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Research Article

Hepatoprotective Activity of *Bergenia ciliata* Roots in Ethanol Induced Oxidative Stress and Hepatotoxicity in Rats

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ABSTRACT

Liver disease (LD) is one of the main causes of mortality and modality in worldwide. Around 500 million people worldwide are thought to have chronic hepatitis infections, which cause over a million deaths a year. Treatments for LD must be developed with a new approach to cure or prevent the progression of the disease without any consequences. Currently, this study's purpose is to examine and confirm the methanolic extract of *Bergenia ciliata* root's ability to protect rats from ethanol induced hepatotoxicity. Wistar rats were administered one cc of 30% ethyl alcohol in all the groups except the normal control PO once a day for 40 days in order to cause hepatoxicity. After confirmation of LD, the methanolic extract of *B. ciliata* (MEBC) roots and the standard drug silymarin (0.1 g/kg, b.w., orally) were given twice daily for 21 days. Liver weight, body weight, serum liver enzymes like serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), serum alkaline phosphatase (ALP), total protein, albumin, and antioxidant enzymes were assayed to investigate the hepatoprotective effect, followed by liver histopathology to evaluate the hepatic architecture and all alignments and inflammatory cells. The protective markers of all the rats treated with alcohol showed a substantial increase, and the rats administered MEBC showed a remarkable recovery toward an almost normal level. These findings suggest that MEBC protected the structural integrity of the hepatocellular membrane and ethanol-damaged liver cell's cellular architecture, which was supported by histological analysis. The present study demonstrates that the methanolic extract *B. ciliata* roots possess hepatoprotective property.

Introduction

Liver disease is classified as a high attention in the healthcare system. According to the WHO, over five hundred million people are suffering globally from hepatotoxicity.[1] The liver is a key organ for filtration, digestion, metabolism, detoxification, protein synthesis, and the disposition of endogenous substances.[2] The greater risk of liver injury in today's world is due to excessive alcohol consumption. The consumption of alcohol induces hepatic lesions, which cause steatosis, hepatitis, fibrosis, and cirrhosis. Addiction to alcohol is a serious global health issue with detrimental effects on society, the economy, and medicine. It was the cause of 3.3 million fatalities in 2012 and counting. Chronic addiction to alcohol will affect other organs as well, but the earliest and greatest tissue injury is in the liver due to its metabolism.[3] Chronic liver disorders are a significant public health and financial problem due to the absence of a viable therapy, the rise in cirrhosis cases, and the necessity for liver transplantation. Thus, to lower the morbidity and death linked to chronic liver disease, therapeutic approaches that are both economical and effective are required.[4] Herbal medicines can be used to treat liver problems as they are easily available, safer, cost-effective, and environmentally friendly.[5] In healthcare systems, medicinal herbs have acquired importance throughout the world.[6] Many herbal medicines containing various phytochemicals have been reported to cure or prevent the progression of various disorders like gastrointestinal tract (GIT), cancer, renal disease, and cardiovascular (CVS) disease.[7] Although

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there are still many types of liver illnesses whose causes are unknown, the most common ones are typically brought on by one of the following reasons: alcohol, obesity,[8] genetics, autoimmune disorder,[9] drugs and toxins, cancer,[10] viral hepatitis.[11]

Bergenia ciliata (BC) is a medicinal plant of the family Saxifragaceae known as fringed bergenia, hairy leaf bergenia, or pashanbheda in Hindi. It is a large-leaved, clump-forming, slow-growing perennial. Its natural habitats are wooded regions, shaded ledges of rock, and alpine meadows in the Himalayan region, which stretches from eastern Pakistan to Kashmir, Tibet, and Nepal at heights between 6500 and 10,000 feet. Leaves are ciliate, with the leaf margins being toothed and fringed. The extremely valuable herb *B. ciliata* has long been used as a medication to cure a variety of human ailments. It is also called as 'Zakhmehayat' or 'Pakhanabhed'.[12]

BC is also called a "miracle herb" because of its extensive application in treating a variety of illnesses, including gall bladder stones, gastrointestinal problems, lung infections, heart problems, ophthalmic diseases, hemorrhoids, and kidney infections.[13]

In addition, it has been shown to have characteristics that are antifungal, antiviral, anticancer, antitussive, analgesic, antibacterial, diuretic, antiinflammatory, hepatoprotective, and antimalarial.[14] Phytochemical tests have revealed that this plant contains afzelechin, β-sitosterol, arbutin, gallic acid, b-catechin, gallicin, ergenin, and paashaanolactone.[15] *B. ciliata* contains phytochemicals that have hepatoprotective potential.

