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Original Research Article

Melastoma malabathricum and *Muntingia Calabura* against Colon Cancer Cell Line and DMH-Induced Rat Model: *In Vitro* and *In Vivo* Perspective

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ABSTRACT

Melastoma malabathricum (MM) and *Muntingia calabura* (MC) are traditionally used medicinal plants. The present study examined the anti-colon cancer effects of a water-based extract derived from the combined leaves of both plants (MM-MC). Cell lethality was tested in HCT116 human colon cancer cells, where MM-MC induced cell shrinkage. The impact of the combined extract on apoptosis induction, cell cycle regulation, and oxidative stress was analyzed in HCT116 cells. The extract amplified the expression of Bax and caspase-3, while repressing Bcl-2 and Cyclin B1. Additionally, MM-MC suppressed the activities of antioxidant enzymes (CAT, GSH-Px, SOD) and the total antioxidant capacity (T-AOC), while it increased MDA and total oxidant status (TOS) levels in HCT116 cells. In the 1,2-dimethylhydrazine (DMH)-exposed rats, oral administration of MM-MC at 250 and 500 mg/kg markedly inhibited aberrant crypt foci (ACF) formation, achieving 71.42% and 85.68% inhibition, respectively. The extract also restored normal colonic architecture and decreased the proportion of PCNA-positive nuclei in the distal colon to 11% and 7% in the 250 and 500 mg/kg treatment groups, as opposed to 16% observed in the untreated DMH-induced control group. These findings demonstrate the anti-colorectal cancer potential of the MM-MC extract, highlighting its potential use as a treatment for colon cancer.

Keywords: Colon cancer, Apoptosis, Immunohistochemistry, *Melastoma malabathricum*, *Muntingia calabura*

Introduction

Colon cancer stands as the second most common cause of cancer-related death globally.[1,2] Statistics from the Global Cancer Observatory (GLOBOCAN), a program under the International Agency for Research on Cancer (IARC), demonstrate a continuous rise in colon cancer-related deaths, increasing from 694,000 in 2012 to 881,000 in 2018 and reaching 935,173 by 2020. Cancer cells are characterized by uncontrolled division, resistance to apoptosis, and disruption of cell cycle regulation.[6] A common feature relating to cancer cells is the enhanced level of the cell death–inhibiting protein Bcl2, coupled with the lowered expression of apoptosis-promoting factors, which include Bax and caspases, which enables tumor cells to evade programmed cell death.[5,9] Oxidative stress is another key contributor to tumor development.[7] While cancer cells regulate reactive oxygen species (ROS) to support survival, excessive ROS accumulation damages mitochondria, promotes cytochrome c release, and triggers apoptosis.[8,9] Therapeutic interventions targeting the suppression of cell death-inhibiting proteins, the activation of apoptosis-promoting molecules, and the induction of oxidative stress have therefore become central to cancer research. Although conventional colon cancer treatment methods such as surgery, radiotherapy, and chemotherapy are available, their effectiveness is often restricted by significant side effects, particularly toxicity to healthy tissues.[3] The growing global incidence of colon cancer and the shortcomings of current therapies emphasize the pressing requirement for novel treatment strategies that are both effective and less toxic. Medicinal plants, rich in biologically active compounds and generally associated with fewer adverse effects, have gained recognition as promising sources of anticancer agents. [5] The current study explores the anti-colon cancer activity of *Melastoma malabathricum* (MM) and *Muntingia calabura* (MC), two plants widely used in Southeast Asia for their medicinal properties, including digestive relief and wound healing. Both are abundant in phenol-based compounds and other secondary metabolites. MM exhibits antioxidative,[11,13] anti-inflammatory,[12] and cancer-fighting properties, particularly in combating colon cancer.[14] Its pharmacological effects are attributed to phenolics, saponins, and alkaloids[10,11] Similarly, MC contains tannins, saponins, and alkaloids, which contribute to its anti-inflammatory,[16] antioxidant,[15,16] and anticancer activities.[17] While both plants individually demonstrate moderate cytoprotective effects against colon cancer,[14,17] the therapeutic potential of their combined extract remains largely unexplored. But previous findings show their synergistic effects against gastric ulcers,[18] which suggest that their combination may also be effective in the management of colon-related diseases.

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Materials and Methods

Plant Material Collection and identification

Newly harvested leaves of *Melastoma malabathricum* (Senduduk) and *Muntingia calabura* (Cherry) were harvested from the northern region of Peninsular Malaysia (6.6667ºN, 100.2667ºE). Plant identification and authentication were carried out at Universiti Putra Malaysia's Institute of Bioscience (IBS), where voucher specimens (SK2683/15 for MC and SK2684/15 for MM) were deposited in the plant specimen repository of the Natural Products Laboratory for reference.

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Extraction procedure
Aqueous solutions of MM and MC were prepared in combination using a previous study extraction protocol. [47] The collected leaves were cleaned, air-dried, ground into powder, and sieved through an 18-mesh screen. Twenty kilograms of the powdered leaf mixture (10 kg of each plant) was macerated in 400 L of reverse osmosis (RO) water (plant-to-water ratio 1:20) and stirred at 50°C for 24 hours. Filtration of the extract was carried out using Whatman Grade 1 filter paper, and the filtrate underwent evaporation to concentrate it at 80°C under reduced pressure (620 mm/Hg) for 6 hours, followed by oven drying at 65°C. The dried product was then spray-dried under controlled conditions (inlet 217°C, outlet 128°C, feed flow 30 L/h). The final spray-dried extract was collected hourly, packed in food-grade containers, labeled, and stored at 18–22°C. Extraction yield was recorded at 11.6%, calculated according to a previously established method. [48]

Cell cultivation
The HCT116 cell line, derived from human colon carcinoma (ATCC CCL-247) was sourced from ATCC (Virginia, USA). HCT116 cells were propagated in McCoy's 5A medium fortified with 10% fetal bovine serum (FBS) and 1% antibiotic mixture (penicillin-streptomycin). The cell culture was incubated at 37°C in a humid environment with carbon dioxide (5%).

Examination of HCT116 cell microscopic features
Changes in HCT116 cellular structure following MM-MC treatment were observed using an inverted microscope (Nikon Eclipse TS 100), as indicated in a previous study.[19] Cells were cultured in 6-well plates at a concentration of 2×10^5 cells per well and subsequently incubated with 5-FU (100 µg/mL) or MM-MC (using concentrations of 12.5 to 100 µg/mL) over 72 hours. Morphological features such as cell shrinkage, membrane protrusion, and apoptotic body formation were examined.

Protein expression analysis of HCT116 cells
Protein expression of Bcl2, Bax, caspase-3, and cyclin B1 in HCT116 cells following 72 h of MM-MC exposure was evaluated using the Jess capillary-based Western blot system, as described in a previous study.[20] Cells were broken down with M-PER reagent supplemented with Halt protease inhibitor cocktail (Cat. ABI-78501). The cell suspension was centrifuged at 14,000 xg for 15 minutes at 4ºC. Protein concentration of the supernatant was determined by BCA assay, after which the supernatant was prepared in diluted form in 0.1× sample buffer to 1 mg/mL. Samples were mixed with a fluorescent master mixture comprising buffer and fluorescent standards, and 40 mM dithiothreitol (DTT), then denatured for 5 minutes at 95°C. A total volume of 3 µL per sample was introduced into 12–230 kDa capillary cartridges. Primary antibodies against Bax (Cat. GZ-AF820), Bcl2 (Cat. GZ-AF810), caspase-3 (Cat. NB100-56708), and cyclin B1 (Cat. GZ-AF6000) were diluted in Antibody Diluent 2. Secondary antibodies were used as provided. For each sample, 10 µL of each primary and secondary antibody was applied. The plates were subjected to centrifugation at 1,000 × g for 5 minutes to separate and remove bubbles before insertion into the Jess system, which performed automated protein fractionation, membrane blocking, antibody binding, and signal recognition. Data were analyzed with Compass software, and protein expression was quantified using the area beneath the curve corresponding to the molecular weight of each target protein.

Analysis of oxidative stress indicators in HCT116 cells
The activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), together with levels of malondialdehyde (MDA), total oxidant status (TOS), and total antioxidant capacity (T-AOC), were analyzed with ELISA kits (Elabscience Biotechnology, USA) according to the producer's protocols. Optical density (OD) was determined at the wavelengths specified in the company's protocols, with an ELISA plate reader (RT-2100C, China), and antioxidant enzyme activities or MDA, T-AOC, and TOS levels were calculated accordingly.

