Life Sciences 237 (2019) 116895

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Probiotic supplementation attenuates the aggressiveness of chemically induced colorectal tumor in rats

Sandra Cristina Genaro[a,b,*], Luis Souza Lima de Souza Reis[a,c], Sabrina Karen Reis[d], Eduardo Augusto Rabelo Socca[d], Wagner José Fávaro[d]

[a] Postgraduate Program in Animal Science, University of West Paulist, Presidente Prudente, SP, Brazil
[b] Faculty of Nutrition, University of West São Paulo, Presidente Prudente, SP, Brazil
[c] Faculty of Veterinary Medicine, University of West São Paulo, Presidente Prudente, SP, Brazil
[d] Laboratory of Urogenital Carcinogenesis and Immunotherapy, State University of Campinas, UNICAMP, Campinas-SP, Brazil

ARTICLE INFO

Keywords:
Colorectal cancer
Functional food
Chemotherapy
Aberrant crypt foci
Intestinal microbita

ABSTRACT

To evaluate the effect of a probiotic on the aggressiveness of a chemically induced colorectal tumor in rats. Twenty-five male Fisher 344 rats, 250 g, provided with feed and water *ad libitum*, were randomly divided into 5 groups (5 rats/group): $G_{Control}$, no treatment; G_{Tumor}, tumor induction; $G_{Tumor+5FU}$, tumor induction, 5-Fluorouracil applied; $G_{Tumor+Prob}$, induction of the tumor, supplemented with probiotic; $G_{Tumor+5\text{-}FU+Prob}$, tumor induction, 5-Fluorouracil applied, supplemented with probiotic. For tumor induction 20 mg/kg of 1,2-dimethylhydrazine was applied intraperitoneally over 4 weeks, followed by an interval of 15 days, and then repeated for a further 4 weeks. Five weeks after the final dose of the carcinogen, treatment was initiated with 5-Fluorouracil (15 mg/kg, intraperitoneally/week) and a commercial probiotic (1×10^9 CFU, daily/gavage). Data were analyzed by *One Way* Variance Analysis and means compared by *Dunnett's* test. *GraphPad Prism* statistical software was used. The histopathological analyzes were evaluated by the chi-square test. A 5% type-I error was considered statistically significant. Compared with the G_{Tumor}, the $G_{Tumor+Prob}$ ($p < 0.0373$) and $G_{Tumor+5\text{-}FU+Prob}$ ($p < 0.0003$) demonstrated an attenuated effect on the aggressiveness of the colorectal tumor, with a reduction in the count of Aberrant Crypt foci; and a lower percentage of malignant neoplastic lesions in the $G_{Tumor+Prob}$ (40% low grade tubular adenoma, 40% carcinoma *in situ*, 20% low grade adenocarcinoma) and $G_{Tumor+5\text{-}FU+Prob}$ (40% low grade tubular adenoma and 60% carcinoma *in situ*). Probiotic supplementation has the potential to decrease the formation of aberrant crypts and ameliorate tumor malignancy, enhancing the antitumor effect of 5-Fluorouracil chemotherapy in colic segments.

1. Introduction

Colorectal cancer (CRC) is considered the third leading cause of death in the world [1], with a 60% increase in its incidence by 2030, resulting in 1.1 million deaths [2].

Risk factors include family history, genetic syndromes, inflammatory diseases, medications [3], excess body weight, low level of physical activity, food [3,4], alcoholic beverages, and smoking [4]. These factors alter the gut microbiota, favoring the growth of pathogens [5–8], which colonize the environment and provoke a local inflammatory response. In chronic cases, they activate carcinogenic components, producing mutagenic compounds [3,4,9] through cell proliferation, apoptosis, and autophagy, resulting in failure in the normal control of mucosal renewal and repair [10].

In the evolutionary process of colorectal cancer, it is possible to notice anomalies in Lieberkühn crypts [6], which present enlarged size, hyperchromatic cells, epithelial thickening, greater peri-cryptic space, elongated and stratified nuclei, and mucin depletion, denominated Aberrant Crypt Foci (ACF) [11]. ACF are important biomarkers in the diagnosis of CRC as they are the first precancerous lesions to appear during carcinogenesis [7], involved in the adenoma-carcinoma sequence [8].

One of the treatments performed for colorectal cancer is chemotherapy [12] and 5-Fluorouracil (5-FU) is a widely used antineoplastic, which has great impact on the division of tumor cells [13], however, due to the various side effects it is necessary to interrupt

* Corresponding author. Postgraduate Program in Animal Science, University of West Paulist, Presidente Prudente, SP, Brazil.
E-mail addresses: sgenaro@unoeste.br (S.C. Genaro), luisreis@unoeste.br (L.S. Lima de Souza Reis), reis.sabrinakaren@gmail.com (S.K. Reis), eduardo_socca@yahoo.com.br (E.A. Rabelo Socca), wjfavaro@gmail.com (W.J. Fávaro).

https://doi.org/10.1016/j.lfs.2019.116895
Received 20 April 2019; Received in revised form 15 September 2019; Accepted 20 September 2019
Available online 11 October 2019
0024-3205/

treatment for a period, making the treatment last longer than expected [14].

In addition to conventional treatment, probiotic supplementation may be considered a tool for the maintenance and restoration of the gut microbiota. In vitro studies, in animal models, have demonstrated benefits in the restoration of homeostasis [15,16], reducing the population of bacteria associated with colonic diseases [17], inhibiting procarcinogenic enzymes, or stimulating the immune system of the host [18]. Its action also promotes a reduction in the side effects caused by antineoplastic treatment such as diarrhea, mucositis [19], and neuropathic pain [20,21], among others.

It is likely that the action of probiotics may minimize the development and progression of colorectal cancer through immunomodulation, modifying the composition and metabolic activity of the gut microbiota; binding and degradation of carcinogenic components in the intestinal lumen; production of short chain fatty acids (SCFA) and conjugated linoleic acid (CLA), which have anti-carcinogenic activity; inhibition of cell proliferation; and induction of apoptosis in cancer cells [22–24]. Studies with probiotics have also demonstrated their ability to decrease the number and size of aberrant crypts in experimental models with induced colon cancer [1,25–27], even though the mechanism by which this occurs is not yet fully understood.

Most experiments are performed in vitro animal models, because experimentation in humans has several requirements to preserve the physical and psychoemotional integrity of the investigated, in addition to limitations that may hinder or Unfeasible Research [28].

