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Hippocampal synaptic plasticity restoration and anti-apoptotic effect underlie berberine improvement of learning and memory in streptozotocin-diabetic rats

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ABSTRACT

Chronic diabetes mellitus initiates apoptosis and negatively affects synaptic plasticity in the hippocampus with ensuing impairments of learning and memory. Berberine, an isoquinoline alkaloid, exhibits anti-diabetic, antioxidant and nootropic effects. This study was conducted to evaluate the effect of berberine on hippocampal CA1 neuronal apoptosis, synaptic plasticity and learning and memory of streptozotocin (STZ)-diabetic rats. Long-term potentiation (LTP) in perforant path-dentate gyrus synapses was recorded for assessment of synaptic plasticity and field excitatory post-synaptic potential (fEPSP) slope and population spike (PS) amplitude. PS amplitude and fEPSP significantly decreased in diabetic group versus control, and chronic berberine treatment (100 mg/kg/day, p.o.) restored PS amplitude and fEPSP and ameliorated learning and memory impairment and attenuated apoptosis of pyramidal neurons in the CA1 area, as determined by the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling method. In summary, chronic berberine treatment of STZ-diabetic rats significantly ameliorates learning and memory impairment and part of its beneficial effect could be attributed to improvement of synaptic dysfunction and anti-apoptotic property.

1. Introduction

Diabetes mellitus is a complicated prevalent metabolic disorder (Khan et al., 2012) with functional changes of the nervous system (Northam et al., 2009). Diabetes gradually impairs hippocampus-dependent cognitive functions and synaptic plasticity (Artola, 2008; Reisi et al., 2008; Reisi et al., 2010). Passive avoidance learning and memory deficits develop in streptozotocin (STZ)-diabetic rats (Kucukatay et al., 2007). Impairment of spatial learning in a hippocampus-dependent complex maze has also been reported in such animals (Stranahan et al., 2008). Changes in hippocampal synaptic plasticity have already been described in diabetes (Iwai et al., 2009). Part of deficits in performing the spatial learning and memory tasks in diabetes is attributed to induction of apoptosis of CA1 pyramidal neurons (Ye et al., 2011).

Although conventional agents in conjunction with lifestyle management are being used to control diabetes and its complications (Khan et al., 2012) but there has been no effective long-term treatment for diabetes-induced neuropathy (Choi and Son, 2011). Until now, no specific treatments have been available for the management and/or prevention of cognitive dysfunction in diabetes (Biessels et al., 2007). Berberine is an isoquinoline alkaloid, which is mainly found in such plants as berberis, with anxiolytic, analgesic, anti-inflammatory, antipsychotic, antidepressant, and antiamnesic effects (Imanshahidi and Hosseinzadeh, 2008; Kulkarni and Dhir, 2008a; Kulkarni and Dhir, 2010). A number of clinical and preclinical investigations have shown beneficial effects of berberine in diabetes (Bhutada et al., 2011; Cok et al., 2011; Gu et al., 2010; Zhang et al., 2008). Berberine could also enhance insulin resistance and B cell regeneration (Kong et al., 2009; Zhou et al., 2009), act as a potential antioxidant (Zhou and Zhou, 2011), and being a potent acetylcholinesterase inhibitor (Huang et al., 2010). The protective effect of berberine against hydrogen peroxide-induced apoptosis could be related to its antioxidant activity and imply its therapeutic relevance in oxidative stress-related disorders (Zhu et al., in press). Moreover, antiamnesic effect of berberine through augmenting cholinergic neuronal system activity has already been reported (Peng et al., 1997). Berberine has also beneficial effects on neural health and function and can protect neurons from various brain insults (Zhu and Qian, 2006) and may

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ameliorate diabetes-induced cognitive dysfunction (Bhutada et al., 2011). Neuroprotective effect of berberine in a transgenic mouse model of Alzheimer's disease has recently been justified (Durairajan et al., 2012). Since long-term potentiation (LTP) as a long lasting increase in synaptic strength (Cooke and Bliss, 2006) has long been linked with learning and memory through a contribution to synaptic plasticity (Muller et al., 2002), therefore, we hypothesized whether chronic oral administration of berberine could improve learning and memory deficit and LTP induction and maintenance in dentate gyrus (DG) and attenuate neuronal apoptosis in CA1 area of STZ-diabetic rats.

2. Materials and methods

2.1. Animals

Male albino Wistar rats (Pasteur's Institute, Tehran, Iran), weighing 225–285 g, were housed in an air conditioned colony room on a light/dark cycle (21–23 °C and a humidity of 30–40 %) and supplied with standard pelleted diet and tap water ad libitum. Procedures involving animals and their care were conducted in conformity with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Experimental procedure

The rats ($n=50$) were randomly allocated and similarly grouped into five groups, i.e., control, berberine-treated control (100 mg/kg; BBR100), diabetic, and berberine-treated diabetics (50 and/or 100 mg/kg; BBR50 and BBR100, respectively). Berberine-Cl and STZ (Sigma-Aldrich Co., USA) were dissolved in Cremophore (Sigma-Aldrich Co., USA) and citrate buffer (0.1 M, pH 4.4), respectively. All drug solutions were freshly prepared. STZ was administered at a dose of 55 mg/kg (i.p.). All STZ-treated rats received 5% glucose solution instead of water for 24 h after injection in order to reduce death due to hypoglycemic shock. Control animals received an injection of an equivalent volume of the vehicle. One week after STZ injection, overnight fasting blood samples were collected from the tail vein and glucose concentration was measured using glucose oxidation method (Zistshimi, Tehran). Only those animals with glucose levels higher than 250 mg/dl were selected as diabetic. Berberine was administered p.o. at doses of 50 and/or 100 mg/kg/day started 1 week after STZ injection for a period of 11 weeks. Body weight of the animals was also measured on a weekly basis. Behavioral tests including passive avoidance and Y-maze were performed at the end of the study.

