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Research Article

EFFECT OF *BOERHAAVIA DIFFUSA* AGAINST DIMETHYLNITROSAMINE INDUCED LIVER CIRRHOSIS

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ABSTRACT

Boerhaavia diffusa (Nyctaginaceae) has been used for the chronic liver disease. The worldwide use of *B. diffusa* roots to treat liver disorders was validated when researchers demonstrated, in 1980 and 1991, that its root extract had antihepatotoxic properties[1]. Roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain. In present study alcoholic and aqueous extract of whole plant of *Boerhaavia diffusa* given orally exhibited anticirrhosis activity against Dimethylnitrosamine induced liver cirrhosis in rat's model. The activity was assessed using Increases in life span (ILS), histopathological studies of liver, biochemical and hematological studies. The oral administration of EEBD & AEBD shows significant increase in the survival time (life span), a decrease in cirrhotic nodules. The biochemical and hematological parameters were also corrected by EEBD & AEBD in dimethylnitrosamine induced liver cirrhosis in rats. These observations are suggestive of the protective effect of EEBD & AEBD in dimethylnitrosamine induced cirrhosis in rats. However, out of these two extracts the anti-cirrhosis activity was maximally observed with the ethanol extract of *Boerhaavia diffusa* (EEBD) as compared to the aqueous extract of *Boerhaavia diffusa* (AEBD).

Keywords: *Boerhaavia diffusa*; Dimethylnitrosamine; Anticirrhosis; Antihepatotoxic, EEBD, AEBD.

INTRODUCTION

The approach to new drugs through natural products has proved to be the single most successful strategy for the discovery of new drugs[2]. Many herbal remedies have been employed in various medical systems for the treatment and management of different liver diseases. However, most of the drugs showed limited efficacies due to the development of various side effects. This fostered our attempts to evaluate some plant products against cirrhosis as they are less likely to cause serious side effects. Many Indian spices and plants are quoted to be useful in different types of liver diseases.

Boerhaavia diffusa, commonly known as "Punarnava" in Sanskrit, is an herbaceous plant of the family Nyctaginaceae[3,4]. *Boerhaavia diffusa* is indigenous to India; it is found throughout the warmer parts of the country up to an altitude of 2000 m in the Himalayan region. It grows well on wastelands and in fields after the rainy season[5,6]. Different parts of the *Boerhaavia diffusa* plant have been widely used by indigenous tribes in the traditional system of medicine. In India, number of tribes uses the roots of this plant to treat liver ailments. A decoction of whole plant is taken with milk in early morning to cure jaundice and weakness by tribes of south India. The tribal population in south Garhwal used the whole plant in the treatment of liver enlargement[7]. The roots have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain, abdominal tumours, and cancers[8].

A vast literature collection fails to produce a scientific evidence to prove the anti-cirrhosis activity of *Boerhaavia diffusa*. Hence this study was planned to evaluate the effect of *Boerhaavia diffusa* against Dimethylnitrosamine (DMN) induced cirrhosis in rats.

MATERIALS AND METHODS

The plant *Boerhaavia diffusa* was collected from Algarkovil Temple, Madurai, Tamilnadu. This plant was authenticated by Department of Botany, The American College, Madurai.

The Male wistar albino rats weighing 150-200gms were selected for this study. [Approved by the institution animal ethical committee (Reg.No.KMCP/09/3-27)]. The rat's were housed in clean polypropylene cages having 6 rat's per cage and maintained under temperature controlled room (27±2° C) with photoperiod of 12h light and 12h dark cycle. The animals were fed with commercially available food pellet diet and water *ad libitum*.

Preparation of Drug

The shade dried plant leaves of *Boerhaavia diffusa* was powdered coarsely and about 200g of plant powder was extracted (soxhlet) with 70% ethyl alcohol and aqueous for 72h. The extract was dried in vacuum and resuspended in water before use. The Phytochemical screening proves the presence of flavonoids, alkaloids, steroids, triterpenoids, lipids, carbohydrates, proteins, and glycoprotein's9.

