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The Effects of Thymoquinone and β-aminoisobutyric acid on Brain Tissue of Streptozotocin-Induced Diabetic Rats

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Abstract

The aim of this study is to investigate the effects of timoquinone and β-aminoisobutyric acid (BAIBA) on the brain tissue of streptozotocin-induced diabetic rats. Randomly selected 35 male rats were divided into five groups of 7 animals each at 8 weeks. The groups are respectively; C, D, DT, DB, DTB. Diabetes mellitus (DM) was induced by intraperitoneally injection of a single dose of streptozotocin. Thymoquinone (20 mg/kg/day) and BAIBA (100 mg/kg/day) were administered to diabetic rats by gavage for 5 weeks. In the D group; glutathione (GSH) levels decreased. Again, in this group; relative brain weight, malondialdehyde (MDA), glucose, cholesterol (CH) triglyceride (TG) and creatine kinase BB (CK BB) levels increased. The histological structure of the hippocampus, cortex and cerebellum in diabetic rats was similar to that of the other groups. No histopathological alterations were detected in the central nervous system (CNS) at light microscopic level in any of the groups. It was observed that these biocehemical changes occurring after DM were reversed significantly in DT, DB and DBT groups. Although the protective effects of BAIBA were stronger than thymoquinone, the most effective result was obtained with the combined use of thymoquinone+BAIBA. The biochemical results obtained in this study showed that oxidative stress occurred in the brain tissue of diabetic rats. However, the effects of oxidative stress on the histological structure of brain tissue could not be detected by light microscopic level. The biochemical analysis results suggested that administration thymoquinone and BAIBA could be used as therapeutic agents with the potential to ameliorate brain damage caused by diabetes mellitus because of the antioxidant effects.

Keywords: β-aminoisobutyric acid, brain injury, rat, streptozotocin, thymoquinone.

INTRODUCTION

Glucose homeostasis in the body occurs by secretion of insulin from β cells of the pancreas in response to an increase in glucose concentration in the blood. The increase in insulin secretion inhibits gluconeogenesis in the liver and stimulates glucose entry into target tissues such as liver, muscle and fat (Kahn et al. 2014). DM is a disease characterized by an increase in blood sugar level and develops due to insufficient insulin hormone synthesis and secretion. Insulin deficiency and resistance to insulin also play a role in the formation of DM and changes in protein, carbohydrate and lipid metabolism can occur (Hasselbaink et al. 2003). Insulin resistance is defined as the inadequate response of peripheral tissues to insulin. As a result of this situation; The body's ability to use and metabolize glucose is significantly reduced and hyperglycemia occurs. Chronic hyperglycemia increases the production of reactive oxygen species (ROS) resulting in oxidative stress. ROS causes tissue and organ failure by damaging small blood vessels. Additionally ROS such as hydroxyl and superoxide radicals attack important cellular macromolecules such as carbohydrates, nucleic acids, lipids and proteins, cause cell damage or death (Tolmanet al. 2007; Karandrea et al. 2017; Joudaki and Setorki, 2019).

Streptozotocin is an unstable molecule that accumulates in pancreatic β cells and breaks down into carbonium radicals. Highly reactive carboxylic radicals produce direct and indirect toxic effects on pancreatic islet cells by increasing the formation of ROS (Sadek et al. 2017; Ghanema and Sadek,2012). These toxic effects induce the destruction of pancreatic insulin-producing cells by DNA methylation and experimentally model type 1 diabetes mellitus (T_1DM). This situation mimics the impaired insulin secretion from the pancreas, the etiology of T_1DM and the later stages of type 2 diabetes (T_2DM) (Cruz et al. 2019; Furman, 2015).

BAIBA is a catabolite of antiretroviral thymine analogs zidovudine and stavudine. This β-amino acid can increase fatty acid oxidation and reduce body weight in mice through an increased production of leptin by white adipose tissue (Maisonneuve et al. 2004). BAIBA is degraded in mitochondria to downstream catabolites such as propionyl-CoA, methylmalonyl-CoA and succinyl-CoA. However, unlike these derivatives BAIBA, has low toxicity on mitochondrial DNA replication (Begriche et al. 2008). Recently, it has been discovered that BAIBA is secreted by skeletal muscles after regular exercise via the peroxisome proliferator activated receptor gamma coactivator 1 α. On the other hand, BAIBA as a myokine, converts white adipose tissue into brown adipose tissue, which has an increased glucose metabolism and insulin sensitivity. BAIBA has also been shown to reduce hepatic ERS, apoptosis, and impairments in glucose and lipid metabolism after T_2DM (Shi et al. 2016; Tanianskii et al. 2019). Sawada et al. (2019) observed that BAIBA treatment significantly increased mRNA levels and the levels of antioxidant molecules such as catalase, superoxide dismutase, thioredoxin and gamma-

glutamylcysteine ligases (Sawada et al. 2019). Additionally, Jung et al. (2015) have reported that BAIBA reduces insulin resistance in skeletal muscles, suppresses inflammation and induces fatty acid oxidation (jung et al 2015).

