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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Benfluorex, Friends or Foe? The Effects of Benfluorex on Oxidative Status in the Brain During Experimental Diabetes

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Abstract: Benfluorex is a pharmacological agent with antidiabetic and antihyperlipidemic properties. In this study, the brain's oxidative and non-enzymatic antioxidant status in diabetic and benfluorex administrated diabetic rats have been investigated. For the experimental procedures, three groups of 18 Wistar albino rats were used to control diabetes (induced by streptozotocin), and benfluorex treated diabetic rats (benfluorex administration intragastric 50 mg/kg daily for 21 days). Brain NOx, TBARS, GSH, AA levels, and MPO activity were determined spectrophotometrically. Benfluorex administration was caused that decreased lipid peroxidation and MPO activity while increased non-enzymatic antioxidant and NOx levels. These results showed that benfluorex treatment positively affects lipid peroxidation and the non-enzymatic antioxidant status of the brain during diabetes..

Keywords: Benfluorex, brain, diabetes, lipid peroxidation, non-enzymatic antioxidants, nitric oxide.

Benfloreks: Dost mu, Düşman mı? Deneysel Diyabette, Benfloreksin Beyindeki Oksidatif Olaylara Etkileri

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Öz: Benflureks, antidiyabetik ve antihiperlipidemik özelliklere sahip farmakolojik bir ajandır. Bu çalışmada, uygulanan diyabetik sıçanlarda benfloreks tedavisinin beynin oksidatif ve enzimatik olmayan antioksidan durumu üzerindeki etkileri araştırılmıştır. Deneysel prosedürler için, kontrol, diyabet (streptozotosin ile indüklenen) ve benfloreks ile tedavi edilmiş diyabetik sıçanlar (benfloreks 21 gün boyunca intragastrik 50 mg/kg dozda uygulanmıştır) olarak 18 Wistar albino sıçanından oluşan üç grup kullanıldı. Beyin NOx, TBARS, GSH, AA seviyeleri ve MPO aktivitesi spektrofotometrik olarak belirlendi. Benfloreks uygulaması, lipid peroksidasyonunu ve MPO aktivitesini azaltırken; enzimatik olmayan antioksidan seviyelerini ve NOx düzeylerini arttırmıştır. Bu sonuçlar, benfloreks tedavisinin, diyabet sırasında beyinde lipid peroksidasyonunu ve enzimatik olmayan antioksidan durumu olumlu etkilediğini göstermiştir.

Anahtar kelimeler: Benfloreks, beyin, diyabet, lipid peroksidasyonu, enzimatik olmayan antioksidanlar, nitrik oksit.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which is one of the most important causes of morbidity and mortality worldwide (Ong et al., 2018). Therefore, the diabetes-related in vivo and in vitro studies remain its importance and popularity. DM becomes resulting from that either deficiency in insulin secretion from the beta (β) cells of the pancreatic islets of Langerhans or breakdown in the susceptibility of the insulin hormone or both of together exists. Streptozotocin (STZ, 2-deoxy-2-[3-methyl-3-nitrosoureido]-D glucopyranose) is one of the most used pharmacological agents in order to create experimental diabetes. The diabetic effects of STZ are

caused by the devastation of β pancreatic cells by natural killer (NK) cells (Szkudelski, 2001).

It is a known fact that increased Reactive Oxygen Species (ROS) production breaks down tissue homeostasis and it causes tissue damage. Particularly, lipids are vulnerable to ROS damage. Resulting of diabetic processes, hyperglycemia stimulates ROS generation from a variety of sources such as cytochrome P450 monooxygenases, nitric oxide synthase (NOS), oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase, lipoxygenase, and glucose auto-oxidation (Pandey et al., 2010). ROS formation leads to devastation and injury on cell membranes by lipid peroxidation (Halliwell, 1994). Oxidative stress appears when ROS are produced in excess amounts or when antioxidant defense systems are impaired. Oxidative stress is the one of the causes in the pathogenesis and progression of complications from diabetes (Piero et al., 2014).

In every aerobic organism has antioxidant defense system existing of non-enzymatic antioxidant substances and antioxidant enzymes to protect the physiological activities of organisms against oxidative stress. Ascorbic acid (AA) and Glutathione (GSH) are a part of the non-enzymatic antioxidant that their cycle is known to act a fundamental role in the keeping of cellular redox homeostasis (Noctor et al., 2002).

The brain is one of the major organs of the body which responsible for maintaining of neuronal and hormonal processes and homeostasis. It is vulnerable to oxidative stress induced by diabetes consequence of its rich oxidizable polyunsaturated fatty acids content, high rate of oxygen consumption the existence of redox-active metals (Cu, Fe), and low defending of enzymatic antioxidant (Montilla et al., 2005; Uzar et al., 2012).

