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The ameliorative effects of thymoquinone and beta-aminoisobutyric acid on streptozotocin-induced diabetic cardiomyopathy

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ABSTRACT

Diabetic cardiomyopathy (DCM) is a cardiac dysfunction observed in a patient with diabetes that may lead to heart failure. No specific treatment has yet been tested in DCM. Therefore, in this study, it was investigated that the potential of thymoquinone (TYM) and beta-aminoisobutyric acid (BAIBA) to treat DCM. Five groups (n = 7) were formed, namely control, diabetes, TYM, BAIBA and TYM + BAIBA, with a random selection from 35 adult male rats. Diabetes mellitus was induced by intraperitoneal administration of 50 mg/kg streptozotocin to all groups except the control. After establishing experimental diabetes, TYM (20 mg/kg/day) and BAIBA (100 mg/kg/day) were administered alone or in combination with other groups other than the control and diabetes groups for five weeks by gavage. Serum aspartate aminotransferase, lactate dehydrogenase, creatine kinase-MB, and tissue malondialdehyde levels increased significantly, and tissue glutathione levels decreased in the diabetes group compared to the control group. An increase in the expression of tumor necrosis factor-α in the myocardium and the rate of fibrosis and apoptosis were found in the histopathological analysis. In the TYM and BAIBA groups, all pathological changes observed in the diabetes group improved significantly. The therapeutic effects of these agents on DCM are probably due to their antihyperglycemic, antidiabetic, antioxidant, and anti-inflammatory effects. The present results suggested that TYM and BAIBA have the potential therapeutic effects on DCM that were used alone or combined.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease characterized by a chronic increase in blood glucose level because of impaired insulin production and/or its effects (Kerner et al., 2014; Marso et al., 2016). In 2015, it was estimated that the total number of diabetic patients in the world was approximately 415 million and this number is expected to increase to 642 million in 2040 (Cho, 2015). DM triggers severe clinical complications, such as DCM, hepatopathy, retinopathy, neuropathy and nephropathy (Aktas and Gur, 2021a,b; Chen et al., 2011; Feldman et al., 2019; Ren and Ceylan-Isik, 2004; Zheng et al., 2018). DCM is a dysfunction specific to the patient with diabetes. This disorder may occur independently of other cardiovascular diseases, such as coronary artery disease, hypertension, valvular and congenital heart disease. DCM has two stages, early and late stages. It is characterized by early-stage ventricular hypertrophy and diastolic dysfunction. The late-stage is characterized by systolic dysfunction and fibrosis in the heart (Paolillo et al., 2019). The pathophysiological process of DCM, which may cause pathological disorders, such as heart failure and arrhythmia (Murtaza et al., 2019; Paolillo et al., 2019), is complicated. Chronic hyperglycemia resulting from diabetes triggers oxidative stress by increasing the production of reactive oxygen species (ROS), as well as causing tissue and organ failure by causing damage to small blood vessels (Abouzed et al., 2018; Oyenihi et al., 2017). Excessive ROS production activates apoptotic proteins, cytokine production and transcription factors. This situation results in chronic inflammation and increased apoptosis, which are significant factors in the formation of post-diabetes complications (Miranda-Diaz et al., 2016). Previous studies have reported that oxidative damage, endoplasmic reticulum (ER) stress, apoptosis and proinflammatory responses play a key role in pathological conditions associated with diabetic cardiomyopathy (Althunibat et al., 2019; Li et al., 2019; Xiong et al., 2018). In histopathological and immunohistochemical studies, diabetes has been shown to increase fibrosis (Russo and Frangogiannis, 2016), apoptosis (Zhang and Wei, 2013), and tumor necrosis factor-α (TNF-α) expression (a pro-inflammatory cytokine) in the myocardium (Wang et al., 2015). In biochemical studies

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investigating the effects of DM on the heart tissue, it has been reported that the serum levels of enzymes, such as creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), which are signs of myocardial cell damage increased (Ahmed et al., 2020; Ali et al., 2016).

Streptozotocin is an antibiotic that gives rise to irreversible damage with its direct toxic effect on pancreatic β-cells and is used to create experimental DM (Furman, 2015). THY, found in seeds of N. Sativa, is a phytochemical agent with powerful antioxidant effects. It creates its antioxidant effects by scavenging ROS (Atta et al., 2018). BAIBA is a catabolite of antiretroviral thymine analogs zidovudine and stavudine (Maisonneuve et al., 2004). BAIBA is non-protein amino acid. It was recently discovered that BAIBA is secreted by skeletal muscles after exercise. BAIBA, a myokine, converts white adipose tissue into brown adipose tissue, which improves glucose metabolism and insulin sensitivity. BAIBA lowers body fat mass, positively affects lipid metabolism, increases insulin sensitivity, and also has an anti-inflammatory effect (Shi et al., 2016; Tanianskii et al., 2019).