Thymoquinone is the most important bioactive ingredient found in *Nigella sativa* essential oil (Abdel-Fattah et al. 2000). It is stimulates insulin secretion from pancreatic cells in response to the increase in blood glucose. In addition, it promotes glucose entry into cells and the use of glucose in cells, maintains glucose homeostasis by inhibiting hepatic glucose production. It has also been reported that β cells protect against oxidative stress and support the redox cycle (Karandrea et al. 2017). Thymoquinone has been shown to protect the brain against ischemic damage, reduces epileptic seizures, and most importantly, it reduces cerebral oxidative stress caused by diabetes (Elmaci and Altinoz,2016; Hamdy and Taha, 2009).

Results of studies ivestigating the effects of streptozotocin induced DM on the CNS are variable. Some studies have reported that learning and memory-related changes occur in diabetic individuals leading to dementia and cognitive dysfunction (Gispen and Biessels, 2000). In addition, long-term DM can disrupt the structure of the blood-brain barrier resulting in vascular damage and increase the rate of apoptosis in the CNS (Dai et al. 2002; Jakobsen et al.1987; Gurpinar et al. 2012). However, Guven et al. (2009) reported that no histopathological changes were observed at light microscopic level in the hippocampus, cortex and cerebellum after STZ induced diabetes in rats. Gurpinar et al. (2012) were reported that DM did not cause any significant a histopathological and apoptotic changes in the hippocampus, cortex and cerebellum, except for the damage to vascular endothelium.

In this study, the effects of Thymoquinone and BAIBA, which are reported above to have positive effects on glucose lipid metabolism and the prevention and treatment of diabetes, against pathological changes in the central nervous system caused by DM will be examined.

MATERIALS AND METHODS

Animals

In this study, 35 male Sprague-Dawley rats (210-250 g, 8 weeks old) were obtained from Adıyaman University Experimental Animal Production Application and Research Center**.** Experimental procedures were carried out in the same center also. The animals were accommodated at 24 °C, 65 ± 10% humidity with 12 hours of light: 12 hours dark cycle. During these procedures, water and pellet feed were given to the rats as adlibitum. Ethics committee permission was taken from Adiyaman University Laboratory Animals Local Ethics Committee (Protocol 2019/002). 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, each containing seven rats, were randomly divided into five groups. The groups are respectively; control (C), diabetes (D), diabetes+thymoquinone (DT), diabetes+BAIBA (DB), diabetes+thymoquinone+BAIBA (DTB). A single dose of streptozotocin (50 mg/kg) dissolved in 0.1 M sodium citrate buffer (pH: 4.5) was administered intraperitoneally (i.p.) to all groups except group C. After 72 hours of streptozotocin administration, blood glucose level was measured from the tail vein. Rats whose blood glucose concentrations are detected above 250 mg/dL were considered as diabetic. Diabetic rats except D group received thymoquinone (20 mg/kg/day) and BAIBA (100 mg/kg/day) by gavage for five weeks (Bayat et al. 2019; Liu et al. 2019; Begriche et al. 2008; Pari and Sankaranarayanan, 2009; Randhawa et al.2013). Blood glucose levels 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 ketamine hydrochloride+xylazine 10 mg/kg i.p.). The serum obtained by centrifuging the blood samples at 5,000 x g for 15 minutes were stored at -86 ° C for biochemical analyses. After decapitation one part of the excised brain, was separated for biochemical analysis. The other part was immersed in 10% buffered neutral formalin solution for histopathological analysis and fixed at +4 ºC for 24 hours.

Histological procedures

The fixated tissues were embedded in paraffin through routine histological procedures (Yahyazadeh and Altunkaynak, 2020). Tissues cut from paraffin blocks with microtome with a thickness of 5 µm were stained with hematoxylin & eosin (H & E) methods (Yahyazadeh and Altunkaynak, 2019). Histopathological examinations were performed using Olympus BX-53 microscope and photographs were taken with this microscope camera (DP 80 Olympus, Tokyo, Japan).

