The Protective Effect of Celecoxib on CA1 Hippocampal Neurons and Oxidative Stress in a Rat Model of Parkinson's Disease

Maryam Sarbishegi[1,2], Hamidreza Mahmoudzadeh-Sagheb[3,4], Zahra Heidari[3,4], Farzaneh Baharvand[2]

[1] Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

[2] Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

[3] Infectious Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

[4] Department of Histology, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

Received: 04 Mar. 2018; Accepted: 15 Dec. 2018

Abstract- Several studies point to an important role of neuroinflammation in Parkinson's disease (PD). Cognitive and memory impairments have been known in the early stages of PD. In the present study, we examined the effects of celecoxib (CLX), a selective inhibitor of cyclooxygenase-2 (COX-2), on hippocampus cell loss, passive avoidance memory and antioxidant status in a rat model of PD. We used the subcutaneous injection of 2.5 mg/kg/48h rotenone (ROT) for 4 weeks for induction of PD in a male Wistar rat. Animals were randomized to 4 groups (n=12): Control, sham, PD and PD+CLX group that receive celecoxib (20 mg/kg/day) for 4 weeks. Passive avoidance memory evaluated. We also determined the protective effect of CLX on a number of CA1 neurons in Nissl and TUNEL staining. Total antioxidant capacity (TAC) and malondialdehyde (MDA) a marker of lipid peroxidation in hippocampus assessed. Our findings indicated administration of CLX increase the passive avoidance memory (P<0.05), and by a decrease in apoptosis caused an increase in viable pyramidal neurons in CA1 hippocampus (P<0.01). On the other hand, CLX markedly reduced MDA level and increased TAC in the hippocampus of the PD model animal (P<0.05). It seems CLX with anti-inflammatory and antiapoptotic effect could prevent neurons loss and memory impairment which induced in PD.
© 2019 Tehran University of Medical Sciences. All rights reserved.
Acta Med Iran 2019;57(2):94-102.

Keywords: Celecoxib; Parkinson's disease; Hippocampus; Memory

Introduction

Parkinson's disease (PD) is one of the neurodegenerative diseases accompanied by loss of dopaminergic neurons of the substantia nigra pars compacta in the midbrain (1). Although the dopaminergic cells are vulnerable and damaged in PD, MRI studies revealed that other brain regions such as the hippocampus were also affected (2,3). Hippocampus is an important structure of the human's brain and other vertebrates which is located in the temporal lobe and is crucial for memory, spatial recognition and emotions (4). It has reported that the decrease in hippocampal volume and neuron loss is seen in Parkinson's disease. Memory and cognitive impairment are the non-motor early symptoms which are seen in patients who suffer from PD (5).

It is believed that neuroinflammation and oxidative stress participate in the development of PD. Therefore neuronal protection against inflammation and oxidative stress are effective strategies for PD management (6,7).

Cyclooxygenase-2 (COX-2) is an essential enzyme in inflammatory conditions and products. Prostaglandin E is an important inflammation mediator. COX-2 is expressed in some areas of cerebral cortex and in the hippocampus. COX-2 plays a special role in normal neuronal function and in neurotoxicity (8). It is reported that COX-2 overexpression plays a detrimental role in neurodegeneration disease (9,10). Different studies revealed that dopaminergic neurons show the majority of COX-2 positive cells in PD (9,11).

A previous study demonstrated the expression of COX-2 increased significantly in the hippocampus in a rat model of global cerebral ischemia-reperfusion injury and inhibition of COX-2 has an important role in the protection of cognitive impairments during ischemia and stroke (12).

COX-2 inhibitor was also showed to attenuate cytotoxicity of chronic neuroinflammation in basal

Corresponding Author: F. Baharvand
Department of Anatomy, School of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran
Tel: +98 915 4646884, Fax: +98 33 291124, E-mail address: baharvandfarzaneh@gmail.com

forebrain cholinergic neurons of rats (13).

Celecoxib (CLX), which is one of the non-steroidal anti-inflammatory drugs (NSAIDs) and a COX-2-specific inhibitor, has anti-pyretic, anti-inflammatory, and analgesic activities attributed to the inhibition of prostaglandin (14). Kurhe and *et al*., reported that Celecoxib has potential antidepressant-like effect in depression associated with obesity (15). Reksidler *et al.*, showed that that parecoxib (a cox-2 inhibitor) has a neuroprotective effect on the motor, tyrosine hydroxylase expression, and cognitive e functions in Parkinson's disease in an animal model (16).

Rotenone is a neurotoxin used for induction of PD model in animals. It caused dysfunction of complex-I of the mitochondrial respiratory chain and enhanced oxidative stress and neuroinflammation in the nigrostriatal dopaminergic pathway (17). ROT increased the levels of proinflammatory cytokines (IL-1β, IL-6, and TNF-α), and inflammatory mediators (COX-2 and iNOS) in rat brain tissues (18).

Thus the aim of the present study was the evaluation of neuroprotective effects of celecoxib on hippocampal CA1 neurons, passive avoidance memories (shuttle box) and oxidative stress in Parkinson's disease induced by rotenone in male rats.

