Pharmacological Screening Of Polyherbal Formulation For Hepatoprotective Effect Against Anti Tuberculosis Drugs Induced Hepatotoxicity On Albino Rats

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Abstract:
The present study was designed to evaluate hepatoprotective activity of poly herbal formulation(PHF)(*Andrographis paniculata,Boerhaavia diffusa, Nigella sativa* and *Punica granatum*)on Wistar albino rats using Anti Tuberculosis drugs induced hepatic damaged experimental animals. Isoniazid and Rifampicin-induced was administered a 100 and 50 mg/kg bw dose to inducehepatotoxicity, PHF 200 and 300 mg/kg, p.o, and liv-52 syrup 5ml/kg, p.o.were administered once daily for 28 days. The degree of hepatoprotection was measured liver function serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), Serum alkaline phosphate and Total protein. Oxidative enzymes MDA,GSH,SOD and CATand histopathology of liver. Results obtained PHF significantly P decreases the SGOT, SGPT ALP. The histopathological parameters of liver architecture like architecture of hepatic lobules, swelling of liver cell, fatty changes, focal necrosis, inflammatory cell infiltration around portal areas, kupffer cell hyperplasia etc.The result of this study strongly indicated that the PHF has got a hepatoprotective action against Isoniazid and Rifampicin induced hepatic damage in experimental animals.

Keywords: PHF, Isoniazid and Rifampicin and Liv-52

INTRODUCTION

The Liver is a key organ in the human body, regulating homeostasis and is a frequent target for a number of toxicants [1]. In spite of tremendous systematic advancement in the field of hepatology during recent years, liver problems are on the rise. Regrettably there are only a few drugs with serious side effects available for the treatment of liver ailments [2]. In view of the undesirable side effects of synthetic agents, there is growing focus towards the therapeutic evaluation of medicinal plants using systemic research methodology.

Tuberculosis (TB), an airborne infectious disease, is one of the major public health issues in the world, especially in developing countries. Approximately two million people die of TB and more than 10 million of new active cases are diagnosed every year [3]. Isoniazid (INH) and rifampicin (RIF) are first-line drugs for anti-TB chemotherapy. However, the hepatotoxicity caused by these drugs is a major concern for clinical treatment due to the long therapy duration and concurrent use of several medications[4]. RIF, as a potent inducer of CYP2E1, could aggravate INH-induced hepatotoxicity by increasing the production of toxic metabolites such as hydrazine, which results in a synergistic effect of INH-induced liver damage[5-6].

The whole plant of *Andrographis paniculata*, root of *Boerhaviadiffusa*, seeds of *Nigella sativa* and and Peel part of *Punica granatum* are prepared poly herbal formulation (PHF) The extract reports of these plants, we are developed herbal Formulations developed based on Ayurvedic principles where plants are included for antioxidant activity, hepatoprotactive activity, bio availability enhancement and specific activity in modulation of different liver disease conditions as many of these herbal ingredients are known to have liver modifying activity.

Recent clinical data revealed that *Andrographis paniculata, Boerhaavia diffusa, Nigella sativa* and *Punica granatum* (PHF) had been used in the treatment of liver injuries induced by an anti-TB drug, and the damaged liver function in patients was improved by PHF administration. However, the mechanisms of the protective effect of PHF against liver injury induced by the anti-TB drug are not clear at present. The purpose of this study was to investigate the effect of PHF on anti-TB drug-induced liver injury in rats.

2. MATERIALS AND METHODS

2.1 Plants collection and Preparation of plant extract

The whole plant of *Andrographis paniculata*, root of *Boerhaviadiffusa*, seeds of *Nigella sativa* and Peel part of *Punica*

granatum were collected from the local area of Hyderabad, All the plant materials was identified and authenticated by the department of Botany, Osmania University. The plants voucher specimen number: PARC/2013/2026-2030 was deposited in the center herbarium.
All the medicinal plants and plants part were subjected to surface sterilization using ethanol and then dried in shade. The all the dried plants was subjected to size reduction to a coarse powder by using dry grinder and passed through sieve (20 mesh). All the medicinal plant powdered samples (100 g) were defatted by treating with pet-ether and then extracted with ethanol by using soxhlet apparatus. The solvent was removed under vacuum to get the solid mass. The residue was weighed and stored in air and water proof containers, kept in refrigerator at 4 ^{o}C. From this stock, fresh preparation was made whenever required.

