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RESEARCH ARTICLE

The Effects of the Basil *(Ocimum sanctum)* Treatment on the Tumor Necrosis Factor-α and Interleukin 1β Release in the Kidney Tissue of the Diabetic Rats [1][2]

Sevda ELİŞ YILDIZ [1,a (*)] Buket BAKIR [2,b] Hasan ASKER [3,c] Ebru KARADAĞ SARI [4,d]

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[1] Department of Midwifery, Faculty of Health Sciences, Kafkas University, TR-36100 Kars - TURKEY
[2] Department of Histology and Embryology, Faculty of Veterinary Medicine, Namik Kemal University, TR-59030 Tekirdag, - TURKEY
[3] Department of Histology and Embryology, Faculty of Medicine, Uşak University, Uşak - TURKEY
[4] Department of Histology and Embryology, Faculty of Veterinary Medicine, Kafkas University, TR-36040 Kars - TURKEY
ORCIDs: [a] 0000-0002-3585-6648; [b] 0000-0003-3637-3688; [c] 0000-0002-5703-2164; [d] ORCID:0000-0001-7581-6109

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Abstract

This study aims to examine the changes of the *Ocimum sanctum* treatment on the tumor necrosis factor alpha (TNF-α) and interleukin 1β (IL-1β) in the kidney tissue of the rats, in which the experimental diabetes was induced with streptozotocin (STZ). Forty *Sprague Dawley* male rats were divided into 5 groups: Diabetes, Diabetes + *Ocimum sanctum, Ocimum sanctum,* Control, and Sham. The immunohistochemical localization of TNF-α and IL-1β in the kidney tissue was determined by using the streptavidin-biotin-peroxidase method. Strong TNF-α immunoreactivity was determined in the renal cortex of the rats in the Diabetes and Diabetes + *Ocimum sanctum* groups on 14th days, low immunoreactivity was determined in the rats in *Ocimum sanctum*, Sham, and Control groups. While strong IL-1β immunoreactivity was observed in the renal cortex of the Diabetes group, moderate IL-1β immunoreactivity was observed in the renal cortex of the Diabetes + *Ocimum sanctum* and low immunoreactivity was determined in the *Ocimum sanctum,* Sham, and Control groups. In this study, it was assessed how the polymorphisms, occurring in the cytokine genes of *Ocimum sanctum* in the rats, in which experimental diabetes was induced, and TNF-α and IL-1β, which was demonstrated to have an important role in the complication development in the diabetic patients affected the renal tissue.

Keywords: *Diabetes, Ocimum sanctum, Tumor Necrosis Factor Alpha, Interleukin, Kidney*

Diyabetik Ratlarda Fesleğen *(Ocimum sanctum)* Uygulamasının Böbrek Dokusunda Tümör Nekrozis Faktör-α ve İnterlökin 1β Salınımı Üzerine Etkileri

Öz

Bu çalışmada, fesleğen *(Ocimum sanctum)* uygulamasının, streptozotocin (STZ) ile deneysel diyabet oluşturulan ratların böbrek dokusunda tümör nekrozis faktör alfa (TNF-α) ve interlökin 1β (IL-1β) üzerine meydana getirdiği değişikleri incelemek amaçlanmıştır. Çalışmamızda toplam 40 adet *Spraque Dawley* cinsi erkek rat kullanıldı. Ratlar, Diyabet, Diyabet + *Ocimum sanctum, Ocimum sanctum,* Kontrol ve Sham olmak üzere 5 gruba ayrıldı. TNF-α ve IL-1β'in böbrek dokusundaki immunohistokimyasal lokalizasyonu streptavidin-biotin peroxidaz yöntemi ile belirlendi. Diyabet ve Diyabet + *Ocimum sanctum* uygulanmış grubun dışındaki grupların böbrek korteksinde zayıf düzeyde TNF-α immunoreaktivitesi tespit edildi. Diyabet grubunun böbrek korteksinde güçlü, Diyabet + *Ocimum sanctum* grubunda ise böbrek korteksinde orta düzeyde IL-1β immunoreaktivitesi tespit edilmesine rağmen *Ocimum sanctum,* Sham ve Kontrol grubundaki ratlarda ise zayıf düzeyde immunoreaktivite tespit edildi. Bu çalışma ile deneysel olarak diyabet oluşturulmuş ratlarda *Ocimum sanctum*'un sitokin genlerinde oluşan polimorfizmlerin diyabetli hastalarda komplikasyon gelişiminde önemli rol oynadıkları gösterilen TNF-α ve IL-1β'in böbrek dokusu üzerine ne kadar etkili olduğu değerlendirildi.

