Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1625516

Effect of probiotics on kidney tissue in an experimental diabetes model

Deneysel diyabet modelinde probiyotiklerin böbrek dokusuna etkisi

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Posted date:27.01.2025 Acceptance date:18.03.2025

Abstract

Purpose: Diabetes, a major public health issue, is an endocrine and metabolic disease that causes damage to various tissues, including the kidneys. Probiotics are thought to play a beneficial role in the prevention and treatment of diseases when used in sufficient amounts. This study aims to investigate the effects of probiotic supplementation on kidney tissue damage induced by diabetes.

Materials and methods: A total of 34 rats were divided into five groups. The control group (K, n=5) received PBS, while the probiotic-only group (Pm, n=5) was given probiotics (0.6 mg/kg). In the diabetes group (Dm, n=8), diabetes was induced using streptozotocin (50 mg/kg). The PmD group (n=8) received probiotic supplementation before diabetes induction, and probiotic administration continued after diabetes was induced. In contrast, the DmP group (n=8) first underwent diabetes induction, followed by probiotic supplementation. Kidney tissues were examined histopathologically and immunohistochemically, with Bcl-2 and alpha-SMA antibody expressions evaluated.

Results: No histopathological alterations were observed in the control group. In the Dm group, moderate-to-mild nephrotoxicity was detected, while the probiotic-supplemented diabetes groups exhibited mild nephrotoxicity. Bcl-2 expression was decreased in the Dm group but was found to be higher in the DmP and PmD groups. Conversely, alpha-SMA expression was elevated in the Dm group, whereas it was lower in the DmP and PmD groups.

Conclusion: Histopathological and immunohistochemical analyses indicate that probiotics exert a protective effect against diabetes-induced kidney damage.

Keywords: Diabetes, probiotic, kidney, alpha-SMA, Bcl-2.

Yesil Z, Cetin H, Lafci I, Tan S, Vural BK, Ozdamar S. Effect of probiotics on kidney tissue in an experimental diabetes model. Pam Med J 2025;18:340-355.

Öz

Amaç: Diyabet, önemli bir halk sağlığı sorunu olan endokrin ve metabolik bir hastalıktır. Tüm dokularda olduğu gibi böbrek dokusunda da hasara yol açmaktadır. Probiyotiklerin yeterli miktarda kullanılması, hastalıkların önlenmesi ve tedavisinde faydalı olabileceği düşünülmektedir. Bu çalışmada, diyabetin böbrek dokusunda oluşturduğu hasara karşı probiyotik takviyesinin etkisini araştırmayı amaçladık.

Gereç ve yöntem: Toplam 34 sıçan beş gruba ayrıldı. Kontrol grubuna (K, n=5) PBS, yalnızca probiyotik alan gruba (Pm, n=5) ise probiyotik (0,6 mg/kg) uygulandı. Diyabet grubu (Dm, n=8) streptozotosin (50 mg/kg) ile diyabet oluşturuldu. PmD grubuna (n=8) diyabet oluşturulmadan önce probiyotik takviyesi uygulandı ve diyabet oluştuktan sonra da probiyotik uygulamasına devam edildi. DmP grubunda (n=8) ise önce diyabet oluşturuldu, ardından probiyotik takviyesi uygulandı. Böbrek dokuları histopatolojik ve immünohistokimyasal olarak incelendi; Bcl-2 ve alfa-SMA antikor ekspresyonları değerlendirildi.

Bulgular: Kontrol grubunda histopatolojik değişiklikler gözlenmezken, Dm grubunda orta-hafif nefrotoksisite saptandı. Probiyotik takviyesi alan diyabet gruplarında ise hafif nefrotoksisite gözlendi. Dm grubunda Bcl-2 ekspresyonu azalmış olup, DmP ve PmD gruplarında ise daha yüksek seviyelerde olduğu tespit edilmiştir. Alfa-SMA ekspresyonu ise Dm grubunda artarken, DmP ve PmD gruplarında daha düşük seviyelerde olduğu gözlenmiştir.

Sonuç: Histopatolojik ve immünohistokimyasal değerlendirmeler, probiyotiklerin diyabetin neden olduğu böbrek hasarına karşı koruyucu bir etki gösterdiğini ortaya koymaktadır.

Anahtar kelimeler: Diyabet, probiyotik, böbrek, alfa-SMA, Bcl-2.

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Yeşil Z, Çetin H, Lafcı İ, Tan S, Vural BK, Özdamar S. Deneysel diyabet modelinde probiyotiklerin böbrek dokusuna etkisi. Pam Tıp Derg 2025;18:340-355.

Introduction

Diabetes mellitus (diabetes) is a chronic metabolic disease characterized by insulin deficiency and the associated pathological consequences [1]. Globally, while significant reductions in mortality from non-communicable diseases such as chronic respiratory diseases, cardiovascular diseases, and cancer were observed between 2000 and 2019, diabetes-related mortality increased by 3% during the same period [2]. Approximately 10-20% of patients with diabetes succumb to renal failure, and it has been reported that 50% of patients undergoing dialysis have diabetes [3].

Kidneys are important organs that maintain the acid-base balance of the body by filtering the blood from metabolic wastes [3]. In histopathological evaluation of the tissue in diabetes-induced renal damage, podocyte damage, cell shedding and apoptosis are among the primary pathological findings. Damage to podocytes exacerbates proteinuria in diabetes [4].

Diabetes adversely affects multiple organs, including the heart, blood vessels, kidneys, eyes, and nerves. One of the most well-documented renal complications of diabetes is diabetic nephropathy. Experimental diabetes models have demonstrated that diabetic nephropathy impairs kidney function and leads to histological damage in the glomerular and tubular structures [5].

In addition to conventional pharmacological treatments, medical nutrition therapy plays a key role in diabetes management. Patients who adhere to medical nutrition therapy achieve better blood glucose control and a reduced risk of complications [6]. In addition, the occurrence of gastrointestinal dysfunction, which shows symptoms such as obesity, delayed gastric emptying, diabetic gastroparesis, diarrhea and constipation, is considered a contributing factor in the pathogenesis of type 2 diabetes [7-9]. This suggests that the risk of developing type 2 diabetes may be influenced by factors related to the gut microbiota [10]. Probiotics, defined as live microorganisms that confer health benefits when administered in sufficient amounts, are key components of the gut microbiota. Evidence from animal studies suggests that probiotics may modulate glucose metabolism and enhance insulin sensitivity [11, 12]. Recently, research on the therapeutic potential of probiotics, particularly their antibacterial and anti-inflammatory properties, has gained momentum. The concept that modulating the gut microbiota is crucial for overall health has become increasingly recognized [13].

In this study, we aimed to investigate the effects of probiotic supplementation on the kidneys in a streptozotocin (STZ)-induced diabetic rat model by analyzing biochemical markers and assessing Bcl-2 and alpha-SMA expression via immunohistochemical methods.

Materials and methods

Permission for the study was obtained from Pamukkale University Animal Experiments Ethics Committee with the decision numbered and dated PAUHDEK-2021/07, 24.08.2021.

Experimental study design

The effect size obtained in the reference study [31] was found to be strong (F=0.786). Assuming that an effect size at this level could be obtained, as a result of the power analysis performed for 5 groups, it was calculated that 90% power could be obtained at 95% confidence level when at least 35 rats (at least 7 rats for each group) were included in the study. Since the control and probiotic-only groups were the control group, it was deemed appropriate to use 5 rats each in these groups and 8 animals each in the diabetes groups since animal loss due to diabetes could be seen.

