

HEPATOPROTECTIVE ACTIVITY OF ALCOHOLIC EXTRACTS OF *BOERHAAVIA DIFFUSA* AND *ANISOCHILUS CARNOSUS* AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

An alcoholic extract of stem and leaves of *Boerhaavia Diffusa* (AEBD) and leaves of *Anisochilus Carnosus* (AEAC) was studied for hepatoprotective activity against Carbon Tetrachloride (CCl₄) induced hepatotoxicity in rats. Hepatotoxicity was introduced in Albino rats of either sex by intraperitoneal injection of CCl₄ (in olive oil). Alcoholic extracts of AEBD and AEAC were administered to the experimental rats at the dose levels 150mg and 300mg/kg body weight, 200mg and 400mg/kg body weight of AEBD and AEAC respectively. The hepatoprotective effects of the extracts were evaluated by the assay of liver function biochemical parameters like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Alkaline Phosphatase (SALP), Total and Direct Serum Bilirubin. It was concluded that, the alcoholic extracts of AEBD and AEAC possess hepatoprotective activity against CCl₄ induced hepatotoxicity in rats.

Keywords: Hepatoprotective, Boerhaavia Diffusa, Anisochilus Carnosus, Carbon Tetrachloride.

INTRODUCTION

The liver weighs about 1.4kg (about 3lb) in the average adult. It is located under the diaphragm and occupies most of the right hypochondrium and part of the epigastrium of the abdomen. The liver is almost completely covered by peritoneum and completely covered by a dense connective tissue layer that lies beneath the peritoneum. It is divided into two principal lobes-a large right lobe and a smaller left lobe-separated by the falciform ligament. The right lobe is considered by many anatomists to consist of an inferior quadrate lobe and a posterior caudate lobe. However, on the basis of internal morphology primarily the distribution of blood, the quadrate and caudate lobes more appropriately belong to the left lobe. The falciform ligament is a reflection of the parietal peritoneum, which extends from the undersurface of the diaphragm to the superior surface of the liver, between two principal lobes of the liver. In the free border of the falciform ligament is the ligamentum teres. It extends from the liver to the umbilicus. The ligamentum teres is a fibrous cord derived from the umbilical vein of the fetus.

Bile, one of the liver's products, enters bile capillaries or canaliculi that empty into small ducts. These small ducts eventually merge to form the larger right and left hepatic ducts, which unite to leave the liver as the common hepatic duct. Further on, the common hepatic duct joins the cystic duct from the gallbladder. The two tubes become the common bile duct. The common bile duct and pancreatic duct enter the duodenum in a common duct called hepatopancreatic ampulla.

The liver performs many vital functions, many of which are related to metabolism. A battery of blood tests (liver nattery) is performed to evaluate the status of the liver. Such liver function tests (LFTs) include serum glutamic pyruvic transaminase (SGPT), alphafetoprotein (AFP), alkaline phosphatase, bilirubin, serum glutamic oxalacetic transaminase (SGOT), and lactic dehydrogenase (LDH) ¹.

Patients with severe liver disease characteristically show abnormal end-organ response to drugs. The liver has a large metabolic reserve, and it is only when disease becomes decompensate that important changes in drug handling occur. e.g. chronic viral or alcoholic liver disease, has more impact on hepatic drug metabolizing enzyme activity than primarily cholestatic conditions, e.g. primary biliary cirrhosis, although clearance of drugs eliminated mainly by biliary excretion will be impaired in the latter. Hepatocellular injury (toxic, infectious) leads to decreased activity of

drugs-metabolising enzymes, which is reflected in diminished plasma clearance of drugs that are metabolized.

If liver disease is stable and well compensated, prescribing of most drugs is safe. Particular care should attend evidence of: Impaired hepatic synthetic function, current or recent hepatic encephalopathy, fluid retention and/or renal impairment and drugs with high hepatic extraction, high plasma protein binding, low therapeutic ratio and CNS depressant effect. The spectrum of hepatic abnormalities caused by drugs is broad, and encompasses the whole range of liver lesions from other causes ².

