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BIOSYNTHESIS OF SELENIUM NANOPARTICLES MEDIATED BY SPIRULINA PLATENSIS AND ASSESSMENT OF THEIR IN VIVO ANTICANCER EFFECTS IN WISTAR ALBINO RATS

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ABSTRACT
Selenium nanoparticles were successfully synthesized using Spirulina platensis extract by mixing 10 mL extract with 20 mL of 50 mM selenium solution and reducing with 200 µL of 40 mM ascorbic acid, producing a characteristic ruby red colour. The nanoparticles were further analysed for stability using particle size and zeta potential measurements. Acute oral toxicity studies (OECD 423) revealed no mortality or adverse effects at doses up to 2000 mg/kg. In vivo anticancer evaluation in DMBA-induced rats showed that SP-SeNPs (5 mg/kg) effectively reduced tumor progression, improved body weight, and demonstrated significant protective effects compared to cancer control. The formation of nanoparticles was confirmed by a characteristic ruby red color, indicating the reduction of selenium ions. Characterization studies revealed an average particle size of 84 nm with a zeta potential of −48.9 mV, demonstrating nanoscale formation, high stability, and good dispersion. Acute oral toxicity studies conducted as per OECD 423 guidelines showed no signs of toxicity or mortality at doses up to 2000 mg/kg, and histopathological analysis of liver and kidney tissues confirmed normal architecture, indicating biocompatibility. In vivo studies demonstrated that SP-SeNPs (5 mg/kg) significantly improved body weight compared to the cancer control group. Hematological parameters were restored, with RBC levels reaching 5.8×10^6 cells/µL and WBC levels 8.5×10^3 cells/µL. Inflammatory markers were markedly reduced, with TNF-α decreasing from 118.8 to 67.2 pg/mL, IL-6 from 95.0 to 49.2 pg/mL, and CRP from 6.88 to 3.02 mg/L following treatment. Histopathological evaluation of skin tissues showed near- normal epidermal and dermal architecture in SP-SeNP-treated groups compared to severe alterations in cancer control. Overall, the findings indicate that SP-SeNPs possess significant anticancer, anti-inflammatory, and protective effects, highlighting their potential as a safe and effective therapeutic agent for skin cancer management.

KEYWORDS: Spirulina platensis, Selenium nanoparticles (SeNPs), Skin cancer, DMBA-induced carcinogenesis, Anticancer activity

INTRODUCTION

Skin cancer is one of the most prevalent malignancies worldwide and is associated with several serious complications that affect both patient survival and quality of life [1]. If left untreated, it can progress from localized lesions to invasive and metastatic disease, spreading to lymph nodes and distant organs such as the lungs, liver, and brain. Advanced stages often result in tissue destruction, ulceration, and severe disfigurement, particularly in exposed areas like the face and neck, leading to psychological distress and reduced self-esteem [2]. Chronic inflammation, persistent pain, and secondary infections are also common complications due to impaired skin integrity [3]. Aggressive forms such as melanoma can rapidly metastasize, significantly increasing mortality rates. Conventional treatments, including chemotherapy and radiotherapy, may further cause adverse effects such as immunosuppression, anemia, and organ toxicity [4]. Prolonged disease progression can disrupt normal physiological functions and immune responses [5]. These complications highlight the need for early diagnosis [6], effective therapeutic strategies, and the development of safer, targeted treatments to minimize disease burden and improve patient outcomes.

Nanoparticles have emerged as a promising approach in the treatment of skin cancer due to their unique physicochemical properties and targeted delivery capabilities. Their nanoscale size enables enhanced penetration into tumor tissues, improving drug accumulation at the cancer site while minimizing damage to healthy cells [6]. Nanoparticles can be engineered to carry anticancer drugs, genes, or therapeutic agents, allowing controlled and sustained release, which enhances treatment efficacy and reduces side effects. They exhibit improved bioavailability and stability compared to conventional therapies. Certain nanoparticles, such as selenium nanoparticles, also possess intrinsic antioxidant and anticancer properties, contributing to inhibition of tumor growth and modulation of inflammatory responses [7]. Nanoparticles can be functionalized for targeted therapy, improving specificity toward cancer cells. These advantages make nanoparticle-based systems highly valuable in developing safer, more effective, and innovative strategies for skin cancer management.

MATERIAL AND METHODS

Synthesis of Selenium Nanoparticles Mediated by Spirulina platensis

The selenium nanoparticles were synthesised using the ethanolic extract of Spirulina platensis under controlled conditions (**SP-SeNPs)**. 10 mL of the extract was mixed with 20 mL of 50 mM selenium solution and stirred continuously using a magnetic stirrer to ensure uniform interaction. The reduction process was initiated by the addition of 200 µL of 40 mM ascorbic acid. Formation of selenium nanoparticles was indicated by a characteristic ruby red colour, after which the suspension was centrifuged to collect the nanoparticle pellet, which was dried and stored for further analysis. Particle size distribution and zeta potential of the synthesised nanoparticles were analysed using a Malvern Zetasizer Nano ZS to assess stability and dispersion [8, 9].

