Kafkas Univ Vet Fak Derg
21 (2): 195-201, 2015
DOI: 10.9775/kvfd.2014.12015

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Effects of Kefir, Koumiss, Milk and Yoghurt Administration on Distribution of Plasma Cells and Mast Cells in Mice Spleen [1]

Sevda ELİŞ YILDIZ [1] Funda YİĞİT [2] Berna DUMAN AYDIN [3]
Ebru KARADAĞ SARI [4] Turgay DEPREM [4] Serap KORAL TAŞÇI [4]

[1] *This research was presented at XII National Histology and Embriyology Congress with International Contribution, P:030, 27-30 May, 2014, Ankara, Türkiye*

[1] Kafkas Üniversitesi, Kars Sağlık Yüksekokulu, TR-36100 Kars - TÜRKİYE
[2] İstanbul Üniversitesi, Veteriner Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, TR-34320 İstanbul - TÜRKİYE
[3] Gıda Tarım ve Hayvancılık İl Müdürlüğü, TR-21100 Diyarbakır - TÜRKİYE
[4] Kafkas Üniversitesi, Veteriner Fakültesi, Histoloji ve Embriyoloji Anabilim Dalı, TR-36300 Kars - TÜRKİYE

KVFD-2014-12015 Received: 20.07.2014 Accepted: 04.12.2014 Published Online: 10.12.2014

Abstract

The present study was conducted to investigate the effect of kefir, koumiss, milk and yoghurt administration on plasma cells, which synthesize specific antibodies against antigens, and mast cells, which have a role in the regulation of humoral and cellular events, in the mice spleen tissues. Mice (n=30) were divided into 5 groups. As a result of statistical evaluation, live weights of mice in kefir, koumiss, milk and yoghurt groups were found to be increased compared to the control group. When numbers of plasma cells in spleens of mice treated with kefir, koumiss, milk and yoghurt were checked with stereological methods, significant difference was found to be increased in number of plasma cells of kefir-treated mice compared to other groups at the end of 15 days. At the end of day 15, a significant difference was found to be increased in the number of mast cells in the spleen of milk-treated mice compared to control group. As the number of plasma cells in the spleen is at higher level with kefir, than with koumiss, milk and yoghurt; also there being a significant difference between the numbers of mast cells in the spleens of mice treated with milk compared to control group, this is remarkable for healthy eating/functional food.

Keywords: *Kefir, Koumiss, Milk, Yoghurt, Plasma cell, Mast cell*

Kefir, Kımız, Süt ve Yoğurt Uygulamasının Fare Dalağında Plazma Hücresi ve Mast Hücrelerinin Dağılımı Üzerine Etkileri

Özet

Bu çalışma, kefir, kımız, süt ve yoğurt uygulamasının fare dalak dokusunda, karşılaştıkları antijene spesifik antikor sentezleyen plazma hücreleri ile humoral ve hücresel olayların düzenlemesinde rolü olan mast hücrelerinin sayısal dağılımını belirlenmeyi amaçlanmıştır. Fareler (n=30) her grupta 6 adet olacak şekilde 5 gruba ayrıldı. İstatiksel değerlendirmeler sonucunda kefir, kımız, süt ve yoğurt grubunda bulunan farelerin canlı ağırlıklarının kontrol grubuna göre arttığı tespit edildi. Kefir, kımız, süt ve yoğurt uygulanan farelerin sterelojik yöntemle dalaktaki plazma hücreleri sayısına bakıldığında 15. günün sonunda kefir uygulanan farelerin dalağında plazma hücrelerinin sayısında diğer gruplara göre anlamlı bir artışın olduğu tespit edildi. 15. günün sonunda süt uygulanan farelerin dalağında mast hücrelerinin sayısında kontrol grubuna göre anlamlı bir artışın olduğu belirlendi. Yararlı mikroorganizmalardan oluşan kefirin, dalaktaki plazma hücre sayısına bakıldığında kımız, süt ve yoğurttan daha yüksek düzeyde bulunması; ayrıca süt uygulanan farelerin dalağında mast hücrelerinin sayısında kontrol grubuna göre anlamlı bir farkın olması sağlıklı beslenme/fonksiyonel gıda kapsamında dikkat çekicidir.

