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Effect of Triphala on a Murine Model of Isoniazid and Rifampicin Induced Model of Hepatotoxicity.

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ABSTRACT

The aim of the study was to evaluate the effect of triphala on murine model of isoniazid and rifampicin induced hepatotoxicity. Thirty wistar rats of either sex, weighing 150-250 g were randomly divided into five groups of six animals each – Normal control, hepatotoxic control, Triphala 250mg group, Triphala 500mg group and standard control. Hepatotoxicity was induced in all groups except normal control, by administering isoniazid 100mg/kg body weight intraperitoneally and rifampicin 100mg/kg body weight orally, for 21 days. Triphala 250mg,Triphala 500mg and standard control groups were treated simultaneously with Triphala at doses 250mg/kg/500 mg/kg and Silymarin2.5mg/kg respectively for 21 days. The blood samples were collected by cardiac puncture under ether anesthesia, for biochemical estimation of liver enzymes, total proteins, albumin, total and direct bilirubin. Subsequently, the rats were sacrificed and the liver was dissected for histopathological evaluation. The hepatotoxic group showed significant increase in liver enzymes($P<0.001$) and total and direct bilirubin ($p<0.001$, $p=0.006$ respectively), compared to normal control. Triphala 250mg, Triphala 500mg and Silymarin groups showed statistically significant decrease in liver enzymes and total bilirubin and direct bilirubin compared to hepatotoxic control ($p<0.001$).There was no statistically significant difference in the total protein and albumin among groups. Histopathological evaluation of liver further conferred the hepatotprotective potential of Triphala. To conclude, Triphala at doses of 250mg/kg and 500mg/kg showed hepatoprotective effect in murine model of isoniazid and rifampicin induced hepatotoxicity.

Keywords: Triphala, hepatoprotective, Isoniazid, Rifampicin, murine model.

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INTRODUCTION

Tuberculosis is one of the potential communicable and highly infectious disease and a major health problem in a developing country like India. Over one-third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* and has proven to be fatal to over 2 million people a year [1].Multi-drug resistant strains of *M. tuberculosis*emerging as a co-infection with Aquired Immune Deficiency Syndrome (AIDS) has further escalated the sobriety of the disease. In tune with this the World Health Organization has also declared tuberculosis as a 'Global health emergency'[2].

Isoniazid and rifampicin, two principle drugs used in multidrug regimen treatment of tuberculosis is beset with the limitation of hepatotoxicity as a principle adverse effect. The incidence of hepatotoxicity further increases with the use of both drugs in combination rather than the use of a single drug [3].Moreover, the rate of hepatotoxicity has been reported to be more in the Indian population (8-30%) rather than in the western population [4].

Many herbal drugs as traditional remedies are being currently employed for the treatment of liver disorders [5].The escalation in the use of herbal remedies may be attributed to their effectiveness, minimal side effects in clinical experience and relatively low cost. Triphala is one of the important *rasayana*drugs, used since time immemorial and described in the Ayurveda as a "tridoshicrasayana" (Charaka, 2500 B C), having balancing and rejuvenating effects on the three constitutional elements that govern human life (*vata*, *pitta*, and *kapha)*[6].It is the most commonly used ayurvedic herbal formulation, comprising the fruits of three trees, Indian goose berry (*Emblicaofficinalis*Gaertn, family-Euphobiaceae), Bellericamyrobalan (*Terminaliabelerica* Linn, family-Combretaceae), Chebulicmyrobalan (*Terminaliachebula*Retzr, family-Combretaceae). It is credited with diverse beneficial properties such as anti-aging, anticancer, antibacterial, antiviral, cardioprotective, hepatoprotective, antistress, antidiabetic, antiparasitic, antidiarrheal and antianemic [7-12].

