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*Original Article*

# Dietary silymarin supplementation enhances chemotherapy efficacy of capecitabine and irinotecan and mitigates hepatotoxicity in a mouse model of colon cancer

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## *Abstract*

**Background and purpose:** The flavonoid silymarin (SMN) has shown promise due to its antioxidant, anti-inflammatory, and anticancer properties. SMN has been widely used in preclinical and clinical studies to treat various types of cancer, alone and with chemotherapy agents. Recent research suggests that SMN may increase conventional chemotherapy efficacy and reduce adverse effects. Herein, we investigated the therapeutic efficacy of SMN and its combination with capecitabine (CAP) and irinotecan (IRI) in a mouse model of colon cancer.
**Experimental approach:** Following 1,2 dimethylhydrazine-induced colon cancer, a modified diet supplemented with SMN (2500 ppm) and mono- and combined therapy of CAP and IRI was used. Serum samples were analyzed for lipid profile, liver function, and inflammatory cytokines. Oxidative stress and inflammation markers, including malondialdehyde (MDA), nitric oxide (NO), myeloperoxidase (MPO), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were measured in colonic, hepatic, and circulatory samples. Colonic BAX and Bcl-2 levels were examined *via* western blotting and histopathological analysis of colon sections was conducted.
**Findings/Results:** SMN alone and combined with chemotherapeutic agents significantly mitigated the elevated inflammatory cytokines liver function enzyme levels, and hyperlipidemia. Furthermore, SMN supplementation with chemotherapy agents enhanced antioxidant activity and reduced lipid peroxidation and inflammatory markers. Significant upregulation of BAX and downregulation of Bcl-2 were observed. In addition, treatment regimens ameliorated carcinogen-induced polyp multiplicity, adenoma formation, dysplastic changes, and lymphocytic aggregation.
**Conclusion and implications:** Our results demonstrated that the potential anticancer properties of SMN could enhance chemotherapy efficacy and reduce carcinogen- and chemotherapy-induced hepatotoxicity.

**Keywords:** Colorectal cancer; Combination therapy; Oxidative stress; Silymarin.

## INTRODUCTION

Colorectal cancer (CRC) is a global health concern, ranked as the third most frequently diagnosed cancer and the second leading cause of cancer-related deaths (1). According to WHO reports, CRC is the second most diagnosed cancer in women and the third in men. Familial, environmental, and dietary factors play pivotal roles in the etiology of CRC (2). Animal models of CRC provide a wide area of investigation for a better understanding of the possible pathways triggering CRC induction and studying the effects of dietary and environmental agents on disease prevention and treatment (3). 1,2 dimethylhydrazine (DMH), a strong DNA alkylating agent, is broadly utilized to induce CRC in animal models.

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The metabolism of DMH in the liver produces azoxymethane and methylazoxymethanol, which are further metabolized in the colon to generate diazonium ions after being transported through bile or blood (4,5). These ions cause colon carcinogenesis by forming reactive oxygen species (ROS) leading to lipid peroxidation and oxidative stress. Furthermore, many reports have addressed the impact of high levels of ROS on proliferation, invasion, drug resistance, and metastasis (6,7). Antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase play a crucial role in maintaining redox balance *via* preventing ROS activity and accumulation in the cells (8); nonetheless, higher lipid peroxidation end products such as malondialdehyde (MDA) relative to the antioxidant levels may result in CRC initiation and progression (9). In addition, the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α), as one of the targets of nuclear factor kappa B (NF-κB) was indicated to have an important role in the CRC initiation and progression stages (10).

Combination chemotherapy regimens like FOLFOX, FOLFOXIRI, CAPIRI, and CAPOX along with monoclonal antibodies are the cornerstone of CRC treatment. They aim to improve anticancer effects such as reduced metastatic potential and cancer stem cell populations, and induction of apoptosis, as well as suppressing the possible drug resistance of cancer cells to single drugs (11). Irinotecan (IRI) is an inhibitor of topoisomerase I that prevents DNA replication and induces apoptosis by inhibiting DNA supercoil relaxation. IRI monotherapy was first introduced as a potential agent for the treatment of CRC refractory to 5-fluorouracil (5-FU) (12). IRI in combination with 5-FU is usually used in the clinical treatment of CRC (13). Capecitabine (CAP), an orally administered fluoropyrimidine, is a prodrug of 5-FU that is converted to 5-FU in tumor tissues by thymidine phosphorylase and is shown to be less toxic and as effective and well-tolerated as 5-FU/leucovorin for stage III CRC (14,15). Several investigations have reported the effectiveness of IRI in combination with CAP (CAPIRI) or Xeloda (XELIRI) in patients suffering from CRC as the first or second line of chemotherapy. For instance, Laudani *et al.* indicated the effectiveness and tolerability of CAPIRI as the first-line therapy for metastatic colon cancer, suggesting the replacement of 5-FU with CAP in 5-FU-based regimens (16); however, various side effects were observed despite the therapeutic effects of these agents. Bone marrow suppression, gastrointestinal, and dermatologic toxicity were reported most frequently in patients undergoing treatment with CAP (17). In addition, gastrointestinal toxicity symptoms like diarrhea as one of the main toxic side effects in IRI monotherapy or combination therapy with fluoropyrimidines were documented. Unfortunately, these adverse effects can affect the patient's quality of life and drug dose adjustment (18,19).

Flavonoids have gained great interest in cancer prevention and treatment due to their safety and effectiveness. Silymarin (SMN), a flavonoid extracted from *Silybum marianum*, has been used for treating hepatic problems such as cirrhosis and hepatitis for many years (20). This flavonoid acts as a potent antioxidant through free radical scavenging, increasing the amount of glutathione, and inhibiting lipid peroxidation (21). SMN exerts its anti-inflammatory effects by modulating the genes involved in the NF-κB pathway including interleukin-1, cyclooxygenase-2, inducible nitric oxide synthase, and TNF-α (22). Numerous preclinical and clinical studies have confirmed the anticancer effects of SMN, including induction of apoptosis and suppression of cell proliferation, angiogenesis, and metastasis (23). Due to the lack of investigations about SMN-supplemented chemotherapy regimens, the present study was conducted to evaluate the effect of SMN supplementation on the mono- and combination therapy of CAP and IRI in the mouse model of DMH-induced colon carcinogenesis.

## MATERIALS AND METHODS

### *Chemicals*

DMH and SMN were obtained from Sigma (St. Louis, MO, USA). CAP was purchased from Actero Pharmaceuticals, Iran. IRI was purchased from Mylan, France.

Sulphanilamide, N-(1-naphthyl) ethylene-diamine.2HCL, thiobarbituric acid (TBA), and phosphoric acid (85%) were purchased from Merck (Darmstadt, Germany). n-Butanol was obtained from Carl Roth, GmbH Co. (Karlsruhe, Germany).

### *Animal grouping, housing, and diet*

Fifty-six male BALB/c mice weighing between 25-30 g were obtained from the Central Animal House of Urmia University of Medical Sciences, Urmia, Iran. The study was conducted according to the guidelines of the National Research Council and approved by the Ethics Committee of Urmia University of Medical Sciences (Ethical code: IR.UMSU.REC.1400.170). The animals were housed in plastic cages at a temperature of 25 ℃ under 12/12-h light/dark cycles until the end of the study period. A modified pellet diet containing 2500 ppm SMN was prepared daily for the SMN-supplemented treatment groups. Animals had access to diet and water *ad libitum.*

### *Cancer induction*

DMH was dissolved in a 0.9% saline solution containing 1 mM ethylene-diaminetetraacetic acid (EDTA) and 10 mM sodium citrate, pH 8. CRC in mice was induced by weekly intraperitoneal (i.p.) injection of 20 mg/kg (body weight (BW)) DMH for ten consecutive weeks (24).

