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Hepatoprotective Activity of *Hedyotis corymbosa* (Linn.) Lam. Extract Against Anti-Tubercular Drug Induced Hepatic Damage in Sprague-Dawley Rats

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Abstract

The aim of this study was to investigate the protective actions of hydroalcoholic extract of *Hedyotis corymbosa* against hepatotoxicity caused by different combinations of anti-tubercular drugs. Anti-tubercular drugs isoniazid and rifampicin were used to make the elevated level of ALT, AST, ALP and bilirubin as well as decreased level of albumin and total protein. Hepatoprotective activity of the plant was indicated when it causes the decrease of these marker enzymes and elevated level of albumin and total protein. *H. corymbosa* prevented liver damage caused by anti-tubercular drugs and also from histopathological changes. It can be concluded from the above experiment that hydroalcoholic extract of *H. corymbosa* showed significant hepatoprotective activity against anti-tubercular drugs.

Key words: Isoniazid, rifampicin, hepatotoxicity, anti-tubercular, *Hedyotis corymbosa*.

Introduction

Tuberculosis (TB) continues to be a health problem. Short course combination chemotherapy of isoniazid (INH), rifampin (RMP) and pyrazinamide (PZA) is highly effective in the management of TB. Rifampicin has bactericidal activity against *M. tuberculosis* by inhibiting bacterial DNA dependent RNA polymerase (Houston and Fanning, 1994). Isoniazid is a prodrug activated by bacterial catalase-peroxidase (KatG) and kills actively growing tubercle bacilli by inhibiting the biosynthesis of mycolic acids which are major components of cell wall of *M. tuberculosis* (Timmins and Deretic, 2006). The another prodrug pyrazinamid is activated by bacterial pyrazinamidinase which is only active in acidic conditions (pH: 5.5). The active metabolite of pyrazinamid is pyrazinoic acid that inhibits fatty acid synthesis in *M. tuberculosis* (Zimhony *et al*., 2000). This drug is used in the initial two months of treatment to reduce the duration of therapy and is not used alone. Several adverse reactions of anti-tubercular drugs are reported. The best known toxic drug effect is hepatotoxicity. The frequency and severity of hepatotoxicity is increased when these drugs are used in combination (Petri, 2001; Zimhony *et al*., 2000).

Hedyotis corymbosa (L.) Lam. syn. *Oldenlandia corymbosa* (L.) Lam. (Rubiaceae) is a weedy herb, widely distributed throughout India. It is commonly known as 'Parppatakapullu' in traditional medicine of Kerala. *H. corymbosa* is extensively used in modern Chinese practice for the treatment of viral infections, cancer, syndromes involving "toxic heat", acne, boils, skin ailments, appendicitis, hepatitis, eye diseases and bleeding. They call it 'Peh-Hue-Juwa-Chi-Cao' (Lin *et al*., 2004). The plant is used for

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treating venomous bites. It has anthelmintic, diuretic, depurative, diaphoretic, expectorant, digestive and stomachic properties (Kirtikar and Basu, 1994). It is given in jaundice and other diseases of the liver, heat eruptions, vitiated conditions of pitta, hyperdypsia, giddiness, dyspepsia, flatulence, colic, constipation, helminthiasis, leprosy, skin diseases, cough, bronchitis, necrosis, nervous depression caused by deranged bile and hepatopathy (Warrier *et al.*, 1995). Recently there have been many studies on traditional medicines, attempting to develop new drugs for hepatitis from them. So far, there has been several research reported on hepatoprotective effect of *H. corymbosa* (L.) against D-galactosamine (Gupta *et al*., 2012), carbon tetrachloride (Wang *et al*., 2011) and paracetamol (Sultana *et al.*, 2010) induced liver damage in rats. However, there are no reports on hepatoprotetive effect of *H. corymbosa* (L.) against anti-tubercular drug induced hepatic damage. A search for an alternative drug useful for the prophylactic treatment of hepatotoxicity induced by anti-tubercular drugs still remains important. Efforts to explore hepatoprotective effect of any natural product thus carry a great clinical significance. Therefore, the present study was undertaken for the first time for the hepatoprotective activity of *H. corymbosa* (L.) extract against antitubercular drug induced hepatotoxicity in rats.

Materials and Methods

Plant material: Whole plants of *H. corymbosa* was collected from Jahangirnagar University Campus, Savar, Dhaka-1342.

