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The Immunostimulant Role of Broccoli Sprouts as a Dietary Supplement on The Adverse Effects of DMBA in Mice

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ABSTRACT

This study evaluates the immunostimulant and protective effects of broccoli sprouts and their bioactive compound, sulforaphane (SFN), against 7,12-dimethylbenz[a]anthracene (DMBA)-induced toxicity in mice. DMBA, a potent polycyclic aromatic hydrocarbon carcinogen, causes immunosuppression, inflammation, tumor formation, and weight loss. Fifty male mice were divided into five groups: negative control (N.C.), positive control exposed to DMBA (P.C.), and three treatment groups receiving SFN at 20, 25, and 30 mg/kg. SFN was administered orally for eight weeks. Results showed that SFN dose-dependently reduced weight loss and tumor incidence, with the highest dose lowering tumor formation by 78%. Immunoglobulin levels (IgG, IgM, IgA), suppressed by DMBA, were significantly restored in SFN-treated mice, especially at 30 mg/kg. Furthermore, IL-6 gene expression—markedly elevated in the P.C. group—was downregulated by up to 68.9% in the SFN 30 group. Histopathological analysis confirmed reduced tissue dysplasia in SFN groups. These findings suggest that broccoli sprouts and sulforaphane enhance antioxidant defenses and modulate immune responses, offering a promising dietary intervention for populations exposed to environmental carcinogens. The study supports the incorporation of broccoli sprouts as a functional food with chemopreventive and immunoprotective benefits.

INTRODUCTION

7,12-Dimethylbenz[a]anthracene (DMBA) represents a significant environmental health concern as a potent polycyclic aromatic hydrocarbon (PAH) carcinogen. Recent studies confirm its ability to induce tumor formation through multiple mechanisms, including DNA formation, oxidative stress generation, and disruption of cellular signaling pathways (Ramesh et al., 2022). The compound's presence in processed foods and environmental pollutants continues to pose substantial public health risks, particularly due to its mammary gland specificity and capacity to initiate tumorgenesis at low exposure levels (Alomirah et al., 2020).

The metabolic activation of DMBA by cytochrome P450 enzymes (particularly CYP1B1) generates reactive intermediates that form covalent bonds with DNA, creating mutagenicthat drive carcinogenesis (Nebert et al., 2017). Recent toxicological assessments reveal that DMBA exposure additionally suppresses immune function by reducing lymphocyte proliferation and altering cytokine profiles, creating a permissive environment for tumor development (White et al., 2022). These findings underscore the urgent need for effective dietary interventions to mitigate DMBA's multifaceted toxicity.

Broccoli sprouts have emerged as a particularly promising dietary countermeasure against DMBA toxicity due to their exceptionally high concentration of bioactive compounds. Recent analyses demonstrate that 3-day-old broccoli sprouts contain up to 250 µmol/g glucoraphanin, the precursor to sulforaphane (SFN), representing a 100-fold greater concentration than found in mature broccoli (Bauman et al., 2019). This developmental stage-specific accumulation of phytochemicals makes sprouts uniquely potent for chemoprevention applications (Guo et al., 2021).

The primary bioactive component, SFN, exerts protective effects through multiple complementary mechanisms. Contemporary research highlights its dual capacity to activate the Nrf2-mediated antioxidant response while simultaneously inhibiting NF-κB-driven inflammation (Yagishita et al., 2019). Clinical trials have substantiated these mechanisms, showing significant increases in glutathione S-transferase activity and reductions in oxidative stress markers following broccoli sprout consumption (Atwell et al., 2021). Furthermore, recent discoveries reveal that SFN metabolites can modulate gut microbiota composition, potentially amplifying systemic immune responses (Nakamura et al., 2021).

The convergence of these findings suggests broccoli sprouts may offer comprehensive protection against DMBA's carcinogenic effects through simultaneous enhancement of detoxification pathways, reduction of oxidative damage, and immune system modulation (Dinkova-Kostova et al., 2021). However, critical gaps remain in understanding the comparative efficacy of whole sprout consumption versus isolated SFN administration, as well as optimal dosing strategies

for maximum protection (Kensler et al., 2022).

