Possible correlation between *Lactobacillus paracasei* X12 intake and tumor characteristics in the rat model of colorectal cancer

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**Abstract**

**Introduction:** There are many different therapeutic approaches to control colorectal cancer (CRC) which is recognized as one of the deadliest diseases in the world today. One of the most recent of which is the probiotic interventions to change the gut microbiome. Probiotics can be related to the control of gastrointestinal cancers in a variety of ways. Therefore, the present study aimed to investigate the relationship between supplement use with macroscopic and physiological changes of tumor in 1,2-dimethylhydrazine (DMH) -induced rats.

**Methods:** The male Wistar rats were divided into three groups. *Lactobacillus paracasei* X12 was administrated (40 weeks) to the DMH-induced rats. DMH injection (30 mg/kg BW) was used for 12 weeks to induce CRC. The “AgNOR method” was applied for the evaluation of cell proliferation. Real-time polymerase chain reaction (PCR) was used to measure the gene expression of apoptotic markers.

**Results:** The findings of this study indicated that *L. paracasei* X12 intake prevented weight loss caused by CRC (*P*<0.001), and probiotic consumption could significantly prevent tumor growth. Additionally, a significant correlation was observed between apoptosis markers and weight of animals, and a strong negative correlation (*P*<0.000) between apoptosis parameters and tumor characteristics (incidence, volume, and multiplicity of adenomas). A close association between cell proliferation and tumor characteristics was illuminated as well (*P*<0.001).

**Conclusion:** This study revealed a strong correlation between tumor incidence and growth and probiotic intake in CRC. Moreover, it could be believed that cancer prevention is a far more essential and cost-effective way than its cure.

Introduction

In the history of deadly diseases, colorectal cancer (CRC) has been thought as one of the leading causes of death in the world, which has led to the emergence of a variety of problems and bearing heavy burdens on the global public health.¹² There are still many concerns regarding cancer treatment in that the definite cause of cancer has not yet been discovered, and consequently, there is no definitive cure for it as well.¹³ Gastrointestinal cancers, especially cancers of the large intestine, are at the heart of such frequent and deadly cancers.¹ Therefore, preventive intervention research to reduce the incidence and progression of CRC appears to be much more significant than therapeutic implications.¹⁶

Recent developments in the fields of diets and nutritional interventions have opened new doors to a renewed interest in studying their association with cancers, especially gastrointestinal diseases.⁶⁷ Therefore, special attention to dietary interventions can be one of the most critical factors in reducing or controlling CRC cancers.⁶

On the other hand, the gut microbiome - as a novel and mysterious target - can provide many benefits for the host.⁸ Recent literature has suggested that interventions that can alter the composition of the gut microbiome can have beneficial effects on the cancers.⁹ On this note, there is a great deal of evidence linking microbiome and CRC.⁹¹⁰ Nutritional interventions like probiotics such as *Lactobacillus* species have beneficial effects on the host.¹¹

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Therefore, there is no consequence that recently, the use of probiotics for the prevention and even treatment of chronic diseases, including cancers, is at the heart of the concerns.14,15

The indefinite growth and proliferation of tumor cells in CRC results in a severe weight loss16 as well as an increase in the apoptosis resistance, which is a crucial mechanism in reducing the growth and development of tumors.17 Also, there is clarifying evidence that the use of probiotics may have beneficial effects on apoptotic pathways18 and the prevention of severe weight loss.19

Numerous studies have reported the beneficial roles of probiotics in improving CRC in vivo and in vitro.18,20,21, however, the generalizability of much-published research on this issue is problematic in that challenges remain in their mechanisms of action, and their apparent role in the control and recovery of cancer has not yet been identified.22 What is more, there are some debates witnessed on the part of probiotics in CRC.23

One of the most significant current discussions indicated that the consumption of *Lactobacillus paracasei* could reduce the incidence of cancers in vitro and in vivo.24-26 Besides, the controversy about the beneficial effects of *L. paracasei* has been reflected in the reports of various diseases, including its tumor-suppressive effects.26-27 Based on the reports from other studies and our previous study, the present study aimed to investigate the correlation between the weight changes and the rate of apoptosis with tumor characteristics (size, incidence, multiplicity, and volume of the tumors) following the consumption of *L. paracasei* X12.

