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# Evaluation the skin regeneration by using Kefir production in local dogs

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**Abstract:**

The aim of this study was to investigate the effect of kefir productions on infected wound healing. Twenty mature dogs were used . The animals were divided into two equal groups (control and treated groups). The animals were anesthetized by ketamine and xylazine as general anesthesia. In control group the dogs were anesthetized and preparation the site of operation was done in the upper part of back , then Surgical incision was done to made induced skin wound in size 3*4 cm. While in treated groups same procedure mention above with and treated this groups after infected the surgical wound with *staphylococcus Aurous* bacteria from first day. The clinical parameters included temperature , respiratory, and heart rates. The scar present large quantitate in control group when compared with treated group have a good and best results. Histopathological Examinations was done by taken the skin biopsies on 15th, and 30th, days postoperatively , the histopathological results of this study revealed that the wound healing in treated group faster and better and the epithelial cells with more maturity and the skin near normal when compare with control group.

**Keywords:** Kefir, Regeneration , Skin, Dogs

## INTRODUCTION

Kefir is a popular traditional Middle Eastern beverage . Consumption of it, that leads to a 'good-feeling (1). It originated in the Caucasus Mountains in the former Soviet Union (Central Asia ). So has been consumed for thousands years (2). Kefir grains described by the tribes in the Northern Caucasian mountain region of Russia (3). Historically ; kefir grains were considered as gifts from Allah among the Muslim peoples of the Caucasian Mountains . They were passed down from generation to generation among the tribes of Caucasus and considered a source of family wealth . Traditional authentic kefir can be prepared by culturing fresh or pasteurized milk with kefir grains in homes all over the world (4).

In recent years fermented milk have had a strong influence on health . They are considered to be beneficial with therapeutic effects and various other properties . Researchers have identified yet another fermented milk drink ,however: The word 'kefir ' is derived from the Turkish word 'keif' that means 'good feeling ' (5). The drink originated in the Caucasus Mountains of Russia ,which are between the Black and the Caspian Seas . The mesophilic bacteria and yeasts have important role in produced Kefir by the fermentation of lactic acid and alcohol (6). So Kefir can be prepared by inoculating milk with kefir grains which are a combination of bacteria and yeasts in a symbiotic matrix . Most microorganisms present in kefir are non-pathogenic bacteria, especially Lactobacillus sp. and yeasts. Kefir is enriched with amino acids , vitamins, carbon dioxide, acetone, alcohol and all of this which have been shown to have health benefits . Recently, the antibacterial, immunologic and antitumor effects of kefir were studied on human beings (7).

The antibacterial effect of the kefir against many pathogenic organisms results from the inherent formation of organic acids, hydrogen peroxide, acetaldehyde, carbon dioxide, and bacteriocins. (8). As well as, hydrogen peroxide is another metabolite produced by some bacteria as an antimicrobial compound (9).

Probiotics are live microorganisms which , when administered in adequate amounts, confer a health benefit on the host (10). As is the case with the fermented dairy product sreferred to above, probiotics are consumed in foods containing these organisms insufficiently large quantities to pass safely to the gastrointestinal tract but can also come in the form of supplements consisting of live organisms such as pills. Although not as widely popular as other fermented dairy products , such as yogurt and cheese, kefir has been consumed and associated with health benefits for 100s of years ; originally by communities in the Caucasian mountains . The be verage itself typically has as lightly viscous texture with tart and acidic flavor , low levels of alcohol, and in some cases slight carbonation . Kefir is made with cow's milk but it can be made with milk from other sources such as goat, sheep, buffalo, or soy milk (11, 12). the important features that distinguish it from many other fermented dairy products is the requirement for the presence of a kefir grain in fermentation and the presence and importance of a large population of yeasts (13, 14).

The antimicrobial properties of kefir may lead to its use for nontraditional applications . Indeed, when rats bearing open wounds inoculated with S. aureus were treated with age made from kefir grains , it was found that the wound shealed at amuch faster rate than was observed in control rats (15). Gels made from kefir and kefir grains were found to be more effective at shealing wound size in P. aeruginosa contaminated third degree burns than a traditional silver sulfadiazine treatment in a rat model of burn woundsh (16, 17). a study using a rabbit model for contaminatedy open wound also found that gel made from kefir grain resulted in quicker healing times and quicker clearing of infection (18). These decreased healing times are likely result from multiple factors. like the ability of kefir to inhibit the growth of bacterial and fungal cells, thus leading to a cleaner wound , as shown to be the case in some studies (18, 16).

