RESEARCH PAPER

Assessment of Anticancer Potential of Novel Indole Derivatives in DMBA-Induced Skin Carcinogenesis Model

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ABSTRACT

Skin cancer remains a major global health burden, driven primarily by oxidative stress, inflammation, and genetic alterations caused by chemical carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA). The present study aimed to evaluate the chemopreventive potential of newly synthesized indole derivatives against DMBA-induced skin carcinogenesis in Swiss albino mice. Following tumor induction with DMBA, animals were treated orally with indole derivatives (ID-1, ID-2, and ID-3) for 16 weeks. Tumor incidence, latency, and burden were recorded, alongside biochemical, histopathological, and molecular analyses. Treatment with indole derivatives significantly reduced tumor number, size, and volume compared to the DMBA control. Enhanced antioxidant enzyme levels (SOD, CAT, GSH) and decreased lipid peroxidation indicated restoration of oxidative balance. Inflammatory biomarkers (COX-2, TNF-α, IL-6) were markedly suppressed, while apoptotic markers (Bax, caspase-3) were upregulated and Bcl-2 downregulated, suggesting activation of intrinsic apoptosis. Histopathological observations confirmed normalization of epidermal architecture and reduced dysplastic lesions. Molecular docking revealed strong binding affinities of indole derivatives, particularly ID-3, with COX-2, NF-κB, and Bcl-2 proteins, supporting their multitarget anticancer mechanism. These findings collectively highlight the potent chemopreventive and therapeutic potential of indole derivatives in mitigating DMBA-induced skin carcinogenesis through antioxidant, anti-inflammatory, and pro-apoptotic mechanisms. Collectively, the multifaceted mechanisms—antioxidant defense, anti-inflammatory modulation, DNA protection, and apoptosis induction—underscore the potential of indole derivatives as promising chemopreventive agents against chemical carcinogen-induced skin cancer. Future studies involving molecular dynamics, pharmacokinetics, and clinical validation are warranted to establish their therapeutic applicability and safety profile for translational cancer prevention..

Keywords: Indole derivatives, Skin carcinogenesis, DMBA, Antioxidant defense, Apoptosis, COX-2 inhibition..

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INTRODUCTION

Cancer remains one of the most formidable health challenges of the 21st century, characterized by uncontrolled cellular proliferation, genomic instability, and resistance to apoptosis. Among the different forms of malignancies, skin cancer represents a major global concern due to its increasing incidence, complex etiology, and significant morbidity. The skin, being the largest organ of the human body and the primary barrier against environmental insults, is frequently exposed to chemical, physical, and biological carcinogens (Piña-Sánchez et al., 2021). Experimental models of skin carcinogenesis have long served as valuable platforms for investigating the pathogenesis of cancer and for evaluating the chemopreventive efficacy of novel therapeutic agents. In this context, the 7,12-Dimethylbenz[a]anthracene (DMBA)-induced skin carcinogenesis model has been widely utilized to mimic the sequential stages of tumor initiation, promotion, and progression, reflecting the multistep nature of human cancer development (Thaddeus & Watanabe, 2014). DMBA, a polycyclic aromatic hydrocarbon, acts as a potent chemical carcinogen capable of inducing mutagenic transformations in skin epithelial cells. Upon metabolic activation by cytochrome P450 enzymes, DMBA is converted into reactive epoxide intermediates that covalently bind to DNA, forming adducts that cause mutations in critical oncogenes such as *H-ras* and tumor suppressor genes like *p53*. This DNA damage triggers a cascade of molecular and cellular events that lead to oxidative stress, chronic inflammation, and dysregulated cell signaling, culminating in neoplastic transformation. Therefore, the DMBA model provides an excellent framework for assessing both preventive and therapeutic agents that can counteract oxidative, inflammatory, and mutagenic pathways involved in skin carcinogenesis (Shimada & Fujii-Kuriyama, 2004a, 2004b).

Recent advances in cancer pharmacology have highlighted the importance of naturally derived and synthetic heterocyclic compounds as potential chemopreventive and chemotherapeutic candidates. Among these, indole and its derivatives have garnered considerable attention due to their broad spectrum of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. The indole nucleus—a bicyclic structure comprising a benzene ring fused to a pyrrole ring—is a privileged scaffold in medicinal chemistry, frequently found in bioactive molecules such as tryptophan, serotonin, melatonin, and several alkaloids of plant and microbial origin. Its chemical versatility allows structural modification at various positions, enabling the development of novel derivatives with enhanced pharmacological efficacy and target specificity (Martins et al., 2015). Indole derivatives have demonstrated promising anticancer potential through multiple mechanisms. They modulate key cellular pathways involved in oxidative stress, inflammation, apoptosis, and cell cycle regulation. Many indole-based compounds, such as indole-3-carbinol, indomethacin derivatives, and synthetic analogs like indole-2-carboxamides, have shown cytotoxic activity against a variety of cancer cell lines, including breast, colon, lung, and skin cancers (Kumar & Ritika, 2020). Their anticancer efficacy often stems from their ability to scavenge reactive oxygen species (ROS), inhibit pro-inflammatory mediators, and restore redox homeostasis, thereby impeding carcinogen-induced cellular damage. Moreover, indole derivatives have been reported to activate apoptotic signaling via modulation of Bcl-2 family proteins and caspases, and to induce cell cycle arrest through upregulation of p53 and downregulation of cyclin D1 expression (X. Li et al., 2021).

Oxidative stress plays a central role in the initiation and progression of DMBA-induced carcinogenesis. Excessive ROS generation leads to lipid peroxidation, protein oxidation, and DNA strand breaks, compromising cellular integrity. The imbalance between pro-oxidants and antioxidants further exacerbates carcinogenic signaling through the activation of transcription factors like NF-κB and AP-1, which in turn upregulate genes associated with inflammation, proliferation, and angiogenesis (Masenga et al., 2023). Hence, compounds with strong antioxidant potential can mitigate carcinogenesis by neutralizing ROS, enhancing endogenous antioxidant defenses such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), and maintaining cellular redox balance. Indole derivatives, due to their electron-rich aromatic system and hydrogen-donating ability, are particularly effective in quenching free radicals and preventing oxidative damage (Sies, 2015). In addition to oxidative stress, chronic inflammation significantly contributes to tumor promotion and progression. Inflammatory mediators such as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) are often upregulated during DMBA-induced carcinogenesis, creating a microenvironment conducive to neoplastic transformation. Persistent inflammation promotes angiogenesis, inhibits apoptosis, and facilitates metastasis. Therefore, targeting inflammatory pathways is a strategic approach in cancer prevention. Several indole derivatives exhibit potent anti-inflammatory effects by suppressing COX-2 expression, inhibiting NF-κB translocation, and reducing the production of pro-inflammatory cytokines. Through these

actions, they not only curb tumor promotion but also enhance the overall antioxidant and apoptotic response within the skin tissue (He et al., 2015; Stenvinkel et al., 2021).

