Investigation of Anticancer Activity of *Lannea coromandelica* Ethanol Bark Extract in 7, 12-Dimethylbenz (a) Anthracene Induced Cancer in Swiss Albino Mice

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ABSTRACT

The present study was aimed with the focus of ethanol bark extract of *Lannea coromandelica* (LC) to assess an anticancer property in two stage carcinogenesis model using 7, 12-Dimethylbenz (a) anthracene (DMBA) and croton oil induced cancer in Swiss albino mice for 16 weeks. Extract of LC bark and Flonida 5% cream of 5-Fluorouracil (5-FU) as standard drug was applied topically every day for 16 weeks following DMBA application. The topical treatment of extract significantly reduces various tumour parameters. Effect of extract on Superoxide dismutase (SOD) and Catalase (CAT) level revealed that extract showed significant results (*$p<0.05$) as compared to standard group. The malondialdehyde (MDA) levels in the extract-treated group were nearly the same as those observed in the standard group, and both groups exhibited lower values than the normal control group. In addition, reduced glutathione (GSH) activity in the extract-treated group was increased compared with the carcinogen-treated control group. Histopathological alterations in carcinogen treated control group observed in the form of hyperplasia, dyskeratosis, and well-differentiated squamous cell carcinoma, presence of keratinized pearls, whereas these were found to be reduced after administration of extract and standard drug. An overall finding shows that LC bark extract has potent anticancer activity and provides a scientific basis for its chemopreventive property.

Keywords: Anticancer, histopathology, 5FU, *Lannea coromandelica*, MDA, SOD.

1. INTRODUCTION

The most frequent malignant disease in humans and the second leading cause of mortality globally is skin cancer (Chinembiri et al., 2014). Different types of skin cancer go by different names depending on where the cell originated and how it behaved clinically (Orthaber et al.,2017). There are approximately one million or more new cases diagnosed year, and the figure is growing. Currently, radiation therapy, chemotherapy, immunotherapy, and surgical excision are the primary therapies for skin cancer (Awale et al., 2009). Despite progress in the field of drug research, the major adverse effects of currently available synthetic pharmaceuticals need the creation of new plant-based therapies. Due to the significant financial burden that cancer causes in poorer nations, numerous research teams there are examining the potential cytotoxic properties of therapeutic plants (Brennan and Davey-Smith, 2022; Elshazly et al., 2023; Amer et al., 2025) The fact that up to 80% of people in poor countries utilize herbal medicine to treat a range of illnesses indicates how popular it is to employ herbal extracts in addition to contemporary therapy (Nguyen et al., 2021). Therefore, a more advanced strategy is

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needed to reduce unintended toxicity while utilizing natural compounds that may function through various routes.

Animal models used to study human skin cancer often involve the application of environmental carcinogens to induce tumor formation. A commonly used skin carcinogen is DMBA, which is frequently utilized in preclinical research to investigate the mechanisms of skin cancer development and assess potential treatments. When applied topically, DMBA, in combination with a promoter agent like croton oil, induces the formation of skin tumors (Saha and Hait, 2012). This two-stage carcinogenesis model is widely regarded as an effective system for testing the impact of experimental drugs on tumor growth at various stages of initiation and progression (Able et al., 2009).

India is a country gifted with a traditional medical system and is home to several significant plant species that are employed in traditional medicines. The need for newer herbal medications against cancer is developing (Varicola et al., 2012). The plant has active ingredients in different portions that have distinct pharmacological effects in addition to secondary metabolites that are essential in treating multiple diseases (Bhide et al., 2016, Patil S. 2021). Tropical species include *Lannea coromandelica* (Family: Anacardiaceae) (LC), often known as the Indian ash tree. Based on existing data, this specific plant has been used in traditional medicine to cure many diseases (Sathish et al., 2010). Many research papers were successful in their attempts to verify the plant's claims. Numerous traditional applications of the plant are still awaiting scientific validation (Islam et al., 2022). Numerous chemical components have been found and isolated by a variety of investigations (Selvaraj et al., 2015; Das and Singirikonda, 2023) with the help of a vast numbers of secondary metabolites viz. flavonoids, phenolic acids, tannins, terpinoids, sterols, fatty acids and their ester derivatives (Sivaraj et al., 2018). Because of the presence of the active constituents, the LC plant showed various pharmacological activities like antioxidant (Alam et al., 2017), antimicrobial (Kaur et al., 2013), anticancer (Weerapreeyakul et al., 2016), anti-inflammatory, wound healing activity (Sathish et al., 2010), hepatoprotective (Sobeh et al., 2018) etc.

Authors have already done the phytochemical analysis and anticancer activity of various extracts of *L. coromandelica* bark by in-vitro and molecular docking approach on B16F10 melanoma cell line. The findings of study indicated that the various concentrations of ethanolic extract of *L. coromandelica* bark have significant anticancer activity by inducing cytotoxicity and apoptosis in B16F10 melanoma cell line. Along with that the molecular docking studies reveals that phytochemicals which are present in *L. coromandelica* bark shows strong binding affinity with a targeted protein human tyrosinase related protein (TYRP1) (Nargatti and Wadkar, 2024). But this study only provides preliminary data, not the actual biological process which will happen in animal models. Hence, the present study was aimed to evaluate the potential anticancer effects of ethanolic extract of LC using model of DMBA and croton oil induced two stage carcinogenesis in swiss albino mice.

2. MATERIALS AND METHODS

2.1 *Materials*

DMBA were procured from Sigma-Aldrich, Mumbai, India. Ethanol (AR Grade- 99.9%) was procured from Loba Chemie Mumbai. Flonida 5% cream of 5-FU was purchased locally.