In the study, 10-week-old Wistar rats weighing between 250-300 g, were used (n=34). The animals were kept in specially designed cages under the supervision of a veterinarian and maintained in a temperature-controlled environment with 50%±5% humidity under laboratory conditions simulating a 12-hour day-night cycle. The rats were given standard rat

pellet food and tap water ad libitum. The rats were randomly assigned to five groups: control group (group K, n=5), probiotic group (group Pm, n=5), diabetes group (group Dm, n=8), probiotic + diabetes group (group PmD, n=8) and diabetes + probiotic group (group DmP, n=8). VSL#3 was used as probiotic. VSL#3 contains 3 genera, 7 species and 8 strains. The species included 4 Lactobacilli, 3 Bifidobacteria and 1 Streptococcus strains (Table 1).

Table 1. Probiotic microorganisms contained in VSL#3

Streptococcus thermophilus BT01
Lactobacillus plantarum BP06
Lactobacillus acidophilus BA05
Lactobacillus helveticus BD08
Lactobacillus paracasei BP07
Bifidobacterium animalis subsp. lactis BL03
Bifidobacterium animalis subsp. lactis BL04
Bifidobacterium breve BB02

During the experiment, 200 µl PBS (phosphate buffered saline solution) was given to group K by gavage 5 days a week for 4 weeks. After a five-week break, the same procedure was repeated for an additional four weeks, starting from week 10. In Group Pm, 0.6 mg/kg VSL#3 probiotic was dissolved in 400 µl PBS and administered by gavage for each rat 5 days a week for the first 4 weeks [14]. Probiotic (reminder dose) was administered twice a week for the next 5 weeks. Starting from the 10th week, probiotics were given 5 times a week for 4 weeks. To induce diabetes, a single dose of streptozotocin (STZ, 50 mg/kg) was administered. Seventy-two hours later, blood samples were collected from the tail veins of STZ-treated rats, and blood glucose levels were measured using a commercial glucometer (Accu-Chek Performa) [9]. Diabetes was induced in group Dm rats by STZ administration at the 10th week of the study. Group PmD rats were administered probiotic (0.6 mg/kg VSL#3) 5 times a week for the first 4 weeks and twice a week for the following 5 weeks and diabetes was induced in the 10th week. After diabetes was induced, probiotic administration was continued 5 times a week for 4 weeks. No treatment was applied to group DmP for the first 9 weeks. Diabetes was induced in the 10th week of our experiment and probiotic solution was administered by gavage (0.6 mg/kg VSL#3) for 4 weeks starting from the 10th week. Body weights of the rats were measured weekly. After the completion of the 13th week of the experiment, blood glucose levels of the rats were measured by glucometer from blood samples taken from the lateral tail vein. One day later, the rats were anaesthetized with 90 mg/kg ketamine + 10 mg/kg xylazine intraperitoneally. For biochemical analysis, intracardiac blood samples were collected and centrifuged in yellow-capped biochemistry tubes. Urea, Blood Urea Nitrogen (BUN) and creatinine levels were determined from serum by immunoassay method (Beckman coulter Access 2).

Animal care, injection and gavage applications, sampling procedures were carried out at Pamukkale University Experimental Animals Research Unit (DEHAB).

Histopathological analysis

The kidneys excised from rats were weighed and fixed in a 10% formalin solution. For histopathological examination, the tissues were processed and embedded in paraffin. Subsequently, 5-micron thick sections were obtained using a microtome and stained histologically with hematoxylin-eosin (HE) and PAS stains (Histomed BS-0046, Lot: 092016-001).

Immunohistochemical analysis

Bcl-2 antibody [(Elabscience E-AB-64067 host: Rabbit) (1:100)], an anti-apoptotic marker and alpha-SMA antibody [(Elabscience E-AB-34268 host: Rabbit) (1:100)], a marker of myofibroblasts, were used for immunohistochemical analysis. Secondary antibody (Thermo-Scientific cat no: 37624) and DAB chromogen kit (Thermo Scientific) were then used. Nuclear staining was performed using hematoxylin. Both histochemically and immunohistochemically stained sections were examined and photographed with Olympus BX51 and Olympus DP72 brand/model devices. The immunoreactivity intensity of the sections was evaluated in 10 different areas at x40 magnification with Image-J program and the mean values were calculated for each group.

Histopathological evaluation of kidney damage

Sections taken from the kidney tissues of the groups were coded and evaluated in a blinded manner by two independent histologists. The semiquantitative evaluation of renal tissues was performed based on previously published criteria and graded according to the severity of tissue damage (Table 2) [15].

Table 2. Criteria for histopathological evaluation of kidneys [15]

Value	Glomerular damage	Acute tubular necrosis	Tubulointerstitial inflammatory infiltrates
0	None	None	None
1	less than 25% of glomeruli exhibit non-specific damage characteristics	less than 25 per cent of all renal parenchymal tubules	leukocytes confined within the interstitium
2	25-50% of glomeruli exhibit non-specific damage characteristics	25-50% of all renal parenchymal tubules	leukocytes infiltrating interstitium and tubular epithelial cells
3	50-75% of glomeruli exhibit non-specific damage characteristics	50-75% of all renal parenchymal tubules	
4	more than 75% of glomeruli exhibit non-specific damage	more than 75% of all renal parenchymal tubules	

(A) no nephrotoxicity: 0-1, (B) mild nephrotoxicity: 2-4, (C) moderate nephrotoxicity: 5-7, (D) severe nephrotoxicity: 8-10

Statistical analysis

Data were analysed with SPSS 23.0 software package. Continuous variables were given as mean ± standard deviation, median (minimum-maximum values). Shapiro-Wilk test was used to determine the suitability of the data for normal distribution. One-way analysis of variance (ANOVA) with Tukey's post hoc test was applied for comparisons among independent groups when parametric test assumptions were met. When parametric assumptions were not satisfied, the Kruskal–Wallis test was employed, followed by the Mann–Whitney U test with Bonferroni correction for post hoc comparisons. A *p*-value of <0.05 was considered statistically significant.

Results

Weight gain was observed in all experimental groups from the beginning of the study. In the tenth week, a decrease in body weight was noted in the diabetes groups. Although not statistically significant, the average weight loss in the DmP group was lower than in the other diabetes (Dm and PmD) induced groups. When the mean body weights were compared, no statistically significant difference was found between the groups (Table 3, Figure 1).

Although the difference between the mean kidney weights among the groups was not statistically significant, it was noteworthy that the mean kidney weight of Group Pm was higher than in the other groups (Table 4).

Table 3. Weight measurement results of rats (g)

Groups	Week 1	Week 2	Week 3	Week 4	Week 10	Week 11	Week 12	Week 13
K	289.4±19.42	298.4±22.17	314.2±25.78	312.8±34	371.6±55.8	378± 56.67	380±48.89	379±46.45
Pm	294.6±25.54	306.2±26.15	317.8±27.79	321.8±30.7	357.4±43.65	366.2±45.16	368±47.14	366.6±44.8
Dm	290.38±19.65	308.88±21.86	321.88±24.4	335.25±21.91	396.75±26.75	388.75±31.22	366.75±29.08	336.5±31.1
DmP	293.75±24.36	309±27.23	329±30.47	341±33.82	391.13±47.32	388.5±45.3	384.63±48.71	377±51.8
PmD	293.5±34.71	308.25±36.99	322±43.26	327.63±40.81	394.5±51.23	383±50.94	367.75±55.43	333.25±51.8
p	0.996 F= 0.047	0.965 F= 0.141	0.94 F= 0.193	0.597 F= 0.702	0.513 F= 0.837	0.911 F= 0.244	0.919 F=0.23	0.188 F=1.653

