

Evaluation of Betaine Neuroprotective Effects on 6-Hydroxy Dopamine Induced Hemi Parkinsonism in Male Wistar Rats

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Abstract

BACKGROUND: Parkinson's disease (PD) is one of the prevalent debilitating neurodegenerative disorders. Accordingly, researchers are working on methods to modify PD progression. Previously, the neuroprotective effects of betaine, as a methyl donor agent in homocysteine metabolism, have been demonstrated in animal models of chronic cerebral hypoperfusion and memory deficits.

OBJECTIVES: It was aimed to investigate the neuroprotective effects of betaine in an animal model of PD.

METHODS: In male Wistar rats under two-week course of oral betaine administration (50, 100, and 200 mg/kg per day), the behavioral, biochemical, and histological evaluations were conducted one week following unilateral nigral 6-OHDA injection.

RESULTS: Betaine administration with dose of 200 mg/kg, one week before and after 6-OHDA lesioning, was associated with a meaningful reduction in the plasma levels of homocysteine (Hcy) in comparison with the control and sham groups ($P < 0.05$). Our evaluations revealed a remarkable improvement in motor asymmetry induced by apomorphine in the rats under treatment of betaine 200 mg/kg. Moreover, in this group, a significant decrease of malondyaldehyde (MDA) concentrations was detected in the brain tissues, as well as a significantly diminished neuronal cell loss (percent) in substantia nigra pars compacta ($P < 0.05$). The results of 50 and 100 mg/kg betaine groups were not significant.

CONCLUSIONS: Altogether, our findings indicate the antioxidant neuroprotective effects of betaine in this animal model of PD and it is in concordance with betaine properties in decreasing the plasma levels and possible neurotoxic effects of Hcy.

KEYWORDS: betaine, homocysteine (Hcy), 6-Hydroxydopamine (6-OHDA), malondyaldehyde (MDA), Parkinson's disease (PD),

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Introduction

After Alzheimer's disease, Parkinson's disease (PD) is the second most-prevalent neurodegenerative disorder, which affects approximately 2-3% of humans more than 65 years of age (Poewe et al., 2017). Clinically, the core presentation of PD is bradykinesia in combination with rigidity and/or rest tremor (Postuma et al., 2015). Besides, the majority of PD patients are suffering the non-motor problems including sleep disorders, cognitive impairment, mood and affect disorders, autonomic dysfunction, and sensory symptoms (Chaudhuri & Schapira, 2009).

From the pathophysiological view, PD is mainly characterized by dopaminergic neurodegeneration in substantia nigra pars compacta (SNpc) and dopamine depletion in striatum (Golpich et al., 2015; Poewe et al., 2017). The dopaminergic neurodegeneration in PD is caused by different underlying mechanisms. In this regard, oxidative stress (OS) performs an important role. OS is a result of a disequilibrium between the produced levels of reactive oxygen species (ROS) and the reactive intermediates detoxifying system's ability which can lead to cell injury (Dias et al., 2013). It has been illustrated that, nigral dopaminergic neurons with high levels of dopamine and Fe²⁺ are vulnerable to OS. Since dopamine is an unstable molecule, its auto-oxidation forms quinones and free radicals that result in OS. In addition, metals, oxygen, and several enzymes such as tyrosinase can catalyze dopamine auto-oxidation. Moreover, dopamine metabolism can be mediated by other enzymes such as monoamine oxidase (MAO-A and MAO-B) and catechol-o-methyl transferase (COMT) in the catecholaminergic neurons. In PD with progressing of neuronal degen-

eration, MAO-B located in glial cells will increase and become a predominant enzyme in dopamine metabolism (Dias et al., 2013; Fowler et al., 1997). During dopamine degradation by MAO-B, the produced hydrogen peroxide can react with Fe²⁺ leading to formation of hydroxyl radicals and increasing the occurrence of OS (Hermida-Ameijeiras et al., 2004).

It has been shown that, under OS conditions, oxidative damages take place at the various compartments of cells generating lipid peroxidation, protein oxidation, DNA and RNA oxidation; subsequently, resulting in cell death. In addition, OS may be linked to other processes such as mitochondrial dysfunction, neuroinflammation, excitotoxicity, and nitrosative stress as the cascade of consequences associated with cell injury in PD (Ambrosi et al., 2014; Blesa et al., 2015). The link between OS and PD progression is the theoretical base of developing the animal models of PD with toxins such as 6-OHDA, MPTP, rotenone, paraquat in which the toxins induce oxidative stress (Dias et al., 2013).

Another agent that can induce cellular OS is homocysteine (Hcy), a non-essential amino acid with sulfhydryl group. Hcy is an intermediate product derived during methionine metabolism that can pass across blood brain barrier (BBB). Increased plasma levels of Hcy, hyperhomocysteinemia, is considered as a risk factor for a number of peripheral and cardio cerebrovascular diseases, cancers and neurodegenerative disorders (Ganguly & Alam, 2015; Zhang et al., 2015). Hcy can increase production of ROS along with inhibiting the enzymatic activity of antioxidants (Lehotsky et al., 2014; Skovierova et al., 2016). Although in several

studies it has been reported that 10-30% of PD patients have presented with hyperhomocysteinemia, it is not clear whether Hcy is a causative agent of PD onset or not. In this respect, according to the neurotoxic properties of Hcy, there are several suggestions to hypothesize that Hcy may contribute to pathophysiology of PD (Obeid et al., 2009; Sharma et al., 2015; Skovierova et al., 2016; Zoccolella et al., 2010).

