



Received on 17 September, 2016; received in revised form, 24 November, 2016; accepted, 25 November, 2016; published 01 April, 2017

## NEUROPROTECTIVE EFFECTS OF *OCIMUM SANCTUM*, LINN. EXTRACT ON MPTP-INDUCED OXIDATIVE AND NITROSATIVE STRESS MARKERS IN MALE MOUSE BRAIN

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### Keywords:

*Ocimum sanctum* hydromethanolic extract, MPTP, protein oxidation, nitrosative stress, endogenous antioxidant pool, *Mucuna pruriens*

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**ABSTRACT:** The present study focuses on the 'antioxidant approach' as a therapeutic intervention for Parkinson's disease (PD). It deals with understanding of the effects of *Ocimum sanctum* extract on oxidative and nitrosative stress biomarkers, responsible for neurodegeneration. The standardized hydromethanolic extract of *O. sanctum*, Linn (Os HM) at the dose of 50, 100 and 200 mg/kg were evaluated for their neuroprotective effects against MPTP induced Parkinsonism. Single divided doses of MPTP were used for induction of parkinsonism in male mice. L-DOPA rich and free fractions of *Mucuna pruriens* and Bromocriptine were considered as positive standards. The antioxidant effect and thus the neuroprotective potential was estimated based upon the levels of protein oxidation products, endogenous enzymatic and non-enzymatic antioxidant pool of glutathione, nitric oxide, superoxide dismutase and catalase. The results revealed that Os HM extract, particularly at the doses of 100 and 200 mg/kg, significantly depleted the protein carbonyl content, restored the decreased levels of GSH. Os HM prevented the observed compensatory response with superoxide dismutase levels against MPTP treatment. It also significantly reduced the MPTP-induced nitric oxide therefore the nitrosative stress levels. Thus, the study demonstrated that Os HM can correct the disrupted homeostasis and redox imbalances due to excess reactive oxygen and nitrogen species generated by the neurotoxin MPTP.

**INTRODUCTION:** Parkinson disease (PD) is one of the fatal and major neurodegenerative disorder manifested by depletion in dopamine levels due to degeneration of nigrostriatal dopaminergic neurons. PD is a multifactorial disorder wherein oxidative stress due to the reactive oxygen species (ROS) from both endogenous and exogenous sources is involved in its etiology<sup>1</sup>. The overburden of free radicals causes imbalance in homeostatic phenomenon between oxidants and antioxidant defenses in the body leading to oxidative stress<sup>1,2</sup>.

Further, the increased level of nitric oxide (NO) has also been implicated in its pathogenesis<sup>3</sup>. Nitric oxide is a highly fat-soluble free radical with numerous promiscuous roles. Several studies have demonstrated that inflammation correlates with the level of NO<sup>3,4</sup>.

Researches in the recent past have accumulated enormous evidence advocating enrichment of body systems with antioxidants to correct the vitiated homeostasis and prevent the onset as well as treat the disease fostered due to free radicals<sup>5</sup>. Glutathione, a potent antioxidant plays an essential role in the dopamine turnover and pathogenesis of PD<sup>6</sup>. The estimation of the activity of antioxidant enzymes such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) can be used to assess the therapeutic effects of different antioxidant agents<sup>5,2</sup>.

<p><b>QUICK RESPONSE CODE</b></p> 	<p><b>DOI:</b> 10.13040/IJPSR.0975-8232.8(4).1694-00</p>
<p>Article can be accessed online on: <a href="http://www.ijpsr.com">www.ijpsr.com</a></p>	
<p>DOI link: <a href="http://dx.doi.org/10.13040/IJPSR.0975-8232.8(4).1694-00">http://dx.doi.org/10.13040/IJPSR.0975-8232.8(4).1694-00</a></p>	

*Ocimum sanctum* L. is also known as *Ocimum tenuiflorum* or Tulsi. It belongs to the family Lamiaceae, and is known for its healing properties. *O. sanctum* is an adaptogen, which can balance different processes in the body, and is considered helpful in adapting to stress<sup>7</sup>. Substantial evidence for *Ocimum sanctum*, L's (Os) antioxidant properties provides an approach to serve as nerviness<sup>8</sup> in the prevention and treatment of stress-related degenerative diseases<sup>9</sup>. Evidence presented from both *in vitro* and *in vivo* studies support that amongst various neurotoxins that are used in animal models, (1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine) MPTP exposure generates ROS resulting in oxidative stress<sup>10</sup>.

Thus the present study is aimed at investigating the influence of the antioxidant-enriched herb *Ocimum sanctum*, on the oxidative stress biomarkers in a modified MPTP model.

## MATERIALS AND METHODS:

**Preparation of extract:** The fresh leaves of *Ocimum sanctum* were collected from Vasai, Maharashtra. The identification and authentication of the above plant materials was done by Dr. Harshad Pandit, Reader, Department of Botany, Guru Nanak Khalsa College, University of Mumbai, Matunga, Mumbai, India. The separated leaves were subjected for shade drying at room temperature. They were sun dried and powdered to coarse size 40# with the aid of domestic mixer. The powdered plant leaves were defatted with petroleum ether. It was further subjected to soxhlet extraction at 60-80°C for 3-4 days with 70% methanol to get hydromethanolic extract of *Ocimum sanctum* (Os HM).