### *Study design*

Following one week of acclimatization, mice were randomly allocated into seven groups, eight each. The treatment schedule was as follows: control group (group 1), animals were administered the vehicle (0.9% saline solution containing 1 mM EDTA and 10 mM sodium citrate, i.p.) weekly for 10 weeks and received a normal commercial diet; DMH group (group 2), the mice were weekly injected 20 mg/kg (BW) DMH i.p. for ten weeks and were fed with a normal commercial pellet diet; DMH + SMN (group 3), animals were fed with a modified diet containing SMN (2500 ppm) prepared daily for eight consecutive weeks post CRC initiation ($11^{th}$ - $18^{th}$ week) (25); DMH + SMN-CAP (group 4), animals daily received CAP orally at 200 mg/kg (BW) dissolved in sterile normal saline for eight weeks (26); DMH + SMN-IRI (group 5), IRI was injected i.p. to mice at 50 mg/kg (BW) once a week for eight weeks (27); DMH + CAPIRI (group 6), mice received daily oral administration of CAP at 100 mg/kg (BW) and weekly injections of IRI at 25 mg/kg (BW) i.p. for eight weeks; DMH + SMN-CAPIRI (group 7), mice received CAP orally at 100 mg/kg (BW) daily and were given weekly injections of IRI at 25 mg/kg (BW) i.p. for eight weeks.

Tumor induction in groups 3-7 was done similarly to group 2. Additionally, groups 3-5, and 7 daily received the SMN-supplemented diet during the chemotherapy period. Figure 1 shows the time course of the experiment.

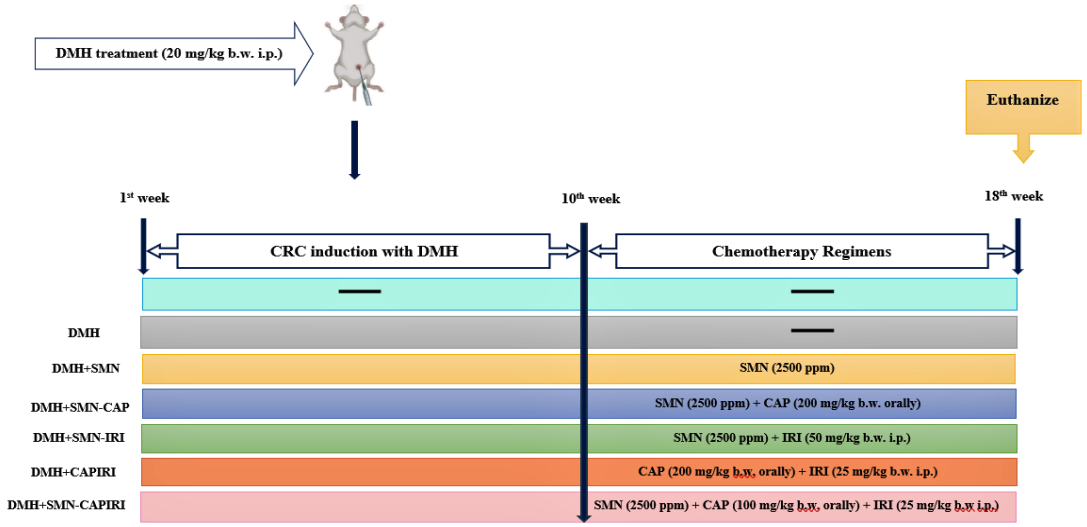


**Fig. 1.** Time course and treatment design. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

***Animal euthanasia, blood sample collection, and tissue preparation***

At the end of the 18th week, blood samples from all animals were collected through cardiac puncture while they were under anesthesia with a ketamine-xylazine cocktail (ketamine: 90 mg/kg and xylazine: 9 mg/kg, i.p.). The blood samples were left to coagulate at room temperature for 15 min and then were centrifuged at 3000 *g* for 10 min to obtain the sera. After blood collection, the animals were euthanized using an overdose injection of sodium pentobarbital (200 mg/kg, BW, i.p.), and immediately the colon and liver tissues were dissected. Following the dissection, colons were opened longitudinally and flushed with physiological saline for further macroscopic evaluation of the polyps. Later, the colon tissues were divided into two parts. One part was washed with chilled saline and was kept at -70 ℃ for biochemical and enzymatic examinations. The second part was preserved in 10% buffered formaldehyde for further histopathological analysis. The same procedure was done to the liver tissues.

***Measurement of polyp incidence***

To measure the polyp incidence in the colon, following the cleansing and flushing of the colon, the number of the present polyps in every mouse was counted precisely to determine the average number of polyps per group as well as the percentage of the polyp incidence. The average number of polyp-bearing mice was calculated as the ratio of the total number of polyps to the number of polyp-bearing mice in each experimental group. Moreover, the polyp incidence percentage was calculated as the percentage of the ratio of the total number of polyps in every treatment group to the same value of the DMH group.

***Biochemical estimations***

The serum levels of lipid profile indices including total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL), high-density lipoprotein (HDL), liver function enzymes including alanine aminotransferase (ALT), aspartate amino-transferase (AST), as well as lactate dehydrogenase (LDH) as a marker of tissue damage were measured commercially utilizing colorimetric diagnostic kits (Pars Azmoon Inc., Tehran, Iran) by biochemistry auto-analyzer system (BT3000, Italy).

***Determination of circulatory, colonic, and hepatic antioxidant enzymes***

The enzymatic activities of SOD and GPx in the serum, colon, and liver specimens were measured using commercially available kits (Zellbio GmbH, Hinter den Gärten 5689173 Lonsee, Germany, CAT No. ZB-SOD-96A & ZB-GPX-96A) according to the manufacturer's instructions.

***MDA measurements in colon and liver specimens***

To evaluate the lipid peroxidation rate, the MDA content of colon and liver samples was examined *via* TBA reaction as described previously (28). Briefly, colon samples were homogenized in ice-cooled KCl (150 mM). Thereafter, the mixture was centrifuged at 3000 *g* for 10 min; 0.5 mL of the supernatant was mixed with 3 mL phosphoric acid (1% V/V) and after vortex mixing, 2 mL of 6.7 g LTBA was added to the samples. The samples were heated at 100 °C for 45 min and then chilled on ice. After adding 3 mL n-butanol, the samples were centrifuged at 3000 *g* for 10 min. The absorbance of the supernatant was measured spectrophotometrically at 540 nm and expressed as the samples' nmol/mg protein.

***Nitric oxide determination in colon and liver Samples***

To assess nitrosative stress, the nitric oxide (NO) content of the colonic and hepatic homogenates was measured using the Griess reaction (29) in which, NO is transformed into nitrite as a more stable metabolite that is then converted to $HNO_2$ in an acidic environment. $HNO_2$ forms a diazonium salt in reaction with sulphanilamide, which reacts with N-(1-naphthyl) ethylene-diamine.2HCl to generate an azo dye detectable at 540 nm. The NO content of the examined specimens was expressed as nmol/mg of protein in the sample.

***Colonic and hepatic myeloperoxidase activity assessment***

Myeloperoxidase (MPO) activity was measured in the colonic and hepatic homogenates as previously described (30). Briefly, the colon specimens were homogenized in 10 mM potassium phosphate buffer (pH 7.0), containing 0.5% hexadecyl trimethyl ammonium bromide, and centrifuged at 20000 *g* for 30 min at 4 ℃. A solution of 1.6 mM tetramethyl benzidine and 0.1 mM $H_2O_2$ was added and reacted with an aliquot of the supernatant. One unit of MPO activity was defined as degrading 1 µmol of $H_2O_2$ / min at 37 ℃. The color change was measured at 650 nm spectrophotometrically and the MPO activity was expressed as units/mg of the tissue sample.

***Inflammatory cytokines assessment in serum samples***

The serum levels of C-reactive protein (CRP) and TNF-α were determined using mice-specific R&D systems kits (614 McKinley Place NE Minneapolis, MN 55413, USA; TNF-α CAT No: DY410, CRP CAT No. DY1829) according to the manufacturer's instructions and were expressed as pmol/mL.

***Protein content estimation***

Lowry method was utilized to measure the total protein content of the samples (31).

***Histopathological examinations***

Fixed colon tissues from all groups were embedded in paraffin. Then, paraffin-embedded tissues were cut into 5-µm sections and stained with hematoxylin and eosin (H&E) and later evaluated with a light microscope (Nikon, Tokyo, Japan) for any histoarchitecture changes in the colonic mucosa focusing on the middle and distal colonic sections.

