J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg.,47(2): 438-449, 2023
Doi: 10.33483/jfpau.1225760

ORIGINAL ARTICLE / ÖZGÜN MAKALE

NEPHROPROTECTIVE EFFECT OF *ALOE VERA* EXTRACT WITH REGULATION OF OXIDATIVE STRESS, APOPTOSIS AND AQUAPORIN 3 EXPRESSION LEVELS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

ALOE VERA EKSTRAKTININ STREPTOZOTOSİN İNDÜKLÜ DİYABETIK RATLARDA OKSİDATİF STRESS, APOPTOZİS VE AQUAPORİN 3 EKSPRESYON DÜZEYİNİ DÜZENLEYEREK NEFROPROTEKTİF ETKİSİ

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Submitted / Gönderilme : 28.12.2022
Accepted / Kabul : 14.02.2023
Published / Yayınlanma : 20.05.2023

ABSTRACT

Objective: *In this study we examined the protective activity of Aloe vera with considering anti-oxidant, anti-apoptotic properties, and the status of Aquaporin 3 (AQP3) channel protein.*
Material and Method: *Twenty-one adult female rats were divided into three groups (n=7); Control, Diabetes, Treatment. Control group did not expose to any application. Animals in Diabetes and Treatment were exposed to experimental diabetes with administration of streptozotocin. Rats in Treatment received 300 mg/kg Aloe vera extract daily for 14 days. Rats were sacrificed and kidney samples were used for analyses.*
Result and Discussion: *Analyses indicated that lowest malondialdehyde (MDA) and luminol levels in control group were increased significantly (P<0.05) in diabetic animals. Severe pathological changes observed in Diabetes group while microscopic examinations. Bax, Caspase-3 and apoptotic index (AI) were elevated significantly (P<0.05) in this group compared to Control. Oxidative stress, apoptotic protein expression levels and TUNEL Assay positive cell ratio were down-regulated in Treatment group. When AQP3 levels were measured, immunopositivity reduced significantly (P<0.05) in cortical kidney of Diabetes group which is normalized significantly in Treatment group.This study reporting anti-diabetic potency of Aloe vera extract has capability to avoid streptozotocin induced diabetic renal injury via regulating anti-apoptotic and anti-oxidant cellular signaling. Furthermore, Aloe vera consumption in diabetes might regulate AQP3 levels. Although we observed promising results, more studies are required to explore anti-diabetic, anti-hyperglycemic and nephroprotective activity of Aloe vera.*

Keywords: *Aloe vera, apoptosis, aquaporin 3, diabetes, kidney*

ÖZ

Amaç: *Bu çalışmada Aloe vera'nın koruyucu aktivitesini antioksidan, anti-apoptotik özellikleri ve Aquaporin 3 (AQP3) kanal proteininin durumu dikkate alınarak inceledik.*
Gereç ve Yöntem: *Yirmi bir yetişkin dişi sıçan Kontrol, Diyabet, Tedavi olmak üzere üç gruba (n=7) ayrıldı. Kontrol grubu herhangi bir uygulamaya maruz bırakılmadı. Diyabet ve Tedavideki hayvanlar, streptozotosin uygulanarak deneysel diyabete maruz bırakıldı. Tedavideki sıçanlara 14 gün boyunca günde 300 mg/kg Aloe vera özütü verildi. Sıçanlar sakrifiye edildi ve analizler için böbrek örnekleri kullanıldı.*
Sonuç ve Tartışma: *Analizler control grubundaki en düşük malondialdehit (MDA) ve luminal düzeylerinin diyabetik hayvanlarda anlamlı olarak arttığını (P<0.05) gösterdi. Diyabet grubunda mikroskobik incelemelerde ciddi patolojik değişiklikler gözlendi. Bax, Kaspaz-3 ve apoptotic indeks (AI) bu grupta Kontrol'e gore anlamlı olarak yüksek bulundu (P<0.05). Tedavi grubunda oksidatifstres, apoptotik protein ekspresyon seviyeleri ve TUNEL Assay pozitif hücre oranı aşağı regüle oldui. AQP3 düzeyleri ölçüldüğünde, Tedavi grubund aanlamlı olarak normalize edilen immünopozitiflik, Diyabet grubunun kortikal böbreğinde önemli ölçüde azaldı (P<0.05). Bu, Aloe veraekstraktının anti-diyabetik gücünü bildiren, anti-apoptotik ve antioksidan hücresel sinyallemeyi düzenleyerek streptozotosin kaynaklı diyabetik böbrek hasarını önleme yeteneğine sahip olduğunu göstermektedir. Ayrıca, diyabette Aloe vera tüketimi AQP3 seviyelerini düzenleyebilir umut verici sonuçlar gözlememize rağmen, Aloe vera'nın anti-diyabetik, anti-hiperglisemik ve nefroprotektif aktivitesini keşfetmek için daha fazla çalışmaya ihtiyaç vardır.*