2.2 Preliminary phytochemical analysis :
All the different extracts were then subjected to preliminary phytochemical analysis to assess the presence of various phytoconstituents .

2.3 Preparation of PHF.
The extracts of the plants parts of *Andrographispaniculata,Boerhaaviadiffusa, Nigella sativa* and *Punica granatum* were employed to prepare formulation in different proportions as to prepare Herbal syrup formulation as given in table1.

S.no	Name of Ingredients	Ingredients % w/w
1	*Andrographispaniculata* extract	2 g
2	*Boerhaaviadiffusa* extract	1 g
3	*Nigella sativa* extract	3 g
4	*Punica granatum* extract	2g
5	Sorbitol	5.0 g
6	Sucrose	12 g
7	Carbox methyl cellulose (CMC)	2.0 g
8	Olive Oil	1 g
9	Distilled Water	100 ml

Table1: composition of Poly Herbal Syrup Formulation

2.4 Animals selections
Experimental Animals
Albino mice 20-25gm was purchased from Sanzyme lab Pvt. Animal feed was procured fromMahaveera enterprises, Hyderabad. Animals were accommodated in recommended laboratory environment at 25°C under 12 hr light-dark cycle. All the experimental animals had free access to chow and water *ad libitum*. The research protocol was approved by (HSKCP/IAEC, Clear/1/2020-12/R&D87)

2.5 Evaluation of Hepato Protective Activity:
Hepatic injury:
A dose of 50 mg/kg and 100 mg/kgb.w Isoniazid (INH) and Rifampicin (RIF)respectively in Aqueous 1% CMC through per oral route for 28days.

Albino rats weighing around 160-180 gm used, the study design is divided into 6 groups, six rats in each as describes in table 2.

Group No	No. of Rats	Groups	Treatment Dose
1	6	Control-Aqueous 1% CMC	10ml/kg b.w
2	6	Positive control – INH + RIF	50 mg/kg +100 mg/kg btw,
3	6	INH + RIF+ PHF	50 mg/kg +100 mg/kg btw+200mg/kg
4	6	INH + RIF+ PHF	50 mg/kg +100 mg/kg btw+3 00mg/kg
5	6	INH + RIF + *Liv-52*	50 mg/kg +100 mg/kg btw +5mlg/kg

Table 2: treatment of PHF on anti TB drugs induced liver injury

Group 2 animals received INH + RIF and Group 3 to 6 received as per protocol INH+ RIF, PHF and LIV-52 for 28 days of treatment. On 29th day all animals were sacrificed following by anesthesia, collected the blood and weight liver. Blood was withdrawn and their serum was separated by centrifugation at 3000 rpm at 30^{o}C for 15 min. This was subsequently analysed for various biochemical parameters including serum transaminases viz. SGOT,SGPT and total protein .[7-9]

2.6 In-vivo antioxidant activity

The *in-vivo* antioxidant activity of the PHF was carried out in Anti TB drugs intoxicated rats. The liver samples collected were washed with chilled normal saline, weighed and 10% (w/v) liver homogenate was generated in ice cold 0.15 M KCl solution using a motor driven Teflon pestle. The suspension was centrifuged at 2000 rpm at 4°C for 10 min and the clear supernatant was used for the estimation of the following antioxidant markers SOD, MDA,GSH and CAT. [7-10]

2.7 Histopathological Investigation:

The liver tissues were excised out, washed with the cold saline, fixed in 10% buffered formalin for 12 hours and processed and stained with hematoxylin and eosin dye for photomicroscopic observations[11].

2.8 Statistical Analysis:

The data analysed using statistical package of social version 17.0 (SPSS) software. Descriptive statistics used to present data in terms of mean± SEM employing ANOVA, followed by Tukey's Multiple Comparison Test post hoc test.Analyzed Data was by using Graph Pad Prism software (version 8.4.2 V; SanDiego, CA).Differences were considered significant whenever the P value are reported as mean ± Score data expressed in terms of mean ± S.E.M, n=6. (ANOVA) followed by Tukey Multiple Comparison Test,$P<0.001$ vs Normal group, $P<0.01$, $P<0.001$ vs group.