Anahtar sözcükler: *Şeker Hastalığı, Fesleğen, Tümör Nekrozis Faktör Alfa, İnterlökin, Böbrek*

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(*) **Corresponding Author**

Tel: +90 474 225 1567 Fax : +90 474 225 1265
E-mail: sevdaelis36@hotmail.com (S. Eliş Yıldız)

Introduction

Diabetes mellitus (DM) is a common metabolic disorder that involves glucose, amino acids, and fatty acids. Either insulin deficiency or insulin resistance may cause diabetes and partial deficiency of the insulin or insulin resistance in the peripheral tissues [1]. The reactive oxygen species (ROS) increase as a result of the oxidative stress and insulin resistance develops with the cytokines secretions from the activated macrophage and monocytes in large amounts [2]. The cytokines play a significant role in both the natural and specific inflammatory response. Most of the polymorphisms that will be able to show up in the cytokine genes are in the form of single nucleotide polymorphism (SNP) in the regulatory regions. These variations increase the sensitivity against various diseases by affecting the cytokine genes expression levels [3,4]. The cytokines detection is important for prognosis and diagnosis of different diseases related with inflammation, immunology and atherosclerosis and the most important ones are interleukins (ILs) and TNF-α [5]. TNF-α is produced as a pro-hormone substance constituting 233 amino acids and is a strong cytokine taking place among Type 1 proinflammatory cytokines secreted by the macrophage and T lymphocytes. The TNF-α has important functions such as stimulating growth, cytotoxicity, and angiogenesis in Type 2 diabetes and its microvascular complications [6]. It was specified by various studies performed that amount of the proinflammatory cytokines such as IL-1β and TNF-α was correlated with increased complications in diabetic patients [3,4].

The interest to use plants for treatments progressively is in increase and treatment with plants is become prevalent. The tendency to the pharmacological and toxicological studies related to plants started to increase in our country. *Ocimum sanctum* has fertility prevention, anticancer, antidiabetic, antifungal and antimicrobial influences [7]. The basil leaf exhibits antidiabetic activity, as was revealed in one study, it was observed that aqueous extract of Tulsi significantly lowered the blood glucose level in diabetic rats [8]. The basil leaf pulverized by foods was given to healthy and diabetic rats for one week, it was observed that the basil decreased the fasting blood glucose [9]. In a clinical trial in which the basil was acquired, it was determined that it had positive influences on the serum glucose level after both the preprandial and postprandial in 40 Type 2 diabetic patients since it was made by mixing water and fresh leaf powder and used as a support product for 4 weeks [10]. Medicinal plants are increasingly being used as a DM management strategy. In this context, traditional herbal medicine is used across the world for disease treatment [11]. It is argued as a result of the studies performed that gene polymorphisms of the cytokines such as TNF-α and IL-1β are associated with the complication development in diabetic patients.

We investigated the immunohistochemical localization of TNF-α and IL-1 β in the kidneys of healthy and diabetic rats to which *Ocimum sanctum* had been administered. We also investigated the effects of *Ocimum sanctum* on structural changes in the kidney.

Material and Methods

Animals

Approval was received by the Animal Experiments Local Ethics Committee of Kafkas University (Decision no: KAU-HADYEK/2016-108). In this study, 40 male rats, aged 4 to 5 month weighing 200-300 g, that were not copulated previously and used in any study, were used. The rats used in the study were harbored in a standard cage in 12 h light and 12 h dark environment at 22±2°C ambient temperature and fed by *ad-libitum* and tap water.

Preparation of Ocimum Sanctum Extract

Ten g of basil in powder form was taken and dissolved in 100 mL of ethanol. The mixture obtained was left to shake on shaker for 24 h at room temperature. After the time expired, the mixture was filtered with the help of filter paper. The solvent content was removed in a Rotary evaporator at low pressure and low temperature. Extracts were stored at -20°C until experiment [12].