Anahtar Kelimeler: *Aloe vera, apoptoz, aquaporin 3, böbrek, diyabet*

INTRODUCTION

Diabetes mellitus (DM) is one of the mostly encountered metabolic disorders in individuals of undeveloped and developing countries. DM affects kidneys, retina, cardiovascular system and neural system structure due to prolonged hyperglycemia [1]. In 2019 it was reported that the globally prevalence of DM will increase to 578 million people until 2030 and 700 million people until 2045 [2]. DM is divided into subgroups of insulin dependent Type 1 (T1DM) and Type 2 (T2DM) diabetes mellitus which is a result of insulin resistance [3,4]. T1DM results with diabetic nephropathy and renal dysfunction. Underlying mechanism of diabetic nephropathy is linked with increased oxidative stress due to over accumulation of reactive oxygen species (ROS) in kidney [5]. Degenerated renal corpuscles and tubular system dysfunction are the other observed pathologic problems in diabetic nephropathy

which are appear due to ROS and hyperlipidemia [6]. Although insulin therapy is still a reliable modality to control glycaemia to reduce disease complications, therapy protocol may change among the patients due to lifestyle and/or severity of the disease [7]. In literature some findings reported that over and/or long term insulin consumption may lead to other diseases such as hypoglycemia, weight gain, lipohypertrophy, local allergic reactions [8-10]. In recent years, some studies also reported possible cancer risk in insulin therapy received patients and possible contribution of insulin treatment on progress of malign diseases such as endometrial cancer [11,12]. For these reasons, numerous researchers investigated natural compounds and plant-based traditional supplements to control diabetic hyperglycemia and reduce insulin dependence of the DM patients [13-15]. Phytomedical compounds are used in treatment or control of various diseases [16]. Phenolic compound containing plant based control of hyperglycemia aims to increase deposition of blood sugar and targets antioxidant activity to protect organs which are more tendency to be affected in diabetic complications [17]. *Aloe vera* can be considered as one of these phenolic compounds containing medical plants. *Aloe vera* is a succulent that used in cosmetics, traditional medicine and drug industry. Due to containing large amount of bioactive compounds and antioxidant substances, protective activity of *Aloe vera* on various diseases examined. Phenolic compounds such as catechin, genistic acid, quercetin and much more idicates a large antioxidant spectrum of this plant [18]. Some published studies reported phenolic compounds in *Aloe vera* have capability to chelate metal ions thus down-regulate oxidation mechanisms [19]. Furthermore, hypoglycemic and hypolipidemic activities of *Aloe vera* is believed this plant to be a novel treatment modality in diabetes mellitus. Although, numerous studies reported beneficial effects of *Aloe vera* treatment in various diseases, possible effects of *Aloe vera* on streptozotocin induced diabetic nephropathy hasn't been clearly understood yet. For that reasons we aim to investigate the possible effects of *Aloe vera* against streptozotocin induced diabetic nephropathy with considering oxidative stress, apoptosis related protein expression and DNA fragmentation ratio, and water/glycerol transporter channel protein Aquaporin 3 levels in tissue.

MATERIAL AND METHOD

Study Design

All experimental procedure of this study was performed with approval of the Local Experimental Animal Ethics Committee of a University (approval date & no: 14.12.2021 & 2020/09). Twenty-one mature female rats between 169-230 gm were obtained from Experimental Animal Unit of a University and animals were randomly divided into three groups (n=7) as follows; Control, Diabetes, Treatment. Rats were kept in standard animal cages in 22 ± 2°C and 12 h light-dark cycle. Tap water and standard pellet food provided to the animals ad libitum. Animals in Control were not exposed to any application during the experiment. Rats in Diabetes and Treatment were subjected to 40 mg/kg streptozotocin for induction of Type 1 diabetes with intraperitoneal route. Following the injection all of the animals in this study received 10% glucose containing water for 48 hours to avoid hypoglycemia. Seventy-two hours post streptozotocin injection, fasting blood glucose levels in all groups were measured from tail vein and animals with glucose level >240 considered as diabetic. Rats in Treatment group received 300 mg/kg *Aloe vera* extract daily by oral gavage. The administered dose of *Aloe vera* was chosen according to a previously published article that reported anti-hyperglycemic activity [20]. All animals were sacrificed by exsanguination after 14 days of confirmation of experimental diabetes. Left kidney samples were fixed in 10% formaldehyde for microscopic examinations, right kidney of the animals were frozen in -80°C for the measurement of biochemical analyses.