RESULTS:

3.1 Preliminary Phytochemical Screening:

The preliminary phytochemical studies were performed for testing different phytochemical constituents present in polyherbal formulation. The observations showed the presence of alkaloids, flavonoids, steroids, tannins and phenolics, which were found to be more in methanolic extract.

3.2 Effect of PHF on Liver Injury Induced by Anti-TB Drug

Anti-TB drug-induced liver injury was indicated by the elevation of serum SGOT,SGPT and ALP significantly increased, TP significantly decreased in rats. Treatment with PHF 200 and 300 significantly reduced the increase of serum SGOT, SGPT and ALP in a dose-dependent manner, TP significantly increase compare to control groups as shown in figure1

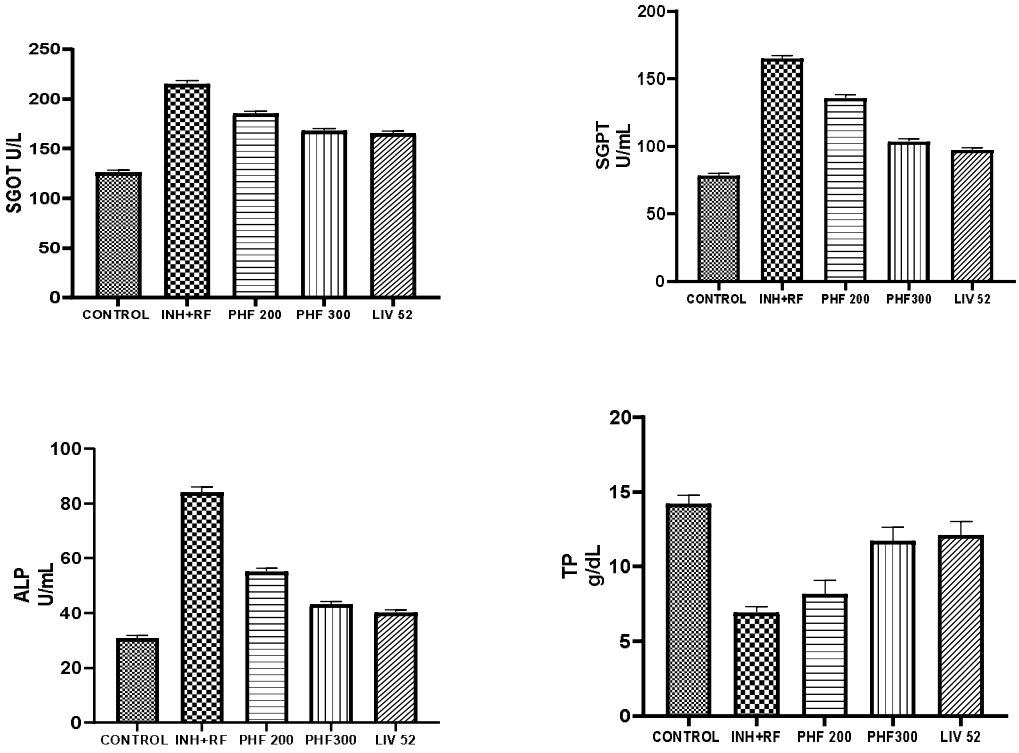

Figure: 1 showing effect of PHF on liver function on anti TB drugs induced

3.3 Effect of PHF on Oxidative Stress Induced by Anti-TB Drug

The liver MDA level, as a marker of lipid peroxidation, was significantly increased in rats treated with the anti-TB drug alone, while was simultaneously decreased. In addition, the activities of liver antioxidant enzymes including catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), were also reduced by treatment with the anti-TB drug in

rats. PHF treatment with 200 and 300 showed a significant inhibition of the formation of liver MDA and the depletion of liver glutathione (GSH) contents, as well as the reduction of antioxidant enzymes to the certain extents (figure 2).

