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Histopathological Effect of Probiotics after Intra-Peritoneal Injection of Ehrlith Ascites Tumor Cells

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Abstract

Background: Some functional foods such as probiotics play a beneficial role functions in the treatment of some health problems and ameliorate the oxidative stress mechanisms and other risks which improving the quality of life. Cancer is one of the most important health problems.

Methods: For this study forty four female Swiss albino mice were used. These mice were subdivided into four groups (negative control, positive control and two induced carcinogenic groups fed on probiotics). Yoghurt and fermented kidney beans used as probiotics. Cancer induced by intra-peritoneal injection of 2×106 Ehrlich ascites tumor cells. The tumer will examine histopathologically.

Results: Feeding on one of the two tested probiotics lowers the number and size of the tumor cells, with signs of degeneration and necrosis. Feeding on fermented kidney beans was more effective than yogurt in which the neoplastic cells became smaller in size, degenerated and lost their nuclei.

Conclusion: In this study the two tested probiotics (yoghurt and fermented kidney beans) used as biofunctional foods to reduce the high risk of these disease.

Keywords: Cancer; Ehrlith ascites; Fermented kidney beans; Yoghurt

Introduction

Functional food, i.e. foods that promise the consumer not just a full stomach but also some "added benefit" [1]. The term "functional foods" comprises some bacterial strains and products of plant and animal origin containing physiologically active compounds beneficial for human health and reducing the risk of chronic diseases. Probiotics are the best examples of functional foods. Probiotics considered functional foods because they provide health benefits beyond the traditional nutrition function [2]. Probiotic bacteria are defined as live microbial food ingredients that are beneficial to the health of the host. Probiotics occur naturally in fermented food products such as yoghurt, kefir, sauerkraut, and soybean. Numerous health benefits have been attributed to probiotics, including effects on gastrointestinal tract function and diseases, immune function, hyperlipidemia, cancer diseases, hypertension, and allergic conditions [3]. Lactic Acid Bacteria (LAB) are present in many foods such as yoghurt and are frequently used as probiotics to favor some biological functions in the host [4]. In the present work tested fermented kidney beans which produced by following the same methods of the production of yoghurt for their therapeutic effects. The development of the cancer is a multistage process that occurs when the accumulation of mutations in certain proto-oncogenes and tumor suppressor genes leads to cancer initiation. DNA damage in these genes could lead to mutations and therefore, LAB have been investigated extensively in model systems for their ability to prevent mutations [5]. The human probiotic kills colorectal adenocarcinoma cells through apoptosis in vitro via its metabolites, the Short Chain Fatty Acids (SCFA), acetate and propionate [6]. Another possible explanation for the preventive effect of probiotics on carcinogenesis is their effect on other bacteria in the intestine. Probiotics may suppress the growth of bacteria that convert procarcinogens into carcinogens, thereby reducing the amount of carcinogens in the intestine [4]. There is a strong correlation between orally administered probiotics and suppression of the low-grade inflammation that can lead to restoration of normal local immune functions. Results suggested that oral administration of microencapsulated probiotic L. acidophilus exerted anti-timorous activity, which consequently leads to reduced tumor outcome [7]. The aim of the present study was to investigate the beneficial effects of the two tested probiotics (yoghurt and fermented kidney beans) as potential role to providing good quality of life in case of induced intra-peritoneal cancer in female mice.

To achieve the objective, the present work was included the microscopical examination of cancer section of carcinogenic mice in comparison with healthy mice.

Material and Methods

Animals groups and Experimental design

This work was carried out on (44) apparently healthy Swiss female albino mice, with a body weight (25) gm ± (5) gm. These mice were divided into four groups, (11) mice for each. Each group fed on a diet (25 gm/day), its content was differed from group to another group as the following:

Group (1) (negative control) (healthy group): mice fed on balanced diet according to American Institute of Nutrition-93 [AIN-93] and adjusted by [8].

Group (2) (positive control) (untreated carcinogenic mice): mice fed on balanced diet.

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Group (3) (carcinogenic mice fed on yogurt): mice fed on balanced diet with 50% yogurt (as a probiotics).

Group (4) (carcinogenic mice fed on fermented kidney been): mice fed on balanced diet with 50% fermented kidney been (as a probiotics) (which fermented with Lactobacillus spp. And Streptococcus spp).

At ten days from the beginning of the experiment, groups (2, 3 and 4) will inject Intra Peritoneum (IP) by 2 × 106 cells (as single dose) from Ehrlith Ascites tumor cells resistant to endoxan. From National Cancer Institute (NCI), Cairo University. The tumor cells present in the abdomen of female Swiss albino mice (infected mice).