Biochemical evaluation

CK BB, CH, TG and glucose levels in the blood were analysed by using routine enzymatic methods with the Abbott Arcitech analyzer 16000 using GluC glucose kit, Ref no; 3L82-42 (Transasia Biomedicals Limited, Solan HP, in technical collaboration with GluC Diagnostics Mannheim Gmbh, Germany).

Oxidative stress biomarkers

MDA level in brain tissue was measured. The lipid peroxidation was analyzed 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 2500 x g for 10 minutes, samples were read by spectrophotometer at a wavelength of 532 nm (Placer et al. 1966). GSH levels in the brain tissues were determined according to Sedlak and Lindsay method (Sedlak and Lindsay, 1968). The sample was washed with 50% TCA and centrifuged at 1000 x g for 5 minutes. 2 mL of thermo fisher scientific (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 read by spectrophotometer at 412 nm wavelength.

Statistical analysis

Data such as glucose level were analyzed 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

oneway ANalysis Of VAriance (ANOVA). Post hoc LSD; Kruskal-Wallis test was used for biochemical parameters (serum CH, TG, CK BB and relative brain weight) for nonparametric values. In addition, Kruskal-Wallis test was used to evaluate the semiqualified evaluation of histopathological scores. Differences in the parameters measured among the groups were analyzed by Kruskal-Wallis test. A Mann-Whitney U test was used to compare dual groups. $P \leq 0.05$ values were considered statistically significant.

RESULTS

Histopathological analysis: The histological structure of the cortex, hippocampus and cerebellum in the control group and other groups were examined in detail at light microscopic level. Histological structures of the control group and the other groups were similar, and no histopathological changes were observed in any of the groups.

Biochemical analysis: In the present study, serum glucose level was increased in comparison to control group. This data of the DTB group was similar to the control group, but also they were largely normalized in the DT and DB groups significantly (Table 1). The results of glucose levels stated in this study were used in our other articles on the effects of thymoquinone and BAIBA use on heart, liver and kidneys against streptozotocin-induced diabetes (articles in press). In the D group, relative brain weight decreased; while serum TG, CH and CK BB amount increased as compared the C group These data's started to normalize in the DT and DB groups. This normalization was more successful in the DBT group compared to the other two groups in table 2 significantly.

Oxidative stress biomarkers: As shown in table 2 tissue GSH amount decreased and MDA amount increased in D group compared to C group significantly. In the DT and DB groups, this was reversed, respectively. The DTB group was more successful than the other two treatment groups significantly. The use of thymoquinone and BAIBA greatly reduced post-diabetic oxidative stress.

Table 1. Comparision of serum biochemical parameters (body weight and glucose) among the study population.

Stage of study	Control	STZ	STZ+TQ	STZ+BAIBA	STZ+TQ+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 ± 24 $^{\alpha}$

Changes in glucose 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. $p \leq 0\ 05$. C: Control; D: Diabetes; DT: Diabetes+thymoquinone; DB: Diabetes+BAIBA; DBT:Diabetes+thymoquinone+BAIBA $^{£,€,¥,\beta,\alpha}$ in each column, different superscript characters mean significant differences at $p < 0\ 05$ in different groups.

Table 2. Changes in of relative brain weight, CH, TG, CK BB, MDA and GSH experimental rats.

Stage of study	Control	STZ	STZ+TQ	STZ+BAIBA	STZ+TQ+BAIBA
Brain (g/100 g BW)	0.92± 0.01 ^{a,b,c,d}	0.66±0.01 ^{b,c,d,e}	0.74±0.00 ^{a,c,d,e}	0.82±0.00 ^{a,b,d,e}	0.89±0.00 ^{a,b,c,e}
CH (mg/dL)	50.86 ±1.12 $^{b,\ c,\ d}$	74.71±0.68 $^{a,\ c,\ d,\ e}$	66.86±0.67 $^{a\ b,\ d,\ e}$	60.43±1.04 $^{a\ b,c,e}$	53.00±0.78 ,^{b,c,d}
TG (mg/dL)	69.00 ±4.37 ^{b,c,d,e}	12.57±1.83 ^{a,c,d,e}	39.86±2.40 ^{a,b,d,e}	50.00±2.16 ^{a,b,c,e}	63.57±2.69 ^{b,c,d}
CK BB (mg/dL)	22.71±0.96 ^{b,c,d,e}	67.14 ±1.71^{a,c,d,e}	54.86 ±1.62 ^{a,b,d,e}	44.00±1.49 ^{a,b,c,e}	31.14±2.42 ^{a,b,c,d}
MDA (nmol/g tissue)	245..86 ± 2.17 ^{b,c,d,e}	412.29 ± 2.95 ^{a,c,d,e}	380.43±1.66 ^{a,b,d,e}	337.57±3.57 ^{a,b,c,e}	270.86±2.04^{a,b,c,d}
GSH (mg/g tissue)	234.14±1.77 ^{b,c,d}	153.71±1.42 ^{a,c,d,e}	172.86±1.86 ^{a,b,d,e}	196.86±0.8 ^{a,b,c,e}	235.14±1.83 ^{b,c,d}

