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Does Bosentan Protect Diabetic Brain Alterations in Rats? The Role of Endothelin-1 in the Diabetic Brain

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Abstract: Diabetes mellitus (DM) is a major problem all over the world, affecting more people in recent years. Individuals with diabetes are more prone to disease than non-diabetics, especially vascular complications. The aim of this study was to examine the roles of the endothelin (ET)-1 in brain damage formed in a streptozocin (STZ)-induced diabetes model, and the effect of bosentan, which is the non-specific ET1 receptor blocker in the prevention of the diabetes-induced brain damage. To examine the effects of bosentan (50 mg/kg and 100 mg/kg) in this study, the rats were given the drug for 3 months. The rats were divided into four groups: the sham group (n = 10), the diabetic control group (n = 10), the group of diabetic rats given bosentan 50 mg/kg (n = 10) and the group of diabetic rats given bosentan 100 mg/kg (n = 10). Diabetes was induced in the rats by STZ (60 mg/kg i.p.). On day 91, all rats were killed. Brain tissues of the rats were measured by molecular, biochemical and histopathological methods. Antioxidant levels in the therapy groups were observed as quite near to the values in the healthy group. In this study, while the brain eNOS levels in the diabetic groups decreased, the ET1 and iNOS levels were found to be increased. However, in the diabetes group, hippocampus and cerebellum, pericellular oedema and a number of neuronal cytoretraction were increased in neuropiles, whereas these results were decreased in the therapy group. Based on all of these results, ET1 will not be ignored in diabetes-induced cerebral complications.

Diabetes mellitus (DM) is a severe disease which eventually results in death. During diabetes mellitus, tissues cannot uptake glucose and very severe complications such as nephropathy, retinopathy, neuropathy and peripheral vascular complications occur [1]. Many new mechanisms have been suggested during the formation and progress of these complications. There are studies in many areas, from inflammation to cellular membrane disorders, of the mechanisms of diabetes-induced complications [1–3]. There are also many studies on the receptors which are responsible for the formation of diabetes and diabetes-induced complications in relation to our topic: angiotensin receptors, urotensin receptors, glucagon-like peptide 1 receptors and peroxisome proliferator-activated receptor-α [4,5].

Previous studies have shown the importance of angiotensin in diabetes-induced liver damage [6,7], and there are many recent studies showing that endothelin-1 receptors play important roles in diabetes. One study showed that cell proliferation and, at the same time, ET1 production increased in type 2 diabetes [8,9]. Diabetes-related, clinical and experimental studies showed that ET1 plasma levels increased [10,11]. A study by Mastumoto *et al.* showed that the expression of both ET1 and the receptors increased in the streptozotocin (STZ)-induced diabetes model [12]. In another study, giving endothelin-1 to individuals with insulin resistance reduced endothelin-1-induced vasodilatation [13]. ET1 levels were determined to be raised in diabetic patients with retinopathy, diabetic hypertensive patients and patients with microalbuminuria [10,14]. All these studies show that ET1 and the receptors play important roles in diabetes-induced complications.

With respect to our subject, diabetes mellitus leads to slow and progressive brain damage [15,16]. There are studies showing that it leads to diabetes-induced neuropathic cognitive disorders [17]. In addition, rapidly progressive learning, memory and cognitive disorders were observed in type 2 diabetes patients with insulin resistance [18,19]. Similarly, hippocampal plasticity-based cognitive dysfunction was seen in STZ-induced diabetic rats [20]. Although endothelin-1 is quite important in the etiopathology of these complications, we wonder whether endothelin-1 receptors play any role in diabetes-induced neurological complications.

The purpose of this study was to examine the changes in the brain with respect to endothelin-1 in diabetes and to examine the role of bosentan, which is the non-selective endothelin-1 receptor antagonist [21], in the prevention of diabetes-induced brain damage. Additionally, we aimed to show the levels of iNOS, eNOS and free O_2 radicals which play important roles in tissue damage and promote these results via histopathological analyses.