The acute oral toxicity of SP-SeNPs

The acute oral toxicity of SP-SeNPs was evaluated in Wistar albino rats in accordance with OECD guideline 423 (Acute Toxic Class Method). Healthy rats were fasted overnight and randomly divided into groups (n = 3). A single oral dose of SP-SeNPs (300 mg/kg body weight) was administered using an oral gavage. Animals were closely observed during the first 4 hours and periodically for 24 hours for signs of toxicity such as behavioral changes, tremors, salivation, and mortality. Based on the observations, a higher dose (2000 mg/kg) was administered to another group. Animals were monitored daily for 14 days to assess clinical signs, body weight, and survival [10, 11].

In-vivo anticancer activity of SP-SeNPs

Healthy male Wistar albino rats (150–180 g) were randomly divided into four groups (n = 6). Group I served as normal control. Group II received a single topical application of 100 µg 12- Dimethylbenz[a]anthracene dissolved in 100 µL acetone on shaved dorsal skin, followed by 1% croton oil (100 µL) thrice weekly for 16 weeks to induce skin carcinogenesis. Group III (standard) received DMBA treatment plus oral administration of 5-fluorouracil (20 mg/kg/week). Group IV received DMB along with Spirulina platensis-mediated selenium nanoparticles (SP-SeNPs) at 5 mg/kg body weight orally, three times per week for 16 weeks. Tumor incidence (%), tumor volume (mm^3), body weight, and latency period were recorded weekly. At the end of the experiment, animals were sacrificed, and skin tissues were collected for histopathological and biochemical analyses to evaluate anticancer efficacy [11].

RESULTS AND DISCUSSION

Synthesis of Selenium Nanoparticles Mediated by Spirulina platensis

The formation of selenium nanoparticles mediated by Spirulina platensis extract was confirmed through a distinct visual colour change during the reaction process. The initial selenium nitrate solution appeared colourless, while the algal extract showed a dark greenish appearance. Upon addition of ascorbic acid as a reducing agent, the reaction mixture gradually developed a characteristic ruby red colour, indicating the successful reduction of selenium ions into elemental selenium nanoparticles.

Figure 1: Visual confirmation of selenium nanoparticle formation.

The control samples exhibited no such prominent colour transition, confirming the essential role of both the extract and reducing agent in nanoparticle synthesis. The intensity of the red coloration suggested effective nanoparticle formation and stability in the reaction medium. The final nanoparticle suspension was homogeneous without visible aggregation, demonstrating good dispersion. These observations provide preliminary evidence for the successful green synthesis of selenium nanoparticles using Spirulina platensis, which can be further validated by physicochemical characterisation techniques.

SP-SeNPs characterization by particle size and zeta potential analysis

The zeta potential analysis of SP-SeNPs revealed a value of −48.9 mV, indicating strong surface charge and excellent colloidal stability. The high negative charge suggests significant electrostatic repulsion between particles, preventing aggregation and promoting uniform dispersion in the medium.

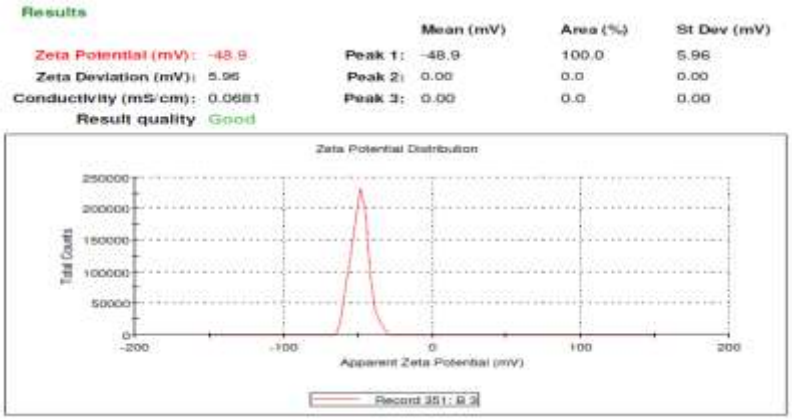

Figure 2: SP-SeNPs characterization by zeta potential analysis.

The single sharp peak with 100% intensity further confirms the homogeneity of the nanoparticle population. The low standard deviation (5.96 mV) reflects consistent particle behavior, while the measured conductivity supports proper dispersion in solution. Findings demonstrate that the synthesised nanoparticles possess good stability and are well-suited for biomedical applications, including anticancer studies, due to their stable physicochemical properties.