2.2 *Procurement of plant and extract preparation*

The plant *Lannea coromandelica* (LC) was collected from the rural Chandgad area of Kolhapur District, Maharashtra, India. The plant material was identified and authenticated by a botanist, Mr. D. L.

Shirodkar, at the Botanical Survey of India (BSI), Pune. Taxonomical identification was confirmed using *Indian Medicinal Plants*, Vol. 3 (Kirtikar and Basu, 2000) as a reference. A voucher specimen of the bark was deposited in the herbarium of the Department of Pharmacognosy (Ref. No. BSI/WRC/Iden.Cer./2022/1804220006437).

The bark was collected, thoroughly cleaned, and shade-dried for three to four weeks. The dried bark was mechanically ground into a fine powder and stored in an airtight container until further use.

For extraction, 500 g of the dried powdered material was subjected to Soxhlet extraction using 99.9% ethanol for 7 hours at 40 °C. The resulting extract was filtered through Whatman filter paper, and the solvent was removed under reduced pressure using a rotary evaporator. The concentrated residue obtained after complete solvent evaporation was collected and stored in a glass bottle under refrigerated conditions (4 °C) for further investigations.

2.3 *Animal care and handling*

For the investigation, 25–30 gm Swiss albino mice were employed. For research purposes, the Appasaheb Birnale College of Pharmacy in Sangli provided the inbred mouse species. The animals were kept in controlled environments with 12-hour light-dark cycles, 50 ± 5% humidity, and 23 ± 2 ℃ temperature. Prior to the study, all the animals underwent a seven-day acclimatization period. Each animal was housed separately in sterile rice husk bedding inside cleaned polypropylene cages, and they were randomly assigned to the experimental, normal, and control groups. They received unrestricted access to water *ad libitum* and regular pellets for their base diet. Prior to the experimental protocol, the animals were acclimated to the laboratory environment for 48 hours in order to reduce any potential non-specific stress. The Appasaheb Birnale College of Pharmacy in Sangli, Maharashtra's Institutional Animal Ethical Committee (IAEC) accepted all of the experiments that were carried out (IAEC/ABCP/15/2021-22, dated 25/01/2022).

2.4 *Grouping of animals*

Initially, 6 animals were used for acute toxicity study as per the OECD guideline (No. 423) and fixed the doses of LC extract.

A total of 35 animals were divided into 5 groups at random in each group total 7 animals (5 x 7 =35) were kept. Except Normal group and Vehicle control group, all other animals received single topical treatment of DMBA (25µg/100µl of acetone) on shaven dorsal skin of mice and two weeks later, croton oil (1% in 100µl of acetone) was applied three times per week until the completion of the experiment (i.e., 16 weeks). (Xiao et al., 2021)

The groups were made as Normal group (Gr-1), Vehicle control group (Gr-2)- received topical treatment of acetone 100µl/mice on shaven dorsal skin of mice, Carcinogen treated control group (Gr-3)- received DMBA 25µg/100µl of acetone and croton oil 1% in 100µl of acetone shaven dorsal skin of mice, Standard group (Gr-4)- received topical application of 5% Flonida cream (5-FU) shaven dorsal skin of mice and Ethanolic extract of LC (Gr-5)- received topical application of LC ethanolic extract 200 mg/k b.w on shaven dorsal skin of mice. All animals received their treatment until the completion of the experiment (i.e., 16 weeks).

2.6 Parameters monitored

a. *Measurement of body weight:* The initial (at starting of experiment) and final (at termination of experiment) body weight of each animal of all groups was carried out upto 16^{th} week.

b. Tumour parameters: The various tumor parameters viz. the number cumulative of papillomas in each week was measured until completion of experiment. Tumour incidence, tumor yield, tumour volume, tumour size, tumour burden, tumour mass, and

average latent period of each group were carried out as per the standard methods (Patil et al.2016, Sharma, 2016, Vellaichamy L, 2009)

b. *Estimation tissue antioxidant biomarkers:* Estimation of Superoxide dismutase activity (SOD) in skin was measured as per method described by Marklund and Marklund, 1974, Catalase activity (CAT) in skin, lipid peroxidation (MDA) in skin and reduced glutathione (GSH) activity in skin is done by method described by Patil CR et al.2016,

c. *Estimation of DNA content of skin tissue homogenate***:** DNA content was estimated according to method of Ali *et al*., (2010).

d. *Histopathology study*: At the conclusion of the experiment on animals, mice were killed by cervical dislocation. Skin samples were taken, and they were preserved in 10% formalin for a subsequent histological analysis. Before being processed, a sample of each animal's skin was taken, preserved in cassettes with labels, and fixed in 10% formalin. Following the prescribed procedure, all skin samples were dehydrated in a graded series of alcohol (from 70% to 100% ethanol) in an automated tissue processor that operates automatically for 16 hours before being embedded using an embedder machine. The microtome was used to segment the tissue blocks at a thickness of 4 mm. After that, the slides were left to dewax before being stained with hematoxylin and eosin (H&E). Under a light microscope, stained slides were inspected with 40x magnification. The microscopic images were taken using Digital micro-imaging adaptor (12 mega pixel) that was connected to SGL-11A Digital microscope with SAGLO soft image analysis software. The thickness of epidermal hyperplasia, keratin pearl, and invasion into the neighboring layer are among the parameters that were noted.

Statistical data analysis

The mean ± standard error of the mean (SEM) was used to present the data from each group. The study utilized ANOVA followed by Dunnett's t-test to examine the data collected from the various groups. For all experiments, *$p<0.05$ and **$p>0.01$ were considered as statistical significance. All statistical analysis was carried out using GraphPad Prism 8.0.

3. RESULTS

The ethanolic extract of LC produced a yield of 27.8% following solvent evaporation.

Over the course of the 14-day trial of acute toxicity study, no animal deaths were noted even between treated and control animals, there was no discernible difference ($p<0.05$) in the changes in body weight or relative organ weight.