Experiment Groups

1. Control Group (n=8): No application was made.

2. Sham Group (n=8): Sodium citrate solution was administered as 50 mg/kg intraperitoneal injection.

3. *Ocimum Sanctum* Group (n=8): *Ocimum sanctum* extract was administered as 200 mg/kg for through the oral gavage 14 days [13].

4. Diabetes Group (n=8): Experimental diabetes was induced by intraperitoneal injection of 50 mg/kg STZ dissolved in 0.1 M citrate buffer, pH 4.5 [14].

5. Diabetes + *Ocimum sanctum* Group (n=8): Experimental diabetes was induced by intraperitoneal injection of 50 mg/kg STZ dissolved in 0.1 M citrate buffer, pH 4.5 and *Ocimum sanctum* extract was administered as 200 mg/kg for 14 days through the oral gavage for the group in which diabetes was created.

Then, cervical dislocation was performed under anesthesia to the rats in each group and kidney tissues samples were taken up for histology and immunohistochemistry.

Determination of Blood Glucose Levels

The blood samples were taken from the tail vein of the animals left hungry for 8 hours before starting to the study (0^{th} day) by using a glucometer (Yasee, GLM-76, Taiwan) in order to determine the preprandial blood glucose levels. Those, of which glucose levels were at the level of 200 mg/dL, were included in the study by measuring the

preprandial blood glucose levels for 8 h on the 3rd day of the STZ practice. *Ocimum sanctum* extract was given by the oral gavage for 14 days starting from the 3rd day of the STZ practice.

Statistical Evaluation

The blood glucose measurements of the rats in all groups were taken on the 0th, 3rd and 14th days. Statistical Package for Social Sciences 20.0 program was used to compare statistically the blood glucose levels between the groups. ANOVA (Variance analysis) and T-test were applied for probable differences. The confidence interval was specified as 0.05 in the statistical analyses. Kolmogorov Smirnov test was performed to measure the normality of the distribution. Since the P value was >0.05, the distrubition was said to be normal.

Histopathological Examination

The kidney tissue samples taken from all experimental animals were fixed in a 10% formaldehyde solution. The tissue sections were taken at thickness of 5 µm from the paraffin blocks prepared. Sections were stained with Crossman's triple staining [15]. Slides were evaluated under a light microscope (Olympus BX51; Olympus Optical Co., Osaka, Japan) and microscopic photos were taken.

Immunohistochemical Examination

The streptavidin-biotin-peroxidase technique, one of the indirect methods, was used to the sections taken to the lams coated by chrome aluminum gelatin. The sections were then incubated in 3% H_2O_2 prepared in 0.1M PBS for 15 min to prevent the endogenous peroxidase activity by agitating in the PBS (0.1 M, Ph, 7.2) after the deparaffinization and rehydration processes. They were then applied heat at the maximum temperature in a microwave oven for 10 min (800 watt) in the citrate buffer solution to bring antigens into the open after washing with the PBS. The blocking solution A was dripped to prevent the nonspecific binding (Histostain-Plus IHC Kit, HRP, broad-spectrum Ref.) after washed by PBS. The anti-TNF-α (Santa Cruz, it was diluted at the rate of 1/500) and anti-IL-1β primary antibody (Santa Cruz, it was diluted at the rate of 1/250) were applied on the sections in a humid environment at the ambient temperature for 1 h. The Broad Spectrum Antibody was dripped on the sections since it was against the type produced by the primary antibody. The HRP streptavidin was incubated at the ambient temperature for 15 min after washing with PBS. The 3,3'-Diaminobenzidine tetrahydrochloride (DAB) substrate solution was added for the chromogen practice and then, Mayer's hemotoxilen was used for the back-groud staining. The slides were examined in a research microscope and their photos were taken. Rabbit serum without primer antibody served as the negative control. The immunohistochemical evaluation was made by considering staining characteristic and staining density of the target cells. The evaluation was made by two independent observers by giving values from 0 to 3 in accordance with the characteristics including nonstaining (-), weak staining (+), moderate staining (++), and severe staining (+++) [16-18].