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Materials and Methods

Animals. A total of 40 male Wistar rats were used in the experiments. Each rat weighed 220–240 g, and all were obtained from the Ataturk University's Experimental Animal Laboratory at the Medicinal and Experimental Application and Research Centre (ATADEM). The animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals, and they were approved by Ataturk University's local animal care committee. The rats were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22 ± 1°C. Standard rat food and tap water were given *ad libitum*.

Chemicals. All chemicals used in our laboratory experiments were purchased from Sigma Chemical Company (Germany). Bosentan (Tracleer, 125 mg Tb) was purchased from Actelion, USA.

Experimental design. The rats were separated into four groups, each composed of 10 individual rats:

Group 1 (sham)**:** control group.

Group 2 (DM)**:** STZ, 60 mg/kg (i.p.)

Group 3 (DM+Bos1)**:** STZ, 60 mg/kg (i.p.) + bosentan, 50 mg/kg oral administration (in 2 ml distilled water).

Group 4 (DM+Bos2)**:** STZ, 60 mg/kg (i.p.) + bosentan, 100 mg/kg oral administration (in 2 ml distilled water).

Induction of type 1 diabetes. Diabetes was induced in the male Wistar albino rats by intraperitoneal administration of streptozotocin (STZ) (Sigma-Aldrich Co., USA) at a single dose of 60 mg/kg body-weight, as described previously [22]. The STZ was dissolved in a 0.1 M acetate buffer (pH 4.5) that was freshly prepared. Rats in the healthy group were injected with only the buffer solution. After the STZ application, the pancreas secretes insulin at high levels; therefore, fatal hypoglycaemia can occur. To prevent this adverse effect, 4–6 hr after STZ treatment, a 5-ml 20% glucose solution was injected intraperitoneally. Then, a 5% glucose solution was added to the drinking water of the rats for 24 hr to prevent possible hypoglycaemia. Seventy-two hours after the STZ injection, the rats were fasted for 12 hr (free access to water), and fasting blood samples were collected via the tail vein and used for measuring the blood glucose levels with the Accu-Chek Active blood glucose monitor [23]. Rats with glucose levels of 250 mg/kg or higher were considered to be diabetic [24].

Drug treatment. The treatment with bosentan was immediately administered after the STZ injection and continued for 3 months. In our study, there were 10 rats in each group. The non-diabetic healthy group and diabetic control group were exposed to only 0.9% saline for 30 days (1 ml/day), and the groups treated with diabetic drugs were exposed to bosentan for 30 days at 50 mg/kg/day and 100 mg/kg/day of dissolved saline at 1 ml/day orally**.** During the experiment, each group was kept in separate standard plastic cages. After the drug administration period, all rats were killed on day 91 after the oral treatment using an overdose of a general anaesthetic (thiopental sodium, 50 mg/kg). Brain tissues were dissected out immediately and transferred into 10% formaldehyde solution for serological and histopathological evaluations.

Biochemical analyses.

Biochemical investigation of brain tissues. After the macroscopic analyses, the rat tissues were kept at −80°C. All tissue samples from each rat were first perfused with PBS/heparin and then ground in liquid nitrogen using a TissueLyser II grinding jar set. Approximately 100 mg of ground tissue was homogenized in 1 ml PBS homogenate buffer in Eppendorf tubes with the TissueLyser II and then centrifuged. Superoxide dismutase activity (SOD) [25], glutathione levels (GSH) [26] and malondialdehyde levels (MDA) [27] from each sample supernatant and the standards were measured in duplicate, according to the modified methods, with an ELISA reader. The average absorbance of each sample and standard were calculated. A standard curve was plotted, and the equation obtained from the absorbance of these standards. The linear SOD, GSH and MDA concentrations were calculated according to this equation. All data were presented as the mean ± standard deviation results based on per mg protein.

