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Original Article

The effect of cerium oxide nanoparticles on memory impairment and antioxidant capacity in streptozotocin-induced diabetic rats

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Abstract

Introduction: Diabetes mellitus is a major chronic metabolic disorder that induces memory and learning impairment. Herein, we investigated the protective effects of cerium oxide nanoparticles (CeO2) against streptozotocin (STZ)-induced memory impairment and antioxidant capacity in the diabetic rats.

Methods: Adult male Wistar rats were assigned into the control, STZ, CeO2 and STZ plus CeO2 groups. Diabetes was induced using STZ and next CeNPs (60mg/kg) was administered for 14 constitutive days. The day after the last administration, spatial memory was assessed using the Morris water maze (MWM). Ultimately, the level of total antioxidant capacity (TAC) was investigated.

Results: Our results showed that STZ significantly decreased the spatial memory and CeO2 could compensate for these changes. Furthermore, TAC increased following administration of CeO2 in the diabetic rats.

Conclusion: The results of this study suggest that CeO2 seems to be able to improve STZ-induced neurotoxicity in the rats.

Keywords:
Streptozotocin;
Cerium oxide nanoparticles;
Spatial memory;
Antioxidant capacity

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Introduction

Diabetes mellitus (DM) as a major chronic metabolic disorder induces hippocampal dysfunction and memory impairment in both human and animal (Kamal et al., 2000; Kodl and Seaquist, 2008). It has been reported that some factors such as oxidative stress, inflammation and the decrease of growth factors are involved in the DM-induced memory impairment (Whitmer, 2007; Stranahan, 2015). Previous studies have demonstrated the generation of DM-induced free radicals (Gerber and Rutter, 2017) and the disruption of the antioxidant defense system (Abou-Seif and Youssef, 2004). Sen et al. (2005) has suggested that diabetic neurotoxicity has been correlated with hyperglycemia that leads to glucose-induced glycation and the structural and functional modification of hemoglobin.

Glycated hemoglobin species has been reported as a

potential source of the free radicals and stress oxidative (Roy et al., 2008). Therefore, it seems that administration of external antioxidants may be an ideal therapeutic agent to improve diabetes-induced memory impairment. Nanoparticles as novel antioxidants due to having unique chemical, biological and mechanical properties and as well scavenging free radicals are an effective strategy for modifying the outcome of DM (Wu et al., 2008; Najafi et al., 2017). Cerium oxide nanoparticles (CeO2) represent free radical scavenging properties (Mohammad et al., 2008), reduce inflammatory function in the microglia and protect injured nerve cells from calcium dysregulation (Callahan et al., 2003; Singh et al., 2007).

Given that the diabetes is associated with oxidative stress and that CeO2 has antioxidant properties, in this study, we examined the effect of CeO2 on the memory impairment in the streptozotocin (STZ)-diabetic rats.

Materials and methods

Drugs and experimental design

All experiments were conducted in the accordance with the ethical committee of the Hamadan University of Medical Sciences (HUMS). Male Wistar rats (250-300g, 10-11 weeks old) were obtained from animal house of HUMS and kept under the standard condition (12/12 h light and dark cycle, 22±2°C and 55±5% relative humidity) with free access to food and water *ad libitum*.

The rats were randomly divided into 4 groups (n=7 per group): 1- intact control group, 2- STZ- treated group which received a single IP injection of STZ (65mg/kg) in the citrate buffer at 7:00 AM. Three days after STZ injection, the blood glucose level was measured and the rats with a blood glucose level of >200 mg/dl was considered as a diabetic model; 3- STZ+CeO2 group was orally (by gavage) treated with 60mg/kg CeO2 after confirmation of diabetes for two weeks (Ranjbar et al., 2018) at 9:00 AM and 4- CeO2 group that orally received 60mg/kg CeO2 for two weeks.

