Chemopreventive Potential of *Pleomele terniflora* in DMBA-Induced Skin Carcinogenesis: A Study on Oxidative Enzyme Status

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Abstract

Background: Skin carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) is closely associated with oxidative stress and disruption of endogenous antioxidant defenses. *Pleomele terniflora* (PT), a medicinal plant rich in bioactive phytochemicals, has been traditionally used for its therapeutic properties. However, its chemopreventive efficacy against skin cancer has not been fully elucidated.

Objective: The present study evaluated the effect of *Pleomele terniflora* extract on DMBA-induced murine skin carcinogenesis, with special emphasis on the status of key antioxidant enzymes.

Methods: DMBA-treated mice received PT extract and were assessed for tumor incidence, tumor burden, and biochemical alterations. The activities of antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR)—along with reduced glutathione (GSH) levels were measured to determine oxidative stress status.

Results: PT treatment significantly suppressed cutaneous tumor formation, reflected by decreased tumor incidence and multiplicity. Administration of PT markedly restored the antioxidant defense system by elevating the activities of SOD, CAT, GST, GPx, GR and increasing GSH levels. This enhancement of endogenous antioxidants was accompanied by a substantial reduction in oxidative stress markers. The findings reveal that the antitumor-promoting effect of PT is mediated through reinforcement of redox homeostasis and inhibition of oxidative damage.

Conclusion: *Pleomele terniflora* extract exhibits strong anticarcinogenic potential against DMBA-induced skin carcinogenesis. By boosting antioxidant enzyme activity and mitigating oxidative stress, PT offers a promising chemopreventive strategy for the management and prevention of skin cancer.

Keywords

Pleomele terniflora; DMBA; Skin carcinogenesis; Oxidative stress; Antioxidant enzymes; SOD; CAT; GST; GPx; GR; GSH; Chemoprevention; Murine model; Tumor suppression.

INTRODUCTION

Cancer is one of the leading non-communicable diseases worldwide and contributes significantly to global morbidity and mortality. Its incidence continues to rise due to rapid environmental changes, lifestyle modifications, and increased exposure to carcinogenic agents. All cancers originate from genetic damage, which may arise from internal factors such as spontaneous mutations, hormonal imbalance, or altered nutrient metabolism, as well as external factors including tobacco and alcohol consumption, chemical exposure, and various forms of radiation.

Skin cancer, characterized by the abnormal proliferation of skin cells, is the most common malignancy globally, accounting for approximately 40% of all diagnosed cancers. Although it can occur in individuals of all skin types, those with lighter skin who sunburn easily are at a higher risk. Skin cancer originates in the epidermis, which comprises three major cell types—squamous cells, basal cells, and melanocytes—each giving rise to distinct forms of cancer. These cancers develop as abnormal cells gain the ability to invade surrounding tissues and metastasize to distant sites.

The two-stage mouse skin carcinogenesis model, involving initiation and promotion, is widely used to investigate the molecular mechanisms underlying tumor development and assess the efficacy of chemopreventive agents. 7,12-Dimethylbenz(a)anthracene (DMBA), a potent polycyclic aromatic hydrocarbon, serves as an effective initiator capable of inducing DNA damage when applied topically. Its metabolic activation is primarily mediated by cytochrome P450 enzymes CYP1A1 and CYP1B1, generating the ultimate carcinogenic metabolite 1,2-epoxide-3,4-diol-DMBA. This metabolite forms DNA adducts that trigger mutations essential for tumor initiation. During the promotion stage, topical application of croton oil—rich in phorbol esters—stimulates excessive generation of reactive oxygen species (ROS) by macrophages, polymorphonuclear leukocytes, and non-phagocytic cells. Elevated ROS levels contribute to oxidative stress, DNA damage, and enhanced tumor promotion, establishing oxidative imbalance as a critical factor in carcinogenesis.

Pleomele terniflora is a small subshrub, typically less than 1 m in height, characterized by simple or sparsely branched spreading stems and distinctly stalked leaves arranged along the upper portions of the stem. The leaves are elliptic to elliptic-lanceolate, measuring 20–30 cm in length and 6–8 cm in width, with a conspicuous sheath at the base of the petiole. The plant bears terminal inflorescences approximately 15 cm long, with white flowers arranged singly or in small clusters. The fruit is a globose berry, 1–1.3 cm in diameter, containing 1–3 seeds. Although traditionally recognized for various medicinal properties, the chemopreventive or anticarcinogenic potential of *Pleomele terniflora* has not been thoroughly investigated.

