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Research Article

EVALUATION OF HEPATOPROTECTIVE EFFECT OF *CYMBIDIUM ALOIFOLIUM (L.) SW EXTRACTS* ON ETHYL ALCOHOL-INDUCED HEPATOTOXICITY IN RODENTS

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ABSTRACT

Objective: Liver diseases are among the leading causes of global morbidity and mortality, with alcohol consumption being a major contributing factor. The search for safer, plant-based hepatoprotective agents has gained significant interest. *Cymbidium aloifolium* is a medicinal orchid traditionally used for its therapeutic benefits, but its hepatoprotective potential remains underexplored. The present study aimed to analyze the phytochemical composition and evaluate the hepatoprotective efficacy of *C. aloifolium* ethyl acetate extract (CAEAE) and hydro-alcoholic extract (CAHAE) in an ethanol-induced hepatotoxicity model.

Methods: Phytochemical screening of both extracts was carried out using standard qualitative methods, while quantitative estimation of total phenolic and alkaloid content was performed. Hepatoprotective activity was assessed in ethanol-induced liver-damaged rats, with Liv-52 serving as the standard reference drug. Serum biochemical parameters, including alkaline phosphatase, alanine transaminase, aspartate aminotransferase, total bilirubin, and total protein, were analyzed to evaluate liver function.

Results: Phytochemical analysis revealed the presence of steroids, alkaloids, phenols, flavonoids, and sugars in both extracts. The hydro-alcoholic extract (CAHAE) exhibited higher phenolic and alkaloid content compared to the ethyl acetate extract (CAEAE). Both extracts significantly reversed ethanol-induced liver damage and restored biochemical markers toward normal levels in a dose-dependent manner. The most pronounced hepatoprotective effect was observed at 400 mg/kg body weight, with CAHAE demonstrating superior activity to CAEAE.

Conclusion: The findings indicate that *C. aloifolium* possesses potent hepatoprotective properties, likely due to its rich phytochemical content, particularly phenols and alkaloids. These results provide scientific validation for the traditional use of *C. aloifolium* and highlight its potential as a natural hepatoprotective agent. Further research is needed to isolate and characterize the active bio-compounds responsible for its protective effects.

Keywords: Liver diseases, Alcohol, Liv52, *Cymbidium aloifolium*, Phytochemicals, Hepatoprotective.

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INTRODUCTION

The liver is an essential organ having different physiological roles in the human body, mainly protein synthesis and detoxification of toxic substances [1]. The liver also produces various biochemicals necessary for digestion, stores energy in the form of glycogen, and maintains the body's sugar level [2]. If the liver is damaged, our body may become prone to different diseases because of the liver's incapability to handle drugs and toxins [3,4]. Toxin accumulation is a result of inadequate metabolism of medications, inhalation of industrial chemicals, solvents, unhealthy lifestyle, and alcohol consumption, causing high mortalities around the world [4,5]. The alcoholic liver damage occurs gradually over the years with hepatic scarring, fibrotic changes, culminating in liver cirrhosis [6]. Different treatment modalities are available to treat liver diseases depending on the stage of damage. The treatment includes lifestyle changes, alcohol abstinence, and over-the-counter medicines for liver damage diseases [7]. As earlier said, inappropriate and chronic use of different medications may affect the function of other organs in the body and show various side effects [8,9]. Of late, new diseases are emerging, and also, the microorganisms are developing resistance to most of the commonly used antibiotics [10,11]. Hence, there is a pressing need to develop new medicines to treat different diseases including liver diseases [12,13].

The people around the world have been using herbal medicines since ancient times to treat different diseases even before the advent of allopathic medicines [14]. At present, in case of failure of allopathic medicine to treat different diseases and their side effects, people are turning back to herbal medications, probably because of their lower cost, fewer side effects, and greater availability [15,16]. In the last few decades, different medicinal plants were identified and various bioactive compounds and their medicinal uses were reported [17-19]. Yet, many medicinal plants are unexplored around the globe and their biological activities are unreported and underreported. Therefore, the present study aimed to evaluate the hepatoprotective activity of one such medicinal plant, i.e., *Cymbidium aloifolium*.

