



## SHORT COMMUNICATION

### Comparison of the Anti-tumoral Effects of Kefir with Buffalo and Cow Yogurt on 1,2-Dimethylhydrazine-induced Colon Cancer in Mice

Ibrahim Demirkan<sup>1</sup>, Tolga Ertekin<sup>2</sup>, Musa Korkmaz<sup>1</sup>, Sebnem Pamuk<sup>3</sup>, Hayati Yuksel<sup>4</sup>, Mehmet Fatih Bozkurt<sup>5</sup>, Mehmet Ozdemir<sup>6</sup>, Muhammad Nasir Bhaya<sup>5,7\*</sup>, Aysun Cevik-Demirkan<sup>8</sup>, Atilla Dogan<sup>1</sup> and Sinan Saracli<sup>9</sup>

<sup>1</sup>Department of Surgery, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye;

<sup>2</sup>Department of Anatomy, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Türkiye;

<sup>3</sup>Department of Food Hygiene, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye;

<sup>4</sup>Department of Pathology, Faculty of Veterinary Medicine, Bingöl University, Bingöl, Türkiye; <sup>5</sup>Department of Pathology, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye; <sup>6</sup>Department of Medical Pharmacology, Faculty of Medicine, Karabük University, Karabük, Türkiye; <sup>7</sup>Department of Pathology, Faculty of Veterinary Sciences, University of Agriculture Faisalabad, Pakistan; <sup>8</sup>Department of Anatomy, Faculty of Veterinary Medicine, Afyon Kocatepe University, Afyonkarahisar, Türkiye; <sup>9</sup>Department of Biostatistics and Medical Informatics, Faculty of Medicine, Balikesir University, Balikesir, Türkiye.

\*Corresponding author: dr.muhammadnasir@uaf.edu.pk

#### ARTICLE HISTORY (25-776)

Received: August 04, 2025  
Revised: October 20, 2025  
Accepted: October 23, 2025  
Published online: November 05, 2025

#### Key words:

Anatolian Water Buffalo

Colon Cancer

Kefir

Mice

Probiotics

Yogurt

#### ABSTRACT

This study was conducted to evaluate the possible anti-tumorigenic effects of Anatolian water buffalo and cow yogurts supplemented with *Lactobacillus plantarum* or *Lactobacillus rhamnosus*, and kefir on the development colon cancer (CC) in the mouse model induced with 1,2-dimethylhydrazine (DMH). A total of 440, eight weeks-old, BALB/c male mice were used in the experiment. Mice were allocated into five (05) main groups and further 22 subgroups (20 mice/group). Animals were administered DMH subcutaneously @ 20mg/kg body weight on weekly basis for 12 weeks. In experimental groups, dairy products were given either before DMH administration, simultaneously or after the tumor formation. Tumors were evaluated by the intensity of proliferating cell nuclear antigen, apoptosis and p53 status. Anatolian water buffalo yogurt supplemented with *L. rhamnosus* showed statistically significant ( $P<0.05$ ) antitumorigenic effects than the other dairy products used (Anatolian water buffalo yogurt supplemented with *L. plantarum*, cow yogurt supplemented with *L. plantarum* or *L. rhamnosus* and kefir). Hence it was concluded that timely consumption of dairy products (buffalo or cow yogurt supplemented with probiotics, or kefir) have the potential either to prevent or decrease the malignancy of CC.

**To Cite This Article:** Demirkan I, Ertekin T, Korkmaz M, Pamuk S, Yuksel H, Bozkurt MF, Ozdemir M, Bhaya MN, Cevik-Demirkan A, Dogan A and Saracli S, 2025. Comparison of the anti-tumoral effects of kefir with buffalo and cow yogurt on 1,2-dimethylhydrazine-induced colon cancer in mice. Pak Vet J. <http://dx.doi.org/10.29261/pakvetj/2025.291>

#### INTRODUCTION

Probiotics are beneficial (non-pathogenic) organisms that increase the native bacterial growth in the gut, thereby exerting a positive effect on the health status of host (Syngai *et al.*, 2016; Savaiano and Hutkins, 2021). *In vitro* effects of *Lactobacillus* on colonic cell lines were exclusively reported elsewhere (Chen *et al.*, 2017). *L. rhamnosus* and *Lactobacillus plantarum* are probiotics and have the capacity to strengthen the immune system, modulate intestinal inflammation and bind to toxic substances. The hepatoprotective (Sozmen *et al.*, 2005), haveunoprotective (Hong *et al.*, 2009; Moreno de Leblanc

*et al.*, 2007), nephroprotective, cytoprotective, antioxidant, anti-inflammatory (Sozmen *et al.*, 2005; Yosef, 2024), antimutagenic, antibacterial, antifungal and antitumoral (Guzel-Seydim *et al.*, 2006; Ozsoy *et al.*, 2021; De Leblanc *et al.*, 2007; Ma'mon *et al.*, 2018; Sepe and Arguello, 2019; Guzel-Seydim *et al.*, 2006) effects of kefir has already been reported in different studies. Previous studies evaluated how the administration of kefir, koumiss, milk, and yoghurt affected the mice's spleen tissues' mast cells, which regulate humoral and cellular processes, and plasma cells, which produce antibodies against antigens (Sevda *et al.*, 2015). Yogurt as a biocontrol alternative method for eliminating different pathogens in the dairy industry has

been reported (Fahim *et al.*, 2023). To achieve sustainability of intestinal stability by accomplishing microbial balance and immunologic barrier within intestine (especially IgA and increase in inflammatory response) probiotics can be added to the diet (Galdeano *et al.*, 2019).