Triphala extract has been reported to be a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, B-sitosterol and flavonoids [13]. Its components are reported to possess anti-inflammatory, antimutagenic, antioxidant, cytoprotective andgastroprotective activity [14-15]. However, a literature search revealed no evidence of the effect of Triphala on isoniazid and rifampicin induced hepatotoxicity. Hence the present study was conducted to evaluate the effects of Triphala on a murine model of isoniazid and rifampicin induced hepatotoxicity.

MATERIALS AND METHODS

Experimental animals

Thirty Wistar rats of either sex, weighing 150-250 g, inbred at the Central Animal House, Kasturba Medical College, Mangalore were used for the study. These animals were randomly divided into five groups of six animals each. All the animals were maintained as per standard guidelines of CPCSEA: they were housed in clean polypropylene cages, three rats per cage, under 12 hour natural light and dark cycles at a temperature of 22 ± 2^{0}C and were given standard chow containing fat 4.15%, protein 22.15%, carbohydrate 4%(supplied by Amruth laboratory animal feed, manufactured by Pranav Agro industries ltd., Sangli) and water ad libitum. The animals were acclimatized to the study conditions for a week before beginning the experiment. The study was conducted after obtaining the permission from the institutional animal ethics committee.

Study Drugs

Isoniazid (S.D Fine Chemicals, Mumbai).Rifampicin (Sigma Aldrich Co.)Silymarin(Signova Co) and Triphala (Natural Remedies Pvt Ltd) were used in the study. Normal saline served as a control.

Study procedure

Induction of experimental hepatotoxicity

Isoniazid and rifampicin (100 mg/kg body weight) solution was prepared separately in sterile distilled water. Rats were treated with isoniazid and rifampicin for 21 days by intra-peritoneal route to induce

hepatotoxicity [16].The test drug Triphala was administered per oral at 250 mg/kg and 500 mg/kg body weight. Silymarin (2.5 mg/kg body weight per oral) was used as a standard drug in this study [16].The normal control animals were fed orally withnormal saline only.

The body weights of all the rats were recorded on day 1 and at the end of the experiment.One hour after administration of drugs on day 21, the blood samples were collected by cardiac puncture under ether anaesthesia, for biochemical estimation and then the rats were sacrificed by cervical dislocation and the liver was dissected for histopathological evaluation.The blood samples were centrifuged for 10 minutes at 3000 rpm to separate the serum for biochemical evaluation.

Enzyme assays

The activities of serum hepatic marker enzymes namely aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were assayed using standard kits from Lupin Laboratories and Pointe Scientific respectively. The results were expressed as units/litre (IU/L).

Protein estimation

The level of total protein was estimated using biuret method and albumin was estimated by bromocresol green method. Total and direct bilirubin levels were also estimated.

Histopathological evaluation

The tissues of liver were fixed in 10% formalin and embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope.

Statistical analysis

The results were expressed as mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA followed by post hoc Dunnett'stest using SPSS version 20.0. P value<0.05 was considered as significant.

RESULTS

Intraperitoneal administration of Isonizid and Rifampicin at a dose of 100 mg kg/ day over a period of 3 weeks produced hepatotoxicity as evidenced by biochemical and histopathological parameters.

Effect of Triphala on biochemical parameters:

The results of effects of Triphala extract on isoniazid and rifampicin intoxicated rats are shown in Table 1, 2 and 3. As shown in table no. 1, on day 21, administration of isoniazid and rifampicin at a dose of 100 mg/kg i.p. each to the hepatotoxic control group significantly ($P<0.001$) elevated liver enzymes i.e, Aspartate transaminase (AST), Alanine transaminase (ALT), and Alkaline phosphatase (ALP) compared to normal control group. Treatment with Triphala at both doses employed i.e.250 mg/kg body weight and 500mg/kg body weight showed significant decrease in AST, ALT, ALP compared to hepatotoxic group signifying hepatoprotective effect of triphala. The standard control group treated with silymarin also showed a similar significant ($p<0.001$), decrease in the liver enzymes. However, there was no statistically significant difference in the total protein and albumin in the hepatotoxic group and triphala and silymarin treated groups as shown in table 2.