**Methods**

**Animals**

Six-week-old male Wistar rats (140-180 g, n=36) were obtained from the Pasteur Institute of Iran. All animal procedures were under the Principles of Laboratory Animal Care (NIH Publication 1986) and were approved by the Animal Experimentation Ethics Committee of Tabriz University of Medical Sciences, Tabriz, Iran. The animals were then housed in standard cages (four rats per cage) in a room with controlled temperature (22–25°C) and humidity (40–60%) under a 12h:12h light-dark cycle. They were also allowed ad libitum access to food and water following which they were randomly assigned to three groups (n=12 per group) as following:

1) Healthy Control (HC), which received single weekly doses of 1 mM EDTA-normal saline. 2) DMH treated rats’ control (DC), which received a single dose of DMH (Sigma, St. Louis, MO, USA) subcutaneously (SC) and sterile normal saline intragastrically. 3) Probiotic treated rats (DP), in which the rats were injected with DMH. Finally, the rats were fed *L. paracasei* X12 daily for forty weeks.

**Experimentally Induced Tumors in Animals**

Animals were given subcutaneous injections of DMH (dissolved in 1 mM EDTA-normal saline, pH 7.0) twice a week for 12 consecutive weeks at a single dose level of 30 mg/kg BW.28

**Study design**

The probiotic was administrated for 40 weeks. Body weight (BW) was measured every eight weeks. Eventually, the rats were anesthetized with sodium pentobarbital (65 mg/kg BW, IP) and were sacrificed by cervical dislocation at the endpoint. After laparotomy, the colon and rectum tissues were removed and cut longitudinally. Following all, they were thoroughly washed with cold saline.

**Preparation of supplement**

Regarding the process of the probiotic development, *L. paracasei* X12 was purchased from TBZMED Biotechnology Research Center (Tabriz, Iran) and was collected after incubation in MRS broth at 37°C and centrifuged at 3000 rpm for four minutes.29 Then, it was washed with sterile phosphate-buffered saline (PBS). Ultimately, the numbers of the viable bacteria were adjusted to 2×10⁹ colony-forming units (CFU)/rat/day and administrated orally every day for each rat in the DP group.

**Surgery and macroscopic measurements of tumors**

The animals were sacrificed under an overdose (65 mg/kg BW) of sodium pentobarbital anesthesia and cervical dislocation at the endpoint.30 After laparotomy, the colon and rectum tissues were removed, their colons were harvested and washed with cold saline, and the macroscopic changes were evaluated (Figure 1). Finally, the tumors count, volume, and multiplicity were counted and measured using Vernier caliper (0.1-mm graduation).

**Real-time PCR analysis**

As previously described, total RNAs of cells were extracted from the rats’ colon and were applied for complementary DNA (cDNA) by PrimeScript RT Reagent Kit (Takara Bio Inc., Tokyo, Japan). cDNA template was the basis of the design, and specific primers were used in this regard as well. Finally, all amplification reactions were carried out on ABI-step I plus (Applied Biosystems, California, USA).

**Cell proliferation**

The “AgNOR method” has been applied for prognostic and diagnostic purposes of tumor pathology. AgNORs were identified by silver nitrate staining. AgNORs staining was performed based on Murray et al.31

**Statistical analysis**

Results were obtained from 8-12 rats in each group, and their related data were analyzed using SPSS (version 24). The repeated-measures ANOVA test was used to examine weight changes. The Pearson correlation coefficient test was also applied to study the correlations between
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Changes in body weight
The weight gain of the animals did not differ notably among the three groups at the beginning of the intervention (Figure 2). In comparison with the control group, the BW of the DC group dramatically decreased at the end of the last weeks (weeks 16, 32, and 40). The administration of L. paracasei X12 could prevent severe weight loss in these weeks (P < 0.001). Interestingly, the most significant weight loss was observed after the 32nd week in the DMH-injected group. Moreover, weight loss in the treated group (DP) was much lower than the DC group (P < 0.001).