New legislations propose the reduction of the number of animals for an experiment, refining the technologies used and statistical tests [29], and it is important to use appropriate animal models for each study [28], as in the case of the Fisher rat F344. Being an isogenic model (inbred), it is ideal to be used in cancer research, toxicology and longevity studies, explaining the sample size of 5 animals per group in the present study.

Considering the increasing levels of colorectal cancer in the world population and the importance of therapies as aid measures in the treatment, the main objective of this study was to evaluate the effect of a commercial probiotic containing *Lactobacillus acidophilus* NCFM®, *Lactobacillus paracasei* Lpc 37™, *Bifidobacterium lactis* Bi-04™, *Bifidobacterium lactis* Bi-07™, and *Bifidobacterium bifidum* Bb-02™, on the aggressiveness of a chemically induced colorectal tumor in rats.

2. Methods

All experiments were carried out with the objective of minimizing the pain and discomfort of the animals. The experiment followed the ethical principles in animal research with approval from the Committee on Ethics in the Use of Animals (CEUA)/UNICAMP (protocol number 4431-1) and UNOESTE (protocol number 3052), following the criteria of the National Council for Control of Animal Experimentation - CONCEA-Conselho Nacional de Controle de Experimentação Animal (2013) [30] and in accordance with the National Council for Control of Animal Experimentation - Guide for the Care and Use of Laboratory Animals (2011) [31].

2.1. Experimental design

In total, 25 male rats of the Fisher F344 strain, healthy, 8 weeks of age (young adult), average body weight of 250.0 g were used.

The rats were provided by the center of Biotericism of the State University of Campinas (Campinas, SP, Brazil) and were kept in the vivarium of the Department of Structural and Functional Biology of the Institute of Biology of Unicamp (IB/Unicamp). The rats were allocated in white polypropylene cages, specific to the bioterium, lined with wood shavings (sawdust), with a maximum of 5 rats per large cages.

The animals were provided with commercial feed (Nuvilab®, Nuvital, Colombo, PR, Brazil) and filtered water *ad libitum*, and maintained at an ambient temperature of 22 ± 2 °C, relative humidity of 55 ± 10%, light and dark cycle 12/12 h, and continuous air circulation. After seven days of adaptation to the experimental conditions, the rats were randomly divided into five experimental groups (n = 5 animals per group): $G_{Control}$, without tumor induction or treatment; G_{Tumor}, colorectal tumor induction; $G_{Tumor+5\text{-}FU}$, induction of colorectal tumor and application of chemotherapy; $G_{Tumor+Prob}$, colorectal tumor induction and probiotic supplementation; $G_{Tumor+5\text{-}FU+Prob}$, colorectal tumor induction, chemotherapy application, and simultaneous supplementation with probiotic.

For induction of colorectal carcinogenesis, the chemical agent 1,2-dimethylhydrazine (DMH, Sigma Aldrich, St. Louis, MO, USA) was used, prepared immediately prior to use. 20 mg of carcinogen were solubilized in 1 mL of 0.9% NaCl solution with 1.5% EDTA and the pH adjusted to 6.5 with 1N NaOH solution when necessary [32] and applied twice weekly on alternate days [33], intraperitoneally, for four consecutive weeks. After an interval of 15 days, DMH applications were repeated for another 4 weeks.

Five weeks after the final administration of DMH, probiotic and chemotherapy administrations were started for 10 consecutive weeks, according to the time diagram, illustrated in Fig. 1.

The chemotherapy protocol was adapted from Bose, Elyagoby, Wong [34], applying a weekly dose of 15 mg of 5-FU/kg body weight (Fauldfluor®, Libbs) intraperitoneally.

The probiotic used in the experiment was 20 bi® (Eurofarma, Brazil), with each 435 mg capsule consisting of 2×10^{10} CFU of the probiotic microorganisms: *Lactobacillus acidophilus* (NCFM®), *Lactobacillus paracasei* (Lpc 37™), *Bifidobacterium lactis* (Bi-04™), *Bifidobacterium lactis* (Bi-07™), and *Bifidobacterium bifidum* (Bb-02™), together with microcrystalline cellulose stabilizer and silicon dioxide antifoaming agent to maintain the same pattern as the therapeutic dose used, each capsule was opened, weighed on a calibrated digital scale, to reach the amount of 1×10^9 Colony Forming Units (CFU), standard for all animals in the study, because each capsule presented a different weight, which could interfere with the result of the work. The contents diluted in physiological saline, given daily by gavage, for 10 consecutive weeks [35]. Administration of the gavage was performed with caution, with the animal immobilized, using a gastric needle appropriate for rats.

All procedures were performed in the morning, at the Biotherm of the Department of Structural and Functional Biology of the Institute of

Groups	4 weeks	15 days	4 weeks	5 weeks	10 weeks	
GTumor+5FU	DMH		DMH		5-FU	Euthanasia
GTumor+Prob	DMH		DMH		Probiotic	Euthanasia
GTumor+5FU+Prob	DMH		DMH		5-FU+Probiotic	Euthanasia
GControl						Euthanasia

Fig. 1. Time diagram.

Biology of Unicamp (IB/UNICAMP).

2.2. Euthanasia and colon harvest

Euthanasia was performed in the Surgery Room of the Department of Anatomy of the Institute of Biology of UNICAMP, at the 16th week after the last application of DMH.

The death of the rats was through barbiturate intoxication, causing depression in the Central Nervous System, with minimal discomfort for the animals; applying a dose of 100 mg thiopental sodium/kg body weight, intraperitoneally [28]. After the confirmation of absence of heart beats, laparotomy was performed to collect the colon of the rats.

2.3. Detection of Aberrant Crypt Foci (ACF)

Soon after death, the colon of the rats was resected, opened longitudinally, washed with physiological solution, and extended on "craft" paper, for macroscopic evaluation of the presence of mucosal surface alterations. Subsequently, the colon was subdivided into three segments of equal sizes, denominated the proximal, medial, and distal segments in relation to the cecum, which were attached to paraffin plates by pins, with the mucosa facing the upper part of the plate, fixed in formalin solution 10% for 24 h, followed by washing with 70% ethanol to remove the fixative and immersed in 0.2% methylene blue solution for 10 min, followed by washing in physiological saline to remove excess dye [36].

