

Introduction: The third most frequently diagnosed cancer and one of the highest causes of tumour deaths worldwide is colorectal cancer (CRC). The main objective of this study was to determine the role of microRNA-224 (miR-224) as well as microRNA-200a (miR-200a) in CRC. Phytic acid (PA) is a natural antitumour product that was reported to inhibit CRC and play a vital role as a chemopreventive agent against CRC.

Material and methods: We induced CRC in albino rats using 1,2-dimethylhydrazine (1,2-DMH). The miR-224, miR-200a, and β -catenin expressions were determined. ELISAs were performed to investigate Bcl-2 expression, caspase-3 activity, and total tissue antioxidants. Finally, histopathological investigations were performed.

Results: We observed a chemoprotective role of PA. PA has a synergistic effect as an antitumour agent with oxaliplatin in CRC treatment. The miR-224, miR-200a, and β -catenin expression, when treated with PA alone or with oxaliplatin, was decreased markedly in comparison with the positive control group. The histopathological investigations of colorectal tissues confirmed our molecular and biochemical findings.

Conclusions: Phytic acid possessed efficient anti-carcinogenic properties alone or with oxaliplatin against 1,2-DMH-induced CRC in rats through pathways of apoptosis, cell proliferation, and antioxidants.

Key words: CRC, phytic acid, miR-224, miR-200a, oxaliplatin.

Contemp Oncol (Pozn) 2021; 25 (2): 118–124
DOI: <https://doi.org/10.5114/wo.2021.106061>

Phytic acid potentiates oxaliplatin effects in colorectal cancer induced by 1,2-DMH: the role of miR-224 and miR-200a

Tarek M. Okda¹, Mohamed A. Katry^{2,3}, Noha M. Ragab⁴,
Abdel-Gawad S. Shalkami⁵

¹Department of Biochemistry, Faculty of Pharmacy, Damanshour University, Egypt

²Department of Pharmacology and Toxicology, Faculty of Pharmacy, Damanshour University, Egypt

³Department of Oral Biology and Diagnostic Sciences, Augusta University, Augusta, USA

⁴Department of Pathology, Medical Research Institute, Alexandria University, Egypt

⁵Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Egypt

Introduction

Colorectal cancer (CRC) is widely diagnosed with cancer, accounting for over 9% of all forms of cancer, and the world's fourth-largest cause of cancer deaths. It poses a significant concern for public health and is a major global cause of morbidity and mortality [1]. While CRC is a disease in the developed world, the rates of CRC in developing countries are increasing [2], which indicate an increased occurrence of CRC risk factors, such as unhealthy diets, obesity, and smoking [3]. CRC has a heterogeneous structure, with a small fraction of the tumours responding to chemotherapy. A molecular understanding is required of the CRC in oncology [4]. A previous study assessed the convenience of patient-derived tumour xenografts as a tool in cancer diagnosis [5].

The rate of CRC incidence has prompted the search for novel treatments furthermore markers cause early detection and the evaluation and monitoring of the prognosis of the drug therapy of choice. MicroRNAs (miRNAs) consider family of the latest research biomarkers. These RNA molecules modulate the other gene expressions and are approximately 20–22 nucleotides long. The strong correlation between tumourigenesis and miRNA expression has led to a substantial amount of research investigating their potential use as biomarkers and therapeutics for cancer. The maintenance of cancer, angiogenesis, and epithelial-mesenchymal transformation are also related to altered miRNA levels, all of which lead to malignancy [6].

MiR-224 and miR-200a are prognostic tumour markers of CRC. MiR-224 activates signalling of Wnt/ β -catenin and directs the nuclear translocation of β -catenin in CRC [7]. Furthermore, an phenotype and bad prognosis have been significant with high levels of miR-224 expression, and miR-224 over-expression stimulated in vitro proliferation of CRC cells [8]. Additionally, miR-224 supports CRC metastasis. In CRC, miR-224 expressions have prognostic value for the survival of CRC patients, alone or with its targets [9]. MiR-200a one of miR-200 families present on chromosome 1, and it is over-expressed in mesenchymal-epithelial transition in CRC. Furthermore, a relation between the miR-200a family and permanence in CRC patients has been confirmed. Thus, miR-200a expression as in surgical samples may be helpful to design treatment. Further studies are necessary to assess the effectiveness of these miRNAs on the CRC treatment [10].

Chemotherapy is an adjuvant treatment for unresectable CRC, to prevent postoperative recurrence, and for systemic chemotherapy. Fluorouracil, oxaliplatin, tegafur, uracil-tegafur, and doxifluridine are anticancer drugs that

have been approved for CRC treatment [11]. Oxaliplatin, a chemotherapeutic platinum-based drug, is used for the treatment of CRC. Platinum-based drugs increase cytotoxicity through oxidative stress, leading to the direct and indirect production of reactive oxygen species (ROS) [12]. Furthermore, its use causes significant gastrointestinal side effects, resulting in dosage limitation and therapy discontinuation [13]. Apoptosis is among the recognized pathways of oxaliplatin [14].

