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Antihepatotoxic Effects of *Boerhaavia diffusa* L. on Antituberculosis Drug, Rifampicin Induced Liver Injury in Rats

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Abstract: The aim of the present study was to investigate the antihepatotoxic effect of aqueous leaf extract of *Boerhaavia diffusa* (BDEX) on rifampicin induced liver injury. The activities of serum hepatic marker enzymes viz., aspartate aminotransferase (AST, 95.30±2.96), alanine aminotransferase (ALT, 51.27±2.52) and alkaline phosphatase (ALP, 167.04±2.59), levels of bilirubin (0.96±0.01), cholesterol (95.88±3.29) and protein (8.43±0.10) were estimated in control rats. Significant elevation of serum hepatic marker enzymes (AST, 254.59±3.10; ALT, 181.95±2.45; ALP, 316.57±2.35), bilirubin (3.46±0.28) and cholesterol (151.09±1.15) whereas protein (5.28±0.07) level decreased in rats treated with rifampicin (1 g kg⁻¹ b. wt. orally one day only). Oral administration of BDEX (250 and 500 mg kg⁻¹ b. wt. once daily for 28 days) and silymarin to rifampicin induced liver injury rats caused significantly (p<0.05) attenuated the aforementioned parameters. The maximum antihepatotoxic effect against rifampicin induced liver injury was achieved with BDEX 500 mg kg⁻¹ b. wt. but doses higher than 500 mg kg⁻¹ b. wt. were less effective. These results are compared to the reference hepatoprotective agent silymarin. These results suggest that BDEX possess the antihepatotoxic activity against rifampicin induced liver injury.

Key words: *Boerhaavia diffusa*, rifampicin, AST, ALT, ALP, bilirubin

INTRODUCTION

Tuberculosis is a hazardous disease, which gradually swallows the life span of human beings. It remains a major public health problem and most deadly infectious disease and also kills approximately two million people every year (Calleja *et al.*, 2004). Tuberculosis is an infectious disease, which can be totally cured by combining antitubercular drugs. Current therapeutic regimens with isoniazid, rifampicin, pyrazinamide, ethambutol and streptomycin have proved successful in treating tuberculosis. However, they are associated to a high rate of adverse effects that can lead to therapeutic failure. Understanding the nature and the severity of these adverse effects allows for their appropriate management (Aouam *et al.*, 2007).

Rifampicin, a complex semisynthetic macrocyclic antibiotic with a molecular weight of 823 derived from *Streptomyces mediteranei* (Charity *et al.*, 2007) is a member of the rifamycin class of antibiotics (Maggi *et al.*, 1966) used for the treatment of tuberculosis and other infectious diseases (Tsankov and Angelova, 2003; Liang *et al.*, 2007). It is categorized one of the first line antituberculosis agents, however various side effects such as hepatotoxicity, allergic rashes, lack of appetite, nausea or immunological disturbance have been reported associated with the administration of the drug (Deol and Khuller, 1997; Gallieni *et al.*, 1999; Tsankov and Angelova, 2003).

Hepatotoxicity is one of the most important adverse drug reactions associated with antituberculosis chemotherapy (Lee, 1995; Rana *et al.*, 2006; Upadhyay *et al.*, 2007). Hepatitis is a

common disease in the world especially in the developing countries. Liver diseases constitute a major problem of worldwide proportions (Gordillo *et al.*, 2007). The liver is the largest organ in the vertebrate body and is the major site of xenobiotics metabolism. Toxic chemicals, drugs and virus infiltration can cause liver injury from ingestion or infection (Lee *et al.*, 2007). The liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions (Wolf, 1999). Additionally, it is the key organ of metabolism and excretion is continuously and variedly exposed to xenobiotics because of its strategic placement in the body. The toxins absorbed from the intestinal tract gain access first to the liver resulting in a variety of liver ailments. Thus liver diseases remain one of the serious health problems. Modern medicines have little to offer for alleviation of hepatic diseases and it is chiefly the plant-based preparations, which are employed for their treatment of liver disorders (Orhan *et al.*, 2007). Despite considerable progress in the treatment of liver diseases by oral hepatoprotective agents, search for newer drugs continues because the existing synthetic drugs have several limitations. Hence there are many researchers of traditional medicines attempting to develop new drugs for hepatitis (Liu, 1989).