Each group represents the mean ± SEM for six rats. $^{a}p < 0\ 01$ *vs* the control group; $^{b}p < 0\ 01$ *vs* the D group; $^{c}p < 0\ 01$ *vs* the DT group; $^{d}p < 0\ 01$ *vs* the DB group; and $^{e}p < 0\ 01$ *vs* the DTB group. C: Control; D: Diabetes; DT:Diabetes+thymoquinone; DB: Diabetes+BAIBA; DBT:Diabetes+thymoquinone+BAIBA; CH, cholestrol; TG, triglyceride; CK BB, creatine kinase BB.

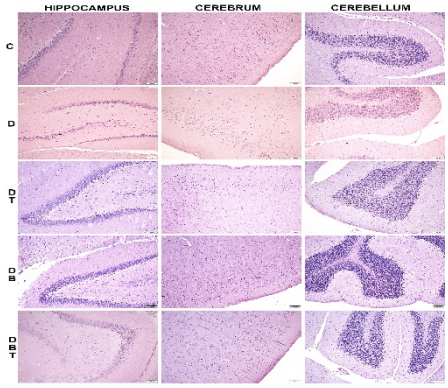

Figure 1. Photomicrographs of H&e staining of brain sections of Control, STZ, TQ, BAIBA and TQ+BAIBA gruoups (X100). No histopathological alterations were observed in any of the groups.

DISCUSSION AND CONCLUSION

Increase in serum glucose level in D group were compatible to result of Joudaki and Setorki (2019) study. In the present study, serum glucose levels in DT and DB groups decreased when compared to D group. This result was consistent with the literature (Shi et al., 2016; Usta and Dede, 2017). In the D group; while relative brain weight decreased compared to the C group, the amount of serum glucose increased. Although these data were almost the same in the DTB group with the C group, they were largely improving in the DT and DB groups (Yajing et al.2012). Thymoquinone was proven to be beneficial in the treatment of DM by lowering blood sugar and increasing insulin secretion and sensitivity, increasing glucose utilization as well a decreasing hepatic glucose production. It has been shown that thymoquinone protects β cells from oxidative stress following streptozotocin treatment (Pari

and Sankaranarayanan, 2009). BAIBA has been shown to reduce hepatic insulin resistance, hepatic gluconeogenesis and blood glucose levels in Type 2 diabetes (Shi et al. 2016). In this study, it was found that serum glucose level decreased in DT, DB and DTB groups. This result is consistent with the data's in the literatures (Shi et al. 2016; Usta and Dede, 2017).

Oxidative stress plays an important role in the pathogenesis of diabetic complications. Oxidative stress increases under diabetic conditions and causes dysfunction in many cell types. There is a balance between ROS and antioxidants in the body. The shift of this balance to ROS is called oxidative stress. Polyunsaturated fatty acids (PUFA) formed as a result of these ROS cause oxidative degradation. These PUFAs are rich in the brain and cause oxygen free radical origin and also play an important role in DNA pathogenesis. MDA is one of the lipid peroxidation products that are an important indicator of oxidative stress. Increase in ROS level results in elevated MDA level causes death of neurons by oxidizing various components of the cellular system such as lipid, proteins and nucleotides. This results in serious complications that cause learning and memory deficits (Al-Enazi 2007; Wang et al. 2018; Ozerol et al. 2009; Ohkawa et al. 1979; Shoji and Koletzko, 2007; Arora and Singh, 2014). Oxidative stress level in the body is regulated by enzymatic and non-enzymatic antioxidant systems. Considered to be the most common and important intracellular protein (thiol), GSH plays a very important role as a free radical (FR) scavenger. Over production of FRs cause depletion endogenous antioxidants reserves in order to reduce the damaging effects of FRs (Ozerol et al. 2009). In this study, the GSH level in the brain tissue decreased in the D group and the amount of MDA increased compared to the C group. In the DT and DB groups these findings were reversed respectively. DBT group was more successful than the other two treatment groups. Use of thymoquinone and BAIBA greatly reduced post-diabetic oxidative stress. These data's are consistent with the literature and confirm the antioxidant effects of thymoquinone and BAIBA (Begriche et al. 2008; Hamdy and Taha, 2009; Wang et al. 2018; Ozerol et al. 2009; Manna et al. 2010; Abdel-Daim et al. 2018; Wang et al. 2017; Oboh et al. 2018).

