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Chemopreventive effects of *Costus comosus* Linn against diethylnitrosamine-induced hepatocellular carcinoma in rats

Rachumallu Venkateswararao, Balasubramanian Arul, Ramalingam Kothai*

Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem-636008, Tamilnadu, India

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ABSTRACT

Introduction: *Costus comosus* is a potential medicinal plant used traditionally to treat various ailments. The present study aimed to evaluate antioxidant status, lipid peroxidation, and the ameliorative effect of its ethanolic leaf extract against diethylnitrosamine (DEN)-induced hepatocellular carcinoma (HCC) in rats.

Methods: HCC was induced by 0.01 % v/v DEN through the drinking water for 16 weeks. The animals were treated with ethanolic leaf extract of *C. comosus* (EECC) at 200 and 400 mg/kg for 16 weeks. In this study, tumour incidence, tumour volume, tumour burden, lipid peroxidation, antioxidant activity, liver marker enzymes, and histological responses were measured in the animals. At the end of the study, rats were sacrificed, their livers were removed and the levels of antioxidant enzymes were measured in the liver homogenate.

Results: In DEN-treated animals, there were 100% tumour occurrences probably due to an imbalance in carcinogen metabolizing enzymes and cellular redox state. The oral administration of ethanolic leaf extract of *C. comosus* therapy at a dose of 200 and 400 mg/kg reduced lipid peroxide levels and restored the increased activities of liver marker enzymes and antioxidant status to near normal. The biochemical findings corroborate histological findings, indicating that the leaf extract has a significant hepatoprotective impact in a dose-dependent manner.

Conclusion: The results of the present study showed the promising anti-carcinogenic effects of ethanolic leaf extract of *C. comosus* against the DEN-induced HCC in rats.

Implication for health policy/practice/research/medical education:
This research work showed the promising chemopreventive effects of ethanolic leaf extract of *Costus comosus* against the DEN-induced hepatocellular carcinoma in rats. Thus, this plant might be considered a candidate for the isolation of compounds, which leads to identifying the novel lead compounds for the development of anticancer drugs.

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Introduction

Hepatocellular carcinoma (HCC) is the most common histopathological type of liver cancer and is responsible for millions of liver cancer cases and fatalities. Over the last few decades, the prevalence of this condition has increased in several countries and is a vital component of the global cancer burden. Asia and Africa have the greatest prevalence rates in the globe, followed by Europe. According to the World Health Organization 2018 reports, the predicted global incidence rate of liver cancer per 100 000 persons per year was 9.3, with a corresponding fatality rate of 8.5. A direct relationship exists between HCC prevalence and age >75 years in most communities. According to the National Cancer Institute, men have 2 to 4-fold higher risks of HCC than women (1).

HCC is a highly complicated disease, with a variety of causes contributing to its development. The hepatitis B and C viruses (HBV and HCV), diabetes, obesity, alcoholic fatty liver disease, and non-alcoholic fatty liver disease are the most significant risk factors for HCC. Tobacco smoking, food pollutants such as aflatoxins, family or genetic factors, and a variety of environmental chemicals that function as carcinogens are all known to enhance the occurrence of HCC. The formation of HCC is triggered by

***Corresponding author**: Ramalingam Kothai,
Email: kothaiarul@yahoo.co.in

hepatic damage, which involves inflammation and results in the necrosis of hepatocytes and the regeneration of new ones. This liver disease initially develops as fibrosis and cirrhosis, and gradually turns into hepatocellular cancer (2).

The treatment of hepatic cancer is a difficult challenge for the medical field to keep people safe from cancer. HCC can be treated with resection, local ablation therapy, liver transplantation, transarterial chemoembolization, hepatic arterial infusion chemotherapy, and systemic targeted medicines like sorafenib based on the tumour size or underlying liver damage (3). As of right now, there is no particular effective drug or therapy option for the treatment of this serious disorder.