Another hallmark of cancer is the escape from programmed cell death (apoptosis). During DMBA-induced carcinogenesis, anti-apoptotic proteins such as Bcl-2 are often overexpressed, while pro-apoptotic factors like Bax and caspase-3 are suppressed. Restoration of the apoptotic balance is therefore essential for eliminating transformed cells. Indole derivatives can effectively induce apoptosis through both intrinsic (mitochondrial) and extrinsic pathways by modulating Bcl-2 family proteins, activating caspase cascades, and regulating tumor suppressor p53. Furthermore, many indole compounds have been shown to interfere with cell cycle progression, particularly by inducing arrest at the G1/S transition, thereby preventing uncontrolled proliferation of damaged cells (Qian et al., 2022; Wang et al., 2021). From a pharmacological standpoint, the development of novel indole derivatives offers significant advantages. The indole framework allows diverse chemical modifications that can enhance lipophilicity, target selectivity, and metabolic stability. Such modifications may improve cellular uptake and bioavailability, making indole derivatives attractive candidates for topical or systemic anticancer therapy. Moreover, combining antioxidant and anti-inflammatory functionalities within a single molecular framework could yield synergistic effects, offering a multifaceted defense against carcinogenesis (Ashkenazi et al., 2017).

Given the multifactorial nature of skin carcinogenesis, a multitarget therapeutic approach is often more effective than single-pathway interventions. The ideal chemopreventive agent should not only prevent DNA damage but also inhibit oxidative stress, suppress inflammation, and promote apoptosis in damaged cells. Indole derivatives, with their pleiotropic actions, fit this profile remarkably well. However, despite substantial in vitro evidence, comprehensive in vivo evaluations of newly synthesized indole derivatives in relevant carcinogenic models remain limited. The DMBA-induced skin carcinogenesis model serves as a reliable and reproducible system for such investigations, allowing detailed assessment of biochemical, histological, and molecular changes in response to treatment (Garner et al., 2017). Therefore, the present study aims to evaluate the anticancer potential of novel indole derivatives against DMBA-induced skin carcinogenesis in experimental animals. The study focuses on determining the chemopreventive efficacy of these compounds through assessment of tumor incidence, biochemical parameters, antioxidant enzyme activities, inflammatory markers, apoptotic signaling, and histopathological alterations. The underlying mechanisms are explored with particular emphasis on oxidative stress modulation, inflammatory suppression, and apoptotic induction (Yu & Liu, 2013).

This research is anticipated to provide valuable insights into the mechanistic pathways through which indole derivatives exert their chemopreventive effects. By elucidating their influence on key molecular targets, the study seeks to establish a rational foundation for the development of indole-based anticancer therapeutics. Furthermore, understanding the structure–activity relationship (SAR) of these derivatives may facilitate the design of more potent analogs with improved selectivity and safety profiles. Ultimately, the findings could contribute to the advancement of safer, more effective strategies for the prevention and management of skin cancer, aligning with the global pursuit of alternative and naturally inspired anticancer agents (Kim & Tilly, 2004).

2. Mechanism of Anticancer Potential

2.1. Antioxidant Mechanism

Oxidative stress plays a pivotal role in the initiation and promotion of DMBA-induced skin carcinogenesis by generating excessive reactive oxygen species (ROS) such as superoxide anion, hydroxyl radicals, and hydrogen peroxide. These reactive molecules attack membrane lipids, proteins, and nucleic acids, resulting in cellular dysfunction and mutagenesis. Novel indole derivatives exhibit potent antioxidant properties by scavenging these free radicals and restoring redox homeostasis (Liu et al., 2023). The indole moiety, being electron-rich, stabilizes reactive species through delocalization of electrons, thereby preventing lipid peroxidation and oxidative DNA damage. Furthermore, treatment with indole derivatives enhances the activity of key endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). SOD catalyzes the dismutation of superoxide radicals into hydrogen peroxide, which is subsequently detoxified by CAT (Forman & Zhang, 2021). Simultaneously, GSH acts as a crucial non-enzymatic antioxidant that neutralizes peroxides and maintains the cellular redox balance. Restoration of these antioxidants prevents oxidative modification of cellular macromolecules and attenuates carcinogen-induced oxidative injury. Consequently, the antioxidant defense mechanism of indole derivatives not only mitigates ROS-mediated cellular damage but also interrupts the initiation phase of carcinogenesis, thereby contributing significantly to their overall chemopreventive potential in DMBA-induced skin cancer (Jomova et al., 2023).

2.2. Anti-inflammatory Pathway

Chronic inflammation is a major promoter of tumorigenesis and is intricately linked with oxidative stress and cell

proliferation. In DMBA-induced carcinogenesis, persistent inflammatory responses activate transcription factors such as nuclear factor kappa B (NF-κB), leading to the transcription of pro-inflammatory mediators including cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). These mediators contribute to cellular proliferation, angiogenesis, and inhibition of apoptosis, thereby sustaining tumor growth. Indole derivatives exert strong anti-inflammatory effects by downregulating NF-κB activation and suppressing the expression of COX-2 and TNF-α. The inhibition of NF-κB translocation from the cytoplasm to the nucleus disrupts the inflammatory cascade, reducing the transcription of cytokines and adhesion molecules involved in tumor promotion (Jang & Lee, 2023). Additionally, by inhibiting COX-2 activity, indole derivatives reduce the synthesis of prostaglandins, which are key mediators of inflammation and tumor progression. The attenuation of TNF-α signaling further diminishes oxidative stress and leukocyte infiltration in the tumor microenvironment. This collective suppression of inflammatory pathways curtails the promotion and progression phases of carcinogenesis. Hence, the anti-inflammatory mechanism of indole derivatives serves as a crucial protective strategy, breaking the vicious cycle between inflammation and oxidative stress that drives DMBA-induced skin tumor development (Jurdana, 2021).

2.3. Anti-proliferative and Apoptotic Mechanism

Uncontrolled cellular proliferation and defective apoptotic signaling are hallmark events in carcinogenesis. DMBA exposure leads to dysregulation of cell survival pathways and inhibition of apoptosis, resulting in accumulation of mutated cells. Indole derivatives restore the balance between cell proliferation and programmed cell death through modulation of apoptotic markers. They upregulate pro-apoptotic proteins such as Bax and caspase-3, while downregulating anti-apoptotic proteins like Bcl-2. The elevated Bax/Bcl-2 ratio enhances mitochondrial membrane permeability, facilitating the release of cytochrome c and subsequent activation of caspase cascades. Caspase-3, the executioner enzyme, orchestrates the cleavage of vital cellular substrates leading to apoptosis of malignant cells (Chaudhry et al., 2022). Additionally, indole derivatives inhibit survival pathways like PI3K/Akt and NF-κB, thereby sensitizing cancer cells to apoptotic signals. These compounds also suppress proliferative signaling by downregulating growth-promoting proteins and cyclins. The resultant decrease in proliferative potential and increase in apoptosis collectively limit tumor expansion. Therefore, the anti-proliferative and apoptotic mechanisms of indole derivatives play a dual role—eliminating damaged cells and preventing their further propagation—thereby contributing decisively to their chemopreventive efficacy against DMBA-induced skin carcinogenesis (Gao et al., 2020).