Immediately after staining of the colon segments, aberrant crypt foci (ACF) were observed and counted using optical microscopy (Leica DM 2500M, coupled to a Leica camera DFC 295, Munich, Germany) with a magnitude of 100×. To differentiate the ACF from colonic polyps, characteristics described in the literature were established, such as larger diameter than the surrounding mucosa, thicker epithelial lining, dilated lumen or slightly enlarged appearance, with a height of less than 2 mm [37].

2.4. Histopathological analysis

The colic tissue of each animal was subjected to sequential washing in 70% ethanol to remove the methylene blue dye. After complete removal of the dye, the tissues were processed in sequential washes of dehydration alcohols, followed by xylol washes for 2 h to be diaphanized, and finally included in plastic polymer (Paraplast Plus® Sigma Chemical Co., St. Louis, MO, USA). The materials were sectioned in a microtome (Microtome CUT5062 SLEE MAINZ, Munich, Germany) with a thickness of 5 μm (μm) and stained with hematoxylin and eosin (HE) for histopathological evaluation.

Reading of the slides was performed with an optical photomicroscope (Leica DM 2500M with Leica DFC 295 camera coupled) at magnitudes of 100× and 400x, for identification of neoplastic lesions.

2.5. Statistical analysis

The data presented normal distribution by the *Shapiro-Wilk* test, so were analyzed by *One Way* Variance Analysis and the means were compared by *Dunnett's* test using GraphPad Prism statistical software. A significance of 5% was considered in all analyzes. Histopathological analyzes were evaluated using the chi-square test. For these analyzes, a 5% type-I error was considered statistically significant.

3. Results

The $G_{Control}$ was used as the normal pattern for comparison of Lieberkühn Crypts, composed of numerous Lieberkühn crypts, presenting no ACF in the colic tissue of the rats (Figs. 1, 2a and b, 2c) and differing significantly from the other groups, which passed through the same period of maturation of the tumor process and presented lesions,

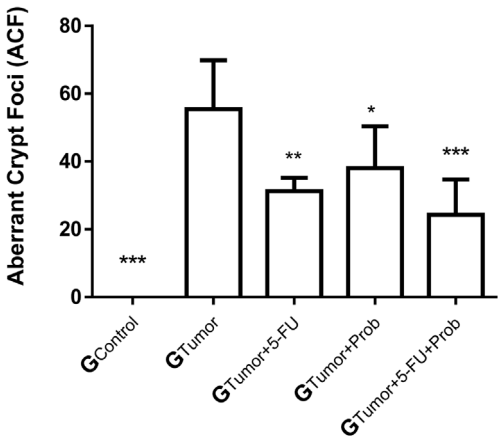

Fig. 2. Count Aberrant Crypt Foci (ACF). *p < 0,0373. **p < 0,0033, ***p < 0,0003. ANOVA of one way, with a Dunnet test a posteriori.

indicating that the protocol used was able to develop ACF.

ACF counts in the proximal, medial, and distal segments were higher in the G_{Tumor} when compared with the $G_{Tumor\ +\ Prob}$ ($p < 0.0033$), $G_{Tumor\ +\ 5\text{-}FU}$ ($p < 0.0373$), and $G_{Tumor+5\text{-}Fu+Prob}$ ($p < 0.0003$), as demonstrated in Fig. 2.

Fig. 3 shows the ACFs detected in the proximal, medial, and distal parts of the colon of the rats of the G_{Tumor}, $G_{Tumor+5\text{-}Fu,}$ and $G_{Tumor+Prob}$ groups and the medial and distal parts in the $G_{Tumor+5\text{-}Fu+Prob}$, identified through their particular characteristics such as cells with greater intensity of coloration, increased size, epithelial thickening, and presenting a wide border [37].

The histopathology of colonic tissues is illustrated in Fig. 4 and the $G_{Control}$ (Fig. 4A) was used for comparison of histological tissues, presenting the normal histological pattern for comparison with the other experimental groups, with the absence of neoplasia, in which the submucosal layer, composed of fibroelastic connective tissue, and the muscular layer, composed of circular and longitudinal muscle fibers can be noted.

In Figures B and C, it is possible to observe tubular adenoma. Polypoid projection for intestinal lumen with lower goblet cell density and discrete atypia (arrow) ($G_{Tumor+5\text{-}FU}$).

Carcinoma *"in situ"* is represented by figures D and E (G_{Tumor}) by the architectural disruption with absence of goblet cells (D) and nuclear hyperchromasia (E) (arrows).

Figure F represents invasive adenocarcinoma, in which Architectural disruption and glands superficially invading the submucosa (arrow) ($G_{Tumor+5\text{-}FU}$).

The frequency distribution of the histopathological analyzes is presented in Table 1. The results demonstrate that the induction of CRC with DMH was effective and led to the occurrence of neoplastic lesions, such as low-grade adenocarcinoma and carcinoma *in situ* in 3 (60%) and 2 (40%) of the animals of the G_{Tumor}, respectively. All rats of the $G_{Tumor+5\text{-}Fu}$ presented carcinoma *in situ*; the groups that used a probiotic demonstrated its effectiveness in inhibiting tumor progression, since in the $G_{Tumor+Prob}$, the lesions were 2 (40%) low-grade tubular adenoma, 2 (40%) carcinoma *in situ*, and 1 (20%) low-grade adenocarcinoma and in the $G_{Tumor+5\text{-}FU+Prob}$, 2 (40%) low grade tubular adenoma and 3 (60%) carcinoma *in situ*.

4. Discussion

The experimental model used in this study was of great clinical importance, since it allowed the reproduction of colorectal cancer, it was possible to test the use of a specific commercial probiotic, besides observing the evolution of CRC.