CC is the most common type of tumors worldwide. It is a major health concern in western countries where it remained the main cause of death and it is associated with an estimated 1.8 million cancer deaths (18%), after lung cancer (9.4%) (Sung *et al.*, 2020). The incidence of CC is also significantly increasing in many countries like Singapore, Korea, Japan, China and Asia (Ng and Wong, 2013). Colon cancers are usually treated by one or combination of surgery, chemotherapy and/or radiotherapy, albeit disputable outcomes. Alternative approaches such as biological therapies i.e. viral therapy, gene therapy (Tripodi *et al.*, 2021) and antineoplastic nano-agents are all still in their early stages. Surgery, partial or total colectomy, is the best option for the treatment of CC (Derikx *et al.*, 2018). The other problem using chemotherapy is the development of drug resistance or cross resistance. The regulation of intestinal microflora is an important factor for the modulation of CC risk (Sankarapandian *et al.*, 2022). The association between immune system and probiotics in CC has been exclusively studied. However, possible interactions between cancer cell markers (proliferating cell nuclear antigen (PCNA), p53 and apoptosis index), probiotic yogurt and kefir appear to be interesting to evaluate (Alves *et al.*, 2020; Byanju and Lamsal 2023). This study evaluated the possible anti-tumorigenic effects of Anatolian water buffalo and cow yogurts (supplemented with probiotics), and kefir on the DMH induced CC in the mouse model.

## MATERIALS AND METHODS

**Ethical Approval:** This study was approved by the local ethical committee of Afyon Kocatepe University, Turkey (No: AKUHADYEK 13-09), and all interventions were carried out under the laboratory animal handling and animal experiments guidelines of the University.

**Preparation of probiotic yogurt and kefir:** After that, preparation of probiotic yogurt and kefir was done. Fresh milk was obtained from Anatolian water buffaloes or Brown Swiss cows raised in the research farm of the University without any antibiotic treatments. The milk sample was pasteurized at 72°C for 2 min, and cooled down at 42°C, then 2% thermophilic cultures were added. Lyophilized *L. plantarum* and *L. rhamnosus* probiotic cultures were purchased from Refik Saydam Hıfzısıhha Başkanlığı (Code no: 050256, Ankara, Türkiye) and CHR-Hansen (No: YC-350; Istanbul, Türkiye), respectively. They were inoculated into milk as 107cfu/mL and incubated at 42-43°C for 4h until the final pH was 4.6. After Brown Swiss cow milk pasteurised, 15-20g kefir granules were added in 1L of the milk and completely mixed and then kept to ferment at 20-22°C for 18-24h. Drained kefir was cooled and kept at 4°C. To achieve standardization, industrial kefir culture (CHR-Hansen BB-12R LAF-3) was used. 2-8% kefir culture was added to pasteurized milk, incubated at 20-22°C for 18-24h until pH was 4.4-4.9 and acidity was 30-40 Soxhlet Henkel (°SH).

Freshly prepared kefir was kept at 3-10°C. Each mouse was administered 1cc of kefir (5.2x10<sup>8</sup> cfu/ml) by oral gavage. To induce colon tumors, mice were administered DMH subcutaneously at a dose of 20mg/kg/body weight on weekly basis for 12 weeks.

**In vivo experiment:** Total of 440 male, 8-week-old male BALB/c mice of 20-30g were experimented. Mice were divided into 5 main groups and further 22 subgroups. Each subgroup consisted of 20 mice. Group 1 (divided into groups 1A and 1B) was further subgrouped as group 1A1 (the mice were fed cyclically with water buffalo yogurt (Wby) supplemented with *Lactobacillus plantarum* (Lp) for two weeks by oral gavage (basal yogurt feeding), then treated with DMH once a week for 12 weeks, cyclic administration of Wby+Lp continued until the end of the experiment; to observe protective effects of Wby+Lp); group 1A2 (animals were fed cyclically with Wby+Lp for two weeks and then subcutaneous DMH were injected once a week for 12 weeks; to observe the effect of Wby+Lp on tumor initiation); group 1A3 (subcutaneous DHM was given once a week for 12 weeks then fed cyclically with oral Wby+Lp upto the end- no basal yogurt feed; to observe antineoplastic effects of Wby+Lp); group 1A4 (only cyclic oral Wby+Lp was given throughout the experiment; control group for Wby+Lp). To evaluate the effects of Wby supplemented with *L. rhamnosus* (Wby+Lr), group 1B subgrouped as 1B1, 1B2, 1B3 and 1B4 and the same procedure was applied changing only probiotic *L. rhamnosus* in place of *L. plantarum* as in group 1A. Group 2 was also divided into 8 subgroups to observe antineoplastic effects of cow yogurt (Cy) supplemented with *L. plantarum* (Cy+Lp; subgroups 2A1, 2A2, 2A3 and 2A4) or *L. rhamnosus* (Cy+Lr; subgroups 2B1, 2B2, 2B3 and 2B4) as in groups 1A and 1B. Group 3 was divided into 4 subgroups (3A, 3B, 3C and 3D) similar to group 1A, however kefir was substituted instead of Wby. Group 4 served as positive control where only subcutaneous DHM was given once a week for 12 weeks to induce tumor growth and group 5 (control group) where mice were given only a conventional balanced diet. The experiment was terminated at the 6<sup>th</sup> month of the study.

