Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2025.311

RESEARCH ARTICLE

Rosmarinus officinalis L. extract with antioxidant and anticancer effects against 1,2-Dimethylhydrazine and N-Nitroso-N-Methylurea-Induced breast and colon carcinogenesis in experimental rat models

Amira M. Alghamdi[1], and Areej S. Jalal[2*]

[1]Department of Biochemistry, Faculty of Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[2]Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia
***Corresponding author:** Asjalal@pnu.edu.sa

ARTICLE HISTORY (25-765)

Received: February 08, 2025
Revised: November 01, 2025
Accepted: November 09, 2025
Published online: December 09, 2025

Key words:
Breast cancer
Colon cancer
Gene expression
Phytochemicals
Rosmarinus officinalis

A B S T R A C T

Cancer is among the leading causes of mortality worldwide, and the treatment of breast and colorectal cancers presents significant challenges. Persistent inflammation and oxidative stress promote tumor growth, highlighting the importance of enhanced prevention and therapeutic strategies in veterinary medicine. Consequently, this research investigates the potential use of *Rosmarinus officinalis* L. extract (RE) as a biotherapeutic agent against chemically induced breast and colon cancers in rats. Carcinogens such as 1,2-dimethylhydrazine (DMH) and N-nitroso-N-methylurea (MNU) were administered to induce colon and breast tumors, respectively. Gas chromatography-mass spectrometry (GC/MS) was employed to identify active compounds within the crude aqueous ethanol extract, which demonstrated high antioxidant activity, achieving 92% DPPH scavenging. The extract also exhibited potent anticancer effects, reducing the viability of HT-29 (colon) and MCF-7 (breast) cancer cell lines by 82% and 88%, respectively. A total of 360 rats were divided into six groups: control (untreated, healthy rats), RE-only (rats receiving RE at 100 mg/kg for 12 weeks), breast cancer (BC) (induced with N-nitroso-N-methylurea [MNU], 50 mg/kg as a single dose), colon cancer (CC) (induced with 1,2-dimethylhydrazine [DMH], 20 mg/kg weekly for ten weeks), RE+BC (rats with MNU induction and RE treatment), and RE+CC (rats with DMH induction and RE treatment). Repeated in vivo experiments with DMH/MNU demonstrated that RE could mitigate precancerous phenotypes, including aberrant crypt foci (ACF) in colonic tissues and hyperplastic alveolar nodules in mammary glands. The therapy notably downregulated pro-apoptotic genes such as BCL2 and IL-1β, while upregulating Casp-3, and restored oxidative balance by normalizing levels of glucose, calcium, LDH, total antioxidant capacity (TAC), catalase (CAT), glutathione peroxidase (GPx), lipid peroxidation (LPO), and total phenolic content (TPC). Histopathological analysis revealed nearly normal tissue architecture, underscoring the protective role of RE against carcinogen-induced damage. These findings suggest that RE is a promising multi-targeted adjunct for the prevention and treatment of cancer, particularly through modulation of oxidative stress, inflammation, and precancerous lesions.

To Cite This Article: Alghamdi AM and Jalal AS, 2025. *Rosmarinus officinalis* L. extract with antioxidant and anticancer effects against 1,2-dimethylhydrazine and n-nitroso-n-methylurea-induced breast and colon carcinogenesis in experimental rat models. Pak Vet J. http://dx.doi.org/10.29261/pakvetj/2025.311

INTRODUCTION

Animal models are frequently utilized in the investigation of chemopreventive interventions in cancer research. Among the most commonly employed chemical inducers are 1,2-dimethylhydrazine (DMH) and N-nitroso-N-methylurea (MNU). DMH is a potent DNA-alkylating hydrazine compound commonly used to induce colorectal cancer in rodents. Following hepatic activation, it generates methylazoxymethanol, which produces carcinogenic metabolites, thereby inducing DNA methylation, oxidative stress, and genetic mutations. These alterations lead to the formation of aberrant crypt foci, mucosal dysplasia, and tumors analogous to human colorectal cancer (Qi *et al.,* 2015; Elsadek *et al.,* 2017; Venkatachalam *et al.,* 2020). Conversely, MNU, a water-soluble nitrosourea, is often employed to induce mammary tumors in rats, particularly in Sprague-Dawley strains. Repeated or single

intraperitoneal injections result in preneoplastic and neoplastic lesions that closely resemble human breast cancer in both histology and gene expression (Ashrafi *et al.,* 2012; Subarmaniam *et al.,* 2023).

There is a growing body of evidence suggesting that oxidative stress and chronic inflammation are pivotal factors in the development and advancement of cancer. Consequently, natural products, particularly phytochemicals derived from medicinal plants, have become the focus of intensified research to identify safe and efficacious antioxidant and anticancer agents (Al-Quwaie *et al.,* 2023; Mueed *et al.,* 2023; Ahmed *et al.,* 2025; El-Saadony *et al.,* 2025). These compounds are capable of modulating molecular pathways associated with the metabolism of carcinogens, attenuating cellular proliferation, inducing apoptosis, and inhibiting proinflammatory signaling pathways (Chunarkar-Patil *et al.,* 2024; Wang *et al.,* 2024). Several plant-derived agents, including curcumin, resveratrol, genistein, and quercetin, exhibit significant potential to target tumor suppressor genes, diminish reactive oxygen species (ROS) accumulation, and restore homeostasis within cellular redox and inflammatory networks (Chunarkar-Patil *et al.,* 2024; Mohamed *et al.,* 2025; Mohammed *et al.,* 2025; Talaat et al., 2025). The multifunctional properties of natural compounds are of particular interest in both chemopreventive strategies and as adjunctive therapies for cancer, owing to their relatively low toxicity and pleiotropic effects (Hegazy *et al.,* 2024; Mueed *et al.,* 2024; Saadany *et al.,* 2025).

Rosemary (*Rosmarinus officinalis* L) is a perennial herb that is widespread throughout the Mediterranean region and has received much interest due to the various bioactive compounds and associated pharmacological effects. Rosemary extract (RE) is a source of major phytochemicals, such as phenolic diterpenes, phenolic acids, triterpenes, flavonoids, and volatile oils. These compounds have different biological purposes, and the main ones are antioxidants, anti-inflammatory and anticancer actions (Aziz *et al.,* 2022; Alharbi *et al.,* 2024; Saadony *et al.,* 2024). Flavonoids are also involved in antioxidant defense, and it is known that ursolic acid has a pro-apoptotic effect and anti-metastatic effect on different cancer cell models (Gahtori *et al.,* 2023).

Over the past decade, comprehensive research studies have confirmed that rosemary extract exhibits broad-spectrum activity against chemically induced cancers in rodents. Its principal bioactive constituents are effective in inhibiting the proliferation of cancer cells, inducing cell cycle arrest, initiating intrinsic apoptosis, reducing oxidative DNA damage, and suppressing persistent inflammatory signals. These benefits have been observed not only in models of colon and mammary cancers but also in skin, liver, and hematologic malignancies. In addition to its chemopreventive and cytoprotective properties, rosemary extract has the potential to enhance the efficacy of existing chemotherapeutic agents, mitigate their toxicity, and improve overall treatment outcomes (Gird *et al.,* 2021; Gahtori *et al.,* 2023; Eghbalpour *et al.,* 2024; Elsayed *et al.,* 2024).

Nevertheless, despite favorable outcomes, numerous obstacles and gaps persist. The majority of available data are based on cell culture and small animal studies; however, extrapolation to larger animal models remains limited. Clarification is needed regarding dose optimization, long-term safety, pharmacokinetic properties, and the specific molecular targets of each rosemary constituent. Additionally, the potential impact of rosemary extract in conjunction with traditional chemotherapies warrants careful investigation. Challenges arise from variability in extract preparation, differences in composition, and diverse methodologies employed in studies, which complicate direct comparisons and hinder meta-analyses. The objective of this study is to critically evaluate the protective and curative properties of rosemary extract against carcinogen-induced colon and breast cancer in rat models, focusing on molecular and histopathological effects. The proposed study aims to (1) investigate the progression of pro-inflammatory, antioxidant, and apoptotic gene expression following exposure to DMH and MNU; (2) quantify the effects of rosemary extract on oxidative stress markers and endogenous antioxidant defenses; (3) assess alterations in tissue architecture and tumor development under exposure conditions; and (4) relate mechanistic insights from recent rodent in vivo studies to the translational potential of dietary-based chemoprevention.

MATERIALS AND METHODS

Collection and identification of samples: Fresh rosemary leaves were collected and dried for 7-10 days in a well-ventilated room with shade. The leaves were crushed into a fine powder and used in various investigations.