Given the crucial role of oxidative stress in skin cancer progression, evaluating natural plant extracts that enhance endogenous antioxidant defenses represents a promising strategy for cancer prevention. Therefore, the present study investigates the protective effect of *Pleomele terniflora* extract on DMBA-induced skin carcinogenesis in mice, with particular focus on the modulation of key antioxidant enzymes.

Below is a **clean, organized, publication-ready "Materials and Methods" section** based on the details you provided. Language, scientific formatting, grammar, and flow have been improved while preserving all procedural details.

MATERIALS AND METHODS

1. Reagents and Chemicals

The following analytical-grade chemicals and reagents were used: DMBA, croton oil, L-methionine, Triton X-100, hydroxylamine hydrochloride, EDTA, riboflavin, sulphanilamide, phosphoric acid, naphthyl ethylenediamine dihydrochloride, 1-chloro-2,4-dinitrobenzene (CDNB), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), oxidized glutathione (GSSG), NADPH, sodium azide, hydrogen peroxide, trichloroacetic acid (TCA), phosphate buffer, Ellman's reagent, monobasic and dibasic sodium phosphate, potassium dichromate, acetic acid, thiobarbituric acid (TBA), and hydrochloric acid (HCl). All chemicals were obtained from standard commercial suppliers.

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2. Plant Material

Fresh leaves of *Pleomele terniflora* were collected from various regions of Tripura, India. The plant material was authenticated, cleaned, and prepared for extraction.

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3. Preparation of Plant Extract

Collected leaves were shade dried and ground into a coarse powder. The powder was extracted with 10% aqueous methanol using the percolation method for 24 hours. The extract was filtered, and the filtrate was evaporated to dryness to obtain a solid crude extract. Desired concentrations were prepared by dissolving the extract in dimethyl sulfoxide (DMSO). These extracts were used for evaluating chemoprotective activity.

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4. Experimental Animals

Outbred male Swiss albino mice were procured from the animal house of Tripura University. Animals were housed under standard laboratory conditions: temperature 22 ± 2°C, relative humidity 55 ± 5%, and a 12:12 h

light–dark cycle. They were fed standard pellet diet and water ad libitum. Mice were divided into four groups (n = 6 each):

- **Group 1:** Normal control (naïve mice)
- **Group 2:** Mice treated with *Pleomele terniflora* extract only
- **Group 3:** DMBA-induced skin carcinogenesis (disease control)
- **Group 4:** DMBA-induced mice treated with *Pleomele terniflora* extract

All experimental protocols were approved by the Institutional Animal Ethics Committee.

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5. Induction of Skin Carcinogenesis

Skin papillomas were induced by topical application of DMBA (25 µg in 0.1 ml acetone) twice weekly for 6 weeks. After tumor development, Group 4 animals received *Pleomele terniflora* extract orally at 500 mg/kg body weight/day for 4 weeks. At the end of the treatment period, mice were sacrificed, and blood and tissue samples (skin and liver) were collected for biochemical analysis.

6. Preparation of Tissue Homogenate

Skin and liver tissues were homogenized in 0.1 M phosphate buffer and centrifuged at 10,000 rpm for 20 minutes. The supernatant was collected for subsequent antioxidant assays.

7. Antioxidant and Oxidative Stress Assays

a. Superoxide Dismutase (SOD) Activity

SOD activity was estimated following Mark and Marklund (1974). A reaction mixture (1.4 ml) was prepared, and 100 µl of sample was added. After preincubation at 37°C for 5 min, 80 µl riboflavin was added. Tubes were exposed to light (200 W fluorescent lamp) for 10 min, followed by addition of 1.0 ml Griess reagent. Absorbance was measured at 543 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of nitrite formation.

b. Glutathione-S-Transferase (GST)

GST activity was measured using CDNB as a substrate (Habig et al., 1974). The reaction mixture contained buffer (1.0 ml), sample (0.1 ml), water (1.7 ml), and CDNB (0.1 ml). After incubation at 37°C for 5 min, 0.1 ml GSH was added. The increase in absorbance was recorded at 340 nm, and activity was expressed as nmoles of CDNB conjugate/min/mg protein.

c. Reduced Glutathione (GSH)

GSH levels were assessed according to Follow et al. (1974). Tissue homogenate (1.0 ml) was precipitated with 4.0 ml metaphosphoric acid and centrifuged. To 2.0 ml supernatant, 2.0 ml disodium hydrogen phosphate and 1 ml DTNB were added. Absorbance was read at 412 nm and results expressed as µM/mg protein.