## MATERIALS AND METHODS

**Anesthesia and drugs**

1. Xylazine : Xyla 2% Castenray, Holland . 50 ml.
2. Ketamine hydrochloride 5%
3. Penicillin – Streptomycin: Penoksa LA, Vilsan Ankara. 100ml.

Twenty mature healthy street dogs from both sexes, weighted (17-28 kg), were used in current study . All animals were free from acquired or congenital disease as presented by their physical and clinical examination . The animals were treated for external and internal parasites by Niclosamide 1250 mg/kg B.W. orally and Ivermactin 200 µg/kg B.W. subcutaneously . The animals were housed in department of surgery and Obstetrics, veterinary medicine collage of Al-Qasim green University under similar condition and feeding . The animals were divided randomly into :

**Experimental Design:**

Dogs were randomly allocated to two groups (10 rabbits/group ) as follows :

1. **First group** : Also named control group

Animals of this group which have the numbers from (1-10), induced skin wound 3*4 cm infected with bacteria without treated with kefir

2. **Second group** : treated group In this group animals were numbered (11-20), induced skin wound 3*4 cm infected with bacteria for first day and treated with kefir

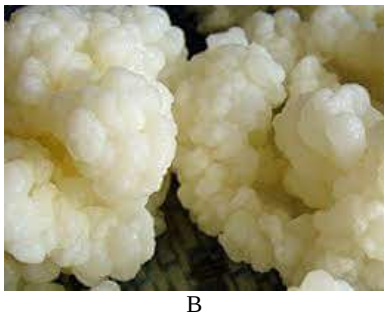
The two groups have subgroups equally to have histopathology at $15^{th}$ day and $30^{th}$ day respectively.

Elevate after clipping and shaving the site of operation **(Fig. 1)** then, inducing skin wound at 3*4 cm in size **(Fig.2),** and give histopathologically in $15^{th}$ day and $30^{th}$ day.

**Kefir preparation:**

The grains (5 g) kept at Lab . Fitofarmacos (Unifenas, MG, Brazil ) were cultured in 100 g/L of molasses for 15 days prior to experiments . The medium was changed at 24 h intervals so it washed with sterile water . Preliminary taxonomic classification of the bacterial isolates was performed on individual colonies by Gram staining (API 20 S system for streptococci, API 20 NE for bacteria) and API 20 AUX for yeasts (API Biomerieux, SA, France). Both kefir grains and suspensions contained significant number of *Leuconostoc* spp., *Lactobacillus lactis*, *Acetobacter* spp., *Saccharomyces cerevisae*, *Kluyveromyces marxianus*and *K. lactis* (19 , 20). $15^{th}$ days later of fermentation , kefir was extracted for kefiran production for use in the antimicrobial and cicatrizing experiments . A kefir gel containing 70% grains freshly dried at 60 ◦C and homogenised with a lanette -based commercially available cream, was used in the rat experiments . But the kefiran (polysaccharide matrix ) was isolated from kefir grains using the method described by (21, 15) .Briefly, the stirred grains were washed with boiling distilleda water for 1 h (one part grains to 100 parts water ). The mixture was then cooled and centrifuged at 16000 g for 15 min. The procedure was repeated with the sediment . The polysaccharide dissolved in the combined supernatants was precipitated by the addition of an equal volume of cold ethanol at 4 ºC overnight . The precipitate was redissolved in hot water (1:100) for 1 h at 70 ◦C with stirring and the precipitation procedurewas repeated twice . Finally the precipitate was dissolved in 100mL distilled water, dialysed against distilled water until the conductivity reached 1.5 _S/cm and lyophilised (Datamed TS-600 conductivity meter, Brazil) (15).

A



B

Figure (1): show the kefir preparation, A& B

**Clinical examination**

The animals were examined physically and clinically for temperature , respiratory rate , heart rate, defecation and urination during a period of one week post operation .

**Histopathological Examinations :**

Skin biopsies were taken on $15^{th}$, and $30^{th}$, day postoperatively. Biopsies were obtained by using scalpel and scissors, Biopsies were fixed in 10 % neutral buffered formalin, then routinely processed and embedded in paraffin as blocks which were cut at 5-6 micrometer and stained by Hematoxyline and Eosin stain and finally examined under light microscope.