Preparation of *Aloe vera* Extract

Plant materials were received and Ethanolic extract was prepared as described previously [21]. Healthy leaves were washed with sterile water and solid gel was removed from leaves. Collected gels were mixed with equal volume of 95% ethanol. Prepared suspension filtered and the extract evaporated. Extract was administered to the animals with dissolving in water prior administration. Plant samples were also deposited in Herbarium of a University Faculty of Pharmacy (shelf number: AEF 30758).

Biochemical Analyses

Tissue samples were homogenized for measurement of tissue lipid peroxidation and chemiluminescence assay that are used to measure total MDA and ROS level in samples. Measurement of these substances was performed as described previously [22]. Lipid peroxidation was evaluated as MDA equivalents using an extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$ and results are expressed as nmol MDA/gm tissue. Total ROS were measured with chemiluminescenceassay which is able to use to detection of hydrogen peroxide, hydroxyl radical and hypochlorite radicals specifically. Measurement performed in a luminometer (Junior LB 9509, EG&G Berthold, Germany) with administration of luminol (5-amino-2,3-dihydro-1,4-phthalazinedione, Sigma) to the tissue samples. Results of chemiluminescence assay are expressed in relative light units per mg (rlu/mg) of tissue. All obtained biochemical results were analyzed for determination of statistically difference among the groups.

Tissue Processing Protocol

Kidneys were fixed in 10% formaldehyde solution for 24 hours. After fixation tissue samples were washed under tap water and dehydrated through increasing alcohol series and embedded into paraffin block following cleared in xylene. Five µm thick sections were obtained with a rotary microtome and sections were stored for the staining of Hematoxylin and Eosin (HE), Periodic acid-Schiff (PAS) and immunohistochemistry.

Histopathological Staining

Tissue sections were deparaffinized in Xylene and rehydrated in decreasing alcohol series. Sections from each samples then stained with HE or PAS. HE staining was performed as described previously [23]. PAS staining performed with a ready to use staining kit (Cat no: 04-130802, Bio-optica MI, IT) and all steps were performed under the recommendations of the manufacturer's instructions. Stained samples were mounted with entellan and examined under a camera attached light microscope and micrographs were captured.

Immunohistochemistry and TUNEL Assay

Tissue sections were deparaffinized, rehydrated and washed in PBS. Samples were brought to citrate buffer (Ph: 6.0) and heated on a hot plate until reaching sub-boiling temperature. The samples then cooled into room temperature and endogenous peroxidase activity was blocked with incubating the samples for 15 minutes in 3% H2O2 that dissolved in methanol. Primary antibodies of Bax (Cat no: sc-7480, Santa Cruz Biotechnology, Dallas, Texas, USA), Caspase 3 (Cat no: sc-56053, Santa Cruz Biotechnology, Dallas, Texas, USA) and AQP3 (Cat no: sc- sc-20811, Santa Cruz Biotechnology, Dallas, Texas, USA) were diluted 1:100, 1:100 and 1:200 respectively in an antibody diluent prior application. Blocking step, secondary antibody and enzyme applications were performed with a ready to use kit of Large Volume Detection System, HRP (Cat no: TP-125-HL, Thermo Fisher Scientific, MA, USA) and all procedures performed according to instructions of the manufacturer. Between all steps samples were washed in two series of PBS except primary antibody application. Primary antibody incubation performed for overnight at +4°C refrigerator. Chromogenic reaction was developed with DAB chromogen. Sections were counterstained in hematoxylin, mounted with entellan and examined under light microscope. TUNEL Assay was performed with In Situ Cell Death Detection Kit, Fluorescein (Cat no: 11684795910; Roche, Basel, CH) and all steps were performed with manufacturer's directions that also described previously [24]. TUNEL Assay sections were mounted with a ready to use mounting media that contains DAPI (Cat no: sc-24941, Santa Cruz Biotechnology, Dallas, Texas, USA). Fluorescein stained sections were examined under a fluorescein attached light microscope and micrographs were captured.