Animal subjects
A total of 36 adult (4–5 weeks old, 100–130 g) male Sprague-Dawley (SD) rats were employed in this experiment. The animals were maintained in well-aerated cages in regulated environmental conditions of 25 ± 2°C and a 12-h light/dark cycle, and allowed to adapt before the experiment. They were provided unrestricted drinking water and a conventional pelleted diet. Ethical clearance for the study protocol was granted by the Institutional Animal Care and Use Committee (IACUC), UPM (Ethics No: UPM/IACUC/AUP-R009/2023), and the experiment was undertaken in line with the Animal Welfare Act 2015.

In vivo study design
Thirty-six rats were randomized into six groups (n = 6). Cancer, a staged process, was induced to the rats using 1,2-dimethylhydrazine (DMH) to evaluate the anticarcinogenic effects of MM-MC.[49] Aside from the normal control (N, Group 1), all animals acquired subcutaneous dosing with 1,2-dimethylhydrazine at 40 mg/kg once weekly within 10 weeks. While the negative control (NC, Group 2) received only DMH, the positive control (PC, Group 3) was treated with 5-fluorouracil (5-FU, 35 mg/kg, i.p.) once weekly for 20 weeks.[50] Treatment groups received MM-MC orally daily for 20 weeks at 50 mg/kg (T1, Group 4), 250 mg/kg (T2, Group 5), or 500 mg/kg (T3, Group 6).[51] At termination, animals were humanely euthanized with a combination of ketamine at 100mg/kg and xylazine at 10 mg/kg, and colonic tissues were collected for microscopic tissue analysis.

Microscopic tissue analysis
Alterations in the collected colon samples were examined following the previous study protocol.[52] The tissues were subjected to fixation in 10% neutral-buffered formalin for 24 hours, after which they were dehydrated through ascending ethanol concentrations, cleared using xylene, and infiltrated in molten paraffin. Tissue sections measuring 5 µm in thickness were subjected to hematoxylin and eosin (H&E) staining, then mounted in DPX. Aberrant crypt foci (ACF) were quantified as early precancerous markers. The colon sections were examined for ACF by counting the crypts in ACF across six selected fields at random per sample by means of an Olympus CX41 light microscope (10× and 40×), and ACF were identified based on their abnormally enlarged size. Total ACF counts and crypt numbers per colon were recorded as described in the previous research report.[53] In addition to ACF, a benign glandular tumor was assessed as an intermediate precancerous marker, at 10× magnification using an Olympus BX41 inverted microscope (Olympus Corporation, Japan), in accordance with previous methodology.[54] Features of polyps relating to benign glandular tumor assessed included glandular distortion,[52] extracellular mucin pulls with malignant cells,[55] and neoplastic invasion extending into or beyond the muscularis propria.[56]

Immunohistochemical (IHC) analysis of PCNA expression
Proliferating Cell Nuclear Antigen (PCNA) protein expression in colonic tissues was evaluated using a commercial 2-step plus Poly-HRP Anti-Rabbit/Mouse IgG Detection IHC kit, following the manufacturer's protocol. Sections (5 µm) of paraffin-embedded tissue were cleared of paraffin, restored with water. Antigen retrieval was performed using sodium citrate buffer (pH 6.0), after which endogenous peroxidase was inhibited using 3% hydrogen peroxide (H_2O_2), and imprecise binding was minimized with normal goat serum. Sections were subjected to rat PCNA monoclonal antibody (E-AB-18205; 1:50) for 1 h, followed by polymer-based secondary antibody and visualization with DAB substrate. Contrast staining was performed with hematoxylin, and slides were mounted with DPX. PCNA-positive nuclei, detected as brown staining, were examined at 20× magnification under an Olympus BX41 microscope. For quantification, 100 cell populations were assessed in ten irregularly selected view areas per section, and the proportion of PCNA-positive nuclei was calculated, as described in a previous study.[57]

Statistical analysis
Data evaluation was conducted with GraphPad Prism (v10.2.0) software. A One-way analysis of variance (ANOVA) with Tukey's

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multiple comparison test was performed to assess distinctions among groups, with a significance level set at $p < 0.05$

Results and Discussion

Impact of MM-MC on the morphology of HCT116 cells

The present study investigated the morphological alterations induced in HCT116 cells after for 72 h incubation with MM-MC. Microscopic examination (Figure 1) showed maintenance of the normal epithelial-like cell morphology, characterized by polygonal shape and intact cell membranes, of the negative control (untreated) group. This suggests no detectable cytotoxic effect was exerted on the cells. In contrast, HCT116 cells treated with the conventional drug 5-fluorouracil (5-FU) exhibited cell shrinkage, a feature typically associated with apoptosis arrest.[66] Exposure to MMMC extract induced morphological changes in HCT116 cells. At 12.5 µg/mL MM-MC, minimal alterations were observed, with most cells retaining their normal shape. At 25 µg/mL, 50 µg/mL, and 100 µg/mL MM-MC, cell shrinkage and membrane rupture were observed in most cells, implying induction of apoptosis by the extract, consistent with earlier findings such as the Gypenosides-induced apoptosis in the Colo 205 cells.[21] The morphological alterations observed in this study align with previous reports demonstrating the cytotoxic potential of *M. malabathricum* [14] and *M. calabura* [17] individually against various cancer cell lines. The combined extract (MM-MC) may therefore act synergistically, enhancing cytotoxicity through the complementary mechanisms of its bioactive compounds. The presence of polyphenols, flavonoids, and phenolic acids in the plants[11,15] could collectively contribute to the disruption of cancer cell integrity and inhibition of cell proliferation, as suggested in prior studies on phytochemical synergy.[63] Future mechanistic studies, such as annexin V/PI staining, caspase activation assays, and mitochondrial membrane potential analysis, are warranted to elucidate the specific pathways involved in MMMC-induced apoptosis of HCT116 cells.

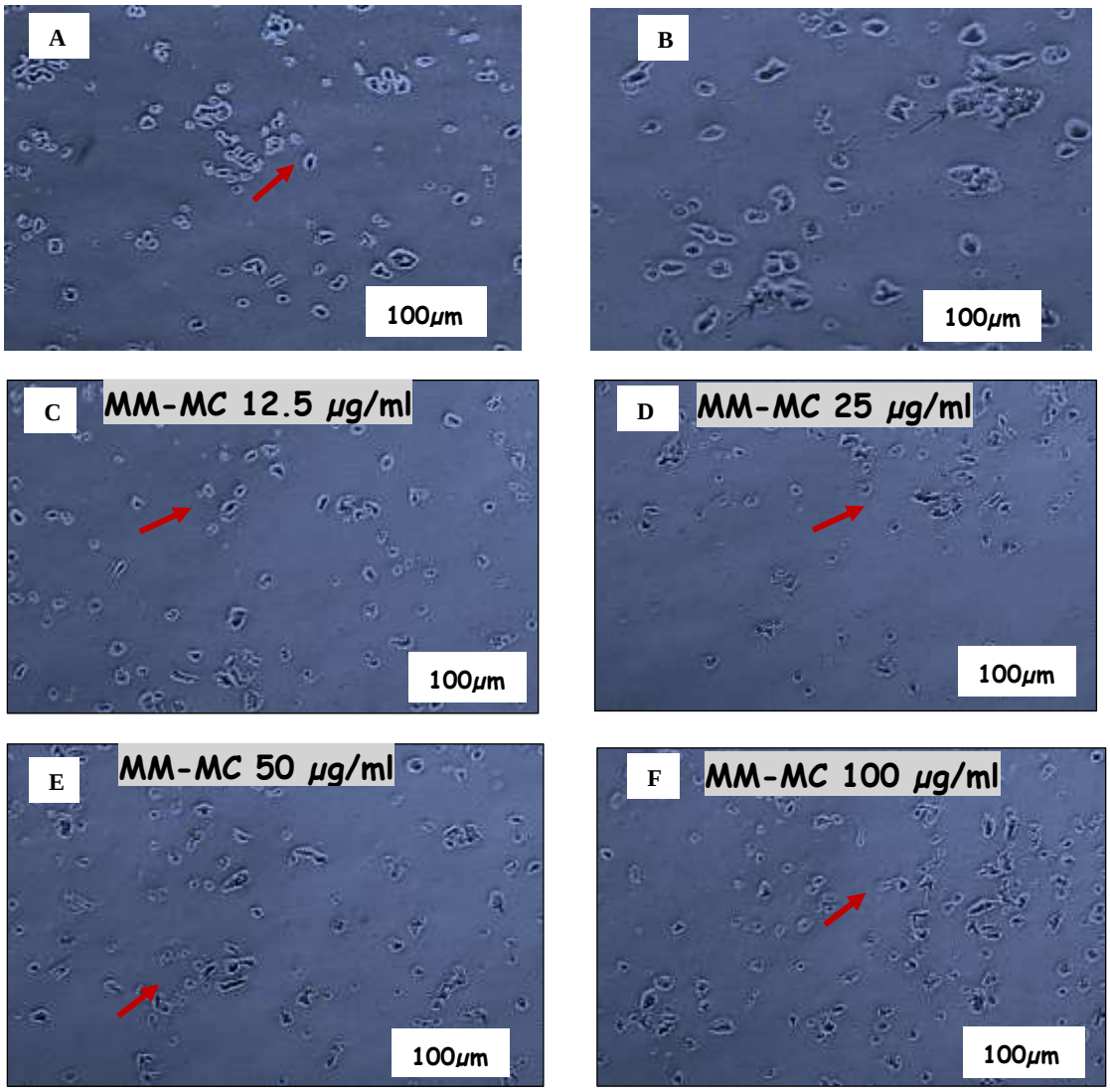

Figure 1: Morphological features of HCT116 cells following 72-hour exposure to MM-MC. Cells were either (A) untreated (control) or treated with (B) 5-FU; (C) 12.5 µg/mL MM-MC; (D) 25 µg/mL MM-MC; (E) 50 µg/mL MM-MC; or (F) 100 µg/mL MM-MC. Red arrows highlight cellular shrinkage (20× magnification).