Materials and Methods

A total of 48 male Wistar male rats (Animal lab of Zahedan University of Medical Sciences, Iran) weighing 300-350 g were included in this experiment. The animals were housed in standard cages at Controlled temperature (22±1° C) with a 12-h light: 12-h dark cycle and supplied with normal commercial chow and tap water. The animals fed with drug or vehicle by gavage tube for 4 weeks except for the control group. The PD model was induced via the subcutaneous (S.C) injection of 2.5 mg/kg/ 48 h of ROT for 28 days as reported earlier (19).

The rats were randomly allocated into 4 groups (n=12) as follows: Group I: (Control) animal were not treated with any drug or injection, group II: vehicle-treated (Sham), group III: the rotenone-injected (PD) and group IV: the rotenone-injected+celecoxib (20mg/kg/daily) (PD+CLX).

The experimental procedures were approved by the Ethics Committee of Zahedan University of Medical Silences, (IR.ZAUMS.REC.1395.238).

Passive avoidance memory

The shuttle box device consisted of two compartments of dark and light (illuminated by a 5 W lamp) with the floor which was covered with steel metal wires stainless (2 mm diameter with 1 cm distance). The compartments were connected by a guillotine door and a device for producing an electrical shock. Each rat was placed inside the shuttle box for 10-minutes in order to adaptation with the device (free access to light and dark chamber). Two days after adaptation, the animal was put inside the lightbox and the latency time of animal's entrance into the dark box was recorded (learning). Upon the entrance of the animal into the dark chamber, a guillotine door was closed and an electrical light shock (1.5 mA for 3 seconds) was applied to the fore and hind paws of the animals. The next day (24 hours later), the animal was placed in the light compartment, and guillotine door was raised and latency time of entering the dark compartment (without electric foot shock) was recorded (as step-through latency) (20).

Tissue processing

At the end of the schedule, a subset of rats in groups (n=6) was perfused transcardially using 4 % paraformaldehyde fixative then the brains removed and immersed in the same fixative solution for one week , processed routinely, and embedded in paraffin after graded ethanol dehydration. Coronal serial (5 µm-thick) sections were cut at the level of hippocampus according to Paxinos atlas (–2.5 mm to –4.5 mm from bregma) (21) and mounted on historic-treated slides for Nissl staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL).

Another subset of animals was killed under deep anesthesia, and the brains were quickly removed. The hippocampus of both sides was isolated on the ice and prepared as a homogenate to determine the MDA level and TAC.

Nissl staining

The coronal sections of brains were stained with 0. 1% cresyl violet, dehydrated through graded alcohols (70, 95, 100% 2x), placed in xylene, and mounted. The intact pyramidal neurons in the CA1 area of the hippocampus on the right side of the brain were counted under a light microscope at a magnification of 400x.

TUNEL staining

The TUNEL staining method was performed according to the Roche protocol (Roche molecular biochemicals kit, Germany). Briefly, de-waxed tissue sections were treated with proteinase K for 30 minutes. The sections were incubated with 3% H_2O_2 for 10 minutes to reduce endogenous peroxidase activity, then

the TUNEL reaction mixture was added, and the sections were incubated for 1 hour. The tissue sections were incubated with a convertor-POD at 37° C for 30 min. Finally, the sections were incubated with diaminobenzidine substrate solution (DAB) for 15 min to achieve visualization of the apoptotic cells and counterstained with hematoxylin for 30 s. Finally, the samples were dehydrated in alcohol, cleared in xylene and mounted for visualization. Cells with deep brown-stained nuclei were counted in the CA1 area of the hippocampus on the right side of the brain under a light microscope at a magnification of 400x (22).

Biochemical analysis

Preparation of hippocampal supernatant

For biochemical analysis, both hippocampus from each rat were quickly isolated, weighted and homogenized (10% w/v) in cold PBS (0.1 M, pH 7.4). The homogenates were centrifuged at 12,000 g at 4° C for 25 minutes. The supernatants were stored at −80° C for measurement of Malondialdehyde (MDA) level and total antioxidant capacity (TAC). The content of protein in the hippocampus homogenate was measured by the method of Bradford (23).

Determination of malondialdehyde (MDA)

MDA level which is a marker of lipid peroxidation was determined by thiobarbituric acid reactive substances (TBARs) according to Ohkawa *et al*., Briefly 200 µl of hippocampal supernatant was added to 500 µL of acetic acid solution (2.5 M HCl, pH 3.4), 200 µL of 8.1 % sodium dodecyl sulfate (SDS) and 500 µL of 0.8 % thiobarbituric acid (TBA) and after vortex mixing sample was heated at 95° C for 1 h. After centrifuging at 3,000 rpm for 10 min, the absorbance of the supernatant was read at 532 nm. The results were expressed as nmol/mg protein (24).

