

A study on the protective activity of kefir against gastric ulcer

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Background/aims: The effect of kefir on peptic ulcer disease was evaluated in an experimental model, with non-steroid anti-inflammatory drugs, together with the determination of gastric mucus secretion by quantitative digital histochemistry. **Materials and Methods:** The experimental group included 28 male albino Wistar rats. After a diet with standard rat bait for 7 days, 14 rats were fed with kefir for 7 days while the others were kept on the same diet. At the 14th day, indomethacin was injected to 7 of the rats fed on kefir and to 7 of the rats on standard rat bait. All the rats were sacrificed after 4 hours. Gastric erosion and ulceration were scored histopathologically. Mucosal mucus was quantified by image analysis, and periodic acid-Schiff stained area percentage was determined. **Results:** Erosion and ulceration were identified only in cases that received indomethacin. In the cases on kefir, erosion was identified in 6 cases (86%) and ulceration in 1 case. Rats fed on standard diet had erosion in 4 cases (57%) and ulceration in 3 (43%), but the difference was statistically insignificant (Mann-Whitney test, $p=0.25$). The stained area percentage for gastric mucus was not different between the four groups (Kruskal-Wallis test, $p=0.313$). **Conclusions:** These findings suggest that kefir does not change gastric mucus secretion. Although statistically insignificant, as there were more cases with ulceration in cases on the rat diet, kefir might have a beneficial effect on peptic ulcer disease induced by non-steroid anti-inflammatory drug. This requires further evaluation in larger series.

Key words: Kefir, probiotics, gastric ulcer, image analysis

Gastrik ülsere karşı kefirin koruyucu aktivitesi üzerine bir araştırma

Amaç: Kefirin nonsteroid anti-inflamatuar ilaç ile peptik ülser hastalığı üzerine, kefirin mide mukus bariyerinin miktarı ve etkinliği üzerindeki etkisi deneyel bir modelle araştırılmıştır. **Gereç ve Yöntem:** 28 Wistar türü erkek sıçan üzerinde yapılan araştırmada 14 denek kontrol olarak 15 gün boyunca standart olarak grup başına 300 gram standart sıçan yemi ile beslenmiş, diğer 14 denek ise 7 gün yem alıp bu günden sonra 300 gram yem ile 450 gram kefir karıştırılarak beslenmiştir. Her iki gruptan 7 deneye subkutan 30 mg/kg indometazin verilip 4 saat sonra sakrifiye edilmiştir. Histopatolojik inceleme ile gastrik ülserasyon gelişimi skorlanmış ve imaj analizi ile mukus miktarı kesitlerde saptanmıştır. **Bulgular:** Ülserasyon skorları karşılaştırıldığında kefir alan ve almayan gruplar arasında fark yoktur. Mikroskopik incelemede sadece indometazin verilen gruplarda gastrik ülserasyon ve erozyon saptanmıştır. Kefir almayan kontrol grubunda 4 (%57) olguda erozyon ve 3 (%43) olguda ülserasyon saptanmıştır. Kefir alan grupta erozyon 6 olguda (%86), ülserasyon 1 (%14) olguda saptanmıştır. Gruplar arasında istatistiksel fark saptanmamıştır (Mann Whitney Test $p=0.25$). Kontrol grubunu oluşturan deneklerde kefir alımı olmaksızın, gastrik mukoza mukus salgısının bulunduğu alan yüzdesi $\% 6.04 \pm 2.94$ olarak bulunmuştur. Kefir alanlarda ise $\% 5.90 \pm 3.15$ 'dir. Kefir almayan ve indometazin alanlarda bu oran $\% 4.65 \pm 1.55$ olup, kefir alan ve indometazin alanlarda ise $\% 3.75 \pm 2.5$ bulunmuştur. Bu da istatistiksel analiz gruplar arasında fark bulunmadığını ortaya koymustur (Kruskal Wallis $p = 0.313$, $r=0.52$). **Sonuç:** Bu bulgular kefirin gastrik mukus miktarını ya da koruyuculuğu artttığını desteklememektedir. İstatistiksel sonuçlar öneksiz çırksa da, nonsteroid anti-inflamatuar ilaç ile ülser yapılan ve kefir ile beslenen grupta sonuçların daha iyi çıkması bu çalışmanın daha kapsamlı olarak yeniden yapılması düşünülebilir.