The particle size analysis of SP-SeNPs showed a Z-average diameter of 84 nm, indicating nanoscale formation suitable for biomedical applications. The presence of a single intensity peak around 89.1 nm confirms a relatively uniform particle population. However, the high polydispersity index (PDI = 0.93) suggests a broad size distribution, indicating some degree of heterogeneity among particles. Despite this, the good intercept value (0.945) reflects reliable measurement quality. The intensity-based distribution further supports the dominance of particles within the nanoscale range. The results demonstrate successful nanoparticle synthesis, though optimization may be required to achieve improved size uniformity and dispersion.

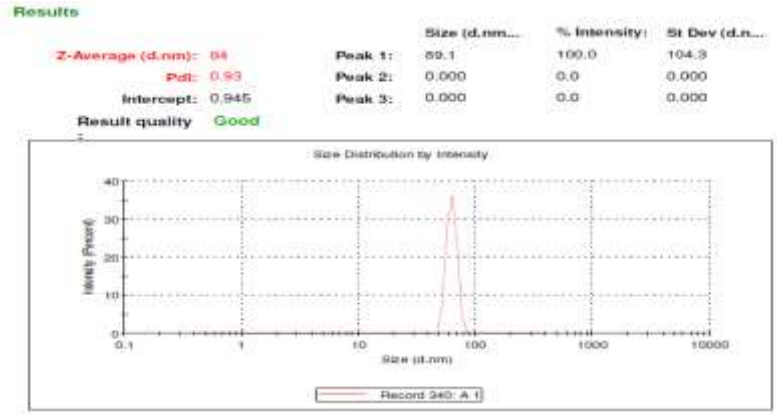

Figure 3: SP-SeNPs characterization by particle size analysis.

Acute oral toxicity study of SP-SeNPs.

The acute oral toxicity assessment of SP-SeNPs demonstrated no observable adverse effects in treated animals, indicating a favorable safety profile. All monitored parameters, including salivation, sleep pattern, and gastrointestinal function, remained within normal limits throughout the study period. The absence of diarrhea, lethargy, or changes in skin, fur, and eye condition suggests that the nanoparticles did not induce external or systemic toxicity. Furthermore, normal respiratory and circulatory functions confirm that vital physiological systems were unaffected. Neurological evaluation showed no impairment, as autonomic and central nervous system activities, along with motor behavior, remained stable. Importantly, no signs of tremors or convulsions were recorded, indicating the lack of neurotoxic effects. Findings suggest that SP-SeNPs are well tolerated at the tested dose levels and do not produce acute toxic manifestations, supporting their potential suitability for further in vivo biomedical applications.

Histopathology of liver and Kidney-Acute toxicity

The histopathological evaluation of kidney and liver tissues in SP-SeNP-treated rats revealed normal structural integrity without significant pathological alterations. The kidney sections showed well-defined glomeruli and intact tubular architecture, with no evidence of necrosis, inflammation, or cellular degeneration. The liver tissues exhibited preserved hepatocellular arrangement with normal sinusoidal spaces and absence of fatty changes, fibrosis, or inflammatory infiltration (Figure 4). These findings indicate that administration of SP-SeNPs did not induce any detectable organ toxicity at the studied dose. The maintenance of normal histoarchitecture in both organs supports the biocompatibility and safety of the synthesized nanoparticles. Furthermore, the absence of tissue damage correlates with the results obtained from the acute toxicity study, reinforcing that SP-SeNPs are non-toxic under experimental conditions. Study

suggests that Spirulina platensis-mediated selenium nanoparticles can be considered safe for further pharmacological and anticancer investigations.

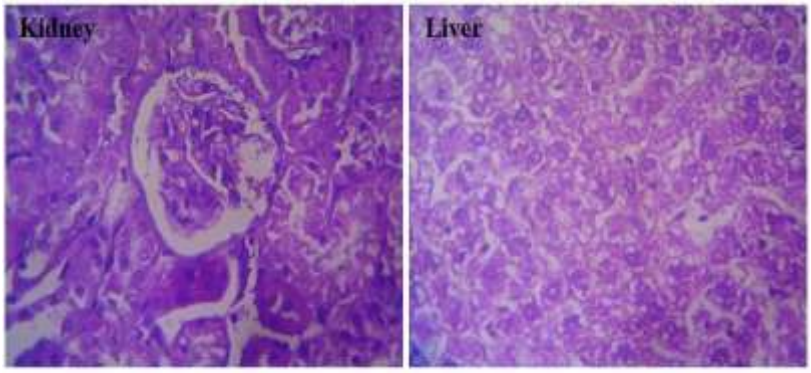

Figure 4: Histopathological sections of kidney and liver tissues from SP-SeNP-treated rats showing normal architecture. The kidney section exhibits intact glomeruli and well-preserved tubular structures, while the liver section shows normal hepatocyte arrangement with clear sinusoids, indicating absence of toxicity (H&E staining, magnification 40×).