Total antioxidant capacity (TAC)

To determination of TAC, the ferric reducing/antioxidant power (FRAP) was used according to by the method of Benzie and Strain. The FRAP method measures the power of antioxidants to reduce the ferric tripyridyl triazine (Fe3+-TPTZ) to a ferrous form (Fe2+). Briefly, FRAP solution was prepared by mixing buffer acetate (300 mMpH 3.6) with TPTZ (2,4,6-tripyridyl-triazine) (10 mM) solution with 10:1 ratio in HCL (40 mM) and 1 mL FeCl3, the 6H2O solution was added just before use. Then, 1.5 mL of the FRAP reagent was added to 50 µL of 10% homogenate and incubated for 10 minutes at 37° C. In addition, FeSO4 was used as a standard to measure the FRAP. The complex between Fe2+/TPTZ formed a blue color that absorbance was measured at 593 nm. The final results were reported as µmol/g tissue (25).

Statistical analysis

Analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test by SPSS software. Data are presented as mean±SEM. The significance level was set at $P<0.05$.

Results

Passive avoidance memory

As seen in figure 1, step-through latency time showed a significant difference between control and PD group, which indicated decreased passive avoidance learning in the PD group compared with the control group ($P<0.001$). 20 mg/kg oral administration of *celecoxib* for four weeks significantly increased step-through latency time in the CLX group compared to the PD group ($P<0.05$).

Nissl staining

As seen in figure 1, the number of surviving neurons in the CA1 hippocampal area was counted and compared among groups. Control and sham group did not show any histopathological abnormalities at CA1area of the hippocampus and pyramidal cells had intact nuclei and normal arrangement. Our results showed that injection of ROT enhanced the neurodegeneration in the hippocampus and decreased the viable pyramidal cells in the PD group compared to the control group ($P<0.001$). Treatment with CLX markedly prevented a decrease in intact pyramidal cells in the CA1 hippocampal area compare to the PD group ($P<0.01$).

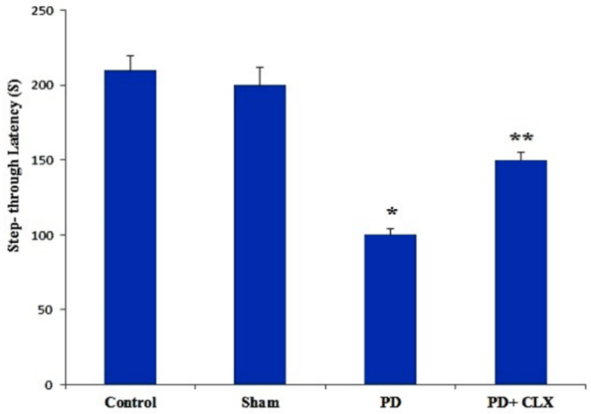

Figure 1. Effects of CLX on step-through latency time in experimental groups. Data are expressed as the mean ± SEM (n = 6) and analyzed using one-way analysis of variance and Tukey's *post-hoc* test. *P<0.001 versus control group, **P<0.05 versus PD group

TUNEL staining

The apoptotic cells in the hippocampal samples were detected by TUNEL staining (Figure 3). In the control and sham groups, TUNEL-positive pyramidal neurons rarely observed in the CA1 area of the hippocampus. In contrast, in the PD group, the numbers of TUNEL-positive neurons significantly increased compared to the control group (P<0.001). CLX treated group showed a decrease in the number of apoptotic neurons in comparison with the PD group (P<0.01).

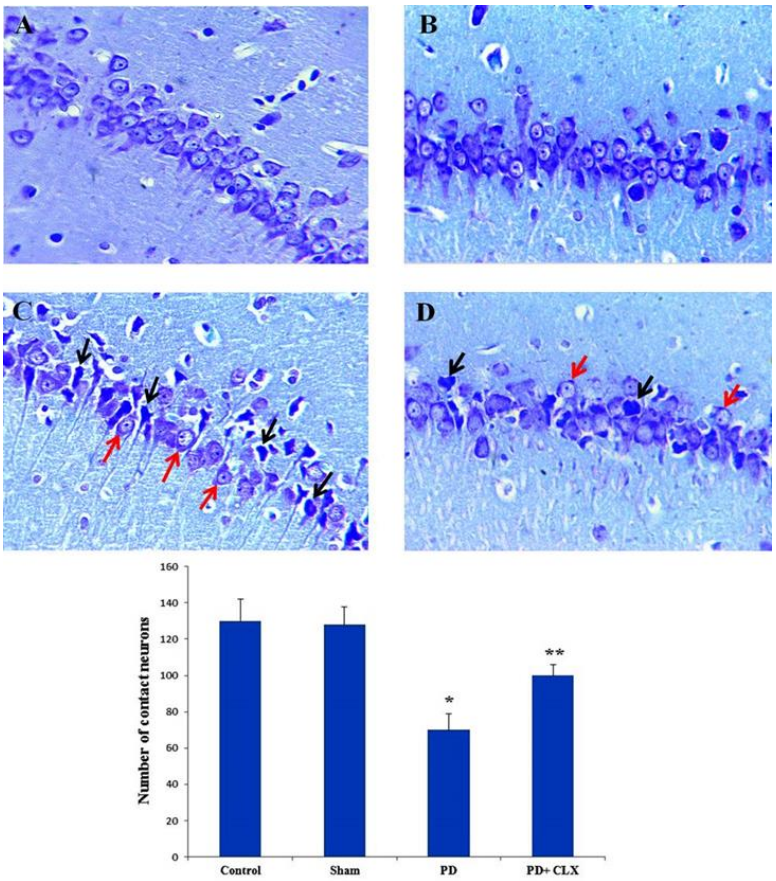

Figure 2. Effects of celecoxib on pyramidal cells count in the hippocampal CA1 of a rat with PD (Nissl staining)