Anahtar sözcükler: *Kefir, Kımız, Süt, Yoğurt, Plazma hücresi, Mast hücresi*

INTRODUCTION

Kefir, obtained from kefir granules or main culture of granules, is a refreshing, slightly acidic fermented dairy product with combination of ethyl alcohol and lactic acid fermentation [1]. Kefir has been reported to have antitumoral effect, effect on immune system and digestive system, effect against lactose intolerance and cholesterol, as well

İletişim (Correspondence)
+90 474 2251567
sevdaelis36@hotmail.com

as relaxing effect [2-6]. Koumiss is a dairy product produced from fermented mare's milk in Central Asia, especially in Turkestan and Mongolia [7]. Koumiss is an old Turkish drink derived from mare's milk. Koumiss ferment also includes yeasts such as Torulasp in addition to lactic acid bacteria such as *Lactobacillus bulgaricus* and *Saccharomycetales* [8]. Milk is a fluid with peculiar smell and flavor which is secreted from milk glands according to animal species at different times in order to feed new breeds and it contains essential amino acids, fat, lactose, essential minerals and vitamins that baby animals have to get until they can feed themselves [9]. Yoghurt is a fermented dairy product obtained by lactic acid fermentation with addition of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* to milk [10]. It has carbohydrates, lipids, quality protein, important minerals and B vitamins and is known to have an important place in human nutrition [11]. Plasma cells typically represent less than 1% of the cells in lymphoid organs, yet they are responsible for all antibody in circulation [12]. Because it has more phagocytic cells, spleen is an important place for defense against antigens reaching blood circulation. Spleen is an important filter for blood and antibody-producing organ as it catches blood borne antigens and reacts quickly [13,14]. Mast cells are found in the skin and in all mucosal tissues at homeostasis, and numbers are elevated in asthmatics lungs and gastrointestinal tract of inflammatory bowel disease [15]. These cells are particularly found in body surfaces such as gastrointestinal tract mucosa, skin and peritoneum in proximity to blood vessels and nerves [16] and mast cells are necessary for the development of allergic reactions, through crosslinking of their surface receptors for IgE leading to degranulation and the release of vasoactive, pro-inflammatory and nociceptive and proteolytic enzymes [17,18].

In this study, it was aimed to determine the effects of kefir, koumiss, milk and yoghurt on number of plasma cells that synthesize specific antibodies against antigens and mast cells that play important role in regulation of immune system and defense of organism in mice spleen tissue.

MATERIAL and METHODS

Experimental Animals

This study, approved by the Animal Testing Local Ethics Committee of Mehmet Akif Ersoy Universty use Committee Protocol (12/03/2014, 11/69). In the study, a total of 30 male mice *(Swiss albino)*, weighing approximately 22-35 g, which were never mated and never used in any studies, were used. Mice were randomly divided into 5 groups each containing 6 mice and fed ad libitum for fifteen days with normal rat chow and tap water, at 22±2°C ambient temperature, 12 h of light/dark cycle in standard cages.

Establishment of Experimental Groups

Group I (Kefir), kefir drink was prepared by adding kefir granules into 3% sterile milk and fermentation for 24 h at 30°C. The obtained kefir was diluted at a 1/3 rate and prepared fresh for every administration. Group II (Koumiss), koumiss obtained from Alaş Koumiss Farm (Kemalpaşa, İzmir) was used in its original form. Group III (Milk), sterile milk was given to rats in their original form, as sold in the market as UHT milk. Sterile milk used during the study contained 3% fat, 3% protein and 4.5% carbohydrates. Group IV (Yoghurt), lyophilized yoghurt culture was added into sterile milk and fermentation was achieved at 43°C for 3-4 h and yoghurt was provided to complete 24 h at +4°C. At the end, yoghurt was applied following 1/3 dilution rate. Group V (Control), this group of mice was fed with mice chow and tap water.

Live Weight Measurements

Live weight of all animals in all groups was weighed before starting 15 days administration and at the end of study. Mice were euthanized by cervical dislocation under anesthesia with diethyl ether and spleen tissues were collected. Statistical Package for Social Sciences 15.0 (SPSS 2006) program was used for statistical comparison of live weight measurements between groups. Using the Wilcoxon Signed Ranks Test, possible differences were detected.

Histological Studies

A portion of the spleen tissue fragments was fixed in alcohol-formalin fixing solution in order to determine plasma cells and pyroninophilic cells, and fixed in 10% formaldehyde solution for 48 h in order to determine mast cells. Then they were passed from graded alcohols, methyl benzoate and benzene series and blocked in paraplast embed. 5 µm sections from these blocks were stained by methyl green-pyronin staining [19] to demonstrate plasma cells and Toluidine Blue (pH 0.5) staining method to demonstrate mast cells [20].