The correlation between weight gain and apoptosis
As shown in Table 1, there are indicative correlations between weight gain and apoptotic parameters, especially in the last weeks. For example, there is a positive correlation between weight loss and the increased level of caspase-8, protein kinase B (Akt-1), and Janus kinase 1 (Jak-1) (P < 0.001). However, no significant correlation was found between BW and concentrations of caspase-3, caspase-9, and Bax. Besides, based on anti-apoptotic markers, the most active correlation was between Bcl-2 and weight gain in the last week.

The correlation between tumor characteristics and weight gain
Using Pearson’s correlation coefficient test, a negative correlation between weight gain and tumor characteristics was observed (Figure 3). Weight loss was strongly correlated with an increase in tumor incidence (P < 0.001). There was also a close association between the number of adenomas and BW (P < 0.001). Eventually, a good correlation could be found in a positive and negative relationship between tumor volume and weight gain.

The correlation between tumor characteristics and apoptosis
As expected, there was an interesting correlation between tumor characteristics and apoptosis, the overall results of which are summarized in Table 2. For example, although there were no significant correlations related to a few markers, strong correlations were observed between caspase-8 and Akt-1 and Jak-1 with tumor incidence rates (P < 0.001). Besides, there was a close relationship between the volume and the number of tumor cells with these parameters (P < 0.001). Equally important, it could be indicated that there is a positive correlation between cell proliferation (AgNOR) with tumor characteristics (incidence, volume, and multiplicity of adenomas) (Figure 4).

Discussion
As mentioned above, there is an ample evidence that probiotic use may have beneficial effects on the prevention or even control of CRC. Therefore, it is the primary
purpose of this paper was to draw attention to the possible relationships and correlations between macroscopic and physiological characteristics of the colorectal tumors and weight changes following the consumption of *L. paracasei* X12 in DMH-induced rats.

In the current study, the use of *L. paracasei* X12 helped to discover that it could prevent severe weight loss in animals. As stated, the presence of tumor cells and their growth has been closely correlated with weight loss. Further, more severe weight loss was observed by increasing the number and size of tumor masses, and these results were reversed following the administration of the supplementation. In a study, Li and Li have shown that the weight of the animals with CRC was dramatically decreased. According to the reports, the main reasons for severe weight loss caused by cancers is the sarcopenia (the loss of muscle mass). Another important cause of weight loss is the increased consumption of glucose by tumor cells. On the same note, one study reported that the weight of mice with colorectal tumors was significantly reduced. In this regard, it was indicated in several reports that decreases of muscle mass were one of the main factors for mortality of CRC.

Contrary to expectations, another implication was the possibility for the weight loss to originate from impaired host metabolism (glucose, lipids, and proteins). As mentioned above, the use of probiotics could improve the weight loss caused by cancer. This finding is consistent with those of many other studies suggesting the beneficial role of probiotics such as *L. paracasei* in enhancing metabolism, appetite, food intake, weight control, and energy, which may be related to the high correlation between *L. paracasei* X12 consumption and weight management in this study. Therefore, it can be assumed that the administration of probiotics in DMH-induced rats could have an active role in improving metabolism and controlling weight gain.

Also, an increase in the number of adenomas or an increase in their volume along with excessive proliferation (increased energy requirement as well as uncontrolled consumption of glucose) was closely associated with weight loss, especially in the last weeks. A high correlation between weight gain at weeks 32 and 40 with apoptosis variables and animals’ weight changes following the consumption of *L. paracasei* X12 in DMH-induced rats could have an active role in improving metabolism and controlling weight gain.

