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Protective Effect of Caffeic Acid Phenethyl Ester on Oxidative Stress in Diabetic Rat Sciatic Nerve

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Abstract: There has been no report which investigates the effects of Caffeic Acid Phenethyl Ester (CAPE) on elevated levels of oxidative stress in sciatic nerve tissues of diabetic rats. Therefore, this study was undertaken to determine whether CAPE, by virtue of its antioxidant properties, could affect lipid peroxidation, nitric oxide (NO), Paraoxonase (PON-1) and the oxidant/antioxidant balance in the sciatic nerve of Streptozotocin (STZ)-induced diabetic rats. The rats were treated as follows: control; this group of rats (n = 9) received isotonic solution. Diabetic (STZ, untreated diabetic): STZ 50 mg kg^{-1} b.wt. was given intraperitoneally for the induction to this group (n = 8). Diabetic+CAPE treatment (STZ+CAPE, CAPE-treated diabetic): diabetic rats (n = 8) received CAPE (10 μmol/kg/day) for a period of 21 days beginning one week after the STZ administration. Biomarkers; Malondialdehyde (MDA), Total Oxidant Status (TOS), total antioxidant status (TAS), PON-1 and NO levels for oxidative stress in sciatic nerve of the rats were measured. We found a significant increase in MDA, NO and TOS levels along with a reduction in TAS levels and PON-1 activity in the sciatic nerves of the STZ-induced diabetic rats (at $p<0.001$). The MDA, TOS and NO levels in sciatic nerve were significantly reduced in the CAPE-treated diabetic group compared to the untreated diabetic group (at $p<0.05$). In conclusion, the results of this study demonstrated that CAPE exhibits protective effects against oxidative damage in the sciatic nerve tissues of diabetic rats.

Key words: Diabetic rat, sciatic nerve, oxidative stress, curcumin

INTRODUCTION

Diabetic Neuropathy (DN) is counted among the most prevalent and life-threatening complications of diabetes and is related with clinically significant morbidities (Abbott *et al*., 2011). Despite extensive study in the field of diabetic neuropathy, its pathophysiology and treatment is still not completely clear. Thereby, the research for newer pharmacological drugs for the treatment of DN continues and drugs must be found which are capable of impeding different pathogenic factors simultaneously (Negi *et al*., 2010, 2011).

Hyperglycemia with multiple pathways induces oxidative stress in DN. Prolonged hyperglycemia is likely to damage dorsal root ganglion mitochondrial DNA and cause long-term nerve dysfunction by overproduction of Reactive Oxygen Species (ROS). ROS is well-known to have a contribution to Schwann cell and neuronal tissue damage in DN. Recently, many experiments have strengthened this hypothesis, including measurement of oxidative stress parameters in sciatic nerve and dorsal root ganglion (Russell *et al*., 2008; Negi *et al*., 2010; Lupachyk *et al*., 2011; Uzar *et al*., 2012a). The increase of ROS is well documented in streptozotocin-diabetic rats (Uzar *et al*., 2012a). DN is related with metabolic changes of abnormal glucose regulation, such as accumulation of sorbitol, incremental levels of glycosylated proteins and impairment of oxidative/antioxidative balance within the peripheral nerve tissue (Figueroa-Romero *et al*., 2008; Lupachyk *et al*., 2011; Uzar *et al*., 2012a). Consumption of natural antioxidant components in the Schwann cells and vascular endothelium of the sciatic nerve may cause neurovascular and metabolic changes in DN (Lupachyk *et al*., 2011). In recent studies, the role of oxidative stress which plays a key role in the

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pathophysiology of DN has been emphasized (Lupachyk *et al*., 2011; Uzar *et al*., 2012b). A better understanding of the involvement of peripheral system could lead to new treatments for preventing the nerve damage caused by diabetes mellitus (Uzar *et al*., 2012a). Protective effects of various antioxidants in experimental DN have been demonstrated by previous investigators (Negi *et al*., 2010; Uzar *et al*., 2012a). It has been reported that antioxidant Caffeic Acid Phenethyl Ester (CAPE) has a potential therapeutic role in preventing diabetic complications related with brain, heart, kidney and liver (Park and Min, 2006; Celik and Erdogan, 2008).