Trimethylglycine, which is known as betaine, can transfer its methyl group to Hcy with the aid of the enzyme betaine homocysteine methyl transferase (BHMT). During this reaction with formation of dimethylglycine from betaine, Hcy is remethylated to methionine similar to another reaction pathway that catalyzes Hcy by methionine synthase using 5-methyltetrahydrofolate. Because BHMT is expressed mostly in liver and kidney, Hcy metabolism by the use of betaine takes place mainly in mentioned tissues (Imbard et al., 2015; Ueland et al., 2005). Considering betaine's effect on reduction of Hcy level, it has been hypothesized that administration of betaine can protect against hazardous effects of Hcy on cell damage. In this respect, previous animal studies have shown that the administration of betaine reduced plasma Hcy level; subsequently prevents oxidative stress in different tissues such as brain, cerebellum, liver, kidney, testis and ovary (Alirezaei, 2015; Alirezaei, et al., 2015; Alirezaei, et al., 2012; Kim et al., 2009; Tan et al., 2015).

In consideration of PD importance as one of the prevalent debilitating disorders, many research studies are conducted to identify the methods for modifying the trajectory of PD progression. In this respect, according to the above mentioned neuroprotective effects of betaine and probable role of Hcy in PD, this study is conducted with the main aim of

examining the effects of betaine administration in an animal model of PD induced by 6-OHDA, as neurotoxin. In this regard, betaine effects will be under study in relation with plasma Hcy levels as well as evaluating brain lipid peroxidation and histological alterations within SNpc.

Material and Methods:

Material

The homocysteine ELIZA kit was prepared from MyBioSource® (MBS703069, USA). Lipid peroxidation assay kit provided from Nalondi™ (NS-15022, Navand Salamat Co. Iran). Betaine, 6-hydroxy dopamine hydrochloride (6-OHDA), apomorphine hydrochloride and ascorbic acid were obtained from Sigma Chemical, St. Louis, Mo., USA.

Betaine solved in water with concentrations of 1, 2 and 4% and stored at 8 °C to perform gavage in different doses. 6-OHDA was solved in saline and 0.2 % ascorbic acid and used freshly. Apomorphine hydrochloride was solved in water with concentration of 0.05% and the fresh solvent was administered intraperitoneally (IP).

Experimental design

Adult male Wistar rats weighing between 190 and 240 grams were provided from the animal house of Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. Animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press). The rats were left in a colony room with controlled temperature under a 12:12 hours of light and dark cycle. The animals were permitted to have ad libitum access to food and tap water. This study was approved by the Institutional Animal Ethics Committee, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; no. 750600/6/12. Drug

administration, surgery, behavioral testing and blood and brain sampling were carried out between 08:00 and 17:00 hours. After at least one week of keeping the animals in the colony room, a total number of 40 rats with less than 30 net rotations following intraperitoneal administration of apomorphine hydrochloride (0.5 mg/kg), were included in our study. The animals were randomly divided into five equal groups ($n = 8$) in which the treated groups were administered betaine or water (vehicle of betaine) by daily gavage for two weeks. In this way, 6-OHDA lesioning was conducted one week after initiation of oral gavage. Our studied groups were as follows: a sham group, a control group gavaged daily with water, a 50 mg/kg betaine group, a 100 mg/kg betaine group and a 200 mg/kg betaine group which received betaine in mentioned doses by performing daily gavage. One hour after the last gavage, the animals were under behavioral assessment by rotation test and about 2 h after the behavioral testing, rats were deeply anesthetized by intraperitoneal injection of ketamine/xylazine (100 mg/kg and 5 mg/kg, respectively) in order to collect blood samples via the left ventricle after performing thoracotomy. Biochemical evaluations of four obtained blood samples in each group were conducted to identify Hcy plasma levels. In order to monitor the OS, five brains in each animal group were removed from the skulls, and immediately frozen and stored at -80°C to measure malondialdehyde (MDA) concentrations. In addition, the brains of three anesthetized rats in each group were prepared for histological assessment by intracardiac formalin 10% perfusion.

6-OHDA lesioning

Intranigral injection of 6-OHDA in the left brain hemisphere was performed via stereo-

taxic surgery in order to conduct 6-OHDA lesioning. After anesthetizing of male Wistar rat with ketamine (100 mg/kg IP) and xylazine (5 mg/kg IP), the animal was placed on the stereotaxic apparatus (Stoelting, USA) with a mounted 30-gauge needle, which was attached to a 10- μl Hamilton syringe. During 5 min, 8 μg 6-OHDA in 5 μl of saline and 0.2 % ascorbic acid was infused into the left SNpc. In our study, the coordinates of injection area were AP -4.8 mm, ML -2.2 mm from bregma fontanel and DV -7.8 mm from the surface of dura matter based on the Paxinos and Watson atlas (Paxinos & Watson, 2007).

Behavioral testing

One week following the 6-OHDA lesioning in laboratory room, each animal was kept in a plexiglass cylindrical container with 33 cm diameter and 35 cm height. After 10 min of habituation, apomorphine hydrochloride was administered intraperitoneally (0.5 mg/kg) and 1 min later, full rotations were counted at 10 min intervals for 1 h. As described previously, the positive scores were assigned to the full rotations contralateral to the 6-OHDA lesioned side (right turns) and the negative scores were assigned to the full rotations ipsilateral to the damaged brain hemisphere (left turns). At last, net number of rotations was defined by subtracting the mentioned values (Fujita et al., 1996; Rezaei & Alirezaei, 2014).