Next day of the dairy product administration, freshly voided stool specimens of mice, three samples from each group, were collected and transferred to the laboratory within five minutes. Determination of the faecal excretion of live probiotics in yogurt and kefir was confirmed by faecal sample inoculation on agars (Mann Rogosa Sharpe®, Oxoid, CM0361B and M17, Oxoid, CM0785B) by the routine manner. The intestinal lactobacilli composition was shown as (log<sup>10</sup> colony-forming units (cfu)/g) counts and colonies were not typed.

Mice were euthanized after the administration of general anesthesia with the dose rate of 15mg/kg xylazine (Rompun®, Bayer, Germany) and 50mg/kg ketamine HCl (Alfamine®, Egevet, Turkey). The specimen was longitudinally opened, and content was carefully removed and washed with saline solution. The presence of tumors was observed, counted and measured by a magnifier. The Swiss-roll technique was performed according to procedure described by Moolenaar and Ruitenberg (1981). Briefly, longitudinally opened colon strips were rolled up, with the mucosa in the outwards direction, using

a clean wooden stick. They were fixed in 10% buffered formalin saline solution and kept at 4°C for 48h for fixation. After paraffin-wax embedding, 5µm thick sections were stained with haematoxylin-eosin stain (H&E) and examined under a light microscope. Tissue samples from colons were processed with routine tissue processing methods. Astler-Coller staging system, a modification of Dukes' classification, was used for histopathological staging of colon tumors (Table. 1).

**Table. 1:** Histopathological staging of colon malignancy (Astler and Coller, 1954).

| Stage | Lesion location                                                               |
|-------|-------------------------------------------------------------------------------|
| A     | Involvement of mucosa                                                         |
| B1    | extending with negative nodes into the muscularis propria without entering it |
| B2    | Penetration of Muscularis propria layer with negative nodes                   |
| C1    | extending with positive nodes into the muscularis propria without penetration |
| C2    | Ppenetration of muscularis propria with positive nodes                        |

5µm sections were paraffinised and rehydrated and were maintained in 3% hydrogen peroxide at 22°C for 10min for blocking of endogenous peroxidase. Slides were shifted in citrate buffer (pH 6.0) solution in a pressure cooker and a 900-watt power microwave applied for 20 min for antigen retrieving. As the primary antibodies anti-PCNA (Abcam, Rabbit polyclonal, ab18197, 1/400 dilution) and anti-p53 (Sigma, Rabbit polyclonal, 1/25 dilution) were used and 2 and 18h incubation periods were performed at room temperature, respectively. Mouse on Mouse Kit (Elite peroxidase kit Vectorlab) was used to mouse primary antibodies, and ABC kit (Vectastain Elite ABC-Peroxidase kit) was applied to rabbit primary antibodies. Visualization was made with 3-amino-9-ethylcarbazole substrate (AEC, Invitrogen). Background was stained with Gill's (I) haematoxylin dye for 2 min. After washing with tap water sections were covered slipped with an aqueous medium. These ready sections were kept in the dark room until analysed. Only nuclear staining in the sections was considered positive. Thus, percentage of positive cells was used as reactivity criteria in this study.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL assay) was performed by getting sections on adhesive slides. Antigen retrieval was done by Proteinase, and 3% hydrogen peroxide were used for the blocking of endogenous peroxidase. Apoptotic kit (In Situ Cell Death Detection Kit, POD, Roche, Mannheim, Germany) was used for the reaction. Visualization was made with 3-amino-9-ethylcarbazole substrate (AEC, Invitrogen). Background was stained with Gill's (I) haematoxylin dye for 2min. After washing with tap water sections were covered with an aqua medium. Ten different fields were counted at 40X magnification (Image J program) for apoptotic cell percentage.

**Statistical analysis:** Correlation analysis was used to observe possible association between immunohistochemical stainings (PCNA, p53) and malignancy of tumors (Astler-Coller staging). Histopathological and immunohistochemical staining of samples were analysed by descriptive statistics (i.e. number and percentage). The ability to provide both categorical and continuing (continuous) variables is one of the advantages of chi-squared automated interaction detection (CHAID)

analysis over other types of analysis. The selected predictor was used to partition the data. To generate further subdivisions for analysis, each of these subgroups underwent independent reanalysis. To construct the contingency table with the maximum significance level in accordance with the chi-squared test, the type of each predictor dictated the acceptable groupings of its categories (Kass, 1980). The data was also described, examined, and summarised using multiple correspondence analysis. The threshold for significance was fixed at  $P < 0.05$ .

## RESULTS

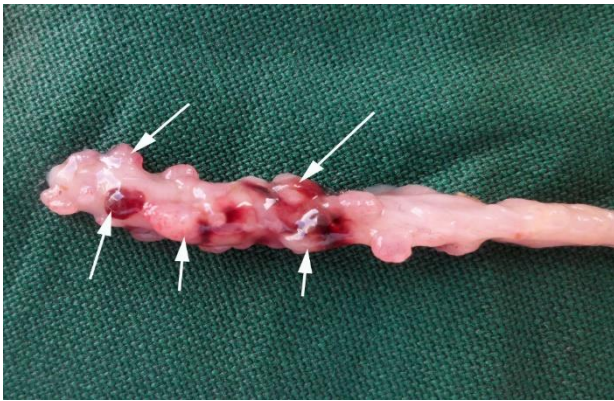
The live lactobacilli levels in stool samples were presented in Table 2. Tumors were macroscopically observed as early as within 4 months in group 4. Lesions were seen in both serosa and mucosa in the colon. Nodular neoplasms of varying size, usually located in the middle colon, were reaching the size to fill the colon lumen in places (Fig. 1). No tumor development was seen either in control group (group 5) or groups that were given only dairy products (groups 1A4, 1B4, 2A4, 2B4, and 3D) by macroscopic evaluations.