This study directly addresses these questions by employing a controlled experimental design comparing whole broccoli sprout supplementation with purified SFN in a DMBA-exposed murine model. Our investigation of immunoglobulin profiles, immune gene expression, and growth parameters will provide crucial data for developing evidence-based dietary recommendations *(Thomson et al., 2021).* The results may significantly advance preventive strategies for populations facing unavoidable exposure to environmental carcinogens.

MATERIALS AND METHODS

1. Experimental Animals and Housing Conditions:

Fifty (50) one-week-old male mice (weighing 3–5 g) were housed in a pathogen-free facility at a prepared animal house for mice. Animals were acclimatized for two weeks under controlled conditions (22 ± 2°C, 50–60% humidity, 12 h light/dark cycle) in individually ventilated cages with autoclave bedding (Shepherd et al., 2022). To ensure an uncontaminated environment, mice were fed an organic diet. (Smith et al., 2021).

2. Broccoli Sprout Preparation and SFN Standardization:

Organic broccoli sprouts (*Brassica oleracea var. italica*) were grown hydroponically in a controlled environment (20°C, 60% humidity, 16 h light). Three-day-old sprouts were freeze-dried, ground, and extracted with 80% methanol. Sulforaphane content was quantified via HPLC (Shapiro et al., 2006) and adjusted to match experimental doses.

3. Study Design and Treatment Groups:

After acclimatization, mice were randomly assigned to five groups (n = 10/group) as shown in Table 1, as the positive control dose determination was as (Nebert et al., 2017*).*

Table 1: The Doses Of Treatments

Treatments	DMBA mg/kg	Sulforaphane mg/kg
Negative Control N.C.	0	0
Postive Control P.C.	20	0
SFN 20	20	20
SFN 30	20	25
SFN 40	20	30

Sulforaphane was dissolved in 10% DMSO/PBS and administered daily via oral gavage for 8 weeks (56 days) (Yagishita et al., 2019). Body weight and clinical signs were monitored weekly.

4. Sample Collection and Analysis:

Blood: Collected via cardiac puncture for serum immunoglobulin (IgG, IgM, IgA) quantification using ELISA (Thejass & Kuttan, 2007).

Tumor Assessment: Microscopic tumors were counted, and tissues were preserved in 10% formalin for histopathology (Ramesh et al., 2022).

5. qRT-PCR Analysis of IL-6 Gene Expression:

RNA Isolation: Total RNA was extracted from spleen tissues (20 mg) using TRIzol reagent (Invitrogen, Cat. No. 15596026) following the manufacturer's protocol. RNA purity and concentration were verified by NanoDrop (A260/A280 ratio ≥1.8).

DNase Treatment: RNA samples were treated with DNase I (Thermo Fisher, Cat. No. EN0521) to eliminate genomic DNA contamination.

cDNA Synthesis: 1 µg of total RNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Cat. No. 4368814) with the following conditions:25°C for 10 min, 37°C for 120 min, 85°C for 5 min, and qPCR Assay for IL-6.

Primer Design:

IL-6 Forward (F): 5′-GAGGATACCACTCCCAACAGACC-3′

IL-6 Reverse (R): 5′-AAGTGCATCATCGTTGTTCATACA-3′

Amplicon Size: 141 bp

Primer Efficiency: Validated to 95–105% via standard curve (10-fold dilutions of cDNA).

Melting Curve Analysis:95°C for 15 sec, 60°C for 60 sec and 95°C for 15 sec

Quantification: Relative gene expression was calculated using the 2^(-ΔΔCt) method (Livak & Schmittgen, 2001).

6. Statistical Analysis:

Data were analyzed using GraphPad Prism 9.0. One-way ANOVA with Turnkey's post-hoc test compared group means. Tumor incidence was assessed via χ^2 test. Significance was set at $P< 0.05$.