According to the World Health Organization (WHO) and United

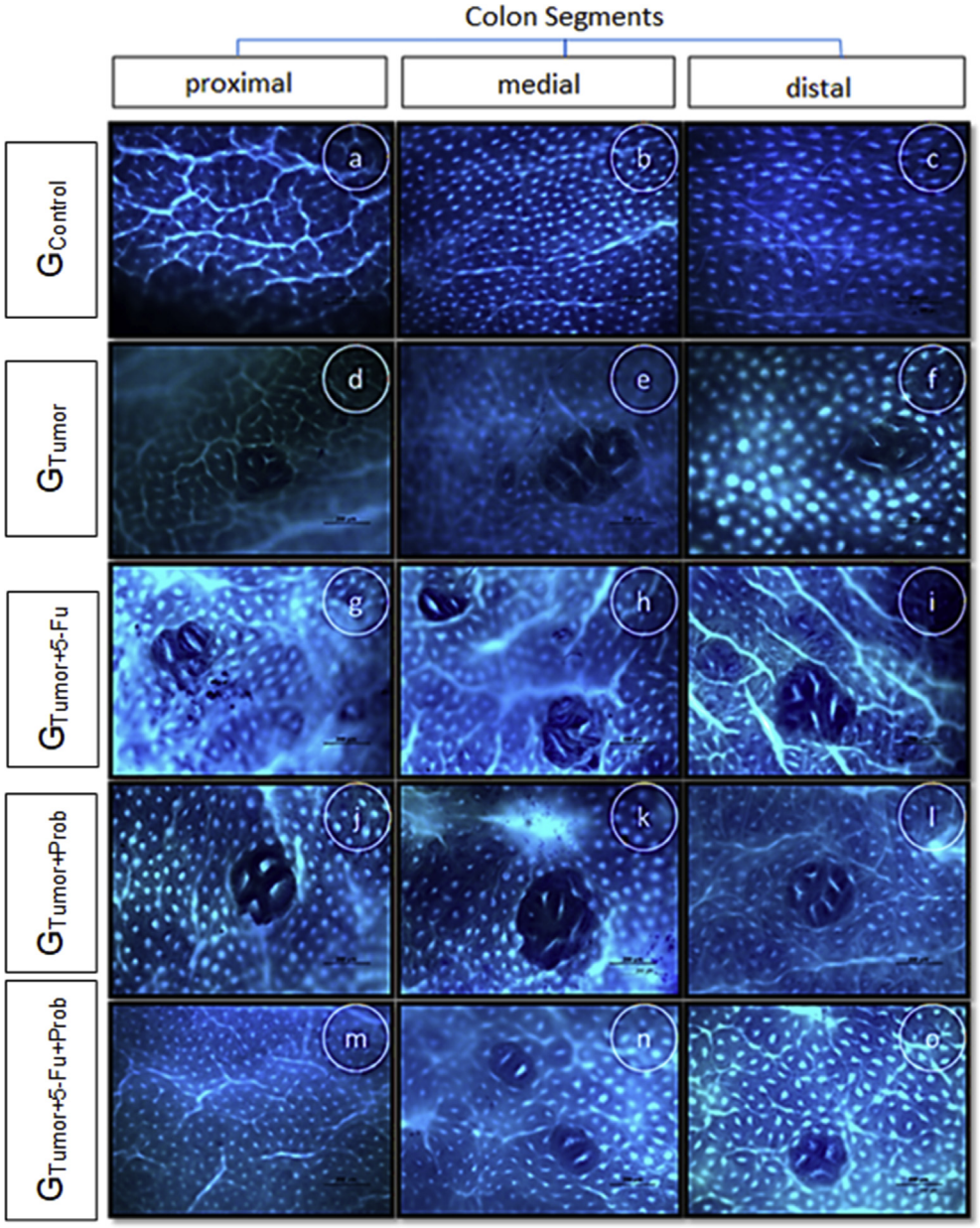

Fig. 3. Detection of Aberrant Crypt Foci (ACF) on the proximal, medial, and distal colic segments of DMH-induced. Magnitude 100×.

Nations Food and Agriculture Organization (FAO), when administered in adequate amounts, probiotics produce beneficial effects on host health, in both animals and humans [38]. As many studies cannot be performed directly on some species, especially the human species, due to ethical, financial issues, among others, animal models such as rats are often used to recreate physiological and pathological mechanisms *in vivo*, in which, later, the results are extrapolated to these species [2,3].

Colorectal cancer has an invasive characteristic in the glandular epithelium of the colon and rectum that begins with an expansive precursor lesion [39,40].

Currently there is a wide search for alternatives that help in the treatment of cancer. Over time, probiotics have emerged, demonstrating more than simply the possibility of modulating the gut microbiota [21]. Due to their safe use, several studies have been carried out with the aim of enhancing the action of chemotherapy in the fight against cancer, as well as attenuating the side effects of conventional treatments [14], reinforcing the hypothesis that probiotics may also be able to minimize the development and progression of CRC, mitigating the aggressiveness of the tumor.

The intestinal epithelium is a rapidly renewing tissue and its homeostasis is maintained by the balance between cell proliferation and apoptosis [36]. In the process of CRC formation in the present work, small clusters of abnormal Lieberkühn crypts with increased size, epithelial thickening, and greater peri-cryptic space were observed through microscopy in the cranial tissues of rats exposed to the DMH carcinogen. These altered crypts were initially described by Bird [41], as aberrant crypt foci, which were later related to early pre-neoplastic lesions, being able to evolve to early adenoma, advanced adenoma and, finally, malignant neoplastic lesions [42], suggesting that the traditional adenoma-carcinoma sequence in tumor progression of the large intestine, be renamed ACF-adenoma-carcinoma [36].

Although the mechanism by which probiotics may inhibit the development of CRC are unknown, it is assumed that by modifying the composition and metabolic activity of the gut microbiota, binding and degradation of compounds with carcinogenic potential, and production of antitumor or antimutagenic compounds in the colon, such as short

