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Research Article

Chemopreventive Potential of *Celastrus paniculatus* Seeds Extract in DMBA Synergistic with Croton Oil-Induced Skin Carcinogenesis in Swiss Albino Murine Model

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ABSTRACT

As one of the leading and fatal public health problems, skin cancer's progression is highly influenced by chronic inflammatory reactions and oxidative stress, accompanied by DNA mutation. Need for multi-option, plant-based alternatives, as first mentioned by Michael Sporn in 1976, offering chemoprevention to prevent initiation or inhibit tumor promotion and progression, and/or chemoprotection by use of plant-derived immunostimulants with the intention of immune system stimulation to either slow down or stop cancer evolution has been established as an acceptable standard treatment is costly and has severe side effects. The present study was designed to assess the chemopreventive effect of *Celastrus paniculatus* seeds against DMBA–induced skin carcinogenesis in Swiss albino mice, followed by croton oil administration. Parameters assessed included tumor incidence, yield, size, average latent period, hematological subsets, oxidative stress markers (LPO, CAT), inflammatory cytokines (TNF-α, IL-6), and histopathology. The disease control group (DMBA + croton oil application) showed 100% tumor incidence, high tumor yield, large papillomas (>8 mm), elevated LPO, reduced CAT, increased TNF-α, and IL-6 levels. The treatment groups showed promising results in contrast to the respective control groups. The oral + topical 400 mg/kg group showed the most pronounced protection, with tumor incidence reduced to 13.4%, tumor yield 0.33/mouse, the smallest papillomas (<2 mm), near-normal skin in histological assessments, restored CAT, reduced LPO levels, and normalized systemic inflammatory marker levels. *C. paniculatus* methanolic seed extract demonstrated dose- and route-dependent chemopreventive effects, with oral + topical 400 mg/kg showing the most promising effects, suggesting potential as well as a safer alternative adjuvant therapy along with the standard care in skin cancer prevention.

Introduction

As one of the leading non-communicable diseases, cancer contributes significantly to global mortality. It arises from uncontrolled cell proliferation and loss of normal regulatory mechanisms, often due to DNA damage caused by environmental carcinogens, radiation, or genetic mutations. This abnormal growth leads to tumorigenesis, and in malignant cases, can metastasize to other organs. The burden of cancer is rising universally, with skin cancer being one of the most common forms. Skin cancers are broadly categorized into basal-cell carcinoma (BCC), squamous-cell carcinoma (SCC), and melanoma. BCC is the most prevalent, with an 80% diagnosis in non-melanoma skin cancer, slow-growing but locally invasive. SCC, the second most common, is more likely to metastasize. Melanoma, although less frequent, is highly aggressive with a high mortality rate. The major risk factor for all three types is UV radiation exposure, especially UVB, which can cause direct DNA damage. Other factors include exposure to chemical carcinogens, chronic inflammation, and genetic predisposition.[1]

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The multistage model of skin carcinogenesis using DMBA as an initiator and croton oil as a promoter is a well-established experimental system to study cancer development. DMBA induces DNA mutations and oxidative stress, while croton oil promotes chronic inflammation, creating an environment conducive to tumor formation. This model allows evaluation of chemopreventive agents that can intervene at various stages of cancer development.[2] Conventional chemotherapeutic agents are effective against skin cancer but often cause significant side effects, including systemic toxicity, and may not completely prevent tumor recurrence. This has driven research toward safer, plant-based alternatives with antioxidant and anti-inflammatory properties.

Celastrus paniculatus, commonly known as "Intellect Tree" or Jyotishmati, is widely recognized in traditional systems of medicine, particularly Ayurveda and Unani, for its use in enhancing memory, alleviating inflammation, and promoting overall vitality.[3-5]

Phytopharmacological studies have demonstrated that its seeds contain bioactive compounds, including alkaloids (celastrine and paniculatine), terpenoids, flavonoids, celapanigin, malkangunin, celapagin[6] and fatty acids (linoleic and oleic). These phytoconstituents exhibit potent antioxidant, anti-inflammatory, and cytoprotective properties. Experimental studies have shown that *C. paniculatus* seed extract can reduce oxidative stress, inhibit pro-inflammatory cytokines, and protect against DNA damage induced by carcinogens.[7,8] An *in-vitro* analysis demonstrated significant free radical scavenging activity and protection against hydrogen peroxide-induced oxidative stress in neuronal cell models. Given the close link between chronic oxidative stress, inflammation, and carcinogenesis, these pharmacological attributes strongly support the hypothesis that *C. paniculatus* seed extract may exhibit meaningful chemopreventive effects in skin cancer models.[9,10]

Given its pharmacological profile, it's sufficient to say *C. paniculatus* has a promising anticancer effect on skin cancer. Evaluating its efficacy in a DMBA synergistic with croton oil-induced skin carcinogenesis paradigm provides an opportunity to assess its effects on tumor incidence, oxidative stress markers, inflammatory mediators, and histopathological changes, and to compare its performance with conventional chemotherapy. This experimental design not only provides insight into its anticancer potential but also establishes a novel pharmacological use of this ethnomedicinal plant, bridging traditional claims with modern chemoprevention research.

Materials And Methods

Plant Collection and Authentication

The seeds of *C. paniculatus* were procured from the authenticated vendor (Local Supplier) during June, Bengaluru, India and validated by Dr. V. Rama Rao, Research Officer, Department of Botany, CARI, and Reg. No. SMPU/CARI/BNG/2025-26/358.