**Table. 2:** The level of live lactobacilli in the stool samples.

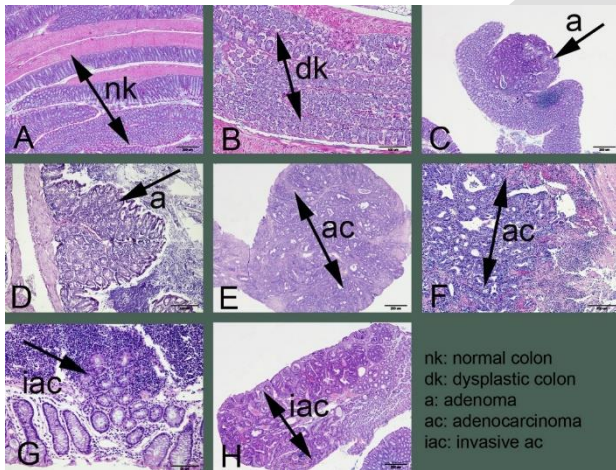
| Groups            | Live lactobacilli level in stool sample |
|-------------------|-----------------------------------------|
| 1A1-1A3           | $4.8 \times 10^6$ cfu/g                 |
| 1A4               | $7.0 \times 10^7$ cfu/g                 |
| 1B1-1B3           | $3.0 \times 10^6$ cfu/g                 |
| 1B4               | $6.0 \times 10^6$ cfu/g                 |
| 2A1-2A3           | $3.5 \times 10^6$ cfu/g                 |
| 2A4               | $6.8 \times 10^7$ cfu/g                 |
| 2B1-2B3           | $2.6 \times 10^6$ cfu/g                 |
| 2B4               | $5.8 \times 10^6$ cfu/g                 |
| 3A-3C             | $3.0 \times 10^6$ cfu/g                 |
| 3D                | $4.8 \times 10^6$ cfu/g                 |
| 4 (DMH group)     | $2.0 \times 10^3$ cfu/g                 |
| 5 (Control group) | $2.0 \times 10^3$ cfu/g                 |

Histopathological findings are presented in Fig. 2. No tumor development was seen either in control group (Fig. 2A). Preneoplastic and neoplastic lesions described in DMH received groups. DMH administrations caused different colon lesions such as dysplasia (Fig. 2B), adenoma (Fig. 2C-D) adenocarcinoma (Fig. 2E-F), and invasive adenocarcinoma (Fig. 2G-H). Adenomas and adenocarcinomas consisted of atypical glands and epithelium. In the neoplastic glands of adenocarcinomas, there were numerous mitotic figures, inflammatory cell infiltration, and pleomorphic cells. Significant nuclear elongation, hyperchromatic vesicular nuclei, anionucleosis, and anisocytosis were all observed in these pleomorphic cells. Anaplastic cell clusters and inflammatory cell infiltration into the submucosa were seen in the region of adenocarcinoma. No tumor development was seen either in groups that were given only dairy products (groups 1A4, 1B4, 2A4, 2B4, and 3D) by microscopic evaluations. Staging of all tumors according to Astler-Coller is presented in Fig. 3. Among all tumors ( $n=92$ ) observed in this study, the predominant lesion was type A (53/92; 57.6%) followed by type B1 (30/92; 32.6%). The development and frequency of tumors were superior in groups where dairy products were given after exposure to the carcinogen. According to results of CHAID analysis, the most effective variable on tumor development was the duration of administration of dairy products. In groups

1A1, 1B1, 2A1, 2B1 and 3A (where dairy products were given prior to carcinogen challenge), in general, the likelihood of non-tumoral observation was 59.7% and the variables were found to be statistically significant ( $P < 0.05$ ). In these groups the ratio of non-tumoral observation was 84.2% for Wby, whereas it was 58.6% for Cy and 28.6% for kefir subgroups. The addition of probiotic to Cy was associated with statistically significant decrease in tumor development i.e. Cy+*Lr* generated less tumor development (73.3%) than Cy+*Lp* (42.9%). In Wby groups 1A1 and 1B1, however, the effect of probiotics addition produced no statistically significant data in the analysis. The reason for this, in these groups, was higher rate of non-tumor observation, 90% for *L. plantarum* and 73.3% for *L. rhamnosus*.



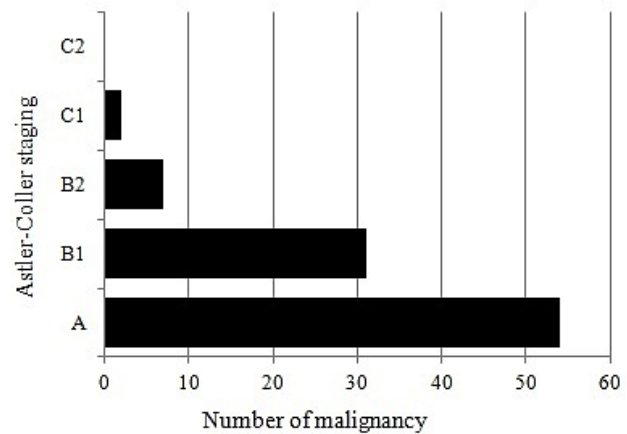
**Fig. 1:** Macroscopic appearance of tumors in different numbers and sizes in the DMH-induced colon in a mouse (arrows).



