

Hepatoprotective activity of hepatoplus on isoniazid and rifampicin induced hepatotoxicity in rats

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Abstract: Present study deals with the hepatoprotective activity of polyherbal formulation Hepatoplus (HP) as an oral supplement to the INH and RIF induced hepatitis in experimental rats. Rats treated with INH and RIF show abnormal liver function with significant increase in serum transaminases, bilirubin and clotting time (CT) and significant decrease in total protein and Albumin, which is brings to near normal levels by HP and LIV 52 treatments. Rats treated with INH and RIF suffer from oxidative stress in the hepatocytes, due to the decrease in Glutathione (GSH), Glutathione peroxidase (GPX), Catalase (CAT), Super oxide dismutase (SOD) and significant increase in Lipid Per oxidation (LPO). HP decreases the oxidative stress and protects the liver cells membrane from LPO. 85% of DNA damage (comet tail) seen with RIF and INH treatment is reduced to 34.1% on HP application. A decrease of hepatocytes mitochondrial dehydrogenase activity is observed in INH and RIF treatment is restored by HP supplementation. Hepatic apoptotic and CYP2E1 gene expressions were also studied, BAX, p53, Caspase 3 and CYP2E1 were significantly up regulated and Bcl2 was down- regulated in INH and RIF treated rats. Concomitant application of HP prevents the modulation of these gene expressions. It is concluded that high dose of HP (100mg/kg) supplemented along with INH and RIF effectively prevents the toxicity induced by INH and RIF, as effective as 100mg/kg of LIV52.

Keywords: Isoniazid, Rifampicin, LIV52, Comet and CYP2E1.

INTRODUCTION

Isoniazid (INH) and Rifampicin (RIF) two front line drugs were commonly used for the treatment of Tuberculosis, results in hepatic dysfunction (Vijaya Padma *et al.*, 1998). The incidence of hepatotoxicity was 2.6%, when INH and RIF are used in combination, whereas it was 1.6% for INH and 1.1% for RIF alone (Jiang *et al.*, 2009). The adverse effects of these two drugs may be due to their synergistic action (Yue *et al.*, 2004). INH is metabolized to Hydrazine (HYZ) and Acetyl hydrazine. Oxidation of these metabolites by cytochrome P450 (CYP 450), generates an electrophilic intermediate and free radicals, which covalently adduct the hepatic macromolecules resulting in acute liver failure (Tayal *et al.*, 2007). RIF is a powerful inducer of CYP2E1 (Thattakudian *et al.*, 2011) and activation of CYP2E1 leads to oxidative stress (Tarasankar *et al.*, 2012). Moreover hydrazine depletes the reserve sources of GSH in hepatocytes, which results in altered mitochondrial permeability and induce apoptosis. Oxidative stress in the hepatocytes results in apoptosis, is one of the attributing mechanism of liver dysfunction caused by INH and RIF (Chowdhury *et al.*, 2006).

The present study is focused on elucidation of the role of Hepatoplus an amber based herbal formulation on oxidative stress, DNA damage and mitochondrial dysfunction in liver, associated with INH and RIF induced toxicity.

MATERIAL AND METHODS

Chemicals and drugs

Rifampicin and Isoniazid were procured from sigma chemicals and all other chemicals used for the study were of analytical grade.

Hepatoplus

Hepatoplus a licensed ayurvedic polyherbal formulation drug, procured from Care and Cure Herbs (Pvt.) Ltd, Chennai, India, in the form of capsule Each capsule has the following composition: *PhyllanthusAmarus* 100mg, *Eclipta Alba* 50mg, *Tephrosia Purpurea* 30mg, *Curcuma Longa* 30mg, *Picrorhiza Kurooa* 20mg, *Withania Somnifera* 100mg, *Pinus Succinifera* (Amber) 37.50mg, *Pistacia Lentiscus* 25mg, *Orchis Masculata* 25mg and *Cycas Circinalis* 62.50mg.

LIV 52

LIV 52 introduced in 1955, is an herbal tablet for liver protection from Himalayan Company India. Since then it is recognized worldwide by health professionals. In India it is a standard drug prescribed by the medical fraternity to treat any liver related diseases. Each tablet contains. Capparis Spinosa 32mg, Cichorium Intybus 32mg, Mandur Bhasma 32mg, Solanum Nigrum 32mg, Terminalia Arjuna 32mg, Cassia Occidentalis 16mg, Achillea Millefolium 16mg, Tamarix Gallica 16mg.