C. aloifolium is an epiphytic orchid medicinal plant that grows around the Indian sub-continent (Fig. 1). Various parts of *C. aloifolium* were reported to have emetic, purgative properties; paste of its parts was used for treating orthopedic injuries [20-23]. However, there were very less reports on the biological activities of *C. aloifolium*. Therefore, the current research was designed to explore the phytochemical components and assess the ability of *C. aloifolium* extracts against alcohol-induced liver toxicity.

METHODS

Chemicals and reagents

The chemicals and reagents used in the current research are of analytical grade. The ascorbic acid from Sigma Aldrich, USA, Liv.52 from Himalaya

Drug Company, and kits used for biomarker enzymes' estimation were from Span Diagnostics, India.

Preparation of *C. aloifolium* extracts

The aerial parts of the plant *C. aloifolium* were collected nearby Araku Valley region, Andhra Pradesh, India. Prof. S. B Padal, Dept. of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India, authenticated the collected specimen (23344). The plant material was collected, shade-dried, and powdered. The powder material was used to prepare both ethyl acetate extract (EAE) and hydro-alcoholic extract (70% ethanol) (HAE) by the maceration technique, respectively. The extracts were prepared to dry under Rotavapor, and for further usage, it was stored in a desiccator.

Percentage yield

The percentage yield of the *C. aloifolium* EAE (CAEAE) was calculated to be 0.3% extract per 10.0 g dry powder, and the percentage yield of the *C. aloifolium* HAE (CAHAE) was calculated to be 3.2% extract per 10.0 g dried powder, respectively.

Phytochemical analysis

The qualitative phytochemical analysis of *C. aloifolium* extracts was carried out using standard test procedures [24,25]. The total alkaloid and phenolic contents were quantified in *C. aloifolium* extracts.

Quantification of phenolic and alkaloid content analysis

Phenolic content analysis

The phenolic content was analyzed using a calorimetric method with Folin–Ciocalteu reagent (FCR). The experiment was described by Shamsa *et al.,* as this method is the colorimetric method, based on chemical reduction of a reagent mixture containing tungsten and molybdenum. To the extract (mg/mL) add FCR (5 mL) and after the 30 min incubation time, color (blue) of the reaction mixture measured at 760 nm, presence of phenolic content will enhance the absorbance and was calculated against the standard graph of gallic acid as standard drug against control [26] and expressed as gallic acid equivalents (mg/g) as mean±standard error of mean (SEM) (n=3).

The calibration curves for gallic acid and atropine were constructed by preparing a series of standard solutions (n=6 concentrations, each measured in triplicate). For gallic acid, standards ranged from 10 to 150 μg/mL and absorbance at 765 nm was measured after reaction with Folin–Ciocalteu reagent. Linear regression of absorbance versus concentration gave the equation A=0.0068 C+0.012A=0.0068\,C+0.012 A=0.0068C+0.012 with R^2=0.998. For atropine, standards ranged from 0.5 to 50 μg/mL and peak area was measured by HPLC-UV (λ=220 nm). The calibration curve was linear over the range with regression equation Area=32500 C+150\text{Area}=32500\,C + 150Area=32500C+150 and R^2=0.9994. LOD and LOQ were calculated as 3.3× and 10× the standard deviation of the blank divided by the slope, respectively.

- Linear range (example for HPLC): 0.5–50 μg/mL.
- Regression: Area=32500C+150.
- Coefficient of determination: R^2=0.9994.

