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Lactobacillus kefiri Suppresses Inflammatory Cytokine Expression and Induces Apoptosis in Breast Cancerous Cells in Rat Model

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ABSTRACT

Background: *Lactobacillus kefiri* (*L. kefiri*), found in kefir, is noted for its health benefits, including potential breast cancer prevention.

Objectives: This study aimed to assess the apoptotic and therapeutic effects of *L. kefiri* on breast carcinoma in rats.

Methods: Five groups of 10 rats each were used: Group 1 (negative control) had no tumors, group 2 where rats in this group were injected subcutaneously with a single dose of 50 mg/kg b.w. of DMBA (in 2 ml of corn oil) into the mammary gland, allowed to develop tumors over a period of 120 days. In the third group (*L. kefiri* oral "Group 3"), tumor-bearing rats received 0.4 ml of *L. kefiri* orally, six times a week for 5 weeks. Lactobacillus injection (Group 4) where tumor-bearing rats received 0.1 ml of *L. kefiri* solution via intraperitoneal injection twice a week for 5 weeks. Finally, the L. *kefiri* mix group (Group 5) where tumor-bearing rats received *L. kefiri* solution through duo ways: orally with 0.4 ml six times per week and intraperitoneally with 0.1 ml per tumor twice a week for five weeks.

Results: DMBA reduced TNFR1, cytochrome c, TRADD, and Bax levels, while increasing Bcl-2 level. *L. kefiri* reversed these effects, promoting apoptosis in cancer cells. It also improved liver and kidney function markers, reduced oxidative stress and enhanced immunity as shown by better antioxidant levels and lower malondialdehyde.

Conclusion: *L. kefiri* showed promise as a natural agent against breast cancer, warranting further research to determine its mechanisms and optimal application for human health.

Keywords*:* *Lactobacillus kefiri*, Apoptosis, Breast cancer, Cytokines, Antioxidant defense mechanism.

INTRODUCTION

In 2018, cancer was the second leading cause of death worldwide, with breast cancer being the deadliest form among women, irrespective of their country's development status [(1)]. Originating from mammary cells, untreated breast cancer can metastasize, making early detection crucial for effective treatment options like radiotherapy or chemotherapy [(2)].

The disease poses significant challenges due to its potential for early metastasis, aggressive spread, therapy resistance, and high mortality rates [(3)].

Apoptosis, essential for cellular balance, is crucial to understand. Dysregulation can lead to abnormal growth and mutations. Managing apoptosis is key in cancer treatment [(4)]. Effective late-stage cancer treatments are scarce, and pain management is vital as it affects survival. Targeting metastatic cells without damaging healthy tissue remains a challenge. [(5)]. Traditional cancer therapies, such as chemotherapy, radiotherapy, and surgery, can significantly impact the patient's quality of life due to their harsh effects. By 2040, therapies should not only improve outcomes but also reduce toxicity [(6)]. Affordable prevention strategies are essential for public health, with probiotics showing potential benefits in the early 21st century [(7)].

Probiotics, beneficial bacteria and yeasts, support digestive health and overall well-being. They help balance gut flora, with some strains showing potential in preventing breast cancer [(8)]. Probiotics can modulate the immune system, influence anti-inflammatory cytokine production, and enhance the body's ability to target cancer cells. Studies suggest that probiotics, especially when used with radiation therapy, may improve the immune response against cancer [(9)].

Kefir, a fermented milk drink rich in probiotics, is made with kefir grains containing beneficial bacteria and fungi. It's linked to health benefits such as reducing inflammation, cancer risk, cholesterol levels, and supporting digestion, gut, and cardiovascular health [(8)].

Lactobacillus species, predominant in kefir grains, play a key role in these effects. Kefir contributes to cancer prevention by inducing apoptosis, enhancing immunity, modifying gut microbiota, reducing DNA damage and tumor growth, and inhibiting carcinogens. Studies confirm kefir's protective impact on various cancers, including leukemia, breast, gastrointestinal, and sarcoma [(10)]. Lactic acid bacteria (LAB), used in probiotics for over a century, are effective in health improvement and disease treatment [(11)].

LABs ferment carbohydrates, producing lactic acid, which was advocated by **Metchnikoff** [(11)] for longevity and health. Probiotics, particularly lactobacilli, are known to balance gut flora and have been effective against pouchitis [(12)]. Kefir, a LAB fermented milk, has demonstrated antioxidant, antimicrobial, anti-

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inflammatory, and wound-healing properties, and has also been shown to benefit osteoporosis in rat models [13].

A doubtlessly beneficial probiotic product referred to as probiotics fermentation technology (PFT) emerges as a singular kefir grain-primarily based formulation. PFT predominantly comprises LAB lines, with about ninety percent *Lactobacillus kefiri* P-IF, in conjunction with 2–3% of any other *L. Kefiri* compound and 3 yeast traces. In vitro research has already tested PFT's anticancer outcomes towards multidrug-resistant (MDR) human myeloid leukemia cells (HL60/AR) and human gastric cancer cells [14].

This evidence aligns with different research displaying that Lactobacillus strains exhibit in vitro outcomes in opposition to bladder and gastric most cancers, as well as inhibitory consequences in animals with breast, intestinal, colon, and oral cancer, and in people with colon, liver, and breast cancer [15]. Lactic acid bacteria have tested anticancer effects through diverse pathways, including inhibiting capacity pathogens and intestine carcinogenesis via binding to and degrading cancer-causing agents.

Additionally, LAB enhances antioxidant activities, produces anti-tumorigenic or anti-mutagenic compounds, and boosts the host's immune response. Probiotics have confirmed the capacity to trigger apoptosis in numerous cancer mobile lines. In the present study, our aim was to clarify the apoptotic influence of *L. Kefiri* on breast cancer cells using an in vivo model and to demonstrate the underlying mechanisms of its action [16].