2.3. Y-maze task

Short-term spatial recognition memory performance was assessed by recording spontaneous alternation behavior in a single-session Y-maze as described before (Bagheri et al., 2011; Baluchnejadmojarad and Roghani, 2011). Spontaneous alternation assessed in Y-maze evaluates hippocampal-dependent memory, constituting a basic mneumonic task, which does not involve learning components and does not isolate memory performance (Canas et al., 2009; Duarte et al., 2012). The maze was made of black-painted Plexiglas. Each arm was 40 cm long, 30 cm high and 15 cm wide. The arm converged in an equilateral triangular central area that was 15 cm at its longest axis. The procedure was basically the same as that described previously as follows: each rat, naive to the maze, placed at the end of one arm and allowed to move freely through the maze during an 8-min session. The series of arm entries was recorded visually. Arm entry was considered to be completed when the base of the animal's tail had been completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of maximum spontaneous alternation was then the total number of arms entered-2 and the percentage is calculated as the ratio of actual to possible alternations (defined as the total number of arm entries-2).

2.4. Single-trial passive avoidance test

This test was conducted 2–3 days after Y-maze task and was conducted as described before (Baluchnejadmojarad and Roghani, 2006, 2011). The apparatus consisted of an illuminated chamber connected to dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator. On the first and second days of testing, each rat was placed on the apparatus and left for 5 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period (5 min), the guillotine door was opened and after the rat entering the dark chamber, the door was closed and an inescapable scrambled electric shock (1 mA, 1 s once) was delivered. In this trial, the initial latency of entrance into the dark chamber was recorded and rats with initial latencies greater than 60 s were excluded from the study. Twenty-four hours later, each rat was placed in the illuminated chamber for retention trial. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (up to a maximum of 600 s as cut-off).

2.5. Electrophysiological experiments

Dentate gyrus LTP was recorded under anesthesia. Twelve weeks after diabetes induction, the rats were anesthetized with urethane (1.5 g/kg) and placed in a stereotaxic apparatus. The rectal temperature was monitored and maintained at 37 ± 0.5 °C with an automatic heating pad. Bipolar stimulating and recording electrodes were made of stainless steel wire (0.125 mm diameter, Advent, UK). It was positioned stereotaxically so as to selectively stimulate the medial perforant path while recording in the dentate gyrus. The electrode stimulating the medial perforant path was implanted 4.2 mm lateral to the true lambda. A recording electrode was implanted ipsilaterally 3.8 mm posterior and 2.2 mm lateral to the bregma.

The electrical signals from the DG were amplified 1000-fold, digitized at 10 kHz, and band-pass filtered at 0.1 Hz–10 kHz. Recording of field potentials was started at least 15 min after placing the stimulation and recording electrodes. All the stimuli were biphasic square wave pulses (200 μs width) and their intensities for baseline recording were set at the current that evoked 40% of the maximum population spike amplitude (PSA). Test stimuli (0.1 Hz) were delivered at 10 s intervals to monitor field excitatory postsynaptic potentials (fEPSP) and population spike (PS). The strength of a field potential was evaluated from the slope of the EPSP and amplitude of the PS. The maximal EPSP slope was obtained on the first positive deflection of the field potential. The PS amplitude was measured by averaging the distance from the negative peak to the preceding peak and the following positive peak.

After stable baseline recording for at least 30 min, the LTP was induced by delivery of high-frequency stimulation (HFS; 10 trains of 15 pulses at 200 Hz separated by 10 s) and after the tetanic stimuli, the baseline stimulation was resumed and recording continued for at least 60 min.

2.6. Histological studies

For this purpose, the rats were deeply anesthetized with a high dose of ketamine (150 mg/kg) and perfused through the ascending aorta with 50 ml of heparinized normal saline followed by 100–150 ml of fixative solution containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Following perfusion, brains were removed from the skull, hippocampal blocks prepared and immersed in 30% sucrose in phosphate buffer at 4 °C for 2–3 days. Then, sections were cut at a thickness of 40 μm on a freezing microtome (Leica, Germany) and collected in phosphate buffer (0.1 M). Every second section was Nissl-stained with 0.1% cresyl violet (Sigma) and alternate sections were used for the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end-labeling (TUNEL) histochemistry. In Nissl-stained sections, neuronal counting was done in CA1 area of the hippocampus in at least three sections at a level range between −3.6 and −4.3 mm from the bregma (according to the coordinates of the stereotaxic atlas of Paxinos and Watson (1986)) in an area of 0.1 mm^2 using an image capturing and analysis system (Bel Engineering, Italy). The process was repeated at least two times for each section and its average was taken as the final value. Counting was done blind to the treatments received.

2.7. Determination of DNA fragmentation by TUNEL histochemistry

In order to detect apoptotic cell death, a TUNEL assay was performed using the In Situ Cell Death Detection Kit (Roche, Germany) according to a previously described method with some modifications (Kang et al., 2006). Briefly, the sections were mounted onto gelatin-coated slides and air-dried overnight at room temperature. The sections were post-fixed in ethanol–acetic acid (2:1) and rinsed. The sections were then incubated with proteinase K (100 μg/ml), rinsed, incubated in 3% H_2O_2, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using a converter-POD and subsequent incubation with DAB (3-3′-diamonobenzidine tetrachloride) and hydrogen peroxide, counterstained with 0.1% cresyl violet, coverslipped and evaluated. A dark brown color indicating DNA breaks developed. Positive TUNEL-stained neurons were counted in an area of 0.1 mm^2 for two sections from each group and counting was done at least two times for each section and its average was taken as the final value. Counting was done blind to the treatments received.

2.8. Data analysis

For electrophysiologicalal comparisons, data were analyzed using repeated measure two-way ANOVA, with Tukey's post-hoc test to discriminate between groups. Body weight and blood glucose data were analyzed by repeated measure one-way ANOVA followed by Tukey's post-hoc test. The non-parametric Kruskal–Wallis test was used to analyze the behavioral data. Histochemical data was evaluated using one-way ANOVA and the Bonferroni post-hoc test. In all calculations, significant level was set at $P<0.05$.

3. Results

3.1. General considerations

Body weight of the vehicle-treated diabetic rats was significantly lower at weeks 4 ($P<0.05$), 8 ($P<0.01$), and 12 ($P<0.005$) as compared to baseline value (week 0). Moreover, BBR50-treated diabetic group showed a less significant reduction of body weight in weeks 4, 8 ($P<0.05$) and 12 ($P<0.01$) relative to baseline value and body weight of BBR50-treated diabetic group was significantly higher than diabetic group in weeks 8 and 12 ($P<0.05$). In addition, BBR100-treated diabetic group also showed a less significant reduction of body weight only in weeks 8 and 12 relative to baseline value ($P<0.05$) and body weight of this group was significantly higher than vehicle-treated diabetic group in weeks 4, 8 ($P<0.05$) and 12 ($P<0.01$) (Fig. 1).