In previous studies, many pharmacological agents, including THY, which have antioxidant, anti-inflammatory effects, have been notified to have protective and therapeutic effects against DCM occurring after DM (Abukhalil et al., 2021; Alabi et al., 2020; Ali et al., 2020; Atta et al., 2018; Wang et al., 2018). This study aims to investigate the protective and therapeutic effects of THY and BAIBA against DCM after streptozotocin-induced DM and compare their potencies.

2. Materials and methods

To conduct the present study, ethics committee approval was obtained from Adiyaman University Laboratory Animals Ethics Committee (Protocol 2019/004) and this study was conducted according to this protocol.

2.1. Animals

Thirty-five male Sprague-Dawley rats (210–250 g, aged eight weeks) obtained from Adiyaman University Experimental Animal Production Application and Research Centre were housed in the same center in an environment where water and pellet feed was given as ad libitum at 24 °C, 12 h dark/12 h light.

2.2. Treatment protocol

Five groups (n = 7), namely control, diabetes, TYM, BAIBA, and TYM + BAIBA, were formed by randomly selecting from 35 male rats.

To induce DM, 50 mg/kg streptozotocin was dissolved in 0.1 M sodium citrate buffer (Ph: 4.5) and groups (total 28 rats) other than the control group were then administered as intraperitoneal (i.p.) (Bayat et al., 2019; Liu et al., 2019). In this study, 18 rats with blood glucose concentrations above 250 mg/dL 72 h after streptozotocin administration were considered diabetic (Pari and Sankaranarayanan, 2009). The same dose of streptozotocin was administered to the remaining 10 rats. When blood glucose was checked after 24 h, diabetes occurred in three animals, two animals after 48 h, and two animals after 72 h. The third dose of streptozotocin was administered to the remaining three animals. When blood glucose was checked after 24 h, diabetes occurred in one animal, one animal after 48 h and the last animal remaining after 72 h. TYM and BAIBA were started to be administered to animals considered to be diabetic, as detailed below. One rat each from the control, TYM, TYM + BAIBA and streptozocin groups died from the beginning to the end of this study. After the experimental processes were completed, the rats were anesthetized and their blood was taken from the vena cava caudalis (75 mg/kg ketamine hydrochloride + xylazine 10 mg/kg i.p). Blood taken for biochemical analysis was centrifuged at 5000 × *g* for 15 min and stored at −86 °C (El-Shemi et al., 2018). The heart tissue, which was quickly removed from the body and weighed, was divided into two. One half was stored at −86 °C for biochemical analysis, while the other halves were fixed in buffered neutral formalin at +4 °C for 24 h for histopathological analysis. The blood glucose of the rats was measured on the third and last day of the experimental applications (Sharma et al., 2019).

Five groups were formed by randomly selecting seven from 35 rats, as detailed below.

1. Control group: No application was taken during the experimental processes (total 38 days).
2. Diabetes group: Diabetes was induced as described above.
3. TYM group (diabetes + thymoquinone): Diabetes was induced as described above. Dissolved in warm tap water (65 °C), TYM (20 mg/kg) was applied by gavage daily for five weeks after cooling to room temperature (Mabrouk, 2018).
4. BAIBA group (diabetes + BAIBA): Diabetes was induced as described above. Dissolved in tap water, BAIBA (100 mg/kg) was applied by gavage daily for five weeks (Begriche et al., 2008).
5. TYM + BAIBA (diabetes + thymoquinone + BAIBA): Diabetes was induced as described above. TYM and BAIBA were administered to diabetic rats by gavage in doses as described above for five weeks.

2.3. Histopathological analysis

Fixated tissues were embedded in paraffin after undergoing routine histological procedures. The tissues embedded in paraffin were cut with a microtome at a thickness of 5 μm and then stained using Crossman's trichrome (CT) method to investigate any morphological and fibrotic changes in the heart. Blue staining represented collagen accumulation. Histopathological examination was conducted by a blinded histopathological investigator using the Olympus BX-53 microscope and photos were taken by microscope's camera (DP 80 Olympus Japan).