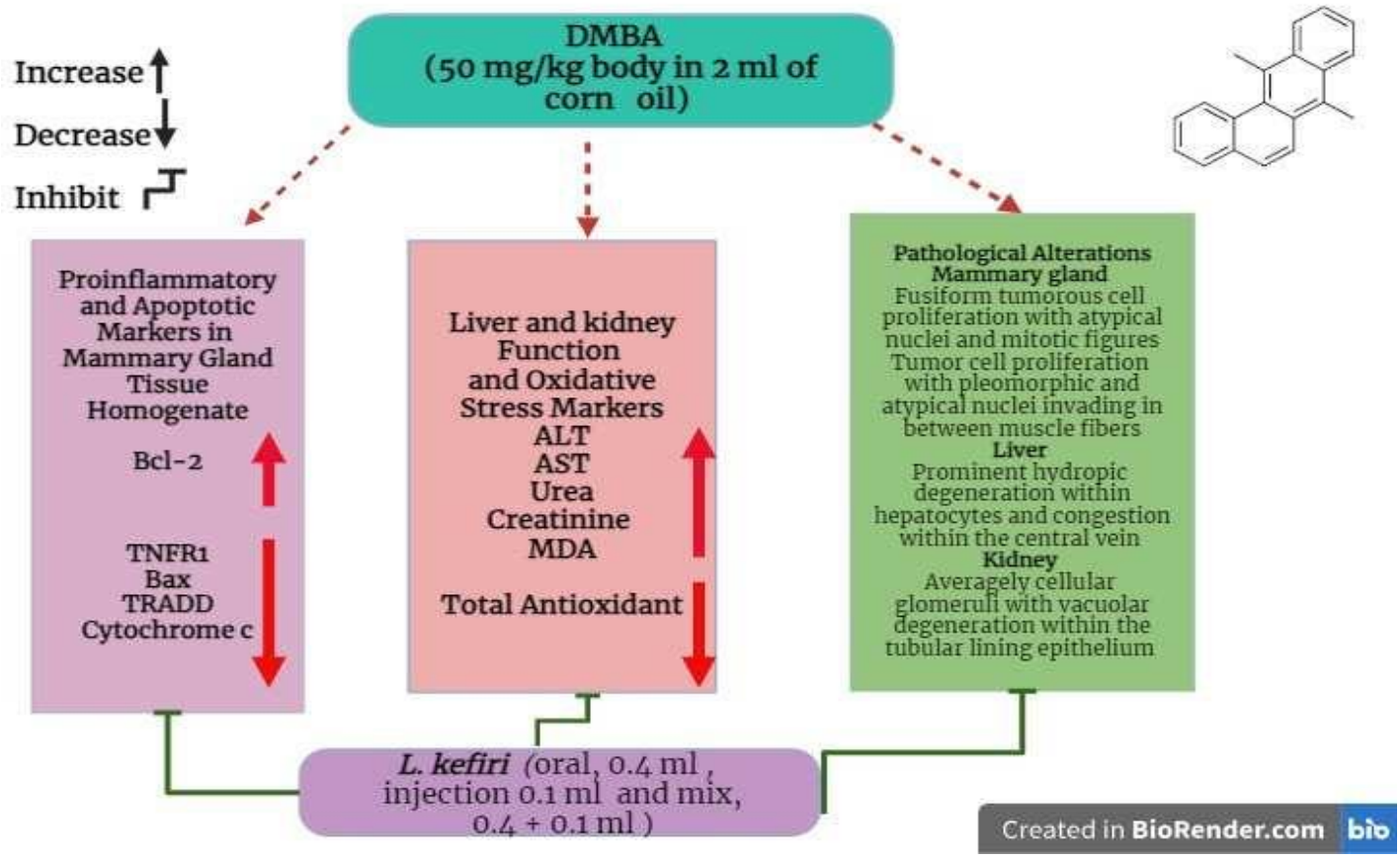

Figure (1): The suppressive effects of *L. kefiri* on DMBA-induced carcinogenesis

MATERIALS AND METHODS

Experimental animals:

The study utilized female Sprague-Dawley rats weighing 120–150 g obtained from the animal house at the National Research Centre in Dokki Giza Egypt. Prior to the experiment the rats were housed in plastic cages for one week to acclimatize to their surroundings with a 12-hour light-dark cycle. During the study the rats had free access to purified water and commercial food. Environmental conditions like temperature, humidity and light levels were closely monitored and maintained.

Tumor induction: After undergoing a period of adaptation lasting one week, the rats were administered DMBA acquired from the esteemed Sigma Chemicals Company (USA). Subsequently, a solitary dosage of 50 mg/kg body weight in 2 ml of corn oil was injected subcutaneously into the mammary gland. The rats that were in good health possessed an average weight ranging from 120 to 150 g. Following this injection, the rats were closely observed for duration of 120 days to assess the onset of tumor growth [17].

***L. kefiri* preparation:** A solution of *Lactobacillus kefiri* was formulated through the dissolution of 30 grams of *Lactobacillus kefiri* in 200 milliliters of distilled water.

Experimental protocol and drug administration:

Five different groups (10 rats for each) were used to assess the effects of *L.kefiri* on tumor growth and development in the rats **(Figure 1)**.

1. **Group 1** (negative control)**:** Normal rats without tumors.
2. **Group 2** (positive control)**:** Rat injected subcutaneously with a single dose of 50 mg/kg b.w. of DMBA in 2 ml of corn oil in the mammary gland [20] and allowed to develop tumors for 120 days.
3. **Group 3** (*L. kefiri* oral)**:** Tumor-bearing rats received 0.4 ml of *L. kefiri* orally six times a week for 5 weeks.
4. **Group 4** (*L. kefiri* injection)**:** Tumor-bearing rats received 0.1 ml/ tumor of *L. kefiri* solution through intraperitoneal injection twice a week for 5 weeks [18].
5. **Group 5** (*L. kefiri* mix)**:** Tumor-bearing rats received *L. kefiri* solution by dual routes: orally with 0.4 ml six times per week and intraperitoneally with 0.1 ml/ tumor twice a week for 5 weeks.