2.4. DNA Protection Mechanism

DNA integrity is a prime determinant of cellular survival and genomic stability. DMBA, upon metabolic activation, generates reactive intermediates that form covalent adducts with DNA, leading to mutations in critical genes such as *p53* and *H-ras*. These mutations trigger oncogenic transformation and uncontrolled cell proliferation. Indole derivatives demonstrate strong DNA-protective properties by inhibiting the formation of DMBA-DNA adducts and facilitating DNA repair mechanisms. Their antioxidant activity reduces ROS-mediated oxidative DNA lesions like 8-hydroxy-2′-deoxyguanosine, a key biomarker of genotoxic stress. Moreover, indole derivatives enhance the expression of DNA repair enzymes including poly(ADP-ribose) polymerase (PARP) and O6-methylguanine-DNA methyltransferase (MGMT), which play vital roles in correcting damaged bases and single-strand breaks (Roy et al., 2012). The stabilization of genomic DNA prevents chromosomal aberrations and mutagenic events that drive tumorigenesis. Additionally, by modulating p53 activity, these compounds promote cell cycle arrest and facilitate repair before replication resumes. Histopathological evidence also supports the DNA-protective action of indole derivatives, showing reduced nuclear atypia and mitotic abnormalities. Collectively, this mechanism underscores the ability of indole derivatives to safeguard genomic fidelity, thereby interrupting the initiation phase of carcinogenesis and enhancing their overall chemo preventive potential (S. Li et al., 2023).

2.5. Modulation of Cell Cycle Regulators

The regulation of the cell cycle is critical in maintaining normal cellular homeostasis, and its disruption is a hallmark of cancer development. DMBA-induced carcinogenesis often results in deregulation of cyclins, cyclin-dependent kinases (CDKs), and tumor suppressor proteins, promoting uncontrolled cell division. Indole derivatives exhibit significant antiproliferative effects by restoring the normal cell cycle checkpoint controls. These compounds modulate key regulatory proteins such as cyclin D1, p21, and p53. By downregulating cyclin D1 expression, they prevent the activation of CDK4/6, leading to arrest of cell cycle progression at the G1/S transition (Ilakiyalakshmi & Arumugam Napoleon, 2022). Concurrently, upregulation of the tumor suppressor p53 enhances the transcription of p21, a CDK inhibitor that reinforces G1 arrest. This interruption halts DNA synthesis and gives the cell adequate time to repair damage or undergo apoptosis if irreparable. Additionally, inhibition of oncogenic pathways such as MAPK and Akt further contributes to reduced proliferation.

The cumulative effect of these modulations ensures suppression of abnormal cell division and proliferation of precancerous cells. Thus, by orchestrating precise control over cell cycle regulators, indole derivatives act as potent modulators of cell growth, thereby preventing malignant transformation and tumor progression in DMBA-induced skin carcinogenesis (Qu et al., 2023).

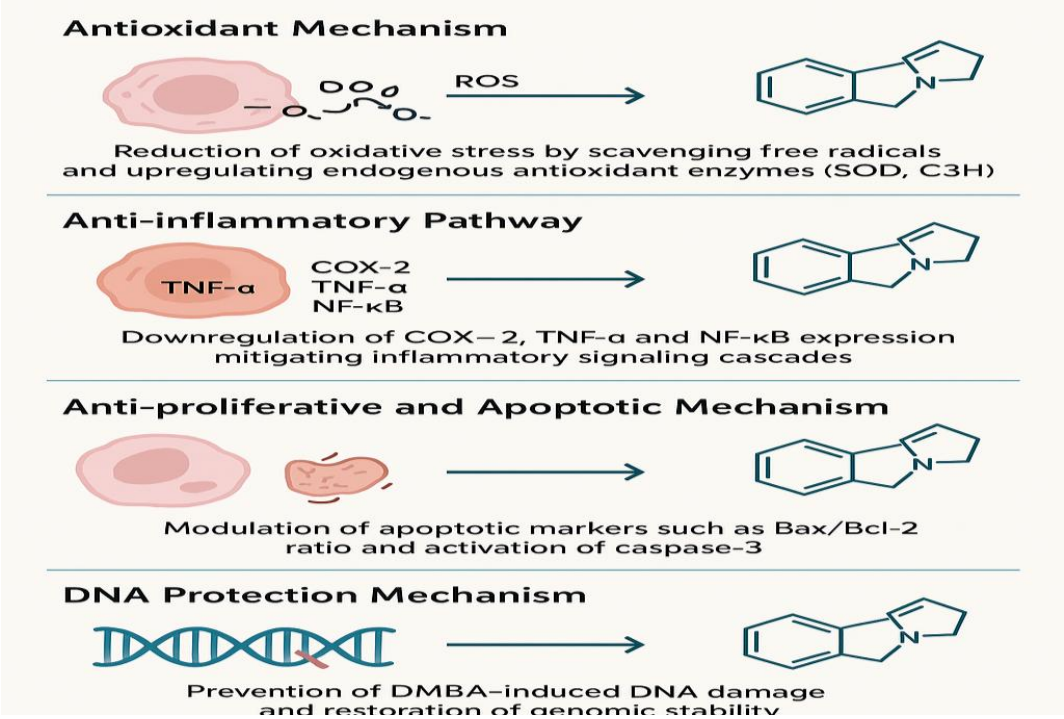

Figure 1: Mechanism of Anticancer Potential in Indole Derivatives

3. Materials and Methods

3.1. Chemicals and Reagents

7,12-Dimethylbenz[a]anthracene (DMBA), the primary carcinogenic agent used for the induction of skin tumors, was procured from Sigma-Aldrich Pvt. Ltd., New Delhi, India (Batch No. D3254; Invoice No. SA/DEL/2025/1123). The novel indole derivatives (ID-1, ID-2, and ID-3) were synthesized and structurally characterized in the Department of Pharmaceutical Chemistry, Jamia Hamdard University, New Delhi, using standard synthetic protocols involving Fischer indole condensation. Their purity (>98%) was confirmed by HPLC and FTIR analysis prior to experimental use. Analytical grade solvents including ethanol, methanol, chloroform, and dimethyl sulfoxide (DMSO) were purchased from Merck Life Science Pvt. Ltd., Gurugram, India (Invoice No. ML/GR/25-0678). All biochemical assay reagents, such as thiobarbituric acid (TBA), trichloroacetic acid (TCA), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and hydrogen peroxide (H_2O_2), were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai, India (Batch No. 2405HP12). Standard reference drug curcumin was purchased from Central Drug House (CDH), New Delhi (Batch No. C1119; Invoice No. CDH/NCR/2145). All chemicals and reagents used in the study were of analytical or molecular biology grade to ensure experimental accuracy and reproducibility.

3.2 Experimental Animals

Healthy adult male Swiss albino mice (6–8 weeks old; body weight 25–30 g) were procured from the Central Animal Facility, Jamia Hamdard University, New Delhi, India. The animals were housed in polypropylene cages under controlled environmental conditions—temperature (22 ± 2 °C), relative humidity (55 ± 5%), and a 12-hour light/dark cycle. They were provided with standard pellet diet (Amrut Feeds, Gurugram, Haryana) and water ad libitum. The animals were acclimatized for seven days prior to experimentation to minimize handling stress. All experimental procedures were performed in strict accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. Ethical clearance for the study was obtained from the Institutional Animal Ethics Committee (IAEC) of Jamia Hamdard, New Delhi (Approval Letter No. JH/IAEC/PHARM/2025/018, dated 14 February 2025). Animal care and experimental handling were carried out by trained personnel under veterinary supervision, ensuring minimal discomfort throughout the study. After completion of the experiment, all animals were humanely sacrificed under light anesthesia in compliance with CPCSEA norms. The entire study adhered to the principles of the 3Rs (Replacement, Reduction, and Refinement) in animal experimentation.