2.3.1. Immunohistochemistry

Tissue slides were left in the oven for one hour at 60 °C to remove the paraffin and increase the tissue adhesion. The paraffinized tissues were cut 5 μm thick. Tissue sections that were deparaffinized in xylol and dehydrated in alcohol series were washed in distilled water and phosphate buffer saline (PBS). The antigen retrieval and immunohistochemistry protocol were performed as in previous studies (Gur and Timurkaan, 2012; Gur et al., 2018). To determine TNF-α expression, tissue sections were incubated with anti-TNF-α primary antibody (ab220210, Abcam) in a humidified chamber at 4 °C for 16–20 h. Before this procedure, TNF-α was diluted at 1:200. Tissue sections were washed with PBS solution after the incubation with biotinylated secondary antiserum for one hour in a 37 °C humid environment and then incubated with streptavidin horseradish peroxidase for one hour under the same conditions. These sections were then immersed in a 3-amino-9-ethyl carbazole (AEC) chromogen substrate for five minutes. PBS was used instead of the primary antibody for negative control. Mayer's hematoxylin was used for counterstaining for three minutes. Then, stained sections were examined using the Olympus BX-53 microscope, and photographs were taken with a camera (DP 80 Olympus Japan) belonging to the same microscope.

2.3.2. TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) analysis

Apoptosis was determined by TUNEL analysis as previously described (Gur et al., 2021a, b). For this purpose, In Situ Cell Death Detection Kit (Roche REF 11684795910, LOT 32806500) was used. The stained tissue sections were examined and photographed using Olympus BX-53 fluorescence microscope and its camera. TUNEL positive cells were detected at a wavelength of 515–565 nm. The apoptotic index was calculated as in a previous study (Karabulut et al., 2016).

2.4. Biochemical evaluation

Glucose, AST, LDH and CK-MB in the serum were measured using commercial kit by the Abbott ARCHITECT c16000 (Abbott Laboratories, Abbott Park, IL, USA).

2.4.1. Oxidative stress biomarkers

Heart malondialdehyde (MDA) levels, which are indicators of lipid peroxidation, were measured according to the concentration of thiobarbituric acid (TBA) reagent species (TBARS). MDA was treated with TBA at pH 2–3 and 95 °C for 15 min. After the residue was centrifuged at 2500 × *g* for 10 min, samples were read by spectrophotometer at a wavelength of 532 nm (Placer et al., 1966). Glutathione (GSH) levels in heart tissues were determined according to Sedlak and Lindsay method (Sedlak and Lindsay, 1968). The sample was washed with 50 % trichloroacetic acid (TCA) and centrifuged at 1000 × *g* for five minutes. 2 mL of Tris-ethylenediaminetetraacetic asid (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′-dithiobis-2 were added. The mixture sample was allowed to stand at room temperature for five minutes and read by spectrophotometer at 412 nm wavelength.

2.5. Statistical analysis

In our study, version 20.0 of SPSS software was used for statistical analysis. Data were presented as means ± SEM. Glucose values of data before and after using preservatives were analyzed by paired samples *t*-test. Groups were compared by paired samples *t*-test at the beginning and end of the experimental procedures. The Shapiro-Wilk test was performed to assess normality for biochemistry parameters (serum AST, LDH, CK-MB, MDA, GSH). Intra-group and inter-group comparisons were made using parametric one-way ANOVA post hoc LSD. Kruskal-Wallis test was used for non-parametric biochemical parameters. Results for the apoptotic index were expressed as mean ± standard deviation. Statistically, a significant difference was determined by Tukey's multiple comparison tests performed after ANOVA. Values for $p \leq 0.05$ were considered statistically significant.

3. Results

3.1. Histopathological analysis

3.1.1. Crossman's trichrome stain

The histological structure of the heart tissue was normal in the control group rats (Fig. 1A). Focal interstitial fibrosis was observed in the heart tissue of diabetic rats (Fig. 1B, B_1). The degree of myocardial fibrosis decreased in the TYM and BAIBA groups than the diabetes group (Fig. 1C, D). Myocardial fibrosis was reduced in TYM + BAIBA group. The histological structure of the heart tissue of the rats in this group was almost the same as the control group (Fig. 1E).

3.1.2. Immunohistochemistry

In the diabetic group, TNF-α-positive staining intensity was higher than the control group (Fig. 2A, B). TNF-α-positive staining intensity in the heart tissue of the BAIBA group was lower than the TYM group but higher than the TYM + BAIBA group (Fig. 2C–E). The TNF-α expression in the TYM + BAIBA group was almost similar to that in the control group. The negative control tissue section was TNF-α negative (Fig. 2F).