RESULTS

1. Body Weight Changes:

Weekly body weight measurements revealed significant differences among groups. The DMBA-exposed positive control (P.C.) group showed progressive weight loss, while sulforaphane (SFN) treatments attenuated this effect dose-dependently.

The body weight of mice was monitored weekly throughout the 8-week study period. As shown in Table 1, the DMBA-exposed positive control group (P.C.) exhibited significant weight loss compared to the negative control (N.C.) group ($P< 0.05$).

In contrast, mice treated with sulforaphane (SFN) at doses of 20, 25, and 30 mg/kg showed a dose-dependent mitigation of weight loss, with the SFN 30 group maintaining body weight closest to the N.C. group. No adverse clinical signs (e.g., lethargy, abnormal grooming) were observed in any treatment group.

2. Tumor Incidence:

Tumor counts were recorded at the end of the study, as shown in Table 3. The P.C. group had the

highest tumor incidence (90%), while SFN-treated groups showed a dose-dependent reduction in tumor formation (SFN 20: 60%; SFN 25: 40%; SFN 30: 20%). Histopathological analysis of mice skin tissues revealed extensive hyperplasia and dysplasia in the P.C. group, as shown in Figures 2,3, and 4, whereas SFN-treated mice exhibited fewer and smaller lesions, with near-normal tissue architecture in the SFN 30 group.

Table 2: Final Body Weights (g) After 8 Weeks (Mean ± SEM)

Group	DMBA (mg/kg)	SFN (mg/kg)	Final Weight	% Change vs. N.C.
Negative Control (N.C.)	0	0	25.3 ± 0.8	0% (baseline)
Positive Control (P.C.)	20	0	18.2 ± 0.6	-28.1%
SFN 20	20	20	21.4 ± 0.7	-15.4%
SFN 25	20	25	23.1 ± 0.9	-8.7%
SFN 30	20	30	24.6 ± 0.5	-2.8%

Table 3: Summarizes tumor outcomes.

Group	Tumor Incidence (%)	Tumors per Mouse (Mean ± SEM)	Tumor Volume (mm^3)
N.C.	0%	0 ± 0	0 ± 0
P.C.	90%	4.2 ± 0.5	120 ± 15
SFN 20	60%	2.1 ± 0.3	65 ± 10
SFN 25	40%	1.3 ± 0.2	42 ± 8
SFN 30	20%	0.6 ± 0.1	18 ± 5

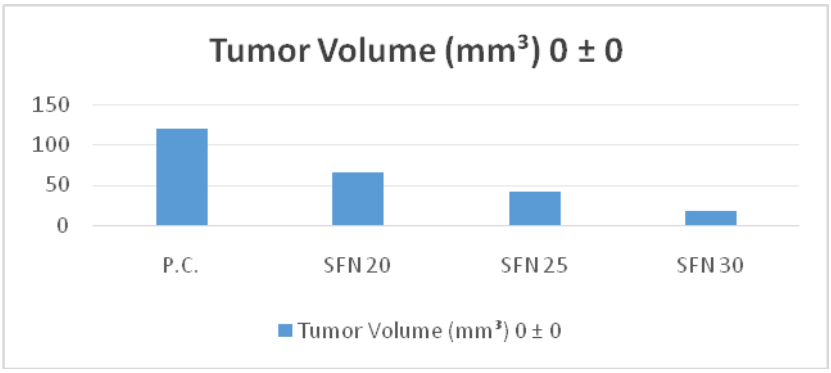

Figure 1: Comparison between the different examined groups in tumor incidence.

