



# Curcumin-Polyallylhydrocarbon Nanocapsules Potently Suppress 1,2-Dimethylhydrazine-Induced Colorectal Cancer in Mice by Inhibiting Wnt/ $\beta$ -Catenin Pathway

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## Abstract

Curcumin (CUR), a natural polyphenol found in *Curcuma longa* (turmeric) rhizomes, has been widely studied for its anticancer activities against various types of tumors, including colorectal cancer (CRC). However, CUR's therapeutic efficacy is limited by its low bioavailability, short half-life, limited absorption, and rapid and extensive metabolism. Recently, the use of biodegradable and non-toxic polymeric nanocapsules, such as those using polyallylhydrocarbon (PAH), has offered promising delivery systems of poorly absorbed drugs, including CUR. The aim of this study was to determine the *in vivo* antiproliferative efficacy of intraperitoneally injected CUR-PAH nanocapsules (100 mg/kg body weight; 5 days/week) using a mouse model of 1,2-dimethylhydrazine (DMH)-induced CRC. Histopathological analysis confirmed that the formulated nanocapsulate systems reduced the major neoplastic features of CRC. At the molecular level, CUR-PAH nanocapsules downregulated the Wnt/ $\beta$ -catenin pathway as determined by quantitative real-time polymerase chain reaction (qRT-PCR) analysis. A statistically significant downregulation in the gene expression levels of Wnt, frizzled (Frz),  $\beta$ -catenin, transcription factor 4 (Tcf4), lymphoid enhancer-binding factor 1 (Lef1), c-Myc, and cyclin D1 ( $P < 0.01$ ), combined with significant upregulation in the gene expression levels of glycogen synthase kinase (GSK3 $\beta$ ) and adenomatous polyposis coli (APC) ( $P < 0.05$ ), was observed upon post-treatment with CUR-PAH nanocapsules. The observed histopathological and molecular antiproliferative effects were completely absent when using free PAH polymer without CUR, confirming that the anticancer efficacy was solely exerted by the encapsulated CUR. These findings suggest the utility of CUR-PAH nanocapsules as an efficient delivery system with promising therapeutic effects against CRC.

**Keywords** Colorectal cancer · 1,2-dimethylhydrazine · Curcumin · Wnt/ $\beta$ -catenin signaling · Nanocapsules

## 1 Introduction

Colorectal cancer (CRC) is the third most prevalent cancer globally, with nearly 1.8 million new cases in 2018 [1]. This disease occurs due to interplay between genetic and environmental factors. One of the major genetic pathways that are aberrantly activated in CRC is the Wnt signaling pathway [2, 3]. Nowadays, several CRC treatment methods are used, including chemotherapy, radiotherapy, and surgery; however, they lead to numerous side effects, are non-selective, have low cure rates, and result in high recurrence rate of CRC [4].

Therefore, there is a global focus on the use of anticancer medicines from natural sources, such as plant extracts and secondary metabolites, due to their high effectiveness, limited side effects, availability, and low cost [5, 6].

Curcumin (diferuloylmethane) (CUR) is the major polyphenolic compound present in the Indian spice turmeric, which is derived from *Curcuma longa* rhizomes [7]. CUR has been widely applied as a food color, additive, and spice, and it has been recently marketed in many countries in different forms, such as tablets, capsules, ointment, and soap [8, 9]. Over the centuries, CUR has been traditionally used for medicinal purposes in different Asian countries. More specifically, it has been a major constituent of Ayurveda, the traditional Indian holistic system, for its ability to treat eye infections, various dermatological conditions, respiratory ailments, liver disorders, inflammatory diseases, digestive disorders, and oral and dental diseases [10]. Moreover, in-depth research studies have shown that CUR possesses myriad pharmacological activities, including antioxidant, anti-inflammatory, anticancer,

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anti-thrombotic, antimicrobial, anti-diabetic, hypolipidemic, and chemopreventive effects [11, 12]. Such pre-clinically unraveled findings have caused a growing global interest in CUR, thereby paving the road for progress toward human clinical trials [13].

A plethora of *in vitro* and *in vivo* studies have revealed the ability of CUR to prevent the incidence, growth, invasion, and metastasis of cancers. In addition, CUR has been widely valued for suppressing different types of cancers, including esophageal, stomach, colorectal, hepatic, head and neck, breast, and prostate cancer [14, 15]. While many clinical studies reported the safety of CUR consumption, unfortunately it was shown to have rapid metabolism rendering it poorly absorbed. Also, CUR was shown to have low serum levels, limited distribution to tissues, and low bioavailability, which collectively reduce its therapeutic efficacy [16, 17]. Thus, various promising strategies have been devised to surmount CUR's low bioavailability. These strategies involve adjuvants, metal complexes, nanoparticles, liposomes, phospholipid and micelle complexes, derivatives, and analogues [18]. Recently, applying nanotechnology for the target delivery of drugs has become a remarkable solution to enhance the bioavailability of therapeutic drugs and biologics, including CUR [19, 20]. Over the past decade, a pronounced progress has been recognized in the development and application of CUR drug delivery nanocapsules, microemulsions, cyclodextrin inclusions, solid dispersions, and nanotubes [18, 21].

Currently, nanocapsules, such as those made up of polyallylamine hydrochloride (PAH) polymers, are increasingly used as potential targeted drug delivery for treating diseases [22, 23]. Due to being degradable and biologically compatible, polymeric nanoparticles are the most preferable method for delivering drugs [24]. Moreover, polymeric nanoparticles are characterized by their ability to load water insoluble molecules in minimal concentrations that are required to induce therapeutic effects [25].

Recently, a study by Slika et al. revealed a successful formulation of polyallylamine hydrochloride-based CUR nanocapsules via self-assembly [26]. The designed nanocapsules were morphous and spherical with a diameter of 80–100 nm. The *in vitro* drug release study showed that pH triggered the maximum release of CUR under basic conditions, and CUR nanoencapsulation improved its physicochemical properties, drug loading and release, solubility, and bioavailability. Additionally, their findings revealed that these nanocapsules selectively and potently suppressed the growth of colon cancer cells. The present study was carried out to expand upon previous studies and provide a better understanding of the potential *in vivo* therapeutic effects of CUR-PAH nanocapsules, using the 1,2-dimethylhydrazine (DMH)-induced mouse model of CRC.