3.1.3. TUNEL analysis

Apoptotic cells in the heart tissue were determined using the TUNEL method. TUNEL-positive cells (green fluorescence) were observed in all of the groups' heart tissues (Fig. 3). Apoptotic index results are given in Fig. 3G. The number of apoptotic cells in the diabetes group was considerably higher than in the control group. The apoptotic cell ratio in the TYM group decreased compared to the diabetes group. The number of apoptotic cells in the BAIBA group was less than the TYM group but more than the TYM + BAIBA group. The proportion of apoptotic cells in the TYM + BAIBA group significantly reduced.

3.2. Biochemical analysis

In the diabetes group, serum glucose, AST, LDH, CK-MB levels statistically significantly increased than the control group (Tables 1 and 2) (p = 0,013, 0,012, 0,003, respectively). Serum AST and CK-MB levels in the TYM + BAIBA group were close to the control group, with no statistically significant difference p = 0,056, 0,060, respectively). In addition, when compared to the diabetes group, the LDH level in the TYM + BAIBA group approached the control group, but this improve-

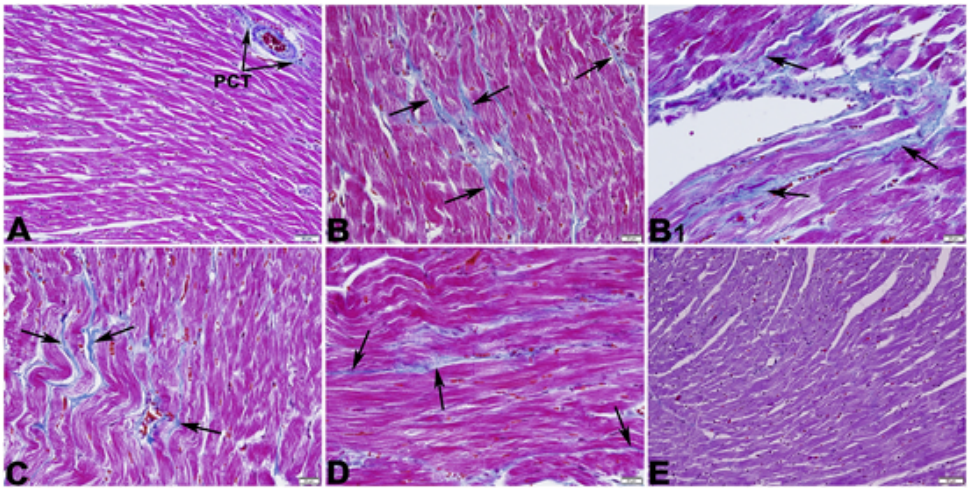

Fig. 1. Crossman's trichrome staining of myocardial sections belonging to control (A), diabetes (B, B_1), TYM (C), BAIBA (D) and TYM + BAIBA (E) groups (A, E × 200; B, B_1, C, D × 400). A Normal collagen distribution between myocardial fibers in the control group. B, Marked increase in the amount of collagen tissue between myocardial fibers (myocardial fibrosis). The degree of myocardial fibrosis significantly decreased in the TYM and BAIBA groups than the diabetes group. arrow: collagen accumulation in the cardiac interstitium (myocardial fibrosis), PCT; perivascular connective tissues.