Plant material extraction and preparation: Three extracts of rosemary leaves were prepared as follows: Two hundred grams of powdered leaves were extracted with petroleum ether, chloroform, or ethanol (ADVET CHEMBIO PVT.LTD, India) for 24h while the mixture was continuously shaken (AOAC, 2012). Whitman's Filter paper (No. 1) was used to filter the extracts. The rotary evaporator [(R210, BÜCHI Labortechnik (Switzerland)] was used to concentrate the filtrate at 46°C. Finally, at 4 °C, the crude extracts were stored for further experiments (Omoregie and Folashade, 2013).

GC/MS analysis: The active compounds present in the three fractions derived from rosemary extracts were analyzed utilizing a Trace GC-1310 gas chromatograph in conjunction with an ISQ mass spectrometer from Thermo Scientific, located in Austin, TX, USA. The separation process employed a TG-5MS capillary column measuring 30 meters in length, with a diameter of 0.25 mm and a film thickness of 0.25 µm. Helium was used as the carrier gas at a consistent flow rate of 1.0 mL per minute. The oven temperature was initially set at 50°C for one minute, then increased at a rate of 5°C per minute until reaching 280°C, where it remained for a duration of 20 minutes. The injector temperature was maintained at 260°C, and an automatic injection of 1 µL of each appropriately diluted sample was performed in split mode using an AS1300 autosampler. Mass spectra were acquired in full-scan mode over the m/z range 40 to 1000, employing electron ionization at 70 eV, with the ion source temperature held constant at 200°C. Compounds were identified by comparing their mass spectra and retention times with those contained in the WILEY 09 and NIST 11 mass spectral libraries (Rao *et al.*, 2017).

Antioxidant Activity: The radical scavenging activity of rosemary extract (RE) was evaluated according to the method described by Abdel-Moneim et al. (2022). Briefly, 0.5 mL of ethanolic DPPH solution was mixed with 1.0 mL of RE and kept in the dark for 30 min. The absorbance of the reaction mixture was then measured at 517 nm using a spectrophotometer. The IC50 value, which represents the extract concentration required to destroy 50% of DPPH radicals, was determined as described by El-Saadony et al. (2022). The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$\%\ Radical\ (DPPH)\ scavenging\ activity = \frac{Abs(control) - Abs(sample)}{Abs(control)} x100$$

Cytotoxicity assay

Cell line and culture: Breast (MCF-7), and colon (HT-29) cancer cell lines were obtained from the VACSERA Tissue Culture Unit. RPMI-1640 Medium with 10% FBS (Fetal Bovine Serum) and antibiotics (gentamycin, 50 µg/ml from Lonza) was used to culture the cell lines.

MTT assay: The MTT (3-(4, 5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) assay was employed to evaluate cell viability (Mosmann, 1983). Cancer cells were seeded at a density of 5×10^4 cells per well in 96-well microtiter plates containing 100 µL of fresh culture RPMI-1640 medium. The plates were incubated at 37°C for 24 hours in a humidified atmosphere with 5% carbon dioxide to facilitate the formation of a partial monolayer. The old growth medium was discarded and replaced with a new medium containing the test compound at various concentrations of REs (20, 40, 60, 80, and 100 µg/mL). Following a 2-day incubation at 37°C with 5% CO_2, the growth medium was removed, and 10 µL of the 12 mM MTT stock solution (5 mg/mL of MTT in PBS) was added to each well. After incubation for 4 hours at 37°C, the MTT reagent was aspirated and replaced with 50 µL of DMSO, which was then mixed thoroughly using a pipette. Control wells contained only the incubation medium without the test compound.

$$\%\ viability = [(ODt/ODc) \times 100]$$

where ODt is the mean optical density of the treated sample and ODc is the optical density of the control, as measured using a microplate reader (SunRise, TECAN, Inc., USA). The positive control for this experiment is the standard anticancer drug, vinblastine.

Animal behavior and design methodology: All procedures adhered strictly to internationally recognized principles and institutional standards for the care and ethical use of laboratory animals. A total of 360 male albino rats of the Sprague-Dawley strain (Serum and Vaccine Centre, Giza, Egypt) were utilized for this experiment. Each rat had an average weight of 150 grams, ranging from 140 to 160 grams. Prior to the commencement of the experiment, all rats were maintained under uniform housing conditions for a period of 7 days, with ambient temperatures maintained between 22°C and 24°C and a 12-hour light/dark cycle. All animal experiments were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Zagazig University and were approved under protocol ZU-IACUC/3/F/457/2025.

Rats were allocated into six experimental groups as follows:

1. Control group: Healthy, untreated rats.
2. RE-group: Rats administered rosemary extract (RE) at a dose of 100 mg/kg for 12 weeks.
3. BC group: Breast cancer-induced rats using N-nitroso-N-methylurea at 50 mg/kg (single intraperitoneal dose).
4. CC group: Colon cancer-induced rats using 1,2-dimethylhydrazine (DMH) at 20 mg/kg per week for 10 weeks.
5. RE+BC group: MNU-induced breast cancer rats treated with RE (100 mg/kg).
6. RE+CC group: DMH-induced colon cancer rats treated with RE (100 mg/kg).

Gene expression of proinflammatory and precancerous genes: Rat livers were utilized for the extraction of RNA. Following extraction, the RNA was dissolved in diethyl pyrocarbonate (DEPC)-treated water. Spectrophotometric analysis was conducted to determine RNA concentration, maintaining an optical density (OD) ratio at 260/280 (Saif and Khan, 2022). A quantity of 3 µg of RNA was employed for semi-quantitative reverse transcription PCR. The PCR thermocycler (Bio-Rad T100TM) was set to 70 °C for 5 minutes to facilitate the denaturation of the plates.

In this process, 0.5 ng of oligo dT primers was used. A mixture of 2 µL of 10X RT buffer, 1 µL of 100 M reverse transcriptase, and 2 µL of 10 mM dNTP was prepared for cDNA synthesis. The incubation was carried out at 42 ºC for one hour, followed by heating to 70 ºC for 10 minutes to deactivate the enzymes. Densitometry was employed to quantify mRNA expression levels, with β-actin mRNA serving as the standard. The $2^{-\Delta CT}$ method was utilized to measure gene expression via real-time PCR. The actin (reference endogenous) gene was used to normalize the expression levels of the genes analyzed in this experiment.

Colon and mammary gland histopathology: The Colon and mammary gland were preserved using 10% neutral buffered formalin immediately after removal from the animals. The fixed tissues were thereafter subjected to known histological tests (Chen *et al.*, 2022).

Oxidative stress markers: Following the experiment, all the rats were anesthetized using an R550 Multi-Output Laboratory Small Animal Anesthesia Machine, which was designed to anesthetize 1-5 small animals simultaneously, such as rats, mice, cats, and rabbits. This machine allows independent adjustment of each anesthesia channel and consequently the regulation of the induction box gas flow (0-2.0 L/min). The liver was excised, and the tissues were rinsed with chilled 0.9% saline solution by weight or volume. Subsequently, the tissues were measured and stored at -70°C. The concentrations of malondialdehyde (MDA) and the enzymatic activities of superoxide dismutases (SOD), glutathione (GSH), and catalase (CAT) were determined following the procedures outlined in reference (Alatawi *et al.,* 2018). Additionally, the total antioxidant capacity (TAC) was evaluated (Pappas *et al.,* 2021).

Statistical analysis: The data were analyzed using SPSS version 17.0. Results are shown as mean ± SE. After performing an ANOVA, comparisons were made with Duncan's multiple-range test. A significance level of $P \leq 0.05$ was set.

RESULTS

Composition of Rosemary Extract: Table 1 provides a summary of the concentrations of active compounds in rosemary extracts prepared using ethanol, ether, and chloroform. The data reveal significant differences in both the concentration and diversity of bioactives across these extracts. The ethanolic extract contains notably higher ($p<0.05$) levels of phenolic acids (e.g., rosmarinic acid: 45.2±2.1 mg/g; carnosic acid: 62.4±3.5 mg/g), as well as certain flavonoids and triterpenoids such as ursolic acid. Most bioactive groups are consistently more abundant in this extract, indicating that alcohol efficiently extracts polar and moderately polar substances. Conversely, nonpolar extracts (ether) demonstrate higher levels of volatile monoterpenoids such as 1,8-cineole (35.4±2.7 mg/g) and sesquiterpenes like β-caryophyllene (11.2±1.0 mg/g), attributed to ether's affinity for polar volatile constituents. Additionally, chloroform extracts present an intermediate profile, which is particularly suited for extracting some moderately polar diterpenes and triterpenes, including methyl carnosate and betulinic acid, reflecting the balanced polarity of chloroform.