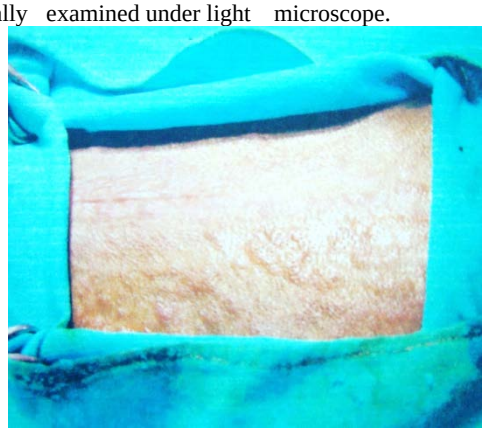


Figure (2): the site of operation

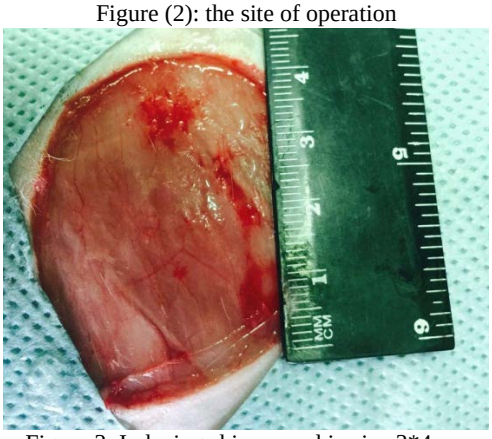


Figure 3: Inducing skin wound in size 3*4 cm

**Clinical and Physical examination :**

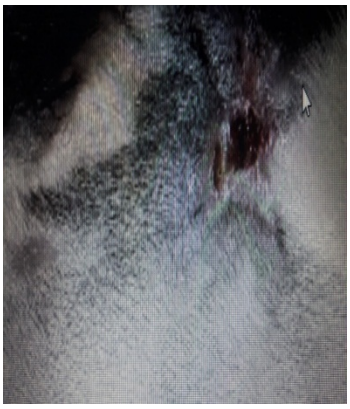
The results of the physical and clinical examination for temperature , respiratory rate , heart rate, defecation and urination during first week post operation revealed that, slight elevated in temperature, respiratory rate and heart rate with seen normal defecation and urination in all animals .Significant convergence in results between the control group since the second days postoperation and treated group but early disappeared in treated group when compared with control group at $3^{rd}$ and $5^{th}$ days postoperation respectively, that may be due to increase the blood flow in operative area. Beside that increase dilatation of blood vessels with increase permeability of capillaries was agreed with other workers (22), whom mentioned that there were no significant changes recorded in these clinical parameters before and after surgical operation .

**Scar Formation**

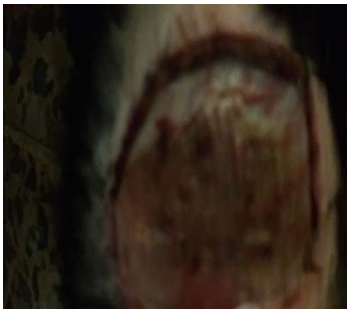
The amount of sca tissue take indication for quality of wound healing, the results of macroscopic evaluation at $15^{th}$ day postoperation showed the formation of scar tissue in control group more than in kefir group that may be due to mechanisms occur to different degrees during the four types of healing . Primary healing occurs when a wound is closed within hours of its creation . Delayed primary healing occurs when the wound is purposefully left open, for some

interval, prior to closure. Healing by secondary intention occurs in wounds that are left to heal with or without topical therapy . Here, dressing changes are performed until the wound closes by contraction and epithelialization . Finally , partial thickness wounds or wounds involving the epidermis and part of the dermis heal by epithelialization that agreement with (23). One of the most potent stimuli for initiation of an inflammatory response and thus adhesions formation is surgical trauma. Routine surgical procedures involve various degree of tissue handling that initials tissue abrasion, desiccation, ischemia ,bleeding , infection and exposure to foreign materials ,any of these factors can initiate inflammatory responses which eventually lead to adhesion formation (24).

During day's five to ten, fibroblasts become aligned with the adhesion , while collagen deposition and organization advance .The relatively few cells present are predominantly fibroblasts . 15$^{th}$ to 30$^{th}$ days after injury , the collagen fibrils become organized into discrete bundles interposed by fibrocytes and a few macrophages. Extensive well-defined adhesions are often covered by mesothelium and contain blood vessels and connective tissue fibers (25).

