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Chemopreventive Potential of Diosgenin Against 1,2-Dimethylhydrazine-Induced Colorectal Cancer in *Albino Wistar* Rats

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KEYWORDS

Colorectal cancer, Diosgenin, Chemoprevention, Oxidative stress, Aberrant crypt foci, Antioxidant, Histopathology

ABSTRACT:

Introduction: Colorectal cancer (CRC) is a leading global health concern, ranking third in incidence and second in cancer-related mortality. Limitations of conventional therapies, such as toxicity and resistance, have driven interest in naturally occurring compounds like diosgenin, a steroidal saponin known for its antioxidant, anti-inflammatory, and anticancer properties.

Objectives: The present study aimed to evaluate the chemopreventive efficacy of diosgenin against 1,2-dimethylhydrazine (DMH)-induced colorectal cancer in Albino Wistar rats. The investigation focused on diosgenin's ability to attenuate preneoplastic lesions, preserve colonic architecture, and mitigate oxidative stress through dose-dependent interventions.

Methods: Rats were divided into five groups: normal control, DMH-treated toxic control, standard treatment (Tamoxifen), and diosgenin-treated groups at low (20 mg/kg) and high doses (80 mg/kg). DMH (20 mg/kg/week) was administered subcutaneously for six weeks. Therapeutic assessment included body weight variation, gastric pH and acidity, oxidative stress markers (GSH, SOD, catalase, TBARS, and PC), aberrant crypt foci (ACF) analysis, scanning electron microscopy (SEM), and histopathological evaluation (H&E staining).

Results: DMH exposure resulted in significant weight loss, increased gastric acidity, elevated oxidative stress, and pronounced ACF formation. Diosgenin, especially at high doses, significantly improved antioxidant status, reduced ACF number, preserved mucosal integrity, and restored near-normal histological features.

Conclusions: Diosgenin exhibits strong chemopreventive potential against DMH-induced colorectal carcinogenesis by restoring antioxidant defences, reducing preneoplastic lesions, and maintaining histological and structural integrity. These findings support diosgenin's role as a promising natural agent in CRC prevention and therapy.

1. Introduction

Cancer remains a significant global health burden, with colorectal cancer (CRC) ranking among the most prevalent and deadly malignancies worldwide. According to WHO, CRC is the third most common cancer and the second leading cause of cancer-related deaths. The high incidence of CRC is attributed to lifestyle factors, dietary habits, and genetic predisposition. Sedentary behaviour, high consumption of processed foods, red meat, alcohol, and smoking are major contributors to CRC development. Additionally, genetic mutations such as APC, KRAS, and p53 alterations play a crucial role in tumor progression [1-3].

Chemotherapy and targeted therapies are commonly used for CRC treatment, but they often come with severe side effects and resistance development. Standard

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treatment regimens include fluoropyrimidines, oxaliplatin, and irinotecan, often combined with targeted therapies such as bevacizumab or cetuximab. However, these treatments can lead to complications such as gastrointestinal toxic centrality, neuropathy, and immunosuppression, highlighting the need for alternative therapeutic approaches [4-7].

Therefore, natural compounds with anticancer potential are being explored for their therapeutic benefits. Diosgenin, a bioactive steroidal saponin found in fenugreek, wild yam, and other plants, has demonstrated multiple pharmacological effects, including anti-inflammatory, antioxidant, and anticancer properties. Studies suggest that diosgenin exerts anticancer effects by modulating signalling pathways, inducing apoptosis, and inhibiting cancer cell proliferation. Diosgenin has been shown to interact with key molecular targets such as NF-κB, PI3K/Akt, and MAPK pathways, which are involved in cancer cell survival and proliferation. Furthermore, it exhibits anti-metastatic properties by inhibiting epithelial-mesenchymal transition (EMT) and reducing angiogenesis [8-10].