Liver injury may be either acute or chronic. Acute liver injury may present with non-specific symptoms of fatigue and abnormal LFTs, or with jaundice and acute liver failure. Chronic liver injury is defined as hepatic injury, inflammation and/or fibrosis occurring in the liver for more than 6 months. In the early stages patients can be asymptomatic with abnormal LFTs. With more severe liver damage, however the presentation can be with jaundice, portal hypertension or other signs of cirrhosis.

Any cause of liver damage can produce acute liver failure, provided it is sufficiently severe. Acute viral hepatitis is the most common cause world-wide, whereas paracetamol toxicity is the most frequent cause in the UK. Acute liver failure occurs occasionally with other drugs, or from Amanita phalloides (mushroom) poisoning, in pregnancy, in Wilson's disease, following shock and, rarely, in extensive malignant disease of the liver. In 10% of cases the cause of acute liver failure remains unknown and these patients often labeled as having non-A-E viral hepatitis or cryptogenic acute liver failure ³.

Boerhaavia Diffusa belongs to the family Nyctaginaceae, which is commonly known as Horse-purslane, Hogweed and Pig weed in English. Atikimamidi, Atima mamidi, Punarnava in Telugu. It is a diffuse herb with stout root-stocks. Leaves are thick-chartaceous in unequal pairs, ovate or elliptic-oblong, subfleshy. Anthocarps club-shaped, 5-ribbed, glandular hairy, top rounded; seeds erect. It is commonly distributed weed along roadsides, fields and waste places throughout the Chittoor district of Andhra Pradesh, India. The whole plant is used for the treatment of jaundice, dyspnoea, constipation, arthritis, anaemia, cardiac diseases and liver diseases.

Anisochilus Carnosus belongs to the family Lamiaceae, which is commonly known as thick-leaved lavender in English. Saugudu ganapa, ritchu-rodha and karpuravalli in Telugu. It is an annual erect herb, stems quadrangular, sparsely pubescent, brownish from

prolonged exposure to sun. Leaves fleshy, broadly ovate, deeply crenate, obtuse or acute, base rounded, verrucose above, and pubescent beneath. It is commonly distributed in rock crevices on hills. On the way from Papanasam to Kumaradhara theertham (tirumala), dhanambanda area in Talakona. The whole plant used as diaphoretic, stimulant, expectorant, liver disorders, cough and cold. Leaf used for cough, dropsy, indigestion and sores in the leg fingers ⁴.

Antibacterial activity of *Boerhaavia Diffusa* L. leaves⁵, Chemopreventive action of *Boerhaavia Diffusa* on DMBA-induced skin carcinogenesis in mice⁶, anti-ulcer activity of *Anisochilus Carnosus* leaf extract in pylorus ligated rats⁷ has been reported. A detailed literature reviews indicated that, the hepatoprotective activity of stem and leaves of *Boerhaavia Diffusa* and leaves of *Anisochilus Carnosus* has not been clinically evaluated so far. In the present study, the hepatoprotective activity of alcoholic extracts of *Boerhaavia Diffusa* and *Anisochilus Carnosus* against CCl₄ induced hepatotoxicity in rats is reported.

MATERIALS AND METHODS

Plant material

The stem and leaves of *Boerhaavia Diffusa* and leaves of *Anisochilus Carnosus* were collected from Sri Venkateswara University campus, Tirumala gardens of Chittoor district of Andhra Pradesh and the same were authenticated by Assistant Professor, Dr.K.Madhava Chetty, Department of Botany, S.V. University, Tirupati, AP. Voucher specimens were deposited at department of pharmacognosy for further reference.

Extraction and Phytochemical screening

The shade dried plant materials were reduced to moderately coarse powder and extracted successively with alcohol using Soxhlet apparatus after defatting. The prepared extracts were subjected to identify the presence of various phytoconstituents^{8,9}.