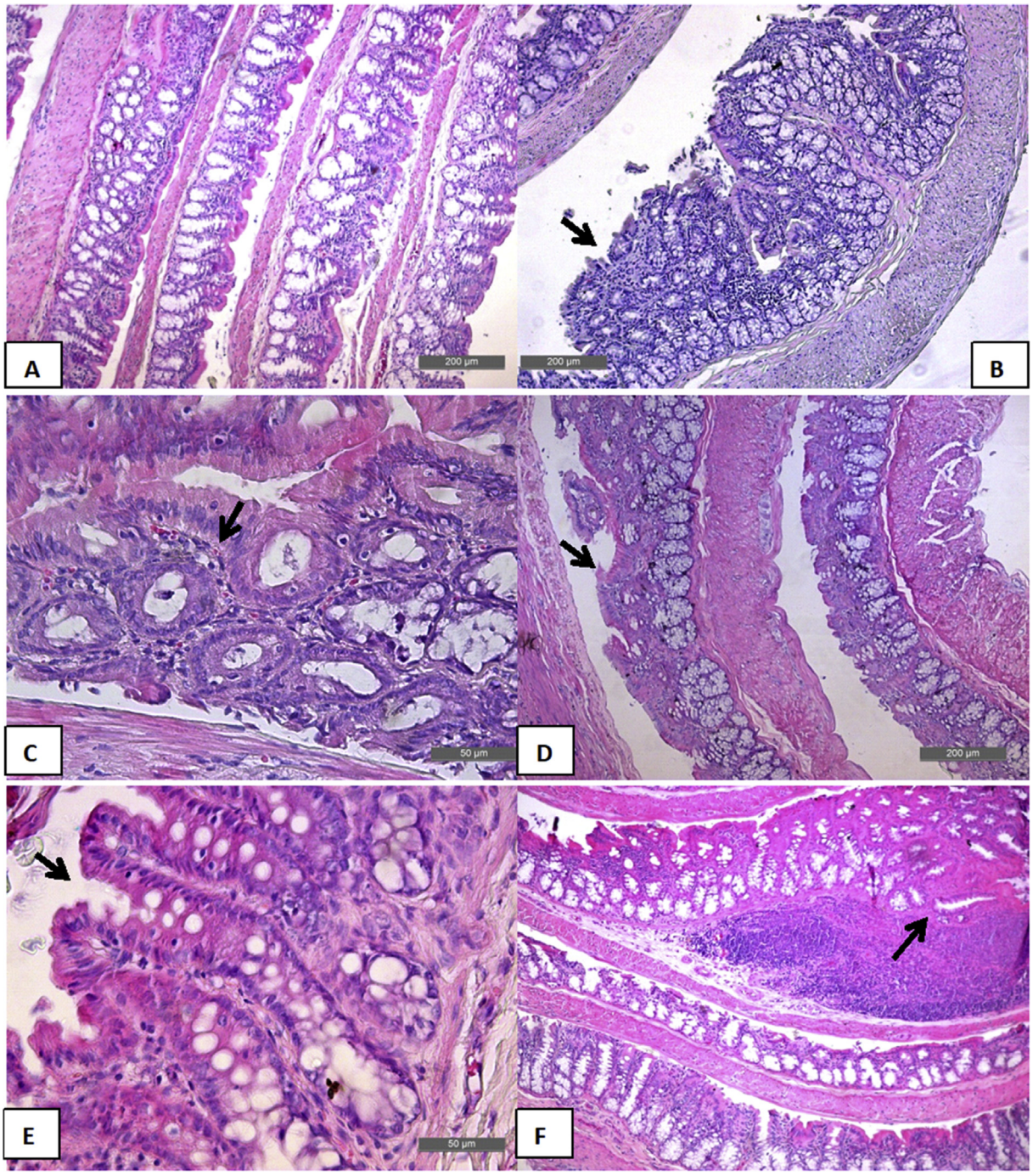

Fig. 4. Histopathological evaluation of the colon of Fisher 344 rats. A – Mucosa with normal pattern. B, C – Tubular adenoma. Polypoid projection for intestinal lumen with lower goblet cell density and discrete atypia. (arrow) ($G_{Tumor+5\text{-}FU}$). D, E – Carcinoma "*in situ*". Architectural disruption with absence of goblet cells (D) and nuclear hyperchromasia (E) (arrows) (G_{Tumor}). F – Invasive adenocarcinoma. Architectural disruption and glands superficially invading the submucosa (arrow) ($G_{Tumor+5\text{-}FU}$). Slides stained with HE. Magnitude: A, B, D, F: ×100; C, E: x400.

Table 1
Percentage of histopathological alterations.

Histopathology	Groups				
	$G_{Control}$ (n = 5)	G_{Tumor} (n = 5)	$G_{Tumor+5\text{-}Fu}$ (n = 5)	$G_{Tumor+Prob}$ (n = 5)	$G_{Tumor+5\text{-}Fu+Prob}$ (n = 5)
Normal	5(100%)*	-	-	-	-
Tubular Adenoma	-	-	-	2(40%)*	2(40%)*
Carcinoma *in situ*	-	2(40%)	5(100%)*	2(40%)	3(60%)*
Low Grade Adenocarcinoma	-	3(60%)*	-	1(20%)	-

* Statistical significance $p < 0.0001$ (Chi-square proportion test).

chain fatty acids (CCFA) and conjugated linoleic acid (CLA); which participate in the immunomodulation process, they can improve the integrity of the intestinal barrier, hamper cellular proliferation, and induce apoptosis in cancer cells [22,23].

The normal Gastrointestinal Tract Microbe consists mainly of lactic bacteria such as *Lactobacillus* and *Bifidobacterium* and, to a lesser extent, *Enterococcus faecium*, which can be used as probiotics [43]. These bacteria can be added to foods and called probiotic foods [44] or selected according to indication and drugs, provided their safety and efficacy are proven [38].

In the literature, numerous experimental studies are found using one or more probiotic strains, in a therapeutic way, containing different dosages to investigate the effects of probiotics on reducing the aggressiveness of precancerous lesions. Among these studies, Uccello et al. [18] showed a positive effect on the modulation of the immune system, with improvement in the integrity of the intestinal mucosa, capable of inhibiting cell proliferation. Irecta-Nájera et al. [42] demonstrated that the preventive administration of *Lactobacillus* ATCC 393 at a dose of 10^6 CFU, administered twice a week, can delay the onset of colon cancer induced in rats by reducing ACF. Chang et al. [46] showed that the consumption of *Lactobacillus acidophilus* KFRI342 decreased the incidence of ACF in rats with induced colon tumor at the dose of 2×10^9 CFU, administered 3 times a week. Verma and Shukla [45], studied different probiotics at the dose of 10^9 CFU administered daily in rats, comparing their protective effects against chemically induced colon carcinogenesis, and concluded that the probiotics *Lactobacillus GG* and *Lactobacillus acidophilus* can be used as the best prophylactic agents for experimental colon carcinogenesis. In another study using probiotics, Asha and Devaraja Gayathri [46] induced CRC in rats and fed them with feed, *Lactobacillus fermentum,* and *Lactobacillus plantarum, at a concentration of* 2×10^8 CFU, isolated or together with the chemotherapeutic Vincristine. As a result, they observed that the probiotic *Lactobacillus fermentum*, administered with Vincristine, demonstrated more effective reduction in the number of ACF, presenting an important antitumor effect resulting from synergistic cytotoxic action.