Alkaloid content analysis

The alkaloid content of the *C. aloifolium* extracts was quantified using Bromocresol Green (BCG) solution with spectroscopic method explained by Shamsa *et al.* [26]. One milliliter of the plant extracts was dissolved in 2N hydrochloric acid (mg/mL), then add 5 mL of BCG solution in a separating funnel and 5 mL of phosphate buffer solution and mixed well. The formed complex in the solution was separated using 5 mL of chloroform using separating funnel. The dissolved alkaloid content absorbance was measured at 470 nm against the control, and concentrations were measured using the standard drug atropine. The results were expressed as mean±SEM (n=3).

Evaluation of toxicity of *C. aloifolium* extracts

The toxicity of *C. aloifolium* extracts (CAEAE and CAHAE) was evaluated at 1000 and 2000 mg/Kg body weight (b.w.) as per the Organization for Economic Cooperation and Development 420 guidelines. The animal studies for the current research were permitted and conducted at Santhiram Medical College and General Hospital – SRMC&GH (897/PO/RE/S/05/CPCSEA) Institutional Ethical Committee. The animals (albino Wistar rats of either sex) which were aged ≈90 days and around 220–250 g were maintained at 12 h dark and light at 24±2°C and relative humidity of 40–70% [27]. The test extract doses were given to different groups of animals (n=6) which were on overnight fasting and kept under observation up to 48 h for any different physiological and psychological changes and finally mortality.

Hepatoprotective activity

The ethyl alcohol-induced liver toxicity model was used to evaluate the hepatoprotective activity of *C. aloifolium* extracts [28]. Different doses of each extract were prepared as 100, 200, and 400 mg/Kg b.w. The animals were separated into 9 different groups (n=6). Groups I served as control, administered with drug vehicle (2% v/v tween 80). Group II served as toxic group, administered with ethanol 3.76 g/Kg p.o. 2 times/day. Group III served as the standard group, administered with Liv,52 (25 mg/Kg b.w) before ethanol administration. Groups IV–IX were served as testing groups, were administered 30 min before ethanol administration and CAEAE (Groups IV–VI), CAHAE (Groups VII–IX) as above-said doses, respectively, for 21 days, 30 min before ethanol administration. The drugs were administered to the animals orally with the help of intragastric gavage. On the 22nd day of the study, blood was collected from each group of animals in an isoflurane anesthetic condition by retro-orbital puncture for the estimation of liver biomarker enzymes such as alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate aminotransferase (AST), total bilirubin (T.Bil), and total protein (T.ptn). After the collection of blood, the animals were sacrificed, and hepatic tissues were collected and stored in 10% formalin solution for histopathological studies.

Statistical analysis

The total alkaloid, phenolic contents, and hepatic biomarker enzyme levels were expressed as mean±SEM, and the percentage (%) protection was calculated with below formula:

$$\% \text{ Protection} = \frac{(\text{Toxic group levels} - \text{Test group levels})}{(\text{Toxic group levels} - \text{Control group levels})} \times 100$$

Using one-way analysis of variance (ANOVA) and Dunnett's multiple comparison test, the results were analyzed.

RESULTS AND DISCUSSION

The current research was intended to identify the phytochemical profile, estimation of alkaloid and phenolic contents in different extracts of *C. aloifolium* (CAEAE, CAHAE), and their hepatoprotective potential. The phytochemical analysis of *C. aloifolium* extracts reveals the presence of different phytochemical constituents (Table 1).

Both the extracts (ethyl acetate and hydro-alcoholic) have the presence of glycosides, terpenoids, alkaloids, carbohydrates, phenols, sterols, flavonoids, and tannins. HAEs gave positive results for oils and saponins and negative results for EAEs. Quinones and amino acids were absent in both extracts.

Phenols and alkaloids were present in both the extracts. As the phenols and alkaloids are wide-spectrum bioactive molecules, standard procedures were carried out for their estimation and found that HAE has more content than EAE (Table 2). The phenolic contents in CAEAE and CAHAE were found to be 20.12±0.25 and 25.59±0.52 mg/g, respectively. The alkaloid contents in CAEAE and CAHAE were found to be17.58±0.39 and 24.12±0.59 mg/g, respectively.