In-vivo anticancer activity of SP-SeNPs Animal body weight

The body weight analysis revealed notable differences among the experimental groups over the study period. Initially, all groups exhibited comparable body weights, indicating uniform baseline conditions. However, by the fourth week, a significant reduction in body weight was observed in the cancer control group, reflecting the adverse effects of DMBA-induced carcinogenesis on overall health and metabolism. In contrast, the normal control group maintained steady weight gain, indicating normal physiological growth. Treatment with 5- fluorouracil showed partial improvement in body weight compared to the cancer control, suggesting some therapeutic benefit. Notably, the group treated with SP-SeNPs demonstrated better weight maintenance and recovery, closely approaching normal levels. This improvement may be attributed to the protective and antioxidant properties of Spirulina platensis-mediated selenium nanoparticles. The findings indicate that SP-SeNPs help mitigate cancer-induced weight loss and support physiological stability in treated animals.

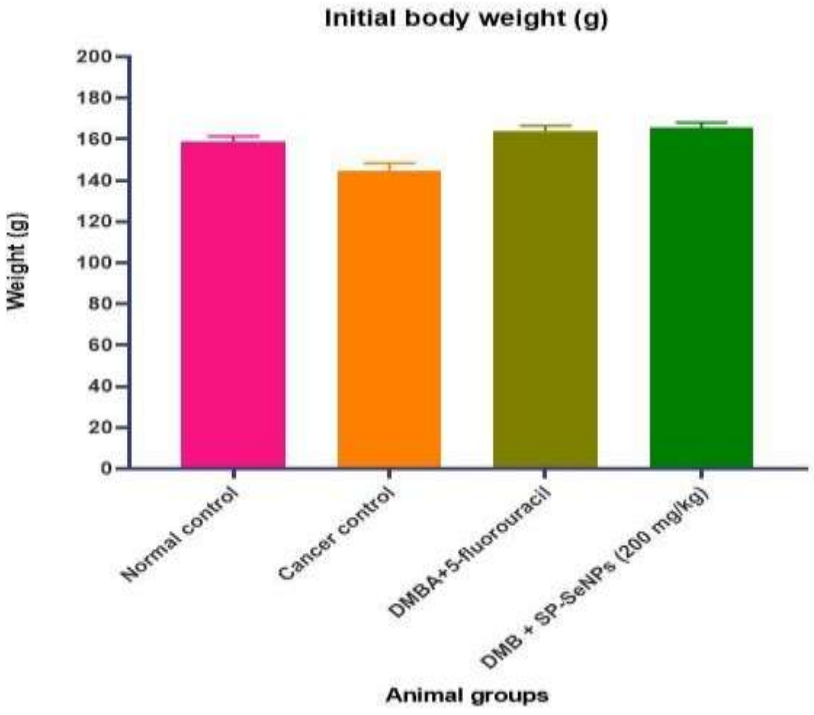

Figure 5: Initial body weight of selected Wister albino rats

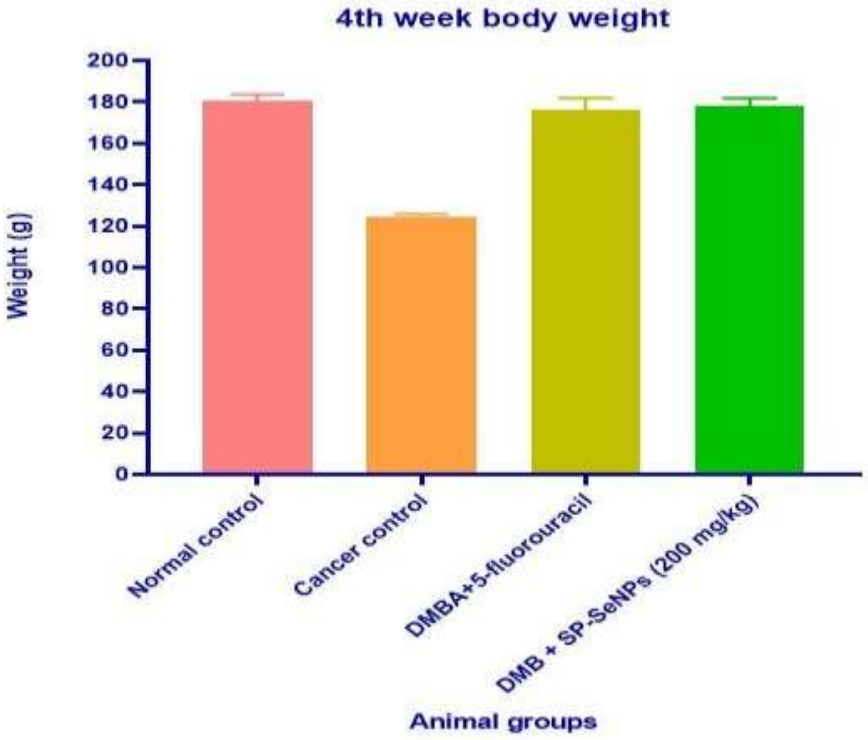

Figure 6: Effect of SP-SeNPs on body weight changes in experimental rats over the study period.