Numerous pharmaceutical agents originate from natural products, and it is fair to assume that there are additional agents in nature that remain undiscovered [15]. Phytic acid (PA) is an omnipresent, natural antitumour substance that is particularly abundant in cereals and legumes in the plant kingdom. PA and its lower phosphorylated forms present in much smaller amounts in most mammalian cells, where they are essential for critical cellular functions. During inositol polyphosphate metabolism, mammalian cells take up exogenous PA by feeding or they develop it. PA is involved in development, inflammation, and neurotransmission regulation [16].

Extracellular PA may be taken up and metabolized by mammalian cells, and the rate and trend of PA metabolism by cancer cells vary according to the cell type [17]. PA has shown antitumour activity in the experimental models of tumorigenesis, which is a fascinating property of PA [18]. The main aim of our study was the first-time determination of miR-224 and miR-200a in CRC. We also evaluated the possible antitumour and protective effects of PA in CRC treatment.

Material and methods

Chemicals

PA powder (PA sodium salt hydrate) and 1,2-dimethylhydrazine (1,2-DMH) were purchased from Sigma-Aldrich. Oxaliplatin was purchased from Sanofi-Aventis Deutschland GmbH (Frankfurt, Germany). Other chemicals were obtained following a quality control examination from commercial sources and had an analytical grade.

Animals

We purchased 36 male rats (150 g) from the Animal Care Unit of Vacsera. The rats under controlled experimental conditions were housed in polyethylene cages (temperature, 25°C ± 1°C; humidity, 50% ± 5%; and light/dark cycle). Food and water were available ad libitum. Every 3 days the

animals were weighed, and their activity was tracked on a regular basis [19]. The Animal Care and Use Committee of the Faculty of Pharmacy at Damanhur University approved all experimental protocols (Ref. 1647ag).

Design and sampling

After 2 weeks of acclimatization, we divided the rats into 6 groups containing 6 rats each. Group I rats (control) were injected with 0.9% normal saline throughout the experiment. Group II rats (positive control) were subcutaneously injected with 1,2-DMH (20 mg/kg) twice weekly for 10 weeks [20]. Group III rats (preventive) were treated orally for 12 weeks with PA (2 weeks before induction with 1,2-DMH, as in group II, followed by 10 weeks of oral treatment with 0.5% w/v PA daily) [21]. Group IV rats (treated with PA) were treated orally with PA (0.5% w/v daily, 8 weeks) after induction with 1,2-DMH, as in group II. Group V rats (treated with oxaliplatin) were treated with intraperitoneal injections (ip) of oxaliplatin (5 mg/kg) after induction with 1,2-DMH, as in group II [22]. Group VI rats (treated with oxaliplatin + PA) were treated with a combination of a single weekly dose of oxaliplatin for 3 weeks (5 mg/kg ip) and oral PA (0.5% w/v) daily for 8 weeks after induction with 1,2-DMH, as in group II. All rats were sacrificed after 18 weeks (Fig. 1).

The rats fasted overnight at the end of the experiment and were anaesthetized by diethyl ether and sacrificed by cervical dislocation. Colorectal tissue was applied for histopathological analysis, and the remainder was homogenized in 0.1 M Tris-HCl buffer (pH 7.4) and centrifuged. The resultant supernatant was used for molecular and biochemical studies.

Blood samples (4 mL) were taken, centrifuged at 5000 rpm, and the serum was collected. Reverse-transcription-polymerase chain reaction (RT-PCR) was used to monitor the miR-224, miR-200a, β -catenin, and β -actin expressions. ELISA was performed to determine the expression of Bcl-2 and caspase-3 activity in colorectal tissues. The total protein content of the colorectal homogenate was estimated by Bradford reagent [23]. The samples were stored at -80°C until use.

RNA preparation and reverse-transcription-polymerase chain reaction

Total tissue RNA was isolated using a Total RNA Omega Bio-tek Kit. Then, RT-PCR was performed by an RT-PCR Mas-