Recent research has also highlighted diosgenin's ability to regulate oxidative stress and modulate inflammatory responses, both of which are critical factors in CRC progression [11,12]. By enhancing antioxidant enzyme activity and reducing pro-inflammatory cytokines such as TNF-α and IL-6, diosgenin contributes to a tumor-suppressive microenvironment. Moreover, diosgenin has been reported to sensitize cancer cells to chemotherapeutic agents, suggesting its potential use in combination therapies to enhance efficacy and reduce drug resistance [10,13].

Moreover, drug repurposing strategies have gained attention in cancer research, where natural compounds like diosgenin are evaluated for their potential use in new therapeutic applications. As a well-tolerated compound with a favourable safety profile, diosgenin presents an opportunity for developing novel treatment regimens against CRC [14,15].

This study aims to investigate the chemopreventive effect of diosgenin on DMH-induced colon cancer in Albino Wistar rats by assessing oxidative stress markers, histological changes, and other relevant parameters. Through this research, we seek to provide insights into the molecular mechanisms by which diosgenin exerts its protective effects and evaluate its potential as an adjunct or alternative therapy for colorectal cancer management.

2. Objectives

The primary objective of the present study was to investigate the chemopreventive potential of diosgenin, a naturally occurring steroidal saponin, against 1,2-dimethylhydrazine (DMH)-induced CRC in Albino Wistar rats. Colorectal cancer remains a major global health challenge, with conventional therapies often hindered by adverse effects and acquired resistance. Therefore, there is a pressing need to identify effective, well-tolerated, and affordable alternative agents that can intervene in the early stages of colorectal carcinogenesis. Diosgenin, known for its antioxidant, anti-inflammatory, and anticancer properties, has emerged as a promising candidate due to its ability to modulate key signalling pathways and inhibit tumor progression.

This study aimed to evaluate the efficacy of diosgenin in preventing or mitigating CRC development by assessing multiple parameters, including ACF formation, oxidative stress biomarkers (GSH, SOD, catalase, TBARS, and protein carbonyl content), and histopathological changes in the colon. Additionally, morphological alterations were examined through SEM and H&E staining to elucidate the structural integrity of colonic tissues. By comparing diosgenin’s effects at low and high doses to those of a standard chemotherapeutic agent (Tamoxifen), the study sought to delineate dose-dependent responses and therapeutic relevance. Ultimately, the objective was to determine whether diosgenin could effectively inhibit the initiation and progression of CRC and serve as a potential adjunct or alternative to existing therapeutic regimens, thereby contributing to the development of novel strategies for colorectal cancer prevention and management.

3. Materials and methods

3.1. Drugs and chemicals

Diosgenin (CAS no. 512-04-9) was bought from Tokyo chemical industry co. ltd. 1, 2 Dimethyl hydrazine (DMH) was acquired from ACROS Organics, Thermo Fisher Logical, New Jersey, US. Any remaining synthetics were of scientific grade and acquired from Himedia Labs, Mumbai, India.

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3.2. Methodology

3.2.1. Animal Model

Wistar albino rats weighing between 100 and 120 grams were procured from the Central Animal Facility and housed under controlled environmental conditions (temperature 25 ± 1°C, 12-hour light/dark cycle), with free access to standard food and water. Following a one-week acclimatization period, the experimental procedures were conducted in accordance with CPCSEA regulations and received ethical clearance from the Institutional Animal Ethics Committee (Approval No. GIPS/IAEC/02/12/2024). The animals were randomly divided into five groups, each consisting of six rats. Group 1 served as the normal control and received subcutaneous saline (2 mL/kg/day). Group 2, the disease control, was given 1,2-dimethylhydrazine (DMH) at 20 mg/kg/week via subcutaneous injection. Group 3 received fluorouracil (5-FU) at a dosage of 20 mg/kg/per day/p.o. 21 days after DMH treatment served as the standard treatment group. In Group 4 (Diosgenin low dose), rats were first administered DMH (20 mg/kg/week, s.c.) for six weeks, followed by oral Diosgenin at 20 mg/kg/day for 21 days after DMH treatment. Group 5 (Diosgenin high dose) followed the same induction protocol but received Diosgenin at 80 mg/kg/day. On day 43, the animals were euthanized using light ether anesthesia, and the intestinal tissues were collected, thoroughly rinsed with ice-cold saline, and preserved for biochemical and oxidative stress analyses [16-18].