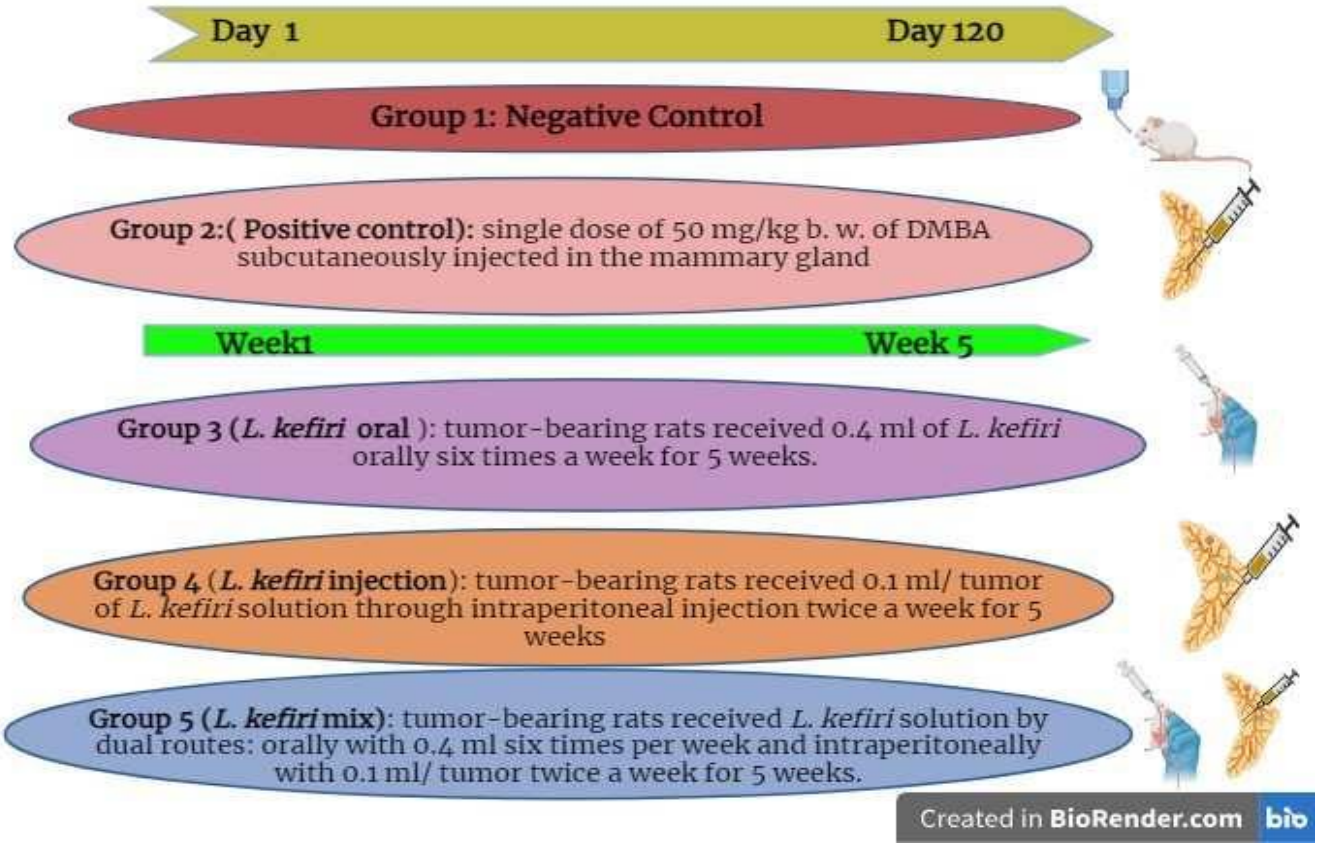

Figure (2): Illustrative diagram depicting the arrangement of animals and the experimental methodology.

Sampling: Blood samples were collected from rats in two phases of the experiment. In the first phase, rats in groups 1 and 2 were euthanized after the induction period. Blood samples were taken from their retro-orbital plexus under diethyl ether anesthesia and placed in clean tubes. This phase included untreated rats with tumors and normal rats without tumors. In the second phase, rats in groups 3, 4, and 5 were euthanized after five weeks of treatment. Blood samples were taken from their retro-orbital plexus under the same anesthesia and placed in clean tubes. This phase included treated rats with tumors. The blood samples were allowed to clot and then cool-centrifuged at 3000 rpm for 10 min using a Hettich centrifuge (Newtown, Connecticut, USA). The sera obtained after centrifugation were stored at −80 °C till used.

Preparation of tissue homogenates: The procedure for preparing tissue homogenates involved sacrificing rats with and without tumor treatment, cutting and weighing breast tissue samples, rinsing them with ice-cold phosphate-buffered saline, mincing and homogenizing them in phosphate-buffered saline using a SONICS homogenizer, and then centrifuging the homogenates at 3000 rpm for 10 minutes. The supernatants were stored at -80 °C for analysis.

Biochemical analyses: Spectrophotometrically (MY 1345003 spectrophotometer, China) and using kits purchased from Reactivos GPL (Barcelona, España), serum alanine aminotransferase and aspartate aminotransferase (ALT and AST) activities were estimated as described by Reitman and Frankel's [19] method. Urea and creatinine levels were estimated according to the methods of Patton and Crouch [20] and **Bowers and Wong** [21], respectively.

Total antioxidants and lipid peroxidation/malondialdehyde (LPO/MDA) levels were measured using Elabscience (Biochemical Assay Kit) according to **Smith *et al.*** [22] and **Ohkawa *et al.*** [23], respectively. Breast tissue homogenate (tumor with or without treatment) were analysed for levels of cytochrome c (Cyt c), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), tumor necrosis factor receptor 1 (TNFR1), B-cell leukemia/lymphoma 2 (Bcl-2) and BCL 2-associated X protein (Bax) were measured using ELISA technique (UV-2401; Shimadzu, Japan) and rats ELISA reagent kits (SinoGeneClon Biotech Co. Ltd, China).

Histopathological preparation:

The experiment ended with the animals being euthanized. Samples of mammary gland tissue, liver, and kidney were taken and fixed in 10% buffered formalin for two days. The tissues were then processed, cleared and embedded in paraffin. Each paraffin block was cut into 5 µm thick sections and stained with hematoxylin and eosin for histopathological analysis [24].