Protein determination. The protein concentrations were determined by the Lowry method using commercial protein standards (Sigma-Aldrich, Total protein kit-TP0300-1KT, USA).

Molecular analyses.

Total RNA extraction and cDNA synthesis. The tissues (20 mg) were stabilized in RNA Stabilization Reagent (RNAlater, Qiagen, Hilden, Germany) and then disrupted using the TissueLyser II (2 × 2 min. for brain, Qiagen). The total RNA was purified using the RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions in QIAcube (Qiagen). The RNA samples were reverse-transcribed into complementary DNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA). From 10 µl, the total RNA was treated with 2 µl 10 X RT Buffer, 0.8 µl 25 X dNTPs mix, 2 µl 10X RT Random Primers, 1 µl MultiScribe Reverse Transcriptase and 4.2 µl DEPC-H_2O. Reverse transcription was carried out at 25°C for 10 min., followed by 120 min. at 37°C, and finally 85°C for 5 min. using the Veriti 96 Well Thermal Cycler (Applied Biosystems). The cDNA concentration and quality were assessed and quantified by the Epoch Spectrophotometer System and Take3 Plate (BioTek, Turkey) [28].

Relative quantification of gene expression. The Relative ET1, eNOS and iNOS expression analyses were performed with StepOnePlus Real Time PCR System technology (Applied Biosystems) using cDNA synthesized from rat brain RNAs. The qPCR was run using the Primer Perfect Probe mix, TaqMan probe-based technology (Primer Design Ltd., Southampton, UK). The results are expressed as the relative fold compared to the control animals. The expression data of the β-actin in each tissue were used as the endogenous control. The primers and probes for β-actin were designed by Primer Design, Southampton, UK. For each tissue, triplicate determinations were performed in a 96-well optical plate for both targets using 9 µl of cDNA (100 ng), 1 µl of Primer Perfect Probe mix and 10 µl of QuantiTect Probe PCR Master mix (Qiagen) in each 20 µl reaction. The plates were heated for 2 min. at 50°C and 10 min. at 95°C, and subsequently, 40 cycles of 15 sec. at 94°C and 60 sec. at 60°C were applied. All data are expressed as the fold change in expression compared to the expression in the other animal groups, using the $2^{-\Delta\Delta Ct}$ method [29].

Histopathological examination. The brain tissue samples were obtained at necropsy and fixed in a 10% formaldehyde solution. Then, routine pathological processing was performed and the tissues were embedded in paraffin wax. From the paraffin wax block of each brain sample, 4–5 µm slices were cut. After haematoxylin and eosin staining (H&E), prepared slides were examined by light microscopy (Olympus BX51, Olympus Co, USA) by a pathologist blinded to the treatment groups.

Statistical analysis. For the statistical analysis of molecular results (ET-1, iNOS, eNOS), we used the GraphPad Prism, version 5.0.

GraphPad Software, Inc., USA Results are presented as the means ± standard deviation (S.D.). Comparisons between the groups were performed using one-way ANOVA and Tukey's multiple comparison test. Significance was accepted at $p < 0.001$ and $p < 0.05$. For the statistical analysis of biochemical results (SOD, GSH, MDA), we used the SPSS, version IBM-20.0. Results are presented as means ± S.D. Comparisons between the groups were performed using one-way ANOVA and Duncan's multiple comparison test. Significance was accepted at $p < 0.05$.

Results

Biochemical results.

SOD activity. As seen in table 1, the SOD activity in the diabetes-induced groups was statistically reduced when compared to the healthy groups in our study. The SOD activity significantly recovered in the groups in which we applied bosentan therapy, and no change was observed in the SOD activity in the different doses of bosentan.