Measurement of Hcy concentration

Within 30 min after blood sampling through left ventricle, in order to prevent the release of red blood cells Hcy, the plasma samples were collected by centrifuging EDTA blood for 15 min at $1000 \times g$. Immediately, the plasma samples were stored at -80°C until the Hcy level measurement. The Hcy concentrations of plasma were quantitatively determined according to the method described by rat

Hcy ELISA kit (My Bio Source, Cat # MBS703069) in which the biochemical technique is based on Hcy antibody-Hcy antigen interactions and a horseradish peroxidase (HRP) colorimetric detection system. The Hcy concentrations were expressed as micromoles per liter ($\mu\text{mol/l}$) of plasma.

Evaluation of lipid peroxidation

Lipid peroxidation as an indicator of oxidative stress in cells can be detected by monitoring of its naturally occurring product, namely MDA (Tsikas, 2017). In this regard, we measured the MDA levels in homogenized brain tissue by the use of lipid peroxidation assay kit (Nalondi™, Iran) based on the manufacturer's instructions. In brief, forebrain and brainstem (1g), containing the mesencephalon, were thawed and manually homogenized in RIPA buffer (Gibco, USA), and the protease inhibitor PMSF (Gibco, USA). Debris was removed by centrifugation and the supernatant was recovered and used for evaluation of MDA concentration and protein content. By utilizing thiobarbituric acid (TBA) at high temperatures, the MDA content in supernatant reacted to TBA and produced a pink to red derivative quantified by colorimetric method at the wavelength of 530–540 nm. The concentrations of MDA were expressed as nanomoles per milligram of tissue protein (nmol/mg protein) (Hamzyan Olia et al., 2017). In addition, total protein concentration in the brain homogenate was determined by BCA protein quantification kit (DNAbiotech Co. Iran).

Histological study

Following formalin perfusion, the brains of rats were removed from the skulls and post-fixed in 10% formalin. The fixed brain tissues were cut coronally from the brain region 3.2 to 4.2 mm anterior of the interaural line containing substantia nigra (Paxinos &

Watson, 2007). These tissue slices were routinely processed for light microscopy and then embedded in paraffin. By means of a microtome (Leica Microsystems, Wetzlar, Germany) the paraffin-embedded blocks were cut into the 5 μm coronal sections and three slides were selected with at least 100 μm interval thickness. Afterwards, sections were deparaffinized by xylene (Merck, Germany), rehydrated through a graded ethanol series; eventually stained by 0.1 % cresyl violet solution (Sigma-Aldrich, St. Louis, Mo, USA). The slides were observed under light microscope equipped with camera (YS2-T, Nikon, Tokyo, Japan) and ImageJ software (NIH, Maryland, USA).

Only Nissl stained neurons with a nucleus, which contains visible nucleolus, within SNpc region were counted by a trained observer who was blind to the groups' status. By comparing the ipsilateral and contralateral cell count difference to the contralateral cell count in each rat, the percentage of neuronal cell loss was determined and considered as sample data to perform analysis (Browne et al., 2017).

Statistical analysis

To analysis of the extracted data between groups, the one-way analysis of variance (ANOVA) bootstrap method was used with Tukey's post hoc test to do multiple comparisons. Our extracted data presented the number of rotations induced by apomorphine as behavioral testing. We also showed the levels of plasma Hcy in parallel to betaine therapy in rats and MDA levels in brain tissue as a lipid peroxidation marker. Moreover, the percentage of neuronal cell loss under the effects of neurotoxin 6-OHDA or its vehicle was analyzed as histological evaluation. All the mentioned data were expressed as mean \pm SEM and the significance level was set at $P < 0.05$.

Results

There was no significant weight loss and mortality detected in any of the groups after operating gavage nor stereotactic surgery.

Rotation numbers

The effect of betaine administration on behavioral presentation was evaluated by analyzing the net number of rotations induced by apomorphine over one hour rotation testing. There was no significant rotation diversity between groups during behavioral assessment at the initiation of our animal research, while the relevant statistical analysis one week after stereotactic surgery was noticeable. Following 6-OHDA lesioning, apomorphine induced significant contralateral rotations in the animals of the control group ($P < 0.0001$) and in the rats, which were under treatment of betaine with doses 50 mg/kg ($P = 0.002$), and 100 mg/kg ($P < 0.0001$) during comparison with the sham group. A significant reduction in rotations was recorded from the rats under two weeks' oral administration of 200 mg/kg betaine in comparison with the control ($P < 0.0001$), betaine

50 mg/kg ($P = 0.001$), and betaine 100 mg/kg ($P < 0.0001$) groups ($F = 14.86$). All data summarized in Fig. 1.

Biochemical evaluations

Treatments of rats with betaine 200 mg/kg significantly decreased the plasma levels of Hcy as compared to the sham and control groups with p values of 0.028 and 0.017 respectively (Fig. 2). The non-significantly reduced plasma Hcy levels were detected in animals under administration of betaine with doses 50 and 100 mg/kg in comparison with the rats in sham and control groups ($F = 5.43$).