Preparation of extract: The whole plants of *H. corymbosa* were washed thoroughly in tap water, shade dried and powdered. The powder (100 g) was successively extracted with 1000 ml of ethanol overnight with constant stirring. The filtrate was then concentrated and the solvent was evaporated under reduced pressure in a rotary evaporator. The yield of the extract was found to be 0.42% (w/v). This crude extract was referred to as HC. For administration, the crude extract was suspended in distilled water to make required concentrations.

Experimental animals: Adult male rats (*Rattus novergicus*: Sprague-Dawley strain) were collected from central animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342. The animals were randomized and separated into normal and experimental groups of body weight ranging from 170 to 230 g.

Experiment design: Animals were divided into following four groups:

Group I: Normal control (n=6, the animals were given normal saline only for 28 days)

Group II: Standard (n=6, the animals were given Silymarin only for 28 days)

Group III: Hepatotoxic control (n=6, the animals were given INH+RIF for 28 days)

Group IV: Treatment group (n=6, the animals were given INH+RIF+HC for 28 days)

Rats were treated as per the treatment protocol. Body weights of these rats were monitored sequentially in control and experimental animals for a period of 28 days.

Dose and route of administration: Isoniazid and rifampicin (50 mg/kg body weight) solutions were prepared separately in sterile distilled water. Rats were treated with isoniazid, co-administered with rifampicin for 28 days by oral route (Hewawasam *et al*., 2003). For hepatotoxic model, 50 mg/kg per day of INH and RIF each was used in the study (Jiang *et al*., 2004). For the hepatoprotective model, 500 mg/kg per day of freshly prepared *H. corymbosa* homogenate along with INH+RIF solution was administered orally and Silymarin was administered at a dose of 50 mg/kg.

Preparation of the samples for biochemical studies: From the post vena cava of the animal, blood samples were collected and immediately blood was transferred to the tubes having heparin. Blood samples were centrifuged for 10 minutes at 3000 rpm to separate serum for biochemical analysis. The liver was dissected out for histopathological examinations.

Assessment of liver functions: Biochemical parameters such as, serum alanine aminotransferase (ALT), serum aspartate aminotransferase (AST),

serum bilirubin (SB), serum alkaline phosphatase (ALP) (Malloy and Evelyn, 1937; Reitman and Frankel, 1957), total protein (TP) (Reinhold, 1953; Lowry *et al*., 1951) and albumin (Lowry *et al*., 1951) were determined by Humalyzer-3500 auto-analyzer using kits manufactured by HUMAN GmbH, Germany in Pharmacology Lab at Jahangirnagr University, Savar, Dhaka, Bangladesh.

Histopathological studies: The liver specimens obtained from the control and treated groups of animals were fixed in 10% buffered formalin for 24 h. The formalin-fixed liver samples were stained with haematoxylin–eosin for photomicroscopic observations of the liver histological architecture (Kalyani *et al*., 2010).

Estimation of *in vitro* antioxidant activity: The method of Ottolenghi (1959) was used to determine the thiobarbituric acid (TBA) values of the samples. Two milliliters of 20% trichloroacetic acid and 2ml of thiobarbituric acid aqueous solution were added to 1ml of sample solution prepared as in ferric thiocyanate (FTC) procedure and incubated in a similar manner. The mixture was placed in boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of the supernatant was measured at 532 nm. Antioxidant activity was based on the absorbance on the final day. The inhibition of lipid peroxidation in percentage was calculated by the following equation:

$$\text{Percent inhibition} = 1 - \frac{A_1}{A_0}$$

Where, A_0 is the absorbance of control and A_1 is the absorbance of sample (Duh *et al.*, 1999).

Statistical analysis: All the grouped data were statistically evaluated with SPSS (Chicago, IL) version 16.5 software. All the results were expressed as mean ± SEM (Standard error of mean) values for six animals in each group. Means were compared by independent sample t-test. Probability (p) value of 0.05 or less ($p<0.05$) was considered as significant.

Results and Discussion

Administration of isoniazid and rifampicin (50 mg/kg, p.o.) induced a marked increase in the serum hepatic levels of AST, ALT, ALP, SB and induced a marked decrease in total protein and albumin level as compared to normal controls indicating liver damage. Pre-treatment of the rats with HC (500 mg/kg) prior to isoniazid and rifampicin administration caused a significant reduction in the values of AST, ALT, ALP and SB but increase in total proein and albumin level. Same result was observed in case of standard drug Silymarin (Table 1).