Similar results were observed in several studies using the same probiotic strains employed separately or in a mix of probiotics, using different therapeutic dosages. It is known that probiotics of the genera *Bifidobacterium*, *Lactobacillus* and yeasts are the most used to benefit the intestine of the host [46]. Chen et al. [47] used *Lactobacillus acidophilus NCFM®* in mice to demonstrate reductions in CRC growth and development, such as the severity of carcinogenesis and structural abnormality of epithelial damage. Sanders and Klaenhammer [48] found the efficacy of *Lactobacillus acidophilus NCFM®* in inhibiting the formation of aberrant crypts in rats that have undergone mutagenesis, decreasing the risk of CRC. Regarding the *Lactobacillus paracasei Lpc-37™* strain, a study by Aljewicz et al. [49] presented immunomodulatory characteristics, acid and bile resistance, adhesion to the intestinal mucosa and ability to inhibit pathogenic microorganisms. *Bifidobacterium bifidum Bb-02* can exert antitumor effects on colorectal cancer development, as reported by Biarc et al. [50]; Wei et al. [51] and Liboredo et al. [52]. Hibberd et al. [53] demonstrated increased butyrate-producing bacteria and reduction of bacteria associated with CRC when colon cancer patients used probiotics containing *Bifidobacterium lactis Bi-04*. Also, the probiotic strain *Bifidobacterium lactis Bi-07* was able to modulate imbalances caused by tumor necrosis factor alfa (TFN-α) in the intestinal microbial fraction associated with enterocyte [54]. Therefore, the mix of probiotics containing *Lactobacillus acidophilus (NCFM®)*, *Lactobacillus Paracasei (Lpc 37™)*, *Bifidobacterium lactis (Bi-04™)*, *Bifidobacterium lactis (Bi-07™)*, and *Bifidobacterium bifidum (Bb-02™)*, showed promising results to assist in the treatment of CRC, because it was able to decrease the number of ACF, reduce the progression of tumor malignancy and potentialize synergistically, the Antitumor cytotoxic action of 5-fluorouracil chemotherapic in the colic segments.

Therefore, further studies should be carried out in this line of research to better understand the mechanisms of action of probiotic microorganisms on the amelioration of aberrant crypt formation and the progression of colorectal tumor malignancy, as well as to prove the efficacy in humans, considering the anatomical, physiological and metabolic differences existing in these two species.

5. Conclusion

Probiotic supplementation decreased the number of formations of aberrant crypts, reduced tumor malignancy progression, and potentiated the antitumor effect of 5-fluorouracil chemotherapy on the colic segments.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors would like to thank the NANOBIOSS/UNICAMP Laboratories, INOMAT/UNICAMP, the NANOREG-MCTIC Program, and the Surgery Science Program/UNICAMP and University of Western São Paulo for financial support and research viability.

References

[1] I. Kahouli, M. Malhotra, S. Westfall, M. Alaoui-Jamali, S. Prakash, The potential of *lactobacillus* probiotic treatments in colorectal cancer (CRC), Cancer Res. 77 (13 Suppl) (2017) 254, https://doi.org/10.1158/1538-7445.AM2017-254.
[2] M. Arnold, M.S. Sierra, M. Laversanne, L. Soerjomataram, A. Jemal, F. Bray, Global patterns and trends in colorectal cancer incidence and mortality, Gut 66 (4) (2017) 683–691, https://doi.org/10.1136/gutjnl-2015-310912.
[3] E. Jones-Mclean, J. Hu, L.S. Greene-Finestone, M. Gro, A DASH dietary pattern and the risk of colorectal cancer in Canadian adults. *Health Promotion and Chronic Disease Prevention in Canada: Research*, Policy Pract. 35 (1) (2015) 12–20.
[4] J. Erdrich, X. Zhang, E. Giovannucci, W. Willett, Proportion of colon cancer attributable to lifestyle in a cohort of US women, Cancer Causes Control 26 (9) (2015) 1271–1279, https://doi.org/10.1007/s10552-015-0619-z.
[5] S. Jahani-Sherafat, M. Alebouyeh, S. Moghim, H. Ahmadi Amoli, H. Ghasemian-Safaei, Role of gut micobiota in the pathogenesis of colorectal câncer: a review article, Gastroenterol. Hepatol. Bed Bench 11 (2) (2018) 101–109.
[6] M. Tovey, F.A. Rizvi, S. Raman, K.E. Wheatley, Rectal cancer presenting as Fusobacterium nucleatumpyogenic liver abscess: a case report, Int. J. Case Rep. Images 2 (6) (2011) 16–19, https://doi.org/10.5348/ijcri-2011-06-39-CR-4.
[7] M. Castellarin, R.L. Warren, J.D. Freeman, L. Dreolini, M. Krzywinski, J. Strauss, et al., Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma, Genome Res. 22 (2) (2012) 299–306, https://doi.org/10.1101/gr.126516.111.
[8] A.D. Kostic, D. Gevers, C.S. Pedamallu, M. Michaud, F. Duke, A.M. Earl, et al., Genomic analysis identifies association of fusobacterium with colorectal carcinoma, Genome Res. 22 (2) (2012) 292–298, https://doi.org/10.1101/gr.126573.111.
[9] Q. Aziz, J. Doré, A. Emmanuel, F. Guarner, E.M. Quigley, Gut microbiota and gastrointestinal health: current concepts and future directions, Neuro Gastroenterol. Motil. 25 (1) (2013) 4–15, https://doi.org/10.1111/nmo.12046.
[10] F. Mariani, P. Sena, L. Roncucci, Inflammatory pathways in the early steps of colorectal cancerdevelopment, World J. Gastroenterol. 20 (29) (2014), https://doi.org/10.3748/wjg.v20.i29.9716 971-31.
[11] B. Gupta, P. Das, S. Ghosh, J. Manhas, S. Sen, S. Pal, et al., Identification of high-risk aberrant crypt foci and mucin-depleted foci in the human colon with study of colon cancer stem cell markers, Clin. Colorectal Cancer 16 (3) (2017) 204–213, https://doi.org/10.1016/j.clcc.2016.09.001.
[12] J. Mishra, J. Drummond, S.H. Quazi, S.S. Karanki, J.J. Shaw, B. Chen, et al., Prospective of colon cancer treatments and scope for combinatorial approach to enhanced cancer cell apoptosis, Crit. Rev. Oncol.-Hematol. 86 (3) (2013) 232–250, https://doi.org/10.1016/j.critrevonc.2012.09.014.
[13] B.A. Chabner, Quimioterapia das doenças neoplásicas: agentes citotóxicos, in: L.L. Brunton, B.A. Chabner (Eds.), Knollmann BC. As bases farmacológicas da terapêutica de Goodman & Gilman. 12, AMGH, Porto Alegre, 2012, pp. 1695–1698.
[14] B. Golkhalkhali, A.S. Paliany, K.F. Chin, R. Rajandram, The roles of adjuvant supplements in colorectal câncer patients on chemotherapy: reaping benefits from metabolic crosstalk, Nutr. Cancer 70 (2) (2018) 184–191, https://doi.org/10.1080/01635581.2018.1412470.
[15] S. Miquel, R. Martin, O. Rossi, L. Bermudez-Humaran, J. Chatel, H. Sokol, et al., Faecalibacteriumprausnitzii and human intestinal health, Curr. Opin. Microbiol. 6 (3) (2013) 255–261, https://doi.org/10.1016/j.mib.2013.06.003.
[16] M.I. Masood, M.I. Qadir, J.H. Shirazi, I.U. Khan, Beneficial effects of lactic acid bacteria on human beings, Crit. Rev. Microbiol. 37 (2011) 91–98, https://doi.org/10.3109/1040841X.2010.536522.
[17] P. Hemarajata, J. Versalovic, Effects of probiotics on gut microbiota: mechanisms of