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Table 1: Effect of Triphala on liver enzymes in isoniazid and rifampicin induced hepatotoxicity in rats

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Normal control	30.66 ±3.99	30.13±0.66	69.5±8.21
Hepatotoxic control	140.0±8.17*	205.66±11.67*	588.81±34.03*
Triphala 250 mg/kg	45.45±14.69#	47.9±17.02#	87.33±13.88#
Triphala 500mg/kg	49.83±16.95#	53.87±13.62#	87.5±11.77#
Standard control	39.7±8.87#	42.52±16.94#	83.5±4.63#

Results are expressed as mean ± standard deviation (n=6); * P<0.05 vs Normal control, #p<0.05 vs hepatotoxic group

Table 2: Effect of Triphala on total protein and albumin levels in isoniazid and rifampicin induced hepatotoxicity in rats

Group	Total protein grams/dl	Albumin grams/dl
Normal control	5.84± 5.8	4.28±0.68
Hepatotoxic control	4.79± 0.77	3.53±0.34
Triphala 250mg/kg	7.11±1.86	4.08±0.16
Triphala 500mg/kg	6.81± 1.41	4.04±0.16
Standard control	5.96±0.96	4.09±0.16

Results are expressed as mean ± standard deviation, * P<0.05 vs Normal control

Table 3: Table 1: Effect of Triphala on total bilirubin and direct bilirubin in isoniazid and rifampicin induced hepatotoxicity in rats

Group	Total bilirubin	Direct bilirubin
Normal control	0.32±02	0.04±0.09
Hepatotoxic control	1.26±0.5*	0.32±0.01*
Triphala 250mg/kg	0.63±0.18#	0.16±0.04#
Triphala 500mg/kg	0.70±18*#	0.17±0.45#
Standard control	0.61±0.17#	0.15±0.04#

Results are expressed as mean ± standard deviation mg/dl, * P<0.05 vs Normal control, #p<0.05 VS hepatotoxic group

As depicted in table no. 3, there was a significant increase in total and direct bilirubin in hepatotoxic group compared to normal control group with p<0.001, p=0.006 respectively. Triphala 250mg/kg body weight, triphala 500 mg/kg body weight and standard control group showed significant decrease in total bilirubin and direct bilirubin compared to hepatotoxic group {(p=0.002, p=0.002), (p=0.003, p=0.002), (p=0.001, p=0.001) respectively} signifying the hepatoprotective properties of triphala and standard therapy silymarin.

Histopathological Evaluation

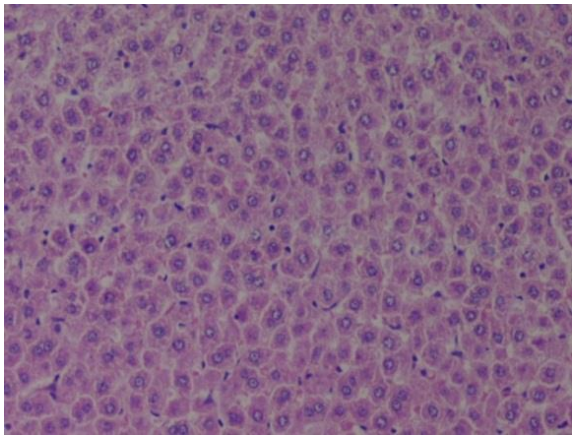

Figure 1: Normal rats (Group I) showed normal hepatocytes with well preserved cytoplasm with normal lobular structural design of the liver

