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# Cromolyn chitosan nanoparticles as a novel protective approach for colorectal cancer

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ABSTRACT

Colorectal cancer is the third most common cancer in the world. Cromolyn is a mast cell stabilizer and was proposed as an anticancer agent; however its high polarity limits its bioavailability by rapid washing from the body. We formulated 10 cromolyn chitosan nanoparticles (CCSNPs)[1] following ionic gelation technique to improve its bioavailability and investigated the protective anticancer effect of the optimum formula against colorectal cancer in dimethylhydrazine-induced model in rats. Rats were divided into seven groups, group-1: normal control, group-2: cromolyn control, group-3: CCSNPs control, groups-4 to 7 received dimethylhydrazine for 16 weeks to induce colorectal cancer. Groups-5 to 7 received cromolyn solution, non-medicated chitosan nanoparticles and CCSNPs, respectively as protective treatments. Optimum CCSNPs (size 112.4 nm, charge +39.9 mV, enclosed 93.6% cromolyn and showed a sustained drug release pattern over 48 h) significantly reduced tumor-signaling molecules and the number of aberrant crypt foci compared to dimethylhydrazine. Histopathological examination of colon samples revealed that CCSNPs exerted an augmented protective anticancer effect by ameliorating tumor pathology compared to cromolyn solution. In conclusion, CCSNPs ameliorated tumor pathology and malignant oncogenic signaling molecules in colorectal cancer tissue. Thus, CCSNPs may provide a novel protective approach in colorectal cancer treatment. Moreover, encapsulating cromolyn in chitosan nanoparticles augmented the protective anticancer effect of the drug.



## 1. Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed cancer in males and the second in females, with an estimated 1.4 million cases and 693,900 deaths occurring in 2012 [1]. People get colon cancer through hereditary (familial) and sporadic means. A 90% of the colon cancer cases in which there is no familial history of colon cancer is resulting from sporadic gene mutation [2]. Dietary factors and environmental agents are the major causes of sporadic gene mutations [3]. The mutation occurs in key genes like the adenomatous polyposis coli (APC) gene, K-Ras oncogene, p53 tumor suppressor gene and various other that mediate DNA mismatch repair [4].

β-catenin is an essential component of cell–cell adhesion complexes and is also considered as a transcriptional cofactor [5]. β-catenin level is stabilized through Wnt-signaling. In the absence of Wnt ligands, β-catenin level is suppressed through binding with protein complex composed of APC, axin and GSK-3β which phosphorylates β-catenin leading to its degradation by the proteasome [6]. In CRC, both aberrant activation of the Wnt-signaling and mutation in APC or β-catenin genes occur and were associated with the majority of human and rat colon tumors [7,8].

KRAS protein plays a central role in controlling the activity of several downstream-signaling pathways such as mitogen-activated protein kinase (MAPK) and AKT pathways that regulate normal cellular proliferation, differentiation and survival. Mutations in KRAS lead to activation of its downstream-signaling pathways resulting in uncontrolled cell growth [9]. In particular, activation of the PI3K/AKT pathway leads to activation of several down-stream targets including anti-apoptotic Bcl-2 and NF-κB [10], thus

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promoting colorectal tumorigenesis.

Oxidative stress is linked to cancer initiation and progression by inducing DNA mutations, DNA damage and epigenetic alterations resulting in the transformation of epithelial cells. It can also lead to chronic inflammation, which in turn, could mediate cancer. Moreover, it activates many transcription factors like NF-κB, PPAR-γ, β-catenin/Wnt as well as signaling pathways such as PI3K/AKT, MAPK pathways which are directly related to cancer pathogenesis [11].

Cromolyn is a mast cell stabilizer and an anti-inflammatory agent used in allergic rhinitis and bronchial asthma [12]. Recently, it was proved that cromolyn, combined with gemcitabine, inhibited tumor growth and metastasis in mice model of pancreatic cancer [13]. Our previous work proved that cromolyn showed anticancer properties against several cancer cell lines including colorectal adenocarcinoma (Caco2). Cromolyn not only significantly reduce the viability of cancer cell lines but also raised apoptotic percentages. Furthermore, it significantly reduced survivin and elevated caspase-3 gene expression [14]. Cromolyn binds with S100 calcium binding protein P (S100P) and blocks its interaction with receptor for advanced glycation end product (RAGE) leading to reduction of tumor growth and invasiveness [13]. Overexpression of S100P occurs in colorectal cancer and is positively correlated with clinical staging, lymph node metastasis and recurrence [15,16]. Activation of RAGE stimulates the extracellular signal-regulated kinase (ErK) and NF-κB activity, hence increasing the progression of pancreatic cancer [17]. Dysregulation of GSK-3β occurs in many cancers including colorectal cancer [18,19]. Cromolyn was also found as a GSK-3β inhibitor [20]. Inhibition of GSK-3β enzyme may be a new strategy for cancer treatment. However, following administration, cromolyn is rapidly washed out of the body due to its highly polarity and water solubility, leading to low drug bioavailability [21].