3.2.2. Weight Variation

Percentage change in body weight was determined using the formula: % Weight change = [(Final weight – Initial weight)/Final weight] × 100

3.2.3. Estimation of pH and total acidity

Following treatment, animals were sacrificed via cervical dislocation. Intestinal contents were extracted and analyzed for pH using a portable digital pH meter (Hanna Instrument Hey 98107)

3.2.4. Antioxidant Parameters (GSH, TBARS, SOD, PC)

3.2.4.1. GSH Levels: - GSH levels were estimated based on Sedlak and Lindsay's modified protocol. A 0.2 ml sample of 10% tissue homogenate was diluted with 1.8 ml distilled water and treated with 3.0 ml precipitating reagent. After 5 minutes, the mixture was filtered. A 2.0 ml filtrate was then reacted with 8.0 ml of 0.3 M phosphate buffer and 1.0 ml of DTNB (0.4%). The resulting yellow color was read at 412 nm. GSH (µM/mg protein) was computed using:

GSH = (310.4 × Ei × OD) / protein (mg); *Ei = 0.542* [19,20].

3.2.4.2. TBARS (Lipid Peroxidation Marker): - TBARS was assessed by incubating a mixture of tissue homogenate (1 ml), TCA (0.5 ml, 30%), and TBA (0.5 ml, 0.8%) at 80°C for 30 minutes. After cooling and centrifugation, the absorbance of the supernatant was measured at 540 nm. TBARS (nM/mg protein) = (V × OD) / (0.56 × protein concentration) [21,22].

3.2.4.3. SOD Activity: - SOD activity was evaluated by measuring inhibition of pyrogallol auto-oxidation. Cytosolic extracts (100 µl) were combined with Tris-HCl buffer to 3 ml and pyrogallol (25 µl) was added. Absorbance was measured at 420 nm for 3 min.

SOD (U/mg protein) = [100 × (A - B) / (A × 50)] / protein [23,24].

3.2.4.4. PC (Protein Carbonyl Content - Oxidative Protein Damage Marker): - Oxidative protein damage was assessed using the DNPH method. A homogenate (300 µl) was precipitated with TCA (1 ml, 10%) and incubated with DNPH. The protein pellet was washed and solubilized in guanidine hydrochloride. Absorbance was recorded at 360 nm. Results were expressed as µM/mg protein [25,26].

3.2.4.5. Catalase Activity: - Catalase activity was determined in homogenates prepared in phosphate buffer (50 mM). The reaction involved H_2O_2 (19 mM) and the rate of absorbance decline at 240 nm was tracked. Catalase Activity = (ΔA/min × total assay volume) / (0.0719 × sample volume × protein concentration) [27].

3.2.5. ACF Analysis

Excised colon tissues were longitudinally cut, fixed flat in 10% buffered formalin, and stained with 0.2% methylene blue. Under 40× magnification, ACF were identified by size, peri cryptal zone, and crypt multiplicity (1, 2, 3, or ≥4 crypts) [28,29].

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3.2.6. Morphological Evaluation (SEM)

Samples for scanning electron microscopy were fixed in glutaraldehyde (2.5%), post-fixed in osmium tetroxide (1%), and dehydrated using acetone gradients. Air-dried specimens were mounted and imaged with a JEOL JSM6490LV SEM [30,31].

3.2.7. H & E staining

Colon sections were fixed in paraformaldehyde, dehydrated, cleared in xylene, and embedded in paraffin. Thin sections (5 µm) were stained with hematoxylin and eosin and analyzed using a digital microscope (N120, BR Biochem, India) at 40× magnification [32,33].

