RESEARCH ARTICLE

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Cancer Chemo-Preventive Potential of *Calligonum polygonoides* Stem Extract Against DMBA Induced Skin Carcinogenesis

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Abstract

Background: Cancer remains a global health challenge, and natural plant-based compounds show potential in prevention. *Calligonum polygonoides* Linn., a drought- and frost-resistant shrub native to the Thar desert, has adaptive resilience and ethnomedicinal applications. This study explores *C. polygonoides* stem extract (CPSE) against DMBA-induced two stage skin carcinogenesis in male Swiss albino mice. **Methods:** Skin carcinogenesis was induced in male Swiss albino mice using a single topical application of carcinogen DMBA (100 µg/animal), followed by the application of promotor croton oil (1% solution) thrice weekly as a tumour promoter, starting two weeks after DMBA initiation. CPSE was orally administered at a dose of 600 mg/kg body weight/day (100 µL per animal). Tumour incidence, yield, and burden were assessed, along with biochemical markers of oxidative stress in skin and liver tissues, including reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), lipid peroxidation (LPO), total protein, and vitamin C levels. **Results:** Mice treated with CPSE demonstrated a significant reduction in tumour incidence, yield, and burden compared to the carcinogen-treated control group. Additionally, CPSE administration significantly modulated oxidative stress and biochemical parameters in both skin and liver tissues. The CPSE-treated group exhibited decreased LPO levels and elevated antioxidant enzyme activities (GSH, SOD, and CAT), along with elevated total protein content and vitamin C levels, indicating enhanced systemic antioxidant defences. **Conclusion:** The findings of this study reveal the significant cancer chemo-preventive potential of *C. polygonoides* stem extract. Its ability to reduce tumour progression and modulate oxidative stress underscores its promise as a natural agent for cancer prevention. These results highlight *C. polygonoides* as a potential source for the development of cost-effective and sustainable cancer prevention strategies.

Keywords: Ethnomedicine- Natural Antioxidants- Oxidative Stress- Skin Papilloma- Swiss albino mice

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Introduction

Cancer is a disease condition characterized by uncontrolled cell growth and the cells invade and spread to distant parts of the body. It's a leading cause of mortality and morbidity globally, with millions of new incidents and deaths annually [1]. Lifestyle factors such as poor dietary habits, smoking, alcohol intake, lack of physical activity, obesity, and chronic stress significantly contribute to cancer risk, accounting for nearly 90–95% of all cases [2]. Preventing cancer through lifestyle modifications is crucial, particularly in low-income and developing countries where access to advanced treatments is limited [3]. Eating a healthy diet packed with whole grains, veggies, fruits, and regular exercise, while avoiding tobacco and limiting alcohol, can significantly lower your cancer risk. Additionally, cancer chemoprevention using synthetic or natural compounds to delay or inhibit tumour development is emerging as a promising approach to address the global cancer burden [4, 5].

Since ancient times, medicinal plants have been recognized and utilized in traditional therapeutic procedures. Human diseases can be prevented and treated with the use of herbal plants [6]. Mixtures of several chemical substances that can operate singly, in combination, or in concert to enhance health are commonly found in medicinal plants [7]. Several plants have been explored for the anticancer potential and reported with significant chemo-preventive potential such as *Vitis vinifera* [8], *Aloysia citrodora* [9], *Moringa oleifera* [10], *Chlorophytum borivilianum* [11], *Syzygium cumini* [12] etc.

Calligonum polygonoides Linn. is a drought- and frost-resistant shrub of the Polygonaceae family, adapted to arid desert conditions [13]. The plant's distribution spans North Africa, Southern Europe, and Western Asia, with its diversity centred in desert regions. In India, it is primarily found in Western Rajasthan and Southern Punjab, thriving

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in districts like Jaisalmer, Barmer, Bikaner, and Jaipur, where it contributes to the desert ecosystem's resilience [14]. Phytochemical screening of *C. polygonoides* revealed a high concentration of tannins, carbohydrates, and phenolic compounds in its roots, stems, flowers, and buds. Additionally, significant amounts of steroids, saponins, and terpenoids were identified in the roots, stems, buds, flowers, and seeds, along with a moderate presence of alkaloids in the roots, buds, and seeds. These findings highlight the rich phytochemical composition of *C. polygonoides* [15]. It has been determined that *C. polygonoides* is a storehouse of several phytochemicals with substantial potential for therapeutic uses. The significance of investigating the therapeutic potential of phyto-compounds from *C. polygonoides* is highlighted by the lengthy history of using plant extracts for therapeutic purposes [16]. This study intended to evaluate the chemo-preventive potential of *C. polygonoides* stem extract using two stage skin carcinogenesis in mouse model system.

Materials and Methods

Chemicals

Analytical-grade chemicals were utilized throughout this study and procured from well-established suppliers in India. The promoter croton oil and the initiator 7,12-dimethylbenz(a)anthracene (DMBA), were acquired from Sigma Chemical Company, USA.

Experimental Animals and Ethical Approval

The experiments were conducted following approval from Institutional Animal Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur, in compliance with CPCSEA guidelines, Government of India. Male Swiss albino mice (7–8 weeks old, 24 ± 2 g) were obtained from the National Institute of Pharmaceutical Education and Research (NIPER) Punjab, India. Mice were housed in polypropylene cages under controlled lighting condition (14 hours light:10 hours dark) and temperature (25 ± 2°C) in the departmental animal house. They were provided with a regular mice diet (Aashirwad Industries, Chandigarh, India) and water ad libitum. Prior to experimentation, the mice were isolated for seven days to acclimatize and screen for any illnesses. Only healthy, acclimatized animals were used for the study.

