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Research Article

Evaluation of Antioxidant and Anti Parkinsonism Activity of Betaine in Experimental Rats

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ABSTRACT

Aim and Objectives: The present study was aimed to evaluate anti parkinsonism effect of Betaine for its Applications in trigger factors in pathogenesis of Parkinson's disease and to understand development of new treatments approaches for PD. Betaine is naturally obtained product. It has antioxidant, neuroprotective activity. Hence, we inspected whether betaine can act as a protective agent in 6-OHDA induced oxidative stress on cerebellum of Sprague-Dawley rats.

Material and Methods Thirty-six adult Sprague-Dawley rats were divided into six groups. Rats were received unilateral 6-hydroxydopamine lesions for induction except normal and rats were treated with respective treatment. At the day of 21 rats were sacrificed. Prepared brain homogenate was used for further Biochemical estimation.

Result: Betaine showed marked rise in SOD and Catalase activity as well as GSH content subsequently decreasing in the lipid peroxidation process. Our result suggests Betaine to be potent antioxidant at dose 12.5 and 25 mg/kg as compared to standard (L-dopa+Benserazide) and pro-inflammatory cytokines viz: TNF- α , IL-1 β and IL-6 were significantly reversed by Betaine as compared to that of standard group (L-dopa+ Benserazide).

Discussion and Conclusion: Betaine showed dose dependent effect by reducing LPO level as increasing SOD, GSH and Catalase activity and marked reduced proinflammatory cytokine, hence we conclude that betaine has good anti parkinsonism activity.

Keywords: 6-OHDA, Antioxidant, Betaine, Pro-inflammatory cytokines.

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INTRODUCTION

Parkinsonism (PD) is slowly progressive neurodegenerative disease caused due to die of brain cell that control body movements. It is described by James Parkinson in 1817. It is mainly characterised clinically by bradykinesia, tremor, rigidity and postural instability¹. Prevalence of PD increase with age near about 1% people with above 60 age affected by parkinsonism². In pathophysiology of PD increase in cytokine level as increases the oxidative stress as well as change in behavioural changes occurs³. 6-Hydroxydopamine (6-OHDA)-induced neurodegeneration is a well known experimental model for PD. It is selectively taken up by the dopamine transporters of dopaminergic neurons, and it induces retrograde neurodegeneration. Thus, 6-OHDA injections into the striatum or medial forebrain bundle induce nigral degeneration that then results in

characteristic behavior abnormalities with change biochemical parameters in experimental animals^{4,5}.

Betaine is found in plants, animals and microorganisms and is a significant component of many foods, including wheat, shellfish, spinach, and sugar beets⁶. It is a zwitter ionic quaternary ammonium compound that is also known as trimethylglycine, glycine betaine, lycine, and oxyneurine. It has physiologic function either as an organic osmolyte to protect cells under stress. The principle role of Betaine is to protect cells against osmotic inactivation in plants and microorganisms. Betaine synthesis is triggered due to exposure to drought, high salinity, or temperature stress, which results in its accumulation in the cells. It increases the water retention of cells, replaces inorganic salts, and protects intracellular enzymes against temperature-induced or osmotically induced inactivation as it is a compatible osmolyte⁷. It has been reported as Betaine is for the number of several conditions of disease

involving brain, heart and kidney. It also has potential as a neuroprotective agent for prevention of LD induced oxidative damage in brain tissue of rats. It has been demonstrated that Betaine may have a potential as a neuroprotective agent for prevention of LD induced oxidative damage in cerebellum and Benserazide mediated hyperhomocysteinemia in rats⁸. It prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum⁷. In suggesting Betaine may have a therapeutic neuroprotective effect of PD. It is also has been used in the treatment of elevated homocysteine¹⁰.

MATERIAL AND METHODS

Animals and groups

Sprague-Dawley male rats with 200-250 gm were used for this study. Animal were procured from National Institute of Bioscience (CPCSEA reg. no. 1139/a/07/CPCSEA) and housed at the Institute Animal House at standard laboratory conditions with a temperature of 25±1°C, relative humidity of 50–65% and 12:12 h dark and light cycle. Animals had free access to food (Standard chaw pellets, Nutrivet life sciences, Pune) and water with *ad libitum*. The experimental protocol (Protocol approval no. SIOP/IAEC/2017/02/03) was approved by the Institutional Animal Ethics Committee (IAEC) constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experimental Animals, India (CPCSEA).

