



## Effect of *Berberis aristata* DC. Against Dimethylnitrosamine Induced Liver Cirrhosis in Rat Model.

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### ABSTRACT

*Berberis aristata* is an edible plant employed in the South Asian Traditional Medicine, particularly its fruits and stem being used as a tonic remedy for liver and heart. *Berberis aristata* exhaustively used by industries and in ayurvedic system of medicine. In present study ethanolic (EEBA) and aqueous extract (AEBA) of whole plant of *Berberis aristata* DC. (Berberidaceae) was analysed for its anti-cirrhosis 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 EEBA shows significant increase in the survival time (life span), a decrease in cirrhotic nodules. The biochemical and hematological parameters were also corrected by EEBA in dimethylnitrosamine induced liver cirrhosis in rats. These observations are suggestive of the protective effect of EEBA and AEBA in dimethylnitrosamine induced cirrhosis in rats. The result obtained were compared with silymarin (100mg/kg; p.o), the standard drug. In conclusion EEBA and AEBA at (200mg/kg, p.o) showed significant anti-cirrhosis activity similar to that standard drug, silymarin. However we observed that ethanolic extract of *Berberis Aristata* is more effective than aqueous extract.

**Key words:** *Berberis aristata*; Dimethylnitrosamine; Anticirrhosis; EEBA, AEBA, Silymarin.

### INTRODUCTION

Hepatic disorder is one of the major causes of death among the adult population globally. Evidence has accumulated that cell death is involved in liver injury and liver diseases. Apoptosis and necrosis underlie many types of liver injury, including fibrosis, alcoholic liver disease and hepatitis<sup>1</sup>. Liver cirrhosis is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury. Scar tissue replaces healthy liver tissue, partially blocking the flow of blood through the liver<sup>2</sup>. Dimethylnitrosamine through metabolic activation by cytochrome P450 2E1 exerts hepatotoxicity and tissue injury. Liver injuries induced by multiple DMN treatments lead to hepatic necrosis, fibrosis, and eventually cirrhosis<sup>3</sup>. In the absence of reliable liver protective drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorder and quite often claimed to offer significant relief. Attempts are being made globally to get scientific evidence for these traditionally reported herbal drugs. 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.

*Berberis aristata* DC (Berberidaceae) known as 'daruharidra' is an evergreen, spinescent shrub with known antichlamydia, antiplatelet, antimicrobial and hepatoprotective activity<sup>4</sup>. *Berberis aristata* an edible plant and the main source of berberine, is indigenous to Indo-Pak subcontinent and has been traditionally used in liver damage<sup>5</sup>. Berberine is a well known alkaloid from *Berberis* species. The compound has been extensively studied and is known to exhibit multiple pharmacological activities, such as antiprotozoal, antihypertensive, choleric, antitumor and antibacterial. Moreover, anti-inflammatory, cardiogenic, anticholinergic, antiarrhythmic, antiplatelet and anti-HIV activities have also been reported. In earlier studies, we demonstrated that this folk medical use had scientifically justified basis, as the crude extract of *B. aristata* leaves and fruits showed hepatoprotection possibly through inhibitory action on hepatic drug metabolizing enzymes<sup>6</sup>.

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

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### 2. MATERIALS AND METHODS

The plant *Berberis aristata* 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*.

#### 2.1 Preparation of Drug

The shade dried whole plant of *Berberis aristata* was powdered coarsely and about 300g of plant powder was extracted (soxhlet) with 70% ethyl alcohol for 72h. The extract was dried in vacuum and resuspended in water before use. The Phytochemical screening proves the presence of alkaloids (berberine, berbamine and oxycanthine), tannins, gum and resin<sup>7</sup>.

#### 2.2 Effect against DMN induced cirrhosis

Animals were divided into four 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 EEBA and AEBA. 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 cirrhotic control, it was not treated with EEBA and AEBA. Group G3 served as the positive control, was treated with 100mg/Kg of Silymarin dissolved in 0.05% carboxy methylcellulose by oral route<sup>8</sup>. Group G4 was treated with EEBA orally in dose of 200mg/kg body weight twice daily and group G5 treated with AEBA at same dose<sup>9,10</sup>. 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.

#### 2.3 Determination of hematological parameters

Apart from above mentioned parameters, the effect of EEBA and AEBA 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.