An expert observer blinded to the sample identity performed the histopathologic evaluation to prevent bias. A CCD digital camera (Olympus SC100) that was connected to the microscope captured digital images of the sections at different magnifications.

Ethical approval:

All animal procedures were conducted in accordance with guidelines from the National Health and Medical Research Council and approved by the Institutional Animal Ethics Committee of the National Research Centre in Giza Egypt (No. 19-204).

Statistical analysis

Using one-way analysis of variance with a paired sample test method and post-hoc analysis, statistical comparisons were performed. *P ≤ 0.05, **P ≤ 0.01 and***P ≤ 0.001.

RESULTS

L. kefiri enhancing apoptotic signaling pathways.

The impact of TNFR1 and cytochrome c

The experimental work showed a significant downregulation of TNFR1 and cytochrome c levels in tumor-bearing animals (DMBA) as compared to the normal/negative control group. In contrast, *L. kefiri* showed a major recovery, presenting high TNFR1 and cytochrome c levels (P ≤ 0.05. 0.01& 0.001) relative to the tumor-bearing animals (Table 1).

Table (1): The Effect of *L. kefiri* on cytochrome c and TNFR1 levels in breast tissue homogenates of normal and cancer- bearing animals.

Parameters **Groups**	**TNFR1** (ng/ml)	**Significance relative to normal**	**Significance relative to tumor**	**Cytochrome c** (pg/ml)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	181.0±5.5	----	###	4.28± 0.3	----	###
G2 (tumor) Positive control	170.6±6.4	***	------	2.56±0.2 •	***	------
G3, *L.kefiri* oral	288.8±6.2	***	###	3.38±0.2	NS	#
G4 *L.kefiri* injection	370.8±3.8	***	###	7.74±0.4	***	###
G5, *L.kefiri* mix (oral& injection)	333.0±5.3	***	###	5.31±0.5	NS	##

Data were presented as mean ± SEM (n=8). Significance relative to normal: *P ≤ 0.05, **P ≤ 0.01 and***P ≤ 0.001; significance relative to tumor: # P ≤ 0.05, ##P ≤ 0.01 and ###P ≤ 0.001 and NS: P ≥ 0.05

The impact of TRADD, Bcl-2 and Bax: In the present research, tumor-bearing animals showed a monumental rise in TRADD and Bax levels concomitantly with a highly remarkable reduction (P ≤ 0.05, 0.01& 0.001) in Bcl-2 levels compared to the negative control group. Of particular note, the *L. kefiri* treatment led to reduction of TRADD and Bax levels with a considerable increase in Bcl-2 expression (P ≤ 0.05. 0.01& 0.001) as compared to that of the DMBA group (Tables 2 & 3). It was outstanding to find that group injected with *L. kefiri* was the most competent of the other *L. kefiri* treatments (oral and mix).

Table (2): The Effect of *L. kefiri* on TRADD level in breast tissue homogenates of normal and cancer- bearing animals

Parameters Groups	**TRADD** (Pg/mL)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	619.05± 6.02	----	#
G2 (tumor) Positive control	347.0± 5.9	*	------
G3, *L.kefiri* oral	589.8± 7.3	NS	#
G4, *L.kefiri* injection	847.9± 5.2	NS	###
G5, *L.kefiri* mix (oral& injection)	700.4±4.2	NS	###

Significance relative to normal: *P ≤ 0.05, **P ≤ 0.01 and***P ≤ 0.001; significance relative to tumor: # P ≤ 0.05, ##P ≤ 0.01 and ###P ≤ 0.001 and NS: P ≥ 0.05.

Table (3): The Effect of *L. kefiri* on Bcl-2 and Bax levels in breast tissue homogenates of normal and cancer- bearing animals

Parameters **Groups**	**Bcl-2** (ng/g)	**Significance relative to normal**	**Significance relative to tumor**	**Bax** (Pg/mL)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	1.36±0.08	----	###	247.6±3.5	----	NS
G2 (tumor) Positive control	4.65±0. 3	***	------	134.8±4.9	***	------
G3 *L.kefiri* oral	0.834±0.2	NS	###	256.0±7.2	NS	###
G4 *L.kefiri* injection	0.850±0.2	*	###	684.0±9.4	***	###
G5 *L.kefiri* mix (oral& injection)	1.36±0.03	*	###	460.0±3.7	***	###

Data are presented as mean± SEM (n=8).Significance relative to normal: *P ≤ 0.05, **P ≤ 0.01 and***P ≤ 0.001; significance relative to tumor: # P ≤ 0.05, ##P ≤ 0.01 and ###P ≤ 0.001 and NS: P ≥ 0.05

***L. kefiri* enhancing liver and kidney function:** Serum ALT and AST enzyme activities as well as levels of urea and creatinine in tumor-bearing animals were reported to be significantly higher ($P \leq 0.05$. 0.01& 0.001) than in the normal control group. The liver and kidney functions were improved in *L.kefiri*-treated groups by normalizing both of the ALT and AST enzyme activities or the urea and creatinine levels ($P \leq 0.05$. 0.01& 0.001) and contrasted with DMBA group (Tables 4 & 5).

Table (4): The Effect of *L. kefiri* on serum ALT and AST activities in normal and cancer- bearing animals

Parameters **Groups**	**ALT** (U/L)	**Significance relative to normal**	**Significance relative to tumor**	**AST** (U/L)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	266.7±5.2	----	###	282.3± 2.9	----------	###
G2 (tumor) Positive control	451.3±5.5	***	------	414.9± 2.3	***	----------
G3 *L.kefiri* oral	298.4±4.1	NS	###	303.6± 2.2	NS	###
G4 *L.kefiri* injection	243.0±4.2	NS	###	278.4± 2.3	NS	###
G5 *L.kefiri* mix (oral& injection)	243.8±4.5	NS	###	279.2± 3.5	NS	###

Data are presented as mean± SEM (n=8). Significance relative to normal: *$P \leq 0.05$, **$P \leq 0.01$ and***$P \leq 0.001$; significance relative to tumor: # $P \leq 0.05$, ##$P \leq 0.01$ and ###$P \leq 0.001$ and NS: $P \geq 0.05$