Table 1. The correlation coefficient among apoptosis variables and animals’ weight

|        | Weight (week 32) | Weight (week 40) |
|--------|------------------|------------------|
| Caspase-8 | r = -0.845**     | r = -0.884**     |
|         | p < 0.001        | p < 0.001        |
| Caspase-3 | r = -0.199       | r = -0.216       |
|         | p = 0.274        | p = 0.236        |
| Caspase-9 | r = 0.296        | r = 0.313        |
|         | p = 0.100        | p = 0.081        |
| Akt-1   | r = -0.854**     | r = -0.894**     |
|         | p < 0.001        | p < 0.001        |
| Bax     | r = -0.149       | r = -0.177       |
|         | p = 0.416        | p = 0.333        |
| Bcl-2   | r = 0.428**      | r = 0.449**      |
|         | p = 0.015        | p = 0.010        |
| Jak-1   | r = -0.820**     | r = -0.858**     |
|         | p < 0.001        | p < 0.001        |
| Bax/Bcl2 | r = 0.408**      | r = 0.431**      |
|         | p = 0.020        | p = 0.014        |

Akt-1: protein kinase B; Bcl-2: B-cell lymphoma 2; Bax: Bcl-2-associated X protein; Jak-1: Janus kinase 1.

Pearson correlation coefficients computed correlations between two variables.

Data were expressed as means ± SD. P < 0.05 was considered statistically significant.

Table 2. The correlation coefficient among apoptosis variables and tumor characteristics

|        | Volume | Incidence | Multiplicity |
|--------|--------|-----------|--------------|
| Caspase-8 | r = 0.846** | r = 0.980** | r = 0.858** |
|         | p < 0.001 | p < 0.001 | p < 0.001 |
| Caspase-3 | r = 0.041 | r = 0.347 | r = 0.087 |
|         | p = 0.823 | p = 0.052 | p = 0.636 |
| Caspase-9 | r = -0.513** | r = -0.248 | r = -0.463** |
|         | p = 0.003 | p = 0.171 | p = 0.008 |
| Akt-1   | r = 0.923** | r = 0.950** | r = 0.948** |
|         | p < 0.001 | p < 0.001 | p < 0.001 |
| Bax     | r = -0.048 | r = 0.254 | r = -0.021 |
|         | p = 0.794 | p = 0.160 | p = 0.910 |
| Bcl-2   | r = -0.256 | r = -0.535** | r = -0.278 |
|         | p = 0.157 | p = 0.001 | p = 0.123 |
| Jak-1   | r = 0.894** | r = 0.955** | r = 0.919** |
|         | p < 0.001 | p < 0.001 | p < 0.001 |
| Bax/Bcl2 | r = -0.230 | r = -0.535** | r = -0.254 |
|         | p = 0.205 | p = 0.002 | p = 0.161 |

Akt-1: protein kinase B; Bcl-2: B-cell lymphoma 2; Bax: Bcl-2-associated X protein; Jak-1: Janus kinase 1.

Pearson correlation coefficients computed correlations between two variables.

Data were expressed as means ± SD. P < 0.05 was considered statistically significant.
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Figure 3. The correlation coefficient between the animal’s weight and tumor characteristics (incidence, volume, and multiplicity of adenomas). (A) Correlation coefficients between volume and of adenomas with bodyweight (week 40). (B) Correlation coefficients between the incidence of adenomas with bodyweight (week 40). (C) Correlation coefficients between the multiplicity of adenomas with bodyweight (week 40). HC: healthy control group; DC: DMH group; DP: treated group by the L. paracasei X12.

Figure 4. The Correlation coefficient between cell proliferation and tumor characteristics (incidence, volume, and multiplicity of adenomas). (A) Correlation coefficients between volume and of adenomas with cell proliferation (AgNOR). (B) Correlation coefficients between the incidence of adenomas with cell proliferation (AgNOR). (C) Correlation coefficients between the multiplicity of adenomas with cell proliferation (AgNOR). HC: healthy control group; DC: DMH group; DP: treated group by the L. paracasei X12.

been demonstrated. These notions are consistent with those of other studies such as that of Baldwin et al. suggesting that L. acidophilus and L. casei consumption could augment apoptosis (40%) by activation of caspase-3 of a CRC cell line. According to the findings of this study, there was also a high correlation between the size and the number of tumors with anti- and apoptotic markers. Considering the evidence, improving immune response probably decreases cell proliferation, gut inflammation suppression, and regulation of metabolism, all of which could be the possible mechanisms involved in this study. However, it cannot be confidently stated that there is a direct relationship between the incidence of a tumor and the rate of apoptosis, and there are supporting reports in this regard as well. Overall, it is difficult to indicate a clear pathway between apoptosis and tumor growth. More research to reveal related mechanisms is warranted.