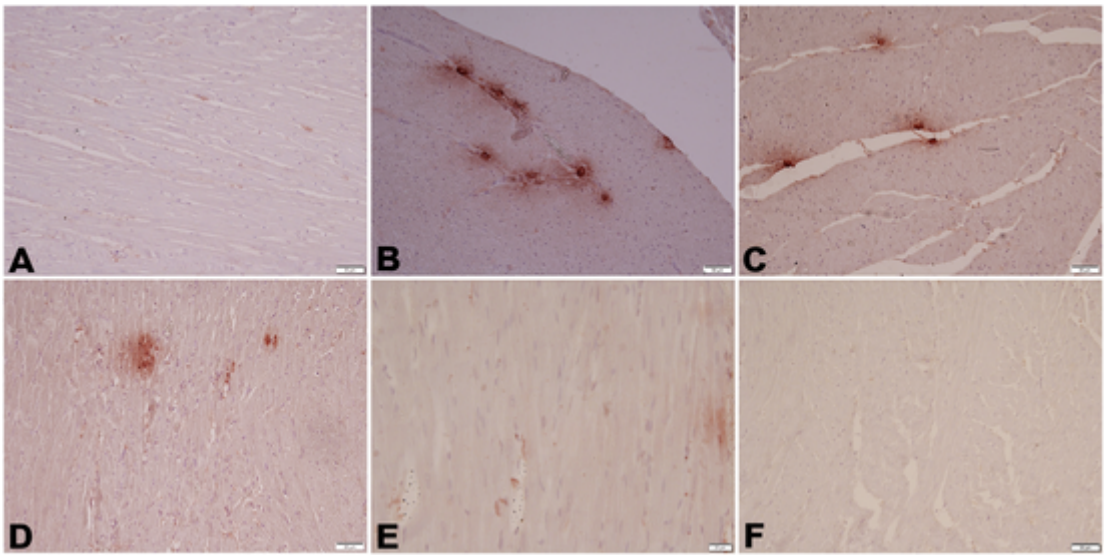

Fig. 2. Immunohistochemical distribution of TNF-α in myocardial sections of control (A), diabetes (B), TYM (C), BAIBA (D) and TYM + BAIBA (E) groups (A, C, D, E × 200; B × 400) In the diabetes group, TNF-α-positive immunostaining intensity appears to be much higher than the control group. In the TYM, BAIBA, and TYM + BAIBA groups, the staining intensity of TNF-α decreased significantly compared to the diabetes group. Negative control (F × 200).

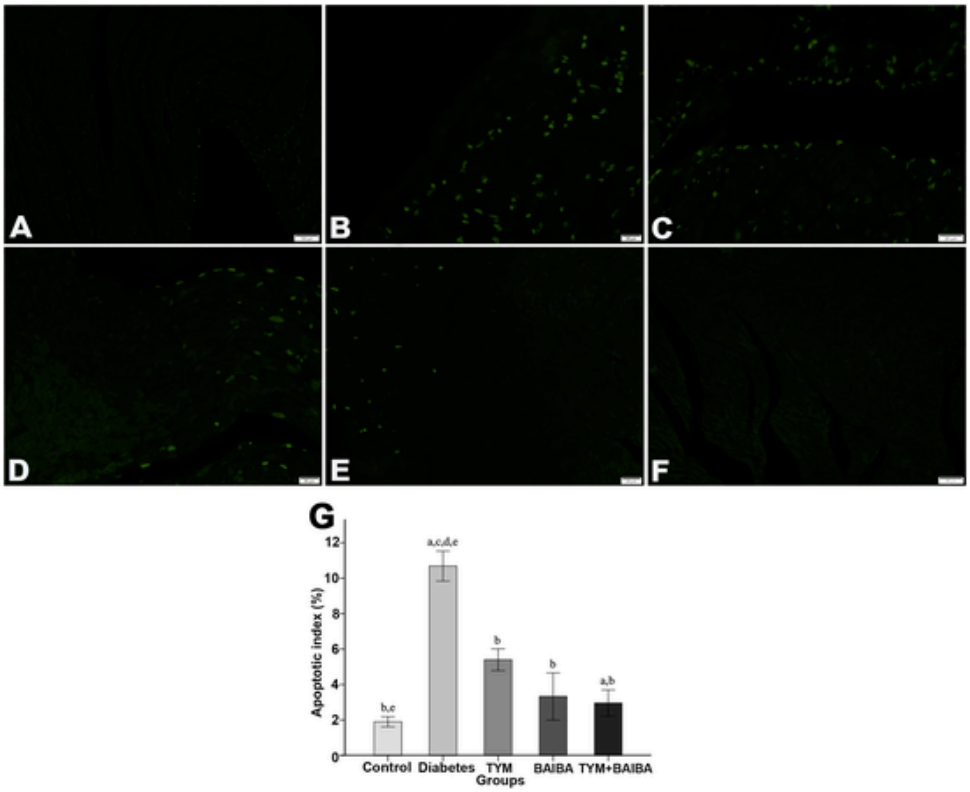

Fig. 3. TUNEL analysis of myocardial tissues in control (A), diabetes (B), TYM (C), BAIBA (D) and TYM + BAIBA (E) groups (A × 100; B, C, D × 400; E × 200). Green fluorescent shows TUNEL-positive cells in heart muscle. Apoptotic cells were mostly observed in the diabetes group. The number of apoptotic cells in the TYM (C), BAIBA (D) and TYM + BAIBA groups was lower than in the diabetes group. arrow: TUNEL positive cells. Negative control (F × 200), Apoptotic index (G); Values are mean ± SD for six rats in each group. a: Significant from control; b: Significant from diabetes; c: Significant from TYM; d: Significant from BAIBA; e: Significant from BAIBA + TYM ($p \leq 0.05$).

ment was not statistically significant (p = 0,014). Although pathological alterations in the diabetes group showed a higher improvement in the BAIBA group than the TYM group, the group with the highest amelioration was the TYM + BAIBA group (Table 2). The data on serum glucose levels in this study were also used in our previous studies (Aktas and Gur, 2021a,b).