**Fig. 2:** Photomicrographs of DMH and dairy products received mice colon sections stained by H&E: A: Control group; normal histological appearance. B: Dysplasia, dysplastic changes both in glands and epithelia. C-D: Adenomas, numerous neoplastic glands partially resembling normal colon glands. E-F: Adenocarcinomas, mitotic figures, inflammatory cell infiltration and pleomorphic cells were abundant in the neoplastic glands of adenocarcinomas. These pleomorphic cells exhibited significant nuclear elongation, hyperchromatic vesicular nuclei, anisonucleosis and anisocytosis. G-H: Invasive adenocarcinomas, in some areas of adenocarcinomas, clumps of anaplastic cells and infiltration of inflammatory cells in the submucosa were observed.

In groups 1A2, 1B2, 2A2, 2B2 and 3B (simultaneous administration of dairy products with carcinogen), it was determined that 77.8% of animals were no tumor bearing and there was statistically significant difference between Wby and kefir with Cy (Wby and kefir clustered within

the same group). In Wby or kefir applied groups, tumors were not observed in 86.1% even though it was 58.3% in Cy groups. In Wby+*Lp* group, no tumor was observed in 78.3% of animals whereas in *L. rhamnosus* added yogurt groups, tumor rate decreased in all animals. Finally, in groups 1A3, 1B3, 2A3, 2B3 and 3C (where dairy products were given after cancer development), observation of no tumor was 27.9%. The Wby and kefir displayed similar effects, but Cy significantly differed from them. No tumor development was 34.3% for Wby and kefir groups and 14.3% for Cy groups. Addition of probiotics produced statistically significant data such as *L. rhamnosus* addition associated with 90.0 % no tumor development whereas *L. plantarum* addition generated tumors in 22.2% cases.

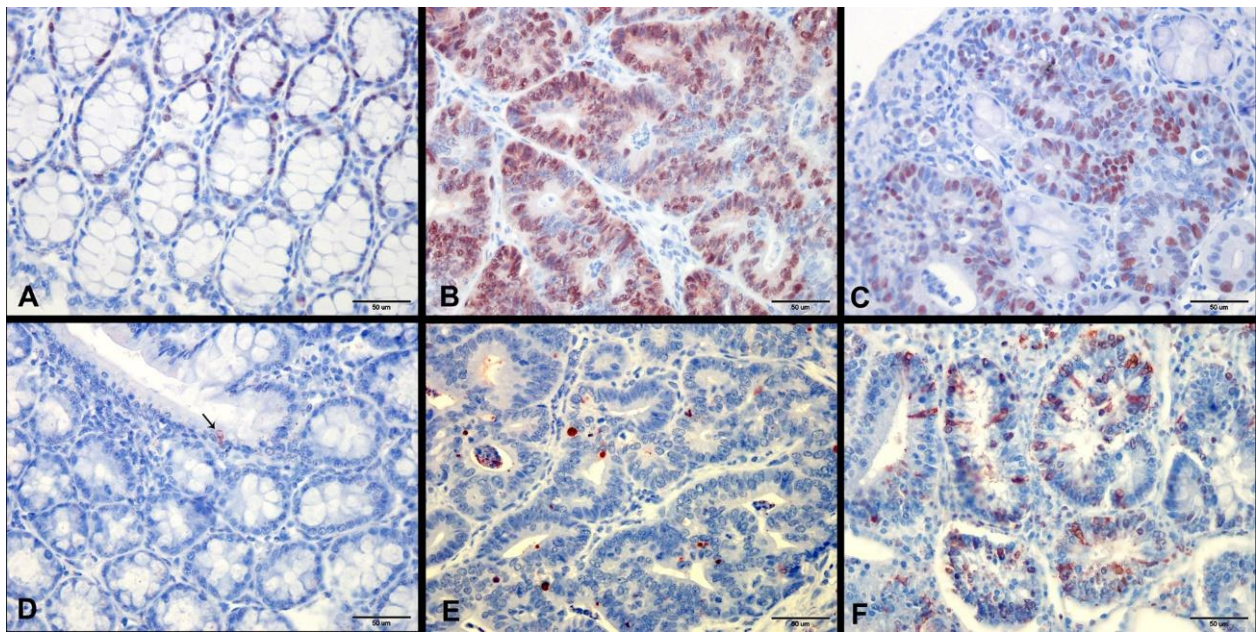


**Fig. 3:** Distribution of tumor types according to Astler-Coller staging.

Immunohistochemical findings are presented in Fig. 4A-F. There was a positive correlation between PCNA, *p53* and apoptosis positivity and presence of tumors according to Spearman's  $\rho$  correlation coefficient ( $\rho = 0.981, 0.939$  and  $0.971$ , respectively). The mean PCNA staining was significantly lower in animals treated with cyclic Wby+*Lr* prior to DMH administration than the other groups ( $P < 0.01$ ). PCNA expression was also present in all DMH-administered animals, suggesting that some degree of proliferation was already initiated by the carcinogen.

When multiple correspondence analyses were considered, the tumor detection, generally, by *p53* staining was observed in groups where tumor challenge followed by administration of Cy+*Lp*. However, the tumor detected by PCNA staining was generally seen in Wby+*Lr* groups in which dairy products were given before tumor initiation. Mice that received yogurt or kefir prior to DMH challenge had profound effect on apoptosis of the colonic epithelial cell in comparison to DMH treated control group ( $P < 0.01$ ).