Preparation of Extraction

Dried seeds were cleaned, ground into coarse powder, and extracted in methanol (99.9% of 750 mL) for 3 to 4 hours by using the soxhlet apparatus for three cycles by reducing the quantity of methanol over time. The mixture was filtered, and the solvent was evaporated under a reduced rotary evaporator to produce an extract, which was refrigerated (2–8°C) until use.[11, 12]

Dose selection

Based on previous toxicity and pharmacological studies, 200 and 400 mg/kg body weight oral doses of *C. paniculatus* seed extract were selected for the present investigation.[13]

Experimental Design

Female mice, Swiss Albino strain, weighing 20 to 25 g were used in this study. Although either sex can be employed in carcinogenesis experiments, females were selected for the present work to maintain uniformity and minimize variability. The animals were sheltered in standard conditions (temperature 22 ± 2°C, RH 50–60%, and a 12 h light/dark cycle) as per CPCSEA guidelines and kept for acclimatization for five days before the experiment in the controlled environment. The protocol was duly approved by IAEC and Sl.no. Sl. No. KCP IAEC/16/24-25/14/10/03/25.

Groupings

The animals were divided into the following manner: (n = 8/each group)

Normal control – water ad libitum

Disease control – DMBA (100 µL in acetone, STAT dose) + Croton oil (1% in acetone, TIW for 28 days)

Standard Dose – 5-Fluorouracil (10 mg/kg s.c., 3×/week for 28 days)

C. paniculatus (CP) – 200 mg/kg oral for 28 days. [13]

C. paniculatus (CP) – 400 mg/kg oral for 28 days. [13]

C. paniculatus (CP) oral + topical – 400 mg/kg oral + topical daily for 28 days.

Objective Parameters

During experimentation, mice were weighed daily and carefully examined once a week for skin tumors, and observations were recorded. The parameters below were taken into account.

Morphological Estimation[14]

- Body mass: Record the changes in body mass from the baseline
- %Tumor frequency
- Cumulative sum of tumors
- Tumor yield: Average number/mouse
- Diameter (mm) of Tumor; No(s) of Papilloma's
- Average latent period

Biochemical Estimation

After the end of the experiment, animals were sacrificed and tumors and skin biopsies were removed. One portion of tissue was used for biochemical estimations, and it was carried out on the skin tissues and test animals in each group. Cancer biomarkers were analysed. Murine skin tissue (DMBA-treated, untreated) was dissected, blotted dry, weighed, and homogenized using PBS in a homogenizer with a teflon glass pestle. Murine skin tissue homogenate was centrifuged at 1000×g for 5 minutes, and the supernatant was used for analysis. [15]

Lipid Peroxidation (LPO)/ Malondialdehyde (MDA) Content

Measured as malondialdehyde (MDA) content in skin tissue homogenates using the thiobarbituric acid reactive substances (TBARS) method, the chromogen (MDA–TBA adduct) absorbance is measured at 532 nm and expressed as nmol MDA/mg protein. [16]

Catalase (CAT) Activity

Determined in tissue homogenates by monitoring the decomposition of H_2O_2 absorbance at 240 nm. Results were expressed as nmol/ml. [17]

Haematological Parameters

Whole blood was analysed using an automated haematology analyser to determine complete blood counts (CBC), including total leukocytes, haemoglobin, neutrophil, lymphocyte, platelets, and red blood cell indices.

Systemic Inflammatory Markers/ Immunomodulation

Derived from hematological indices: neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), and systemic immune-inflammation index (SII = Platelets × Neutrophils/Lymphocytes). [18]

Inflammatory Cytokines (TGF-β and IL-6)

Quantified in tissue homogenates using commercially available sandwich ELISA kits, following the manufacturer's protocols. Concentrations were expressed as pg/ml protein.[19,20]

Histopathology Studies

After sacrificing the animals, one portion of tissue was removed and fixed in 10% formalin fixative for 24 hours. Dehydration of the tissue was done in an ascending series of alcohol, embedded in paraffin wax, and 4-µm-thick sections were prepared and studied using a light microscope.[14]

Statistical Analysis

The data were expressed as mean +/- SEM, n = 8/mice. The experimental groups were analysed by using Prism GraphPad and ANOVA, followed by Tukey's test to compare the significance difference between the treatment groups vs. disease control; p <0.05 was considered significant.

Results

In-vivo Study Parameters for the Anticancer Effect of the Test Drug Against DMBA Synergistic with Croton Oil-induced Skin Carcinogenesis

The *in-vivo* study assessed multiple parameters to assess the anticancer potential of the *C. paniculatus* against DMBA synergistic with croton oil-induced skin cancer in mice (Table 1). Changes in body weight across the treatment groups were monitored as an indicator of systemic health, providing insight into the overall physiological impact of the interventions. The normal control group exhibited a healthy weight gain from 20.96 to 23.00 g. In contrast, the disease control group (DMBA/Croton oil) showed a weight gain from 21.73 to 27.17 g, indicating tumor burden. The standard drug 5-Flu caused only a slight reduction in body

Table 1: *In-Vivo* study parameters for anticancer effect of Test drug against DMBA/Croton oil induced Skin Carcinogenesis