Extensive research has been conducted to discover a biomolecule from a natural source that can inhibit the various stages of cancer. Natural-derived substances have gained great importance in recent years, owing to their widespread use as antioxidants, which protect cellular organelles from oxidative damage. Many plants or plant components, such as *Silybum marianum* (silymarin), *Colchicum autumnale* (Colchicine), *Artemisia annua* (Artemisinin), *Ocimum sanctum* (Eugenol, Orientin), *Ginkgo biloba* (Ginkgetin, Ginkgolide A&B), *Ziziphus mauritiana* (α-linolenic acid, Methylstearat), *Solanum nigrum* (Solamargine, Solasonin), *Bauhinia variegate* (Kaempferol galactoside), *Zingiber officinale* (Gingerol), *Silybum marianum* (Silibinin), *Aegle marmelos* (Skimmianine), *Podophyllum hexandrum* (Podophyllotoxin), *Plumbago zeylanica* (Plumbagin), *Curcuma longa* (Curcumin), *Oldenlandia diffusa* (Ursolic acid), *Gossypium hirsutum* (Gossypol), *Actaea racemose* (Actein), and *Berberis vulgaris* (Berberine, Cannabisin), etc, have been reported for their anticancer activities against liver cancer (4).

N-nitroso compounds are very familiar for their mutagenic, teratogenic, and carcinogenic properties. Diethylnitrosamine (DEN) is a hepatocarcinogen, found in a wide range of products, including milk products, meat products, soft drinks, alcoholic beverages, and cigarette smoke. DEN is a typical chemical carcinogen with the ability to produce cancers in a variety of organs, including the liver, skin, gastrointestinal tract, and respiratory system. In particular, DEN is a full carcinogen in the case of HCC (5).

In this study, DEN was used for the induction of experimental HCC in rats. DEN seems to an essential ecological carcinogen in an environment surrounded by nitrosamines, and it is the primary cause of liver tumours (6). The presence of nitrosamines and their constituents in the human environment, together with the likelihood of their endogenous synthesis in the human body as a result of ingested secondary amines and nitrites, has raised suspicions about their possible role in HCC. It is currently widely used as a standard experimental model for human colorectal cancer (7).

Costus is a genus of an herbaceous perennial plant containing numerous species. Its species have been proved for various activities, such as anti-cancer (8-10), antioxidant (11), hepatoprotective (12-14), and anti-inflammatory (15,16) properties. *Costus comosus* Linn is a rhizome that belongs to the family of Costaceae, also known as red tower ginger. It is a favorite plant in tropical gardens all around the world because of its beautiful colour. Traditionally, the leaves and rhizomes have been used to cure fever, rash, asthma, bronchitis, intestinal worms, diabetes, and liver ailments (17). Its in-vitro antioxidant and antiproliferative effects were also reported (18). Despite these investigations and the widespread use of this plant in traditional medicine, only a few studies, such as phytochemical studies and acute toxicity, were reported. No works have been published on its anticancer activities. Hence, the present study aims to investigate its anticancer activity against DEN-induced HCC in male Wistar rats.

Materials and Methods

Drugs and chemicals

Diethylnitrosamine and silymarin were purchased from Sigma-Aldrich Chemicals Private Limited, Bangalore, India.

The fresh leaves of the *C. comosus* were collected from the Shervaroy hills, Salem, Tamilnadu, in January 2021. The plant materials were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. A voucher specimen (CCKV-1) was deposited in the Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Salem, Tamilnadu, for future reference.

Extraction of plant material

The leaves were dried in the shade at room temperature for 10 days before being finely pulverized and stored in an airtight container for future use. In this experiment, approximately 500 g of coarsely crushed leaves were used for continuous hot percolation with solvent systems in increasing order of polarities, such as pet ether, chloroform, acetone, ethanol, and aqueous solution. Preliminary phytochemical studies were performed to find out the presence of phytoconstituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates in the extracts.

Animals

Healthy adult male Wistar rats, 45 days old and weighing 120 g, were used for the study. The animals were procured from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) listed suppliers of Srivenkateshwara Enterprises, Bangalore, India. The animals were kept in well-ventilated polypropylene cages (12 hours light and 12 hours dark

schedule, 25°C and 55–65% humidity). The rats were given a normal diet of pellets and free access to water.