Given the crucial and determining role of the gut microbiome in gastrointestinal cancers’ association which has received much attention in recent years – a possible explanation could be that the effects of probiotics such as L. paracasei X12 may have been altered by the changes in the gut microbiome combination and population that have been well documented in some studies. The results of this study, which is the continuation of the previous research, represents a strong correlation between weight changes as well as markers of apoptosis with physical characteristics of the tumor. Besides, the consumption of L. paracasei X12 was able to prevent severe weight loss in rats with CRC. It has also been well demonstrated that the use of L. paracasei X12 is closely related to the reduction of tumor cell incidence and suppression of tumor growth. What is more, there was a close relationship between cell proliferation in tumors and weight changes. Although the present study clarified a promising and negative correlation between probiotic consumption with tumor growth, there are some limitations, most notably the lack
of consideration of microbial changes due to the use of \textit{L. paracasei} X12. However, there were some limitations to this study, as well. Perhaps the most notable limitation was the need to investigate and analyze changes in the gut microbiome as an essential part of the host intestine - as it has been reported to have many functions and have not been addressed in this study.

It could be assumed that probiotics consumption appears to be a promising way to reduce the incidence of CRC and even to treat and control it. Further research is needed to elucidate the extent and type of association of the gut microbiome with the spreading and incidence of cancer, especially with signaling pathways. As dietary supplements, probiotics could have great potential in controlling and managing CRC.

**Conclusion**

The most prominent finding to emerge from this study is that there was a high correlation between apoptotic markers, weight gain, and macroscopic characteristics of tumors. Considering the increasing prevalence of CRC in the world community, the heavy burden of cancers on the world public health, and the lack of definitive treatment for this issue, the path is paved for the need for the prevention and the reduction of the incidence of cancer. Therefore, the use of non-pharmacological treatments such as probiotics that have shown promising results could attribute to a small part of this larger goal. Moreover, there is a clear need for more well design researches to clarify more evidence on possible roles of probiotic and its mechanisms for suppression of the colorectal tumors. Clearly, much more research is needed, especially concerning human beings.

**Conflict of Interest**

There is nothing to declare.

**Ethical Approval**

The Research and Ethics Committee of Islamic Azad University, Tabriz Branch, approved this study’s ethicality with no.: 5D997543

**Author’s contributions**

SAM was the writer of the study protocol, study design, keeping rats, and performed the experiments and drafting the manuscript. AYK held the responsibility to analyze and interpret the data. BA was involved in developing and editing the document. All authors have given their final approval of the present version to be published.

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**References**

1. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray E. Global patterns and trends in colorectal cancer incidence and mortality. Gut. 2017;66(4):683-91. doi: 10.1136/gutjnl-2015-310912.

2. Haggar FA, Boushey RP. Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg. 2009;22(4):191-7. doi: 10.1055/s-0029-1242458.

3. Siegel R, Desantis C, Jemal A. Colorectal cancer statistics, 2014. CA Cancer J Clin. 2014;64(2):104-17. doi: 10.3322/caac.21220.

4. Pourhoseingholi MA, Vahedi M, Baghestani AR. Burden of gastrointestinal cancer in Asia: an overview. Gastroenterol Hepatol Bed Bench. 2015;8(1):19-27.

5. Weller DP, Owen N, Hiller JE, Willson K, Wilson D. Colorectal cancer and its prevention: prevalence of beliefs, attitudes, intentions and behaviour. Aust J Public Health. 1995;19(1):19-23. doi: 10.1111/j.1753-4605.1995.tb00291.x.

6. Pericleous M, Mandair D, Caplin ME. Diet and supplements and their impact on colorectal cancer. J Gastrointest Oncol. 2013;4(4):409-23. doi: 10.9797/j.jissn.2078-6891.2013.003.