Results

Blood Glucose Levels

When the groups were compared, significant differences were observed between diabetes and diabetes + *Ocimum sanctum* groups and control, sham and *Ocimum sanctum* groups in 3rd-day. The significant differences were also observed on the 14th day between diabetes and diabetes + *Ocimum sanctum* groups and control, sham and *Ocimum sanctum* groups. When the diabetes and diabetes + *Ocimum sanctum* group was compared, no significant difference was statistically determined on the 3rd and 14th days *(Table 1)*.

Histopathological Results

The kidney tissue samples taken from the rats in all groups were semi-quantitatively evaluated in terms of the tissue damage. Bowman cavities (urinary cavity) were determined as either very decreased or removed as a result of the increased cellularity in the glomerular mesangium in some glomerulus in the kidney tissues of the rats, by which diabetes created. No thickening was seen in the glomerular basal membranes. The casts were specified in some tubulus lumens. It was determined that there was a hydropic and balloony degeneration in some collector (collecting) and distal tubulus epithelium *(Fig. 1-a)*. A vacuolization was observed in the tubulus epithelium cells in the cortical regions.

Bowman cavities were specified as either very decreased or removed as a result of the increased cellularity in the

Table 1. *Statistical evaluations of the rats fasting blood glucose level in accordance with the groups*

Days	Sham	Control	*Ocimum sanctum*	Diabetes	Diabetes + *Ocimum sanctum*
0	87.375 ± 9.66^{aA}	76.000 ± 5.66^{aA}	68.250 ± 7.29^{aA}	81.625 ± 6.21^{aA}	74.875 ± 8.43^{aA}
3	77.125 ± 4.32^{aA}	75.875 ± 5.74^{aA}	67.750 ± 6.94^{aA}	361.500 ± 11.39^{bB}	284.630 ± 41.30^{cB}
14	89.000 ± 10.17^{aA}	79.125 ± 5.11^{aA}	61.375 ± 9.13^{aA}	353.250 ± 26.39^{bB}	261.750 ± 37.83^{cB}

^{a,b,c} The differences between the averages shown by the different letter in the same line are statistically important ($P<0.05$); ^{A,B,C} The differences between the averages shown by the different letter in the column are statistically important ($P<0.05$)

glomerular mesangium in diabetes + *Ocimum sanctum* group rats' kidney tissues. Not any thickening was seen in the glomerular basal membranes. It was specified that the proximal and distal tubules were normal *(Fig. 1-b)*.

The control, sham and *Ocimum sanctum* group rats kidney tissues were determined to be in normal histological form on the 14th day *(Fig. 1-c,d)*.

Immunohistochemical Results

Tumor necrosis factor alpha and IL-1β immunoreactivity were specifically seen on the 14th day in the rats kidney tissues in all groups. TNF-α immunoreactivity was determined at a strong level in the kidney cortex of the diabetes and diabetes + *Ocimum sanctum* groups and at a weak level in the *Ocimum sanctum,* sham and control groups. Although a strong TNF-α immunoreactivity was in the tubulus proximalis and tubulus distalis epithelial cells in diabetes *(Fig. 2-a)* and diabetes + *Ocimum sanctum (Fig. 2-b)* groups, it was determined at a weak level in the *Ocimum sanctum (Fig. 2-c)*, control *(Fig. 2-d)* and sham *(Fig. 2-e)* groups on the 14th day in the rats. TNF-α immunoreactivity was not encountered in glomerulus and vessel endothelium in the kidney tissues in all groups. The semiquantitative analysis results of intergroup for TNF-α immunohistochemical staining were summarized in *Table 2*.