$p<0.05$ was considered significant, F=One-Way Anova Test

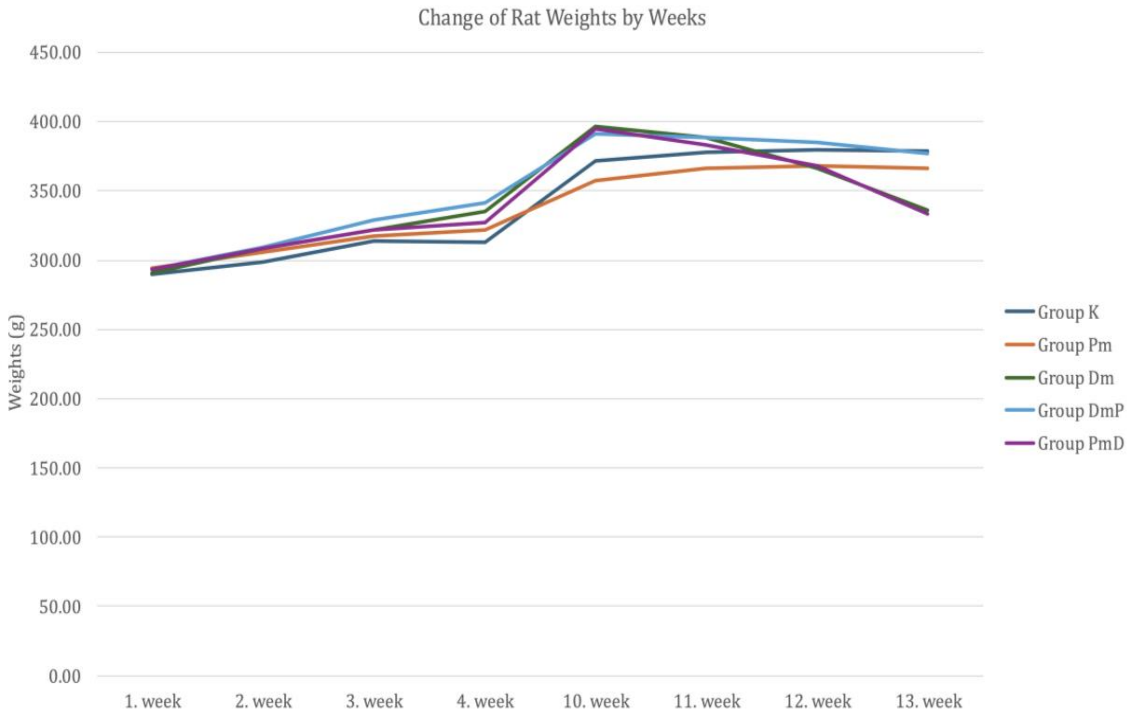


Figure 1. Weekly weight changes of rats according to groups are shown

Table 4. Mean kidney weights of the groups (g)

	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
Right Kidney Weight	1.9±0.38	2.4±0.54	1.96±0.63	1.61±0.33	1.89±0.2	0.063 F=2.511
Left Kidney Weight	1.78±0.33	2.13±0.45	1.67±0.38	2.2±0.5	1.88±0.29	0.083 F=2.294

p<0.05 was considered significant, F=One-Way Anova Test

Blood glucose values measured before sacrification were compared among the groups. The blood glucose levels in the Dm, DmP, and PmD groups were higher than those in the K and Pm groups (Table 5, Figure 2).

The blood glucose levels in the DmP and PmD groups were lower than those in the Dm group. Among the diabetes-induced groups, the PmD group, which received probiotics prior to diabetes induction, exhibited the lowest blood glucose levels. However, the differences in blood glucose levels among these three groups were not statistically significant (Table 5, Figure 2).

Table 5. Blood glucose value measurement results of the groups (mg/dL)

	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
72 hours after STZ induction	109.2±4.55	116.2±2.49	607.13±94.16 k*p*. p*p*	397.13±67.99 k*p*. p*p*	489.5±140.13 k*p*. p*p*	0.0001* kw=25.912
Before sacrification	109.8±5.97	121.2±8.23	544.13±126.87 k*p*. p*p*	454.75±110.41 k*p*. p*p*	431.13±59.55 k*p*. p*p*	0.0001* kw=22.728

k*p*=(*p*<0.05) Statistically significant with the control group (Group K), p*p*=(*p*<0.05) Statistically significant with the probiotic group (Group Pm)

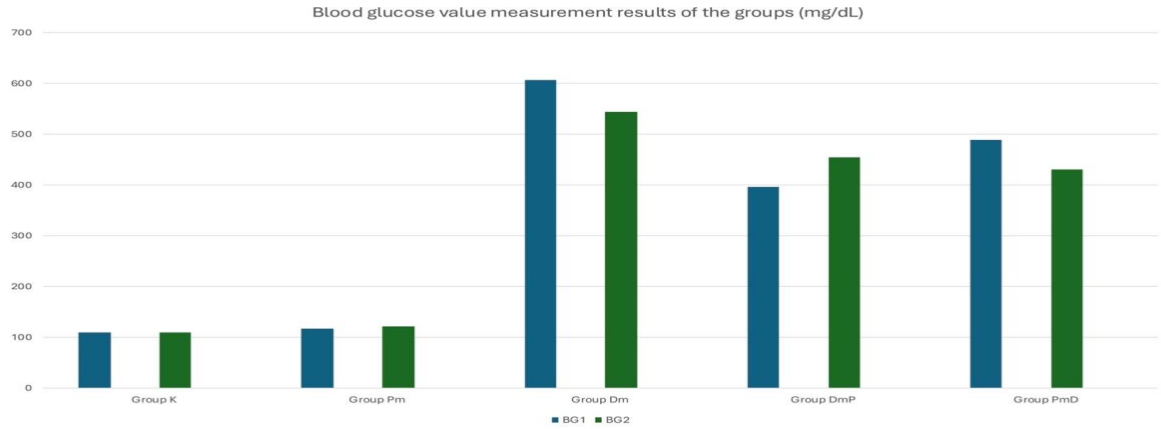


Figure 2. Blood glucose measurement results of the groups (BG1=blood glucose measurement result 72 hours after STZ induction, BG2=blood glucose measurement result just before sacrification)

The differences in mean serum urea, creatinine and BUN levels between the groups were statistically significant. The mean urea concentration in the DM group was significantly higher than groups K, P and DMP. Additionally, the mean urea concentration in the PmD group was significantly higher than in group K. Although not statistically significant, the mean urea concentration in the PmD group was lower than in the DM group. The mean serum BUN concentration in the Dm group differed significantly from those in groups K and P. Although, the mean BUN concentrations in the diabetic groups receiving probiotic supplements (groups DmP and PmD) were lower than in the Dm group, these differences were not statistically significant. When the mean creatinine concentration was compared between the groups, the difference between group Pm and group PmD was statistically significant (Table 6).

Tubular necrosis was observed in the kidney of one rat in the K group. No histopathological changes were detected in the kidney tissue of the remaining rats in the K group (Figure 3 A, F). In the P group, tubular necrosis was observed in two rats, with glomerular damage in one and tubulointerstitial inflammation in another; however, no nephrotoxicity was identified in these rats (Figure 3 B, G). Since both tubular necrosis and tubulointerstitial inflammation were observed in only one rat in the P group, mild nephrotoxicity was determined as a result of scoring (Table 7).

Two of the rats in DM group exhibited moderate nephrotoxicity, while the remaining 6 rats showed mild nephrotoxicity. As a result of scoring, no nephrotoxicity was observed in one rat in the DmP group, moderate nephrotoxicity was identified in one rat and mild nephrotoxicity was observed in the remaining 6 rats. In the PmD group, mild nephrotoxicity was observed in seven rats, with no nephrotoxicity detected in the remaining rat (Table 7).