GSH level. As shown in table 1, the GSH levels of the healthy groups were set as 2.84. In the diabetes-induced groups, on the other hand, a significant reduction was observed in the GSH levels when compared to the healthy group. While no significant difference was observed in the GSH levels between the diabetic groups and the therapy group when bosentan was applied in a 50 mg/kg dose, the use of bosentan in the 100 mg/kg dose significantly increased the GSH levels, and these values were observed as quite near to the values in the healthy group.

MDA level. As seen in table 1, the MDA levels in the healthy groups of our study were recorded as 1.10. The MDA levels which were measured in the brain tissues in the diabetic groups were observed to have increased severely when compared to the healthy group. The MDA values were significantly reduced in the therapy groups of our study; in particular, in the bosentan group (which was given the 100 mg/kg dose), the MDA level was observed to be almost the same as that of the healthy group.

Gene expression results.

ET1, eNOS and iNOS mRNA levels. We firstly investigated the ET1 mRNA expression in the brain tissue of rats using real-time PCR. As shown in fig. 1, the ET1 gene expression increased in the STZ-induced DM groups (1.39 times) when compared to the control groups ($P < 0.001$). Bos1 and Bos2 showed a significantly down-regulatory effect on the ET1 mRNA expression, by 0.59 times and 0.37 times ($p < 0.001$) in the DM+Bos1 and DM+Bos2 groups, respectively. This demonstrated that ET1 expression increased depending on diabetes, and bosentan reduced the ET1 expression in the rat brains.

Also, we evaluated endothelial NOS mRNA expression in the brain tissue of rats using real-time PCR. As shown in fig. 2, the eNOS gene expression decreased in the STZ-induced DM groups (0.71) when compared to the control groups. Bos1 and Bos2 showed a significantly up-regulatory effect on eNOS mRNA expression, by 1.02 and 0.81 times, in the DM+Bos1 groups and DM+Bos2 groups, respectively. These results demonstrate that eNOS expression decreased depending on diabetes and bosentan increased the eNOS expression in the rat brains.

We investigated the iNOS mRNA expression in the brain tissue of rats using real-time PCR (fig. 3). When compared to the control group, the iNOS mRNA expression was significantly higher in the DM group (27.03 times) ($p < 0.001$). This suggested that Bos1 and Bos2 reduced the iNOS expression in

Table 1.
The effects of bosentan on the levels of GSH, MDA and SOD activities in diabetic rat brains.

	SOD (U/mg protein)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
Healthy	13.19 ± 4.42**c**	2.84 ± 1.02**c**	1.10 ± 0.51**a**
DM	6.80 ± 1.40**a**	1.38 ± 0.67**a**	3.17 ± 0.76**c**
DM + Bos1	10.05 ± 2.17**b**	1.68 ± 0.81**a**,**b**	1.87 ± 0.47**b**
DM + Bos2	10.08 ± 1.73**b**	2.36 ± 0.70**b**,**c**	1.49 ± 0.43**a**,**b**

Means in the same column by the same letter are not significantly different to Duncan's test ($p < 0.05$). Results are means ± S.D.

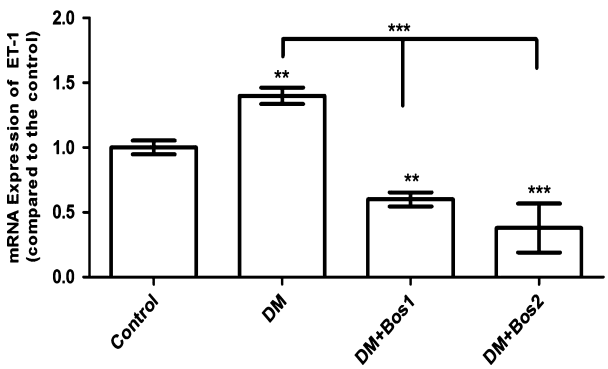

Fig. 1. The effects of Bosentan on changes in tissue ET-1 expression in DM rat brain.

