

Pharmacological Evaluation of *Centella asiatica* Leaves Extract Against DMBA-Induced Skin Carcinogenesis in Wistar Rats

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Received: 27th April, 26; Revised 07th May, 26, Accepted: 13th May, 26; Available Online: 15th May, 26

ABSTRACT

Skin cancer is one of the most common forms of malignancy worldwide, and the search for safer and effective therapeutic agents remains a major area of research. The present study was designed to evaluate the anticancer and chemopreventive potential of *Centella asiatica* leaves extract against 7,12-dimethylbenz[a]anthracene (DMBA)-induced skin carcinogenesis in Wistar rats. Ethanolic extract of *Centella asiatica* leaves was prepared using Soxhlet extraction and subjected to phytochemical screening and antioxidant evaluation. Skin carcinogenesis was induced using DMBA followed by croton oil promotion for 16 weeks. Experimental animals were divided into five groups: normal control, disease control, standard drug-treated group, low-dose extract-treated group, and high-dose extract-treated group. Parameters such as tumor incidence, tumor burden, antioxidant enzyme levels, and histopathological alterations were assessed. Preliminary phytochemical analysis revealed the presence of triterpenoids, flavonoids, tannins, phenolics, glycosides, and saponins. The ethanolic extract showed a percentage yield of 9.0% w/w. Administration of *Centella asiatica* extract significantly reduced tumor incidence, tumor burden, and tumor multiplicity in treated animals compared to the disease control group. The extract also improved antioxidant defense by enhancing superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPx) levels while reducing lipid peroxidation (LPO). Histopathological studies demonstrated restoration of normal skin architecture with reduced inflammatory and neoplastic changes. The findings suggest that *Centella asiatica* possesses significant chemopreventive and anticancer activity against DMBA-induced skin carcinogenesis, primarily through antioxidant, anti-inflammatory, and apoptosis-modulating mechanisms. Therefore, *Centella asiatica* may serve as a promising natural therapeutic agent for the prevention and management of skin cancer.

Keywords: *Centella asiatica*, Skin cancer, DMBA, Anticancer activity, Antioxidant, Chemoprevention, Wistar rat

INTRODUCTION

Cancer is a multifactorial disease characterized by uncontrolled proliferation and abnormal growth of cells due to disruption in normal cellular regulatory mechanisms.¹ Among different forms of cancer, skin cancer is one of the most prevalent malignancies worldwide.² Exposure to ultraviolet radiation, environmental pollutants, tobacco smoke, and chemical carcinogens significantly contribute to the development of skin carcinogenesis.^{3,4}

Skin carcinogenesis is commonly studied using experimental models involving 7,12-dimethylbenz[a]anthracene (DMBA), a potent polycyclic aromatic hydrocarbon known to induce DNA mutations and tumor initiation. DMBA-induced skin carcinogenesis in laboratory animals is widely accepted for evaluating chemopreventive and anticancer agents.⁵

Medicinal plants have gained increasing attention in cancer research because of their diverse pharmacological properties and reduced side effects compared with conventional chemotherapy. *Centella asiatica* (L.) Urban, commonly known as Gotu Kola, is an important medicinal herb belonging to the family Apiaceae.⁷ The plant has been

extensively used in Ayurveda and traditional medicine systems for wound healing, anti-inflammatory, antioxidant, antimicrobial, and neuroprotective activities.⁸ The therapeutic efficacy of *Centella asiatica* is attributed to the presence of bioactive compounds such as asiaticoside, madecassoside, asiatic acid, flavonoids, and phenolic compounds. Several studies have demonstrated that these phytoconstituents exhibit antioxidant and anticancer properties by scavenging reactive oxygen species, inhibiting inflammatory mediators, regulating apoptosis, and suppressing tumor cell proliferation.⁹ Considering these pharmacological properties, the present study was designed to evaluate the protective effect of ethanolic extract of *Centella asiatica* leaves against DMBA-induced skin carcinogenesis in Wistar rats.

MATERIAL AND METHODS

Plant Material Collection and Authentication

Leaves of *Centella asiatica* were collected and authenticated by a qualified botanist. The collected leaves were shade dried, powdered, and preserved for extraction.