#### 2.4 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.

2.5 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.

3. RESULT

Liver cirrhosis is a condition in which the liver slowly deteriorates and malfunctions due to chronic injury. Dimethylnitrosamine (DMN) induced liver cirrhosis in rat is a well established, reproducible model and has several similarities with human liver cirrhosis<sup>11</sup>.

Effects of *Berberis Aristata* on hepatotoxicity and cirrhosis induced by DMN

The prepared formulation were subjected to toxicity study and were found to be safe up to daily dose of 3000 mg/kg of body wt. in rats of either sex with no toxic reaction being observed. The body weight of the experimental animals was monitored throughout the study. The DMN administered animals did not gain body weight during the course of treatment. A significant decrease (P<0.05) was noticed in the mean body weight of the animals on day 28 after the start of DMN administration. Also large fraction of vehicle-treated cirrhotic rats died within the first 3 wk. Tables 1 represent the EEBA and AEBA treatment (orally for 4 wk) improved the survival rate of these rats on day 28 compared with vehicle-treated cirrhotic animals. However the average life span of standard drug Silymarin treatment was found to be 85%. Rats treated with EEBA and AEBA had significantly greater body weight gain on day 28 than that of vehicle-treated ones.

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

Groups	% ILS Life span	Body Wt.(gms) on 28 <sup>th</sup> day	Body Wt.(gms) on 58 <sup>th</sup> day
Control	>>60 days	212.08±2.7	215.78±1.02
Cirrhotic Control	48%	190.28±4.8 <sup>**a</sup>	188.98±3.2 <sup>**a</sup>
Standard	85%	192.95±3.84 <sup>**a</sup>	214.06±1.24 <sup>b**</sup>
Treatment (AEBA)	65%	191.35±1.7 <sup>**a</sup>	211.48±1.63 <sup>b**</sup>
Treatment (EEBA)	75%	192.38±5.04 <sup>**a</sup>	209.16±1.47 <sup>b**</sup>

All values are expressed as mean ± SEM for 6 animals in each group. <sup>\*\*a</sup> – Values are significantly different from control (G1). <sup>\*\*b</sup> – Values are significantly different from cirrhotic control (G2). \*P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul’s multiple range tests. EEBA- Ethanolic extract of *Berberis aristata*. AEBA- Aqueous extract of *Berberis aristata*.

For assessment of anticirrhosis activity, the degree of cirrhosis developed was determined by withdrawing blood and evaluating different parameters on 28<sup>th</sup> day, the elevated levels of ALT, AST, Cholesterol, Bilirubin and decreased level of Total protein and albumin indicates the cirrhosis. The increased level of ALT, AST, Cholesterol and bilirubin is conventional indicator of liver injury<sup>12</sup>. Also productions of cirrhotic nodules in liver of rats of toxic control group indicate the cirrhosis<sup>13</sup>. Table 2 shows that EEBA and AEBA treatment attenuated the elevation of AST, ALT, Cholesterol and Bilirubin activities in the rats treated with DMN. Changes in total protein and albumin levels were blocked completely.

Table 2: Effect of *B. Aristata* on the serum Enzymes and lipid proteins.

Groups	Total Proteins (g %)	Albumin (g/dl)	AST (U/L)	ALT (U/L)	Bilirubin (mg %)	Cholesterol (mg/dl)
Control	9.11 ±0.16	5.21 ±0.17	142.6 ±1.47	86.16±2.42	0.79 ±0.06	89.05 ±15.1
Cirrhotic Control	5.15±0.17 <sup>**a</sup>	2.85±0.11 <sup>**a</sup>	272.25 ±1.41 <sup>**a</sup>	182.1±3.14 <sup>**a</sup>	4.65 ±0.17 <sup>**a</sup>	172.42 ±4.76 <sup>**a</sup>
Standard	8.01 ±0.17 <sup>b**</sup>	4.50 ±0.11 <sup>b**</sup>	184.12 ±2.64 <sup>b**</sup>	101.9 ±2.72 <sup>b**</sup>	0.96±0.17 <sup>b**</sup>	115.18±0.55 <sup>b**</sup>
Treatment (AEBA)	6.35 ±0.07 <sup>b**</sup>	4.05 ±0.08 <sup>b**</sup>	210.6 ±1.13 <sup>b**</sup>	115.15±2.17 <sup>b**</sup>	2.04 ±0.25 <sup>b**</sup>	135.65±1.81 <sup>b**</sup>
Treatment (EEBA)	7.25 ±0.17 <sup>b**</sup>	4.40 ±0.06 <sup>b**</sup>	192.10 ±3.67 <sup>b**</sup>	108.19±1.33 <sup>b**</sup>	1.25 ±0.11 <sup>b**</sup>	121.43±1.51 <sup>b**</sup>