However, to the best of our knowledge, there is no experimental research concerning the protective effects of CAPE against sciatic nerve damage in the diabetic rats. CAPE, a flavonoid-like compound, is one of the major components of honeybee propolis. CAPE has potent biological properties such as antioxidant, anti-inflammatory and anti-apoptotic effects (Ilhan *et al*., 2004; Celik and Erdogan, 2008). Also, previous studies have demonstrated the neuroprotective and antidiabetic effects of CAPE (Ilhan *et al*., 2004; Celik *et al*., 2009).

This finding indirectly points that CAPE may prevent the production of ROS in the peripheral nervous system of STZ-induced diabetic rats. There has been no report which investigates the effects of CAPE on elevated levels of oxidative stress in sciatic nerve tissues of diabetic rats. Therefore, this study was undertaken to determine whether CAPE, by virtue of its anti-oxidant properties, could affect lipid peroxidation, nitric oxide (NO), Paraoxonase (PON-1) and the oxidant/antioxidant balance in the sciatic nerve of Streptozotocin (STZ)-induced diabetic rats. Also, new therapeutic possibilities for peripheral neurological complications related with diabetes will be elucidated.

MATERIALS AND METHODS

This study was approved by the Dicle University Animal Ethics Committee. Twenty-five female Wistar rats (aged 8-12 weeks) each weighing 200-250 g were obtained from the animal laboratory of Dicle University. The rats were housed in groups of four per polypropylene cage. The rats were kept in a temperature-controlled room (21±2°C) for periods of 12 h light and 12 h dark at a certain humidity (60±5%) and they had free access to standard food and water *ad libitum*.

Induction of diabetes: The rats were fasted overnight and diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of streptozotocin (STZ) (50 mg kg^{-1} b.wt.) in 0.1 M cold citrate buffer (pH 4.5). The rats were considered diabetic if their blood glucose levels were above 250 mg dL^{-1} on the seventh day after STZ injection (Uzar *et al*., 2012a) and were used in groups II and III.

Treatment of rats: The rats were divided into three groups and treated as follows. All treatments were done intraperitoneally (Yilmaz *et al*., 2004; Lupachyk *et al*., 2011):

Group I: Non diabetic, control, n = 9, this group was treated with the isotonic solution

Group II: Diabetic control, n = 8, this group was also treated with the isotonic solution

Group III: Diabetic, n = 8, this group was treated with CAPE (10 μmol/kg/day) for 21 days beginning from one week after administration of STZ

Rats were anaesthetize with ketamine hydrochloride 50 mg kg^{-1} i.m. (Ketalar, Eczacibasi, Istanbul, Turkey) 24 h after the last injection. After completion of the 21 day treatment, the rats were sacrificed by cervical dislocation and sciatic tissues were excised. The tissues were washed with ice-cold saline and immediately stored at -50°C for further biochemical analysis.

Biochemical analysis: The excised sciatic nerve samples were weighed and immediately stored at -50°C for biochemical analysis. These tissues were homogenized in five volumes (w/v) with ice-cold saline solution. Assays were performed on the supernatant of the homogenate which was prepared at 14,000 rpm for 30 min at +4°C. The protein concentration of the tissue was measured by the Lowry's method (Lowry *et al*., 1951). Lipid peroxidation level in the sciatic nerve tissue was expressed as MDA. MDA was measured according to the procedure proposed by Ohkawa *et al*. (1979). PON-1 activity was measured spectrophotometrically by a modified Eckerson method (Eckerson *et al*., 1983). NO were determined by Griess' method (Cortas and Wakid, 1990). The TAS of supernatant fractions was evaluated by using a novel automated and colorimetric measurement method developed by Erel (2004). Hydroxyl radicals, the most potent biological radicals, are produced by this method. In the assay, the ferrous ion solution contained in reagent 1 is mixed with hydrogen peroxide which is contained in reagent 2. The subsequently produced radicals, such as brown-colored dianisidine radical cations produced by the hydroxyl radicals, are also potent radicals. Using this method, the antioxidative effect of the sample is measured against the potent-free radical reactions initiated by the produced hydroxyl radicals. The assay has excellent precision values lower than 3%. The TAS results are

expressed as nmol Trolox equivalent/mg protein. The TOS of supernatant fractions was evaluated by using a novel automated and colorimetric measurement method developed by Erel (2005). Oxidants contained in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is increased by glycerol molecules which are abundantly present in the reaction medium. The ferric ion creates a colored complex with xylenol orange in an acidic medium. The color intensity which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide. The units of sciatic nerve tissue TOS and TAS were mmol H_2O_2 meq g^{-1} protein (Uzar *et al*., 2012a).