Effect of MM-MC on Apoptotic and Cell Cycle Markers in HCT116 cells

To elucidate the mechanisms driving the cytotoxic action of MM-MC in HCT116 colon cancer cells, Bax, Bcl2, caspase-3, and cyclin B1 expression were monitored via capillary-based Western blotting. Apoptosis plays a central role in maintaining tissue homeostasis and eliminating malignant cells. Dysregulation of apoptotic proteins, including enhanced levels of anti-apoptotic Bcl-2 and decreased levels of pro-apoptotic Bax and Caspase-3, is a hallmark of colorectal cancer progression. [64,65] In the current study, treatment with MM-MC extract at concentrations between 12.5 and 100 µg/mL and 5-FU significantly ($p<0.05$) downregulated Bcl-2 and upregulated Bax and caspase-3 in

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HCT116 cells with increasing doses of the extract (Figure 2), suggesting an apoptotic event. Similar pro-apoptotic effects have been reported for individual extracts of *M. malabathricum* and *M. calabura*, both rich in phenolics and flavonoids such as quercetin, kaempferol, and gallic acid.[66,67] These bioactive constituents are known to trigger mitochondrial dysfunction and caspase activation in various cancer models, including the effect of gallic acid observed in HCT116 cells.[68] Thus, the synergistic combination of both plant species in the MM-MC extract may enhance apoptotic signaling by simultaneously targeting multiple molecular pathways. Cyclin-B1, a regulatory protein essential for cell division, is frequently overexpressed in colorectal tumors, promoting mitotic entry and cell division.[69] MM-MC treatment contributed to a pronounced decline in Cyclin-B1 expression, suggesting a potential cell cycle arrest**.** These results align with earlier findings revealing similar pro-apoptotic effects of natural agents such as gypenosides,[21] corosolic acid,[22] taxifolin,[23] and *Allium porrum* extracts[24] in colon cancer cells. MM-MC may suppress Bcl2 through p53 activation, inhibition of NF-κB/PI3K pathways, or both,[8] while MM-MC-induced Bax enhancement may result directly from p53 activation or indirectly via oxidative stress.[8] Reduced Bcl2 relieves Bax inhibition, enabling mitochondrial membrane opening, cytochrome c release, and caspase-3 activation.[27] Cyclin B1 diminution may result from p53-driven p21 activation, which inhibits E2F1-dependent CCNB1 gene transcription, leading to cell cycle arrest.[59]

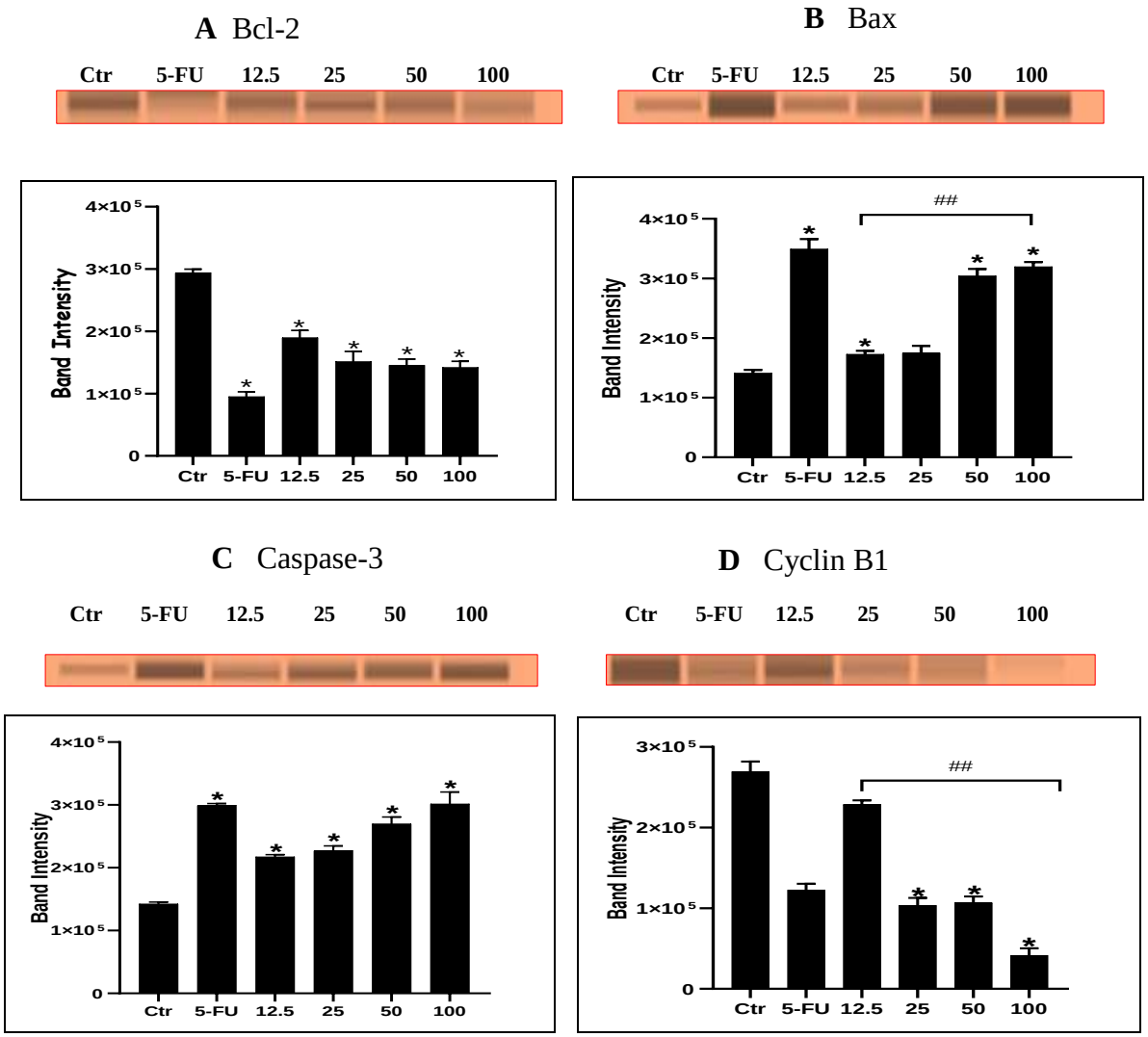

Figure 2: BCL-2, Bax, Caspase-3, and Cyclin-B1 expression in HCT116 cells after 72 h of MM-MC treatment. Data are presented as Mean ± SEM (n = 3). Statistical significance was determined by one-way ANOVA with Tukey's *test (*$p < 0.05$, ##$p < 0.01$; ns, not significant*).*

Influence of MM-MC treatment on oxidative stress profile of HCT116 cells

The current research explored the influence of varying concentrations of MM-MC extract (12.5 -100 µg/mL) on oxidative stress markers in HCT116 colon cancer cells, following 72 h of treatment. Oxidative stress represents a state of oxidoreduction imbalance where oxidants outweigh antioxidants, thereby favoring pro-oxidant activity.[8] Oxidative stress is a recognized cell death activator,[8,25] primarily through p53-mediated activation of Bax, a critical regulator of programmed cell death.[8] Cancer cells maintain a steady-state oxidant level to support survival; however, compounds capable of disrupting this balance by enhancing oxidative stress can promote apoptotic cell death. In the current study, the ELISA-based analysis (Figure 3) revealed significant ($p<0.05$) suppression of the catalytic functions of CAT, SOD, and GSH-Px, and reduction in T-AOC level in HCT116 cells incubated with MM-MC extract as well as 5-FU, compared to the control, suggesting a down-regulation of the endogenous antioxidant system. In contrast, total oxidant status (TOS) and the lipid oxidative degradation marker malondialdehyde (MDA) were notably higher in the cells. MDA serves as a marker of lipid peroxidation and oxidative membrane damage. The elevation in MDA implies induction of lipid peroxidation and ROS-triggered cellular damage. The observed effects

