EVALUATION OF EFFECT OF ETHANOLIC EXTRACT OF PHYLLANTHUS DEBILIS ON ANTITUBERCULAR DRUGS INDUCED HEPATOTOXICITY IN WISTAR RATS.

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ABSTRACT

Background: The whole plant of Phyllanthus debilis has been shown to have hepatoprotective effect against carbon tetrachloride induced hepatotoxicity in rats. This study was undertaken to evaluate its effect on antitubercular drugs induced hepatotoxicity in rats. Methods: The study was conducted in five groups of male wistar rats, each having six animals. Gum acacia and a combination of isoniazid, rifampin and pyrazinamide were administered to the two control groups. The ethanolic extract of the whole plant of Phyllanthus debilis was administered in a dose of 200 and 400 mg/kg to the two test groups, respectively. Silymarin (hepatoprotective, 50 mg/kg, orally) was administered to the fifth group. Serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, tissue malondialdehyde and thiols were estimated. Statistical analysis was done using one-way ANOVA followed by Tukey’s test. Results: The rise in levels of serum enzymes was lower in rats treated with a combination of antitubercular drugs and high dose (400 mg/kg) of ethanolic extract of P debilis as compared to those administered antitubercular drugs alone. Co-administration of high dose of extract of P debilis with antitubercular drugs significantly (p<0.05) decreased MDA levels and increased thiol levels. But the levels of these biochemical parameters were not normalized.
**Conclusion:** Phyllanthus debilis, in high dose, exerted a partial protective effect against antitubercular drugs induced hepatotoxicity in rats.

**Keywords:** Phyllanthus debilis, isoniazid, rifampin, pyrazinamide, hepatoprotective.

**INTRODUCTION**

An important toxicity of first line antitubercular drugs like pyrazinamide, isoniazid and rifampin is hepatic damage. This affects patient compliance which can lead to treatment discontinuation, with chances of development of resistance. *Phyllanthus debilis* belongs to the genus Phyllanthus which has been used traditionally as a herbal medicine. This herb has been shown to possess anti-inflammatory and antioxidant properties. Studies have revealed the hepatoprotective effect of aqueous extract of leaves, root, stem as well as whole plant against carbon tetrachloride induced hepatotoxicity in rats. This study was conducted to evaluate the effect of *Phyllanthus debilis* on antitubercular drugs induced toxicity in wistar rats.

**MATERIALS AND METHODS**

**Animals**

After obtaining approval from the Institutional Animal Ethics Committee, Manipal, the study was conducted at Central animal house, Manipal. Adult, male wistar rats weighing about 200 g were used in the study. Each rat was housed in polypropylene cage under standard conditions of temperature, humidity and light-dark cycle. They were fed with standard rat feed and water *ad libitum*. After overnight fasting of the rats, the study was carried out.

**Drugs and chemicals**

Antitubercular drug, INH and rifampin (Lupin laboratories), pyrazinamide tablet (Pfizer) were used. Chemicals used were 2% gum acacia (Nice Chemicals, Kochi) and ketamine (Neon laboratories, Mumbai). The plant *Phyllanthus debilis* was obtained locally and authenticated by Dr. K Gopalkrishna Bhat, retired Professor of Botany, Poorna Prajna College, Udupi. Analytical grade reagents were used. Kits from Aspen Laboratories Pvt. Ltd., Delhi were used to estimate serum alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels.

**Preparation of ethanolic extract of whole plant of Phyllanthus debilis**

The whole plant was shade dried and ground into a coarse powder with a grinder. About 500 g was soaked in 3L of absolute alcohol for 24h following which reflux condensation was
carried out at 60°C for 3h. Alcohol was drained and process repeated twice. The alcohol was concentrated to obtain the extract which was then evaporated to dryness. The extract was stored in a dessicator.

**Study design**

**The study was conducted using five groups of rats. Each group had six rats:** Isoniazid (INH) 50 mg/kg intraperitoneally (i.p.), rifampin 100 mg/kg (i.p.) and pyrazinamide 350 mg/kg body weight of rat orally (p.o.) were used as hepatotoxic drugs. Silymarin (hepatoprotective) was administered in a dose of 50 mg/kg p.o. The ethanolic extract of Phyllanthus debilis was administered orally in doses of 200 mg/kg and 400 mg/kg. All the drugs were administered using 2% gum acacia suspension to the rats once daily for 14 days. Group I was administered 2% gum acacia, 10 ml/kg p. o. and 10 ml/kg i. p.

Group II was given a combination of (INH + Rifampin + Pyrazinamide)

Group III was treated with (INH + Rifampin + Pyrazinamide) along with extract of Phyllanthus debilis 200 mg/kg

Group IV was treated with (INH + Rifampin + Pyrazinamide) along with extract of Phyllanthus debilis 400 mg/kg

Group V was given (INH + Rifampin + Pyrazinamide) along with silymarin

About 24 h after the last dose of drug administration to each rat, they were sacrificed using excess of ketamine. By cardiac puncture, blood samples were drawn for estimation of serum hepatic enzyme levels - aspartate aminotransferase (AST), alanineaminotransferase (ALT) and alkaline phosphatase (ALP). The liver was dissected out, washed with cold 0.9% saline, suspended in phosphate buffer, then weighed, homogenized and hepatic tissue thiols and MDA were estimated.