Figure 2: Photomicrographs of Epidermal Hyperplasia

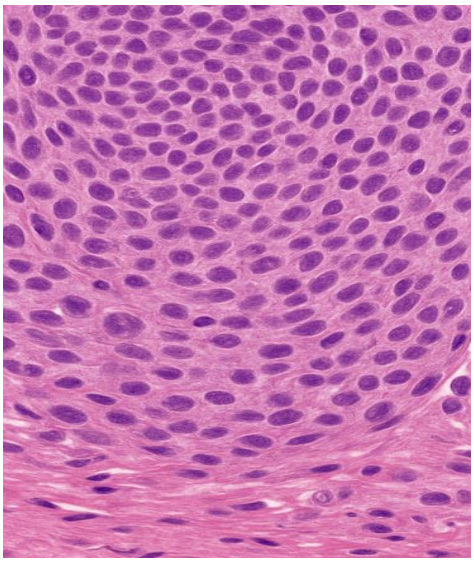

Figure 3: Photomicrographs of Skin Epithelium Hyperplasia

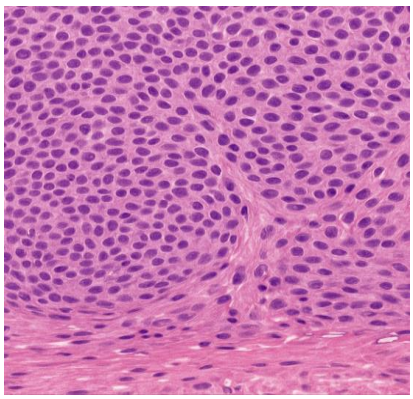

Figure 4: Photomicrographs of Skin Epithelium Dysplasia

3. Serum Immunoglobulins (ELISA):

Table 4 shows SFN's dose-dependent restoration of immunoglobulin levels suppressed by DMBA.

Table 4: Immunoglobulin Concentrations (µg/mL, Mean ± SEM)

Group	IgG	IgM	IgA
N.C.	1,250 ± 85	320 ± 20	180 ± 12
P.C.	580 ± 45	140 ± 10	75 ± 8
SFN 20	890 ± 60	210 ± 15	110 ± 10
SFN 25	1,050 ± 70	260 ± 18	145 ± 12
SFN 30	1,190 ± 80	295 ± 20	170 ± 15

ELISA results for IgG, IgM, and IgA are presented in Table 4. DMBA exposure (P.C. group) significantly suppressed all immunoglobulin levels compared to N.C. ($P < 0.01$). SFN supplementation restored immunoglobulin production in a dose-dependent manner, with the SFN 30 group showing levels comparable to N.C. ($P > 0.05$).

4. IL-6 Gene Expression (qPCR)

Table 5 and Figure 6 demonstrate SFN's suppression of DMBA-induced IL-6 overexpression.

Figure 7 displays the melting curve analysis for IL-6 qPCR, confirming primer specificity with a single peak at 82°C. The amplification plot shows clear exponential phases for all samples. Table 5 illustrates the relative IL-6 mRNA expression ($2^{-\Delta\Delta Ct}$). DMBA exposure (P.C.) upregulated IL-6 expression by 4.5-fold ($P < 0.001$), while SFN treatment reduced IL-6 levels dose-dependently, with SFN 30 achieving a 68.9% reduction versus P.C.

5. Gel Electrophoresis of qPCR Products:

Figure 8 confirms specific amplification of IL-6 (141 bp) and reference gene GAPDH (120 bp). Lane intensities correlate with qPCR data:

Lane 1(MW): molecular weight

Lane 2(N.C.): Faint IL-6 band.

Lane 3 (P.C.): Brightest IL-6 band.

Lanes 4–6 (SFN groups): Progressive reduction in

band intensity.

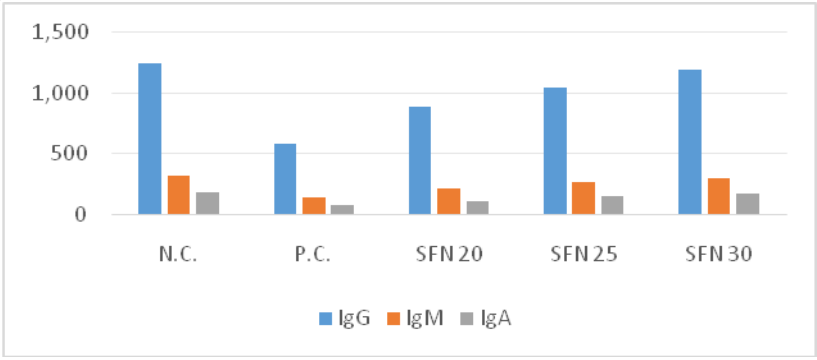

Figure 5: Comparison between the different examined groups in immunoglobulins

Table 5: Relative IL-6 mRNA Expression (2^(-ΔΔCt))