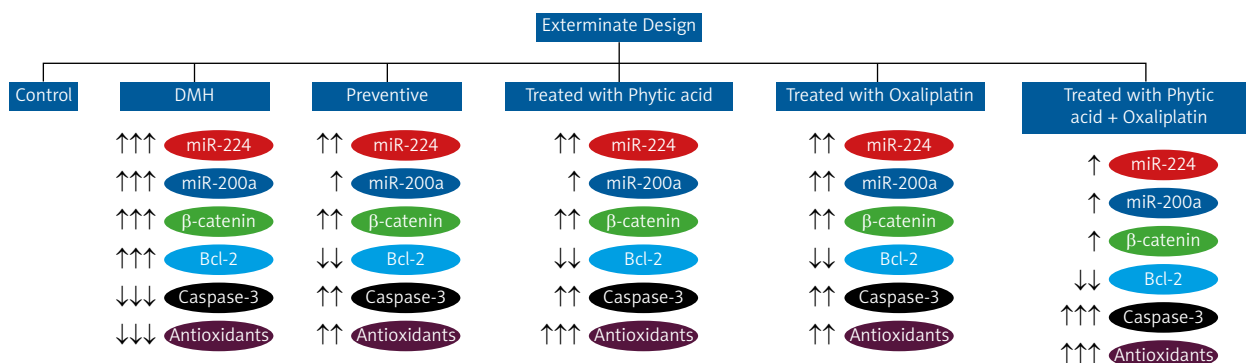


Fig. 1. Design and sampling

Table 1. Primers sequences used for the reverse-transcription-polymerase chain reaction technique

Primer	Tm (°C)	Sequence (5'-3')
miR-224		
Forward	58.69	TCAAGTCACTAGTGGTTCCG
Revers	59.72	GGCTTTGTAGTCACTAGGGC
miR-200a		
Forward	61.94	ACTCTGAGTCATCTCAGTGC
Revers	60.81	GGCCATCTTACAGACAGTGT
β-catenin		
Forward	59.96	AAGCTCCCATTCATCCTTGT
Revers	60.04	CAGACGAGCCACAGTTCCAT
β-actin		
Forward	60	CATGGATGACGATATCGCTG
Revers	60	CATAGATGGGCACAGTGTGG

m – melting temperatures

ter Mix Kit (122020-1109; (BIORON GmbH) and Taq Master (Jena Bioscience), which produces a high RNA yield. The cDNA strand was synthesized according to the instruction manual of the Revert Aid TM cDNA synthesis kit. PCR was completed by a thermal cycler (BOECO, BYQ6041R-158; Hamburg, Germany). The PCR conditions were as follows: pre-denaturing for 5 min at 94°C, 30 cycles of denaturing at 94°C for 30 sec, annealing at the primer-specific temperature for 30 sec, and extension at 72°C for 1 min.

Reference sequences obtained from the NCBI were applied to design primers for miR-224, miR-200a, β-catenin, and β-actin. The primer sequences and melting temperatures are illustrated in Table 1.

The products of PCR were electrophoresed on 1% of agarose gel using TAE buffer. A DNA ladder of 50–1000 kbp was used (Jena Bioscience). The bands were detected using a transilluminator and then photographed.

Determination of Bcl-2 in colorectal tissues using ELISA

We used a Bcl-2 Human SimpleStep ELISA Kit (ab202411; Abcam) for measurement of Bcl-2 protein quantitatively in the extracts of rat tissue. All reagents, working standards, and samples were prepared. The colorectal homogenates were typically prepared with phosphate-buffered saline (PBS). Then, 50 μL of sample or standard were added to the appropriate wells of the ELISA plate. After adding 50 μL of the antibody to each well, the plates were incubated for 1 h. Next, each well washed with 350 μL of 1× Wash Buffer 3 times, and 100 μL of 3,3',5,5'-tetramethylbenzidine substrate was added to each well and incubated for 10 min. Finally, 100 μL of stop solution was added, and the absorbance was read at 450 nm. The Bcl-2 concentration (ng/mL) was calculated by using a typical standard curve.

Determination of caspase-3 levels

We used ELISA Kit of Caspase-3 (MBS262914; MyBioSource, San Diego, USA) for the quantitative determination of caspase-3 in the rat tissue homogenates. Briefly,

the tissue homogenate was prepared using tissue slices washed in 0.01 M PBS. A tissue protein extraction reagent was added, mixed in ice water (1 g: 10 mL), and centrifuged for 10 min at 5000 rpm, and supernatant was removed for use. Each supernatant sample was added to corresponding wells (100 μL/well) of the ELISA plate and incubated at 37°C for 90 min. Biotinylated rat caspase-3 antibody (100 μL/well) was added and incubated at 37°C for 60 min. Then, the enzyme-conjugate was prepared, and 100 μL was added and incubated at 37°C for 30 min to each well. Finally, 100 μL of a coloured reagent was added to wells and incubated at 37°C for 15 min. The absorbance was recorded at 450 nm within 10 min using a reader (1603029; BioTek Instruments, USA).

Estimation of tissue total antioxidants

We used an assay Kit of Total Antioxidant Capacity (ab65329; Abcam) to determine the total tissue antioxidant levels. Briefly, the small kit vials were centrifuged. Then, the sample was centrifuged at 4°C for 5 min. All reagents were equilibrated to room temperature prior to use. Cu²⁺ working solution was freshly prepared before adding 100 μL to all standard and sample wells. The solution was mixed, and the plate was protected from light and incubated at room temperature for 90 min on an orbital shaker. The absorbance at 570 nm was measured using a microplate reader. Trolox was used to standardize the antioxidant results by measuring the other antioxidants in Trolox equivalents. The concentration of total antioxidant capacity (TAC) or Trolox (nmol/mg) was calculated as $Ts/S_v \cdot D$, where Ts = TAC amount in the sample, Sv = sample volume added to the sample well, and D = sample dilution factor.