All values are expressed as mean ± SEM for 6 animals in each group. <sup>\*\*a</sup> – Values are significantly different from control (G1). <sup>\*\*b</sup> – Values are significantly different from cirrhotic control (G2). \*P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul’s multiple range tests. EEBA- Ethanolic extract of *Berberis aristata*. AEBA- Aqueous extract of *Berberis aristata*.

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 EEBA and AEBA significantly increased the RBC, Hb, platelets and significantly decreased the WBC count to near standard level. All these results suggest the effective nature of *Berberis Aristata*.

Table 3: Effect of *B. Aristata* on the Hematological Parameters.

Groups	RBC (millions/mm <sup>3</sup> )	WBC (cells/mm <sup>3</sup> )	Hemoglobin (g/dl)	Platelets (laks/mm <sup>3</sup> )
Control	9.25 ±0.20	12.10±0.18	16.46 ±1.57	1.13±0.03
Cirrhotic Control	6.15±0.12 <sup>**a</sup>	14.66±0.26 <sup>**a</sup>	10.15±0.14 <sup>**a</sup>	0.93±0.03 <sup>**a</sup>
Standard	8.19 ±0.11 <sup>b**</sup>	12.61±0.07 <sup>b**</sup>	14.65±0.31 <sup>b**</sup>	0.99 ±0.01 <sup>b**</sup>
Treatment (AEBA)	7.16 ±0.12 <sup>**a</sup>	13.52±0.14 <sup>**a</sup>	13.65±0.20 <sup>**a</sup>	0.95±0.01 <sup>b**</sup>
Treatment (EEBA)	7.52 ±0.17 <sup>**a</sup>	12.96±0.10 <sup>**a</sup>	14.13 ±0.10 <sup>b**</sup>	0.97±0.01 <sup>b**</sup>

All values are expressed as mean ± SEM for 6 animals in each group. <sup>\*\*a</sup> – Values are significantly different from control (G1). <sup>\*\*b</sup> – Values are significantly different from cirrhotic control (G2). \*P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul’s multiple range tests. EEBA- Ethanolic extract of *Berberis aristata*. AEBA- Aqueous extract of *Berberis aristata*.

Histopathological analysis for hepatic cirrhosis

Dimethylnitrosamine (DMN) exerts hepatotoxic and carcinogenic effects in animals, and induces hepatic necrosis and subsequent fibrosis probably through metabolic activation by cytochrome P450 2E1<sup>14</sup>. EEBA and AEBA markedly reduced the number of cirrhotic nodules and the staining intensities of nodular capsules.

To determine whether cirrhosis could be treated with *Berberis Aristata*, 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.

Histopathological photomicrograph of rat liver from normal control showed normal architecture the intact hepatic cords exhibiting healthy radiating column of hepatocytes and portal tract appearing normal (Fig 1). DMN (G2) treated

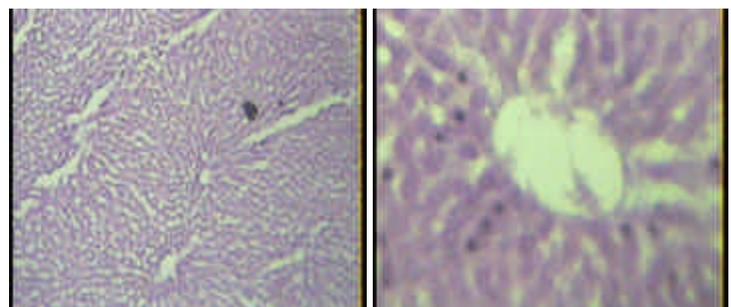


Fig.1. Normal liver intact hepatic cords exhibiting healthy hepatocytes

Fig. 2. CC14 control Micro to macro vesicular fatty changes in the cytoplasm of hepatocytes