Liver disorders have traditionally been treated with herbal remedies. The market offers a wide variety of herbal preparations. Similarly, a large number of herbal medicines have been issued for bodybuilding and cause cholestatic injury. Therefore, it is high time to think about the misleading products available on the market. Thus, the study's objective was to assess the hepatoprotective potential of an extract derived from the roots of *B. ciliata* in rats that had suffered alcohol-induced liver damage.

Materials And Methods

Drugs and Chemicals

All the drugs & chemicals are of pure analytical grade was obtained from the authenticated suppliers.

Collection and Authentication of Plant Material

Roots of *B. ciliata* was procured from Indian Jadi-Booti, Noida, UP-20130, India. They were authenticated by Dr. Noorunnisa Begum, Curator, FRLHT, Yelahanka, Bangalore-560064, and Reference No- 6366.

Plant Material Extraction and Sample Preparation

The roots of *B. ciliata* were washed with running tap water. The roots are removed from the peel, cut into slices, and dried. The dried roots were ground into a fine powder. 50 g of fresh roots were used for extraction with 500 mL of methanol on the soxhlet apparatus. In order to make semisolid residue, the extract was concentrated using a rotating vacuum evaporator and kept at 2 to 8°C for further use.

Phytochemical Screening

The concentrated extracts were used for preliminary screening of main phytoconstituents and found to be: Polyphenols, saponin, terpenoids, sterols, glycosides, carboxylic acids, and flavonoids.

Experimental Animals

In the investigation, Wistar albino rats between 150 and 200 g were used. The care of animals was done according to the guidelines of the CPCSEA. The experiment on animals was conducted in accordance with IAEC and registration number: KCP/IAEC/11/22-23/05/22/12/22.

Design of Experiments

Experimental rats were categorised into 5 groups, n = 8/ group

Group 1: Normal control: Received normal saline (10 mL/ kg/b.w.)

Group 2: Disease control: 1-mL 30% ethyl alcohol, PO [16] once a day for 40 days.

Group 3: Silymarin 0.1 g/kg b. w, orally. [17] twice a day for 21 days after confirmation of hepatotoxicity in rats.

Group 4: *B. ciliata* extract 250 mg/kg b.w, orally, twice daily for 21 days after confirmation of hepatotoxicity in rats.

Group 5: *B. ciliata* extract 500 mg/kg b.w, orally, twice daily for 21 days after confirmation of hepatotoxicity in rats.

Parameters of the Study

At the completion of the treatment period, a dose of 40 to 50 mg/kg b.w. of phenobarbital was used to anesthetize all experimental animals, and a cardiac puncture was used to obtain blood samples from a subset of the animals. For biochemical analysis, samples of serum and plasma were stored at -20°C. Later, the same animals were sacrificed by giving a dose of 80 to 90 mg/kg of phenobarbital. The liver was removed and cleaned with an isotonic solution, and its wet weight was noted. PBS was used to prepare the half portion of liver homogenate, and the supernatant was used for further evaluation for an enzyme study on antioxidants. Another half of the liver was put in 10% formalin for histological examination. The following biochemical parameters were determined:

Effects of extracts on body weight, liver wet weight

Rats' body weight was obtained pre and post-completion of the experiment. Liver wet weight was measured as mentioned above.