Histopathological examinations

Fresh colorectal tissues were washed with 0.9% saline solution and fixed in buffered formalin. Then, the formalin-fixed tissue samples were transferred to 70% ethanol and embedded in paraffin. Finally, paraffin block sections (0.5 μm thick) were double stained with haematoxylin and eosin (HE) and visualized using an optical microscope.

Data analysis

The data were statistically analysed using Graph Pad Prism 4.0 software (GraphPad Software, Inc., San Diego, CA). Values were reported as means ± standard deviation (SD), and *p*-values < 0.05 were considered statistically significant. Multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test.

Results

Expression of miR-224, miR-200a, and β-catenin by reverse-transcription-polymerase chain reaction

Figure 2 shows the mRNA expression of miR-224, miR-200a, and β-catenin expression as determined by RT-PCR for all experimental groups. The expression of miR-224, miR-200a, and β-catenin was increased in the carcinogenic group but significantly decreased in the other

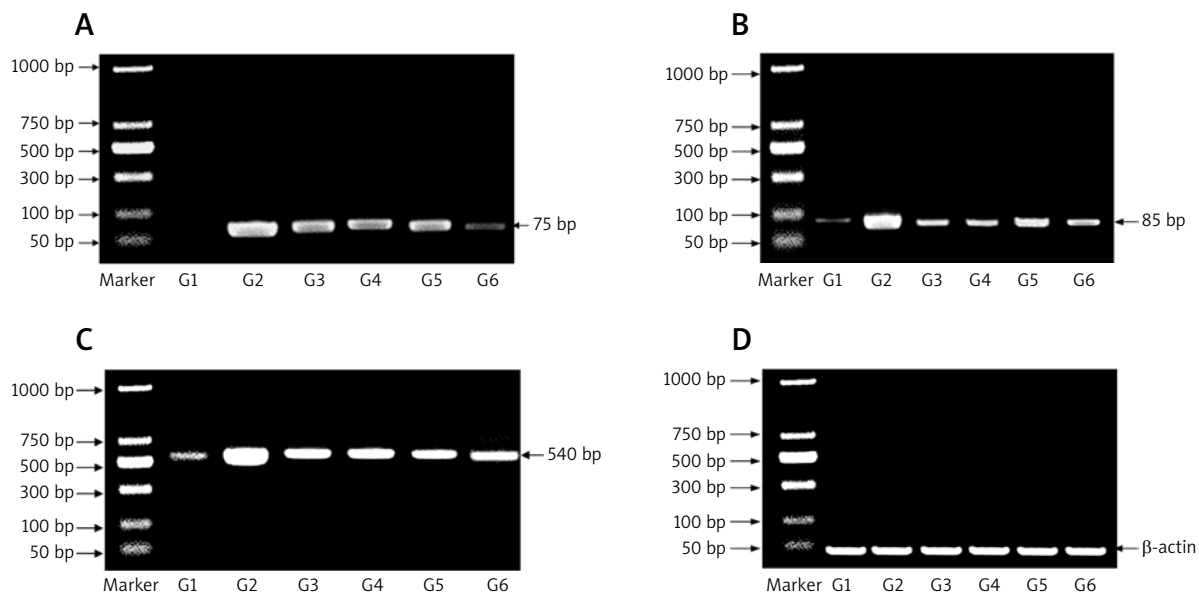


Fig. 2. Expression of miR-224, miR-200a, and β -catenin in colorectal tissue homogenates of all experimental groups, as determined by reverse-transcription-polymerase chain reaction. **A** – miR-224, **B** – miR-200a, **C** – β -catenin, **D** – β -actin

groups (group 3: prophylactic, group 4: treated with PA, group 5: treated with oxaliplatin, group 6: treated with oxaliplatin + PA). β -actin was used as the housekeeping gene in the RT-PCR assays.

Estimation of Bcl-2, Caspase-3, and total antioxidants in colorectal tissues by ELISA

Table 2 illustrates the Bcl-2, Caspase-3, and total antioxidants levels in all experimental groups. Bcl-2 levels were markedly increased in group II but reduced significantly in other treated groups (group 3: prophylactic, group 4: treated with PA, group 5: treated with oxaliplatin, group 6: treated with oxaliplatin + PA). Caspase-3 and total antioxidants were significantly reduced in group II but increased gradually in other groups.