Animals

Male Sprague Dawely rats, weighing 150 to 200g obtained from Sri Ramachandra Medical College,

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Chennai, India, were used for this study, after getting the approval from Institutional Animal Ethics Committee, New Delhi, India. Animals (IACE/ XXII/SRU/168/2011) were housed individually in with well-ventilated polypropylene cages. A 12-h light/12-h dark artificial photoperiod, 22°C ($\pm 3^\circ$) room temperature and 50–70% relative humidity were maintained in the room. Animals had free access to pelleted feed (Nutrilab rodent, Tetragon Chemie Pvt Ltd., India) and reverse osmosis purified water (Rios, USA).

Experimental study protocol: Animals used in the present study were divided in to six, groups of 6 rats each. All the drugs per kg of body weight, were orally administered daily once for a period of 30 days.

Group I Rats receiving carboxyl methyl cellulose (vehicle), served as control. Group II Rats treated with 50 mg/kg of each RIF and INH (Positive control) (Sangameswaran *et al.*, 2012). Group III Rats supplemented 100 mg/kg of LIV 52 along with 50 mg /kg of each RIF and INH (Reference control). Group IV Rats co- administrated 50 mg/kg of Hepatoplus along with 50 mg /kg of each RIF and INH. (HP low dose). Group V rats supplemented 100 mg /kg of Hepatoplus along with 50 mg /kg of each RIF and INH (HP high dose) and Group VI Rats treated with Hepatoplus (HP control) alone 100mg/kg.

Antioxidant assay

After the end of the experimental period, the rats were decapitated under anaesthetic condition. Each liver sample was (blotted dry) placed in a previously weighed ice cold glass homogenizer containing phosphate buffer pH 7.5 .The weight of the sample was determined. After homogenization and centrifugation (3000 g for 10 mts) the supernatant was collected and used for the determination of following assay, CAT (Sinha., 1972) , LPO (Ohkawa *et al.* ,1979), SOD (Kakkar *et al.*, 1984), GPX (Rotruck *et al.*, 1973) and GSH (Moren *et al.*, 1979)

Serum biochemical parameter for Liver function

Serum biochemical parameters such as Alanine Transaminases (ALT), Asparate Transaminases (AST), Total protein (TP), Albumin, Totalbilirubin (TB) were assayed using kits supplied by Merck India Limited, Mumbai, India.

Clotting time

Clotting time determined by capillary tube method (McGowen *et al.*, 1955)

Assay of mitochondria dehydrogenase

Mitochondria were isolated by the method of (Takasawa *et al.*, 1993) and used for the determination of Isocitrate Dehydrogenase (ICD) (King, 1965) α -Ketoglutarate

Dehydrogenase (α -KD) (Reed *et al.*, 1969), Malate Dehydrogenase (MAD) (Mehler *et al.*, 1948), and NADH Dehydrogenase (Minakami *et al.*, 1962).

Gene expression studies

Reverse transcriptase (RT) - PCR (Genet bio kit) was performed to determine the level of mRNA expression. Briefly, 50mg of tissue was homogenized with 500 μ l TRIzol (sigma USA) and the tubes were incubated for 10min and centrifuged at 1000 rpm for 5min. 200 μ l of chloroform was added to the supernatant, allowed to incubate for 5min at room temperature and centrifuged at 12000rcf for 20min. Then 500 μ l of isopropyl alcohol was added to the supernatant to precipitate the total RNA and centrifuged at 12000rcf for 15min following the incubation period of 10min. The supernatant was decanted carefully; the pellet was washed three times with 75% ethanol, centrifuged at 12000 rcf for 15min and the pellet was allowed to air dry. The pellet was resuspended in 20 μ l of RNase free water and stored in -80°C until further use. The isolated RNA was allowed to undergo reverse transcription and polymerization reaction to get cDNA using PCR master cycler gradient. PCR products were electrophoresed at 80V for 30min using 1.0% agarose gel with ethidium bromide and quantified (Bio rad software) using gel documentation unit, Vilber Loumart, France. The forward and reverse primer used for this experiment is summarized in table 1 The following sequence was performed for each PCR reaction: 42°C for 30s, 94°C for 5min (1 cycle); 94°C for 1min, β -actin (55.4°C), Bax (58.8°C), Bcl-2 (56.7°C) and 72°C for 1 min (with 35 cycles); and a final extension phase at 72°C for 5 min.