Anahtar kelimeler: Kefir, probiyotik, gastrik ülser, imaj analiz

INTRODUCTION

Gastric ulceration is related to more than one factor, including *Helicobacter pylori* (*H. pylori*) infection, stress, mucosal mucus secretion, gastric irritants, and gastric acidity. It has been claimed for many years that the consumption of kefir, a probiotic fermented milk product, is beneficial for the treatment of gastric and duodenal ulcer. Upon this observation, Evenshtein (1) described in 1978 that the gastric juice was increased by 89% after consumption of kefir for six weeks. It was also demonstrated that kefir reduced *H. pylori* infection owing to its antibacterial effects in vivo (2,3). On the other hand, to the best of our knowledge, the effect of kefir on the gastric mucosal mucus secretion, which may be a factor inducing ulceration, has not been described yet. It is thought that an increase in gastric mucosal secretion might delay or prevent the disease (4). In the present work, it was intended to evaluate the effect of kefir consumption in an experimental model of acute gastric ulcer, with non-steroid anti-inflammatory drugs (NSAIDs), with determination of gastric mucus secretion by quantitative digital histochemistry.

MATERIALS AND METHODS

Experimental Materials and Animals

The study protocol was approved by the Local Ethics Committee at Dokuz Eylül University Faculty of Medicine. The experimental groups consisted of 28 male albino Wistar rats from Dokuz Eylül University, Medical Science Faculty, Laboratory of the Experimental Animals Department. After a diet with standard rat bait for 7 days, 14 rats were fed with kefir for 7 days while the others were kept on the same diet. At the 14th day of the experiment, 7 rats fed on kefir and 7 rats fed standard rat bait were given indomethacin injection, and after 4 hours (h) all the rats were sacrificed (Table 1).

The rats were weighed on the 1st, 7th and 14th days (5,6).

Production of Kefir

Raw cow's milk supplied from Ege University Agriculture Faculty, Menemen Research and Application Farm, was used in kefir-making. The milk was heated at 90°C for 5 minutes (min), followed by inoculation with the kefir grains provided by Ege University Agriculture Faculty, Dairy Technology Department, Microbiology Laboratory, at a rate of 5% and incubated at 20±3°C until pH 4.7 was attained in glass cups. After incubation, kefir grains were removed by a sterile wire sieve. The filtrate was used as kefir in the rest of the experiment (7).

Raw Milk and Kefir Analysis

In raw milk and kefir, the pH, titratable acidity (expressed as percentage lactic acid), total solids, fat, total nitrogen, ash, and lactose contents were determined according to Turkish Standards Institution Method (8). In the counts of *Streptococcus* spp., M17 agar (OXOID, CM 785), *Lactobacillus* spp. MRS agar (OXOID, CM 361) and total yeasts Dichloran Rose Bengal Chloramphenicol (DRBC) agar (CM 727) were used (9).

Gross Examination

The gastric and duodenal tissues were evaluated by gross examination and scored as follows: 0: no evidence of ulceration, 1: erosion, and 2: ulceration. The gastric and duodenal tissues were formalin-fixed, and for histopathological examination, the gastric wall was sampled, beginning from the cardia-esophageal junction to the duodenum through the lesser curvature, and paraffin-embedded tissue blocks were prepared. The sections from these blocks were stained by hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) (10).