Plant Material and Extract Preparation

The stems of *Calligonum polygonoides* were collected from Chelak Village, Jaisalmer District, Rajasthan, India (26.508917°N, 70.908462°E), during winter, and authenticated with a voucher specimen (RUBL-211762) preserved in the herbarium of the Department of Botany, University of Rajasthan, Jaipur. A hydroalcoholic extract of the stems was prepared using Soxhlet extraction for 72 hours with a 3:1 methanol-water solvent mixture to maximize the yield of bioactive compounds. The *Calligonum polygonoides* stems extract (CPSE) was concentrated by evaporating the solvent, dried, and stored in an airtight container at room temperature. For experiments, the extract was reconstituted in distilled water before oral administration.

Optimum Dose Selection

The optimum dose of CPSE was determined by administering it orally in distilled deionized water (DDW) at varying doses. Healthy Swiss albino mice were divided into five groups and received oral gavage doses of 200, 400, 600, and 800 mg/kg body weight per day for 30 days. During this period, the mice were closely monitored for signs of illness, mortality, behavioural abnormalities, and morphological changes. On the 30th day, the animals were sacrificed via cervical dislocation, and their skin and liver tissues were analysed for lipid peroxidation (LPO) and reduced glutathione (GSH) levels. Based on these evaluations, the optimum dose of CPSE for Swiss albino mice was determined.

Induction of Tumour

Animals were treated with carcinogen for induction of tumour on dorsal surface, for better application of carcinogen interscapular area were shaved prior to application of carcinogen. The carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) was dissolved in analytical grade acetone at a concentration of 2 mg/ml, and 50 µl of this solution was applied topically to the dorsal surface of each mouse to induce tumours. Two weeks after initiation with DMBA three times a week treatment of 100 µl croton oil at 1% v/v concentration was given up to 14 weeks on the same area of dorsal surface of mice where DMBA was applied [17].

Cancer Chemo-prevention Study

To evaluate cancer chemo-preventive potential of CPSE male Swiss albino mice were allotted into five groups, with each group having five mice and the treatment groups given CPSE through oral gavage in DDW at the dose 600mg/kg body weight daily as shown in Figure 1.

Normal Group

Animals were not given any treatment neither carcinogen nor the CPSE treatment.

Carcinogen control Group

Animals were treated with carcinogen and no CPSE treatment was given.

Pre-treatment Group

Animals received carcinogen treatment same as carcinogen control group, in addition to that mice were treated with CPSE for two weeks prior to DMBA application.

Post-treatment Group

Mice of this group first received DMBA and croton oil treatment in similar manner as given to carcinogen control group and CPSE given orally for 16 weeks from next day of DMBA application to till the end of the experiment.

Throughout-treatment Group

Mice of this group treated with CPSE from start of the experiment (before two weeks of DMBA application) to till the termination of the experiment i.e. 18 weeks. DMBA and croton oil treated in similar manner as given