In terms of the measuring MDA levels in brain tissues, the statistical analysis revealed that in sham group and in animals of treatment group with betaine 200 mg/kg, the MDA levels that were high significantly decreased as compared to the control and betaine groups with doses of 50 and 100 mg/kg ($F = 142.53$, $P < 0.0001$). Non-significant upper MDA concentrations were detected in the brain tissues of the rats in betaine 200 mg/kg group versus sham group (Fig. 3).

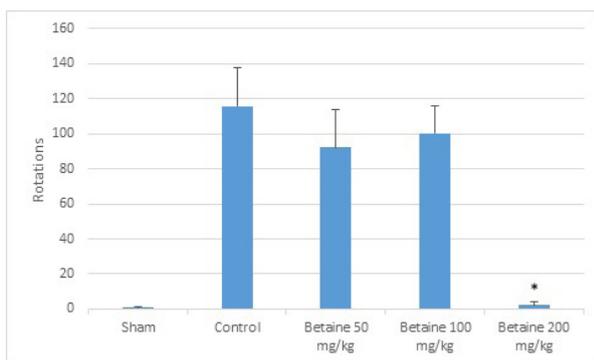


Figure 1. Net number of rotations (mean \pm SEM) in experimental groups ($n = 8$) within one hour following IP injection of apomorphine 0.5 mg/kg, one week after stereotactic surgery. * Treatment with 200 mg/kg of betaine significantly associated with decreased net rotation numbers versus the control, betaine 50 mg/kg, and betaine 100 mg/kg groups ($P < 0.05$).

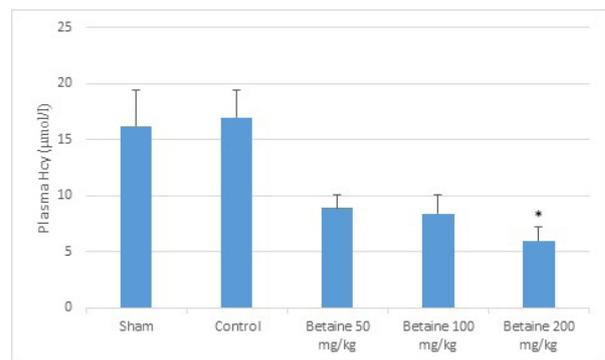


Figure 2. Homocysteine (Hcy) concentrations of plasma (Mean \pm SEM) among our studied groups. * The animals under treatment of two-week course of betaine with dose of 200 mg/kg represented a significant reduction in the plasma levels of Hcy ($\mu\text{mol/l}$) as compared to the animals in sham ($P = 0.028$) and control ($P = 0.017$) groups, without betaine administration.

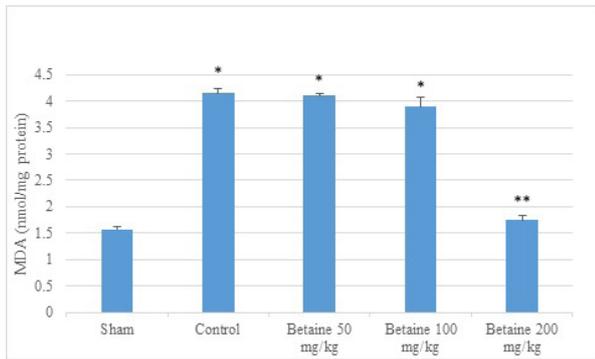


Figure 3. Brain tissue malondialdehyde (MDA) concentrations (Mean \pm SEM) in experimental groups. * The intranigral injection of 6-OHDA in animals within the control group and the animals under treatment of betaine with doses of 50 and 100 mg/kg resulted in significantly elevated brain tissue MDA concentrations in comparison with the sham group ($P < 0.05$). ** MDA levels (nmol/mg protein) in the brain tissues of the rats under treatment of betaine 200 mg/kg, in spite of 6-OHDA administration, were associated with a remarkable reduction in comparing with the control, and betaine 50 and 100 mg/kg groups ($P < 0.05$).

Histological examination

In consideration of histological assessment, our findings yield that the percentage of neuronal cell loss was significantly increased, under the effect of neurotoxin 6-OHDA, in the control, betaine 50 mg/kg, and betaine 100 mg/kg groups in comparison with the sham group ($P < 0.0001$). In the betaine 200 mg/kg group, the percentage of neuronal loss was meaningfully diminished as compared to the control and betaine groups with doses 50 mg/kg and 100 mg/kg ($F = 55.68$, $P < 0.0001$). However, the percentage of neuronal cell loss in rats with treatment of betaine 200 mg/kg was non-significantly higher than that of sham group (Fig. 4).

Discussion

Previously, the neuroprotective effects of betaine have been shown in the animal models for chronic cerebral hypoperfusion and memory deficits (Chai et al., 2013; Nie et

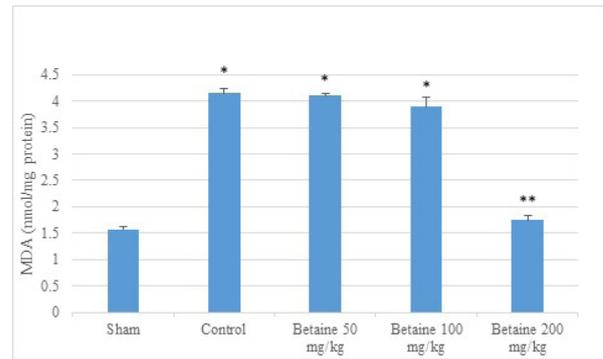


Figure 4. The percent of neuronal loss in SNpc (Mean \pm SEM) was determined by comparing the difference between Nissl- stained neurons number of ipsilateral and contralateral sides of 6-OHDA injection to the neurons number of contralateral side. * Under influence of unilateral neurotoxin administration, the neuronal cell loss percent was significantly increased in the control, and betaine 50 mg/kg and 100 mg/kg animals in comparison with the rats in the sham group, which were unilaterally under the injection of vehicle ($P < 0.05$). ** A significant reduction of neuronal cell loss percent was detected in animals under treatment of betaine with dose of 200 mg/kg versus the control group ($P < 0.05$).