Body weight for all the treated groups was measured initially and after treatments for the period of 16^{th} week. The body weights were significantly decreased in carcinogen treated control group as compared to normal group and vehicle treated group. The topical administration of ethanol LC extract (30.83 ± 1.18**) and standard drug (31.20 ± 0.84) significantly increased the body weight in mice more than all other groups (Figure-1). Tumour parameters like tumour incidence, tumour volume and tumour burden were measured for all groups of animals and the result tabulated in Table 1. Tumour incidence of Standard group was found to be 25% and the LC ethanol extract treated group also shows tumour incidence of 25%, whereas carcinogen treated control group showed 75% with higher tumour volume (90.25± 2.20 mm^3) and tumour burden (1.54 ±0.44 mm^3). The tumour volume and tumour burden of Standard group was found to be 2.20 ±1.20 mm^3 and 0.18 ±0.13 mm^3 whereas LC extract treated group shows tumour volume and tumour burden of 2.24±1.56 mm^3 and 0.38 ± 0.15 mm^3.

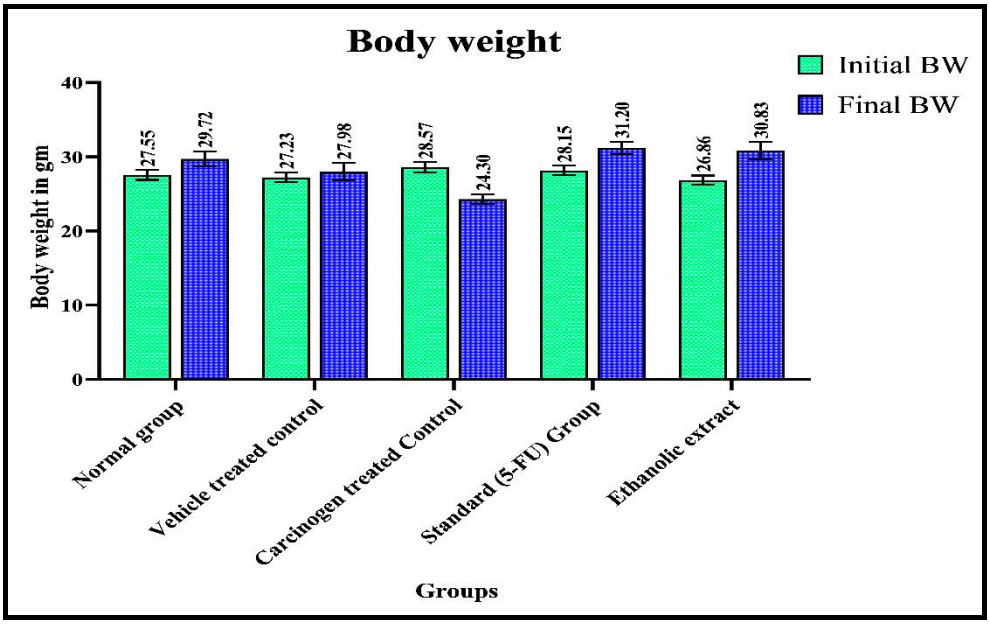

Figure 1: Effect of extract treatment on body weight of mice exposed to DMBA/ croton oil induced Skin cancer

Table-1: Effect of extract treatment on tumour incidence, tumour volume and tumour burden in mice exposed to DMBA/ croton oil induced Skin cancer

Groups	Tumour incidence	Tumour volume (mm^3)	Tumour burden (mm^3)
Normal group	00	00	00
Vehicle treated control	00	00	00
Carcinogen treated Control	75%	90.25± 25.20##	1.54 ±0.44##
Standard (5-FU)	25%	2.20 ±1.20	0.18 ±0.13
Ethanolic extract	25%	2.24±1.56**	0.38 ± 0.15**

• Values were represented as the mean ± SEM (n =7); **p<0.01, significant using one way ANOVA coupled with Dunnett "t" test. (**) indicates all other groups compared with control gr; (##) indicates control group compared with normal group.

Tumor yield, tumor size, and tumor mass were determined for all experimental groups (Table 2). Both the standard-treated and extract-treated groups showed lower values for these parameters compared with the carcinogen-treated control group. The LC ethanolic extract–treated group exhibited a significant reduction in tumor yield (0.09 ± 0.06**) compared with the standard-treated group (0.10 ± 0.04). In contrast, the standard-treated group showed a greater reduction in tumor size (0.70 ± 0.32 mm) and tumor mass (7.45 ± 4.25 mg) compared with the LC ethanolic extract–treated group, which recorded a tumor size of 0.84 ± 0.42** mm and a tumor mass of 8.55 ± 5.52** mg.

Table-2: Effect of extract treatment on Tumour yield, tumour size and tumour mass in mice exposed to DMBA/ croton oil induced Skin cancer

Groups	Tumour yield	Tumour size (mm)	Tumour mass (mg)
Normal group	00	00	00
Vehicle treated control	00	00	00
Carcinogen treated Control	1.28± 0.39##	5.58± 1.62##	41.15± 11.82##
Standard (5-FU)	0.10 ±0.04	0.70 ±0.32	7.45 ±4.25
Ethanolic extract	0.09 ± 0.06**	0.84± 0.42**	8.55± 5.52**

• Values were represented as the mean ± SEM (n =7); **p<0.01, significant using one way ANOVA coupled with Dunnett "t" test. (**) indicates all other groups compared with control gr; (##) indicates control group compared with normal group.

The cumulative number of papillomas was markedly reduced in the LC extract–treated group (7**) compared with the carcinogen-treated control group (78##). Additionally, the average latent period was significantly prolonged in the LC extract–treated group (11.80** weeks) relative to the carcinogen-treated control group, which exhibited an average latent period of 6.5## weeks (Table 3).