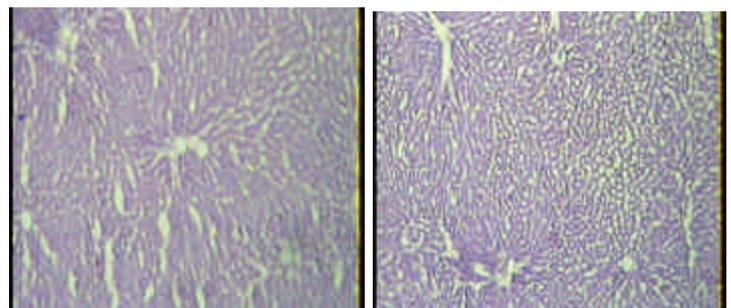


Fig.3. Standard control shows Portal tracts appear normal.

Fig. 4. AEBA control shows mild congestion and haemosiderin pigments.

rats showed, the micro to macro vesicular fatty changes in the cytoplasm of hepatocytes (Fig-2).

Silymarin treated group almost disappeared of liver fibrotic nodules. Standard control section shows parenchyma of liver with central vein and also portal tracts appear normal as compared to cirrhotic control group (Fig-3). AEBA treated group shows mild congestion and haemosiderin pigments (Fig-4). Fig- 5 shows liver fibrotic nodules completely disappeared after EEBA treatment. Only marginal fibrous

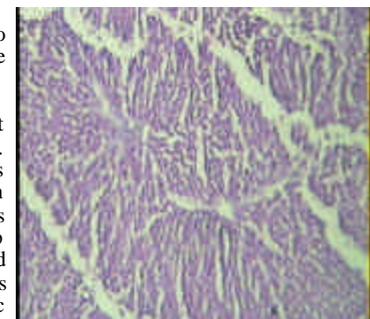


Fig.5. EEBA control shows nodules completely disappeared.

bands were detected. Table 4 anti-cirrhotic effects were further supported by decreases in Knodell score a general marker of LC and inflammation<sup>15</sup>.

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

Groups	Fibrosis score	Knodell score
Control	0	0
Cirrhotic Control	5.18±0.41**a	20.15±2.8**a
Standard	2.55±0.58**b	9.89±1.6**b
Treatment (AEBA)	3.45±0.51**b	15.22±1.8**b
Treatment (EEBA)	2.97±0.67**b	12.49±1.4**b

All values are expressed as mean ± SEM for 6 animals in each group. \*\*a – Values are significantly different from control (G1). \*\*b – Values are significantly different from cirrhotic control (G2). \*P (<0.05). All values are found out by using one way ANOVA followed by Newman Keul's multiple range tests. EEBA- Ethanolic extract of *Berberis aristata*. AEBA- Aqueous extract of *Berberis aristata*.

## DISCUSSION

Dimethylnitrosamine-induced hepatic fibrosis in rats is a reproducible and potentially valuable animal model for studying the pathogenesis of human hepatic fibrosis and cirrhosis. The 28-day course of DMN administration in rats produced centrilobular necrosis and well-developed fibrosis, as present in alcoholic liver diseases. The model has been evaluated previously and demonstrated that it is an excellent animal model for studying the biochemical and pathophysiological as well as molecular alterations associated with the development of hepatic fibrosis and cirrhosis<sup>16, 17</sup>.

Plants have served as a good source of anti-cirrhosis agents, several studies have been conducted on herbs under a multitude of ethnobotanical grounds. A large number of plants possessing anti-cirrhotic properties have been documented<sup>18</sup>.

In this study treatment with *Berberis Aristata* 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.

Treatment with *Berberis Aristata* 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, which resulted in edema and ascites formation in liver cirrhosis, was restored in present study<sup>18</sup>. Hence, *Berberis Aristata* improved liver function.

The plasma transaminase activity is increased with biliary obstruction in cirrhotic patients<sup>19</sup>. 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 of *Berberis Aristata* 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 *Berberis Aristata* possessed protective action on the haemopoietic systems.

However the EEBA have shown more anticirrhosis activity in DMN induced hepatic damage in rat's model as compared to AEBA. The biochemical and

histological studies supported its anticirrhosis properties in rats comparing with the standard drug Silymarin.

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