The toxicity studies of *C. aloifolium* extracts (CAEAE and CAHAE) have not shown any toxic symptoms nor any abnormal physiological, psychological signs on tested animals at different test doses. The results

Table 1: Phytochemical analysis of the *Cymbidium aloifolium* extracts

Name of the Phytochemical constituent	Name of the extract	
	Ethyl acetate	Hydro-alcoholic
Phytosterols	+	+
Terpenoids	+	+
Glycosides	+	+
Saponins	-	+
Flavonoids	+	+
Tannins	+	+
Carbohydrates	+	+
Alkaloids	+	+
Amino acids	-	-
Oils	-	+
Quinones	-	-
Phenols	+	+

+: Present; -: Absent

Table 2: Phenolic and alkaloid contents of the *Cymbidium aloifolium* extracts

Name of the phytochemical constituent (mg/g)	Name of the extract	
	Ethyl acetate	Hydro-alcoholic
Total phenols	20.12±0.25	25.59±0.52
Total Alkaloids	17.58±0.39	24.12±0.59

indicate that the selected *C. aloifolium* extracts were safe for further evaluation of different biological activities.

The hepatoprotective activity of the *C. aloifolium* extracts (CAEAE and CAHAE) at different doses, i.e., 100, 200, and 400 mg/Kg b.w was evaluated on ethyl alcohol-induced hepatotoxicity model in albino Wistar rats. Hepatoprotective activity was shown by the tested extract doses of *C. aloifolium* plant in a dose-dependent manner in reducing liver toxicity. Among the extracts of *C. aloifolium,* HAE had shown more protection compared to EAE. The result of current study reveals that ethyl alcohol, Liv.52, and extracts of *C. aloifolium* (EAE & HAE) treated groups had shown crucial alterations in the hepatic biomarker enzyme levels such as ALP, AST, ALT, T.Bil, and T.ptn compared with the control group [29].

Results were analyzed using two-way ANOVA, followed by Dunnett's multiple comparison test. All groups were compared with toxic group. ***p<0.001; **p<0.01; *p<0.05; ns=Non significance.

The changes in these hepatic biomarker enzyme levels were based on the dose of the treated extracts, and results are shown in Table 3. The ALP, AST, ALT, and T.Bil levels were increased and T.ptn levels were decreased compared to toxic group of animals and the enzymatic levels of Liv.52 treated group were almost similar to the control group. The percentage protection (PP) of tested extracts of *C. aloifolium* at different doses was depicted in Fig. 2. The PP of CAEAE at 100 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels is 7.94, 8.32, 9.02, 8.88, and 8.64. The PP of CAEAE at 200 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels are 16.02, 15.27, 17.46, 17.16, and 16.42. The PP of CAEAE at 400 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels are 27.85, 28.40, 29.73, 28.99, and 27.66. The PP of CAHAE at 100 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels are 13.06, 13.00, 14.05, 14.79, and 12.96. The PP of CAHAE at 200 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels are 25.97, 25.52, 26.92, 27.81, and 25.93. The PP of CAHAE at 400 mg/kg on hepatic biomarker enzymes ALP, AST, ALT, T.Bil, and T.ptn levels are 43.35, 43.19, 44.23, 46.75, and 46.67. The results clearly indicate that the HAE has more PP in restoring the altered liver biomarker enzymes in the hepatic disease conditions.