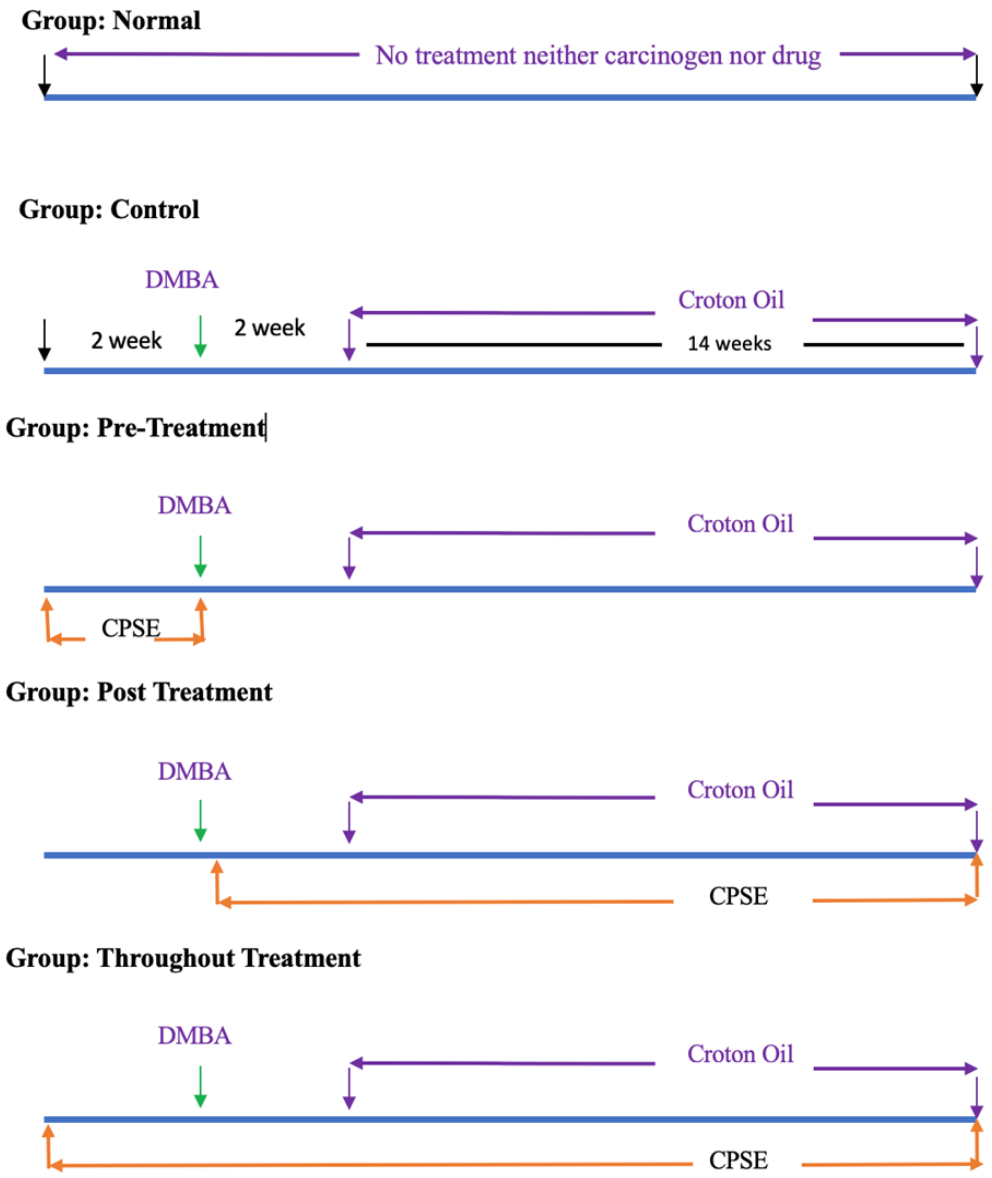

Figure 1. Graphical Representation of Experimental Groups for the Study of Cancer Chemo-Preventive Potential of *C. polygonoides* Stem Extract (CPSE).

to animals of carcinogen control group.

Animals of all the experimental groups were observed regularly for any sign of illness, mortality, behavioural abnormality, morphological alteration and presence of skin papilloma or tumour.

Following morphological and biochemical parameters were studied to evaluate cancer chemo-preventive potential of *C. polygonoides* stem extract.

Morphological parameters

All the animals were carefully examining regularly for any abnormality, appearance of papilloma on dorsal surface of mice. Tumour was defined as lesions with a diameter larger than 1mm that persisted for at least one week. Following tumour indexes were calculated from the experimental data.

Tumour incidence

The percentage incidence was calculated as the number of mice carrying one or more papilloma.

Tumour yield

The average number of papilloma per mouse.

Tumour burden

The average number of papilloma per papilloma-bearing mouse.

Average latent period

The time in which 50% of animals has at least one tumour.

Biochemical study

Sacrificed the animals from each group by cervical dislocation, and the tumour-affected dorsal skin and liver were promptly removed and carefully cleaned with cooled 0.9% saline. Tissue cleaned with cold saline was used for further biochemical analysis. Following biochemical

parameters were studied:

Lipid peroxidation

The thiobarbituric acid reactive substances (TBARS) assay, as described by Ohkhawa et al. [18] was used to measure lipid peroxidation spectrophotometrically. Tissue homogenate was treated with thiobarbituric acid, sodium dodecyl sulphate, and trichloroacetic acid, then heated at 90°C for one hour. The cooled mixture was separated with n-butanol-pyridine (15:1), and optical density was measured at 532 nm. Lipid peroxidation levels were presented as nmol/mg of tissue using 1,1,3,3-tetramethoxypropane (TMP) as the standard.

Reduced glutathione

The method by Moron et al. [19] was used to measure GSH as the total nonprotein sulfhydryl group. Tissue homogenates were treated with 25% trichloroacetic acid (TCA) to precipitate proteins, followed by centrifugation. The supernatant was reacted with 0.6 mM DTNB in 0.2 M phosphate buffer (pH 8.0), and absorbance was recorded at 412 nm using a UV-VIS spectrophotometer. GSH levels were expressed in µmol/g tissue, with reduced GSH as the standard.

Catalase

The Aebi [20] technique was used to measure the catalase activity. The homogenate was prepared using phosphate buffer (50 mM) and centrifuged. Using spectrophotometry, the change in absorbance was detected at 240 nm. The enzyme's activity was measured in U/mg of tissue, where U was defined as µmol of H_2O_2 disappearance/min. Standard curve was obtained by using tetra-methoxy propane (TMP).

Superoxide dismutase

The level of enzyme superoxide dismutase (SOD) was estimated using the Marklund & Marklund, [21] method, which involved quantifying the inhibition of pyrogallol auto-oxidation and expressing the results as units/mg protein. The oxidation of pyrogallol was monitored by measuring the rise in absorbance at 420 nm in a Tris-HCl buffer (50 mM, pH 7.5).

Total proteins

The concentration of protein in the samples was quantified using the Lowry et al. [22] method . This involves the formation of a cupric-protein complex when the sample's protein reacts with basic copper tartrate. The blue colour produced is because of the reduction of phosphomolybdic and phosphotungstic acids in Folin's reagent by aromatic amino acids. The absorbance of the complex was recorded at 620 nm, and protein content was reported in mg/ml, using bovine serum albumin as a standard.