Table-3: Effect of extract treatment on Cumulative number of papillomas, Average latent period in mice exposed to DMBA/ croton oil induced Skin cancer

Groups	Cumulative number of papillomas	ALP (weeks)
Normal group	00	00
Vehicle treated control	00	00
Carcinogen treated Control	78##	6.5##
Standard (5-FU)	6	12.34
Ethanolic extract	7**	11.80**

• Values were represented as the mean ± SEM (n =7); **p<0.01, significant using one way ANOVA coupled with Dunnett "t" test. (**) indicates all other groups compared with control gr; (##) indicates control group compared with normal group.

Tissue antioxidant markers and lipid peroxidation of skin tissue homogenate were determined with respect to SOD level and CAT level. Increased in SOD and CAT levels observed with LC ethanol extract (12.85 and 2.88 U/mg of protein respectively) than Carcinogen treated control group (7.25 and 1.85 U/mg of protein respectively) (Table-5).

Table-4: Effect of extract treatment on level of SOD and CAT in mice exposed to DMBA/ croton oil induced Skin cancer

Groups	SOD (U/mg of protein)	CAT(U/mg of protein)
Normal group	14.20± 1.07	3.45± 0.05
Vehicle treated control	13.37± 1.45	2.96± 0.12
Carcinogen treated Control	7.25± 0.64##	1.85 ± 0.03##
Standard (5-FU)	13.02 ±0.41	2.92 ±0.08
Ethanolic extract	12.85± 0.45**	2.88± 0.02**

• Values were represented as the mean ± SEM (n =7); **p<0.01, significant using one way ANOVA coupled with Dunnett "t" test. (**) indicates all other groups compared with control gr; (##) indicates control group compared with normal group.

Thereafter, MDA level was decreased in LC ethanol extract (2.72 ± 0.02** nM of MDA/mg of protein) than Carcinogen treated control group (3.66 ± 0.01## nM of MDA/mg of protein) (Figure-2) whereas, GSH level was increased in the LC ethanol extract group (44.23 ± 0.17** nmol/mg protein) than Carcinogen treated control group (24.17 ± 0.32## nmol/mg protein) (Figure-3).

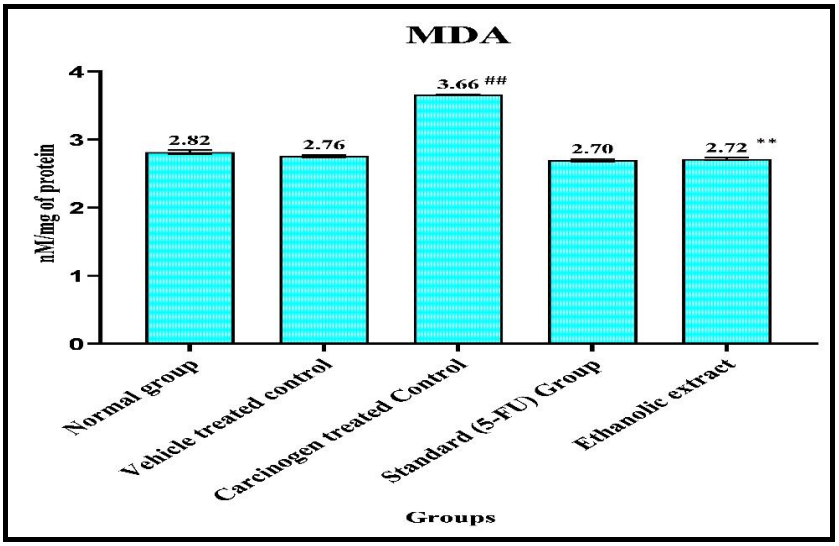

Figure 2: Effect of extract treatment on level of MDA in skin tissue homogenate

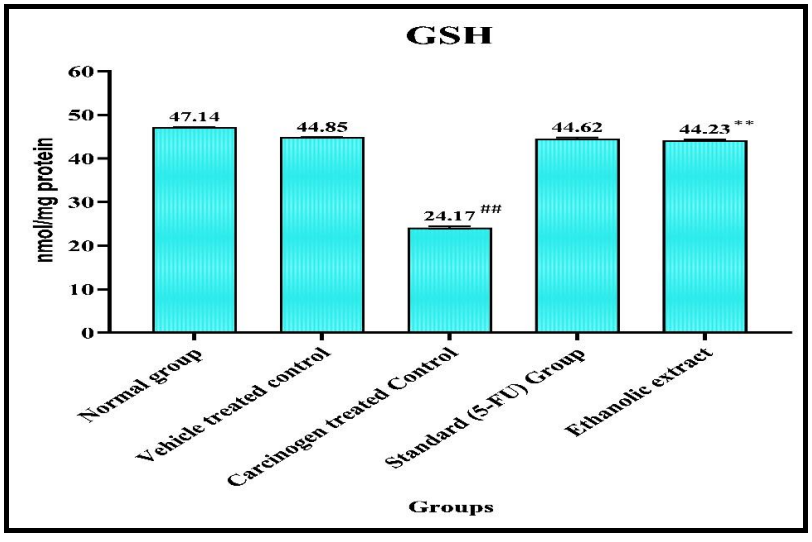

Figure 3: Effect of extract treatment on level of GSH in skin tissue homogenate

Finally, DNA content of the skin was estimated after 16th week and resulted the same trend as earlier results. LC ethanol extract treated animals showed high DNA content (38.80 0.75** mg/g) and nearly to the normal level as determined in normal group (41.95 ± 1.00 mg/g) but was more higher than carcinogen treated control group (27.15 0.42## mg/g) (Figure-4).