Preparation of animals

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for 12 hours; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P.col/28/2021/IAEC/VMCP) and were cleared by the same. In accordance with CPCSEA rules for the care of laboratory animals, as well as the ethical guideline for the examination of experimental pain in conscious animals, all studies were carried out in the early hours of the morning. For oral drug delivery in experimental animals, the conventional orogastric cannula was employed as a guideline.

Pharmacological studies

Experimental design

Thirty healthy male Wistar rats weighing 120-130 g were randomly divided into 5 groups with six animals in each group. Group I served as normal control, which received 0.1 ml of normal saline orally for 120 days. Group II served as disease control, which received 0.01% v/v DEN through the drinking water for 16 weeks (19). Similarly, Groups III, IV, and V animals received a 0.01% v/v DEN in drinking water for 16 weeks. Group III served as positive control and received silymarin (30 mg/kg) (20) for 16 weeks. Groups IV and V received EECC (200 and 400 mg/kg, p.o) for 16 weeks (19). During the study, body weight, food and water intake, and normal morphology of the animals were monitored periodically.

On the last day, after an overnight fast, the bodyweight of all animals was measured and sacrificed under light ether anaesthesia. Blood samples were taken from the retro-orbital plexus. To determine the occurrence of nodules, the rat livers were examined at the end of the 16-week study for the presence of nodules, and then meticulously numbered using a visual macroscopic inspection technique. The livers were then excised, weighed, cleansed with ice-cold saline, and homogenized with three volumes (w/v) of the required buffer using a Potter-Elvehjem homogenizer with a Teflon tube before centrifuging it for 20 minutes at 4°C at 1200 rpm. The supernatants were used to make biochemical estimates of the samples.

Evaluation of blood parameters

Haematological parameters comprising red blood cells (RBCs), white blood cells (WBC), and haemoglobin (Hb) were determined by the procedure described by Verma et al (21).

Estimation of biochemical parameters

Pepatic parameters, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), γ-glutamyl transpeptidase (γ GT) were determined by previously described methods (22,23). Other non-hepatic biochemical markers such as albumin, globulin, proteins, and total bilirubin were also determined by standard procedures.

Estimation of antioxidant and oxidative stress parameters

Various enzymatic antioxidant parameters, such as catalase (CAT) (24), superoxide dismutase (SOD) (25), glutathione peroxidase (GPx) (26)], glutathione reductase (GR) (27), and glutathione-S-transferase (GST) activities (28) were determined in liver homogenates.

Malondialdehyde (MDA) was measured in liver tissue to identify the lipid peroxidation profile (29).

Histopathological evaluation

Liver samples were fixed in 10% formalin and then embedded in paraffin wax for histological analysis. The embedded liver blocks were sliced into 3–5 mm sections with the help of an ultramicrotome and processed for haematoxylin and eosin (H & E) staining. The pathological alterations in liver tissue were assessed under light microscopy.

Statistical analysis

The results were expressed as the mean ± SEM and evaluated statistically using one-way ANOVA followed by Tukey's multiple comparison tests, which were performed using the GraphPad Instat software. Differences were considered statistically significant when $P<0.05$.

Results

Percentage yield and Phytochemical screening

The colour of the ethanolic extract was dark green. The ethanolic extract of *C. comosus* had a powder consistency and a distinctively pleasant aromatic odour. The percentage yield of ethanolic extract was 14.24%. Phytochemical evaluation of leaf extract of *C. comosus* showed the presence of the following phytoconstituents: alkaloids, sterols, carbohydrates, glycosides, fixed oils and fats, phenolic compounds, proteins and amino acids, terpenoids, saponins, tannins, gums, mucilage, and flavonoids.

General observations on body weight, food, and drink intake

There were no significant changes in the morphology of the animals during the entire period of the study (Table 1). The body weight of DEN-induced animals was considerably lower ($P<0.001$) than that of untreated control (group I) animals, whereas the bodyweight of animals treated with EECC (200 and 400 mg/kg) (group IV, and V) was close to group III. Throughout the trial,

there was no difference in food and water consumption between the control and experimental animals.