Existence of human beings on the earth is made possible because of the vital role played by plant kingdom. Besides providing basic requirements of man, the plants offer unique protection to mankind by providing innumerable drugs to prevent and treat various disorders (Manjunatha *et al.*, 2004). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, through relatively little knowledge about their mode of action. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine (Gupta *et al.*, 2004).

The plant *Boerhaavia diffusa* (BDEX) Linn. belongs to the Nyctaginaceae family. It is a perennial diffuse herb with stout root stock and many procumbent branches, leaves simple, opposite, short-petioled in unequal pairs, ovate-oblong, acute or obtuse, rounded or subcordate at base, glabrous above and whitish beneath, flowers in irregular coloured, small, short stalked in irregular clusters of terminal panicles at the ends of branches, fruits, highly viscid, easily detachable, one-seeded, indehiscent with a thin pericarp. It is popularly known as Mukukrattai in Tamil, Spreading Hogweed in English, Common name Punarnava and found throughout India as a weed in wastelands and roadsides. The plant was found to contain alkaloids, flavonoids, glycosides, tannins, saponins, proteins (Orisakwe *et al.*, 2003), tricontanol hentriacontane, β -sitosterol, sucrose, hypoxanthine 9-L-arabinoside, moulding hormone, β -ecdysone and many more active compounds (Prajapathi *et al.*, 2004). Silymarin is a standardized mixture of antioxidant flavonolignans (silybin and silibinin) extracted from the medicinal plant *Silybum marianum* (Shalan *et al.*, 2005). It is a free radical scavenger and a membrane stabilizer that prevents lipid peroxidation and its associated cell damage in some experimental models (Soto *et al.*, 1998). Silymarin was proved to have a protective effect against experimental hepatotoxicity by regulating the actions of the ultra structures of liver cells and improving the performance of hepatic enzymes and bile production (Hagymasi *et al.*, 2002; Lucena *et al.*, 2002). There is no available report on the effect of BDEX on rifampicin induced liver damage. Therefore, the present investigation to evaluate the antihepatotoxic effect of aqueous extract of BDEX on rifampicin induced liver injury in rats.

MATERIALS AND METHODS

Plant Material

B. diffusa leaves were collected from Chidambaram in Cuddalore district of Tamil Nadu, India in the month of October 2006 during the early hours of the day. The plant was identified and authenticated at the herbarium of Botany Directorate, Faculty of Science, Annamalai University. The leaves were shade dried and powdered. The powdered leaves were kept in airtight container in a deep freeze until the time of use.

Preparation of Extract

One hundred gram of *B. diffusa* leaf powder was mixed with 1000 mL of distilled water and stirred magnetically overnight (12 h) at 37°C. This was repeated three consecutive times. The residue was removed by filtration and the extract evaporated to dryness at a lower temperature (<40°C) under reduced pressure in a rotary evaporator. The residual extract was dissolved in normal saline and used in the study. The yield of the extract was approximately 13.5 g.

Experimental Design and Animals Management

Male albino Wistar rats weighing 150-180 g were procured from the Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and were maintained in polypropylene cages in an air-conditioned cooling room (22±1°C) under a 12/12 h light/dark cycle. A standard pellet diet (Hindustan Lever Ltd., Mumbai, India) and water were provided *ad libitum*. All studies involving animals were done according to NIH guidelines, after getting the approval of the Institute's Animal Ethics Committee.

The rats were divided into 6 groups of 6 rats each. Group 1 received physiological saline (10 mL kg⁻¹ b. wt. orally) as normal control; group 2 received rifampicin (1 g kg⁻¹ b. wt. orally one day only) as treated group; group 3 received BDEX (250 mg kg⁻¹ b. wt. orally once daily for 28 days) to rifampicin treated group; group 4 received BDEX (500 mg kg⁻¹ b. wt. orally once daily for 28 days) to rifampicin treated group; group 5 received silymarin (25 mg kg⁻¹ b. wt. orally once daily for 28 days) to rifampicin treated group; group 6 received BDEX (500 mg kg⁻¹ b. wt. orally once daily for 28 days) alone.