Preparation of Extract

The powdered plant material was subjected to Soxhlet extraction using ethanol as solvent. Extraction was performed at controlled temperature conditions. The extract was filtered and concentrated under reduced pressure using a rotary evaporator at 40°C. The obtained crude extract was stored in airtight containers for further study.

Preliminary Phytochemical Screening

The extract was subjected to qualitative phytochemical screening for identification of major phytoconstituents including alkaloids, flavonoids, tannins, glycosides, saponins, phenolics, phytosterols, and carbohydrates using standard methods.

Experimental Animals

Male Wistar rats weighing 150–200 g were used for the study. Animals were maintained under standard laboratory conditions with free access to food and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) in accordance with CPCSEA guidelines.

Acute Toxicity Study

Acute oral toxicity study was carried out according to OECD guideline 423. The extract was found to be safe up to 2000 mg/kg body weight.

Induction of Skin Carcinogenesis

Skin carcinogenesis was induced using DMBA and croton oil.

- Initiation: A single topical application of DMBA (100 µg dissolved in 100 µL acetone) was applied to shaved dorsal skin.
- Promotion: Croton oil (1% in acetone) was applied three times weekly for 16 weeks.

Experimental Design

Animals were divided into five groups containing five animals each:

Group	Treatment
Group I	Normal control (vehicle only)
Group II	Disease control (DMBA + croton oil)
Group III	Standard drug (5-Fluorouracil cream)
Group IV	<i>Centella asiatica</i> extract 200 mg/kg
Group V	<i>Centella asiatica</i> extract 400 mg/kg

Evaluation Parameters

The following parameters were evaluated:

Tumor Parameters: Tumor incidence, Tumor multiplicity, Tumor burden, Tumor diameter, Tumor weight

Biochemical Parameters: Superoxide dismutase (SOD), Catalase (CAT), Reduced glutathione (GSH), Glutathione peroxidase (GPx), Lipid peroxidation (LPO)

Histopathological Studies

Skin and liver tissues were fixed in 10% formalin, processed, and stained using hematoxylin and eosin stain for microscopic examination.

RESULTS

Percentage Yield of Extract

The Soxhlet extraction of *Centella asiatica* leaves using ethanol yielded 45 g extract from 500 g powdered plant material, corresponding to 9.0% w/w yield.

Phytochemical Screening

Table 1: Phytochemical Screening

S. No	Phytochemical Constituent	Test Performed	Result
1	Alkaloids	Mayer's / Wagner's Test	Present (+)
2	Flavonoids	Alkaline Reagent Test	Present (+)
3	Tannins	Gelatin Test	Present (+)
4	Saponins	Froth Test	Present (+)
5	Glycosides	Keller-Killani Test	Present (+)
6	Phenolic compounds	Ferric Chloride Test	Present (+)
7	Terpenoids	Salkowski Test	Present (+)
8	Steroids	Libermann-Burchard Test	Present (+)

Preliminary phytochemical analysis revealed the presence of: Triterpenoids, Flavonoids, Tannins, Saponins, Glycosides, Phenolic compounds, Phytosterols. These constituents are known for antioxidant and anticancer activities.

Effect on Tumor Parameters

Table 2: Impact on Tumor Incidence across Experimental Groups

Group	Number of Animals	Animals with Tumors	Tumor Incidence (%)
Normal Control	5	0	0%
DMBA Control	5	5	100%
Standard Drug	5	1	30%
Extract Low Dose	5	3	60%
Extract High Dose	5	2	40%

Table 3: Detailed Morphological Parameters (Mean ± SEM)

Group	Tumor Yield (Mean Count)	Tumor Burden (Mean Size)
Normal Control	0	0
DMBA Control	8.5 ± 1.2	7.8 ± 1.0
Standard Drug	2.1 ± 0.5	1.8 ± 0.4
Extract Low Dose	4.5 ± 0.8	3.9 ± 0.7
Extract High Dose	2.8 ± 0.6	2.3 ± 0.5

The disease control group showed significant tumor incidence and tumor burden following DMBA induction. Treatment with *Centella asiatica* extract significantly reduced:

- Tumor incidence
- Tumor multiplicity
- Tumor burden

- Tumor size

The high-dose extract group demonstrated greater protective activity compared with the low-dose group.