7. Garla P, Waitsberg DL, Tesser A. Nutritional therapy in gastrointestinal cancers. Gastroenterol Clin North Am. 2018;47(1):231-42. doi: 10.1016/j.gtc.2017.09.009.

8. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. Physiol Rev. 2010;90(3):859-904. doi: 10.1152/physrev.00045.2009.

9. Ahn J, Sinha R, Pei Z, Dominianini C, Wu J, Shi J, et al. Human gut microbiome and risk for colorectal cancer. J Natl Cancer Inst. 2013;105(24):1907-11. doi: 10.1093/jnci/djt300.

10. Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petsiroso JF, Chen GY, et al. The gut microbiome modulates colon tumorigenesis. mBio. 2013;4(6):e00692-13. doi: 10.1128/mBio.00692-13.

11. Vargas AJ, Thompson PA. Diet and nutrient factors in colorectal cancer risk. Nutr Clin Pract. 2012;27(5):613-23. doi: 10.1177/0884536612454885.

12. Morshed M, Hashemi R, Moazzen S, Saehekar A, Hosseinifar ES. Immunomodulatory and anti-inflammatory effects of probiotics in multiple sclerosis: a systematic review. J Neuroinflammation. 2019;16(1):231. doi: 10.1186/s12974-019-1611-4.

13. Morshed M, Saghafi-Azl M, Hosseinifar ES. The potential therapeutic effects of the gut microbiome manipulation by symbiotic containing-Lactobacillus plantarum on neuropsychological performance of diabetic rats. J Transl Med. 2020;18(1):18. doi: 10.1186/s12976-019-02169-y.

14. Zhu Y, Michelle Luo T, Jobin C, Young HA. Gut microbiota and probiotics in colon tumorigenesis. Cancer Lett. 2011;309(2):119-27. doi: 10.1016/j.canlet.2011.06.004.

15. Azcárate-Peril MA, Sikes M, Bruno-Bárcena JM. The intestinal microbiota, gastrointestinal environment and colorectal cancer: a putative role for probiotics in prevention of colorectal cancer? Am J Physiol Gastrointest

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Liver Physiol. 2011;301(3):G401-24. doi: 10.1152/ajpli.00110.2011.

16. Mohri Y, Inoue Y, Tanaka K, Hiro J, Uchida K, Kusunoki M. Prognostic nutritional index predicts postoperative outcome in colorectal cancer. World J Surg. 2013;37(11):2688-92. doi: 10.1007/s00268-013-2156-9.

17. Watson AJ. Apoptosis and colorectal cancer. Gut. 2004;53(11):1701-9. doi: 10.1136/gut.2004.027040.

18. Konishi H, Fujima Y, Tanaka H, Ueno N, Moriiichi K, Sasaiima J, et al. Probiotic-derived ferrichrome inhibits colon cancer progression via JNK-mediated apoptosis. Nat Commun. 2016;7:12365. doi: 10.1038/ncomms12365.

19. Hosseinifard ES, Morshedi M, Bayafa-Valentia K, Saghaifi-Asl M. The novel insight into anti-inflammatory and anxiolytic effects of psychobiotics in diabetic rats: possible link between gut microbiota and brain regions. Eur J Nutr. 2019;58(8):3361-75. doi: 10.1007/s00394-019-01924-7.

20. Li W, Li CB. Effect of oral Lactobacillus casei containing endostatin on 1, 2-dimethylhydrazine-induced colon tumor in rats. World J Gastroenterol. 2005;11(46):7242-7. doi: 10.3748/wjg.v11.i46.7242.

21. Paolillo R, Romano Carratelli C, Sorrentino S, Mazzola N, Rizzo A. Immunomodulatory effects of Lactobacillus plantarum on human colon cancer cells. Int Immunopharmacol. 2009;9(11):1265-71. doi: 10.1016/j.intimp.2009.07.008.

22. Ambalam P, Raman M, Purama RK, Doble M. Probiotics, prebiotics and colorectal cancer prevention. Best Pract Res Clin Gastroenterol. 2016;30(1):119-31. doi: 10.1016/j.bpcg.2016.02.009.