CH is biosynthesized in all animal cells and it is an important structural component of all living cell membranes. CH acts as a precursor for biosynthesis of steroid hormones, bile acids and vitamin D. CH is transported in the blood as lipoprotein complex. CH extracted from tissues is transported to the liver via high density lipoproteins, then excreted in bile (Narwal et al. 2019). Human body converts excess calories immediately into TG which are stored in fat cells. In the case of energy requirement between meals. TGs are released from the fat cells in response to hormonal stimulation. A high TG synthesis occurs especially when high carbohydrate foods are consumed. High TG levels in the blood increases the risk of stroke, heart attack, and heart disease (Triglycerides, 2020). A creatine kinase isoform, CK-BB, found in the CNS catalyzes the transfer of phosphate groups from ATP to creatine phosphate. It also plays a role in energy transfer in tissues with high-energy requirement such as brain. CK-BB is found in astrocytes and therefore released when brain tissue is damaged. It has been reported that serum CK BB levels increase in various brain injury cases, including ischemia, and trigger neurodegenerative events that lead to neuronal losses (Sharma et al. 2017). In previous studies, increased serum concentrations of CH, TG and CK BB have been reported in streptozotocin-induced DM (Zhou et al. 2019). Consistent with previous reports, serum concentrations of CH, TG and CK BB increased in the D group compared to the C group in this study. This increase is indicative of brain damage. In the DT and DB groups, these data began to normalize and this normalization was more successful in the DTB group than in the other two groups. The results obtained in this study are consistent with studies reporting that the increase in serum CH and TG levels after diabetes was greatly improved after thymoquinone administration (Begriche et al. 2008; Shi et al. 2016; Abdel-Daim et al. 2018).

Results of studies examining the histopathological effects of DM on brain tissue are controversial. In some studies, it has been reported that DM causes neuro-pathological changes in brain tissue (Klein et al. 2004; Patrick and Campbell, 1990). Hernandez-Fonseca et al. (2009) reported histopathological lesions such as edema, increased apoptosis rate, and vacualization in neurons and glial cells at the light microscopic level, in the cerebral cortex, hypothalamus and cerebellum of diabetic rats. However, in one of the studies, it was reported that no significant histopathological and apoptotic changes observed after diabetes, except for vascular endothelial damage in brain regions such as the hippocampus, cortex and cerebellum (Gurpinar et al. 2012) In another study investigating the effects of diabetes on the same brain regions, it was stated that no histopathological changes were detected (Guven et al. 2009). The possible reason for the differences between the results of these studies is the duration of diabetes. Generally, experimental diabetes models of longer than six weeks (Hernandez-Fonseca et al. 2009; Baydas et al. 2002; Baydas et al. 2003; Kamal et al. 1999) have been reported to result in pathological changes in brain tissue. On the other hand, no pathological changes were reported in studies using short diabetes models (Gurpinar et al. 2012; Güven et al. 2009). The biochemical findings obtained in this study, in which the effects of five-week diabetes on the brain were investigated, showed that oxidative stress was formed in the brain tissue. However, light microscopic examinations showed that there were no histopathological alterations in the cortex, hippocampus or cerebellum regions.

This study showed that thymoquinone and BAIBA application was effective in alleviating the pathological changes in serum and brain tissue at the biochemical level as a result of DM induced by streptozotocin. The possible cause of the healing effects of these agents may be inhibition of lipid peroxidation and stimulation of antioxidant enzymes. The findings obtained in this study can be interpreted as that the damage caused by oxidative stress in the brain tissue is at a low level that cannot be detected by the histological methods used in this study. In future studies, it is thought that extending the duration of experimental diabetes and performing ultrastructural analysis may enable the histopathological effects of diabetes on brain tissue to be determined more precisely.