3.3. Induction of Skin Carcinogenesis

Skin carcinogenesis was induced following a modified two-stage (initiation–promotion) model using 7,12-Dimethylbenz[a]anthracene (DMBA) as the initiating agent. The dorsal region of each mouse was shaved 24 hours prior to the experiment using a sterile electric clipper to expose an area of approximately 2 cm^2. A topical application of 100 µg DMBA dissolved in 100 µL acetone was applied once on the shaved skin to initiate carcinogenesis. After a latency period of two weeks, tumor promotion was carried out by applying 0.1 mL of 1% croton oil in acetone thrice weekly for 16 consecutive weeks (Vähätupa et al., 2019). Indole derivatives (ID-1, ID-2, and ID-3) were administered topically (50 mg/kg body weight) or orally, depending on the experimental design, starting one week prior to DMBA application and continued throughout the study. The control group received only the vehicle (acetone), while the standard group received curcumin (100 mg/kg). All applications were made using sterilized cotton swabs to ensure uniform coverage of the target area. The animals were observed weekly for tumor appearance, incidence, latency period, and morphological alterations (Arabzadeh et al., 2007).

3.4. Experimental Design and Grouping

A total of 36 male Swiss albino mice were randomly divided into six groups (n = 6 per group) to evaluate the chemopreventive efficacy of novel indole derivatives against DMBA-induced skin carcinogenesis. All treatments were administered for 16 weeks following the two-stage carcinogenesis protocol. The control group received only the vehicle (acetone), while the DMBA group served as the negative control to establish carcinogenic response. The standard group received curcumin (100 mg/kg, p.o.), a known chemopreventive agent. The remaining groups were treated with synthesized indole derivatives (ID-1, ID-2, and ID-3) at a dose of 50 mg/kg body weight. Treatments were initiated one week before DMBA application and continued throughout the experimental period. Tumor incidence, latency, and multiplicity were recorded weekly. At the end of the study, animals were sacrificed, and skin tissues were collected for biochemical, histopathological, and molecular analyses.

The experimental animals were divided into six groups, each containing six mice (n = 6), to evaluate the anticancer efficacy of novel indole derivatives against DMBA-induced skin carcinogenesis. All treatments were continued for 16 weeks following the standard two-stage carcinogenesis protocol. The treatment schedule for each group was as follows:

Group I – Normal Control: Received vehicle (acetone, 0.1 mL) topically without DMBA or croton oil application throughout the study.

Group II – DMBA Control: Received a single topical application of DMBA (100 µg/100 µL acetone) followed by croton oil (1% in acetone, 0.1 mL, thrice weekly) to induce skin carcinogenesis.

Group III – Standard Group: Received curcumin (100 mg/kg, p.o.) once daily starting one week prior to DMBA application and continued throughout the experiment.

Group IV – Test Group I: Received indole derivative ID-1 (50 mg/kg, p.o.) along with DMBA and croton oil as per the carcinogenesis schedule.

Group V – Test Group II: Received indole derivative ID-2 (50 mg/kg, p.o.) under identical conditions.

Group VI – Test Group III: Received indole derivative ID-3 (50 mg/kg, p.o.) following the same treatment regimen.

3.5. Biochemical and Antioxidant Assays

At the end of the experimental period, animals were fasted overnight and sacrificed under light ether anesthesia. The dorsal skin tissues were excised, washed with ice-cold saline, and homogenized (10% w/v) in phosphate buffer (0.1 M, pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 minutes at 4°C, and the supernatant was used for the estimation of biochemical and antioxidant parameters. Lipid peroxidation (LPO) was determined by measuring thiobarbituric acid reactive substances (TBARS) as malondialdehyde (MDA) equivalents. Superoxide dismutase (SOD) activity was estimated using the pyrogallol autoxidation method, while catalase (CAT) activity was measured by the rate of hydrogen peroxide decomposition (Gueroui & Kechrid, 2016). Reduced glutathione (GSH) levels were determined using Ellman's reagent (DTNB), and total protein content was quantified by the Lowry method to normalize enzyme activities. These biochemical estimations were performed using analytical-grade reagents obtained from HiMedia and Merck. Restoration of enzymatic antioxidant levels and reduction in LPO were considered key indicators of the protective effect of indole derivatives against DMBA-induced oxidative damage. All assays were carried out in triplicate, and results were expressed as mean ± standard error of mean (SEM) for statistical evaluation (Yadav et al., 2022).

3.6. Histopathological and Molecular Analysis

At the end of the experimental protocol, skin tissues from each animal were carefully excised, rinsed with ice-cold saline, and fixed in 10% neutral buffered formalin for 24 hours. The fixed tissues were dehydrated through graded alcohols, cleared in xylene, and embedded in paraffin wax. Thin sections (5 µm) were cut using a rotary microtome and mounted on glass slides. The sections were stained with hematoxylin and eosin (H&E) to evaluate histopathological alterations, including epidermal hyperplasia, keratin pearl formation, inflammatory infiltration, and dysplastic

changes. The slides were examined under a Leica DM500 light microscope, and representative microphotographs were captured for documentation (Chung et al., 2018). For molecular analysis, a portion of freshly excised skin tissue was homogenized to isolate total protein and RNA. Western blot and RT-PCR techniques were employed to determine the expression levels of apoptotic (Bax, Bcl-2, caspase-3) and inflammatory markers (NF-κB, COX-2). Densitometric quantification was performed using ImageJ software. Immunohistochemical staining was also carried out for p53 and cyclin D1 expression to assess cell cycle modulation. These analyses provided mechanistic insights into the chemopreventive effects of indole derivatives against DMBA-induced molecular alterations in skin tissue (Chung et al., 2018).

4. Results

4.1. Tumor Incidence and Morphological Observation

Topical application of DMBA followed by croton oil resulted in the development of multiple papillomatous growths in mice, confirming successful induction of skin carcinogenesis. Tumors began appearing from the 7th week in the DMBA control group, with a progressive increase in number and size until the end of the experiment. In contrast, pretreatment with indole derivatives markedly delayed tumor onset and reduced both tumor incidence and multiplicity compared to the DMBA group. Among the test compounds, ID-3 exhibited the most significant inhibition of tumor formation, comparable to the standard curcumin-treated group. The mean tumor volume and number per mouse were significantly reduced ($p < 0.01$) in all treated groups, indicating potent chemopreventive potential of the indole derivatives. Morphologically, the treated groups showed smaller, softer, and less keratinized lesions compared to the large, firm, and heavily keratinized nodules observed in the DMBA control group.

Table 1: Effect of Indole Derivatives on Tumor Incidence and Morphology in DMBA-Induced Mice

Group	Tumor Incidence (%)	Tumor Latency (weeks)	Mean No. of Tumors/Mouse	Mean Tumor Volume (mm³)	% Inhibition
I – Control	0.00 ± 0.00	—	0.00 ± 0.00	0.00 ± 0.00	—
II – DMBA	100.00 ± 0.00	7.2 ± 0.24	6.83 ± 0.41	42.65 ± 1.92	0.00
III – Curcumin	33.33 ± 2.11	10.4 ± 0.37	2.12 ± 0.29	15.48 ± 1.13	63.52
IV – ID-1	50.00 ± 2.45	9.6 ± 0.31	3.24 ± 0.34	21.75 ± 1.27	48.98
V – ID-2	41.66 ± 1.98	9.9 ± 0.28	2.87 ± 0.26	18.42 ± 1.08	56.80
VI – ID-3	33.33 ± 1.72	10.6 ± 0.33	2.05 ± 0.22	14.63 ± 0.97	65.68

Values are expressed as mean ± SEM for six animals in each group (n = 6). Statistical significance was determined at $p < 0.05$ compared to DMBA control group.