After 6 weeks the mice were slaughtered to obtain the samples (small and large intestine) for the histopathological examination. The samples were gently washed in saline and fixed in 10% neutral buffered formalin and Bouin's solution. The specimens were dehydrated in ascending grade of alcohol, clearing in xylene and imbedded in paraffin wax. 3 – 5 µm thick, sections were obtained and subjected to haematoxylin and eosin as general histological stain according to [9,10].

Preparation of yoghurt

Yoghurt was prepared as follows according to [11].

The fresh bufflo's milk was heated at 90°C for 10 min.

Boiled milk was allowed to stand until its temperature reached to 43°C.

Milk was inculated by using the organisms Streptococcus thermophilus and Lactobacillus delbrueckii subsp. (0.04%).

Inculated warm milk samples were put into 100ml plastic containers.

Samples were incubated (incubator ILL.6160, made in China) at 43°C for 3 hrs (pH 4.6 ± 0.1).

Fermented milk (yoghurt) was kept at refrigerator for 3 hrs at 4.5 ± 0.5°C and then used fresh.

Preparation of fermented kidney beans

Fermented kidney beans were prepared as follows according to [11].

Precleaned crude kidney beans presented from local market in Ismailia were cleaned from any foreigen materials and washed using tap water.

Cleaned kidney beans were soaked for 8 hours using tap water, on which the beans were washed and resoaked again at new tap water each hour.

Soaked kidney beans were heated at 100°C for 1/2 hr.

After heat treatment kidney beans were grinded by home mixture (National Quicki mini Blender, made in China) to produce the selary.

Selary of kidney beans were allowed to stand until its temperature reached 43°C.

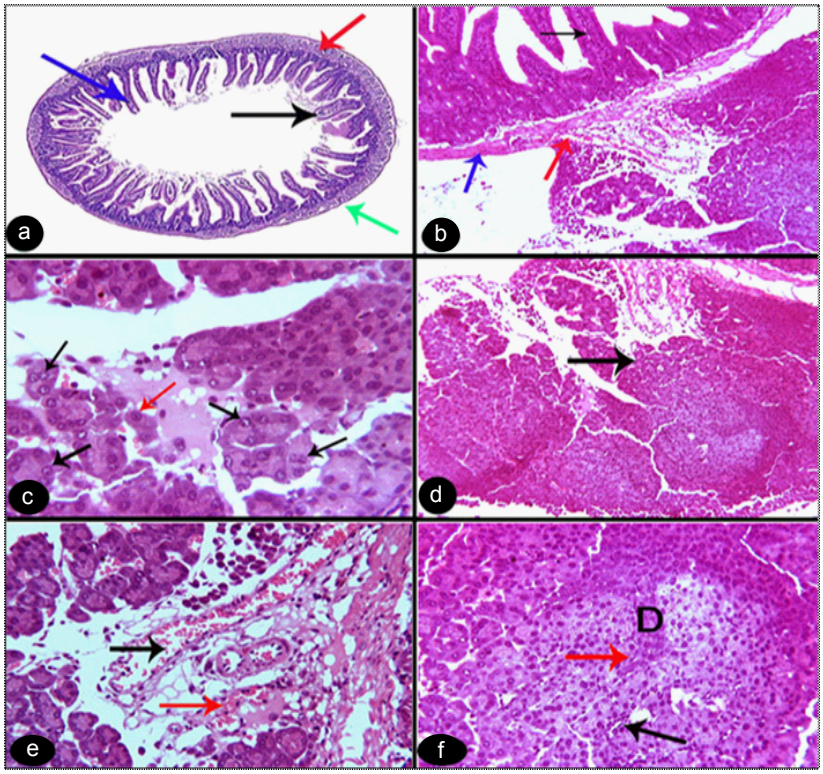

Figure 1: (a) a photomicrograph of group (1) showing normal histological structure of the jejunum, intestinal villi (black arrow), lamina propria (blue arrow), crypt of Lieberkühn (red arrow) and tunica muscularis (green arrow). Stain: H&E. Mag. X 4. (b): a photomicrograph of a section in the intestine group (2) showing intestinal villi (black arrow), tunica muscularis (red arrow) and blood vessels (blue arrow). Stain: H&E. Mag. X 10. (c): a photomicrograph of group (2) showing cells with hyperchromatic nuclei with typical (red arrow) and atypical (black arrow) mitotic figures. Stain: H&E. Mag. X 40. (d): a photomicrograph of group (2) showing cells with different shape and size, mostly ovoid to polyhedral in shape (arrow). Stain: H&E. Mag. X 10. (e): a photomicrograph of group (2) showing congested blood vessels (black arrow) and focal hemorrhage in the stroma (red arrow). Stain: H&E. Mag. X 25. (f): a photomicrograph of group (2) showing the center of the neoplastic mass with some degenerated and necrotic cells (D), some cells with pycknotic (black arrow) or karryolytic (red arrow) nuclei. Stain: H&E. Mag. X 25.

