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Potentiating Effect of Piperine on Hepatoprotective Activity of *Boerhaavia diffusa* to Combat Oxidative Stress

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Abstract: The hydro alcoholic extract of roots of *Boerhaavia diffusa* (HEBD) was evaluated for its hepatoprotective activity against CCl_4 and Rifampicin - Isoniazid combination induced hepatotoxicity at two dose levels 150 and 300 mg kg^{-1}. HEBD exhibited a significant protective action on the liver evident by a reduction in the elevated levels of serum lysosomal enzymes namely Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transminase (SGOT), Alkaline Phosphatase (ALP) in both CCl_4 and Rifampicin-Isoniazid induced hepatotoxicity. Thus HEBD showed a dose dependent hepatoprotective activity. In addition, the hepatoprotective activity of *Boerhaavia diffusa* was evaluated for possible potentiation in the presence of piperine based on recent research which has reported the latter enhancing bioavailability of certain drugs and nutritional compounds. Piperine was checked for potentiation, if any, at two dose levels 10 and 20 mg, respectively. Piperine was found to produce a dose dependent potentiation of the hepatoprotective activity of *Boerhaavia diffusa*.

Key words: Carbon tetrachloride, hepatotoxicity, isoniazid, rifampicin

INTRODUCTION

Liver has a pivotal role in the regulation of physiological processes. It is involved in several vital functions such as storage, secretion, metabolism and detoxification of a variety of drugs and xenobiotics. Liver diseases are mainly caused by either toxic chemicals (certain antibiotics, peroxidised oil, aflatoxin, carbon-tetrachloride, chlorinated hydrocarbons etc.), excess consumption of alcohol, infections or autoimmune disorders. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages in liver (Rafatullah *et al*., 2008). Though liver diseases are amongst the important diseases affecting mankind, no remedy is available to majority of them at present. *Boerhaavia diffusa* L. (Nyctaginaceae) commonly known as Punarnava in the Indian system of medicine, is a perennial creeping herb found throughout India. The roots are reputed to be diuretic and laxative and are given for the treatment of ascites and jaundice. The roots have also been found to be anti-inflammatory (Bhalla *et al*., 1971), antifibrinolytic (Jain and Khanna, 1989), anticonvulsant, hepatoprotective (Chakraborti and Handa, 1989; Nandave *et al*., 2007). A large number of compounds have been isolated from the roots of *Boerhaavia diffusa* L., namely punarnavine, β-sitosterol, β-D glucoside, tetracosanoic, hexacosanoic, stearic, palmitic, arachidic acid, hentriacontane, ursolic acid and punarnanavoside (Misra and Tewari, 1971).

Only a few plants are really very promising hepatoprotective agents. Realizing the importance and common use of the roots of *Boerhaavia diffusa* in the treatment of liver disorders by several tribes in India, it was decided to investigate the hepatoprotective activity of *Boerhaavia diffusa* roots. Piperine is reported to increase the bioavailability of valuable phytochemicals present in other spices and can boost the activity of biochemically active compounds such as phenytoin (Velpandian *et al*., 2001), co-enzyme Q (Vladimir and Majeed, 2000), beta-carotene (Vladimir and Majeed, 1999), curcumin (Shoba and Joy, 1998) and a variety of other spices by up to several hundred percent, depending on the molecule concerned.

Since no effective treatment has been established for liver disorders, it is very crucial to find out the manner in which liver can be best treated. The possibility of enhancing the existing activity of *Boerhaavia diffusa* with piperine thereby increasing its efficacy might open new

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vistas in the treatment of liver disorders. In general, such an approach would ensure increase in efficacy of herbal formulations used to treat oxidative stress in the liver and also help combat tuberculosis more effectively which has remained a life threatening disease. An attempt was hence made to evaluate the hepatoprotective activity of roots of *Boerhaavia diffusa* and subsequently investigate the effect of piperine, if any, on its bioactivity.