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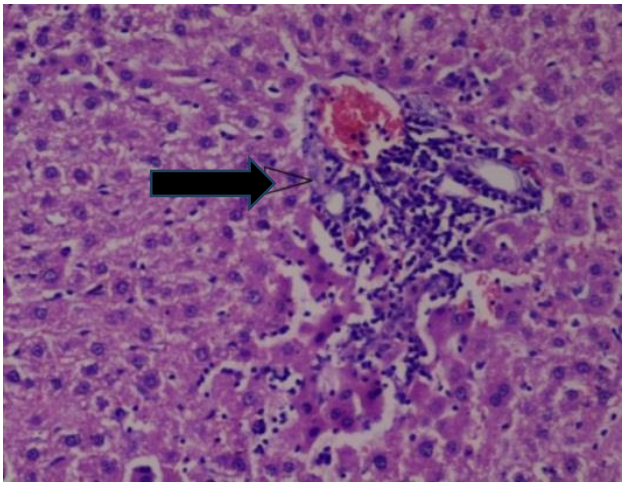

Figure 2: Hepatocytes of Isoniazid and Rifampicin treatedrats (Group 2) showing periportal dense inflammation, interphase hepatitis and sinusoidal inflammation

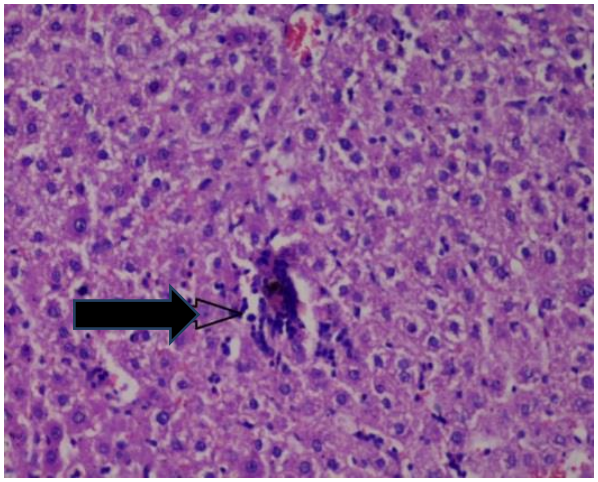

Figure 3: Hepatocytes of rats treated with Isoniazid and Rifampicin along with Triphala 250mg/kg (Group 3) showing less sinusoidal infiltration and focal cell death

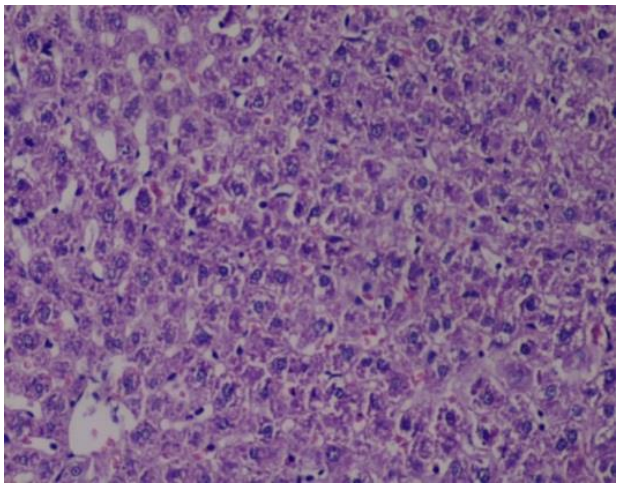

Figure 4: Hepatocytes of rats treated with Isoniazid and Rifampicin along with Triphala 500mg/kg (Group 4) showing minimal sinusoidal infiltration

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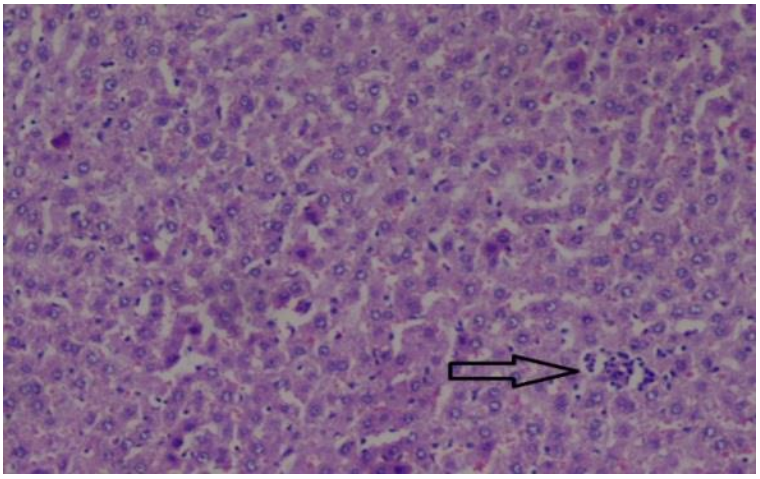