Biological activities of extracts of rosemary: Table 2 contrasts the antioxidant activity of *Rosmarinus officinalis* (rosemary) extracts utilizing three solvents—ethanol, ether, and chloroform—at various concentrations based on DPPH radical scavenging. The highest antioxidant activity was observed in the ethanolic extract across all concentrations, reaching a peak of 92.3% at 100 mg/mL. It possesses an IC50 value of 25±1.4 mg/mL, which is significantly lower than those of the ether (41.63±1.9 mg/mL) and chloroform (55.92±2.2 mg/mL) extracts, thereby indicating greater potency. The extract demonstrated moderate activity, whereas the chloroform extract exhibited the least activity yet remained noteworthy. These findings underscore ethanol as an effective solvent for extracting potent antioxidant compounds from rosemary, likely attributable to its capacity to extract both polar and moderately nonpolar phytochemicals.

Table 3 demonstrates that all three rosemary extracts exhibit dose-dependent anticancer activity against both MCF-7 (breast adenocarcinoma) and HT-29 (colon carcinoma) cell lines. The ethanolic extract displayed the highest efficacy, followed by the chloroform and ether extracts. Specifically, the ethanolic extract achieved significant inhibition, approximately 85-88%, at a concentration of 100 µg/mL, compared to 74-79% for the ether extract and 80-84% for the chloroform extract. These findings are consistent with the elevated levels of phenolic acids (such as rosmarinic and carnosic acids) and flavonoids (including apigenin and luteolin) present in the ethanolic extract, which are known to induce apoptosis via the generation of reactive oxygen species (ROS) and the activation of *caspase*-3.

Fig 1 demonstrates the cytotoxic effects of various rosemary extracts (ethanolic, ether, and chloroform) in comparison to doxorubicin on two cancer cell lines, HT-29 (colorectal) and MCF-7 (breast). Control images for both cell lines show a high population of healthy, adherent cancer cells (Fig 1A). When cell density is modified and morphological alterations are observed following treatment with rosemary extracts at 100 mg/mL, it becomes evident that both ethanolic and ether extracts significantly impact cell density (Fig 1 B, C D).

Table 1: Active Compounds in Rosemary Extracts (GC/MS Analysis)

Compound	Rosemary extract (mg/g)			Primary biological activities
	Ethanolic extract	Ether extract	Chloroform extract	
			Phenolic Acids & Diterpenes	
Rosmarinic Acid	45.2±2.1a	12.5±1.3c	18.7±1.8b	Antioxidant, anti-inflammatory, anticancer
Carnosic Acid	62.4±3.5a	28.6±2.4c	35.2±2.9b	Neuroprotective, anticancer, antimicrobial
Carnosol	15.8±1.2a	8.3±0.9b	12.6±1.1ab	Anticancer, anti-angiogenic
Methyl Carnosate	4.2±0.4a	2.1±0.2c	3.0±0.3b	Antioxidant, hepatoprotective
			Triterpenoids	
Ursolic Acid	18.6±1.5a	9.4±0.8c	14.3±1.2b	Anticancer, anti-diabetic
Oleanolic Acid	5.1±0.4a	2.8±0.3b	4.0±0.4ab	Anti-inflammatory, hepatoprotective
Betulinic Acid	3.2±0.3a	1.5±0.2c	2.4±0.3b	Anticancer (melanoma, glioblastoma)
			Monoterpenes	
α-Pinene	5.3±0.4c	9.1±0.8a	6.8±0.6b	Antimicrobial, anti-inflammatory
β-Pinene	3.7±0.3c	6.5±0.5a	4.2±0.4b	Anticancer, bronchodilator
Myrcene	2.5±0.2c	4.8±0.4a	3.1±0.3b	Sedative, analgesic
Limonene	4.8±0.4c	8.3±0.7a	5.9±0.5b	Anticancer (breast, colon)
			Monoterpenoids	
1,8-Cineole	22.6±1.8b	35.4±2.7a	18.9±1.5c	Expectorant, anti-proliferative
Camphor	8.5±0.7c	14.2±1.2a	10.3±0.9b	Antipruritic, anticancer
Linalool	4.2±0.3c	7.8±0.6a	5.1±0.4b	Anxiolytic, anticancer
Borneol	3.6±0.3c	6.2±0.5a	4.5±0.4b	Anti-inflammatory, neuroprotective
Bornyl Acetate	2.1±0.2c	4.7±0.4a	3.0±0.3b	Antispasmodic, anti-angiogenic
Verbenone	1.5±0.1c	3.2±0.3a	2.0±0.2b	Anticancer (lung, prostate)
			Flavonoids	
Apigenin	7.3±0.6a	2.1±0.2c	4.5±0.4b	Anticancer, anti-anxiety
Luteolin	5.8±0.5a	1.7±0.2c	3.6±0.3b	Anti-inflammatory, anticancer
Genkwanin	1.2±0.1a	0.6±0.1c	0.9±0.1b	Antimicrobial, anti-proliferative
			Sesquiterpenes	
β-Caryophyllene	6.4±0.5c	11.2±1.0a	8.3±0.7b	Anti-inflammatory (CB2 agonist)
α-Humulene	3.1±0.3c	5.6±0.5a	4.0±0.4b	Anticancer, anti-allergic
			Other Compounds	
Salvigenin	0.8±0.1a	0.3±0.05c	0.6±0.1b	Antioxidant, vasorelaxant
Cirsimaritin	0.9±0.1a	0.4±0.05c	0.7±0.1b	Antimicrobial, anti-diabetic

n=3; data are presented as mean±SE.

Table 2: Antioxidant Activity of *Rosmarinus officinalis* extracts Against DPPH Radicals

Concentration (µg/mL)	DPPH Inhibition (%) (Mean±SE)		
	Ethanolic extract	Ether extract	Chlorform extract
20	41.33±2.1	39.22±1.1	31.3±1.9
40	59.65±2.5	52.33±1.5	49.31±1.4
60	66.2±1.8	61.51±1.3	59.35±1.0
80	84.5±2.3	81.33±1.8	76.58±1.1
100	92±3.2	88.36±2.1	80.11±1.2
IC50 (µg/mL)	25±1.4	41.63±1.9	55.92±2.2

n=3; data are presented as mean±SE. Different lowercase letters in the same column indicate significant variation at P<0.05.

Table 3: Anticancer Activity of Rosemary Extracts Against MCF-7 and HT-29 Cell Lines

Extract	Concentration (µg/mL)	MCF-7 (Breast Cancer) % Inhibition	HT-29 (Colon Cancer) % Inhibition
Ethanolic	20	18.5±2.1	22.3±1.8
	40	35.2±3.0	41.6±2.7
	60	54.7±4.2	58.9±3.5
	80	72.4±5.1	76.3±4.8
	100	85.6±6.3	88.2±5.9
Ether	20	15.2±1.8	19.7±1.6
	40	32.6±2.8	38.4±2.5
	60	49.8±3.9	55.2±3.7
	80	67.3±5.0	70.8±4.9
	100	80.1±6.0	83.5±5.8
Chloroform	20	12.4±1.5	15.8±1.3
	40	28.9±2.4	34.2±2.1
	60	45.3±3.6	50.1±3.2
	80	61.7±4.8	65.4±4.5
	100	74.2±5.7	78.6±5.3

(SE = Standard error; MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay).

These fractions induce significant cellular rounding, detachment, and clear evidence of cell death, which cannot be distinguished from the effects observed with the globally potent chemotherapeutic agent, doxorubicin, administered at the same dosage (Fig. 1E). The chloroform extract also demonstrates a cytotoxic effect, although to a considerably lesser extent ($p<0.05$), resulting in a higher number of viable cells remaining post-treatment compared to other treatments. This phenomenon is observed in both the MCF-7 and HT-29 cell lines and underscores the potent anticancer properties of the ethanolic and ether-soluble phytochemicals present in rosemary.

Gene expression regulation: Figure 2 demonstrates that the mRNA fold-changes (mean ± SE) of the primary markers associated with apoptosis, oxidative stress, and inflammation exhibit variation across six experimental conditions. Treatment with rosemary extract results in a reduction of BCL-2 levels by 1.1-2.1-fold in T4 (corresponding to an 88% reduction), 1.8-2.1-fold in T5 (approximately 80% reduction), and 1.8-2.1-fold in T6 (approximately 79% reduction). In Figure 2B, Nrf-2 levels increase by 7.5- and 8.2-fold in T2 and T3, respectively, and are subsequently reduced to 1.15-, 1.45-, and 1.85-fold in T4, T5, and T6, corresponding to reductions of 85%, 82%, and 78%. In Fig. 3C, the OH marker (OH) elevates to 8.2- and 9.1-fold in cancerous cells; following treatment with OH, levels decrease to 1.2- (T4), 1.9- (T5), and 2.0-fold (T6). Figs. 2D and E illustrate that pro-inflammatory cytokines IL-1β and TNF-α, which increase approximately 8- and 9-fold in cancer models, are reduced to near baseline levels (1.1-2.2-fold) upon extract treatment, indicating the extract's anti-inflammatory properties. Lastly, pro-apoptotic genes BAX and Caspase-3, initially elevated 3- to 2-fold, are maintained at moderate levels (1.1-1.9-fold) following treatment in Figs. 3F and G, supporting effective apoptosis without excessive basal activation. In summary, rosemary extract in breast cancer treatment (T4) achieves the highest normalization, reducing levels by approximately 85-90% in comparison to T2, T3, T5, and T6 treatments.