Cell Counts and Statistical Analysis

For stereological analysis of plasma cells in spleen tissue preparations; digital camera (MBF/Bioscience, Qimaging), automatically controlled sample stepper, light microscope (Leica, DM400B) and the software program (MBF Bio-science, Stereo investigator, version 9) were used. Each region was determined to be 200.000 μm^2 in 5 different regions of spleen preparations obtained from each animal. In this field, number of plasma cells was determined in a 1 mm^2 area on the entire surface with a random systematic with 70 µm x70 µm step interval and 900 μm^2 unbiased counting frame [21]. 100 square ocular micrometer (eye piece graticule) was used in order to determine the numerical distribution of mast cells in spleen preparations stained with Toluidine Blue. The area of 100 square ocular micrometer was calculated with the help of micrometer slide at hundred (100) lens zoom of Olympus CX22-type

light microscope. Mast cell count was done with 100x lens magnification in 25 randomly selected different regions of each section. Then all the obtained data was converted to number of mast cells per 1 mm^2 unit area [19]. SPSS 15.0 was used for statistical analysis of plasma and mast cell counts in the spleen and One-Way ANOVA and Duncan's multiple comparison tests were performed.

RESULTS

Live Weight Results

As a result of the statistical data, live weights of kefir, koumiss, milk and yoghurt groups were increased compared to the control group *(Table 1)*. When groups were inter-compared, a significant difference was detected regarding live weight gain in kefir-control, koumiss-control, milk-control and koumiss-yoghurt groups *(Table 2)*.

Table 1. *A comparison of the live weight between groups*
Tablo 1. *Gruplar arasında canlı ağırlıklarının karşılaştırılması*

Groups	Number (n)	Mean ± Standard Deviation
Kefir	6	37.71±3.34
Koumiss	6	38.80±2.85
Milk	6	39.08±3.84
Yoghurt	6	36.63±2.26
Control	6	33.58±3.76

Histological Findings

In spleen tissue of all groups, specific plasma cells were detected as a result of methyl green-pyronin staining *(Fig. 1, 2, 3)*. Plasma cells were determined to be found in greater amounts in the red pulp compared to white pulp. Considering the number of plasma cells, distribution was observed to differ between the groups. When number of plasma cells in 1 mm^2 of spleen was examined by stereological methods at the 15th day a significant difference was found in number of plasma cells in the kefir group compared to koumiss, milk, yoghurt and the control group ($P<0.05$). Also increased number of plasma cells was found in koumiss, milk and yoghurt groups compared to control group ($P<0.05$) *(Table 3)*.

Specific mast cells were detected in spleen tissues of all groups *(Fig. 4, 5)*. Mast cells were determined in the red pulp in greater amount than in white pulp of spleen. It was observed that they especially concentrated around blood vessels. Considering the number of mast cells, significant differences between groups were observed regarding distribution. When number of mast cells in 1 mm^2 of spleen was examined by stereological methods at the 15th day groups, a significant difference was found in number of mast cells in the milk group compared to other groups ($P<0.05$). The largest increase in the number of mast cells of groups was determined to be in the milk group ($P<0.05$) *(Table 4)*.

Table 2. *Comparison of inter-group live weights with Wilcoxon signed-range test*
Tablo 2. *Gruplar arası canlı ağırlıkların Wilcoxon signed rang testi ile karşılaştırılması*

Test	Kefir Koumiss (g)	Kefir Milk (g)	Kefir Yoghurt (g)	Kefir Control (g)	Koumiss Milk (g)	Koumiss Yoghurt (g)	Koumiss Control (g)	Milk Yoghurt (g)	Milk Control (g)	Yoghurt Control (g)
Z value	-0.94	-0.52	-0.73	-2.20	-0.11	-1.99	-1.99	-0.73	-1.57	-1.57
P value	0.35	0.60	0.46	0.03*	0.92	0.046*	0.046*	0.46	0.03*	0.12