23. Doss Reis SA, da Conceição LL, Siqueira NP, da Silva LL, Peluzio MD. Review of the mechanisms of prebiotics and colorectal cancer prevention. Best Pract Res Clin Gastroenterol. 2016;30(1):119-31. doi: 10.1016/j.jnutbio.2013.09.006.

24. Huang L, Shan YJ, He CX, Ren MH, Tian PJ, Song W. Effects of L. paracasei subsp. paracasei X12 on cell cycle of colon cancer HT-29 cells and regulation of mTOR signalling pathway. J Funct Foods. 2016;21:431-9. doi: 10.1016/j.jff.2015.12.024.

25. Choudrou P, Karapetsas A, Kioussis DE, Kiousi DE, Tsela D, Tiptiri-Kourpeti A, Anestopoulos I, et al. Lactobacillus paracasei K5 displays adhesion, anti-proliferative activity and apoptotic effect of L. paracasei subsp. paracasei X12 on cell cycle of colon cancer HT-29 cells and regulation of mTOR signalling pathway. J Funct Foods. 2016;21:431-9. doi: 10.1016/j.jff.2015.12.024.

26. Forsyth GM, Simeoli R, Iacono A, Santoro A, Amero P, Picielli O, et al. Effects of a Lactobacillus paracasei B21060 based symbiotic on steatosis, insulin signaling and toll-like receptor expression in rats fed a high-fat diet. J Nutr Biochem. 2014;25(1):81-90. doi: 10.1016/j.jnutbio.2013.09.006.

27. El-Khadragy MF, Nabil HM, Hassan BN, Tohamy AA, Waar HF, Yhia HM, et al. Bone marrow cell therapy on 1, 2-dimethylhydrazine (DMH)-induced colon cancer in rats. Cell Physiol Biochem. 2018;45(3):1072-83. doi: 10.1159/000487349.

28. Morshedi M, Valentina KB, Hosseinifard ES, Shahabi P, Abbasi MM, Ghorbani M, et al. Beneficial psychological effects of novel psychobiotics in diabetic rats: the interaction among the gut, blood and amygdala. J Nutr Biochem. 2018;57:145-52. doi: 10.1016/j.jnutbio.2018.03.022.

29. Valentina KB, Morshedi M, Saghaifi-Asl M, Shahabi P, Abbasi MM. Beneficial impacts of Lactobacillus plantarum and inulin on hypothalamic levels of insulin, leptin, and oxidative markers in diabetic rats. J Funct Foods. 2018;46:529-37. doi: 10.1016/j.jff.2018.04.069.

30. Murray PG, Boldy DA, Crocker J, Ayres JG. Sequential demonstration of antigens and AgNORs in frozen and paraffin sections. J Pathol. 1989;159(2):169-72. doi: 10.1002/path.1711590212.

31. Cederholm TE, Bauer JM, Boirie Y, Schneider SM, Sieber CC, Rolland Y. Toward a definition of sarcopenia. Clin Geriatr Med. 2011;27(3):341-53. doi: 10.1016/j.cger.2011.04.001.

32. Liefers JJ, Bathe OE, Fassbender K, Winget M, Baracos VE. Sarcopenia is associated with postoperative infection and delayed recovery from colorectal cancer resection surgery. Br J Cancer. 2012;107(6):931-6. doi: 10.1038/bjc.2012.350.

33. Miyamoto Y, Baba Y, Sakamoto Y, Ohuchi M, Tokunaga R, Kurashige J, et al. Sarcopenia is a negative prognostic factor after curative resection of colorectal cancer. Ann Surg Oncol. 2015;22(8):2663-8. doi: 10.1245/s10434-014-4281-6.

34. Elf SE, Chen J. Targeting glucose metabolism in patients with cancer. Cancer. 2014;120(6):774-80. doi: 10.1002/cncr.28501.