intestinal immunomodulation and neuromodulation, Ther. Adv. Gastroenterol. 6 (1) (2013) 39–51, https://doi.org/10.1177/1756283X12459294.
[18] M. Uccello, G. Malaguarnera, F. Basile, V. D'agata, M. Malaguarnera, G. Bertino, et al., Potential role of probiotics on colorectal cancer prevention, BMC Surg. 12 (Suppl 1) (2012), https://doi.org/10.1186/1471-2482-12-S1-S35.
[19] R. Levit, G. Savoy de Giori, A. de Moreno de LeBlanc, J.G. LeBlanc, Protective effect of the riboflavin-overproducing strain Lactobacillus plantarum CRL2130 on intestinal mucositis in mice, Nutrition 54 (2018) 165–172, https://doi.org/10.1016/j.nut.2018.03.056.
[20] V. Castelli, P. Palumbo, M. d'Angelo, N.K. Moorthy, A. Antonosante, M. Catanesi, Probiotic DSF counteracts chemotherapy induced neuropathic pain, Oncotarget 9 (46) (2018) 27998–28008, https://doi.org/10.18632/oncotarget.25524.
[21] C.W. Chang, C.Y. Liu, H.C. Lee, Y.H. Huang, L.H. Li, J.C. Chiau, et al., *Lactobacillus casei* variety *rhamnosus*probiotic preventively attenuates 5-fluorouracil/oxaliplatin-induced intestinal injury in a syngeneic colorectal cancer model, Front. Microbiol. 9 (2018) 983, https://doi.org/10.3389/fmicb.2018.00983.
[22] P. Gourbeyere, S. Denery, M. Bodinier, Probiotic, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions, J. Leukoc. Biol. 89 (5) (2011) 685–695, https://doi.org/10.1189/jlb.1109753.
[23] D.E. Serban, Gastrointestinal cancers: influence of gut microbiota, probiotics and prebiotics, Cancer Lett. 345 (2014) 258–270, https://doi.org/10.1016/j.canlet.2013.08.013.
[24] Z.Y. Chen, Y.M. Hsieh, C.C. Huang, C.C. Tsai, Inhibitory effects of probiotic lactobacillus on the growth of human colonic carcinoma cell line HT-29, Molecules 22 (1) (2017) E107, https://doi.org/10.3390/molecules22010107.
[25] E.S. Chong, A potential role of probiotics in colorectal cancer prevention: review of possible mechanisms of action, World J. Microbiol. Biotechnol. 30 (2) (2014) 351–374, https://doi.org/10.1007/s11274-013-1499-6.
[26] K. Desrouillères, M. Millette, M. Jamshidian, B. Maherani, O. Fortin, M. Lacroix, Cancer preventive effect of a specific probiotic fermented milk components and cell walls extracted from a biomass containing L. acidophilus CL1285, L. casei LBC80R, and L. rhamnosus CLR2 on male F344 rats treated with 1,2-dimethylhydrazine, J. Funct. Foods 26 (2016) 373–384, https://doi.org/10.1016/j.jff.2016.08.005.
[27] M. Djaldetti, H. Bessler, Probiotic strains modulate cytokine production and the immune interplay between human peripheral blood mononucear cells and colon cancer cells, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 364 (3) (2017), https://doi.org/10.1093/femsle/fnx014.
[28] D.J. Fagundes, M.O. Taha, Modelo animal de doença: critérios de escolha e espécies de animais de uso corrente, Acta Cir. Bras. 19 (1) (2004) 59–65, https://doi.org/10.1590/S0102-86502004000100010.
[29] V.L.S.S. Castro, Uso de animais de experimentação e legislação correlata: orientações sobre estudos com peixes e roedores, Jaguar: Embrapa Meio Amiente (2013) (Documentos; 99) [Acesso em 2019 jan 17]. Disponível emhttp://www.fepar.edu.br/images/editais/comite_etica_animal/UsodeAnimaisdeExperimentacaoeLegislacaoCorrelata-OrientacoessobreEstudoscomPeixeseRoedores.pdf.
[30] Conselho Nacional de Controle de Experimentação Animal (Brasil), Diretrizes da prática de eutanásia do CONCEA, (2013).
[31] Guide for the Care and Use of Laboratory Animals, The National Academic Press, Washington, 2011.
[32] L.L.S. Larangeira, M.O. Taha, A. Ferme, R. Lemos, H. Plapler, Localização de lesões tumorais induzidas pela 1,2-dimetilhidrazina e seu grau de atipia no colon de ratos, Acta Cir. Bras. 13 (3) (1998), https://doi.org/10.1590/S0102-86501998000300008.
[33] M.A.M. Rodrigues, L.A.G. Silva, D.M.F. Salvadori, J.L.V. de Camargo, M.R. Montenegro, Aberrant crypt foci and colon cancer: comparison between a short- and medium-term bioassay for colon carcinogenesis using dimethylhydrazine in Wistar rats, Braz. J. Med. Biol. Res. 35 (3) (2002) 351–355, https://doi.org/10.1590/S0100-879X2002000300010.
[34] A. Bose, A. Elyagoby, T.W. Wong, Oral 5-fluorouracil colon-specific delivery through *in vivo* pellet coating for colon cancer and aberrant crypt foci treatment, Int. J. Pharm. 468 (1–2) (2014) 178–186, https://doi.org/10.1016/j.ijpharm.2014.04.006.
[35] Agência Nacional de Vigilância Sanitária (Brasil), Probióticos, [Acesso em: 2018 jun 12]. Disponível em: http://portal.anvisa.gov.br.
[36] M.C. Dias, N.F. Vieiralves, M.I. Gomes, D.M. Salvadori, M.A. Rodrigues, L.F. Barbisan, Effects of lycopene, synbiotic and their association on early biomarkers of rat colon carcinogenesis, Food Chem. Toxicol. 48 (3) (2010) 772–780, https://doi.org/10.1016/j.fct.2009.12.003.