Quantification of Microscopic Examinations

Immunohistochemistry samples were quantified by threshold analysis feature of Image J software (NIH) with considering 3 randomly selected cortical areas from each animal kidney section. Immunodensity was obtained with comparing DAB positive are to the total tissue section. Measured

immunodensity levels were expressed as ratio. TUNEL Assay analyses performed under fluorescence attached microscope system. For determination of Apoptotic Index (AI), randomly selected 21 renal corpuscles from each group were used and green fluorescence positive cell count in each renal corpuscle were compared with blue and only DAPI stained total glomerular cell nuclei. All obtained datasets for immunohistochemistry and TUNEL Assay were analyzed statistically.

Statistical Analysis

Statistical analyzes were performed with SPSS Statistics Version 24.0 (IBM, NY USA) software. All obtained data were analyzed statistically to determine whether the differences among the groups are significant or not. For that purpose, all obtained datasets were analyzed with the one-way analysis of variance (ANOVA). Multiple comparisons were performed with post-hoc Tukey test. All results were shown as mean ± SD and $P<0.05$ considered as significant.

RESULT AND DISCUSSION

Our analyses indicated that tissue MDA and total ROS level in the kidney of Control group was 9,46 ± 2,44 nmol/gm and 48,93 ± 2,53 rlu/mg respectively. The tissue level of MDA and total ROS were increased significantly in streptozotocin induced Diabetes group to 21,39 ± 8,37 nmol/gm and 60,67 ± 10,96 rlu/mg. Statistical analyses indicated that the difference between Control and Diabetes were significant ($P<0.05$). When we measure the tissue MDA and total ROS levels in Treatment group, it was observed that the lipid peroxidation status alleviated to 12,29 ± 3,74 nmol/gm and 54,53 ± 8,12 rlu/mg. The statistical evaluation indicated that MDA and ROS levels in Treatment group was similar ($P>0.05$) to both of these groups. Graphical demonstrations of the statistical results are shown in Figure 1.

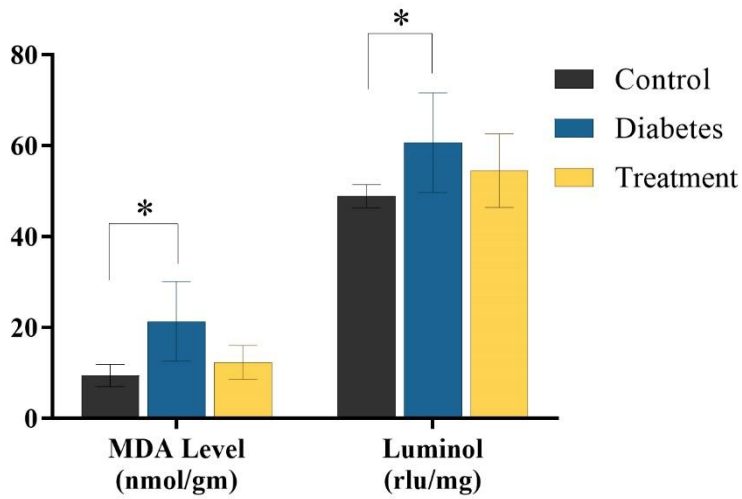

Figure 1. Tissue MDA and luminol level in the groups. The superscript between the groups indicate statistically significance (*$P<0.05$).

Morphological structure in control group was normal. Renal cortex was filled with renal glomerulus, proximal and distal tubules and upper portions of the collecting tubular system. In kidneys of Diabetes group, there were severe degenerations. Most of the glomerulus in this group was irregular. In PAS stained sections, increase in the thickness of glomerular basement membrane and depletion in microvilli structure and brush border of convoluted portion of proximal tubules were clearly visible. Vascular congestion and partially dilated vascular structure was widespread in kidney of this group. Intertubular edema was widespread. However, the mentioned kidney pathology was alleviated in *Aloe vera* exposed animals. Irregular renal glomerulus was less common. Vascular structure was normal and basement membrane thickening is not observed. Representative HE and PAS stained tissue sections are

shown in Figure 2.