Liver morphology

In this study, the total number of rats, the incidence of liver nodules, and the average number of nodes per node bearing liver were measured. The effect of EECC on nodule incidence was shown in Table 1. It was significantly increased in the group II DEN treated animals when compared to normal group animal's ($P<0.001$) and showed the tumour formation. Group III animals were treated with silymarin, which significantly reduced the nodule formation when compared to group II animals ($P<0.001$). Meanwhile, administration of EECC at 200 and 400 mg/kg, b.w reduced the nodule formation in a dose-dependent manner when compared with DEN treated animals ($P<0.001$).

Effects of EECC on haematological factors

The effects of DEN and EECC on haematological markers, such as WBC, RBC, and Hb, were investigated. DEN groups confirmed that WBC levels were enhanced and the levels of RBC and Hb were reduced (Figure 1). The EECC at 200 and 400 mg/kg restored all of the DEN-induced hepatocarcinogenesis mediated by haematological factors. Treatment with silymarin in DEN-induced cancer groups resulted in a significant ($P<0.001$) alteration of the haematological profile that was comparable to the control group.

Effects of EECC on hepatic parameters

The results related the effects of EECC on liver functions are shown in Table 2. The serum levels of liver enzymes such as ALT, AST, ALP, LDH, and γ GT were remarkably elevated, while the non-hepatic serum markers such as proteins, albumin, globulin, and total bilirubin levels were considerably decreased in the DEN treated groups compared to the control group animals. The EECC-treated animals showed a significant decline in the liver enzyme levels and improved the non-hepatic markers in a

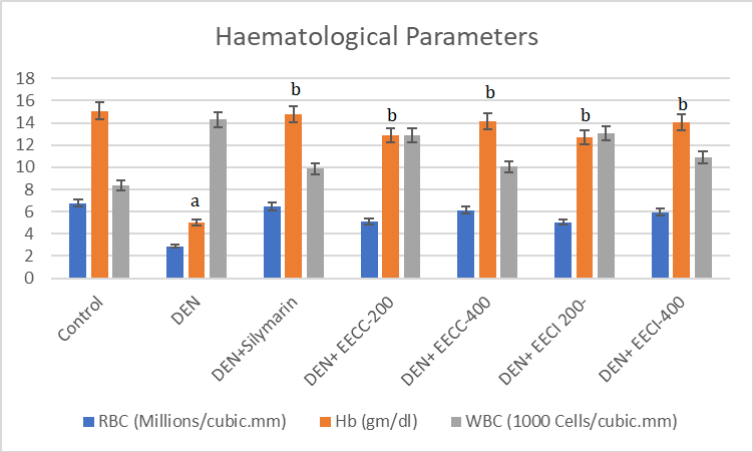

Figure 1. Effects of ethanolic leaf extract of *Costus comosus* on haematological parameters in diethylnitrosamine-induced hepatocellular carcinoma in rats. Values are expressed as mean ± SEM of 6 animals. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. a: indicates that group II (negative control) was compared with group I (control) ($P<0.001$). b: indicates that group III, IV, and V were compared with group II ($P<0.001$). Abbreviations: EECC: Ethanolic leaf extract of *Costus comosus*; DEN: Diethylnitrosamine; RBC: Red blood cells; Hb: Haemoglobin; WBC: White blood cells.

Table 1. Effects of ethanolic leaf extract of *Costus comosus* on body weight and liver morphology in diethylnitrosamine-induced hepatocellular carcinoma in rats

Groups	Tumour incidence rate	%Tumour incidence	Total number of nodules	Initial body weight (g)	Final body weight (g)	Liver weight (g)	Relative liver weight (g)
I Control	0/6	-	-	122.33±0.88	277.33±0.80	9.15±0.12	3.30±0.05
II DEN (0.01 % v/v)	6/6	100	115	120.17±0.60	230.16±0.60[a]	17.78±0.15[a]	7.66±0.08[a]
III DEN+Silymarin (30 mg/kg)	2/6	33.33	16	124.17±0.94	272.83±1.35[b]	9.98±0.17[b]	3.66±0.07[b]
IV DEN+ EECC (200 mg/kg)	4/6	66.66	38	123.66±0.95	250.67±0.95[b]	13.6±0.22[b]	5.43±0.09[b]
V DEN+ EECC (400 mg/kg)	3/6	50	23	124.33±0.33	264.33±0.33[b]	11.66±0.12[b]	4.41±0.05[b]