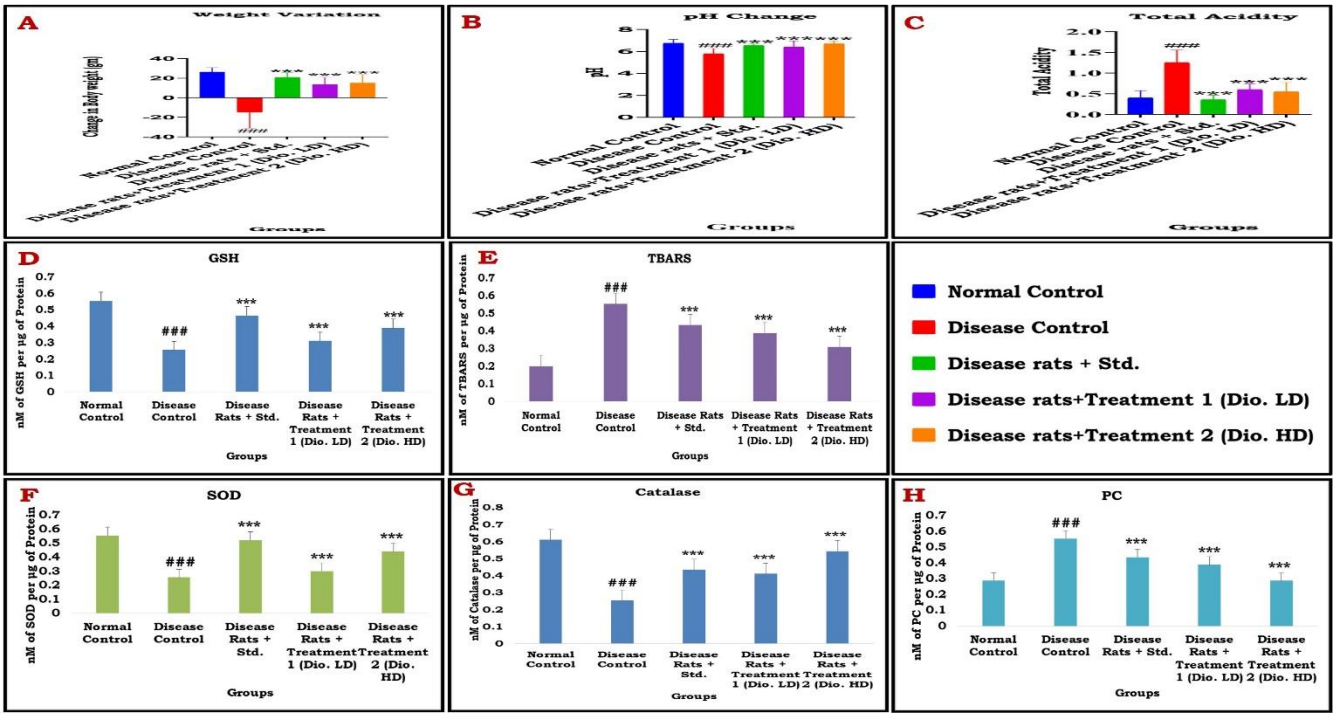
4. Results

4.1 Body Weight Changes: - The disease control group (DMH-treated) exhibited a significant decline in body weight compared to the normal control group, indicating systemic toxicity and disease progression (Fig. 1A). The standard treatment group (5-FU) showed partial restoration of body weight. Both diosgenin-treated groups (20 mg/kg and 80 mg/kg) demonstrated dose-dependent improvements, with the high-dose group (80 mg/kg) showing body weight values nearly equivalent to the normal control.

4.2 Gastric pH and Total Acidity

DMH administration significantly reduced gastric pH, indicating increased gastric acidity (Fig. 1B). This acidic shift was partially reversed in the standard treatment group. Diosgenin-treated groups also showed dose-dependent normalization of gastric pH, with the high-dose group approaching control levels. Total gastric acidity was markedly increased in the disease control group (Fig. 1C). Treatment with 5-FU significantly reduced acidity. Diosgenin administration led to a dose-dependent decrease in total acidity, with the 80 mg/kg group showing a more pronounced effect.