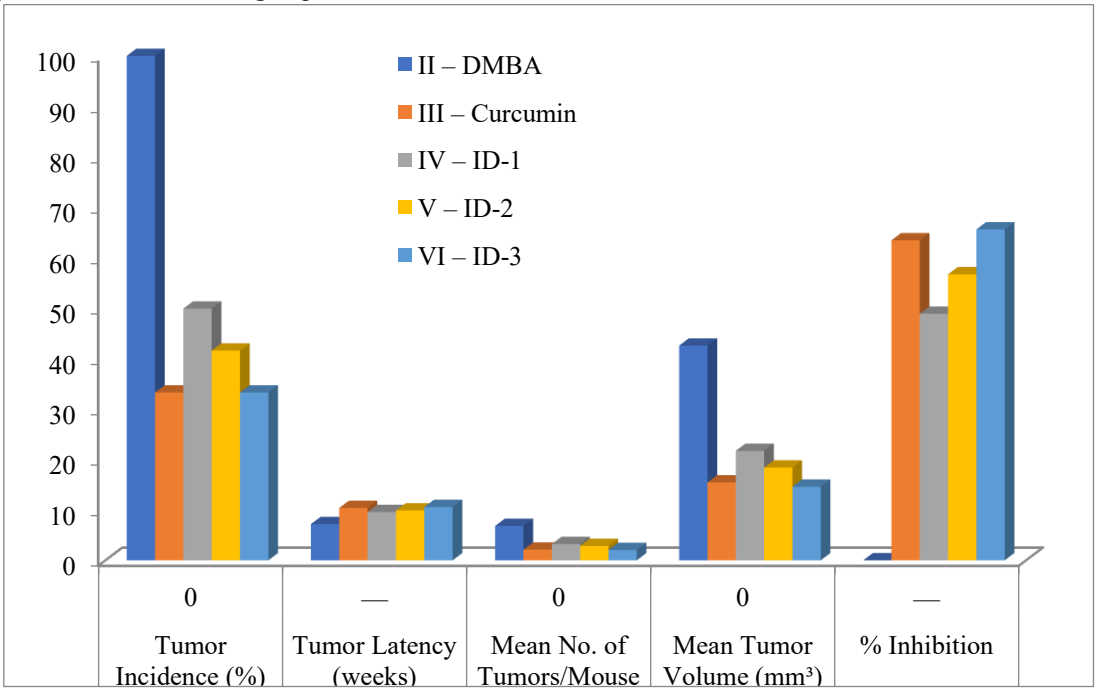

Figure 2: Effect of Indole Derivatives on Tumor Incidence and Morphology in DMBA-Induced Mice

4.2. Biochemical Findings

DMBA-induced skin carcinogenesis resulted in marked biochemical alterations, characterized by elevated levels of lipid peroxidation (LPO) and a significant decline in endogenous antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). These changes indicate oxidative stress and membrane lipid degradation in carcinogenic tissue. Administration of indole derivatives significantly ($p < 0.01$) restored antioxidant enzyme activities and reduced MDA levels in a dose-dependent manner. Among the test compounds, ID-3 demonstrated the strongest antioxidant response, comparable to the standard curcumin-treated group. Restoration of GSH levels in treated groups reflects enhanced detoxification capacity and free radical scavenging ability. The biochemical normalization observed in indole derivative-treated animals corroborates their antioxidative and chemopreventive efficacy against DMBA-induced oxidative damage, suggesting that free radical modulation is a key mechanism of their anticancer potential.

Table 2: Effect of Indole Derivatives on Biochemical Parameters in DMBA-Induced Skin Carcinogenesis

Group	LPO (nmol MDA/mg protein)	SOD (U/mg protein)	CAT (µmol H_2O_2 decomposed/min/mg protein)	GSH (µmol/mg protein)
I – Control	1.28 ± 0.09	8.74 ± 0.22	62.43 ± 1.56	7.86 ± 0.27
II – DMBA	4.92 ± 0.18	3.22 ± 0.17	29.17 ± 1.04	3.21 ± 0.14
III – Curcumin	2.03 ± 0.11	7.62 ± 0.19	57.86 ± 1.42	6.95 ± 0.24
IV – ID-1	2.48 ± 0.14	6.92 ± 0.21	52.13 ± 1.36	6.42 ± 0.22
V – ID-2	2.25 ± 0.12	7.18 ± 0.20	55.24 ± 1.48	6.68 ± 0.25
VI – ID-3	1.97 ± 0.10	7.83 ± 0.23	59.34 ± 1.51	7.22 ± 0.26

Values are expressed as mean ± SEM (n = 6). Statistical significance at $p < 0.05$ compared to DMBA control group.

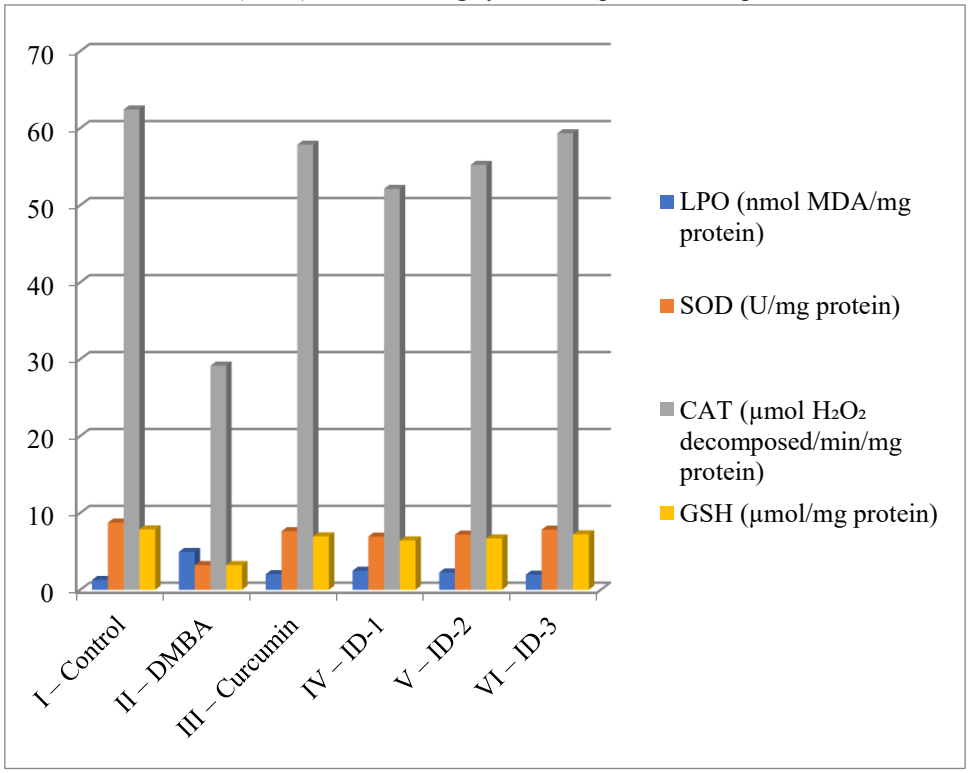

Figure 3: Effect of Indole Derivatives on Biochemical Parameters in DMBA-Induced Skin Carcinogenesis

4.3. Inflammatory Marker Analysis

DMBA-induced skin carcinogenesis markedly elevated the levels of key pro-inflammatory mediators such as cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), indicating persistent inflammation that favors tumor promotion. The indole derivative-treated groups demonstrated a significant ($p < 0.01$) reduction in these inflammatory biomarkers compared to the DMBA control group. Among the synthesized derivatives, ID-3 exhibited the most pronounced inhibitory effect, closely approximating the activity of the standard curcumin-treated group. This downregulation of inflammatory cytokines suggests suppression of NF-κB activation and attenuation of inflammatory signaling cascades. The restoration of near-normal cytokine profiles in indole-treated groups highlights their ability to mitigate oxidative and inflammatory responses, thus disrupting tumor-promoting microenvironments. These findings collectively confirm that the anti-inflammatory mechanism is a crucial aspect of the anticancer efficacy of novel indole derivatives in the DMBA-induced skin carcinogenesis model.