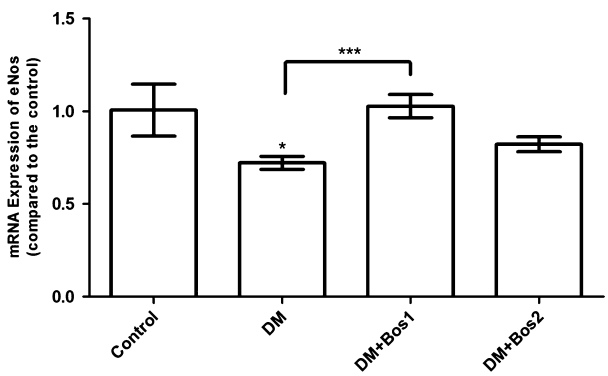

Fig. 2. The effects of Bosentan on changes in tissue eNOS expression in DM rat brain.

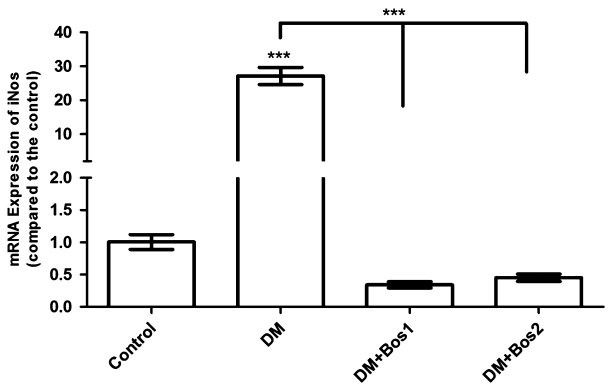

Fig. 3. The effects of Bosentan on changes in tissue iNOS expression in DM rat brain.

the rat brains. The DM+Bos1 group had reduced expression of iNOS by 0.33 times, and the DM+Bos2-showed a reduced mRNA expression of iNOS by 0.44 times, compared to the control group.

Histopathological results.

In the healthy control group, no histopathological changes were seen in the brain regions (fig. 4A–D); they were all in normal appearance. The observed significant changes in the histology of the various brain regions from the DM group, especially in the hippocampus and cerebellum, were increased in the number of neuronal cytoretraction (cell shrinkage) and pericellular oedema in neuropiles. The hippocampus showed the most noticeable changes among the various brain regions in the DM group. Additionally, the Purkinje cells in the cerebellum were the most affected cell population in all groups. The brain sections from the diabetic rats showed oedema (fig. 4O) and degenerative changes in the neurons of the hippocampus (fig. 4N, P) and the Purkinje cells in the cerebellum (fig. 4R). Although a number of neuronal cytoretraction (arrow) are seen in the hippocampus, formed of a majority of normal neurons are seen in the DM+Bos2 (fig. 4I, K). Also, pericellular oedema in neuropiles is seen slightly in the hippocampus sections of these groups (DM+Bos2) (fig. 4). For cerebellum, neuronal cytoretraction was decreased and the normal neurons were not shrunk (DM+Bos2 group). In the low-dose group of bosentan (50 mg/kg), neuronal cytoretraction and normal neurons (arrow heads), from mild to severe pericellular oedema in neuropil and neuronal cytoretraction, were present in the hippocampus regions (fig. 4E–G). For cerebellum of low-dose bosentan (50 mg/kg), neuronal cytoretraction was also present (fig. 4G) which was lower than that of the DM group (fig. 4R).

Discussion

In this study, we examined the roles of endothelin-1 in the brain damage formed in an STZ-induced diabetes model and the effect of bosentan, which is the non-specific endothelin-1 receptor blocker in the prevention of the induced damage. To summarize the results obtained from our study, type-1 diabetes led to many changes in the brain, both in histopathological and in molecular terms. These changes were greater in the hippocampus and cerebellum, and oedema and neurodegenerative changes occurred in the diabetic hippocampal neurons. However, these changes are rarely seen in diabetic rats treated with bosentan. Additionally, when looking at the biochemical and molecular aspects of this recovery, increased MDA and decreased SOD and GSH values in diabetes were recovered in the bosentan therapy groups. Likewise, while the iNOS expression significantly increased, dependent on diabetes, the increase tended to recover in the therapy groups. In parallel with these histopathological and biochemical values, we showed that the amount of endothelin increased in the brain, dependent on diabetes. Bosentan application, however, decreased this increasing amount of endothelin to normal levels.