Nissl staining was used to observe pyramidal cells in different groups . Survival cells were quantified at 400× magnification. Red arrows show the viable cells and black arrows show the pycnotic cells. The graph shows the number of survival hippocampal CA1 neurons. Data are expressed as the mean ± SEM (n = 6) and analyzed using one-way analysis of variance and Tukey's *post-hoc* test. *P < 0.001 versus control group, **P < 0.01 versus PD group. A: control group; B: sham group; C: PD group; D: PD+CLX group

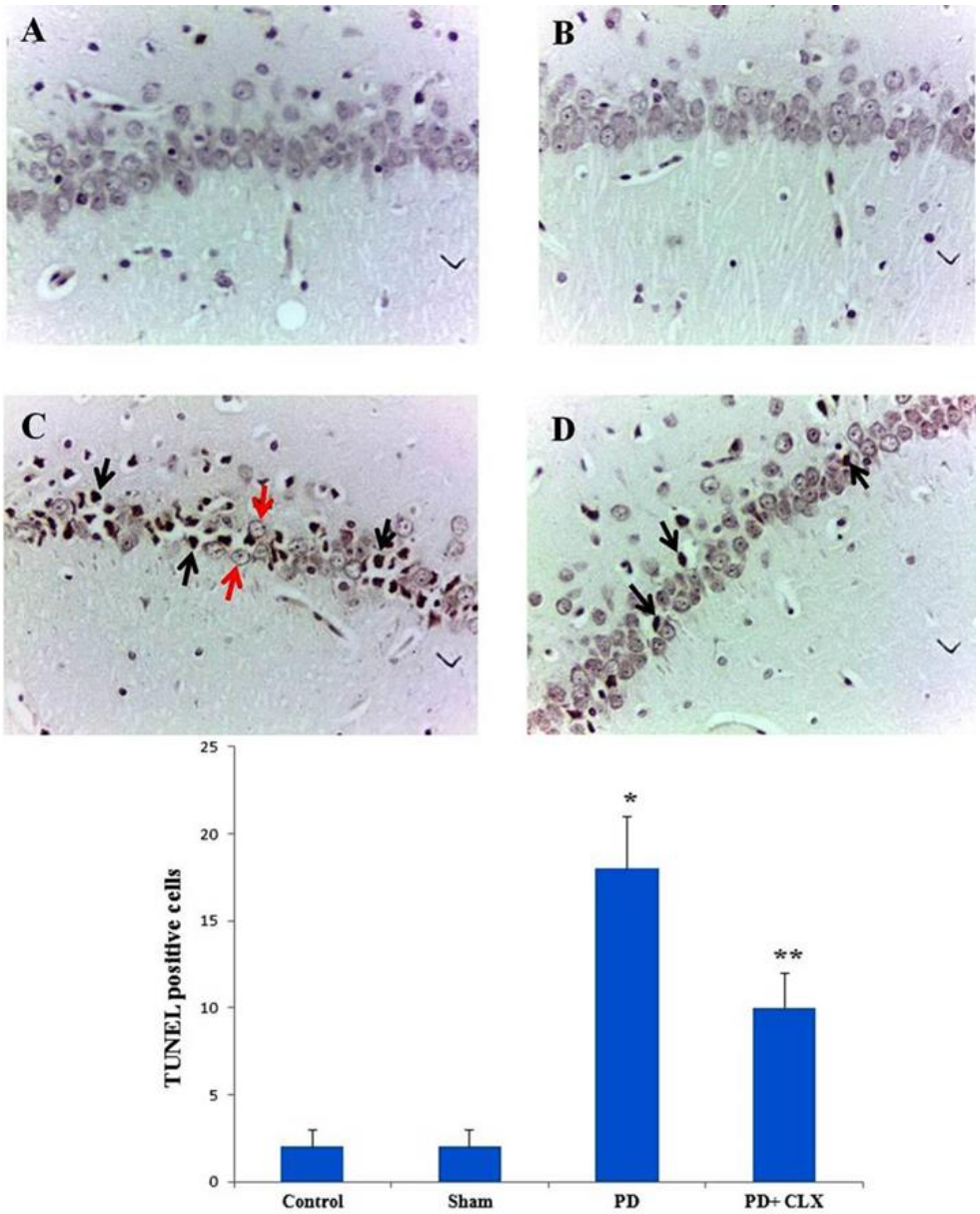

Figure 3. Effects of celecoxib on cell apoptosis in the hippocampal CA1 of the rat with PD (TUNEL staining)