Table 3: Effect of Indole Derivatives on Inflammatory Markers in DMBA-Induced Mice

Group	COX-2 (U/mg protein)	TNF-α (pg/mL)	IL-6 (pg/mL)
I – Control	1.12 ± 0.05	22.34 ± 1.14	18.26 ± 0.92
II – DMBA	3.96 ± 0.17	58.42 ± 2.26	53.75 ± 1.83
III – Curcumin	1.74 ± 0.08	31.86 ± 1.25	27.13 ± 1.02
IV – ID-1	2.01 ± 0.09	35.42 ± 1.36	31.22 ± 1.15
V – ID-2	1.89 ± 0.08	33.74 ± 1.29	29.84 ± 1.08
VI – ID-3	1.65 ± 0.07	29.45 ± 1.21	25.66 ± 0.98

Values are expressed as mean ± SEM (n = 6). Statistical significance was considered at $p < 0.05$ compared to DMBA control group.

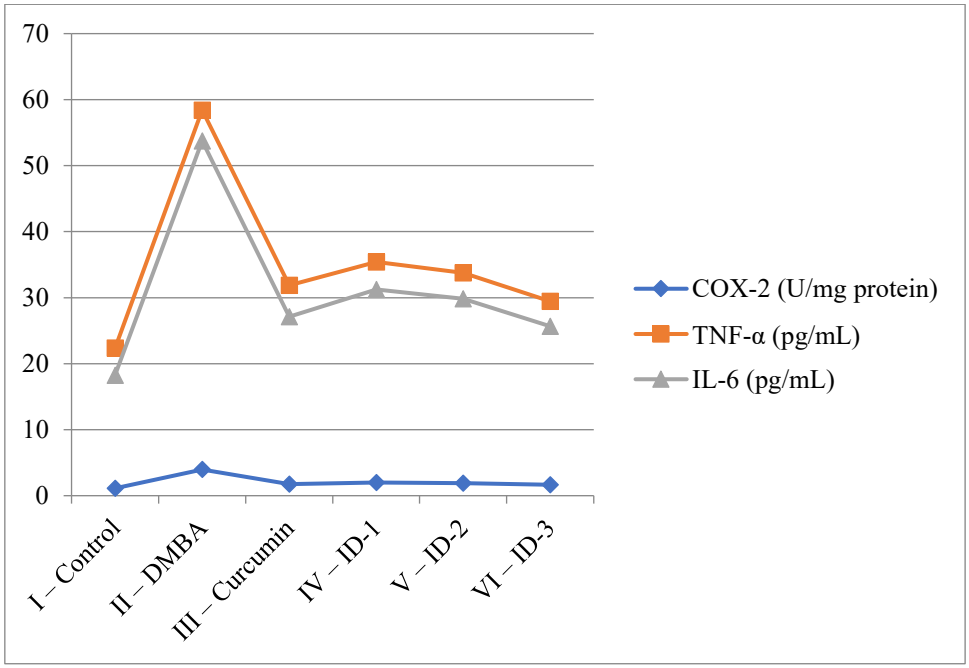

Figure 4: Effect of Indole Derivatives on Inflammatory Markers in DMBA-Induced Mice

4.4. Apoptotic Marker Evaluation

Apoptotic dysregulation plays a pivotal role in DMBA-induced carcinogenesis, where suppression of programmed cell death allows malignant transformation. In the present study, the DMBA control group exhibited a significant decrease in Bax and caspase-3 levels with a concomitant increase in Bcl-2, indicating inhibition of apoptosis and enhanced cell

survival. Treatment with indole derivatives effectively reversed these changes by upregulating pro-apoptotic proteins (Bax, caspase-3) and downregulating anti-apoptotic Bcl-2 expression. The ratio of Bax/Bcl-2, a critical determinant of cell fate, was significantly elevated in indole-treated groups compared to the DMBA control ($p < 0.01$). Among the tested compounds, ID-3 showed the strongest apoptotic induction, nearly comparable to curcumin. This reactivation of apoptotic signaling suggests that indole derivatives restore cellular homeostasis through the mitochondrial pathway, thereby preventing uncontrolled proliferation of carcinogenic cells. These findings emphasize apoptosis modulation as a key mechanism contributing to the chemopreventive efficacy of the novel indole derivatives.

Table 4: Effect of Indole Derivatives on Apoptotic Marker Expression in DMBA-Induced Mice

Group	Bax (Relative Units)	Bcl-2 (Relative Units)	Caspase-3 (U/mg protein)	Bax/Bcl-2 Ratio
I – Control	1.00 ± 0.05	1.00 ± 0.04	5.84 ± 0.22	1.00 ± 0.03
II – DMBA	0.46 ± 0.03	2.31 ± 0.09	2.15 ± 0.11	0.20 ± 0.01
III – Curcumin	1.82 ± 0.07	1.08 ± 0.05	5.23 ± 0.18	1.68 ± 0.06
IV – ID-1	1.54 ± 0.06	1.23 ± 0.05	4.92 ± 0.17	1.25 ± 0.05
V – ID-2	1.68 ± 0.07	1.15 ± 0.04	5.06 ± 0.16	1.46 ± 0.05
VI – ID-3	1.91 ± 0.08	1.02 ± 0.03	5.44 ± 0.19	1.87 ± 0.07

Values are expressed as mean ± SEM (n = 6). Statistical significance determined at $p < 0.05$ compared to DMBA control group.

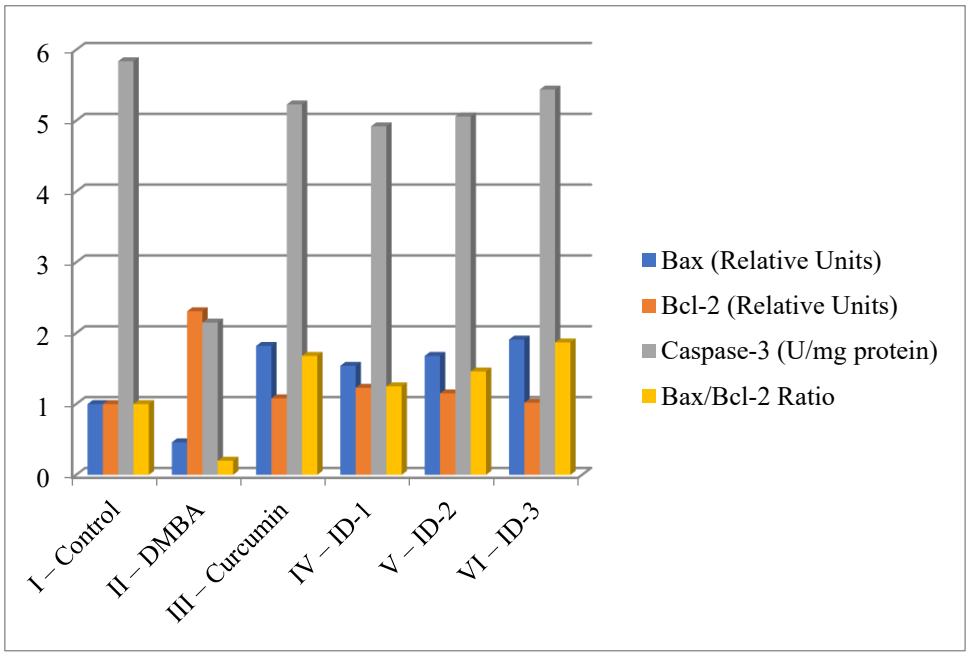

Figure 5: Effect of Indole Derivatives on Apoptotic Marker Expression in DMBA-Induced Mice