al., 2016). Here, we demonstrated the neuroprotective efficacy of betaine with dose of 200 mg/kg, but not 50 and 100 mg/kg, in an animal model of PD induced by 6-OHDA. Within our research, we evaluated the betaine effects from multiple perspectives comprising behavioral, biochemical and histological aspects.

A prominent number of neurons within SNpc are dopaminergic (Ip et al., 2017; Nair-Roberts et al., 2008). Destructing these dopaminergic neurons by administration of neurotoxins can, to some extent, mimic the PD pathophysiology, which has been processed as an animal model of PD in diverse research (Fabricius et al., 2017). In this view, unilateral intranigral injection of 6-OHDA, as the most commonly used neurotoxin in rodents, exhibits prominent motor asymmetry caused by unilateral nigral dopaminergic neuronal loss and dopamine depletion in striatum. Unilateral dopamine depletion in stri-

tum subsequently alters the striatal dopamine D1 and D2 receptors sensitivity, unilaterally. The motor asymmetry, which is developed by change in sensitivity of mentioned receptors, can be assessed by inspecting the number of rotations under exposure of dopamine agonists that stimulate striatal dopamine receptors either directly (i.e. apomorphine) or indirectly (i.e. amphetamine) (Konieczny et al., 2017). In this research, while performing rotation test by apomorphine one week after unilateral, left side, intranigral injection of 6-OHDA, a significantly decreased rotation number has been observed in rats with two weeks treatment of betaine 200 mg/kg compared to the control group (Fig. 1). The amelioration of turning behavior can be ascribed to the probable neuroprotective properties of betaine, in mentioned dose, against the neurodegenerative effects of 6-OHDA. On the one hand, the possible neuroprotection induced by betaine eventuated in preserving a more number of dopaminergic neurons that can lead to maintaining left nigrostriatal dopamine at a level in which the sensitivity of striatal dopamine receptors was not different enough from the contralateral side to generate marked turning behavior, under stimulation of apomorphine.

Moreover, our histological evaluations, carried out in the control group, showed the partial lesioning of Nissl-stained neurons in SNpc. In this regard, the histological study revealed a significant reduction in neuronal loss percentage in betaine 200 mg/kg group in comparison with the control group (Fig. 4). As mentioned before, it has been demonstrated that the prominent number of Nissl-stained neurons within SNpc are dopaminergic and 6-OHDA, as a selective catecholaminergic neurotoxin, has toxic effects on dopaminergic cells (Konieczny et

al., 2017; Nair-Roberts et al., 2008). In relevance, since we obtained more number of intact Nissl-stained neurons along with attenuated rotational behavior in betaine 200 mg/kg group, it can be concluded that betaine may protect the dopaminergic neurons toward neurotoxic effects of 6-OHDA.

As hinted above, several lines of evidence indicate that OS has an important role in inducing dopaminergic neuronal degeneration in PD and in animal model of PD under the influence of 6-OHDA. OS can induce cellular damages at the various compartments generating lipid peroxidation, protein, DNA and RNA oxidation (Blesa et al., 2015; Dias et al., 2013). During lipid peroxidation, MDA can be formed, as secondary products, among producing the many different aldehydes. Measuring MDA tissue levels, as a convenient biomarker for lipid peroxidation, has been widely used in different research (Ayala et al., 2014). Accordingly, in the present study, we evaluate the MDA levels in homogenized brain tissue with respect to the monitoring of OS, induced by neurotoxin. Our findings significantly revealed the decreased levels of MDA in betaine 200 mg/kg group in comparison with the control group which can show the possible antioxidant role of betaine, with depicted dose, against OS induced by 6-OHDA. This result is in concordance with our mentioned behavioral and histological evaluations.

With taking into account Hcy properties which lead to cell damage via different pathways such as inducing OS, and the influence of betaine on reducing hyperhomocysteinemia, it has been hypothesized that betaine can protect cells by detracting the Hcy levels (Alirezai et al., 2015). Analysis of the extracted data in the present research indicated a significant reduction in concentrations of

plasma Hcy in animals under administration of betaine with dose of 200 mg/kg as compared to the control and sham groups. Statistically significant decreased levels of Hcy, under exposure of betaine, were associated with a meaningful reduction of MDA concentrations in brain tissues as well as a significantly diminished neuronal cell loss percentage in SNpc and remarkable improvement in motor asymmetry recorded during behavioral testing. However, as pointed out above, the findings of clinical trials concerning the influence of Hcy on pathophysiology of PD are controversial, several researches have verified a close relationship between hyperhomocysteinemia and PD (Xie et al., 2017; Zoccolella et al., 2010). Likewise, the results of our study on this model of PD highlighted the role of Hcy in PD pathogenesis.