Figure 1. Protective Effect of Diosgenin on Body Weight, Gastric Acidity, and Oxidative Stress Biomarkers in DMH-Induced Colorectal Carcinoma in Wistar Rats. (A) Percentage change in body weight: DMH-induced (disease control) rats exhibited significant weight loss compared to the normal control group. Treatment with 5-FU and diosgenin (low and high doses) significantly improved body weight, indicating mitigation of cachexia. **(B) Gastric pH:** A significant reduction in pH was observed in the disease control group, indicating enhanced gastric acidity. Diosgenin, particularly at high dose, effectively restored pH towards normal levels. **(C) Total acidity:** Elevated acidity in the disease control group reflects gastrointestinal disruption.



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Treatment with 5-FU and diosgenin markedly reduced total acidity, with the high-dose diosgenin group showing pronounced normalization. **(D–H) Oxidative stress and antioxidant markers:** DMH significantly decreased GSH, SOD, and catalase activity, while increasing TBARS and protein carbonyl (PC) levels, indicative of oxidative damage. Both diosgenin and standard treatment restored antioxidant enzyme levels and attenuated oxidative injury. Data are expressed as mean ± SEM (n = 6), ###p < 0.001: Highly significant vs. Normal Control; ***p < 0.001: Highly significant vs. Disease Control.

4.3 Antioxidant Enzymes and Oxidative Stress Markers

DMH exposure led to significant reductions in endogenous antioxidant enzyme levels—GSH, SOD, and catalase—in the disease control group (Fig. 1D–F). Simultaneously, oxidative stress biomarkers TBARS and PC were markedly elevated (Fig. 1G, 1H). Treatment with 5-FU partially restored antioxidant enzyme levels and decreased TBARS and PC. Diosgenin-treated groups demonstrated dose-dependent restoration of antioxidant enzymes and a significant reduction in oxidative stress markers, especially at the high dose.

4.4 Aberrant Crypt Foci (ACF) Analysis

No ACF were observed in the normal control group (Fig. 2A). The DMH-treated group showed a significant increase in ACF, confirming carcinogenic induction. Diosgenin treatment (both LD and HD) and standard therapy significantly reduced ACF count, with the high-dose diosgenin group showing the greatest reduction.

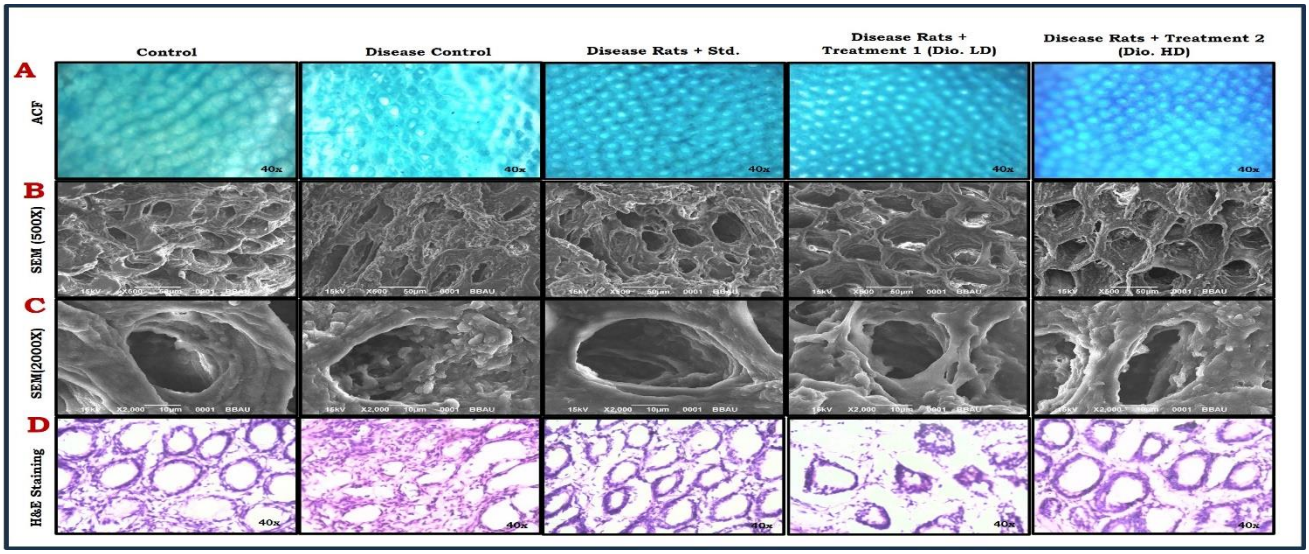
4.5 Scanning Electron Microscopy (SEM) Analysis