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Conflict of Interest
The authors declare that there is no conflict of interest in the content of the article

REFERENCES

Abdel-Daim MM, Shaheen HM, Abushouk AI, Toraih EA, Fawzy MS, Alansari WS, Aleya L, Bungau S. 2018. Thymoquinone and diallyl sulfide protect against fipronil-induced oxidative injury in rats. Environ Sci Pollut Res Int, 25(24): 23909-16.

Abdel-Fattah AM, Matsumoto K, Watanabe H. 2000. Antinociceptive effects of Nigella sativa oil and its major component, thymoquinone, in mice. Eur J Pharmacol, 400: 89-97.

Al-Enazi MM. 2007. Effect of thymoquinone on malformations and oxidative stress-induced diabetic mice. Pak J Biol Sci. 10(18):3115-3119.

Arora, M., Singh, UK. 2014. Oxidative Stress: Meeting Multiple Targets in Pathogenesis of Diabetic Nephropathy. Current drug targets. Curr Drug Targets, 15: 531-8.

Bayat M, Dabbaghmanesh MH, Koohpeyma F, Mahmoodi M, Montazeri-Najafabady N, Bakhshayeshkaram M. 2019. The effects of soy milk enriched with lactobacillus casei and omega-3 on the tibia and L5 vertebra in diabetic rats: a stereological study. Probiotics Antimicrob Protein, 11: 1172-81.

Baydas G, Canatan H, Turkoglu A. 2002. Comparative analysis of the protective effects of melatonin and vitamin E on streptozotocin-induced diabetes mellitus. Journal of Pineal Research, 32: 225-30.

Baydas G, Nedzvetskii VS, Tuzcu M. 2003. Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. European Journal of Pharmacology, 462(1-3): 67-71.

Begriche K, Massart J, Abbey-Toby A, Igoudjil A, Letteron P, Fromenty B. 2008. Beta-aminoisobutyric acid prevents diet-induced obesity in mice with partial leptin deficiency. Obesity (Silver Spring), 16: 2053-67.

Crowley LV. 1967. The Reitman-Frankel colorimetric transaminase procedure in suspected myocardial infarction. Clin Chem, 13: 482-7.

Cruz B, Flores RJ, Uribe KP, Espinoza EJ, Spencer CT, Serafine KM, Nazarian A, O'Dell LE. 2019. Insulin modulates the strong reinforcing effects of nicotine and changes in insulin biomarkers in a rodent model of diabetes. Neuropsychopharmacology, 44(6): 1141-51.

Dai J, Vrensen GF, Schlingemann RO. 2002. Blood-brain barrier integrity is unaltered in human brain cortex with diabetes mellitus. Brain Research, 954: 311-6.

Elmaci I, Altinoz MA. 2016. Thymoquinone: An edible redox-active quinone for the pharmacotherapy of neurodegenerative conditions and glial brain tumors. A short review. Biomed Pharmacother, 83: 635-40.

Furman BL. 2015. Streptozotocin-Induced Diabetic Models in Mice and Rats. Curr Protoc Pharmacol, 70: 5.47.1-20.

Ghanema IIA, Sadek KM. 2012. Olive Leaves Extract Restored the antioxidant Perturbations in Red Blood Cells Hemolysate in Streptozotocin Induced Diabetic Rats. International Journal of Animal and Veterinary Sciences, 6(4): 124-30.

Gispen WH, Biessels GJ. 2000. Cognition and synaptic plasticity in diabetesmellitus. Neuroscience, 23(11): 542-9.

Gurpinar T, Ekerbicer N, Uysal N, Barut T, Tarakci F, Tuglu MI. 2012. The effects of the melatonin treatment on the oxidative stress and apoptosis in diabetic eye and brain. TheScientificWorldJournal, 2012: 498489.

Guven A, Yavuz O, Cam M, Comunoglu C,Sevinc Ö. 2009. Central nervous system complications of diabetes in streptozotocin-induced diabetic rats: A histopathological and immunohistochemical examination. Int J Neurosci, 119(8): 1155-69.

Hamdy NM, Taha RA. 2009. Effects of Nigella sativa oil and thymoquinone on oxidative stress and neuropathy in streptozotocin-induced diabetic rats. Pharmacology, 84(3): 127-34.

Hasselbaink DM, Glatz JFC, Luiken, JJFP, Roemen TH, Van der Vusse GJ 2003. Ketone bodies disturb fatty acid handling in isolated cardiomyocytes derived from control and diabetic rats, Biochem J, 371: 753-60.