***Western blotting analysis***

To evaluate the effect of SMN treatment strategies on crucial anti-apoptotic and pro-apoptotic genes involved in CRC, the protein levels of BAX and Bcl-2 in colon samples of all test groups were estimated *via* western blotting. First, radioimmunoprecipitation assay buffer (RIPA; Sigma-Aldrich, USA) was utilized to prepare colon tissue protein samples for the test. The Bradford method was used to determine the protein concentration of the colon samples. Later, the proteins were separated *via* 10% sodium dodecyl-polyacrylamide gel electrophoresis (SDS-PAGE), followed by transferring the separated proteins onto a polyvinylidene difluoride membrane (PVDF; Sigma-St. Louis, MO, USA). The PVDF membrane was subsequently placed in a buffer containing 5% bovine serum albumin at room temperature for 2 h, followed by the addition of polyclonal primary antibodies (Santa Cruz, UK) of BAX and Bcl-2 and incubation at 4 ℃ overnight. This step was followed by adding secondary antibodies (Santa Cruz, UK), and incubating at room temperature for 2 h. Eventually, enhanced chemiluminescence (BIO-RAD, USA) was applied for antigen-antibody visualization, and Image J software (National Institute of Health, Bethesda, Maryland, USA) was utilized for densitometry analysis.

***Statistical analysis***

Kolmogorov-Smirnov test was used to assess the normality of data and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was performed to compare the groups of interest using GraphPad Prism software (version 9). Data are presented as mean ± SD. *P*-values < 0.05 were considered statistically significant.

## RESULTS

***General observations***

All mice except for the CAPIRI and DMH groups survived until the end of the study period. Three mice in the CAPIRI group and one mouse in the DMH group died during the experimental period. The data regarding the body weight changes and the growth ratio are presented in Table 1. DMH-exposed mice displayed significant weight loss at the end of the study compared to the control group. Except for SMN alone and SMN-IRI, other treatment strategies did not significantly affect the weight changes compared to the DMH group. A significant difference in weight gain was seen between the CAPIRI and

SMN-CAPIRI groups. Additionally, the growth rate was calculated as the difference between the final and initial weight gain divided by the total days (126) of the study. The results indicated that DMH led to a remarkable reduction in the weight gain and growth rate parameters; while, SMN alone, SMN-IRI, and SMN-CAPIRI experimental groups showed significant changes in the weight gain and growth rate compared to the DMH group. SMN-CAPIRI could significantly improve the growth rate in comparison with CAPIRI, as well.

### *Effects of SMN-added chemotherapy regimens on colonic polyp incidence*

The results showed DMH exposure led to a significant increase in the average number of polyp-bearing mice compared to the control group; while all treatment regimens could significantly ameliorate polyp formation. In addition, the highest percentage of tumor incidence belonged to the DMH group (100%) and the polyps were usually observed at the distal segment of the colon. SMN alone could remarkably reduce tumor incidence to half of the DMH group. Further, the tumor incidence in SMN-CAP was lower than SMN alone and SMN-IRI. The highest reducing effect was observed in SMN-CAPIRI (4%) followed by CAPIRI (8%) suggesting their ability for optimum prevention of DMH-induced CRC (Table 2 and Fig. 2).

**Table 1.** Changes in body weight and growth ratio. Data represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI groups.

| Groups | Initial body weight (g) (1st week) | Final body weight (g) (18th week) | Weight gain (g) | Growth rate |
|---|---|---|---|---|
| **Control** | 27.62 ± 1.68 | 44.42 ± 2.36 | 16.80 ± 2.30 | 0.133 ± 0.018 |
| **DMH** | 29.12 ± 1.12 | 38.13 ± 1.19$^{*}$ | 9.26 ± 1.46$^{*}$ | 0.073 ± 0.011$^{*}$ |
| **DMH + SMN** | 27.75 ± 1.28 | 41.92 ± 1.18$^{\#}$ | 13.55 ± 1.62 | 0.107 ± 0.010$^{\#}$ |
| **DMH + SMN-CAP** | 28.62 ± 1.31 | 40.75 ± 2.48 | 12.12 ± 2.79 | 0.096 ± 0.022 |
| **DMH + SMN-IRI** | 28.87 ± 1.45 | 43.01 ± 2.95$^{\#}$ | 14.14 ± 3.28$^{\#}$ | 0.112 ± 0.026$^{\#}$ |
| **DMH + CAPIRI** | 28.61 ± 1.34 | 37.45 ± 1.13 | 8.85 ± 2.17 | 0.070 ± 0.017 |
| **DMH + SMN-CAPIRI** | 28.25 ± 1.58 | 41.29 ± 1.36$^{\&}$ | 14.42 ± 2.53$^{\#, \&}$ | 0.110 ± 0.017$^{\#, \&}$ |

DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

**Table 2.** Effects of treatment strategies on colon polyps. Data of the average number of polyp-bearing mice represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group.

| Groups | Total number of mice | Total number of polyps | Average number of polyp-bearing mice | Percentage of polyps incidence |
|---|---|---|---|---|
| **Control** | 8/8 | 0 | 0 | 0 |
| **DMH** | 7/8 | 23 | 3.28 ± 0.95* | 100 |
| **DMH + SMN** | 8/8 | 12 | 1.50 ± 0.53$^{\#}$ | 52 |
| **DMH + SMN-CAP** | 8/8 | 9 | 1.12 ± 0.35$^{\#}$ | 39 |
| **DMH + SMN-IRI** | 8/8 | 11 | 1.37 ± 0.51$^{\#}$ | 47 |
| **DMH + CAPIRI** | 5/8 | 2 | 0.40 ± 0.54$^{\#}$ | 8 |
| **DMH + SMN-CAPIRI** | 8/8 | 1 | 0.12 ± 0.35$^{\#}$ | 4 |

DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

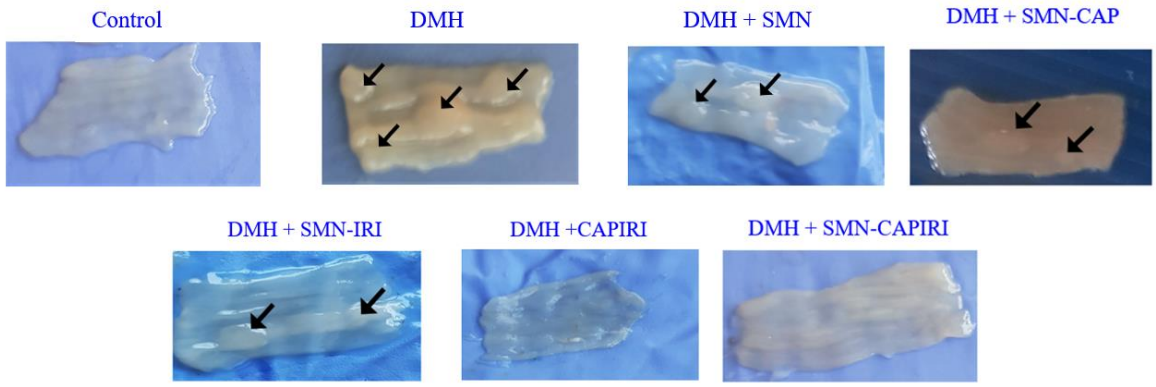


**Fig. 2.** Macroscopic evaluation of the polyps. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan

**Table 3.** Effect of SMN-supplementation on chemotherapy regimens on the lipid profile and hepatic enzyme changes. Data represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences among DMH + SMN, DMH + SMN-CAP, and DMH + SMN-IRI; $^{\$}P < 0.05$ significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI.