Microscopic Evaluation of Gastric Mucosa

Hematoxylin and eosin-stained sections were evaluated for any evidence of erosion or ulceration and scored as follows: 0: no evidence of erosion or ulceration, 1: erosion identified by loss of the superficial layer of mucosa replaced by inflamma-

Table 1. The distribution of the experimental rats

	Group 1	Group 2	Group 3	Group 4
Nutrition	Kefir + standard bait + tap water	Standard bait + tap water	Kefir + standard bait tap water	Standard bait + tap water
Formation of ulcer	Indomethacin	Indomethacin	-	-
Duration to sacrifice	14 days + 4 hours	14 days + 4 hours	14 days +	14 days +

tory cells and/or fibrin, and 2: ulceration with loss of mucosal layer. Additionally, *H. pylori* was searched in every section (10,11,12). In the sections of the whole gastric mucosa, painted hematoksiilen eosin was processed and researched. In those parts there were not gastric erosion and ulceration, but erosion and ulceration was scored to come into (Fig. 1a - b, 2).

Determination of Gastric Mucus by Quantitative Digital Histochemistry

For a standard measurement of the gastric mucosal mucus, PAS-stained sections from the small curvature were selected for measurement, and images were taken subsequently from the gastroesophageal junction to the duodenum. All the gastric mucosal mucus could thus be quantified at the lesser curvature, excluding the zones with extensive erosion and ulceration, where mucosa could not be observed. Digital images were obtained from the selected areas using light microscopy (Olympus, BX51, Japan) at original magnification of x40. Images were captured by a digital color video camera (Olympus DP70). The stained mucosal areas in the captured images were quantified by measuring the high power fields using a software (Mustafa Sakar, Izmir, Turkey). The magenta color was selected; the image analysis program calculated the stained area percentage (SAP), as described previously (11-13). On the image when the magenta color was selected, neighbors to that and designated color neighbors were covered by the analysis programme automatically, and was expressed as the proportion of the selected area to the total area (Fig. 3 a - b) (12).

Statistical Evaluation

Statistical evaluation was performed using the Statistical Package for the Social Sciences for

Windows software package (SPSS ver. 11.0 for Windows; Chicago, IL). SPSS version 11.0 was used for statistical analysis and the Mann-Whitney U test for comparisons between groups. The relationship between variables was investigated using Pearson's rank correlation (14).

RESULTS

The chemical and microbiological properties of raw milk and kefir are summarized in Table 2.

The average weight of the rats was 188.04 ± 19.66 g, 210.46 ± 21.20 g and 219.39 ± 22.59 g on the 1st, 7th and 14th day, respectively, and there was a significant difference between groups ($p < 0.000$, one way ANOVA). There was a significant difference the first and second as well as between the first and third measurements ($p < < 0.000$ and $p < < 0.000$, Mann-Whitney U test), but there was no significant difference between the second and third measurements ($p = 0.14$, Mann-Whitney U test).