Upon examination of the kidney sections of group Dm were examined, areas of inflammation, impaired glomerular membrane structures, enlarged Bowman's space, vacuolisation and tubular dilatation, tubular epithelial shedding and brushy edge loss were identified (Figure 3 C, H). In the kidney sections of group DmP, it was found that glomerular structures were preserved, but some tubules were damaged and pignotic nuclei were formed (Figure 3 D, I). In the kidney sections of group PmD, although glomerular and tubule structures were more smooth, tubular dilatation areas and pignotic nuclei were still observed (Figure 3 E, J). When the thickness of Bowman's capsule basement membrane was evaluated semiquantitatively in the kidney sections of the diabetic groups, an increase in thickness was noted. In the diabetic groups (DmP, PmD) given probiotic supplementation, it was observed that the basement membrane thickening was less than in group Dm (Figure 3 H-J).

Table 6. Mean serum creatinine. urea and BUN values of the groups (mg/dL)

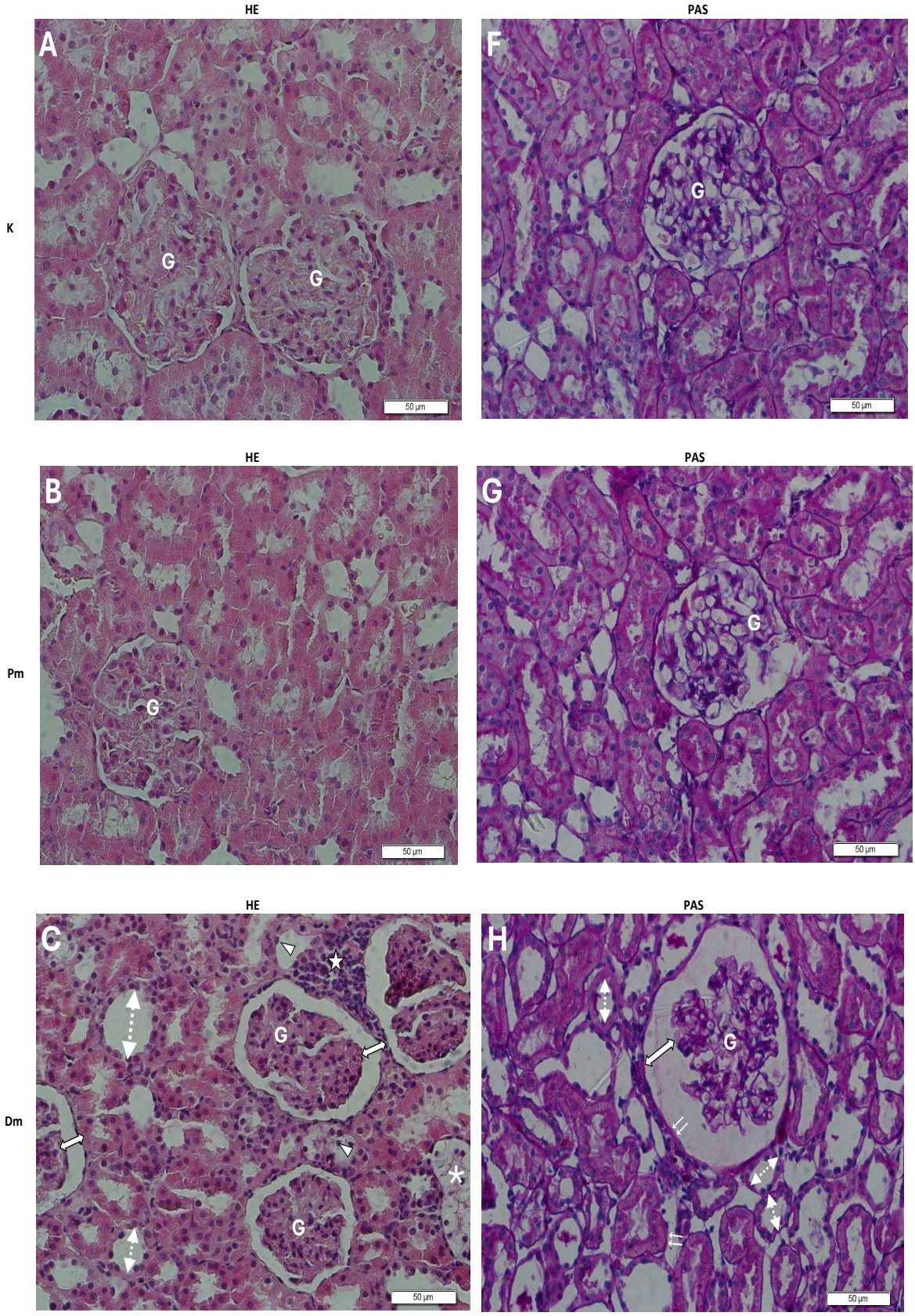
	Group K	Group Pm	Group Dm	Group DmP	Group PmD	*p*
Urea	47.2±5.17	54±16.81	94.63±16.74 k*p*. p*p*	71.13±1.73 d*p*	77.88±5.99 k*p*	0.0001* kw=23.631
BUN	22.06±2.38	25.26±7.8	44.15±7.93 k*p*. p*p*	33.74±1.94	34.8±2.6	0.0001* kw =22.381
Creatinine	0.36±0.03	0.34±0.03	0.38±0.05	0.39±0.06	0.44±0.07 p*p*	0.028* kw=10.87

k*p*=(*p*<0.05) Statistically significant with the control group (Group K), p*p*=(*p*<0.05) Statistically significant with the probiotic group (Group Pm) d*p*=(*p*<0.05) Statistically significant with diabetes group (Group Dm)

Table 7. Histopathological evaluation of kidneys

Animal No	Glomerular Damage	Tubular Necrosis	Tubulointerstitial Inflammation	Total Score	Scoring Scale
K1	0	0	0	0	A
K2	0	1	0	1	A
K3	0	0	0	0	A
K4	0	0	0	0	A
K5	0	0	0	0	A
Pm1	0	1	0	1	A
Pm2	0	1	0	1	A
Pm3	1	0	0	1	A
Pm4	0	0	1	1	A
Pm5	0	1	1	2	B
Dm1	1	2	1	4	B
Dm2	2	1	1	4	B
Dm3	2	2	1	5	C
Dm4	1	2	1	4	B
Dm5	2	1	1	4	B
Dm6	2	1	1	4	B
Dm7	2	2	0	4	B
Dm8	2	2	1	5	C
DmP1	1	1	0	2	B
DmP2	1	1	0	2	B
DmP3	0	1	1	2	B
DmP4	2	2	1	5	C
DmP5	0	1	1	2	B
DmP6	2	0	1	3	B
DmP7	1	1	0	2	B
DmP8	0	0	1	1	A
PmD1	0	1	1	2	B
PmD2	0	1	1	2	B
PmD3	1	1	0	2	B
PmD4	1	0	1	2	B
PmD5	0	1	0	1	A
PmD6	1	0	1	2	B
PmD7	0	1	1	2	B
PmD8	1	1	0	2	B