Group		*Body Weight (gm)*		*Tumor Incidence (%)*	*Cumulative of no's Tumours*	*Tumor Yield*	*ALP*	*No. of Papilloma with Tumor size (mm)*		
		Initial	*Final*					*<2*	*2-4*	*4>*
1	Saline treated mice (Normal control; 2ml/ kg.b.w)	20.96 ± 0.57	23.00 ± 0.58	-	-	-	-	-	-	-
2	DMBA/Croton oil (Disease control)	21.73 ± 1.06	27.17 ± 0.74	100	27	4	4.2	8	4	3
3	5-Fluorouracil – STD	22.09 ± 0.57	19.17 ± 0.60	32.5	8	2	5.4	4	2	-
4	CP orally (200mg/kg.b.w)	20.26 ± 1.38	25.00 ± 0.577	40	12	1.8	6.3	5	3	-
5	CP orally (400mg/kg.b.w)	22.24 ± 0.54	24.50 ± 0.76	23.5	7	0.56	6.5	3	-	-
6	CP orally (400mg/kg.b.w) + Topical	21.24 ± 0.54	22.50 ± 0.76	13.4	3	0.33	7.5	2	-	-

Values are expressed as Mean ± SEM, (n = 6 mice in each group).

weight, suggesting some level of drug-induced toxicity. *C. paniculatus* treatment groups, however, showed favourable weight gain.

Tumor incidence was 100% in the DMBA control group, confirming successful tumor induction. In contrast, treatment with 5-Flu reduced the incidence to 32.5%. *C. paniculatus* showed a dose-dependent reduction, with oral administration at 200 mg/kg reducing incidence to 40%, and at 400 mg/kg to 23.5%. The oral plus topical application of *C. paniculatus* group exhibited the lowest tumor incidence of 13.4%, indicating the most effective tumor prevention. Correspondingly, the average tumor size was largest in the DMBA control group (8 mm) and decreased in the treated groups, with oral plus topical application of *C. paniculatus* group showing the smallest tumors (2 mm). The cumulative number of tumors also declined significantly from 27 in the DMBA group to 3 in the *C. paniculatus* oral (400 mg/kg) plus topical application group.

Tumor yield, measured as tumors per mouse, followed a similar trend. While the DMBA group averaged 4 tumors per mouse, this was reduced to 2 with 5-Flu, 0.56 with oral *C. paniculatus* at 400 mg/kg, and 0.33 with oral plus topical *C. paniculatus*, demonstrating strong tumor suppression. ALP showing the most normalization (7.5), suggesting reduced tissue injury and tumor activity.

Additionally, papilloma analysis revealed that the DMBA group had a higher number of large papillomas (>8 mm), whereas *C. paniculatus*-treated groups had smaller papillomas (<2 mm) and fewer large ones. This indicates that *C. paniculatus* not only reduced tumor incidence and size but also limited the progression and aggressiveness of papillomas. The *in-vivo* study illustrated that the oral plus topical application of the *C. paniculatus* (400 mg/kg b.w.) exhibited the most potent anticancer activity in the DMBA synergistic with Croton oil-induced skin cancer murine model. In contrast to the disease control group, this group exhibited the lowest tumor incidence, the smallest average tumor size, the lowest cumulative number of tumors, and the most favourable body weight gain.

Assessment of the Test drug in Complete blood Profiles and subsets

Lymphocyte levels were markedly reduced in the disease control group (27.55 ± 2.55%) compared to the normal control (52.50 ± 2.5%), indicating immunosuppression induced by DMBA/Croton oil. Treatment with 5-Flu partially restored lymphocyte counts (41.50 ± 1.5%). Oral administration of *C. paniculatus* extract at 200 mg/kg significantly increased lymphocytes (57.50 ± 2.5%, $^{**}p < 0.01$ vs. disease control), while the 400 mg/kg PO dose and the combined oral + topical formulation produced the highest values (79.15 ± 0.85% and 81.20 ± 3.8%, $^{***}p < 0.001$), approaching or exceeding normal control levels (Table 2 and Fig. 1). These findings suggest that *C. paniculatus* treatment reverses carcinogen-induced lymphocyte depletion in a dose-dependent manner, indicating potent immunorestorative effects. Platelet counts were significantly elevated in the disease control group (4.05 ± 0.15 × 10^5/µL) in comparison to the normal

Table 2: Assessment of Test drug in Complete blood Profiles and subsets

Groups/Parameters	*NC (Saline)*	*DC (DMBA + Croton Oil)*	*STD Drug (5-FU)*	*C. paniculatus 200mg/kg*	*C. paniculatus 400 mg/kg*	*C. paniculatus 400 mg/kg P.O. + Topical*
Haemoglobin (g/dl)	16.90±0.5	13.30±0.7	16.33±0.87	15.50±1.0	16.00±1.4	15.55±0.95
RBC (Cells/mcL)	9.810±0.31	8.075±0.625	11.94±0.46**	8.970±0.15	8.435±0.06	8.225±0.07
Neutrophils (%)	47.50±2.5	38.25±1.75	40.00±5.0	42.50±2.5	20.00±5.0	22.50±7.5
Lymphocytes (%)	52.50±2.5	27.55±2.55	41.50±1.5	57.50±2.5	79.15±0.85	81.20±3.8
Platelet counts	1132±18	963.5±13.50	739.5±40.50	1212±12	688.0±12	1265±35
WBC (Cells/µL)	2.460±0.04	4.070±0.19	0.9250±0.53*	2.650±0.49	3.890±0.23	5.535±0.24
Systemic Inflammation Markers						
NLR	0.9091±0.09	1.406±0.19	0.9608±0.085	0.7424±0.075	0.2546±0.05	0.2721±0.08
Platelet lymphocyte Ratio (PLR)	21.59±0.68	35.23±2.77	17.88±1.62	21.13±1.12	8.692±0.058	15.59±0.29
Systemic Inflammation Index (SII)	1027±86.55	1352±167.6	707.0±24.51	900.7±100.7	175.9±42.90	347.0±111.8

Values are expressed as a Mean ± S.E.M (n = 3).