Table 1: Effect of MEBC on body weight

Groups	*Body weight (gm) pre-treatment (A)*	*Body weight (gm) post-treatment (B)*	*Body weight gain (B-A)*
NC (Normal saline (10 mL/kg)	165.3 ± 9.262	210 ± 7.881	44.7 ± 1.381
DC (1-mL, 30% ethyl alcohol)	189.3 ± 16.75	176.3 ± 5.45	(-)13 ± 11.3
Std. drug - Silymarin (0.1 g/kg)	158.0 ± 22.12	198.0 ± 20.82	40 ± 1.3
MEBC 250 mg/kg	174.7 ± 24.97	186.0 ± 26.15	11.3 ± 1.18
MEBC 500 mg/kg	197.3 ± 21.67	228.3 ± 29.46	31 ± 7.79

The data is shown as mean ± SEM (n = 8). Abbreviations: NC- Normal Control, DC- Disease Control, Std- Standard, MEBC- Methanolic Extract of *Bergenia ciliata.*

Liver function test

Serum glutamic pyruvic transaminase (SGPT), serum oxaloacetic transaminase (SGOT), alkaline phosphatase, total protein, and albumin: The blood samples were obtained by cardiac puncture and centrifuged at 3000 rpm for 15 minutes to get serum and evaluated as per the guidance mentioned in the diagnostic kit (Auto analyzer).

Liver tissue antioxidant enzyme study – SOD, LPO

The assay for the antioxidant enzyme study was done as previously reported. Briefly, the isolated liver was homogenized, spun down, and the supernatant was estimated for lipid peroxidase (LPO) and superoxide dismutase (SOD), whose antioxidant activities were assessed using standard methods.[18]

Histopathological studies – Liver tissue

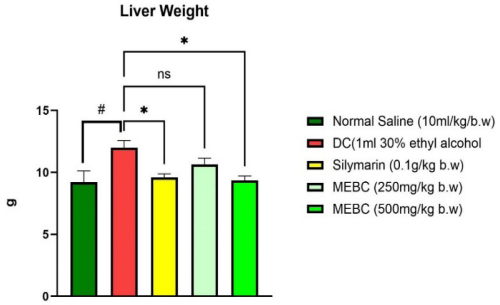
It has been done as previously reported. Briefly, liver tissue was isolated after the animals were sacrificed and preserved in 10% formalin and, followed by processes like tissue purification, embedding in paraffin, sectioning to an approximate thickness of 5 mm, and staining with H&E.[19]

Statistical Evaluation

The data were expressed as mean ± SEM, n = 8 rats/ group. Software Graph Pad Prism *vs* 5 was used to plot the graph. 1- ANOVA and followed by Tukey's test were used to determine the significant differences between the groups. A *p-value* of less than 0.05 was deemed significant when comparing the control (untreated) group against all other groups.