(A) no nephrotoxicity: 0-1. (B) mild nephrotoxicity: 2-4. (C) moderate nephrotoxicity: 5-7. (D) severe nephrotoxicity: 8-10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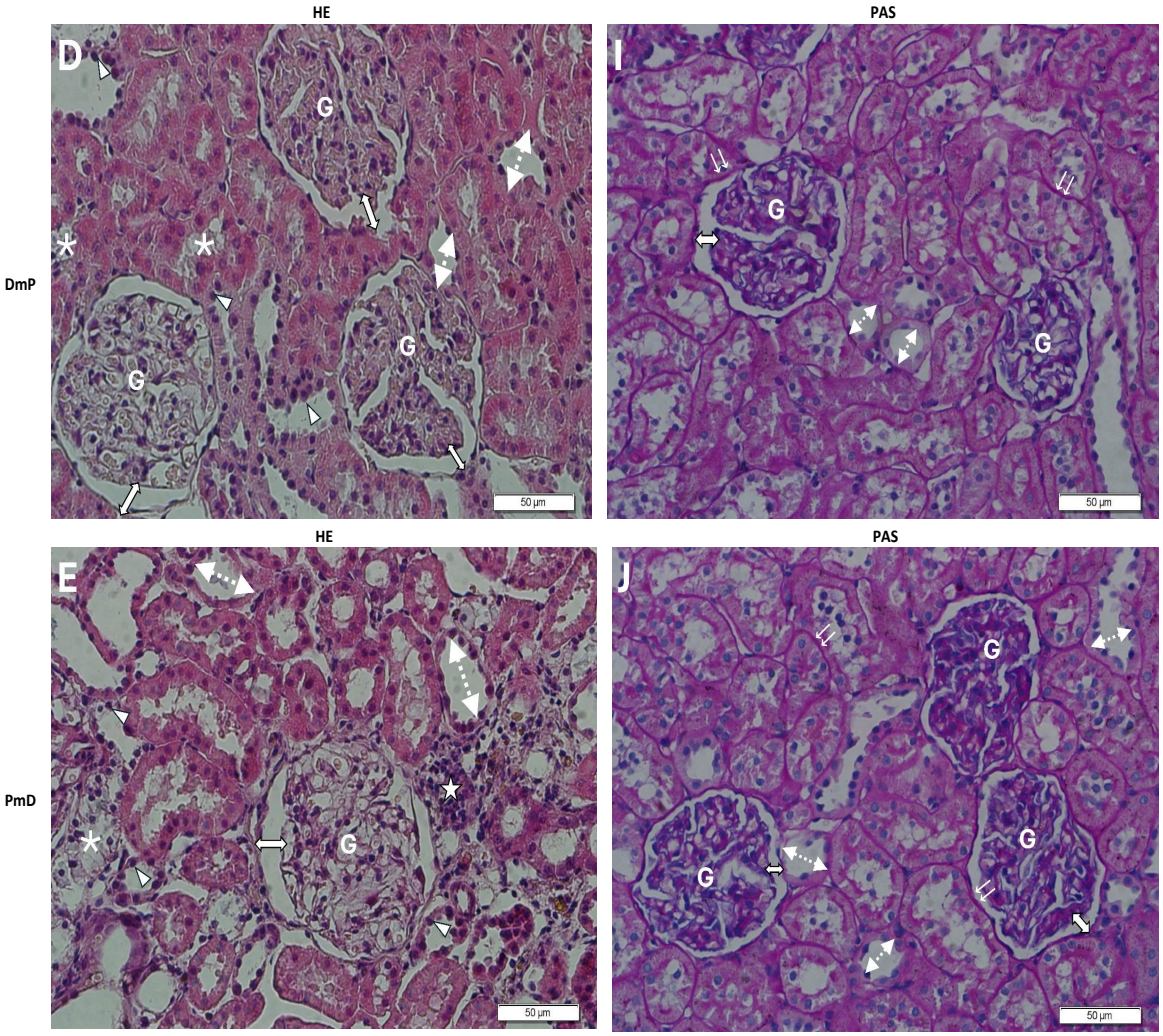
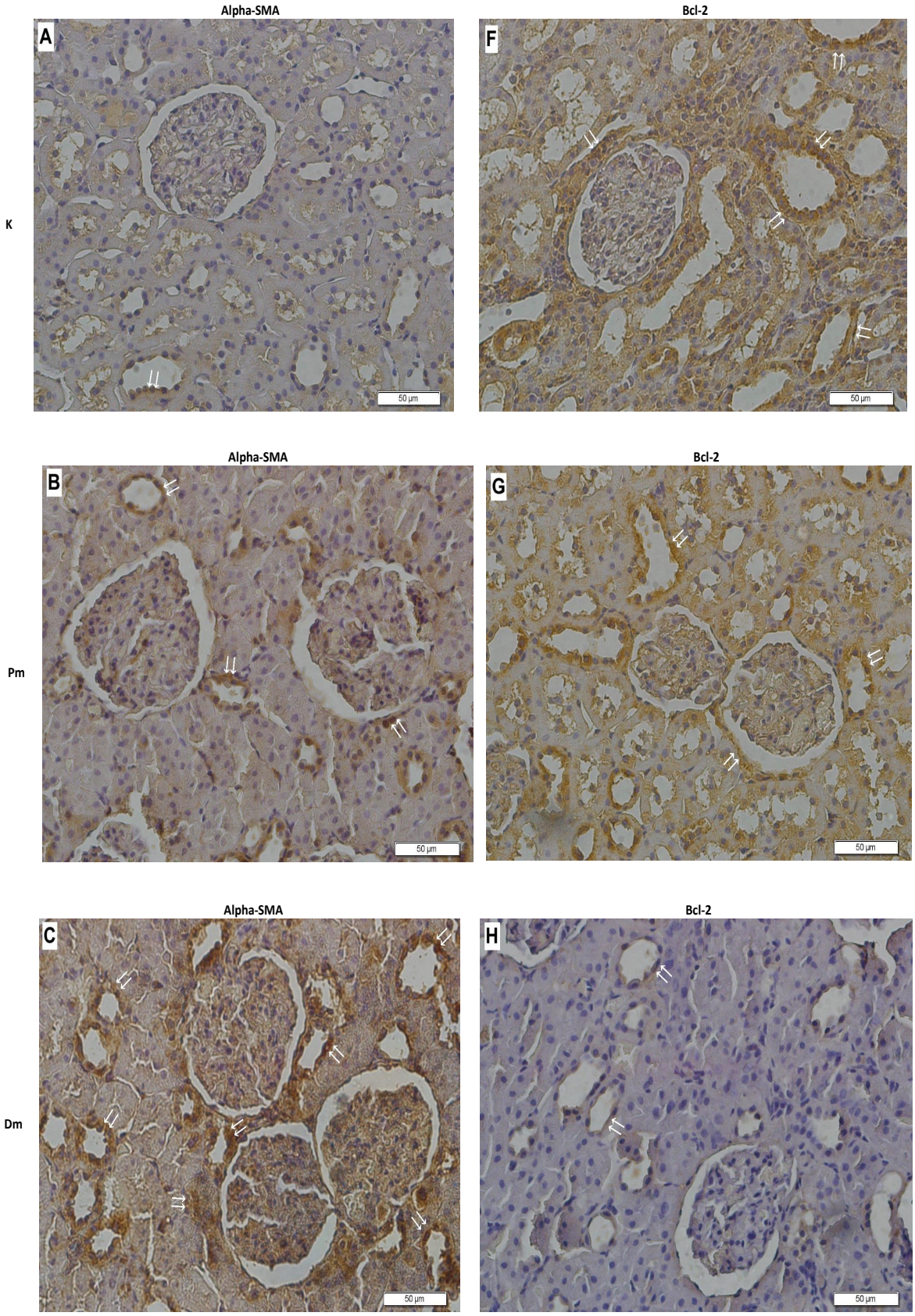


Figure 3. Glomerular structures (G) and tubules appear normal in tissues of K(A, F) and Pm(B, G) groups. Infiltration areas (star), pignotic nuclei (triangle), tubular dilatation areas (dashed two-way arrow), vacuolisation of tubule cells (asterix sign) and enlargement of Bowman's space (two-way arrow) are prominent in the renal tissue of the DM(C, H) group. In DmP(D, I) and PmD(E, J) groups, kidney tissue was less damaged. Thickening of the Bowman capsule and tubule basement membrane (double arrow) [HE staining (A-E); PAS staining (F-J) 40X]