MATERIALS AND METHODS

Animals: Albino Wistar rats of either sex, in the weight range of 100-125 g, maintained on natural light/dark cycle, at a temperature of 25±2°C, commercial pellet diet and water *ad libitum* were used in the study. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional Animal Ethics Committee (Animal House Registration No. 25/1999/CPCSEA) which conforms to the Indian National Science Academy Guidelines for the use and care of experimental animals in research.

Plant materials and drugs: Roots of *Boerhaavia diffusa* were collected from Yucca enterprises, Dadar, Mumbai, in September 2007, coarsely powdered in a micro-pulveriser and subjected to Soxhlet extraction, using a hydro alcoholic (70% ethanol) solvent at a temperature of 70°C. The extract was concentrated by drying it over a water bath at a temperature of 70°C. Silymarin obtained from Sigma Aldrich, Mumbai, was used as a reference standard for this study. Rifampicin and Isoniazid were obtained as gift samples from Mc leods Pharmaceuticals and Ajanta Pharma Ltd., Mumbai, respectively. All serum marker enzyme estimations were carried out using the Cobas Integra- 400 auto-analyzer at Accutest Laboratories, Navi Mumbai with the help of standard diagnostic kits from Roche diagnostics. All reagents and chemicals used were of analytical grade.

Experimental

Effect of Piperine on CCl_4 induced hepatotoxicity (Shenoy *et al.*, 2001; Rafatullah *et al.*, 2008): Forty two rats of either sex were divided into 7 groups containing 6 rats each:

Group 1: Control group which was administered distilled water 1 mL kg^{-1} i.p. for 10 days.

Group 2: Toxicant control group which was administered the toxicant CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Group 3: Standard group which was administered the standard Silymarin-100 mg kg^{-1} p.o. for 10 days, an hour before administration of the toxicant-CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Group 4: Treatment group receiving HEBD at 150 mg kg^{-1} p.o. for 10 days, an hour before administration of the toxicant-CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Group 5: Treatment group receiving HEBD at 300 mg kg^{-1} p.o. for 10 days, an hour before administration of the toxicant-CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Group 6: Potentiation group which was administered 10 mg piperine concomitantly with 150 mg kg^{-1} of HEBD p.o. for 10 days, an hour before administration of the toxicant- CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Group 7: Potentiation group which was administered 20 mg piperine concomitantly with 150 mg kg^{-1} of HEBD p.o. for 10 days, an hour before administration of the toxicant-CCl_4 in olive oil (1:1)-1 mL kg^{-1} i.p. from the 4th to the 10th day.

Duration of study: Ten days.

Procedure: The rats were sacrificed on the 11th day under light ether anaesthesia. Blood withdrawal (4 mL) was carried out by cardiac puncture; serum was separated by centrifugation at 3000 rpm using the pathological centrifuge. Estimation of biochemical parameters namely serum marker enzymes-SGOT, SGPT, ALP was done by using the auto-analyzer.

Effect of Piperine on Anti- tubercular drug induced hepatotoxicity (Juvekar *et al.*, 2000): Forty two rats of either sex were divided into 7 groups containing 6 rats each:

Group 1: Control group which was administered 1% CMC 100 mg kg^{-1} p.o. for 30 days.

Group 2: Toxicant control group which was administered the toxicant rifampicin 50 mg kg^{-1} + isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Group 3: Standard group which was administered the standard Silymarin-100 mg kg^{-1} p.o. for 30 days, an hour before receiving the toxicant rifampicin 50 mg kg^{-1} + isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Group 4: Treatment group receiving HEBD at 150 mg kg^{-1} p.o. for 30 days an hour before receiving the toxicant rifampicin 50 mg kg^{-1} +isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Group 5: Treatment group receiving HEBD at 300 mg kg^{-1} p.o. for 30 days, an hour before receiving the toxicant rifampicin 50 mg kg^{-1} + isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Group 6: Potentiation group which was administered 10 mg piperine concomitantly with 150 mg kg^{-1} of HEBD p.o. for 30 days, an hour before receiving the toxicant rifampicin 50 mg kg^{-1} +isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Group 7: Potentiation group which was administered 20 mg piperine concomitantly with 150 mg kg^{-1} of HEBD p.o. for 30 days, an hour before receiving the toxicant rifampicin 50 mg kg^{-1} +isoniazid 50 mg kg^{-1} in 1% CMC p.o. for 30 days.