TUNEL staining was used to observe differences in apoptosis (×400) in different groups. TUNEL-positive cells were quantified at 400× magnification. Black arrows show the TUNEL-positive cells and red arrows shows TUNEL-negative cells. The graph shows the number of apoptotic cells hippocampal CA1 neurons. Data are expressed as the mean ± SEM (n = 6) and analyzed using one-way analysis of variance and Tukey's *post-hoc* test. *P < 0.001 versus control group, **P < 0.01 versus PD group. A: control group; B: sham group; C: PD group; D: PD+CLX group

Effect of CLX on hippocampal MDA level

As seen in figure 4 A injection of ROT significantly increased MDA level in the hippocampus of the PD group compared to the control group (P<0.001). On the other hand, the CLX treated group showed a reduction of MDA level compared to the PD group (P<0.05).

Effect of CLX on hippocampal TAC level

A significant decrease in TAC level was observed in the hippocampus of the PD group compared to the control group (P<0.001). Treatment with CLX 20 mg/kg significantly prevented of reduction of TAC level in comparison to the PD group (P<0.05) (Figure 4 B). There was no significant difference in the TAC level between the control and the sham groups (P>0.05).

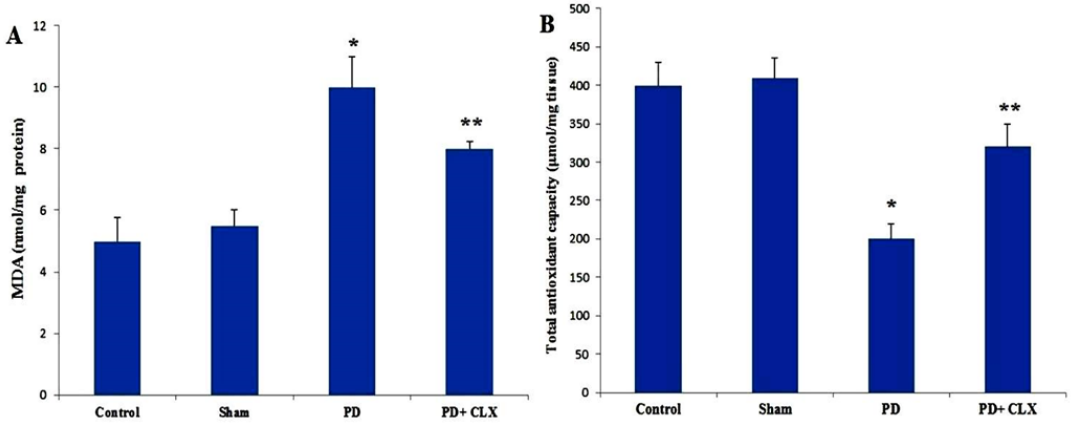

Figure 4. Effects of CLX on MDA (A) and TAC level (B) in the hippocampus of animals. ROT increased MDA level and decreased antioxidant enzymes level in all animals. Pretreatment with CLX prevented the increase in the level of MDA and improved TAC level. Statistical analysis by one way ANOVA followed by Tukey's post hoc multiple comparison tests. $P<0.05$ considered significance level. Data expressed as mean±SEM (n=6).*$P<0.001$ versus control group and **$P<0.05$ versus PD group A: control group; B: sham group; C: PD group; D: PD+CLX group

Discussion

The current study demonstrated neuroprotective effects celecoxib on CA1 hippocampal neurons and oxidative damages following PD induced by rotenone in rats. Our finding showed that celecoxib decrease apoptosis by neuronal death improved the passive avoidance memory and antioxidant status in the rat model of PD.

PD is a progressive neurodegenerative disease, which involved a neuronal loss in midbrain and some area of the brain, especially in hippocampal tissue (26).

Learning and cognitive impairment in PD patients have been approved by different studies, and it has shown that hippocampal neuronal damages in PD are resulting in memory deficits which is the primary and non-motor symptoms (27).

The effect of PD on memory has been examined by MRI pictures which are showed atrophy in the hippocampus CA1 region (3).

Heijer *et al.*, has demonstrated an increase in the hippocampal atrophy in patients with type 2 diabetes mellitus, and it is accompanied by weakness in verbal episodic memory (28), thus it is suggested that prevention of cell death or stimulation of neuronal proliferation in hippocampus can be a hopeful strategy for PD treatment (7).

Because of inflammatory mechanisms contributing to neuronal damage in neurodegenerative diseases such as PD, some researchers suggested that the use of anti-inflammatory agents may decrease or inhibit the progress of PD (7,29). Inflammatory processes caused enhancement in COX-2 expression which leads to neuro-degeneration in SNC and hippocampus (30).

Yang stated that increase of COX-2 in the hippocampus of diabetic rats caused impairment in spatial memory, hippocampal cells loss and long-term potentiation (LTP) (31).