The expression of *p53* in low degree neoplasms was in nuclear locations. However, it was both nuclear and cytoplasmic in high neoplastic malignancy. Cytoplasmic *p53* expression was significant in groups 3 and 4, while it was only nuclear in groups 1 and 2. Vicinity of *p53* positive cells were surrounded by massive mononuclear cell infiltration. The *p53* expression was lower in groups 1A1 (90%) and 1A2 (60%) than 1A3 (30%); groups 1B1 (80%) and 1B2 (100%) than 1B3 (60%). However, groups 2A1 (70%) and 2A3 (80%) showed more positivity than 2A2 (60%) whereas it was 40% for 2B1, 70% for 2B2 and 100% for 2B3. In kefir group's positivity was 80% for group 3A, 70% for group 3B and 90% for group 3C.



**Fig. 4:** Microscopic images of PCNA and TUNEL immunohistochemical staining. A: Very weak and limited PCNA staining in nuclei of intestinal glandular epithelial cells in group 5 (control group). B: Copious staining of PCNA in tumor cell nuclei of group 4 (DMH group). C: Decreased PCNA staining in tumor cell nuclei in dairy product administered groups (group 1B given for repretantation). D: Apoptosis in a single intestinal glandular epithelial cell in control group (arrow). E: A number of apoptotic cells in tumor cells of group 4. F: Increased number of apoptotic cells in group 1B.

The effects of yogurt or kefir on tumor development were not significant once tumor occurred ( $P > 0.05$ ). Chi-square test revealed statistically significant differences within groups between the consumption of yogurt or kefir before or simultaneous exposure to DMH ( $P < 0.01$ ). When three dairy products were compared, Anatolian water buffalo yogurt showed superior antitumorigenic effects. In addition, *L. rhamnosus* generated statistically significant difference in comparison to *L. plantarum* probiotic ( $P < 0.05$ ).

## DISCUSSION

The addition of probiotic bacteria in different dairy products has become increasingly popular in today's human diet policy (Galdeano *et al.*, 2019). The health and nutritional benefits ascribed to probiotic bacteria include the regulation of intestinal functions, alteration of physico-chemical environment in the colon milieu (Hansen *et al.*, 2019), alleviation of the symptoms of lactose intolerance, qualitative and quantitative changes in the gut microflora, serum cholesterol reduction, alleviation of constipation (Wan *et al.*, 2019), production of anti-mutagenic (Guzel-Seydim *et al.*, 2006) and anti-tumorigenic factors (De Leblanc *et al.*, 2007; Ma'mon *et al.*, 2018; Sepe and Arguello, 2019; Guzel-Seydim *et al.*, 2006) interaction and degradation of potential carcinogens (Perdigon *et al.*, 2002) and improvement of host immune system efficacy (i.e. enhancement of local IgA production (Kailasapathy and Chin, 2000)). To produce therapeutic benefits, lowest levels for probiotic bacteria in yogurt products must have  $10^6$  viable cells per mL or g in the final preparation (Mortazavian *et al.*, 2012). In our study viable and live organisms, either in yogurt and kefir preparations or in fecal samples, were observed to be at satisfactory level.

Probiotics may prevent CC by lowering intestinal pH, modifying enzymes like  $\beta$ -glucosidase that change pro-

carcinogens into proximate carcinogens, reducing ras-P21 oncoprotein expression, and producing sodium butyrate through lactose fermentation, which is a potent growth inhibitor and inducer of phenotype differentiation and apoptosis (Jung *et al.*, 2007). The second most widely available milk source in many countries around the world is water buffaloes (Borghese and Mazzi, 2005). The data about the use of dairy products for the treatment of animal model colon cancer was also published in review studies (Nacimento *et al.*, 2021). It was reported that significant differences were found between the contents of cow and buffalo milk. Concentrations of vitamins (A, B<sub>6</sub>, C, E, niacin and biotin), minerals (calcium, magnesium, phosphorus, sodium and zinc), total protein, totals of saturated, monounsaturated and polyunsaturated fatty acids are predominantly higher in buffalo milk than cow milk (Medhammar *et al.*, 2012). This has been reported in this study, that showed superior effects on the prevention of CC with respect to other dairy products.

Lactic acid bacteria strains like *L. plantarum* and *L. rhamnosus* have been shown to be more successful in protecting the colon's microbial balance, preserving the essential nutrients the bacteria produce, removing harmful substances from food, preventing decay, and destroying pathogens (Bengmark, 2000). As a result, probiotics have been frequently added to yoghurt products to increase their therapeutic potential and create a market for them as functional foods (Badgeley *et al.*, 2021; Abiden *et al.*, 2024). Like the well-known probiotic strain *L. rhamnosus*, *L. plantarum* also suppresses pathogen adherence. Additionally, it might reduce the expression of interleukin 8 (IL-8) and tumour necrosis factor alfa (TNF- $\alpha$ ) in cells treated with *E. coli* (Ko *et al.*, 2007). However, in our study *L. rhamnosus* showed superior ability to inhibit CC with respect to *L. plantarum*. Kefir is reported to induce some antitumor activity (Dos *et al.*, 2019). However, in our study, kefir showed non-significant tumor inhibition