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were dose-dependent, with the most pronounced alterations detected at 100 µg/mL. Collectively, these findings demonstrate the pro-oxidative effect of MM-MC extract in HCT116 cells, thereby implicating oxidative stress-mediated pathways in the cytotoxic activity of MM-MC. Previous studies have shown that many natural compounds exert anticancer effects by elevating ROS levels beyond the tolerable threshold of cancer cells, thus sensitizing them to apoptosis.[70] Therefore, MM-MC may contain phytochemicals, such as flavonoids, phenolics, and tannins, that modulate redox signaling pathways, suppress antioxidant defenses, and amplify ROS-mediated cytotoxicity.[67] Collectively, these results suggest that MM-MC exerts its anticancer effects in HCT116 cells by promoting oxidative stress through suppression of cellular antioxidant systems and enhancement of lipid peroxidation. The findings corroborate the growing evidence that targeting redox homeostasis represents an effective strategy for the selective elimination of cancer cells while minimizing toxicity to normal cells. Comparable results have been documented in other studies. For example, *Vernonia cinerea* extract decreased the enzymatic performance of SOD, CAT, and GSH-Px in SW620 and HT29 cells.[26] Similarly, *Allium porrum* treatment suppressed antioxidant enzyme activities in HT-29 cells,[24] while quercetin isolated from *Toona sinensis* leaves reduced CAT and GPx activities and increased MDA levels in SW620 cells.[27]

Effect of MM-MC on DMH-mediated colon carcinoma in rats

Aberrant crypt foci (ACF) are recognized as preliminary indicators of colon carcinogenesis, often preceding adenoma and carcinoma development,[60] and characterized by distortion of typical glandular morphology,[28] decline in goblet cell number and mucin, and submucosal and muscularis propria infiltration by non-functional epithelial cells.[29] Evidence from this study suggests that administration of MM-MC extract significantly reduced the number of ACF.

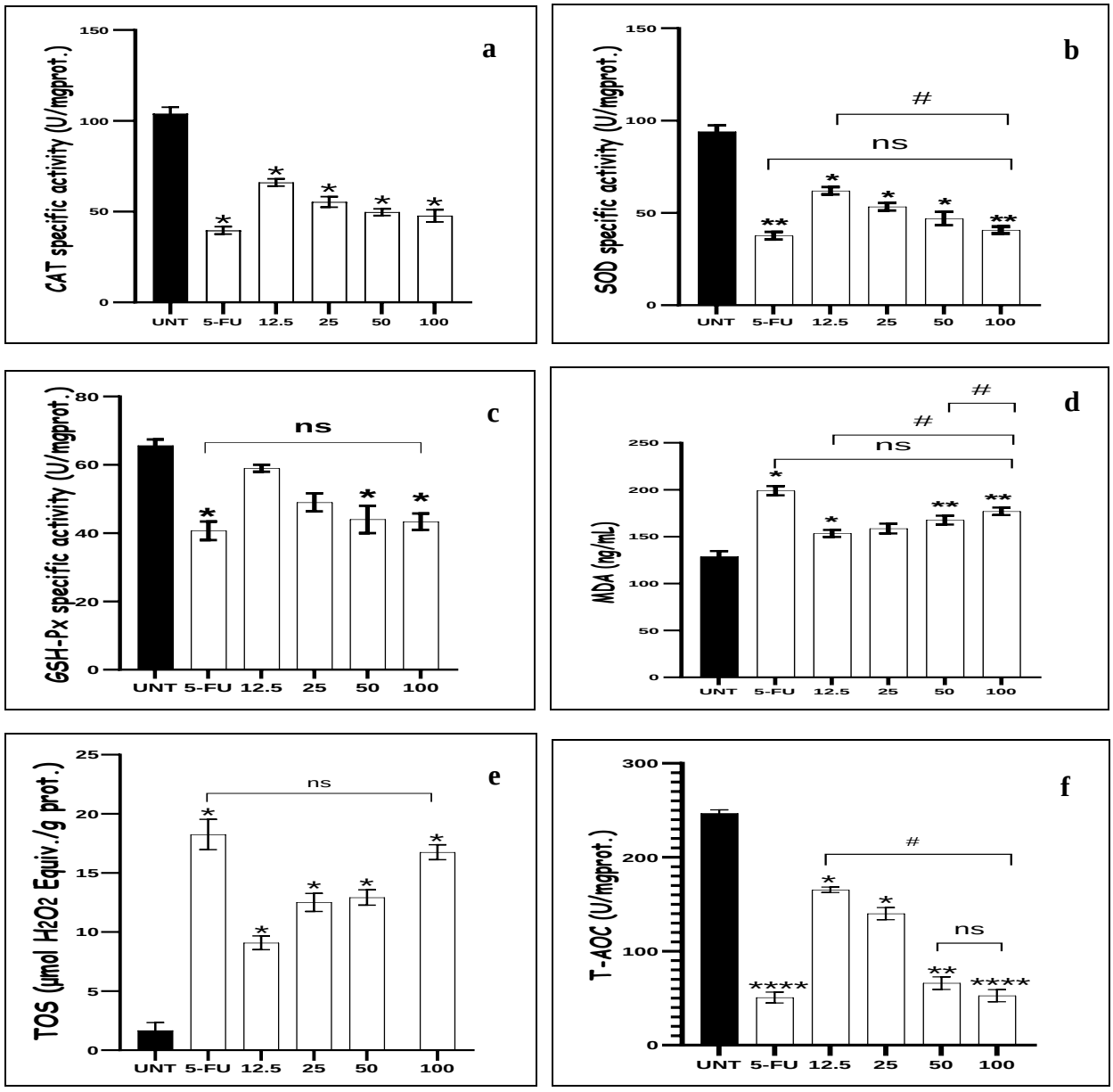

Figure 3: Enzymatic activities of CAT (a), SOD (b), and GSH-Px (c), and levels of MDA (d), TOS (e), and T-AOC (f) in HCT116 cells after 72 h exposure to MM-MC (12.5–100 µg/mL) or untreated (UNT) (negative control). Data are shown as Mean ± SEM (n = 3). Significance was assessed by one-way ANOVA with Tukey's test: *p < 0.05, **p ≤ 0.01, ****p ≤ 0.001 vs. control; ns = not significant; #p < 0.05.

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As presented in Table 1, no ACFs were evident in the normal control (Group N), confirming the absence of early premalignant changes. In contrast, the DMH-exposed negative control group (Group NC) showed a rise in the number of ACF, confirming successful induction of preneoplastic lesions. Nevertheless, treatment with MMMC markedly reduced the number of ACF, suggesting inhibition of the early stage of colon tumorigenesis. The positive control group (5-FU + DMH, Group PC) exhibited a 71.4% reduction in ACFs, with only two lesions detected. Among the treatment groups, the largest dose of MM-MC extract (500 mg/kg; Group T3) produced the most pronounced effect, with an 85.7% ACF reduction and only one ACF recorded. The intermediate dose (250 mg/kg; Group T2) achieved a 71.4% reduction with two ACFs, whereas the lowest dose (50 mg/kg; Group T1) did not reduce lesion incidence, with seven ACFs. The findings of this study, as in Figure 4, demonstrated that administration of MM-MC preserved the normal histoarchitecture of the colon in DMH-induced rats.

