Assessment of Hepatoprotective Potential of Dendrobium bicameratum, a Bryophyte from Eastern Ghats, Odisha

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Abstract: An investigation was conducted to assess the hepatoprotective potential of hydroalcoholic and aqueous extracts of *Dendrobium bicameratum* in Wistar rats with carbon tetrachloride (CCl_4)-induced hepatic damage. The extent of liver injury was evaluated by measuring serum biomarkers, including SGOT, SGPT, total albumin, total protein, IL-1β, IL-6, and TNF-α. Administration of hydroalcoholic (HADBM) and aqueous (ADBM) extracts at 200 mg/kg, as well as silymarin (100 mg/kg), demonstrated significant ($P < 0.05$) hepatoprotective effects by reducing the levels of serum hepatoprotective and inflammatory mediators. These findings suggest that Dendrobium bicameratum extracts exhibit pronounced hepatic protection, comparable to the standard hepatoprotective agent silymarin.

Key words: *Dendrobium bicameratum*, hepatoprotective, SGOT, SGPT, total albumin, total protein

Introduction

The liver is a crucial organ that regulates various essential biochemical and physiological processes, encompassing homeostatic balance, growth, energy metabolism, nutrient distribution, xenobiotic detoxification, and immune defense. Due to its central role in these vital functions, the liver is particularly vulnerable to injury from hepatotoxic substances.[1] Plant-based medicines have historically played a significant role in the treatment of human diseases. In recent years, there has been a growing interest in natural and herbal remedies for the treatment of various ailments in humans and animals globally.[2]

Despite significant progress in modern medicine, liver diseases persist as a major global health concern. Consequently, the quest for novel therapeutic agents remains an active area of research.[3] Induction of liver injury by CCl4 has been used vastly as a model for investigation of hepatoprotective agents. [4,5,6]. Bryophytes, comprising liverworts and mosses, are a rich source of naturally occurring bioactive compounds. These non-vascular plants have been found to exhibit a range of biological activities, including antifungal, antibacterial, antiviral, anti-inflammatory, and antioxidant properties.[7]

The objective of the present study was to investigate the hepatoprotective effect of *Dendrobium bicameratum* on CCl_4-induced rats. In our study we investigated the hepatoprotective effect on different levels, serum parameters, inflammatory parameters and histopathological changes of liver. In addition, silymarin, a clinically proven hepatoprotective effect, was used as a reference hepatoprotective agent in our study.[8,9,10]

Materials and methods

Plant materials

Dendrobium bicameratum collected from Mahendra Giri Gajpati district, India. Authenticated by Dr Pramod Kumar Dash. “RPRC, Bhubaneswar, Odisha, India”. A coupon sample was submitted (specimen no: 17979).

Extraction

A hydroalcoholic extraction was performed by combining 500 g of coarse powder with an ethanol-water mixture (8:2 v/v) and subjecting it to continuous agitation on an orbital shaker for 48 hours. Hydroalcoholic extraction was carried out by maceration process.

Animals and experimental design

Wistar albino rats weighing 150-200 g were housed in polypropylene cages under controlled environmental conditions: temperature (25 ± 2°C), relative humidity (50 ± 5%), and a 12-hour light-dark cycle. The rats were acclimatized to these conditions for one week prior to the experiment and throughout the study period. A standard rodent pellet diet and water were provided ad libitum. The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), approval number CIP/IAEC/2019/157.

Acute Toxicity (Single Dose)
The acute oral toxicity study was conducted in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD 423).

Induction of hepatotoxicity
Hepatotoxicity was induced through intraperitoneal (i.p.) administration of carbon tetrachloride (CCl_4) at a dose of 0.5 ml/kg body weight, formulated as a 20% solution in olive oil. This treatment was repeated twice weekly for a period of four weeks.[11]

Blood Sample Collection and Biochemical Analysis
On day 28th, the animals were humanely euthanized, and blood samples were collected in heparinized tubes. The blood was allowed to clot, and then centrifuged at 3000 rpm for 15 minutes at 4°C to isolate the serum. The resulting serum was stored at -80°C for further analysis.