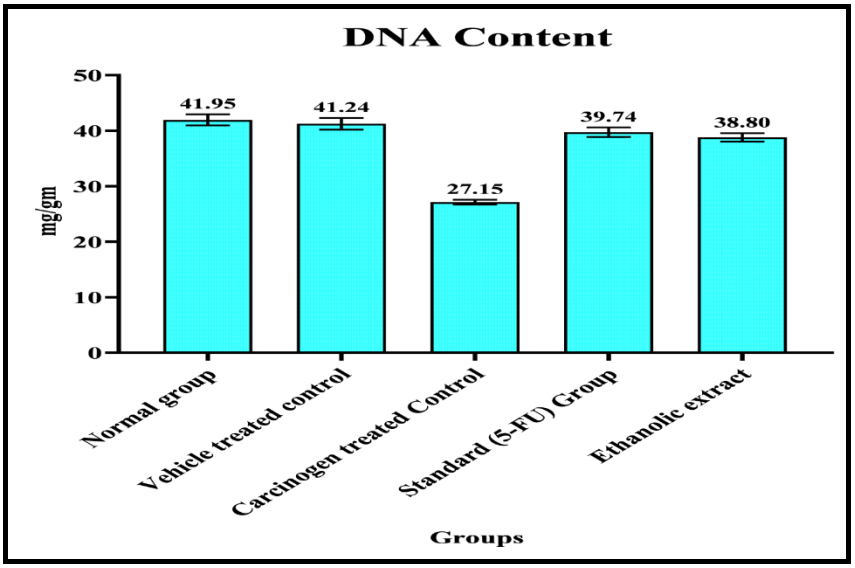

Figure 4: Effect of extract treatment on DNA content of skin in mice exposed to DMBA/ croton oil induced Skin cancer

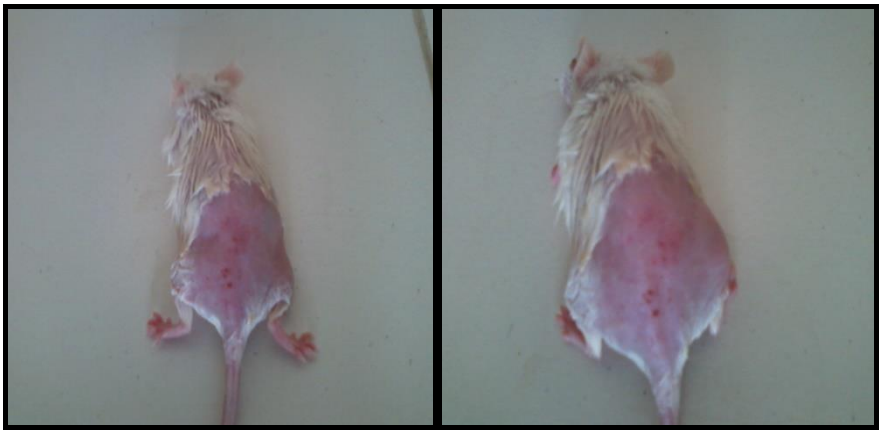

Figure 5: Normal control &Vehicle control group: No any signs of papilloma and ulceration

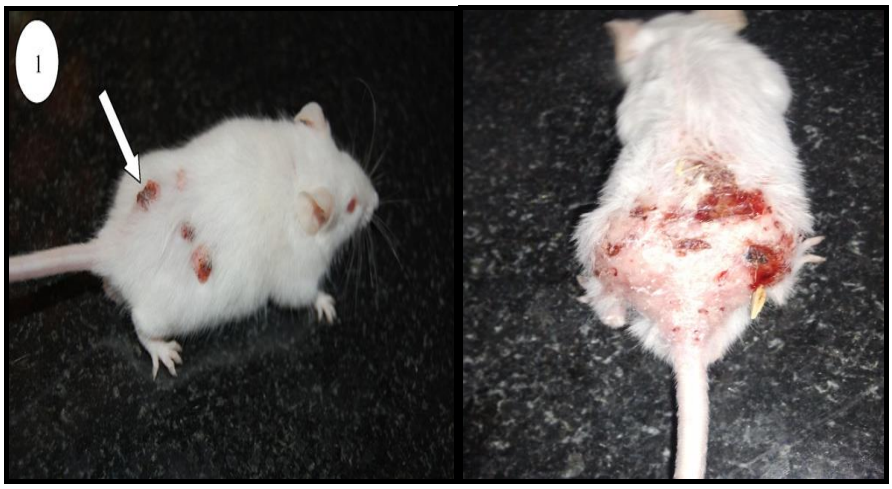

Figure 6: DMBA control group: The occurrence of maximum number of multiple papilloma with ulceration

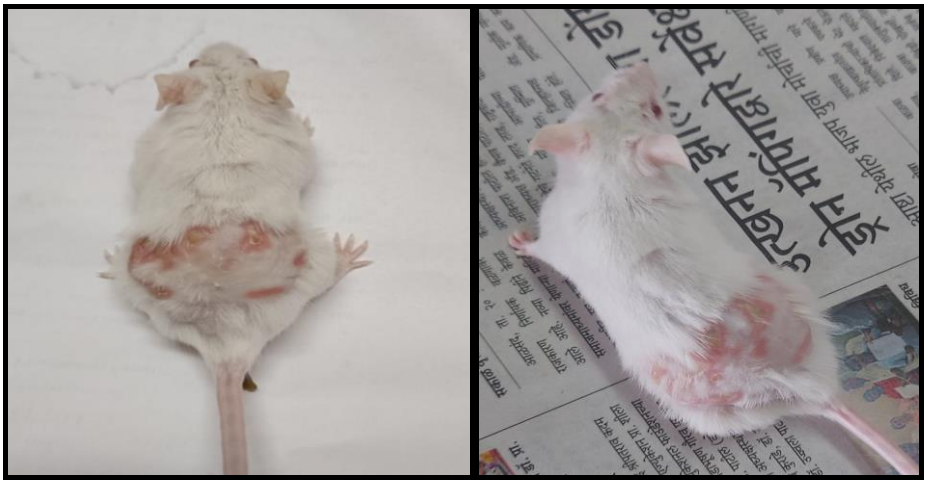

Figure 7: Standard (5-FU) + DMBA/ croton oil group: The occurrence of multiple papillomas and extent of ulceration is decreased as compared to DMBA control group