** P<0.05*

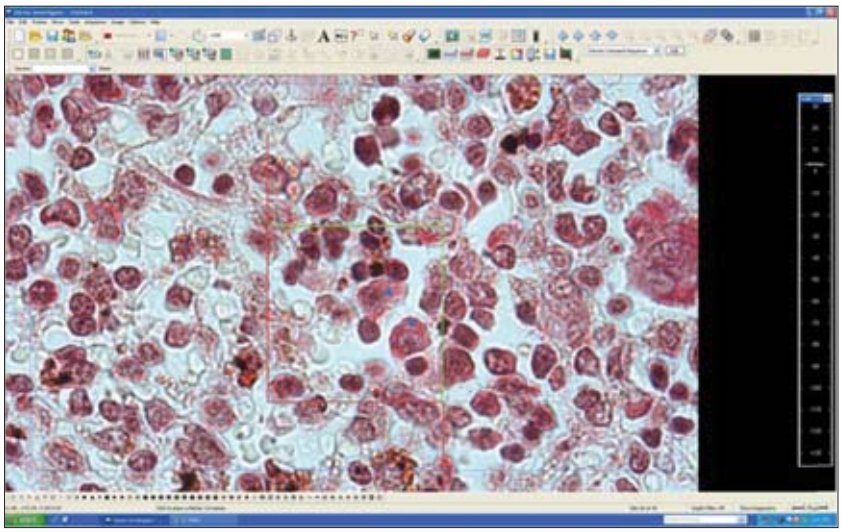


Fig 1. Specific plasma cells in mice spleen tissue as a result of methyl green-pyronin staining, 1000x

Şekil 1. Fare dalak dokusunda metil green-pironin boyaması sonucu spesifik plazma hücreleri, 1000x

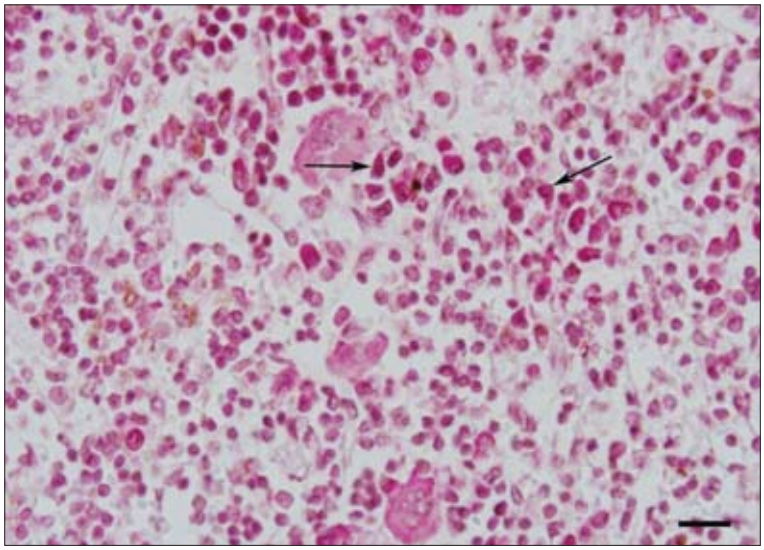


Fig 2. Specific plasma cells in mice spleen tissue as a result of methyl green-pyronin staining, Bar: 50 µm

Şekil 2. Fare dalak dokusunda metil green-pironin boyaması sonucu spesifik plazma hücreleri, Bar: 50 µm

Fig 3. Specific plasma cells in mice spleen tissue as a result of methyl green-pyronin staining, Bar: 10 µm

Şekil 3. Fare dalak dokusunda metil green-pironin boyaması sonucu spesifik plazma hücreleri, Bar: 10 µm

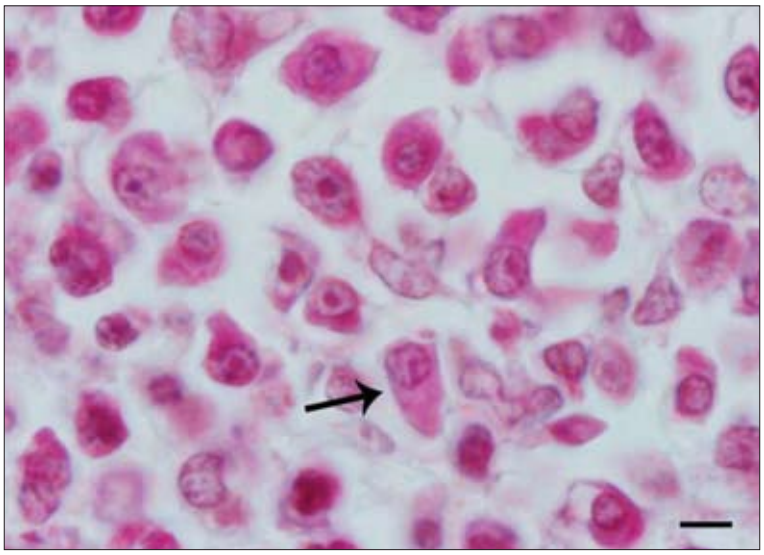


Table 3. *The number of plasma cells in the spleen*
Tablo 3. *Dalakta plazma hücre sayısı*