The hepatoprotective effect of *H. corymbosa* was confirmed by histopathological examination of the liver tissue of control and treated animals. The histological architecture of isoniazid and rifampicin treated liver sections showed massive hepatic necrosis with dilated blood vessels in comparison with normal control. However, administration of *H. corymbosa* (500mg/kg) causes blood vessels dilation and proliferation of bile canaliculi. It also normalize the defects in the histological architecture almost to the level of the control groups, showing its potent hepatoprotective effects of the plant extract and Silymarin (Figures 1-4). The antioxidant activity exhibited by *H. corymbosa* in TBA methods (*in vitro*) is represented in table 3.

Tuberculosis is a leading public health problem worldwide, particularly in developing countries. About one third of world's population has latent tuberculosis and approximately 9 million cases of active tuberculosis emerge annually resulting in 2–3 million deaths (Adhvaryu *et al*., 2007). Out of 1.86 billion people estimated to be infected with the tuberculosis bacillus, an estimated 1.3 billion infected people are living in developing countries, such as India and China (Santhosh *et al*., 2006). In view of the seriousness of the problem World Health Organization (WHO) declared it to be a global emergency in 1993. Active tuberculosis will kill about two out of every three people if untreated.

Table 1. Effects of alcoholic extract of *H. corymbosa* on rat serum parameters after INH + RIF administration.

Group	ALT (IU/L)	AST (IU/L)	ALP (KA Units/100 ml)
Control	17.33 ± 3.39	23.83 ± 4.31	144.50 ± 5.28
Silymarin	21.23 ± 3.12 [a]	31.152 ± 4.11 [a]	151.25 ± 6.78 [a]
INH+RIF	56.67 ± 3.98	78.00 ± 5.55	177.83 ± 17.07
INH+RIF+HC	33.50 ± 7.87[a]	52.33 ± 9.71[a]	163.17 ± 11.87

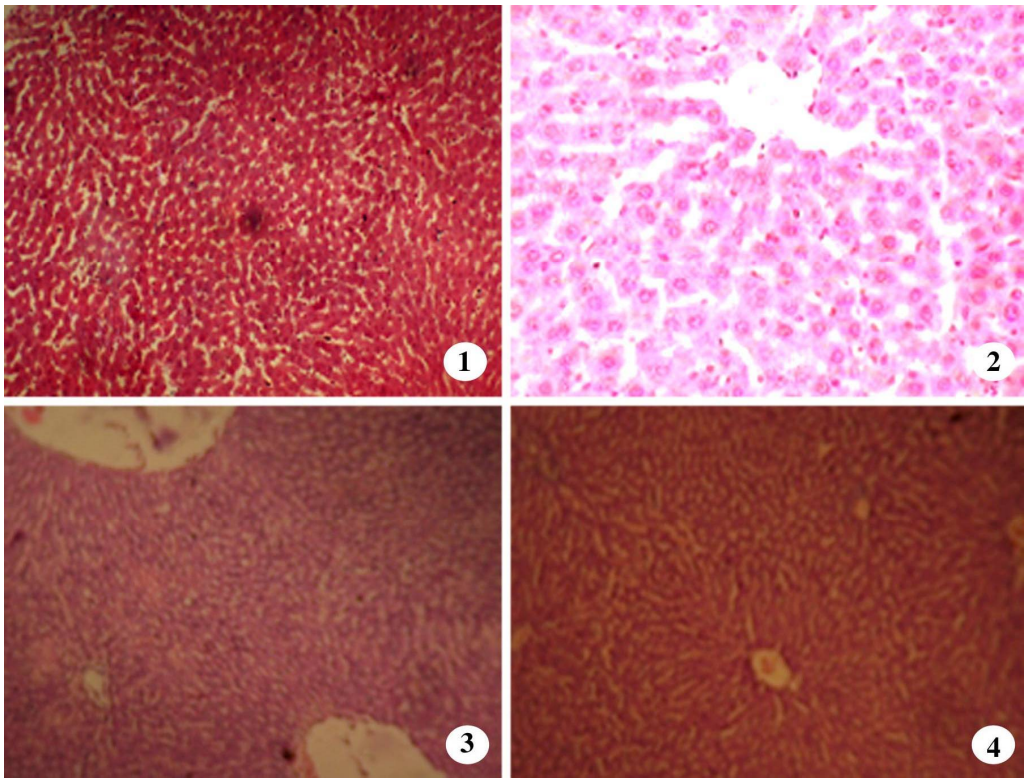
Results are represented as mean ± SEM (n = 6). [a] $p \leq 0.001$, compared to INH+RIF

ALT- Alanine transaminase, AST- Aspartate aminotransferase, ALP- alkaline phosphatase.