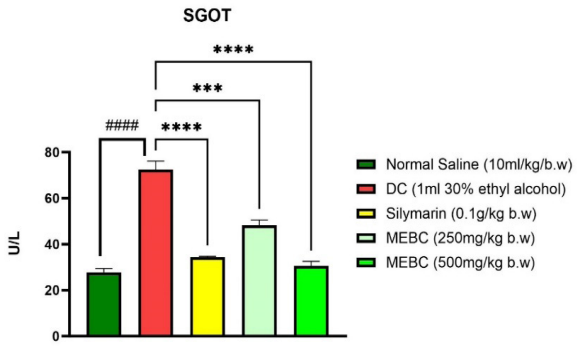
Results And Discussion

Liver damage or injury can happen due to any reason; it could be a chemical, drug, addiction to alcohols, virus, or autoimmune disease. Since it is therapeutically relevant, ethanol was induced to develop hepatotoxicity. A variety of dose-related harmful consequences are produced in the liver by ethanol. Since the liver is where most ethanol is processed, those who misuse alcohol regularly run the risk of developing alcoholic liver disorders. Furthermore, hepatic Kupffer cells that are exposed to ethanol both acutely and chronically produce more cytokines, particularly TNF-alpha, which plays a major part in liver damage. Besides the development of a fatty liver, also called steatosis, liver enlargement and protein buildup are two additional early indicators of excessive ethanol use that are frequently observed in alcoholics and heavy drinkers. Here is the crude extract for its hepatoprotective effect using an *in-vivo* rat model. A preliminary phytochemical analysis of the methanolic extract of *B. ciliata* had previously shown the presence of varieties of phytoconstituents. The root contains a large number of flavonoids and alkaloids that have hepatoprotective action. The plant has opted to participate in this investigation for this reason. The effect of MEBC on body weight was significantly increased by 31% compared to hepatotoxicity rats (-13%) Table 1. Similarity seen in liver weight as * $p < 0.05$ compared to positive control 30% ethyl alcohol. Fig.1. LD is indicated by deviation in serum levels of the enzymes SGOT, SGPT, ALP, and protein. The levels of SGOT and SGPT were significantly balanced by the administration of an extract of MEBC (***$p < 0.001$, **** $p < 0.0001$, Fig. 2) and SGPT (****$p < 0.000$, Fig. 3), ALP (***$p < 0.001$, ****$p < 0.0001$, Fig. 4), total protein (**$p < 0.01$, *** $p < 0.001$, Fig. 5), & albumin (***$p < 0.001$, Fig. 6). The liver toxicants are suggested by the suppression of high ALP expression and a concurrent high in the TP level. This demonstrates that MEBC has a kind of phytoconstituents that can reduce free radicals or inflammatory markers that reduce liver damage, delaying the progression of hepatotoxicity. This is an indicator that the plasma membrane has stabilized and that the ethanol-induced damage to the hepatic tissue has been repaired. As seen in the antioxidant enzymes study, the levels of LPO (**$p < 0.01$, *** $p < 0.001$, Fig. 7)



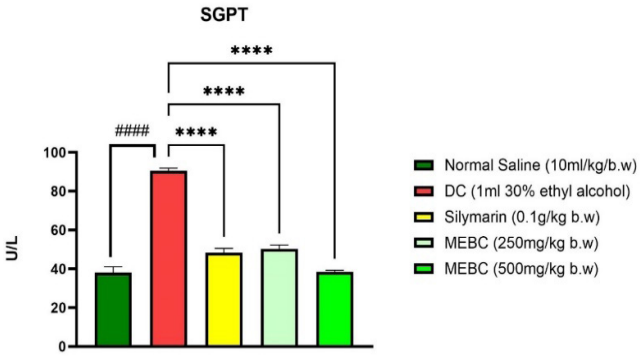
The data is shown as mean ± SEM (n = 8). #$p < 0.05$, compared with NC, normal saline *$p < 0.05$, compared with DC, 30% ethyl alcohol

Fig. 1: Effect of MEBC on wet liver weight



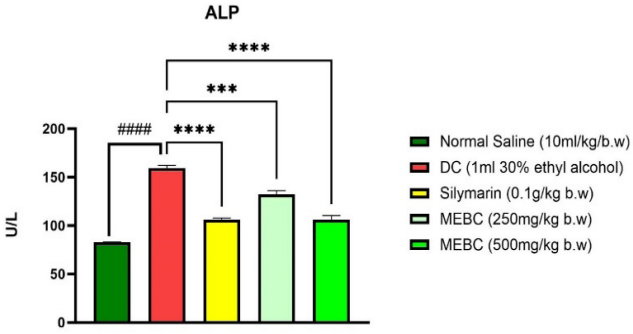
The data is shown as mean ± SEM (n = 8). #### $p < 0.0001$ compared with NC, normal control *** $p < 0.001$, **** $p < 0.0001$ compared with DC, 30% ethyl alcohol

Fig. 2: Effect of MEBC on SGOT levels in ethyl alcohol-induced hepatotoxicity



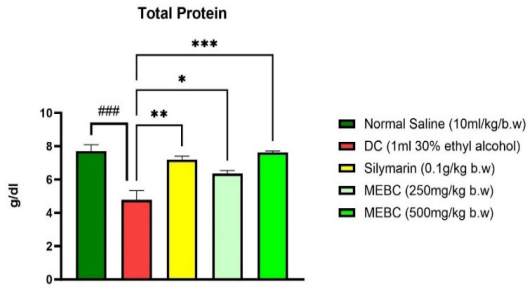
The data is shown as mean ± SEM (n = 8). #### $p < 0.0001$ compared with NC, normal control **** $p < 0.0001$ compared with DC, 30% ethyl alcohol