Oxidative stress modulation: Table 4 comprehensively illustrates the considerable modulatory effect of rosemary extract on oxidative stress and antioxidant defenses in rat models of breast and colon cancer. In comparison to the cancer-induced groups (T2 and T3), treatment with rosemary extract (T4) yielded significant improvements across all evaluated markers of oxidative stress and antioxidant status. Statistically, lactate dehydrogenase (LDH)—a marker indicative of tissue damage—was significantly reduced ($p<0.05$) by 55.4% relative to T2 and by 51.9% relative to T3, signifying a meaningful reduction in cellular injury. The total antioxidant capacity (TAC) increased twofold in comparison to T2 (a 100% increase) and by 77.8% relative to T3, reflecting a restoration of the overall antioxidative capacity. Furthermore, key antioxidant enzymes were markedly upregulated: catalase (CAT) levels increased by 116.7% over T2 and 85.7% over T3, while glutathione peroxidase (GPx) levels were elevated by 110% and 90.9%, respectively, in these comparisons.

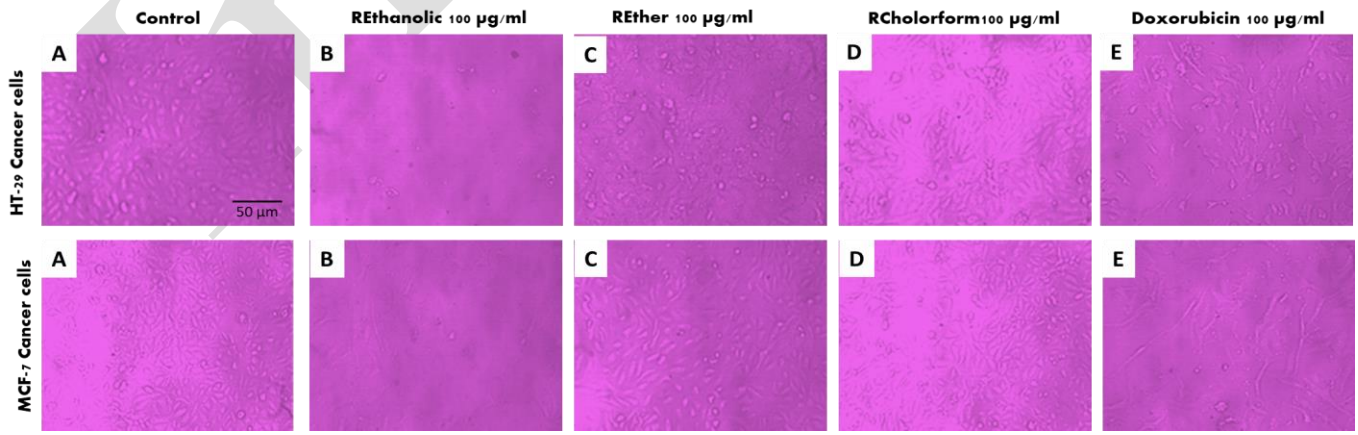

Fig 1: Microscopic images demonstrating the inhibitory effects of rosemary extract on the viability of cancer cell lines: HT-29 colon cancer and MCF-7 breast cancer cells. Images show cells treated with different rosemary extracts and a chemotherapeutic control: (A) Untreated control cells, (B) Cells treated with ethanolic rosemary extract, (C) Cells treated with ether rosemary extract, (D) Cells treated with chloroform rosemary extract, (E) Cells treated with Doxorubicin (positive control). Images were captured at 400× magnification with a scale bar of 50 µm.

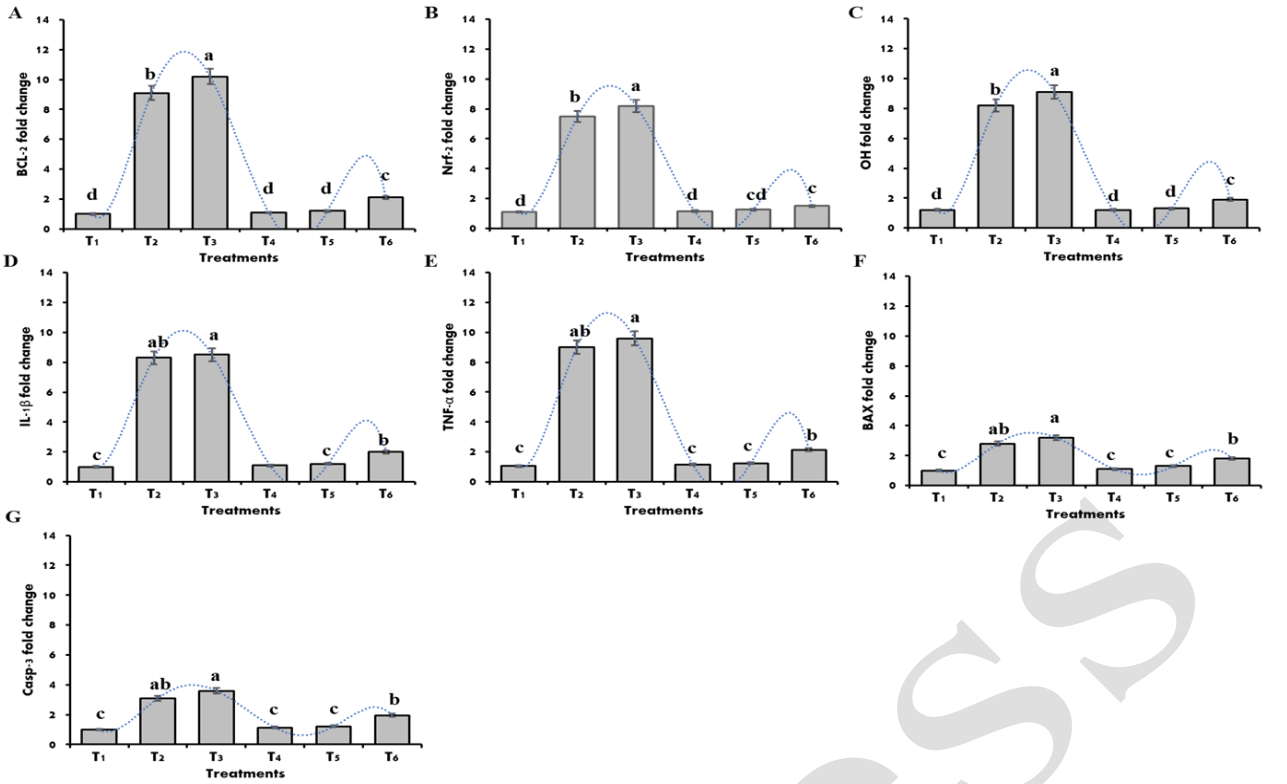

Fig 2: The effects of dietary rosemary extract (RE) on proinflammatory cytokines and cancer-related gene expression are presented as mean±SE for the following markers: (A) BCL-2, (B) Nrf-2, (C) OH, (D) IL-1β, (E) TNF-α, (F) BAX, and (G) Caspase-3. Experimental groups include: T1, control rats; T2, breast cancer (BC) rats induced by MNU; T3, colon cancer (CC) rats induced by DMH; T4, rats treated with RE only; T5, RE-treated BC rats; and T6, RE-treated CC rats.