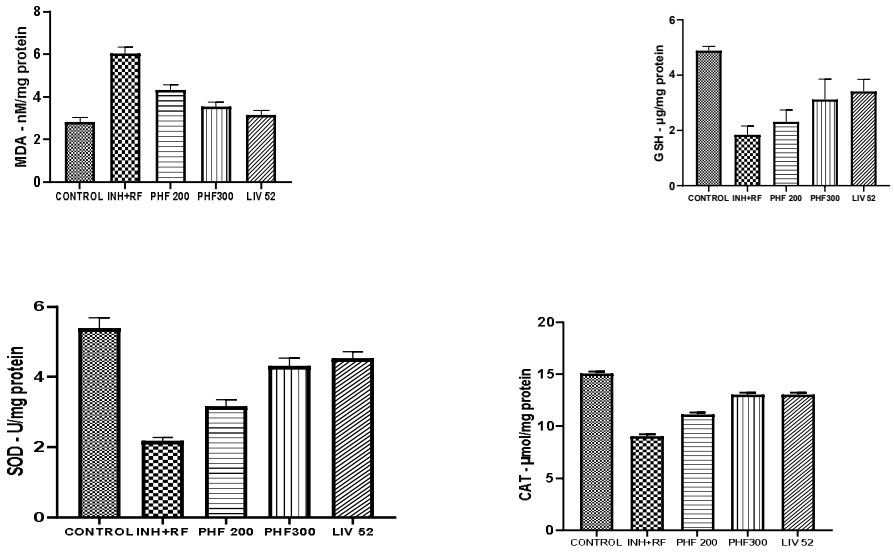

Figure 2: showing Effect of PHF on Oxidative Stress Induced by Anti-TB Drug induced

3.4 Histopathological Study of Liver

The histological profile of the hepatic tissue of the placebo control animals showed a normal lobular architecture. Normal hepatocytes were arranged in single cell cords radiating away froma central vein (A). Group II rats, treatedwith distilled water and Isoniazid and Rifampicin, showed disturbed liver architecture, exhibiting central lobular necrosis with tiny vacuoles, and fatty infiltrations (B).Group III and IV animals, pretreated with Liv 52 respectively, and subsequently treated with Isoniazid and Rifampicinretained normal hepatic tissue architecture, so received significant protection from Isoniazid and Rifampicin-induced hepatic damage (C and D). Group V animals, treated with Liv-52 syrup alone, did not show any significant hepatic tissue architectural changes (E) (Fig.3)