Fig. 3: Effect of MEBC on SGPT levels in ethyl alcohol-induced hepatotoxicity



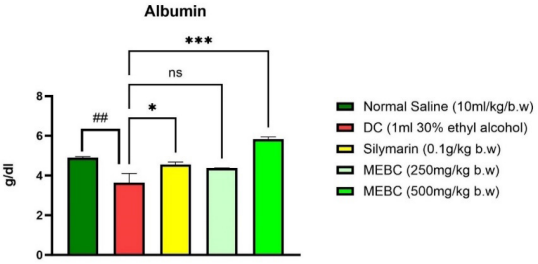
The data is shown as mean ± SEM (n = 8). #### $p < 0.0001$ compared with NC, normal control *** $p < 0.001$, **** $p < 0.0001$ compared with DC, 30% ethyl alcohol

Fig. 4: Effect of MEBC on ALP levels in ethyl alcohol induced hepatotoxicity



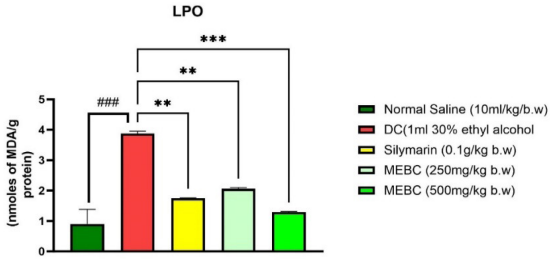
The data is shown as mean ± SEM (n = 8). ### $p < 0.001$, compared with NC, normal control * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, compared with DC, 30% ethyl alcohol

Fig. 5: Effect of MEBC on total protein levels in ethyl alcohol induce hepatotoxicity



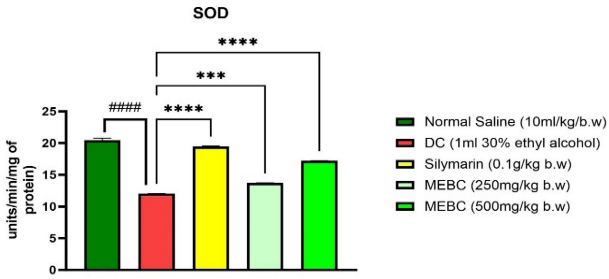
The data is shown as mean ± SEM (n = 8). ## $p < 0.01$, compared with NC, normal control * $p < 0.05$, ns $p > 0.05$, *** $p < 0.001$, compared with DC, 30% ethyl alcohol

Fig. 6: Effect of MEBC on albumin levels in ethyl alcohol induced hepatotoxicity



The data is shown as mean ± SEM (n = 8). ### $p < 0.001$, compared with NC, normal control ** $p < 0.01$, *** $p < 0.001$, compared with DC, 30% ethyl alcohol

Fig. 7: Effect of MEBC on LPO levels in ethyl alcohol-induced hepatotoxicity



The data is shown as mean ± SEM (n = 8). #### $p < 0.0001$ compared with NC, normal control *** $p < 0.001$, **** $p < 0.0001$ compared with DC, 30% ethyl alcohol