Table 1: ACF count and inhibition of DMH-induced rats after MM-MC treatment

Group	Treatment	ACF count	Total no. of ACF/Colon	ACF inhibition
N	Norma	0	0.00 ± 0.00	
NC	Negative control (DMH only)	7	1.75 ± 1.18	0 %
PC	Positive control (DMH + 5-FU)	2	0.50 ± 0.29	71.42 %*
T1	DMH + 50 mg/kg	7	1.75 ± 1.43	0 %
T2	DMH + 250 mg/kg MMMC	2	0.50 ± 0.29	71.42 %*
T3	DMH + 500 mg/kg MMMC	1	0.25 ± 0.25	85.68 %*

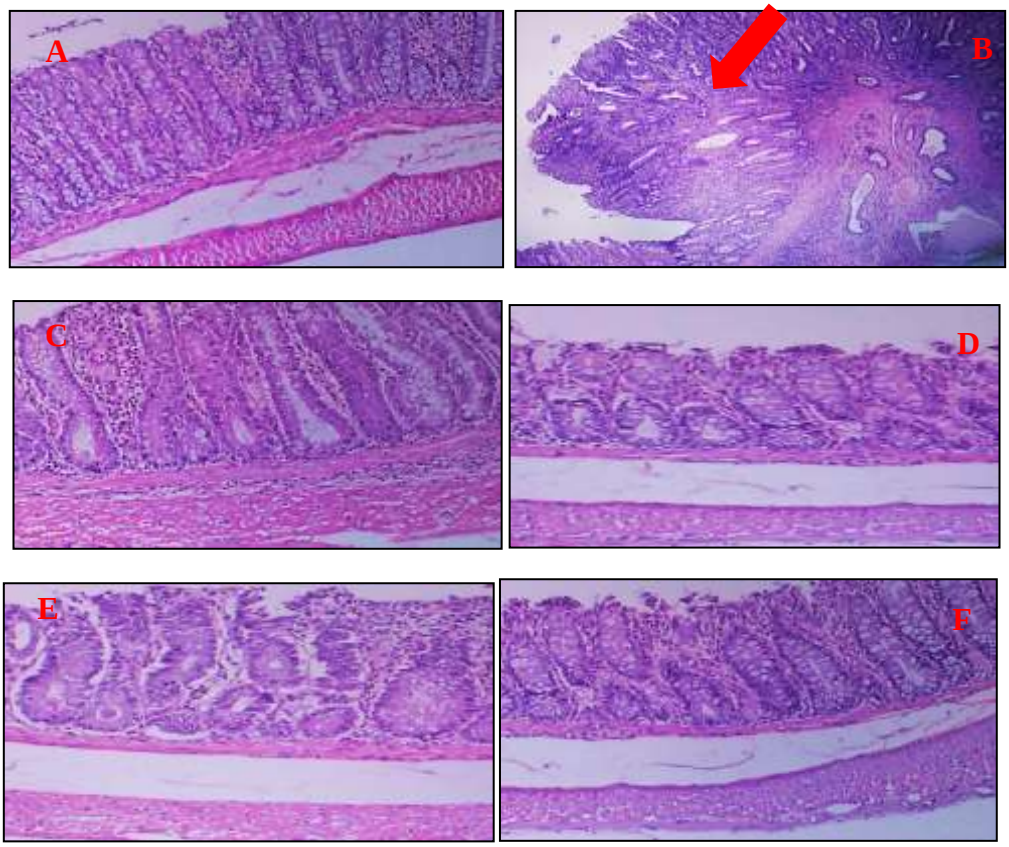

Figure 4: Histology of colonic tissues from DMH-administered rats after MM-MC treatment (H&E, 10×). **(A) Normal control, (B) NC (C)** DMH + 5-FU (PC), (D) T1: DMH + 50 mg/kg MM-MC, (E) T2: DMH + 250 mg/kg MM-MC, (F) T3: DMH + 500 mg/kg MM-MC as Colon tissue from the DMH-only group shows mucous membrane ulceration, indicated by red arrow (Magnification: 10X).

Colon sections from the untreated control exhibited intact mucosa-associated morphology with preserved glandular structure. In contrast, the colon tissue of the negative control group showed adenomas, characterized by mucosal distortion, crypt loss, and cellular atypia, confirming the early stage of colon carcinogenesis as described in the previous report.[71] However, MM-MC treatment at all tested dosages (50,250, and 500mg/kg) attenuated these histopathological lesions and produced a near-normal mucosal morphology comparable to that of the 5-FU-treated group and the normal control. The observed reduction in ACF number and the restoration of normal crypt architecture indicate that MM-MC effectively suppressed DMH-induced colon carcinogenesis at the initiation and promotion phases. This effect could be attributed to the synergistic phytochemical composition of *M. malabathricum* and *M. calabura*, both of which are rich in polyphenols, flavonoids, anthocyanins, and triterpenoids with potent free radical scavenging and inflammation-reducing effects.[72] These bioactive constituents may neutralize DMH-derived free radicals, reduce oxidative DNA damage, and modulate key molecular pathways associated with cell proliferation and apoptosis. Furthermore, the improvement in colon architecture observed in MM-MC-treated groups

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could be linked to the extract's ability to regulate oxidative stress and inflammatory responses. Previous studies have reported that abnormal accumulation of ROS and lipid peroxidation promotes ACF formation and mucosal dysplasia.[73] By enhancing the catalytic functions of antioxidant enzymes and reducing peroxidation of lipid molecules, MM-MC may have provided cytoprotective effects that prevented further epithelial damage. Similar protective effects have been reported with other agents [30-32]

Effect of MM-MC on colonic PCNA expression in DMH-exposed rats

Proliferating cell nuclear antigen (PCNA) is a recognized indicator for cell multiplication and DNA synthesis, and its overexpression is closely associated with neoplastic transformation and tumor progression in colon carcinogenesis,[33-38] thereby serving as a useful biomarker for detecting precancerous and cancerous lesions.[39] The present research assesses the influence of MM-MC on PCNA expression in DMH-administered rats, using immunopathological analysis. The findings (Figure 5) revealed a significant upregulation of PCNA expression in the negative control group (DMH alone), confirming successful induction of aberrant colonic cell proliferation and tumorigenesis. In contrast, treatment with MM-MC at varying doses, as well as the standard chemotherapeutic agent 5-FU, markedly reduced PCNA expression, suggesting a suppressive effect on colonocyte proliferation. Compared to other treatment concentrations, the most pronounced effect of the extract was observed at 500 mg/kg (Figure 6).

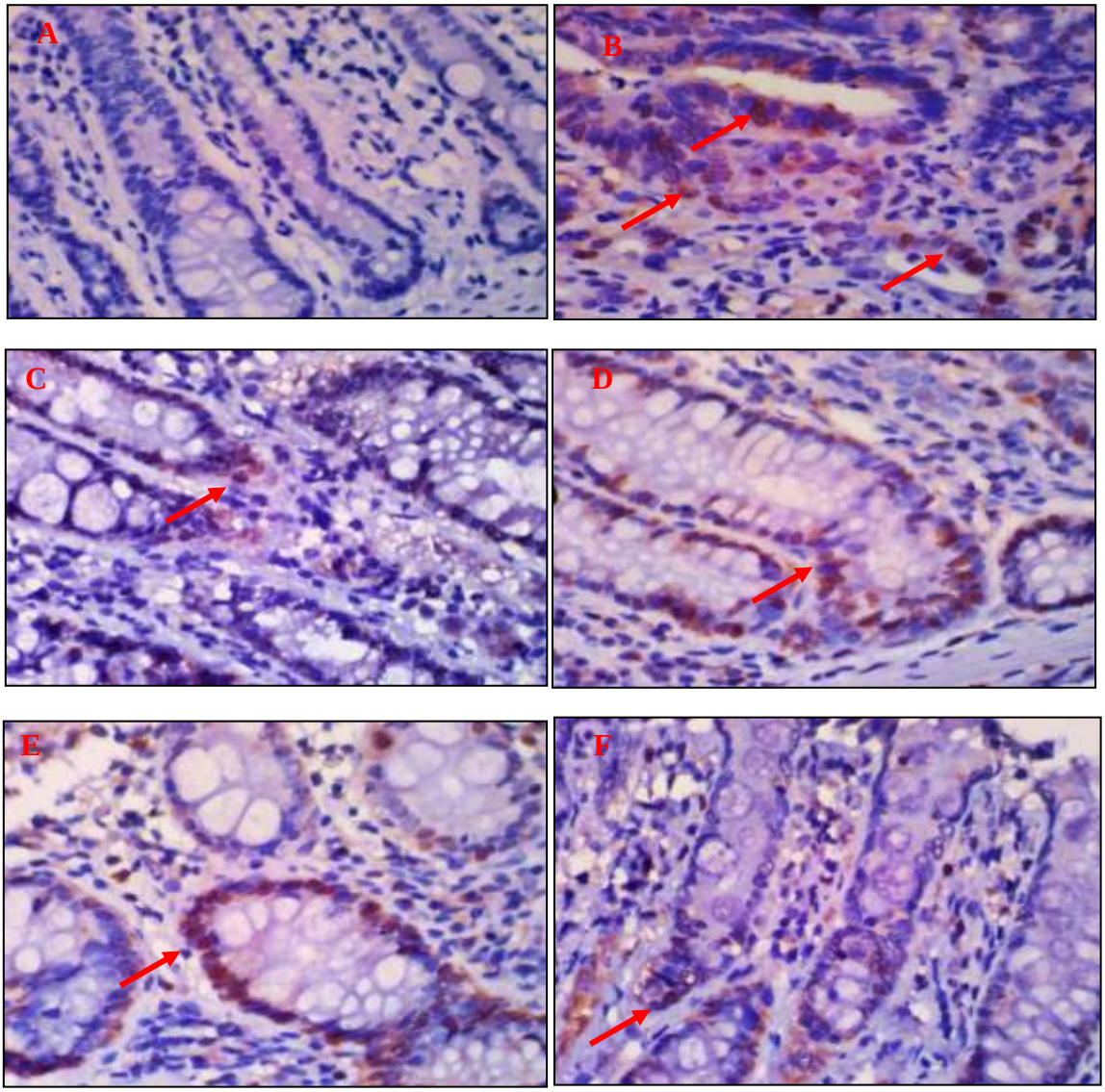

Figure 5: PCNA expression in colonic sections of DMH-exposed rats following MM-MC treatment. (A) Normal control; (B) NC; (C) DMH + 5-FU (PC); (D) T1: 50 mg/kg MM-MC; (E) T2: 250 mg/kg MM-MC; (F) T3: 500 mg/kg MM-MC. PCNA-positive nuclei appear brown (red arrows) (20× magnification)