At 500X magnification, the control group exhibited intact crypts. In contrast, the disease control group showed severe epithelial disruption and irregularities. Diosgenin-treated groups showed improved mucosal surface, with the high-dose group displaying near-normal architecture (Fig. 2B). At 2000X magnification, the disease group revealed mucosal erosion and crypt distortion. Diosgenin treatment restored mucosal integrity in a dose-dependent manner, with the HD group showing the best-preserved architecture (Fig. 2C).

4.6 Histopathological Evaluation (H&E Staining)

The control group showed normal histology with intact crypts (Fig. 2D). The disease control group displayed hyperplasia, inflammatory infiltration, and epithelial damage. The 5-FU group showed partial histological improvement. Diosgenin-treated groups revealed dose-dependent histological restoration, with the high-dose group exhibiting nearly normal colonic structure.

Figure 2. Comparative Microscopic Evaluation of Tissue Morphology: The figure presents a multi-modal microscopic assessment of biological tissue samples. **(A)** Optical microscopy image illustrating surface topography and general cellular organization**. (B)** SEM image at 500x magnification showing the microstructural framework and porosity of the tissue. **(C)** SEM image at 2000x magnification highlighting detailed surface architecture and cellular interactions**. (D)** Histological section stained for cellular visualization, revealing tissue organization and potential pathological changes.



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5. Discussion

The results of the present study demonstrate the chemopreventive efficacy of diosgenin against DMH-induced colon carcinogenesis in Albino Wistar rats. The significant decline in body weight observed in the disease control group is a classic indicator of cancer-induced cachexia, a multifactorial syndrome marked by increased energy expenditure, metabolic disruption, and systemic inflammation. Diosgenin treatment counteracted this weight loss in a dose-dependent manner, with high-dose diosgenin restoring weight to levels comparable with the normal group, indicating strong protective effects likely tied to its anti-inflammatory and antioxidative properties [34–36].

Gastric parameters also supported the therapeutic benefits of diosgenin. DMH significantly acidified the gastric environment, reducing pH and increasing total acidity. This may reflect carcinogen-induced mucosal damage and disrupted acid regulation [11]. Both standard and diosgenin treatments reversed these effects, with high-dose diosgenin most effectively normalizing gastric pH and reducing acidity. This suggests a gastroprotective role for diosgenin, potentially involving modulation of acid secretion and preservation of gastric mucosa [36].

Oxidative stress plays a central role in colorectal cancer progression. The depletion of antioxidants (GSH, SOD, catalase) and elevation of TBARS and PC in DMH-treated rats confirm enhanced oxidative burden [11,37]. Diosgenin demonstrated potent antioxidant effects by restoring enzymatic levels and reducing oxidative damage markers in a dose-dependent manner. These actions may involve free radical scavenging, lipid peroxidation inhibition, and modulation of redox-sensitive signaling pathways such as Nrf2 [4,36].