Abbreviations: EECC, ethanolic leaf extract of *Costus comosus;* DEN, diethylnitrosamine.
Values are expressed as mean± SEM of 6 animals. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. a: indicates that group II (negative control) was compared with group I (control) ($P<0.001$). b: indicates that Group III, IV, and V were compared with group II ($P<0.001$).

Table 2. Effect of ethanolic leaf extract of *Costus comosus* on biochemical parameters in diethylnitrosamine-induced hepatocellular carcinoma in rats

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	LDH (IU/L)	γ GT (IU/L)
I Control	120.12±0.42	39.23±0.57	35.60±0.88	75.49±0.81	5.61±0.15
II DEN (0.01 % v/v)	277.28±0.52[a]	109.63±1.48[a]	94.46±0.62[a]	151.25±0.67[a]	15.20±0.14[a]
III DEN + silymarin (30 mg/kg, p.o)	126.07±0.66[b]	43.38±0.57[b]	38.72±0.72[b]	76.54±0.90[b]	6.83±0.12[b]
IV DEN + EECC (200 mg/kg, p.o)	186.60±0.40[b]	85.05±0.40[b]	69.66±0.88[b]	94.36±0.62[b]	9.53±0.13[b]
V DEN + EECC (400 mg/kg, p.o)	134.50±0.54[b]	48.80±0.49[b]	41.92±0.96[b]	81.40±0.63[b]	7.14±0.33[b]

Abbreviations: EECC, ethanolic leaf extract of *Costus comosus;* DEN, diethylnitrosamine; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; γ GT, γ-glutamyl transpeptidase
Values are expressed as mean± SEM of 6 animals. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. a: indicates that group II (negative control) was compared with group I (control) ($P < 0.001$). b: indicates that groups III, IV, and V were compared with group II ($P < 0.001$).

dose-dependent manner. Animals treated with silymarin had significantly lower levels of liver enzymes, close to the levels found in the control animals.

Effect of EECC on anti-oxidant enzymes

Antioxidant enzymes are the most essential defensive mechanism against oxidative stress-induced cell damage. Animals in the DEN group had considerably lower levels of antioxidant indices, such as SOD, CAT, GPx, and GST, which were shown to be significantly improved ($P < 0.001$) by EECC in a dose-dependent manner (Table 3). Following treatment with silymarin, all of these enzymes underwent significant changes and were comparable with the control group.

Effect of EECC on oxidative stress

When compared to normal control rats, DEN-induced animals showed an elevated level of LPO. EECC administration resulted in a dose-dependent reduction in LPO, confirming its antioxidant properties, while the lipid peroxidation caused by DEN in hepatic tissue, was considerably improved by silymarin (Table 3).

Histopathological evaluation

Hepatocytes with uniform polyhedral shape were detected in the liver tissue of the control animals, with small uniform nuclei spread throughout the cytoplasm. Cell necrosis was observed in DEN-induced cancer animals, and the cytoplasm took on an uneven shape. Multiplication was identified in the portal area of hepatic stellate cells, which indicated the presence of focal proliferation (Figure 2). A significant improvement in histological characteristics of hepatic tissue was observed in the silymarin groups, with improved cytoplasm, fewer necrotic cells, altered hepatocytes, and altered hepatocellular architecture being observed. The EECC treated animals at 200 and 400 mg/kg showed an improvement by reducing the number of inflammatory cells, regularising the cytoplasm shape, reducing the number of bi-nucleated cells, and restoration of the architecture of hepatic tissue with altered hepatocytes in a dose-dependent manner.