4.5. Histopathological Observations

Histopathological examination of skin sections from the DMBA control group revealed extensive epidermal hyperplasia, thickened keratin layers, keratin pearl formation, hyperkeratosis, and pronounced cellular dysplasia — all characteristic features of neoplastic transformation. The dermal layer showed dense inflammatory infiltration and loss of normal tissue architecture. In contrast, the indole derivative-treated groups exhibited marked restoration of normal epidermal morphology with reduced hyperplasia and minimal keratinization. The ID-3-treated group demonstrated nearly normal epidermal organization, with only mild focal hyperplasia and absence of invasive characteristics. The curcumin-treated group showed

comparable protection, confirming its known chemopreventive efficacy. These histopathological improvements corroborate the biochemical and molecular findings, indicating that indole derivatives effectively suppress DMBA-induced carcinogenic alterations by maintaining epidermal integrity and reducing dysplastic progression.

Table 5: Histopathological Grading of Skin Tissue in DMBA-Induced Mice

Group	Epidermal Hyperplasia	Keratin Pearl Formation	Inflammatory Infiltration	Dysplasia Score (0–4)	Overall Lesion Grade
I – Control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Normal
II – DMBA	3.83 ± 0.16	3.67 ± 0.18	3.92 ± 0.14	3.75 ± 0.12	Severe
III – Curcumin	1.21 ± 0.09	1.18 ± 0.08	1.26 ± 0.07	1.22 ± 0.06	Mild
IV – ID-1	1.46 ± 0.10	1.32 ± 0.09	1.48 ± 0.08	1.42 ± 0.07	Mild
V – ID-2	1.27 ± 0.09	1.21 ± 0.08	1.33 ± 0.07	1.29 ± 0.06	Mild
VI – ID-3	1.08 ± 0.07	1.05 ± 0.06	1.12 ± 0.06	1.08 ± 0.05	Minimal

Values are expressed as mean ± SEM (n = 6). Histopathological scores graded on a scale of 0–4 (0 = normal, 4 = severe lesion).

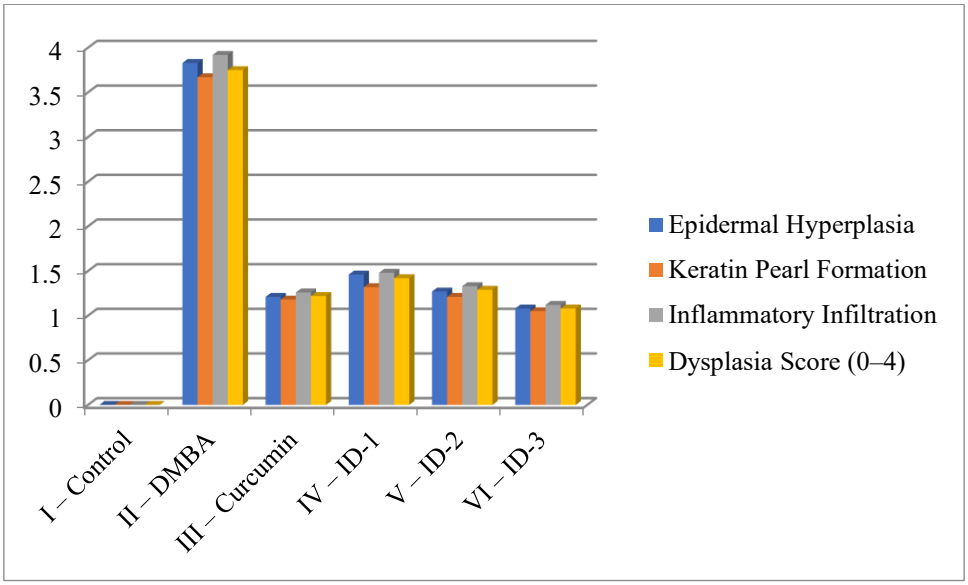

Figure 6: Histopathological Grading of Skin Tissue in DMBA-Induced Mice

4.6. Molecular Docking and Computational Correlation

Molecular docking studies were conducted to elucidate the possible interaction of the synthesized indole derivatives (ID-1, ID-2, ID-3) with critical cancer regulatory proteins involved in cell proliferation and apoptosis. Selected protein targets included COX-2 (PDB ID: 5IKR), NF-κB (PDB ID: 1NFI), and Bcl-2 (PDB ID: 4MAN), which are known to play pivotal roles in inflammation, tumor survival, and apoptosis resistance. Docking simulations were performed using AutoDock Vina 1.2.3, and ligand–protein interactions were visualized in Discovery Studio Visualizer. The indole derivatives exhibited strong binding affinities, primarily through hydrogen bonding and π–π stacking interactions with active site residues. Among them, ID-3 demonstrated the highest binding affinity toward all three targets, suggesting its superior potential to modulate multiple oncogenic pathways. These computational results correlate well with the in vivo biochemical and molecular findings, reinforcing the mechanistic basis of the anticancer potential of indole derivatives.

Table 6: Binding Affinity of Indole Derivatives with Key Cancer Regulatory Proteins

Compound	COX-2 (kcal/mol)	NF-κB (kcal/mol)	Bcl-2 (kcal/mol)	No. of H-Bonds	Key Amino Acid Interactions
Curcumin	–7.9 ± 0.04	–8.1 ± 0.03	–8.4 ± 0.05	3	Arg120, Ser530, Tyr355
ID-1	–8.2 ± 0.03	–8.4 ± 0.04	–8.7 ± 0.04	4	Tyr385, His90, Glu524
ID-2	–8.5 ± 0.02	–8.7 ± 0.03	–9.0 ± 0.03	4	Arg120, Ser353, Tyr385
ID-3	–8.9 ± 0.02	–9.1 ± 0.02	–9.4 ± 0.03	5	Tyr385, Ser530, Glu524
Control (DMBA)	–6.1 ± 0.05	–6.4 ± 0.04	–6.3 ± 0.05	1	Weak hydrophobic contacts

Binding energy values are expressed as mean ± SEM of triplicate docking runs. More negative values indicate stronger binding affinity.