Table 1: Experimental design of hepatoprotective activity

Group	Treatment
Normal Control	No treatment
Negative Control	0.5 ml/kg b.w., 20% CCl_4/olive oil
Standard	CCl_4 + Silymarin (100 mg/kg)
HADBM (200 mg/kg)	CCl_4 + HADBM (200 mg/kg)
ADBM (200 mg/kg)	CCl_4 + ADBM (200 mg/kg)

Analysis of liver enzymes and other Biomarkers: Blood samples were obtained via cardiac puncture from each animal. The serum was then isolated and refrigerated at 2-8 °C pending analysis of (SGOT) Serum Glutamic-Oxaloacetic Transaminase, (SGPT) Serum Glutamic-Pyruvic Transaminase, Total albumin and total protein, liver inflammatory mediators like IL_{1B} (pg/mg), IL_6 (pg/mg), TNF-α (pg/mg). Additionally, liver tissues were harvested for histopathological examination.

Histopathology of the liver:
Liver tissues were fixed in 10% neutral buffered formalin, dehydrated, and embedded in paraffin wax. Thin sections (5-6 µm) were cut using a microtome, stained with haematoxylin-eosin (H&E), and examined under a light microscope.

Statistical analysis
Results were expressed as Means ± Standard Error Mean (SEM). Statistical analysis was carried out using one-way analysis of variance followed by Dunnett's multiple comparison test (Graph Pad Prism 5 Software). Statistical significance was set at ($P < 0.05$)

RESULTS

The Effect of *D bicameratum* Extract on Clinical Signs and Behaviors
The acute oral toxicity profile of the hydroalcoholic extract of *D. bicameratum* was evaluated. the results showed no evidence of toxic symptoms, behavioral changes, or mortality throughout the study duration. Furthermore, no adverse effects, clinical signs of toxicity, stress, or alterations in appearance or behavior were observed in the treated animals.

The Effect of *D bicameratum* Extract on Body Weight.
As shown in Table 2, the body weight significantly decreased in group treated with CCl_4 no change in body weight observed group treated with Silymarin (100 mg/kg) and both extract of *D bicameratum* extract 200 mg/kg.

Table:2 The Effect of *D bicameratum* Extract on Body Weight.

	Body weight (gm)				
Group	**Initial**	**Final**	**Change in bw**	**bw gain or loss (%)**	**bw gain or loss**
Normal Control	145 ± 1.53	158 ± 1.43	13	8.9	Gain
Negative control (CCl4)	138 ± 1.63	119 ± 1.23	-19	13.7	Loss
Standard (Silymarin 100 mg/kg)	141 ± 1.73	148 ± 1.43	7	4.9	Gain
HADBM (200 mg/kg)	150 ± 1.43	155 ± 1.33	5	3	Gain

ADBM (200 mg/kg)	149 ± 1.29	152 ± 1.35	3	2	Gain

Values are expressed as Mean ± SEM (n = 5); Values with * indicate significant differences (P < 0.05) between negative control and test, standard drug treated group. ** indicate significant differences (P < 0.05) with respect to Normal control (NC) and Negative control CCL_4 induced group

Table: 3 Effect of *Dendrobium bicameratum* extract on LFT of CCl_4 induced and normal albino rats

Group	SGOT (IU/L)	SGPT (IU/L)	Total albumin (gm/dl	Total Protein (gm/dl)
Normal Control	132.19 ± 0.68**	71.28 ± 0.34**	5.04 ± 0.09**	15.4 ± 0.07**
Negative control (CCl4)	176.08± 0.39	110.41 ± 0.73	3.54 ± 0.04	6.66 ± 0.05
Standard (Silymarin 100 mg/kg)	132.96± 0.52*	82.19 ± 0.74*	4.86 ± 0.04*	14.5 ± 0.10*
HADBM (200 mg/kg)	141.48± 0.36*	91.5 ± 1.76*	4.76 ± 0.07*	10.54 ± 0.22*
ADBM (200 mg/kg)	148.62± 0.34*	96.04± 0.26*	4.72± 0.09*	7.78 ± 0.02*

Values are expressed as Mean ± SEM (n = 5); Values with * indicate significant differences (P < 0.05) between negative control and test, standard drug treated group. ** indicate significant differences (P < 0.05) with respect to Normal control (NC) and Negative control CCL_4 induced group