Fig. 8: Effect of MEBC on SOD levels in ethyl alcohol-induced hepatotoxicity

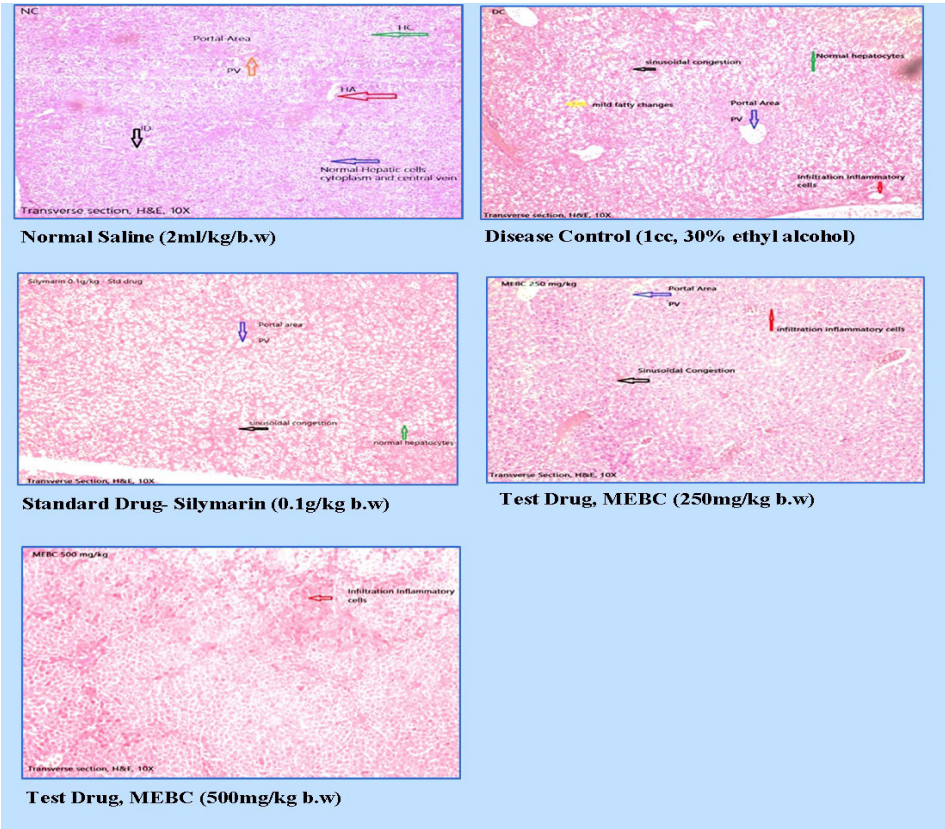


Fig. 9: A Normal liver has well-arranged hepatocytes (Green arrow), a well-circulating hepatic artery (Red arrow), a central vein (Blue arrow), a portal vein (Orange arrow), and an interlobular duct (Black arrow). The portal area includes the portal vein, hepatic artery, and bile duct. Disease liver has shown normal hepatocytes (Green arrow), portal vein (Blue arrow), and irregular circulating portal vein (Blue arrow). The main changes were small vacuoles in some hepatocytes, some inflammation infiltration cells (Red arrow), mild fatty changes (Yellow arrow) and sinusoidal congestion (Black arrow). Silymarin-treated liver shows normal hepatocytes (Green arrow), congested portal vein (Blue arrow), well-circulating hepatic artery and interlobular duct. No inflammation infiltration cells, and seen sinusoidal congestion (black arrow). MEBC 250 mg/kg treated liver shows normal hepatocytes, obstructed portal vein (Blue arrow), well circulating hepatic artery and interlobular duct. The main changes were sinusoidal congestion (Black arrow), and some inflammation infiltration cells (Red arrow). MEBC 500 mg/kg treated liver shows normal hepatocytes, portal vein, well-circulating hepatic artery and the interlobular duct. The main changes were some inflammation infiltration cells (Red arrow) seen.

and SOD (***$p < 0.001$, **** $p < 0.0001$, Fig. 8) drastically dropped with the administration of MEBC. These findings suggest that MEBC protected the structural integrity of the hepatocellular membrane and liver cells' cellular architecture, which was shown in a biopsy of liver tissue (Fig. 9). However, herbal supplements work slowly on the root cause, so for better results, it is recommended to have them with the primary treatment available for liver disease.

Conclusion

A study on the hepatoprotective activity of *B. ciliata* has demonstrated a significant dose-dependent hepatoprotective effect. The liver weight and body weight were restored in test-drug-treated rats. The hepatoprotective effect was shown to be more promising results at 500 mg/kg, as significant changes were noted in terms of hepatoprotective markers, i.e., SGPT, SGOT, ALP, TP, and albumin, followed by antioxidant enzymes and histology of the liver, as confirmed in all treated animals. The hepatoprotective activity of *B. ciliata* in an experimental model could be due to antioxidant active polyphenolic components like flavonoids and saponins. It would be the best choice to use it as an adjuvant therapy or combine it with the primary treatment.

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