Vitamin C

The liver and skin tissues were weighed, homogenized in 20 mg/mL acetate buffer, extracted with cold 4% trichloroacetic acid, then centrifuged, and filtered to complete the process. The ascorbic acid was measured using the Roe & Kuether, 1943 method [23].

Statistical Analysis

The data from all the experimental groups were presented as mean ± standard deviation. The student's t-test was employed to statistically compare all the mentioned parameters across the various groups, and the significance levels of the biochemical parameters were determined. The $p<0.001$ considered very highly significant, $p<0.01$considered as highly significant, $p<0.05$ considered as significant and $p>0.05$ considered as non-significant.

Results

Optimum Dose Selection

Animal of all the groups well tolerated the CPSE up to dose of 800mg/kg body weight, no mortality or morbidity was observed. Animals treated with 600mg/kg body weight shows lowest LPO level in skin (3.38±0.22) and liver (5.44±0.24) compared to skin (5.67±0.15) and liver (10.35±0.31) of 200, skin (4.67±0.11) and liver (8.09±0.23) of 400, skin (3.83±0.26) and liver (6.53±0.19) of 800mg/kg body weight (Figure 2). GSH content was higher in skin (8±0.32) and liver (14.37±0.64) of 600mg/kg body weight animal group compared to skin (6.46±0.37) and liver (9.77±0.59) of 200, skin (7.24±0.27) and liver (12.37±0.28) of 400, skin (7.23±0.24) and liver (13.31±0.18) of 800mg/kg body weight animal groups (Figure 3). Therefor 600mg/kg body weight dose of CPSE was selected for study of cancer chemo preventive potential of CPSE.

Morphological parameters

A significant reduction in tumour incidence was observed across all CPSE-treated groups pre-treatment group (50%), post-treatment group (75%) and throughout-treatment group (16.66%), compared to 100% in the control group (Figure 4). The tumour yield for groups pre-treatment, post-treatment, and throughout-treatment found 0.66, 1.75, and 0.33 respectively, compared to 2 for the control group. The tumour burden for groups pre-treatment group, post-treatment group, and throughout-treatment group was 1.33, 2.33, and 2, respectively, compared to 2.4 in the control group (Figure 5). The average latent period for CPSE-treated groups pre-treatment group, post-treatment group, and throughout-treatment group was 16, 15 and 16 weeks respectively, compared to a shorter average latent period of 11 weeks in the carcinogen control group (Figure 6). Photograph of the experimental mice from each group is shown in Figure 7.

Biochemical study

Lipid peroxidation

Carcinogen control group showed significant increase in LPO level compared to normal group's skin (5.67±0.51) and liver (4.94±0.79) tissue. Animals receiving CPSE showed significant reduction in lipid peroxidation content in skin of all three groups, throughout-treatment group (2.31 ± 1.36 nmole/mg tissue), pre-treatment group (3.46 ± 1.22 nmole/mg tissue), post-treatment group

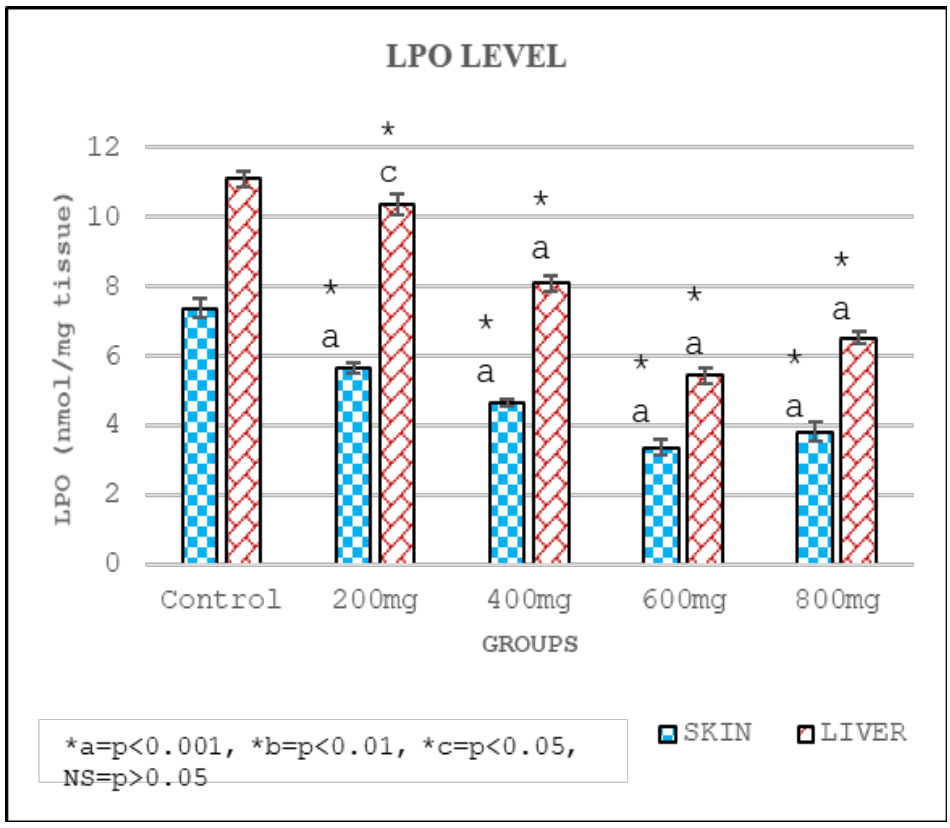

Figure 2. Effect of Different Concentration of CPSE on Lipid Peroxidation (LPO) Level in Skin and Liver of Mice

(4.61±0.93 nmole/mg tissue) compared to carcinogen control group (9.65±0.44 nmole/mg tissue). Similarly decrease in liver LPO level was observed across all three groups: throughout-treatment group (1.55 ± 0.44 nmol/mg tissue), pre-treatment group (2.25 ± 1.94 nmol/mg tissue), and post-treatment group (4.80 ± 0.31 nmol/mg tissue), compared to the carcinogen control group (7.09 ± 1.45 nmol/mg tissue) (Supplementry Figure 1).