Fig. 1: *Cymbidium aloifolium*

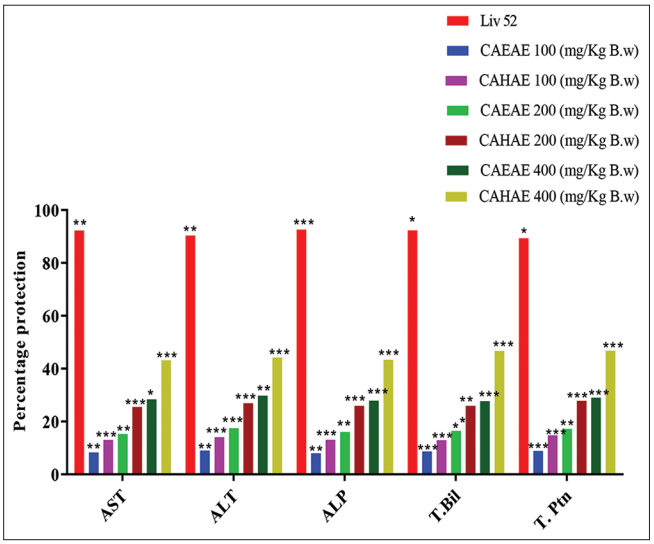

Fig. 2: Percentage protection produced by different extracts of *Cymbidium aloifolium* at different doses

Results were analyzed using one-way ANOVA, followed by Dunnett's comparison test and the significance levels (e.g., *p<0.05, **p<0.01, ***p<0.001 vs. Group II).

The histopathological studies clearly indicate that the restoration of damaged liver tissue in ethanol-induced liver toxicity animal groups treated with the *C. aloifolium* extracts at different doses was shown in Fig. 3. There was no abnormality observed in the portal triad containing bile duct, portal vein appeared normal, and in the control group, hepatocytes appear normal in the portal, peri-portal, and centri-lobular region [30,31]. Diffused macro and micro-vesicular fatty degeneration along with inflammation of hepatocytes was observed in centri-lobular/peri-portal region in ethyl alcohol (Group-II) treated animals. The changes observed in hepatic biomarker enzyme levels of ethyl alcohol treated groups were due to damage to hepatic cells, as shown in Table 2. The extracts of *C. aloifolium* were given to the ethyl alcohol treated animals, and the given extracts showed good restoration depending on their doses of the liver damage due to the ethanol toxicity. Fig. 3g and h shows multi-focal necrosis in the hepatocytes of portal and peri-portal region and moderate peribiliary inflammation and fibrosis in the portal triad region in CAEAE at 200 mg/Kg b.w group. Fig. 3i and 3j shows normal hepatocytes at the portal and peri-portal and centri-lobular region in CAEAE at 400 mg/Kg b.w treated group. Fig. 3k and l

Table 3: Effect of the *Cymbidium aloifolium* extracts on liver biomarker enzymes' levels

Name of the drug	Name of enzymes				
	Aspartate aminotransferase (U/L)	Alanine transaminase (U/L)	Alkaline phosphatase (U/L)	Total bilirubin (mg/dL)	Total protein (g/dL)
Group I–Control	85.00±1.03	46.33±1.28	129.00±0.82	0.24±0.01	6.97±0.07
Group II–Ethanol	327.33±2.16	159.00±0.63	483.83±2.47	2.17±0.06	4.15±0.05
Group III–Liv-52 (25 mg/kg)	103.83±0.65***	57.17±0.60***	155.17±1.42***	0.39±0.04***	6.67±0.08***
Group IV–CAEAE 100 mg/kg	307.17±1.51*	148.83±0.60*	455.67±1.87*	2.00±0.04*	4.40±0.07*
Group V–CAEAE 200 mg/kg	290.33±1.61**	139.33±1.02**	427.00±2.73**	1.85±0.07**	4.63±0.06**
Group VI–CAEAE 400 mg/kg	258.50±2.70***	125.50±1.31***	385.00±1.53***	1.63±0.06***	4.97±0.06***
Group VII–CAHAE 100 mg/kg	295.83±2.46**	143.17±1.38**	437.50±2.06**	1.92±0.07**	4.57±0.06**
Group VIII–CAHAE 200 mg/kg	265.50±1.89***	128.67±1.43***	391.67±2.60***	1.67±0.07***	4.93±0.07***
Group IX–CAHAE 400 mg/kg	222.67±2.62***	109.17±0.54***	330.00±1.46***	1.27±0.08***	5.47±0.07***