Discussion

The present study investigated the anticancer effect of ethanolic leaf extract of *C. comosus* against DEN-induced HCC. *C. comosus* is a perennial plant that belongs to the costaceae family, commonly known as red tower ginger. Traditionally, this plant has been used to cure fever, rash, asthma, bronchitis, intestinal worms, diabetes, and liver ailments. Previous studies reported the presence of bio-active components namely alkaloids, sterols, carbohydrates, glycosides, fixed oils, fats, phenolic

Table 3. Effect of ethanolic leaf extract of *Costus comosus* on antioxidant enzymes in diethylnitrosamine-induced hepatocellular carcinoma in rats

Groups	SOD	CAT	GPx	GST	MDA
I Control	1.37±0.01	0.89±0.01	12.49±0.09	0.49±0.02	22.13±0.64
II DEN (0.01 % v/v)	0.59±0.02[a]	0.55±0.01[a]	7.70±0.01[a]	0.28±0.01[a]	47.99±0.71[a]
III DEN + silymarin (30 mg/kg, p.o)	1.32±0.01[b]	0.84±0.02[b]	12.27±0.04[b]	0.42±0.01[b]	21.07±0.75[b]
IV DEN+ EECC (200 mg/kg, p.o)	0.88±0.02[b]	0.61±0.01[b]	9.05±0.05[b]	0.37±0.02[b]	33.85±0.80[b]
V DEN+ EECC (400 mg/kg, p.o)	1.25±0.08[b]	0.71±0.01[b]	11.17±0..16[b]	0.41±0.02[b]	22.31±0.94[b]

Abbreviations: EECC, ethanolic leaf extract of *Costus comosus;* DEN, diethylnitrosamine; CAT, catalase; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde.
Values are expressed as mean ± SEM of 6 animals. Data were analyzed by one-way ANOVA followed by Tukey's multiple comparison tests. a: indicates that group II (negative control) was compared with group I (control) ($P < 0.001$). b: indicates that group III, IV, and V were compared with group II ($P < 0.001$).

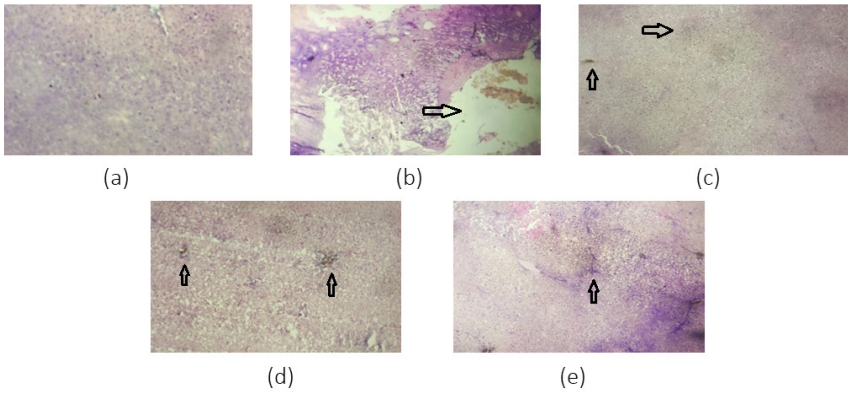

Figure 2. Effect of ethanolic leaf extracts of *Costus comosus* on histopathological studies. a: Control liver, b: DEN induced hepatocellular carcinoma, c: DEN+ Silymarin, d: DEN+ EECC-200 mg/kg, e: DEN+ EECC-400 mg/kg. Fig 2(a) shows the normal architecture of liver tissue of control animals. 2(b) shows cell necrosis and the presence of focal proliferation in the DEN-induced hepatocellular carcinoma animals, 2(c) shows the improved hepatic architecture with fewer necrotic cells, 2(d) and 2(e) show the improvement by the reduction of inflammatory cells and restoring the architecture of hepatic tissue in a dose-dependent manner. DEN: Diethylnitrosamine; EECC: Effect of ethanolic leaf extract of *Costus comosus*.