Benfluorex was an amphetamine derivative drug widely medicated by Type II diabetes patients through worldwide due to both lipid-lowering and antihyperglycemic effects until withdrawn in 2010 (Tribouilloy et al., 2012). In contrast to other antidiabetic drugs, the nonexistence of gastrointestinal side effects of benfluorex has increased the possibility of its preference (Moulin et al., 2009). Nevertheless, after the publication of several reports suggesting the link between the administration of benfluorex and serious cardiac valve regurgitation, it was withdrawn from the European market in 2010 (Rafel Ribara et al., 2003; Noize et al., 2006; Boutet et al., 2009; Frachon et al., 2010; Tribouilloy et al., 2010; Le Ven et al., 2011). The drug-induced cardiac side effects of benfluorex are caused by serotoninergic mechanisms via its metabolite norfenfluramine which has the ability to activate 5-HT_{2B} serotonin receptors in the heart valve, where it plays a role in the synthesis of glycosaminoglycans and collagen (Rothman et al., 2000; Roth, 2007; Tribouilloy et al., 2012). Besides known their cardiac and pulmonary adverse effects, amphetamines and their derivatives also could produce several neurological changes such as intracerebral vasculitis, ischemic stroke, and cerebral hemorrhage (Galvan-Arzate & Santamaria, 2002). On the other hand, there is no finding pointing to the effect of benfluorex on brain oxidant-antioxidant status during diabetes.

In this context, the present study was carried out to put forth the effects of benfluorex treatment on brain oxidative events throughout the diabetic process.

MATERIALS AND METHODS

Animals and Groups: Male adult Wistar albino rats were use in experiments (18, weighing 190–200 g). All animals were held in a temperature-controlled room with 12 hours of light and 12 hours of the dark cycle, in separate cages with access to water and food. All animal studies were performed in accordance with international ethical rules, and all animal procedure were approved by the Animal Experimentation Ethics Committee of Giresun University (Report no: 2019/06).

The animals have divided into three different groups as 1) Control (n=6) 2) Diabetes (n=6) and 3) Diabetes+benfluorex (n=6), randomly. Rats of the control group were given only an injection of 0.1 M, 1 ml citrate buffer at pH 4.5. On the Diabetes group, disease model was created by intraperitoneal injection of a single dose of STZ (Serva 35503) (45 mg/kg body weight) dissolved in 0.1 M, 1 ml citrate buffer at pH 4.5 (2). Rats were accepted diabetic if their fasting blood glucose (FBG) levels exceeded 200 mg/dl at 48 h after STZ injection. Rats of Diabetes+benfluorex group were treated with benfluorex (Sigma B-7522) intragastric (IG) 50 mg/kg daily for 21 days (Brindley et al., 1988; Serradas et al., 1993). The equal volume of tap water was IG delivered to control and diabetes groups for the same period.

After FBGs and body weights were measured, on day 22 of the experimental protocol, rats were sacrificed with taken blood from the heart under anesthesia. The brain tissues were removed rapidly and instantly freeze in liquid nitrogen and stuck at –80 °C until use.

Determination of TBARS: Lipid peroxidation in tissues was determined by the formation of TBARS (Casini et al., 1986). Brain tissues homogenized in cold trichloroacetic acid and centrifuged at 3000 rpm for 15 minutes. The supernatant then added to the tube containing an equal amount of thiobarbituric acid of 0.67% (w/v) and boiled at 100°C for 15 minutes. The absorbance of the samples determined at 535 nm.

Determination of GSH: The GSH levels of tissues were measured by the modified Elman method (Aykaç et al., 1985). Brain tissue samples homogenized in trichloroacetic acid solution and homogenate then centrifuged at 3000 rpm for 10 min. Supernatant added on the tube containing 0.3 mol/L $Na_2HPO_4 \cdot 2H_2O$ solution. After, the dithiobisnitrobenzoate solution added in the tube and the absorbance was determined at 412 nm.

Determination of NOx: The Griess method was used to determine total nitric oxide (NOx) values in the brain tissues (Green et al., 1982). Tissue samples were homogenized in cold phosphate-buffered saline (pH = 7.5) then centrifuged at 3000 rpm for 5 min. Supernatant added to a tube containing 0.3 M NaOH. After 5% (w/v) ZnSO4 was added for deproteinization, mixture incubated for 5 min at room temperature, then centrifuged at 14,000 rpm for 5 min. The nitrate levels in brain tissues were measured spectrophotometrically by method of Miranda et. al. (Miranda et al., 2001). The Griess reaction was used to determine nitrite levels of tissues.

Determination of AA: Total ascorbate levels of brain tissues were measured by the modified Roe and Keuther method (Berger et al., 1989). Brain tissues homogenized on cold in 35% perchloric acid and centrifuged at 12,000 rpm for 3 min. Supernatants were combined with color reagent (0.6% copper sulfate, 5% thiourea, and 2,4-dinitrophenlyhydrazine at 1:1:20, v/v/v) and mixtures incubated 3 h at 37°C in a water bath. After they were cooled to 0°C, 65% (v/v) sulfuric acid was added and absorbances were measured at 515 nm.