Comet assay

Comet assay was performed as per Dhawan *et al.*, 2002, with minor modifications. Briefly, a small piece of a tissue was placed in 1 ml of cold HBSS containing 20 mM EDTA in 10% DMSO and tissue was minced into fine pieces, allowed to settle, removed and mixed 5-10 μ l of this with 75 μ l of low melting point agarose. This was poured onto the base slide, which is coated with normal melting point agarose and low melting point agarose. The slides were placed in lysing solution and subsequently microgel slides were electrophoresed for 30 minutes at 30v. The slides were stained with ethidium bromide and visualized under microscope.

STATISTICAL ANALYSIS

All the data were expressed as Mean $\pm$ SEM. Statistical significance of data was analysed by one-way analysis of variance (ANOVA) followed by Turkey –Kramer multiple comparison test, using Graph pad prism software package for windows (Version 5). P values <0.05 are considered as significant.

RESULTS

Liver function test

Assay of serum biochemical markers for liver function test using serum were carried out in order to study the efficacy of HP formulation as supplementary agent for INH and RIF. Results are shown in table 2. Rats treated with INH and RIF show significant increases of ($P \leq 0.001$) serum AST, ALT and bilirubin, sign of liver injury. HP Supplement to the rats (Groups IV and V) shows significant decrease of serum transaminases and bilirubin in a dose dependent manner. Rats treated with LIV 52 in-group III also revealed liver protective action by decreasing AST, ALT and bilirubin levels. Significant decrease of serum Total Protein ($P \leq 0.001$) and Albumin ($P \leq 0.05$) level in-group II, indicate impaired of liver function. HP or LIV52 supplementation improves the liver function by promoting the synthesis of protein, thus maintaining normal total protein and albumin levels.

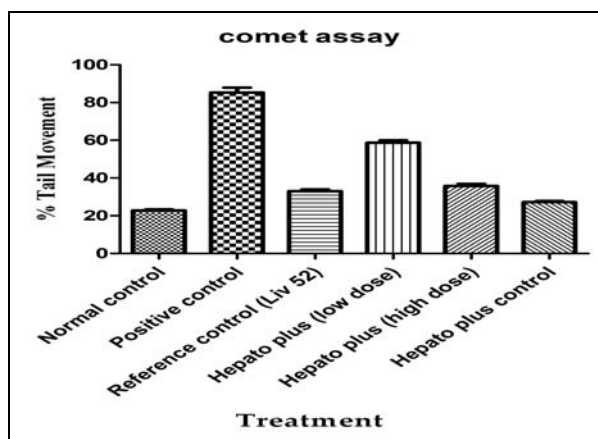


Fig. 1: Effect of Hepatoplus on DNA damage (Comet assay)

DNA Damage: Normal control, 22%, Positive control (ISO+RIF) 85.27%, Reference control (LIV52) 33%, HP (low dose) 58.67%, HP (high dose) 34.1% and HP control 27%.

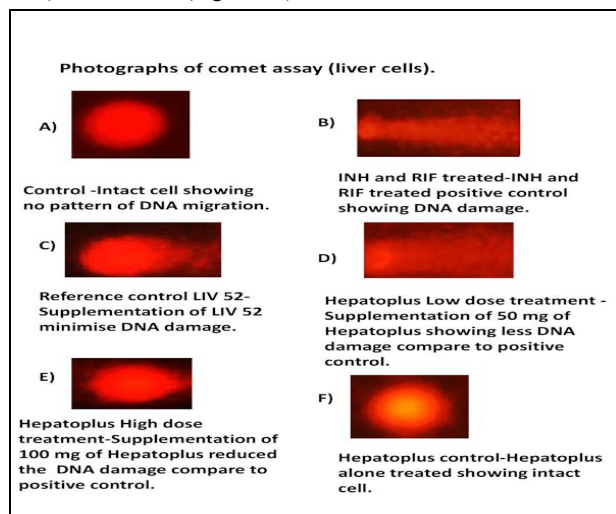


Fig. 1.1: Liver cells.

Effect of Hepatoplus on INH and RIF induced oxidative stress

In a trial to evaluate the ability of HP to protect the liver from oxidative stress induced by INH and RIF, the data obtained are presented in a table 3. Rats treated with INH and RIF in-group II show significant reduction of hepatic GSH ($P \leq 0.05$), when compared to control, which was retained at near normal levels in group V. The increase in hepatic GSH was better in HP group V, when compared to LIV 52 treated rats in Group III. INH and RIF treated rats show 51% reduction of liver GPX (Group II ($P \leq 0.001$), where as rats treated with HP in group IV and V show significant increase (70% and 89%) in rat liver GPX in dose dependent manner, when compared to Group II. LPO is a marker for oxidative stress. Rats treated with INH and RIF show significant increase of LPO in rats in-group II ($P \leq 0.001$). Co administration of HP 50mg /kg to rats group IV ($P \leq 0.01$) did not exhibit remarkable change. But HP at 100mg/kg dosage on Group V yields better results when compared to LIV52 administration (Group III). CAT and SOD form the mutual antioxidant system. Rats in Group II show significant reduction of both hepatic CAT ($P \leq 0.05$) and SOD ($P \leq 0.001$), when compared to normal rats (Group I). Administration of 100mg HP to rats in Group V shows significant increase, when compared to Group II.