## 2 Materials and Methods

### 2.1 Chemicals

1,2-Dimethylhydrazine dihydrochloride (DMH) was obtained from ACROS Organics<sup>TM</sup> (Thermo Fisher Scientific, NJ, USA). All primers were purchased from BIO-RAD® (CA, USA). All other chemicals used were of high analytical grades.

### 2.2 Curcumin-PAH Nanocapsules

CUR-PAH nanocapsules were self-assembled based on the nanoprecipitation method. In brief, 1:2 CUR/PAH ratio was prepared by mixing 20 mg of PAH dissolved in water and 10 mg of CUR dissolved in acetone. Then, the mixture was placed on a hot plate at 60°C to totally evaporate acetone. Afterwards, the suspension was centrifuged at 15,000 rpm for 15 min, and the supernatant was used for further analysis. The full characterization of the used nanocapsules was studied by Slika et al. [26].

### 2.3 Animals

Six-week-old female albino Balb/c mice weighing 20–25 g were provided by Beirut Arab University's animal facility. They were housed in plastic cages with *ad libitum* access to tap water and standard mouse diet under standard laboratory conditions of light (12-h light/dark cycle), controlled room temperature, and humidity. All procedures involving animals were in compliance with the guidelines of the Institutional Review Board (IRB) at Beirut Arab University (approval number 2018A-0033-S-M-0245).

### 2.4 Experimental Design

A total of 24 mice were randomly divided into four experimental groups of six mice each.

- Group A (Control): Mice were injected intraperitoneally (i.p.) with 20 mg/kg of saline (0.9% NaCl) once a week over 18 weeks.
- Group B (DMH only): Mice received DMH (20 mg/kg body weight) in saline i.p. once a week for 12 weeks and were left with no further treatment for additional 6 weeks receiving only water.
- Group C (DMH + free PAH): Mice received DMH for 12 weeks, as in group B, but then were post-treated with free PAH i.p. at a dose of 100 mg/kg for 6 weeks (5 days/week).
- Group D (DMH + CUR-PAH post-treatment): Mice received DMH for 12 weeks, as in group B, but then were post-treated with CUR-PAH nanocapsules (100 mg/kg) for 6 weeks.

At the end of the experiments, animals were fasted overnight and then sacrificed.

## 2.5 Histopathological Analysis

After dissection, colons were excised, opened longitudinally, and flushed with saline, and part of them was immediately fixed in 10% formalin at room temperature for 24 h and sent to Specialized Medical Laboratories (Beirut, Lebanon). Histopathological examination was performed using hematoxylin and eosin (H&E) staining.

## 2.6 Tissue Homogenization

The distal region of the colon with tumors was manually homogenized on ice at a ratio of 1 g per 5 mL of phosphate buffered saline (PBS) buffer (pH 7.4). The homogenization buffer contained 1 mM of phenylmethylsulfonyl fluoride (PMSF)—a commonly used protease inhibitor. Centrifugation of samples was subsequently done at 15,000 rpm for 15 min at 4°C. Eventually, the supernatants were collected and stored at –80°C for later use.

## 2.7 Molecular Assays

Total RNA was purified from colon tissue homogenates using Aurum<sup>TM</sup> total RNA mini kit (BIORAD®, CA, USA) according to the recommendations of the manufacturer. The integrity and size distribution of total RNA were checked by agarose gel electrophoresis and ethidium bromide staining. Reverse transcription was done using QuantiTect® Reverse Transcription Kit (QIAGEN®, MD, USA). Two micrograms of total RNA was reverse transcribed into cDNA in a volume of 40 µL at 42°C for 25 min, and the reaction was terminated at 95°C for 3 min.

Wnt5a, frizzled receptor (Frz)-8,  $\beta$ -catenin (Ctnnb), adenomatous polyposis coli (APC), glycogen synthase kinase (GSK3- $\beta$ ), transcription factor 4 (Tcf4), lymphoid enhancer-binding factor 1 (Lef1), c-Myc, cyclin D1, and GAPDH (housekeeping gene) genes were quantified by quantitative real-time polymerase chain reaction using QuantiFast® SYBR® Green PCR Kit (QIAGEN®, MD, USA). These genes were amplified from 2 µL of template cDNA in a final volume of 20 µL PCR reaction mixture which contains 10 µL of 2x QuantiFast SYBR Green PCR Master Mix, 6 µL RNase-free water, and 2 µL of each primer (1 µM). Cycling was done with a denaturation step at 95 °C for 5 min, followed by 45 cycles of denaturation at 95 °C for 10 s and annealing/extension at 57 °C for 30s. The PCR primer sets used are enlisted in Supplementary Table 1. All primer sets used in this study for PCR amplification were selected based on previously published work [27]. Experiments were carried out in

triplicate, and the relative gene expression was calculated using the  $2^{-\Delta\Delta CT}$  gene dosage ratio formula.

## 2.8 Statistical Analysis

All statistical analyses were performed using Microsoft Excel and GraphPad prism software (Version 7). Statistical significance was tested using one-way ANOVA followed by Tukey test for carrying out multiple comparisons. A *P*-value < 0.05 was considered significant.