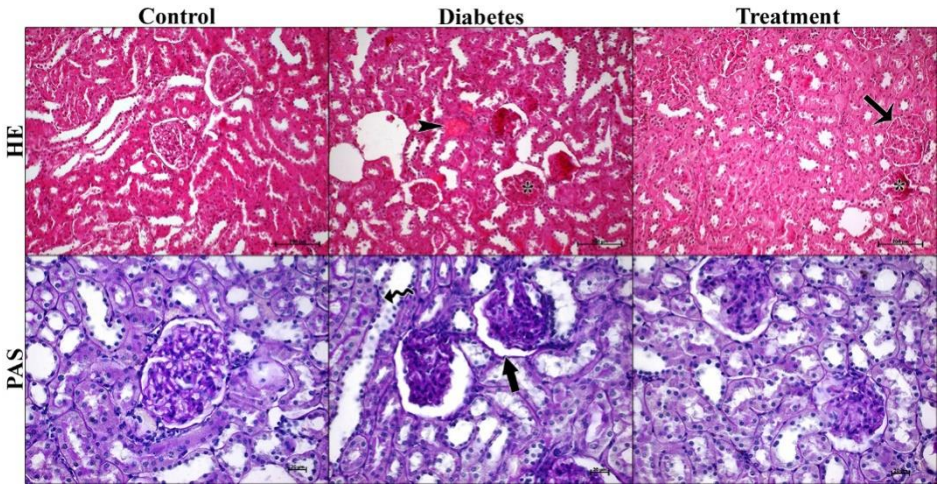

Figure 2. Light micrographs of the Control, Diabetes and Treatment groups. Irregular glomerular nephrons (*), edematous parenchyma (arrow head), decrease in filtration space (arrow), increase in thickness of glomerular basement membrane (thick arrow), depletion of brush border in proximal convoluted tubules (curved arrow). Staining: H&E, PAS, Bar: 200 µm in H&E and 20 µm in PAS sections.

Representative micrographs of the immunohistochemistry stainings are shown in Figure 3. The Baximmunodensity was lowest in Control group and it increased significantly in Diabetic animal kidney (P<0.05). Bax level in Treatment group alleviated, but it was stand between Control and Diabetes groups

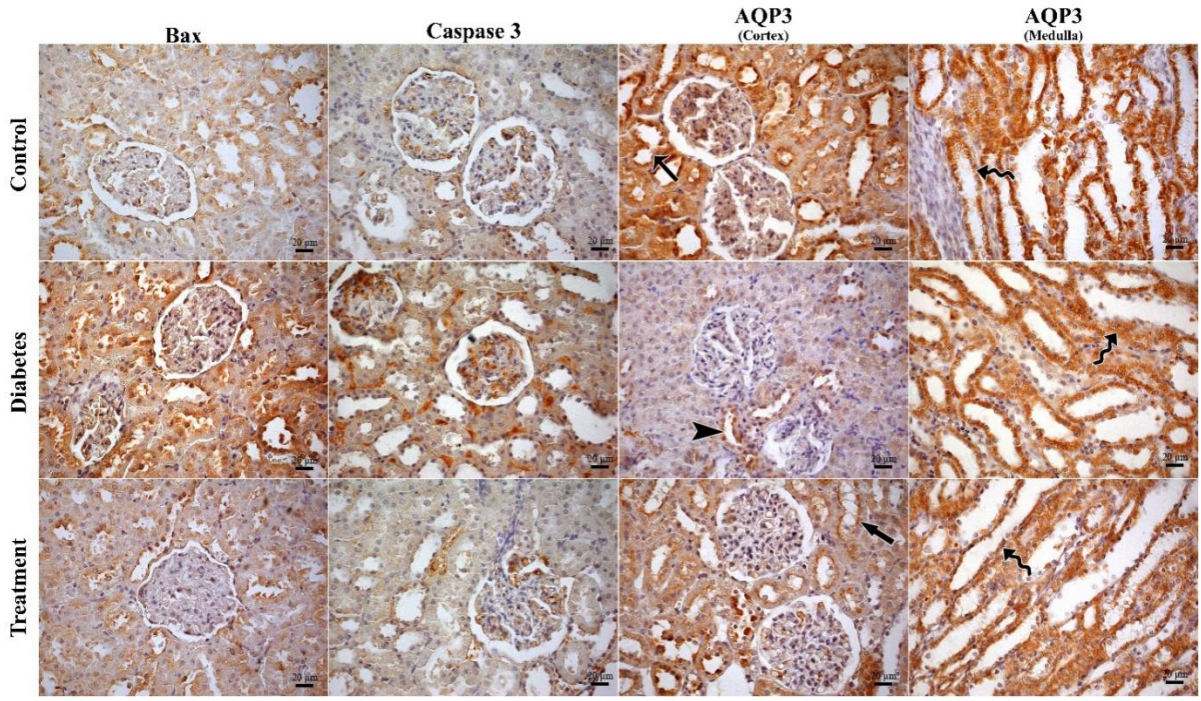

Figure 3. Representative Immunohistochemistry micrographs of Bax, Caspase 3 and Aquaporin 3 in groups. Varying AQP3 immunopositivity in proximal tubules of control (arrow), diabetes (arrow head) and treatment (thick arrow) groups, intense AQP3 immunopositivity in medullary collecting tubules (curved arrow). Bar: 20 µm.

(P>0.05). Caspase 3 immunodensity was dramatically (P<0.01) increased in diabetic animals compared to the rats in Control group. On the other hand, Caspase 3 immunodensity in Treatment group was significantly alleviated and it was similar (P>0.05) to the rest of the groups. Cortical AQP3 immunopositivity in Control group was the highest and it down-regulated in Diabetic rat kidney significantly (P<0.01). In *Aloe vera* treated animals, AQP3 immunpositivity was up-regulated but density in this group was still significantly different than Diabetes (P<0.05) and Control (P<0.01) groups. When AQP3 levels were evaluated in renal medulla, there weren't any significance (P>0.05) among the groups observed.

When TUNEL Assay sections were evaluated, TUNEL positivity was very slight in Control group, but positivity observed almost all renal cell types (Figure 4). AI in Diabetes group increased dramatically in renal glomerulus cells (P<0.01). In Treatment group, AI alleviated significantly (P<0.01) compared to the Diabetes group, but mean positive cell ration in this group was still significantly different (P<0.05) than Control group. Statistical results of immunohistochemistry threshold analyses and TUNEL Assay are shown in Table 1 and Figure 5.

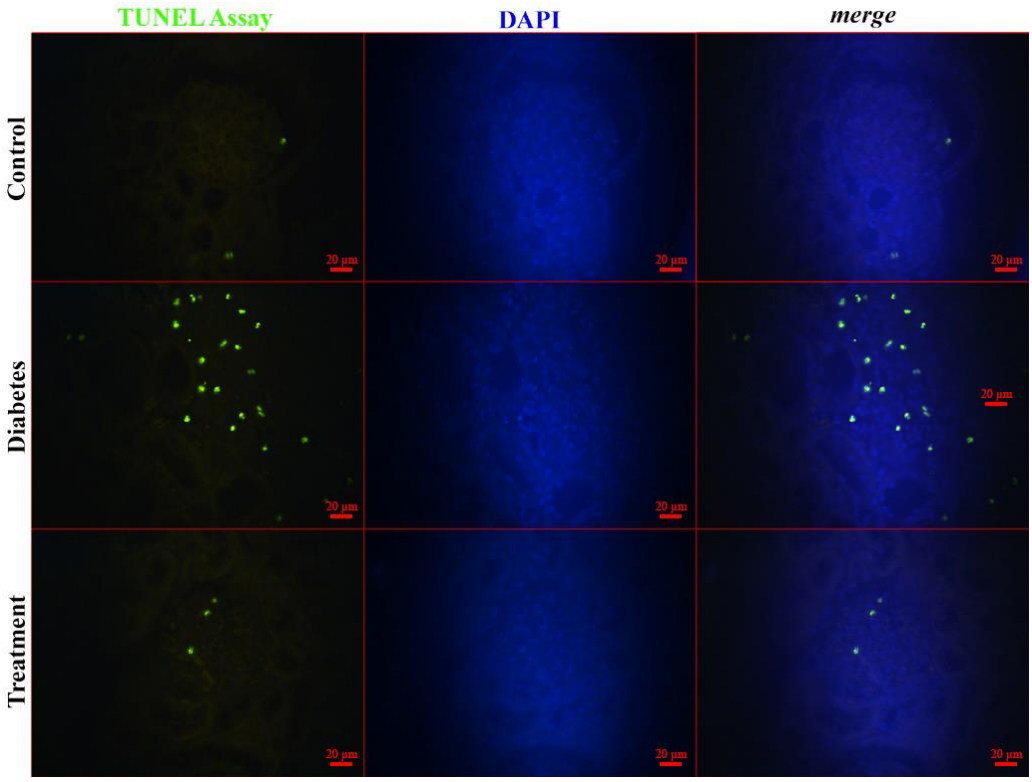

Figure 4. Representative TUNEL Assay micrographs of Control, Diabetes and Treatment groups. Green fluorescence probes indicate DNA fragmentation in kidney sections. Samples counterstained with DAPI.Bar: 20 µm.

Table 1. Statistical analyses result of immunohistochemistry and TUNEL Assay. Different superscripts among the groups indicate significantly differences between the groups ($^{a\text{-}b}P$<0.05, $^{a\text{-}c}P$<0.05, $^{b\text{-}c}P$<0.05).

	Bax	Caspase 3	AQP3 (Cortex)	AQP3 (Medulla)	AI
Control	26,68±3,63^{a}	23,48±4,31^{a}	69,00±10,94^{a}	84,29±10,92^{a}	4,22±2,36^{a}
Diabetes	30,50±5,13^{b}	32,91±8,79^{c}	49,02±12,34^{c}	80,50±9,39^{a}	10,79±3,71^{c}
Treatment	27,57±3,37^{ab}	28,24±3,93^{b}	57,64±9,22^{b}	83,87±10,31^{a}	6,40±2,27^{b}

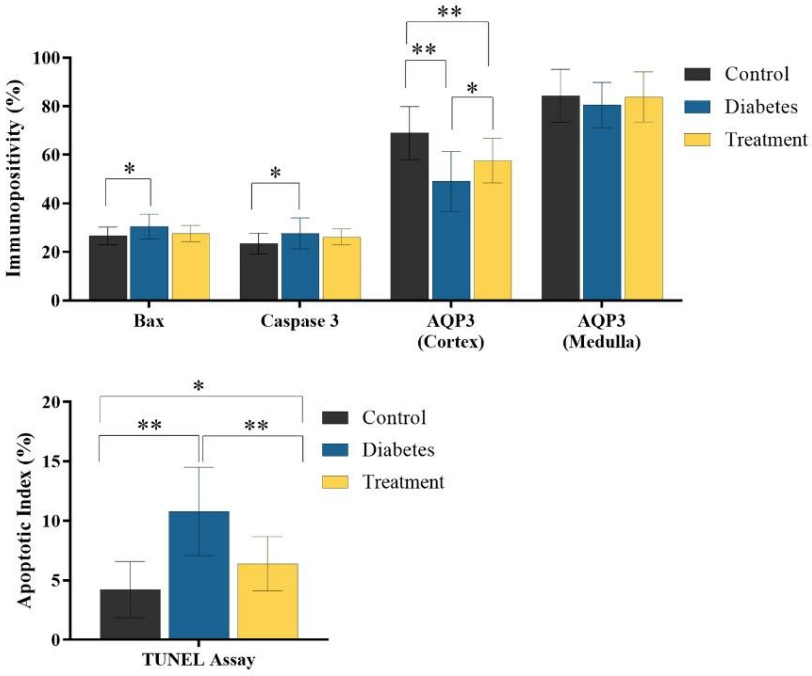

Figure 5. Graphical demonstration of immunohistochemistry and TUNEL Assay statistical analyses. Different superscripts between colums indicate significantly difference **P*<0.05, ***P*<0.01.

DM is one of the most observed metabolic disorders among the individuals. Although there are clinical applications such as insulin administration to balance blood sugar, researchers are still looking for the alternative modalities. Although many organs are affected from the hyperglycemia, the kidney are the most affected organs in DM [25]. When we review literature that is possible to reach numerous studies examined the anti-diabetic activity of herbal medicine [26]. If oxidative stress and DM evaluated as a whole, some researchers reported that DM and oxidative stress have synergistic actions over each other and each of them has ability to intensify the other one [27-30]. Although there is a limited data for the anti-diabetic activity of *Aloe vera*, in a previously published study hypoglycemic activity of Ethanolic*Aloe vera* extract investigated both in normal fasting and streptozotocin induced diabetic rats [21]. Results of this study reported that *Aloe vera* administration down-regulated blood glucose level in normal fasting animals which indicates hypoglycemic activity. Moreover, this experiment demonstrated that Ethanolic extract of *Aloe vera* increased glucose tolerance as well. Clinical experiments also reported promising results that hypoglycemic and triglyceride level regulatory activity of *Aloe vera* in T2DM patients [31]. *Aloe vera* was also found protective on pancreatic β cells besides the blood glucose and triglyceride regulatory and insulin secretion enhancing effect in streptozotocin induced diabetic rats [32]. Previously published in vitro studies also reported functional activities of *Aloe vera* in glucose metabolism. In one of these, *Aloe vera* increased Glucose transporter type 4 (GLUT4) protein gene expressions and glucose deposition [33]. In literature, most of the *Aloe vera* related experiments concluded that rich phenolic compounds and glucose depositing activity of *Aloe vera* is possible underlying hypoglycemic activity of this phenolic plant. Although kidneys are one of the most affected organs in diabetes, literature on effects of *Aloe vera* against diabetic nephropathy is limited [34]. In one of the previously published article, *Aloe vera* extract alleviated ROS level and oxidative stress in streptozotocin induced diabetic kidney [35]. Results of this study also reported diabetic nephropathy related microscopic degenerations were attenuated in treated animals. Results of this study and our current experiment are consistent. We observed severe degenerations in streptozotocin induced diabetic kidney and microscopic degenerations were reduced significantly. We also reported that *Aloe vera* oral supplementation protected kidney tissue with exerting an influence on apoptotic protein expression

