Investigation The Protective Effects of Kefir in Experimental Diabetes Mellitus and Nonalcoholic Liver Fattened Rats

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Abstract: In this study the protective effect of kefir against liver tissue damage at experimental Type 2 Diabetes Mellitus (T2DM) and Nonalcoholic Fatty Liver Disease (NAFLD) was investigated. A total of 30 Wistar albino rabbit female rats were used. Rats were divided into 5 groups each group had 6 animals. Any application was done to control group animals. A single dose of 80 mg / kg intraperitoneal Streptozotocin was given to the animals to form T2DM, and a high fat rat was fed to the animals throughout the experiment to generate NAFLD. The animals in the experimental group were fed high fat rat diet, high fat rat diet + 30 ml / kg kefir (oral gavage), high fat rat diet + Streptozotocin 80 mg / kg intraperitoneal (IP), high fat rat diet + 30 ml / kg kefir Oral gavage) + Streptozotocin 80 mg / kg (IP). A decrease in blood glucose levels was observed with the addition of kefir compared to the T2DM group. Also increased serum AST, ALT, total protein, cholesterol, triglyceride levels were decreased by the addition of kefir in the T2DM and NAFLD groups. Histopathological findings also support biochemical results. In the liver of kefir-added group, close to normal histological structure was observed. As a result, it was concluded that consumption of kefir beverage would be beneficial against T2DM and NAFLD, which cause serious damage to the liver.

Keywords:*Liver, Kefir, Nonalcolic fatty liver disease, Type 2 diabetes mellitus.*

Deneysel Diabetes Mellitus ve Nonalkolik Karaciğer Yağlanması Oluşturulan Ratlarda Kefirin Koruyucu Etkilerinin Araştırılması

Özet: Bu çalışmada kefirin deneysel olarak oluşturulan Tip 2 Diabetes Mellitus (T2DM) ve Nonalkolik Karaciğer Yağlanmasında (NAFLD) karaciğer dokusunda oluşan hasara karşı koruyucu etkileri araştırıldı.Toplam 30 adet Wistar albino ırkı dişi rat kullanıldı. Ratlar her grupta 6 adet olmak üzere 5 gruba ayrıldı. Kontrol grubu hayvanlara herhangi bir uygulama yapılmadı. T2DM oluşturmak amacıyla hayvanlara tek doz 80 mg/kg intraperiteonal Streptozotocin, NAFLD oluşturmak için de hayvanlara yüksek yağlı rat yemi deneme boyunca verildi. Deneme grubundaki hayvanlara sırasıyla yüksek yağlı rat yemi, yüksek yağlı rat yemi+30 ml/kg kefir (oral gavaj), yüksek yağlı rat yemi+Streptozotocin 80 mg/kg intraperiteonal (İP), yüksek yağlı rat yemi+30 ml/kg kefir (oral gavaj)+ Streptozotocin 80 mg/kg (İP) verildi. T2DM oluşturulan gruba kıyasla kan glikoz düzeylerinde kefir eklenmesi ile düşme gözlendi. Ayrıca T2DM ve NAFLD grubunda serumda artan AST, ALT, total protein, kolesterol, trigliserit değerlerinin kefir eklenmesi ile azaldığı gözlendi. Histopatolojik bulgular da biyokimyasal sonuçları desteklemektedir. Kefir eklenen grubun karaciğerlerinde normale yakın histolojik yapı gözlendi. Sonuç olarak karaciğer dokusunda ciddi hasarlara neden olan T2DM ve NAFLD ye karşı kefir içeceğinin tüketilmesinin yararlı olacağı kanısına varıldı.

Anahtar Kelimeler:*Karaciğer, Kefir, Nonalkolik karaciğer yağlanması, Tip 2 diabetes mellitus.*

Introduction

Diabetes mellitus is a disease with acute and chronic complications (Powers, 2005). Of course, the most serious health problems result in chronic degenerative complications (Kahn et al., 2005). These complications include atherosclerosis, microangiopathy, nephropathy and neuropathy (Brownlee, 2001; Vlassara et al., 1984; Yabe-Nishimura, 1998), and pancreatic necrotic degenerative changes in the liver (Mir and Darzi, 2009; Sağkan Öztürk et al., 2015). Lipoidozis in the liver due to alcohol or non-alcohol can be detected. Liver fatigue is considered as a disease in itself. If liver lipoidozis has done without alcohol it named as "Non-alcoholic fatty liver disease (NAFLD)". This histopathological findings are similar to alcoholic liver disease. NAFLD itself includes some subgroups. First non-alcoholic fatty liver (NAFL) (hepatic steatosis is seen withoutinflammation) and second non-alcoholic steatohepatitis (NASH) (because of hepatic steatosis is associated with hepatic