35. Wala S, Kamal R, Kanwar SS, Dhawan DK. Cyclooxygenase as a target in chemoprevention by probiotics during 1,2-dimethylhydrazine induced colon carcinogenesis in rats. Nutr Cancer. 2015;67(4):603-11. doi: 10.1080/01625192.2015.1017788.

36. Hendler R, Zhang Y. Probiotics in the treatment of colorectal cancer. Medicines (Basel). 2018;5(3). doi: 10.3390/medicines5030101.

37. Bjerg AT, Kristensen M, Ritz C, Holst JJ, Rasmussen C, Leser TD, et al. Lactobacillus paracasei subsp paracasei L. casei W8 suppresses energy intake acutely. Appetite. 2014;82:111-8. doi: 10.1016/j.appet.2014.07.016.

38. Zheng J. Energy metabolism of cancer: glycolysis versus oxidative phosphorylation (Review). Oncol Lett. 2012;4(6):1151-7. doi: 10.3892/ol.2012.928.

39. Moreno-Sánchez R, Martin-Hernández A, Saavedra E, Pardo JP, Ralph SJ, Rodríguez-Enriquez S. Who controls the ATP supply in cancer cells? Biochemistry lessons to understand cancer energy metabolism. Int J Biochem Cell Biol. 2015;60:1-10. doi: 10.1016/j.biocel.2014.01.025.

40. Zhang M, Fan X, Fang B, Zhu C, Zhu J, Ren F. Effects of Lactobacillus salivarius Ren on cancer prevention and intestinal microbiota in 1, 2-dimethylhydrazine-induced rat model. J Microbiol. 2015;53(6):398-405. doi: 10.1007/s12275-015-5046-z.

41. Evans GI, Vousten KH. Proliferation, cell cycle and apoptosis in cancer. Nature. 2001;411(6835):342-8. doi: 10.1038/35077213.

42. Iyer C, Kusters A, Sethi G, Kunnumakkara AB, Aggarwal BB, Versalovic J. Probiotic Lactobacillus reuteri promotes TNF-induced apoptosis in human myeloid leukemia-derived cells by modulation of NF-kappaB and MAPK signalling. Cell Microbiol. 2008;10(7):1442-52. doi: 10.1111/j.1462-5822.2008.0137.x.

43. Guzy C, Paclik D, Schirbel A, Sonnborn U, Wiedenmann

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B, Sturm A. The probiotic *Escherichia coli* strain Nissle 1917 induces gammadelta T cell apoptosis via caspase- and FasL-dependent pathways. Int Immunol. 2008;20(7):829-40. doi: 10.1093/intimm/dxn041.

45. Baldwin C, Millette M, Oth D, Ruiz MT, Luquet FM, Lacroix M. Probiotic *Lactobacillus acidophilus* and *L. casei* mix sensitize colorectal tumoral cells to 5-fluorouracil-induced apoptosis. Nutr Cancer. 2010;62(3):371-8. doi: 10.1080/01635580903407197.

46. Uccello M, Malaguarnera G, Basile F, D'Agata V, Malaguarnera M, Bertino G, et al. Potential role of probiotics on colorectal cancer prevention. BMC Surg. 2012;12 Suppl 1:S35. doi: 10.1186/1471-2482-12-s1-s35.

47. Kumar M, Kumar A, Nagpal R, Mohania D, Behare P, Verma V, et al. Cancer-preventing attributes of probiotics: an update. Int J Food Sci Nutr. 2010;61(5):473-96. doi: 10.3109/09637480903455971.

48. Arthur JC, Gharaibeh RZ, Uronis JM, Perez-Chanona E, Sha W, Tomkovich S, et al. VSL#3 probiotic modifies mucosal microbial composition but does not reduce colitis-associated colorectal cancer. Sci Rep. 2013;3:2868. doi: 10.1038/srep02868.

49. Li J, Sung CY, Lee N, Ni Y, Pihlajamäki J, Panagiotou G, et al. Probiotics modulated gut microbiota suppresses hepatocellular carcinoma growth in mice. Proc Natl Acad Sci U S A. 2016;113(9):E1306-15. doi: 10.1073/pnas.1518189113.