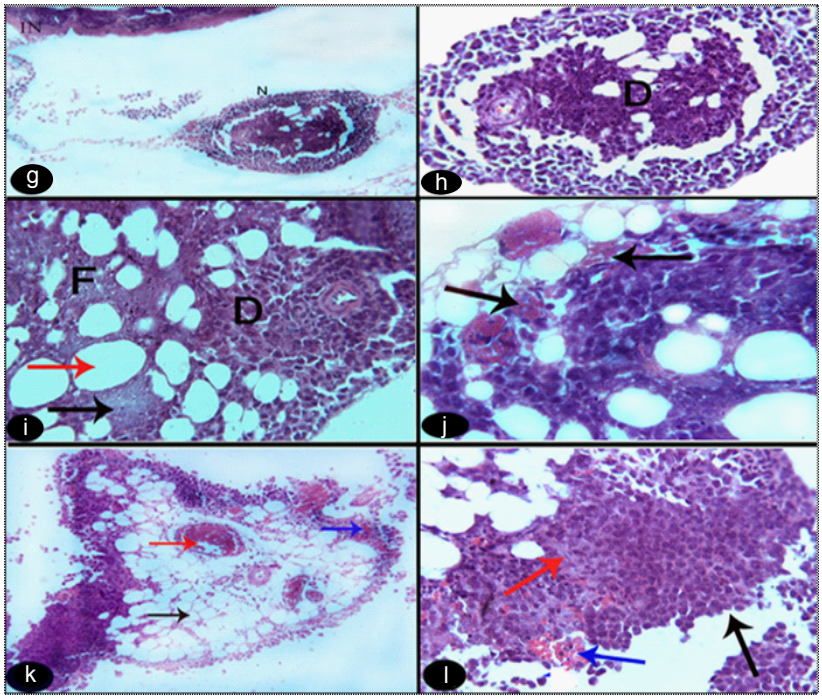

Figure 2: (a) a photomicrograph of group (3) showing neoplastic mass (N) away from the intestinal wall (IN). Stain: H&E. Mag. X 10. (b): a photomicrograph of group (3) showing moderate cell proliferation, localized size with signs of degeneration and necrosis (D). Stain: H&E. Mag. X 25. (c): a photomicrograph of group (3) showing some cells lacked their nuclei (black arrow). Focal (F) to diffuse (D) areas of necrosis were observed and the necrotic cells replaced by empty cavities (red arrow). stain: H&E. Mag. X 25. (d): a photomicrograph of group (3) showing focal hemorrhage (black arrow). Stain: H&E. Mag. X 40. (e): a photomicrograph of group (4) showing empty cavities (black arrow), congested blood vessels (red arrow) and focal hemorrhage (blue arrow). Stain: H&E. Mag. X 10. (f): a photomicrograph of group (4) showing degenerated cells (black arrow), Small focal areas of necrosis (red arrow) and focal hemorrhage (blue arrow). Stain: H&E. Mag. X 25.

Selary were inculated by the using organism's streptococcus thermophilus and lactobacillus delbrueckii subsp. (0.04%).

Inculated samples were put into 100ml plastic containers.

Samples were incubated (incubator ILL.6160, made in China) at 43°C for 6 hrs (pH 6 ± 0.1).

Fermented kidney beans were kept at refrigerator at 4.5 ± 0.5°C for 3 hrs and then used fresh.

Results

The results obtained from these studies revealed that:

Group (1) normal control-healthy group

The negative control group showed the normal histological structure of the intestine as intestinal villi, lamina propria contain the crypt of Lieberkühn, tunica muscularis and tunica adventitia (Figure 1a).

In the other three carcinogenic groups and after the intra peritoneal injection of Ehrlith Ascites tumor cells it will develop a tumor on the tunica adventitia of the small or large intestine as described.