Effect on Antioxidant Enzymes

Table 4: Effect on Antioxidant Enzymes (Mean \pm SEM)

Group	SOD (U/mg)	CAT (U/mg)	GSH (μ mol/g)	LPO (MDA/mg)
Normal Control	8.2 \pm 0.5	45.1 \pm 2.2	22.4 \pm 1.2	1.8 \pm 0.2
DMBA Control	2.1 \pm 0.4	12.5 \pm 1.8	6.8 \pm 0.9	9.4 \pm 1.1
Standard Drug	7.1 \pm 0.6	38.4 \pm 2.5	18.2 \pm 1.4	2.5 \pm 0.4
Extract High Dose	6.4 \pm 0.5	32.8 \pm 2.1	15.9 \pm 1.1	3.2 \pm 0.5

Administration of *Centella asiatica* extract significantly enhanced antioxidant enzyme levels compared with disease control animals., Increased SOD activity, Increased CAT activity, Elevated GSH and GPx levels, Reduced lipid peroxidation. These findings indicate restoration of antioxidant defense mechanisms.

Histopathological Findings

Histopathological examination of disease control animals showed severe epidermal hyperplasia, keratin pearl formation, inflammatory infiltration, and neoplastic alterations.

Animals treated with *Centella asiatica* extract showed:

- Reduced epidermal hyperplasia
- Improved tissue architecture
- Reduced inflammatory infiltration
- Lower neoplastic changes

The high-dose group exhibited near-normal histological appearance.

DISCUSSION

The present study demonstrated significant chemopreventive and anticancer activity of ethanolic extract of *Centella asiatica* against DMBA-induced skin carcinogenesis in Wistar rats. DMBA is a potent carcinogen capable of generating reactive oxygen species and inducing DNA mutations that initiate tumor formation. Repeated application of croton oil further promotes proliferation of initiated cells, leading to tumor development. In the present investigation, disease control animals developed pronounced skin tumors accompanied by oxidative stress and histopathological abnormalities.¹⁰ Treatment with *Centella asiatica* extract significantly reduced tumor incidence and tumor burden, suggesting inhibition of tumor initiation and progression. The observed anticancer activity may be attributed to the presence of triterpenoids such as asiaticoside and madecassoside along with flavonoids and phenolic compounds.¹¹

Oxidative stress plays a major role in carcinogenesis. The significant increase in antioxidant enzymes such as SOD, CAT, GSH, and GPx in extract-treated groups indicates enhancement of endogenous antioxidant defense systems. Reduction in lipid peroxidation further confirms the free radical scavenging activity of the extract.

Histopathological findings supported the biochemical observations, demonstrating restoration of normal tissue architecture and reduction in neoplastic alterations. These effects may result from anti-inflammatory, antioxidant, and apoptosis-inducing properties of *Centella asiatica*.

Previous studies have also reported that asiatic acid and related triterpenoids induce apoptosis through caspase activation, inhibit angiogenesis, suppress inflammatory mediators such as NF- κ B and COX-2, and regulate cell signaling pathways involved in cancer progression.

Overall, the study supports the traditional use of *Centella asiatica* and highlights its potential as a natural chemopreventive agent in skin cancer management.

CONCLUSION

The present investigation demonstrated that ethanolic extract of *Centella asiatica* possesses significant anticancer and chemopreventive activity against DMBA-induced skin carcinogenesis in Wistar rats. The extract effectively reduced tumor incidence, tumor burden, and oxidative stress while improving antioxidant enzyme status and histopathological features. The anticancer activity of *Centella asiatica* may be attributed to its rich phytochemical composition, particularly triterpenoids, flavonoids, and phenolic compounds, which exert antioxidant, anti-inflammatory, and apoptosis-modulating effects. These findings provide scientific evidence supporting the therapeutic potential of *Centella asiatica* as a natural and safer alternative for the prevention and management of skin cancer. Further studies involving molecular mechanisms and clinical evaluation are recommended to establish its role in cancer therapeutics.

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