With respect to serum glucose level, diabetic rats had an elevated serum glucose level relative to baseline data (week 0) of the same group ($P<0.0005$) and treatment of diabetic rats with berberine at both doses (50 and 100 mg/kg) for 11 weeks caused a significant reduction of serum glucose level in weeks 8 ($P<0.05$ for BBR50 and $P<0.01$ for BBR100) and 12 ($P<0.01$) as compared to the diabetic group. Moreover, BBR100-treated control group had a significantly lower serum glucose level in weeks 4, 8, and 12 as compared to week 0 data of the same group ($P<0.05$) (Fig. 2).

3.2. Spatial recognition memory in Y-maze

Fig. 3 shows the results for the performance of rats in Y-maze task, in which short-term spatial recognition memory as an alternation behavior can be examined. In this respect, the alternation score of the vehicle-treated diabetic rats was lower than that of the control group ($P<0.01$). Meanwhile, although

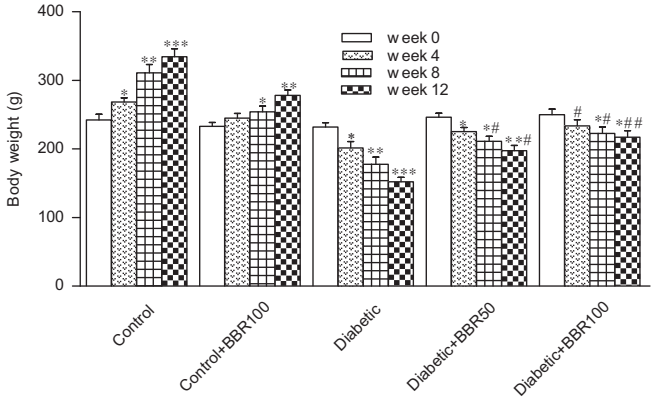

Fig. 1. Body weight in different weeks. $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.005$ (as compared to week 0 in the same group); $^{\#}P<0.05$, $^{\#\#}P<0.01$ (versus diabetic in the same week).

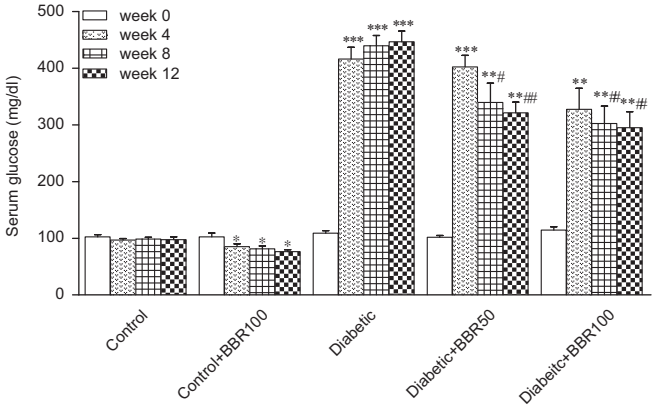

Fig. 2. Blood glucose concentration in different weeks (means+S.E.M.). $^{*}P<0.05$, $^{**}P<0.001$, $^{***}P<0.0005$ (vs. week 0 in the same group); $^{\#}P<0.05$, $^{\#\#}P<0.01$ (versus diabetic in the same week).

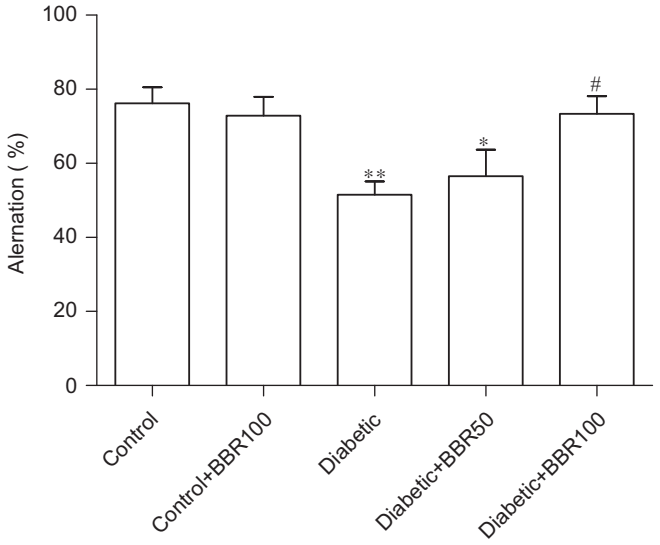

Fig. 3. Alternation behavior of treated-control and diabetic rats in Y-maze task. $^{*}P<0.05$, $^{**}P<0.01$ (as compared to control); $^{\#}P<0.01$(as compared to diabetic).

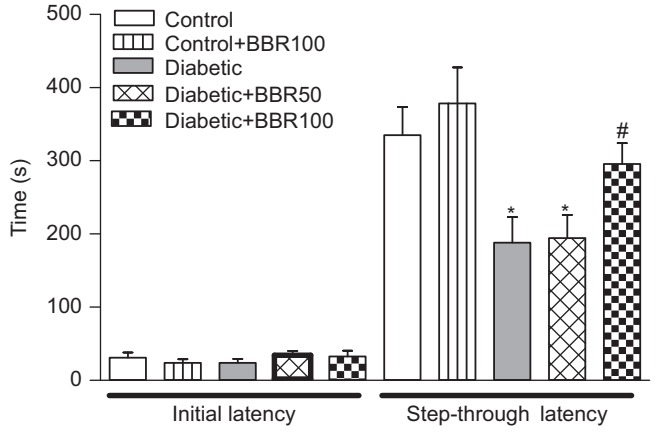

Fig. 4. Initial and step-through latencies of treated control and diabetic groups in single-trial passive avoidance test. $^{*}P<0.01$ (as compared to control); $^{\#}P<0.05$ (as compared to diabetic).

BBR50-treated diabetic rats showed a higher alternation score as compared to diabetic group, but this difference was not statistically significant. In contrast, BBR100-treated diabetic group had a significantly higher alternation score as compared to diabetics ($P<0.05$). In our study, locomotor activity of the animals was also assessed by counting the total number of arms visited by each rat to avoid the compounding effect of locomotor activity on memory processes in experimental groups. In this respect, although total number of arms entered by the vehicle- and berberine (50 and 100 mg/kg)-treated diabetic rats was lower than that of the control group, but the existing difference was not statistically significant (data not shown).