Chemicals and Drugs

Betaine, Apomorphine hydrochloride hemihydrates, Desipramine HCL, 6-Hydroxydopamine Hydrochloride, Benserazide Sigma Aldrich), Ketamine (Themis Medicare), Xylazine (Indian Immunological LTD. Telangana.), L-Dopa, Dopamine (Aqua Fine Injecta PVT. LTD. Pune), Haloperidol (RPG Lifescience).

Experimental Protocol

Two weeks after surgery, rats were randomly divided into six groups based on the number of contralateral rotations

Group 1: Normal- received vehicle for 21 days

Group 2: Control- Rats received unilateral 6-hydroxydopamine

Group 3: Standard - received 6- hydroxydopamine + L-Dopa 10mg/kg along with Benserazide 2.5 mg/kg orally

Group 4 to 6: Test-I, II and III- received 6-hydroxydopamine + 12.5mg/kg, 25 mg/kg, 50 mg/kg betaine orally for 21 days.

6-Hydroxydopamine (6-OHDA) lesioning

Rats received unilateral 6- hydroxydopamine lesions medial forebrain bundle to destroy DA neurons. Desipramine HCL (25mg/kg, i.p.) was given prior to the 6-OHDA injection to protect norepinephrine (NE) neurons. Rats were anesthetized with Ketamine (80 mg/kg, i.p.) and then placed in a stereotaxic apparatus (Inco Ambala, India). The coordinates for 6-OHDA injections were AP: -2.5mm, ML: +2.0 mm, DV: -9 mm relative to bregma with the incisor bar positioned 3.3 mm below the interaural line. Using a 10µl Hamilton syringe attached to a 26 gauge needle, 6-OHDA (12 µg) dissolved in 0.9% NaCl + 0.1% ascorbic acid was infused through a small burr hole in the skull at a rate of 2 µl/min for a total volume of 4 µl. The needle was withdrawn 1 m later. Two weeks after the surgery rats were challenged with apomorphine hydrochloride (0.2 mg/kg, s.c.) and contralateral rotation

was monitored. Animals showing fewer than 20 rotations per 5 min were excluded from further studies¹⁰⁻¹².

Behavioural Parameter

Grid test

Gridiron of 30 cm wide and 35 cm high with a space of 1.2 cm between each wire was used. Each rat was hung by all four paws on the vertical grid and stopwatch was started as the rat was held on the grid. A stopwatch was stopped and time taken by the rats was noted as descent latency. The maximum cut of time was 180 seconds¹³.

Catalepsy Bar test

A rat was placed with both forepaws on a bar, which was 10 cm above the surface in half rearing position (Catalepsy Bar Test, VJ Instruments India). The maximum cut of time for 180 seconds¹⁴.

Locomotor Activity

Spontaneous locomotor activity was assessed in the Actophotometer. Rats were placed in Digital Photoactometer the center allowed to freely explore it for 5 min. Evaluate the total no. of counts as an indicator of spontaneous locomotor activity¹⁵.

Evaluation of the oxidative stress

Estimation of Reduced Glutathione

GSH was estimated by the method with add 0.1 ml supernatant into 0.9 ml of 0.001 mol/l 5, 5'-dithiobis-2-nitrobenzoic acid (in 0.1 mol/l phosphate buffer, pH 8.0). The yellow color that developed was read immediately at 412 nm (endpoint) using PowerWave™ XS Microplate Spectrophotometer. GSH (Sigma Chemicals, USA) was used as the standard. The amount was expressed as mg of GSH /g of wet tissue¹⁶.

Estimation of lipid peroxidation

Lipid peroxidation in the brain was determined by measuring MDA content using the method reported by 0.5 ml homogenate added into the mixture of 3ml of 1% w/v Phosphoric acid and 1ml of 0.6% w/v Thiobarbituric acid. Heat for 45 min (boiling water bath 85°C), cool immediately in ice bath. Add 4ml n-butanol and vortex and centrifuge at 5000 rpm for 10 min. Take the absorbance of organic layer at 535 nm (endpoint method) using PowerWave™ XS microplate spectrophotometer. 1, 1, 3, 3-tetraethoxypropane (Sigma Chemicals, USA) was used as the standard MDA and levels were expressed as µg/g tissue¹⁷.

Estimation of catalase

Catalase activity was estimated with the supernatant (0.5 mL) was added to a quartz cuvette containing 2 mL 10 mM H₂O₂ prepared in potassium phosphate buffer (50 mM, pH 7.0). The change in absorbance was monitored at 240 nm from 00 sec. to 210 sec. using a Shimadzu spectrophotometer (UV-1201, Japan). Catalase levels were expressed as U/mg protein¹⁶.