3.2.1. *Oxidative stress biomarkers*

In Table 2, while the MDA level in the diabetes group increased significantly compared to the control group (p = 0,024), the GSH level decreased (p = 0,041). Although these values showed a higher improvement in the BAIBA group than the TYM group, the highest success was pointed out in the TYM + BAIBA group. MDA and GSH levels in the TYM + BAIBA group approached the control group with no statisti-

Table 1
Comparison of serum glucose levels among the groups.

Parameters	Control	Diabetes	TYM	BAIBA	TYM + BAIBA
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 ± 24α

Changes in the glucose levels of experimental rats. Values are expressed as mean ± SEM of six animals. The groups were compared with the paired-samples *t*-test at initial and final treatment p ≤ 0 05. TYM: thymoquinone; BAIBA: beta-aminoisobutyric acid. ¥, α in each column, different superscript characters mean significant differences at p < 0 05 in different groups.

cally significant difference (p = 0,057, 0,017, respectively). The use of TYM + BAIBA almost completely prevented post-diabetic oxidative stress.

4. Discussion

DCM is a diabetic complication that occurs independently of other cardiovascular disorders, such as heart valve diseases, congenital heart disease, hypertension and coronary diseases (Seferovic et al., 2018). DCM, which is characterized by diastolic and systolic dysfunction, ventricular hypertrophy, and cardiac fibrosis, is an important cause of heart failure (Murtaza et al., 2019; Paolillo et al., 2019). The pathophysiology of DCM has not been fully elucidated yet. However, in DCM formation, factors, such as hyperglycemia, insulin resistance, ER stress, oxidative stress, apoptosis, fibrosis and increased TNF-α were detected to be effective (Alabi et al., 2020; Althunibat et al., 2019; Liu et al., 2019; Miranda-Diaz et al., 2016; Russo and Frangogiannis, 2016; Xiong et al., 2018). In previous studies, it has been shown that TYM and BAIBA have effects, such as antioxidant (BAIBA increases the number of antioxidant molecules), anti-hyperglycemic, hypolipidemic, anti-inflammatory, hepatoprotective, anti-diabetic. In addition to this, TYM and BAIBA have reducing effects on the amount of TNF-α (Abdelrazek et al., 2018; Awad et al., 2016; Bashandy et al., 2015; Farkhondeh et al., 2017; Sawada et al., 2019; Shi et al., 2016). Therefore, in the present study, the protective and therapeutic effects of TYM and BAIBA alone and together against DCM were investigated.

The serum glucose level measured at the end of this study was statistically significantly higher in the diabetes group than in the control group. Serum glucose level decreased in the TYM group than in the diabetes group. The serum glucose level in the BAIBA group was lower than the TYM group and higher than the TYM + BAIBA group. This result means that the anti-hyperglycemic effect caused by the combined use of TYM and BAIBA is stronger. It has been reported that TYM increases insulin secretion, insulin sensitivity, and glucose consumption and decreases hepatic glucose production. TYM has also been reported to protect β-cells from oxidative stress after streptozocin administration (Aktas and Gur, 2021a,b; Karandrea et al., 2017; Pari and Sankaranarayanan, 2009; Shi et al., 2016). Shi et al. (2016) proved that the BAIBA treatment, which is applied in mice with type II diabetes, reduced hepatic ER stress, hepatic insulin resistance, hepatic gluconeogenesis, blood glucose levels, and glucose/lipid metabolic disorder. In addition, Jung et al. (2015) reported that BAIBA reduces insulin resistance in skeletal muscles, suppresses inflammation, and induces fatty acid oxidation. The blood glucose-reducing effect of TYM and BAIBA in the present study is probably a result of the effects of these agents reported above and is consistent with the literature (Aktas and Gur, 2021a,b; Karandrea et al., 2017; Pari and Sankaranarayanan, 2009; Shi et al., 2016).

Heart damage and the death of cardiomyocytes may cause the membrane integrity of cardiomyocytes to break down, leading to the release of intracellular enzymes, such as CK-MB, LDH and AST, into the circulatory system (Al Hroob et al., 2019). Studies conducted in diabetic patients (Ali et al., 2016) and streptozotocin-induced diabetic animals (Ahmed et al., 2020; Wang et al., 2015) have shown that serum levels of these enzymes increase, which is a sign of myocardial damage. The increase in serum enzyme levels observed in the diabetes group in this study is consistent with the above literature and is an indicator of cardiac muscle damage caused by diabetes. These enzyme levels, which increased in the diabetes group, decreased significantly in the TYM + BAIBA group and approached the control group. The combined use of TYM and BAIBA was more successful in reducing enzyme levels than their separate use. Alam et al. (2018) reported that the use of TYM significantly reduced CK-MB, LDH, and AST levels in serum as a result of doxorubicin-induced cardiomyopathy (Alam et al., 2018). On the other hand, Atta et al. (2018) reported that TYM uses significantly decreased CK-MB and troponin I level in serum after streptozotocin-induced diabetes (Atta et al., 2018). Studies have shown that TYM's membrane-stabilizing effects prevent the enzymes in the cytosol from entering the circulation (Galaly et al., 2014; Lebda et al., 2011). This effect of TYM may be one of the reasons for the decrease in enzyme levels in the diabetes group as a result of TYM application in this study. Shi et al. (2016) reported that BAIBA application reduced serum AST levels (Shi et al., 2016). This result is consistent with the data in the present study.

Hyperglycemia and insulin resistance can increase myocardial ROS formation in diabetic animals (Russo and Frangogiannis, 2016). Oxidative stress, defined as the disruption of the balance between the production and elimination of ROS, plays a critical role in the development of DCM and heart failure (Wang et al., 2015). Oxidative stress leads to lipid peroxidation. In this case, it results in biomolecular damage. MDA is a lipid peroxidation product formed due to oxidative stress (Ohkawa et al., 1979; Shoji and Koletzko, 2007). At the molecular level, the GSH system plays an important role in cellular defense against oxidants. GSH depletion may lead to tissue damage by disrupting cell defense (Alam et al., 2018). In the present study, the MDA level in the heart muscle tissue in the diabetic group increased statistically significantly than the control group, while the GSH level, which is a non-enzymatic antioxidant, decreased. This result, which indicates that oxidative stress increases in the heart muscle after diabetes, is consistent with the results of previous studies (Abukhalil et al., 2021; Mostafa et al., 2021; Othman et al., 2017; Wang et al., 2018). In this study, MDA level decreased and GSH level increased in TYM, BAIBA, and TYM + BAIBA groups compared to the diabetes group. The antioxidant effect that oc-

Table 2
Changes in AST, LDH, CK-MB, MDA and GSH levels of experimental rats.

Parameters	Control	Diabetes	TYM	BAIBA	TYM + BAIBA
AST (U/L)	70.29 ± 4.66^{b,c,d}	118.57 ± 7.69^{a,d,e}	104.00 ± 7.14^{a,e}	91.86 ± 4.05^{a,b,e}	74.14 ± 4.71^{b,c,d}
LDH (U/L)	102.71 ± 7.49^{b,c,d,e}	150.00 ± 4.92^{a,e}	141.00 ± 3.50^{ae}	132.86 ± 5.75^{a}	121.00 ± 7.65^{a,b,c}
CK MB (U/L)	827.43 ± 56.50^{b,c,d}	1332.00 ± 72.53^{c,d,e}	1164.29 ± 52.00^{a,b,e}	1041.57 ± 19.34^{a,b,e}	853.57 ± 32.30^{b,c,d}
MDA (nmol/g tissue)	144.14 ± 3.12^{b,c,d}	178.00 ± 6.75^{a,d,e}	167.43 ± 3.42^{a,e}	160.00 ± 6.91^{a,b}	146.29 ± 4.44^{b,c}
GSH (mg/g tissue)	10.71 ± 0.36^{b,c,d,e}	6.57 ± 0.48^{a,d,e}	6.86 ± 0.49^{a,e}	8.00 ± 0.48^{a,b}	8.43 ± 0.57^{a,b,c}

Each group represents the mean ± SEM for six rats. ap < 0 01 vs the control group; bp < 0 01 vs the Diabetes group; cp < 0 01 vs the TYM group; dp < 0 01 vs the BAİBA group; and ep < 0 01 vs the TYM + BAIBA group. TYM, thymoquinone; BAIBA, beta-aminoisobutyric; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CK-MB, creatine kinase; MDA, Malondialdehyde; GSH, glutathione.

curred with the combined use of TYM and BAIBA was stronger than their individual use. In previous studies, it has been shown that TYM use after diabetes significantly decreases the level of MDA in the heart muscle and increases the level of GSH (Alam et al., 2018; Atta et al., 2018; Randhawa et al., 2013). Shi et al. (2016) reported that BAIBA reduced insulin resistance and impairment in glucose metabolism in diabetic mice. Sawada et al. (2019) reported that BAIBA increased the amount of ROS scavenging antioxidants. In this study, the reduction of oxidative stress in the heart muscle because of the use of BAIBA and TYM coincides with the above-mentioned effects of TYM and BAIBA.