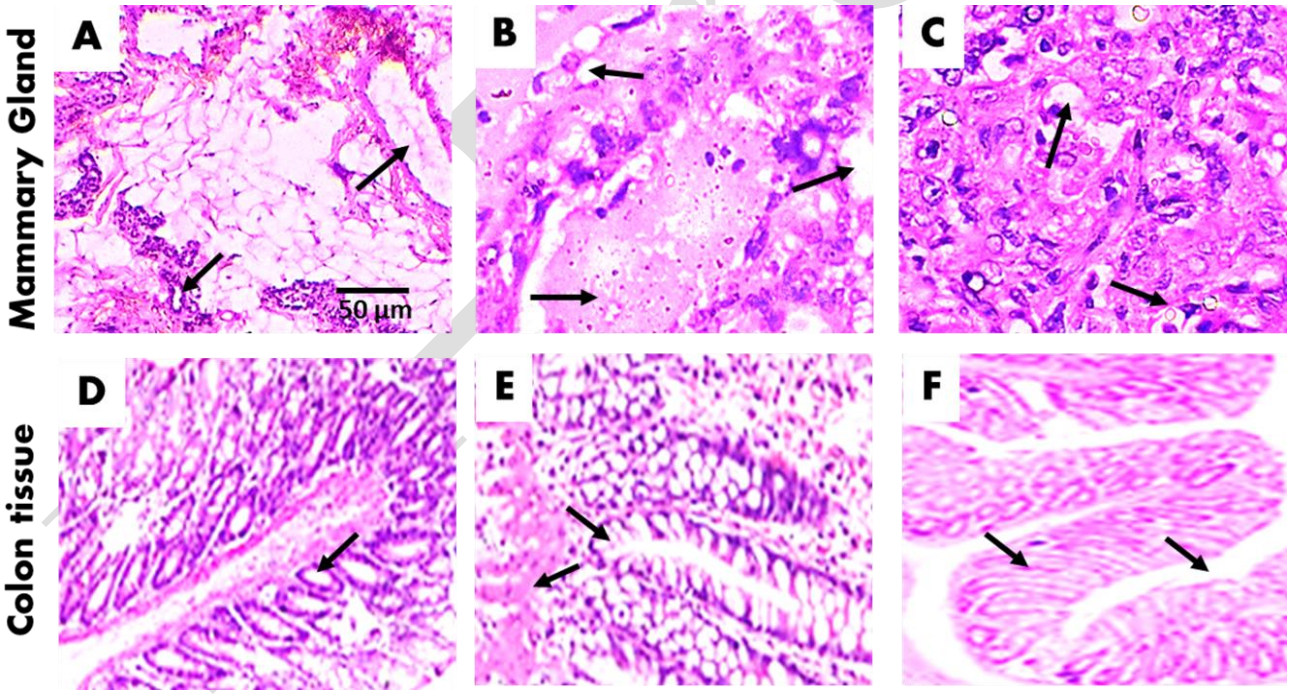

Fig 3: Hematoxylin & Eosin–Stained Micrographs of Rat Mammary and Colon Tissues Demonstrating Carcinogen-Induced Preneoplastic Lesions and theirenuation by Rosemary Extract Panels (100x) A–C: Mammary gland sections. (A) Normal alveolar architecture in untreated control; (B) Hyperplastic alveolar nodules following N-nitroso-N-methylurea (MNU) exposure; (C) Restoration of alveolar morphology and reduction of epithelial hyperplasia after concurrent rosemary extract treatment. Panels D–F: Colon tissue sections. (D) Uniform colonic crypts in untreated control; (E) Aberrant crypt foci (ACF) induced by 1,2-dimethylhydrazine (DMH) characterized by enlarged, dysplastic crypts; (F) Significant reduction in ACF number and severity with preservation of goblet cell population following rosemary extract administration.

Meanwhile, rosemary treatment decreased lipid peroxidation (LPO), a marker of oxidative membrane damage, by 67.7% compared to T2 and 63.8% compared to T3. Total phenolic content (TPC), which indicates antioxidant phytochemicals, also doubled (increased by 100%) relative to T2 and rose by 77.8% compared to T3. Overall, these results demonstrate that rosemary extract not only reduces the oxidative and cellular stress commonly

seen in cancer initiation but also effectively restores antioxidant defenses in both breast and colon cancer models. The magnitude of these improvements, often exceeding 75%, highlights rosemary's strong chemopreventive properties and supports previous findings of its cytoprotective and antioxidative effects.

Table 4: Effects of Rosemary Extract (RE) on Oxidative Stress and Antioxidant Markers

Parameter	T1	T2	T3	T4	T5	T6
LDH (U/L)	120±10	280±20*	260±18*	125±12	160±15#	150±14#
TAC (mmol/L)	1.5±0.2	0.8±0.1*	0.9±0.1*	1.6±0.2	1.3±0.1#	1.4±0.1#
CAT (U/mg protein)	25±3	12±2*	14±1*	26±3	20±2#	22±2#
GPx (U/mg protein)	20±2	10±1*	11±1*	21±2	18±1#	19±1#
LPO (nmol MDA/mg protein)	2.0±0.3	6.5±0.5*	5.8±0.4*	2.1±0.2	3.2±0.3#	3.0±0.2#
TPC (mg GAE/g)	15±1	8±0.5*	9±0.6*	16±1	14±1#	15±1#

LDH: Lactate dehydrogenase (marker of tissue damage), TAC: Total antioxidant capacity, CAT: Catalase, GPx: Glutathione peroxidase, LPO: Lipid peroxidation (MDA=malondialdehyde), TPC: Total phenolic content (GAE = gallic acid equivalents), *: Significant increase/decrease vs. control ($P<0.05$). #: Significant improvement vs. cancer groups ($P<0.05$). T1, control rats; T2, breast cancer (BC) rats induced by MNU; T3, colon cancer (CC) rats induced by DMH; T4, rats treated with RE only; T5, RE-treated BC rats; and T6, RE-treated CC rats.

DISCUSSION

Plant-derived natural compounds have garnered significant interest for their safety and efficacy as antioxidant and anticancer agents. Compounds such as phenolics, flavonoids, terpenoids, and alkaloids are free radical scavengers with substantial potential to prevent oxidative cellular damage—a key contributor to cancer development. These compounds are particularly suitable due to their favorable safety profile and multifaceted mechanisms of action when used as adjuncts to traditional therapies. They can be incorporated into the food industry, cosmetics, and nutraceutical products as natural antioxidants to prevent oxidative damage and promote healthful living (Abdelbaky *et al.,* 2025; Dai *et al.,* 2025). Natural compounds play a role in modulating inflammatory processes, apoptosis, cell proliferation, and metastasis by inhibiting inflammation, inducing apoptosis, and preventing metastasis, which has been demonstrated to be advantageous in cancer prevention (El-Sayed *et al.,* 2024; Dai *et al.,* 2025). The widespread applicability of these compounds underscores their potential in medical and commercial domains as convenient, low-toxicity therapeutic and preventive agents. Ongoing research aims to facilitate their integration into clinical guidelines and product formulations to enhance their utility.

Rosemary extract contains bioactive compounds, among which carnosic acid and rosmarinic acid are recognized for their potent antioxidant and anticancer properties. Recent studies demonstrate that these polyphenols inhibit cancer cell proliferation, enhance apoptosis, and increase the sensitivity of tumor cells to chemotherapeutic agents such as 5-fluorouracil. Moreover, standardized rosemary extract, containing these acids at defined levels, has been shown to reduce viability in colon, breast, and pancreatic cancer cell lines at relatively low concentrations. It is important to emphasize that the efficacy of the whole extract generally surpasses that of its isolated constituents, a phenomenon attributed to the synergistic effects of its diverse bioactive components (Moore *et al.,* 2016; Bouammali *et al.,* 2023; Ijaz *et al.,* 2023).

Furthermore, ursolic acid and betulinic acid, which are predominantly present in ethanolic and chloroform extracts, exhibit cytotoxic properties against various cancers. They induce apoptosis and inhibit tumor growth, potentially through the formation of reactive oxygen species (ROS) and cell cycle arrest. These triterpenes are integral to the antiproliferative activity of rosemary, especially within complex mixtures (Perez-Sanchez *et al.,* 2019; Jordamovic *et al.,* 2023).

Furthermore, additional constituents such as 1,8-cineole, α-pinene, and camphor have demonstrated cytotoxic and antiproliferative effects across various tumor cell lines. The primary effects exerted by these molecules in cancer cells include the induction of oxidative stress, which has been shown to be effective when utilized independently or in conjunction with conventional chemotherapeutic agents. Volatile fractions from these compounds have been proven to possess anticancer properties in models of breast, colon, ovarian, and cervical cancers (Silva *et al.,* 2021; e Silva *et al.,* 2022; Alaboudi *et al.,* 2025). Moreover, flavones such as apigenin and luteolin, predominantly extracted using ethanol, are associated with anti-inflammatory, antioxidant, and anticancer effects, potentially disrupting pathways essential for cancer cell survival and angiogenesis. The antioxidant and anticancer properties of rosemary extract have been extensively documented in prior research, attributable to key bioactive compounds including carnosic acid, carnosol, and rosmarinic acid. These phenolic diterpenes exhibit potent free radical scavenging activity, chelating reactive oxygen species and inhibiting lipid peroxidation, thereby reducing oxidative stress and DNA damage (Saad *et al.,* 2025). Evidence suggests that rosemary extract functions as a superior antioxidant compared to certain synthetic antioxidants in various in vitro studies (Nieto *et al.,* 2018).