## 3 Results

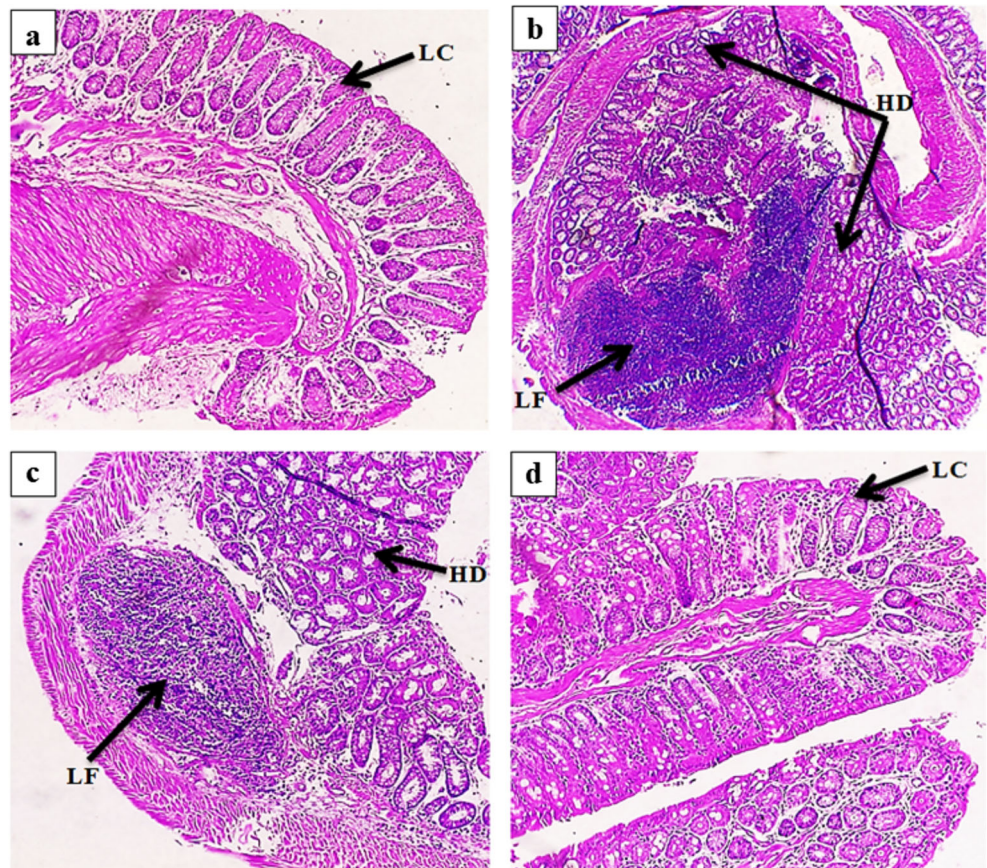
### 3.1 Histopathological Analysis

The histopathological examination of the colons from different groups is shown in Fig. 1. In the control group, a regular histoarchitecture of the colon with its abundant Lieberkühn crypts and goblet cells lining the crypts was observed (panel a). In the DMH control group, there is a clear presence of colon tumors and histological features of adenocarcinoma (panel b). In this group, high-grade dysplasia and abnormal structures of Lieberkühn crypts were noted. Also, neoplastic invasion to the muscular layers of the intestine and formation of hyperplastic gland-like structures called lymphoid follicles were reported. Similar effects were noticed in the colonic tissues of mice receiving DMH and post-treated with free PAH polymers, as evident in panel c, indicating that the free polymers did not exert any therapeutic effect against DMH damage. On the other hand, upon post-treatment of DMH-injected mice with CUR-PAH nanocapsules, most of the pre-malignancy parameters such as dysplasia and hyperplasia were mitigated (panel d). Additionally, the nanocapsules restored the normal histoarchitecture of Lieberkühn crypts.

### 3.2 Expression Levels of Wnt Pathway Regulators

As shown in Fig. 2, significant downregulation in the expression levels of Wnt5a (12-fold, *P* < 0.01), Frz (8.1-fold, *P* < 0.01), Ctnnb (15.8-fold, *P* < 0.01), Tcf4 (15.6-fold, *P* < 0.01), and Lef1 (20.2-fold, *P* < 0.01) and upregulation in the expression levels of GSK3 $\beta$  (0.5-fold, *P* < 0.05) and APC (0.62-fold, *P* < 0.05) were observed in mice post-treated with CUR-PAH nanocapsules (group D) compared to those receiving DMH (group B). A similar trend was observed in the CUR-PAH nanocapsules treated group when compared to the free PAH nanocapsules treated group, suggesting limited effects of free PAH nanocapsules (group C).

**Fig. 1** Histopathological analysis of colonic tissues from controls and DMH-treated mice (H&E  $\times$  100). Histological architecture of the colon from control mice (**a**), DMH-injected mice (**b**), DMH-injected mice treated with free polymers (**c**), and DMH-injected mice post-treated with CUR-PAH nanocapsules (**d**). Lieberkühn crypts CL ( $\rightarrow$ ); hyperplastic lymphoid follicles LF ( $\rightarrow$ ); high-grade dysplasia HD ( $\rightarrow$ )



### 3.3 Expression Levels of Cell Cycle Regulators

As shown in Fig. 3, the mRNA levels of cell cycle regulators c-Myc and cyclin D1 were significantly upregulated upon DMH treatment (group B) compared to the control group. However, the levels of these genes were significantly downregulated by 22.5- and 16.7-fold ( $P < 0.01$ ), respectively, upon post-treating the mice with CUR-PAH nanocapsules (group D), while the free PAH polymer had no effect on the expression of those genes (group C).