Duration of study: Thirty days.

Procedure: The rats were sacrificed on the 31st day under light ether anaesthesia. Blood withdrawal (4 mL) was carried out by cardiac puncture; serum was separated by centrifugation at 3000 rpm using the pathological centrifuge. Estimation of biochemical parameters namely serum marker enzymes - SGOT, SGPT, ALP were estimated by using the auto-analyzer.

Statistical analysis: All the values are expressed as Mean±SEM and data analysed by one-way ANOVA, using Graphpad INSTAT. The level of significance was found out by Dunnett's test wherein all the groups are compared against control. *$p<0.05$ was considered to be significant.

RESULTS

In CCl_4 induced hepatotoxicity the administration of the toxicant CCl_4 showed a distinct rise in the levels of serum marker enzymes namely SGOT, SGPT and ALP as shown in group 2 of Table 1. The drug treatment (HEBD) was carried out at 2 dose levels 150 and 300 mg kg^{-1} both of which along with the standard (silymarin) treated group showed a significant reduction in the elevated enzyme levels ($p<0.01$). However the treatment group at 300 mg kg^{-1} showed values of enzymes comparable to the control group as shown by the statistical analysis ($p<0.01$). Taken together these data suggest a dose dependent hepatoprotective activity of HEBD.

Table 1: Effect of HEBD on CCl_4 induced hepatotoxicity

	Biochemical parameters		
	ALP	SGOT	SGPT
Group No.	(IU L^{-1})		
1 (Control)	132.26±4.013	134.26±8.833	43.36±2.662
2 (Toxicant)[a]	404.17±7.327*	4080.33±184.78*	3014.56±62.56*
3 (Standard)[b]	232.60±1.622*	444.70±60.033*	562.86±109.22*
4 (Treatment group-HEBD-150 mg kg^{-1})[b]	336.59±8.019*	1950.56±160.64*	2067.10±57.85*
5 (Treatment group-HEBD-300 mg kg^{-1})[b]	165.63±21.5*	185.90±3.072*	59.06±3.903*

All values are expressed as Mean±SEM, N = 6. [a]As compared to control group. [b]As compared to toxicant control group. Analysis by One way ANOVA followed by Dunnett's test, *Significant at $p<0.01$

Table 2: Effect of HEBD on Rifampicin- Isoniazid induced hepatotoxicity

	Biochemical parameters		
	ALP	SGOT	SGPT
Group No.	(IU L^{-1})		
1 (Control)	110.93±3.753	104.80±2.201	28.36±0.577
2 (Toxicant)[a]	267.26±1.045*	203.73±6.351*	44.46±0.4619*
3 (Standard)[b]	137.40±3.724*	133.36±1.472*	32.83±1.796*
4 (Treatment group-HEBD-150 mg kg^{-1})[b]	211.40±7.534*	180.20±0.664	42.26±0.2714
5 (Treatment group-HEBD-300 mg kg^{-1})[b]	115.36±4.099*	102.56±13.856*	27.53±0.5485*

All values are expressed as Mean±SEM, N = 6. Analysis by One way ANOVA followed by Dunnett's test. [a]As compared to control group. [b]As compared to toxicant control group, *Significant at $p<0.01$

In Rifampicin-Isoniazid induced toxicity (Table 2) a considerable elevation in the levels of serum enzymes was observed in group 2 receiving only toxicant i.e., Rifampicin and Isoniazid.