Table (5): The Effect of *L. kefiri* on serum urea and creatinine levels in normal and cancer- bearing animals

Parameters **Groups**	**Urea** (mg/dl)	**Significance relative to normal**	**Significance relative to tumor**	**Creatinine** (mg/dl)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	50.0± 0.3	----	###	0.520± 0.02	----------	#
G2 (tumor) Positive control	75.8± 2.1	***	------	0.920±0.04	***	----------
G3 *L.kefiri* oral	64.8± 1.2	***	###	0.550±0.01	NS	###
G4 *L.kefiri* injection	52.6± 0.9	NS	###	0.424±0.03	*	###
G5 *L.kefiri* mix (oral& injection)	55.8± 1.2	*	###	0.476±0.02	NS	###

Data are presented as mean± SEM (n=8). Significance relative to normal: *$P \leq 0.05$, **$P \leq 0.01$ and***$P \leq 0.001$; significance relative to tumor: # $P \leq 0.05$, ##$P \leq 0.01$ and ###$P \leq 0.001$ and NS: $P \geq 0.05$.

***L. kefiri* Empowering antioxidant protection:** In table (6), tumor-bearing animals caused marked increase in oxidative stress ($P \leq 0.05$, 0.01 and 0.001). To elucidate this rise, MDA levels increased, and the total antioxidant capacity decreased compared to the normal control group. However, *L. kefiri*-treated animals showed a surprising recovery of MDA and total antioxidants levels. Additionally, *L. kefiri* not only improved the immune system but also effectively suppressed the oxidative stress compared to DMBA group.

Table (6): The Effect of *L. kefiri* on MDA and total antioxidant levels in breast tissue homogenates of normal and cancer-bearing animals

Parameters **Groups**	**MDA** (nmol/g)	**Significance relative to normal**	**Significance relative to tumor**	**Total antioxidant** (U/mL)	**Significance relative to normal**	**Significance relative to tumor**
G1 (normal) Negative control	5.32± 0.3	----	###	13.50± 0.6	----------	##
G2 (tumor) Positive control	15.35±0.3	***	------	7.24± 0.5	***	----------
G3 *L.kefiri* oral	11.20±0.4	NS	###	11.9± 0.7	NS	###
G4 *L.kefiri* injection	5.50± 0.2	**	###	14.02± 0.2	**	###
G5 *L.kefiri* mix (oral& injection)	10.38± 0.5	*	###	12.48± 0.6	NS	###

Data are presented as mean± SEM (n=8).Significance relative to normal: *P ≤ 0.05, **P ≤ 0.01 and***P ≤ 0.001; significance relative to tumor: # P ≤ 0.05, ##P ≤ 0.01 and ###P ≤ 0.001 and NS: P ≥ 0.05.

Histopathological alterations: Mammary gland tissue of control female rats **(Figure 3 A)** revealed a benign mammary duct lined by benign double epithelial and myoepithelial cell layers, against mature fat cells. DMBA-injected rats **(Figures 3 B** and **D)** showed fusiform tumorous cell proliferation with atypical nuclei and mitotic figures. Tumor cell proliferation with pleomorphic and atypical nuclei **(Figures 3 C, E** and **F)** invading in between muscle fibers.

Lactobacillus-injected rat's revealed benign, regular mammary duct against mature fat cells, a picture approximating control **(Figure 3 G)** and no tumor was found. Oral *Lactobacillus* rats **(Figure 3 H & I)** revealed residual tumor fusiform cell proliferation infiltrating in between muscle bundles with atypical and bizarre nuclei showing prominent nucleoli. Figure (3 **J-L),** DMBA-injected rats treated with combined oral and injected *Lactobacillus*, showed scattered sporadic **(J, K)** residual atypical cells amidst ordinary mammary acini. Residual atypical fusiform cell **(Figure 3 L)** proliferation in patches studded by inflammatory cells.

Liver tissue sections of control rats showed ordinary liver cells that were polyhedral with centrally rounded nuclei and abundant cytoplasm. Liver cells were disposed in plates radiating from the central vein **(Figures 4 A** and **B)**. DMBA-injected rats showed prominent hydropic degeneration within hepatocytes and congestion within the central vein **(Figure 4 C)**. Oral Lactobacillus rats showed congestion within the sinusoids and residual minimal hydropic degeneration within liver cells **(Figure 4 D)**. Rats treated with combined oral and injection *Lactobacillus* showed a dilated, congested central vein and minimal residual hydropic degeneration within hepatocytes **(Figure 4 E)**. Animals treated with injected *Lactobacillus* showed regular polyhedral hepatocytes with ample cytoplasm and centrally rounded regular nuclei, picture approximating control **(Figure 4 F)**.

Kidney tissue sections of the control rat showed averagely cellular glomeruli surrounded by renal tubules lined by ordinary low cuboidal epithelium **(Figure 5 A)**.

Kidney tissue sections of DMBA-injected rats showed averagely cellular glomeruli with vacuolar degeneration within the tubular lining epithelium **(Figures 5 B** and **C)**. DMBA-injected rats treated with oral *Lactobacillu*s showed ordinary, averagely cellular glomeruli with congestion within renal interstitium and surrounding ordinary tubules, picture approximating control **(Figure 5 D)**.

Rats treated with injected *Lactobacillus* showed averagely sized cellular glomeruli and ordinary surrounding tubules lined by low cuboidal epithelium; picture approximating control **(Figure 5 F)**. Combined oral and injection *Lactobacillus* rats showed averagely cellular glomeruli with congestion within renal interstitium and surrounding ordinary tubules; picture approximating control **(Figure 5 G)**.