| Groups | TC (mg/dL) | TG (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | ALT (U/L) | AST (U/L) |
|---|---|---|---|---|---|---|
| **Control** | 83.33 ± 6.13 | 59.45 ± 2.97 | 77 ± 10.5 | 17.16 ± 2.19 | 11.25 ± 1.70 | 64. 20 ± 3.83 |
| **DMH** | 116.40 ± 3.04$^{*}$ | 115.2 ± 10.48$^{*}$ | 49 ± 3.16$^{*}$ | 27.40 ± 3.50$^{*}$ | 57.00 ± 11.22$^{*}$ | 231.8 ± 29.36$^{*}$ |
| **DMH + SMN** | 97.30 ± 6.77$^{\#}$ | 81.33 ± 11.13$^{\#}$ | 65.6 ± 4.87$^{\#}$ | 15.72 ±1.86$^{\#,\&}$ | 16.10 ± 3.39$^{\#}$ | 88 ± 3.16$^{\#,\&}$ |
| **DMH + SMN-CAP** | 99.66 ± 11.5$^{\#}$ | 96.76 ± 6.1$^{\#}$ | 69.80 ± 3.49$^{\#}$ | 24.58 ± 3.82$^{\#}$ | 39.40 ± 8.41$^{\#}$ | 221.4 ± 5.41 |
| **DMH + SMN-IRI** | 100.80 ± 5.63$^{\#}$ | 91.56 ± 14.86$^{\#}$ | 63.80 ± 5.26 | 23.00 ± 4.19$^{\#}$ | 25.00 ± 2.84$^{\#}$ | 215.20 ± 9.28 |
| **DMH + CAPIRI** | 96 ± 8.03$^{\#}$ | 68.31±6.09$^{\#}$ | 70.60 ± 8.82$^{\#}$ | 19.14 ± 2.00$^{\#}$ | 53.5 ± 7.39$^{\#}$ | 199.2 ± 26.75$^{\#}$ |
| **DMH + SMN-CAPIRI** | 88 ± 7.35$^{\#}$ | 62.42 ± 6.16$^{\#}$ | 73.20 ± 5.80$^{\#}$ | 16.38 ± 2.02$^{\#}$ | 25.7 ± 3.43$^{\#,\$}$ | 131.50 ± 14.06$^{\#,\$}$ |

DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; TC, total cholesterol; LDL, low-density lipoprotein; HDL, high-density lipoprotein

### *Effects of SMN-added chemotherapy regimens on the lipid profile*

Table 3 presents all alterations in lipid profile indices. Our results indicated that DMH significantly increased serum TC levels compared to the control group. All treatment regimens could markedly reduce TC levels, especially CAPIRI and SMN-CAPIRI, which restored TC alteration to normal levels; nonetheless, no significant changes were found among groups 3, 4, and 5 in TC levels.

DMH also elevated TG levels significantly relative to the control group. In all groups, there was a significant decrease in serum TG levels compared with the DMH group. There were no significant changes among groups 3, 4, and 5 in TG levels. Despite a decrease in TG levels due to SMN-CAPIRI treatment relative to CAPIRI, the change was not statistically significant ($P > 0.05$). HDL levels declined significantly in the sera of DMH-treated mice in comparison with the control mice. While all other treatments considerably increased the level of HDL compared to the DMH group, except for the DMH + SMN-IRI treatment. Moreover, DMH elevated LDL levels significantly compared to the control group; however, it was reduced considerably by all chemotherapy regimens respective to the DMH group. SMN alone reduced LDL levels more than SMN-added monotherapy regimens . No significant differences between SMN-CAP and SMN-IRI as well as CAPIRI and SMN-CAPIRI were observed. Notably, CAPIRI and especially SMN-CAPIRI treatment groups could normalize the alterations in lipid profiles.

**Table 4.** Effect of SMN-supplementation on chemotherapy regimens on circulatory inflammatory cytokines, antioxidant enzymes, and LDH. Data represent mean ± SD. *$P$ < 0.05 indicates significant differences between DMH and control, #$P$ < 0.05 versus DMH-treated group, &$P$ < 0.05 significant differences among DMH + SMN, DMH + SMN-CAP, and DMH + SMN-IRI; $$P$ < 0.05 significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI.

| Groups | TNF-α (pmol/mL) | CRP (pmol/mL) | SOD (U/mL) | GPx (U/mL) | LDH (U/L) |
|---|---|---|---|---|---|
| **Control** | 422.1 ± 2.38 | 1553.16 ± 67.28 | 34.57 ± 0.65 | 127.9 ± 1.23 | 285.2 ± 47.65 |
| **DMH** | 673.6 ± 2.84* | 2133.09 ± 49.16* | 19.80 ± 1.34* | 87.04 ± 1.73* | 1908 ± 203.5* |
| **DMH + SMN** | 561.3 ± 2.02# | 1914.81 ± 36.46# | 24.28 ± 0.74# | 104.8 ± 1.79# | 797.1 ± 98.46#,& |
| **DMH + SMN-CAP** | 598.6 ± 4.43#,& | 1690.23 ± 29.95#,& | 28.57 ± 0.77#,& | 118.1 ± 1.36#,& | 1531 ± 115.9# |
| **DMH + SMN-IRI** | 564.2 ± 4.48# | 1878.13 ± 22.68# | 26.65 ± 0.54#,& | 112.8 ± 0.99#,& | 1263 ± 181.5# |
| **DMH + CAPIRI** | 580.2 ± 6.33# | 1884.11 ± 14.17# | 30.48 ± 1.12# | 117.3 ± 0.43# | 1802 ± 141.9 |
| **DMH + SMN-CAPIRI** | 496.4 ± 7.37#,$ | 1828.95 ± 15.5# | 32.64 ± 1.21#,$ | 123.5 ± 1.33#,$ | 1396 ± 90.81#,$ |

DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan; TNF-α, tumor necrosis factor- α; CRP, C-reactive protein; SOD, superoxide dismutase; GPx, glutathione peroxidase; LDH, lactate dehydrogenase

### *Effects of SMN-added chemotherapy regimens on circulatory inflammatory cytokines and LDH levels*

Data regarding the serum levels of TNF-α, CRP, and LDH are presented in Table 4. The results indicated that the serum TNF-α and CRP levels elevated significantly in the DMH group in comparison with the control group; whereas all chemotherapy regimens significantly decreased TNF-α levels compared with the DMH group. SMN alone and SMN-IRI were able to produce a greater reduction than SMN-CAP in TNF-α levels; while this effect was reversed in CRP levels. Additionally, SMN-CAPIRI caused a significant decrease in TNF-α levels and an insignificant decrease in CRP levels compared to CAPIRI treatment.

Furthermore, serum LDH levels increased significantly in the DMH-injected mice in comparison with the control mice. All treatment regimens significantly reduced LDH levels compared to the DMH group; however, this was insignificant in the CAPIRI group. SMN alone and SMN-IRI decreased LDH levels compared to SMN-CAP. There was also a significant difference between CAPIRI and SMN-CAPIRI-treated groups.

### *Effects of SMN-added chemotherapy regimens on the colonic MDA and NO content and MPO activity levels*

The changes in the levels of MDA and NO as well as MPO activity are depicted in Fig. 3A-C, respectively. The results showed that DMH treatment led to a considerable rise in the MDA level of colon tissue in comparison with the control group; whereas, it was reduced significantly in all treatment groups compared to the DMH group. SMN-CAP induced the highest reduction in MDA levels compared to SMN alone and SMN-IRI. The levels of MDA decreased more in the SMN-CAPIRI group than in the CAPIRI-treated mice. SMN-CAPIRI reduced MDA levels significantly as compared to CAPIRI.

DMH caused a considerable increase in colonic NO levels, which were significantly reversed by all chemotherapy regimens. SMN alone had the best-decreasing effect in comparison with SMN-CAP and SMN-IRI; while no significant changes were observed between SMN-CAP and SMN-IRI. Furthermore, SMN-CAPIRI had a significantly greater impact on reducing the NO levels than CAPIRI.

Levels of colonic MPO activity were assessed, as well. Our findings demonstrated that DMH led to a significant elevation in MPO activity in the colonic homogenates compared to the control group; whereas, it decreased significantly in all chemotherapy groups. Evaluation of the effects of SMN alone and in combination with CAP and IRI revealed that SMN-CAP had the strongest attenuating effect on the colonic MPO activity. In addition, adding SMN to the CAPIRI combination therapy resulted in a significant decrease in colonic MPO activity levels.