Table 2. The levels of albumin, total protein and total bilirubin after the treatment of rats with antitubercular drugs and *H. corymbosa.*

Group	Bilirubin (mg/dl)	Total Protein (mg/dl)	Albumin (mg/dl)
Control	0.33 ± 0.15	6.26 ± 0.29	4.35 ± 0.44
Silymarin	0.31 ± 0.04 [a]	6.08 ± 0.12 [a]	4.32 ± 0.09 [a]
INH+RIF	1.22 ± 0.16	4.85 ± 0.62	3.68 ± 0.31
INH+RIF+HC	0.55 ± 0.19[a]	5.61 ± 0.41[b]	4.09 ± 0.12[b]

Results are represented as mean ± SEM (n = 6). [a] $p \leq 0.001$, compared to I+R, [b] $p \leq 0.05$, compared to INH+RIF



Figures 1-4. 1.Section of control rat liver, showing normal architecture of hepatic cell. 2. Section of Silymarin rat liver, showing almost normal architecture of hepatic cell. 3. Section of INH-RIF treated rat liver, showing massive hepatic necrosis with dialated blood vessels. 4. Section of *H. corymbosa*. (500 mg/kg) treated rat liver, showing marked improvement over INH + RIF group

Table 3. IC_{50} values of the plant extract and standard.

Sample/Standard	IC_{50} (µg/ml)
H. corymbosa	361.63
BHT	53.36

Drug-induced hepatotoxicity is a potentially serious adverse effect of the currently used anti-tubercular chemotherapeutic regimens containing INH, RMP and PZA. All these drugs are potentially hepatotoxic independently and when given in combination their toxic effects are enhanced in a synergistic manner. The conversion of monoacetyl hydrazine, a metabolite of INH to a toxic metabolite via cytochrome P_{450} leads to hepatotoxicity. RMP-induces cytochrome P450 enzyme causing an increased production of toxic metabolites from acetyl hydrazine (AcHz). RMP also increase the metabolism of INH to isonicotinic acid and hydrazine, both of which are hepatotoxic. The plasma half life of AcHz (metabolite of INH) is shortened by RMP and AcHz is quickly converted to its active metabolite by increasing the oxidative elimination rate of AcHz, which is related to the higher incidence of liver necrosis caused by INH and RMP in combination (Hussain *et al*., 2003).