3.3. Passive avoidance test

Fig. 4 shows the performance of treated control and diabetic rats in passive avoidance paradigm as indicated by initial and step-through latencies. Regarding initial latency, there was no significant difference among the groups. With respect to step-through latency, vehicle-treated diabetic rats developed a significant impairment in retention and recall in passive avoidance test ($P<0.01$) relative to control one, as it was evident by a lower step-through latency and berberine treatment at a dose of 100 mg/kg significantly improved it in comparison with diabetic group ($P<0.05$). Furthermore, retention and recall of BBR100-treated control rats was slightly and non-significantly better versus vehicle-treated control group.

3.4. Electrophysiological data

As illustrated in Fig. 5, the effect of berberine treatment on LTP induction and maintenance in dentate gyrus of control and diabetic rats were determined. A repeated measure ANOVA followed by post hoc test revealed that the PS-LTP after tetanization (HFS) was significantly lower in the diabetes group relative to the control group ($P<0.001$).There was also no change in the maintenance of PS-LTP in the groups. Moreover, in BBR100-treated diabetic animals, tetanic stimulation induced a significant increase of PS amplitude ($P<0.001$), but no significant difference was observed in BBR50-treated diabetic group as compared to diabetic one.

The EPSP-LTP after tetanization was significantly lower in the diabetes group with respect to the control group ($P<0.001$). Although high frequency stimulation (200 Hz) of medial perforant path produced a long-lasting synaptic potentiation in both BBR50- and BBR100-treated diabetic animals up to 60 min after HFS, but only BBR100-treated animals showed a significant synaptic potentiation of EPSP ($P<0.001$) as compared to control group. There was also no significant difference between BBR100-treated control and vehicle-treated control groups.

3.5. Nissl staining

In this study, the number of neurons per unit area in the CA1 area was counted and compared among groups (Fig. 6). Our results showed that berberine treatment of control group did not produce any significant change and 12-week diabetes was not followed by any noticeable and significant reduction in this regard. In addition, berberine treatment of diabetic group at both doses (50 and/or 100 mg/kg) did not produce any significant change.

3.6. TUNEL histochemistry

The apoptotic cells in the hippocampal slices were detected by TUNEL staining (Fig. 7). In the control and BBR100-treated control groups, TUNEL-positive pyramidal neurons were not observed in the CA1 area of the hippocampus. However, a significant number of TUNEL-positive neurons appeared in the hippocampal CA1 area of the diabetic group and berberine treatment of diabetic rats only at a dose of 100 mg/kg led to a significantly lower number of TUNEL-positive neurons as compared to vehicle-treated diabetics ($P<0.05$).

4. Discussion

This study showed that STZ-induced diabetes significantly impairs learning and memory and synaptic plasticity in DG and initiates apoptosis of CA1 pyramidal neurons, and berberine treatment at a dose of 100 mg/kg significantly ameliorates these impairments.

Although the multifactorial pathogenesis of cognitive and memory impairments in type 1 diabetes has not yet completely been understood, several factors such as disturbance in glucose metabolism and its ensuing hyperglycemia (Iwai et al., 2009), increased oxidative stress burden (Frier, 2011; Kucukatay et al., 2007), an imbalance in nitric oxide production (Comin et al., 2010), impaired nerve growth factor signaling (Chae et al., 2009)

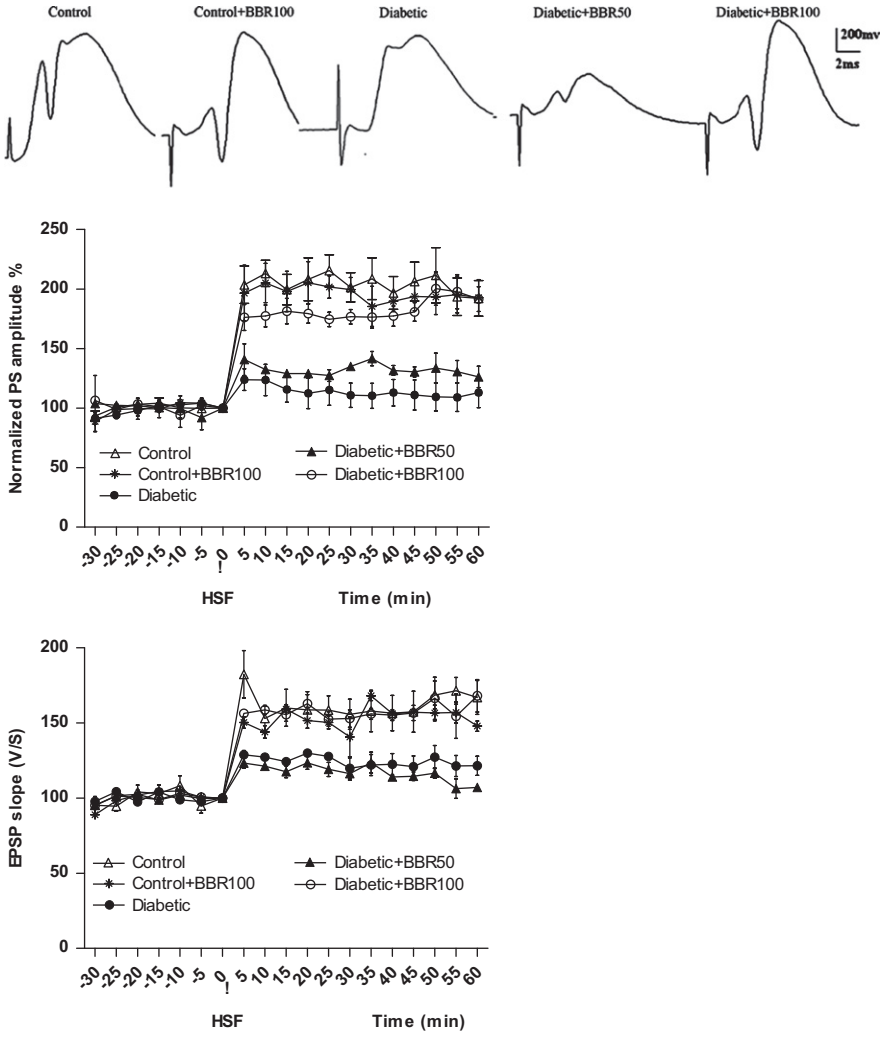

Fig. 5. The normalized PS amplitude and EPSP slope in different groups before and after HFS. In this respect, PS amplitude and EPSP slope was lower in untreated diabetic and BBR50-treated diabetic animals and they recovered in BBR100-treated diabetic group. Specimen recordings show changes in LTP recording for 60 min after HFS, each recording is the average of 10 consecutive recordings in 100 s with an interval of 10 s. Upper panel shows recorded traces from different groups.