Some studies suggested that non-steroidal anti-inflammatory drugs (NSAIDs) such as celecoxib by inhibition of proinflammatory cytokines expression cascades have a neuroprotective effect in PD and Alzheimer disease and reduce the risk of memory deficit (32). The NSAIDs inhibit the cascade of proinflammatory cytokines expression. Yu *et al.*, reported an elevation of COX-2 expression in the hippocampus of a rat model of global cerebral ischemia-reperfusion injury; they showed the beneficial role of COX-2 inhibitors in the protection of cognitive impairment during ischemia (12). Cox-2 overexpression increased in hippocampus and cortex of patients suffering from senile memory abnormalities (33). According to a study conducted by Small *et al*., daily administration of celecoxib in people with age-associated memory decline increase regional brain metabolism and improve cognitive performance (34).

The result of Yang's study showed that celecoxib increased the upregulation of BDNF-TrkB (Brain-derived neurotrophic factor -tyrosine receptor kinase B) in the hippocampus of diabetic rats, which resulted in improving memory and learning deficit. BDNF/TrkB signaling pathway plays an essential role in enhanced learning and memory (31). Decreased mRNA expression of BDNF is shown in the hippocampus of PD rats (7). On the other hand, the results of some studies demonstrated that COX-2 up-regulation contributed to neuronal death in neurodegenerative diseases and Cox-2 inhibitors decrease neuronal loss in the brain (35). Other studies suggest that memory function is closely attributed to the

numbers and functional capability of pyramidal cells in the hippocampus CA1 region and hippocampal synaptic plasticity impairment and apoptosis in CA1 pyramidal neurons contribute to cognitive deficits (36,37). In line with these reports, here we showed a decrease in intact pyramidal cells in CA1 hippocampus in Nissl staining in a model of PD group which is accompanied by a decrease in passive avoidance memory in the shuttle box. Our results showed that the celecoxib-treated group had a number of surviving pyramidal cells in the CA1 area and also elevation in passive avoidance memory compare to the PD group.

Ye *et al*., reported that part of impairment in performing the spatial learning and memory tasks in PD is contributed to apoptosis of CA1 pyramidal neurons (38). The hippocampus is vulnerable to different injuries, such as inflammation induced by neurotoxins (39).

We used ROT injection for PD induction, and this neurotoxin induces neuro-inflammation which causes neuronal apoptosis in the nigrostriatal, cerebral cortex, and hippocampus (40). Our findings demonstrated significant enhancement of TUNEL positive cells in the CA1 hippocampus area in PD group compared to the control group. This observation is in line with a previous study showing that rotenone-induced caspase-3-mediated apoptosis (40). Smith *et al*., stated that during inflammation, NSAIDs by inhibition of inflammatory cytokines induction, inhibit caspase catalytic activity and reduce cell death (14).

According to the results, COX-2 inhibition has a protective effect on CA1 hippocampal neurons. This effect can be attributed to the anti-inflammatory mechanism of celecoxib which mitigates microglial activation and decrease expression of proinflammatory cytokines resulted in apoptosis cell inhibition (41).

Also, in this study, we showed a decrease in the total antioxidant capacity and an increase in MDA level in the hippocampus of PD rats. MDA is a lipid peroxidation marker, which is formed by the attack to free radicals to the cell membrane lipids and causing cellular dysfunction. We also previously reported a significant decrease of antioxidant enzymes activity and an increase of MDA level in the midbrain of a rat model of PD (42).

It is well known that free radicals are involved in the pathogenesis of PD. The increase of oxidative stress and a decrease in antioxidant status are reported in patients with PD (43,44).

ROT which was used in this experiment is a high-affinity inhibitor of complex I of the electron transport which causes the production of reactive oxygen species (ROS) and oxidative stress in dopaminergic neurons in substantia nigra (45).

It has been shown that the hippocampus receives the dopaminergic projection from the substantia nigra by mesolimbic pathway; therefore hippocampus might uptake the toxin and oxidative stress in dopaminergic neurons, and the result would be neuronal loss and memory impairment (46) (43).

We evaluated the effect of celecoxib on hippocampus lipid peroxidation and total antioxidant capacity of PD rats. The results indicated that administration 20 mg/kg/daily CLX for 4 weeks improved MDA level and reverse the decrease of TAC in the hippocampus of PD rats compared to the control group.

As mentioned already microglial activation is one of the sources of ROS generation in neuro-inflammation disease such as PD. Since celecoxib with anti-inflammatory effect could inhibit the microglial activation, therefore it shows antioxidant property (41) (47).

In line with our results, Santiago et al. demonstrated that celecoxib enhanced antioxidant defenses and attenuated oxidative stress in the hippocampus of depressed rats (48).

It is concluded that celecoxib via its anti-inflammatory, anti-apoptotic, and antioxidant effect promotes neuron survival and enhances the memory in this PD model. Consequently, the use of celecoxib may have application in the alleviation of hippocampal neuronal death, learning, and memory abnormalities in PD patients. However, further researches are still required.