Reduced glutathione

Animals receiving carcinogen treatment showed significant decrease in GSH level compared to normal

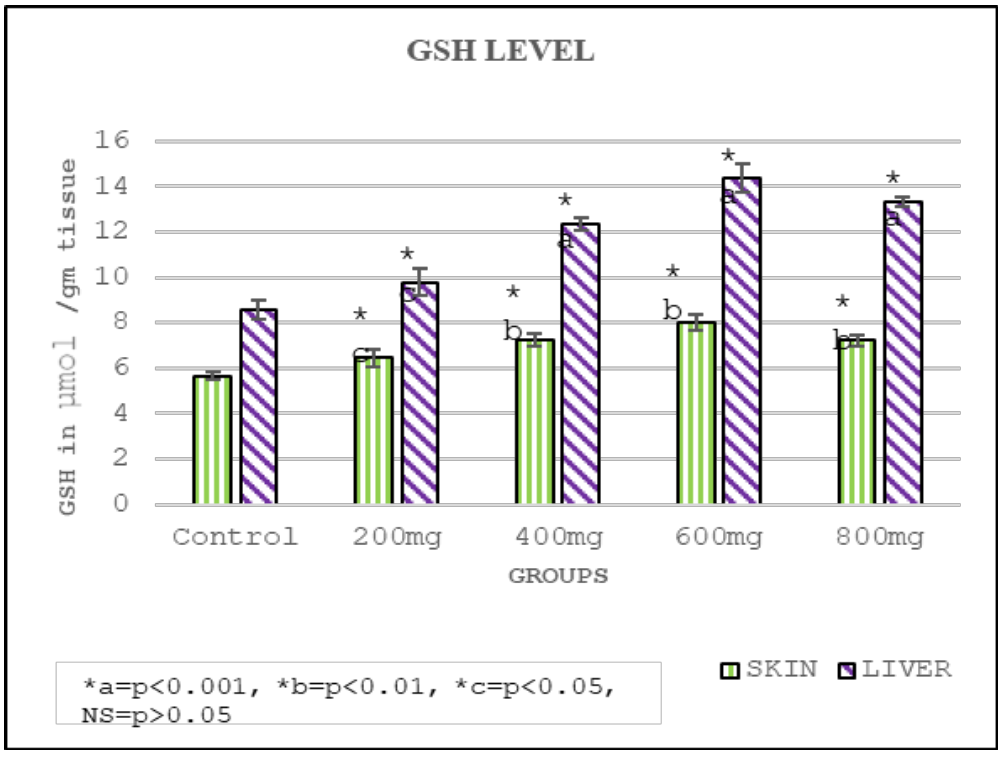

Figure 3. Effect of Different Concentration of CPSE on GSH Level in Skin and Liver of Mice

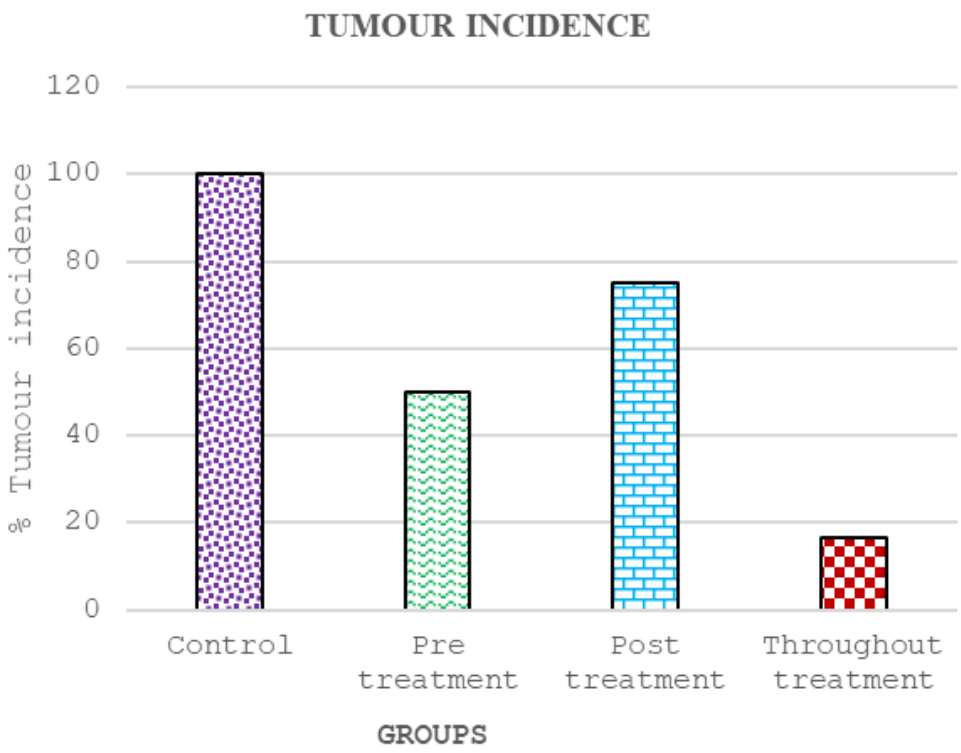

Figure 4. Variations in the Tumour Incidences after CPSE Treatment Compared to the Control Group