Learning and memory assessment

The day after the last administration, the rats were assessed for spatial memory using the Morris water maze (MWM) test as our previous protocol (Soleimani Asl et al., 2015). In brief, the rats were trained for four days in the MWM apparatus with four quadrants (north, east, south and west). There was an invisible platform located 1cm below the water in the northeast quadrant. Each training day included two blocks, with four trials (60s). There was a 5min rest time between two blocks that rats were allowed to rest on the platform. The day after the last learning trial, a single 60s probe trial was given without a platform. Finally, a visible test was performed with a platform in the northwest quadrant that was covered with aluminum foil. The time taken to reach the hidden platform (escape latency), the length of the swim path (traveled distance) and the time spent in the target quadrant were record by a video camera linked to a computer.

Measurement of total antioxidant capacity (TAC)

We used FRAP assay to determine TAC based on our previously published protocol (Ranjbar et al., 2018).

Statistical analysis

Data were presented as mean±SEM and analyzed using SPSS 20. Statistical analysis was performed using two-way analysis of variance (for the escape latency and traveled distance during the training days) and one- way analysis of variance for the percentage of entrance to the target quadrant and the concentration of TAC. Tukey's multiple comparison tests were used to analyze the significance of the differences between the groups, when appropriate. $P<0.05$ was considered significant

Results

Effect of CeO2 nanoparticles on the acquisition memory deficit in the STZ- induced diabetic rats

Two-way analysis of variance of the escape latency, with the treatment as one factor and the training days as the second factor, showed a significant effect of treatment [$F(3, 7945)= 34.17$, $P<0.001$] and training days [$F(3, 9723)= 41.82$, $P<0.001$]. Furthermore, there was a significant interaction between the treatment and training days [$F(9, 633.0)= 2.72$, $P<0.01$]. Further analysis with One-way ANOVA showed that the control group took less time to find the hidden platform (escape latency) than the STZ-treated group ($P<0.001$, Fig. 1). Moreover,