Acknowledgments

This paper is part of the MSc thesis supported by the Zahedan University of Medical Sciences (project number: K/170)

References

1. Halliday GM, McCann H. The progression of pathology in Parkinson's disease. Ann NY Acad Sci 2010;1184:188-95.
2. Good M. Spatial memory and hippocampal function: where are we now? Psicológica 2002;23:109-138
3. Laakso M, Partanen K, Riekkinen P, Lehtovirta M, Helkala E-L, Hallikainen M, et al. Hippocampal volumes in Alzheimer's disease, Parkinson's disease with and without dementia, and in vascular dementia An MRI study. Neurology 1996;46:678-81.
4. Bird CM, Burgess N. The hippocampus and memory: insights from spatial processing. Nat Rev Neurosci

2008;9:182-94.

5. Camicioli R, Moore MM, Kinney A, Corbridge E, Glassberg K, Kaye JA. Parkinson's disease is associated with hippocampal atrophy. Movement Disord 2003;18:784-90.
6. Kaur K, Gill JS, Bansal PK, Deshmukh R. Neuroinflammation-A major cause for striatal dopaminergic degeneration in Parkinson's disease. J Neurol Sci 2017;381:308-14.
7. Yang J, Song S, Li J, Liang T. Neuroprotective effect of curcumin on hippocampal injury in 6-OHDA-induced Parkinson's disease rat. Pathol Res Pract 2014;210:357-62.
8. Breder CD, Dewitt D, Kraig RP. Characterization of inducible cyclooxygenase in rat brain. J Comp Neurol 1995;355:296-315.
9. Teismann P, Tieu K, Choi D-K, Wu D-C, Naini A, Hunot S, et al. Cyclooxygenase-2 is instrumental in Parkinson's disease neurodegeneration. Proceed Nat Acad Sci 2003;100:5473-8.
10. Cowley T, Fahey B, O'Mara S. COX-2, but not COX-1, activity is necessary for the induction of perforant path long-term potentiation and spatial learning in vivo. Euro J Neurosci 2008;27:2999-3008.
11. Feng Z-H, Wang T-G, Li D-D, Fung P, Wilson B, Liu B, et al. Cyclooxygenase-2-deficient mice are resistant to 1-methyl-4-phenyl1, 2, 3, 6-tetrahydropyridine-induced damage of dopaminergic neurons in the substantia nigra. Neurosci lett 2002;329:354-8.
12. Yu L, Yang B, Wang J, Zhao L, Luo W, Jiang Q, et al. Time course change of COX2-PGI 2/TXA 2 following global cerebral ischemia-reperfusion injury in rat hippocampus. Behav Brain Funct 2014;10:42.
13. Smith CE, Soti S, Jones TA, Nakagawa A, Xue D, Yin H. Non-steroidal anti-inflammatory drugs are caspase inhibitors. Cell Chem Biol 2017;24:281-92.
14. Kurhe Y, Mahesh R, Gupta D. Effect of a selective cyclooxygenase type 2 inhibitor celecoxib on depression associated with obesity in mice: an approach using behavioral tests. Neurochem Res 2014;39:1395-402.
15. Reksidler AB, Lima MM, Zanata SM, Machado HB, da Cunha C, Andreatini R, et al. The COX-2 inhibitor parecoxib produces neuroprotective effects in MPTP-lesioned rats. Eur J Pharmacol 2007;560:163-75.
16. Tapias V, Cannon JR, Greenamyre JT. Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson's disease model. J Neurosci Res 2010;88:420-7.
17. Javed H, Azimullah S, Khair SBA, Ojha S, Haque ME. Neuroprotective effect of nerolidol against neuroinflammation and oxidative stress induced by rotenone. BMC Neurosci 2016;17:58.
18. Rahimmi A, Khosrobakhsh F, Izadpanah E, Moloudi MR, Hassanzadeh K. N-acetylcysteine prevents rotenone-induced Parkinson's disease in rat: An investigation into the interaction of parkin and Drp1 proteins. Brain Res Bull 2015;113:34-40.
19. Tamburella A, Micale V, Mazzola C, Salomone S, Drago F. The selective norepinephrine reuptake inhibitor atomoxetine counteracts behavioral impairments in trimethyltin-intoxicated rats. Eur J Pharmacol 2012;683:148-54.
20. Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. San Diego: Elsevier Academic Press, 2005.
21. Sarbishegi M, Heidari Z, Mahmoudzadeh-Sagheb H, Valizadeh M, Doostkami M. Neuroprotective effects of Withania coagulans root extract on CA1 hippocamus following cerebral ischemia in rats. Avicenna J Phytomed 2016;6:399-409.
22. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248-54.
23. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
24. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70-6.
25. Choi AY, Choi JH, Lee JY, Yoon K-S, Choe W, Ha J, et al. Apigenin protects HT22 murine hippocampal neuronal cells against endoplasmic reticulum stress-induced apoptosis. Neurochem Int 2010;57:143-52.
26. Carlesimo G, Piras F, Assogna F, Pontieri F, Caltagirone C, Spalletta G. Hippocampal abnormalities and memory deficits in Parkinson disease A multimodal imaging study. Neurology 2012;78:1939-45.
27. den Heijer T, Vermeer S, Van Dijk E, Prins N, Koudstaal PJ, Hofman A, et al. Type 2 diabetes and atrophy of medial temporal lobe structures on brain MRI. Diabetologia 2003;46:1604-10.
28. Kim M, Cho K-H, Shin M-S, Lee J-M, Cho H-S, Kim C-J, et al. Berberine prevents nigrostriatal dopaminergic neuronal loss and suppresses hippocampal apoptosis in mice with Parkinson's disease. Int J Mol Med 2014;33:870-8.
29. Gupta A, Kumar A, Kulkarni S. Targeting oxidative stress, mitochondrial dysfunction and neuroinflammatory signaling by selective cyclooxygenase (COX)-2 inhibitors mitigates MPTP-induced neurotoxicity in mice. Prog Neuro Psychopha 2011;35:974-81.
30. Yang Y, Gao L. Celecoxib Alleviates Memory Deficits by Downregulation of COX-2 Expression and Upregulation

of the BDNF-TrkB Signaling Pathway in a Diabetic Rat Model. J Mol Neurosci 2017;62:188-98.

31. Minghetti L. Cyclooxygenase-2 (COX-2) in inflammatory and degenerative brain diseases. J Neuropath Exp Neur. 2004;63:901-10.
32. Ho L, Purohit D, Haroutunian V, Luterman JD, Willis F, Naslund J, et al. Neuronal cyclooxygenase 2 expression in the hippocampal formation as a function of the clinical progression of Alzheimer disease. Arch Neurol 2001;58:487-92.
33. Small GW, Siddarth P, Silverman DH, Ercoli LM, Miller KJ, Lavretsky H, et al. Cognitive and cerebral metabolic effects of celecoxib versus placebo in people with age-related memory loss: randomized controlled study. Am J Geriat Psychiat 2008;16:999-1009.
34. Jun-Qing Y, Bei-Zhong L, Bai-Cheng H, Qi-Qin Z. Protective effects of meloxicam on aluminum overload-induced cerebral damage in mice. Eur J Pharmacol 2006;547:52-8.
35. Li ZG, Zhang W, Grunberger G, Sima AA. Hippocampal neuronal apoptosis in type 1 diabetes. Brain Res 2002;946:221-31.
36. Kalalian-Moghaddam H, Baluchnejadmojarad T, Roghani M, Goshadrou F, Ronaghi A. Hippocampal synaptic plasticity restoration and anti-apoptotic effect underlie berberine improvement of learning and memory in streptozotocin-diabetic rats. Eur J Pharmacol 2013;698:259-66.
37. Ye L, Wang F, Yang R-H. Diabetes impairs learning performance and affects the mitochondrial function of hippocampal pyramidal neurons. Brain Res. 2011;1411:57-64.
38. Chung DW, Yoo K-Y, Hwang IK, Kim DW, Chung JY, Lee CH, et al. Systemic administration of lipopolysaccharide induces cyclooxygenase-2 immunoreactivity in endothelium and increases microglia in the mouse hippocampus. Cell Mol Neurobiol 2010;30:531-41.
39. Abdel-Salam OM, Omara EA, El-Shamarka ME-S, Hussein JS. Nigrostriatal damage after systemic rotenone and/or lipopolysaccharide and the effect of cannabis. Comp Clin Pathol 2014;23:1343-58.
40. De Simone R, Ajmone-Cat MA, Minghetti L. Atypical antiinflammatory activation of microglia induced by apoptotic neurons. Mol Neurobiol 2004;29:197.
41. Sarbishegi M, Gorgich EAC, Khajavi O, Komeili G, Salimi S. The neuroprotective effects of hydro-alcoholic extract of olive (Olea europaea L.) leaf on rotenone-induced Parkinson's disease in rat. Metab Brain Dis 2018;33:79-88.
42. Sriraksa N, Wattanathorn J, Muchimapura S, Tiamkao S, Brown K, Chaisiwamongkol K. Cognitive-enhancing effect of quercetin in a rat model of Parkinson's disease induced by 6-hydroxydopamine. Evid Based Complement Alternat Med 2012;2012:823206.
43. Sarrafchi A, Bahmani M, Shirzad H, Rafieian-Kopaei M. Oxidative stress and Parkinson's disease: new hopes in treatment with herbal antioxidants. Curr Pharm Design 2016;22:238-46.
44. Martinez TN, Greenamyre JT. Toxin models of mitochondrial dysfunction in Parkinson's disease. Antioxid Redox Sign 2012;16:920-34.
45. Swanson L. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 1982;9:321-53.
46. Bartels AL, Leenders KL. Cyclooxygenase and neuroinflammation in Parkinson's disease neurodegeneration. Curr Neuropharmacol 2010;8:62-8.
47. Santiago RM, Barbiero J, Martynhak BJ, Boschen SL, da Silva LM, Werner MF, et al. Antidepressant-like effect of celecoxib piroxicam in rat models of depression. J Neural Transm 2014;121:671-82.