Nanotechnology is employed in cancer prevention, diagnosis and treatment by increasing the solubility and/or bioavailability of anticancer drugs since they can deeply infiltrate tumors with high specificity [22]. Chitosan (CS) is a natural biodegradable polymer. Owing to their cationic nature, bioadhesive and permeability-enhancing properties; chitosan nanoparticles (CSNPs) served as optimum drug delivery systems for anticancer drugs like doxorubicin and quercetin [23,24].

In this study, we aimed to formulate cromolyn chitosan nanoparticles (CCSNPs) and characterize them in terms of size, charge, drug entrapment and release. The protective effect of the optimum formula was compared to that of cromolyn solution in dimethylhydrazine (DMH)-induced CRC model in rats by investigating different tumor-signaling markers. Moreover, histopathological examination of the rats' colon was carried out.

## 2. Materials and methods

### 2.1. Materials

Cromolyn sodium was a gift from (SigmaTec Pharmaceutical Industries, Cairo, Egypt). Chitosan (low molecular weight, LMW and high molecular weight, HMW), sodium tripolyphosphate (TPP), DMH and Pluronic® F-127 were procured from (SigmaAldrich Company, USA). All other chemicals were of pure analytical grade.

### 2.2. Animals

Male Wistar albino rats (130–150 g), supplied by the Animal House, Faculty of Pharmacy, Cairo University, were used. Animals were housed in separate cages at ambient conditions under a 12 h light/dark cycle, fed with commercial pellet diet and had free access to water. Animals were handled and cared for in accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publications No. 8023, revised 1978) and approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University (BC 1176).

### 2.3. Preparation of CCSNPs

Ten different CCSNPs were prepared by ionic gelation technique using TPP as gelating agent [25] (Table 1). Briefly, different concentrations of CS (LMW and HMW) were dissolved in 8 mL of 1% (v/v) acetic acid pH 3.5. Cromolyn solution (prepared by dissolving different concentrations of cromolyn in 1 mL distilled water) was then added to the CS solution and stirred at 600 rpm for 5 min. If present, Pluronic® was dissolved with cromolyn and added to CS solution. Finally, TPP solution (prepared by dissolving different concentrations of TPP in 1 mL distilled water) was added to the CS and homogenized (WiseMix™ HG15D, Daihan Scientific Co, Ltd, Seoul, Korea) at 12,000 rpm for 5 min. Formulations F6-F10 were subjected to a further mixing step using bath sonicator (S30H Elmasonic, Elma Schmidbauer GmbH, Singen, Germany) for 5 min. The cromolyn loaded CS NPs were separated from free drug by ultracentrifugation at 18,000 rpm for 20 min. The NPs were washed with distilled water, recentrifuged at the same previous conditions, frozen at −20 °C for 24 h followed by lyophilization (Novalyphe-NL 500, Savant, Halprook, NY, USA) with a condenser temperature of −45 °C and under vacuum of $7 \times 10^{-2}$mBAR for 24 h. Dried NPs were redisprersed in distilled water and stored at 4 °C for further use.

### 2.4. Characterization of NPs

#### 2.4.1. Size

The particle size distribution and polydispersity index (PDI) of the CSNPs were determined by dynamic light scattering using a Zetasizer instrument (Nano ZS, Malvern Instruments, UK) operating with a 633 nm laser at 25 °C with an angle of detection of 173°. NPs were diluted with filtered distilled water in the ratio 1:10 and results were expressed as an average diameter of the NPs suspension (z-average mean) against percent sample volume. All measurements were performed in triplicate, and the results were reported in terms of mean diameter ± SD.

#### 2.4.2. Zeta potential (ZP)

Electrophoretic mobility measurements were performed by a Zetasizer instrument (Nano ZS, Malvern Instruments, UK) at 25 °C for 120 s using a combination of laser Doppler velocimetry and phase analysis light scattering to measure particle electrophoretic mobility. NPs were diluted with phosphate buffer saline (pH 7.4) in the ratio 1:10 and the average ZP of three samples of each formulation was determined.

#### 2.4.3. Entrapment efficiency (EE %)

Cromolyn loaded NPs were first separated from the free drug by centrifugation at 18,000 rpm for 20 min. The amount of free cromolyn sodium in the supernatant was estimated by UV spectrophotometric method at 326 nm with reference to a preconstructed calibration curve ($R^2 = 0.998$, $n = 3$). The percent entrapment efficiency (EE%) of cromolyn sodium in nanoparticles was determined in triplicate and calculated following the equation [26]: