BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
© / ISSN 0717 7917 / www.blacpma.ms-editions.cl

Artículo Original / Original Article

Investigation of the protective and therapeutic effects of β-aminoisobutyric acid (BAIBA) and Thymoquinone in the diabetic nephropathy

[Investigación de los efectos protectores y terapéuticos del ácido β-aminoisobutírico (BAIBA) y la timoquinona en la nefropatía diabética]

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Section
Biological activity

Received: 16 May 2020
Accepted: 14 September 2020
Accepted corrected: 6 October 2020
Published: 30 May 2021

Citation:
Aktaş I, Gür FM.
Investigation of the protective and therapeutic effects of β-aminoisobutyric acid (BAIBA) and Thymoquinone in the diabetic nephropathy.
Bol Latinoam Caribe Plant Med Aromat
20 (3): 303 - 314 (2021).
https://doi.org/10.37360/blacpma.21.20.3.22

Abstract: In this study, against streptozotocin (STZ) induced diapetic nephropathy (DN); it is aimed to investigate the use of thymoquinone (TQ) and β-aminoisobutyric acid (BAIBA) and to compare the effects of these agents. With random selection of 35 male rats, five groups (seven rats in each group) were constituted as follows: Control, STZ, STZ + TQ, STZ + BAIBA, STZ + TQ + BAIBA. In the STZ group; body weight, glutathione (GSH) and insulin levels decreased, relative kidney weight, malondialdehyde (MDA), glucose, blood urea nitrogen (BUN) and creatinine (Cr) levels were increased. Also, in kidney tissue; histopathological changes (such as thickening of the capsular, glomerular and tubular basement membranes, increased mesangial matrix amount, increased cytoplasmic vacuolization in some of the tubular epithelial cells, increased tumor necrosis factor-alpha (TNF-α) expression, and inflammatory cell infiltrations in interstitial tissue) were detected. It was observed that these changes occurring after diabetes mellitus (DM) reversed significantly in TQ, BAIBA and TQ + BAIBA groups.

Keywords: β-aminoisobutyric acid; Diabetic nephropathy; Rat; Streptozotocin; Thymoquinone.

Resumen: En este estudio, contra la nefropatía diapética (ND) inducida por estreptozotocina (STZ); tiene como objetivo investigar el uso de timoquinona (TQ) y ácido β-aminoisobutírico (BAIBA) y comparar los efectos de estos agentes. Con la selección aleatoria de 35 ratas macho, se constituyeron cinco grupos (siete ratas en cada grupo) como sigue: Control, STZ, STZ + TQ, STZ + BAIBA, STZ + TQ + BAIBA. En el grupo STZ; el peso corporal, los niveles de glutatión (GSH) y de insulina disminuyeron, el peso relativo de los riñones, el malondialdehído (MDA), la glucosa, el nitrógeno ureico en sangre (BUN) y los niveles de creatinina (Cr) aumentaron. Además, en tejido renal; se detectaron cambios histopatológicos (como engrosamiento de las membranas basales capsular, glomerular y tubular, aumento de la cantidad de matriz mesangial, aumento de la vacuolización citoplasmática en algunas de las células epiteliales tubulares, aumento de la expresión del factor de necrosis tumoral alfa (TNF-α) e infiltraciones de células inflamatorias en tejido intersticial). Se observó que estos cambios que ocurren después de la diabetes mellitus (DM) se revirtieron significativamente en los grupos TQ, BAIBA y TQ + BAIBA.

Palabras clave: Ácido β-aminoisobutírico; Nefropatía diabética; Rata; Estreptozotocina; Timoquinona.

INTRODUCTION

DN, one of the most important complications of DM; It shows a progressive course, resulting in end-stage renal failure (ESRF) (Tziomalos & Athyros, 2015; Hadjadj *et al*., 2016). Although the pathogenesis of DN due to DM is not known precisely, it has been reported that this is associated with hyperglycemia oxidative stress and hypertension formed by DM (Xu *et al*., 2005; Roshan, 2013; Arora & Singh, 2014). Chronic hyperglycemia; It increases the production of reactive oxygen species (ROS) and triggers oxidative stress, causing damage to small blood vessels, causing tissue and organ failure. ROS such as hydroxyl and superoxide radicals attack cell cellular macromolecules such as carbohydrates, nucleic acids, lipids and proteins, causing cell damage or death (Xu *et al*., 2005; Tolman *et al*., 2007; Ros *et al.,* 2011; Karandrea *et al.,* 2017; Joudaki & Setorki, 2019). In addition, pro inflammation due to hyperglycemia and oxidative stress caused an increase in TNF-α, a pro-inflammatory cytokine (Cayakar, 2018). In many studies, it has been observed that the amount of TNF-α increases in DN kidney tissue (Mc Carthy *et al*., 1998; Giribabu *et al.,* 2017). Microalbuminuria, decreased creatinine clearance and glomerular filtration rate are early clinical signs of DN. In addition, kidney tissue in the following stages; histopathological changes such as capsular, glomerular and tubular basement membrane thickening, mesangial matrix enlargement, loss of podocytes, degeneration in the tubular epithelium and macrophage infiltration are observed. ESRF, the last stage of the disease, is characterized by irreversible structural changes such as glomerulosclerosis and tubulointerstitial fibrosis (Kanwar *et al*., 2008; Kanter, 2009; Roshan & Stanton, 2013; Al-Trad *et al.,* 2016; Shaterzadeh-Yazdi *et al.,* 2018). In patients with type 1 and type 2 diabetes, using drugs with blocking effect on the renin-angiotensin-aldosterone system, controlling hypertension, as well as using drugs to control dyslipidemia and hyperglycemia are the most effective treatment strategies in reducing the progression of DN and cardiovascular mortality (Gross *et al*., 2005; Skupien *et al*., 2012; Levey *et al*., 2014).

TQ is the most important bioactive ingredient found in black seed (*Nigella sativa*) essential oil in the ratio of 18.4-24% (Abdel-Fattah *et al.,* 2000). BAIBA is a catabolite of antiretroviral thymine analogs zidovudine (AZT) and stavudine (d4T) (Maisonneuve *et al.,* 2004). It has recently been discovered that BAIBA, a non-protein amino acid, is secreted by skeletal muscles after regular exercise through the peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). BAIBA, a myocin, transforms white adipose tissue into brown adipose tissue, which improves glucose metabolism and insulin sensitivity (Shi *et al*., 2016; Tanianskii *et al.,* 2019). In scientific studies; TQ and BAIBA have been shown to have antioxidant, antihyperglycemic, hypolipidemic, anti-inflammatory, hepatoprotective, antidiabetic properties, as well as reducing the amount of TNF-α, which is a pro-inflammatory cytokine, and hepatic glyconeogenesis (Bashandy *et al.,* 2015*;* Awad *et al.,* 2016*;* Shi *et al.,* 2016*;* Farkhondeh *et al.* 2017*;* Abdelrazek *et al.,* 2018; Sawada *et al.,* 2019). In addition, in recent studies, it has been reported that the use of TQ against the adverse effects of STZ induced DM in the kidneys demonstrates renoprotective and therapeutic effects that reduce oxidative stress. In a study conducted by Wang *et al*. (2017) reported that BAIBA treatment can improve pathological changes in the kidneys and that BAIBA has the potential to be used as a medicine in the treatment of renal fibrosis diseases (Kanter, 2009; Al-Trad *et al*., 2016; Shaterzadeh-Yazdi *et al.,* 2018). STZ is an antibiotic produced by *Streptomyces achromogenes*, cause the destruction of β cells in the pancreas and used to create experimental DM (Furman 2015; Bathina *et al.,* 2017; Joudaki & Setorki, 2019). The mechanism of the action of STZ on pancreatic β-cells has not been completely understood yet; however, it is believed that oxidative stress plays an important role in the development of complications from STZ (Sadek *et al.,* 2017). STZ is an unstable molecule that accumulates in pancreatic β-cells and breaks down into carbonium radicals. Carboxylic radicals, which are highly reactive, produce toxic effects directly or indirectly by increasing ROS formation on the pancreatic islet cell (Ghanema & Sadek, 2012; Sadek *et al.,* 2017). STZ has been reported to cause β-cell damage by inducing DNA fragmentation and methylation (Cardinal *et al.,* 2001). STZ is taken by pancreatic β-cells via GLUT2 glucose transporter causes degenerative changes in these cells, resulting in decreased insulin secretion and ultimately hyperglycemia (Eleazu *et al*., 2013).

In the current study, DN was developed because of DM induced by STZ; It is aimed to investigate the protective and therapeutic effects of TQ and BAIBA and to compare their potency against the biochemical changes in the blood and kidneys and histopathological changes in the kidney tissue.

MATERIAL AND METHODS

Chemicals

TQ (> 98%), STZ (> 98%), BAIBA (> 97%) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Hydrochloric acid (HCl), trichloroacetic acid (TCA), thiobarbuturic acid (TBA), paraffin and all other chemicals were purchased from Sigma-Aldrich (Germany). Xylene, hematoxylin, eosin and ethanol were obtained from Merck (Germany).

Animals

Thirty-five male Sprague-Dawley rats (210-250 g, aged 8 weeks) which are supplied from Adiyaman University Experimental Animal Production Application and Research Centre were used and experimental procedures were performed in the same centre. Ethics committee permission was taken from Adiyaman University Laboratory Animals Ethics Committee (Protocol 2019/001). The obtention and manipulation of test rats, and the following procedures were properly authorized by the institution's ethics guidelines.

Treatment protocol

A total of 35 male rats were randomly selected; control, STZ, STZ + TQ, STZ + BAIBA, STZ + TQ + BAIBA were divided into five groups (n = 7). 50 mg/kg single dose of STZ was dissolved in 0.1 M sodium citrate buffer (pH: 4.5) and then it was administered as i.p. (Bayat *et al.*, 2019; Liu *et al*., 2019). After 72 hours of STZ administration, blood glucose level was measured from the tail vein. Rats with blood glucose concentrations above 250 mg/dL were considered diabetic. Diabetic rats received TQ (20 mg/kg/day) and BAIBA (100 mg/kg/day) by gavage for 5 weeks (Begriche *et al*., 2008; Pari & Sankaranarayanan, 2009; Randhawa *et al*., 2013). Water and food consumption of rats were measured daily, and blood glucose levels and body weights were measured on the third and last day of experimental applications (Sharma *et al.,* 2019). After the experimental procedures were completed, blood samples were taken from the vena cava caudalis of anesthetized rats (75 mg/kg + xylazine 10 mg/kg i.p). Blood serums were obtained by centrifuging blood samples at 5,000 x g for 15 minutes and stored at -86°C for biochemical analysis (El-Shemi *et al.*, 2018). The rats were decapitated. After weighing the excised kidneys, one part was separated for biochemical analysis and the other part was immersed in 10% buffered neutral formalin solution for histopathological analysis and fixed at +4ºC for 24 hours. Kidney tissues separated for biochemical analyses were stored at -86°C.

Histological procedures

Fixated tissues were embedded in paraffin through routine histological procedures. Tissues cut from paraffin blocks with microtome thickness of 5 µm were stained with hematoxylin & eosin (H & E) and PAS methods. Histopathological examinations were performed using Olympus BX-53 microscope and photographs were taken with this microscope camera (DP 80 Olympus, Tokyo, Japan).

Immunohistochemistry

Sections were incubated for 1 hour at 60°C oven to provide stronger adhesion of the slides to the tissue, followed by a series of xylol and alcohol. After removal of endogenous peroxidase, tissue sections were incubated for 10 minutes in 3% hydrogen peroxide (H_2O_2) (prepared with methanol) and then washed in distilled water (5 minutes). The antigen retrieval protocol began by transferring tissue sections to the plastic coplin jar containing 0.01 M citrate buffer (pH 6.0). The coplin jar was placed at the midpoint of the rotating platform of the microwave oven and heated four times in succession for five minutes at 600 W. The amount of buffer in the coplin jar was checked every five minutes to complete the reduced fraction with distilled water. The sections removed from the microwave were then allowed to cool to room temperature for 20 minutes. Antigen retrieval was terminated by washing the cooled tissue sections in phosphate buffer saline (PBS) for 5 minutes. After antigen retrieval protocol, sections washed in PBS were incubated with 10% normal rabbit serum at room temperature (10 minutes) to prevent nonspecific antibody binding. To determine TNF-α expression, tissue sections were incubated with rabbit anti-TNF-α primary antibody in a humidified chamber at 4°C for 16-20 hours. Prior to this procedure, TNF-α was diluted 1:200. The sections were then incubated with biotinylated secondary antiserum for 1 hour followed by streptavidin horseradish peroxidase for 1 hour at 37ºC in a humid environment and washed with PBS solution for 10 minutes before each incubation. Sections were then immersed in AEC (3-amino-9-ethylcarbozole) chromogen substrate (5 minutes), washed with distilled water, stained with hematoxylin (2 minutes) and covered with mounting medium. Then, they were examined under a microscope and photographed.

Biochemical evaluation

Serum glucose mg/dL, BUN mg/dL and Cr mg/dL levels were measured using the Abbott ARCHITECT c16000 (Abbott Laboratories, Abbott Park, IL) and specific commercial kits. Serum insülin ng/mL was measured by the 800DXL analyzer using a commercial kit (Immulite, Surrey, UK)

Oxidative stress biomarkers

MDA was measured in kidney tissue. The amount of lipid peroxidation was measured according to the concentration of TBA reagent species (TBARS). MDA was treated with TBA at pH 2-3 and 95°C for 15 minutes. After the residue was centrifuged at 2,500 x g for 10 minutes, samples were analysed by spectrophotometer at a wavelength of 532 nm (Placer *et al.*, 1966).

GSH levels in kidney tissues were determined according to Sedlak and Lindsay (1968) method. The sample was washed with 50% TCA and centrifuged at 1,000 x g for 5 minutes. 2 mL of Tris-EDTA buffer (0.2 M, pH = 8.9 and 0.1 mL of 0.01 M 5,5'-dithio-bis-2) was added by taking 0.5 mL of the supernatant from the supernatant. 0.5 mL of the supernatant was removed from the supernatant-nitrobenzoic acid and 2 mL of Tris-EDTA buffer (0.2 M, pH: 8.9) and 0.1 mL of 0.01 M 5,5'-dithio-bis-2 were added. The mixture sample was allowed to stand at room temperature for 5 minutes and analysed by the spectrophotometer at 412 nm wavelength.

Statistical analysis

SPSS software, version 20.0 was used for statistical analysis. Data were mean ± SEM. Data's such as body weight and glucose level were analysed by the paired samples t-test. The groups were compared at the beginning and end of the study by paired samples t-test. Shapiro - Wilk test was performed to evaluate normality. Inter-group and intra-group comparisons were made using parametric one-way ANOVA post hoc LSD; Kruskal-Wallis test was used for biochemical parameters (serum insulin, BUN, Cr and relative kidney weight) for nonparametric values. In addition, Kruskal-Wallis test was used to evaluate the semi-qualified evaluation of histopathological scores. Differences in the parameters measured among the groups were analysed by Kruskal-Wallis test. A Mann-Whitney U test was used to compare dual groups. $p \leq 0.05$ values were considered statistically significant.

RESULT

Biochemical evaluation

In the STZ group of the present study, body weight decreased with respect to comparing control group, while serum glucose consumption were increased. These data of the TQ + BAIBA group are strongly similar than the control group, but also they were largely normalized in the TQ and BAIBA groups (Table No. 1).

In the STZ group, serum and insülin amount decreased; while relative kidney weight, serum BUN and Cr amount increased with respect to comparing the control group. These data started to normalize in the TQ and BAIBA groups, and this normalization was more successful in the TQ + BAIBA group compared with the other two groups (Table No. 2).

Oxidative stress biomarkers

As it is shown in Table No. 2; tissue GSH amount decreased and MDA amount increased in STZ group compared with control group. In the TQ and BAIBA groups, this was reversed, respectively. The TQ + BAIBA group was more successful than the other two treatment groups. The use of TQ and BAIBA greatly reduced post-diabetes oxidative stress.

Histopathological results

PAS staining

In the kidneys of the control group rats; histological appearance of the renal corpuscle, glomerulus, proximal and distal convulated tubules, and other parenchymal and intestinal structures was normal. However, in the kidney tissues of the STZ group rats; histopathological changes were determined such as thickening of capsular, glomerular and tubular basement membranes, increased mesangial matrix amount, cytoplasmic vacuolization in some tubular epithelial cells and inflammatory cell infiltrations in interstitial tissue (Figure No. 1). In the kidney tissues of TQ and BAIBA groups, it was observed that histopathological changes shaped in the STZ group improved significantly. The histological appearance of the kidney tissues of the TQ + BAIBA group rats was almost the same as the control group. The therapeutic effect resulting from the use of TQ and BABA was stronger than their use alone (Figure No. 1).

Table No. 1
Comparision of serum biochemical parameters (body weight and glucose) among the study population

	Stage if study				
	Control	STZ	STZ+TQ	STZ+BAIBA	STZ+TQ+BAIBA
Initial weight (g)	220 ± 2	215 ± 2	219 ± 2	216 ± 2	217 ± 2
Final weight (g)	239 ± 6 $^{£}$	199 ± 3$^{€}$	206 ± 1$^{¥}$	207 ± 2 ^{β}	214 ± 2 ^{α}
Initial glucose mg/dL)	80 ± 1	318 ± 33	330 ± 37	319 ± 31	305 ± 35
Final glucose (mg/dL)	94 ± 5	335 ± 29	330 ± 32$^{¥}$	254 ± 34	205 ± 24 ^{α}

Changes in the body weight and glucose of experimental rats. Values are expressed as mean ± SEM of seven animals. The groups were compared with the paired-samples t-test at initial and final treatment. $p \leq 0$ 05. STZ: streptozotocin; TQ: thymoquinone; BAIBA: beta-aminoisobutyric acid. STZ, 50 mg/kg; STZ + TQ, 50 mg/kg STZ + 20 mg/kg TQ; STZ + BAIBA, 50 mg/kg STZ + 100 mg/kg BAIBA; STZ + TQ + BAIBA, 50 mg/kg STZ + 20 mg/kg TQ + 100 mg/kg BAIBA. $^{£,€,¥,\ β,α}$ in each column, different superscript characters mean significant differences at $p < 0$ 05 in different groups

Table No. 2
Changes in of relative kidney weight, insulin, BUN, Cr, MDA, GSH experimental rats

	Stage if study				
	Control	STZ	STZ+TQ	STZ+BAIBA	STZ+TQ+BAIBA
R.Kidney W. (g/100 g BW)	0.43 ± 0.001^{b,c,d,e}	0.93 ± 0.01^{a,c,d,e}	0.76 ± 0.01^{a,b,d,e}	0.64 ± 0.01^{a,b,c,e}	0.50 ± 0.01^{a,b,c,d}
Insulin (ng/mL)	17 ± 0.96^{b,c,d,e}	7 ± 0.84^{a,c,d,e}	10 ± 1^{a,b,d,e}	13 ± 0.81$^{a,\ b,\ c}$	15 ± 1.19$^{a,\ b,\ c}$
BUN (mg/dL)	19.29 ± 0.52^{b,c,d}	25.35 ± 0.56^{a,c,d,e}	23.26 ± 0.26^{a,b,d,e}	21.34 ± 0.28^{a,b,c,e}	19.19 ± 0.25^{b,c,d}
Cr (mg/dL)	0.18 ± 0.05^{b,c,d}	0.28 ± 0.06^{a,c,d,e}	0.24 ± 0.05^{a,b,d,e}	0.21 ± 0.03$^{a,\ b,\ c}$	0.19 ± 0.08^{b,c,d}
MDA (nmol/g tissue)	184.71 ± 0.86^{b,c,d,e}	244.00 ± 0.87^{a,c,d,e}	235.00 ± 0.90^{a,b,d,e}	223.14 ± 0.80^{a,b,c,e}	193.86 ± 1.05^{a,b,c,d}
GSH (mg/g tissue)	0.187 ± 0.01^{b,c,d,e}	0.125 ± 0.01^{a,c,d,e}	0.147 ± 0.01^{a,b,d,e}	0.163 ± 0.01^{a,b,c,e}	0.176 ± 0.01^{a,b,c,d}

Immunohistochemistry

TNF-α-positive immune staining intensity was significantly higher in the STZ group than that of the control group (Figure No. 2). Although the TNF-α-positive immune staining intensity in the STZ + BAIBA group kidney tissues was lower than that of the STZ + TQ group, it was more than the STZ + TQ + BAIBA group. The staining intensity in the STZ + TQ + BAIBA group was almost the same as the control group. Co-administration of TQ and BAIBA significantly reduced TNF-α expression in kidney tissue compared with the STZ group (Figure No. 2). TNF-α-positive immunostaining was not formed in any of the tissue sections used as the negative control (Figure No. 2N).

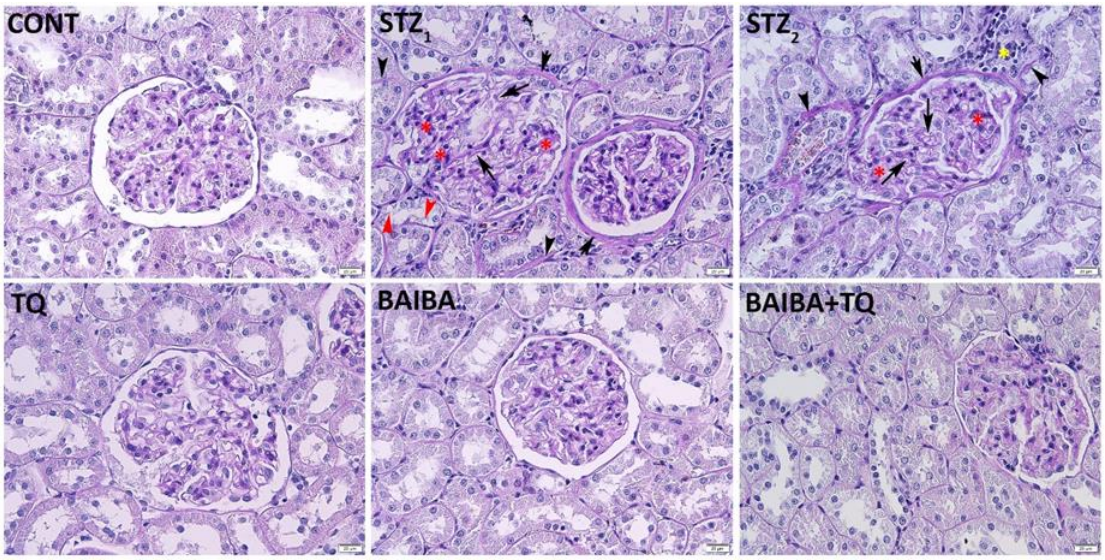

Figure No. 1

Photomicrographs of PAS staining of kidney sections of Control, STZ, TQ, BAIBA and TQ+BAIBA gruoups (x200). In STZ group kidney sections; Thickening of capsular, glomerular and tubular basement membranes, increased mesangial matrix amount, cytoplasmic vacuolization in some tubular epithelial cells and inflammatory cell infiltrations in interstitial tissue are observing. Pathological changes observed in STZ group improved significantly in TQ, BAIBA and TQ + BAIBA groups. ➡: thickening of the glomerular basement membrane (GBM), ➧: thickening of the capsular basal membrane (CBM), ➤: thickening in tubular basement membrane (TBM), ➤: cytoplasmic vacuolization in tubular epithelial cells, *: increased mesangial matrix, *: inflammatory cell infiltrations.

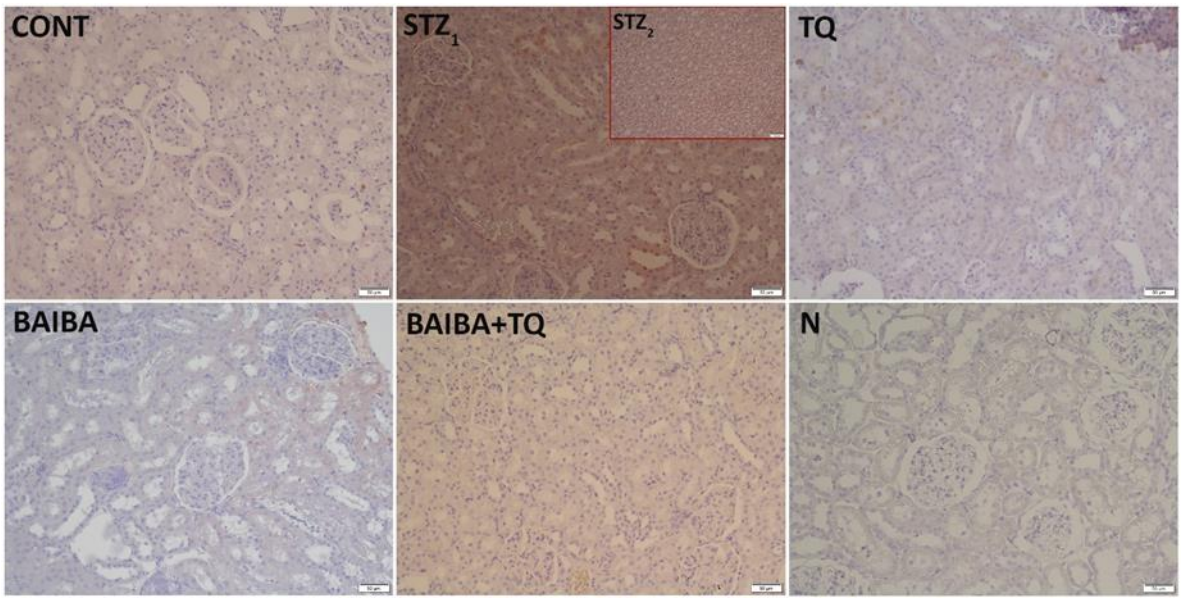

Figure No. 2

Immunohistochemical distribution of TNF-α in kidney sections of Control, STZ, TQ, BAIBA and TQ+BAIBA gruoups (x200). In the STZ (STZ1: cortex; STZ2: medulla) group, TNF-α-positive immunostaining intensity appears to be much higher than the control group. In the Photomicrographs belonging to the TQ, BAIBA and TQ + BAIBA groups, it is seen that the TNF-α-positive immunostaining intensity decreased significantly compared to the STZ group

DISCUSSION

Kidneys are one of the organs where harmful effects of DM are seen. STZ is an antibiotic produced by *Streptomyces achromogenes*, causing irreversible damage with a direct toxic effect on pancreatic β-cells and used to create experimental DM (Salih *et al*., 2014; Dhanavathy, 2015; Furman, 2015; Bathina *et al*., 2017; Joudaki & Setorki, 2019). Diabetic nephropathy is the main reason of morbidity and death in diabetic people. Effective therapeutic agents are needed to stop the progression of this disease and to improve kidney damage (Shaterzadeh-Yazdi *et al.*, 2018).

In the current study, in the STZ group; while insulin level and body weight decreased compared with the control group, serum glucose amount and relative kidney weight increased. These findings overlap with the literature (Kanter, 2009; Ulu *et al*., 2012; Omran, 2014; Sameni *et al*., 2016; Rivoira *et al*., 2018; Khalilpour *et al.*, 2019**)** While these data were almost identical with the control group in the TQ + BAIBA group, they were largely normalized in the TQ and BAIBA groups. Studies have shown that hyperglycemia causes an increase in kidney weight by increasing the amount of extracellular matrix in kidney tissue (Seyer-Hansen *et al*., 1980; Yajing *et al*., 2011; Sameni *et al*., 2016). This information explains the increase in relative kidney weight in the STZ group in the current study. The probable cause of body weight loss in STZ group rats is that because the glucose in the blood cannot be used by the body cells as a source of metabolic energy, the cells use amino acids formed by the breakdown of fats and structural proteins as energy sources (Giribabu *et al*., 2017; Joudaki & Setorki, 2019). It has been proven that TQ lowers blood sugar and is beneficial in the treatment of DM by increasing insulin secretion and sensitivity, increasing glucose utilization and also decreasing hepatic glucose production. It has also been shown that TQ protects β cells against oxidative stress following STZ treatment (Pari & Sankaranarayanan, 2009; Karandrea *et al*., 2017). BAIBA has been shown to reduce hepatic insulin resistance, hepatic gluconeogenesis and blood glucose level in the Type 2 diabetes (Shi *et al*., 2016). In this study, it was found that the serum glucose level decreased in the TQ and BAIBA groups. This result coincides with the data in the literature (Kanter, 2009; Omran, 2014; Shi *et al.*, 2016; Usta & Dede, 2017).

In previous studies, serum BUN and Cr amounts increased in DN induced by STZ (Omran, 2014; Giribabu *et al.*, 2017; Khalilpour *et al*., 2019). Similarly, in the present study, serum BUN and Cr amounts increased in the STZ group compared with the control group. The increase of BUN and Cr amounts in the serum is an indicator of dysfunction and damage in the kidneys and is compatible with the histopathological findings obtained (Omran, 2014; Giribabu *et al*., 2017; Khalilpour *et al*., 2019). In the TQ and BAIBA groups, this data started to normalize and in the TQ + BAIBA group, this normalization was more successful than the other two groups. In studies conducted, it was reported that the increase in serum BUN and Cr levels in DN induced by STZ restored to a great extent after TQ application (Kanter, 2009; Omran, 2014; Ozer *et al*., 2020). These results are in line with the data in the current study.