Hernandez-Fonseca JP, ´Rincon J, Pedreanez A, Viera N, Arcaya JL, ´ Carrizo E, Mosquera J. 2009. Structural and Ultrastructural Analysis of Cerebral Cortex, Cerebellum, and Hypothalamus from Diabetic Rats. Hindawi Publishing Corporation Experimental Diabetes Research, ID 329632: 1-12.

Jakobsen J, Knudsen GM, Juhler M. 1987. Cation permeability of the bloodbrain barrier in streptozotocin-diabetic rats. Diabetologia, 30: 409-13.

Joudaki R, Setorki M. 2019. The protective effect of Satureja bachtiarica hydroalcoholic extract on streptozotocin-induced diabetes through modulating glucose transporter 2 and 4 expression and inhibiting oxidative stress. Pharm Biol, 57: 318-27.

Jung TW, Hwang HJ, Hong HC, Yoo HJ, Baik SH, Choi KM. 2015. BAIBA attenuates insülin resistance and inflammation induced by palmitate or a high fat diet via an AMPK-PPARd-dependent pathway in mice. Diabetologia, 58: 2096-105.

Kahn SE, Cooper ME, Del Prato S. 2014. Pathophysiology and treatment of type 2 diabetes: perspectives on the past, present, and future. Lancet, 383: 1068-83.

Kamal A, Biessels GJ, Urban IJ, Gispen WH. 1999. Hippocampal synaptic plasticity in streptozotocin-diabetic rats: impairment of long-term potentiation and facilitation of long-term depression. Neuroscience, 90: 737-45.

Karandrea S, Yin H, Liang X, Slitt AL, Heart EA. 2017.Thymoquinone ameliorates diabetic phenotype in diet-induced obesity mice through the activation of SIRT-1-dependent pathways. PLoS One, 12(9): 1-19.

Klein JP, Hains BC, Craner MJ, Black JA, Waxman SG. 2004. Apoptosis of vasopressinergic hypothalamic neurons in chronic diabetes mellitus. Neurobiol Dis, 15: 221-8.

Liu YD, Zhang SC, Xue J, Wei ZQ, Shen BX, Ding LC. 2019. Caffeine improves bladder function in streptozotocin-induced diabetic rats. Neurourol Urodyn, 38: 81-6.

Mabrouk A. 2018. Therapeutic effect of thymoquinone against lead-induced testicular histological damage in male Wistar rats. Andrologia, 50(6): e13014.

Maisonneuve C, Igoudjil A, Begriche K, Letteron P, Guimont MC, Bastin J, Laigneau JP, Pessayre D, Fromenty B. 2004. Effects of zidovudine, stavudine and beta-aminoisobutyric acid on lipid homeostasis in mice: possible role in human fat wasting. Antivir Ther, 9: 801-10.

Manna P, Das J, Ghosh J, Sil PC. 2010. Contribution of type 1 diabetes to rat liver dysfunction and cellular damage via activation of NOS, PARP, IkappaBalpha/NF-kappaB, MAPKs, and mitochondria-dependent. Free Radic Biol Med, 48(11): 1465-84.

Narwal V, Deswal R, Batra B, Kalra V, Hooda R, Sharma M, Rana JS. 2019. Cholesterol biosensors: A review. Steroids, 143:6-17.

Oboh G, Oyeleye SI, Akintemi OA. Olasehinde TA. 2018. Moringa oleifera supplemented diet modulates nootropic-related biomolecules in the brain of STZ-induced diabetic rats treated with acarbose. Metab Brain Dis, 33(2): 457-66.

Ohkawa H, Ohishi N, Yagi K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95: 351-8.

Ozerol E, Bilgic S, Iraz M, Cigli A., Ilhan A., Akyol O. 2009. The protective effect of erdosteine on short-term global brain ischemia/reperfusion injury in rats. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 33(1): 20–4.

Pari L, Sankaranarayanan C. 2009. Beneficial effects of thymoquinone on hepatic key enzymes in streptozotocin-nicotinamide induced diabetic rats. Life Sci, 85: 830-4.

Patrick AW, Campbell IW. 1990. Fatal hypoglycemia in ınsulin-treated diabetes-mellitus – clinical-features and neuropathological changes. Diabetic Med, 7: 349–54.

Placer ZA, Cushman LL, Johnson BC. 1966. Estimation of product of lipid peroxidation (malonyl dialdehyde) in biochemical systems. Anal Biochem, 16(2): 359-64.