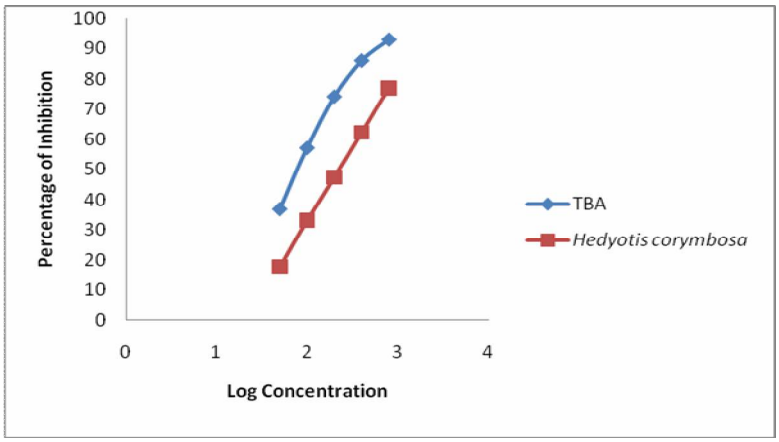


Figure 5. Lipid peroxidation inhibition capacity of *Hedyotis corymbosa* and BHT

Rats have been used successfully to investigate INH and RIF-induced hepatotoxicity models (Tasduq *et al*., 2005; Pal *et al*., 2006; Victorrajmohan *et al*., 2005; Rana *et al*., 2006). Therefore, we selected rats to study the hepatotoxic effect of antitubercular drugs and hepatoprotective action of *H. corymbosa*. The doses of the drugs used (INH: 50 mg/kg and RIF: 50 mg/kg) are very high compared to those used in the treatment of tuberculosis in human subjects. However, higher doses of drugs are required in animal models to produce hepatotoxicity, because rats metabolize the drugs at a faster rate and the duration of treatment is much shorter compared to the treatment of tuberculosis in humans. Biochemical tests have been done to follow hepatocellular integrity and liver injury. In this study, the injection of INH and RIF caused a significant elevation in the activities of ALT, AST and ALP. Serum total bilirubin was also increased two fold, indicating membrane damage in the liver (Table 2). Decrease in albumin and total protein levels proved that administration of drugs caused impairment of liver function to synthesize albumin (Table 2).

Pre-treatment of the rats with *H. corymbosa* extract at 500 mg/kg, resulted in a significant protection of INH+RIF induced elevation of serum marker enzymes. *H. corymbosa* appears to be effective in reducing the injurious effect of INH+RIF

observed in the present study. This was an indication of stabilization of plasma membrane, as well as repairment of hepatic tissue damage, caused by INH+RIF. The results are in agreement with the commonly accepted view that serum level of transaminase returns to normal with healing of hepatic parenchyma and the regeneration of hepatocytes (Thabrew *et al*., 1987). Further, the stimulation of hepatic regeneration was known to make the liver more resistant to damage by toxins (Lesch *et al*., 1970). The hepatoprotective effect of *H. corymbosa* was further confirmed by histopathological examination of the liver. The histological observation basically supported the results from the serum assays as *H. corymbosa* administration reversed to a large extent of hepatic lesions produced by INH+RIF.

From the aerial parts of *H. corymbosa* nine iridoid glycoside derivatives were isolated (Otsuka *et al*., 1991). The iridoid glycosides isolated from *Picrorrhiza kurroa* showed marked protective action on liver against CCl4-intoxicated rats by enhancing the choleretic activity and also reduced the levels of SGOT and SGPT (Handa *et al*., 1986). *H. corymbosa* has also been reported to contain oleanolic acid, ursolic acid and γ-sitosterol (Khastgir *et al*., 1960). Ursolic acid exhibited potent hepatoprotective effects (Shukla *et al*., 1992a,b; Liu, 1995). Oleanolic acid has been reported to increase the antioxidant components in the liver, such as glucuronosyl transferase towards acetaminophen in mice. It also increased/maintained the hepatic glutathione, which plays an important role in protecting acetaminophen-induced liver injury (Zhang and Li, 1992; Liu, 1995). Inhibition of lipid peroxidation by oleanolic acid is also proposed to play a role in preventing CCl_4 and d-galactosamine plus endotoxin-induced liver injury (Balanehru and Nagarajan, 1991; Zhang and Li, 1992; Liu *et al*., 1993). Preventing liver lesions from progressing to fibrosis and cirrhosis, and repairing parenchymal cell damage by stimulating liver regeneration are important mechanisms for hepatoprotection. Perhaps the triterpenoids and iridoid glycosides present in *H. corymbosa* are responsible for the marked hepatoprotective effects, observed in the present study.

In TBA method, formation of malonaldehyde is the basis for evaluating the extent of lipid peroxidation. At low pH and high temperature (100^0C) malonaldehyde binds TBA to form a red complex that can be measured at 532 nm. The increase of the amount of red pigment formed correlates with the oxidative rancidity of the lipid. Most of the hepatotoxic chemicals including isoniazid and rifampicin damage liver mainly by inducing lipid peroxidation directly or indirectly. In higher animals, lipid peroxidation was known to cause destabilization and disintegration of the cell membrane, leading to liver injury, arteriosclerosis and kidney damage (Rael *et al*., 2004). Peroxy radicals are important agents that mediate lipid peroxidation thereby damaging cell membrane. The IC_{50} values of the plant extract and standard are presented in table 3. % inhibition of lipid peroxidation was found to rise with increasing concentration of plant extract (Figure 4).

In conclusion, the findings of this study demonstrates that *H. corymbosa* has potent hepatoprotective activity upon INH+RIF-induced hepatic damage in rats and antilipid peroxidative activity. The present study thus justifies the traditional use of *H. corymbosa* in the treatment of liver diseases. This study also points out that *H. corymbosa* warrants detailed investigation for developing promising hepatoprotective agents.

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