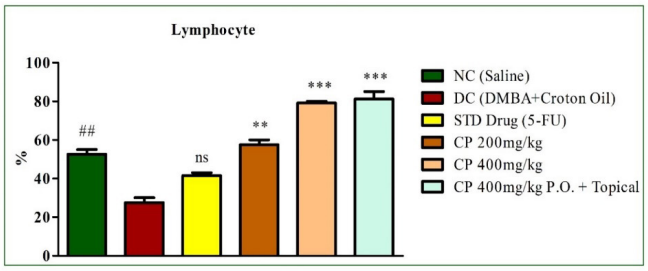


Fig. 1: Lymphocytes. Values are expressed as a 3). ##p<0.01 compared to disease control (DMBA + Croton Oil) vs normal control, ***p<0.001, and nsp>0.05 compared to Disease control vs Treatment groups. *Abbreviations: NC: Normal control, DC: Disease control, STD: Standard, CP: C. paniculatus*

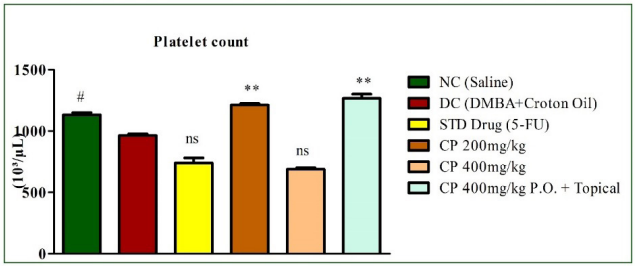


Fig. 2: Platelet counts. Values are expressed as a Mean ± S.E.M (n = 3). #p<0.05 compared to disease control (DMBA + Croton Oil) vs normal control, **p<0.01 and nsp>0.05 compared to Disease control vs Treatment groups

control ($3.12 \pm 0.13 \times 10^5/\mu L$, #$p < 0.05$), reflecting carcinogen-induced systemic inflammation. Treatment with 5-Flu and *C. paniculatus* (200 and 400 mg/kg, PO or oral + topical) reduced platelet counts toward normal levels. The reductions in treatment groups were not statistically significant (ns) when compared to the disease control, but the trend indicates a potential normalization effect, particularly at higher doses (Table 2 and Fig. 2). The NLR was markedly elevated in the disease control group, indicating carcinogen-induced systemic inflammation and immune imbalance. Treatment with 5-Flu and *C. paniculatus* extract (200 and 400 mg/kg) lowered NLR values compared to the disease control, with a significant decrease (**$p < 0.01$) observed in the high-dose oral + topical group. Other treatment groups showed a downward trend, but the changes were not statistically significant, suggesting partial but not complete normalization of the NLR in those groups (Table 2 and Fig. 3). The PLR was markedly elevated in the disease control group, indicating carcinogen-induced systemic inflammation and immune imbalance. Treatment with 5-Flu and *C. paniculatus* extract (200 and 400 mg/kg) reduced PLR values compared to the disease control, with a significant decrease (***$p < 0.001$) observed in the high-dose oral + topical application of *C. paniculatus* group (Table 2 and Fig. 4). SII was markedly elevated in the disease control group, indicating severe

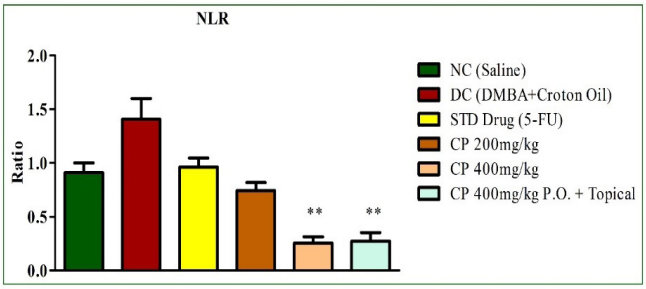


Fig. 3: NLR. Values are expressed as a Mean ± S.E.M (n = 3). **p<0.01 compared to Disease control (DMBA + Croton oil) vs Treatment groups. The rest other groups were shown non-significant though it has decrease in the treatment but not significantly changes

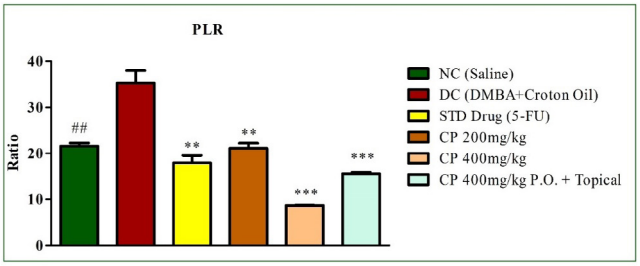


Figure 4: PLR. Values are expressed as a Mean ± S.E.M (n = 3). ##p<0.01 compared to disease control (DMBA + Croton Oil) vs normal control, **p<0.01 and ***p<0.001 compared to Disease control vs Treatment groups

carcinogen-induced systemic inflammation. Treatment with *C. paniculatus* extract reduced SII values compared to the disease control, with a significant decrease (**$p < 0.01$) observed in the high-dose oral + topical group. Other treatment groups showed a downward trend, but the changes were not statistically significant, suggesting partial improvement in systemic inflammatory status (Table 3 and Fig. 5).