Randhawa MA, Alghamdi MS, Maulik SK. 2013. The effect of thymoquinone, an active component of Nigella sativa, on isoproterenol induced myocardial injury. Pak J Pharm Scİ, 26: 1215-9.

Rivoira M, Rodríguez V, Picotto G, Battaglino R, Tolosa de Talamoni N. 2018. Naringin prevents bone loss in a rat model of type 1 Diabetes mellitus. Arch Biochem Biophys, 637: 56-63.

Sadek KM, Lebda MA, Nasr SM, Shoukry M. 2017. Spirulina platensis prevents hyperglycemia in rats by modulating gluconeogenesis and apoptosis via modification of oxidative stress and MAPK-pathways. Biomed Pharmacother, 92: 1085-94.

Sawada M, Yamamoto H, Ogasahara A, Tanaka Y, Kihara S. 2019. βeta-aminoisobutyric acid protects against vascular inflammation through PGC-1beta-induced antioxidative properties. Biochem Biophys Res Commun, 516: 963-8.

Sharma G, Ashhar MU, Aeri V, Katare DP. 2019. Development and characterization of late-stage diabetes mellitus and -associated vascular complications. Life Sci, 216: 295-304.

Sharma R, Rosenberg A, Bennett ER, Laskowitz DT, Acheson SK. 2017. A blood-based biomarker panel to risk-stratify mild traumatic brain injury. PLoS One, 12: e0173798.

Sedlak J, Lindsay RH. 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem, 25(1): 192-205.

Shi CX, Zhao MX, Shu XD, Xiong XQ, Wang JJ, Gao XY, Chen Q, Li YH, Kang YM, Zhu GQ. 2016. βeta-aminoisobutyric acid attenuates hepatic endoplasmic reticulum stress and glucose/lipid metabolic disturbance in mice with type 2 diabetes. Sci Rep, 6: 21924.

Shoji H, Koletzko B. 2007. Oxidative stress and antioxidant protection in the perinatal period. Curr Opin Clin Nutr Metab Care, 10: 324-8.

Tanianskii DA, Jarzebska N, Birkenfeld AL, O'Sullivan JF, Rodionov RN. 2019. Beta-Aminoisobutyric Acid as a Novel Regulator of Carbohydrate and Lipid Metabolism. Nutrients, 11: 524.

Tolman KG, Fonseca V, Dalpiaz A, Tan MH. 2007. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. Diabetes Care, 30: 734-43.

Triglycerides. Why do they matter? https://www.mayoclinic.org/diseases-conditions/high-blood-cholesterol/in-depth/triglycerides/art-20048186 erişim tarihi. 25.07.2020.

Usta A, Dede S. 2017. The Effect of Thymoquinone on Nuclear Factor Kappa B Levels and Oxidative DNA Damage on Experimental Diabetic Rats. Pharmacogn Mag, 13: 458-61.

Wang H, Qian J, Zhao X, Xing C, Sun B. 2017. β-Aminoisobutyric acid ameliorates the renal fibrosis in mouse obstructed kidneys via inhibition of renal fibroblast activation and fibrosis. J Pharmacol Sci, 133: 203-13.

Wang J, Wang L, Zhou J, Xing C, Sun B. 2018. The protective effect of formononetin on cognitive impairment in streptozotocin (STZ)-induced diabetic mice. Biomed Pharmacother, 106: 1250-57.

Yahyazadeh A, Altunkaynak BZ. (2020). Effect of Luteolin on Biochemical, Immunohistochemical, and Morphometrical Changes in Rat Spinal Cord following Exposure to a 900 MHz Electromagnetic Field. Biomed Environ Sci, 33(8): 593-602.

Yahyazadeh A, Altunkaynak BZ. (2019). Protective effects of luteolin on rat testis following exposure to 900 MHz electromagnetic field. Biotech Histochem, 23:1-10.

Yajing L, Chen M, Xuan H, Fuliang H. 2012. Effects of encapsulated propolis on blood glycemic control, lipid metabolism, and insulin resistance in type 2 diabetes mellitus rats. Evid Based Complement Alternat Med, 2012: 1-8.

Zhou G, Yan M, Guo G, Tong N. 2019. Ameliorative Effect of Berberine on Neonatally Induced Type 2 Diabetic Neuropathy via Modulation of BDNF, IGF-1, PPAR-γ, and AMPK Expressions. Dose Response, 17(3): 1559325819862449