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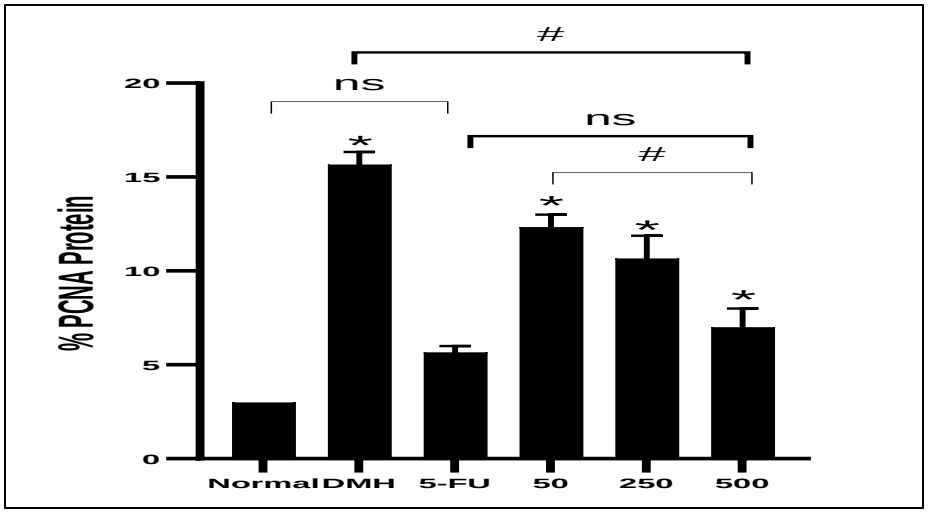

Figure 6: PCNA expression in the colonic tissues of DMH-administered rats following MM-MC treatment. The number of PCNA-positive nuclei per ×40 field was quantified and averaged from ten fields per rat. Data are expressed as Mean ± SEM (n = 3). Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test: *$p < 0.05$; ns = not significant, relative to the normal control group, #$p < 0.05$.

The percentage of PCNA-positive nuclei in the distal colon significantly reduced from 16% observed in the untreated DMH-induced control group (NC) to 11% and 7% in the 250 and 500 mg/kg MM-MC treatment groups, respectively. The reduction in PCNA is likely associated with the impact of MM-MC in suppressing the cellular replication cycle and activating apoptosis, thereby diminishing the cellular requirement for PCNA. This observation could be due to the phytochemicals present in *M. malabathricum* and *M. calabura*. Both plants are known to contain abundant polyphenols, flavonoids, and anthocyanins with antioxidant and anticancer properties.[61] These bioactive compounds have been reported to modulate important signaling pathways such as PI3K/Akt, MAPK, and NF-κB, thereby minimizing cell proliferation and activating apoptosis.[62] These findings align with earlier reports demonstrating that plant-derived compounds,[40-43] similarly repressed PCNA expression in DMH-exposed models of colon cancer [44-46].

Conclusion

Aqueous extract prepared from the combined leaves of *Melastoma malabathricum* and *Muntingia calabura* (MM-MC) reduced the proliferation of HCT116 human colon cancer cells by eliciting intrinsic apoptosis, arresting the cell cycle, and promoting oxidative stress. In vivo, MM-MC counteracted DMH-triggered structural abnormalities in the colon and reduced the number of PCNA-marked cells, thereby preventing tumor development. These suggest that MM-MC could represent a potential treatment for colonic carcinoma.

Conflict of interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics. GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71(3):209–249.
2. Morgan E, Arnold M, Gini A, Lorenzoni V, Cabasag CJ, Laversanne M, Vignat J, Ferlay J, Murphy N, Bray F. Global Burden of Colorectal Cancer in 2020 and 2040: Incidence and Mortality Estimates from GLOBOCAN. Gut. 2023; 72(2):338-344.
3. Bhargavi R, Shreyas VD. Colorectal Cancer: Etiology, Pathogenesis and Current Treatment. J Innov Pharm Biol Sci. 2020; 7(4):20-24.
4. Wang H, Khor TO, Shu L, Su ZY, Fuentes F, Lee JH, Kong AN. Plants vs. Cancer: A Review on Natural Phytochemicals in Preventing and Treating Cancers and their Druggability. Anticancer Agents Med Chem. 2012; 12(10):1281-1305.
5. Dzobo K. The Role of Natural Products as Sources of Therapeutic Agents for Innovative Drug Discovery. Compr Pharmacol. 2022; 2:408–422.
6. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell. 2011; 144(5):646-674.
7. Seril DN, Liao J, Yang GY, Yang CS. Oxidative Stress and Ulcerative Colitis-Associated Carcinogenesis: Studies in Humans and Animal Models. Carcinogenesis. 2003; 24(3):353-362.
8. Nelson VK, Nuli MV, Mastanaiah J, Saleem TSM, Birudala G, Jamous YF, Alshargi O, Kotha KK, Sudhan HH, Mani RR, Muthumanickam A, Niranjan D, Jain NK, Agrawal A, Jadon AS, Mayasa V, Jha NK, Kolesarova A, Slama P, Roychoudhury S. Reactive Oxygen Species Mediated Apoptotic Death of Colon Cancer Cells: Therapeutic Potential of Plant Derived Alkaloids. Front Endocrinol. 2023; 14:1201198
9. El-Seedi HR, El-Said AMA, Khalifa SAM, Göransson U, Bohlin L, Borg-Karlson AK, Verpoorte R. Biosynthesis, Natural Sources, Dietary Intake, Pharmacokinetic Properties and Biological Activities of Hydroxycinnamic Acids. J Agric Food Chem. 2012; 60(44):10877–10895.
10. Zakaria ZA, Zainol ASN, Sahmat A, Salleh NI, Hizami A, Mahmood ND, Nasir N, Mamat SS, Kamisan FH, Mohtarrudin N, Abdul Hamid SS, Tohid SF, Teh LK, Salleh MZ. Gastroprotective Activity of Chloroform Extract of *Muntingia calabura* and *Melastoma malabathricum* Leaves. Pharm Biol. 2016; 54(5):812–826.