Blood parameters RBC

The evaluation of red blood cell (RBC) levels showed noticeable variations among the experimental groups. The normal control group maintained stable RBC counts, reflecting normal hematological status. In contrast, the cancer control group exhibited a slight reduction in RBC levels, indicating the negative impact of DMBA-induced carcinogenesis on erythropoiesis. The group treated with 5-fluorouracil showed a further decrease in RBC count, which may be attributed to the known myelosuppressive effects of the chemotherapeutic agent. However, the group treated with SP-SeNPs demonstrated a marked improvement in RBC levels compared to both cancer and standard-treated groups. This suggests that Spirulina platensis- mediated selenium nanoparticles may possess protective effects against hematological alterations. The improved RBC count indicates enhanced recovery of blood parameters and reduced toxicity, highlighting the potential of SP-SeNPs in mitigating cancer-induced hematological disturbances.

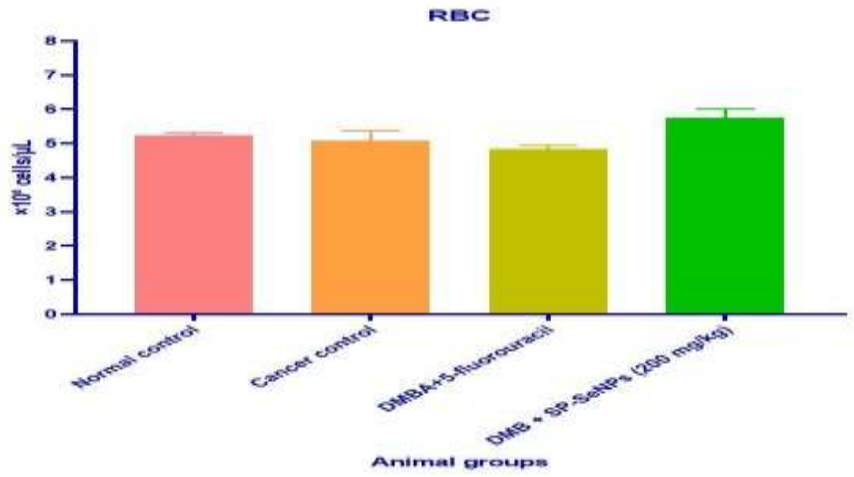

Figure 7: Effect of SP-SeNPs on red blood cell (RBC) levels in different experimental groups.

WBC

The white blood cell (WBC) count showed notable variations among the experimental groups, reflecting immune response changes during the study. The normal control group exhibited standard WBC levels, indicating normal immune function. In the cancer control group, a reduction in WBC count was observed, suggesting immunosuppression associated with DMBA-induced carcinogenesis. The group treated with 5-fluorouracil showed a slight improvement compared to the cancer control, although values remained below normal, likely due to the cytotoxic effects of the drug. In contrast, the SP-SeNPs-treated group demonstrated a more regulated WBC count, closer to normal levels. This indicates a potential immunomodulatory effect of Spirulina platensis-mediated selenium nanoparticles. The ability of

SP-SeNPs to maintain balanced leukocyte levels suggests their role in supporting immune function while reducing the adverse hematological effects associated with cancer progression and treatment.

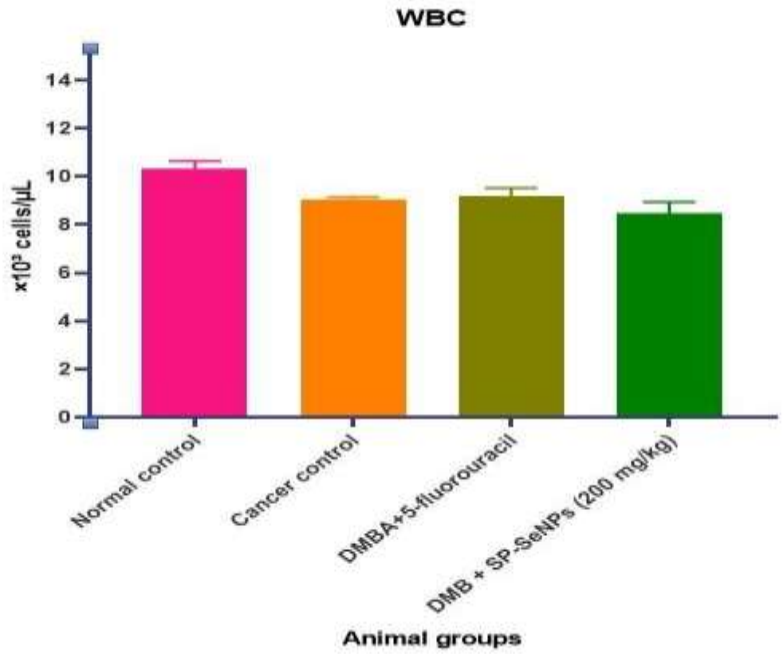

Figure 8: Effect of SP-SeNPs on white blood cell (WBC) levels in different experimental groups.