With regard to anticancer effects, rosemary extract and its constituents have been demonstrated to promote cell proliferation, induce apoptosis, and influence the expression of cancer-related genes in various human cancer cell lines, including those of breast, colon, ovarian, and pancreatic cancers (Hoca *et al.,* 2025). Research indicates that rosemary enhances the activity of chemotherapeutic agents through a synergistic mechanism. Furthermore, the anti-inflammatory and pro-apoptotic effects of the extract substantiate its potential in chemopreventive applications (Allegra *et al.,* 2020). In summary, these findings support the consideration of rosemary extract as a promising natural compound in the field of cancer prevention and therapy.

It is also believed that the antioxidant activity of rosemary is chiefly attributed to its high content of phenolic diterpenes, such as carnosic acid and carnosol, as well as phenolic acids like rosmarinic acid (Chun *et al.,* 2014). The mechanism of action of these compounds occurs through multiple pathways, wherein rosemary constituents possess the capacity to interrupt the chain reaction of lipid oxidation, particularly within biological membranes and

food systems, by inhibiting peroxyl radicals (Gad and Sayd, 2015; Nieto et al., 2018). Moreover, certain active components demonstrate the ability to chelate metal ions (e.g., Fe^{2+}, Cu^{2+}), thereby preventing Fenton-type reactions that generate highly reactive hydroxyl radicals. Additionally, some constituents, such as carnosic acid, are reported to stimulate endogenous antioxidant enzymes, including superoxide dismutase and glutathione peroxidase (Gad and Sayd, 2015). Furthermore, rosemary extracts can modulate cellular defenses by activating redox-sensitive transcription factors like Nrf2, thereby enhancing cellular resistance to oxidative stress (Nieto *et al.,* 2018).

Numerous prior studies have established that rosemary extracts, particularly those prepared using polar solvents such as ethanol or methanol, exhibit consistent and potent antioxidant activity. Notably, recently research has found that ethanolic and methanolic extracts outperform less polar solvents due to their elevated levels of phenolic compounds and flavonoids (Al-jaafreh, 2024). Standard antioxidant assessments, including DPPH, FRAP, and ABTS assays, consistently demonstrate that rosemary extracts are comparable or superior to conventional synthetic antioxidants such as BHA and BHT in preventing lipid peroxidation in oils and model systems (Chen *et al.,* 2014; Jafari *et al.,* 2022). The constituents, including carnosic acid, carnosol, rosmarinic acid, and other active components, can account for up to 90 % of the overall antioxidant effect, underscoring the importance of solvent selection in enhancing bioactivity of the extract (Jafari *et al.,* 2022). Recent investigations emphasize that the efficacy of rosemary in food preservation and in cellular systems is intricately linked to its phenolic composition and overall antioxidant capacity (Song *et al.,* 2023).

Rosemary extract exhibits significant anticancer effects through a multi-target mechanism. These phytochemicals influence several vital cellular pathways in a mechanistic manner: Rosemary extract induces programmed cell death in cancer cells via activation of both caspase-dependent and caspase-independent pathways. Studies have demonstrated increased PARP cleavage, elevated levels of pro-apoptotic factors, and decreased expression of anti-apoptotic genes following rosemary exposure (Elsayed *et al.,* 2024). This effect is attributable to the intervention of rosemary extract (particularly at concentrations of 20-100 µg/mL), which causes cell cycle arrest at the G2/M or G1 phase, thereby inhibiting tumor cell proliferation (Chan *et al.,* 2021). Furthermore, the extract enhances intracellular reactive oxygen species (ROS) levels in cancer cells, rendering them susceptible to oxidative damage, while concurrently regulating the Nrf2 antioxidant pathway, culminating in cell death (Bouammali et al., 2023). Rosemary also impedes signaling cascades, such as the Wnt/β-catenin pathway (notably in breast and colon cancer models), and diminishes the expression of cholesterol-modulating genes and oncogenes like β-catenin and K-ras (Eghbalpour *et al.,* 2024). Additionally, the extract augments the efficacy of chemotherapeutic agents such as 5-fluorouracil and cisplatin, thereby overcoming drug resistance and enhancing cytotoxicity in cancer cell lines (Moore *et al.,* 2016).

Rosemary extract also inhibits the proliferation of various cancer cell lines, including colon, breast, prostate, and leukemia cells, at low micromolar concentrations. The IC50 values for rosemary extract generally range from 20 to 50 µg/mL (Raad *et al.,* 2024). Oral administration of rosemary extract demonstrates a significant reduction in tumor volume in animal xenograft models, further supporting its cellular effects and indicating potential in vivo anti-carcinogenic properties (Elsayed *et al.,* 2024). Recent research reveals that the direct downregulation of Wnt/β-catenin signaling, along with the upregulation of endoplasmic reticulum stress and unfolded protein response genes, are critical mechanisms in breast and colon cancer pathogenesis (Eghbalpour *et al.,* 2024). Furthermore, combinatorial therapy studies suggest that rosemary extract may synergize with standard chemotherapeutic agents, enhancing their efficacy and reducing adverse effects (Jaglanian *et al.,* 2020).

To date, recent and cumulative studies highlight rosemary's unique ability to disrupt cancer cell survival on multiple levels, inducing apoptosis, preventing cancer cell proliferation, sensitizing cells to drugs, and altering oxidative and epigenetic landscapes. This multifunctional activity supports its potential as an adjunct treatment in cancer therapy (Eghbalpour *et al.,* 2024; Elsayed *et al.,* 2024).

DMH exposes rat colon cancer models to the development of aberrant crypt foci and pre-neoplastic lesions and is associated with dramatic increases in oxidative markers (lipid peroxidation and hydroxyl radicals) and the increased expression of proliferation and survival genes. When rosemary extract or its active ingredient rosmarinic acid is supplemented, the number of polyps is reduced by half, and oxidative stress indicators are decreased by more than 20 times. Rosemary extract at the molecular level normalizes the expression of proinflammatory cytokines (IL-1b and TNF-a) and antioxidant regulatory genes like Nrf-2, bringing them down to non-cancerous baseline levels (Moore *et al.,* 2016; Ilhan *et al.,* 2022; Zhao *et al.,* 2022; Azhar et al., 2023; Czerwinska and Radziejewska, 2024). Additionally, in mammary carcinogenesis induced by MNU—a well-established rodent model that mimics the histopathology and gene expression changes of breast cancer—rosemary extract exhibits strong anti-proliferative and pro-apoptotic effects. MNU causes a significant rise in the expression of anti-apoptotic factors such as cyclin D1 and p21, leading to alveolar hyperplasia and epithelial disorganization (Ashrafi *et al.,* 2012; Eghbalpour *et al.,* 2024; Darra *et al.,* 2025). Treatment with rosemary extract not only suppresses these pathogenic genes and restores the balance between pro- and anti-apoptotic signals but also regulates tissue architecture, reducing the size and cellularity of hyperplastic nodules, thereby promoting tissue normalization. The extract's effectiveness appears linked to its ability to disrupt Wnt/b-catenin and other pro-survival signaling pathways, induce cell cycle arrest, promote caspase-mediated apoptosis, and modify Nrf-2-mediated antioxidant defenses (de Oliveira *et al.,* 2023; e Silva *et al.,* 2022).

Recent studies reinforce the antioxidative and anti-inflammatory effects of rosemary, showing a notable reduction in malondialdehyde (MDA), a lipid peroxidation marker, and increased levels of natural antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx) in cancer-affected animals treated with rosemary extract (Pérez-Sánchez *et al.,* 2019;

Mansour and Mousa, 2022; Abdelrazik *et al.,* 2023). These changes are linked not only to decreased oxidative DNA damage but also to improved tissue structure, reduced dysplasia, and fewer preneoplastic lesions in the colon and mammary tissues. Histological analysis confirms the restoration of crypt and alveolar architecture, indicating healthier tissue profiles in treated animals (Moore *et al.,* 2016; Eghbalpour *et al.,* 2024).

These findings not only substantiate the significant chemopreventive and therapeutic properties of rosemary extract against DMH- and MNU-induced colon and breast carcinogenesis in rats but also align with the expanding corpus of mechanistic evidence. This evidence emphasizes the targeting of inflammatory, oxidative, and apoptotic dysregulation as primary mechanisms underlying rosemary's anticancer activity. Such robust multi-pathway effects support the potential use of rosemary extract as a safe and effective adjunct dietary supplement in the prevention or treatment of hormone- or toxin-induced carcinogenesis in mammalian tissues (Eghbalpour *et al.,* 2024; Elsayed *et al.,* 2024).