group's skin (5.53±1.93) and liver (4.73±0.30) tissue. Animals receiving CPSE showed significant increase in reduced glutathione content in skin of all three groups throughout-treatment group (6.17±1.17 µmol/gm tissue), pre-treatment group (5.91±0.35 µmol/gm tissue), post-treatment group (3.11±0.62 µmol/gm tissue) compared to carcinogen control group (2.12±0.31 µmol/gm tissue). An increase in liver GSH content was observed across all three groups: throughout-treatment group (5.66 ± 1.28 µmol/g tissue), pre-treatment group (5.40 ± 1.09 µmol/g tissue), and post-treatment group (2.68 ± 0.12 µmol/g tissue), compared with carcinogen control group (2.07 ± 0.33 µmol/g tissue) (Supplementary Figure 2).

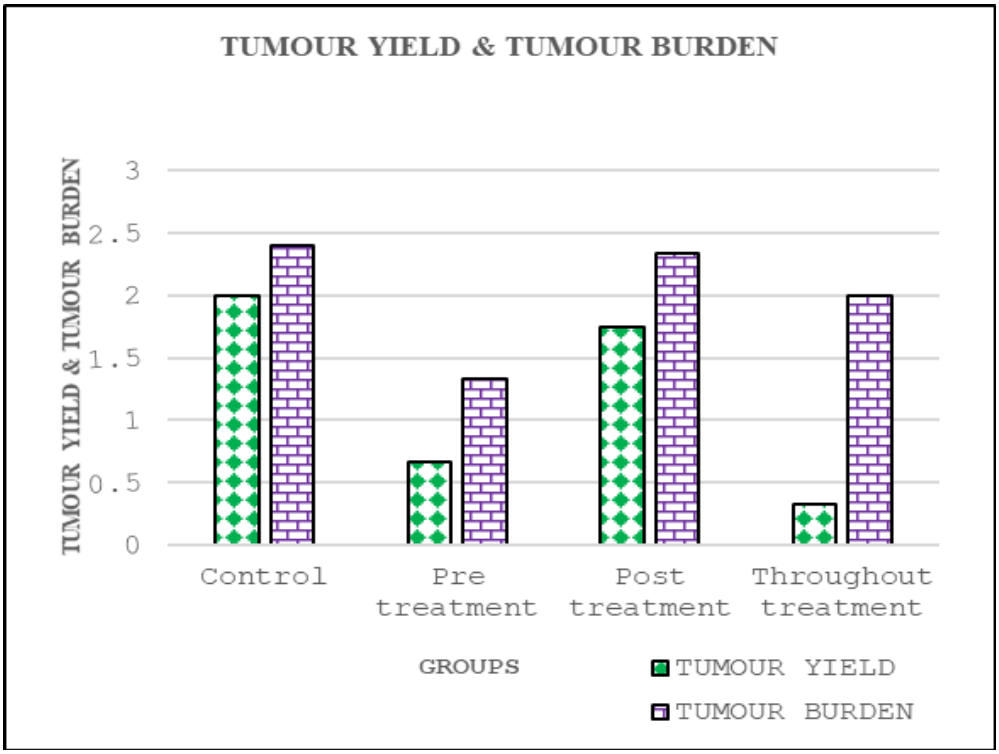

Figure 5. Variations in the Tumour Yield and Tumour Burden after CPSE Treatment Compared to the Control Group