Figure 5: Liver sections of rats treated with Isoniazid and Rifampicin along with silymarin –positive control (Group 5) showing near normal liver morphology

Figure no 1 shows the histopathological features of the liver in the normal control group fed with normal saline. As evidenced the normal liver architecture was preserved in the saline treated group with no evidence of portal inflammation, balloon degeneration and hepatic necrosis whereas hepatocellular disintegration with periportal and sinusoidal inflammation and interphase hepatitis was observed in the hepatotoxic control group (Figure no 2). Treatment with Triphalaat both doses employed showed considerable hepatoprotection signified by minimal sinusoidal infiltration and focal cell death (Figure no 3 & 4). The silymarin treated group also showed significant hepatoprotection with only focal cell drop out and maintenance of normal liver architecture to a large extent.(figure no 5).

DISCUSSION

The administration of isoniazid and rifampicin the most common antitubercular medications, produces many changes in the liver morphology and metabolism as liver is the liver is the main detoxifying site for these antitubercular drugs. These antitubercular drugs induce hepatotoxicity by a multiple step mechanism. The exact mechanism responsible for liver injury caused by these drugs is not clear. Isoniazid is acetylated and then hydrolyzed, resulting in isonicotinic acid and monoacetylhydrazine; the later compound can be activated to a toxic species by cytochrome P-450 [17]. *In vitro* studies indicate that metabolic oxidation of acetylhydrazine leads to a reactive acylating species that binds covalently to microsomal protein. Acetylhydrazine and hydrazine acting as acetylating agents by binding covalently with liver cell macromolecules have been implicated in causing hepatocyte injury [18]. Rifampicin, a powerful inducer of mixed-function oxidase, increases the hepatotoxicity of isoniazid by enhancing the production of toxic metabolites from acetylhydrazine [19-20].A similar genetically determined acetyltransferase activity as is seen in humans has been reported in rats. The murine models have been deemed to be more sensitive to isoniazid-induced hepatotoxicity due to a high amidase activity, resulting in release of large amount of acetylhydrazine, which is a potential hepatotoxicant [21].

Free radical formation which stimulates lipid peroxidation and source for destruction and damage to the cell membrane is said to contribute to the oxidative damage induced byantitubercular drugs [22]. Alterations of various cellular defense mechanisms consisting of enzymatic and non-enzymatic components [reduced glutathione (GSH)] have been reported in Isoniazid and rifampicin-induced hepatoxicity [23].

Previous studies have concluded that phenolic acids, flavonoids and tannins are themost commonly found polyphenolic compounds in the plant extracts of Triphala. These polyphenols have been implicated in the antioxidant properties of Triphala which reduces oxidative stressby converting the reactive oxygen free radicals to non-reactive products [24]. Potentiation of the antioxidant defensemachinery of the host so as to guard the liver against theseoffending drugs could be an ideal approach to prevent hepatotoxicity caused by drugs.Triphaladue to its excellent antioxidant activity could be an important drug in our armamentarium to combat this free radical-induced hepatotoxic injury. In the present study, both biochemical and histopathological evaluation results conclude that Triphala at 250mg/kg and 500 mg/kg doses effectively

showed hepatoprotective effect in the murine model employed in the study. Further studies to isolate the active principles and studies to corroborate the hepatoprotective activity of these principles might shed more light on using Triphala as an effective hepatoprotective against antitubercular drug induced hepatotoxicity in humans.

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