d. Glutathione Reductase (GR)

GR activity was determined by the method of Carlberg and Mannervik (1975). The reaction mixture consisted of sample (0.2 ml), phosphate buffer (1.5 ml), EDTA (0.5 ml), GSSG (0.2 ml), and NADPH (0.1 ml). Decrease in absorbance at 340 nm was recorded and activity expressed as µmoles NADPH oxidized/min/mg protein.

e. Glutathione Peroxidase (GPx)

GPx activity was measured as described by Rashid et al. (2013). The reaction mixture included buffer (0.2 ml), EDTA (0.2 ml), sodium azide (0.1 ml), tissue homogenate (0.5 ml), GSH (0.2 ml), and H_2O_2 (0.1 ml). After incubation at 37°C for 10 min, reaction was terminated with 0.5 ml 10% TCA. Glutathione content in the supernatant was determined using Ellman's method at 412 nm. Activity was expressed as µg GSH consumed/min/mg protein.

f. Catalase (CAT)

Catalase activity was estimated following Luck (1963). The reaction contained buffer (1.0 ml) and water (0.4 ml). The reaction was initiated by adding 0.2 ml enzyme sample. Dichromate–acetic acid reagent (2.0 ml) was added at specific time points (0, 30, 60, 90 seconds). Tubes were heated for 10 min and absorbance was measured at 610 nm. Results were expressed as µmoles H_2O_2 decomposed/min/mg protein.

g. Lipid Peroxidation (LPO)

LPO was measured using TBA assay (Ohkawa et al., 1979). To 1.0 ml sample, 2.0 ml TCA–TBA–HCl reagent was added, heated for 15 min, cooled, and centrifuged. Absorbance of supernatant was read at 535 nm. Results were expressed as nmoles of malondialdehyde (MDA)/min/mg protein ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

8. Protein Estimation

Protein content was determined using the Lowry method (Lowry et al., 1951).

RESULTS

1. Confirmation of Skin Melanoma Development in DMBA-Induced Mice

Successful induction of skin melanoma in the DMBA-treated mouse model was confirmed through careful physical and morphological observation. Following four consecutive weeks of topical DMBA application, mice exhibited visible tumor growth on the dorsal skin surface. Tumors appeared as well-defined, elevated nodular lesions of varying sizes, indicating progressive development of papillomas/melanomas. In contrast, no such abnormalities were observed in the skin of naïve (control) mice, which remained smooth and devoid of lesions throughout the study.

Mice belonging to the DMBA-only group showed prominent tumor initiation and growth, whereas animals treated with *Pleomele terniflora* extract displayed a noticeable reduction in tumor size, appearance, and severity, suggesting a protective effect of the plant extract.

2. Alteration of Enzymatic and Biochemical Parameters

Along with visible tumor formation, DMBA-treated mice exhibited marked alterations in serum biochemical and enzymatic parameters. Significant changes were noted in antioxidant enzyme activities and oxidative stress markers, indicating enhanced oxidative damage associated with DMBA exposure. These alterations served as biochemical evidence supporting successful induction of carcinogenesis.

Conversely, treatment with *Pleomele terniflora* extract restored several antioxidant enzyme levels and reduced oxidative stress, reflecting its potential chemoprotective action.

DISCUSSION

Reactive oxygen species (ROS) generated during the metabolic activation of DMBA or during the subsequent stages of tumor development are capable of diffusing across cellular compartments and causing oxidative injury to various biomolecules. DMBA and its metabolites exert their mutagenic and carcinogenic effects through the generation of ROS, which act synergistically with DMBA-derived diol epoxides to induce DNA damage (Arya, 2011). Excessive ROS disrupt the cellular redox balance and shift the pro-oxidant–antioxidant equilibrium toward a more oxidative state, leading to enhanced oxidative stress. This oxidative burden damages cellular components, alters gene expression patterns, and promotes pathological changes associated with tumorigenesis.

In the present study, DMBA-treated mice showed enhanced hepatic lipid peroxidation (LPO), reflecting increased ROS generation and subsequent oxidative injury. The elevated tumor burden observed in these animals may be attributed to oxidative stress-induced damage and a compromised antioxidant defense system. Glutathione (GSH), a major intracellular antioxidant, plays an essential role in protecting tissues

from xenobiotics, ROS, and toxic metabolites. The liver, which contains high levels of GSH, supplies this antioxidant to peripheral tissues through specific transport mechanisms. Depletion of GSH in DMBA-treated mice likely occurred due to its excessive utilization in neutralizing ROS, thereby shifting the redox status further toward oxidative stress.