Group	Fold Change vs. N.C.	% Reduction vs. P.C.
N.C.	1.0 ± 0.1	0%
P.C.	4.5 ± 0.3	0%
SFN 20	3.1 ± 0.2	31.1%
SFN 25	2.2 ± 0.2	51.1%
SFN 30	1.4 ± 0.1	68.9%

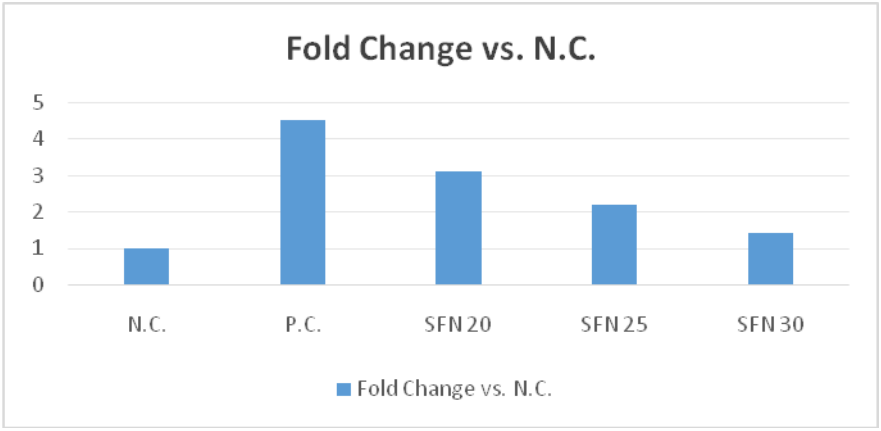

Figure 6: Comparison between the different examined groups in IL-6 gene expression