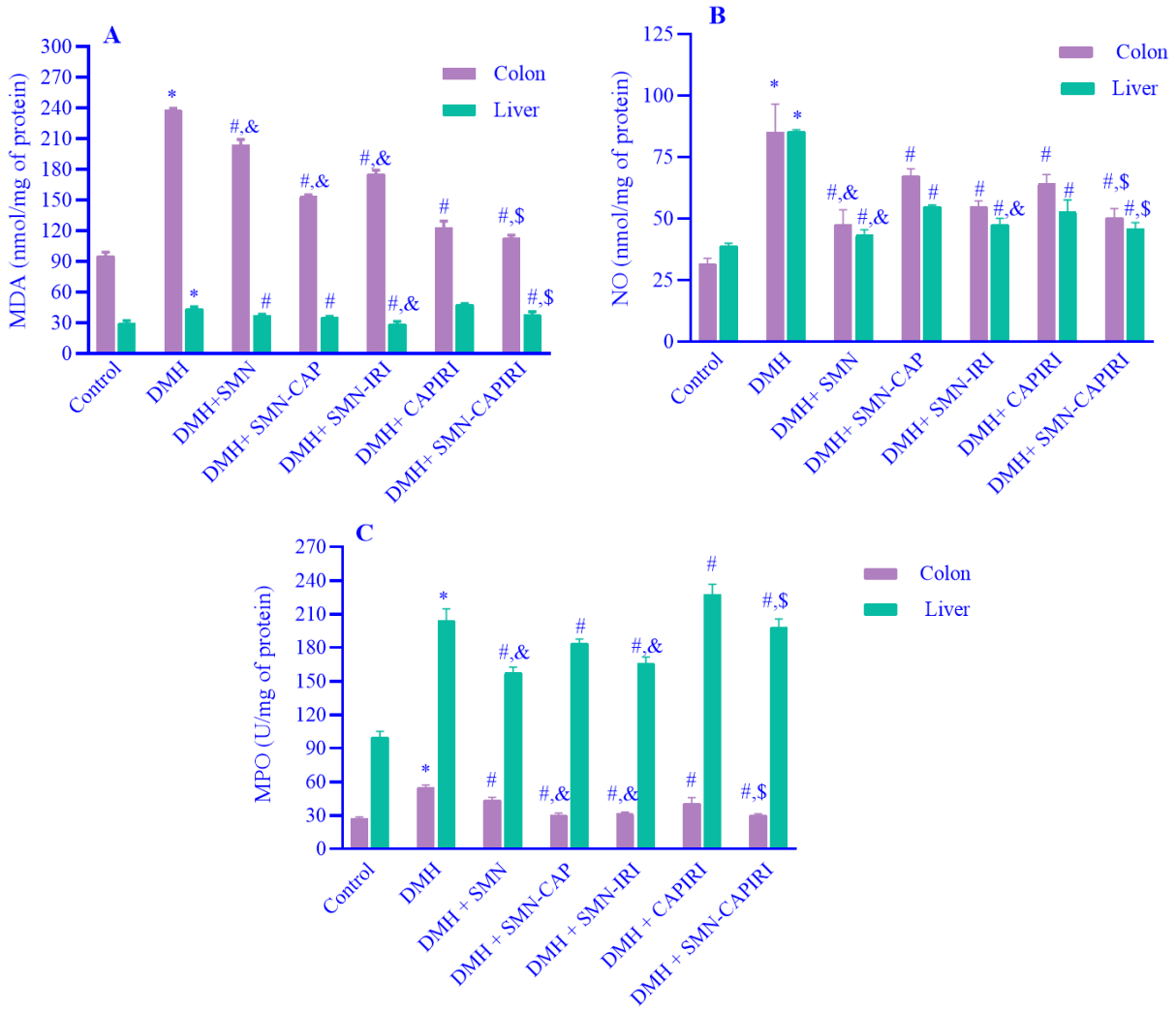


**Fig. 3.** Effect of SMN-supplementation on chemotherapy regimens on the colonic and hepatic (A) MDA, (B) NO, and (C) MPO levels. Data represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences among DMH + SMN, DMH + SMN-CAP, and DMH + SMN-IRI. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan; MDA, malondialdehyde; NO, nitric oxide; MPO, myeloperoxidase.

***Effects of SMN-added chemotherapy regimens on circulatory and colonic antioxidant enzymes***

The antioxidant changes are presented in Table 4 and Fig. 4A and B. SOD activity in the colon and serum was significantly lowered in the DMH-treated mice compared to the control mice; however, the circulatory and colonic SOD levels elevated significantly in all chemotherapy groups relative to the DMH-administered group. SMN-CAP caused a significant increase in the colonic SOD levels in comparison with SMN-IRI and SMN-alone; while the changes in the circulatory SOD levels between SMN-CAP and SMN-IRI showed no significant differences. In addition, circulatory and colonic SOD levels were significantly increased by the SMN-CAPIRI regimen compared to CAPIRI.

Similarly, DMH led to a remarkable reduction in circulatory and colonic GPx activity, which was reversed by all treatment regimens. The circulatory and colonic GPx activity in the SMN-CAP group showed a significant increase compared to SMN-IRI and SMN alone. Moreover, there were significant differences in the circulatory and colonic GPx activity between SMN-CAPIRI and CAPIRI regimens.

***Effects of SMN-added chemotherapy regimens on liver***

We examined the liver function enzymes and inflammation and oxidative stress markers of the liver to assess the liver changes during carcinogenesis and chemotherapy. The alterations regarding the liver examinations are shown in Tables 3 and 4 and Figs. 3 and 4.

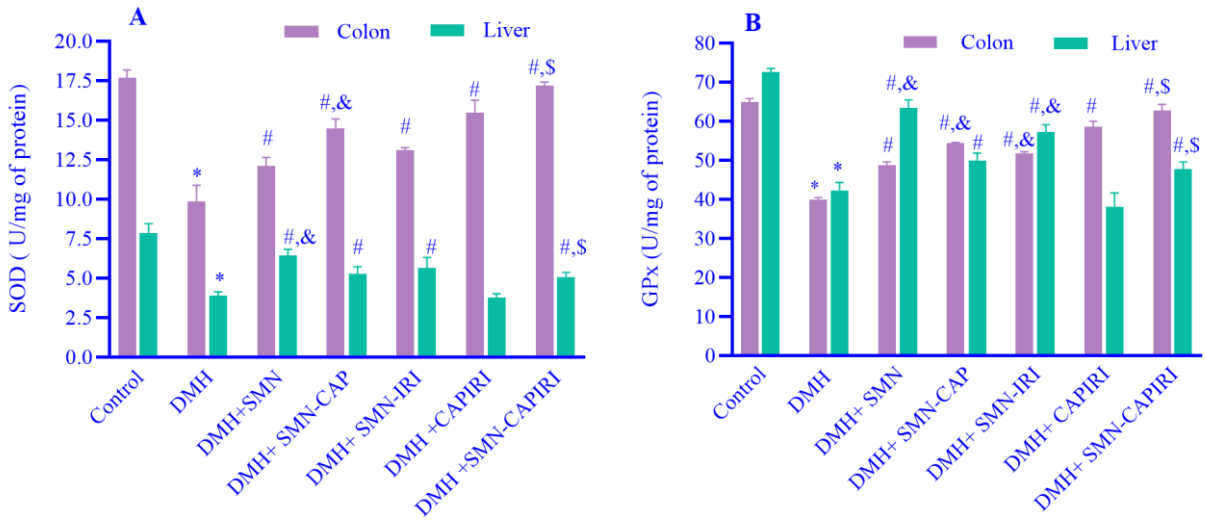


**Fig. 4.** Effect of SMN-supplementation on chemotherapy regimens on the colonic and hepatic (A) SOD and (B) GPx levels. Data represent mean ± SD. *$P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences among DMH + SMN, DMH + SMN-CAP, and DMH + SMN-IRI; $^{\$}P < 0.05$ significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan; SOD, superoxide dismutase; GPx, glutathione peroxidase.

Our results indicated that DMH exposure significantly increased serum transaminases, ALT and AST, compared to their respective control groups. All treatment regimens, except for DMH + SMN-CAP and DMH + SMN-IRI groups, could restore the alterations significantly. SMN alone had the highest effect on reducing ALT levels, whereas the least decreasing effect was observed in the CAPIRI regimen. SMN-CAPIRI compared to CAPIRI could significantly decline the transaminase levels.