compounds, proteins, amino acids, terpenoids, saponins, tannins, Gums, mucilage, and flavonoids (17). This study, mainly focused on the effects of EECC on DEN-induced hepatocarcinogenesis and their changes in the body weight, food, and water intake, the morphology of the liver, and the incidence of nodules in the liver, changes in haematological, liver antioxidant enzymes, and oxidative stress levels. DEN-induced hepatocarcinoma is one of the most widely used animal models for hepatic cancer, which allows researchers to study the effects of anticancer drugs at various stages of neoplastic alteration and progression (30). DEN is a liver toxin that causes liver cancer and interferes with the normal functioning of the liver. The repeated interaction of the toxicant with the liver results in inflammation, cirrhosis, scarring of the liver, and ultimately cancer. End-stage liver cancer is characterized by impaired liver function, increased liver enzyme values, and death at the end. Losing weight is an indication of cancer induction induced by DEN (31). In this study, it was observed that EECC at 200 and 400 mg/kg doses considerably caused an increase in weight gain by increase in appetite or by blocking the action of DEN-induced liver damage and inflammation. No significant changes were also observed in the food and water intake of animals. These results were similar to those of a previous investigation (32).

The existence of preneoplastic nodules in liver tissue has already been proven in many publications that hepatocytes with prominent hyperplasia, cell proliferation, and enzymatic markers are the cause of the nodules. There is evidence in the literature that the establishment of hepatocyte nodes plays an important role in the progression of hepatic cancer (33). EECC at 200 and 400 mg/kg inhibited the growth of hepatic nodules and decreased the amount of hepatic blood marker enzymes in test animals throughout the experiment. EECC was also effective in removing the liver damage caused by DEN.

The most sensitive indications of hepatocyte injury are liver enzymes, such as AST and ALT, ALP, LDH, and γ GT. Due to liver dysfunction and a halt in the manufacturing of these serum markers enzymes, the onset of hepatocellular damage can be observed, along with a change in the penetrability of the liver cell membrane. The increase in the level of hepatic serum enzymes after DEN administration may be attributable to the leakage of these cytosolic enzymes into the bloodstream, which came as a result of hepatic destruction following DEN administration.

Serum proteins, such as albumin and globulin, are the most essential factors for determining the liver's secretory capacity. Decreased levels of serum albumins, proteins, globulins, and elevated levels of bilirubin indicated mild haemolysis and bile obstruction. EECC at high doses was successful in eliminating the liver toxicity caused by the DEN. The increased level of globulin suggests a change in liver physiology (34).

Bilirubin is a metabolic product of Hb that occurs in hepatocytes after conjugation with glucuronic acid. The measurement of blood bilirubin plays an important role in determining hepatic capacity, and any abnormal increase in serum bilirubin levels indicates hepatobiliary diseases and inflammation of liver function (35). In a dose-dependent manner, treatment with EECC reduced the amount of bilirubin in the blood, and the liver's normal function was restored.

The assessment of haematological parameters can be used to determine the degree of the deleterious effect of hazardous substances (20). The DEN group rat showed an increase in WBC counts, which is linked to pathological conditions and liver malignancy. The decreased RBC and Hb levels lead to anaemia (36). Pre-treatment with EECC at 200 and 400 mg/kg doses showed a significant reduction of WBC levels and a considerable increase of RBC and HB when compared to DEN group. This

suggests the protective effects of EECC against DEN-induced haematological changes.

Enzymatic antioxidant parameters are involved in scavenging superoxide anion to produce hydrogen peroxide, resulting in a reduction in the harmful effects of these radicals. SOD, CAT, GPx, and GST are important antioxidant enzymes. Reductions in these parameters may have an array of harmful effects (37). In this study, these hepatic enzymes were significantly reduced in DEN-treated animals, while EECC treatment reversed these levels near to the ones in the standard group.

Conclusion

The results of the present study showed a promising chemopreventive effect of *C. comosus* ethanolic extract against DEN-induced HCC in rats. This may be due to the antioxidant properties of the flavonoids, tannins, and polyphenols present in it. In the future, isolation and characterization studies are required for further evidence of its chemopreventive activity.

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Authors' contribution

RK and BA designed the study and wrote the manuscript with an interpretation of the results. RV carried out the animal studies. All authors read and approved the final version and agreed to publish it.

Conflict of interests

The authors declare no conflict of interest.

Ethical considerations

The experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P. Col/28/2021/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals.

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