When the kidney sections of the experimental groups were treated with Bcl-2 antibody, an anti-apoptotic marker, a 2.7% decrease in Bcl-2 expression was observed in the kidney sections of the Dm group. It was determined that Bcl-2 expression of DmP (6.2%) and PmD (8.6%), which were among the groups receiving probiotic supplements, increased compared to group Dm and showed an effect against diabetes. It was concluded that Bcl-2 expression percentages of group K and group Pm (respectively 14.3%,10.5%) were higher than the groups in which diabetes was induced (Figure 4). However, when the expression percentages were compared across the groups, it was found that it was not statistically significant (p=0.075)

Immunohistochemical evaluation of the kidney sections from our experimental groups, using alpha-SMA as a marker for myofibroblasts, revealed lower alpha-SMA expression in the kidney sections of group K (3.9%) and group Pm (8.2%) compared to group DM (13.3%). Among the diabetes groups receiving probiotic supplements, DmP (8%) and PmD (10.3%) exhibited lower alpha-SMA expression compared to group DM (Figure 4). However, no statistically significant differences were observed in the expression percentages between the groups (p=0.285).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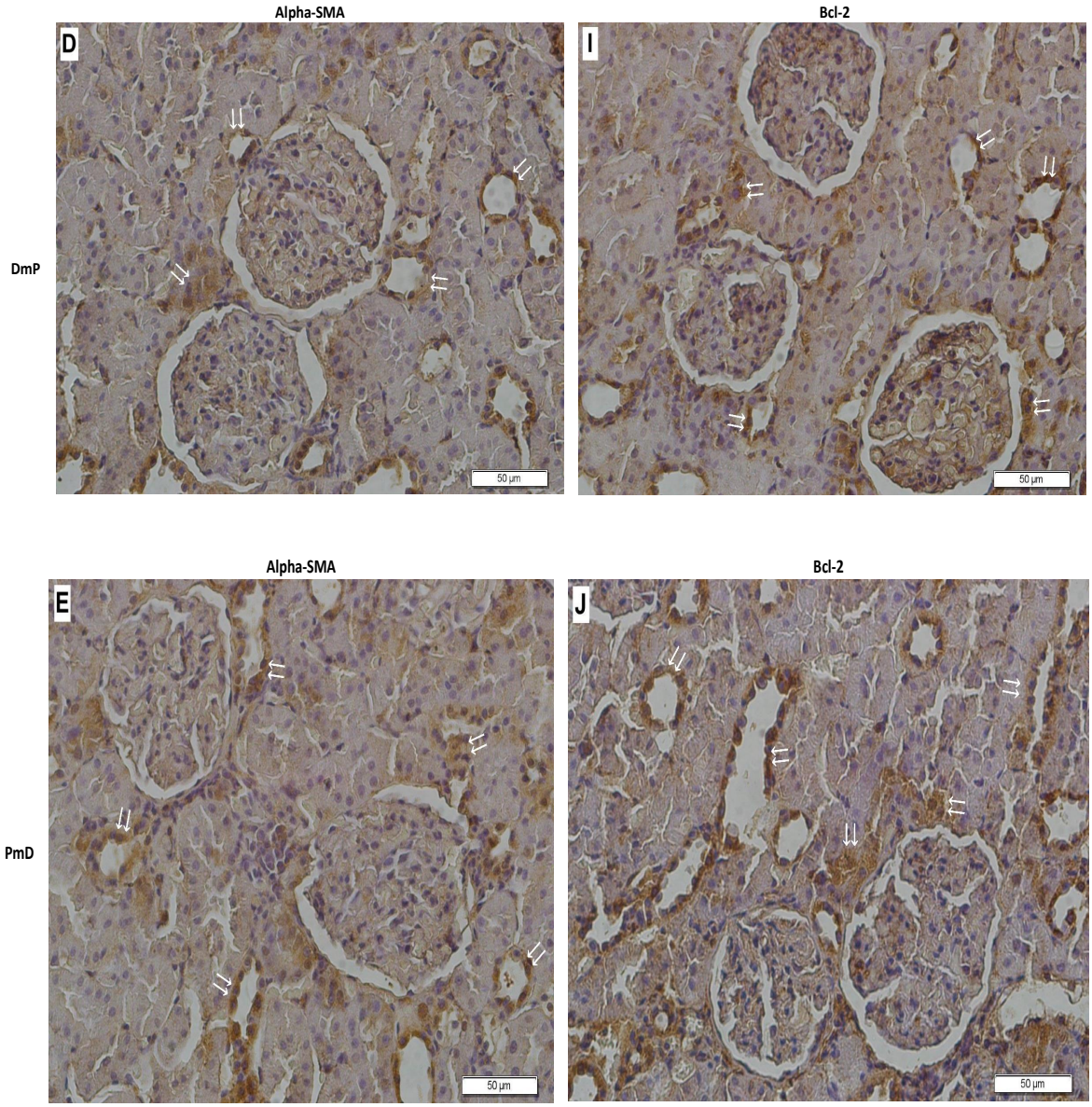

Figure 4. Immunohistochemical alpha-SMA expression was highest in DM group (C) and lowest in K (A) and Pm groups (B). Alpha-SMA expression was decreased in DmP (D) and PmD groups (E) compared to Dm group. Bcl-2 expression is highest in K (F) and P groups (G) and lowest in DM group (H). Bcl-2 expression was increased in DmP (I) and PmD groups (J) compared to Dm group (40x)

Discussion

Probiotics are known to regulate the intestinal microbiota, thus preventing or reducing inflammation by enhancing immune function. Additionally, probiotics are suggested to be effective in lowering blood sugar levels by reducing insulin resistance. It is emphasized in studies that probiotics may be an effective method in the prevention or treatment of Type 2 diabetes [16, 17]. Research has demonstrated that, in diabetes, the levels of *Lactobacillus* and *Bifidobacteria* in the intestinal microbiota decrease, while *Enterococci*, which are associated with increased insulin resistance, rise [18].

One study found that supplementation with *Lactobacillus plantarum* reduced food intake and blood glucose levels in a Type 2 diabetes model in mice [19]. Another study reported that *Lactobacillus gasseri*, derived from human

breast milk, did not result in weight loss in mice with Type 2 diabetes [20]. Additionally, some studies have found that certain bacterial strains cause weight gain, while others lead to weight loss [21]. VSL#3 probiotic supplementation has been shown to increase total fat and visceral fat [22].

The studies show that the use of probiotics containing a combination of several strains is more effective than the use of single-strain probiotics. For example, a 12-week study demonstrated that body weight decreased in individuals as a result of the use of probiotic supplements containing different strains [23]. Jones et al. [22] reported that VSL#3 probiotic supplementation caused an increase in total adiposity and trunk adiposity in subjects. In our study, the mean body weight of group Pm, which was given only probiotic, increased less compared to the control group. This result is in parallel with various studies [21, 23].

In our study, when the average weights of the groups Dm, DmP and PmD were compare during the 4-week diabetes process, weight reductions were determined despite no statistically significant differences were found. No specific effect of probiotic supplementation on weight gain or loss was determined. However, it was observed that the average body weight of the DmP group was higher than that of the Dm and PmD groups, though this difference was not statistically significant. Based on this result, we suggest that probiotic supplementation may reduce the rate of weight loss typically observed in diabetic patients.

It was reported that kidney weights increased in the diabetic mouse model in which probiotic supplement obtained from camel milk was given [24]. In a study, when the damage caused by cisplatin in kidney tissue was evaluated in rats given probiotic supplementation at different doses, it was concluded that the ratio of kidneys to body weight was close to the control in the group given low dose probiotic supplementation, but this ratio was higher in the group receiving high dose probiotic supplementation [25]. In our study, it was observed that the kidney weights of group Pm given probiotic supplementation were higher, although not significantly. However, probiotic supplementation did not have a significant effect on kidney weight changes in the diabetic groups.