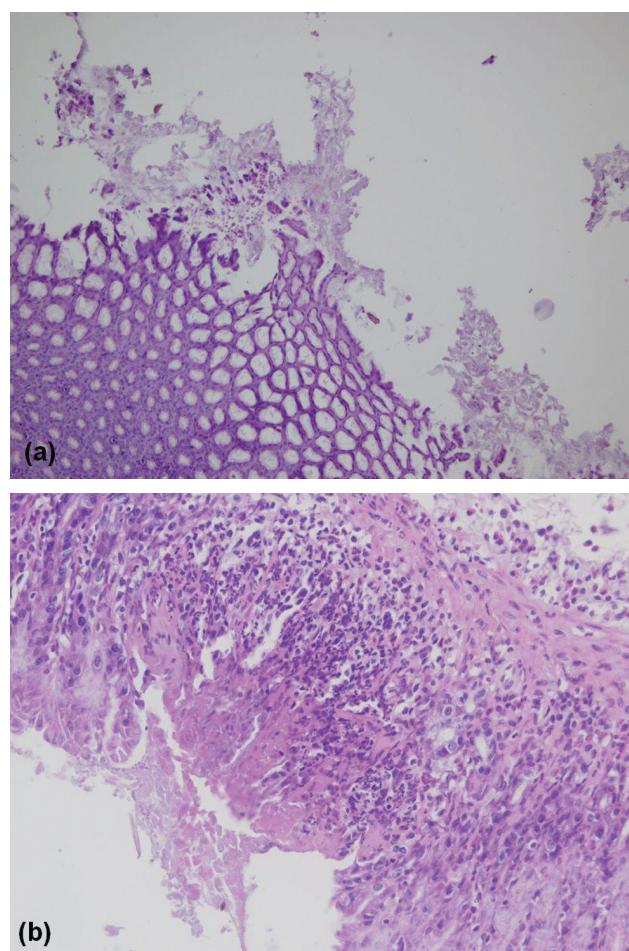


Figure 1. **a.** The gastric mucosa with superficial erosion. **b.** Gastric ulcer, with total loss of the mucosa (H&E, original magnification x10).

Table 2. The chemical and microbiological properties of raw milk and kefir samples (n=7)

	Raw milk	Kefir
pH	6.7 ± 0.04	4.5 ± 0.06
Total solids (%)	11.30 ± 0.8	10.78 ± 0.85
Acidity (lactic acid), %	0.13 ± 0.03	0.79 ± 0.15
Milk fat (%)	3.2 ± 0.2	3.15 ± 0.35
Lactose (%)	3.8 ± 0.3	3.45 ± 0.20
Protein (%)	3.5 ± 0.2	3.40 ± 0.15
Ash (%)	0.80 ± 0.05	0.75 ± 0.04
<i>Lactococcus</i> spp. (cfu/ml)	-	2.25×10^8
<i>Streptococcus</i> spp. (cfu/ml)	-	1.5×10^8
Yeast (cfu/ml)	-	1.3×10^6

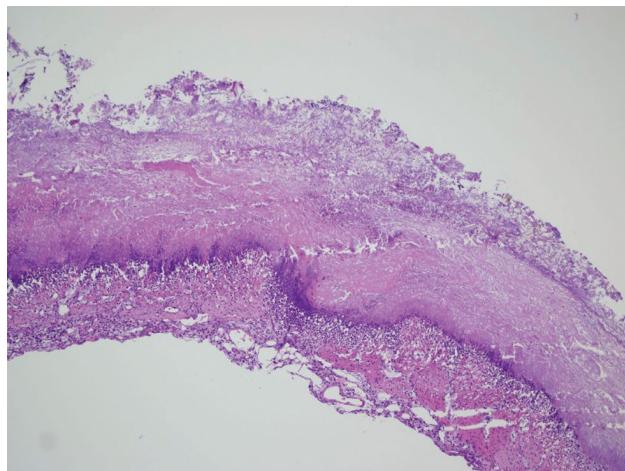


Figure 2. Ulceration damaging the whole mucosa and destroying the superficial muscle layer (H&E original magnification $\times 4$).