11. Lestari OA, Palupi NS, Setiyono A, Kusnandar F, Yuliana ND. *In vitro* Antioxidant Potential and Phytochemical Profiling of *Melastoma malabathricum* Leaf Water Extract. Food Sci Technol (Brazil). 2022; 42(1): e92021.
12. Kumar V, Sachan R, Rahman M, Rub RA, Patel DK, Sharma K, Gahtori P, Al-abbasi FA, Alhayyani S, Anwar F, Kim HS. Chemopreventive Effects of *Melastoma malabathricum* L. Extract in Mammary Tumor Model via Inhibition of Oxidative Stress and Inflammatory Cytokines. Biomed Pharmacother. 2021; 137:111298.
13. Zakaria ZA, Kamsani NE, Azizah R, Sulistyorini L. Anticarcinogenic Activity of Methanol Extract of *Melastoma malabathricum* Leaves is Attributed to the Presence of Phenolics Compounds and the Activation of Endogenous Antioxidant System. Bol Latinoam Caribe Plant Med Arom. 2022; 21(1):66–80.
14. Kamsani NE, Zakaria ZA, Md Nasir NL, Mohtarrudin N, Mohamad ANB. Safety Assessment of Methanol Extract of *Melastoma malabathricum* L. Leaves Following the Subacute and Subchronic Oral Consumptions in Rats and Its Cytotoxic Effect Against the HT29 Cancer Cell Line. Evid-Based Complement Alternat Med. 2019; 4:1-4.
15. Nasir Md NL, Kamsani NE, Mohtarrudin N, Md Tohid, SF, Zakaria ZA. Safety Evaluation of Orally-Administered Methanol Extract of *Muntingia Calabura* Linn. Leaves: A Sub-Chronic Toxicity Study in Sprague Dawley Rats. Pak J Pharm Sci. 2020; 33(5):2009–2016.
16. Zakaria N, Mohd YN, Zakaria Z, Widera D, Yahaya BH. Inhibition of NF-κB Signaling Reduces the Stemness Characteristics of Lung Cancer Stem Cells. Front Oncol. 2018; 17(8):166.
17. Zakaria ZA, Nasir NLM, Kamsani NE, Khaza'ai H, Sulistyorini L, Azizah R. Ethyl Acetate Partition Obtained from The Methanol Extract of *Muntingia Calabura* Leaf Exerts Effective *In vitro* Antiproliferative Activity Against the HT-29 Colon Cancer. Bol Latinoam Caribe Plant Med Arom. 2022; 21(5):654–670.
18. Halim SZ, Zakaria ZA, Omar MH, Mohtarrudin N, Wahab IRA, Abdullah MNH. Synergistic Gastroprotective Activity of Methanolic Extract of a Mixture of *Melastoma malabathricum* and *Muntingia calabura* Leaves in Rats. BMC Compl Altern Med, 2017; 17(1):488.
19. Nelson VK, Sahoo NK, Sahu M, Sudhan HH, Pullaiah CP, Muralikrishna KS. In vitro anticancer activity of *Eclipta alba* whole plant extract on colon cancer cell HCT-116. BMC Complement Med Ther. 2020; 20(1):355.
20. Pham TL, He J, Kakazu AH, Calandria J, Do KV, Nshimiyimana R, Petasis NA, Bazan HEP, Bazan NG. Elovanoid-N32 or RvD6-Isomer Decrease ACE2 and Binding of S Protein RBD After Injury or INFγ in the Eye. Exp Eye Res. 2020; 199:108201.
21. Chen J, Kung-Wen L, Jau-Hong L, Chin-Chung Y and Jing-Gung C. Gypenosides Induced Apoptosis in Human Colon Cancer Cells through the Mitochondria-Dependent Pathways and Activation of Caspase-3. Anticancer Res. 2006; 26:(6B):4313-4326.
22. Sung B, Kang YJ, Kim DH, Hwang SY, Lee Y, Kim M, Yoon JH, Kim CM, Chung HY, Kim ND. Corosolic Acid Induces Apoptotic Cell Death in HCT116 Human Colon Cancer Cells through a Caspase-dependent pathway. Int J Mol Med. 2014; 33(4):943–949.
23. Razak S, Afsar T, Ullah A, Almajwal A, Alkholief M, Alshamsan A, Jahan S. Taxifolin, a Natural Flavonoid Interacts with Cell Cycle Regulators Causes Cell Cycle Arrest and Causes Tumor Regression by Activating Wnt/ B -Catenin Signaling Pathway. BMC Cancer. 2018; 18(1):1043.
24. Alshammari GM, Balakrishnan A, Subash-Babu P, Al-khalifa A, Abdullah AA, ElGasim Ahmed YA, Naif AL, Hamed AG, Albekairi NA. Alpha-linolenic Acid Rich *Allium porrum* Methanolic Extract Potentially Inhibits HT-115 Human Colon Cancer Cells Proliferation via Mitochondria-mediated Apoptotic Mechanism. J King Saud Univ Sci. 2022; 34(1):101736.
25. Perillo B, di Donato, M, Pezone A, di Zazzo E, Giovannelli P, Galasso G, Castoria G, Migliaccio A. ROS in Cancer Therapy: The Bright Side of the Moon. Exp Mol Med. 2020; 52(2):192–203.
26. Pakpisutkul J, Suwapraphan J, Sripayak N, Sitkhuntod N, Loyrat S, Yahayo W, Supabphol R. The Effects of *Vernonia cinerea* Less Extracts on Antioxidant Gene Expression in Colorectal Cancer Cells. Asian Pac J Cancer Prev. 2022; 23(11):3923–3930.
27. Zhang Y, Guo Y, Wang M, Dong H, Zhang J, Zhang L. Quercetrin from *Toona sinensis* Leaves Induces Cell Cycle Arrest and Apoptosis Via Enhancement of Oxidative Stress in Human Colorectal Cancer SW620 Cells. Oncol Rep. 2017; 38(6)3319–3326.
28. Brenner SA, Zacheja S, Schäffer M, Feilhauer K, Bischoff SC, Lorentz A. Soluble CD14 Is Essential for Lipopolysaccharide-Dependent Activation of Human Intestinal Mast Cells from Macroscopically Normal as Well as Crohn's Disease Tissue. Immunol. 2024; 143(2):174–183.
29. Raskov H, Pommergaard HC, Burcharth J, Rosenberg J. Colorectal Carcinogenesis--Update and Perspectives. World J Gastroenterol. 2014; 20(48):18151-18164.
30. Mrudula K, Hemant U. Chemoprevention of DMH Induced Colon Carcinogenesis by Combination Administration of Lactobacillus acidophilus, Calcium and Moringa oleifera Leaves Extract. Int J Pharm Qual Assur. 2023; 14(4):1002-1010.
31. Sayed A, Youssef EA, Mahmoud SA, Youssry S, Abdel-Mawla AA. Effect of Niclosamide on Colorectal Cancer Induced by Dimethylhydrazine in Albino Mice. Egypt J Basic Appl Sci. 2023; 10(1):846–860.
32. Wang C, Qiao X, Wang J, Yang J, Yang C, Qiao Y, Guan Y, Wen A, Jiang L. Amelioration of DMH-induced colon cancer by eupafolin through the reprogramming of apoptosis-associated p53/Bcl2/Bax signaling in rats. Eur J Inflamm. 2022; 20: 1–15.
33. Yang Q, Ou C, Liu M, Xiao W, Wen C, Sun F. NRAGE Promotes Cell Proliferation by Stabilizing PCNA in a ubiquitin-proteasome Pathway in Esophageal Carcinomas. Carcinogenesis. 2014; 35:1643–1651.
34. Ban QJ, Ali HH, Hussein AG. (2012). Immunohistochemical Expression of PCNA and CD34 in Colorectal Adenomas and Carcinomas Using Specified Automated Cellular Image Analysis System: A Clinicopathologic Study. Saudi J Gastroenterol. 2012; 18(4):268–276.
35. Claudio TR, Tanaka S, Haruma K, Yoshihara M, Sumii K, Kajiyama G. Proliferating Cell Nuclear Antigen Expression at The Invasive Tumor Margin Predicts Malignant Potential of Colorectal Carcinomas. Cancer. 1994; 73(3):575–579.
36. Liu R, Sun K, Wang Y, Jiang Y, Kang J, Ma H. The Effects of Proliferating Cell Nuclear Antigen and P53 in Patients with Oral Squamous Cell Carcinoma: A Systematic Review and Meta-Analysis. Annals Transl Med. 2021; 9(23):1739–1739.
37. Zhou H, Huang T, Xiong Y, Peng L, Wang R, Jun Zhang G. (2018). The Prognostic Value of Proliferating Cell Nuclear Antigen Expression in Colorectal Cancer: A Meta-analysis. Medicine (Baltimore). 2018; 97(50): e13625.
38. Nakae S, Nakamura T, Ikegawa R, Yoshioka H, Shirono J, Tabuchi Y. Evaluation of Argyrophilic Nucleolar Organizer Region and Proliferating Cell Nuclear Antigen in Colorectal Cancer. J Surg Oncol. 1998; 69(1):28–35.
39. Hajrezaie M, Hassandarvish P, Moghadamtousi SZ, Gwaram NS, Golbabapour S, Hussien AN, Almagrami AA, Zahedifard M, Rouhollahi E, Karimian H, Fani S, Kamalidehghan B, Majid NA, Ali HM, Abdulla MA. Chemopreventive Evaluation of a Schiff Base Derived Copper (II) Complex Against Azoxymethane-Induced Colorectal Cancer in Rats. PLoS One. 2014; 9(3): e91246.
40. Hajrezaie M, Shams K, Moghadamtousi SZ, Karimian H, Hassandarvish P, Emtyazjoo M, Zahedifard M, Majid NA, Ali HM, Abdulla MA. Chemoprevention of Colonic Aberrant Crypt Foci by Novel Schiff Based Dichloride (4-methoxy-2-{[2-(piperazin-4-Ium-1-Yl) ethyl] iminomethyl} phenolate) Cd Complex in Azoxymethane-induced Colorectal Cancer in Rats. Sci Rep. 2015; 23(5):12379.
41. Madka V, Kumar G, Pathuri G, Zhang Y, Lightfoot S, Asch AS, Mohammed A, Steele VE, Rao CV. Bisphosphonates Zometa and