The enzyme levels of the standard silymarin treated group were found to be considerably reduced ($p<0.01$) and comparable to control. However the drug treatment at 150 mg kg^{-1} showed that only the reduction in the ALP levels were found to be significant. In comparison the drug treatment at 300 mg kg^{-1} also showed statistically significant reduction in levels of enzymes comparable to control with ($p<0.01$). Like CCl_4 these data also suggest dose dependent hepatoprotective activity of HEBD.

Figure 1 shows the potentiation of HEBD activity by piperine in CCl_4 induced hepatotoxicity which was found to be dose dependent with both groups showing significant reduction in the enzyme levels ($p<0.01$). In Rifampicin-Isoniazid induced hepatotoxicity (Fig. 2), piperine -10 mg was found to be quite significant ($p<0.01$) for reduction in the ALP levels and the group but less significant ($p<0.05$) for SGOT and SGPT. However piperine -20 mg was found to be extremely significant with a marked reduction in levels of all enzymes ($p<0.01$). Thus the above data suggests that piperine was found to potentiate the hepatoprotective activity of HEBD in a dose dependent manner.

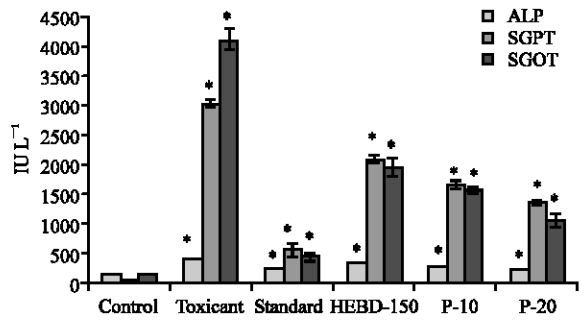

Fig. 1: Effect of Piperine and HEBD treatment on biochemical parameters in CCl_4 induced hepatotoxicity, All values are expressed as Mean±SEM, N = 6. Analysis by one way ANOVA followed by Dunnett's test. *Significant at $p<0.01$ in which all values are compared against toxicant control group. The toxicant control group is compared against the control group

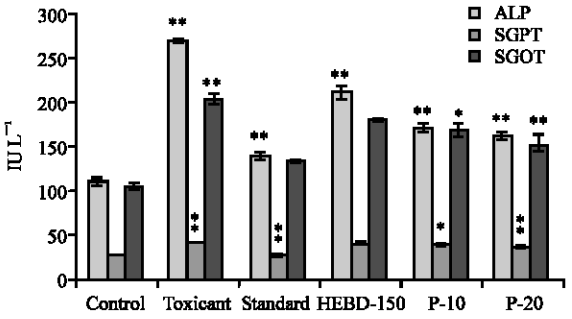

Fig. 2: Effect of Piperine and HEBD treatment on biochemical parameters in Rifampicin- Isoniazid induced hepatotoxicity, All values are expressed as Mean±SEM, N = 6. Analysis by one way ANOVA followed by Dunnett's test. *Significant at $p<0.05$, **Significant at $p<0.01$ in which all values are compared against toxicant control group. The toxicant control group is compared against the control group