Aberrant crypt foci, recognized as early biomarkers of colon carcinogenesis, were substantially reduced by diosgenin treatment, particularly at higher doses, underscoring its chemopreventive efficacy [35]. SEM and histopathological analyses further validated these findings. Diosgenin preserved mucosal integrity, minimized crypt distortion, and alleviated inflammation and hyperplasia, reflecting its protective action against DMH-induced morphological alterations [38].

Collectively, these findings position diosgenin as a potent natural compound with anti-inflammatory, antioxidant, and anti-carcinogenic activities. Its ability to restore physiological parameters, maintain redox balance, and preserve tissue architecture supports its therapeutic potential in colorectal cancer prevention. Further mechanistic studies and clinical investigations are warranted to establish its utility in translational oncology.

Refrences

[1] Ali A, Akhtar J, Ahmad U, Basheer AS, Jaiswal N, Jahan A. Armamentarium in drug delivery for colorectal cancer. Crit Rev Ther Drug Carrier Syst. 2022;40(1):1-48.

[2] Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-91.

[3] Xi Y, Xu P. Global colorectal cancer burden in 2020 and projections to 2040. Transl Oncol. 2021;14(10):101174.

[4] Khanal P, Patil VS, Bhandare VV, Patil PP, Patil BM, Dwivedi PSR, et al. Systems and in vitro pharmacology profiling of diosgenin against breast cancer. Front Pharmacol. 2022;13:1052849.

[5] Parveen S, Fatma M, Mir SS, Dermime S, Uddin S. JAK-STAT signaling in autoimmunity and cancer. ImmunoTargets Ther. 2025;14:523-54.

[6] Hammond WA, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol. 2016;8(1):57-84.

[7] Kumar A, Gautam V, Sandhu A, Rawat K, Sharma A, Saha L. Current and emerging therapeutic approaches for colorectal cancer: a comprehensive review. World J Gastrointest Surg. 2023;15(4):495-519.

[8] Kumar S, Praveen BM, Sudhakara A, Sherugar P, Puttaiahgowda YM. Extraction of diosgenin using different techniques from fenugreek seeds: a review. Steroids. 2025;214:109543.

[9] Semwal P, Painuli S, Abu-Izneid T, Rauf A, Sharma A, Daşan SD, et al. Diosgenin: an updated pharmaological review and therapeutic perspectives. Oxid Med Cell Longev. 2022;2022:1035441.

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[10] Sethi G, Shanmugam MK, Warrier S, Merarchi M, Arfuso F, Kumar AP, et al. Pro-apoptotic and anti-cancer properties of diosgenin: a comprehensive and critical review. Nutrients. 2018;10(5):pii:E645.

[11] Khan I, Mahfooz S, Saeed M, Ahmad I, Ansari IA. Andrographolide inhibits proliferation of colon cancer SW-480 cells via downregulating Notch signaling pathway. Anticancer Agents Med Chem. 2021;21(4):487-97.

[12] Yadav A, Kumar A, Rastogi N, Siddiqui MH. Microsatellite instability in north Indian colorectal cancer patients and its clinicopathological correlation. S Afr J Surg. 2022;60(1):22-7.

[13] Ren QL, Wang Q, Zhang XQ, Wang M, Hu H, Tang JJ, et al. Anticancer activity of diosgenin and its molecular mechanism. Chin J Integr Med. 2023;29(8):738-49.

[14] Wainwright CL, Teixeira MM, Adelson DL, Braga FC, Buenz EJ, Campana PRV, et al. Future directions for the discovery of natural product-derived immunomodulating drugs: an IUPHAR positional review. Pharmacol Res. 2022;177:106076.

[15] Al Khzem AH, Gomaa MS, Alturki MS, Tawfeeq N, Sarafroz M, Alonaizi SM, et al. Drug repurposing for cancer treatment: a comprehensive review. Int J Mol Sci. 2024;25(22):pii:E12456.