Cancer and Inflammatory Markers Tumor necrosis factor-alpha (TNF-α)

The levels of tumor necrosis factor-alpha (TNF-α) showed significant variation among the experimental groups, reflecting inflammatory responses associated with carcinogenesis and treatment. The normal control group exhibited low TNF-α levels, indicating minimal inflammatory activity. In contrast, the cancer control group showed a marked elevation in TNF- α levels, confirming the presence of enhanced inflammatory and tumor-promoting conditions induced by DMBA. Treatment with 5-fluorouracil resulted in a noticeable reduction in TNF-α levels compared to the cancer control, suggesting its partial anti-inflammatory and anticancer effect. Importantly, the SP-SeNPs-treated group demonstrated a further decrease in TNF-α levels, approaching near-normal values. This indicates a strong anti-inflammatory potential of Spirulina platensis-mediated selenium nanoparticles. The reduction in TNF-α suggests that SP-SeNPs may effectively suppress pro-inflammatory cytokine production, thereby contributing to their protective and therapeutic role against cancer progression.

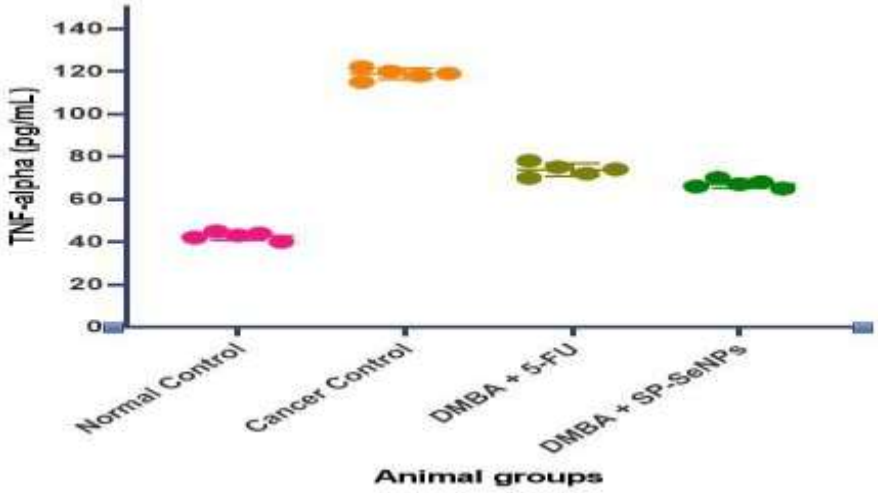

Figure 9: Effect of SP-SeNPs on tumor necrosis factor-alpha (TNF-α) levels in experimental groups.

Interleukin-6 (IL-6)

The levels of interleukin-6 (IL-6) varied significantly among the experimental groups, indicating changes in inflammatory status during the study. The normal control group showed low IL-6 levels, reflecting minimal inflammatory activity under normal physiological conditions. In contrast, the cancer control group exhibited a substantial increase in IL-6 levels, confirming enhanced inflammatory response associated with DMBA-induced carcinogenesis. Treatment with 5-fluorouracil resulted in a reduction of IL-6 levels compared to the cancer control group, indicating its moderate

anti-inflammatory effect. Notably, the group treated with SP-SeNPs demonstrated a further decrease in IL-6 levels, approaching values closer to the normal control. This suggests that Spirulina platensis-mediated selenium nanoparticles possess significant anti-inflammatory properties. The ability of SP-SeNPs to suppress IL-6 levels highlights their potential role in modulating cytokine-mediated inflammation and contributing to the inhibition of cancer progression.