DISCUSSION

Carbon tetrachloride induced hepatotoxicity is well documented regarding its toxic effects on the liver. Following CCl_4 administration the toxin CCl_4 is biotransformed by cyt. P-450 to produce the trichloromethyl free radical (CCl_3^*). This is turn elicits lipid peroxidation of membrane lipids in the presence of oxygen radical generated by metabolic leakage from mitochondria. All these events culminate in functional and morphological changes leading to loss of integrity of cell membranes which is evidenced by the rise in levels of serum marker enzymes- SGOT, SGPT, ALP, damage of hepatic tissue due to the reduced activity of the anti-oxidant enzymes and disturbance of Ca^{2+} homeostasis (Recknagel *et al*., 1989). SGPT is an enzyme present in hepatocytes (liver cells). When a cell is damaged, it leaks this enzyme into the blood, where it is measured. SGPT rises dramatically in acute liver damage. SGOT is similar to SGPT in that it is another enzyme associated with liver parenchyma cells. It is raised in acute liver damage but also present in red cells, cardiac and skeletal muscle and is therefore not specific to the liver (Rafatullah *et al*., 2008). ALP is present in cells lining the biliary ducts. ALP levels rise with infiltrative diseases of the liver. As evidenced in Table 1 and 2 the treatment groups lower the levels of SGPT, SGOT and ALP which are observed to be higher in the toxicant groups thus indicating damage to the hepatocellular membrane.

The use of isoniazid (INH) and rifampicin in the treatment of tuberculosis is limited by their potential for hepatotoxicity. The incidence of hepatotoxicity is higher with isoniazid and rifampicin combination than with isoniazid or rifampicin alone (Lal *et al*., 1972). A meta-analysis has shown an incidence rate of liver toxicity of 2.6% with isoniazid and rifampicin co administration, but only 1.1% with rifampicin alone and 1.6% with isoniazid alone (Wing-Wai and Chi-Chiu, 2007). The conversion of monoacetyl hydrazine, a metabolite of INH, to a toxic metabolite via cytochrome P450 leads to hepatotoxicity. Patients on concurrent rifampicin therapy have an increased incidence of hepatitis, which has been postulated to be due to rifampicin-induced cytochrome P450 enzyme-induction, causing an increased production of toxic metabolites from acetyl hydrazine (AcHz) (Ellard and Gammon, 1976).

Rifampicin increases the metabolism of INH by acetylation and hydrolysis to isonicotinic acid and hydrazine, both of which are hepatotoxic on activation by cyt. P450. The plasma half life of AcHz (metabolite of INH) is shortened by rifampicin. AcHz is quickly converted to its active metabolites i.e., reactive acylating species which bind covalently to the liver macromolecules causing hepatocyte injury. Thus there is a higher incidence of liver necrosis caused by INH and rifampicin in combination (Bhupinder *et al*., 2007).

Since both CCl_4 and rifampicin-isoniazid combination involve activation by cyt.P-450, subsequent damage to the hepatocellular membrane by the toxic intermediate and increase in lipid peroxidation, the possible hepatoprotective mechanism of *Boerhaavia diffusa* would be inhibition of cyt.P 450 leading to inhibition of the lipid peroxidation, stabilization of the hepatocellular membrane and enhancement of protein synthesis.

Bano *et al.* (1987) have demonstrated that Piperine can enhance blood levels of drugs like vasicine, sparteine, phenytoin, propranolol, theophylline, rifampicin when co administered with them. Piperine has been reported to enhance the bioavailability of a number of drugs by a non-specific and non-competitive inhibition of metabolic enzymes. It has been shown to inhibit both the monooxygenases and the conjugating (phase II) enzyme UDP-glucuronyl-transferase (Atal *et al.*, 1985). Thus the effect of potentiation of piperine may be attributed to the inhibition of metabolizing enzymes which metabolise the active constituent of *Boerhaavia diffusa* which is punarnavine, thus accounting for the increase in the hepatoprotective activity of *Boerhaavia diffusa.*

On the basis of the obtained results in this study it can be concluded that the roots of *Boerhaavia diffusa* exert a protective effect against CCl_4 and Rifampicin-Isoniazid induced hepatocellular damage. The flavonoids present in *Boerhaavia diffusa* may probably prevent the accumulation of excessive free radicals and protect the liver against CCl_4 and Rifampicin-Isoniazid intoxication. It was also found that piperine had a dose dependent potentiating effect on the hepatoprotective activity of *Boerhaavia diffusa* which can have important clinical implications in the future treatment of liver disorders.

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