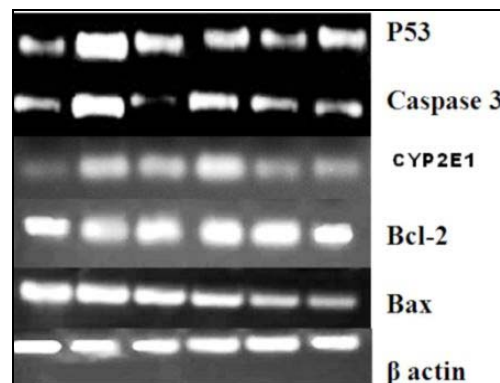


Fig. 2: Effect of Hepatoplus on CYP2E1 and Apoptotic m RNA expression in rat liver.

Lane1 – Normal control, Lane 2- Positive control, Lane 3- Reference control (Liv 52), Lane 4- Hepato plus (low dose), Lane 5- Hepato plus (high dose), Lane 6- Hepato plus control.

Effect of Hepatoplus on rat mitochondrial enzymes.

Effect of HP on rat liver mitochondrial enzymes treated with INH and RIF is shown in table. 4 Rats treated with INH and RIF (group II) show reduction of mitochondrial α -KD ($P \leq 0.05$), MAD ($P \leq 0.01$), ICD and NADH dehydrogenase activities, which remained normal on HP supplemented groups. HP administration results are on par with LIV52 the standard drug results in Group III.

Effect of Hepatoplus on DNA damage (Comet Assay).

Fig. 1.1 shows the Effect of HP on INH and RIF induced DNA damage in rat liver .The relative percentage of comet tail movement (fig. 1) and the amount of DNA

damage was calculated using CASP lab software. Rats treated with INH and RIF in-group II show significant DNA damage (85.27%) when compared to control in-group I (22%). Administration of HP to rats shows significant reduction of DNA damage in rats in Group IV (58.67%) and Group V (34.1%). Administration of 100mg HP to rats provided results as effective as LIV 52(33%) result in-group III.

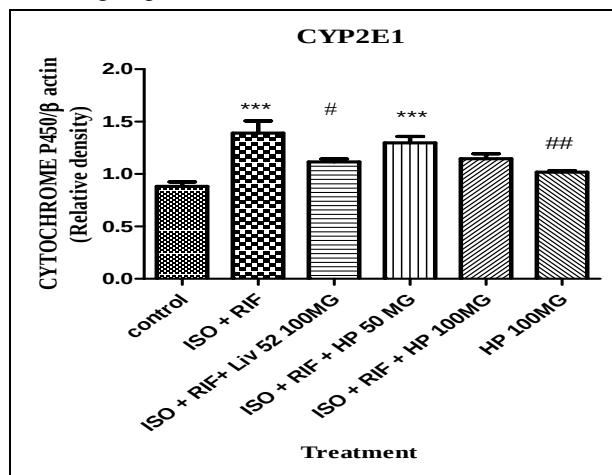


Fig. 3(a): Effect of Hepatoplus on Isoniazid and Rifampicin induced CYP2E1 mRNA expression in rat liver.

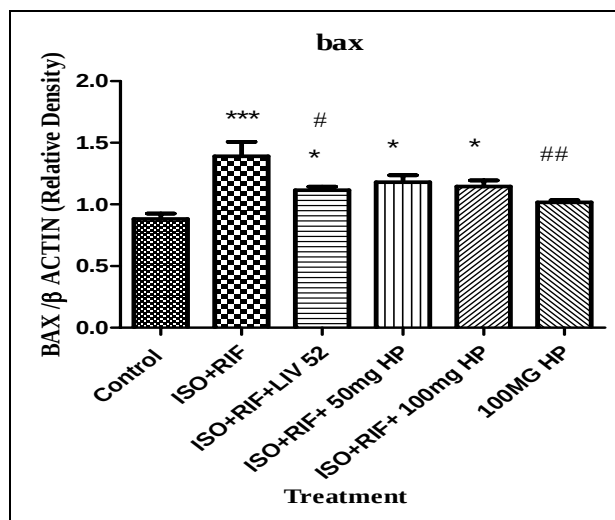


Fig. 3(b): Effect of Hepatoplus on Isoniazid and Rifampicin induced BAX mRNA expression in rat liver.