Assessment of Test Drug on Antioxidant Enzyme Activity

LPO levels were significantly elevated in the disease control group (###$p < 0.001$ vs. normal control), reflecting severe oxidative stress induced by DMBA/Croton oil. Treatment with *C. paniculatus* extract reduced LPO in a dose-dependent manner. The high-dose oral + topical group

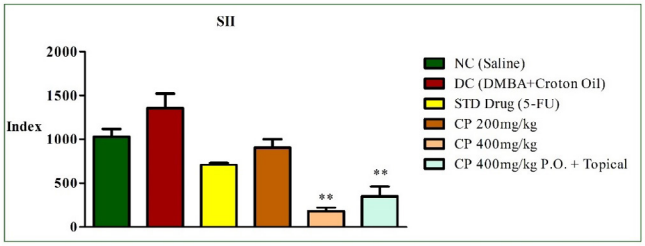


Fig. 5: SII. Values are expressed as a Mean ± S.E.M (n = 3). **p<0.01 compared to Disease control (DMBA + Croton oil) vs Treatment groups. The rest other groups were shown non-significant though it has decrease in the treatment but not significantly changes

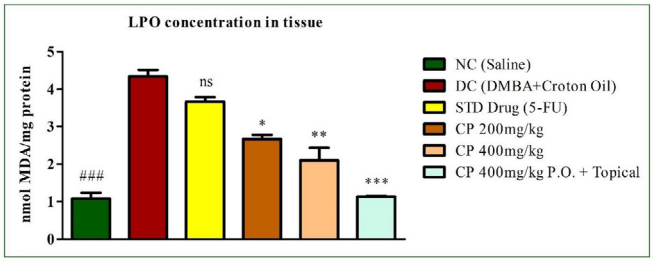


Fig. 6: LPO. Values are expressed as a Mean ± S.E.M (n = 3). ###p<0.001 compared to disease control (DMBA + Croton Oil) vs normal control, nsp>0.05, *p<0.05, **p<0.01, and ***p<0.001 compared to Disease control vs Treatment groups

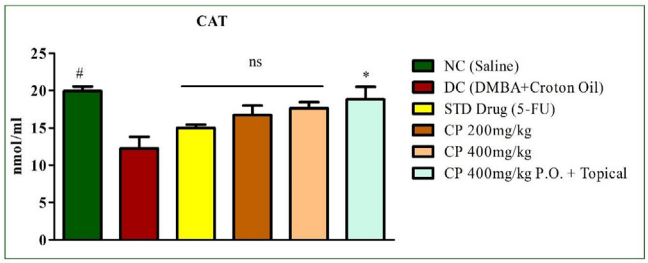


Figure 7: CAT. Values are expressed as a Mean ± S.E.M (n = 3). #p<0.05 compared to disease control (DMBA + Croton Oil) vs normal control, nsp>0.05 and *p<0.05 compared to Disease control vs Treatment groups

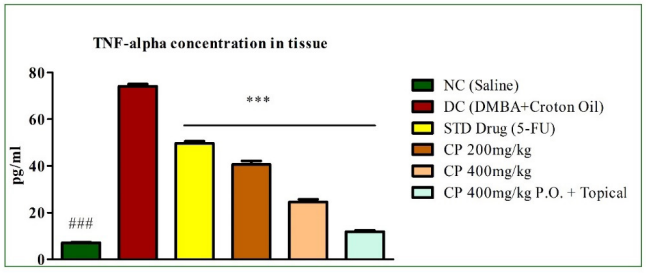


Figure 8: TNF-alpha. Values are expressed as a Mean ± S.E.M (n = 3). ###p<0.001 compared to disease control (DMBA + Croton Oil) vs normal control, ***p<0.001 compared to Disease control vs Treatment groups

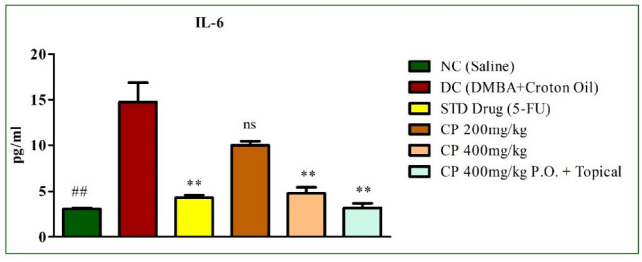


Fig. 9: IL-6. Values are expressed as a Mean ± S.E.M (n = 3). ##p<0.01 compared to disease control (DMBA + Croton Oil) vs normal control, nsp>0.05 and **p<0.01 compared to Disease control vs Treatment groups

showed the greatest reduction (***p < 0.001 vs. disease control), followed by 400 mg/kg CP oral (**p < 0.01) and 200mg/kg CP oral (*p<0.05). Although some reductions in 5-Fluorouracil were not statistically significant, the overall trend indicates that *C. paniculatus* markedly suppresses lipid peroxidation (Fig. 6). CAT activity was significantly less in the disease control group (#p < 0.05 vs. normal control), indicating reduced antioxidant defense following DMBA/Croton oil exposure. Treatment with *C. paniculatus* extract increased CAT activity, with the 400 mg/kg *C. paniculatus* oral + topical group showing a significant improvement (*p < 0.05 vs. disease control). Other treatment groups showed an upward trend, but changes were not statistically significant, suggesting partial restoration of antioxidant capacity (Fig. 7).