[16] Witchey SK, Al Samara L, Horman BM, Stapleton HM, Patisaul HB. Perinatal exposure to FireMaster® 550 (FM550), brominated or organophosphate flame retardants produces sex- and compound-specific effects on adult Wistar rat socioemotional behavior. Horm Behav. 2020;126:104853.

[17] Shaik SM, Shaik AH, E MP, Al Omar SY, Mohammad A, Kodidhela LD. Combined cardioprotective ability of syringic acid and resveratrol against isoproterenol-induced cardiotoxicity in rats via attenuating NF-κB and TNF-α pathways. Sci Rep. 2020;10(1):3426.

[18] Sugimoto M, Takahashi Y, Sugimura YK, Tokunaga R, Yajima M, Kato F. Active role of the central amygdala in widespread mechanical sensitization in rats with facial inflammatory pain. Pain. 2021;162(8):2273-86.

[19] Sedlák J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.

[20] Sedlak J, Lindsay RH. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968;25(1):192-205.

[21] Aguilar Diaz De Leon J, Borges CR. Evaluation of oxidative stress in biological samples using the thiobarbituric acid reactive substances assay. J Vis Exp. 2020;(159):10.3791/61323.

[22] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8.

[23] Weydert CJ, Cullen JJ. Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat Protoc. 2010;5(1):51-66.

[24] Kim SJ, Han D, Moon KD, Rhee JS. Measurement of superoxide dismutase-like activity of natural antioxidants. Biosci Biotechnol Biochem. 1995;59(5):822-6.

[25] Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357-63.

[26] Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol. 1994;233:357-63.

[27] Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-6.

[28] Roncucci L, Medline A, Bruce WR. Classification of aberrant crypt foci and microadenomas in human colon. Cancer Epidemiol Biomarkers Prev. 1991;1(1):57-60.

[29] McGinley JN, Thompson MD, Thompson HJ. A method for serial tissue processing and parallel analysis of aberrant crypt morphology, mucin depletion, and beta-catenin staining in an experimental model of colon carcinogenesis. Biol Proced Online. 2010;12(1):9032.

[30] Morini S, Braun M, Onori P, Cicalese L, Elias G, Gaudio E, et al. Morphological changes of isolated

rat pancreatic islets: a structural, ultrastructural and morphometric study. J Anat. 2006;209(3):381-92.

[31] Shehadat SA, Gorduysus MO, Hamid SSA, Abdullah NA, Samsudin AR, Ahmad A. Optimization of scanning electron microscope technique for amniotic membrane investigation: a preliminary study. Eur J Dent. 2018;12(4):574-8.

[32] Yang S, Zhang B, Zhao X, Zhang M, Zhang M, Cui L, et al. Enhanced efficacy against drug-resistant tumors enabled by redox-responsive mesoporous-silica-nanoparticle-supported lipid bilayers as targeted delivery vehicles. Int J Mol Sci. 2024;25(10):5553.

[33] Rastogi S, Ansari MN, Saeedan AS, Singh SK, Mukerjee A, Kaithwas G. Novel furan chalcone modulates PHD-2 induction to impart antineoplastic effect in mammary gland carcinoma. J Biochem Mol Toxicol. 2024;38(4):e23679.

[34] Tisdale MJ. Cachexia in cancer patients. Nat Rev Cancer. 2002;2(11):862-71.

[35] Bird RP. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett. 1995;93(1):55-71.

[36] Shishodia S, Aggarwal BB. Diosgenin inhibits osteoclastogenesis, invasion, and proliferation through the downregulation of Akt, I kappa B kinase activation and NF-kappa B-regulated gene expression. Oncogene. 2006;25(10):1463-73.

[37] Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421-31.

[38] Nolte T, Brander-Weber P, Dangler C, Deschl U, Elwell MR, Greaves P, et al. Nonproliferative and proliferative lesions of the gastrointestinal tract, pancreas and salivary glands of the rat and mouse. J Toxicol Pathol. 2016;29(1 Suppl):1S-125S.