Effect against DMN induced cirrhosis

Animals were divided into five group's viz. G1, G2, G3, G4 and G5 of six each. For comparison, G1 designated as normal control group was used which was neither injected with DMN nor treated with EEBD and AEBD. To induce Liver cirrhosis, DMN dissolved in sterile saline was intraperitoneally injected (10 µl/kg) to rats three times per week for 3 week, and then on the fourth week, the rats were subjected to three consecutive daily DMN injections and housed for 5 days without further treatment. Cirrhotic rats were randomly distributed to four groups (n=6 per treatment group). As the group G2 was reserved as cirrhosis control, it was not treated with EEBD and AEBD. Group G3 served as the positive control, was treated with 50mg/Kg of Silymarin dissolved in 0.05% carboxy methylcellulose by oral route[10]. Group G4 & G5 was treated with AEBD and EEBD orally[11]. The treatment was continued for 28 days. The mortality and body weight were monitored during the 4 week of treatment. Surviving animals were sacrificed on day 29 & following parameters were estimated,

1. Derived parameter {Body weight & Life span (%)}
2. Hepatic morphology was assessed by light microscopy.

Determination of hematological parameters

Apart from above mentioned parameters, the effect of EEBD and AEBD on hematological parameters was also studied in the rats of all groups. Blood was collected from the all rat in the groups by puncturing retro-orbital plexus and counted for RBC, WBC, Platelets and Haemoglobin.

Blood chemistry

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol, bilirubin, total proteins, and albumin in plasma were analyzed using Spectrum, an automatic blood chemistry analyzer.

Statistical analysis

The results are expressed as mean ± SEM. The evaluation of the data was done using one way ANOVA followed by Newman – Keul's multiple range tests. Difference below P<0.05 implied significance.

RESULT

Liver cirrhosis is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury[12]. Dimethylnitrosamine (DMN) induced liver cirrhosis in rat is a well established, reproducible model and has several similarities with human liver cirrhosis[13].

Anticirrhosis effects of *Boerhaavia diffusa* against DMN

Table 1 shows that large fraction of vehicle-treated cirrhotic rats died within the first 3 wk. EEBD and AEBD treatment (orally for 4 wk) improved the survival rate of these rats to 75% and 65% respectively on day 28 compared with 48% in vehicle-treated cirrhotic animals.

However the average life span of standard drug SILYMOL treatment was found to be 85%. Rats treated with EEBD and AEBD had significantly greater body weight gain on day 28 than that of vehicle-treated ones.

Table 1: Effect of B. Diffusa on the life span and body weight of cirrhosis induced rats.

Groups	% ILS Life span	Body Wt.(gms) on 30th day	Body Wt.(gms) on 58th day
Control	>>60 days	216.08±2.7	219.78±1.02
Cirrhotic Control	48%	194.28±4.8^{a**}	192.98±3.2^{a**}
Standard	85%	194.95±3.84 ^{a**}	213.06±1.24^{b**}
Treatment (AEBD)	65%	197.35±1.7^{a**}	206.48±1.63^{b**}
Treatment (EEBD)	75%	199.38±5.04^{a**}	209.16±1.47^{b**}

All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1). **b – Values are significantly different from cirrhotic control (G_2). *P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests. EEBD- Ethanolic extract of *Boerhaavia diffusa*. AEBD- Aqueous extract of *Boerhaavia diffusa*.

Table 2 shows that the level of serum albumin is regarded as an important index of liver function. The decreased synthesis of albumin in the liver accompanies edema and ascites formation. The plasma albumin, which was significantly decreased to 48% of control in cirrhotic rats, was restored by EEBD and AEBD to 92% and to 80% respectively of healthy control animals.

In the present study, ALT and AST activities were elevated to certain extents in cirrhotic rats. The plasma AST activity was significantly decreased by 60-65% and 40% with EEBD and AEBD treatment respectively.

The total bilirubin content was assessed as representative index for the liver function[14]. Table 2 shows DMN caused an 8-fold increase in the bilirubin content, relative to control, whereas EEBD and AEBD almost completely prevented an increase in the plasma total bilirubin by DMN.

Table 2 shows that treatments with DMN caused 40% to 48% decreases in the total plasma protein and albumin contents, which were restored to 80% to 90% by EEBD and in fewer amounts by AEBD. There is rise in total serum cholesterol in Cholestasis, probably due to retention of cholesterol which is normally excreted in bile. In this study cholesterol level of cirrhotic group raises two folds (165.43±5.66) relative to control group EEBD and AEBD decrease the level of cholesterol by 60% at 40% respectively.

Table 3 shows RBC, Hb, platelets were decreased and WBC count was significantly increased in the cirrhotic control group compared to the normal control group. Treatment with EEBD and AEBD significantly increased the RBC, Hb, platelets and significantly decreased the WBC count to near standard level. All these results suggest the effective nature of *Boerhaavia diffusa.*