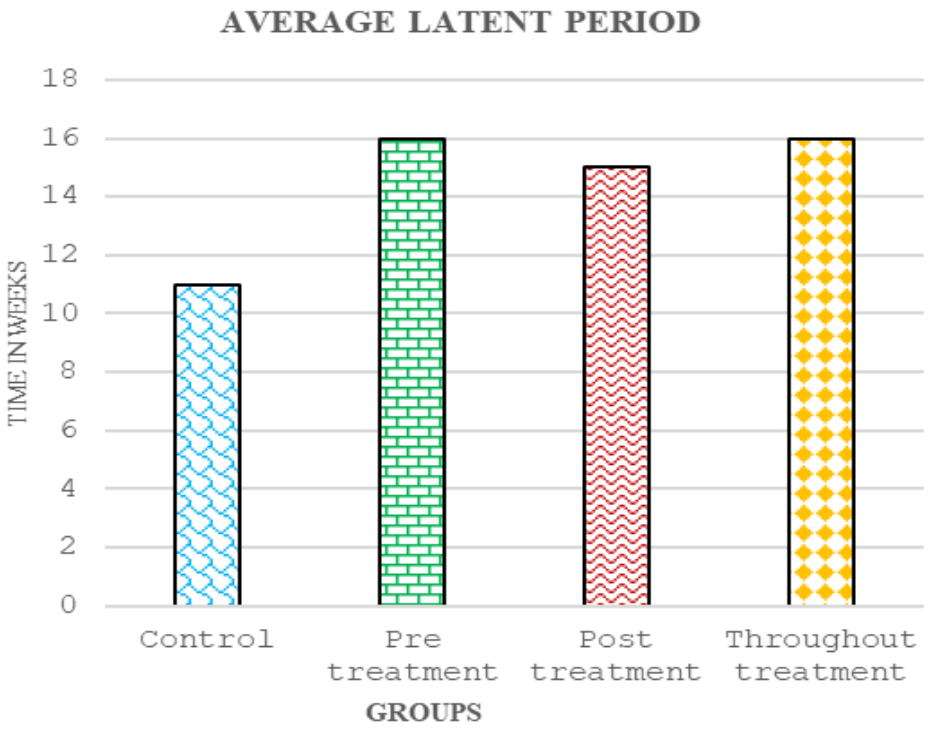

Figure 6. Variations in Average Latent Period of Papilloma Genesis after CPSE Treatment Compared to the Control Group

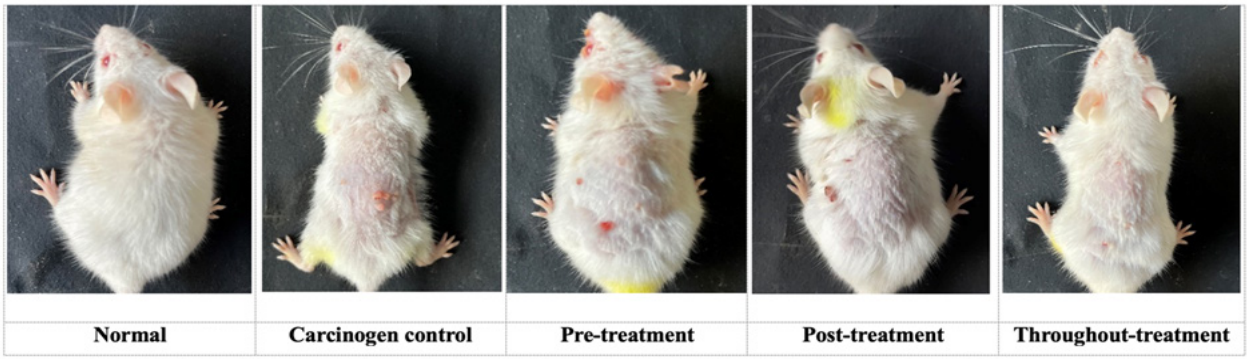

Figure 7. Photographs Showing Papilloma on Dorsal Surface of Mice from Different Experimental Groups.

Catalase

The catalase content in the carcinogen control group was significantly lower compared to the normal group's skin (69.50±33.69) and liver (57.88±11.27). Animals receiving CPSE showed significant increase in catalase activity in skin of all three groups throughout-treatment group (72.16±20.57), pre-treatment group (97.5±31.30), post-treatment group (60.89±7.14) compared to carcinogen control group (22.45±7.36). Compared to the carcinogen control group (21.54±5.88), all three groups showed increased catalase activity in liver during throughout-treatment (72.19±11.27), pre-treatment (86.50±16.84), as well as post-treatment (63.84±2.96) expressed as U/mg tissue (Supplementary Figure 3).

Superoxide dismutase

Animals treated with carcinogen exhibited a substantial reduction in superoxide dismutase (SOD) content compared to the normal group's skin (24.93±1.69) and liver (10.79±0.47). Animals receiving CPSE showed significant increase in SOD activity in skin of all three groups throughout-treatment group (20.94±0.34), pre-treatment group (17.60±0.70), post-treatment group (9.2±0.86) compared to carcinogen control group (4.88±0.23). In comparison to the carcinogen control group (5.35±0.15), the liver SOD activity increased in all three groups throughout-treatment group (18.77±0.26), pre-treatment group (15.77±0.46), post-treatment group (10.29±0.37) (Supplementary Figure 4).

Total proteins

Carcinogen control group exhibited a reduction in total protein content compared to the normal group's skin (21.90±5.48) and liver (15.49±1.67). Animals receiving CPSE showed significant increase in total protein content in skin of all three groups throughout-treatment group (25.45±5.06), pre-treatment group (24.41±2.84), post-treatment group (18.58±0.59) compared to carcinogen

control group (13.31±1.34). Rise in total protein content of liver was observed in all three groups: throughout-treatment group (16.23 ± 2.56), pre-treatment group (15.68 ± 2.01), and post-treatment group (14.68 ± 1.98), compared to the carcinogen control group (14.40 ± 1.39) (Supplementary Figure 5).

Vitamin C

The carcinogen control group had a lower vitamin C content compared to the normal group's skin (0.42±0.05) and liver (0.32±0.01). Animals receiving CPSE showed increase in Vitamin C level in skin of all three groups throughout-treatment group (0.46±0.05), pre-treatment group (0.46±0.12), post-treatment group (0.36±0.01) in comparison with carcinogen control group (0.33±0.06). Compared to the carcinogen control group (0.27±0.01), all three groups showed an increase in their liver's vitamin C levels: throughout-treatment group (0.40±0.02), pre-treatment group (0.30±0.01), and post-treatment group (0.31±0.03) (Supplementary Figure 6).