Diabetes-induced brain damage is quite important, in that diabetes mellitus leads to slow and progressive brain damage [15,16]. Studies have shown that it leads to neuropathic cognitive disorders [17]. In addition, rapidly progressive learning, memory and cognitive disorders were observed in type 2 diabetes patients with insulin resistance [18,30]. Similarly, pathological changes were seen in the hippocampal area. These changes were lower in the therapy groups treated only with bosentan. An impaired cerebral blood flow can be responsible for diabetes-induced brain damage. Cerebral blood flow is extremely important, and there are myogenic, neuronal and ligand-mediated mechanisms in its regulation [31]. Additionally, endothelin-1 was found to be responsible for the cerebrovascular diseases [32,33]. In previous studies, the application of endothelin-1 receptor antagonists created a cerebroprotective effect in diabetes-induced cerebrovascular dysfunction [34]. In our study, the reason why the endothelin-1 receptor antagonist was useful, particularly for the hippocampus and cerebellum, in diabetes-induced brain damage may have originated from the vasculoprotective effect. In diabetes-induced vasculopathies, not only vascular complications but also the changes that occur secondarily in the brain tissue should be considered.

The most important reason why endothelin-1 creates vasculopathy is its association with NO [35]. NO and ET-1 are major endothelium-derived factors that act in opposition to each other, and in control of the vascular tonus [36]. In previous studies, the increase in NO release not only decreased the amount of ET1 but also decreased ET-1-induced vasoconstriction [37]. NO is basically produced by 3 main enzymes: iNOS, eNOS and nNOS [38]. eNOS has primarily been held responsible for cerebellar artery basal tonus [39], and another study showed that there was ET-1 release in the normal cerebral endothelium [40]. In our study, while the brain eNOS expression levels in the diabetic groups decreased, the ET-1 expression levels were found to be increased. Based on these findings, while diabetes-induced endothelial structure changes decreased the normal eNOS release, it increased ET-1 expression. Consequently, we suppose that it leads to brain damage dependent on ET-1-induced vasoconstriction, and studies have