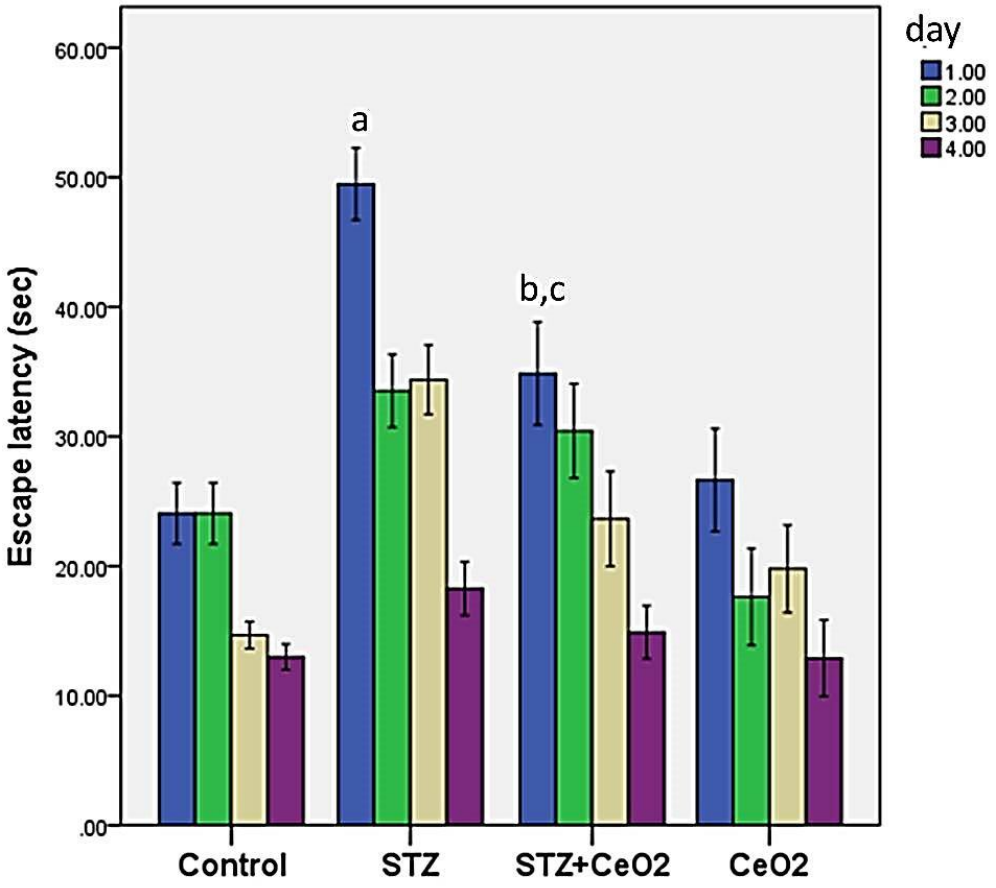

Fig.1. The mean of the escape latency in the control, streptozotocin (STZ), STZ+cerium oxide nanoparticles (CeO2) and CeO2 groups. Data are expressed as mean±SEM ([a] *P*<0.001 and [b] *P*<0.05 vs. control group, respectively; [c] *P*<0.001 vs. STZ group).

administration of CeO2 resulted in a reduction in escape latency compared with the STZ-treated group that was significant (*P*<0.001). In relation to the distance traveled, there was a significant effect for the training days [F(3, 27.82)= 47.61, *P*<0.001] and the treatment [F(3, 2724)= 46.61, *P*<0.001]. As well, a significant interaction was found between the treatment and training days [F(9, 4237)= 7.25, *P*<0.001]. One- way ANOVA analysis showed that administration of the SZT significantly increased the traveled distance compared to the control group(*P*<0.001, Fig. 2). The rats that received CeO2 following the STZ showed less traveled distance compared with the STZ- treated group (*P*<0.05).

The effect of CeO2 nanoparticles on the retention memory deficits in STZ- induced diabetic rats

To assess the deficits in the retention memory, the percentage of the entrance to the target quadrant in the probe trial session was analyzed in comparison to the other quadrants. Our results showed that STZ administration reduced the entrance to the target quadrant in comparison to the control group (*P*<0.05, Fi. 3). CeO2 -treated group spent more time in the target quarter than the STZ group (*P*<0.05).

The effect of CeO2 nanoparticles on the hippocampus TAC levels in the STZ- induced diabetic rats

The analysis of variance showed that the TAC of the rats exposed to the STZ was less compared to the control group (*P*<0.05 for both of them, Table 1). CeO2 treatment caused a significant increase in the TAC when compared to the STZ- treated group (*P*<0.05).

Discussion

The results of this study showed that STZ- treated rats exhibited spatial memory dysfunction, in addition to the diminished antioxidant capacity and thiol content. Interestingly, administration of CeO2 nanoparticles for 2 weeks ameliorated the memory impairment and enhanced the antioxidant capacity. A