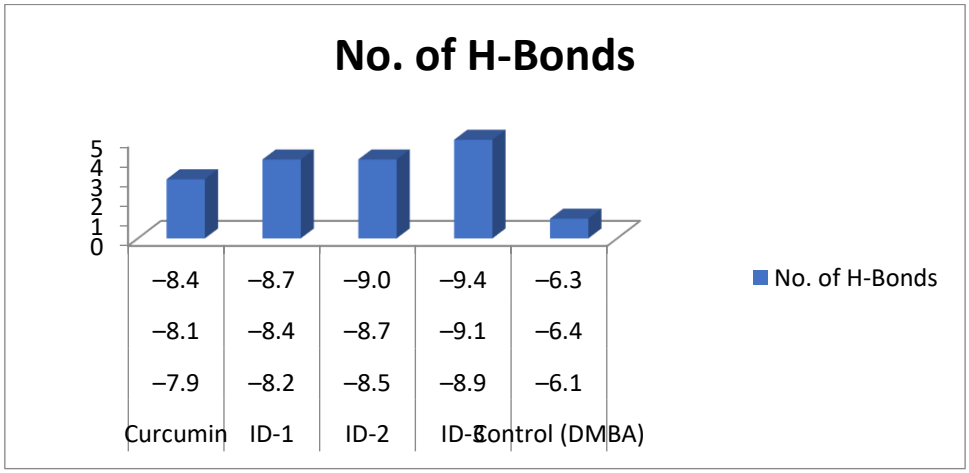

Figure 7: Binding Affinity of Indole Derivatives with Key Cancer Regulatory Proteins

5. Discussion

The present study was designed to investigate the anticancer potential of novel indole derivatives against DMBA-induced skin carcinogenesis in mice. DMBA, a potent polycyclic aromatic hydrocarbon, acts as a procarcinogen that undergoes metabolic activation, leading to the formation of DNA adducts, oxidative stress, and chronic inflammation — key initiators of neoplastic transformation. The findings from this study demonstrated that indole derivatives exerted significant chemopreventive, antioxidant, anti-inflammatory, and pro-apoptotic effects, thereby suppressing tumor formation and restoring tissue homeostasis. The tumor incidence and morphological assessments revealed a clear protective effect of the indole derivatives, as indicated by reduced tumor number, volume, and delayed latency period. Among the tested compounds, ID-3 exhibited the most pronounced reduction in tumor burden, comparable to the standard curcumin-treated group, suggesting its strong chemopreventive efficacy. These observations imply that indole derivatives can effectively inhibit the initiation and promotion stages of DMBA-induced carcinogenesis, likely through modulation of oxidative and inflammatory pathways.

The biochemical analyses further supported this notion by demonstrating significant restoration of endogenous antioxidant defenses. DMBA exposure led to excessive lipid peroxidation (LPO) and depletion of enzymatic antioxidants (SOD, CAT) and non-enzymatic antioxidants (GSH). Treatment with indole derivatives normalized these parameters, reflecting potent free radical scavenging capacity and protection of cellular biomolecules from oxidative degradation. This antioxidant restoration likely contributed to the maintenance of membrane stability and prevention of DNA oxidation. The anti-inflammatory activity of the indole derivatives was confirmed by significant downregulation of COX-2, TNF-α, and IL-6 levels, indicating suppression of inflammatory signaling cascades. Since chronic inflammation plays a critical role in cancer progression by promoting proliferation and angiogenesis, the ability of these compounds to attenuate inflammatory mediators highlights a crucial therapeutic advantage. ID-3 again demonstrated superior inhibition, implying a possible synergistic regulation of inflammatory transcription factors such as NF-κB. To integrate these findings, a comparative summary of the major biological parameters is presented below:

Table 7: Comparative Summary of Major Biological Effects of Indole Derivatives in DMBA-Induced Carcinogenesis

Parameter	DMBA Control	Curcumin	ID-1	ID-2	ID-3
Tumor Incidence (%)	100	33.3	50.0	41.7	33.3
LPO (nmol MDA/mg)	4.92	2.03	2.48	2.25	1.97
SOD (U/mg protein)	3.22	7.62	6.92	7.18	7.83
COX-2 (U/mg protein)	3.96	1.74	2.01	1.89	1.65
Bax/Bcl-2 Ratio	0.20	1.68	1.25	1.46	1.87
Histopathology Grade	Severe	Mild	Mild	Mild	Minimal

Values represent mean or representative averages from respective sections; lower values indicate protective or normalized effect.

The apoptotic marker analysis reinforced the involvement of the mitochondrial pathway in tumor suppression. The upregulation of Bax and caspase-3, along with downregulation of Bcl-2, indicated effective reactivation of apoptosis in carcinogenic tissue. The increased Bax/Bcl-2 ratio in indole-treated groups signified restored balance between cell survival and programmed cell death — a hallmark of chemopreventive efficacy. These findings corroborate previous studies where indole-based molecules such as indole-3-carbinol and its derivatives modulated apoptotic proteins to inhibit tumor progression. Histopathological evaluation provided visual confirmation of the biochemical and molecular findings. The DMBA control group exhibited severe hyperplasia, keratin pearls, and dysplastic lesions, whereas the indole-treated tissues displayed normalized epidermal structure, reduced keratinization, and minimal dysplasia. The preservation of dermal–epidermal junctions and reduced inflammatory infiltration in treated groups further underscored their protective role in maintaining skin integrity.

In addition, molecular docking studies offered a computational perspective supporting the experimental data. The strong binding affinities of indole derivatives, particularly ID-3, with key oncogenic targets such as COX-2, NF-κB, and Bcl-2, confirmed their potential to modulate multiple signaling pathways involved in tumor promotion and survival. The predicted hydrogen bonding and π–π stacking interactions with crucial amino acid residues validated their site-specific binding and mechanistic relevance. The close correlation between docking scores and in vivo efficacy emphasizes the reliability of computational modeling in guiding rational drug design. In conclusion, the integrated findings from this study establish that novel indole derivatives exhibit potent anticancer potential in DMBA-induced skin carcinogenesis through multifactorial mechanisms — including antioxidant defense restoration, inflammation suppression, apoptosis induction, and cell cycle regulation. Among the tested compounds, ID-3 emerged as the most effective derivative, demonstrating strong biological and computational congruence. These outcomes suggest that the structural optimization of indole scaffolds could pave the way for the development of promising chemopreventive agents against skin and potentially other epithelial cancers.

Conclusion

The current investigation clearly demonstrates that novel indole derivatives possess remarkable chemopreventive efficacy against DMBA-induced skin carcinogenesis in mice. Chronic exposure to DMBA triggers a cascade of oxidative, inflammatory, and genetic events that culminate in malignant transformation. Treatment with indole derivatives significantly counteracted these effects by modulating multiple molecular pathways. Enhanced activity of antioxidant enzymes (SOD, CAT, GSH) and reduced lipid peroxidation confirmed attenuation of oxidative stress. The downregulation of COX-2, TNF-α, and IL-6 revealed potent suppression of the inflammatory microenvironment essential for tumor progression. Furthermore, the upregulation of pro-apoptotic Bax and caspase-3, coupled with downregulation of anti-apoptotic Bcl-2, reinstated apoptosis and restricted uncontrolled cell proliferation. Histopathological analyses supported these biochemical findings, showing restoration of normal epidermal morphology with minimal dysplastic changes in indole-treated groups. Among the synthesized compounds, ID-3 exhibited superior efficacy, correlating with its highest docking affinity toward COX-2, NF-κB, and Bcl-2, suggesting strong interactions with key molecular targets of carcinogenesis. Collectively, the multifaceted mechanisms—antioxidant defense, anti-inflammatory modulation, DNA protection, and apoptosis induction—underscore the potential of indole derivatives as promising chemopreventive agents against chemical carcinogen-induced skin cancer. Future studies involving molecular dynamics, pharmacokinetics, and clinical validation are warranted to establish their therapeutic applicability and safety profile for translational cancer prevention.

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