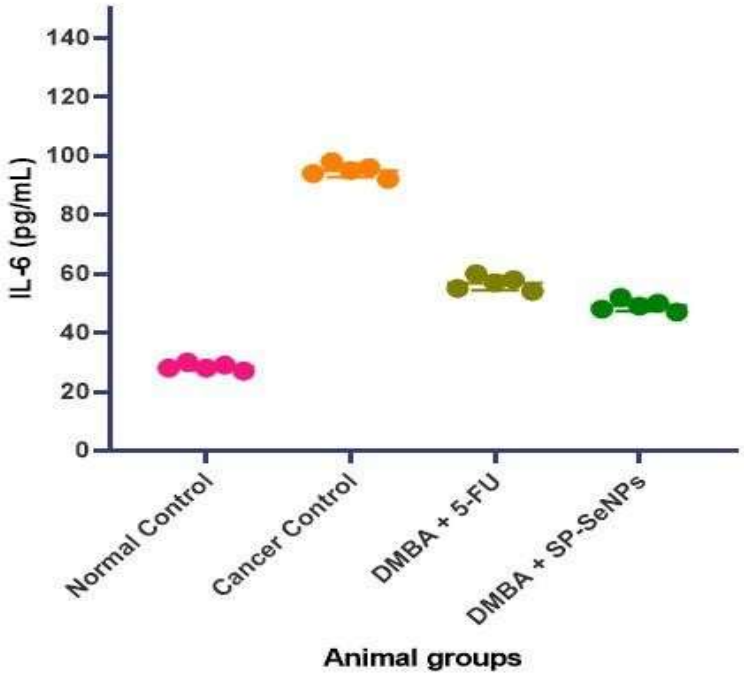

Figure 10: Effect of SP-SeNPs on interleukin-6 (IL-6) levels in experimental groups.

C- reactive protein (CRP)

The levels of C-reactive protein (CRP) showed marked differences among the experimental groups, indicating variations in systemic inflammation. The normal control group exhibited low CRP levels, reflecting a normal physiological state with minimal inflammatory activity. In contrast, the cancer control group demonstrated a significant elevation in CRP levels, confirming the presence of inflammation associated with DMBA-induced carcinogenesis. Treatment with 5-fluorouracil resulted in a reduction in CRP levels compared to the cancer control group, suggesting its partial effectiveness in controlling inflammation. Notably, the SP- SeNPs-treated group showed a further decrease in CRP levels, approaching near-normal values. This indicates a strong anti-inflammatory effect of Spirulina platensis-mediated selenium nanoparticles. The reduction in CRP suggests that SP-SeNPs may effectively suppress systemic inflammation, thereby contributing to their protective role against cancer progression and improving overall physiological conditions.

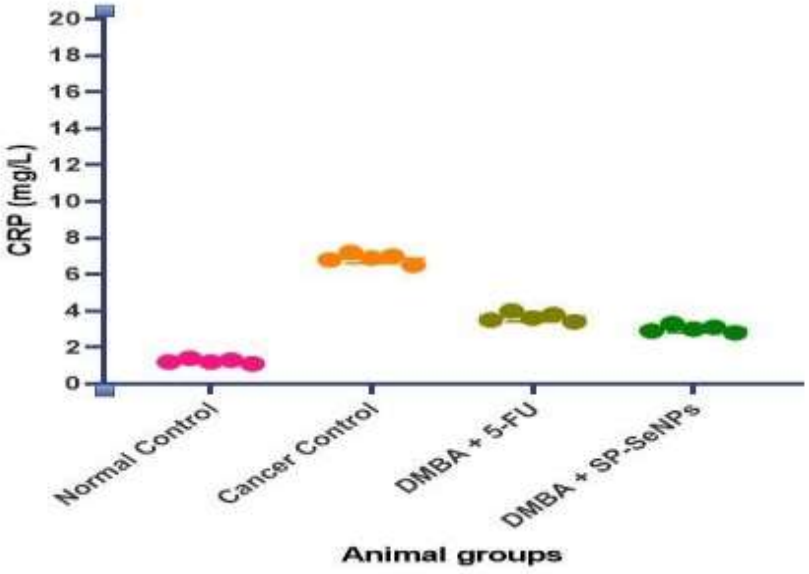

Figure 11: Effect of SP-SeNPs on C-reactive protein (CRP) levels in experimental groups.

Histopathology

The histopathological examination of skin tissues revealed distinct structural variations among the experimental groups.

The normal control group (G-I) exhibited intact epidermal and dermal architecture with well-organized cellular arrangement, indicating healthy skin morphology. In contrast, the cancer control group (G-II) showed severe pathological alterations, including disorganized epidermis, hyperkeratosis, cellular atypia, and dense inflammatory infiltration, confirming successful induction of skin carcinogenesis by DMBA. The group treated with 5- fluorouracil (G-III) demonstrated partial restoration of tissue structure, with reduced abnormal cell proliferation and mild improvement in epidermal organization, although some residual pathological features were still evident. Notably, the SP-SeNPs-treated group (G-IV) showed marked improvement in skin histology, with near-normal epidermal thickness, reduced keratinization, and minimal inflammatory changes. The restoration of tissue architecture in this group suggests enhanced protective and therapeutic effects. These findings indicate that Spirulina platensis-mediated selenium nanoparticles effectively mitigate DMBA-induced histological damage, likely through their antioxidant and anti-inflammatory properties, thereby supporting their potential as a promising agent in skin cancer management [12].