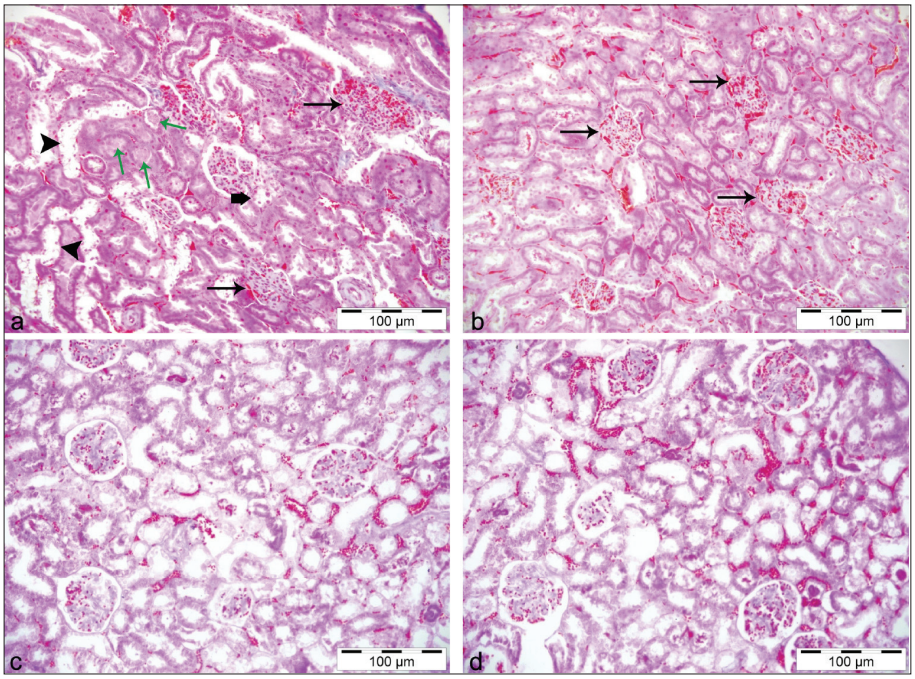

Fig 1. Rat kidney tissues. **a-** Diabetes group. Bowman cavities: *black arrows,* tubulus lumens: *green arrows.* Hydropic and balloony degeneration in some collector (collecting): *arrowhead* and distal tubulus epithelium: *thick arrow;* **b-** Diabetes + *Ocimum sanctum* group. Bowman cavities: *black arrows;* **c-** Control group; **d-** *Ocimum sanctum* group. Triple Staining