Assessment of Test Drug in Inflammatory Markers

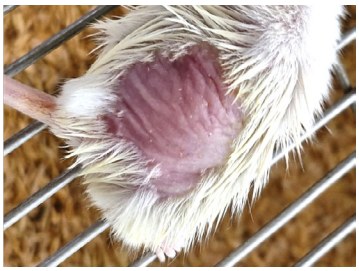
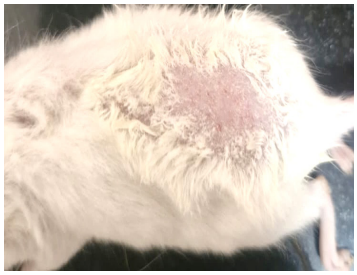
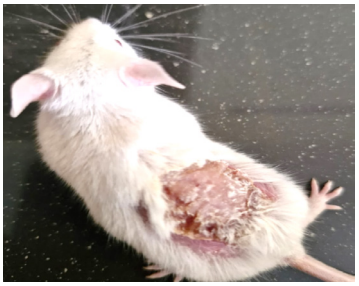
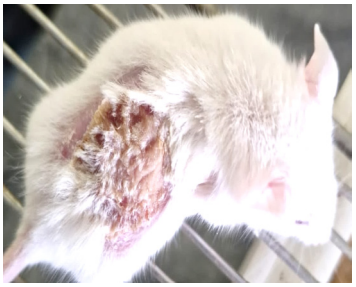
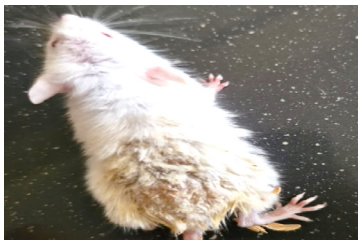
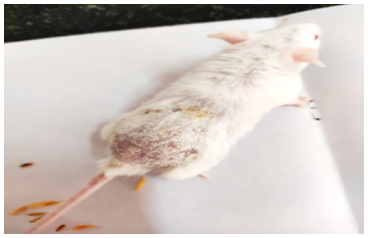
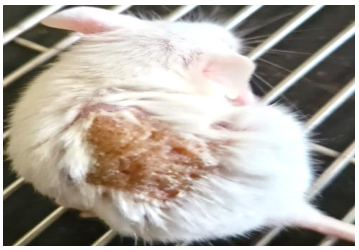
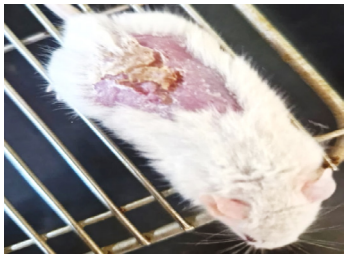
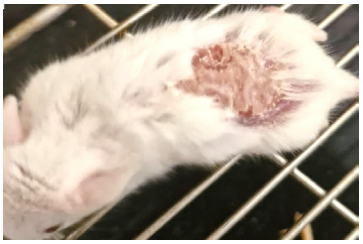
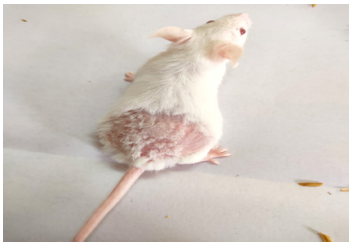
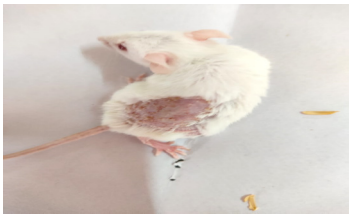
TNF-α levels were markedly elevated in the disease control group (###p < 0.001 vs. normal control), indicating a strong carcinogen-induced inflammatory response. Treatment with *C. paniculatus* extract and 5-Flu significantly reduced TNF-α levels (***p < 0.001 vs. disease control) in all treatment groups, with the greatest reduction observed in the 400 mg/kg *C. paniculatus* oral + topical application group. These results suggest that *C. paniculatus* possesses potent anti-inflammatory activity, effectively suppressing pro-inflammatory cytokine production (Fig. 8). IL-6 levels were significantly elevated in the disease control group (##p <0.01 vs. normal control), indicating carcinogen-induced inflammatory activity. Treatment with *C. paniculatus* extract at 400 mg/kg oral + topical application group significantly reduced IL-6 (**p < 0.01 vs. disease control). At the same time, other treatment groups also showed a downward trend, especially in 5-Flu and *C. paniculatus* 200 mg/kg, showing non-statistical significance (Fig. 9). These findings suggest that *C. paniculatus* can modulate inflammatory cytokines, with the most pronounced effect at higher doses and combined administration routes.

Assessment Study with Pictographs

The pictographs illustrate (Fig. 10) the comparative anticancer efficacy of the *C. paniculatus* against DMBA synergistic with Croton oil-induced skin cancer in the murine model over a treatment period. The normal control group, treated with saline, showed no signs of skin damage or lesions throughout the study, indicating healthy and intact skin architecture. In contrast, the disease control group (DMBA + Croton oil) exhibited progressive tumor development, with pronounced lesions, swelling, and ulcerations evident by the 8th week, confirming the successful induction of skin carcinogenesis. Treatment with the standard anticancer drug 5-Fluorouracil demonstrated significant protective effects, showing reduced lesion severity.