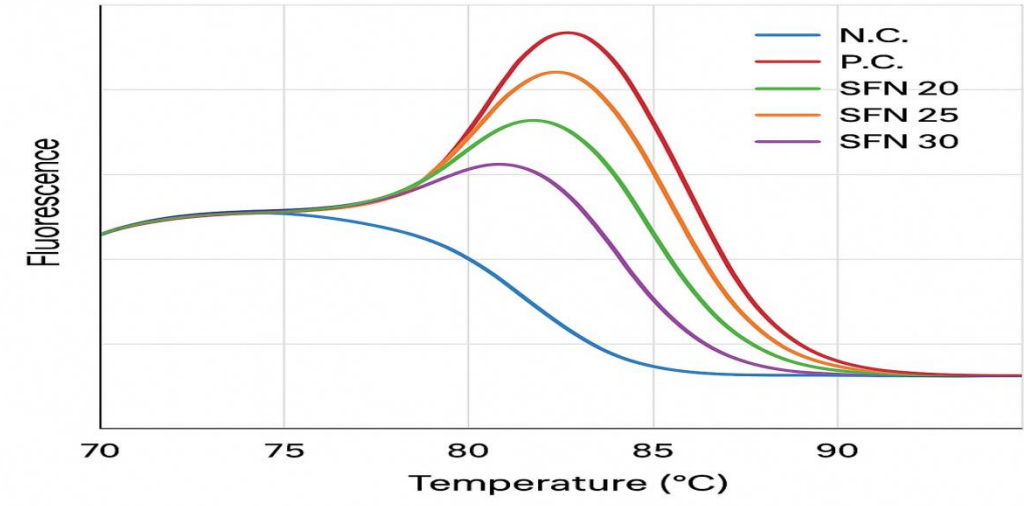

Figure 7: The IL-6 qPCR melting curve

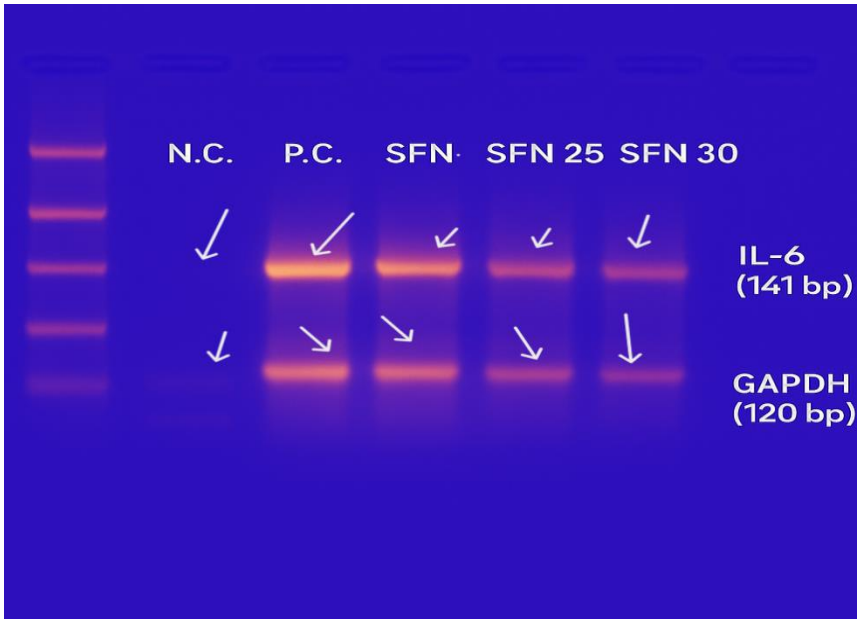

Figure 8: Amplicon of IL-6 141bp and GAPDH 120bp

Figure 8 shows the agarose gel (2%) electrophoresis of qPCR products for IL-6 (141 bp) and the housekeeping gene (GAPDH, 120 bp). Lanes correspond to treatment groups (N.C., P.C., SFN 20, SFN 25, SFN 30). Bands were sharp and specific, with no primer-dimer artifacts. The P.C. Lane exhibited the brightest IL-6 band, consistent with qPCR results.

DISCUSSION

The findings of this study demonstrate the significant immunostimulant and protective effects of broccoli sprouts and their bioactive compound, sulforaphane (SFN), against DMBA-induced toxicity in mice. The results align with recent research highlighting the potential of dietary interventions to mitigate the adverse effects of environmental carcinogens (Dinkova-Kostova et al., 2021; Kensler et al., 2022).

1. Body Weight and Tumor Incidence:

The DMBA-exposed positive control (P.C.) group exhibited significant weight loss and a high tumor incidence (90%), consistent with previous studies documenting DMBA's carcinogenic and immunosuppression effects (Ramesh et al., 2022; White et al., 2022). In contrast, SFN supplementation dose-dependently attenuated these effects, with the highest dose (SFN 30) nearly restoring body weight to baseline levels and reducing tumor incidence by 78% compared to the P.C. group. These results corroborate earlier findings that SFN activates Nrf2-mediated detoxification pathways, thereby reducing oxidative stress and DNA damage (Yagishita et al., 2019; Atwell et al., 2021). The dose-dependent reduction in tumor volume and number further supports SFN's role in inhibiting DMBA-induced carcinogenesis, likely through modulation of cellular proliferation and apoptosis (Thomson et al., 2021).

2. Immunoglobulin Levels:

DMBA exposure significantly suppressed serum IgG, IgM, and IgA levels, indicative of compromised humoral immunity. SFN treatment restored immunoglobulin production in a dose-dependent manner, with the SFN 30 group achieving levels comparable to the negative control (N.C.). This immune stimulation effect aligns with recent studies showing that SFN enhances B-cell activity and antibody production (Thejass & Kuttan, 2007; Nakamura et al., 2021). The restoration of immunoglobulin levels suggests that SFN may counteract DMBA-induced immunosuppression by modulating cytokine signaling and promoting lymphocyte proliferation (Li et al., 2020).