levels. For that reason it's possible to say that *Aloe vera* supplementation not only has potency to down-regulate diabetes related metabolic complications with regulating blood glucose level as reported previously [21] but also affecting pro-apoptotic Bax and Caspase 3 expression levels. In another in vitro study it was reported that DNA fragmentations are increased in streptozotocin induced diabetic kidney tubular cells [36]. Furthermore, authors of this study concluded that inhibition of ferroptosis might be a potent therapeutic strategty to downregulate apoptotic cellular death in diabetic nephropathy. AI has tendency to increase dramatically in tubular system and glomerular structure within a short time as reported in two published studies [37,38]. In one of these of these studies, Sohn et al. also reported that administration of Aster koraiensis extract reduced renal AI in experimental diabetic animals. These results are consistent with our experiment if we consider relationship between AI and streptozotocin induced diabetic nephropathy. We also observed a significant decrease in AI of renal structure of *Aloe vera* treated diabetic animals.

When literature is reviewed it's possible to reach that water/glycerol transporter channel protein levels are affected in streptozotocin induced diabetic kidney [39]. Moreover, AQ3 is not only acting as glycerol and hydrogen peroxide transport channel protein, but also contributes cellular proliferation and apoptosis through regulating various cellular signaling [40].In another detail, some previously published studies and textbooks highlight that AQP3 is to be expressed in cortical basolateral membrane of collecting ducts and medullary collecting ductal systems [41,42]. However, probably as a result of upgraded sensitivity of immunodetection techniques and gene analyses, recently published studies are reporting some conflicting results that AQP3 is not only expressed in cortical collecting ducts but also proximal tubules [43, 44]. In one of these studies, Leung et al. reported down-regulated AQP3 level in streptozotocin induced diabetic kidney. However, in another study, Nejsum et al. reported streptozotocin induced diabetes up-regulated AQP3 level as a response to polyuria [45]. It is possible to reach the localization and expression levels of AQP3 in diabetic nephropathy is not defined clearly. When we compare results of our current experiment with literature, it's possible to reach partially conflicting and consistent results. First of all, we observed AQP3 is expressed in proximal convoluted cortical tubules besides the cortical collecting duct. Furthermore, results of our experiment indicated that AQP3 expression is down-regulated at renal cortex, but not affected at renal medulla in streptozotocin induced diabetic kidney. We also observed that treatment with *Aloe vera* alleviated this suppression in renal cortex. We believe that the conflicting results of AQP3 level in literature might be related with varying of experimental design of the conflicting studies and evaluation method of AQP3. The study that reported AQP3 is up-regulated in diabetic kidney is performed with intravenous administration of streptozotocin, but in the conflicting studies the induction of diabetes was performed with intraperitoneal administration route.

As a conclusion, results of our current experiment indicated that *Aloe vera* extract has potency to protect kidney in hyperglycemic animals with down regulating oxidative stress, apoptosis related protein expressions, DNA fragmentation and apoptosis. Furthermore, in our immunohistochemical analyzes we observed that AQP3 level is down-regulated in hyperglycemic diabetic animals renal cortex. We also observed that treatment with *Aloe vera* normalized AQP3 expressionin cortical structures. Although our study is expressing promising results, the anti-diabetic, anti-apoptotic and anti-hyperglycemic activity and clinically applicability of *Aloe vera* should be evaluated in more detailed studies to understand possible modality potential of the *Aloe vera*.

ACKNOWLEDGEMENTS

The authors thank to Rohlat Seyrek for her valuable contributions.

AUTHOR CONTRIBUTIONS

Concept: U.S., S.S.; Design: U.S., S.S.; Control: U.S., B.C.G., M.Y., O.U.D., S.S.; Sources: U.S., B.C.G., D.S.A., S.B.B., M.Y., O.U.D., S.S.; Materials: U.S., M.Y., O.U.D., S.S.; Data Collection and/or Processing: U.S., B.C.G., D.S.A., S.B.B., M.Y., O.U.D.; Analysis and/or Interpretation: U.S., M.Y.; Literature Review: U.S., B.C.G.; Manuscript Writing: U.S., B.C.G.; Critical Review: U.S., B.C.G., D.S.A., S.B.B., M.Y., O.U.D., S.S.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

Experimental animal tissue samples of this study was obtained with approval of the Local Experimental Animal Ethics Committee of Dicle University (Approval date & no: 14.12.2021 & 2020/09).

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