Group (2) positive control-untreated carcinogenic mice

The positive control group showed neoplastic mass this mass showed direct adhesion to the tunica adventitia of the intestine and there were direct communication between the blood supply of the tunica muscularis of the intestine and the neoplastic mass (Figure 1b). The neoplastic mass consisted of numerous tumor cells that exhibited excessive proliferation, large size and with signs of malignancy. The cells contained hyperchromatic nuclei with typical and a typical mitotic figures (Figure 1c). The cells were varied in shape and size and mostly ovoid to polyhedral in shape (Figure 1d).

The neoplastic mass showed congested blood vessels and focal hemorrhage in the stroma (Figure 1e). The center of the neoplastic mass contained some degenerated and necrotic cells. The cells were swollen, vacuolated with weak staining affinity together with pycknotic or karryolytic nuclei (Figure 1f).

Group (3) carcinogenic mice fed on yogurt

The carcinogenic mice fed on balanced diet with 50% yogurt showed a neoplastic mass located away from the intestinal wall (Figure 2a) consisted of a comparatively lower number of tumor cells, that exhibited moderate proliferation with signs of degeneration and necrosis (Figure 2b). The neoplasic mass was consisted of numerous pleomorphic neoplasitic cells with a considerable number of tumor cells. Some cells showed enlarged and hyperchromatic nuclei while others lacked their nuclei or necrosed (Figure 2c). Focal to diffuse areas of necrosis were observed between the neoplasitic cells. The necrotic cells were replaced by empty cavities (Figure 2d). Some of the blood vessels were congested and focal hemorrhage was evident (Figure 2e).

Group (4) carcinogenic mice fed on fermented kidney been

The neoplasitic mass of group (4) lacked the characteristic architecture and appeared much smaller size than that in yogurt group. Some necrotic neoplastic cells replaced by empty cavities with congested blood vessels, focal hemorrhage and others showed pycknotic or

karryolytic nuclei (Figure 2f). The majorities of the neoplastic cells were degenerated and lost their nuclei. Small focal areas of necrosis were observed between the neoplastic cells. Also, wide-spread necrosis and focal hemorrhage were detected (Figure 2f). Some necrotic neoplastic cells replaced by empty cavities with congested blood vessels, focal hemorrhage, the others showed pycknotic or karryolytic nuclei.

Discussion

The result obtained from this study revealed that, the benefit effect of the probiotics on the induced cancer, in which the probiotics not cure the case but improve it these results agree with [7].

In group (3) the effect of the yogurt on the neoplastic mass was clear by lowering the number and size of the tumor cells, with signs of degeneration and necrosis. Focal to diffuse areas of necrosis were also observed between the neoplasitic cells. The necrotic cells were replaced by empty cavities. All these signs indicated that the using of yogurt before the infection by the tumor cells will help in the progress of the case.

But in group (4) the effect of the fermented kidney been was more pronounced than that of the yogurt, in which the neoplasitic mass lacked the characteristic architecture and appeared of much smaller size than those in yogurt group, and the majority of the neoplastic cells were degenerated and lost their nuclei.

The precise mechanisms by which the probiotics do this action on the cancer are unknown, but the authors suggested that such mechanisms may be due to one of the following theories:

Alteration of the metabolic activities of the intestinal microflora, binding and degrading potential carcinogens, quantitative or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogens and promoters (eg: bile acids metabolizing bacteria) or production of antitumourigenic or antimutagenic compounds [12]. But [13-15] suggest that, the anti-carcinogenic effect of the probiotics may be the result of direct removal of procarcinogens, or activation of the body's immune system. Yogurt exhibits antitumor activity through its immunomodulatory activity, by reducing the inflammatory immune response, which was increased when carcinogen was administered, T lymphocytes may a key role in preventive of colon cancer [16,17].

The antitumorigenic influence of the probiotics may involved in modifying gut PH and increasing the net production of the short chain fatty acid (SCFA) (mainly acetate, probionate and butyrate) which kill the colorectal carcinoma cells [6,14].

The probiotics antagonizing the pathogens through production of anti-microbial and antibacterial compounds such as bacteriocins, cytokines and butyrate, or competing with the pathogens for its available nutrients, receptors, and growth factors. [14].

Yogurt decrease the risk of the induced cancer via apoptosis and moderate cell proliferation, (influence the balance between mitosis and apoptosis) [18,19].

Bacterial enzymes: beta-glucosidase, beta-glucuronidase and urease may contribute to the development of the cancer by generating carcinogens. Areduction of activity of these enzymes by certain lactic acid bacteria (which present in the probiotics) is considered to be beneficial [15].

So from the aforementioned results we concluded that, the use of the probiotics will improve the healthy condition of the body to resist the infection with the tumor cells.

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