inflammation, histologically undistinguishable from alcoholic steatohepatitis) (Bellentani, 2010; Shet et al., 1997; Sonsuz, 2007). Kefir, a natural probiotic beverage, is a fermented dairy product of Caucasian origin (Korolev, 1988; Kubo et al., 1992, Zubillaga et al., 2001). Many benefit effects of kefir such as anti-inflammatory (Ozsoy, 2016), antibacterial, antitumoral, immunological (Furukawa et al., 1990), antioxidant and antifungal (Hoolihan, 2001), cholesterol lowering (Matsuu et al., 2003) and anti-apoptotic (Mumford, 2007) effects had been reported previously.

The aim of this study was to investigate the protective effects of kefir against hepatocellular injury in experimentally generated T2DM and NAFLD. This study also was proposed tocontribute to both public health and animal health by ensuring that not only veterinarians but also human physicians can access the results of the study.

Materials and Methods

For this study, approval was obtained from Mustafa Kemal University Animal Experiments Local Ethics Committee with decision no. 2015 / 10-7.

In the study a total of 30, 4-5 months age Wistar albino female rats were used. Rats were divided into 5 groups. One control (C), four experimental group those consisted of 6 rats. Animals were weighed and recorded at the beginning and end of the study. At the end of the experiment, body weight and body weight gains were determined. The animals were kept in plastic cages.The kefir was prepared by adding 3% of kefir seed and sterilized at 30 ° C for 24 hours. During the five-week trial, the control group received standard commercial rat diet and all groups received drinking water *ad libitum*. All experimental groups were fed pellet containing 35% vegetable oil (canola oil) to form NAFLD. Group 1 received high fat rat diet, Group 2 received high fat rat diet + 30 ml / kg kefir (oral gavage, fresh, daily), Group 3 received high fat rat diet + Streptozotocin 80 mg / kg intraperitoneally (IP), Group 4 received high fat rat diet + 30 ml / kg kefir (oral gavage, fresh, daily) + Streptozotocin 80 mg / kg (IP).

Blood glucose was checked with blood sugar test strips (Accu-chek sensor of Roche Diagnostics, Germany). At the end of the study blood samples were taken from 30 animal into EDTA tubes. AST, ALT, total protein, cholesterol, triglyceride assays were performed in a special laboratory with an autoanalyzer after the blood was centrifuged at 3000 rpm for 5 minutes. For histopathological examination, animals were euthanized by decapitation method under anesthesia. Liver tissue samples were removed and fixed in 10% buffered formalin, followed by tissue patching according to routine methods. Sections taken at 5 µm thickness were stained with Hematoxylin Eosin (H & E) (Luna, 1968). Microphotographs were taken after examination under light microscope. One way ANOVA with SPSS program was used in the statistical analysis of the study (Dawson, 2001).

Results

The body weights of the groups fed with high fat dietwere found to be higher than the controls (summarized in Table 1). The highest glucose level was found as 281.68 mg / dl in the third group (only T2DM). In Group 4 (T2DM+Kefir), this level was decreased to 237.20 mg / dl. In the control and first and second experimental groups, the sugar level was found within the physiological limits. Increased serum AST, ALT, total cholesterol, and triglyceride values in the T2DM and NAFLD groups decreased with the addition of kefir (summarized in Table 2).

Table 1. The initial and final body weights of groups.

	Control	1.Group	2.Group	3.Group	4.Group	P
BW İnitial (g)	236,68±4,49	232,52±3,64	236,87±6,67	235,5±6,20	234,52±2,87	0,98
BW Final (g)	246,54±5,03	253,24±5,43	264,67±7,84	266,24±5,55	259,28±2,85	0,103

No significant difterinceaman groups.

Table 2. Blood glucose and biochemical blood parameters in groups.