At the end of the experiment all the rats were sacrificed by decapitation. Blood samples were collected for evaluating the serum marker enzymes, bilirubin, cholesterol and protein.

Biochemical Analysis

Blood samples were taken into centrifuge tube with rubber caps, labelled and centrifuged at 3000 rpm for 15 min. Serum biochemical parameter such as ALT, AST, ALP, bilirubin, cholesterol and protein levels were estimated according to standard methods (Reitman and Frankel, 1957; King and Armstrong, 1980; Malloy and Evelyn, 1937; Zlatkis *et al.*, 1953; Lowry *et al.*, 1951).

Statistical Analysis

Data are expressed as mean±SD. Statistical significance was analyzed by one way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) using SPSS version 10.0.

RESULTS AND DISCUSSION

Table 1 shows the levels of serum hepatic marker enzymes such as AST, ALT and ALP in normal and experimental groups of rats. There was a significant elevation noticed in the levels of serum hepatic marker enzymes in antitubercular drug rifampicin administered rats as compared to that of normal rats. Oral administration of BDEX (250 and 500 mg kg⁻¹ b. wt.) and silymarin significantly reduced the antitubercular drug, rifampicin induced rise in the levels of serum hepatic marker enzymes in group 3 group 4 and group 5 rats as compared to those group 2 rats, indicating the antihepatotoxic role of BDEX. Oral administration of aqueous extract of BDEX alone (group 6) was found to produce no significant elevation in serum hepatic marker enzymes in normal rats indicating the nonhepatotoxic nature.

Significant increases in the levels of bilirubin and cholesterol whereas protein level decreased in group 2 antitubercular drug, rifampicin administered rats as compared to that of normal rats. The rats administered with BDEX (250 and 500 mg kg⁻¹ b. wt.) and silymarin showed significantly near

Table 1: Serum hepatic marker enzyme activities in control and experimental groups

| Groups | AST | ALT | ALP |
|--|--------------------------|--------------------------|--------------------------|
| | (UL ⁻¹) | | |
| Control | 95.30±2.96 ^a | 51.27±2.52 ^a | 167.04±2.59 ^a |
| Rifampicin (1 g kg ⁻¹ b. wt.) | 254.59±3.10 ^e | 181.95±2.45 ^e | 316.57±2.35 ^e |
| Rifampicin + BDEx (250 mg kg ⁻¹ b. wt.) | 147.28±1.42 ^d | 76.23±2.48 ^d | 232.84±1.78 ^d |
| Rifampicin + BDEx (500 mg kg ⁻¹ b. wt.) | 125.44±1.07 ^b | 66.87±1.39 ^b | 205.90±2.63 ^b |
| Rifampicin + silymarin (25 mg kg ⁻¹ b. wt.) | 139.34±0.91 ^c | 70.33±2.19 ^c | 216.88±0.94 ^c |
| BDEx (500 mg kg ⁻¹ b. wt.) alone | 95.57±3.09 ^a | 52.07±2.34 ^a | 166.49±3.61 ^a |

All the values are mean±SD of six observations, Values which are not sharing common superscript (a-e) differ significantly at 5% level (p<0.05), Duncan's Multiple Range Test (DMRT)

Table 2: Serum bilirubin, cholesterol and protein levels in control and experimental groups

| Groups | Bilirubin | Cholesterol | Protein |
|--|------------------------|--------------------------|------------------------|
| | (mg dL ⁻¹) | | |
| Control | 0.96±0.01 ^a | 95.880±3.29 ^a | 8.43±0.10 ^a |
| Rifampicin (1 g kg ⁻¹ b. wt.) | 3.46±0.28 ^e | 151.09±1.15 ^e | 5.28±0.07 ^e |
| Rifampicin + BDEx (250 mg kg ⁻¹ b. wt.) | 1.50±0.03 ^b | 140.87±2.33 ^d | 6.68±0.19 ^d |
| Rifampicin + BDEx (500 mg kg ⁻¹ b. wt.) | 1.40±0.02 ^b | 116.54±2.25 ^b | 7.78±0.12 ^d |
| Rifampicin + silymarin (25 mg kg ⁻¹ b. wt.) | 1.47±0.02 ^b | 125.34±1.37 ^c | 7.02±0.15 ^c |
| BDEx (500 mg kg ⁻¹ b. wt.) alone | 0.95±0.02 ^a | 96.590±2.07 ^a | 8.48±0.11 ^a |