Determination of MPO activity: MPO activity was measured by method of Glowick and Kaplan (1955). Tissue samples homogenized in cold phosphate buffer (pH 7.5). After centrifuged at 3000 g for 10 minutes at 4°C, supernatants were added to tubes containing 0.5 M phosphate buffer, 30% H_2O_2, 1% o-dianisidin, H_2O (10:1:2:3 v/v/ v/v). Mixtures incubated at 37°C for 30 minutes then HCl was added. One unit (U) of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 410 nm and 37°C.

Statistical analysis: All data were submitted as the mean ± standard deviation. Evaluation of the values for all experimental groups was used by ANOVA variance analysis and nonparametric Mann–Whitney U test. The p-value less than 0.05 was well-considered significant.

RESULTS

The NOx levels of the brain in the control group were found to be 145.63 ± 14.88 µmol g^{-1}, while, they were 101.46 ± 7.61 µmol g^{-1} in the diabetes group and 115.06 ± 9.30 µmol g^{-1} in the benfluorex treatment group. In Figure 1, NOx levels are shown as diagrams. While the NOx levels were found to be significantly reduced in both groups than in the control group ($p<0.05$). In the benfluorex treatment group, NOx level was found to be significantly increased than the diabetes group ($p<0.05$).

The brain TBARS value in the control group was found to be 261.47±32.18 nmol g^{-1}, while they were 430.07±46.12 nmol g^{-1} in the diabetes group, and 262.65±44.74 nmol g^{-1} in the benfluorex treatment group. In Figure 2, TBARS values are shown as diagrams. TBARS level of the diabetes group was found to be elevated significantly compared to the control group ($p<0.05$). However, benfluorex treatment was significantly reduced TBARS levels compared to the diabetes group ($p<0.05$).

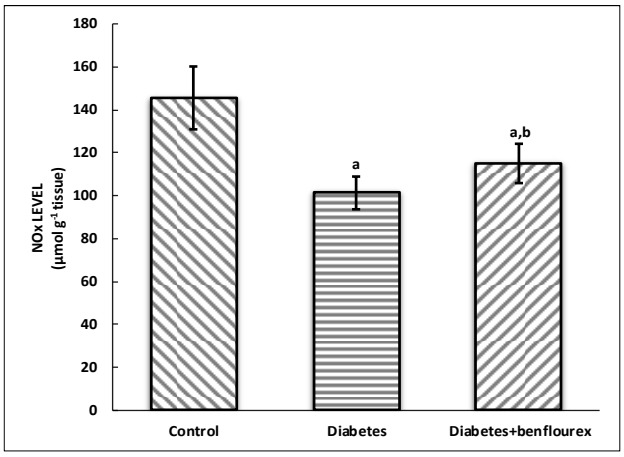

Figure 1. The effects of benfluorex administration during diabetes on the brain NOx levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

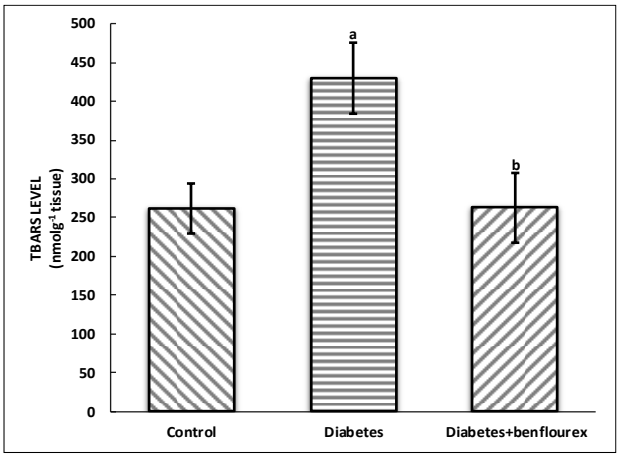

Figure 2. The effects of benfluorex administration during diabetes on the brain TBARS levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

GSH levels of the brain were found to be respectively 3.86±0.36 µmol g^{-1}, 2.96 ± 0.49 µmol g^{-1} and, 4.48 ± 0.89 µmol g^{-1} in the control group, in the diabetes group and, in the benfluorex treatment group. In Figure 3, GSH levels are shown as diagrams. GSH level was found to be significantly decreased in the diabetes group compared to the control ($p<0.05$). Whereas with benfluorex administration, GSH level was shown to be significantly increased compared to the diabetes group ($p<0.05$).

The brain AA levels in the control group was 3.49±0.29 mg g^{-1}, while they were 2.98±0.21 mg g^{-1} in the diabetes group, and 3.48±0.16 mg g^{-1} in the benfluorex treatment group. In Figure 4, AA levels are shown as diagrams. AA level was found to be significantly reduced in the diabetes group compared to the control ($p<0.05$). Although in the benfluorex treatment group AA level was found to be significant enhancements than diabetes group ($p<0.05$).