Histopathological results

We examined HE-stained slides to evaluate changes in the colorectal cells in all rat groups. Normal architecture and histological structure were observed in the colorectal section of rats in the control group (Fig. 3 A). On the other hand, the positive control group showed proliferation of the epithelial layer tumour cells infiltrating the submucosa

(Fig. 3 B), with distorted, swollen nuclei and tightly packed glands causing invasive adenoma. The tumour cells were polyhedral with hyperchromatic nuclei. The preventive group showed a more or less natural histological appearance, with some villi and goblet cells with simple columnar epithelium (Fig. 3 C). Additionally, the regeneration of villi and epithelial linings were observed in response to PA treatment as well as reduced penetration of cancer cells into the connective tissue (Fig. 3 D). The oxaliplatin group showed colorectal necrosis and strong mast cell infiltration (Fig. 3 E). The group treated with PA and oxaliplatin showed normal morphology with some inflammation (Fig. 3 F).

Discussion

Colorectal cancer is a common cancer type in many countries. Because it has few symptoms, it is usually discovered in the final stages [24]. Although CRC has many tumour markers, its diagnosis remains problematic. miRNAs are small, non-coding RNAs that act as suppressors of many cancer types. miR-224 was found to play a primary role in several types of cancer and may be prognostically and biologically important in CRC [25].

Our study determined the presence of miR-224 and miR-200a in CRC. We found that miR-224 expression

Table 2. Levels of Bcl-2, Caspase-3, and total antioxidants in all experimental groups

	Control	Positive control	Prophylactic	Phytic acid	Oxaliplatin	Phytic + oxaliplatin
Bcl-2 levels (ng/mg proteins)	5.29 ± 1.12	31.51 ± 2.47*	28.88 ± 1.62*	22.98 ± 1.32 *#	17.43 ± 1.21*#	9.76 ± 1.67*##
Caspase-3 (ng/ mg proteins)	4.58 ± 0.95	2.92 ± 0.83 *	6.18 ± 0.75 *#	6.06 ± 1.44 *#	8.25 ± 0.91 *#	12.11 ± 1.43 *#
Total antioxidants (nmol/mg)	14..17±0.93	4..75±0.73 *	8.49 ±1.27 *#	7.18±0.98 *#	7.46±1.61 *#	9.88±1.29 *#

Data were expressed as mean ± SD of Bcl-2: B-cell lymphoma-2, Caspase-3, and total antioxidants. * – values differ significantly from group I (control group) ($p < 0.05$), # – values differ significantly from group II (positive control) ($p < 0.05$). Statistical analysis was determined by one-way analysis of variance (ANOVA) followed by Tukey's test as a multiple comparison post-ANOVA test

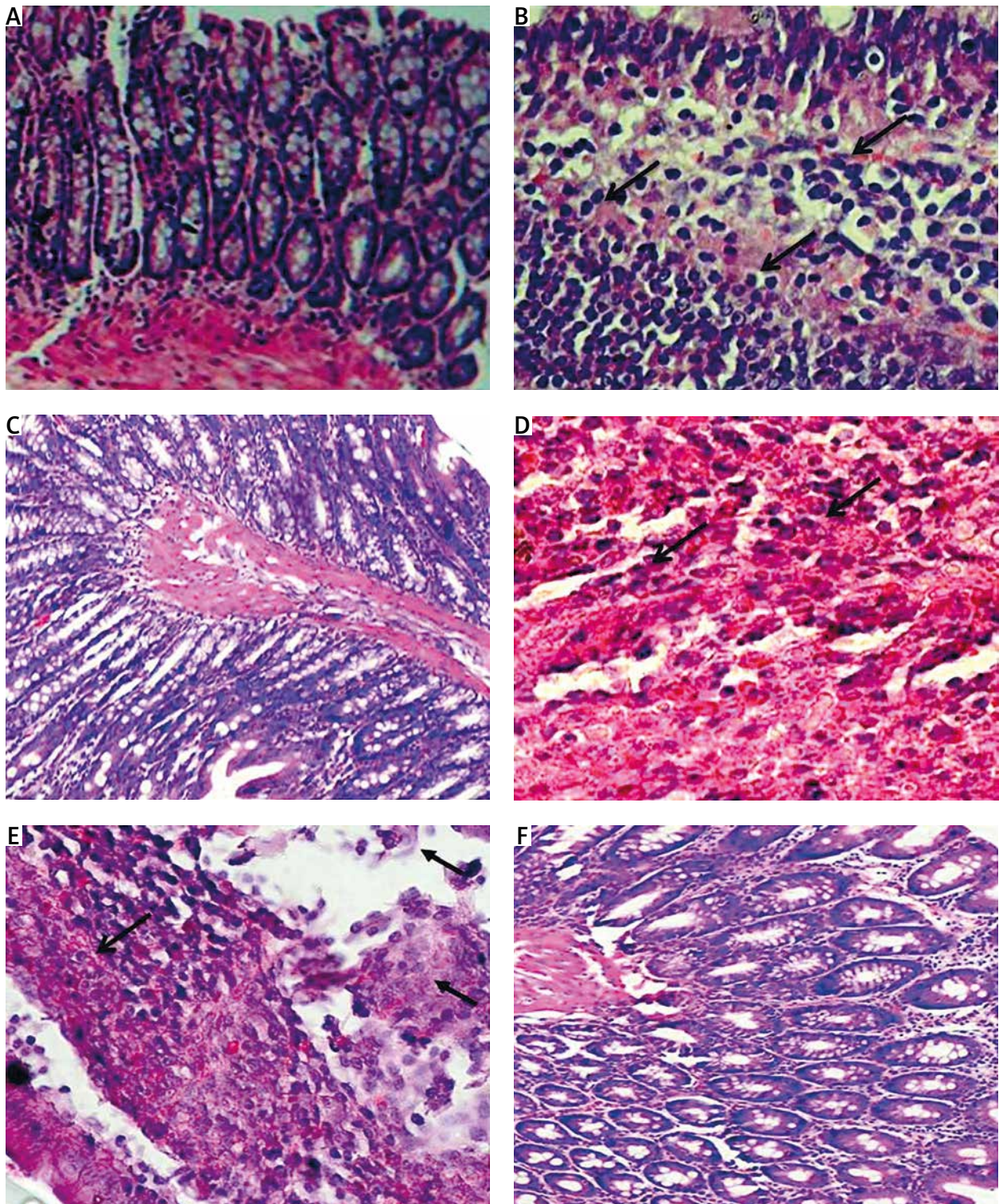


Fig. 3. Histopathological analysis showing the effect of phytic acid (PA) alone or in combination with oxaliplatin. **A** – normal architecture and histological structure are shown in the colorectal section of rats in the control group, **B** – positive control group showing proliferation of epithelial layer tumour cells infiltrating the submucosa, **C** – preventive group showing some villi and goblet cells with simple columnar epithelium, **D** – regeneration of villi and epithelial linings and reduced penetration of cancer cells into the connective tissue following PA treatment, **E** – oxaliplatin group showing colorectal necrosis and strong mast cell infiltration (arrows), **F** – normal morphology and some inflammation in the experimental group treated with PA and oxaliplatin. Slides were stained using haematoxylin and eosin ($\times 40$)

markedly increased in CRC and enhanced its prognosis. Furthermore, our results confirmed that miR-224 and miR-200a were significantly increased in rats with CRC. Moreover, the levels of miR-224 and miR-200a were mark-

edly up-regulated in the carcinogenic group (untreated CRC) compared with the groups treated with PA alone or in combination with oxaliplatin. MiR-224 levels consistently increase with cancer severity, and miR-224 promotes CRC

metastasis of *in vitro* and *in vivo* by regulating SMAD4. Furthermore, miR-224 levels may have a prognostic role in the survival of patients with CRC [9].

Similarly, we found that miR-200a expression was increased with cancer progression. Both miR-224 and miR-200a have a diagnostic and prognostic effect on CRC by reducing inflammation and exerting antitumour effects through different mechanisms. In our study, apoptosis was evaluated by determining the levels of Bcl-2 and caspase-3 expression.

β -catenin plays a role in regulating cell-cell adhesion and is a crucial component of the Wnt pathway. In our study, β -catenin overexpression was associated with CRC. We determined the expression of β -catenin in colorectal tissue in rats in different experimental groups using RT-PCR. β -catenin expression levels were increased in CRC and decreased in the groups treated with PA alone or in combination with oxaliplatin. The expression of β -catenin in azoxymethane-induced rat colon tumours relative to adjacent healthy colon mucosa was found to be pronounced by immunostaining and immunoblotting in both colon adenoma and carcinoma tissues as well as in the cytoplasm and nucleus of the cancer cells. Furthermore, antibody binding in healthy colon epithelial cells was restricted to membranes at intercellular borders [26].

Oxaliplatin is a chemotherapeutic agent used as the drug of choice in CRC treatment. It stimulates cytotoxicity by increasing the levels of ROS and oxidative stress. Because oxaliplatin has severe gastrointestinal side effects, the use of natural products, such as PA, reduces the dose of oxaliplatin as well as its side effects and cytotoxicity. The apoptosis pathway is the main mechanism of action for oxaliplatin as a chemotherapeutic drug.

PA is a natural product that is present in grains and nuts. Our study found that PA might inhibit CRC and play a vital role in the chemoprevention of CRC. Furthermore, PA treatment could reduce the expression of tumour markers such as miR-224 and miR-200a. Our findings also suggested that PA had a synergistic action with oxaliplatin in CRC treatment. In our study, we attempted to identify some of the pathways involved in the effect of PA as a preventive and therapeutic agent for CRC. It has been shown that PA has a potent chemopreventive and antitumour action against CRC. The development and progression of CRC include defects of the intestinal mucosal barrier and inflammation processes. The effect of PA on the intestinal mucosal barrier and proinflammatory cytokines was investigated, and PA was found to have beneficial effects on the intestinal mucosal barrier and reduce serum proinflammatory cytokine levels [27].

Our study found that the Bcl-2 level increased with the incidence of CRC and decreased when rats were treated with PA alone or in combination with oxaliplatin. Bcl-2 is an apoptotic regulator that suppresses apoptosis in several cell types (antiapoptotic agent). As such, it is a crucial protein involved in cell control and is critically related to a number of cancers through mutation or damage to the structure or function of the Bcl-2 proteins. The fusion protein's unregulated expression induces an unusually high

amount of Bcl-2, disrupting apoptosis regulation and leading to unregulated cell growth. The functional interface of Bcl-2/p53 represents a central regulator of apoptosis that can be triggered in colorectal carcinoma cells by targeting Bcl-2 [28].

Apoptosis is an important pathway that clarifies cancer incidence and its regulation. We investigated the expression of caspase-3 as an apoptotic agent. The levels of caspase-3 were markedly reduced in CRC induced with 1,2-DMH and significantly increased in rats treated with PA alone or in combination with oxaliplatin. These findings showed that PA interacted with the apoptosis pathway in the treatment of CRC in rats.

Besides its anticancer properties, PA is also of nutritional value. The anticancer activity of PA against hepatocellular carcinoma cells has been investigated using expression studies of the apoptosis-regulating caspase-3 genes by RT-PCR and DNA fragmentation assays to assess apoptotic activity. Hepatocellular carcinoma cells treated with PA showed caspase-3 up-regulation and Bcl-2 down-regulation, and apoptosis was evident by the presence of fragmented genomic DNA in PA-treated cells. Furthermore, a reducing power assay showed that PA had antioxidant activity. PA had biological activity, stimulated apoptosis in cancer cells, and showed growth inhibition [29].

Our study found that PA is useful because of its possible protective role against CRC in rats. PA treatment alone or in combination with oxaliplatin increased total antioxidant levels and decreased oxidative stress in rats with CRC. Our results correlated with a previous study showing that PA acted as an antioxidant against the harmful effects of generated toxins and played an important role in reducing oxidative stress levels. Because these antioxidants work together, a change in any of them can disturb the balance and trigger cell damage that leads to cancer [30].

The ROS levels were increased in 1,2-DMH-induced CRC rats, resulting in an imbalance between the generation and removal of ROS and leading to oxidative stress-related malignancy. Antioxidants are reducing agents that become oxidized and prevent the production of free radicals. In some cancer cases, the level of antioxidants produced is insufficient to totally overcome the free radicals. Free radicals may be generated by the action of 1,2-DMH in CRC models [31]. Our histopathological examinations confirmed our biochemical and molecular findings. The histopathological analysis of CRC cells showed logical evidence of the potential effect of PA alone or in combination with oxaliplatin against CRC.

Conclusions

Our study revealed that PA possessed efficient anticarcinogenic properties against 1,2-DMH-induced CRC in rats when used as a preventive agent or as a treatment agent alone or in combination with oxaliplatin. PA inhibits CRC in rats through pathways of apoptosis, cell proliferation, and antioxidants.

The authors declare no conflict of interests.