Estimation of superoxide dismutase

SOD activity was determined with slight modifications. To 150 µl of tissue supernatant, 2.85 ml of 0.1 M phosphate buffer (pH 8.4) and 50 µl of 7.5mM pyrogallol were added and absorbance was measured at 420 nm for 3 min at 30 s intervals. Enzyme levels were expressed as U/mg protein¹⁷.

Evaluation of Pro-inflammatory cytokines.

Proinflammatory cytokine were determined in brain tissue homogenate. Cytokines like Tumor Necrosis Factor- alpha (TNF- α), Interleukin-1-beta (IL-1 β), Interleukin-6 (IL-6) was estimated by sandwich ELISA by using commercially available ELISA kits¹⁸.

Statistical analysis

Data was analysed by One way ANOVA followed by Bonferroni multiple comparison test. Data were expressed as mean $\pm$ SEM

RESULTS

Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on Locomotor Activity.

Decreased locomotion count was observed in control group ($^ap<0.001$) as compared to the normal. Whereas significant increase in locomotion count was observed from day 7 onwards in Test-II groups as compared to control group ($^dp<0.001$) as shown in table 1.

Table 1: Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on Locomotor Activity

Groups	Day 0	Day 7	Day 14	Day 21
Normal	764 $\pm$ 2.057	749.5 $\pm$ 1.84	709.5 $\pm$ 21.07	780.33 $\pm$ 1.98
Control	710.5 $\pm$ 2.09 ^a	405.83 $\pm$ 0.68 ^a	385.8 $\pm$ 0.76 ^a	206.83 $\pm$ 0.73 ^a
Standard	759.5 $\pm$ 1.92 ^c	566 $\pm$ 0.87 ^d	649.33 $\pm$ 3.97 ^d	777.67 $\pm$ 2.026 ^d
Test-I	750.83 $\pm$ 1.82 ^b	379.67 $\pm$ 0.85 ^{ns}	362.5 $\pm$ 0.78 ^{ns}	180.167 $\pm$ 0.37 ^{ns}
Test-II	754.5 $\pm$ 1.83 ^b	555.16 $\pm$ 0.72 ^d	630.67 $\pm$ 4.06 ^d	758.33 $\pm$ 2.30 ^d
Test-III	740.5 $\pm$ 1.80 ^{ns}	369.3 $\pm$ 0.98 ^{ns}	349.83 $\pm$ 0.67 ^{ns}	169.667 $\pm$ 0.46 ^{ns}

Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on the cataleptic score.

The graph shows significant increase in cataleptic score in 6-OHDA treated animals in control ($^ap<0.001$) group as

compare to normal group. Whereas significant decrease in cataleptic score was observed in test test-I, test-II and test-III group on day 0 and 7 ($^dp<0.001$), as compare to control group as shown in table 2.

Table 2: Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on the cataleptic score

Groups	Day 0	Day 7	Day 14	Day 21
Normal	2.83 $\pm$ 0.19	3.83 $\pm$ 0.34	3.33 $\pm$ 0.29	2.83 $\pm$ 0.28
Control	26.17 $\pm$ 0.48 ^a	27.33 $\pm$ 0.51 ^a	28.5 $\pm$ 0.65 ^a	30 $\pm$ 0.83 ^a
Standard	12.83 $\pm$ 0.56 ^d	19.33 $\pm$ 0.40 ^d	22.83 $\pm$ 0.54 ^c	26.67 $\pm$ 0.46 ^{ns}
Test-I	13.83 $\pm$ 0.51 ^d	17.17 $\pm$ 0.45 ^d	22.5 $\pm$ 0.30 ^c	25.17 $\pm$ 0.71 ^b
Test-II	17.17 $\pm$ 0.51 ^d	21.5 $\pm$ 0.44 ^c	24.17 $\pm$ 0.58 ^{ns}	27 $\pm$ 0.65 ^{ns}
Test-III	17.83 $\pm$ 0.46 ^d	19 $\pm$ 0.27 ^d	25.17 $\pm$ 0.59 ^{ns}	29.5 $\pm$ 0.78 ^{ns}

Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on decent latency.

The graph shows effect of Betaine on descent latency i.e. the time taken by the rats to remove the grip from the iron grid. The 6-OHDA treatment significantly ($^ap<0.001$)

reduced descent latency on day 0, 7, 14 and 21 as compare to normal. Whereas significantly ($^dp<0.001$) increase in decent latency was observed in test-I, test-II and test-III on day 0 onwards up to day 21 except test-III on day 14. As shown in table 3.