	Control	1.Group	2.Group	3.Group	4.Group	P
Glucose mg/dl	111,29±4,96^{b}	121,04±4,59^{b}	107,33±3,16^{b}	272,98±11,49^{a}	257,20±4,32^{a}	0,000
Total protein mg/dl	57,64±0,82	59,57±1,26	59,36±1,43	59,60±0,59	60,29±1,93	0,68
Triglyceride mg/dl	68,46±2,02^{c}	83,70±3,43^{ab}	65,66±3,18^{c}	91,80±3,61^{a}	74,55±4,76^{bc}	0,000
Cholesterol mg/dl	32,51±1,43^{b}	35,05±1,29^{ab}	34,37±1,11^{b}	38,62±1,55^{a}	33,53±1,05^{b}	0,03
AST IU/l	60,11±2,07	63,58±1,88	61,39±1,01	66,63±1,24	61,73±1,62	0,07
ALT IU/l	41,12±2,40	46,09±1,62	42,03±1,27	47,73±2,36	43,08±0,99	0,08

a,b,c: Means within a row followed by the different superscripts differ significantly (p<0.001).

Macroscopically, there was no pathological change in the liver color and consistency of the control group animals. Especially, the liver of NAFLD group was more yellowish in color and the consistency was crispy and greasy. T2DM and kefir groups showed a decrease in oily appearance compared to group 2. Normal histological structure was observed in the liver tissues of the control group (Figure 1). In the T2DM group, passive hyperemia of the liver, prominence in Kupffer cells, degenerative changes in hepatocytes, and few fat vacuoles were observed (Figure 2A). In the liver of NAFLD group, it was observed that thefat vacuoles in hepatocytes were more diffuse and larger than the T2DM group. Also similarly, degenerative changes in hepatocytes, and increased in the number of Kupffer cells was occured (Figure 2B). When compared with liver tissues in the T2DM group and the NAFLD group, kefir groups showed less degenerative changes in liver hepatocytes and less favorable vacuolization and showed normal histological appearance similar to the control group (Figures 2C and 2D).

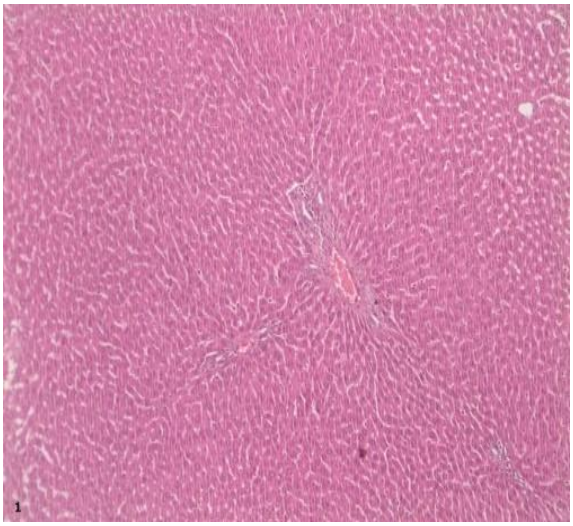

Figure 1. Control group; normal histological architecture of liver, H & E X10.