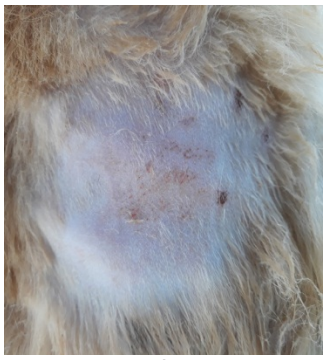


A

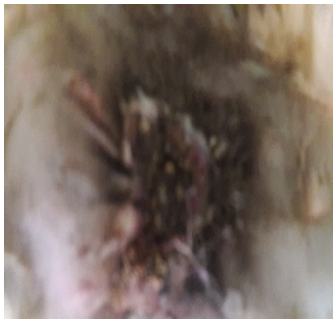


B

Figure (4): show the scar tissue in group A: treated group and group B: control group at 15$^{th}$



A



**B**

Figure (5): show the scar tissue in group A: treated group and group B: control group at 30$^{th}$

**Hsitopathological Findings :**

The histopathological findings of the skin after inducing of wound at 3*4 cm in size were as following :

**Control Group :**

**At 15$^{th}$ Day Postoperation**:

The histopathological examination in control group at 15$^{th}$ day Post operation showed complete sloughing of epidermis marked hemostasis in the dermis, preffusing edpose tissue infiltration of inflammatory cell with perfuse collagen. Fig. 6& 7

**At thirty Day Postoperation** :

While, the control group at 30$^{th}$ day post operation, showed weak healing characterized by present of narrow incision .high proliferation of epidermal layers articularly epithelia cell and stratum pizza preffusing collagen high infiltration of inflammatory cell on the derma also there is congestion on blood vessel. Fig. 8& 9

**Treated Group:**

**At 15$^{th}$ Day Postoperation** :

The histopathological examination in treated group at fifteenth day Post operation showed down word hyperplasia of stratum pizza present of granulation tissue which characterized by formation of new blood vessels and fibrosis. Fig. 10&11

**At thirty Day Postoperation**:

While show in the treated group at 30$^{th}$ day post operation, showed complete healing characterized by proliferation epidermal layers and down word hyperplasia of stratum pizza present of granulation tissue which characterized by formation of new blood vessels and fibrosis but in another figure we are show showings good epithelization ,with mid keratin formation well developed collagen fiber formation with excellent. Restoration of adnexa. Fig. 12, 13& 14

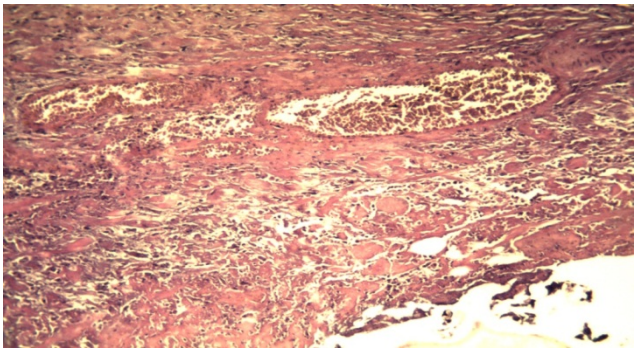


Figure 6: Histopathological section of dog´s skin for control group at 15$^{th}$ days showing extensive necrosis ( ), dilation and congestion blood vessels ( ), with inflammatory cells infiltration (100X)

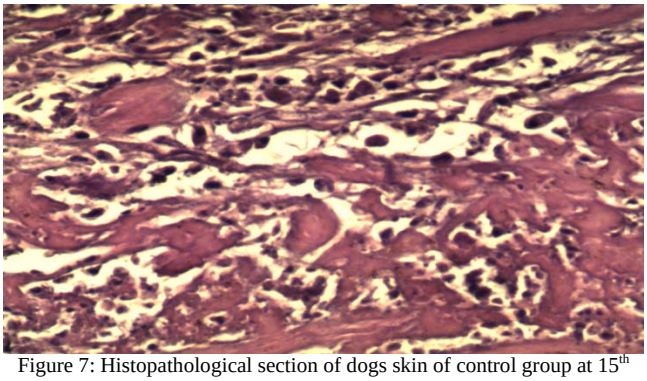


Figure 7: Histopathological section of dogs skin of control group at 15th days showing early granulation tissue formation have highly cellular with fibroblast proliferation and thick collagenous fiber also seen 40X

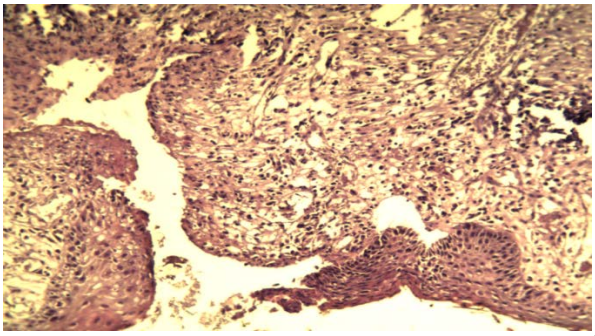


Figure 8: Histopathological section of dogs skin of control group at 30th days showing early epithelization with deep ulcer formation (100X).