Cell	Control X±S	Koumiss X±S	Yoghurt X±S	Milk X±S	Kefir X±S	Significant
Plazma cells	417.5±39.0^{c}	543.5±31.4^{b}	583.5 ±33.1^{b}	551.1±49.0^{b}	732.3±35.3^{a}	P<0.001

^{a,b,c} *Differences between average values, represented by different letters in the same row, is important (P<0.05)*

DISCUSSION

Teruya et al.[22] found that kefir both increase the growth and live weight, in parallel to study of Carnevell et al.[23], in a study on the protective effects of kefir and fermented milk in case of X-ray irradiation-induced intestinal damage. Also in this study, similar to studies [23,24] reporting that beneficial microbial food increase live weight, it was detected that kefir, koumiss, milk and yoghurt administration increases live weight and that this increase is statistically significant (P<0.05). Live weight gain in kefir, koumiss, milk and yoghurt group being more compared to the control group suggests that it arise from kefir, koumiss, milk and yoghurt containing different beneficial organisms [25]. Beyond the inherent high nutritional value as a source of protein and calcium, kefir is believed to be beneficial to health in the countries where kefir is an essential part of the diet culture for a long time [26,27]. Effects of kefir on antioxidant and growth factors have been shown in several studies [22,28-33] found that the protective effect of kefir is more than vitamin E in oxidative damage induced by CCl_4 in mice, by reducing lipid peroxidation and increasing reduced glutathione and glutathione peroxidase levels. In recent years in the mentality of a healthy diet, nutrition support with antioxidant-rich foods to prevent damages causing

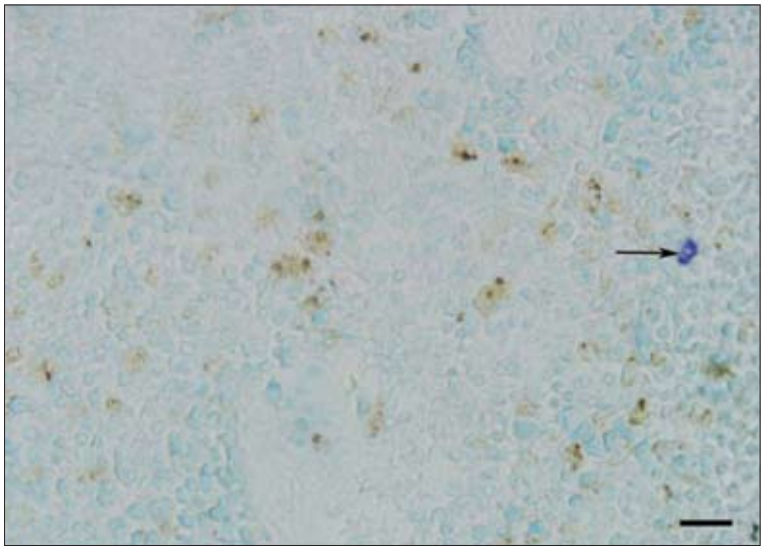
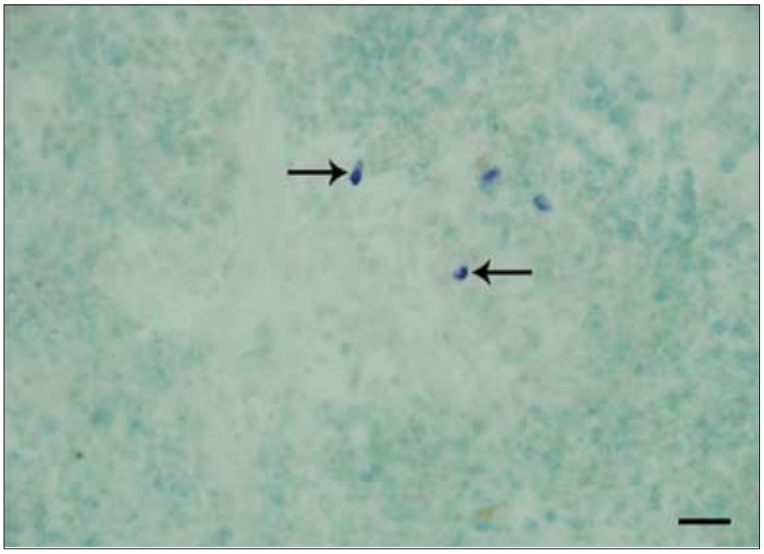


Fig 4. Specific mast cells in mice spleen tissue as a result of Toluidine Blue, Bar: 50 µm