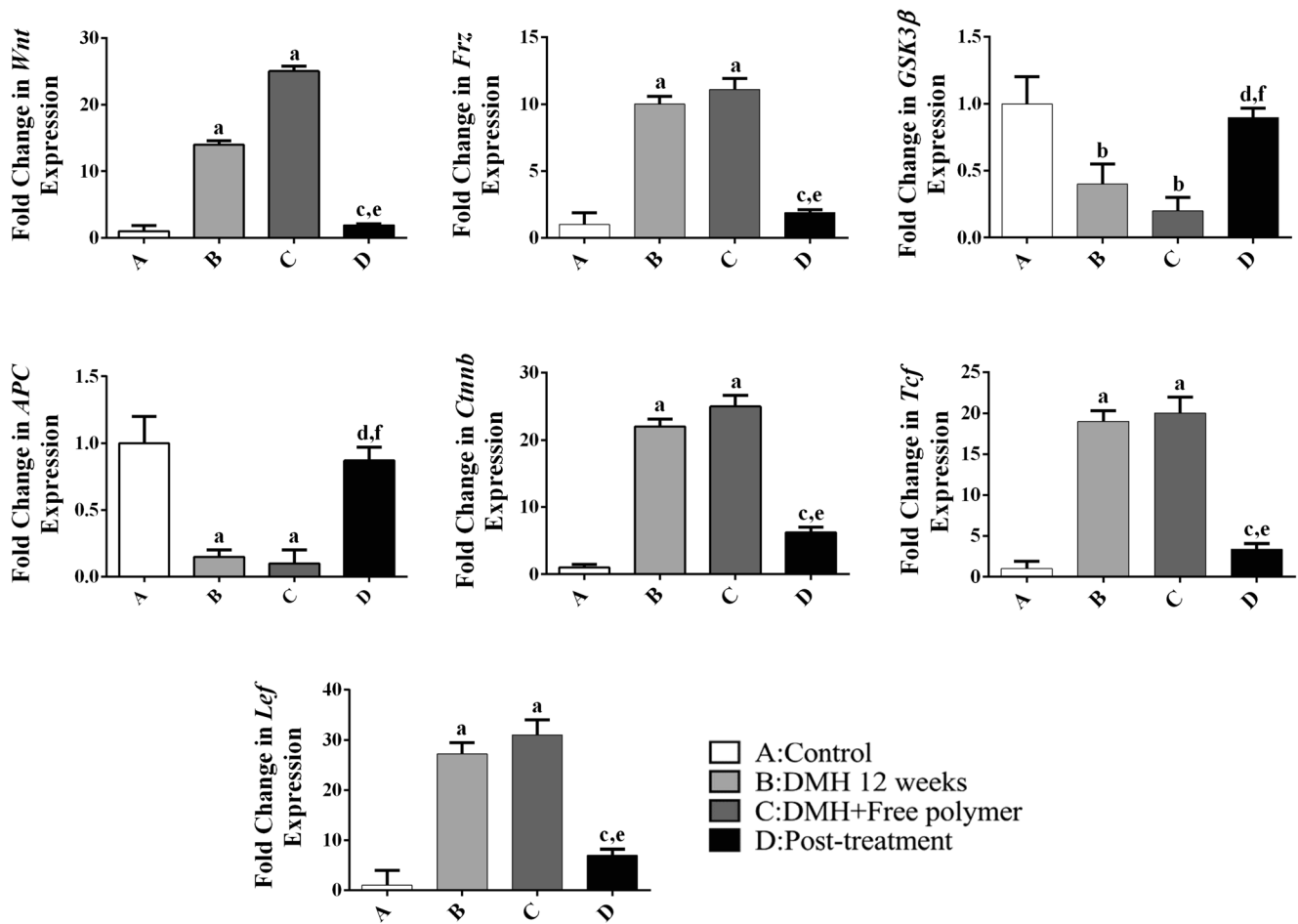
## 4 Discussion

Chemotherapeutic agents are the most commonly used drugs in the treatment of human CRC; however, they are well-documented to have numerous side effects, exert limited response, and induce drug resistance [28]. In contrast, the potent antitumor activities of CUR, a natural polyphenol found in turmeric, have received global attention [10, 29]. Due to the bioavailability problems of CUR, the use of nanotechnology has been applied to increase drug delivery; one of the promising nanotechnological methods is the formation of polymeric nanocapsules [30, 31]. Herein, an 18-week model of CRC was induced in mice by DMH, a common in vivo model for

the experimental study of human CRC [32]. Our study involved investigating the post-treatment efficacy of CUR-PAH nanocapsules as well as of the free PAH polymers on this model.

Interestingly, in comparison with DMH alone or with free polymer, the post-treatment of DMH induced CRC with CUR-PAH nanocapsules resulted in an inhibition of the formation of preneoplastic aspects, dysplasia, and hyperplasia in lymphoid follicles, which collectively are the major microscopic features occurring in this DMH model of carcinogenesis [32]. CUR-PAH nanocapsules induced partial regeneration of epithelial linings, thus proving their efficacy against CRC. Such observations are consistent with the previous studies where CUR was shown to exert anticancer activities against multiple human cancer cell lines including colorectal cancer [33], modulated oxidative stress and aberrant crypt foci in DMH-induced CRC in mice [34], chemo-prevented DMH-induced CRC in albino rat model [35], and abolished inflammation-associated CRC in DMH-initiated and DSS-promoted mouse model [36].

The growth and progression of CRC is multigenic and involves alteration in several signaling pathways. In this view, CRC is characterized by aberrant activation of Wnt/ $\beta$ -catenin signaling pathway [37]. Mechanistically, upon binding of Wnt ligand to the Frz receptor, a series of downstream signaling

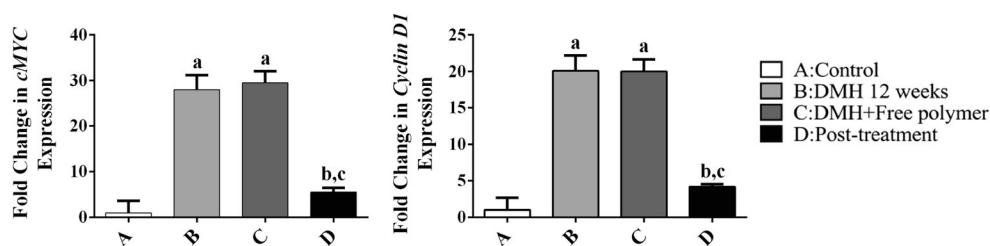


**Fig. 2** qRT-PCR findings show the modulatory effects of DMH, alone and with free polymer, and post-treatment with CUR-PAH nanocapsules, on the relative mRNA expression of Wnt (A), Ctnnb (B), GSK3 $\beta$  (C), APC (D), Tcf (E), and Lef (F) genes. **a**  $P < 0.01$  versus control group; **b**  $P < 0.05$  versus control group; **c**  $P < 0.01$  versus DMH group; **d**  $P < 0.05$  versus DMH group; **e**  $P < 0.01$  versus DMH + free polymer group; and **f**  $P < 0.05$  versus DMH + free polymer group