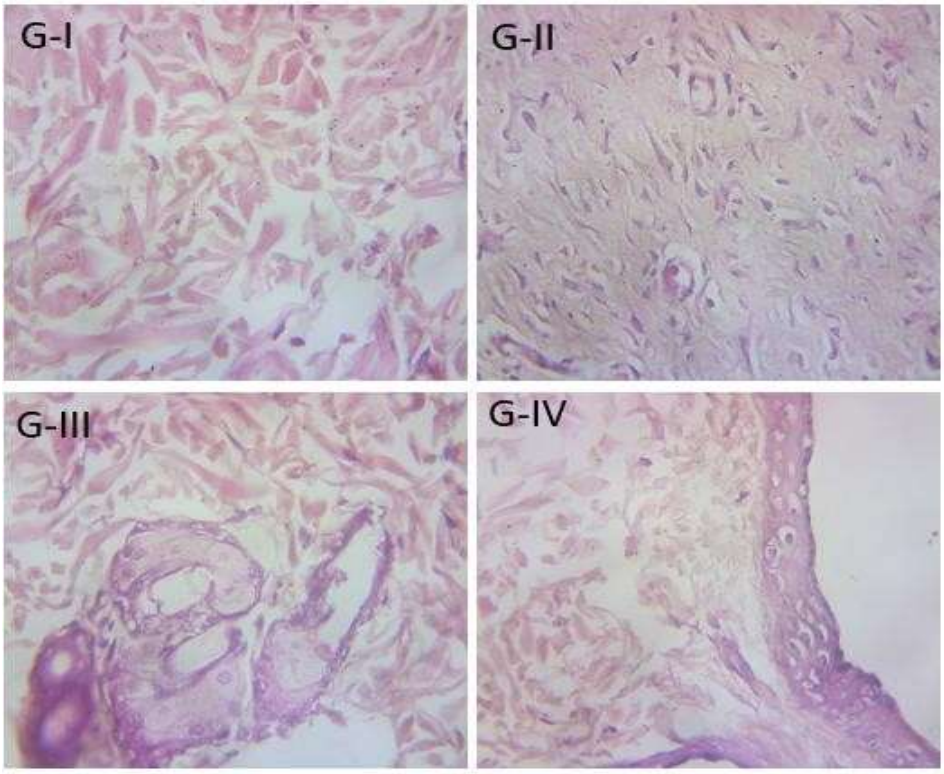

Figure 12: Histopathological analysis of skin tissues from experimental groups (H&E staining, 40×). (G-I) Normal control showing intact epidermal and dermal architecture; (G-II) Cancer control showing hyperkeratosis, cellular atypia, and disorganized tissue structure; (G-III) DMBA + 5-fluorouracil showing partial restoration with reduced abnormalities; (G-IV) DMBA + SP-SeNPs (200 mg/kg) showing near-normal architecture with minimal pathological changes, indicating effective protective and therapeutic activity.

CONCLUSION

Spirulina platensis-mediated selenium nanoparticles were successfully synthesised through a green and eco-friendly approach, as confirmed by visual observation and physicochemical characterization. The nanoparticles exhibited good stability, nanoscale size, and favorable dispersion properties, making them suitable for biomedical applications. Acute toxicity and histopathological studies demonstrated that SP-SeNPs are safe and biocompatible, with no significant adverse effects on vital organs. In vivo anticancer evaluation revealed that SP- SeNPs effectively improved body weight, restored hematological parameters, and reduced inflammatory markers such as TNF-α, IL-6, and CRP. Histological findings confirmed significant protection against DMBA-induced skin damage. SP-SeNPs show promising potential as a safe and effective therapeutic agent for skin cancer management.

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DATA AVAILABILITY: Data generated while conducting the research are available to the corresponding author upon reasonable request.

Abbreviations

SP-SeNPs – Spirulina platensis-mediated Selenium Nanoparticles
SeNPs – Selenium Nanoparticles
DMBA – 12-Dimethylbenz[a]anthracene
OECD – Organization for Economic Cooperation and Development
RBC – Red Blood Cells
WBC – White Blood Cells
TNF-α – Tumor Necrosis Factor-alpha
IL-6 – Interleukin-6
CRP – C-Reactive Protein
H&E – Hematoxylin and Eosin
PDI – Polydispersity Index
FE-SEM – Field Emission Scanning Electron Microscopy
HRTEM – High Resolution Transmission Electron Microscopy
ATR-FTIR – Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy
XRD – X-ray Diffraction
ALT – Alanine Aminotransferase
AST – Aspartate Aminotransferase
ALP – Alkaline Phosphatase

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