References

1. Hagggar FA, Boushey RP. Colorectal cancer epidemiology: Incidence, mortality, survival, and risk factors. *Clin Colon Rectal Surg* 2009; 22: 191-197.
2. Favoriti P, Carbone G, Greco M, et al. Worldwide burden of colorectal cancer: a review. *Updates Surg* 2016; 68: 7-11.
3. Center MM, Jemal A, Ward E. International trends in colorectal cancer incidence rates. *Cancer Epidemiol Biomarkers Prev* 2009; 18: 1688-1694.
4. Schütte M, Risch T, Abdavi-Azar N, et al. Molecular dissection of colorectal cancer in pre-clinical models identifies biomarkers predicting sensitivity to EGFR inhibitors. *Nat Commun* 2017; 8: 14262.
5. Guilhamon P, Butcher LM, Presneau N, et al. Assessment of patient-derived tumour xenografts (PDXs) as a discovery tool for cancer epigenomics. *Genome Med* 2014; 6: 1-10.
6. Thomas J, Ohtsuka M, Pichler M, Ling H. MicroRNAs: clinical relevance in colorectal cancer. *Int J Mol Sci* 2015; 16: 28063-28076.
7. Li T, Lai Q, Wang S, et al. MicroRNA-224 sustains Wnt/ β -catenin signaling and promotes aggressive phenotype of colorectal cancer. *J Exp Clin Cancer Res* 2016; 35: 1-11.
8. Liao W-T, Li T-T, Wang Z-G, et al. microRNA-224 promotes cell proliferation and tumor growth in human colorectal cancer by repressing PHLPP1 and PHLPP2. *Clin Cancer Res* 2013; 19: 4662 LP-4672.
9. Ling H, Pickard K, Ivan C, et al. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. *Gut* 2016; 65: 977-989.
10. Diaz T, Tejero R, Moreno I, et al. Role of miR-200 family members in survival of colorectal cancer patients treated with fluoropyrimidines. *J Surg Oncol* 2014; 109: 676-683.
11. Watanabe T, Itabashi M, Shimada Y, et al. Japanese Society for Cancer of the Colon and Rectum (JSCCR) Guidelines 2014 for treatment of colorectal cancer. *Int J Clin Oncol* 2015; 20: 207-239.
12. Kopetz S, Lesslie DP, Dallas NA, et al. Synergistic activity of the Src family kinase inhibitor dasatinib and oxaliplatin in colon carcinoma cells is mediated by oxidative stress. *Cancer Res* 2009; 69: 3842 LP-3849.
13. McQuade RM, Carbone SE, Stojanovska V, et al. Role of oxidative stress in oxaliplatin-induced enteric neuropathy and colonic dysmotility in mice. *BR J Pharmacol* 2016; 173: 3502-3521.
14. Arango D, Wilson AJ, Shi Q, et al. Molecular mechanisms of action and prediction of response to oxaliplatin in colorectal cancer cells. *Br J Cancer* 2004; 91: 1931-1946.
15. Cragg GM, Grothaus PG, Newman DJ. Impact of natural products on developing new anti-cancer agents. *Chem Rev* 2009; 109: 3012-3043.
16. Shamsuddin AM. Anti-cancer function of phytic acid. *Int J Food Sci Technol* 2002; 37: 769-782.
17. Verghese M, Rao DR, Chawan CB, et al. Anticarcinogenic effect of phytic acid (IP6): apoptosis as a possible mechanism of action. *LWT Food Sci Technol* 2006; 39: 1093-1098.
18. Yim D, Singh RP, Agarwal C, et al. A novel anticancer agent, decursin, induces G1 arrest and apoptosis in human prostate carcinoma cells. *Cancer Res* 2005; 65: 1035 LP-1044.
19. Corbett TH, Griswold DP, Roberts BJ, et al. Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. *Cancer Res* 1975; 35: 2434 LP-2439.
20. Sumiyoshi H, Wargovich MJ. Chemoprevention of 1, 2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. *Cancer Res* 1990; 50: 5084-5087.
21. Saad N, Esa NM, Ithnin H. Suppression of β -catenin and cyclooxygenase-2 expression and cell proliferation in azoxymethane-induced colonic cancer in rats by rice bran phytic acid (PA). *Asian Pac J Cancer Prev* 2013; 14: 3093-3099.
22. Gonzalez-Aparicio M, Alzuguren P, Mauleon I, et al. Oxaliplatin in combination with liver-specific expression of interleukin 12 reduces the immunosuppressive microenvironment of tumours and eradicates metastatic colorectal cancer in mice. *Gut* 2011; 60: 341-349.
23. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-254.
24. Lokeshkumar B, Sathishkumar V, Nandakumar N, et al. Anti-oxidative effect of myrtenal in prevention and treatment of colon cancer induced by 1, 2-dimethyl hydrazine (DMH) in experimental animals. *Biomol Ther* 2015; 23: 471-478.
25. Zhang G Jun, Zhou H, Xiao H, et al. Up-regulation of miR-224 promotes cancer cell proliferation and invasion and predicts relapse of colorectal cancer. *Cancer Cell Int* 2013; 13: 1-10.
26. Takahashi M, Fukuda K, Sugimura T, Wakabayashi K. β -catenin is frequently mutated and demonstrates altered cellular location in azoxymethane-induced rat colon tumors. *Cancer Res* 1998; 58: 42-46.
27. Liu C, Chen C, Yang F, et al. Phytic acid improves intestinal mucosal barrier damage and reduces serum levels of proinflammatory cytokines in a 1,2-dimethylhydrazine-induced rat colorectal cancer model. *Br J Nutr* 2018; 120: 121-130.
28. Jiang M, Milner J. Bcl-2 constitutively suppresses p53-dependent apoptosis in colorectal cancer cells. *Genes Dev* 2003; 17: 832-837.
29. Al-Fatlawi AA, Al-Fatlawi AA, Irshad M, et al. Rice bran phytic acid induced apoptosis through regulation of Bcl-2/Bax and p53 genes in HepG2 human hepatocellular carcinoma cells. *Asian Pacific J Cancer Prev* 2014; 15: 3731-3736.
30. Suzuki N, Miller G, Morales, J et al. Respiratory burst oxidases: the engines of ROS signaling. *Curr Opin Plant Biol* 2011; 14: 691-699.
31. Martin RCG, Li Y, Liu Q, et al. Manganese superoxide dismutase expression as a function of genotype and lung cancer pathology. *Cancer Invest* 2010; 28: 813-819.

Address for correspondence

Tarek M. Okda

Lecturer of Biochemistry and Molecular Biology
Faculty of Pharmacy
Damanhour University
Damanhour, Egypt
Phone: +2010 633-72335.
e-mail: tarekokda@yahoo.com

Submitted: 22.10.2021

Accepted: 26.12.2020