More importantly, our findings demonstrate that CUR encapsulation showed the potency of CUR as a multilevel-repressor of Wnt/ $\beta$ -catenin signaling pathway as well as of its downstream oncogenes c-Myc and cyclin D1. Therefore, our findings implicate the potency of encapsulated CUR as an effective anticancer agent in the treatment of CRC.

events occur leading to a dissociation of the destruction complex and dephosphorylation of  $\beta$ -catenin and stabilization in the cytosol. This causes the translocation of  $\beta$ -catenin into the nucleus wherein it acts as a transcription factor for some oncogenes such as c-Myc and cyclin D1 [38]. In fact, about 80% of all human CRCs are characterized by aberrant activation of the Wnt/ $\beta$ -catenin signaling [39]. On the basis of this fact, it is clear that targeting and repression of this crucial pathway holds great therapeutic potential in treating CRC [38, 40].

Appealingly, the literature is rich in similar studies that prove the potency of CUR against CRC, and it has been shown to significantly repress the overexpressed Wnt/ $\beta$ -



**Fig. 3** qRT-PCR findings show the modulatory effects of DMH, alone and with free polymer, and post-treatment with CUR-PAH nanocapsules, on the relative mRNA expression of c-Myc (A) and cyclin D1 (B) genes. **a**  $P < 0.01$  versus control group; **b**  $P < 0.01$  versus DMH group; and **c**  $P < 0.01$  versus DMH + free polymer group

More importantly, our findings demonstrate that CUR encapsulation showed the potency of CUR as a multilevel-repressor of Wnt/ $\beta$ -catenin signaling pathway as well as of its downstream oncogenes c-Myc and cyclin D1. Therefore, our findings implicate the potency of encapsulated CUR as an effective anticancer agent in the treatment of CRC.

catenin signaling pathway, which also supports our observations [41–43]. Moreover, several studies assessed the effect of either CUR alone or CUR nanoparticles on the modulation of the Wnt/ $\beta$ -catenin pathway in other pathophysiological conditions. For instance, CUR has been reported to exert its anti-tumor activities via inhibiting several pro cancer processes and signaling pathways including the Wnt/ $\beta$  catenin pathway in breast cancer [44, 45], prostate cancer cells [46], hepatocellular carcinoma [47], lung cancer [48–50], endometrial carcinoma cells [51], gastric carcinoma cells [52], and melanoma cancer cells [53].

Consistent to our findings, various CUR nanoformulations have been designed to enhance the delivery, bioavailability, and the therapeutic effectiveness of CUR against CRC [54, 55]. Examples on CUR nanoformulations for CRC treatment include liposomes, micelles, polymeric nanoparticles/nanocapsules, nanogels, dendrimers, cyclodextrin complexes, solid lipid nanoparticles, and gold nanoparticles [30]. A study by Li et al. proved an effective in vitro and in vivo antitumor activity of CUR liposomes against CRC where liposomal CUR inhibited the growth of Lovo and Colo25 cells at lower IC50 compared to free CUR, induced apoptosis, and reduced several angiogenic factors [56]. Another study by Tefas et al. showed that the encapsulation of CUR into doxorubicin liposomes remarkably reduced C26 cell proliferation compared free CUR [57]. In addition, CUR-chitosan nanoparticles enhanced the delivery of CUR to colonic HT29 cells via mucoadhesion which prolonged the contact time of CUR with these cells [58]. These nanoparticles exerted potent anticancer effects, reduced cell viability and IC50, induced cell apoptosis, and cell cycle arrest at G2/M phase [59]. Similarly, CUR-poly(lactic acid/glycolic acid) (PLGA)-lecithin-PEG nanoparticles exerted higher in vitro cytotoxicity effects against HT29 colon cells compared to free CUR leading to enhanced bioavailability [60]. Also, a study by Xiao et al. assessed the in vitro cytotoxic effects of CUR-PLGA-chitosan nanoparticles against Colon-26 cells, whereby these nanoparticles demonstrated a better cellular uptake of CUR and apoptotic effects compared to its free form [61].

Besides, the literature includes numerous studies on the effectiveness of CUR polymeric nanocapsules against CRC [62]. Klippstein and colleagues revealed that PLGA-based polymeric CUR nanocapsules exhibited high loading efficiency, targeted delivery, and exert therapeutic activity against colon cancer in mice. These CUR-loaded nanocapsules induced apoptosis and blocked the cell cycle which led to significantly smaller tumor volumes compared to empty nanocapsules [63]. Also, Le and Kim showed that folate-PEG/Hyd-CUR/C18-g-PSI micelles exhibited site specific delivery of CUR to colon cancer cells via Wnt/ $\beta$ -catenin signaling pathway [64]. Likewise, Lotfi-Attari et al. reported the efficacy of CUR polymeric nanoparticles in inhibiting human CRC cells [65].

Correspondingly, Udompornmongkol and Chiang showed that CUR-loaded polymeric nanoparticles exerted significant anti-colorectal cancer activities [66]. Several studies have reported the therapeutic effects of CUR-loaded biodegradable polymeric micelles against colon cancer in vitro and in vivo [67]. Furthermore, novel polymeric CUR nanocarriers were shown to suppress azoxymethane-initiated colon cancer in rats [68] and in colon-tumor-bearing mice [69].

Finally, it is noteworthy that the CUR-PAH nanocapsules used in our study were originally self-assembled via the process of nanoprecipitation, and CUR release profiles were assessed under various conditions where the best release was attained at alkaline conditions which resemble the colonic regions. In addition, these CUR-PAH nanocapsules allowed targeted delivery of CUR to colonic cells in vitro and exhibited significant and selective cytotoxicity against Caco-2 cells [26].