Furthermore, DMH led to a noticeable increase in the hepatic MDA levels and MPO activity compared to the control group. The treatment regimens significantly reduced the evaluated factors compared to the DMH group except for CAPIRI which caused elevations in MDA and MPO amounts. Contrary to the colonic MDA values, SMN-IRI reduced the liver MDA levels better in comparison to SMN-CAP and SMN alone; while, concerning MPO activity, the most improving effect was seen in SMN alone group compared to SMN-supplemented monotherapies. Moreover, there was a significant difference between SMN-CAP and SMN-IRI in MDA and MPO levels. Adding SMN to CAPIRI chemotherapy reduced MDA and MPO levels markedly compared to CAPIRI.

Hepatic NO levels in the DMH group showed a dramatic increase compared to the control group. Surprisingly, all treatment strategies significantly diminished NO levels when compared to the DMH group. SMN alone had the most pronounced decreasing effect relative to the SMN-added monotherapy agents. Furthermore, SMN-IRI compared to SMN-CAP as well as SMN-CAPIRI compared to CAPIRI led to a significantly greater decrease in hepatic NO levels. A significant decrease in SOD and GPx activity levels in DMH-exposed mice was evident in the liver homogenates; whilst all treatment groups, except for CAPIRI, enhanced the antioxidant activities. In both enzymes, SMN alone exhibited the most pronounced effect on increasing the enzyme activities. SMN-IRI caused a significant and insignificant increase in the SOD and GPx levels relative to SMN-CAP, respectively. Moreover, a significant elevation in the antioxidant activity of liver specimens was shown in SMN-CAPIRI as compared with CAPIRI.

### *Effects of DMH and SMN-added chemotherapy regimens on colon histopathology*

The histopathological assessment of the control group exhibited the normal structure of the colon mucosa. DMH exposure caused

pedunculated adenoma and high-grade dysplastic changes, including goblet cell depletion, nuclear pleomorphism, and stratification. In addition, lymphocytic aggregation and infiltration were observed in the DMH group. Nevertheless, treatment with SMN alone and in combination with CAP and IRI resulted in a remarkable elevation in the number of goblet cells as well as a reduction in the stratified and inflamed areas, thus, an amelioration of the dysplastic changes was apparent. Notably, the changes in SMN-added chemotherapy agents were obvious in the lower parts of the mucosa adjacent to the muscular layer. Moreover, CAPIRI and SMN-CAPIRI regimens resulted in almost complete resolution of the dysplastic changes. The minor traces of inflammation in the CAPIRI group were eliminated in the colon sections of the SMN-CAPIRI group (Fig. 5).

***Effects of DMH and SMN-added chemotherapy regimens on the protein expression of BAX and Bcl-2***

BAX levels were found to be reduced dramatically in DMH-administered mice in comparison with the control group mice. The treatment regimens significantly increased BAX levels. SMN addition to CAPIRI significantly upregulated BAX expression levels when compared to CAPIRI; nonetheless, no significant changes were observed among SMN alone and SMN-added monotherapy agents (Fig. 6).

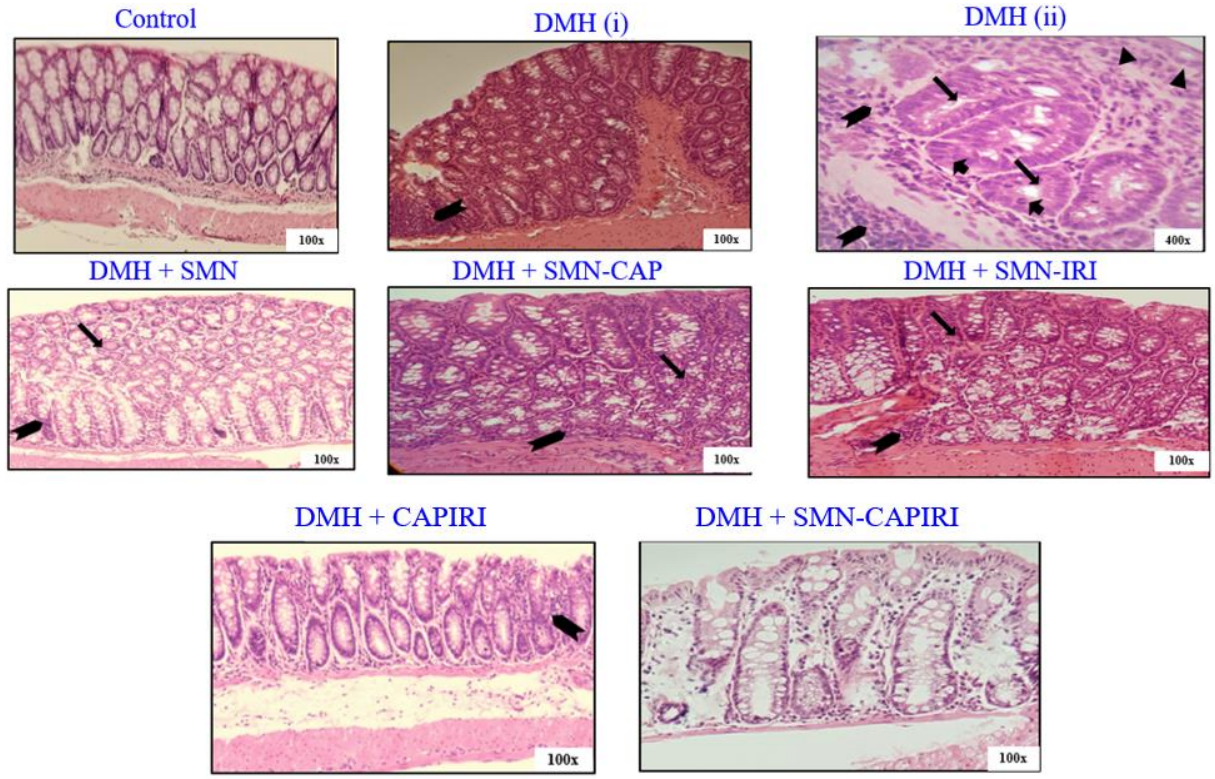


**Fig. 5.** Photomicrographs of histologic (H&E) cross sections of the colon samples from the control, DMH, DMH + SMN, DMH + SMN-CAP, DMH + SMN-IRI, DMH + CAPIRI, and DMH + SMN-CAPIRI groups examined after 18 weeks of treatment schedule. Control section shows the normal mucosal and submucosal architecture (×100). DMH (i) demonstrates pedunculated adenoma formation, high-grade dysplasia and significant lymphocytic aggregation (100×). DMH (ii) demonstrates stratification with multiple cell layers (▶) goblet cell depletion indicating reduced mucin production (⬆), lymphocytic infiltration(⬆), and pleomorphism with variations in size and shape of nuclei (⬆) (400×). DMH + SMN: Mild dysplastic changes in terms of goblet cell depletion (⬆) and attenuation of inflammation (⬆) (100×) DMH + SMN-CAP, DMH + SMN-IRI: mild dysplastic changes in terms of goblet cell depletion (⬆) and attenuation of inflammation (⬆) (100×). DMH + CAPIRI: Almost normal histology of the mucosal and submucosal layers, light inflammation (⬆) preserved glandular structures, suggesting effective treatment (100×). DMH+ SMNCAPIRI: Normal histology of the mucosal and submucosal layers with well-preserved crypt architecture and absence of inflammatory cells or dysplastic changes, indicating the most effective therapeutic outcome (100×). DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