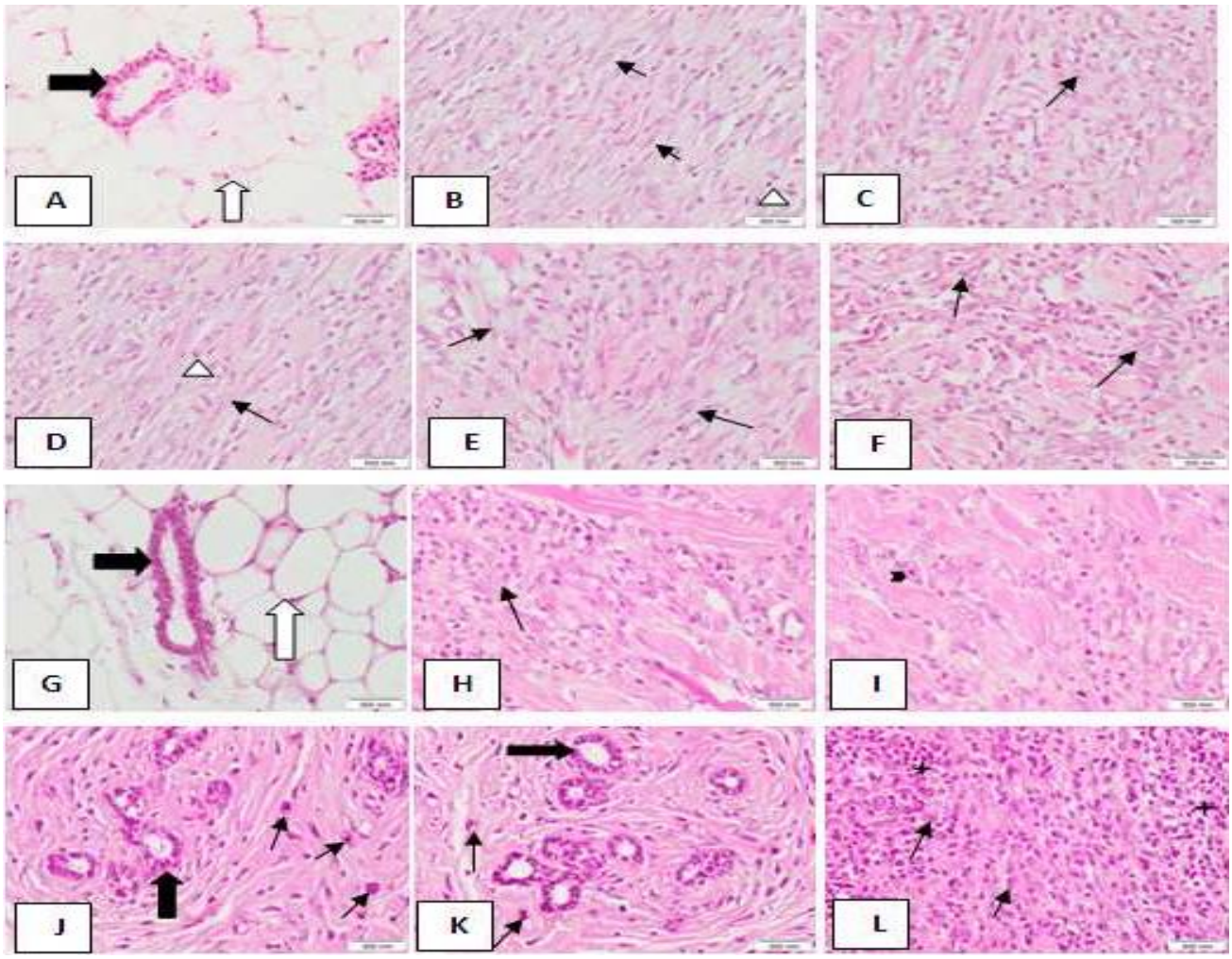

Figure (3): Mammary gland tissue of control female rats (**A)** revealed a benign mammary duct lined by benign double epithelial and myoepithelial cell layers (thick black arrow), against mature fat cells (thick white arrow). (**B** & **D)** DMBA-injected rats showed fusiform tumor cell proliferation with atypical nuclei (thin black arrows) and mitotic figures (white triangles). (**C, E & F)** Tumor cell proliferation with pleomorphic and atypical nuclei invading in between muscle fibers (thin black arrows). (**G)** Rats treated with injected Lactobacillus revealed benign, regular mammary ducts (thick black arrow) against mature fat cells (thick white arrow), a picture approximating control, and no tumor was found. (**H** & **I**) Oral Lactobacillus-treated rats with revealing residual tumor fusiform cell proliferation infiltrating in between muscle bundles (thin black arrows) with atypical and bizarre nuclei showing prominent nucleoli (black arrow head). (**J-L)** Animals treated with combined oral and injected *Lactobacillus* showed (**J** & **K)** scattered sporadic residual atypical cells (thin black arrows) amidst ordinary mammary acini (thick black arrows). **(L)** Residual atypical fusiform cell proliferation in patches (thin black arrows) studded by inflammatory cells (black stars), (H & E x200).

