits protein synthesis[24]. On concurrent treatment with Liv.100 to the cellular thiol status is not significantly altered. Earlier reports with Liv.52 showed significant enhancement in the GSH level in animals treated with it and also normalize radiation-induced alterations in GSH levels[25].

The decreased activities of SOD and CAT the primary antioxidant enzymes, observed in the anti-tubercular drugs treated rats may be due to the interaction of the accumulated free radicals with the associated metal ions or with the active amino acids of these enzymes[26].

During hepatotoxicity these enzymes are structurally and functionally impaired by free radicals resulting in liver damage. In Group IV rats the restoration of the activities of the antioxidant enzymes could be due to the ability of Liv.100 to scavenge reactive oxygen species within the lipid region of the membrane[5].

The results indicate that simultaneous treatment with Liv.100 in animals offers protection to the liver against anti-tubercular drugs induced toxicity.

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REFERENCES

1. Parthasarathy R, Raghupati Sarma G, Janardhanam B, Ramachandran P, Santha T, Sivasubramanian S, et al. Hepatic toxicity in South Indian patients during treatment of tuberculosis with short-course regimens containing iso niazid, rifampicin and pyrazinamide. *Tubercle* 1986;67:99-108.

2. Skakun NP SlivkaYun. The correction of the hepatotoxicity of antitubercular preparations with tocopherol acetate and riboxtn *Eksp Klin farmakol 1992;55:52-4.*

3. Skakun NP, Tabachuk OP. The comparative action of isoniazid, rifampicin and ethambutol on liver function. *Eksp Klin Farmakol 1992;55:45-7.*

4. Pandey S, Gujarati VR, Shanker K, Singh N, Dhawan KN. Hepato protective effect of Liv.52 against CCl_4 induced lipid peroxidation in livers rats. *Indian J Exp Biol 1994;32:674-5.*

5. Suja V, Latha SS, Shyamala Devi CS. Protective effect of Liv.52 and Liv.100, ayurvedic formulations on lipid peroxidation in rat liver homogenate - An *in vitro* study. *Indian J Exp Biol* 1997;35:50-2.

6. King J. Methods for determination of enzyme activity. In : Practical Clinical Enzymology. London. D Von Nostrand Co Ltd, 1965;106-15.

7. Moron MS, Bepierre JW, Mannerwick B. Levels of glutathione, glutathione reductase and glutathione-S-transferase in rat lung and liver. *Biochem Biophys Acta* 1979;582:3170-85.

8. Misra HP, Fridovich I. The role of superoxide anion in the auto oxidation of epinephrine and a simple assay for superoxide dismutase. *J Biol Chem* 1972;247:3170-85.

9. Beers RF, Sizer IW, A spectrophotometric method for measuring the breakdown of the hydrogen peroxide by catalase. *J Biol Chem* 1952;195:133-40,

10. Rotruck JT, Pope AL, Ganther H, Awanson AB, Hafeman DG, Hoeckstra WG. Selenium - biochemical role as a component of glutathione peroxidase. *Science* 1979; 179: 588-9.

11. Habig WH, Papst MJ, Jacoby WB. Glutathione-S-transferase the first enzymatic step in mercapturic acid formation. *J Biol Chem* 1974:249:7130-9.

12. Pinto RE, Bartley W. The effect of age and sex on glutathione reductase and glutathione peroxidase, activities on aerobic glutathione oxidation in rat liver homogenate. *Biochem J* 1969;112:109-15.

13. Bonting SL. Presence of enzyme system in mammalin tissues. In: Bilter EE, editor Membrane and Ion Transport.

London. Wiley Inter Science, 1970; 257-Q.

14. Okhawa H. Ohishi N,Yagi K. Reaction of linoleic acid hydroperoxides with thio-barbituric acids. *Anal Biochem* 1979;95:351-4.

15. Lowry OH, Rosebrough NJ, Farr A, Randall R. Protein determination with the folin reagent. *J Biol Chem* 1951;195:133-40.

16. Tappel AL. Measurement of and projection from *in vivo* lipid peroxidation. In: Pryor WA, edltior. Free radicals in Biology. New York. Academic Press, 1980; 147-9.

17. Zimmerman HJ, Seef LB. The functions and tests of the liver. In: Diagnostic enzymology. Philadelphia. Pergamon Press 1970: I-4.

18. Comporti M. Lipid peroxidation and cellular damage in toxic liver injury. *Lab Invest* 1985;5@3:599-623.

19. Gubdjarson S, Hallgrimson J, Skuladottir G. Properties of transport adenosine triphosphatases In: Peters H, Gresham GA, Paoetti R. Arterial Pollution. New York. Plenum Publishing Crop, 1983; 101-7.

20. Saxena A, Garg NK. Effect of Liv.52 on membrane lipids in carbon tetrachloride induced hepatotoxicity in rats. *Indian J Exp* Biol 1981;19:859-62.

21. Poliodoro G, Ilio CDI, Arduini A, Robere GLa, Federici G. Superoxide dismutase, reduced glutathione and TBA reactive products in erythrocytes of patients with multiple sclerosis. *Int J Biochem* 1984;16:505-10.

22. Crippin JS. Acetaminophen hepatotoxicity: potentiation by isoniazid. *Am J Gastroenterol 1993;88:590-2.*

23. Kumar S, Gangulay NK, Kohli KK. Inhibition of cellular glutathione biosynthesis by rifampicin in *Mycobacterium smegmatis. Biochem Int* 1992;26:469-78.

24. Lil JL, Stantman FW, Lardy HA. Antioxidant enzyme systems in rat liver and skeletal muscle. *Arch Biochem Biophys 1988;263:150-60.*

25. Saini MR, Saini N. Liv.52 protection against radiation induced lesions in mammalian liver. *Radiobiologia, Radiotherapia 1985;26:379-84.*

26. Fee JA, Briggs RG. Studies on the reconstitution of bovine erythrocyte superoxlde dismutase. V Preparation and properties of derivatives in which both zinc and copper sites contain copper. *Biochem Biophys Acta 1975;400:439-50.*