compared to cow or buffalo yogurts. The reason why kefir showed weak anti-tumorigenic effect may be that kefir bears effective properties against carcinogens that are taken by oral route rather than parenteral administration. This mechanism should be exclusively described, if any. Moreover, kefir has significant dose dependent antitumor activity against multiple cancer cell types (Walia *et al.*, 2018), that may be associated with different outcomes between different studies. Leblanc and Perdigon (2004) reported that yogurt feeding before carcinogen injections showed rapid regulatory cytokine release and apoptosis stimulation in the large intestine, this shows that yogurt supplementation alone was incapable of inhibiting tumor appearance and development. Yogurt must be cyclically administered after DMH to inhibit tumor growth. Walia *et al.* (2018) concluded that consumption of dairy products may have beneficial effect on CC only if they are consumed before or during exposure to the carcinogen but not subsequently. This discrepancy may be associated with the use of different cultures and ratio among studies as Leblanc and Perdigon (2004) used *L. delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (approximately  $2 \times 10^9$  cfu/mL), Walia *et al.* (2018) used *L. casei* subsp. *rhamnosus* strain GG ( $2 \times 10^{10}$  cfu/mL) and we used *L. plantarum* and *L. rhamnosus* ( $8 \times 10^8$  cfu/mL) cultures.

It was reported elsewhere that PCNA was generated by cells during the late S-phase and G-phase of the replicative stage as a result it could be a good candidate as a phase index marker for proliferating cells (Yamamoto *et al.*, 2019). Thus, all mitotic activities and tumor grades were closely correlated with PCNA positivity. Not surprisingly *p53* positivity showed similar patterns as TUNEL and PCNA positivity or vice versa. Thus, this indicates that *p53* without disruption may accomplish its duties in cells. Moreover, once the malignancy started, they attracted cytotoxic T-lymphocytes to the region.

**Conclusions:** In conclusion, our findings demonstrated that, in this experimental murine model, yogurt and kefir feeding favoured apoptosis and interfered with the initiation or early promotional stages of DMH-induced CC. Moreover, Anatolian water buffalo yogurt supplemented with *L. rhamnosus* showed more significant antitumorigenic effects than the other dairy products (Anatolian water buffalo yogurt supplemented with *L. plantarum*, cow yogurt supplemented with *L. plantarum* or *L. rhamnosus* and kefir). This study findings provide baseline information and could be used to develop and implement a tool to evaluate further therapeutic effects of these dairy products against CC. Thus, it will be of interest to compare regression of tumor size using different preparations of dairy products.

**Authors contribution:** ID: Designation the study, project administration, writing-original draft, writing-reviewing and editing. TE, MK, ACD and AD: Experimentation and data collection. SP: Preparation of dairy products, HY, MFB and MNB: Evaluation of pathological specimens, MO: DMH preparation, and SS: Data analysis. All authors have read and approved the final manuscripts.

**Acknowledgements:** The authors want to acknowledge Professor Hikmet Keles, Department of Pathology, the

Afyon Kocatepe University Afyonkarahisar, Türkiye, for helping in immunohistochemical evaluation.

**Funding:** The study was financially funded by the Scientific and Technological Council of Türkiye (TUBITAK; Grant no: TOVAG 1090306).

## REFERENCES

- Abedin MM, Chourasia R, Phukon LC, 2024. Lactic acid bacteria in the functional food industry: Biotechnological properties and potential applications. Crit Rev Food Sci Nutr 64:10730-48.
- Alves dos Santos K, Clemente dos Santos IC, Santos Silva C, *et al.*, 2020. Circulating exosomal miRNAs as biomarkers for the diagnosis and prognosis of colorectal cancer. Int J Mol Sci 22: 346.
- Badgeley A, Anwar H, Modi K, *et al.*, 2021. Effect of probiotics and gut microbiota on anti-cancer drugs: Mechanistic perspectives. Biochim Biophys Acta Rev Cancer 1875:188494.
- Bengmark S, 2000. Colonic food: pre- and probiotics. Am J Gastroenterol 95:S5-S7.
- Borghese A and Mazzi M, 2005. Buffalo population and strategies in the World. Buffalo Product Res 67:1-39.
- Bryanju B and Lamsal B 2023. Protein-rich pulse ingredients: preparation, modification technologies and impact on important technological and quality characteristics, and major food applications. Food Rev Int 39: 3314-43.
- Chen ZY, Hsieh YM, Huang CC, *et al.*, 2017. Inhibitory effects of probiotic *Lactobacillus* on the growth of human colonic carcinoma cell line HT-29. Molecules 22:107.
- De LeBlanc AD, Matar C, Farnworth E, *et al.*, 2007. Study of immune cells involved in the antitumor effect of kefir in a murine breast cancer model. J Dairy Sci 90:1920-28.
- Derikx LA, de Jong ME and Hoentjen F, 2018. Short article: Recommendations on rectal surveillance for colorectal cancer after subtotal colectomy in patients with inflammatory bowel disease, Eur J Gastroenterol Hepatol 30:843-46.
- Dos Reis SA, da Conceição LL, eDias MM, *et al.*, 2019. Kefir reduces the incidence of pre-neoplastic lesions in an animal model for colorectal cancer. J Function Foods 53:1-6.
- Fahim KM, Ali ZI, Ahmed LI, *et al.*, 2023. Evaluating the antagonistic effect of *Lactobacillus acidophilus* against Shiga toxinogenic and non-toxicogenic *Escherichia coli* strains in bioyogurt. J Dairy Res 90:82-7.
- Galdeano CM, Cazorla I, Dumit JML, *et al.*, 2019. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metabol 74:115-24.
- Guzel-Seydim ZB, Seydim AC, Greene AK, *et al.*, 2006. Determination of antimutagenic properties of acetone extracted fermented milks and changes in their total fatty acid profiles including conjugated linoleic acids. Int J Dairy Technol 59:209-15.
- Hansen ME, Rubel MA, Bailey AG, *et al.*, 2019. Population structure of human gut bacteria in a diverse cohort from rural Tanzania and Botswana. Genome Biol 20:16.
- Hong WS, Chen HC, Chen YP, *et al.*, 2009. Effects of kefir supernatant and lactic acid bacteria isolated from kefir grain on cytokine production by macrophage. Int Dairy J 19:244-51.
- Jung JJ, Jeung HC, Lee JO, *et al.*, 2007. Putative chemosensitive genes in colorectal cancer cell lines for anticancer agents. Oncol Report 18:593-99.
- Kailasapathy K and Chin J, 2000. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immunol Cell Biol 78:80-8.
- Ko JS, Yang HY, Chang JY, *et al.*, 2007. *Lactobacillus plantarum* inhibits epithelial barrier dysfunction and interleukin-8 secretion induced by tumor necrosis factor- $\alpha$ . World J Gastroenterol 13: 1962-65.
- Leblanc AM and Perdigon G, 2004. Yogurt feeding inhibits promotion and progression of experimental colorectal cancer. Med Sci Mon 10:96-104.
- Ma'mon MH, Nuirat A, Zihlif MA, *et al.*, 2018. Exploring the influence of culture conditions on kefir's anticancer properties. J Dairy Sci 101:3771-77.
- Medhammar E, Wijesinha-Bettoni R, Stadlmayr B, *et al.*, 2012. Composition of milk from minor dairy animals and buffalo breeds: a biodiversity perspective. J Sci Food Agri 92: 445-74.
- Mortazavian M, Mohammadi R and Sohrabvandi S, 2012. Delivery of probiotic microorganisms into gastrointestinal tract by food products. In: Brzozowski, T, Editor. New Advances in the Basic and