In conclusion, this study suggests that CUR-PAH nanocapsules significantly inhibit CRC by modulating the Wnt/ $\beta$ -catenin pathway. In fact, the positive outcomes from the current study may push toward developing CUR nanoformulations for CRC patients. The major limitation in this study is that our conclusions regarding CUR-PAH nanocapsules ability to modulate the Wnt/ $\beta$ -catenin signaling pathway and to regulate the cell cycle were solely based on mRNA levels. Thus, this study confirms that the exerted nanocapsules' control of expression is at the transcriptional level only, which might not be exactly the same on the translational protein level. Therefore, further investigations at the protein level are warranted in future studies. Moreover, biocompatibility and toxicity assessments and detailed in vitro and in vivo evaluations should be validated in order to ensure the safety and effectiveness of these nanoformulations.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s12668-021-00842-5>.

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## Declarations

**Conflict of Interest** None.

**Research Involving Humans and Animals Statement** We declare that the present study involved animals. All experimental procedures were approved by the Institutional Review Board (IRB) at Beirut Arab University (approval number 2018A-0033-S-M-0245).

**Informed Consent** None.

## References

- Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer Journal for Clinicians*, *68*(6), 394–424.
- Polakis, P. (2012). Wnt signaling in cancer. *Cold Spring Harbor Perspectives in Biology*, *4*(5), a008052.
- Novellasademunt, L., Antas, P., & Li, V. S. (2015). Targeting Wnt signaling in colorectal cancer. A review in the theme: Cell signaling: Proteins, pathways and mechanisms. *American Journal of Physiology. Cell Physiology*, *309*(8), C511–C521.
- Van der Jeught, K., Xu, H.-C., Li, Y.-J., Lu, X.-B., & Ji, G. (2018). Drug resistance and new therapies in colorectal cancer. *World Journal of Gastroenterology*, *24*(34), 3834–3848.
- Rafieian-Kopaie, M., & Nasri, H. (2015). On the occasion of World Cancer Day 2015; the possibility of cancer prevention or treatment with antioxidants: the ongoing cancer prevention researches. *International Journal of Preventive Medicine*, *6*, 108.
- Newman, D. J., & Cragg, G. M. (2007). Natural products as sources of new drugs over the last 25 years. *Journal of Natural Products*, *70*(3), 461–477.
- Kocaadam, B., & Şanlıer, N. (2017). Curcumin, an active component of turmeric (*Curcuma longa*), and its effects on health. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2889–2895.
- Gupta, S. C., Sung, B., Kim, J. H., Prasad, S., Li, S., & Aggarwal, B. B. (2013). Multitargeting by turmeric, the golden spice: From kitchen to clinic. *Molecular Nutrition & Food Research*, *57*(9), 1510–1528.
- Prasad, S., Tyagi, A. K., & Aggarwal, B. B. (2014). Recent developments in delivery, bioavailability, absorption and metabolism of curcumin: the golden pigment from golden spice. *Cancer Research and Treatment*, *46*(1), 2–18.
- Hewlings, S. J., & Kalman, D. S. (2017). Curcumin: A review of its' effects on human health. *Foods*, *6*(10), 92.
- Rahmani, A. H., Alsahli, M. A., Aly, S. M., Khan, M. A., & Aldebasi, Y. H. (2018). Role of curcumin in disease prevention and treatment. *Advanced Biomedical Research*, *7*, 38.
- Boroumand, N., Samarghandian, S., & Hashemy, S. I. (2018). Immunomodulatory, anti-inflammatory, and antioxidant effects of curcumin. *Journal of Herbmmed Pharmacology*, *7*(4), 211–219.
- Willenbacher, E., Khan, S. Z., Mujica, S. C. A., et al. (2019). Curcumin: New insights into an ancient ingredient against cancer. *International Journal of Molecular Sciences*, *20*(8), 1808.
- Tomeh, M. A., Hadianamrei, R., & Zhao, X. (2019). A review of curcumin and its derivatives as anticancer agents. *International Journal of Molecular Sciences*, *20*(5), 1033.
- Vallianou, N. G., Evangelopoulos, A., Schizas, N., & Kazazis, C. (2015). Potential anticancer properties and mechanisms of action of curcumin. *Anticancer Research*, *35*(2), 645–651.
- Schneider, C., Gordon, O. N., Edwards, R. L., & Luis, P. B. (2015). Degradation of curcumin: From mechanism to biological implications. *Journal of Agricultural and Food Chemistry*, *63*(35), 7606–7614.