* p < 0.05 vs. Group II, ** p < 0.01 vs. Group II, *** p < 0.001 vs. Group II. CAHAE: *Cymbidium aloifolium* hydro-alcoholic extract

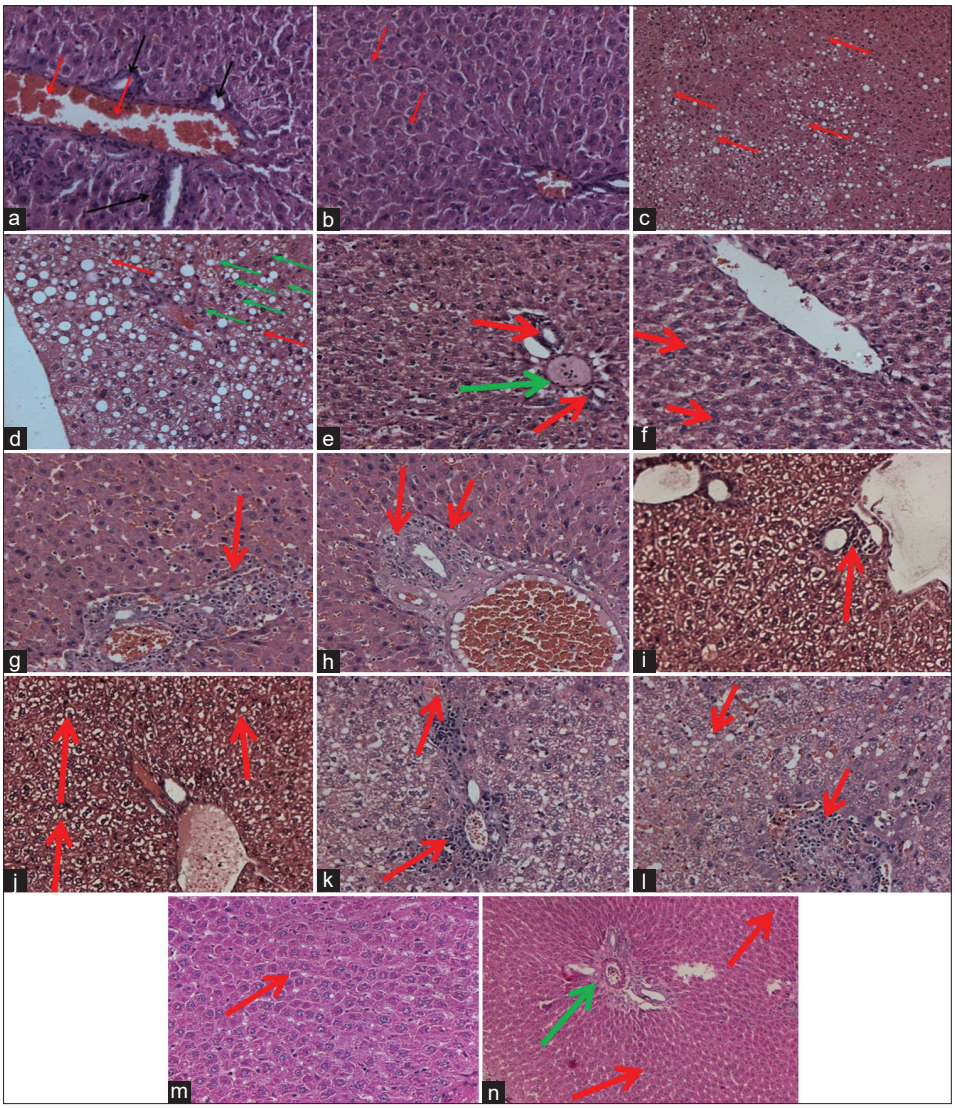

Fig. 3: Histopathological studies of *Cymbidium aloifolium* extracts at different doses and Liv.52 on ethanol-induced liver toxicity. (a and b) are of Group-I (control group) – No abnormality detected; Portal triad containing portal vein, hepatic artery, and bile duct appeared normal. (c and d) are of Group-II (treated with Ethanol) – Diffused macro and micro vesicular fatty degeneration along with hepatocytes ballooning was observed in centri lobular/peri-portal region of some lobules appear in toxic group. (e and f) are of Group-III (treated with Liv 52 25 mg) – Hepatocytes are appeared normal in portal, peri-portal, and centri-lobular region of liver in standard (Liv 52) treated group. (g and h) are of Group-V (treated with *Cymbidium aloifolium* ethyl acetate extract [CAEAE] at 200 mg/Kg b.w) – Moderate peribiliary inflammation and fibrosis noticed in portal triad region were observed in CAEAE 200 mg/kg b.w. treated group. (i and j) are of Group-VI (treated with CAEAE at 400 mg/Kg b.w) - Foci of peribiliary infiltration of inflammatory cells are observed in CAEAE 400 mg/kg b.w. treated group. (k and l) are of Group-VIII (treated with CAHAE at 200 mg/Kg b.w) - Foci of centri lobular necrosis were observed in hepatocytes of liver in CAHAE 200 mg/kg b.w. treated group. (m and n) are of Group-IX (treated with CAHAE at 400 mg/Kg b.w) - Mild peri-portal connective tissue proliferation or fibrosis, infiltration of inflammatory cells in the liver was observed in CAHAE 400 mg/kg b.w. treated group. Red arrow indicate- Portal vein, hepatic artery, bile duct. Green arrow indicate- peri portal and centrilobular region of liver. Black arrow indicate- Portal triad containing portal vein