Statistical analysis: The data was analyzed with Statistical Package for the Social Sciences for Windows version 11.5 (SPSS, Chicago, IL, USA). The variables between the groups were tested by Mann-Whitney U test. A value of $p<0.05$ indicates a significant difference. Data are expressed as Mean±SD.

RESULTS AND DISCUSSION

The MDA, TOS, TAS, PON-1 and NO levels in the sciatic nerve tissues are provided in Table 1. There was a significant depletion in the TAS levels and PON-1 activity in the sciatic nerve tissues of the diabetic group compared to the control groups (for both parameters at $p<0.001$). However, the CAPE-treated diabetic rats showed significantly increased TAS levels and PON-1 activity in the sciatic nerve tissues compared to the untreated diabetic rats ($p = 0.038$, $p = 0.026$, respectively). As can be seen in Table 1, the levels of MDA, TOS and NO in the sciatic nerve tissues increased in the untreated diabetic rats when compared to control rats (for both parameters at $p<0.001$). However, MDA, TOS and NO levels were significantly reduced in the CAPE treated diabetic group as compared to untreated diabetic group ($p<0.05$).

The oxidant/antioxidant balance in diabetic rat sciatic nerve tissues was investigated. The results have shown that the levels of MDA, TOS and NO in the sciatic tissues of the diabetic rats significantly increased when compared to control rats, but the level of TAS and PON-1 activity in this tissue significantly decreased as compared to control rats. However, the levels of MDA, TOS and NO in this tissue were reduced by CAPE treatment when compared to diabetic rats which received no CAPE treatment, but the TAS levels and PON-1 activity have increased. Our study demonstrated that CAPE has a property for protecting the oxidative damage in sciatic tissues of STZ induced diabetic rat.

Diabetes Mellitus (DM) can affect the peripheral nervous systems. It is acknowledged that oxidative stress is a contributor to the occurrence of neurological complications in DM (Hoeldtke *et al*., 2011; Uzar *et al*., 2012b). Previous experimental and human trials have suggested that increased oxidative stress is an important factor in the pathogenesis of DN (Negi *et al*., 2010; Uzar *et al*., 2012b). For instance, increased lipid peroxidation impairs membrane function by decreasing membrane fluidity. Previous studies have demonstrated the increased lipid peroxidation in clinical and experimental diabetes (Hoeldtke *et al*., 2011; Uzar *et al*., 2012b). In this study, lipid peroxidation was determined by measuring MDA, an end product of lipid peroxidation in membrane components of cells. The level of MDA significantly increased in the untreated diabetic rat, indicating an increased oxidative stress due to overproduction of ROS in sciatic nerve tissues. The increase in lipid peroxidation might be a reflection of a decrease in enzymatic and non-enzymatic antioxidants of the defense systems in the diabetic rats. Similar to previous studies, we found increased MDA levels in sciatic nerve of diabetic rats (Kamboj *et al*., 2010; Uzar *et al*., 2012a).

PON-1 is an anti-oxidant enzyme. PON-1 activity is related with high-density lipoprotein and has been demonstrated to reduce the susceptibility of low-density lipoprotein to lipid peroxidation. The occurrence of diabetic complications is usually related with low PON-1 activity, resulting from the excessive use of PON-1 enzyme. Also, PON-1 expression may be down-regulated by complications related with oxidative stress (Inoue *et al*., 2000; Stefanovic *et al*., 2010). Similar to a previous study, we found that PON-1 activity decreased in sciatic nerve of diabetic rats (Uzar *et al*., 2012a). However, we found that CAPE reduced lipid peroxidation and increased PON-1 activity in sciatic nerve of diabetic rats. In addition, the reduced MDA levels and increased