Table 2: Effect of *Boerhaavia diffusa*. on serum Enzymes and lipid proteins

Groups	Total Proteins (g %)	Albumin (g /dl)	AST (U/L)	ALT (U/L)	Bilirubin (mg %)	Cholesterol (mg/dl)
Control	7.18 ±0.16	4.46 ±0.19	145.6 ±1.57	78.61±3.62	0.65 ±0.08	85.08 ±11.7
Cirrhotic Control	4.05 ±0.15**	2.18±0.14^{a**}	278.25 ±1.62^{a**}	179.9±4.04^{a**}	4.87 ±0.12^{a**}	165.43 ±5.66^{a**}
Standard	6.21 ±0.17^{b**}	3.60 ±0.10^{b**}	191.16 ±2.83^{b**}	103.4 ±2.72^{b**}	0.91±0.23^{b**}	118.68±0.85^{b**}
Treatment (AEBD)	5.5 ±0.07^{b**}	3.90 ±0.08^{b**}	218.6 ±1.73^{b**}	117.65±2.57^{b**}	2.78 ±0.25^{b**}	133.05±1.31^{b**}
Treatment (EEBD)	5.85 ±0.22**	4.25 ±0.06^{b**}	199.10 ±3.76^{b**}	110.11±1.33^{b**}	1.74 ±0.11^{b**}	125.91±1.40^{b**}

All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1). **b – Values are significantly different from cirrhotic control (G_2). *P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests. EEBD- Ethanolic extract of *Boerhaavia diffusa*. AEBD- Aqueous extract of *Boerhaavia diffusa*.

Table 3: Effect of *Boerhaavia diffusa* on Hematological Parameters

Groups	RBC (millions/mm^3)	WBC (cells/mm^3)	Hemoglobin (g/dl)	Platelets (lakhs/mm^3)
Control	8.10 ±0.10	11.80±0.18	16.46 ±1.57	1.13±0.03
Cirrhotic Control	5.05 ±0.14**	14.66±0.26^{a**}	10.15±0.14^{a**}	0.93±0.03^{a**}
Standard	7.16 ±0.12^{b**}	12.36±0.07^{b**}	14.65±0.31^{b**}	0.99 ±0.01^{b**}
Treatment (AEBD)	6.56 ±0.12^{b**}	12.80±0.14^{b**}	13.65±0.20^{b**}	0.95±0.01^{b**}
Treatment (EEBD)	6.70 ±0.18**	12.51±0.10^{b**}	14.13 ±0.10^{b**}	0.97±0.01^{b**}

All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1). **b – Values are significantly different from cirrhotic control (G_2). *P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests. EEBD- Ethanolic extract of *Boerhaavia diffusa*. AEBD- Aqueous extract of *Boerhaavia diffusa*.

Histopathological analysis

Dimethylnitrosamine (DMN) exerts hepatotoxic and carcinogenic effects in animals, and induces hepatic necrosis and subsequent fibrosis probably through metabolic activation by cytochrome P450 2E1. EEBD and AEBD markedly reduced the number of cirrhotic nodules and the staining intensities of nodular capsules[15].

To determine whether cirrhosis could be treated with *Boerhaavia diffusa*, we histopathologically examined the formation of cirrhotic nodules, extent of liver fibrosis, intralobular hepatocytes degeneration, and portal inflammation of surviving cirrhotic rats after 4 wk of vehicle or drug treatment.

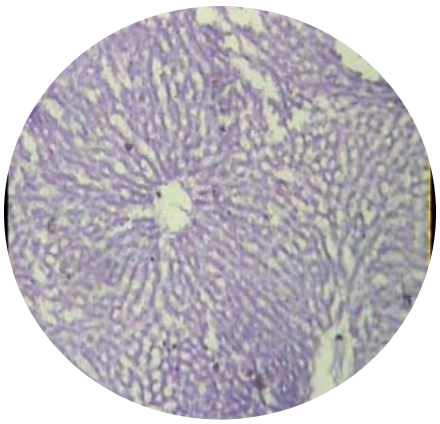

Fig. 1: (Normal control) It shows of liver parenchyma with central vein and radiating column of hepatocytes. Portal tracts appear normal. No evidance of cirrhosis seen

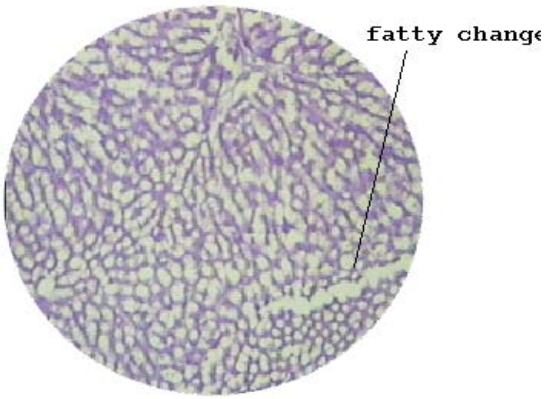

Fig. 2: (Cirrhotic control) It shows Section shows liver parenchyma with foci showing fatty change. This indicates liver cirrhosis occurrence. Masson's trichrome staining revealed that extracellular matrix was heavily accumulated around and within thick multiple fibrotic nodules, particularly in proximity to portal spaces in the liver of cirrhotic rats. DMN treatment caused thick multiple fibrotic nodules