All values expressed as mean $\pm$ SEM where N=6. If P values is ≤ 0.05 * and ≤ 0.001 ***, significantly different when compared to control group I. If P values is < 0.05 # and < 0.01 ## statistically significant, when compared to Group II.

Hepatic CYP2E1 and apoptosis mRNA expression

The effect of HP on mRNA of CYP2E1 and other proapoptotic and antiapoptotic (Bcl2, BAX Caspase 3 & p53) genes are shown in fig. 2. The semi quantitative analysis of hepatic CYP2E1 gene expression is shown in fig. 3(a). Rats in Group II show significant ($P \leq 0.001$) up

regulation of CYP2E1. Administration of HP to rats in-group V ($P \leq 0.05$) shows significant decrease of CYP2E1. Whereas in-group IV not exhibit remarkable change. In LIV52 treated rats CYP2E1 gene expression is significantly reduced. Semi quantitative analysis of hepatic proapoptosis BAX gene expression was significantly upregulated in rats, treated with INH and RIF in-group II ($P \leq 0.001$) is shown in fig. 3. (b). Supplementation of HP to rats in-group IV and V shows down regulation of BAX ($P \leq 0.05$) gene expression. Liver Bcl-2 ($P \leq 0.01$) is significantly down regulated in group II rats (fig. 3. c). HP and LIV52 treatment protect the liver by up regulating Bcl-2 expression. Semi quantitative analysis of hepatic apoptotic gene p53 and caspases 3 gene expressions is shown in fig. 3. (d) and (e) respectively. Rats treated with INH and RIF show significant up regulation of p53 ($P \leq 0.001$) and caspases 3 ($P \leq 0.001$). Whereas, administration of HP prevents apoptosis in the liver cells by down regulating p53 and caspase 3 gene expressions.

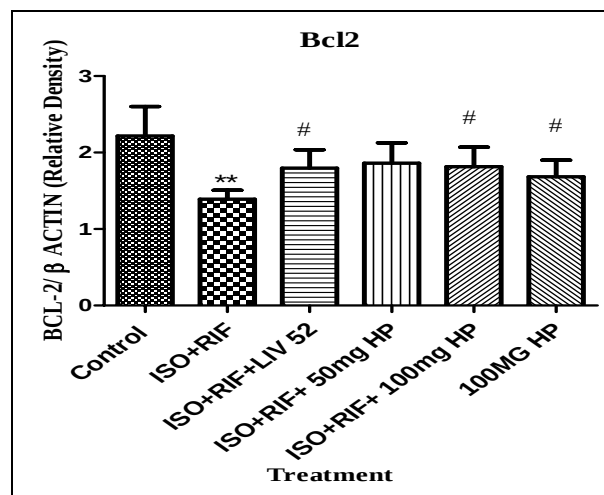


Fig. 3(c): Effect of Hepatoplus on Isoniazid and Rifampicin on Bcl-2 mRNA expression in rat liver.

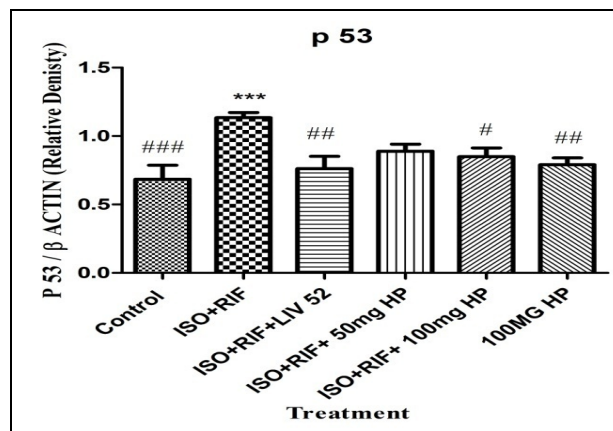


Fig. 3(d): Effect of Hepatoplus on Isoniazid and Rifampicin induced p53 mRNA expression in rat liver.