Şekil 4. Fare dalak dokusunda Toluidine Blue boyaması sonucu spesifik mast hücreleri, Bar: 50 µm

Fig 5. Specific mast cells in mice spleen tissue as a result of Toluidine Blue, Bar: 50 µm

Şekil 5. Fare dalak dokusunda Toluidine Blue boyaması sonucu spesifik mast hücreleri, Bar: 50 µm



Tablo 4. *Dalakta mast hücre sayısı*
Table 4. *The number of mast cells in the spleen*

Cell	Control X±Sx	Koumiss X±Sx	Yoghurt X±Sx	Milk X±Sx	Kefir X±Sx	Significant
Mast cell	4.56±0.32^{c}	6.40±0.71^{b}	6.11 ±0.42^{bc}	8.12±0.46^{a}	5.95±0.63^{bc}	P<0.001

$^{a, b, c}$ Differences between average values, represented by different letters in the same row, is important (P<0.05)

oxidative stress in the body has risen to the forefront. In this direction, antioxidant substances may avoid oxidation damages by preventing the formation of free radicals or stopping or decreasing the activity of formed free radicals [34]. In our study, the number of plasma cells in the spleen of mice treated with kefir is significantly higher than mice in koumiss, milk, yoghurt, and control group. Likewise plasma cell number of mice treated with koumiss, milk and yoghurt is significantly greater than control group. When the groups were compared, plasma cell counts in spleen, which has a very important role in organism's defense by containing antigen presenting cells to T and B lymphocytes and macrophages, being significantly more in kefir group than koumiss, milk and yoghurt group is suggesting that kefir has an effect on natural immune system, modulates the immune system and also it has ability to stimulate immune cells.

Koumiss is rich in fatty acids as linoleic acid and arachidonic acid. These fatty acids being high enhance the importance in nutritional terms. Healing effect of koumiss is brought about intermediates as a result of fermentation of vitamins C, carbondioxide, lactic acid and alcohol contained in mare milk [35]. In our study, koumiss, milk and yoghurt were found to significantly increase plasma cell count in spleen, which response by starting

immune response that then activates T and B cells against blood antigens, compared to control group. Mare milk has essential features for human nutrition due to high amounts of polyunsaturated fatty acids, low cholesterol content and different protein structure [36]. Kefir, koumiss, milk and yoghurt, comprising useful microorganisms, are thought to potentiate the mucosal defenses, induce mucosal antibody response and cellular immune response and play immune modulatory role by stimulating cell activity.

It wasn't come across so many literature about mast cells population and functions in mammalian lymphoid organs. In our study, the number of mast cells in the spleen of mice treated with milk was significantly higher than the control group. Although number of mast cells of mice treated with kefir, koumiss and yoghurt were more than the control group, no statistically significant difference was detected. In this study, mast cells in the spleen of mice treated with milk showed numerical differences. Mast cells are the effector cells of the immune system found in all of connective tissue. They play a very critical role in allergic reactions [37,38]. Despite the entire literature search, no study investigating the effect of milk administration on mast cells in the spleen in a mice model and anti-allergic effects of milk was observed. The role of mast cells in allergic tissue inflammation is well known. IL-4 plays a role in mast cell proliferation and release of IgE-dependent mast cell mediators. Activated mast cells secrete Th2-type cytokines such as IL-3, IL-5, and IL-13 and leads to the accumulation of eosinophils and other inflammatory cells [39]. Milk, necessary in all stages of human life, is thought to cause contraction of smooth muscles and neurogenic vasodilatation by increasing vascular permeability of histamine, the most important and best-known mast cell mediator, and have profound effects on natural and acquired immune system by their effects on T lymphocytes.

In conclusion; as the number of plasma cells in the spleen is at higher level with Kefir, consisting of beneficial bacteria, than with koumiss, milk and yoghurt; also there being a significant difference between the numbers of mast cells in the spleens of mice treated with milk compared to control group, this is remarkable for healthy eating/ functional food. As there are limited researches on this subject, new researches will undoubtedly be important.

REFERENCES

1. Karatepe P, Yalçın H, Patır B, Aydın I: Kefir ve kefirin mikrobiyolojisi. *Elekt Mikrobiyol Derg*, 10 (1): 1-10, 2012.