All the values are mean±SD of six observations, Values which are not sharing common superscript (a-e) differ significantly at 5% level (p<0.05), Duncan's Multiple Range Test (DMRT)

normal levels of bilirubin, cholesterol and protein in group 3, group 4 and group 5 rats as compared to that of group 2 hepatotoxicity induced rats. The rats administered with BDEx alone (group 6) did not show any adverse effects indicating that BDEx is non-toxic (Table 2).

Liver is the key organ in the metabolism, detoxification and secretory functions in the body and its disorders are numerous with no effective remedies, however, the search for new medicines is still ongoing (Jamshidzadeh *et al.*, 2005). Many folk remedies from plant origin have been long used for treatment of liver diseases (Luper, 1999). Liver injury in a patient on antituberculosis treatment often presents the clinician with a difficult problem of management (Dossing *et al.*, 1996). Management of liver diseases is still a challenge to the modern medicine. In Ayurveda, various herbal and herbomineral preparations are extensively used for the treatment of various liver disorders (Praveen Reddy *et al.*, 1992).

Assessment of liver function can be made by estimating the activities of serum AST, ALT and ALP, which are enzymes originally present in higher concentration in cytoplasm (Wells, 1988), when there is hepatopathy, these enzymes leak into blood stream in conformity with the extent of liver damage (Plaa and Charbonneau, 1994; Venukumar and Latha, 2004). Indicators of hepatocellular integrity most commonly measured in clinical toxicology studies are the enzymes AST, ALT and bilirubin levels (Ballet, 1997). ALT is frequently included in biochemical profiles for the purpose of assessing hepatic injury (Williamson *et al.*, 1996).

An elevation in the levels of the serum marker enzymes is generally regarded as one of the most sensitive index of the hepatic damage (Kapil *et al.*, 1995). ALP is a membrane bound glycoprotein enzyme, with high concentrations in sinusoids and endothelium. ALP reaches the liver mainly from bone. It is excreted into the bile so its elevation in serum occurs in hepatobiliary diseases (Burtis and Ashwood, 1986). The elevation of alkaline phosphatase indicates the disturbed excretory function of liver (Kothavade *et al.*, 1996). Alkaline phosphate is a non-specific tissue enzyme widely spread, mainly in the bones, liver and biliary canaliculi (Poole and Lesile, 1989; Ringler and Dabich, 1979).

In the present study, administration of rifampicin treated rats showed an increase in the activities of AST, ALT and ALP when compared with control rats. Oral administration of aqueous extract of BDEx (250 and 500 mg kg⁻¹ b. wt.) and silymarin to rifampicin treated rats showed an inhibition in the elevated activities of serum AST, ALT and ALP when compared with rifampicin alone treated

rats. Similarly administration of garlic to isoniazid and rifampicin treated rats showed significantly decrease the elevated activities of AST, ALT and ALP (Pal *et al.*, 2006). Lenaerts *et al.* (2005) have reported that elevated levels of serum hepatic marker enzymes were noticed in isoniazid, rifampicin and pyrazinamide treated mice. Administration of silymarin to rifampicin, isoniazid and pyrazinamide combination treated rats showed significantly inhibits the increased activities of AST, ALT and ALP (Tasduq *et al.*, 2005).