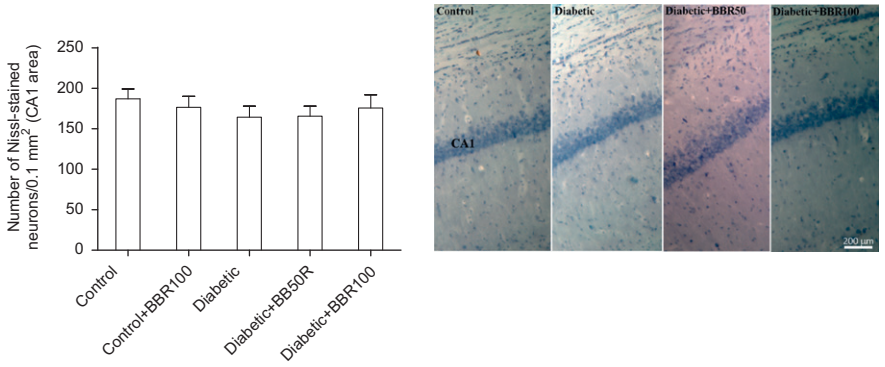

Fig. 6. Number of Nissl-stained neurons in CA1 area of hippocampus (left panel) and photomicrographs of coronal sections through the hippocampus showing Nissl-stained neurons in experimental groups (right panel; $n=5$ for each group).

and enhanced processing of amyloid-β (Aβ) precursor protein resulting in increased Aβ generation (Cai et al., 2011) have been implicated. Long-term diabetes is also associated with an elevated Bax/Bcl2 ratio, increased caspase 3 activity and neuronal apoptosis in the hippocampus and this is functionally well-correlated with impaired learning and memory. These changes are preceded by significant decreases in the hippocampal expression of insulin-like growth factor (IGF) subtypes (Li et al., 2002). IGF-1 could itself protect ganglionic neurons against apoptosis in a high-glucose milieu (Russell and Feldman, 1999). In diabetic rats, TUNEL positive neurons as well as neuronal loss are predominantly found in the CA1 region (Li et al., 2002). Since the

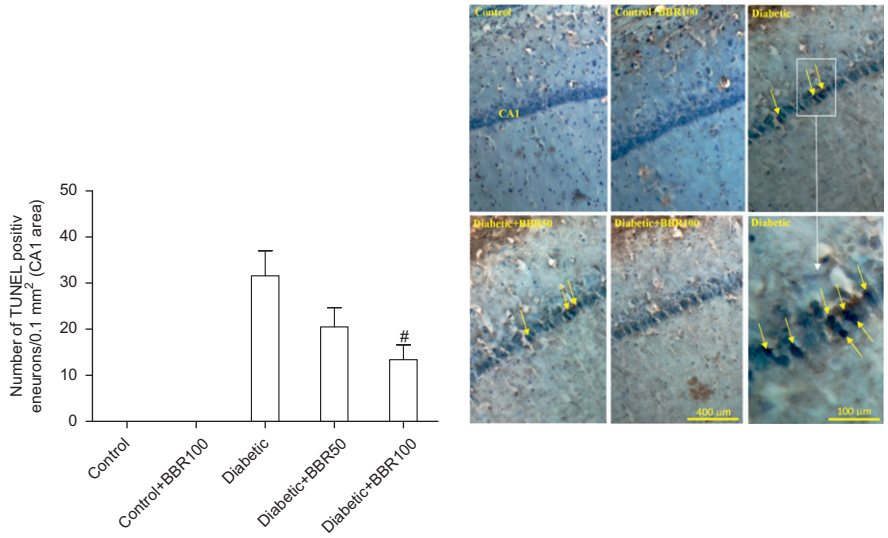

Fig. 7. Number of terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive neurons in the CA1 area (left panel) and photomicrographs of TUNEL-positive neurons (right panel). $^{\#}P < 0.05$ (in comparison with diabetic; $n=4$ for each group). The undefined photomicrographs have a scale bar of 400 μm.

hippocampal CA1 is especially susceptible and closely related to learning and memory, apoptotic cell death of this limbic structure is likely to contribute to cognitive deficits (Li et al., 2002) and impaired hippocampal synaptic plasticity (Biessels et al., 1996; Biessels et al., 1998) in diabetic rats. In our study, we did not observe a significant decline in the number of CA1 pyramidal neurons in Nissl staining. Previous reports have shown that such a decline need more time (Li et al., 2002). Furthermore, it has been reported that after 5 weeks of STZ-injection, some ultrastructural changes indicative of apoptosis and including chromatin aggregates and clumps within the nucleus and swollen mitochondria using transmission electron microscopy occur in CA1 pyramidal neurons (Ye et al., 2011). For this reason, these changes have not been detected by Nissl staining in our study.

Since chronic hyperglycemia type I diabetic patients has been associated with learning impairments (Brands et al., 2005), partial restoration of cognitive functions observed in diabetic animals in our study may be partly due to the ability of berberine to attenuate hyperglycemia. In addition, oxidative damage is associated with cognitive dysfunction (Fukui et al., 2002), therefore, treatment with antioxidants could be a therapeutic approach. It has been suggested that use of antioxidants and neuroprotective agents may decrease the risk of memory deficits (Rasoolijazi et al., 2007). Thus, antioxidant properties of berberine (Bhutada et al., 2011) may provide an underlying mechanism for part of its nootropic effect in diabetic rats in our study. Meanwhile, type I diabetes causes apoptosis-induced neuronal loss in the hippocampus and to a lesser extent in the frontal cortex of rats which is associated with cognitive impairment in diabetes (Li et al., 2002). Since berberine could inhibit apoptosis in some conditions (Hu et al., 2012) and there were a lower number of apoptotic neurons in CA1 area in this study, this may have attenuated learning and memory abnormalities in diabetic rats. In support of this idea, chronic STZ-diabetes accompanies hippocampal neuronal damage (Sherin et al., 2012) and berberine could protect against this (Hong et al., 2012). Furthermore, insulin deficiency and diabetes itself promote the processing of Aβ-precursor protein resulting in increased Aβ generation, neuritic plaque formation, and spatial memory deficits in mice (Wang et al., 2010). Berberine could act as an inhibitor of amyloid formation and could disaggregate preformed amyloid fibrils (Durairajan et al., 2012). Finally, due to significant role of IGF1 in prevention of apoptosis, berberine-containing medicines could affect the related pathways in diabetes in favor of cell survival (Zhao et al., 2012).