Experimental animals

Wistar albino rats of either sex weighing between 200-250gm were obtained from Venkateswara Enterprises, Bangalore, Karnataka, India. The animals were housed under standard environmental conditions, one week before the test and also during the experiment as per the rules and regulations of the Institutional animal ethics committee. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional animal ethical committee (1423/PO/a/11/CPCSEA).

Acute toxicity studies

Acute toxicity studies were performed for the extracts of AEBD and AEAC as per stair case method¹⁰. For the hepatoprotective studies, the amount of dose administered were adjusted on the basis of observation during the toxicity studies.

CCl₄ Induced hepatotoxicity

The entire animals were fasted over night and administered with respective drugs as per the mentioned dosage schedule. Animals were divided into seven groups of six rats in each group.

Group I: Normal control animals received 2ml/kg of 1%NaCMC p.o.

Group II: Toxic control animals received 0.7ml/kg of CCl₄ in a 50% olive oil solution i.p. once daily for 7 days.

Group III: Drug control animals received simultaneously 1ml/kg of Liv-52 p.o. and 0.7ml/kg of CCl₄ i.p. once daily for 7 days.

Group IV: Treated animals received simultaneously AEBD 150mg/kg in 1% NaCMC p.o. and 0.7ml/kg of CCl₄ i.p. once daily for 7 days.

Group V: Treated animals received simultaneously AEBD 300mg/kg in 1% NaCMC p.o. and 0.7ml/kg of CCl₄ i.p. once daily for 7 days.

Group VI: Treated animals received simultaneously AEAC 200mg/kg in 1% NaCMC p.o. and 0.7ml/kg of CCl₄ i.p. once daily for 7 days.

Group VII: Treated animals received simultaneously AEAC 400mg/kg in 1% NaCMC p.o. and 0.7ml/kg of CCl₄ i.p. once daily for 7 days.

On the 8th day, the animals were euthanized by decapitation under ether anaesthesia and blood was collected from retro-orbital and allowed to a lot for 45mins at room temperature. Serum was separated by centrifugation and subjected to various biochemical estimations of SGOT, SGPT, SALP, Serum total bilirubin and direct bilirubin¹¹⁻¹³.

STATISTICAL ANALYSIS

Experimental results were expressed as mean±SEM (n=6). Statistical analysis was performed with one way ANOVA.

RESULTS AND DISCUSSION

The comparative efficacy of the extracts tested for their hepatoprotective activity along with the relationship between the dose, which are depicted in table-1 and in the form of a bar diagram in figure-1&2. In group II animals, Carbon tetrachloride intoxication in normal rats produced elevated levels of serum biochemical parameters as follows: SGOT (292.57±3.12), SGPT (257.70±2.18), SALP (251.33±1.99), Total bilirubin (2.03±0.04) and Direct bilirubin (1.59±0.07) compare to control group I animals having SGOT (154.77±2.31), SGPT (95.90±1.66), SALP (187.12±1.98), Total bilirubin (0.83±0.05) and Direct bilirubin (0.19±0.03) indicating acute hepatocellular damage and biliary obstruction.

Table 1: Effect of AEBD and AEAC on biochemical estimation of SGOT, SGPT, SALP, Total Bilirubin and Direct Bilirubin.

Groups	SGOT (IU/L)	SGPT (IU/L)	SALP (IU/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
I Normal Control	154.77±2.31	95.90±1.66	187.12±1.98	0.83±0.05	0.19±0.03
II Toxic Control	292.57±3.12	257.70±2.18	251.33±1.99	2.03±0.04	1.59±0.07
III Standard Liv-52	193.03±3.15**	115.80±1.95**	189.73±2.94**	0.87±0.06**	0.29±0.05**
IV AEBD 150mg/kg	277.60±3.40*	241.92±2.84*	240.20±2.71*	1.39±0.23*	1.20±0.20
V AEBD 300mg/kg	218.87±5.12**	122.80±4.72**	199.68±3.63**	1.15±0.17**	0.55±0.08**
VI AEAC 200mg/kg	270.33±3.34**	241.65±2.63*	234.90±2.75**	1.36±0.11*	1.12±0.16*
VII AEAC 400mg/kg	201.50±4.33**	124.43±4.95**	196.25±2.45**	1.09±0.14**	0.43±0.04**

Each value represents the mean±SEM, n=6. * p<0.05, ** p<0.01, Statistical significant test for comparison was done by ANOVA. Groups III to VII are compared with group II.