Table 1: Biochemical parameters in the sciatic nerve tissue of control, diabetic and diabetic+CAPE groups of rats

Groups	Malondialdehyde (nmol g^{-1} protein)	Total oxidant status (mmol H_2O_2 meq g^{-1} protein)	Total antioxidant status (mmol Trolox meq g^{-1} protein)	Paraoxonase (IU mg^{-1} protein)	Nitric oxide (μmol g^{-1} protein)
Control	18.0±2.9	11.6±3.2	0.17±0.02	0.112±0.018	0.21±0.07
Diabetic control	29.9±3.9	20.8±1.4	0.07±0.03	0.042±0.007	0.38±0.10
Diabetic+CAPE	26.4±2.5	17.4±1.2	0.10±0.02	0.060±0.015	0.27±0.08

CAPE: Caffeic acid phenethyl ester

PON-1 activity with CAPE treatment of diabetic rats indicate that the CAPE might be an agent that can protect the sciatic nerve against diabetic oxidative stress by scavenging free radicals. These findings demonstrated the anti-peroxidative effects of CAPE. These protective effects of CAPE against oxidative stress are in agreement with previous studies. This finding was supported by previous studies, where CAPE treatment significantly reduced diabetes increased MDA levels in brain, heart and liver tissues of rats (Yilmaz *et al*., 2004; Okutan *et al*., 2005; Celik and Erdogan, 2008).

Determination of TOS may show the levels of all free oxidant radicals caused by STZ induced diabetes. Also, the number of all antioxidants in tissue samples makes it difficult to measure each antioxidant separately (Erel, 2004; Gurbuz *et al*., 2011). Therefore, TAS may be an important factor in providing protection from neurological damage caused by STZ induced diabetes (Uzar *et al*., 2012a). We demonstrated the increased TOS levels and decreased TAS levels in sciatic nerve of the diabetic rats as compared to the control rats. The increased TOS levels may be resulting from the overproduction and/or decreased excretion of oxidants (Erel, 2005; Asilturk *et al*., 2011). Because of an increase in total oxidants and a decrease in total antioxidants, the oxidant/antioxidant imbalance occurred in sciatic nerve tissues of the diabetic rats in this study. Treatment of diabetic rats with CAPE caused a decrease in TOS and increase in TAS levels when compared to untreated diabetic rats. These finding indicated that oxidant/antioxidant imbalance improved in favor of antioxidant status with the CAPE treatment of the diabetic rats. Our study is the first experimental research to demonstrate the protective effectiveness of CAPE against diabetes-induced oxidative stress in sciatic nerve of rats.

Increased NO has been documented in the circulation of diabetic patients in several clinical studies, but some have suggested that this is a non-specific response to inflammation. NO is a free radical. Increased nitrite levels can be related to the diabetic neurologic complications. The excessive production of NO can cause oxidative stress on sciatic nerve by forming peroxynitrite with superoxide amion (Kuhad and Chopra, 2009; Ozkul *et al*., 2010; Hoeldtke *et al*., 2011; Uzar *et al*., 2012a).

In this study, NO levels were significantly increased in sciatic nerve of diabetic rats compared to the control group (Hoeldtke *et al*., 2011). This finding may be supported by increased inducible nitric oxide synthase in the rat sciatic nerve. Also, it was found in this study that there was a significant decrease in NO levels when the diabetic rats were treated with CAPE. This finding was supported by previous study, where CAPE treatment significantly reduced diabetes increased NO levels in brain of rats (Celik and Erdogan, 2008).

CONCLUSION

It may be concluded that STZ-induced diabetes increases the oxidative stress in sciatic tissue of rats. CAPE decreases lipid peroxidation, total oxidants and NO and increases total antioxidants and PON-1 activity in sciatic nerve of STZ induced diabetic rats. The regulating role of CAPE might be associated with its antioxidant and anti-inflammatory effects. Available results indicated that CAPE should be considered to prevent oxidative stress in the diabetic sciatic nerve. In addition, there is a need for further studies in order to confirm the protective effect of CAPE on oxidative stress in sciatic nerve resulting from STZ-induced diabetes.

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