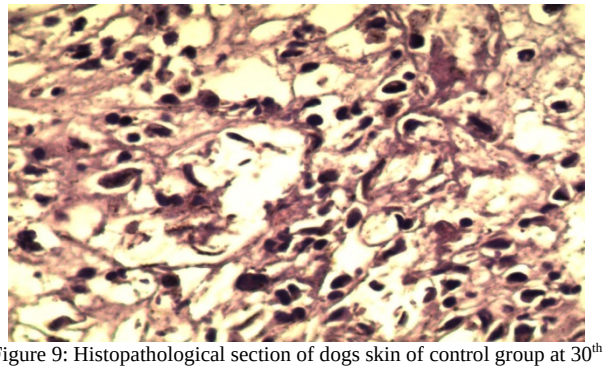


Figure 9: Histopathological section of dogs skin of control group at 30th days showing well developed granulation tissues formation, characterized by less cellular, with proliferation of fibroblast and less vasculation

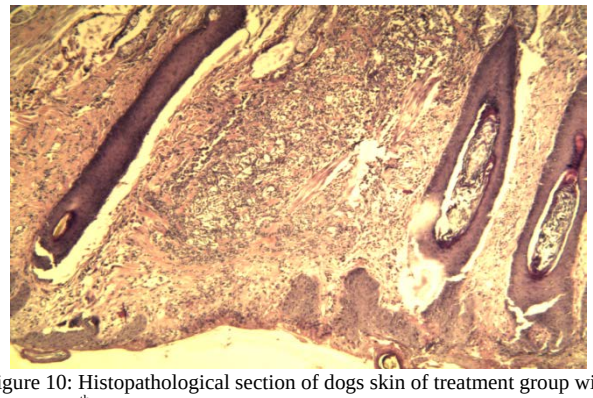


Figure 10: Histopathological section of dogs skin of treatment group with kefir at 15th days showing well epithelization with restoration of adnexa 100X

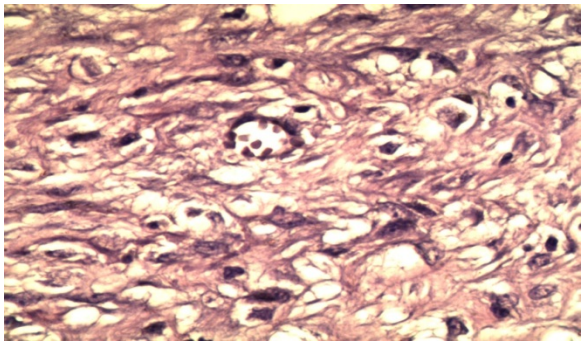


Figure 11: Histopathological section of dogs skin of treatment group with kefir at 15th days showing well epithelization with restoration of adnexa with well development of granulation tissue 100X

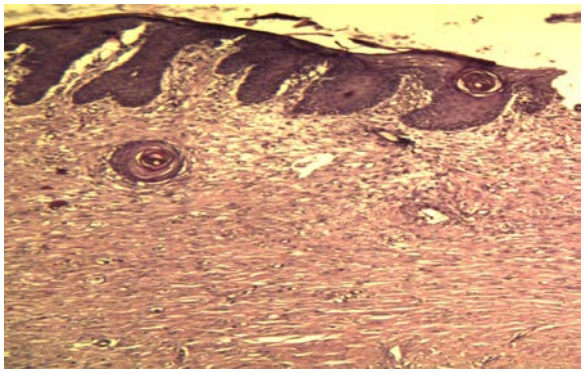


Figure 12: Histopathological section of dogs skin of treatment group with kefir at 30th days showings excellent epitheliazation with very good restoration of adnexa similar to normal tissue