shows peri portal inflammation along with infiltration of lymphocytes and moderate degeneration (necrosis) in hepatocytes of liver in CAHAE at 200 mg/Kg b.w treated group. Fig. 3m and n shows the normal morphology of hepatocytes of the liver and normal morphology of portal region with bile duct and hepatic artery in CAHAE at 400 mg/Kg b.w treated group. The histopathological and enzymatic level analysis of *C. aloifolium* extracts against ethanol liver toxicity showed good liver protection as the standard drug Liv 52 and Fig. 3e and f shows normal portal triad with portal vein, bile duct, and normal appearance of hepatocytes in peri-portal and centri-lobular region in Liv.52 treated group.

The extracts of *C. aloifolium* (EAE & HAE) have shown different bioactive phytochemical constituents such as flavonoids, phenols, and alkaloids. As said above, there is a need to innovate new medicines to current emerging diseases and to fight against multi-resistant microorganisms [32]. In recent decades, many researchers are doing research in innovation of new bioactive molecules from natural resources or from their derivatives [33,34]. In recent times, different bioactive molecules have been reported against different diseases including hepatotoxicity as it is one of the crucial causes of deaths around the world nowadays [35,36]. The Liv.52 is one of such herbal medicines identified used to restore the damaged liver tissue against different hepatotoxins. The main components of Liv.52 were Chicory **(**Kasni) and Caper Bush **(**Himsra), both of which have different phytochemical constituents such as phenols, steroids, and flavonoids. Similarly, the *C. aloifolium* extracts had shown liver protection and antioxidant activity [37,38]. The biological activities of *C. aloifolium* extracts might be due to the presence of similar compounds of Liv52, as the phytochemical results showed the presence of flavonoids in both extracts.

The biochemical findings clearly demonstrate the protective effects of CAEAE and CAHAE extracts against ethanol-induced hepatotoxicity. In the ethanol-treated group (Group II), there was a marked elevation in serum AST, ALT, ALP, and T.Bil, along with a significant decline in T.ptn levels. These changes reflect severe hepatic damage, membrane leakage, and impaired synthetic function caused by chronic ethanol exposure. In contrast, the control group (Group I) exhibited normal enzyme and protein values, confirming the absence of hepatic stress.