Hepatoprotective Activity of *D bicameratum* Extract: Compared to the baseline, CCl_4 therapy caused a substantial reduced body weight % (13.7%); in contrast, treatment with Silymarin, HADBM & ADBM 200 mg/kg extracts resulted in no decrease in body weight and no change in, respectively, as indicated in Table 2. **The Effect of *D bicameratum* Extract on Biochemical Markers.** The biochemical profile was investigated using a semiauto analyzer after treatment with HADBM and ADBM 200 mg/ kg/day for 28 days. Treatment with 200 mg/kg of *D bicameratum* extract significantly decrease in SGOT, SGP, Total albumin, Total Protein level when compared with the CCl_4 induced liver cells damage group. Standard drug Silymarin also significantly decreased the activities of SGOT, SGPT, Total albumin and Total protein, respectively, are presented in Table 3.

Figure:1. a, b, c Effect of *Dendrobium bicameratum* extract on inflammatory mediators: IL-1B, IL-6, TNF-α in CCl_4 induced rat model

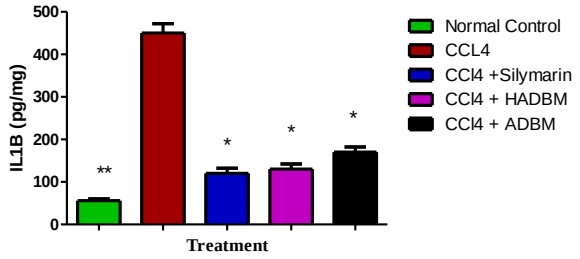

Figure 1.a

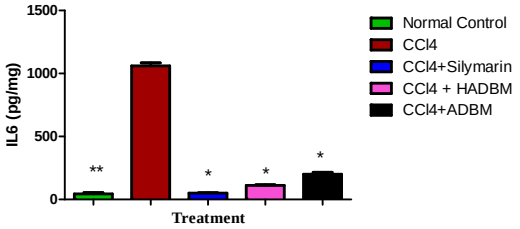

Figure 1.b

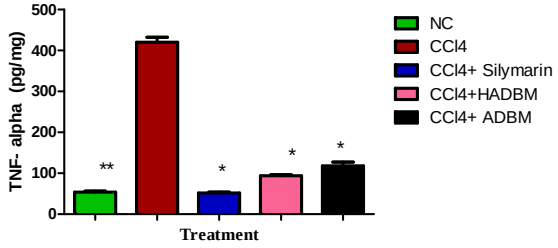

Figure 1.c

Values are expressed as Mean ± SEM (n = 5); Values with * indicate significant differences (P < 0.05) between negative control and test, standard drug treated group. ** indicate significant differences (P < 0.05) with respect to Normal control (NC) and Negative control CCL_4 induced group

The Effect of *D bicameratum* Extract on inflammatory markers

Exposure to carbon tetrachloride (CCl_4) can induce hepatocyte damage, leading to acute inflammation characterized by elevated levels of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α.[12,13] The present study demonstrates that administration of *D. bicameratum* extracts (hydroalcoholic and aqueous) at a dose of 200 mg/kg significantly reduces the levels of IL-1β, IL-6, and TNF-α. These findings suggest that *D. bicameratum* possesses anti-inflammatory properties, which can mitigate acute inflammation induced by CCl_4 exposure. The phenolic compounds present in *D. bicameratum* may contribute to its protective effects by safeguarding cellular structures against oxidative stress and damage.

Prolonged exposure to low doses of carbon tetrachloride (CCl_4) has been shown to induce hepatocyte inflammation, characterized by elevated production of tumor necrosis factor-alpha (TNF-α). Normally, healthy hepatocytes exhibit resistance to TNF-α-mediated damage; however, they are more susceptible to disruptions in RNA and protein synthesis. The inflammatory response triggered by CCl4 exposure can exacerbate liver damage.[14] The tumor necrosis factor-alpha (TNF-α) levels in carbon tetrachloride (CCl_4)-induced experimental animals were substantially elevated compared to the treatment group, with notable differences observed between the induced, standard treatment, and test drug-treated groups (Figure 1 a, b, c). Administration of *D. bicameratum* extracts resulted in a marked alteration in TNF-α levels, indicating a potential anti-inflammatory effect.