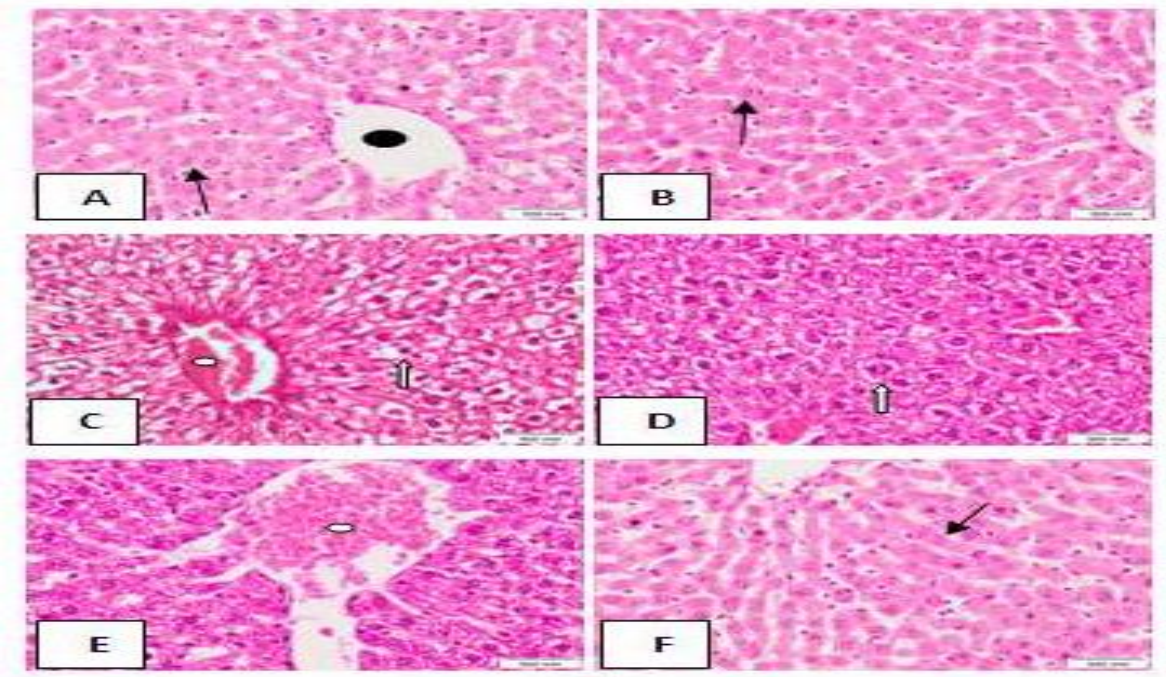

Figure (4): Liver tissue sections of (**A** & **B)** control rats showed ordinary hepatocytes (thin black arrows) disposed in plates, radiating from the central vein (black oval shape). (**C)** DMBA-injected rats showed congested central veins (black oval shapes) and prominent hydropic degeneration within hepatocytes (white arrow). (**D)** Rats treated with oral *Lactobacillus* showed congestion within the sinusoids and minimal residual hydropic degeneration within liver cells (white arrow). (**E)** Combined oral and injected *Lactobacillus*-treated animals showed a dilated, congested central vein (white oval shape) and minimal residual hydropic degeneration within hepatocytes. (**F)** Animals treated with injected *Lactobacillus* showed regular hepatocytes, approximating control (H & E × 200).

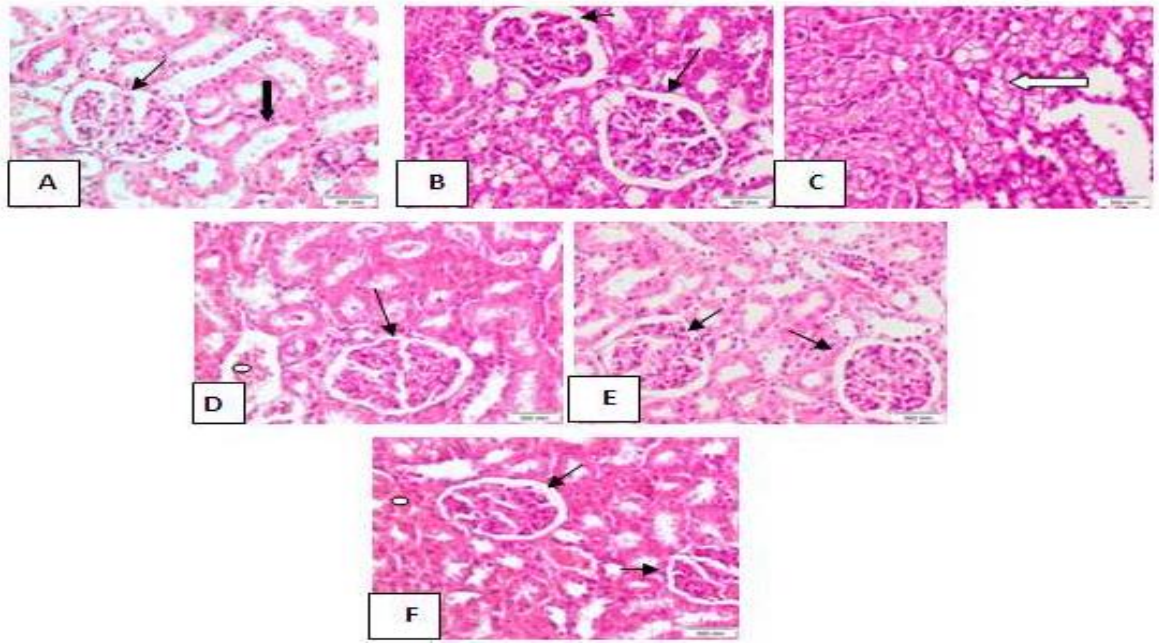

Figure (5): Kidney tissue sections of (**A)** control rat showed an ordinary glomerulus with average cellularity (thin black arrow) surrounded by tubules lined by an ordinary low cuboidal epithelium (thick black arrow). (**B** & **C)** DMBA-injected rats showed **(B)** averagely cellular glomeruli (thin black arrows) and (**C)** vacuolar degeneration within epithelium lining tubules (white arrow). (**D)** Rats treated with oral *Lactobacillus* showed an ordinary glomerulus with average cellularity (thin black arrow) and congestion within the renal interstitium (white oval shape; approximating control). (**E)** *Lactobacillus*-injected rats showed averagely cellular glomeruli (thin black arrows) and surrounding ordinary tubules, approximating control. (**F)** Combined oral and injection of *Lactobacillus*-treated animals showed averagely cellular glomeruli (thin black arrows) and congestion within the renal interstitium (white oval shape); approximating control (H & E × 200).

DISCUSSION

In the present study, the molecular expression level of TNFR1, cytochrome c, TRADD and Bax was shown to be significantly downregulated, while Bcl-2 level was significantly enhanced in the tumor-bearing animals. Likewise, *L. kefiri* was tolerated better, it had apoptotic effect, upregulated TNFR1, cytochrome c, TRADD and Bax and reduced Bcl-2 level. The findings align with others who also showed that the primary etiology of cancer in humans is often ascribed to mutations in the genetic material. Malignancies often exhibit resistance to mitogenic stimuli, an undesirable increase in cell numbers, and the capacity to harm neighboring cells and tissues, which are the most common characteristics. Prior research indicates that only a small percentage of cancer patients, around 5-10%, have genetic mutations as the cause, with external influences responsible for 90-95% of cancer instances [(25)]. Numerous theories propose potential mechanisms for the prevention and management of cancer through the use of probiotics. These mechanisms involve alterations in the composition of the intestinal microbiota, reinforcement of intestinal barrier functions, protection against DNA damage in the gut lining, and the breakdown of potential carcinogens. Furthermore, probiotics have been shown to boost the immune response and regulate inflammation. The control of cancer development and inflammation is facilitated by the anti-inflammatory effects of probiotics, which modulate the release of inflammatory molecules like interleukins, interferons, and cytokines [(26)].