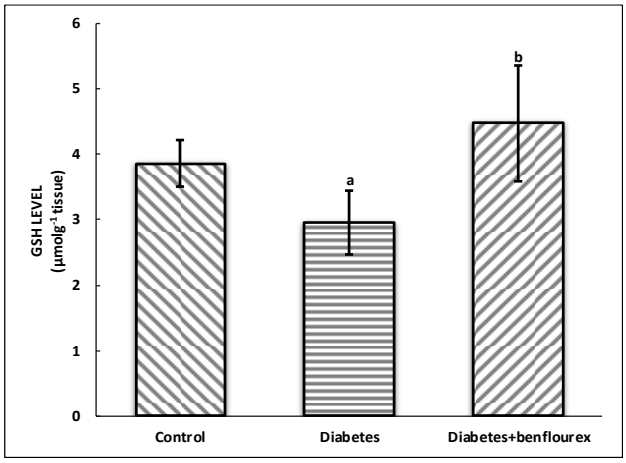

Figure 3. The effects of benfluorex administration during diabetes on the brain GSH levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

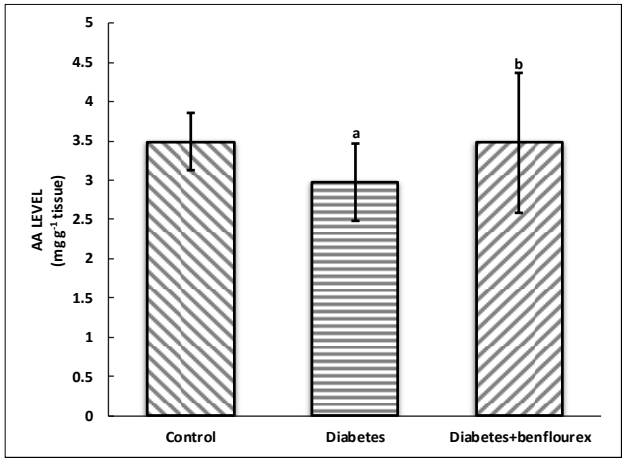

Figure 4. The effects of benfluorex administration during diabetes on the brain AA levels.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

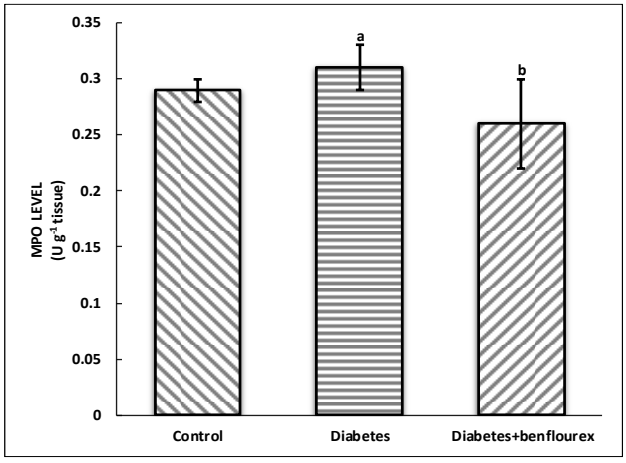

Figure 5. The effects of benfluorex administration during diabetes on the brain MPO activities.
[a] $p < 0.05$ compared to the control group; [b] $p < 0.05$ compared to the diabetes group.

MPO activity of the brain was found to be respectively 0.29±0.01 U g^{-1} tissue, 0.31 ± 0.02 U g^{-1} tissue and, 0.26 ± 0.04 U g^{-1} tissue in the control group, in the diabetes group and, in the benfluorex treatment group. In Figure 5, MPO activities are shown as diagrams. MPO activity of the diabetes group was found to be increased significantly compared to the control group ($p<0.05$). But, benfluorex treatment was significantly reduced MPO activity compared to the diabetes group ($p<0.05$).

DISCUSSION

This was the first study to show brain oxidative status, non-enzymatic antioxidants levels and MPO activity between diabetic rats who were treated with benfluorex compared with untreated diabetic rats.

Several studies have indicated that the altered oxidative state due to hyperglycemia may be induced to the diabetic nerve damage (Aragno et al., 2000; Ateş et al., 2006; Ateş et al., 2007; Zhang et al., 2008). One of the underlying causes of this damage has been that increased intracellular glucose concentration, therefore, the excessive formation of ROS originating from auto-oxidation. (Piero et al., 2014). Different investigators have been revealed that lipid peroxidation was elevated in the brain, arising from diabetes (El-Akabawy & El-Kholy, 2014; Muriach et al., 2014; Ogunyinka et al., 2016; Ibrahim, 2016; Fheem & Askary, 2017; Gürel-Gökmen et al., 2018). The results of this study showed that TBARS levels were increased in the brains of diabetic animals than in controls as demonstrated in other studies. Additionally, in the current results, non-enzymatic antioxidants GSH and AA levels, the inverse of rising lipid peroxidation, were found decreased in the diabetic group. Antioxidant defense system including antioxidant enzymes and non-enzymatic antioxidant compounds may be affected by diabetic processes (Kurutaş, 2016). Evaluation of TBARS and non-enzymatic antioxidant levels of the diabetic group has been suggested that excessive ROS production via both hyperglycemia and increased auto-oxidation of glucose during diabetes may cause lipid peroxidation. Also, it has been thought that of GSH and AA radical scavenging properties may be used to prevent from oxidative damage.