2. Guzel-Seydim ZB, Greene AK, Taş T: Determination of antimutagenic properties of some fermented milks including changes in the total fatty acid profiles including CLA. *Int J Dairy Tech,* 59 (3): 209-215, 2006. DOI: 10.1111/j.1471-0307.2006.00265.x

3. Hertzler SR, Clancy SM: Kefir improves lactose digestion and tolerance in adults with lactosemal digestion. *J Am Dietetic Assoc*, 103 (5): 582-587, 2003. DOI: 10.1053/jada.2003.50111

4. Liu JR, Chen MJ, Lin CW: Antimutagenic and antioxidant properties of milk-kefir and soymilk-kefir. *J Agric Food Chem*, 53 (7): 2467-2474, 2005.

5. Otles S, Cagindi O: Kefir: A probiotic dairy composition, nutritional and therapeutic aspects. *Pakistan J Nutrition*, 2 (2): 54-59, 2003. DOI: 10.3923/pjn.2003.54.59

6. Sarkar S: Potential of kefir as a dietetic beverage: A review. *Br Food J*, 109 (4): 280-90, 2007. DOI: 10.1108/00070700710736534

7. Kurdal E: Kımız. *Atatürk Üniv Ziraat Fak Derg,* 24 (1): 233-225, 1993.

8. Dilanyan SH: Utilisation of mares', ewes', camels and yaks' milk in the USSR. Report Int. Comm. Dairying in warmcountries. Brussels, Belgium: *Int Dairy Federation*, 1959.

9. Demirci M: Süt Teknolojisine Giriş: Yayın No: 105, Ders Notu: 68, Trakya Üniv. Tekirdag Ziraat Fakültesi, Tekirdağ, 1992.

10. Perdigon G, De Moreno de Le Blanc A, Valdez J, Rachid M: Role of yoghurt in the prevention of colon cancer. *Eur J Clin Nutr*, 56 (3): 65-68, 2002. DOI: 10.1038/sj.ejcn.1601490

11. Plessas S, Bosnea L, Alexopoulos A, Bezirtzoglou E: Potential effects of probiotics in cheese and yoghurt production. *Eng Life Sci*, 12 (4): 433-440, 2012. DOI: 10.1002/elsc.201100122

12. Kallies A, Hasbold J, Tarlinton DM, DietrichW, Corcoran LM, Hodgkin PD: Plasma cell ontogeny defined by quantitative changes in Blimp-1 expression. *J Exp Med*, 200, 967-977, 2004. DOI: 10.1084/jem.20040973

13. Junqueira LC, Carneiro J: Temel Histoloji. Solakoğlu S, Aytekin Y (Çeviri Editörleri), 99-274, Nobel Tıp Kitap Evleri, İstanbul, 2009.

14. Berne RM, Levy MN, Koeppen BM, Stanton BA: Fizyoloji. Türk Fizyolojik Bilimler Derneği. 5th ed., 520-521, Güneş Tıp Kitapevleri, Ankara, 2008.

15. Amin K: The role of mast cells in allergic inflammation. *Respir Med,* 106 (1): 9-14, 2012. DOI: 10.1016/j.rmed.2011.09.007

16. Mierke CT, Ballmaier M, Werner U, Manns MP, Welte J, Bischoff SC: Human endothelial cells regulate survival and proliferation of human mast cells. *J Exp Med,* 192 (6): 801-812, 2000. DOI: 10.1084/jem.192.6.801

17. Kobayashi H, Ishizuka T, Okayana Y: Human mast cells and basophils as sources of cytokines. *Clin Exp Allergy,* 30 (9): 1205-1212, 2000. DOI: 10.1046/j.1365-2222.2000.00808.x

18. Galli SJ, Wedemeyer J, Tsai M: Analyzing the roles of mast cells and basophils in host defense and other biological responses. *Int J Hematol*, 75: 363-369, 2002.

19. Böck P: Romeis Mikroskopische Technik. 17th ed., 561, Urban and Schwarzenhferg Verlag, München, 1989.

20. Enerback L: Mast cells in rat gastrointestinal mucosa. I. effect of fixation. *Acta Pathol Microbiol Scand,* 66 (3): 289-302, 1966.

21. Yigit F, Aktas A, Daglioglu S: Effects of bisphenol A and diethyl-stilbestrol on the involution of bursa of fabricius in the hens. *Istanbul Univ Vet Fak Derg,* 39 (2): 168-174, 2013.

22. Teruya K, Myojin-Maekawa Y, Shimamoto F, Watanabe H, Nakamichi N, Tokumaru K, Tokumaru S, Shirahataa S: Protective effects of the fermented milk kefir on X-Ray irradiation- induced intestinal damage in B6C3F1 mice. *Biol Pharm Bull,* 36 (3): 352-359, 2003.