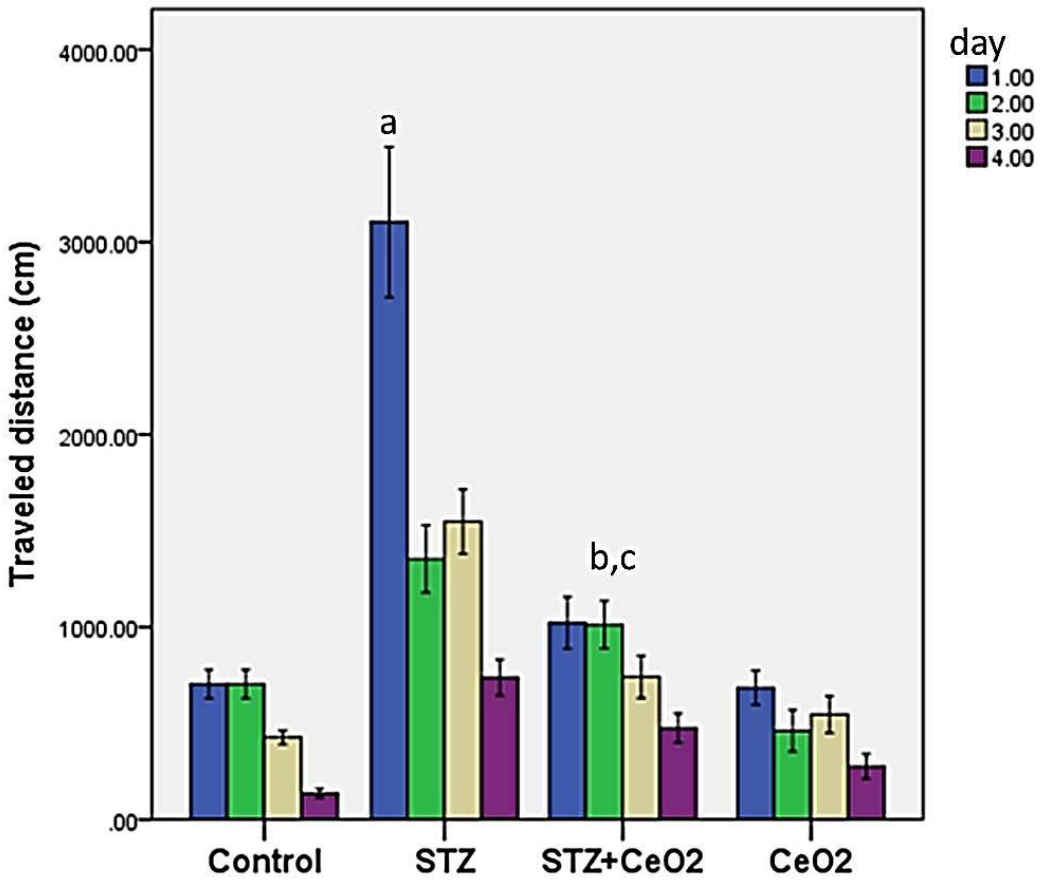

Fig.2. The mean of the traveled distance in the control, streptozotocin (STZ), STZ+cerium oxide nanoparticles (CeO2) and CeO2 groups. Data are expressed as mean±SEM ([a] $P<0.001$ vs. control group; [b] $P<0.05$ and [c] $P<0.001$ vs. STZ group, respectively).

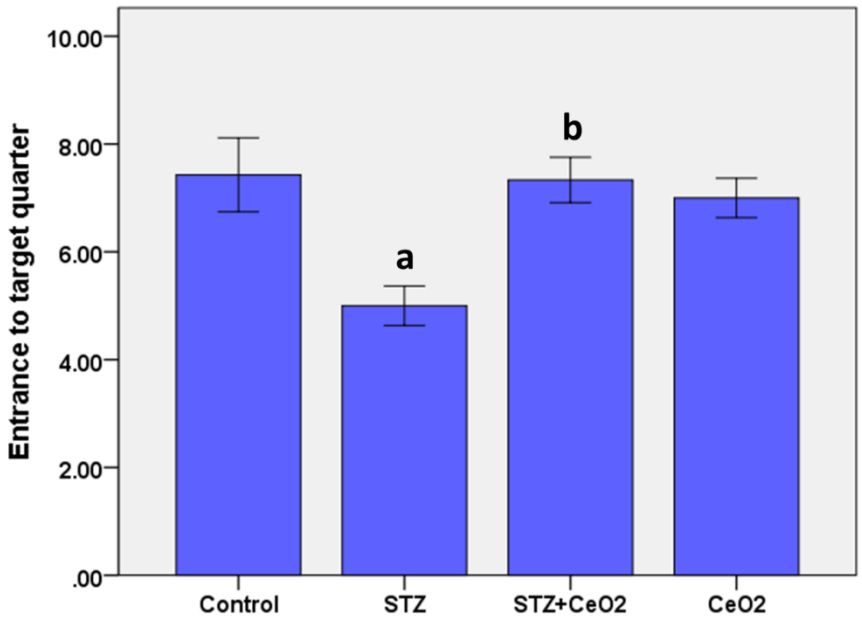

Fig.3. The percentage of the entrance to the target quadrant in the control, streptozotocin (STZ), STZ+cerium oxide nanoparticles (CeO2) and CeO2 groups. Data are expressed as mean±SEM ([a] $P<0.05$ vs. control group; [b] $P<0.05$ vs. STZ group).