Among the test groups, *C. paniculatus* administered orally at 200 mg/kg body weight produced moderate improvements with partial inhibition of tumor progression. A higher oral dose of 400 mg/kg b.w. led to better therapeutic outcomes, evidenced by smaller and less aggressive lesions by the 8th

Fig. 10: Pictograph showing the anticancer effect of Test drug against DMBA/Croton oil Induced Skin carcinogenesis in mice;

Group	*4th Week*	*8th week*	*Interpretation*
Normal control (saline treated			Serves as a baseline, healthy and intact skin
DMBA/Croton oil (Disease control) DMBA (100g / 100l of acetone), croton oil (1% in 100l of acetone)			Visible onset of lesions, severe tumor growth, significant skin damage
5-Fluorouracil			Visible improvement, reduction in lesion
CP orally (200mg/kg.b.w)			Moderate to slight improvement, stabilization lesion development
CP orally (400mg/kg.b.w)			Fewer lesions to marked reduction in tumor size, suggest dose dependent efficacy
CP orally (400mg/kg.b.w) + Topical			Most effective with minimal lesions.

week, suggesting a dose-dependent effect. Notably, oral plus topical application of *C. paniculatus* at 400 mg/kg b.w. provided the most substantial protection, with nearly normal skin appearance and minimal tumor formation.

Histological Examination Evaluation

Histopathological analysis further validated the anticancer efficacy of the *C. paniculatus* against DMBA, synergistic with Croton oil-induced skin cancer in the murine model (Fig. 11). The healthy skin section exhibited well-organized epidermal and dermal layers with intact cellular architecture, indicating healthy tissue morphology. In contrast, the disease control group treated with DMBA/Croton oil showed marked disruption in tissue architecture, including irregular epidermal proliferation, hyperplasia, and complete loss of follicular structures, signifying aggressive tumor development. The standard drug 5-Fluorouracil group showed haemorrhagic changes, necrosis, and dense inflammatory infiltration, which, while indicative of treatment response, also reflected drug-induced tissue damage. In the groups treated with the *C. paniculatus*, oral administration at 200 mg/kg b.w. demonstrated a moderate decline in necrosis and cell death compared to the disease control, with partial preservation of skin architecture and reduced progression of abnormal cell growth. A higher oral dose of 400 mg/kg b.w. showed greater improvement, characterized by more organized tissue layers and a further reduction in tumor-associated histological abnormalities. Notably, the group treated with *C. paniculatus* via oral plus topical application at 400 mg/kg b.w. exhibited near-normal histological features, with well-preserved cellular morphology and minimal signs of necrosis or abnormal proliferation.

Discussion

The present study demonstrated that *C. paniculatus* seed extract possesses strong chemopreventive effects in a DMBA synergistic with Croton oil-induced murine skin carcinogenesis model. Treatment produced a clear dose-dependent response, with the oral plus topical high-dose group (400 mg/kg) showing the greatest effect, reducing tumor incidence to 13.4%, lowering cumulative tumor number from 27 to 3, and minimizing tumor yield to 0.33 aligns with and extends prior evidence that two-stage (DMBA/croton oil) skin carcinogenesis is highly susceptible to agents that blunt promotion-phase inflammation and PKC-driven signaling. Croton oil's phorbol esters are canonical tumor promoters via PKC activation, epidermal hyperproliferation, and sustained inflammation, so agents with antioxidant/anti-inflammatory activity can markedly suppress papillomagenesis.[21,22]

For instance, curcumin in the DMBA/Croton oil model showed greater efficacy when administered by both oral and topical routes compared to either alone, owing to enhanced local and systemic chemopreventive actions. Similarly, bartogenic acid from *Barringtonia racemosa* exhibited potent antitumor activity in the same model when delivered via both routes, reinforcing the concept that localized suppression of promoter-phase signaling (through topical application) combined with systemic antioxidant and immunomodulatory effects (via oral administration) yields synergistic benefits. [23]

The observed reduction in lipid peroxidation and restoration of antioxidant enzymes in the present study is consistent with findings from natural product-based interventions such as *Withania somnifera* and Aloe vera, which suppress oxidative stress and inflammatory mediators to inhibit tumor promotion. Additionally, the normalization of haematological parameters and improvement in NLR, PLR, and SII indices suggest that *C. paniculatus* may exert immunomodulatory benefits, a mechanism less frequently reported in earlier studies but gaining recognition as an important factor in cancer chemoprevention.

Mechanistically, reductions in LPO and improvements in CAT dovetail with *C. paniculatus* documented antioxidant profile *in-vivo,* while the immunomodulatory shift (↓neutrophils, ↑lymphocytes, improved NLR) is consistent with reports of *C. paniculatus*-mediated anti-inflammatory/immunoregulatory effects. Together, these pathways are exactly those most relevant to promotion-phase restraint in this model.[24,25] The haematology and subsets (NLR, PLR, SII) are well-established systemic inflammation surrogates linked to worse cancer outcomes when elevated; their normalization in *C. paniculatus* groups strengthens the claim of immunomodulatory benefit alongside antioxidant effects.[26,27] When benchmarked against a curcumin study in the same model, *C. paniculatus* 13.4% incidence compares favorably to curcumin's best combined-route result (≈33%), with similar antioxidant signatures (↓TBARS, ↑SOD/CAT), but additional immune normalization in the current study suggests *C. paniculatus* may couple redox control with stronger immunomodulation.[28] Tumor promotion hinges on PKC-driven epidermal hyperproliferation, COX-2 upregulation, and NF-κβ/IL-6 signaling. Phytochemicals that blunt these axes typically reduce papillomagenesis and prolong latency.[29] *C. paniculatus* exhibits strong free-radical scavenging and enzyme-restorative effects (↓LPO, ↑CAT), and reports from inflammatory models show reductions in TNF-α and IL-6, aligning with favourable shifts in NLR/PLR/SII.[30] These effects are biologically plausible given *C. paniculatus* phytochemical complexity, which together can act on multiple carcinogenesis stages. For instance, eugenol, a phenolic constituent of clove oil, has been shown to exert chemopreventive effects primarily through downregulation of COX-2 expression, suppression of pro-inflammatory cytokines (IL-6 and TNF-α), and enhancement of apoptotic signaling pathways, including upregulation of Bax and caspase activity while