Altogether, the extracted data in the present manuscript postulates the antioxidant neuroprotective effects of betaine in our applied model of PD. Here, betaine represents the neuroprotective properties along with detracting the plasma levels of Hcy. In this view, by consideration of Hcy toxic effects, it is suggested that betaine applies the neuroprotection through effects on Hcy metabolism. Nevertheless, it is not clear whether betaine performs its neuroprotective role solely via Hcy methylation or through additional pathways from Hcy metabolism. This issue remains to be determined in the future research. Moreover, in order to have a better outline, the possible neuroprotective effects of betaine on underlying mechanisms of PD progression, in addition to OS, should be under evaluation.

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Conflicts of Interest

The author declared no conflict of interest.

Informed Consent

This manuscript does not contain any studies with human subjects performed by any of the authors.

Human and Animal Rights

All experiments were executed according to the Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethics Committee.

References

- Alirezaei, M. (2015). Betaine protects cerebellum from oxidative stress following levodopa and benserazide administration in rats. *Iran J Basic Med Sci*, 18(10), 950–957. <http://doi.org/10.22038/IJBMS.2015.5457> PMID: 26730328
- Alirezaei, M., Khoshdel, Z., Dezfoulian, O., Rashidipour, M., & Taghadosi, V. (2015). Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. *J Physiol Sci*, 65(3), 243–252. <http://doi.org/10.1007/s12576-015-0360-0> PMID: 25665954
- Alirezaei, M., Niknam, P., & Jelodar, G. (2012). Betaine elevates ovarian antioxidant enzyme activities and demonstrates methyl donor effect in non-pregnant rats. *Int J Pept Res Ther*, 18(3), 281–290. <http://doi.org/10.1007/s10989-012-9300-5>
- Ambrosi, G., Cerri, S., & Blandini, F. (2014). A further update on the role of excitotoxicity in the pathogenesis of Parkinson's disease. *J Neural Transm (Vienna)*, 121(8), 849–859. <http://doi.org/10.1007/s00702-013-1149-z>

- Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid Med Cell Longev*, 1–31. <http://doi.org/10.1155/2014/360438> PMID: 24999379
- Blesa, J., Trigo-Damas, I., Quiroga-Varela, A., & Jackson-Lewis, V. R. (2015). Oxidative stress and Parkinson's disease. *Front Neuroanat*, 9, 91. <http://doi.org/10.3389/fnana.2015.00091>
- Chai, G. S., Jiang, X., Ni, Z. F., Ma, Z. W., Xie, A. J., Cheng, X. S., Liu, G. P. (2013). Betaine attenuates Alzheimer-like pathological changes and memory deficits induced by homocysteine. *J Neurochem*, 124(3), 388–396. <http://doi.org/10.1111/jnc.12094> PMID: 23157378
- Chaudhuri, K. R., & Schapira, A. H. (2009). Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. *Lancet Neurol*, 8(5), 464–74. [http://doi.org/10.1016/S1474-4422\(09\)70068-7](http://doi.org/10.1016/S1474-4422(09)70068-7) PMID: 19375664
- Committee for the Update of the Guide for the Care and Use of Laboratory Animals; National Research Council. (2010). *Guide for the Care and Use of Laboratory Animals: Eighth Edition. Guide for the Care and Use of Laboratory Animals.* <http://doi.org/10.2307/1525495>
- Dias, V., Junn, E., & Mouradian, M. M. (2013). The role of oxidative stress in parkinson's disease. *J Parkinsons Dis*, 3(4), 461–91. <http://doi.org/10.3233/JPD-130230> PMID: 24252804
- Fabricius, K., Barkholt, P., Jelsing, J., & Hansen, H. H. (2017). Application of the Physical Disector Principle for Quantification of Dopaminergic Neuronal Loss in a Rat 6-Hydroxydopamine Nigral Lesion Model of Parkinson's Disease. *Front Neuroanat*, 11, 109. <http://doi.org/10.3389/fnana.2017.00109> PMID: 29276478
- Fowler, J. S., Volkow, N. D., Wang, G. J., Logan, J., Pappas, N., Shea, C., & MacGregor, R. (1997). Age-related increases in brain monoamine oxidase B in living healthy human subjects. *Neurobiol Aging*, 18(4), 431–5. [http://doi.org/10.1016/S0197-4580\(97\)00037-7](http://doi.org/10.1016/S0197-4580(97)00037-7)
- Fujita, M., Nishino, H., Kumazaki, M., Shimada, S., Tohyama, M., & Nishimura, T. (1996). Expression of dopamine transporter mRNA and its binding site in fetal nigral cells transplanted into the striatum of 6-OHDA lesioned rat. *Mol Brain Res*, 39(1–2), 127–136. [http://doi.org/10.1016/0169-328X\(96\)00018-6](http://doi.org/10.1016/0169-328X(96)00018-6)
- Ganguly, P., & Alam, S. F. (2015). Role of homocysteine in the development of cardiovascular disease. *Nutr J*, 14, 6. <http://doi.org/10.1186/1475-2891-14-6> PMID: 25577237
- Golpich, M., Rahmani, B., Mohamed Ibrahim, N., Dargahi, L., Mohamed, Z., Raymond, A. A. li, & Ahmadiani, A. (2015). Preconditioning as a potential strategy for the prevention of Parkinson's disease. *Mol Neurobiol*, 51(1), 313–30. <http://doi.org/10.1007/s12035-014-8689-6> PMID: 24696268
- Hamzyan Olia, JB; Khadem Ansari, MH; Yaghmaei, P; Ayatollahi, H; Khalkhali, H. (2017). Evaluation of oxidative stress marker in patients with human papillomavirus infection. *Ann Trop Med Public Health*, 10, 1518–23. http://doi.org/10.4103/ATMPH.ATMPH_464_17
- Hermida-Ameijeiras, Á., Méndez-Álvarez, E., Sánchez-Iglesias, S., Sanmartín-Suárez, C., & Soto-Otero, R. (2004). Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: Role of ferrous and ferric ions. *Neurochem Int*, 45(1), 103–16. <http://doi.org/10.1016/j.neuint.2003.11.018> PMID: 15082228
- Imbard, A., Benoist, J.-F., Esse, R., Gupta, S., Lebon, S., de Vriese, A. S., ... Blom, H. J. (2015). High homocysteine induces betaine depletion. *Biosci Rep*, 35, 4. <http://doi.org/10.1042/BSR20150094> PMID: 26182429
- Ip, C. W., Cheong, D., Volkmann, J. (2017). Stereological Estimation of Dopaminergic Neuron Number in the Mouse Substantia Nigra Using the Optical Fractionator and Standard Microscopy Equipment. *J Vis Exp*, (127). <http://doi.org/10.3791/56103> PMID: 28892024
- Karicheti, V., Browne, K. D., Burell, J. C., Cullen, D. K. (2017). Characterization of Parkinson