LTP is considered as a major synaptic mechanism for evaluating long-term synaptic plasticity in rodents. Post-tetanic LTP has been considered to be a physiological form of synaptic plasticity and its occurrence either in cortical or in sub cortical areas has been regarded as a cellular substrate for learning and memory (Bliss and Collingridge, 1993). In accordance to earlier studies, LTP induction and maintenance significantly impair in diabetic animals after tetanic stimulation (Reisi et al., 2008) and berberine treatment could prevent the abnormal changes in hippocampal synaptic plasticity induced by diabetes. It has been reported that negative regulation of LTP by diabetes may be due to the modulation of endogenous nitric oxide synthesis as NO is released following HFS (Chetkovich et al., 1993), and endogenous NO is essential for the maintenance of HFS-induced hippocampal LTP (Bon and Garthwaite, 2003). LTP itself appears to be highly dependent on NOS (Holscher, 2002). There is some evidence that berberine can modulate NO synthesis (Kulkarni and Dhir, 2007). Another effect of berberine in restoration of LTP induction and maintenance may be due to modulation of neurotransmitters release. In this respect, induction of hippocampal LTP is modulated by neurotransmitter systems like dopaminergic one (Abe et al., 2009) and berberine could increase the concentrations of some neurotransmitters like dopamine in the brain (Kulkarni and Dhir, 2008b). In addition, activity of glucagon-like peptide 1 (GLP-1) reduces in diabetes, a substance that has important roles in cognition, synaptic plasticity, and neurogenesis (Mossello et al., 2011). GLP-1 mimetics could enhance the induction of LTP by the activation of GLP-1 receptors on neurons (Porter et al., 2010). Berberine could enhance GLP-1 release and biosynthesis (Yu et al., 2010) and this could have improved LTP induction in diabetic rats. Berberine could also block potassium channels of hippocampal CA1 neurons (Wang et al., 2004) and this blockade leads to the suppression of apoptosis and a substantial increase in the rate of cell survival (Zarch et al., 2009) and this may be followed by the recovery of LTP.

5. Conclusions

In summary, chronic berberine treatment of STZ-diabetic rats significantly ameliorates learning and memory impairment and part of its beneficial effect could be attributed to its improvement of synaptic dysfunction and anti-apoptotic property and this may have an application in clinical settings to ameliorate cognitive decline of diabetics.

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References

Abe, K., Fujimoto, T., Akaishi, T., Misawa, M., 2009. Basolateral amygdala D1- and D2-dopaminergic system promotes the formation of long-term potentiation in the dentate gyrus of anesthetized rats. Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 552–556.

Artola, A., 2008. Diabetes-, stress- and ageing-related changes in synaptic plasticity in hippocampus and neocortex—the same metaplastic process? Eur. J. Pharmacol. 585, 153–162.

Bagheri, M., Joghataei, M.T., Mohseni, S., Roghani, M., 2011. Genistein ameliorates learning and memory deficits in amyloid beta(1–40) rat model of Alzheimer's disease. Neurobiol. Learn. Mem. 95, 270–276.

Baluchnejadmojarad, T., Roghani, M., 2006. Effect of naringenin on intracerebroventricular streptozotocin-induced cognitive deficits in rat: a behavioral analysis. Pharmacology 78, 193–197.

Baluchnejadmojarad, T., Roghani, M., 2011. Chronic epigallocatechin-3-gallate ameliorates learning and memory deficits in diabetic rats via modulation of nitric oxide and oxidative stress. Behav. Brain Res. 224, 305–310.

Bhutada, P., Mundhada, Y., Bansod, K., Tawari, S., Patil, S., Dixit, P., Umathe, S., Mundhada, D., 2011. Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav. Brain Res. 220, 30–41.

Biessels, G.J., Kamal, A., Ramakers, G.M., Urban, I.J., Spruijt, B.M., Erkelens, D.W., Gispen, W.H., 1996. Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. Diabetes 45, 1259–1266.

Biessels, G.J., Kamal, A., Urban, I.J., Spruijt, B.M., Erkelens, D.W., Gispen, W.H., 1998. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain Res. 800, 125–135.

Biessels, G.J., Kerssen, A., de Haan, E.H., Kappelle, L.J., 2007. Cognitive dysfunction and diabetes: implications for primary care. Primary Care Diabetes 1, 187–193.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Bon, C.L., Garthwaite, J., 2003. On the role of nitric oxide in hippocampal long-term potentiation. J. Neurosci. 23, 1941–1948.

Brands, A.M., Biessels, G.J., de Haan, E.H., Kappelle, L.J., Kessels, R.P., 2005. The effects of type 1 diabetes on cognitive performance: a meta-analysis. Diabetes Care 28, 726–735.

Cai, Z., Zhao, Y., Yao, S., Bin Zhao, B., 2011. Increases in beta-amyloid protein in the hippocampus caused by diabetic metabolic disorder are blocked by minocycline through inhibition of NF-kappaB pathway activation. Pharmacol. Rep. 63, 381–391.

Canas, P.M., Porciuncula, L.O., Cunha, G.M., Silva, C.G., Machado, N.J., Oliveira, J.M., Oliveira, C.R., Cunha, R.A., 2009. Adenosine A2A receptor blockade prevents synaptotoxicity and memory dysfunction caused by beta-amyloid peptides via p38 mitogen-activated protein kinase pathway. J. Neurosci. 29, 14741–14751.

Chae, C.H., Jung, S.L., An, S.H., Park, B.Y., Wang, S.W., Cho, I.H., Cho, J.Y., Kim, H.T., 2009. Treadmill exercise improves cognitive function and facilitates nerve growth factor signaling by activating mitogen-activated protein kinase/extracellular signal-regulated kinase1/2 in the streptozotocin-induced diabetic rat hippocampus. Neuroscience 164, 1665–1673.