- Burgos-Morón, E., Calderón-Montaña, J. M., Salvador, J., Robles, A., & López-Lázaro, M. (2010). The dark side of curcumin. *International Journal of Cancer*, *126*(7), 1771–1775.
- Bansal, S. S., Goel, M., Aqil, F., Vadhanam, M. V., & Gupta, R. C. (2011). Advanced drug-delivery systems of curcumin for cancer chemoprevention. *Cancer Prevention Research*, *4*, 1158–1171.
- Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal*, *26*(1), 64–70.
- Slika, L., & Patra, D. (2020). A short review on chemical properties, stability and nano-technological advances for curcumin delivery. *Expert Opinion on Drug Delivery*, *17*(1), 61–75.
- Mahmood, K., Zia, K. M., Zuber, M., Salman, M., & Anjum, M. N. (2015). Recent developments in curcumin and curcumin based polymeric materials for biomedical applications: a review. *International Journal of Biological Macromolecules*, *81*, 877–890.
- Janeesh, P. A., Sami, H., Dhanya, C. R., Sivakumar, S., & Abraham, A. (2014). Biocompatibility and genotoxicity studies of polyallylamine hydrochloride nanocapsules in rats. *RSC Advances*, *4*(47), 24484–24497.
- Li, H., Zheng, H., Tong, W., & Gao, C. (2017). Non-covalent assembly of poly(allylamine hydrochloride)/triethylamine microcapsules with ionic strength-responsiveness and auto-fluorescence. *Journal of Colloid and Interface Science*, *496*, 228–234.
- Kopecek, J. (2013). Polymer-drug conjugates: origins, progress to date and future directions. *Advanced Drug Delivery Reviews*, *65*(1), 49–59.
- Prabhu, R. H., Patravale, V. B., & Joshi, M. D. (2015). Polymeric nanoparticles for targeted treatment in oncology: Current insights. *International Journal of Nanomedicine*, *10*, 1001–1018.
- Slika, L., Moubarak, A., Borjac, J., Baydoun, E., & Patra, D. (2019). Preparation of curcumin-poly (allyl amine) hydrochloride based nanocapsules: Piperine in nanocapsules accelerates encapsulation and release of curcumin and effectiveness against colon cancer cells. *Materials Science and Engineering: C*, *109*, 110550.
- El Joumaa, M., Taleb, R., Rizk, S., & Borjac, J. (2020). Protective effect of *Matricaria chamomilla* extract against 1,2-dimethylhydrazine-induced colorectal cancer in mice. *Journal of Complementary and Integrative Medicine*, *17*(3), 20190143.
- Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: a Cancer Journal for Clinicians*, *68*(1), 7–30.
- Gupta, S. C., Patchva, S., Koh, W., & Aggarwal, B. B. (2012). Discovery of curcumin, a component of golden spice, and its miraculous biological activities. *Clinical and Experimental Pharmacology & Physiology*, *39*(3), 283–299.
- Yallapu, M. M., Nagesh, P. K. B., Jaggi, M., & Chauhan, S. C. (2015). Therapeutic applications of curcumin nanoformulations. *The AAPS Journal*, *17*(6), 1341–1356.
- Martínez-Ballesta, M., Gil-Izquierdo, Á., García-Viguera, C., & Domínguez-Perles, R. (2018). Nanoparticles and controlled delivery for bioactive compounds: Outlining challenges for new "smart-foods" for health. *Foods*, *7*(5), 72.
- Washington, M. K., Powell, A. E., Sullivan, R., et al. (2013). Pathology of rodent models of intestinal cancer: progress report and recommendations. *Gastroenterology*, *144*(4), 705–717.
- Ismail, N. I., Othman, I., Abas, F., Lagis, N. H., & Naidu, R. (2019). Mechanism of apoptosis induced by curcumin in colorectal cancer. *International Journal of Molecular Sciences*, *20*(10), 2454.
- Bounaama, A., Djerdjouri, B., Laroche-Clary, A., Le Morvan, V., & Robert, J. (2012). Short curcumin treatment modulates oxidative stress, arginase activity, aberrant crypt foci, and TGF- $\beta$ 1 and HES-1 transcripts in 1,2-dimethylhydrazine-colon carcinogenesis in mice. *Toxicology*, *302*(2), 308–317.
- Youssef, K. M., Ezzo, A. M., El-Sayed, M. I., Hazzaa, A. A., El-Medany, A. H., & Arafa, M. (2015). Chemopreventive effects of curcumin analogs in DMH-induced colon cancer in albino rats model. *Future Journal of Pharmaceutical Sciences*, *1*(2), 57–72.
- Murakami, A., Furukawa, I., Miyamoto, S., Tanaka, T., & Ohigashi, H. (2013). Curcumin combined with turmerones, essential oil components of turmeric, abolishes inflammation-associated mouse colon carcinogenesis. *BioFactors*, *39*(2), 221–232.
- Cheng, X., Xu, X., Chen, D., Zhao, F., & Wang, W. (2019). Therapeutic potential of targeting the Wnt/ $\beta$ -catenin signaling pathway in colorectal cancer. *Biomedicine & Pharmacotherapy*, *110*, 473–481.