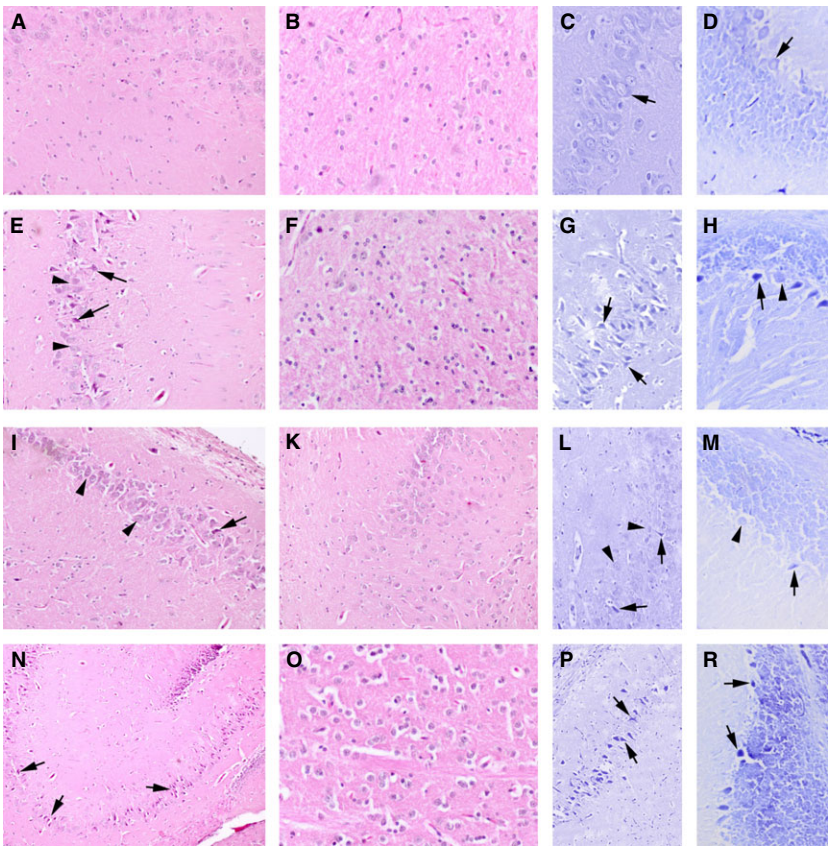

Fig. 4. The histopathological changes of the brain regions. (A) and (B) Photomicrographs of hippocampus in the healthy control group. The normal appearance of neurons and neuropiles (H&E). The magnification is 100X. (C) Photomicrographs of hippocampus in the healthy control group. Cresyl violet staining. Arrows indicate the normal staining of neuron. The magnification is 200X. (D) Photomicrographs of cerebellum in the healthy control group. Cresyl violet staining. Arrows indicate the normal staining of neuron. The magnification is 200X. (E) Photomicrographs of hippocampus in the DM+Bos1 group. Neuronal cytoretraction (arrows) and normal neurons (arrow heads) are seen (H&E). The magnification is 100X. (F) Photomicrographs of hippocampus in the DM+Bos1 group. Mild to severe pericellular oedema *in neuropil is seen* (H&E). The magnification is 100X. (G) Photomicrographs of hippocampus in the DM+Bos1 group. Cresyl violet staining. Arrows indicate mild to severe neuronal cytoretraction. The magnification is 200X. (H) Photomicrographs of cerebellum in the DM+Bos1 group. Cresyl violet staining. Arrows indicate the neuronal cytoretraction. Arrow head shows the normal neuron that was not shrunk. The magnification is 200X. (I) Photomicrographs of hippocampus in the DM+Bos2 group. A very small number of neuronal cytoretraction (arrow) and formed of a majority of normal neurons (arrow heads) are seen (H&E). The magnification is 200X. (K) Photomicrographs of hippocampus in the DM+Bos2 group. Pericellular oedema*s in neuropil are seen slightly* (H&E). The magnification is 100X. (L) Photomicrographs of hippocampus in the DM+Bos2 group. Cresyl violet staining. Arrows indicate slightly neuronal cytoretraction. Arrow head shows the normal neurons. The magnification is 200X. (M) Photomicrographs of cerebellum in the DM+Bos2 group. Cresyl violet staining. Arrows indicate the decreased neuronal cytoretraction. Arrow head shows the normal neuron that was not shrunk. The magnification is 200X. (N) Photomicrographs of hippocampus in DM group. Increased number of neuronal cytoretraction (arrows) (H&E). The magnification is 100X. (O) Photomicrographs of hippocampus in the DM group. Pericellular oedemas are severe in neuropiles (H&E). The magnification is 200X. (P) Photomicrographs of hippocampus in the DM group. Cresyl violet staining. Arrows indicate severe neuronal cytoretraction. The magnification is 100X. (R) Photomicrographs of cerebellum in the DM group. Cresyl violet staining. Arrows indicate increased neuronal cytoretraction. The magnification is 200X.

already shown that ET-1 was responsible for both type 1 and type 2 diabetes-induced vascular complications [41,42]. When we look through the studies on the association between diabetes and eNOS, it was, however, observed that eNOS activity increased in some studies but decreased in others [43,44]. Additionally, when we look through the studies demonstrating decreased eNOS activity, it was shown that high insulin and increasing oxidative stress decreased eNOS [43,45], and these results support our study. These data suggest a decreased eNOS expression and increased ET-1 expression during diabetes-induced brain damage. Bosentan treatment increased eNOS expression as a protective mechanism and so decreased ET-1 expression.