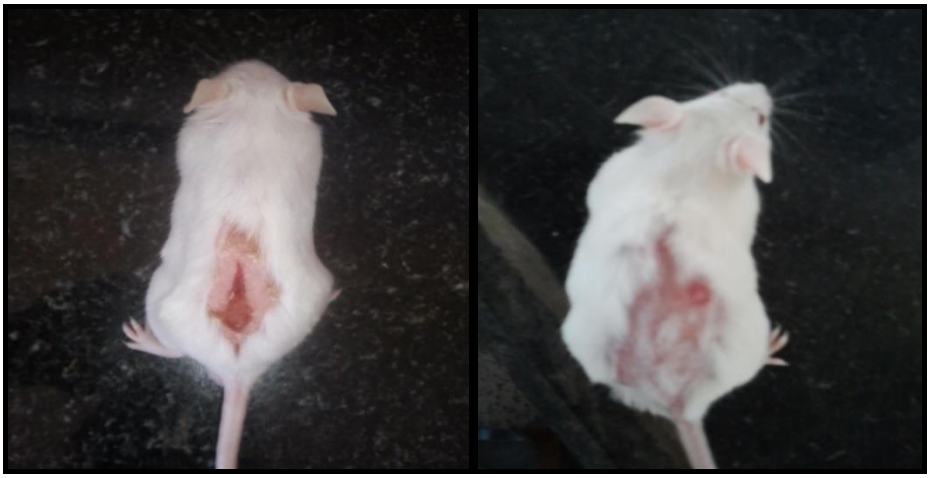

Figure 8: Ethanolic extract of LC+ DMBA/ croton oil group: The occurrence of multiple papillomas and extent of ulceration is decreased as compared to DMBA control group

Histopathological changes on DMBA induced skin cancer

Histological changes in the skin and tumors of mice from the treatment groups were examined using hematoxylin and eosin (H&E)–stained paraffin sections. Normal skin with an intact epithelium was observed in both the normal group and the vehicle control group. These groups showed a uniform arrangement of the epidermal and dermal layers, with a normal keratin layer covering the epidermis. No evidence of hyperplasia, hyperkeratosis, papilloma formation, or thickening of the keratin layer was observed in either the normal or vehicle control groups (Figures 9 and 10).

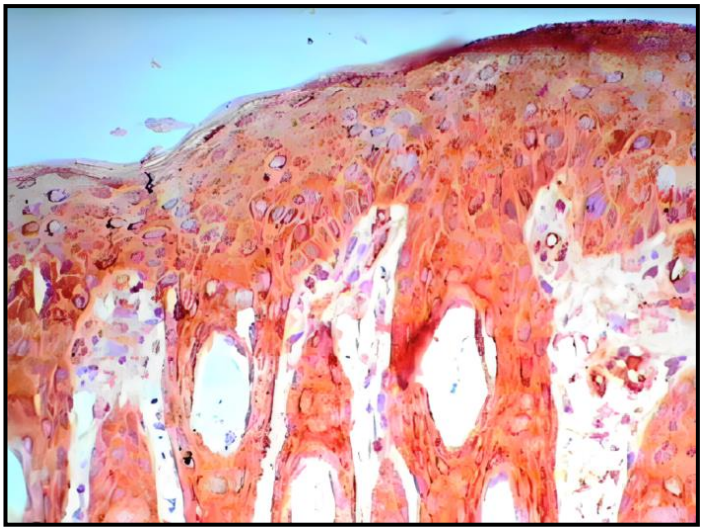

Figure 9: Microphotograph of Normal group (H&E magnification x40)

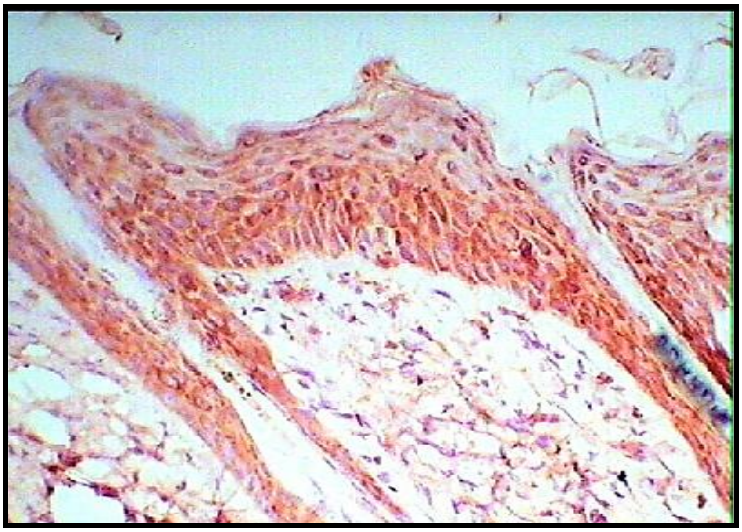

Figure 10: Microphotograph of Vehicle control group

Histological sections from the DMBA/croton oil–treated group revealed well-differentiated squamous cell carcinoma, dyskeratosis of the epidermis, abnormal thickening of the epidermal layer, and marked leukocyte infiltration, along with the deposition of keratinocyte pearls within the epidermis. Pronounced hyperkeratosis, characterized by thickening of the keratinized layer over the epidermis, was also evident in this group (Figure 11).