In the histopathological examinations of the heart tissue of the diabetes rats group, it was observed that diabetes caused an increase in connective tissue, TNF-α expression, and apoptosis rate in the myocardial tissue. These findings showing that DCM is formed in the heart muscle are in line with the results of previous studies investigating the effects of diabetes on the myocardium (Abukhalil et al., 2021; Al-Rasheed et al., 2017; Mostafa et al., 2021; Wang et al., 2018, 2015; Wang et al., 2020; Xiong et al., 2018; Youssef et al., 2021). These pathological lesions occurring in the myocardial tissue showed improvement in the TYM group, the improvement effect in the BAIBA group was higher than the TYM group and lower than the TYM + BAIBA group. The histological structure of the myocardium, TNF-α expression level, and apoptosis rate in the myocardium were almost the same as in the control group in the TYM + BAIBA group, where the improvement effect was highest. Atta et al. (2018) reported that TYM application attenuates DCM occurring in streptozotocin-induced diabetic rats (Atta et al., 2018). Liu et al. (2016) declared that TYM improves cardiovascular function in streptozotocin-induced diabetic rats by reducing oxidative stress, apoptosis, and inflammation (Liu et al., 2016). Pei et al. (2018) reported that TYM administration inhibited doxorubicin-induced cardiac fibrosis and apoptosis (Pei et al., 2018). Studies have shown that BAIBA reduces apoptosis, TNF-α expression, impairment in glucose metabolism and insulin resistance, and increases the amount of antioxidants (Jung et al., 2015; Sawada et al., 2019; Shi et al., 2016). In another study, where we examined the effectiveness of TYM and BAIBA against pathological changes in liver tissue after streptozotocin-induced diabetes, we found that oxidative stress level and AST level increased in the diabetes group. In the TYM and BAIBA groups, these pathological changes that occurred in the diabetes group greatly improved (Article in press). The results obtained in the present study are consistent with the results of the studies mentioned above and confirm the antidiabetic, antioxidant, antihyperglycemic, and TNF-α reducing effects of TYM and BAIBA.

Hyperglycemia and insulin resistance resulting from diabetes may increase myocardial ROS formation (Russo and Frangogiannis, 2016). Excessive ROS production activates cytokine production, apoptotic proteins and transcription factors. This situation results in chronic inflammation and increased apoptosis, which are important factors in the formation of post-diabetes complications (Alabi et al., 2020; Miranda-Diaz et al., 2016). Systemic inflammation triggers the aggregation of leukocytes and causes the release of proinflammatory cytokines and chemokines, such as interleukin (IL)-1, IL-6, and TNF-α (Alabi et al., 2020). TNF-α stimulation increases proliferation and collagen synthesis in cardiac fibroblasts (Russo and Frangogiannis, 2016). Also, constant hyperglycemia results in increased production of advanced glycation end products (AGEs) (Goldin et al., 2006). AGEs increase myocardial collagen and promote fibrosis, causing diastolic and systolic dysfunction (Shabab et al., 2021). The histopathological and biochemical results determined in the diabetes group in the present study are parallel with the data in the literature on the pathogenesis of DCM.

5. Conclusion

As it can be understood from the results of the literature synthesized above on the pathogenesis of DCM, the major cause of DCM is hyperglycemia, insulin resistance and subsequent ROS increase. The therapeutic effects of TYM and BAIBA against DCM are probably because of the antihyperglycemic antidiabetic, antioxidant and anti-inflammatory effects of these agents. This study has shown that TYM and BAIBA are pharmacological agents that have the potential to treat DCM, and their healing power increases even more with their combined use.

Author contributions

FMG contributed to the planning of the study, the writing of the article, and the making and evaluation of all histopathological analyzes in the article. IA contributed to the planning of the study, as well as the biochemical and statistical analysis.

Data availability statements

The data that support the findings of this study are available on request from the corresponding author.

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Declaration of Competing Interest

The authors report no declarations of interest.

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