- Clinical Gastroenterology. London: In Tech Open Ltd 121-146.
- Nascimento-Goncalves E, Mendes BA, Silva-Reis R, *et al.*, 2021. Animal models of colorectal cancer: From spontaneous to genetically engineered models and their applications. *Vet Sci* 8:59.
- Ng SC and Wong SH, 2013. Colorectal cancer screening in Asia. *Br Med Bull* 105:29-42.
- Ozsoy B, Cantekin Z, Yalcin S, *et al.*, 2021. Effects of kefir on blood parameters and intestinal microflora in rats: An experimental study. *Ratlarda kefirin bağırsak mikrobiyotası ve bazı kan parametrelerindeki rolü: Deneysel çalışma. Kafkas Univ Vet Fak Derg* 27 (1):111-15.
- Perdigon G, De Moreno de Leblanc A, Valdez J, *et al.*, 2002. Role of yoghurt in the prevention of colon cancer. *Eur J Clin Nutr* 56:S65-S68.
- Sankarapandian V, Venmathi Maran BA, Jogalekar P, *et al.*, 2022. An update on the effectiveness of probiotics in the prevention and treatment of cancer. *Life* 12:59.
- Savaiano DA and Hutkins, RV, 2021. Yogurt, cultured fermented milk, and health: A systematic review. *Nutr Rev* 79:599-614.
- Sepe L and Argüello A, 2019. Recent advances in dairy goat products. *Asian-Australasian J Anim Sci* 32:1306.
- Sevda EY, Funda Y, Berna DA, *et al.*, 2015. Effects of Kefir, Koumiss, Milk and Yoghurt Administration on Distribution of Plasma Cells and Mast Cells in Mice Spleen. *Kafkas Univ Vet Fak Derg* 21:195.
- Sozmen M, Erginsoy SD, Cenesiz S, *et al.*, 2005. The protective effect of kefir and vitamin C on azoxymethane induced toxicity and induction of metallothionein in mice. *Scand J Lab Anim Sci* 32:211-20.
- Sung H, Ferlay J, Siegel RL, *et al.*, 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *Ca J Clin* 71: 209-49.
- Syngai GG, Gopi R, Bharali R, *et al.*, 2016. Probiotics- the versatile functional food ingredients. *J Food Sci Tech* 53: 921-33.
- Tripodi L, Vitale M, Cerullo V, *et al.*, 2021. Oncolytic adenoviruses for cancer therapy, *Int J Mol Sci* 22: 2517.
- Walia S, Kamal R, Dhawan DK, *et al.*, 2018. Chemoprevention by probiotics during 1,2-dimethylhydrazine-induced colon carcinogenesis in rats. *Dig Dis Sci* 63: 900-909.
- Wan ML, Ling KH, El-Nezami H, *et al.*, 2019. Influence of functional food components on gut health. *Crit Rev Food Sci Nutr* 59: 1927-36.
- Yamamoto T, Nishita T and Taga A, 2019. Dark-colored maple syrup treatment induces S-phase cell cycle arrest via reduced proliferating cell nuclear antigen expression in colorectal cancer cells. *Oncol Lett* 17: 2713-20.
- Yousef FM, 2024. Nephroprotective Impact of Fermented Kefir Laban in Type I Diabetic Rats: Antioxidant and Anti-inflammatory Pathways. *Egyptian J Vet Sci* 11:1-8.