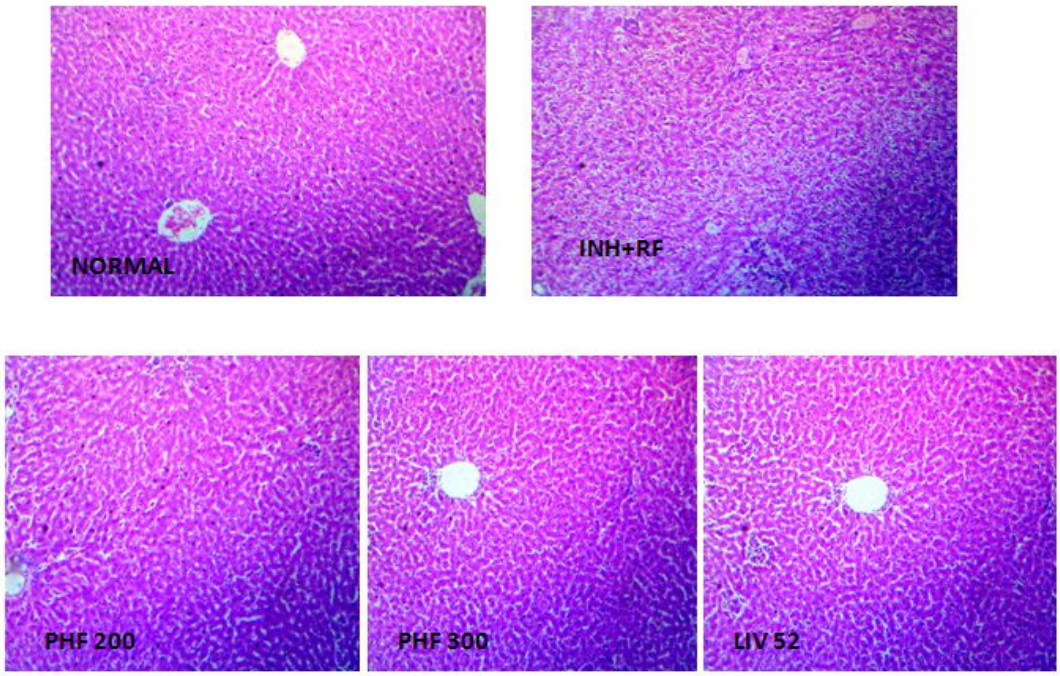

Figure 3: Effect of PHF on Histopathological of liver changes in albino rat.

4 DISCUSSION

The extraction of plant material and its analysis play a significant role in the quality control of plant material.[12]Crude ethanolic extract of the plant materials of Andrographis *paniculata,Boerhaavia diffusa, Nigella sativa* and *Punica granatum* contains both primary and sceondary phytoconstituents.

Hepatic injury has been severe concern during treatment of tuberculosis. Administration of isoniazid and rifampicin causes aberrations and dysfunction in liver as these drugs are bio transformed in liver. Thus, liver organs are affected when substance is metabolized and generates more toxic products like free radicals which leads to induction of oxidative stress, inhibition of antioxidant defense system, inflammation of cells give rise to condition like necrosis.[13] Involvement of oxidative stress in hepatotoxicity caused by combination of isoniazid and rifampicin has already been established. Previous researchers reported the role of oxidative stress in mechanism of isoniazid and rifampicin-induced hepatic inflammation.[14]

Thus, main focus of the present study was to explore a PHF effective therapeutic agent to reverse antituberculosis drug-induced hepatic injury. PHF contain *Andrographis paniculata,Boerhaavia diffusa, Nigella sativa* and *Punica granatum* it contain a flavonoid, steroids and poly phenols that has attracted attention in the last few decades due to its ability to curb oxidative stress under various pathological conditions. Liv 52 was used as standard drugs in respect to PHF as it has been used for centuries for treatment of Liver Disorders.

Lipid peroxidation is the crucial index of oxidative stress. The process involves free radical chain reaction mechanism resulting in cell damage, as free radicals steal electrons from lipids in cell membranes. Elevated level of lipid peroxides in the liver and kidney reflected the cellular damage. Although ATD itself does not generate free radicals directly but generates free radicals via metabolic pathway by producing reactive metabolites such as hydrazine and diacetylhydrazine; both of these metabolites lead to formation of free radicals and cause severe cellular injury.[15] PHF could prevent formation of free radicals by reducing production of reactive metabolites and exerted protective and antioxidant effects as reported in previous findings.[16-17] It may quench free radicals due to its contain 4-hydroxyl group in β-ring that possess electron donating properties and inhibits lipid peroxidation.[18] The GSH is a sulfhydryl peptide which plays essential role in cellular defense against toxicity. Exposure to ATD caused decline in GSH level which might increase the susceptibility of the liver free radical damage.[19] SOD is a metalloprotein that catalyze breakdown of superoxide anion into oxygen and hydrogen peroxide, whereas CAT is a hemeprotein which catalyze conversion of hydrogen peroxide to water and oxygen protects cell from oxidative damage by H_2O_2 and OH. Lower level of SOD and CAT indicated impairment of antioxidant defense system due to administration of ATD.[20-21] PHF restored antioxidant enzymes by scavenging free radical due to presence of its hydroxyl group and reduced hepato-renal damage. These observations were similar to other reports.[22]

5 CONCLUSION

Present findings demonstrated that PHF 200 and 300 dose potentially effective in reversing all the liver function, oxidative stress biochemical and histological in the liver. It can be suggested that PHF contain *Andrographis paniculata,Boerhaavia diffusa, Nigella sativa* and *Punica granatum* it contain a flavonoid, steroids and poly phenols may be useful to lessen or cure liver injury caused during anti TB drugs treatment regimen. However, further study on many other aspects of pharmacological action of PHF needs to be performed.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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