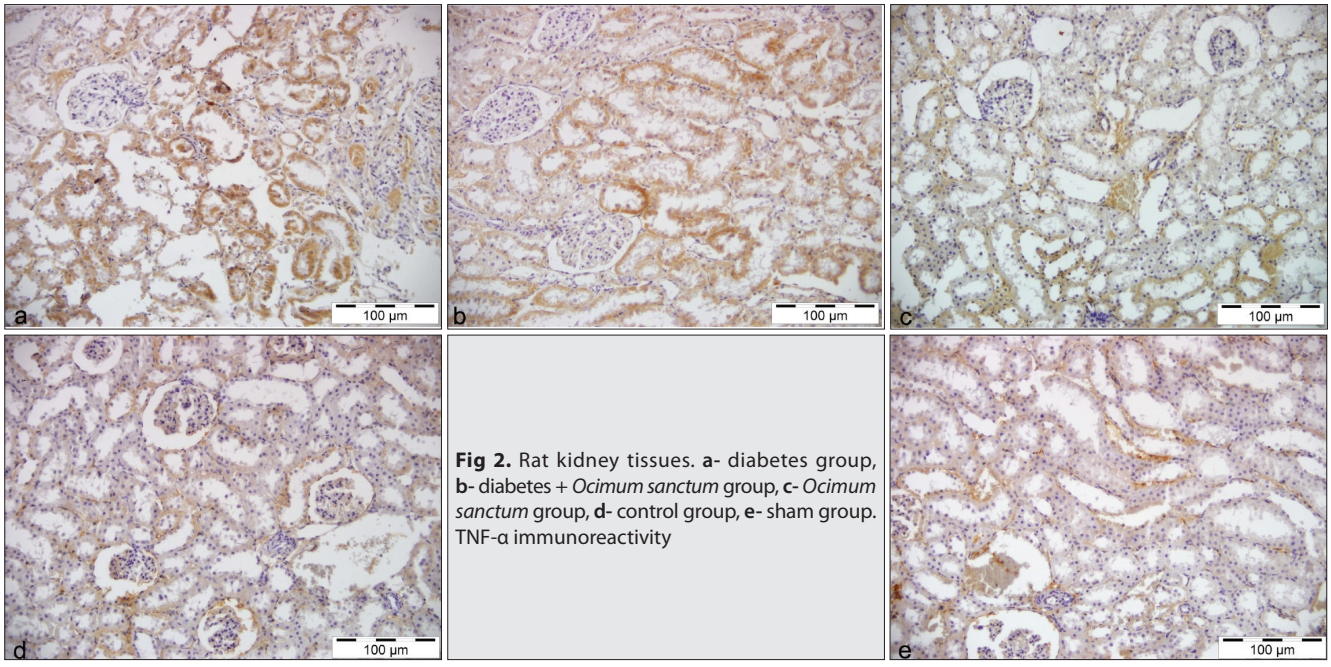

Fig 2. Rat kidney tissues. **a-** diabetes group, **b-** diabetes + *Ocimum sanctum* group, **c-** *Ocimum sanctum* group, **d-** control group, **e-** sham group. TNF-α immunoreactivity

Table 2. *The semiquantitative analysis results of intergroup TNF-α immunoreactivity on the 14th day*					
Cell Type	**Diabetes Group**	**Diabetes + *Ocimum sanctum* Group**	***Ocimum sanctum* Group**	**Sham Group**	**Control Group**
Tubulus proximalis	+++	+++	+	+	+
Tubulus distalis	+++	+++	+	+	+
Glomerulus	-	-	-	-	-
Vessel Endothelium	-	-	-	-	-

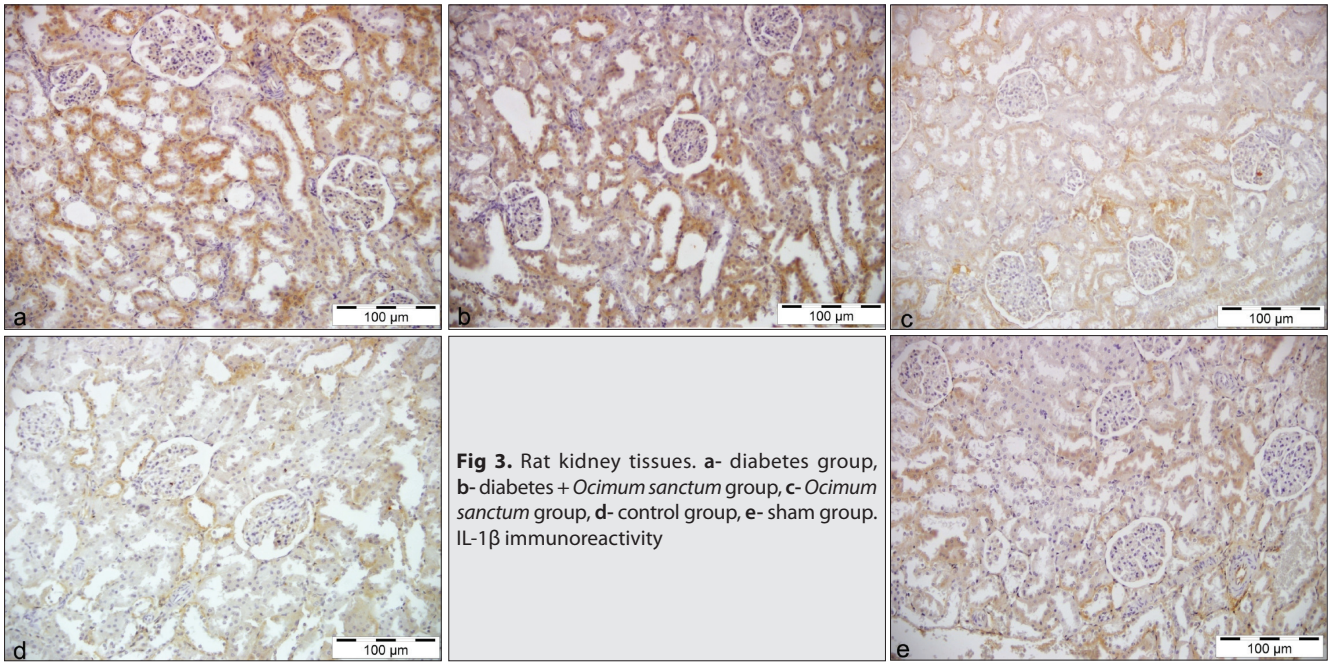

Fig 3. Rat kidney tissues. **a-** diabetes group, **b-** diabetes + *Ocimum sanctum* group, **c-** *Ocimum sanctum* group, **d-** control group, **e-** sham group. IL-1β immunoreactivity