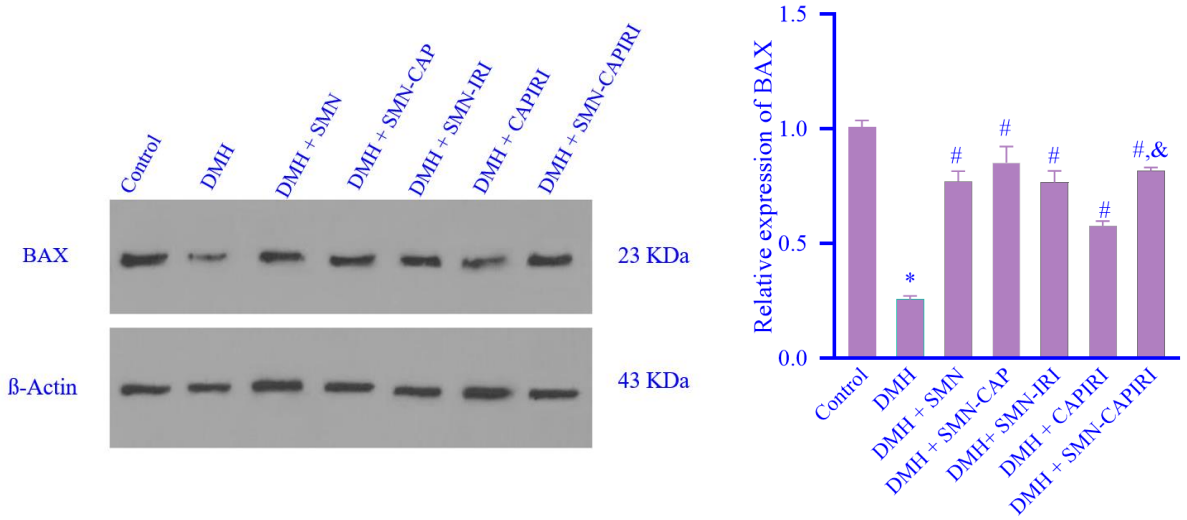


**Fig. 6.** Effect of SMN-supplementation on chemotherapy regimens on the relative expression of BAX. Data represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI groups. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

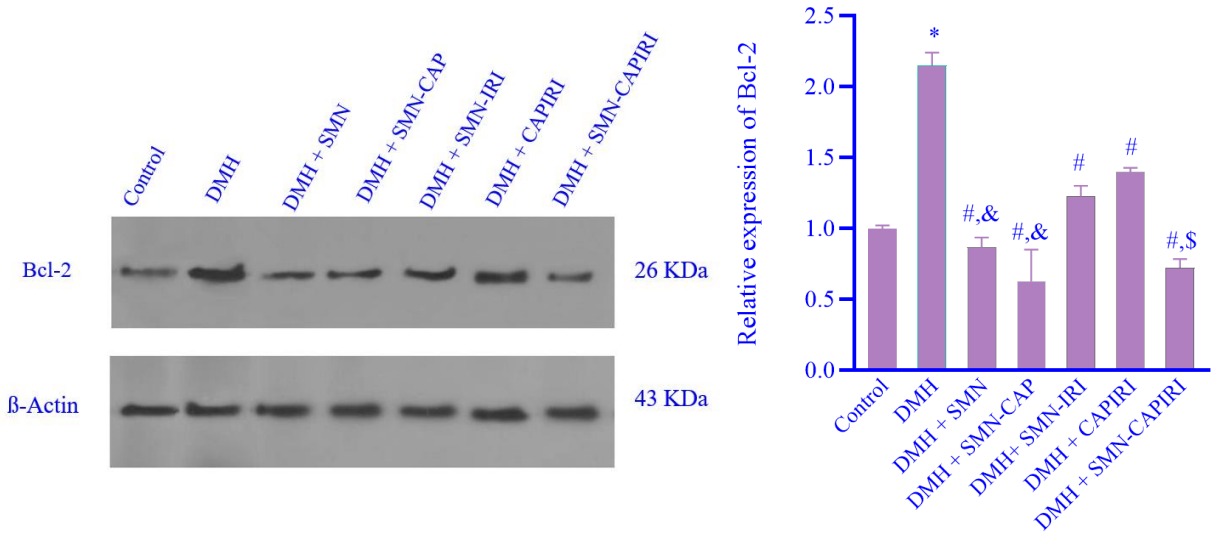


**Fig. 7.** Effect of SMN-supplementation on chemotherapy regimens on the relative expression of Bcl-2. Data represent mean ± SD. $^{*}P < 0.05$ indicates significant differences between DMH and control, $^{\#}P < 0.05$ versus DMH-treated group, $^{\&}P < 0.05$ significant differences between DMH + CAPIRI and DMH + SMN-CAPIRI groups. DMH, 1, 2 Dimethylhydrazine; SMN, silymarin; CAP, capecitabine; IRI, irinotecan.

On the other hand, DMH administration caused a remarkable elevation in Bcl-2 expression levels, which was reversed significantly in all treated groups. SMN-CAP had a better effect compared with SMN-IRI while no significant changes were seen between SMN alone and SMN-CAP. SMN-CAPIRI was able to downregulate the expression of Bcl-2 noticeably relative to CAPIRI and almost near to the control group (Fig. 7).

## DISCUSSION

Previous reports have documented the close association between inflammation and CRC development. Thus, patients enduring ulcerative colitis are at high risk of CRC progression (32). A combination of chemotherapy agents is extensively utilized for various types of cancer including CRC. For CRC, adjuvant fluoropyrimidine-based (5-FU or CAP) chemotherapy combined with oxaliplatin and/or IRI is frequently used (33); however, organ toxicities, mainly hepatotoxicity, caused by every single drug may accumulate and therefore, affect the treatment process negatively. Recent studies have postulated that the incorporation of dietary supplements, particularly flavonoids, alongside

conventional chemotherapy medications, may confer protective effects while mitigating toxicity (34). Given the reported anti-tumor and anti-inflammatory properties of SMN, it has been used widely in cancer prevention and treatment investigations. In the current study, the possible potentiating effects of SMN supplementation on monotherapies and combination therapy with CAP and IRI in the mouse CRC model were evaluated.

The body weight and growth ratio of the DMH-exposed mice were significantly reduced throughout the experimental period compared to the control mice, which has also been reported by Thangaraj *et al.*(35). Decreased body weight and food intake are associated with altered metabolism, polyp development, and inflammation of the colon, which indicates the importance of body weight changes in cancer research (36). SMN-supplemented IRI and CAPIRI regimens significantly improved weight gain in mice.

Previous investigations indicated the association between dyslipidemia and increased risk of CRC (37). Additionally, colorectal polyp formation was reported to increase in cases of elevated serum levels of TG and TC (38). In agreement with previous studies, we found dyslipidemia signs including remarkably increased serum levels of TG, TC, LDL, and decreased levels of HDL in mice treated with DMH (39). Lipid profile imbalances improved noticeably with the SMN-containing diet along with the CAPIRI combination therapy, signifying the protective effect of SMN when added to the monotherapy and combination chemotherapy regimens of CAP and IRI, which may be related to the decreased number of colorectal polyps in SMN-CAPIRI group that was comparable with the control group. In line with our results, supplementation with silibinin, the major active component of SMN, in DMH-induced rat colon cancer could resolve the hyperlipidemia of the carcinogen group remarkably confirming the anti-hyperlipidemic properties of silibinin (40).

Oxidative stress is characterized by an elevation in ROS and RNS production along with a decrease in antioxidant levels, which is involved in the onset and progression of CRC (41). Previous studies have indicated that MDA is one of the main products of lipid peroxidation and a potential mutant attributed to the occurrence of CRC (5). Meanwhile, colon cells exposed to DMH were indicated to possess the characteristics of cancerous cells due to the increased levels of lipid peroxidation (42). Furthermore, NO is one of the mediators of the major CRC pathways, *e.g.* Wnt/ β-catenin and higher levels of NO were seen in the inflamed and cancerous areas due to the over-expression of inducible nitric oxide synthase in inflammation-induced CRC (43). SOD and GPx are the major enzymatic antioxidants that are directly involved in ROS elimination reactions. SOD exerts its antioxidant activity through scavenging superoxide onions (44). GPx as one of the members of the glutathione system, is responsible for the elimination of hydrogen peroxide (45). Various reports have suggested that DMH can decrease the levels of antioxidant enzymes such as SOD, catalase, glutathione reductase, and GPx (5,46). Similarly, our results demonstrated the signs of impaired redox balance in favor of oxidants, since DMH increased the colonic levels of MDA and NO and concomitantly decreased the circulatory and colonic SOD and GPx activities; while all treatment strategies alleviated the oxidative stress conditions. SMN-CAP improved serum and colonic oxidative stress markers (MDA, SOD, and GPx) most pronouncedly among SMN alone and SMN-containing monotherapies; whereas SMN alone had the greatest alleviating effect on colonic NO levels. SMN supplementation in the diet could also improve the effects of the CAPIRI regimen. SMN has been shown to exert its antioxidant activity by scavenging free radicals and affecting antioxidant systems linked to SOD and glutathione. These effects are due to the phenolic nature of SMN, which makes it capable of stabilizing free radicals and ROS *via* donating electrons (47). Moreover, Sangeetha *et al.* showed that silibinin could act as a chemopreventive substance through modulating lipid peroxidation and the antioxidant defense in DMH-induced CRC (48).