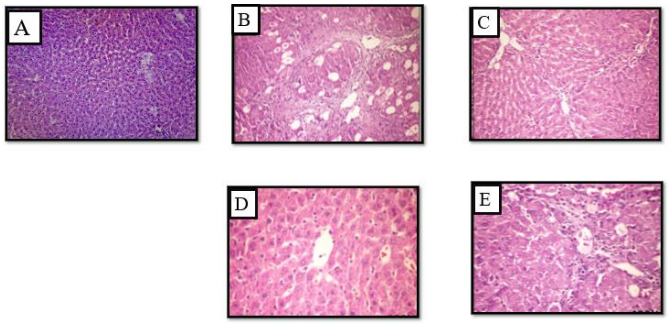

Figure: 2. A, B, C, D, E Effects of *D bicameratum* and silymarin on histopathological changes induced by CCl4 exposure in Wistar rats. A. Normal control group, B. Rats treated with CCl4, C. animals treated withCCl_4 and Silymarin D. CCl_4+ HADBM (200 mg/kg), E. CCl_4+ ADBM (200 mg/kg). All sections were stained with Hematoxylin

Histopathological Findings of *D. bicameratum*: To assess the possible protective effects of *D bicameratum* on liver cells damage induced by CCl_4, histopathological analysis of the liver tissue was performed. The results indicated that CCl_4 treatment led to the emergence of fatty liver cells, necrosis, hyperplasia, infiltration, and inflammation, in contrast to the normal liver cells treated with silymarin and aqueous and hydroalcoholic extract of *D bicameratum* 2. A, B, C, D, E.

DISCUSSION

This study aimed to investigate the hepatoprotective properties of aqueous and hydroalcoholic extract of *D bicameratum* against CCl_4 induced liver damage in rats. This study revealed that the *D bicameratum* extract protects against CCl_4-induced hepatotoxicity and is non-harmful. the protective effects of the extracts are attributed to their antioxidant components, such as phenolics, which protect cells from damage.[15,16] In line with some studies, our results indicated that the hydroalcoholic and aqueous extract of *D bicameratum.*

A marked reduction in SGOT, SGPT, Total protein and albumin levels was observed in the group treated with CCl_4 and they were significantly decreased (P < 0.05) when compared with the normal control group. Rats treated with CCl_4 (Negative control) developed significant liver damage and it was well indicated by elevated levels of hepato specific enzymes like SGOT, SGPT (P < 0.05) in serum and decrease in serum total protein and albumin level. The groups received the pre-treatment of HADBM and ADBM at dose level of 200 mg/kg body weight significantly controlled the change in the biochemical parameters. The extract at dose levels of 200 mg/kg exhibited significant increase (P < 0.05) in the serum total protein level as compared to CCl_4 induced group and the effect was comparable with the standard group (P < 0.05) treated with silymarin.

The *D bicameratum* extract exhibited efficacy in safeguarding against CCl_4 induced hepatotoxicity, as evidenced by the notable decreases in SGOT, SGPT, Total albumin, total Protein, IL_{1B}, $IL_{6,}$ TNF- α levels. Furthermore, the normal hepatocellular histology observed in the extract-treated group compared to that in the CCl_4-treated group indicated the effectiveness of the *D bicameratum* extract. These findings were further confirmed by the presence of fatty liver and necrosis in the liver tissues of the CCl_4 treated.

The effects of the *D bicameratum* extract on liver histological sections were examined. Histological sections of the group treated with *D bicameratum* showed hepatoprotective effects. The results indicated that the CCl_4 treated group exhibited fatty liver, necrosis, hyperplasia, infiltration, and inflammation, while the liver cells in the *D bicameratum* treated groups appeared healthy.

Conclusion

In this study, we evaluated the hepatoprotective effects of the *D bicameratum* extract against CCl_4-induced toxicity. *D bicameratum* protects against CCl4-induced hepatotoxicity. Treatment with the *D bicameratum* extract decreased the levels of SGOT, SGPT, Total albumin, Total protein, IL_{1B}, $IL_{6,}$ TNF-α thereby protecting against CCl_4 induced hepatocellular damage. Based on these findings, *D bicameratum* appears to be a safe, effective, and promising natural product for use in herbal medicine. Further research is needed to underlying mechanisms by which *D bicameratum* protects against cellular damage.

Conflict of Interest:

The authors of this study certify that they have no competing interests or financial relationships that could influence the research findings or interpretation. The authors declare that there are no conflicts of interest related to this study.

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