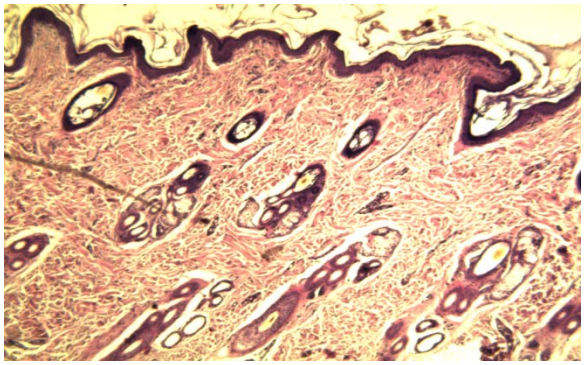


Figure 13: Histopathological section of dogs skin of treatment group with kefir at 30th days showings excellent epitheliazation with very good restoration of adnexa with mild keratin formation similar to normal tissue

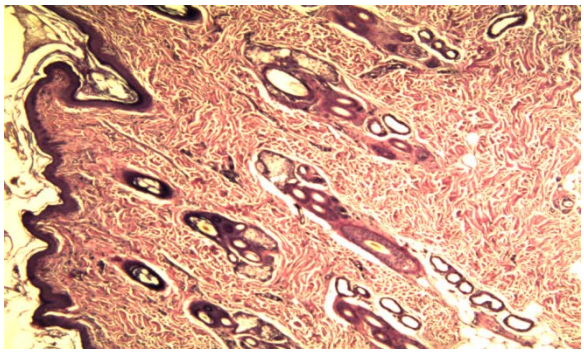


Figure 14: Histopathological section of dogs skin of treatment group with kefir at 30th days showings good epithelization ,with mid keratin formation well developed collgen fiber foemation with excellent. Restoration of adnexa .

When we are show the histopathological results that obtained the treated group with kefir production have highly good results and the skin similar to normal skin; may be this due to the chemical compound of kefir productions like lactic acid, acidic acid polysaccharide and other chemical present in kefir preparation because there are have antimicrobial, anti-inflammatory action and have wound healing properties . So all this observe in this study .

However ; kefir gel therapy with longer culture fermentation time improves clinical outcomes after wound injury when compare with control group and this agree with( 26, 27)

As kefir is a probiotic mixture of a diverse spectrum of bacteria and yeasts (28), it can stimulate innate immune responses in defense against pathogens (29, 18). ( 27 and 30) stated that the anti-inflammatory properties of polysaccharide present in kefir extract may be that influential in the process of wound healing . The lactic acid, acetic acid, polysaccharides and other chemicals present in kefir considered important factors for wound healing properties observed in a study by (26). In 2005 ( 31) conducted a study on rats, treating them with a simple kefir formulation made from dried grains .The results showed better wound healing properties compared with those treated with the clostebol–neomycin emulsion . Similarly, In 2005 (32) and his team proved that rats treated with 70 % kefir gel made with kefiran, showed a faster reduction of the infected-induced wound compared with clostebol–neomycin emulsion. A study by( 26) also showed that kefir had better wound-healing

Kefir can be an amazin example of coevolution of a microbial consortium .It has acquired astrong resistance against several microorganisms, as well as improving the natural immunity of mammals from early times . It is reasonable to think of the consortium as a potential naturally-occurring drug, able to decrease a large variety of illness afflictions (33). In 2003, (34) and his team reported that several strains of Lactobacillus spp. isolated from kefir in various countries have good adhesion to Caco-2 cells. These strains were resistant to low pH and bile acid and had antimicrobial activity against common enteropathogenic bacteria, which are popular criteria required by probiotic bacteria. In addition, prebiotics are considered non-digestible but fermentable oligosaccharides, involving health promotion for the host (35).These compounds are known to provide improvements in nutritional status, in addition to health benefits such as protection against carcinogenesis, mutagenesis , prevention of injuries caused by free radicals, control of intestinal flora, and gastrointestinal resistance . Importantly kefir is able to produce peptide and sugar prebiotics , e.g., lactacin, bacteriocins, and kefiran (36).

Scientific studies indicate kefir to be a complex probiotic, which is a combination of bacteria and yeasts. Kefir has certainly been shown to contain various functional properties such as antimicrobial, anti-carcinogenic, probiotic and others .It provides healthful benefits in the cholesterol lowering effects and improved lactose tolerance in humans. This fermented milk appears to have a great potential and this should inspire researchers to carry out further studies on kefir in order to analyze the hidden therapeutic and functional properties which have not been revealed to date (33).

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