Inflammation is a physiologic reaction of the body to tissue damage; however, chronic inflammation may predispose cell mutation and proliferation, which may result in cancer

initiation. Likewise, inflammatory cytokines such as IL-6, TNF-α, CRP, and IL-1 are associated with chronic inflammation (10). TNF-α is frequently used to describe colitis-associated CRC in animal models and high serum levels of TNF-α are attributed to an increased risk of colorectal adenomas. It also plays a key role in tumor initiation *via* its involvement in leukocyte recruitment, angiogenesis, and invasion and contributes to epithelial-to-mesenchymal transition (49). CRP is another inflammatory cytokine that plays a crucial role in the acute phase of inflammation. Moreover, it has been reported that higher serum levels of CRP were linked to an increased risk of colon cancer (50,51). MPO, on the other hand, is an enzyme that is mostly found in neutrophils and serves as a quantitative index of inflammation in a variety of tissues, such as the intestines (52). Zhao *et al*. indicated that oral administration of SMN could inhibit inflammatory reactions by enhancing antioxidant enzyme levels as well as reducing cytokine levels (53). In addition, in azoxymethane-induced CRC, dietary administration of silibinin could downregulate nitric oxide synthase and cyclooxygenase-2 (54).

To further investigate the anti-inflammatory effects of SMN supplementation, we evaluated TNF-α, CRP, and MPO levels as the markers of inflammation. The results revealed that DMH-exposed mice had remarkably higher serum levels of CRP and TNF-α along with colonic MPO activity compared to the mice in the control group. SMN alone drastically reduced the amounts of these factors, which was reported previously (55). Incorporating SMN to the CAPIRI chemotherapy regimen resulted in a significant reduction in TNF-α and MPO as well as an insignificant reduction in CRP levels, reflecting the potential anti-inflammatory effects of SMN; however, we obtained different results from SMN-added monotherapies which could be attributed to the different mechanisms of action of drugs and various responses to the treatment strategies. All these observations elucidate the ability of SMN to potentiate the effects of chemotherapy agents by ameliorating oxidative stress and inflammation along with possibly augmenting mechanisms of tissue repair in the colonic mucosa.

It is well evidenced that dysplastic aberrant crypt foci are the earliest detectable lesions indicating CRC development (56). In this regard, the microscopic observation of the DMH-influenced colon tissues exhibited dysplastic changes and adenomas, as well as the infiltration and accumulation of inflammatory cells, which is supported by previous study (57). SMN treatment alone and in combination with CAP and IRI ameliorated the dysplastic changes in comparison with the DMH group. SMN supplementation with CAPIRI also maintained the normal appearance of colon tissue. These findings could be correlated with the antiproliferative effects of SMN, which elevated the antitumor effects of the chemotherapy agents. The antiproliferative property of SMN could be associated with its ability to improve the resistance of colon cells to lipid peroxidation, restore the activity of colonic antioxidant enzymes, and inhibit the activity of cell growth-contributing inflammatory cytokines such as TNF-α (58), which may strengthen the effects of chemotherapy drugs.

Apoptosis is a crucial process in intestinal turnover, especially in the colon. Thus, inhibition of apoptosis is associated with the transformation of colon epithelium to carcinoma (59). Bcl-2 family proteins are key regulators of apoptosis. Bcl-2 plays a key role in the early stages of CRC due to the overexpression of Bcl-2 in adenomas rather than carcinomas and inhibits apoptosis in cancer cells by suppressing BAX (60). The expression of the pro-apoptotic protein, BAX, was proved to reduce during the CRC initiation and progression phases. Thus, the crosstalk between these proteins and apoptosis can determine the response to therapy. DMH resulted in the upregulation of Bcl-2 and downregulation of BAX, hence, an increase in the Bcl-2 to BAX ratio in comparison with the control group, which was previously reported by Wang *et al*. (61). Besides, *in vitro* and *in vivo* studies supported that the chemoprotective effects of SMN could be relevant to modulating the expression of BAX and Bcl-2 proteins (62,63). Silibinin was also reported to elevate the mRNA and protein levels of BAX and decrease Bcl-2 levels in the azoxymethane-induced CRC model in rats (64). SMN treatment was shown to significantly decrease

Bcl-2 levels and increase BAX levels in a xenograft model of CRC in mice (65).All treatment groups significantly reversed the alterations caused by DMH in apoptosis-related proteins. SMN-CAPIRI in comparison with CAPIRI resulted in a significant decrease and increase in Bcl-2 and BAX expression levels, respectively. Although no significant changes were observed between SMN and SMN-added monotherapy regimens in BAX levels, SMN-CAP considerably downregulated Bcl-2 levels compared to SMN alone and SMN-IRI. These observations supported that the apoptotic properties of SMN could reinforce the effects of chemotherapy agents on BAX and Bcl-2 which further ameliorated the histopathological changes.

The liver is the major organ where most toxic agents such as drugs, pollutants, carcinogens, and mutagens undergo metabolism. It is well-documented that free radicals generated from the hepatic metabolism of DMH induce liver necrosis, fatty acid infiltration, and the secretion of transaminases into the circulation (66). Furthermore, most patients undergoing chemotherapy were reported to develop liver steatosis, which can turn into inflammatory liver damage (67). Increased levels of transaminases and LDH are indicators of hepatocellular damage and the possibility of liver metastases (68). In the current study, DMH treatment elevated ALT, AST, and LDH enzyme levels. In addition, a considerable increase in the levels of inflammation and oxidative stress indices (MDA, NO, and MPO) along with a significant reduction in the enzymatic antioxidant levels were observed, which was also indicated by Goyal *et al* (69). These changes were markedly resolved by SMN alone and SMN-containing regimens, implying the hepatoprotective and antioxidant effect of SMN alone and combined with chemotherapy agents. These findings could be linked to the modulatory ability of SMN on the free radicals formed during the liver metabolism of toxic substances (47). The hepatoprotective and cardioprotective effects of silibinin were evidenced against doxorubicin toxicity (70). Moreover, SMN could decrease the elevated levels of liver enzymes and improve the histopathological changes in the liver induced by epirubicin in mice (71). Despite the significantly improving effects of CAPIRI on the colon examinations, we did not observe the same results in the liver examinations, which could be associated with hepatotoxicity caused by this regimen exacerbated by DMH metabolism, since the hepatotoxicity of CAP and IRI was previously recorded (67).

## CONCLUSIONS

Our results indicated that SMN supplementation could enhance the therapeutic activity of CAP and IRI and reduce the toxicity caused by monotherapy and combination therapy with these drugs. We also recognized that the SMN-IRI combination, despite the long interval and low dose of administration in comparison with SMN-CAP, indicated almost the same efficacy with lower colonic, circulatory, and hepatic toxicity. Thus, SMN-IRI could be considered an appropriate alternative for SMN-CAP by adjusting the dose and administration interval. We also evidenced the potentiating ability of SMN on CAPIRI as a treatment in patients with colon cancer with obviously decreased side effects of chemotherapy on the liver. Therefore, the results of the present study in mice give us hope that SMN could be incorporated as an adjunct to standard cancer chemotherapies.

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***Conflict of interest statement***

The authors declared no conflict of interest in this article.

***Authors' contributions***

S. Hassani, H. Malekinejad, and F. Kheradmand designed the research; S. Hassani and H. Malekinejad performed the experiments and analyzed the data. S. Hassani, H. Malekinejad, and F. Kheradmand drafted, reviewed, and revised the paper. M.H. Khadem Ansari and A. Abbasi contributed to performing the experiments and reviewed and revised the paper. The finalized article was read and approved by all authors.

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