Table 3: Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on decent latency

Groups	Day 0	Day 7	Day 14	Day 21
Normal	23.17 $\pm$ 0.20	37.33 $\pm$ 0.90	33.5 $\pm$ 0.83	32.33 $\pm$ 0.75
Control	5.83 $\pm$ 0.19 ^a	4.33 $\pm$ 0.20 ^a	3.83 $\pm$ 0.12 ^a	3 $\pm$ 0.23 ^a
Standard	21.67 $\pm$ 0.39 ^d	27.83 $\pm$ 0.59 ^d	31.33 $\pm$ 0.39 ^d	28.67 $\pm$ 0.49 ^d
Test-I	25.83 $\pm$ 0.67 ^d	26.17 $\pm$ 0.39 ^d	31.67 $\pm$ 0.91 ^d	32.33 $\pm$ 0.84 ^d
Test-II	16 $\pm$ 0.3 ^d	27.67 $\pm$ 1.02 ^d	28.83 $\pm$ 0.51 ^d	30.17 $\pm$ 1.04 ^d
Test-III	23.33 $\pm$ 0.52 ^d	24 $\pm$ 0.23 ^d	24.67 $\pm$ 0.36 ^d	25.5 $\pm$ 0.31 ^d

Effect of betaine on activities of Effect of Betaine (12.5, 25 and 50 mg/kg., p.o.) on antioxidant.

The control group shows significantly decrease in catalase activity as compare to normal ($^ap<0.01$). Betaine test-I, test-II and test-III counteracted the deleterious effect of 6-OHDA by increasing level of this antioxidant. SOD activity is increased by test-II, which is less significant ($^cp<0.01$).

MDA content is significantly increased in control as compare to normal ($^ap<0.001$). MDA content significantly decreased by Betaine test-I, test-II and test-III ($^dp<0.001$). GSH content is found to be decreased in control as compare to normal ($^ap<0.01$) Betaine test-II increased GSH content as compared to control ($^cp<0.01$) as shown in table 4.

Table 4: Effect of Betaine (12.5, 25 and 50 mg/kg, p.o.) on antioxidant

Groups	Conc. of Catalase (U/mg of protein)	Conc. of SOD (U/mg of protein)	MDA (μ g/mg protein)	GSH μ g/mg of protein
Normal	22.03 $\pm$ 1.39	22.79 $\pm$ 1.077	0.08 $\pm$ 0.0022	4.70 $\pm$ 0.11
Control	3.151 $\pm$ 0.16 ^a	8.388 $\pm$ 0.26 ^a	0.86 $\pm$ 0.016 ^a	2.96 $\pm$ 0.033 ^a
Standard	11.09 $\pm$ 0.27 ^d	16.38 $\pm$ 0.77 ^{ns}	0.14 $\pm$ 0.0076 ^d	4.57 $\pm$ 0.10 ^c
Test-I	20.83 $\pm$ 0.47 ^d	14.36 $\pm$ 0.12 ^{ns}	0.19 $\pm$ 0.001 ^d	2.52 $\pm$ 0.14 ^{ns}
Test-II	21.61 $\pm$ 0.54 ^d	22.08 $\pm$ 0.54 ^c	0.10 $\pm$ 0.0011 ^d	4.38 $\pm$ 0.11 ^c
Test-III	20.82 $\pm$ 0.32 ^d	14.60 $\pm$ 0.17 ^{ns}	0.19 $\pm$ 0.00151 ^d	2.50 $\pm$ 0.15 ^{ns}

Effect of Betaine (12.5, 25 and 50 mg/kg, p.o.) on pro-inflammatory cytokines.

The effect of Betaine on pro-inflammatory cytokines in control groups showed a significantly (^a $p < 0.001$) increased

in TNF- α , IL-1 β and IL-6 level as compared to normal groups. Twenty one days of Betaine treatment in test-I, test-II and test-III is decreased significantly (^d $p < 0.001$) in TNF- α , IL-1 β and IL-6 level as compared to control groups as shown in table 5.

Table 5: Effect of betaine on pro-inflammatory cytokines

Sr. No.	Groups	IL-6	IL-1 β	TNF- α
1	Normal	50.61 $\pm$ 0.23	50.56 $\pm$ 0.21	77.40 $\pm$ 0.74
2	Control	162.68 $\pm$ 1.48 ^a	143.62 $\pm$ 0.64 ^a	145.35 $\pm$ 0.76 ^a
3	Standard	51.54 $\pm$ 0.07 ^d	51.37 $\pm$ 0.09 ^d	86.61 $\pm$ 0.87 ^d
4	Test-I	59.21 $\pm$ 0.63 ^d	71.28 $\pm$ 0.31 ^d	97.24 $\pm$ 2.11 ^d
5	Test-II	143.78 $\pm$ 0.71 ^d	58.50 $\pm$ 0.63 ^d	121.02 $\pm$ 0.43 ^d
6	Test-III	142.80 $\pm$ 0.54 ^d	70.96 $\pm$ 0.30 ^d	119.69 $\pm$ 0.57 ^d