It has been demonstrated that several vascular complications related to diabetes may result from changes in the production and action of endothelially derived NO (Avogaro et al., 2006). In the literature, contradictory data about the amount of NO in diabetes have been reported. In the animal model of STZ-induced diabetes, some investigators have found to be increased NO levels in the cerebral cortex, hippocampus, cerebellum, brain stem, and spinal cord (Ateş et al., 2007; Xu et al., 2015), although in another study it was reported that NO levels were decreased in the hippocampus (Kino et al., 2004). However, Gurel-Gokmen et al. (2018) have observed that NO levels did not change in the brains of diabetic animals induced by STZ. According to the results of this study, the NOx levels of the brain during diabetes were found

decreased significantly compared to the controls. This situation may be elucidated by the raised catabolism of NO or the reduced production of NO by endothelial dysfunction due to diabetes.

Myeloperoxidase (MPO) is an important pro-oxidant enzyme that is physiologically released in circulating neutrophils, monocytes and some tissue macrophages including microglia as also described an inflammatory marker (39,42). When discharged to the extracellular environment as a component of inherent host defense, tissue damage can occur via MPO-derived oxidants (Lazarević-Pasti et al., 2015). MPO can also debilitate lipoprotein function, initiate endothelial dysfunction, and disrupt synthase of inducible NO (Avogaro et al., 2006). Consistent with other findings, the activity of MPO was found increased in diabetes group, and also at the same time, NO level was found the decrease in diabetic animals because of MPO's probably effect of triggering endothelial dysfunction.

Benfluorex was shown to have hypolipidemic and antihyperglycemic effects in diabetic animal models and in humans (Ravel & Laudignon, 1996). However, there has been never examined the relationship between benfluorex administration with oxidative events in the brain during diabetes. In the current study, the consequences of benfluorex treatment on the brain oxidative status were found to be interesting. According to the results of this study, benfluorex application was reduced TBARS level and MPO activity, but it was increased NO, GSH and AA levels. In the previous studies, benfluorex has been shown to reduce hepatic glucose production, ameliorate binding of insulin to its receptor, and increase aerobic glucose utilization in skeletal muscle (Bianchi et al., 1993; De Feo et al., 1993; Riccio et al., 1993; Kohl et al., 2002). Also, that has been showed that benfluorex affects the expression of genes encoding enzymes related to both glucose and fatty acid metabolism, resulting in inhibition of mitochondrial β-oxidation, that resulted reduces in gluconeogenesis (Kohl et al., 2002). On the other hand, it has been known that fenfluramine derivatives including benfluorex initiate serotonergic mechanisms via increasing synaptic levels of 5-HT. Because, norfenfluramine, a metabolite of benfluorex, has a strong agonistic effect for the 5-HT_{2B} receptor (Rothman et al., 2000). Furthermore, it has been established that 5-HT_2 receptor activation promotes glycogenolysis, which halted the process of gluconeogenesis. (Darvesh, & Gudelsky, 2003). The cumulative effect of all these metabolic paths constitutes both the antidiabetic and antihyperlipidemic effects of benfluorex. Based on our results, it may be suggested that hypolipidemic properties of benfluorex decrease lipid peroxidation, which as a result of changing lipid metabolism due to inhibition of beta-oxidation. In addition, its antidiabetic effects, in turn, may alleviate some metabolic and physiologic abnormalities associated with diabetes such as endothelial dysfunction, preternatural inflammation and, tissue damage via excessive ROS production. These facts about benfluorex affect mechanism may explain that the reason for decreased levels of TBARS and MPO activity, and also increased levels of NOx, GSH and AA in the brain during experimental diabetes.

CONCLUSION

Consequently, in this study, besides the known life-threatening cardiac side effects; from a different perspective, the effects of benfluorex on the brain during diabetes were examined. Taken together, the present study suggests that brain tissue with its inflammatory enzymatic process, ROS production, lipid peroxidation, and its non-enzymatic antioxidant capacity were affected by benfluorex treatment while experimental diabetes.

REFERENCES

Aragno, M., Parola, S., Tamagno, E., Brignardello, E., Manti, R., Danni, O. & Boccuzzi, G. (2000). Oxidative derangement in rat synaptosomes induced by hyperglycemia: restorative effect of dehydroepiandrosterone treatment. *Biochemistry & Pharmacology*, ***60***(3), 389-395. doi: 10.1016/s0006-2952(00)00327-0.