- Model : Cellular Loss Following Unilateral Lesion of the Substantia Nigra with 6-OHDA. Chares River Laboratories International, Inc.
- Kim, S. K., Seo, J. M., Chae, Y. R., Jung, Y. S., Park, J. H., & Kim, Y. C. (2009). Alleviation of dimethylnitrosamine-induced liver injury and fibrosis by betaine supplementation in rats. *Chem Biol Interact*, 177(3), 204–211. <http://doi.org/10.1016/j.cbi.2008.09.021> PMID: 18930038
- Konieczny, J., Czarnecka, A., Lenda, T., Kamińska, K., & Antkiewicz-Michaluk, L. (2017). The significance of rotational behavior and sensitivity of striatal dopamine receptors in hemiparkinsonian rats: A comparative study of lactacystin and 6-OHDA. *Neuroscience*, 340, 308–318. <http://doi.org/10.1016/j.neuroscience.2016.10.070> PMID: 27826109
- Lehotsky, J., Petras, M., Kovalska, M., Tothova, B., Drgova, A., & Kaplan, P. (2014). Mechanisms Involved in the Ischemic Tolerance in Brain: Effect of the Homocysteine. *Cell Mol Neurobiol*, 35(1), 7–15. <http://doi.org/10.1007/s10571-014-0112-3> PMID: 25194713
- Nair-Roberts, R. G., Chatelain-Badie, S. D., Benson, E., White-Cooper, H., Bolam, J. P., & Ungless, M. A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*, 152(4), 1024–31. <http://doi.org/10.1016/j.neuroscience.2008.01.046> PMID: 18355970
- Nie, C., Nie, H., Zhao, Y., Wu, J., & Zhang, X. (2016). Betaine reverses the memory impairments in a chronic cerebral hypoperfusion rat model. *Neurosci Lett*, 615, 9–14. <http://doi.org/10.1016/j.neulet.2015.11.019> PMID: 26592482
- Obeid, R., Schadt, A., Dillmann, U., Kostopoulos, P., Fassbender, K., & Herrmann, W. (2009). Methylation status and neurodegenerative markers in Parkinson disease. *Clin Chem*, 55(10), 1852–1860. <http://doi.org/10.1373/clinchem.2009.125021> PMID: 19679632
- Park, M. J., Kim, S. R., Huh, H., Jung, J. H., & Kim, Y. C. (1994). Betaine attenuates glutamate-induced neurotoxicity in primary cultured brain cells. *Arch Pharm Res*, 17(5), 343–347. <http://doi.org/10.1007/BF0297417>
- Paxinos, G., & Watson, C. R. (2007). *The Rat Brain in Stereotaxic Coordinates*. Academic Press, San Diego, 3rd.
- Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkman, J., ... Lang, A. E. (2017). Parkinson disease. *Nat Rev Dis Primers*, 3, 17013. <http://doi.org/10.1038/nrdp.2017.13> PMID: 28332488
- Postuma, R. B., Berg, D., Stern, M., Poewe, W., Olanow, C. W., Oertel, W., ... Deuschl, G. (2015). MDS clinical diagnostic criteria for Parkinson's disease. *Mov Disord*, 30(12), 1591–601. <http://doi.org/10.1002/mds.26424> PMID: 26474316
- Rezaei, M., & Alirezai, M. (2014). Protective effects of *Althaea officinalis* L. extract in 6-hydroxydopamine-induced hemi-Parkinsonism model: Behavioral, biochemical and histochemical evidence. *J Physiol Sci*, 64(3), 171–176. <http://doi.org/10.1007/s12576-014-0305-z> PMID: 24464760
- Sharma, M., Tiwari, M., & Tiwari, R. K. (2015). Hyperhomocysteinemia: Impact on Neurodegenerative Diseases. *Basic Clin Pharmacol Toxicol*, 117(5), 287–296. <http://doi.org/10.1111/bcpt.12424> PMID: 26036286
- Skovierova, H., Vidomanova, E., Mahmood, S., Sopkova, J., Drgova, A., Cervenova, T., ... Lehotsky, J. (2016). The molecular and cellular effect of homocysteine metabolism imbalance on human health. *Int J Mol Sci*, 17(10). <http://doi.org/10.3390/ijms17101733> PMID: 27775595
- Tan, D., Wang, Y., Bai, B., Yang, X., & Han, J. (2015). Betanin attenuates oxidative stress and inflammatory reaction in kidney of paraquat-treated rat. *Food Chem Toxicol*, 78, 141–146. <http://doi.org/10.1016/j.fct.2015.01.018> PMID: 25662031
- Tsikas, D. (2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal Bio-*