Table 1: Primer sequences

Gene Marker	Forward primer	Reverse primer
Bcl-2	5'- CGGGAGATCGTGATGAAGT-3'	5'- CCACCGAACTCAAAGAAGG-3'
Bax	5'-GAGTGTCTCCGCGCAATTG-3'	5'-TGGTGAGCGAGGCGGTGAG-3'
Caspase 3	5'-AATTCAAGGGACGGGTCATG-3'	5'-GCTTGTGCGCGTACAGTTTC-3'
CYP2E1	5'-GGA TGT GAC TGA CTG TCT CC -3'	5'TGG GGT AGG TTG GAA GGG AC-3' (Czekaj et al., 2006)
P53	5'- CCCACCATGAGCGTTGCT-3'	5'- CCACCCGGATAAGATGTTGG-3'
β -actin	5'-GACATGGAGAAAATCTGGCA-3'	5'-AATGTCACGCACGATTTC-3'

Table 2: Effect of Hepatoplus and LIV 52 on Serum Biochemical parameter for liver function

	Control	ISO + RIF	ISO + RIF + LIV 52	ISO + RIF + HP 50 Mg	ISO + RIF + HP 100 Mg	HP 100Mg
AST (U/L)	66.51 $\pm$ 3.72	145.0 12.64***	81.19 $\pm$ 5.26###	103.0 $\pm$ 11.55#	77.88 $\pm$ 10.94###	68.48 $\pm$ 5.18###
ALT (U/L)	41.39 $\pm$ 2.81	77.85 $\pm$ 4.83***	46.32 $\pm$ 1.93###	48.66 3.13###	45.67 $\pm$ 1.52###	38.76 $\pm$ 2.52###
TP mg/dl	6.57 $\pm$ 0.22	4.14 $\pm$ 0.22***	6.79 $\pm$ 0.03###	5.73 $\pm$ 0.44#	6.59 $\pm$ 0.24###	6.25 $\pm$ 0.14##
Albumin mg/dl	2.48 $\pm$ 0.13	1.75 $\pm$ 0.18*	2.40 $\pm$ 0.11#	2.38 $\pm$ 0.06 #	2.57 $\pm$ 0.08 ##	2.48 $\pm$ 0.10#
Bilirubin mg/dl	0.36 $\pm$ 0.082	0.85 $\pm$ 0.10***	0.40 $\pm$ 0.04##	0.46 $\pm$ 0.04#	0.40 $\pm$ 0.06##	0.31 $\pm$ 0.06###
CT /Sec	81.33 $\pm$ 4.48	132.20 $\pm$ 4.28***	83.50 $\pm$ 3.22###	94.83 $\pm$ 2.97###	85.50 $\pm$ 2.40###	82.67 $\pm$ 3.16###

Table 3: Effects of Hepatoplus and LIV52 on rat Liver antioxidant profile

	Control (Group I)	ISO + RIF (Group II)	ISO + RIF + LIV 52 (Group III)	ISO + RIF + HP 50 Mg (Group IV)	ISO + RIF + HP 100 Mg (Group V)	HP 100Mg (Group VI)
GSH (Mg/g Tissue)	2.46 $\pm$ 0.19	1.46 $\pm$ 0.17 *	1.64 $\pm$ 0.14	1.50 $\pm$ 0.12*	2.28 $\pm$ 0.22	2.29 $\pm$ 0.31#
GPX (mcg of GSH cons/ mt/ mg ptn)	259 $\pm$ 11.00	133.70 $\pm$ 9.46 ***	216.3 $\pm$ 20.32 ##	182.7 $\pm$ 19.29 **	223.0 $\pm$ 7.966 ##	247.90 $\pm$ 9.60 ###
LPO (mcg/g)	12.62 $\pm$ 0.25	36.07 $\pm$ 1.25 ***	16.04 $\pm$ 1.19###	19.17 $\pm$ 1.38 **###	15.68 $\pm$ 0.97 ###	12.86 $\pm$ 1.22 ###
CAT (mm of H ₂ O ₂ cons/mt/mg ptn)	0.70 $\pm$ 0.04	0.39 $\pm$ 0.04*	0.63 $\pm$ 0.08 #	0.42 $\pm$ 0.02*	0.64 $\pm$ 0.08	0.70 $\pm$ 0.05 #
SOD (U/mt/mg ptn)	17.08 $\pm$ 1.47	4.332 $\pm$ 0.77 ***	13.46 $\pm$ 0.97 ###	11.55 $\pm$ 1.48*##	15.79 $\pm$ 1.05 ###	17.37 $\pm$ 0.90###

Table 4: Effect of Hepatoplus and LIV 52 on liver mitochondrial dehydrogenase activity