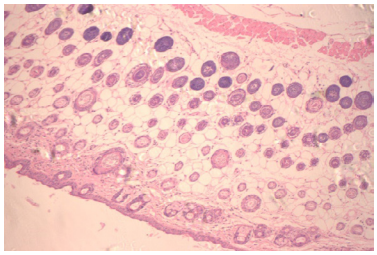
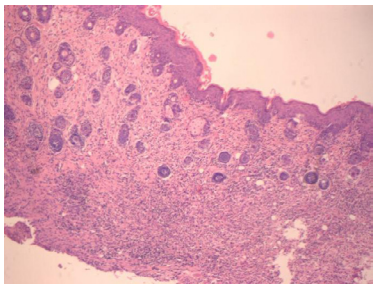
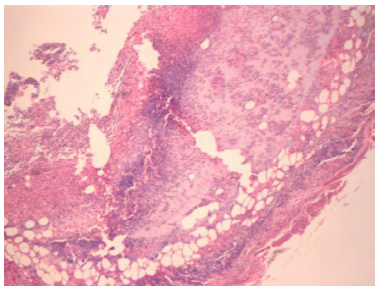
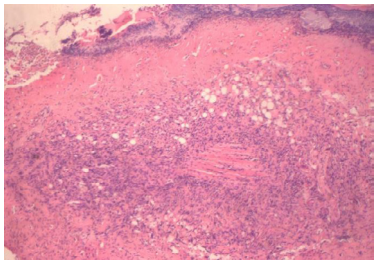
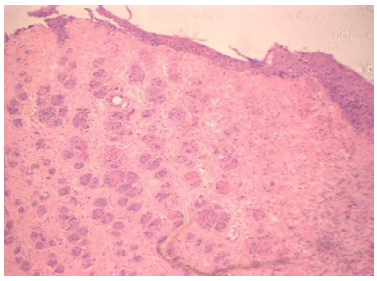
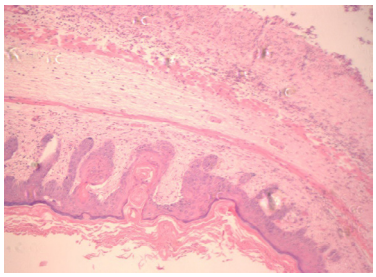
Normal Skin		INTERPRETATION Well organized, normal epidermal, dermal layer, intact hair follicles, no cellular abnormalities. Transverse section, H&E, 10X
DMBA/Croton oil (Disease control)		INTERPRETATION Disrupted architecture, irregular thick epidermal, loss of organization, loss of hair follicles. Showed hyperplasia. Transverse section, H&E, 10X
5-Fluorouracil – STD Drug		INTERPRETATION Necrotic patches, inflammatory cell infiltration, damage cellular components, shows some tissue-level improvement but is associated with drug-induced toxicity. Transverse section, H&E, 10X
CP orally (200mg/kg.b.w) – Test Drug		INTERPRETATION Partial improvement in tissue architecture, signs of necrosis, irregular epidermal layer, reduces progression to some extent. Transverse section, H&E, 10X
CP orally (400mg/kg.b.w) – Test Drug		INTERPRETATION Improved epidermal layer, reduced necrosis, cellular disintegration, tumor growth inhibited Transverse section, H&E, 10X
CP orally (400mg/kg.b.w) + Topical application– Test Drug		INTERPRETATION Appears normal with minimal necrosis. Hair follicles are visible to some extent. Prevent progression and helps maintain normal organization. Transverse section, H&E, 10X

Figure 11: Image of Skin showing the anticancer effect of Test Drug against DMBA/Croton oil Induced Skin carcinogenesis in mice;

downregulating Bcl-2.[31,32] Similarly, *Terminalia chebula* extracts significantly attenuated skin tumor incidence by restoring redox balance, lowering lipid peroxidation, and simultaneously reducing NF-κβ/IL-6/TNF-α signaling, culminating in reduced inflammatory infiltration and epidermal hyperplasia.[33]

These mechanisms strongly parallel our observations with *C. paniculatus*, where reduced lesion burden and size (<2 mm), diminished epidermal hyperplasia/inflammation on histology, and improved haematological inflammatory indices (NLR, PLR, SII) point toward a coordinated suppression of oxidative stress and inflammatory signalling. The convergence of evidence across these plants suggests that *C. paniculatus* exerts its chemopreventive action through a multi-target strategy, integrating antioxidant defense, cytokine modulation, and apoptotic regulation to effectively block the tumor promotion stage. This makes *C. paniculatus* a promising candidate for further development as both a chemopreventive agent and an adjuvant in skin cancer management.

Conclusion

The study establishes that *C. paniculatus* seed extract, particularly when administered both orally and topically at a high dose, exerts potent chemopreventive effects against DMBA synergistic with croton oil-induced skin carcinogenesis in a murine model. Its efficacy is evident from significant reductions in tumor incidence, size, and multiplicity; restoration of antioxidant enzymes; suppression of pro-inflammatory cytokines; and preservation of normal skin histology. Compared to standard 5-FU therapy, *C. paniculatus* demonstrated comparable or maybe superior tumor suppression with the most permissibility. These results highlight *C. paniculatus* as a promising candidate for developing safer, plant-based chemopreventive agents in skin cancer management.

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Conflict of Interest

None.

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