Oxidative stress is the shift of balance between ROS and antioxidants in the body to the side of ROS. MDA, one of the lipid peroxidation products, is an important indicator of oxidative stress (Ohkawa *et al*., 1979; Shoji & Koletzko, 2007; Sameni *et al*., 2016). The level of oxidative stress in the body is regulated by enzymatic and non-enzymatic antioxidant systems. GSH is a non-enzymatic antioxidant. Oxidative stress plays an important role in the pathogenesis of DNA (Xu *et al.*, 2005; Roshan & Stanton, 2013; Arora & Singh, 2014). In this study, the amount of GSH in the kidneys decreased and the amount of MDA increased in the STZ group compared with the control group. This result, which shows that oxidative stress increases, is compatible with other literatures showing that oxidative stress increases in DN (Seyer-Hansen *et al*., 1980; Sameni *et al*. 2016; Giribabu *et al*., 2017). These changes in MDA and GSH levels were reversed in TQ and BAIBA groups. The antioxidant effect caused by the use of TQ + BAIBA was stronger than their use alone. The results obtained are in line with the literature and confirm the antioxidant effects of TQ and BAIBA (Begriche *et al*., 2008; Manna *et al*., 2010; Wang *et al.* 2017; Ozer *et al*., 2020).

In studies carried out, STZ-induced diabetes in the kidneys due to dysfunctions such as microalbuminuria, decreased Cr clearance and glomerular filtration rate, capsular, glomerular and tubular basement membrane thickening, mesangial matrix enlargement, loss of podocytes, degeneration in tubular epithelium, macrophage infiltration and TNF-apoptosis apiltus caused histopathological changes, such as an increase in the ratio, and even

reported to cause irreversible structural disorders such as glomerulosclerosis and tubulointerstitial fibrosis resulting in ESRF (Kanwar *et al*., 2008; Roshan & Stanton, 2013; Al-Trad *et al*., 2016; El-Shemi *et al*., 2017; Giribabu *et al*., 2017; Shaterzadeh-Yazdi *et al*., 2018). In the current study, in the kidney tissues of the STZ group rats; histopathological changes such as thickening of capsular, glomerular and tubular basement membranes, increased mesangial matrix amount, cytoplasmic vacuolization in some of the tubular epithelial cells, inflammatory cell infiltrations in the interstitial tissue and increased TNF-α expression were observed. These findings are consistent with the results of studies showing that DM causes nephropathy in the kidneys.

The development of protective or curative treatment strategies against kidney damage shaped in diabetic patients depends on the full clarification of the pathogenesis of DN. Although the pathogenesis of DN has not been completely clarified yet, studies have shown that hyperglycemia, oxidative stress, inflammation and dyslipidemia that play after DM play a critical role in the pathogenesis of DN (Kanter, 2009; Sayed; 2012; Omran, 2014; Al-Trad *et al*., 2016; Ulabay *et al*., 2019). The results obtained in the current study showed that TQ and BAIBA were effective in preventing functional and histopathological changes in kidney tissue after diabetes caused by STZ. The therapeutic effect caused by the combination of TQ and BAIBA was stronger than their use alone. While the effect of BAIBA's reduction on TNF-α expression in kidney tissue was strong compared with TQ, it was weak compared with the combination of BAIBA and TQ. In a study conducted by Wang *et al*. (2017) it was reported that BAIBA treatment could reverse the accumulation of the extracellular matrix and fibrosis in the kidneys, and BAIBA has the potential to be used as a medicine in the treatment of renal fibrosis diseases. In the current study, normalization of histopathological changes characterized by thickening of capsular, glomerular and tubular basement membranes in the STZ group confirms the reversible effect of BAIBA's extracellular matrix accumulation in kidney tissues. In many studies investigating the effectiveness of TQ against STZ-induced DN, TQ; it has been reported that it improves pathological changes in kidney tissue after diabetes with its antioxidative, antininflammatory and antihyperglycemic effects. The data obtained on the use of TQ in the current study are consistent with the results of these studies (Kaatabi *et al.*, 2015; Darakhshan *et al.*, 2015; El-Shemi *et al.*, 2018; Shaterzadeh-Yazdi *et al*., 2018).

CONCLUSION

The present study was showed that the treatment of TQ and BAIBA improved biochemical and histological alterations in the serum and kidneys of STZ-induced DM rats. Although the healing effect of BAIBA was stronger than TQ, it was weak compared with TQ + BAIBA. This finding showed a synergistic effect between TQ and BAIBA. Also, the action mechanism of these agents may be inhibition of lipid peroxidation and stimulation of antioxidant enzymes. This suggests that TQ and BAIBA may be effective in the treatment of DN. Further studies are needed to fully elucidate the mechanism of therapeutic effect of these agents.

ACKNOWLEDGEMENTS

This study was supported by a grant from Adiyaman University Research Foundation (Project SHMYOMAP/2018-0001).

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