Determination of serum bilirubin represents an index for the assessment of hepatic function and any abnormal increase in the levels of bilirubin in the serum indicate hepatobiliary disease and severe disturbance of hepatocellular function (Martin and Friedman, 1992). In the present investigation, the rats treated with rifampicin showed significantly increased levels of bilirubin as compared to control rats. This result agreement induced hepatitis is characterised by increased levels of bilirubin in serum (Mitchell *et al.*, 1995; Rao and Mishra, 1996, 1997; Lenaerts *et al.*, 2005). Administration of BDEx (250 and 500 mg kg⁻¹ b. wt.) and silymarin to rifampicin treated rats showed decrease the increased bilirubin level when compared to rifampicin alone treated rats. The BDEx mediated reduction of the increased bilirubin level suggests the possibility of the extract being able to stabilise biliary dysfunction. Similarly administration of garlic to isoniazid and rifampicin treated rats showed significantly lowered bilirubin level (Pal *et al.*, 2006). Rao and Mishra (1998) have reported that administration of monomethylfumarate isolated from *Fumaria indica* to CCl₄, paracetamol and rifampicin treated rats showed significant inhibition of the elevated serum bilirubin. Administration of silymarin to rifampicin, isoniazid and pyrazinamide treated rats showed significant decline of the increased bilirubin level (Tasduq *et al.*, 2005). Buzzelli *et al.* (1993) reported that silymarin improved liver function tests related to hepatocellular necrosis and/or increases membrane permeability. Ramadan *et al.* (2002) reported that the protective effect of silymarin was attributed to its antioxidant and free radicals scavenging properties. Horvath *et al.* (2001) suggested that silibinin modulates the cellular immunoresponse and restores impaired liver function through its antioxidant capacity.

Lipids are the most important cellular entities which are not only the constituents of cell membrane but also involved in many cellular functions, metabolic processes and are vital for energy production. In the present study serum cholesterol was increased in rifampicin treated rats when compared to control rats. Any liver disease shows that an increased blood cholesterol level (McIntyre and Rosalki, 1992). The significant increase of serum cholesterol may be due to the inability of the liver to remove cholesterol from circulation. The major disorder encountered in antitubercular drugs induced hepatitis is fatty accumulation in the liver, which develops either due to excessive supply of lipids to the liver or interference with lipid deposition. The pathogenesis is multifactorial, reflecting complex biosynthetic, enzymatic and catabolic derangement in lipoprotein metabolism (Santhosh *et al.*, 2006). The abnormal cholesterol deposition is favoured by the dangerous tendency of cholesterol to passive exchange between the plasma lipoproteins and the cell membranes (Brown and Goldstein, 1986). Administration of BDEx (250 and 500 mg kg⁻¹ b. wt.) and silymarin to rifampicin treated rats showed that decrease the cholesterol content when compared to rifampicin alone treated rats. Similarly administration of chitosan (polysaccharide of marine origin is prepared from the shells of crustaceans) to antitubercular drugs treated rats showed decrease the elevated levels of cholesterol (Santhosh *et al.*, 2006). It was found that feeding of animals on silymarin-phospholipid complex normalized lipid metabolism and inhibited atherosclerosis (Horvath *et al.*, 2001).

Proteins are important organic constituents of the animal cells playing a vital role in the process of interactions between intra and extra cellular media. The depletion in the protein levels might be because of their metabolism to liberate energy during toxicity. The protein level was decreased due to the hepatotoxin intoxication. The reduction is attributed to the damage produced and localised in the endoplasmic reticulum which results in the loss of P₄₅₀ loading to its functional failure with a decrease in protein synthesis (Sureshkumar and Mishra, 2006). In the present study, serum protein level was

decreased in rifampicin-intoxicated rats when compared to control rats. Oral administration of BDEx (250 and 500 mg kg⁻¹ b. wt.) and silymarin to rifampicin treated rats showed increase the level of protein when compared to rifampicin alone treated rats.

CONCLUSIONS

From this study, conclusively state that *Boerhaavia diffusa* (BDEx) aqueous extracts has antihepatotoxic effects on serum hepatic marker enzyme activities and bilirubin level as well as improving cholesterol and protein due to rifampicin induced hepatotoxicity. Phytochemical constituents like flavonoids, tannin and beta-sitosterol are present in the plant BDEx. Flavonoids and tannin and also beta-sitosterol possessing antioxidant and antihypercholesterol properties, respectively. So these biomolecules may be involving minimize the formation of active metabolite, 25-desacetyl rifampin. The active metabolite, 25-desacetyl rifampin formed from rifampicin that may be responsible for liver damage. So the aforementioned active constituents like flavonoids, tannin and beta-sitosterol are responsible for the antihepatotoxic activity of BDEx and these plant extracts are ameliorate the antituberculosis drug induced hepatic problems. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antihepatotoxic effect of *Boerhaavia diffusa* (BDEx).

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