23. Carnevali, O, Vivo L, Sulpizio R, Gioacchini GI, Olivotto I, Silvi S, Cresci A: Growth improvement by probiotic in European seabassjuveniles (*Dicentrarchuslabrax*, L.) with particular attention to IGF-1, myostatin and cortisol gene expression. *Aquacult,* 258 (1-4): 430-438, 2006. DOI: 10.1016/j.aquaculture.2006.04.025

24. Kabir SML, Rahman MM, Rahman MB, Rahman MM, Ahmed SU: The dynamics of probiotics on growth performance and immune responce in broiler. *Int J Poult Sci,* 3 (5): 361-364, 2004.

25. Farnworth ER: Kefir a complex probiotic. *Food Sci Technol Bull: Funct Foods,* 2 (1): 1-17, 2005. DOI: 10.1616/1476-2137.13938

26. Vinderola G, Perdigon G, Duarte J, Thangavel D, Farnworth E, Matar C: Effects of kefir fractions on innate immunity. *Immunobiology*, 211 (3): 149-156, 2006. DOI: 10.1016/j.imbio.2005.08.005

27. Yaman H, Elmalı M, Kamber U: Observation of lactic acid bacteria and yeast populations during fermentation and cold storage in cow's,

ewe's and goat's milk kefirs. *Kafkas Univ Vet Fak Derg,* 16 (Suppl-A): S113-S118, 2010.

28. Lin MY, Change FJ: Antioxidativeeffect of intestinal bacteria *bifido bacterium longum* ATCC 15708 and *lacto bacillus acidophilus* ATCC 4356. *Dig Dis Sci*, 45 (8): 1617-1622, 2000. DOI: 10.1023/A:1005577330695

29. Güven A, Güven A, Gülmez M: The Effect of kefir on the activities of GSH-Px, GST, CAT, GSH and LPO levels in Carbon Tetrachloride-induced mice tissues. *J Vet Med*, 50 (8): 412-416, 2003. DOI: 10.1046/j.1439-0450.2003.00693.x

30. Sozmen M, Erginsoy SD, Cenesiz S, Devrim AK: The protective effect of kefir and vitamin C on Azoxymethane induced toxicity and induction of metallothionein in mice. *Scand J Lab Anim Sci,* 32 (4): 211-220, 2005.

31. Liu JR,Wang SY, Lin YY, Lin CW: Antitumoractivity of milk kefir and soy milk kefir in tumor-bearing mice. *Nutr Cancer*, 44 (2): 183-187, 2002.

32. Sari EK, Bakir B, Aydin BD, Sozmen M: The effects of kefir, koumiss, yoğurt and commercial probiotic formulations on PPARα and PPAR-β/δ expressions in mouse kidney. *Biotech Histochem,* 84 (4): 287-295, 2014. DOI: 10.3109/10520295.2013.844274

33. Güven A, Güven A, Gülmez M, Beytut E, Erişir M: Aterosklerotik farelerde kefir ve yoğurdun lipidperoksidasyonuna ve antioksidan enzimlere etkileri. *Kafkas Univ Vet Fak Derg,* 9 (1): 79-83, 2003.

34. Singh S, Singh RP: *In vitro* methods of assay of antioxidants: An overview. *Food Rev Int*, 24 (4): 393-415, 2008.

35. Yaygın H: Kımızın nitelikleri ve sağlık ile ilgili özellikleri. *Gıda*, 16 (2): 111-115, 1991.

36. Kucukcetin A, Yaygin H, Hinrichs J, Kulozik U: Adaptation of bovine milk towards mares' milk composition by means of membrane technology for koumiss manufacture. *Int Dairy J,* 13 (12): 945-951, 2003. DOI: 10.1016/S0958-6946(03)00143-2

37. Frungieri MB, Weidinger S, Meineke V, Köhn FM, Mayerhofer A: Proliferative action of mast-celltryptase is mediatedby PAR2, COX2, prostaglandins, and PPAR gamma: Possible relevance to human fibrotic disorders. *Proc Natl Acad Sci USA,* 12, 99 (23): 15072-15077, 2002.

38. Kurum A, Özen A, Karahan S, Özcan S: Investigation of mast cell distribution in the ovine oviduct during oestral and luteal phases of the oestrous cycles. *Kafkas Univ Vet Fak Derg*, 20 (6): 915-920, 2014. DOI: 10.9775/kvfd.2014.11398

39. Untersmayr E, Jarolim JJ: Mechanism of type I food allergy. *Pharmacol Ther*, 112 (3): 787-789, 2006.