Another result in our study is with regard to the iNOS expression levels. iNOS is normally either not released or released little, in basal terms [46]. iNOS production increases within hours in many instances, such as inflammation, and it significantly increases the amount of NO [47,48]. Likewise, it was also shown that high glucose levels significantly increased iNOS activity [49,50]. Additionally, diabetes induced increasing iNOS activity which damaged cellular proteins, inhibited the lipid and protein functions in the neurons and also dis-

rupted haemostasis [51]. In another study, the amount of iNOS in the hippocampus was found to be high in rats induced with diabetic encephalopathy [52]. In our study, the expression of brain iNOS significantly increased due to diabetes, and this increase was recovered by bosentan therapy. In another study, which was carried out in relation to our subject, it was shown that ET-1 and iNOS expression was induced in brain astrocyte cells [53]. One previous study also showed decreasing effects of bosentan on iNOS expression [28]. All these studies and our findings suggest an increased iNOS expression during diabetes-induced brain damage, and bosentan treatment decreased iNOS expression as a protective mechanism against this damage.

Likewise, it was shown that oxidative stress increased in many organs, dependent on diabetes [54,55]. A previous study showed the superoxide scavenger effect of the superoxide dismutase enzyme in cerebral arterioles dependent on type 1 diabetes [56]. In another study, it was shown that free O_2 radicals increased in diabetic rat brains, and these increased O_2-free radicals were decreased using an ET-1 antagonist [57]. Long-term oxidative damage leads to morphological damage and memory disorders in many parts of the brain [58,59], and one previous study showed that diabetes-induced hippocampal neuron damage was prevented by antioxidant therapy [60]. As an indicator of lipid peroxidation and oxidative stress, MDA levels were found to be quite high in the diabetic rats. In our study, the MDA levels were also high in diabetic brains, and bosentan application led to a significant decrease in the MDA levels. In one clinical study, serum MDA levels and endothelin-1 levels were found to be high in patients with type 2 diabetes [61]. Furthermore, it was determined that in acetaminophen-induced hepatotoxicity and over torsion-induced ischaemia–reperfusion injury, increased MDA levels were decreased by bosentan application, and there was histopathological improvement in the related tissues [62,63]. These studies support increased MDA levels during diabetes and protective effects of bosentan. The increase in lipid peroxidation, on the other hand, leads to a decrease in a very important endogenous antioxidant substance, like glutathione [64,65]. Glutathione was found to be low in the diabetic rat groups in our study. Additionally, there are studies showing that the decrease in the glutathione level can lead to geriatric cognitive dysfunctions [66,67]. In another study related to our subject, SOD and GSH amounts were found to be low in the brain, and these amounts increased upon therapy and, consequently, diabetes-induced cognitive disorders recovered [68]. The SOD activity of our study decreased in the diabetic rats. Other studies showed that the SOD activity increased dependent on diabetes [69] and bosentan administration improved antioxidant status [62,63]. In summary, the bosentan application improved all antioxidant parameters in our study.

Conclusion

Finally, we determined that endothelin-1 was increased in the diabetic rat brain. In addition to showing the roles of ET-1 in diabetes-induced cerebral artery changes, we also showed that it can play a role in diabetes-induced brain damage. By antagonizing ET-1 receptors with bosentan, we showed that there may be an association between diabetes-induced, increased iNOS, decreased eNOS and ET-1 expression. ET-1 antagonism during diabetes-induced brain damage tended to recover histopathologically and biochemically. Based on all of these results, we hope that ET-1 will not be ignored in diabetes-induced cerebral complications.

Conflict of interest

The authors declare no conflict of interest.

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