Restoration of histological abnormalities induced by DMBA/croton oil was observed in the Standard (5-FU) + DMBA/croton oil–treated group. The skin histology in this group appeared normal and closely resembled that of the vehicle control group (Figure 12).

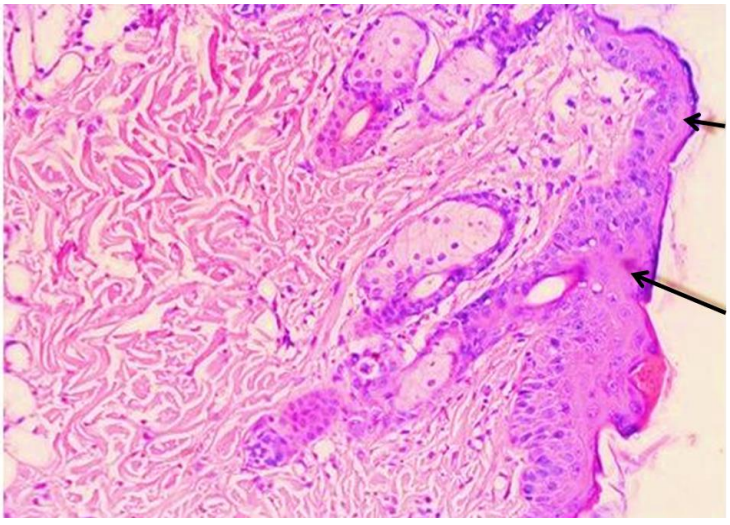

Figure 11: Microphotograph of DMBA/ croton oil control group

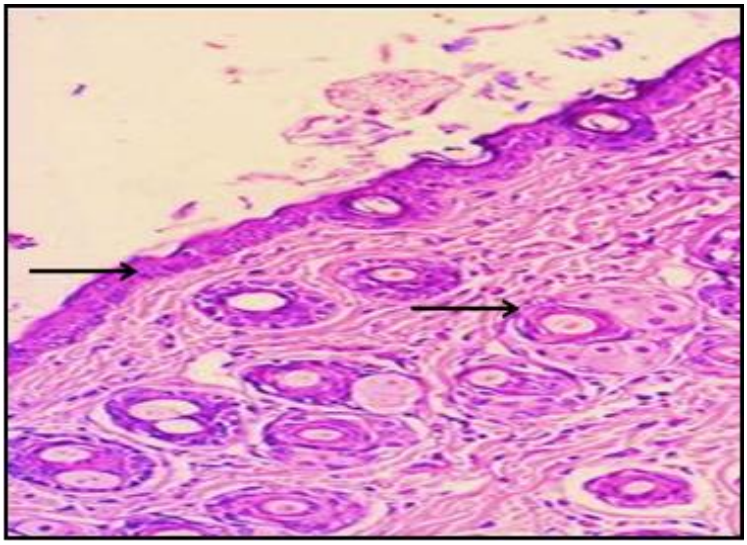

Figure 12: Microphotograph of Standard (5-FU) + DMBA/ croton oil group

In the group treated with the ethanolic extract of *LC*, the extent of lesions, hyperkeratosis, dyskeratosis, and hyperplasia was markedly reduced compared with the DMBA/croton oil–treated group. The epidermis was uniformly arranged in the skin tumor sections, and leukocyte infiltration was reduced, with fewer keratinocyte pearls observed. Additionally, a significant reduction in the size of keratinocyte pearls was noted (Figure 13).

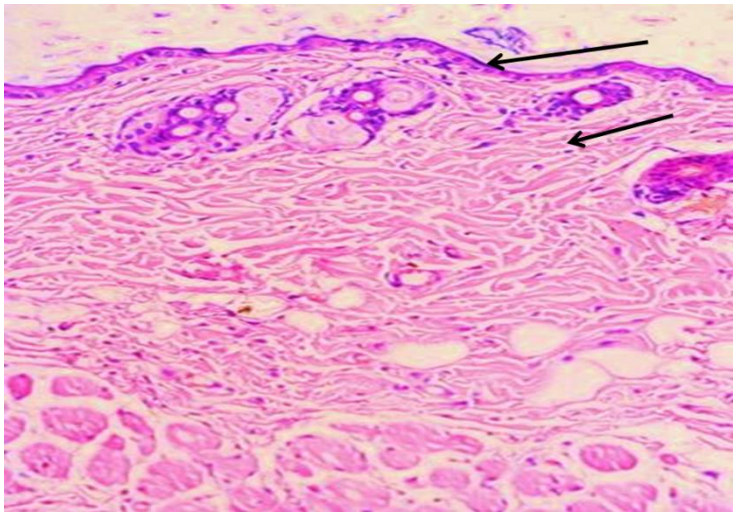

Figure 13: Microphotograph of Ethanolic extract of LC + DMBA/Croton oil group

4. DISCUSSION

The choice of solvent for the extraction of crude products is crucial, as it significantly influences both the efficiency of the extraction process and the quality of the final product. Among commonly used solvents, water and alcohol play major roles in extraction procedures. Although water is widely recognized as a universal solvent due to its inert properties, its effectiveness is limited when the target compounds are insoluble in water. In such cases, alcohol is a more suitable alternative. Alcohol offers several advantages over water: it is chemically neutral and ensures compatibility of the extracted products with other substances; it requires minimal heat for concentration of alcoholic preparations; and it selectively dissolves the active components of the plant material (Ahmad, 1998; Nargatti, 2024). Numerous studies have also reported the extensive use of ethanol as a solvent for plant extraction, highlighting its effectiveness in isolating bioactive compounds (Das et al., 2010; Borges et al., 2020; Das et al., 2022). In the present study, ethanol was selected as the extraction solvent because it is non-toxic, readily available, and capable of dissolving many bioactive constituents present in *LC* bark.