Discussion

The study demonstrated that *Calligonum polygonoides* stem extract (CPSE) effectively reduced oxidative stress and provided protection against DMBA and croton oil induced skin cancer. CPSE treatment significantly decrease the incidence and number of cutaneous papilloma, with the highest papilloma inhibition observed in the treatment group receiving CPSE throughout the experiment. This protective effect may be attributed to the inhibition of DMBA activation or the suppression of ROS generation, delaying the promotion phase of carcinogenesis [24]. CPSE extract significantly inhibited the progression of DMBA and croton oil induced skin carcinogenesis in mice, demonstrating a marked reduction in both tumour yield, tumour burden and incidence. Literature supports that naturally occurring compounds can prevent carcinogenesis by reducing neoplasia expression or blocking the activation of carcinogens from their precursors [25]. Animals treated with CPSE throughout the study showed the most significant benefits, although tumour incidence was more effectively reduced during the pre-treatment phase than the promotion phase. Additionally, CPSE treatment delayed the latency period of tumour development compared to the carcinogen control group it suggests that CPSE significantly hinder the mechanism of carcinogenesis. Earlier studies have demonstrated that certain plants have chemoprotective qualities by interfering with the various phases of multistep skin carcinogenesis, including tumour promotion [26]. Therefore, it can be assumed that CPSE may function similarly to raise the average latency duration of tumour development, prolonging the promotional stage by deferring tumour formation and lowering the frequency of tumours in mice's skin.

Antioxidant Glutathione (GSH) acts as a key to combat reactive oxygen species (ROS). In the DMBA-treated control group, decreased GSH levels indicate oxidative stress, further exacerbated by reduced activity of antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD) in malignant lesions. This imbalance, along with low GSH levels, increases tissue susceptibility to lipid peroxidation [27]. Frequent intake of fruits and vegetables has been correlated to a lower risk of developing cancer [28]. Many compounds, both synthetic and natural, may be chemo-preventive against cancer by preventing mutagenesis, hyperproliferation, differentiation, or apoptosis [29]. The current study shows that oral CPSE administration during papilloma genesis significantly lowers the incidence of DMBA croton oil induced skin papilloma in mice after 18 weeks without harmful side effects. Flavonoids and other phytochemicals' antioxidant qualities may protect tissues from lipid peroxidation and oxygen-derived free radicals linked to cancer and chronic inflammation [30]. An analysis of the total flavonoid, total phenolic, and antioxidant activity of several parts of C. polygonoides revealed that rich source of these phytoconstituents [31]. Similarly, the current study's findings demonstrated a large decrease in lipid peroxidation in liver and skin of the groups which received CPSE, as well as a significant increase in GSH and catalase levels. As antioxidants, flavonoids and other phytochemicals included in CPSE may therefore help to prevent diseases like cancer that are brought on by free radicals.

The two primary stages of carcinogenesis initiation and propagation are known to be brought on by lipid peroxidation, a free radical chain reaction. It's a very damaging process. Lipid peroxidation increases during the carcinogenic process, resulting in the formation of increasingly complex and reactive chemicals including 4-hydroxynonenal and malondialdehyde (MDA). These lipid peroxidation products have been shown to be carcinogenic and mutagenic. As a result, substances that might lower free radical generation in vivo might be thought of as having chemo-preventive potential [32]. In the current study, mice given DMBA, and croton oil had considerably lower levels of LPO after receiving CPSE, which in turn resulting in a lower incidence of skin tumours. Generally thought as the first line of defence versus free radical stress, antioxidants can help lower the chances of oxidative damage caused by carcinogenesis. Together, the antioxidant enzymes catalase and SOD offer a protective barrier against reactive oxygen species (ROS) [33]. Similarly, the CPSE-treated group to the control group, the current study demonstrates a considerable increase in GSH, SOD, and catalase levels.

CPSE supplementation restored the oxidant-antioxidant balance in tumour-bearing mice by reducing ROS generation, delaying carcinogenesis, and inhibiting DMBA activation. DMBA-treated mice exhibited decreased antioxidant capacity and increased lipid peroxidation, linked to ROS overproduction. CPSE's chemo-preventive action is attributed to its secondary metabolites, such as tannins, alkaloids, saponins, flavonoids, and phenols, which enhance antioxidant defence and mitigate oxidative stress.

In conclusion, According to the findings of the current investigation, oral administration of the antioxidant-rich stem extract of *C. polygonoides* may be an essential component of a healthy lifestyle strategy to increase

resistance to skin cancer. The active components in CPSE may have prevented chemically induced skin carcinogenesis significantly in Swiss albino mice by scavenging free radicals, reducing oxidative stress.

Author Contribution Statement

Mr. Gyan Prakash Meghwal conducted the experiments, analysed the results, and contributed to manuscript preparation. Mr. Mahendra Kumar Jeengar, Ms. Shivani Jangir, and Mr. Kamlesh Kumar Sharma assisted in performing the experiments and contributed to manuscript preparation. Dr. Priyadarshi Meena and Dr. Dev Dutt Patel conceptualized and designed the experimental plan, reviewed the results, and critically revised the manuscript.

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General

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Approval

This study is part of the PhD thesis and approved by the departmental research committee (DRC), Department of Zoology, University of Rajasthan, Jaipur.

Ethical Declaration

This study was ethically approved from Institutional Animal Ethical Committee, Department of Zoology, University of Rajasthan, Jaipur (Protocol approval no. UDZ/2021/17 dated 18 Dec. 2021) and management were carried out in compliance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Government of India, India.

Conflict of Interest

Authors declare no conflict of interest.

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