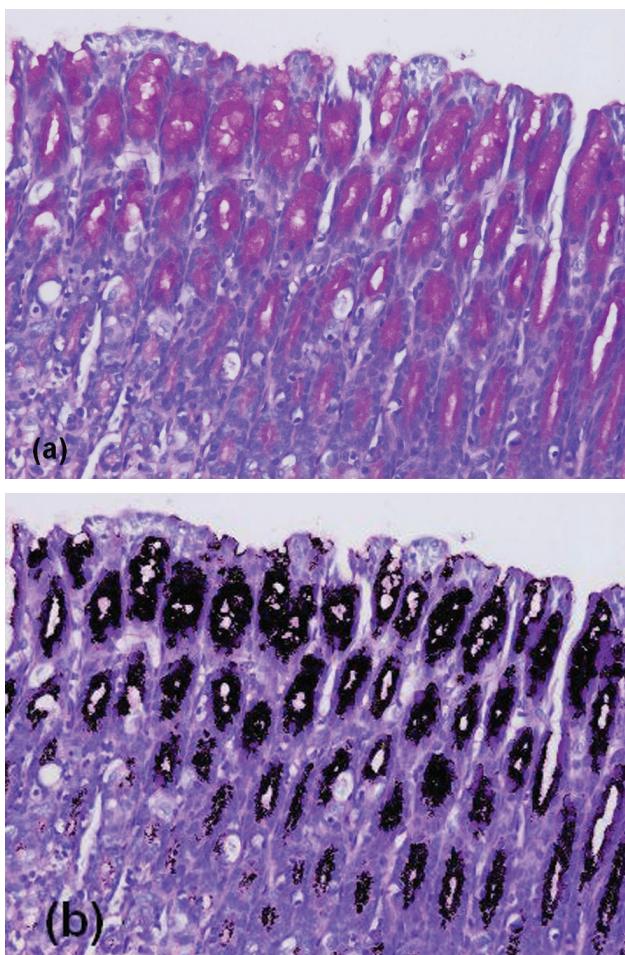


Figure 3 a-b. The demonstration of the stained gastric mucus and the image analysis for measuring the stained area percentage (PAS $\times 10$).

When the body masses of groups on standard diet and those receiving kefir were compared, no significant difference was noted in the last two measurements ($p=0.10$ and $p=0.19$, respectively). Gross and microscopic examinations did not reveal erosion or ulceration for the cases that did not receive indomethacin. There was erosion in 4 (57%) and ulceration in 3 (43%) of the cases fed on standard diet, while erosion was identified in 6 (86%) and ulceration in 1 (14%) of the cases receiving kefir (Table 3). There was no significant difference between the groups ($p=0.25$, Mann-Whitney U test).

The average SAP for all cases was $5.09 \pm 2.64\%$ (range: 0.99-12-10%). The mean SAP for cases receiving standard diet and kefir diet, but not indomethacin, was 6.40 ± 2.94 and 5.90 ± 3.15 , respectively, and for the groups receiving indomethacin, these values were 4.65 ± 1.55 and 3.75 ± 2.5 (Table 3). There was no significant difference between the groups ($p=0.313$, Kruskal-Wallis test). Overall, when the 14 cases on standard diet were compared with the cases on kefir diet, there was no significant difference between the groups in terms of SAP values ($p=0.32$).

DISCUSSION

Peptic ulcer disease is a result of the loss of balance between gastric mucosal protection and gastric acidity. The mucus layer spares the gastric mucosal surface and pit epithelium from the digestive effect of the gastric juice.

The leading etiologic factor for the development of peptic ulcer disease, as well as of gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma is *H. pylori* infection. The bacteria act on the 1500 proteins, which induces epithelial cell apoptosis, metaplasia, atrophy, dysplasia, and carcinoma, as well as ulceration following multiple pathways. The latter is located at the duodenum predominantly, in the setting of antral *H. pylori* infection, with increased gastric acidity and gastric metaplasia of the duodenal mucosa (15). Many drugs as well as natural products have been evaluated for the eradication of *H. pylori* since the identification by Marshall and Warren and Lichtenberger *et al.* (16,24).

Researches on fermented milk and probiotics have proven the health benefits of these products. Kefir is a probiotic fermented milk product originating from the Caucasian mountains. It is a cultured milk, in which both acid and alcohol fermentation is developed. Kefir has a refreshing and slightly

Table 3. The mean values of body mass measurements and histopathological evaluation results of the four experimental groups of rats