[37] M. Lopez-Ceron, M. Pellise, Biology and diagnosis of aberrant crypt foci, Colorectal Dis. 14 (4) (2012) e157–e164, https://doi.org/10.1111/j.1463-1318.2011.02925.x.
[38] C. Hill, F. Guarner, G. Reid, G.R. Gibson, D.J. Merenstein, B. Pot, et al., Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic, Nat. Rev. Gastroenterol. Hepatol. 11 (8) (2014 Aug) 506–514, https://doi.org/10.1038/nrgastro.2014.66.
[39] T. Uchiyama, H. Takahashi, H. Endo, S. Kato, E. Sakai, K. Hosono, et al., Number of aberrant crypt foci in the rectum is a useful surrogate marker of colorectal adenoma recurrence, Dig. Endosc. 24 (5) (2012) 353–357, https://doi.org/10.1111/j.1443-1661.2012.01289.x.
[40] J.J. Liang, I. Bissett, M. Kalady, A. Bennet, J.M. Church, Importance of serrated polyps in colorectal carcinogenesis, Anz J. Surgey 83 (5) (2013) 325–330, https://doi.org/10.1111/j.1445-2197.2012.06269.x.
[41] R.P. Bird, Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings, Cancer Lett. 37 (2) (1987) 147–151.
[42] C.A. Irecta-Nájera, M. Del RosarioHuizar-López, J. Casas-Solís, P. Castro-Félix, A. Santerre, Protectiveeffect of Lactobacillus casei on DMH-Inducedcoloncarcinogenesis in mice, Probiotics Antimicrob Proteins 9 (2) (2017) 163–171, https://doi.org/10.1007/s12602-017-9253-2.
[43] J.J. Sebastian Domingo, Revision del papel de los probioticos en la patologia gastrointestinal del adulto, J. Gastroenterol. Hepatol. 40 (2017) 417–429, https://doi.org/10.1016/j.gastrohep.2016.12.003.
[44] Saad Susana Marta Isay, Probióticos e prebióticos: o estado da arte. *Revista Brasileira de Ciências Farmacêuticas*, [Internet] (2006), pp. 1–16, https://doi.org/10.1590/S1516-93322006000100002 Mar [cited 2019 Aug 01] ; 42(1) http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-93322006000100002&lng=en Available from:.
[45] A. Verma, G. Shukla, Probiotics Lactobacillus rhamnosus GG, Lactobacillus acidophilus suppresses DMH-Induced pro carcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in spraguedawley rats, Nutr. Cancer 65 (1) (2013) 84–91, https://doi.org/10.1080/01635581.2013.741746.
[46] A. Asha, D. Gayathri, Synergistic impact of Lactobacillus fermentum, Lactobacillus plantarum and vincristine on 1,2-dimethylhydrazine-induced colorectal carcinogenesis in mice, Exp. Ther. Med. 3 (6) (2012) 1049–1054, https://doi.org/10.3892/etm.2012.536.
[47] C.C. Chen, W.C. Lin, M.S. Kong, H.N. Shi, W.A. Walker, C.Y. Lin, C.T. Huang, Y.C. Lin, S.M. Jung, T.Y. Lin, Oral inoculation of probiotics Lactobacillus acidophilus NCFM suppresses tumour growth both in segmental orthotopic colon cancer and extra-intestinal tissue, Br. J. Nutr. 107 (11) (2012) 1623–1634, https://doi.org/10.1017/S0007114511004934.
[48] M.E. Sanders, T.R. Klaenhammer, Invited review: the scientific basis of lactobacillus acidophilus NCFM functionality as a probiotic, J. Dairy Sci. 84 (2) (2001 Feb) 319–331. Available from: http://dx.doi.org/10.3168/jds.S0022-0302(01)74481-5.
[49] M. Aljewicz, G. Cichosz, Protective effects of Lactobacillus cultures in Dutch-type cheese-like products, [Internet], LWT - Food Sci. Technol. 63 (1) (2015) 52–56, https://doi.org/10.1016/j.lwt.2015.03.054 Available from:.
[50] J. Biarc, I.S. Nguyen, A. Pini, F. Gossé, S. Richert, D. Thiersé, A. Van Dorsselaer, E. Leize-Wagner, F. Raul, J.P. Klein, M. Schöller-Guinard, Carcinogenic properties of proteins with pro-inflammatory activity from Streptococcus infantarius (formerly S. bovis), Carcinogenesis 25 (2004) 1477–1484, https://doi.org/10.1093/carcin/bgh091.
[51] C. Wei, A.Y. Xun, X.X. Wei, J. Yao, J.Y. Wang, R.Y. Shi, G.H. Yang, Y.X. Li, Z.L. Xu, M.G. Lai, et al., Bifidobacteria expressing tumstatin protein for antitumor therapy in tumor-bearing mice, Technol. Cancer Res. Treat. 15 (2016) 498–508, https://doi.org/10.1177/1533034615581977.
[52] J.C. Liboredo, L.R. Anastácio, C. Pelúzio Mdo, F.X. Valente, L.C. Penido, J.R. Nicoli, M.I. Correia, Effect of probiotics on the development of dimethylhydrazine-induced preneoplastic lesions in the mice colon, Acta Cir. Bras. 28 (2013) 367–372, https://doi.org/10.1590/S0102-86502013000500008.
[53] A.A. Hibberd, A. Lyra, A.C. Ouwehand, et al., Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention, BMJ Open Gastroenterol. 4 (2017) e000145, , https://doi.org/10.1136/bmjgast-2017-000145.
[54] M. Centanni, S. Turroni, S. Rampelli, E. Biagi, S. Quercia, C. Consolandi, M. Severgnini, P. Brigidi, M. Candela, Bifidobacterium animalis ssp. lactis BI07 modulates the tumor necrosis factor alpha-dependent imbalances of the enterocyte-associated intestinal microbiota fraction, FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 357 (2015) 157–163, https://doi.org/10.1111/1574-6968.12515357 (2014) 157–163.