38. Krishnamurthy, N., & Kurzrock, R. (2018). Targeting the Wnt/β-catenin pathway in cancer: Update on effectors and inhibitors. *Cancer Treatment Reviews*, *62*, 50–60.
39. Zhan, T., Rindtorff, N., & Boutros, M. (2017). Wnt signaling in cancer. *Oncogene*, *36*(11), 1461–1473.
40. Shang, S., Hua, F., & Hu, Z.-W. (2017). The regulation of β-catenin activity and function in cancer: therapeutic opportunities. *Oncotarget*, *8*(20), 33972–33989.
41. Dou, H., Shen, R., Tao, J., et al. (2017). Curcumin suppresses the colon cancer proliferation by inhibiting Wnt/β-catenin pathways via miR-130a. *Frontiers in Pharmacology*, *8*, 877.
42. Wang, M., Jiang, S., Zhou, L., et al. (2019). Potential mechanisms of action of curcumin for cancer prevention: Focus on cellular signaling pathways and miRNAs. *International Journal of Biological Sciences*, *15*(6), 1200–1214.
43. Zhang, Z., Chen, H., Xu, C., et al. (2016). Curcumin inhibits tumor epithelial-mesenchymal transition by downregulating the Wnt signaling pathway and upregulating NKD2 expression in colon cancer cells. *Oncology Reports*, *35*(5), 2615–2623.
44. Song, X., Zhang, M., Dai, E., & Luo, Y. (2019). Molecular targets of curcumin in breast cancer (Review). *Molecular Medicine Reports*, *19*(1), 23–29.
45. Li, X., Wang, X., Xie, C., et al. (2018). Sonic hedgehog and Wnt/β-catenin pathways mediate curcumin inhibition of breast cancer stem cells. *Anti-Cancer Drugs*, *29*(3), 208–215.
46. Choi, H. Y., Lim, J. E., & Hong, J. H. (2010). Curcumin interrupts the interaction between the androgen receptor and Wnt/β-catenin signaling pathway in LNCaP prostate cancer cells. *Prostate Cancer and Prostatic Diseases*, *13*(4), 343–349.
47. Hu, P., Ke, C., Guo, X., et al. (2019). Both glypican-3/Wnt/β-catenin signaling pathway and autophagy contributed to the inhibitory effect of curcumin on hepatocellular carcinoma. *Digestive and Liver Disease*, *51*(1), 120–126.
48. Wang, J. Y., Wang, X., Wang, X. J., et al. (2018). Curcumin inhibits the growth via Wnt/β-catenin pathway in non-small-cell lung cancer cells. *European Review for Medical and Pharmacological Sciences*, *22*(21), 7492–7499.
49. Zhu, J. Y., Yang, X., Chen, Y., et al. (2017). Curcumin suppresses lung cancer stem cells via inhibiting Wnt/β-catenin and sonic hedgehog pathways. *Phytotherapy Research*, *31*(4), 680–688.
50. Lu, Y., Wei, C., & Xi, Z. (2014). Curcumin suppresses proliferation and invasion in non-small cell lung cancer by modulation of MTA1-mediated Wnt/β-catenin pathway. *In Vitro Cellular & Developmental Biology. Animal*, *50*(9), 840–850.
51. Feng, W., Yang, C. X., Zhang, L., Fang, Y., & Yan, M. (2014). Curcumin promotes the apoptosis of human endometrial carcinoma cells by downregulating the expression of androgen receptor through Wnt signal pathway. *European Journal of Gynaecological Oncology*, *35*(6), 718–723.
52. Zheng, R., Deng, Q., Liu, Y., & Zhao, P. (2017). Curcumin inhibits gastric carcinoma cell growth and induces apoptosis by suppressing the Wnt/β-catenin signaling pathway. *Medical Science Monitor*, *23*, 163–171.
53. Srivastava, N. S., & Srivastava, R. A. K. (2019). Curcumin and quercetin synergistically inhibit cancer cell proliferation in multiple cancer cells and modulate Wnt/β-catenin signaling and apoptotic pathways in A375 cells. *Phytomedicine*, *52*, 117–128.
54. Wong, K. E., Ngai, S. C., Chan, K.-G., Lee, L.-H., Goh, B.-H., & Chuah, L.-H. (2019). Curcumin nanoformulations for colorectal cancer: A review. *Frontiers in Pharmacology*, *10*, 152.
55. Lee, W. H., Loo, C. Y., Young, P. M., Traini, D., Mason, R. S., & Rohanizadeh, R. (2014). Recent advances in curcumin nanoformulation for cancer therapy. *Expert Opinion on Drug Delivery*, *11*(8), 1183–1201.
56. Li, L., Ahmed, B., Mehta, K., & Kurzrock, R. (2007). Liposomal curcumin with and without oxaliplatin: effects on cell growth, apoptosis, and angiogenesis in colorectal cancer. *Molecular Cancer Therapeutics*, *6*(4), 1276–1282.
57. Tefas, L. R., Sylvester, B., Tomuta, I., et al. (2017). Development of antiproliferative long-circulating liposomes co-encapsulating doxorubicin and curcumin, through the use of a quality-by-design approach. *Drug Design, Development and Therapy*, *11*, 1605–1621.
58. Chuah, L. H., Roberts, C. J., Billa, N., Abdullah, S., & Rosli, R. (2014). Cellular uptake and anticancer effects of mucoadhesive curcumin-containing chitosan nanoparticles. *Colloids and Surfaces. B, Biointerfaces*, *116*, 228–236.
59. Li, L., Xiang, D., Shigdar, S., et al. (2014). Epithelial cell adhesion molecule aptamer functionalized PLGA-lecithin-curcumin-PEG nanoparticles for targeted drug delivery to human colorectal adenocarcinoma cells. *International Journal of Nanomedicine*, *9*, 1083–1096.
60. Xiao, B., Si, X., Han, M. K., Viennois, E., Zhang, M., & Merlin, D. (2015). Co-delivery of camptothecin and curcumin by cationic polymeric nanoparticles for synergistic colon cancer combination chemotherapy. *Journal of Materials Chemistry B*, *3*(39), 7724–7733.
61. Umerska, A., Gaucher, C., Oyarzun-Ampuero, F., et al. (2018). Polymeric nanoparticles for increasing oral bioavailability of curcumin. *Antioxidants*, *7*(4), 46.
62. Klippstein, R., Wang, J. T.-W., El-Gogary, R. I., et al. (2015). Passively targeted curcumin-loaded PEGylated PLGA nanocapsules for colon cancer therapy in vivo. *Small*, *11*(36), 4704–4722.
63. Le, T. T., & Kim, D. (2019). Folate-PEG/Hyd-curcumin/C18-g-PSI micelles for site specific delivery of curcumin to colon cancer cells via Wnt/β-catenin signaling pathway. *Materials Science & Engineering C-Materials*, *101*, 464–471.
64. Loffi-Attari, J., Pilehvar-Soltanahmadi, Y., Dadashpour, M., et al. (2017). Co-delivery of curcumin and chrysin by polymeric nanoparticles inhibit synergistically growth and hTERT gene expression in human colorectal cancer cells. *Nutrition and Cancer*, *69*(8), 1290–1299.
65. Udompornmongkol, P., & Chiang, B. H. (2015). Curcumin-loaded polymeric nanoparticles for enhanced anti-colorectal cancer applications. *Journal of Biomaterials Applications*, *30*(5), 537–546.
66. Yang, X., Li, Z., Wang, N., et al. (2015). Curcumin-encapsulated polymeric micelles suppress the development of colon cancer in vitro and in vivo. *Scientific Reports*, *5*, 10322.
67. Gou, M., Men, K., Shi, H., et al. (2011). Curcumin-loaded biodegradable polymeric micelles for colon cancer therapy in vitro and in vivo. *Nanoscale*, *3*(4), 1558–1567.
68. Alizadeh, A. M., Khaniki, M., Azizian, S., Mohaghheghi, M. A., Sadeghizadeh, M., & Najafi, F. (2012). Chemoprevention of azoxymethane-initiated colon cancer in rat by using a novel polymeric nanocarrier-curcumin. *European Journal of Pharmacology*, *689*(1-3), 226–232.
69. Chaurasia, S., Chaubey, P., Patel, R. R., Kumar, N., & Mishra, B. (2016). Curcumin-polymeric nanoparticles against colon-26 tumor-bearing mice: cytotoxicity, pharmacokinetic and anticancer efficacy studies. *Drug Development and Industrial Pharmacy*, *42*(5), 694–700.

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