	Standard diet indomethacin -	Standard diet indomethacin +	Kefir diet indomethacin -	Kefir diet indomethacin +
Body mass 1 (gram)	202.14	198.57	184.28	168.57
Body mass 2 (gram)	211.28	223.0	207.14	201.85
Body mass 3 (gram)	219.42	224.57	217.14	216.42
Erosion	none	4 (57%)	none	6 (86%)
Ulceration	none	3 (43%)	none	1 (14%)
Stained area percentage (%)	6.40±2.94	4.65±1.55	5.90±3.15	3.75±2.5

sharp taste and aroma. The original starter of kefir is also called kefir grain. Kefir grain, resembling florets of a cauliflower, are white-yellowish in color, 2 to 4 mm in diameter. The surface of the grain is rough and convoluted. They are held together in clusters as an immobilized system of a complex community of yeast and bacterial cells embedded in a microbe-produced polysaccharide matrix along with some milk fat and denatured milk protein (7,17). Kefir grain mainly consists of lactose-fermenting bacteria *Lactobacillus* and *Leuconostoc species* (*Lactobacillus casei*, *L. brevis*, *L. acidophilus*, *L. kefir*, *Leuconostoc mesenteroides*, and *Leu. mesenteroides* subsp. *dextranicum*) as well as *Lactococcus lactis*, *Lactococcus lactis* subsp. *cremoris* and other lactic acid bacteria. The other characteristic organisms are lactose-fermenting or non-lactose-fermenting yeasts, mainly including *Candida kefir*, *Kluyveromyces marxianus* subsp. *marxianus*, *Torulaspora delbrueckii*, and *Saccaromyces cerevisiae*. The main products produced by kefir grains are lactic acid, carbon dioxide, alcohol, diacetyl and acetaldehyde as well as protein degradation products. It is known that kefir was used in cancer treatment in Russia and is still being used in patients with gastric problems (3,18-20).

Kefir and other fermented probiotics milk products may have an antibacterial effect on *H. pylori* infection (3,21). On the other hand, there is no satisfactory data about the effect of kefir on other mechanisms, which may contribute to the progression of the available milieu for the development of peptic ulcer. The second most frequent factor for the development of peptic ulcer disease is NSAIDs. They induce gastric injury by intracellular accumulation and inhibition of prostaglandin synthesis (22,23). NSAIDs also reduce the hydrophobicity of the mucus gel layer by an insult to the

surface-active phospholipids. It has been shown that this can be prevented by pre-associating a NSAID with zwitterionic phospholipids (24).

The present study is based on the hypothesis that kefir may have other effects on the gastric milieu in addition to its antibacterial properties. The experimental model depends upon consumption of kefir with suitable properties. In Table 2, the analysis results of the kefir, which were only for 1-2 days, were in agreement with the findings of other researchers regarding the general composition and microbiologic appearance of kefir; especially from the perspective of probiotic effect, *Lactobacillus* and *Streptococcus* genus showed that they were of sufficient degree considering the number (5,25-27). The gross composition of raw milk used in the production of kefir was close to the average milk composition (1,3,7,17,26,27).

The experimental model questions the effect of kefir consumption on the prevention of NSAID-induced peptic ulcer, and if positive results were obtained, this would lead to a series of new questions about the mechanism. We did not find a statistically significant difference between groups on rat bait and kefir considering lesions of erosion and ulceration, but the number of cases with ulceration was more for cases that did not receive kefir. This finding may deserve evaluation in a larger number of cases, as the statistically insignificant results may have been caused by the low number of cases in this series.

The increase in the amount of mucus secretion may reduce the NSAIDs ulcerogenic effect on the gastric mucosa (28). The amount of gastric mucus was quantified by image analysis based on PAS histochemistry. There was no significant difference between the group on the kefir diet and the group on the standard rat bait ($p>0.05$).

The results of the present study argue against the role of kefir consumption on prevention of NSAID-induced gastric ulceration by increasing gastric mucus. As the number of cases with ulceration was less in cases on kefir in this series, the effects

of kefir on indomethacin-induced gastric injury might be evaluated in larger series with the other mucosa-saving effects of kefir, like prostaglandin E2 secretion, which is suggested as a mechanism for decreasing alcohol-induced injury (29).

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