It is thought that probiotics may regulate glycemic control and inflammatory response of probiotics [26]. In different studies conducted in humans, it has been reported that probiotic supplementation decreases fasting blood glucose and insulin resistance [16, 27-30]. In animal models of diabetes, it was also reported that blood glucose level decreased in the groups given probiotic supplementation, with a more pronounced effect observed in those receiving higher doses of probiotics [19, 31, 32]. In our study, there was no statistical significance when the blood glucose levels of the diabetes groups were compared. However, blood glucose levels in the diabetic groups receiving probiotic supplementation were lower than those in the diabetes group, though the difference was not statistically significant. This shows that probiotic supplementation may reduce blood glucose level in experimental diabetes model.

Diabetes in mice is known to cause an increase in serum creatinine values [33]. It was found that creatinine and urea concentrations increased in rats with diabetes, but creatinine and urea values decreased significantly with probiotic food supplementation, approaching control levels [34]. Another study reported no difference in creatinine values between the diabetes group and the group induced with diabetes and supplemented with kefir; however, a decrease in serum urea concentration was observed [35]. In our study, no statistically significant increase in mean serum creatinine concentrations was observed when comparing the control group to the diabetes-induced groups. However, the creatinine value in the PmD group was significantly higher than that of the Pm group. The group with the highest mean serum urea concentration was DM. The mean serum urea concentration of group DmP and PmD, to which we gave probiotic supplementation, was lower than group Dm. Notably, the serum urea level in the DmP group was statistically lower than that in the DM group.

The mean BUN concentration was higher in the groups in which diabetes was induced. The BUN level in the Dm group was statistically higher than in both the K and Pm groups. Although there was no statistically significant difference when the mean BUN concentrations of the diabetic groups were evaluated, we found that the BUN values of the probiotic

supplemented groups DmP and PmD were lower compared to group Dm. These findings suggest that probiotic supplementation in diabetes may have a positive effect on serum BUN, urea and creatinine levels.

Various studies have demonstrated that histopathological findings such as glomerular membrane thickening, a marked increase in Bowman's interval, tubular atrophy, tubular dilatation, tubular epithelial vacuolization, epithelial shedding, brush-like edge loss, inflammation areas and necrosis-like damage are present in experimental diabetes models [31-33, 36, 37]. In our study, tubular atrophy and dilatation, vacuolization of cells, pycnotic nuclei, epithelial shedding, thickening of the basement membrane, increase in Bowman interval and areas of inflammation were observed in diabetic kidney tissues. When evaluated semi-quantitatively, an increase in the thickness of the basement membrane of Bowman's capsule was observed in the kidneys of the diabetic groups. However, it was observed that basement membrane thickening was less in the diabetic groups given probiotic supplements. In addition, histopathological findings caused by diabetes were found to be less in the groups given probiotic supplementation. There are studies indicating that probiotic supplementation [31] and kefir supplementation [35] have ameliorative effects against diabetes damage. However, histopathological findings were found to persist in the kidney tissue of diabetic rats given probiotic yoghurt supplementation for 7 days [32].

There are studies reporting that Bcl-2 expression, which is a regulator of apoptosis, decreased in diabetic kidneys [25, 38, 39] and increased with probiotic supplementation [25]. In this study, it was observed that Bcl-2 expression decreased in diabetic kidneys and Bcl-2 expression increased in the diabetic groups (DmP and PmD) given probiotic supplementation as a result of the evaluations made with the Image-J program compared to the diabetes group. Bcl-2 expression was observed to be higher especially in distal tubules.

It was found that alpha-SMA expression in kidney tissues increased with the progression of diabetic nephropathy [40]. Additionally, a study reported that the expression of alpha-SMA in renal arteriole walls decreased in rats given probiotic supplementation for 5 weeks against renal damage caused by hyperuricemia induced by oxonic acid [41]. In our study, it was found that alpha-SMA expression increased with diabetes in kidney tissue. In parallel with other studies [24, 41], it was observed that alpha-SMA expression decreased in diabetic groups receiving probiotic supplementation.

In conclusion, when DM, DMP and PmD groups were compared with K and Pm groups, kidney damage occurred in diabetic groups. It was observed that this damage decreased in diabetic groups receiving probiotics. In this respect, it was determined that probiotic supplementation may have both protective and therapeutic aspects. Nevertheless, we think that more detailed and long-term studies on this subject will be important in clarifying the issue.

Limitations of the study: More experimental data and molecular research are needed for the data to be applicable.

Financial support: This study was supported by Pamukkale University Scientific Research Projects Coordination Office (BAP Project no: 2021SABE018).

Authors contributions: Conceptualization: H.C., S.O., Literature Review: H.C., I.L., S.O., S.T., Design: H.C., S.T., Data Collection: H.C., I.L., S.T., Analysis and Interpretation: H.C., S.O., I.L., B.K.V., Manuscript Writing: H.C, I.L, Critical Review: H.C., S.O.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2013;36 Suppl 1(Suppl 1):S67-S74. doi:10.2337/dc13-S067
2. World Health Organization Available at: https://www.who.int/data/gho/publications/world-health-statistics. Accessed 25.06.2024
3. Me TA. Hastalıklarda beslenme tedavisi. *İstanbul: Hatipoğlu Matbaacılık*; 2013;1:277-304.
4. Kanchan G, Dagle P, Pushpalata C, Joshi Y, Kadam V. A review on some nephroprotective medicinal plants. *International Journal of Pharmaceutical Sciences and Research*. 2012;2012(8):2451. doi:10.13040/ijpsr.0975-8232.3(8).2451-54