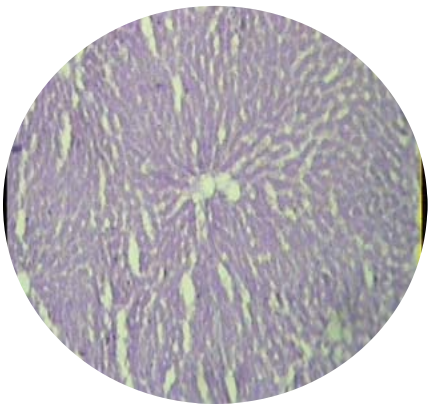

Fig. 3: Treatment of cirrhotic rats with 50 mg/kg of silymarin for 4 wk almost disappeared of liver fibrotic nodules. Section shows parenchyma of liver with central vein and radiating column of hepatocytes also portal tracts appear normal as compared to cirrhotic control group

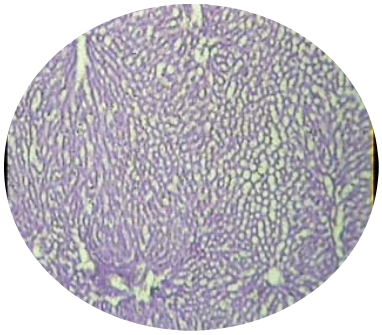

Fig. 4: It shows treatment of cirrhotic rats with AEBD for 4 wk notably decreased the intensities of liver fibrotic nodules

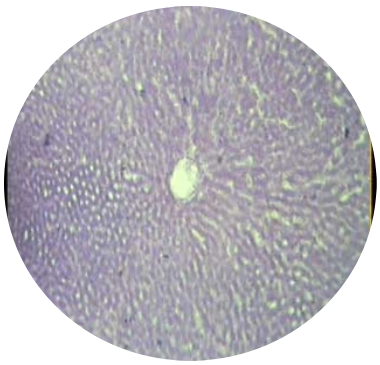

Fig. 5: It shows Liver fibrotic nodules completely disappeared after EEBD treatment. Only marginal fibrous bands were detected

In Table 4 anti-cirrhotic effects were further supported by decreases in Knodell score a general marker of LC and inflammation[15].

Table 4: Fibrosis and Knodell scores in the livers of rats

Groups	Fibrosis Score	Knodell Score
Control	0	0
Cirrhotic Control	4.8±0.41**a	18.5±2.8**a
Standard	2.7±0.58**b	9.5±1.6**b
Treatment (AEBD)	3.8±0.51**b	14.1±1.8**b
Treatment (EEBD)	3.1±0.67**b	11.9±1.4**b

All values are expressed as mean ± SEM for 6 animals in each group. **a – Values are significantly different from control (G_1). **b – Values are significantly different from cirrhotic control (G_2). *P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests. EEBD- Ethanolic extract of *Boerhaavia diffusa*. AEBD- Aqueous extract of *Boerhaavia diffusa*.

DISCUSSION

Plants have served as a good source of anti-cirrhosis agents, several studies have been conducted on herbs under a multitude of ethanobotanical grounds. A large number of plants possessing anti-cirrhotic properties have been documented[16].

Laboratory cirrhotic rats produced by DMN administrations simulate the clinical features of human LC such as mortality, ascites, hepatic parenchymal cell destruction, formation of connective tissue, and nodular regeneration, providing a preclinical model to evaluate therapeutic efficacy of drug and underlying mechanism. In this study treatment with *Boerhaavia diffusa* markedly reduced the number of cirrhotic nodules and the staining intensities of nodular capsules. Anticirrhotic effects were further supported by decreases in Knodell score, a general marker of LC and inflammation[17].

Treatment with *Boerhaavia diffusa* effectively increases the life span of cirrhotic rats as well as it prevents the loss of body weight compare to cirrhotic control group. The decreased synthesis of albumin was restored in present study. Hence, *Boerhaavia diffusa* improved liver function.

The plasma transaminase activity is increased with biliary obstruction in cirrhotic patients[18]. In the present study, the plasma AST and ALT activity in treatment control group was decreased significantly.

We monitored the plasma total bilirubin content as a liver function test. Our treatment prevented an increase in the total plasma bilirubin level induced by DMN, which represented the protective efficacy against DMN-induced liver injury.

Also this treatment was active in restoring the total plasma proteins and albumin contents in rats treated with DMN over a 4-week period.

The reversal of Hb content, RBC, Platelets and WBC by the present treatment towards the value of the normal group clearly indicate that *Boerhaavia diffusa* possessed protective action on the haemopiotic systems.

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