Chetkovich, D.M., Klann, E., Sweatt, J.D., 1993. Nitric oxide synthase-independent long-term potentiation in area CA1 of hippocampus. Neuroreport 4, 919–922.

Choi, S.Z., Son, M.W., 2011. Novel botanical drug for the treatment of diabetic neuropathy. Arch. Pharm. Res. 34, 865–867.

Cok, A., Plaisier, C., Salie, M.J., Oram, D.S., Chenge, J., Louters, L.L., 2011. Berberine acutely activates the glucose transport activity of GLUT1. Biochimie 93, 1187–1192.

Comin, D., Gazarini, L., Zanoni, J.N., Milani, H., de Oliveira, R.M., 2010. Vitamin E improves learning performance and changes the expression of nitric oxide-producing neurons in the brains of diabetic rats. Behav. Brain Res. 210, 38–45.

Cooke, S.F., Bliss, T.V.P., 2006. Plasticity in the human central nervous system. Brain 129, 1659–1673.

Duarte, J.M., Agostinho, P.M., Carvalho, R.A., Cunha, R.A., 2012. Caffeine consumption prevents diabetes-induced memory impairment and synaptotoxicity in the hippocampus of NONcZNO10/LTJ mice. PLoS One 7, e21899.

Durairajan, S.S., Liu, L.F., Lu, J.H., Chen, L.L., Yuan, Q., Chung, S.K., Huang, L., Li, X.S., Huang, J.D., Li, M., 2012. Berberine ameliorates beta-amyloid pathology, gliosis, and cognitive impairment in an Alzheimer's disease transgenic mouse model. Neurobiol. Aging 33, 2903–2919.

Frier, B.M., 2011. Cognitive functioning in type 1 diabetes: the Diabetes Control and Complications Trial (DCCT) revisited. Diabetologia 54, 233–236.

Fukui, K., Omoi, N.O., Hayasaka, T., Shinnkai, T., Suzuki, S., Abe, K., Urano, S., 2002. Cognitive impairment of rats caused by oxidative stress and aging, and its prevention by vitamin E. Ann. N. Y. Acad. Sci. 959, 275–284.

Gu, Y., Zhang, Y., Shi, X., Li, X., Hong, J., Chen, J., Gu, W., Lu, X., Xu, G., Ning, G., 2010. Effect of traditional Chinese medicine berberine on type 2 diabetes based on comprehensive metabonomics. Talanta 81, 766–772.

Holscher, C., 2002. Different strains of rats show different sensitivity to block of long-term potentiation by nitric oxide synthase inhibitors. Eur. J. Pharmacol. 457, 99–106.

Hong, J.S., Chu, Y.K., Lee, H., Ahn, B.H., Park, J.H., Kim, M.J., Lee, S., Ryoo, H.S., Jang, J.H., Lee, S.R., Park, J.W., 2012. Effects of berberine on hippocampal neuronal damage and matrix metalloproteinase-9 activity following transient global cerebral ischemia. J. Neurosci. Res. 90, 489–497.

Hu, J., Chai, Y., Wang, Y., Kheir, M.M., Li, H., Yuan, Z., Wan, H., Xing, D., Lei, F., Du, L., 2012. PI3K p55gamma promoter activity enhancement is involved in the anti-apoptotic effect of berberine against cerebral ischemia-reperfusion. Eur. J. Pharmacol. 674, 132–142.

Huang, L., Shi, A.D., He, F., Li, X.S., 2010. Synthesis, biological evaluation, and molecular modeling of berberine derivatives as potent acetylcholinesterase inhibitors. Bioorg. Med. Chem. 18, 1244–1251.

Imanshahidi, M., Hosseinzadeh, H., 2008. Pharmacological and therapeutic effects of *Berberis vulgaris* and its active constituent, berberine. Phytother. Res. 22, 999–1012.

Iwai, T., Suzuki, M., Kobayashi, K., Mori, K., Mogi, Y., Oka, J., 2009. The influences of juvenile diabetes on memory and hippocampal plasticity in rats: improving effects of glucagon-like peptide-1. Neurosci. Res. 64, 67–74.

Kang, J.O., Kim, S.K., Hong, S.E., Lee, T.H., Kim, C.J., 2006. Low dose radiation overcomes diabetes-induced suppression of hippocampal neuronal cell proliferation in rats. J. Korean Med. Sci. 21, 500–505.

Khan, V., Najmi, A.K., Akhtar, M., Aqil, M., Mujeeb, M., Pillai, K.K., 2012. A pharmacological appraisal of medicinal plants with antidiabetic potential. J. Pharm. Bioallied Sci. 4, 27–42.

Kong, W.J., Zhang, H., Song, D.Q., Xue, R., Zhao, W., Wei, J., Wang, Y.M., Shan, N., Zhou, Z.X., Yang, P., You, X.F., Li, Z.R., Si, S.Y., Zhao, L.X., Pan, H.N., Jiang, J.D., 2009. Berberine reduces insulin resistance through protein kinase C-dependent up-regulation of insulin receptor expression. Metab. Clin. Exp. 58, 109–119.

Kucukatay, V., Agar, A., Gumuslu, S., Yargicoglu, P., 2007. Effect of sulfur dioxide on active and passive avoidance in experimental diabetes mellitus: relation to oxidant stress and antioxidant enzymes. Int. J. Neurosci. 117, 1091–1107.

Kulkarni, S.K., Dhir, A., 2007. Possible involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant activity of berberine chloride. Eur. J. Pharmacol. 569, 77–83.

Kulkarni, S.K., Dhir, A., 2008a. On the mechanism of antidepressant-like action of berberine chloride. Eur. J. Pharmacol. 589, 163–172.

Kulkarni, S.K., Dhir, A., 2008b. On the mechanism of antidepressant-like action of berberine chloride. Eur. J. Pharmacol. 589, 163–172.

Kulkarni, S.K., Dhir, A., 2010. Berberine: a plant alkaloid with therapeutic potential for central nervous system disorders. Phytother. Res. 24, 317–324.

Li, Z.G., Zhang, W., Grunberger, G., Sima, A.A., 2002. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res. 946, 221–231.