Table 1: The effects of CeO2 on the total antioxidant capacity (TAC) level in the control, streptozotocin (STZ), STZ+cerium oxide nanoparticles (CeO2) and CeO2 groups. Data are expressed as mean±SEM ([a] *P*<0.05 vs. control group; [b] *P*<0.001 vs. STZ group).

Groups	TAC (Mm)
Control	0.27±0.0057
STZ	0.22±0.0166 [a]
STZ+CeO2	0.32±0.0182[b]
CeO2	0.30±0.0132

lot of evidence has been shown that diabetes is an important risk factor for the hippocampus-dependent memory dysfunction both in the rodents and human (Kumari et al., 2000; Stranahan et al., 2008). It appears to be a multifactorial process that involved in the STZ- induced memory pathogenesis. Oxidative damage in the hippocampus following STZ administration plays an important role in the Diabetes-induced neurotoxicity (Trudeau et al., 2004).

Our results showed that STZ treatment caused an acquisition and retention memory deficit, which was consistent with Agrawal et al. (2011) study that suggested intracerebroventricular administration of STZ induced the memory deficit in the rats. In another study, STZ treatment caused a significant impairment in the spatial memory in the MWM, an increased malondialdehyde, and a decreased glutathione levels in the brain (Saxena et al., 2011) that confirms the results of the current study that STZ treatment caused a decrease in the antioxidant capacity. As mentioned above, oxidative damage and the decreased antioxidant power may be a major pathway in the STZ- induced memory dysfunction, antioxidant thereby can improve hyperglycemia-induced spatial memory deficit (Moradkhani et al., 2015; Shahidi et al., 2016). It seems that the administration of external antioxidants for induction of endogenous antioxidant enzyme is a critical approach to ameliorate STZ- induced neurotoxicity.

Our results showed that administration of CeO2 nanoparticles considerably improved the STZ-induced memory impairment and increased antioxidant capacity that is consistent with the studies that found an effective role of CeO2 in the attenuation of oxidative damage in the liver (Amin et al., 2011) and brain (Dowding et al., 2014). Najafi et al. (2017) have reported that CeO2 administration in the diabetic rats reversed the reduction in the total thiol molecules (TTM), TAC and ADP/ATP ratio. They showed that CeO2 improved morphological abnormalities of dorsal root ganglia neurons in the diabetic model rat. Furthermore, Dillon et al. (2011) demonstrated that CeO2 nanoparticles could protect dopaminergic neurons and preserve striatal dopamine in the mouse model of Parkinson's disease. CeO2 nanoparticles as a novel regenerative antioxidant have high hydrogen and oxygen-absorbing capacity that reacts with H_2O_2 and their associated radical species. Further, CeO2 nanoparticles prevent oxidation, reduce nitrogen oxide emissions and protect cells from free radical challenge (Bailey et al., 2003).

Conclusion

Overall, oxidative stress and ROS have been reported to have a potential role in the diabetes neurotoxicity (Pop-Busui et al., 2006) and administration of CeO2 in diabetic rats could efficiently increase thiol content and TTM thereby indicates the antioxidant capacity of CeO2 nanoparticles. Thus the effects of CeO2 on the memory impairment of the diabetic rats seems that results from the increasing antioxidant power and alleviations of oxidative damage in the hippocampus and it is likely to be a potential treatment for the adverse effects of Diabetes disease. The most important limitation of this study is big F value that can be due to the number of animals included in the study.

Acknowledgments

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Conflict of interest

None of the authors of this paper have a financial interest to report.

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