DISCUSSION

In present study we have evaluated aanti-parkinsonism effect of betaine in experimental rats. Parkinsonism is neurodegenerative disease associated with loss of dopaminergic neurons in substantia nigra pars compacta¹⁹. PD is characterized by tremor, akinesia and muscle rigidity. it has been proved that in PD there is increase in the content of TNF- α , IL-1 β , IL-6 and many more in striatal dopaminergic region of Parkinson's brain. Numerous studies support that administration of 6-OHDA induces neuronal toxicity. It is a hydroxylate analogue of dopamine which uses the same transport system as dopamine and nor- epinephrine to produce specific degeneration of catecholaminergic neurons. On ICV administration, 6-OHDA causes degeneration of dopaminergic neurons with dramatic loss of DA in striatum. The toxic mechanism of this compound is dependent on its oxidation with concomitant production of ROS, para and semiquinolone products^{201,21}. Brain being deficient in oxidative defence mechanism is at greater risk of cellular damage caused by free radicals. 6-OHDA undergo metabolism and forms ROS. The para and semiquinolone products formed during oxidation of 6-OHDA are capable of inducing cellular damage through reaction with nucleophile such as protein and DNA, they do not appear to be primarily responsible for toxic effects. ROS formed during its metabolism has shown to exhibit potential role in neurotoxicity²². This acute model has been used in the efficacy testing of many pharmacological, anti-Parkinsonism drugs. In order to understand the mechanism of action, we evaluated the effects of Betaine in 6-OHDA induced PD as evident by the grip strength, locomotion and catalepsy score (akinesia with muscle rigidity) parameters. 6-OHDA treatment showed deterioration of above mentioned behavioural parameters in rats. Betaine restored these behavioural changes significantly. Administration of Betaine showed significant improvement in grip strength, catalepsy and restored

locomotor activity in rats; these effects were comparable to that of standard group (L-dopa + Benserazide). Brain homogenates of 6-OHDA treated rats were subjected to biochemical estimation in terms of levels/ activity. Analysis of post-mortem brains from PD rats confirms the high level of oxidative stress in the SNpc marked by increase iron concentrations, decreased levels of GSH and increased lipid peroxidation. Betaine has showed marked rise in SOD and Catalase activity as well as GSH content subsequently decreasing in the lipid peroxidation process. Our result suggests Betaine to be potent antioxidant at dose 12.5 and 25 mg/kg as compared to standard (L-dopa+Benserazide). Elevated inflammatory cytokines in the brain, cerebral spinal fluid (CSF) and plasma of PD patients supports the existence of functional interconnections between the immune and nervous systems²³. Recent reports indicate that a pro-inflammatory event in the periphery can induce chronic, self-propelling neuroinflammation in the brain, and that systemic cytokines are critical for CNS effects in response to peripheral immune activation show that the entry of pro-inflammatory factors, such as TNF- α , to the brain will cause the activation of microglia to produce more inflammatory factors²⁴. The factors may cause neuronal death, suggesting a clinical implication for the link between peripheral inflammation and neuroinflammation²⁵. The stimulation of immune-competent cells and hyper production of cytokines are considered to play a role in the development and progression of multiple neurodegenerative diseases. Peripheral inflammation may amplify the neuro-inflammation contributing to disease pathogenesis²⁶. The augmented expression of pro-inflammatory cytokines viz: TNF- α , IL-1 β and IL-6 were significantly reversed by Betaine as compared to that of standard group (L-dopa+ Benserazide). As per literature review, Betaine is a safer drug possessing wide therapeutic window. It can also act as antioxidant agent versus oxidative stress mediated by antiparkinsonian drugs viz L- dopa and L-dopa+ Benserazide. Betaine also demonstrated a methyl donor

effect to reduce hyperhomocysteinemia. It appears that antioxidant and methyl donor properties of Betaine are promising particularly in management of plasma total homocysteine (tHcy) and oxidative stress in dopaminergic neurons of the brain. Betaine can be better adjuvant in pharmacotherapy of PD and/or to manage extrapyramidal side effects of anti-parkinsonian drugs like L-dopa.

CONCLUSION

The present study provides proof of concept for potential use of Betaine as an adjuvant in pharmacotherapy of PD. Additionally Betaine is safer drug with wide therapeutic window.

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