Ates, O., Caylı, S.R., Altınoz, E., Altinoz, E., Kocak, A., Durak, M.A., Turkoz, Y. & Yologlu, S. (2007). Central nervous system protection by resveratrol in streptozotocin-induced diabetic rats. *Journal of Clinical Neuroscience*, ***14***(3), 256-260. DOI: 10.1016/j.jocn.2005.12.010

Ates, O., Yucel, N., Cayli, S.R., Altinoz, E., Yologlu, S., Kocak, A., Cakir, C.O. & Turkoz, Y. (2006). Neuroprotective effect of etomidate in the central nervous system of streptozotocin-induced diabetic rats. *Neurochemical Research*, ***31***(6), 777-783. DOI: 10.1007/s11064-006-9076-0

Avogaro, A., Fadini, G.P. & Gallo, A. (2006). Endothelial dysfunction in type 2 diabetes mellitus. *Nutrition, Metabolism and Cardiovascular Diseases*, ***16***(1), 39-45. DOI: 10.1016/j.numecd.2005.10.015

Aykac, A.G., Uysal, M., Yalcın, A.S., Koçak-Toker, N., Sivas, A. & Oz, H. (1985). The effect of chronic ethanol ingestion on hepatic lipid peroxide, glutathione, glutathione peroxidase and glutathione transferase in rats. *Toxicology*, ***36***(1), 71-76. DOI: 10.1016/0300-483x(85)90008-3

Berger, J., Shepart, D., Morrow, F. & Taylor, A. (1989). Relationship between dietary intake and tissue levels reduced and total vitamin C in nonscorbutic guinea pig. *The Journal of Nutrition*, ***119***(5), 734-740. DOI: 10.1093/jn/119.5.734

Bianchi, R., Bongers, V., Bravenboer, B. & Erkelens, D.W. (1993). Effects of benfluorex on insulin resistance and lipid metabolism in obese type II diabetic patients. *Diabetes Care*, ***16***(4), 557-559. DOI: 10.2337/diacare.16.4.557

Boutet, K., Frachon, I., Jobic, Y., Gut-Gobert, C., Leroyer, C., Carlhant-Kowalski, D., Sitbon, O., Simonneau, G. & Humbert, M. (2009). Fenfluramine-like cardiovascular side-effects of benfluorex. *European Respiratory Journal*, ***33***(3), 684-688. DOI: 10.1183/09031936.00086308

Brindley, D.N., Akester, H., Derick, G.P., Irvine, C.D., Patmore, R.D., Spencer, H., Yule-Smith, A., Finnerty, C., Saxton, J., Macdonald, I.A. & et al. (1988). Effects of chronic administration of benfluorex to rats on the metabolism of corticosterone, glucose, triacylglycerol, glycerol and fatty acid. *Biochemistry & Pharmacology*, ***37***(4), 695-705. DOI: 10.1016/0006-2952(88)90144-x

Casini, A., Ferrali, M. & Pompella, A. (1986). Lipid peroxidation and cellular damage in extrahepatic tissue of bromobenzene-intoxicated mice. *American Journal of Pathology*, ***123***(3), 520-531. PMID: 3717304

Darvesh, A.S. & Gudelsky, G.A. (2003). Activation of 5-HT2 receptors induces glycogenolysis in the rat brain. *European Journal of Pharmacology*, ***464***(2-3), 135-140. DOI: 10.1016/s0014-2999(03)01432-8

De Feo, P., Lavielle, R., De Gregoris, P. & Bolli, G.B. (1993). Anti-hyperglycemic mechanisms of benfluorex in type II diabetes mellitus. *Diabetes Metabolic Reviews*, ***9***(1), 35-41. DOI: 10.1002/dmr.5610090507

El-Akabawy, G. & El-Kholy, W. (2014). Neuroprotective effect of ginger in the brain of streptozotocin-induced diabetic rats. *Annals of Anatomy*, ***196***(2-3), 119-128. DOI: 10.1016/j.aanat.2014.01.003

Faheem, N.M. & El Askary, A. (2017). Neuroprotective role of curcumin on the hippocampus against the structural and serological alterations of streptozotocin-induced diabetes in Sprague Dawley rats. *Iranian Journal of Basic Medical Sciences*, ***20***(6), 690-699. DOI: 10.22038/IJBMS.2017.8839

Frachon, I., Etienne, Y., Jobic, Y., Le Gal, G., Humbert, M. & Leroyer, C. (2010). Benfluorex and unexplained valvular heart disease: a case-control study. *PLoS ONE*, ***5***(1), e10128. DOI: 10.1371/journal.pone.0010128

Galvan-Arzate, S. & Santamaria, A. (2002). Neurotoxicity of diethylpropion: neurochemical and behavioral findings in rats. *Annual New York Academy of Sciences*, ***965***(1), 214-224. PMID: 12105097.

Glowick, S.P. & Kaplan, S.D. (1955). Methods in enzymology. *Academic Press, New York*, pp 769-782.

Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S. & Tannenbaum, S.R. (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Analitic Biochemistry*, ***126***(1), 131-138. https://doi.org/10.1016/0003-2697(82)90118-x

Gurel-Gokmen, B., Ipekci, H., Oktay, S., Alev, B., Ustundag, U.V., Ak, E., Akakın, D., Sener, G., Emekli-Alturfan, E., Yarat, A. & Tunali-Akbay, T. (2018). Melatonin improves hyperglycemia induced damages in rat brain. *Diabetes Metabolism Research and Reviews*, ***34***(8), e3060. DOI: 10.1002/dmrr.3060

Halliwell, B. (1994). Free radicals, antioxidants, and human disease: curiosity, cause, or consequence? *Lancet*, ***344***(8924), 721-724. DOI: 10.1016/s0140-6736(94)92211-x

Ibrahim, D.S. (2016). Neuroprotective effect of *Cucumis melo var. flexuosus* leaf extract on the brains of rats with streptozotocin-induced diabetes. *Metabolic Brain Disease*, ***32***(1), 69-75. DOI: 10.1007/s11011-016-9886-y

Kino, M., Yamato, T. & Aomine, M. (2004). Simultaneous measurement of nitric oxide, blood glucose, and monoamines in the hippocampus of diabetic rat: an in vivo microdialysis study. *Neurochemistry International*, ***44***(2), 65-73. DOI: 10.1016/s0197-0186(03)00125-6

Kohl, C., Ravel, D., Girard, J. & Pégorier, J.P. (2002). Effects of benfluorex on fatty acid and glucose metabolism in isolated rat hepatocytes: from metabolic fluxes to gene expression. *Diabetes*, ***51***(8), 2363-2368. DOI: 10.2337/diabetes.51.8.2363

Kurutas, E.B. (2016). The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutrition Journal*, ***15***(1), 71-99. DOI: 10.1186/s12937-016-0186-5

Lazarević-Pasti, T., Leskovac, A. & Vasić, V. (2015). Myeloperoxidase Inhibitors as Potential Drugs. *Current Drug Metabolism*, ***16***(3), 168-190. DOI: 10.2174/1389200216031508121206 40

Le Ven, F., Tribouilloy, C., Habib, G., Gueffet, J.P., Maréchaux, S., Eicher, J.C., Blanchard-Lemoine, B., Rousseau, J., Hénon, P., Jobic, Y. & Etienne, Y. (2011). Valvular heart disease associated with benfluorex therapy: results from the French multicentre registry. *European Journal of Echocardiography*, ***12***(4), 265-271. DOI: 10.1093/ejechocard/jeq172

Miranda, K.M., Espey, M.G. & Wink, D.A. (2001). A rapid simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide*, ***5***(1), 62-71. DOI: 10.1006/niox.2000.0319

Montilla, P., Barcos, M., Munoz, M.C., Bujalance, I., Munoz-Castaneda, J.R. & Tunez, I. (2005). Red wine prevents brain oxidative stress and nephropathy in stretozotocin-induced diabetic rats. *Journal of Biochemistry and Molecular Biology*, ***38***(5), 538-544. DOI: 10.5483/bmbrep.2005.38.5.539

Moulin, P., Andre, M., Alawi, H., Dos Santos, L.C., Khalid, A.K., Koev, D., Moore, R., Serban, V., Picandet, B. & Francillard, M. (2009). Efficacy of benfluorex in combination with sulfonylurea in type 2 diabetic patients: an 18 to 34-week, open-label, extension period. *Diabetes & Metabolism*, ***35***(1), 64-70. DOI: 10.1016/j.diabet.2008.10.002

Muriach, M., Flores-Bellver, M., Romero, F.J. & Bracia, J.M. (2014). Diabetes and the brain: oxidative stress, inflammation, and autophagy. *Oxidative Medicine and Cellular Longevity*, ***2014***(1), 1-9. DOI: 10.1155/2014/102158

Noctor, G., Gomez, L., Vanacker, H. & Foyer, C.H. (2002). Interactions between biosynthesis, compartmentation, and transport in the control of glutathione homeostasis and signalling. *Journal of Experimental Botany*, ***53***(372), 1283-1304. DOI: 10.1093/jexbot/53.372.1283

Noize, P., Sauer, M., Bruneval, P., Moreau, M., Pathak, A., Bagheri, H. & Montastruc, J.L. (2006). Valvular heart disease in a patient taking benfluorex. *Fundamental & Clinical Pharmacology*, ***20***(6), 577-578. DOI: 10.1111/j.1472-8206.2006.00441.x

Ogunyinka, B.I., Oyinloye, B.E., Osunsanmi F.O., Opoku, A.R. & Kappo, A.P. (2016). Modulatory influence of *Parkia biglobosa* protein isolate on testosterone and biomarkers of oxidative stress in brain and testes of streptozotocin-induced diabetic male rats. *International Journal of Physiology, Pathophysiology and Pharmacology*, ***8***(3), 78-86. PMID: 27785334