Treatment with the standard hepatoprotective drug Liv.52 (Group III) produced substantial restoration of enzyme levels toward normal ranges, validating the reliability of the experimental model. All doses of CAEAE and CAHAE showed dose-dependent improvements in liver function parameters when compared with the ethanol group. Lower doses (100 mg/kg) of both extracts produced only mild reductions in AST, ALT, ALP, and bilirubin, indicating partial hepatoprotection. However, the 200 mg/kg doses showed better modulation of these enzymes, suggesting an increasing ability to stabilize hepatocyte membranes and reduce oxidative stress.

The highest doses (400 mg/kg) of both extracts demonstrated the most pronounced protective effects. Among them, CAHAE at 400 mg/kg (Group IX) exhibited the greatest reduction in liver enzymes and bilirubin, with values approaching those of the Liv.52 group. In addition, T.ptn levels increased progressively with dose, reflecting improved hepatic synthetic capacity. These findings suggest that CAHAE may possess comparatively stronger hepatoprotective constituents than CAEAE.

Overall, the results indicate that both extracts significantly mitigate ethanol-induced hepatic injury in a dose-dependent manner, with CAHAE showing slightly superior restorative potential, particularly at higher doses. The improvements in biochemical markers support the hypothesis that the extracts exert their protective effects through antioxidant and membrane-stabilizing mechanisms.

Ethanol metabolism is essentially a furnace that floods hepatocytes with reactive oxygen species – acetaldehyde-driven free radicals, lipid peroxides, and protein-damaging oxidants. Into this biochemical storm, the extracts of *C. aloifolium* appear to act like molecular umbrellas, slowing the oxidative downpour through multiple antioxidant avenues.

CAHAE contains the highest levels of both phenols (25.59 mg/g) and alkaloids (24.12 mg/g**)**, notably higher than CAEAE. Phenolic compounds are molecular "electron donors," able to neutralize unstable radicals before they trigger lipid peroxidation. Alkaloids, on the other hand, often act as mitochondrial guardians, modulating redox cycling and stabilizing hepatocyte membranes.

Parameter	Ethanol	Liv. 52	Cymbidium aloifolium hydro-alcoholic extract 400 mg/kg
Aspartate aminotransferase (U/L)	327.33	103.83	222.67
Alanine transaminase (U/L)	159.00	57.17	109.17
Alkaline phosphatase (U/L)	483.83	155.17	330.00
Total bilirubin (mg/dL)	2.17	0.39	1.27
Total protein (g/dL)	4.15	6.67	5.47

CONCLUSION

The present study was intended to evaluate the hepatoprotective activity of *C. aloifolium* against ethyl alcohol causing hepatotoxicity based on its traditional usage in the treatment of different diseases including inflammation. The present study provides a scientific evidence of its medicinal values and the presence of different bioactive molecules in it. Additional research is needed and worthy in the evaluation of various biological functions and isolating specific components from *C. aloifolium.* The hepatoprotective landscape revealed by this study points to C. aloifolium as a promising natural reservoir of antioxidant and membrane-stabilizing phytochemicals. The hydro-alcoholic extract (CAHAE), enriched with significantly higher levels of phenols and alkaloids, demonstrated the greatest biological impact – producing a 43–47% restoration in key liver function markers at 400 mg/kg. This potency not only surpassed the activity of the EAE but also approached the efficacy of Liv-52, as seen in both biochemical normalization and histopathological repair.

These findings suggest that the hepatoprotective effect of *C. aloifolium* is closely tied to its phytochemical richness, particularly the synergistic presence of phenolic antioxidants and alkaloid constituents. By attenuating ethanol-induced oxidative stress, stabilizing hepatocyte membranes, and improving protein biosynthesis, CAHAE positions itself as a compelling botanical candidate for further isolation, purification, and characterization of its active hepatoprotective compounds.

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AUTHOR CONTRIBUTIONS

U. Praveen Kumar – Laboratory work, preparation of manuscript
M. Manikanta **–** Final review and supervision of project
Naresh Dumala **–** Statistical work, reviewing, and editing

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