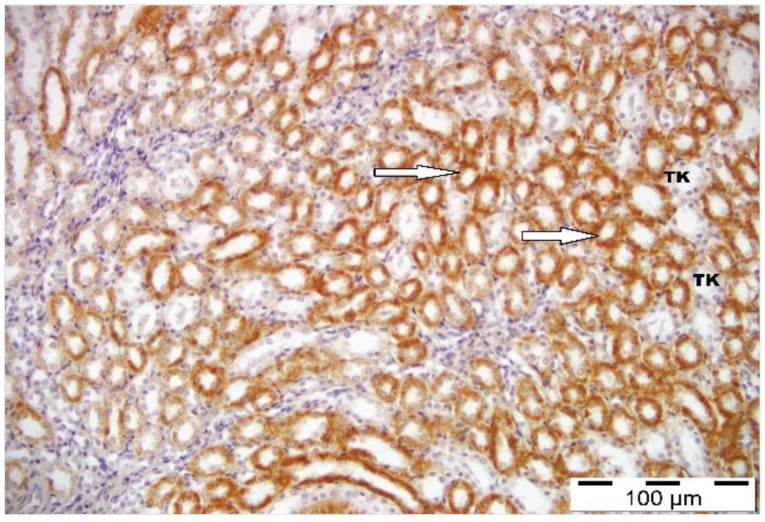

Fig 4. Rat kidney tissues in the diabetes group. *Arrow:* Loop of Henle, TK: Tubulus Collectivus. IL-1β immunoreactivity

Interleukin 1β immunoreactivity was determined at a strong level in diabetes groups kidney cortex and at a moderate level in diabetes + *Ocimum sanctum* groups kidney cortex. Although a strong immunoreactivity was determined in the rats tubulus proximalis and tubulus distalis in diabetes group on the 14th day *(Fig. 3-a)*, the immunoreactivity was at a moderate level in diabetes + *Ocimum sanctum* group *(Fig. 3-b)* and at a weak level in the rats in the *Ocimum sanctum (Fig. 3-c)*, control *(Fig. 3-d)* and sham *(Fig. 3-e)* groups. Strong immunoreactivity was determined in the rats loop of henle cells in diabetes group on the 14th day *(Fig. 4)*. No IL-1β immunoreactivity was detected in the glomeruli and vessel endothelium in the kidney of rats in all groups. The semiquantitative analysis results of intergroup for IL-1β immunohistochemical staining were summarized in *Table 3*.

Table 3. *The semiquantitative analysis results of intergroup IL-1β immunoreactivity on the 14th day*

Cells	Diabetes Group	Diabetes + *Ocimum sanctum* Group	*Ocimum sanctum* Group	Sham Group	Control Group
Tubulus proximalis	+++	++	+	+	+
Tubulus distalis	+++	++	+	+	+
Loop of Henle	+++	++	+	+	+
Vessel endothelium	-	-	-	-	-
Glomerulus	-	-	-	-	-

Discusion

Ocimum sanctum had an influence of decreasing high blood glucose, regulating lipid metabolism and suppressing blood sugar and would be able to have a therapeutical role in Type 2 diabetes disease by showing an influence like insulin in the rats [19,20]. Antora et al.[21] applied *Ocimum sanctum* extract to diabetic rats and they prepared that the application of *Ocimum sanctum* extract had an oral hypoglycemic activity. Leaf extract of *Ocimum sanctum* significantly decreases blood glucose in glucose induced hyperglycemic and STZ-induced diabetic rats [22]. In our study no significant difference was found in blood glucose on the 14th day in the diabetes + *Ocimum sanctum* group. Not being a statistical difference even though the blood glucose decreased in the *Ocimum sanctum* + diabetes group in our study to the contrary of the studies performed [23,24] make us think that it showed up depending on the dose, gender and day.