When compared to the CCl₄ toxic control group, the group treated with AEBD at doses 150&300 mg/kg in CCl₄ intoxicated rats exhibited a significant reduction of SGOT (277.60±3.40&218.87±5.12), SGPT (241.92±2.84&122.80±4.72), SALP (240.2±2.71&199.68±3.63), Total bilirubin (1.39±0.23&1.15±0.17) and Direct bilirubin (1.20±0.20&0.55±0.08) levels. When compared to the CCl₄ toxic control group, the group

treated with AEAC at doses of 200&400 mg/kg in CCl₄ intoxicated rats exhibited a significant reduction of SGOT (270.33±3.34&201.50±4.33), SGPT (241.65±2.63&124.43±4.95), SALP (234.9±2.75&196.25±2.45), Total bilirubin (1.36±0.11&1.09±0.14) and Direct bilirubin (1.12±0.16&0.43±0.04) levels.

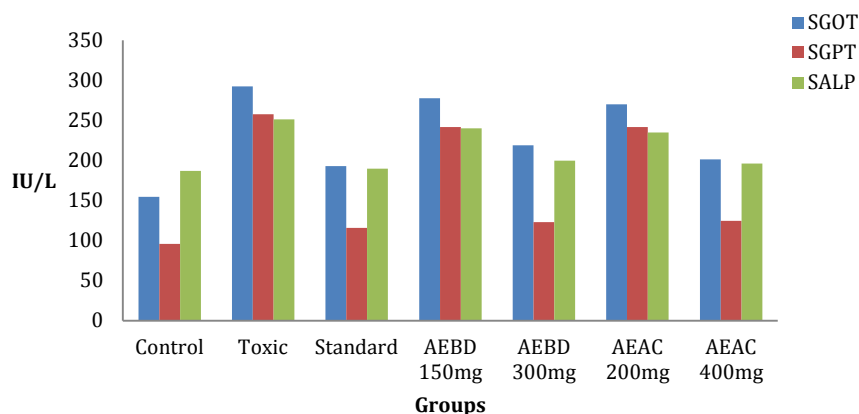


Figure 1: Effect of AEBD and AEAC on Biochemical Parameters

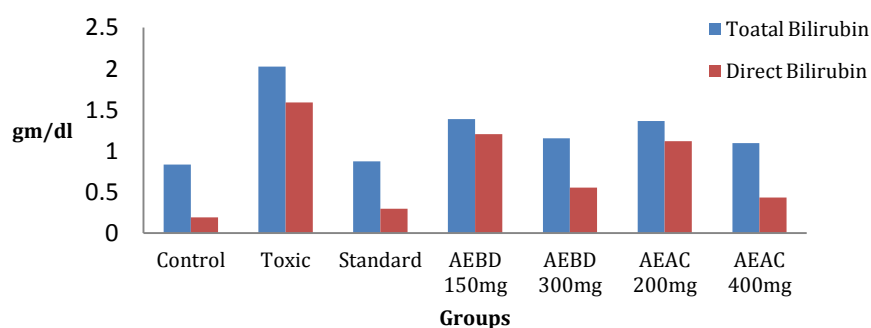


Figure 2: Effect of AEBD and AEAC on Biochemical Parameters

CONCLUSION

In the present study, the stem and leaves of alcoholic extract of *Boerhaavia Diffusa* and leaves of alcoholic extract of *Anisochilus Carnosus* was extensively investigated for its hepatoprotective potential against carbon tetrachloride induced hepatotoxicity. At the end of this study, a strong conclusion can be drawn that, the extracts AEBD and AEAC possess hepatoprotective activities more or less depending on the dose levels.

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