5. Lu L, Zhong Z, Gu J, Nan K, Zhu M, Miao C. ets1 associates with KMT5A to participate in high glucose-mediated EndMT via upregulation of PFN2 expression in diabetic nephropathy. *Mol Med*. 2021;27(1):74. Published 2021 Jul 8. doi:10.1186/s10020-021-00339-7
6. American Diabetes Association. Diabetes and employment. *Diabetes Care*. 2009;32 Suppl 1(Suppl 1):S80-S84. doi:10.2337/dc09-S080
7. Horowitz M, Harding PE, Maddox AF, et al. Gastric and oesophageal emptying in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia*. 1989;32(3):151-159. doi:10.1007/BF00265086
8. Azpiroz F, Malagelada C. Diabetic neuropathy in the gut: pathogenesis and diagnosis. *Diabetologia*. 2016;59(3):404-408. doi:10.1007/s00125-015-3831-1
9. Wu SL. Staging of type 2 diabetes mellitus. *Genet Mol Res*. 2015;14(1):2118-2121. Published 2015 Mar 20. doi:10.4238/2015.March.20.22
10. Qin J, Li Y, Cai Z, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490(7418):55-60. doi:10.1038/nature11450
11. Kobyliak N, Conte C, Cammarota G, et al. Probiotics in prevention and treatment of obesity: a critical view. *Nutr Metab (Lond)*. 2016;13:14. Published 2016 Feb 20. doi:10.1186/s12986-016-0067-0
12. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury?. *Kidney Int*. 1999;56(5):1627-1637. doi:10.1046/j.1523-1755.1999.00721.x
13. Shi R, Ye J, Fan H, et al. *Lactobacillus plantarum LLY-606* supplementation ameliorates hyperuricemia *via* modulating intestinal homeostasis and relieving inflammation. *Food Funct*. 2023;14(12):5663-5677. Published 2023 Jun 19. doi:10.1039/d2fo03411e
14. Sang LX, Chang B, Wang BY, Liu WX, Jiang M. Live and heat-killed probiotic: effects on chronic experimental colitis induced by dextran sulfate sodium (DSS) in rats. *Int J Clin Exp Med*. 2015;8(11):20072-20078. Published 2015 Nov 15.
15. Al Shabanah OA, Aleisa AM, Al Yahya AA, et al. Increased urinary losses of carnitine and decreased intramitochondrial coenzyme A in gentamicin-induced acute renal failure in rats. *Nephrol Dial Transplant*. 2010;25(1):69-76. doi:10.1093/ndt/gfp457
16. Ruan Y, Sun J, He J, Chen F, Chen R, Chen H. Effect of Probiotics on Glycemic Control: A Systematic Review and Meta-Analysis of Randomized, Controlled Trials. *PLoS One*. 2015;10(7):e0132121. Published 2015 Jul 10. doi:10.1371/journal.pone.0132121
17. Kamarlı H. Pre- Probiyotikler ve Diyabet. Bes Diy Derg [Internet]. 2019;47:92-101. Erişim adresi: https://beslenmevediyetdergisi.org/index.php/bdd/article/view/1320. Erişim tarihi 22 Ocak 2025
18. Cai X, Wang L, Wang X, Liu S. Effect of high dietary fiber low glycemic index diet on intestinal flora, blood glucose and inflammatory response in T2DM patients. *Biomed. Res*. 2017;28:9371-9375.
19. Li X, Wang N, Yin B, et al. Effects of Lactobacillus plantarum CCFM0236 on hyperglycaemia and insulin resistance in high-fat and streptozotocin-induced type 2 diabetic mice. *J Appl Microbiol*. 2016;121(6):1727-1736. doi:10.1111/jam.13276
20. Yun SI, Park HO, Kang JH. Effect of Lactobacillus gasseri BNR17 on blood glucose levels and body weight in a mouse model of type 2 diabetes. *J Appl Microbiol*. 2009;107(5):1681-1686. doi:10.1111/j.1365-2672.2009.04350.x
21. Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D. Comparative meta-analysis of the effect of Lactobacillus species on weight gain in humans and animals. *Microb Pathog*. 2012;53(2):100-108. doi:10.1016/j.micpath.2012.05.007
22. Jones RB, Alderete TL, Martin AA, et al. Probiotic supplementation increases obesity with no detectable effects on liver fat or gut microbiota in obese Hispanic adolescents: a 16-week, randomized, placebo-controlled trial. *Pediatr Obes*. 2018;13(11):705-714. doi:10.1111/ijpo.12273
23. Sudha MR, Ahire JJ, Jayanthi N, Tripathi A, Nanal S. Effect of multi-strain probiotic (UB0316) in weight management in overweight/obese adults: a 12-week double blind, randomised, placebo-controlled study. *Benef Microbes*. 2019;10(8):855-866. doi:10.3920/BM2019.0052
24. Lu L, Zhong Z, Gu J, Nan K, Zhu M, Miao C. ets1 associates with KMT5A to participate in high glucose-mediated EndMT via upregulation of PFN2 expression in diabetic nephropathy. *Mol Med*. 2021;27(1):74. Published 2021 Jul 8. doi:10.1186/s10020-021-00339-7
25. Sengul E, Gelen SU, Yıldırım S, Çelebi F, Çınar A. Probiotic bacteria attenuates cisplatin-induced nephrotoxicity through modulation of oxidative stress, inflammation and apoptosis in rats. *Asian Pacific Journal of Tropical Biomedicine*. 2019;9(3):116-122.
26. Miraghajani M, Zaghian N, Mirlohi M, Feizi A, Ghiasvand R. The Impact of Probiotic Soy Milk Consumption on Oxidative Stress Among Type 2 Diabetic Kidney Disease Patients: A Randomized Controlled Clinical Trial. *J Ren Nutr*. 2017;27(5):317-324. doi:10.1053/j.jrn.2017.04.004
27. Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition*. 2012;28(5):539-543. doi:10.1016/j.nut.2011.08.013

28. Ostadrahimi A, Taghizadeh A, Mobasseri M, et al. Effect of probiotic fermented milk (kefir) on glycemic control and lipid profile in type 2 diabetic patients: a randomized double-blind placebo-controlled clinical trial. *Iran J Public Health*. 2015;44(2):228-237.

29. Kobyliak N, Falalyeyeva T, Mykhalchyshyn G, Kyriienko D, Komissarenko I. Effect of alive probiotic on insulin resistance in type 2 diabetes patients: Randomized clinical trial. *Diabetes Metab Syndr*. 2018;12(5):617-624. doi:10.1016/j.dsx.2018.04.015

30. Razmpoosh E, Javadi A, Ejtahed HS, Mirmiran P, Javadi M, Yousefinejad A. The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo controlled trial. *Diabetes Metab Syndr*. 2019;13(1):175-182. doi:10.1016/j.dsx.2018.08.008

31. Wang Y, Dilidaxi D, Wu Y, Sailike J, Sun X, Nabi XH. Composite probiotics alleviate type 2 diabetes by regulating intestinal microbiota and inducing GLP-1 secretion in db/db mice. *Biomed Pharmacother*. 2020;125:109914. doi:10.1016/j.biopha.2020.109914

32. Khalil NA, Eltahan NR, Elaktash HM, Aly S, Sarbini SR. Prospective evaluation of probiotic and prebiotic supplementation on diabetic health associated with gut microbiota. *Food Bioscience*. 2021;42:101149.

33. Glastras SJ, Chen H, Teh R, et al. Mouse Models of Diabetes, Obesity and Related Kidney Disease. *PLoS One*. 2016;11(8):e0162131. Published 2016 Aug 31. doi:10.1371/journal.pone.0162131

34. Negm El Dein A, Ezzat A, Aly HF, Awad G, Farid M. Lactobacillus-fermented yogurt exerts hypoglycemic, hypocholesterolemic, and anti-inflammatory activities in STZ-induced diabetic Wistar rats. *Nutr Res*. 2022;108:22-32. doi:10.1016/j.nutres.2022.10.003

35. Punaro GR, Maciel FR, Rodrigues AM, et al. Kefir administration reduced progression of renal injury in STZ-diabetic rats by lowering oxidative stress. *Nitric Oxide*. 2014;37:53-60. doi:10.1016/j.niox.2013.12.012

36. Gilbert RE, Cooper ME. The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of glomerular injury?. *Kidney Int*. 1999;56(5):1627-1637. doi:10.1046/j.1523-1755.1999.00721.x

37. Vardı N, Iraz M, Öztürk F, Uçar M, Gül M, Eşrefoğlu M, Otlu A. Deneysel Diyabetin Sıçan Böbreklerinde Meydana Getirdiği Histolojik Değişiklikler Üzerine Melatoninin İyileştirici Etkileri. *J Turgut Ozal Med Cent.* Haziran 2005;12(3):145-152.

38. Kikuchi Y, Nonoguchi H, Machida K, Wakamatsu S, Koga H, Tomita K. Regulation of the apoptosis-related genes, Bax and Bcl-2, in the early stage of diabetes mellitus. *Nephrology*. 2002;7(6):294-302.

39. Li A, Yi B, Han H, et al. Vitamin D-VDR (vitamin D receptor) regulates defective autophagy in renal tubular epithelial cell in streptozotocin-induced diabetic mice via the AMPK pathway. *Autophagy*. 2022;18(4):877-890. doi:10.1080/15548627.2021.1962681

40. Manaer T, Yu L, Nabi XH, Dilidaxi D, Liu L, Sailike J. The beneficial effects of the composite probiotics from camel milk on glucose and lipid metabolism, liver and renal function and gut microbiota in db/db mice. *BMC Complement Med Ther*. 2021;21(1):127. Published 2021 Apr 22. doi:10.1186/s12906-021-03303-4

41. García Arroyo FE, Gonzaga G, Muñoz Jiménez I, et al. Probiotic supplements prevented oxonic acid-induced hyperuricemia and renal damage. *PLoS One*. 2018;13(8):e0202901. Published 2018 Aug 24. doi:10.1371/journal.pone.0202901