Fosamax Synergize with Metformin to Prevent AOM-induced Colon Cancer in F344 Rat Model. Cancer Prev Res. 2020; 13(2):185–194.
42. Moghadamtousi SZ, Rouhollahi E, Karimian H, Fadaeinasab M, Firoozinia M, Ameen Abdulla M, Kadir HA. The Chemopotential Effect of *Annona muricata* Leaves Against Azoxymethane-induced Colonic Aberrant Crypt Foci in Rats and the Apoptotic Effect of *Cetogenin Annomuricin* E in HT-29 Cells: A Bioassay-Guided Approach. PloS One. 2015; 10(4): e0122288.
43. Shwter AN, Abdullah NA, Alshawsh MA, El-Seedi HR, Al-Henhena NA, Khalifa SA, Abdulla MA. Chemopreventive Effect of *Phaleria Macrocarpa* on Colorectal Cancer Aberrant Crypt Foci *In Vivo*. J Ethnopharmacol. 2016; 4(193):195–206.
44. Xu G, Ren G, Xu X, Yuan H, Wang Z, Kang L, Yu W, Tian K. Combination of Curcumin and Green Tea Catechins Prevents Dimethylhydrazine-Induced Colon Carcinogenesis. Food Chem Toxicol. 2010; 48(1):390-395.
45. Ahmed KA, Shareef SH, Faraj TA, Abdulla MA, Najmaldin SK, Agha NFS, Kheder RK. Chemoprotective Effect of Arbutin on Azoxymethane-induced Aberrant Crypt Foci in Rat Colon Via Modulation of PCNA/Bax Protein. Braz Journal Med Biol Res. 2024; 1(57): 13306-13316.
46. Kilari BP, Kotakadi VS, Penchalaneni J. Anti-Proliferative and Apoptotic Effects of *Basella Rubra* (L.) Against 1,2-Dimethylhydrazine-Induced Colon Carcinogenesis in Rats. Asian Pac J Cancer Prev. 2016; 17(1):73–80.
47. Zakaria ZA, Nor Hazalin NAM, Zaid SNHM, Ghani MA, Hassan MH, Gopalan HK, Sulaiman MR. Antinociceptive, anti-inflammatory and antipyretic effects of *Muntingia calabura* aqueous extract in animal models. J of Nat Med. 2007; 61(4): 443–448.
48. Nguyen NVT, Duong N, Nguyen KH, Bui N, Pham T, Nguyen KT, Le PH, Kim K. Effect of Extraction Solvent on Total Phenol, Flavonoid Content, and Antioxidant Activity of *Avicennia Officinalis*. Biointerface Res Appl Chem. 2021; 12, 2678-2690.
49. Jisha N, Vysakh A, Vijeesh V, Anand PS, Latha MS. Methanolic Extract of *Muntingia Calabura* Mitigates 1,2-Dimethyl Hydrazine Induced Colon Carcinogenesis in Wistar Rats. Nutr Cancer. 2021; 73(11–12): 2363–2375.
50. Almagrami AA, Alshawsh MA, Saif-Ali R, Shwter A, Salem SD, Abdulla MA. Evaluation of Chemopreventive Effects of *Acanthus ilicifolius* against Azoxymethane-Induced Aberrant Crypt Foci in the Rat Colon. PLoS One. 2014; 9(5): e96004.
51. Nasir NLM, Kamsani NE, Mohtarrudin N, Othman F, Tohid SFM, Zakaria ZA, Md Nasir NL, Kamsani NE, Mohtarrudin N, Othman F, Md. Tohid SF, Zakaria ZA. Anticarcinogenic activity of *Muntingia calabura* leaves methanol extract against the azoxymethane-induced colon cancer in rats involved modulation of the colonic antioxidant system partly by flavonoids. Pharm Biol. 2017; 55(1): 2102–2109.
52. Subarmaniam T, Mahmad Rusli RN, Perumal KV, Yong YK, Hadizah S, Othman F, Salem K, Shafie NH, Hasham R, Yin KB, Abdul Kadir KK, Bahari H, Zakaria ZA. 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. 2023; 24(6)5224-5241.
53. Bird RP. Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett. 1995; 93(1): 55–71.
54. Norazalina S, Norhaizan ME, Hairuszah I, Norashareena MS. Anticarcinogenic efficacy of phytic acid extracted from rice bran on azoxymethane-induced colon carcinogenesis in rats. Exp Toxicol Pathol. 2010; 62(3): 259–268.
55. Fenoglio-Preiser CM, Noffsinger AE, Stemmermann GN, Lantz PE, Isaacson PG. Gastrointestinal pathology: An Atlas and Text. 3rd ed. Lippincott Williams and Wilkins. 2008.
56. Compton CC, Greene FL. The staging of colorectal cancer: 2004 and beyond. CA Cancer J Clin. 2004; 54(6): 295-308.
57. Umesalma S, Nagendraprabhu P, Sudhandiran G. Antiproliferative and apoptotic-inducing potential of ellagic acid against 1,2-dimethyl hydrazine-induced colon tumorigenesis in Wistar rats. Mol Cell Biochem. 2014; 388(1–2): 157–172
58. Wani AK, Akhtar N, Mir TG, Singh R, Jha PK, Mallik SK, Sinha S, Tripathi SK, Jain A, Jha A, Devkota HP, Prakash A. Targeting Apoptotic Pathway of Cancer Cells with Phytochemicals and Plant-Based Nanomaterials. Biomolecules. 2023; 13(2): 194-228.
59. Pucci B, Kasten M, Giordano A. Cell Cycle and Apoptosis. Neoplasia. 2000; 2(4): 291-299.
60. Tanaka, T. Colorectal carcinogenesis and oxidative stress: Role of natural antioxidants. Cancer Lett. 2020; 475: 88–96.
61. Saufi SM, Mastuki SN, Faudzi SMM, Saad N. Bioactive constituents of *Muntingia calabura* and their biological properties. *Plants*. 2021;10(4): 812.
62. Tengku MTS, Zainal ANI, Zainol MI, Ismail N. Phytochemicals as regulators of signaling pathways in colon cancer: A mechanistic review. Front Pharmacol. *2020;13*, 902175.
63. Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT**.** Natural products in drug discovery: advances and opportunities. Nat Rev Drug Discov. 2021*; 20*(3): 200–216.
64. Elmore S**.** Apoptosis: A review of programmed cell death. Toxicol Pathol. 2007; 35(4): 495–516.
65. Youle RJ, Strasser A. The BCL-2 protein family: opposing activities that mediate cell death. Nat Rev Mol Cell Biol. 2008;9(1):47–59.
66. Zakaria ZA, Mohd SMH, Cheema MS, Kader AA, Teh LK, Salleh MZ**.** Antinociceptive activity of methanolic extract of *Muntingia calabura* leaves: further elucidation of the possible mechanisms. *BMC Complement Altern Med*. 2014;14:63.
67. Zakaria ZA, Rofiee MS, Mohamed AM, Teh LK, Salleh MZ, Sulaiman MR**.** In vitro antiproliferative and antioxidant activities and total phenolic contents of the extracts of *Melastoma malabathricum* leaves. J Acupunct Meridian Stud. 2011; **4**(4): 248–256.
68. Yang C, Xie X, Tang H, Dong X, Zhang X, Huang F. *Transcriptome analysis reveals GA induced apoptosis in HCT116 human colon cancer cells through calcium and p53 signal pathways.* RSC Adv. 2018; 8:12449-12458
69. Wang A, Yoshimi N, Ino N, Tanaka T, Mori H. "Overexpression of cyclin B1 in human colorectal cancers." J Cancer Res Clin *Oncol*. 1997;123(2):124–127.
70. Perillo B, Di Donato M, Pezone A, Erika Di Z, Pia G, Giovanni G, Gabriella C, Antimo M. ROS in cancer therapy: The bright side of the moon. Exp Mol Med. 2020; 52(2): 192–203.
71. Takahashi M, Wakabayashi K. Gene mutations and altered gene expression in azoxymethane-induced colon carcinogenesis in rodents. Cancer Sci. 2004; 95(6): 475–480.
72. Rahman MM, Fakir MSA, Rahman MM. Antioxidant and anticancer activities of *Muntingia calabura*. Pharm Biol. 2020; 58(1): 1–12.
73. Perše M. Oxidative stress in the pathogenesis of colorectal cancer: cause or consequence? Biomed Res Int. 2013; 725710