Mossello, E., Ballini, E., Boncinelli, M., Monami, M., Lonetto, G., Mello, A.M., Tarantini, F., Baldasseroni, S., Mannucci, E., Marchionni, N., 2011. Glucagon-like peptide-1, diabetes, and cognitive decline: possible pathophysiological links and therapeutic opportunities. Exp. Diabetes Res. 2011, 281674.

Muller, D., Nikonenko, I., Jourdain, P., Alberi, S., 2002. LTP, memory and structural plasticity. Curr. Mol. Med. 2, 605–611.

Northam, E.A., Rankins, D., Lin, A., Wellard, R.M., Pell, G.S., Finch, S.J., Werther, G.A., Cameron, F.J., 2009. Central nervous system function in youth with type 1 diabetes 12 years after disease onset. Diabetes Care 32, 445–450.

Paxinos, G., Watson, C., 1986. The Rat Brain in Stereotaxic Coordinates, 2nd ed. Academic Press, San Diego.

Peng, W.H., Hsieh, M.T., Wu, C.R., 1997. Effect of long-term administration of berberine on scopolamine-induced amnesia in rats. Jpn. J. Pharmacol. 74, 261–266.

Porter, D.W., Kerr, B.D., Flatt, P.R., Holscher, C., Gault, V.A., 2010. Four weeks administration of Liraglutide improves memory and learning as well as glycaemic control in mice with high fat dietary-induced obesity and insulin resistance. Diabetes Obes. Metab. 12, 891–899.

Rasoolijazi, H., Joghataie, M.T., Roghani, M., Nobakht, M., 2007. The beneficial effect of (−)-epigallocatechin-3-gallate in an experimental model of Alzheimer's disease in rat: a behavioral analysis. Iran Biomed. J. 11, 237–243.

Reisi, P., Babri, S., Alaei, H., Sharifi, M.R., Mohaddes, G., Lashgari, R., 2008. Effects of treadmill running on short-term pre-synaptic plasticity at dentate gyrus of streptozotocin-induced diabetic rats. Brain Res. 1211, 30–36.

Reisi, P., Babri, S., Alaei, H., Sharifi, M.R., Mohaddes, G., Noorbakhsh, S.M., Lashgari, R., 2010. Treadmill running improves long-term potentiation (LTP) defects in streptozotocin-induced diabetes at dentate gyrus in rats. Pathophysiology 17, 33–38.

Russell, J.W., Feldman, E.L., 1999. Insulin-like growth factor-I prevents apoptosis in sympathetic neurons exposed to high glucose. Horm. Metab. Res. 31, 90–96.

Sherin, A., Anu, J., Peeyush, K.T., Smijin, S., Anitha, M., Roshni, B.T., Paulose, C.S., 2012. Cholinergic and GABAergic receptor functional deficit in the hippocampus of insulin-induced hypoglycemic and streptozotocin-induced diabetic rats. Neuroscience 202, 69–76.

Stranahan, A.M., Lee, K., Pistell, P.J., Nelson, C.M., Readal, N., Miller, M.G., Spangler, E.L., Ingram, D.K., Mattson, M.P., 2008. Accelerated cognitive aging in diabetic rats is prevented by lowering corticosterone levels. Neurobiol. Learn. Mem. 90, 479–483.

Wang, F., Zhao, G., Cheng, L., Zhou, H.Y., Fu, L.Y., Yao, W.X., 2004. Effects of berberine on potassium currents in acutely isolated CA1 pyramidal neurons of rat hippocampus. Brain Res. 999, 91–97.

Wang, X., Zheng, W., Xie, J.W., Wang, T., Wang, S.L., Teng, W.P., Wang, Z.Y., 2010. Insulin deficiency exacerbates cerebral amyloidosis and behavioral deficits in an Alzheimer transgenic mouse model. Mol. Neurodegener. 5, 46.

Ye, L., Wang, F., Yang, R.H., 2011. Diabetes impairs learning performance and affects the mitochondrial function of hippocampal pyramidal neurons. Brain Res. 1411, 57–64.

Yu, Y., Liu, L., Wang, X., Liu, X., Xie, L., Wang, G., 2010. Modulation of glucagon-like peptide-1 release by berberine: in vivo and in vitro studies. Biochem. Pharmacol. 79, 1000–1006.

Zarch, A.V., Toroudi, H.P., Soleimani, M., Bakhtiarian, A., Katebi, M., Djahanguiri, B., 2009. Neuroprotective effects of diazoxide and its antagonism by glibenclamide in pyramidal neurons of rat hippocampus subjected to ischemia-reperfusion-induced injury. Int. J. Neurosci. 119, 1346–1361.

Zhang, Y.F., Li, X.Y., Zou, D.J., Liu, W., Yang, J.L., Zhu, N., Huo, L., Wang, M.A., Hong, J., Wu, P.H., Ren, G.G., Ning, G., 2008. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. J. Clin. Endocrinol. Metab. 93, 2559–2565.

Zhao, H.L., Sui, Y., Qiao, C.F., Yip, K.Y., Leung, R.K., Tsui, S.K., Lee, H.M., Wong, H.K., Zhu, X., Siu, J.J., He, L., Guan, J., Liu, L.Z., Xu, H.X., Tong, P.C., Chan, J.C., 2012. Sustained antidiabetic effects of a berberine-containing Chinese herbal medicine through regulation of hepatic gene expression. Diabetes 61, 933–943.

Zhou, J.Y., Zhou, S.W., 2011. Protective effect of berberine on antioxidant enzymes and positive transcription elongation factor b expression in diabetic rat liver. Fitoterapia 82, 184–189.

Zhou, J.Y., Zhou, S.W., Tang, J.L., Zhang, K.B., Guang, L.X., Huang, Y.P., Xu, Y., Ying, Y., Zhang, L., Li, D.D., 2009. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur. J. Pharmacol. 606, 262–268.

Zhu, F.Q., Qian, C.Y., 2006. Berberine chloride can ameliorate the spatial memory impairment and increase the expression of interleukin-1 beta and inducible nitric oxide synthase in the rat model of Alzheimer's disease. BMC Neurosci. 7, 78.

Zhu, X., Guo, X., Mao, G., Gao, Z., Wang, H., He, Q., Li, D. Hepatoprotection of berberine against hydrogen peroxide-induced apoptosis by upregulation of sirtuin 1. Phytother. Res., http://dx.doi.org/10.1002/ptr.4728, in press.