Ong, S.E., Koh, J.J.K., Toh, S.E.S., Chia, K.S., Balabanova, D., McKee, M., Perel, P. & Legido-Quigley, H. (2018). Assessing the influence of health systems on Type 2 Diabetes Mellitus awareness, treatment, adherence, and control: A systematic review. *PLoS One*, ***29***;13(3), e0195086. DOI: 10.1371/journal.pone.0195086

Pandey, K.B., Mishra, N. & Rizvi, S.I. (2010). Protein oxidation biomarkers in plasma of type 2 diabetic patients. *Clinical Biochemistry*, ***43***(4-5), 508-511. DOI: 10.1016/j.clinbiochem.2009.11.011

Piero, M.N., Nzaro, G.M. & Njagi, J.M. (2014). Diabetes mellitus – a devastating metabolic disorder. *Asian Journal of Biomedical and Pharmaceutical Sciences*, ***04***(40), 1-7. DOI: 10.15272/ajbps.v4i40.645

Rafel Ribera, J., Casanas Munoz, R., Anguera Ferrando, N., Batalla Sahún, N., Castro Cels, A. & Pujadas Capmany, R. (2003). Valvular heart disease associated with benfluorex. *Revista Espanola de Cardiologia*, ***56***(2), 215-216. DOI: 10.1016/s0300-8932(03)76849-3

Ravel, D. & Laudignon, N. (1996). Research prospects with benfluorex. *Journal of Diabetes Complications*, ***10***(5), 246-254. DOI: 10.1016/1056-8727(96)00045-1

Riccio, A., Vigili de Kreutzenberg, S., Dorella, M., Da Tos, V., De Biasi, L., Marescotti, M.C., Tiengo, A. & Del Prato, S. (1993). Mechanism(s) of the blood glucose lowering action of benfluorex. *Diabetes Metabolic Reviews*, ***9***(1), 19-27. DOI: 10.1002/dmr.5610090505. PMID: 8299485

Roth, B.L. (2007). Drugs and valvular heart disease. *New England Journal of Medicine*, ***356***(1), 6 -9. DOI: 10.1056/NEJMp068265

Rothman, R.B., Baumann, M.H., Savage, J.E., Rauser, L., McBride, A., Hufeisen, S.J. & Roth, B.L. (2000). Evidence for possible involvement of 5-HT(2B) receptors in the cardiac valvulopathy associated with fenfluramine and other serotonergic medications. *Circulation*, ***102***(23), 2836-2841. DOI: 10.1161/01.cir.102.23.2836

Serradas, P., Blondel, O., Bailbe, D. & Portha, B. (1993). Benfluorex normalizes hyperglycemia and reverses hepatic insulin resistance in STZ-induced diabetic rats. *Diabetes*, ***42***(4), 564-570. DOI: 10.2337/diab.42.4.564

Szkudelski, T. (2001). The mechanism of alloxan and streptozotocin action in β cells of rat pancreas. *Physiological Research*, ***50***(1), 536-546. PMID: 11829314.

Tribouilloy, C., Rusinaru, D., Henon, P., Tribouilloy, L., Leleu, F., Andréjak, M., Sevestre, H., Peltier, M. & Caus, T. (2010). Restrictive organic mitral regurgitation associated with benfluorex therapy. *European Journal of Echocardiography*, ***11***(7), 614-621. DOI: 10.1093/ejechocard/jeq027

Tribouilloy, C., Rusinaru, D., Maréchaux, S., Jeu, A., Ederhy, S., Donal, E., Réant, P., Arnalsteen, E., Boulanger, J., Ennezat, PV., Garban, T. & Jobic, Y. (2012). Increased risk of left heart valve regurgitation associated with benfluorex use in patients with diabetes mellitus: a multicenter study. *Circulation*, ***126***(24), 2852-2858. DOI: 10.1093/ejechocard/jeq027

Uzar, E., Alp, H., Cevik, M.U., Fırat, U., Evliyaoglu, O., Tufek, A. & Altun, Y. (2012). Ellagic acid attenuates oxidative stress on brain and sciatic nerve and improves histopathology of brain in streptozotocin-induced diabetic rats. *Neurological Sciences*, ***33***(3), 567-574. DOI: 10.1007/s10072-011-0775-1

Xu., L., Zhu, J., Yin, W. & Ding, X. (2015). Astaxanthin improves cognitive deficits from oxidative stress, nitric oxide synthase and inflammation through upregulation of PI3K/Akt in diabetes rat. *International Journal of Clinival & Experimental Pathology*, ***8***(6), 6083-6094. PMID: 26261486

Zhang, W.J., Tan, Y.F., Yue, J.T., Vranic, M. & Wojtowicz, J.M. (2008). Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. *Acta Neurologica Scandinavica*, ***117***(3), 205-210. PMID: 17854417.