Lee et al.[25] specified that diabetes created glomerular enlargement, sclerosis and tubulointerstitial fibrosis in the rat kidney. Also, the glomerular and tubular basal membrane thickenings occurred by the accumulation of extracellular matrix components in nephropathy in the experimental diabetic and pathologic cases were emphasized various researchers [26,27]. In our study, Bowman cavities (urinary cavity) were determined as either very decreased or removed as a result of the increased cellularity in the glomerular mesangium in some glomerulus of the kidney tissues of the rats, for which diabetes created. No thickening was seen in the glomerular basal membranes. The casts were specified in some tubulus lumens. It was determined that there was a hydropic and balloony degeneration in some collector (collecting) and distal tubulus epithelium. Vacuolization was observed in the tubulus epithelium cells in the cortical regions. Bowman cavities (urinary cavity) were specified as either very decreased or removed as a result of the increased cellularity in the glomerular mesangium in diabetes + *Ocimum sanctum* group rats' kidney tissues. No thickening was seen in the glomerular basal membranes. It was specified that the proximal and distal tubules were normal. The tubulus lumens enlargement due to the necrosis occurred in the epithelium cells of the proximal, distal and collector tubules that we followed up in diabetic group to which the STZ was given, relieving in diabetes group to which the *Ocimum sanctum* was applied and prevention of the pathologic changes showed up by diabetes in the *Ocimum sanctum* make us think that it will be able to be related to the characteristics of stabilizing the cell membrane and protecting the components from any damage by means of the high lipophilic nature.

The TNF-α is a cytokine causing immune, metabolic and inflammatory events and playing a role in the insulin resistance development [28]. TNF-α is thought to play a role in various diseases including Type 2 diabetes [28] and is an important cytokine in diabetes pathogenesis [29]. The TNF-α's regulating role, especially in the lipid and glucose metabolism, gains importance in the metabolic syndrome and diabetes since it plays a key role in energy metabolism [29]. It was specified in many studies performed that the TNF-α levels increased after the STZ injection and TNF-α played a role in the complications development in diabetes [30,31]. In our study, the TNF-α immunoreactivity was determined at a strong level in the rats kidney cortex in diabetes and diabetes + *Ocimum sanctum* groups and at a weak level in the *Ocimum sanctum,* sham and control groups kidney cortex in parallel with the studies performed. Although a strong immunoreactivity was determined on the 14th day in the rats tubulus proximalis and tubulus distalis cells in diabetes and diabetes+*Ocimum sanctum* groups, the TNF-α immunoreactivity was observed at a weak level in the *Ocimum sanctum*, sham and control groups. In our study, the TNF-α may make us think that it played an important role in diabetic nephropathys pathogenesis since the TNF-α immunohistochemically increased in the rats kidney tissues in diabetes and diabetes + *Ocimum sanctum* groups.

It was specified that the IL-1β and TNF-α are the central inflammatory cytokines and their amounts are correlated with the increased complications in diabetic patients [32]. Although the cytokines influence was not exactly revealed on diabetic nephropathy, there are some data that they have important influences in some experimental studies performed [32,33]. In the immunohistochemical study we performed, the IL-1β immunoreactivity was determined at a strong level in the rats kidney cortex in diabetes group and at a moderate level in diabetes + *Ocimum sanctum* groups kidney cortex. Although a strong immunoreactivity

was determined on the 14th day in the henle loop, tubulus proximalis and tubulus distalis cells in diabetes group in the rats. The immunoreactivity was detected at a moderate level in diabetes + *Ocimum sanctum* group and at a weak level in the *Ocimum sanctum*, control and sham groups for the rats. Although a strong IL-1β immunoreactivity was determined in the rats kidney tissues in diabetes group, detecting the IL-1-β immunoreactivity at a moderate level in diabetes + *Ocimum sanctum* groups kidney tissues is in nature that it supports the studies performed for being an antidiabetic influence [34,35].

In conclusion, although the *Ocimum sanctum* application did not make a statistically significant influence on the blood glucose, it was observed that it caused an observable decrease and reduced the kidney damage caused by diabetes in the rats, to which STZ was applied. However, it was determined that it did not immunohistochemically show an influence on the TNF-α secretion but made an influence on IL-1β. The plants use in the treatment of many diseases will be able to be encouraging for the future and long-term uses and further studies are required in order to show the *Ocimum sanctum's* positive influence on diabetic complications.

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Author Contribution

S.E.Y. and E.K.S. conceived the original idea, designed and supervised the project. S.E.Y, B.B and H.A performed experiments. All authors wrote the manuscript.

Declaration of Interest

The authors report no conflicts of interest.

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