Furthermore, the extract was evaluated for toxicity in mice. Acute toxicity studies were conducted to determine the safe dosage and assess the level of toxicity. Several plant extracts have previously been tested for toxicity, and based on such evaluations, appropriate doses were established for experimental use (Ugwah-Oguejiofor et al., 2019; Das et al., 2022).

In the present study, DMBA was used as a cancer-inducing agent. It is a polycyclic aromatic hydrocarbon and plays a vital role in the rapid initiation of skin cancer through DNA damage. Numerous studies have reported the initiation of skin cancer by DMBA (El-Habashi, 2016; Bashir et al., 2022). DMBA was selected for the present study because it affects not only normal cells and tissues surrounding the tumor but also the immune system, stroma, and vasculature, thereby triggering tumor growth, progression, and response to therapy. After the 16th week, various parameters were evaluated.

Many medicinal herbs have been shown to possess anticancer properties in both experimental studies and traditional medicinal practices. The use of experimental tumor models represents an essential preclinical stage in the development and evaluation of cancer chemotherapy regimens. Notably, the distinct lethal effect of the extract observed in this study appears to be associated with its chemical composition and possibly with the characteristics of the tumor cells.

In the present study, the ethanolic extract of *LC* bark was used to evaluate antitumor activity in carcinogen-induced mice. The extract significantly reduced tumor yield, tumor size, and tumor mass. In addition, the bark extract decreased tumor incidence, tumor volume, tumor burden, cumulative number of papillomas, and increased the average latent period when compared with the carcinogen-treated control group. The same result was also reported earlier research (Pandey, 2017; Manimaran et al., 2024). Above all, our findings indicated that decreased MDA level and increased GSH levels in skin were substantially impacted in cancer treatment. The elevated MDA levels in the skin homogenate may be related to lipid peroxidation, which is crucial for skin. The same result was also reported earlier (Ghafoor, 2023). MDA may be a reliable marker for researching lipid peroxidation in tissues and hence in the study lipid peroxidation was estimated before and after the experimentations. Levels of SOD and CAT in mice were increased and the same was also revealed earlier **(**Mahmoud et al., 2023**)**. The generation of ROS, which is induced by DMBA, results in DNA damage, lipid peroxidation, and the death of cells with antioxidant systems. The present study revealed that recovery of the damaged DNA occurred after the 16th week of the study, and the repair was comparable to that of the normal control group of animals.

5. CONCLUSION

Numerous causes, such as the belief that herbal medicines are safe and efficient and the ongoing research into medicinal plants, have contributed to the rapid rise in the use of herbal therapies worldwide. Ultimately, these in vivo trials demonstrate the efficacy of the LC bark ethanol extract over all other treatments examined, and the extract was able to reduce skin cancer in comparison to the reference medication, 5-FU. Therefore, LC plant is effective in the development of a new and potential anti-cancer drug.

Financial support:

Self Financed project

Ethical issues:

All experiments were certified and reviewed by the Institutional Animal Ethical Committee members (approval number: IAEC/ABCP/15/2021-22, dated 25/01/2022). Experiment was performed according to the guidelines given by the Committee for Control and Supervision of Experiments on Animals in New Delhi, India.

Conflict of Interest:

None reported.

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دراسة النشاط المضاد للسرطان لمستخلص لحاء نبات لانييا كوروماندیليكا الإيثانولي في سرطان الفئران البيضاء السويسرية المُستحث بواسطة 7،12–ثنائي ميثيل بنز (أ) أنثراسين

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ملخص

هدفت هذه الدراسة إلى تقييم الخصائص المضادة للسرطان لمستخلص لحاء نبات لانييا كوروماندیليكا (LC) باستخدام نموذج التسرطن ثنائي المراحل، وذلك عن طريق تحفيز السرطان في فئران سويسرية بيضاء لمدة 16 أسبوعًا باستخدام 7،12–ثنائي ميثيل بنز (أ) أنثراسين (DMBA) وزيت الكروتون. تم تطبيق مستخلص لحاء LC وكريم فلونيدا 5% من 5–فلوروبوراسيل (5–FU) كدواء معياري موضعيًا يوميًا لمدة 16 أسبوعًا بعد تطبيق DMBA. أدى العلاج الموضعي بالمستخلص إلى انخفاض ملحوظ في العديد من مؤشرات الورم. أظهر تأثير المستخلص على مستوى إنزيمات ديسموتاز الفائق (SOD) والكاتالاز (CAT) نتائج معنوية (p<0.05*) مقارنةً بالمجموعة المعيارية. كان مستوى مالونديالدهيد (MDA) في المستخلص مماثلاً تقريبًا لمستوى المجموعة المعيارية، بل إن قيم هذه المجموعات كانت أقل من قيمة المجموعة الطبيعية. ارتفع نشاط الجلوتاثيون المختزل (GSH) في المجموعة المعالجة بالمستخلص مقارنةً بمجموعة التحكم المعالجة بالمادة المسرطنة. ولوحظت تغيرات نسيجية مرضية في مجموعة التحكم المعالجة بالمادة المسرطنة، تمثلت في فرط التنسج، وخلل التقرن، وسرطان الخلايا الحرشفية جيد التمايز، ووجود لآلئ متقرنة، بينما انخفضت هذه التغيرات بعد إعطاء المستخلص والدواء القياسي. وتُشير النتائج الإجمالية إلى أن مستخلص لحاء نبات LC يتمتع بنشاط قوي مضاد للسرطان، مما يُوفر أساسًا علميًا لخصائصه الوقائية الكيميائية.

الكلمات الدالة: مضاد للسرطان، علم الأنسجة المرضية، FU5، Lannea coromandelica، MDA، SOD.

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