- chem, 524, 13-30. <http://doi.org/10.1016/j.ab.2016.10.021> PMID: 27789233
- Ueland, P. M., Holm, P. I., & Hustad, S. (2005). Betaine: A key modulator of one-carbon metabolism and homocysteine status. *Clin Chem Lab Med*, 43(10), 1069-75. <http://doi.org/10.1515/CCLM.2005.187> PMID: 16197300
- Xie, Y., Feng, H., Peng, S., Xiao, J., & Zhang, J. (2017). Association of plasma homocysteine, vitamin B12 and folate levels with cognitive function in Parkinson's disease: A meta-analysis. *Neurosci Lett*, 636, 190-195. <http://doi.org/10.1016/j.neulet.2016.11.007> PMID: 27840145
- Zhang, D., Wen, X., Wu, W., Guo, Y., & Cui, W. (2015). Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: Meta-analysis of 83 case-control studies involving 35,758 individuals. *PLoS One*, 10(5), e0123423. <http://doi.org/10.1371/journal.pone.0123423> PMID: 25985325
- Zoccolella, S., dell'Aquila, C., Specchio, L. M., Logroscino, G., & Lamberti, P. (2010). Elevated homocysteine levels in Parkinson's Disease: is there anything besides L-dopa treatment? *Curr Med Chem*, 17(3), 213-21. <http://doi.org/10.2174/092986710790149774> PMID: 20214564

ارزیابی اثرات محافظتی بتائین بر پارکینسون یکطرفه القاء شده توسط ۶ - هیدروکسی دوپامین در رت های نر نژاد ویستار

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چکیده

زمینه مطالعه: بیماری پارکینسون یکی از بیماری های ناتوان کننده و شایع با منشأ تخریب نورونی می باشد. بر این اساس، امروزه محققان به دنبال یافتن روش های درمانی مؤثر در جلوگیری از روند پیشرفت این بیماری هستند. در تحقیقات به عمل آمده اثرات محافظتی بتائین (به عنوان عامل دهنده گروه متیل در مسیر متابولیسم هموسیستئین) در مدل های تحقیقاتی کاهش مزمن خونرسانی مغز و اختلال حافظه، نشان داده شده است.

هدف: این مطالعه با هدف بررسی اثرات محافظتی بتائین در مدل حیوانی بیماری پارکینسون یکطرفه، شکل گرفته است.

روش کار: سوپهدر این پژوهش ارزیابی های رفتاری، بیوشیمیایی و بافت شناسی بر روی رت های نر نژاد ویستار که تحت تجویز دو هفته ای بتائین به صورت خوراکی با دوزهای روزانه ۵۰، ۱۰۰ و ۲۰۰ mg/kg بودند به دنبال تزریق نوروتوکسین ۶- هیدروکسی دوپامین در ناحیه متراکم جسم سیاه نیمکره چپ (به منظور القاء پارکینسون یکطرفه) انجام شد.

نتایج: مراحل تجویز بتائین در حیوانات با دوز روزانه ۲۰۰ mg/kg از یک هفته قبل تا یک هفته پس از تزریق استریوتاکسیک ۶- هیدروکسی دوپامین، با کاهش معنادار سطوح پلاسمایی هموسیستئین نسبت به حیوانات گروه های شم و کنترل همراه بود. در این گروه، نسبت به گروه کنترل، کاهش معناداری در تحلیل داده های استخراج شده از آزمون رفتاری چرخش، ملاحظه شد. همچنین، بررسی های بیوشیمیایی بافت مغزی حیوانات، که به منظور ارزیابی میزان استرس اکسیداتیو نورون ها در اثر تزریق نوروتوکسین ۶- هیدروکسی دوپامین به عمل آمد، نشانگر کاهش معنادار غلظت های مالون دی آلدئید در گروه بتائین ۲۰۰ mg/kg نسبت به گروه های شم و کنترل بود. در نهایت، درصد نورون های تخریب شده ناحیه متراکم جسم سیاه نیمکره چپ در حیوانات گروه بتائین ۲۰۰ mg/kg با کاهش معناداری نسبت به گروه کنترل، همراه بود ($P < 0.05$). در بررسی های مشابه انجام شده در گروه های بتائین ۵۰ و ۱۰۰ mg/kg تغییرات معناداری نسبت به گروه کنترل، ملاحظه نگردید.

نتیجه گیری نهایی: یافته های این پژوهش بیانگر اثرات آنتی اکسیدانی و محافظتی بتائین در مدل تحقیقاتی پارکینسون یکطرفه می باشد که با ویژگی بتائین در کاهش سطوح پلاسمایی هموسیستئین و در نتیجه کاهش اثرات سمی احتمالی هموسیستئین هم خوانی دارد.

واژه های کلیدی:

بیماری پارکینسون، بتائین، هموسیستئین، مالون دی آلدئید، ۶- هیدروکسی دوپامین