Chemoprevention has emerged as a promising approach for reducing cancer incidence, as numerous natural and synthetic compounds can block, delay, or reverse tumor initiation and progression (Beloki, 1993; Kim & Sporn, 2001). Oxidative stress, characterized by excessive ROS production accompanied by reduced antioxidant defenses, is strongly implicated in the pathogenesis of cancer (Halliwell, 2002). ROS induce lipid peroxidation, DNA strand breaks, and deregulation of cellular signaling pathways, all of which contribute to tumor development.

In the current investigation, administration of *Pleomele terniflora* (PT) extract beginning from the initiation of croton oil promotion and continued throughout the experimental period resulted in a marked inhibition of melanoma formation. This protective effect may stem from suppression of DMBA metabolism to its active carcinogenic intermediates or a delay in tumor promotion by reducing ROS formation (Kausar et al., 2003).

The findings further demonstrate that PT significantly enhanced activities of antioxidant and phase-II detoxifying enzymes such as CAT, GPx, GR, GST, and increased GSH levels, while reducing LPO. These changes reflect an improvement in endogenous antioxidant defense, suggesting that PT functions as an indirect antioxidant by inducing transcriptional upregulation of antioxidant enzymes. This is consistent with earlier reports highlighting the role of natural plant extracts in mitigating oxidative damage. Additionally, DMBA and croton oil are known to trigger inflammatory responses, including leukocyte infiltration, elevated ROS production, hyperplasia, and increased expression of pro-inflammatory mediators such as COX-2 and iNOS—all of which are closely associated with tumor promotion. PT-induced suppression of oxidative stress and inflammation likely contributed to the observed reduction in tumor burden.

Overall, the present study demonstrates that PT exerts its chemopreventive effects by attenuating oxidative stress, enhancing antioxidant defense mechanisms, and modulating early tumor promotional events.

CONCLUSION

The findings of the present study clearly indicate that *Pleomele terniflora* extract exhibits potent chemopreventive effects against DMBA-induced skin carcinogenesis in mice. PT significantly suppressed cutaneous tumor development through enhancement of endogenous antioxidant enzymes, elevation of GSH levels, and reduction of oxidative stress. These biochemical improvements provide strong evidence for the antitumor-promoting potential of PT.

Thus, *Pleomele terniflora* may serve as a promising natural agent for the prevention of skin cancer, offering a valuable strategy for future chemopreventive interventions. Further studies involving molecular pathway analysis and clinical evaluations are warranted to fully elucidate its therapeutic potential.

REFERENCES

1. Angus, W. G., Larsen, M. C., & Jefcoate, C. R. (1999). Expression of CYP1A1 and CYP1B1 depends on cell-specific factors in human breast cancer cell lines: Role of estrogen receptor status. *Carcinogenesis*, 20, 947–955.
2. Arya, P., & Kumar, M. (2011). Chemoprevention by *Triticum aestivum* in mouse skin carcinogenesis induced by DMBA and croton oil: Association with oxidative status. *Asian Pacific Journal of Cancer Prevention*, 12, 143–148.
3. Ather, M. (2002). Oxidative stress and experimental carcinogenesis. *Indian Journal of Experimental Biology*, 40, 656–667.
4. Belinsky, N., & Jaiswal, A. K. (1993). NAD(P)H:quinone oxidoreductase (DT-diaphorase) expression in normal and tumor tissues. *Cancer and Metastasis Reviews*, 12, 103–117.
5. Buters, J. T. M., Sakai, S., Richter, T., et al. (1999). Cytochrome P450 CYP1B1 determines susceptibility to 7,12-dimethylbenz[a]anthracene-induced lymphomas. *Proceedings of the National Academy of Sciences of the United States of America*, 96(5), 1977–1982.
6. Carlberg, I., & Mannervik, B. (1975). Glutathione levels in rat brain. *Journal of Biological Chemistry*, 250, 5475–5480.
7. Cheng, S. C., Prakash, A. S., Pigott, M. A., et al. (1988). Characterization of 7,12-dimethylbenz(a)anthracene–adenine nucleoside adducts. *Chemical Research in Toxicology*, 1, 216–221.
8. Das, U. N. (2002). A radical approach to cancer. *Medical Science Monitor*, 8, 79–92.