The results in Fig 2 support and build on previous findings that rosemary extract has broad-spectrum anticancer activities by altering major apoptotic, oxidative stress and inflammatory pathways, which are in turn associated with caspase-mediated cell death through the upregulation of caspase-3, -8, -9, and Bax and downregulation of Bcl-2 in MCF-7 and HepG2 cells (Alaboudi *et al.,* 2025). Likewise, such a significant normalization of Nrf-2 in the current study is consistent with the results of de Oliveira Silva *et al.,* (2023) who demonstrated that carnosic acid and carnosol in rosemary are strong activators and subsequent modulators of Nrf-2/ARE in HCT116 colon cells, and thus impair redox homeostasis and induce apoptosis in tumor cells. The coinciding attenuation of the hydroxyl radical marker (OH) and β-actin is also comparable to the previous studies, which state that rosemary polyphenols reduce oxidative DNA damage and cytoskeleton repair in colon cancer models (Moore *et al.,* 2016).

The fact that pro-inflammatory cytokines IL-1b and TNF-α were suppressed by rosemary extract from untreated cancer cells to about 1.1-2.2-fold is a close reflection of the anti-inflammatory impact of rosemary diterpenes on lipopolysaccharide-induced IL-1b/TNF-α release in macrophage models, which were inhibited by rosemary extract through NF-kB signaling (Tong *et al.,* 2017). The intermediate downregulation of pro-apoptotic BAX and Caspase-3 (down to 1.1-1.9-fold) suggests that rosemary extract not only disrupts the survival pathways, but it also regulates the activation of executioner caspases to guarantee a controlled apoptotic cell death. This observation is consistent with Jaglanian *et al.,* who established that rosemary extract causes a significant rise in cleaved PARP and caspase activation in MDA-MB-231 cellular breast cancer cells, and it is more cytotoxic than conventional chemotherapeutic reagents such as paclitaxel (Jaglanian *et al.,* 2020).

Conclusions: This article demonstrates that rosemary extract possesses significant chemopreventive and therapeutic properties in both colon and breast cancer models in rats. It mitigates carcinogen-induced increases in proinflammatory cytokines, anti-apoptotic genes, and oxidative stress, as well as improving tissue histology. Rosemary extract reduces the expression of oncogenic genes such as BCL-2, Nrf-2, IL-1b, and TNF-a, indicating its potent anti-inflammatory and pro-apoptotic effects. Additionally, it diminishes oxidative injury and enhances antioxidant defenses, resulting in a notable reversal of early carcinogenic changes, particularly in breast cancer models. These findings support the potential application of rosemary extract as a low-toxicity, natural, multi-targeted anticancer agent, warranting further research for clinical application.

Competing interests: The authors declare that they have no competing interests.

Authors contribution statement: Conceptualization, AMA, and ASJ, formal analysis, AMA, and ASJ, investigation, AMA, and ASJ, data curation, AMA, and ASJ, writing original draft preparation, AMA, and ASJ, writing final manuscript and editing, AMA, and ASJ, visualization and methodology, AMA, and ASJ. All authors have read and agreed to the published version of the manuscript.

Acknowledgements: The project was funded by KAU Endowment (WAQF) at king Abdulaziz University, Jeddah, Saudi Arabia. The authors, therefore, acknowledge with thanks WAQF and the Deanship of Scientific Research (DSR) for technical and financial support. The authors gratefully acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R366), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

Funding: The project was funded by KAU Endowment (WAQF) at king Abdulaziz University, Jeddah, Saudi Arabia. The authors, therefore, acknowledge with thanks WAQF and the Deanship of Scientific Research (DSR) for technical and financial support. This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2025R366), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

REFERENCES

Abdelbaky AS, Mohamed AM, Abu-Talib MM, et al., 2025. Eco-Friendly fungicide alternative: Phytochemical profiling and antifungal efficacy of green-extracted Acalypha marginata formulations. J Agric Food Res 2:102412.

Abdel-Moneim A-ME, El-Saadony MT, Shehata AM, et al., 2022. Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. Saudi J Biol Sci 29:1197-1209.

Abdelrazik E, Hassan HM, Hamza E, et al., 2023. Beneficial role of rosemary extract on oxidative stress-mediated neuronal apoptosis in rotenone-induced attention deficit hyperactivity disease in juvenile rat model. Acta Biomed 94(3):2023104.

Ahmed AE, Al-Qthanin RN, Ahmed H, et al., 2025. The protective effect of rosemary, sage, green-coffee, and oregano extract on mitigating the respiratory distress in wistar albino rats exposed to corn oil fumes inhalation. J Agric Food Res 23:102187.

Alaboudi KA, Aziz IM, Almosa AA, et al., 2025. In vitro and in silico pharmacological effects of Rosmarinus officinalis leaf methanolic extracts and essential oils. Sci Rep 15:10699.

Alatawi FS, Faridi UA, Alatawi MS, 2018. Effect of treatment with vitamin D plus calcium on oxidative stress in streptozotocin-induced diabetic rats. Saudi Pharm Journal 26:1208-1213.

Alharbi YM, Elzahar KM, Qahl SH, et al., 2024. Nutritional significance, antimicrobial, antioxidants, anticancer, and antiviral activities of

lemongrass leaves extract and its application as hepatoprotective agent against CCl4-induced hepatic injury in rats. An Acad Bras Ciênc 96(3):e20230646.

Al-jaafreh AM, 2024. Evaluation of antioxidant activities of rosemary (Rosmarinus officinalis L.) essential oil and different types of solvent extractions. Biomed Pharmacol J 17:323-339.

Allegra A, Tonacci A, Pioggia G, et al., 2020. Anticancer activity of Rosmarinus officinalis L.: mechanisms of action and therapeutic potentials. Nutrients 12(6): 1739.

Al-Quwaie DA, Allohibi A, Aljadani M, et al., 2023. Characterization of Portulaca oleracea whole plant: Evaluating antioxidant, anticancer, antibacterial, and antiviral activities and application as quality enhancer in yogurt. Molecules 28:5859.

AOAC, 2012. Official Methods of Analysis (18th ed.), Association of Official Analytical Chemists, Arlington, VA., USA.

Ashrafi M, Bathaie S, Abroun S, 2012. High expression of cyclin D1 and p21 in N-nitroso-N-methylurea-induced breast cancer in wistar albino female rats. Cell J 14 (3): 193-202.

Azhar MK, Anwar S, Hasan GM, et al., 2023. Comprehensive insights into biological roles of rosmarinic acid: implications in diabetes, cancer and neurodegenerative diseases. Nutrients 15:4297.

Aziz E, Batool R, Akhtar W, et al., 2022. Rosemary species: a review of phytochemicals, bioactivities and industrial applications. S Afr J Bot 151:3-18.

Bouammali H, Zraibi L, Ziani I, et al., 2023. Rosemary as a potential source of natural antioxidants and anticancer agents: A molecular docking study. Plants 13:89.

Chan EWC, Wong SK, Chan HT, 2021. An overview of the chemistry and anticancer properties of rosemary extract and its diterpenes. J Herbmed Pharmacol 11:10-19.

Chen J-C, Fang C, Zheng R-H, et al., 2022. Environmentally relevant concentrations of microplastics modulated the immune response and swimming activity, and impaired the development of marine medaka Oryzias melastigma larvae. Ecotoxicol Environ Saf 241:113843.

Chen X, Zhang Y, Zu Y, et al., 2014. Antioxidant effects of rosemary extracts on sunflower oil compared with synthetic antioxidants. Int J Food Sci Technol 49:385-391.

Chun K-S, Kundu J, Chae IG, et al., 2014. Carnosol: a phenolic diterpene with cancer chemopreventive potential. J Cancer Prev 19:103.

Chunarkar-Patil P, Kaleem M, Mishra R, et al., 2024. Anticancer drug discovery based on natural products: from computational approaches to clinical studies. Biomedicines 12:201.

Czerwińska K, Radziejewska I, 2024. Rosmarinic acid: A potential therapeutic agent in gastrointestinal cancer management—A review. Int J Mol Sci 25:11704.

Dai X, Alnadari F, Nasiru MM, et al., 2025. Novel anthocyanin-enriched film drying from camphor fruit waste enhances the physicochemical and quality properties of beef and reduces oxidation compared to conventional methods. Results Eng 27:106797.

Darra R, Majdalawieh AF, Mahasneh A, et al., 2025. Anti-proliferative effects of Rosmarinus officinalis L. (Rosemary) against human breast and liver carcinoma cells. Food Biosci 65:106164.

de Oliveira e Silva AM, Pereira RO, Oliveira AKdS, et al., 2023. Ameliorative effects of aqueous extract from rosemary on oxidative stress and inflammation pathways caused by a high-fat diet in C57BL/6 mice. Appl Physiol Nutr Metab 49:459-472.

eSilva GdS, de Jesus Marques JN, Linhares EPM, et al., 2022. Review of anticancer activity of monoterpenoids: Geraniol, nerol, geranial and neral. Chem Biol Interact 362:109994.

Eghbalpour K, Eghbalpour N, Khademi S, et al., 2024. Suppressive potential of Rosmarinus officinalis L. extract against triple-negative and luminal a breast cancer. Int J Mol Cell Med 13(2): 198-209.