Probiotics has also been revealed to be effective against colorectal and intestinal cancers by some studies while others revealed their effectiveness in oral cancer and breast cancer. Lactic acid bacteria have garnered significant attention due to their immune-modulatory properties, which have been associated with the suppression and regression of carcinogenesis. This effect is believed to be the result of a complex interaction between these microorganisms or their metabolites and the immune and epithelial cells [(14)].

Probiotic strains have the ability to regulate the production of anti-inflammatory cytokines and prostaglandins, thereby reducing the presence of carcinogens in the body. Additionally, certain probiotics can activate phagocytes, leading to the elimination of early-stage cancer cells [(27)].

Previous in vitro investigations demonstrated that the PFT probiotic exerts an anticancer effect on diverse cancer cells through distinct mechanisms. For instance, PFT induces apoptosis in murine metastatic breast cancer (4 T1) cells and myeloid leukemia cells via a hole-piercing mechanism [(16)]. Additionally, it impacts AGS human gastric cancer cells by reducing the polarization of mitochondrial membrane potential (MMP) and Bcl2 expression. Furthermore, PFT induced a modulation of apoptotic regulators, specifically, an upregulation of P53 (tumor suppressor protein) and Bax expression, along with a downregulation of Bcl2 expression. Additionally, the Bax/Bcl2 ratio increased significantly. Notably, there was a substantial decrease in mitochondrial polarization, and caspase-3 expression more than doubled after PFT treatment, suggesting that PFT triggers apoptosis through the mitochondrial-dependent pathway [(15)].

According to **Weir *et al.* [(28)],** the results highlighted the interplay between the immune and endocrine systems; evident through the activation of immune cells (increased IgA (+) cells and decreased Bcl-2 (+) cells) and altered [(29)] cytokine production (increased IL-10 and decreased IL-6). Numerous research studies have demonstrated that lactic acid bacteria (LAB) exhibit antitumor effects through various mechanisms. These mechanisms include binding to mutagens and eliminating carcinogens within the colon [(30)]. Additionally, LAB induces apoptosis in cancer cells and modulates different components of the immune system, including NK (natural killer) cells, dendritic cells, B cells and T cells. The distinct characteristics of the *L. kefiri* P-IF strain potentially contributes to its capacity to trigger apoptosis in the MDR HL60/AR leukemic cell line [(31)]. The composition of bacterial and microorganism cell surfaces comprises diverse polysaccharide-peptide complexes, which exert specific stimulatory effects on host cells, either promoting or inhibiting cell growth [(32)]. Delving deeper into the distinctive cell wall components and properties of *L. kefiri* P-IF could enhance our understanding of how cell surface proteoglycans induce apoptosis. The presence of *L. kefiri* P-IF in the gut could potentially mitigate harmful galactose levels, thereby offering protective benefits [(33)].

Returning to the current study, the histopathological findings from the liver and kidney specimens revealed near normal tissue in the *L.kefiri* oral, injection, and mixed groups. Among these groups, the injected group exhibited the greatest efficacy when compared to the negative control. In tumor-bearing animals, there was a significant increase in oxidative stress. This was evident through elevated malondialdehyde (MDA) levels and a decrease in the total antioxidant capacity as compared to the normal control group. However, animals treated with *L. kefiri* exhibited a remarkable recovery, with restored MDA and total antioxidant levels. Notably, *L. kefiri* not only enhanced the immune system but also effectively suppressed oxidative stress when compared to the DMBA group. In tumor-bearing animals, there was a marked increase in oxidative stress, leading to elevated serum ALT and AST enzyme activities, as well as higher levels of urea and creatinine, compared to the normal control group.

However, animals treated with *L. kefiri* exhibited a surprising recovery. Their liver and kidney functions improved significantly, with normalized ALT and AST enzyme activities, as well as urea and creatinine levels, in contrast to the DMBA group. The current findings are consistent with previous studies that have demonstrated the ability of lactobacillus as a probiotic to elicit biological changes that hinder the development of cancer. Upon exposure to *Lactobacillus*, there is a rise in the synthesis of certain biological components including cytokines, interleukins (IL-2, IL-12), antioxidants (SOD, CAT, GSH), endogenous microbial flora, interferons (IFN-g), and immune cells (TH cells, NK cells), while other elements such as DNA damage, pathogens, inflammation, ulcers, tumor size, cancer-specific proteins, polyamine levels, and procarcinogenic enzymes are reduced [(34)]. The host's primary defense mechanism involves an antioxidative system, as free radicals have been linked to various cellular impairments and subsequent metabolic conditions such as cancer. Through the generation of short-chain fatty acids like butyrate, propionate, acetate, and conjugated linoleic acid, which serve as anti-carcinogenic agents, probiotic microorganisms can inhibit the proliferation of cancer cells and stimulate apoptosis. Elevated short-chain fatty acids concentration within the intestinal lumen prompts the secretion of anti-inflammatory cytokines, suppresses inflammatory pathways, and enhances the antioxidant system. The administration of probiotics hinders the proliferation of pathogenic or detrimental microbes through antagonistic impacts, diminishing the functions of these bacterial enzymes, and averting cancer. Prebiotics have the potential to inhibit carcinogenesis through the alteration of gene expressions [(35)].

CONCLUSION

According to our results, *Lactobacillus kefiri* showed promise as a potential therapeutic intervention that is capable of reducing tumor incidence rates and inducing inhibition of tumor growth. This effect is achieved by triggering apoptosis through the mitochondrial-dependent pathway in breast cancer cells. Furthermore, it not only halts tumor cell proliferation but also enhances the functionality of the immune system.

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