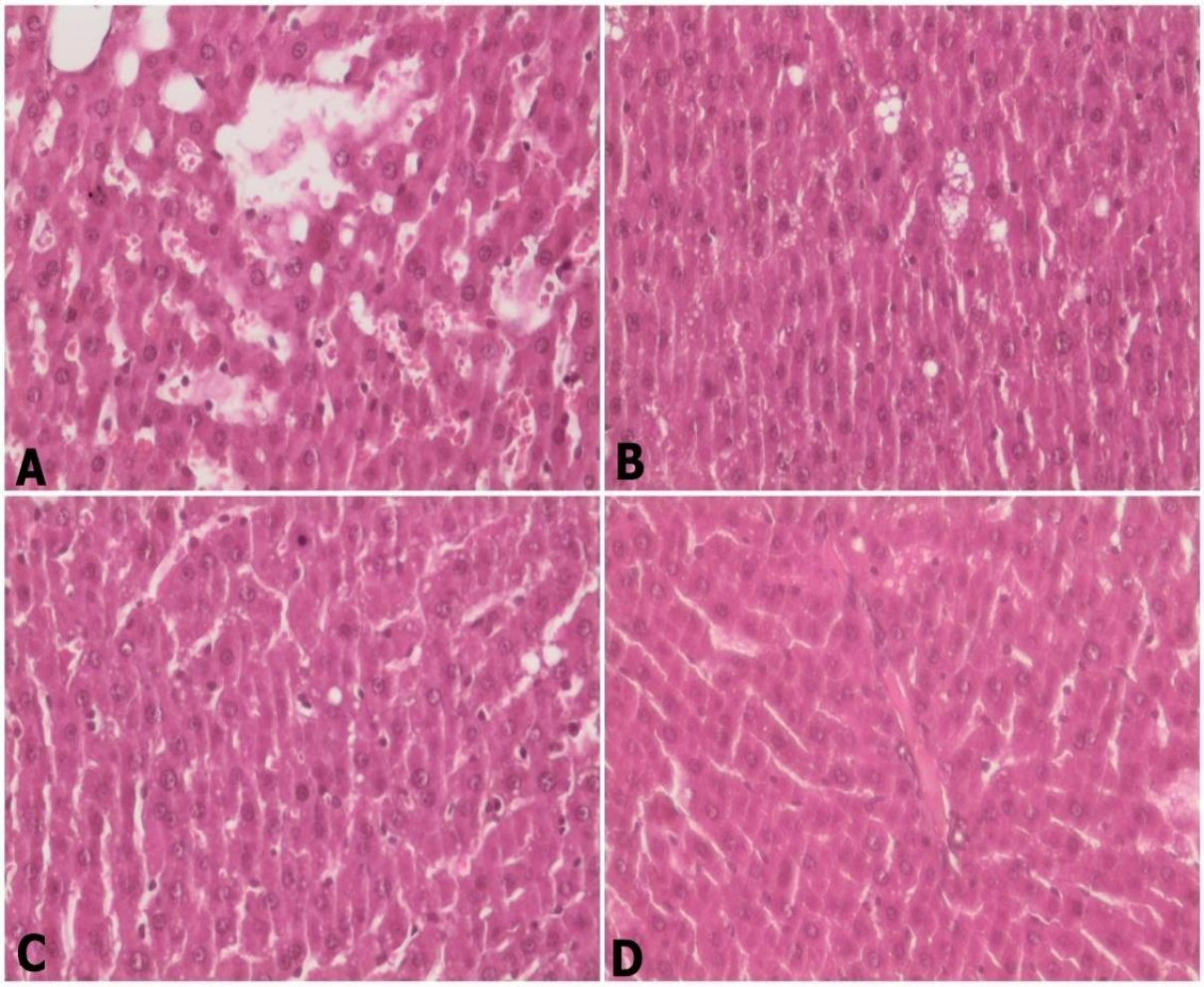

Figure 2. A) T2DM group; degenerative changes with fat vacuoles at hepatocytes, H & E X40. **B)** NAFLD group; degenerative changes with large fat vacuoles at hepatocytes, H & E X40. **C)** T2DM+Kefir group; some degenerative hepatpcytes and few fat vacuoles, H & E X40. **D)** NAFLD+Kefir group; seems nearly control group, H & E X40.

Discussion and Conclusion

The weight gain in the trial groups was an expected situation when previous studies (Akbarzadeh et al. 2007; İşbilen et al., 2007) were considered. The antioxidant, hepatoprotective (Hoolihan, 2001; Ozsoy, 2016), cholesterol lowering (Matsuu et al., 2003; St-Onge et al., 2000) effects of kefir again was proven by decreased serum AST, ALT, total cholesterol, and triglyceride levels in kefir added groups of our study. Bunar et al (2014) reported that with kefir administration in diabetic rats a significant reduce in blood glucose. But in our study the high blood glucose level in the T2DM group was not as impressed as we expected in the kefir group. Diabetes mellitus with acute and chronic complications and degenerative changes in the liver responsible for metabolism and excretion have been reported in previous studies (Sağkan Öztürk et al. 2015; Mir and Darzi, 2009). In diabetic liver degenerative changes in hepatocytes (Benjamin et al. 2006; Mir and Darzi, 2009), severe lipidesin and vacuolization in bile dutepitelyum (Charles, 2006) can be seen. Degeneration and fat vacuoles in hepatocytes were observed as similar histopathological changes in the study.There are also recent studies on non-alcoholic liver fat deposition. According to histopathological findings, microvesicular lipoidosis / macroveicular lipoidosis / mixed type lipoidosis can be found in many places (Sonsuz, 2007). Similarly, in the study, fat vacuoles were observed in the experimental NAFLD group's livers.

In conclusion in this study we observed that with kefir administration the lowest liver damage was observed.This situation was proven by blood sera parameters and histopathologically. Also there was a slight decrease of blood glucose with kefir administration in T2DM group. May be this protective effect can be improved by increasing the amount of kefir. We think that consumption of kefir is increasing day by day as people with addition of new innovation to the study that explains the antioxidant immunological, antitumoral and cholesterol-lowering effects of kefir.

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