	Control (Group I)	ISO + RIF (Group II)	ISO + RIF + LIV 52 (Group III)	ISO + RIF + HP 50 Mg (Group IV)	ISO + RIF + HP 100 Mg (Group V)	HP 100Mg (Group VI)
ICD mcg of α kg liberated/mt/mg	1.01 $\pm$ 0.05	0.53 $\pm$ 0.06	0.79 $\pm$ 0.07	0.70 $\pm$ 0.16	0.80 $\pm$ 0.15	1.02 $\pm$ 0.11#
α -KD mcg PF formed/mt/mg ptn	10.08 $\pm$ 0.62	6.60 $\pm$ 0.58*	9.60 $\pm$ 0.92#	8.49 $\pm$ 1.26	9.24 $\pm$ 0.46	9.90 $\pm$ 0.52
NADH dehydrogenase nm NADH Decomposed /mt/mg ptn	0.47 $\pm$ 0.02	0.27 $\pm$ 0.03	0.41 $\pm$ 0.05	0.39 $\pm$ 0.05	0.47 $\pm$ 0.08	0.46 $\pm$ 0.03
MAD nm NADH decompesd/mt/mg ptn	5.20 $\pm$ 0.40	2.60 $\pm$ 0.14**	5.11 $\pm$ 0.09#	3.97 $\pm$ 0.35	5.77 $\pm$ 0.83##	5.28 $\pm$ 0.65 ##

All values expressed in form of mean $\pm$ SEM were N=6. If P values is $\leq$ 0.05 * and $\leq$ 0.001*** significantly different when compared to control group I. If P values is $<$ 0.05[#], $<$ 0.01^{##}, $<$ 0.001^{###} statistically significant when compared to Group II.

All values are as mean $\pm$ SEM where N=6. If P values is $\leq$ 0.05*, values is $<$ 0.01^{##}, $<$ 0.001^{###} statistically significant, when compared to Group II.

DISCUSSION

Tuberculosis is a major health problem. Currently INH and RIF are the two major chemotherapeutic agents used for the treatment, associated with hepatotoxicity (Satya *et al.*, 2006). It is well documented that both INH and RIF generate lipid peroxides, inducing the formation of MDA and develop oxidative insult in the cell. As a result, integrity of liver cell membrane is lost and liver is damaged (Vishal *et al.*, 2010). Aberrant index of ALT and AST in systemic circulation is a clear sign of hepatocellular injury (Sude *et al.*, 2008). In the present investigation HP reduces the formation of lipid peroxide and maintains liver cell integrity. Thus reducing the ALT and AST level in the serum. During hepatic cellular damage, the liver cell is unable to excrete bilirubin; hence bilirubin piles up in the serum (Anita singh *et al.*, 2011). Additive usage of HP prevents the liver cell damage and retains the normal bilirubin excretion. Total protein and albumin levels are indicators of liver function, since majority of plasma proteins and albumin are synthesized in the liver (Thapa and Walia 2007). Abatement of serum total protein and albumin level indicate alarming liver damage in rats treated with INH and RIF. HP supplement restores the normal function of liver by increasing the total protein and albumin levels. Clotting time is used to demarcate normal and deviant liver, since liver is synthesizing clotting factors. During liver damage, clotting factors are not synthesized properly, hence clotting time is prolonged (Jody 2005). In the present study HP treatment brings down the liver toxicity and sustains normal clotting time.

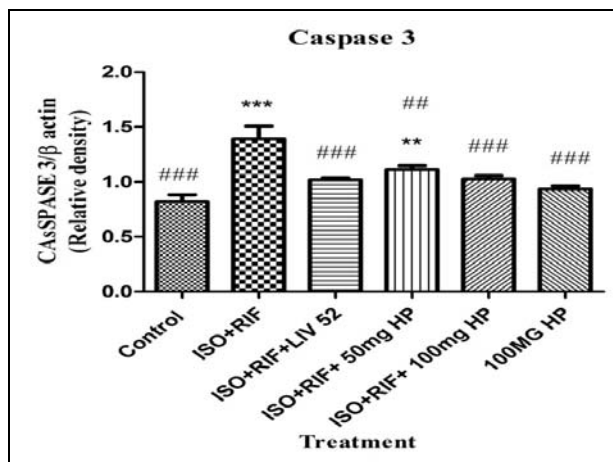


Fig. 3(e): Effect of Hepatoplus on Isoniazid and Rifampicin induced Caspase 3 mRNA expression in rat liver.

All values are as mean $\pm$ SEM where N=6. If P values is ≤ 0.05 *, significantly different when compared to control group I. If P values is < 0.01 ##, < 0.001 ### statistically significant, when compared to Group II.

Oxidative stress due to reactive metabolites of INH and RIF result in liver dysfunction (Jeyakumar *et al.*, 2009).