El-Saadony MT, Alkafaas SS, Saad AM, et al., 2025. Medicinal plants: nutritional, immunological and therapeutic role in treating cancer-related malnutrition: a comprehensive review. Cancer cell Int 25:266.

El-Saadony MT, Saad AM, Elakkad HA, et al., 2022. Flavoring and extending the shelf life of cucumber juice with aroma compounds-rich herbal extracts at 4oC through controlling chemical and microbial fluctuations. Saudi J Biol Sci 29:346-354.

Elsadek BE, Abdel Aziz MA, M El-Deek SE, et al., 2017. Combination therapy with quercetin and 5-fluorouracil ameliorates 1, 2-dimethylhydrazine induced carcinogenesis in the colon of wistar rats. Bull Egypt Soc Physiol Sci 37:227-244.

Elsayed DH, Said NI, Said AA, et al., 2024. Anticarcinogenic activity of rosemary loaded solid lipid nanoparticle against solid tumor in mice. Labyrinth: Fayoum J Sci Interdisciplinary Stud 2:8-13.

El-Sayed HS, Saad AS, Tawfik WA, et al., 2024. The role of turmeric and black pepper oil nanoemulsion in attenuating cytokine storm triggered by duck hepatitis A virus type I (DHAV-I)-induced infection in ducklings. Poult Sci 103:103404.

Gad AS, Sayd AF, 2015. Antioxidant properties of rosemary and its potential uses as natural antioxidant in dairy products—A review. Food Nutr Sci 6:179-193.

Gahtori R, Tripathi AH, Kumari A, et al., 2023. Anticancer plant-derivatives: deciphering their oncopreventive and therapeutic potential in molecular terms. Futur J Pharm Sci 9:14.

Gird CE, Costea T, Mitran V, 2021. Evaluation of cytotoxic activity and anticancer potential of indigenous Rosemary (Rosmarinus officinalis L.) and Oregano (Origanum vulgare L.) dry extracts on MG-63 bone osteosarcoma human cell line. Rom J Morphol Embryol 62:525.

Hegazy AM, Morsy AM, Salem HM, et al., 2024. The therapeutic efficacy of neem (Azadirecta indica) leaf extract against coinfection with Chlamydophila psittaci and low pathogenic avian influenza virus H9N2 in broiler chickens. Poult Sci 103:104089.

Hoca M, Becer E, Yakubu A, et al., 2025. The cytotoxic activity of rosemary essential oil on PANC-1 cells. Cyprus J Med Sci 10(1):65-68.

Ijaz S, Iqbal J, Abbasi BA, et al., 2023. Rosmarinic acid and its derivatives: current insights on anticancer potential and other biomedical applications. Biomed Pharmacother162:114687.

Ilhan N, Bektas I, Susam S, et al., 2022. Protective effects of rosmarinic acid against azoxymethane-induced colorectal cancer in rats. J Biochem Mol Toxicol 36:e22961.

Jafari SZ, Jafarian S, Hojjati M, et al., 2022. Evaluation of antioxidant activity of nano-and microencapsulated rosemary (Rosmarinus officinalis L.) leaves extract in cress (Lepidium sativum) and basil (Ocimum basilicum) seed gums for enhancing oxidative stability of sunflower oil. Food Sci Nutr 10:2111-2119.

Jaglanian A, Termini D, Tsiani E, 2020. Rosemary (Rosmarinus officinalis L.) extract inhibits prostate cancer cell proliferation and survival by targeting Akt and mTOR. Biomed Pharmacother 131:110717.

Jordamović N, Pehlivanović B, Nikšić H, et al., 2023. Anti-proliferative and anti-inflammatory activity of triterpene extracts from plant species belonging to Lamiaceae family. Bol latinoam Caribe Plantas Med Aromát 22 (6): 864 – 878.

Mansour DS, Mousa AM, 2022. Ameliorative potential role of Rosmarinus officinalis extract on toxicity induced by etoposide in male albino rats. Braz J Biol 84:e258234.

Mohamed M, Saad AM, Mohammed DDM, et al., 2025. Curcumin, an active component of turmeric: biological activities, nutritional aspects, immunological, bioavailability, and human health benefits-a comprehensive review. Front Immunol 16:1603018.

Mohammed T, Saad AM, Mohammed DM, et al., 2025. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits-a comprehensive review. Front Immunol 16:1491777.

Moore J, Yousef M, Tsiani E, 2016. Anticancer effects of rosemary (Rosmarinus officinalis L.) extract and rosemary extract polyphenols. Nutrients 8:731.

Mosmann T, 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55-63.

Mueed A, Aljahdali SM, Albalawi M, et al., 2024. Phenolic compounds and biological activities of berberis fruit: Enhancing role on physiochemical and antioxidant properties of yogurt. Lwt Food Sci Techo 211:116834.

Mueed A, Shibli S, Al-Quwaie DA, et al., 2023. Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. Frontin Nutr 10:1125106.

Nieto G, Ros G, Castillo J, 2018. Antioxidant and antimicrobial properties of rosemary (Rosmarinus officinalis, L.): A review. Medicines 5(3): 98.

Omoregie EH, Folashade KO, 2013. Broad spectrum antimicrobial activity of extracts of Jatropha curcas. J Appl Pharm Sci 3:083-087.

Pappas A, Tsiokanos A, Fatouros IG, et al., 2021. The effects of spirulina supplementation on redox status and performance following a muscle damaging protocol. Int J Mol Sci 22:3559.

Pérez-Sánchez A, Barrajón-Catalán E, Ruiz-Torres V, et al., 2019. Rosemary (Rosmarinus officinalis) extract causes ROS-induced necrotic cell death and inhibits tumor growth in vivo. Sci Rep 9:808.

Qi G, Tang B, Zhou L, et al., 2015. Effects of high-fat diet on 1, 2-dimethylhydrazine-induced aberrant crypt foci and colorectal tumours in rats. Biomed Rep 3:289-294.

Rao KVR, Mani P, Satyanarayana B, et al., 2017. Purification and structural elucidation of three bioactive compounds isolated from

Streptomyces coelicoflavus BC 01 and their biological activity. Biotech 7:24.

Saad AM, Mohammed DDM, Alkafaas SS, et al., 2025. Dietary polyphenols and human health: Sources, biological activities, nutritional and immunological aspects, and bioavailability–A comprehensive review. Front Immunol 16:1653378.

Saadany MT, Saad AM, Alkafaas SS, et al., 2025. Chitosan, derivatives, and its nanoparticles: Preparation, physicochemical properties, biological activities, and biomedical applications–A comprehensive review. Int J Biol Macromol 313: 142832.

Saadony MT, Yang T, Saad AM, et al., 2024. Polyphenols: Chemistry, bioavailability, bioactivity, nutritional aspects and human health benefits: A review. I nt J Biol Macromol 277:134223.

Saif GB, Khan IA, 2022. Association of genetic variants of the vitamin D receptor gene with vitiligo in a tertiary care center in a Saudi population: a case-control study. Ann Saudi Med 42:96-106.

Silva BI, Nascimento EA, Silva CJ, et al., 2021. Anticancer activity of monoterpenes: A systematic review. Mol Biol Rep 48:5775-5785.

Song X, Sui X, Jiang L, 2023. Protection function and mechanism of rosemary (Rosmarinus officinalis L.) extract on the thermal oxidative stability of vegetable oils. Foods 12:2177.

Subarmaniam T, Mahmad Rusli RN, Perumal KV, et al., 2023. The potential chemopreventive effect of Andrographis paniculata on 1, 2-dimethylhydrazine and high-fat-diet-induced colorectal cancer in sprague dawley rats. Int J Mol Sci 24:5224.

Talaat M, Saad AM, Sitohy M, et al., 2025. Chitosan nanoparticles: Green synthesis, biological activities, and sustainable frontiers in targeted drug delivery and cancer nanomedicine–A comprehensive review. Materials Today Bio 25:102358.

Tong X-p, Ma Y-x, Quan D-n, et al., 2017. Rosemary extracts upregulate Nrf2, Sestrin2, and MRP2 protein level in human hepatoma HepG2 cells. J Evid Based Complementary Altern Med 2017:7359806.

Venkatachalam K, Vinayagam R, Arokia Vijaya Anand M, et al., 2020. Biochemical and molecular aspects of 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. Toxicol Res 9:2-18.

Wang M, Li Y, Pan T, et al., 2024. Plant natural compounds in the cancer treatment: A systematic bibliometric analysis. Heliyon 10(14): e34462.

Zhao J, Xu L, Jin D, et al., 2022. Rosmarinic acid and related dietary supplements: Potential applications in the prevention and treatment of cancer. Biomolecules 12(10):1410.