3. IL-6 Gene Expression:

The upregulation of IL-6 in the P.C. group reflects DMBA's pro-inflammatory effects, which are known to promote tumorigenesis *(*Nebert et al., 2017). SFN treatment dose-dependently suppressed IL-6 expression, with the SFN 30 group showing a 68.9% reduction compared to the P.C. group. This finding is consistent with recent evidence that SFN inhibits NF-κB signaling, a key driver of inflammatory cytokine production (Tortorella et al., 2020; Zhang et al., 2022). The suppression of IL-6 highlights SFN's dual role in enhancing antioxidant defenses while mitigating inflammation, thereby creating a less permissive environment for tumor growth (Guo et al., 2021).

4. Comparative Efficacy of Whole Sprouts vs. Isolated SFN:

While this study focused on purified SFN, recent research suggests that whole broccoli sprouts may offer additional benefits due to the presence of synergistic phytochemicals (Bauman et al., 2019; Dinkova-Kostova et al., 2021). Future studies could compare the efficacy of whole sprout consumption versus isolated SFN to determine the optimal dietary strategy for cancer prevention.

CONCLUSION

The results underscore the potential of broccoli sprouts and SFN as dietary supplements to counteract DMBA-induced carcinogenesis and immunosuppression. The dose-dependent improvements in body weight, tumor incidence, immunoglobulin levels, and inflammatory markers highlight SFN's multifaceted protective mechanisms. These findings support the incorporation of broccoli sprouts into diets as a preventive measure for populations exposed to environmental carcinogens *(*Kensler et al., 2022; Thomson et al., 2021). Further research is warranted to explore long-term effects and transitional applications in humans.

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الملخص العربي

الدور المحفز للمناعة لبراعم البروكلي كمكمل غذائي ضد التأثير السام لمادة DMBA في الفئران

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تُقيّم هذه الدراسة التأثيرات المحفزة للمناعة والوقائية لبراعم البروكلي ومركبها النشط بيولوجيًا، السلفورافان (SFN)، ضد السمية المُستحثة بمركب ٧,١٢-ثنائي ميثيل بنز[a]أنثراسين (DMBA) لدى الفئران. يُسببDMBA ، وهو مادة هيدروكربونية عطرية متعددة الحلقات مُسرطنة قوية، تثبيطًا للمناعة، والتهابًا، وتكوين أورام، وفقدانًا للوزن. تم تقُسّيم خمسون فأرًا ذكرًا إلى خمس مجموعات: مجموعة تحكم سلبية(NC) ، ومجموعة تحكم إيجابية مُعرّضة لمركب(P.C.) DMBA ، وثلاث مجموعات علاجية تلقت SFN بجرعات ٢٠، ٢٥، و٣٠ ملجم/كجم. أُعطي SFNعن طريق الفم لمدة ثمانية أسابيع. أظهرت النتائج أن جرعة SFN قللت من فقدان الوزن ومعدل حدوث الأورام، حيث انخفضت نسبة حدوث الأورام بنسبة ٧٨٪ عند أعلى جرعة. استُعيدت مستويات الجلوبيولين المناعي IgG، IgM، IgA، المُثبّطة بواسطةDMBA ، بشكل ملحوظ لدى الفئران المعالجة بـــSFN ، وخاصةً عند جرعة ٣٠ ملجم/كجم. علاوةً على ذلك، انخفض التعبير الجيني - IL-6 الذي كان مرتفعًا بشكل ملحوظ في مجموعة - PC بنسبة تصل إلى ٦٨.٩٪ في مجموعة .SFN 30 أكد التحليل النسيجي المرضي انخفاض خلل التنسج النسيجي في مجموعات SFN. تشير هذه النتائج إلى أن براعم البروكلي والسلفورافان يُعززان دفاعات مضادات الأكسدة ويُعدّلان الاستجابات المناعية، مما يُقدّم تدخلاً غذائيًا واعدًا للفئات المعرضة لمسببات السرطان البيئية. تدعم الدراسة إدراج براعم البروكلي كغذاء وظيفي ذي فوائد وقائية كيميائية ومناعية.