HYZ, the principal metabolite of INH is highly reactive with sulfhydryl group, which results in depletion of GSH in liver cell and induces oxidative stress to the hepatic cell (Wessam *et al.*, 2013). Supplementation of HP restricts the sulfhydryl groups in the hepatic cell and maintain GSH content. Decreased levels of GSH reduce hepatic GPX activity (Sodhi *et al.*, 1997). In our study HP protects the liver from oxidative damage by increasing GPX activity. More over elevation of MDA (malonaldehyde), which is a clear manifestation of excessive free radical formation and LPO (Zeinab *et al.*, 2012). LPO leading to formation of free radicals and result in oxidative insult (Viswanthaswamy *et al.*, 2010). It's found that HP as an adjuvant effectively prevents the formation of excessive free radicals and reduced MDA formation in the liver, thus decreasing the LPO in rats. CAT and SOD are mutually supporting front line antioxidant defence enzymes (Hussain *et al.*, 2012). Excessive formation of superoxide anion, inactivates SOD, which in turns leads to inactivation of CAT (Kumar *et al.*, 2010). In this study supplementation of HP decreases the formation superoxide anion, restoring CAT and SOD antioxidant potential in liver.

Administration of INH and RIF results in mitochondrial dysfunction and ROS production (Chowdhury *et al.*, 2006). Increased ROS production inhibits the activities of TCA cycle enzymes and NADH dehydrogenase, leading to inefficient electron transport and oxidative damage to mitochondria. Inhibition of these enzymes may hamper mitochondrial substrate oxidation, which results in decrease in reducing equivalents, leading to depletion of energy production (Sudheesh *et al.*, 2009). Earlier studies also indicate that administration of INH and RIF decreases the activity of specific dehydrogenases (Prabakaran *et al.*, 2000). HP adjuvant effectively prevents TCA cycle enzymes and NADH dehydrogenase in the liver cells in rats exposed to INH and RIF, by inhibiting ROS production. Hepatic CYP2E1 is responsible for the production of hepatic toxins, from isoniazid. CYP2E1 generates free radicals independent of substrate ligand, which causes lipid peroxidation and DNA strands breakage. Rate of Liver damage is proportional to the increase in CYP2E1 gene expression (Yue *et al.*, 2009; Ravi *et al.*, 2010). Concomitant application of HP prevents the formation free radicals by inhibiting CYP2E1 gene expression.

DNA is another major target for Anti TB drug, by inducing oxidative stress and inhibiting DNA repair processing, thus it results in DNA damage (Sangameswaran *et al.*, 2012). Comet assay, is a sensitive tool to analyse DNA damage by chemical and physical agents (El-Hussein *et al.*, 2004). In the present study HP prevented the DNA damage induced by INH and RIF by reversing the inhibition of DNA repairing process and maintaining antioxidant status in the liver.

Accumulation of DNA damage (Sunita *et al.*, 2008), oxidative stress and mitochondrial dysfunction is associated with apoptosis (Labbe *et al.*, 2008). During oxidative stress and DNA damage, cell produces intracellular signals to activate mitochondria mediated apoptosis. Under normal physiological conditions outer membrane of mitochondria display bcl-2 molecules that inhibit apoptosis. When cells are under stress conditions Bax a proapoptotic molecule migrates to mitochondria, inhibiting the protective effect of bcl-2 (Kaszuba *et al.*, 2012). In the present observation administration of INH and RIF induce mitochondria mediated apoptosis by decreasing the expression of Bcl-2 gene, correlates with earlier studies (Chen *et al.*, 2011). Concurrent application of HP to rats prevents mitochondria mediated apoptosis by restoring the protective role of bcl-2 in the liver cells. DNA damage or cells under stress conditions activate p^{53} . Activated p^{53} translocates from the nucleus to the cytoplasm. Then it modulates the action of bcl2 and bax on the mitochondrial membrane. This results in activation of effector caspase that induce cell death (Saqib *et al.*, 2012). It's observed that additive HP effectively prevents the cell from stress condition and inhibits effect of INH and RIF on p^{53} . Activation of caspase 3 is a crucial downstream event in apoptosis (Wang *et al.*, 2010). In the present study HP supplement prevents cells from apoptosis by down regulating caspase 3 expression. The hepato protective effect of HP may be due to the presence of active ingredient like Phyllanthin, hypophyllanthin, ambrin and Withaferin etc. in the formulation.

CONCLUSION

Concurrent application of Hepatoplus in a dose dependent manner is effectively protects the liver from oxidative stress, DNA damage, mitochondrial dysfunction and apoptosis induced by INH and RIF, as same that of standard drug LIV 52.

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