**Statistical analysis:** One-way ANOVA followed by Tukey’s test was used. Results were expressed as mean ± SD. The level of significance was at P < 0.05.

**RESULTS**

Hepatotoxicity by antitubercular drugs resulted in significantly (P < 0.05) elevated levels of serum AST, ALT and ALP levels as compared to control. The rise in levels of serum enzymes was lower in rats treated with a combination of antitubercular drugs and high dose (400 mg/kg) of ethanolic extract of P debilis compared to those administered hepatotoxic drugs alone (Table 1). Lower dose (200 mg/kg) of the plant extract could not prevent the rise in serum enzyme levels caused by antitubercular drugs. The standard drug, silymarin,
prevented a rise in serum hepatic enzyme levels when coadministered with antitubercular drugs.

**Table 1: Effect of coadministration of antitubercular drugs with ethanolic extract of Phyllanthus debilis on serum AST, ALT and ALP levels in rats**

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Drug</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>ALP (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Gum acacia</td>
<td>157.35±30.10</td>
<td>63.65±5.39</td>
<td>262.38±15.64</td>
</tr>
<tr>
<td>II</td>
<td>INH + Rifampin + Pyrazinamide</td>
<td>347.08±28.51*</td>
<td>78.35±5.80*</td>
<td>347.57±19.53*</td>
</tr>
<tr>
<td>III</td>
<td>INH + Rifampin + Pyrazinamide + <em>P debilis</em> (low dose)</td>
<td>322.28±13.84</td>
<td>72.36±8.71</td>
<td>331.82±7.80</td>
</tr>
<tr>
<td>IV</td>
<td>INH + Rifampin + Pyrazinamide + <em>P debilis</em> (high dose)</td>
<td>291.51±18.75**</td>
<td>64.58±4.69**</td>
<td>314.24±18.57**</td>
</tr>
<tr>
<td>V</td>
<td>INH + Rifampin + Pyrazinamide + Silymarin</td>
<td>145.32±10.22**##</td>
<td>57.60±3.45**##</td>
<td>256.76±19.74**##</td>
</tr>
</tbody>
</table>

n= number of rats in each group
*p < 0.05 vs Group I; **p < 0.05 vs Group II; ¶p < 0.05 vs Group III
#p < 0.05 vs Group IV

As compared to control group, treatment with antitubercular drugs caused a significant (p<0.05) rise in hepatic MDA but a significant fall in thiol levels. Treatment of rats with antitubercular drugs along with high dose of extract of *P debilis* significantly (p<0.05) decreased MDA levels but increased thiol levels (**Table 2**). Lower dose (200 mg/kg) of the plant extract did not significantly alter hepatic MDA and thiol levels.

**Table 2: Effect of coadministration of antitubercular drugs with ethanolic extract of Phyllanthus debilis on hepatic MDA and thiol levels in rats**

<table>
<thead>
<tr>
<th>Group (n=6)</th>
<th>Drug</th>
<th>MDA (nmol / mg)</th>
<th>Thiol (nmol / mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2% gum acacia</td>
<td>85.15±8.01</td>
<td>3.34±0.05</td>
</tr>
<tr>
<td>II</td>
<td>INH + Rifampin + Pyrazinamide</td>
<td>156.26±10.75*</td>
<td>1.29±0.09*</td>
</tr>
</tbody>
</table>
n= number of rats in each group
* p < 0.05 vs Group I; ** p < 0.05 vs Group 2; †p < 0.05 vs Group 3;
#p< 0.05 vs Group IV

**DISCUSSION**

In this study, the effect of ethanolic extract of whole plant *Phyllanthus debilis* on hepatic damage caused by a combination of isoniazid, pyrazinamide and rifampin was evaluated. Antitubercular drugs induced hepatocellular damage involves generation of free radicals. The free radicals cause lipid peroxidation which cause destruction of cell membranes. Damage to the membrane results in leakage of enzymes resulting in their elevated levels in serum. A result of lipid peroxidation is the formation of MDA. Generation of free radicals results in consumption of antioxidants like hepatic thiols resulting in a lowering of their levels.

The extract of *Phyllanthus debilis*, using various solvents, have been found to consist of lignans, phytosterols and glycosides. In animals, lignans have been shown to exert a protective effect against hepatotoxins. Studies have demonstrated that polyphenols present in *Phyllanthus debilis* have free radical scavenging activity. This could have led to a decrease in oxidative stress as evidenced by a decrease in hepatic MDA and an increase in thiols in rats treated with the extract. Moreover, the plant has debelalactone which was reported to exhibit antihepatotoxic action against carbon tetrachloride induced hepatotoxicity in rats. This compound may also have contributed to the protective effect of the plant against antitubercular drugs induced hepatotoxicity.

**CONCLUSION**

*Phyllanthus debilis*, in high dose, exerted a protective effect against antitubercular drugs induced hepatotoxicity but the protection was partial as it did not normalize serum enzymes, hepatic MDA and thiols levels.
REFERENCES