**Chemicals:** 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine hydrochloride (MPTP-HCl) was purchased from Sigma-Aldrich (USA). Bromocriptine mesylate was gifted by Inga Labs, Andheri. Probenecid was obtained as a gift sample from Kamud Pharmaceuticals, Navi Mumbai. All other chemicals and reagents used in the experiments were of analytical grade.

**Acute toxicity studies:** The acute toxicity of HM extracts of Os was determined as per OECD guideline 423<sup>11</sup>.

**Screening of neuroprotective property of extracts of *O. sanctum*, L in MPTP induced Parkinson's disease animal model:** A modification of MPTP model i.e. MPTPp was used in the present study for screening of neuroprotective potential in experimental animals. MPTPp utilized a combination of MPTP neurotoxin and probenecid as an adjuvant<sup>12, 13</sup>.

**Animals:** Swiss albino male mice weighing about 25-30g were procured from Haffkine's Biopharmaceuticals, Parel, Mumbai-400 012. The animals were maintained at 22±3°C with 50-70 % relative humidity and 12:12 h of light and dark cycles. The animals were fed with a standard pellet diet procured from Amrut, India (Amrut Laboratories Pranava Agro Industries Ltd, Sangli) and water ad libitum.

**Experimental protocol:** The experimental procedures and protocol for this study was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Institute of Chemical Technology (ICT), Matunga, Mumbai. The pharmacological experiments were performed as per norms laid by Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA), Government of India. IAEC protocol approval number is UICT/PH/IAEC/0910/02.

## Treatment:

- 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): MPTP was dissolved in 0.9 % saline. 15 mg/kg of MPTP was administered intraperitoneally (*i.p.*), twice, 4 h apart.
- Probenecid solution: 250 mg/kg of probenecid was dissolved in DMSO and administered *i.p.*
- Bromocriptine: 10 mg/kg of bromocriptine was administered *i.p.* Because of the solubility characteristics of bromocriptine, all *i.p.* injections were given in 0.2 ml of 20% ethanol in saline.
- Hydromethanolic extracts of *O. sanctum*, L (Os HM): Os HM was reconstituted in distilled water and administered perorally.
- Mucuna pruriens*, L: The hydromethanolic and butanolic extracts of *M. pruriens* were reconstituted in distilled water and 0.2 % w/v of Na-CMC respectively to administer 0.2 ml of

the solution to each animal at a dose of 100 mg/kg.

**Experimental Design:** Animals were randomly divided into following groups containing 6 animals each (N=6):

**Group I-** Control (vehicle), received 0.9 % saline, *p.o.*

**Group II** (Negative control)- MPTPp treatment, MPTP, 15 mg/kg, *i.p.*, twice with 4 h interval followed by probenecid, 250 mg/kg b.w, *i.p.*

**Group III-** 50 mg/kg of Os HM extract, *p.o.*

**Group IV-** 100 mg/kg of Os HM extract, *p.o.*

**Group V-** 200 mg/kg of Os HM extract, *p.o.*

**Group VI** (Herbal positive control group)- 100 mg/kg of *M. pruriens* HM extract, *p.o.*

**Group VII** (Herbal positive control group) - 100 mg/kg of *M. pruriens* butanolic extract, *p.o.*

**Group VIII** (Synthetic positive control group)- 10 mg/kg bromocriptine mesylate, *i.p.*

The animals in the vehicle control group received 0.9 % saline while MPTP in combination with, probenecid (250 mg/kg, *i.p.*) was administered in the negative control group. The animals of group III, IV, V, VI, VII and VIII were pre treated with their respective treatments for 7 days and then MPTP was administered on the 7<sup>th</sup> day followed by probenecid. At the end of the experiment the animals were sacrificed. Safety precautions for the use of MPTP during chemical preparation and animal injections were carried out appropriately.

#### **Isolation of mouse brain and tissue processing:**

The whole body was perfused by cardiac perfusion technique, brains removed was and rinsed in ice-cold isotonic saline. The brain tissue was homogenized immediately in Teflon homogenizer under the cold condition with equal volume (1 ml) of ice-cold 0.1 M phosphate buffer saline (pH 7.4). The homogenate was cold centrifuged at 4°C to obtain 10 % w/v brain tissue homogenate. The aliquots of brain homogenate were stored at -20°C until further processing for the estimation of the selected biomarkers.

**Measurement of protein content:** The protein content in the brain homogenate was carried out according to Lowry protein assay<sup>14, 15</sup>. Briefly, 100 µl of brain homogenate sample was taken in a test tube. 1.0 ml of Lowry stock reagent was added to

each tube and was incubated for 30 min at room temperature. 100 µl of Folin's reagent was further added to each tube and incubated for 30 min at room temperature. The absorbance was read at 595 nm. The protein content in brain tissue was expressed as µg/mg of tissue.

#### **Measurement of oxidative stress parameters:**

##### **1. Measurement of protein oxidation products as protein carbonyl content:**

The oxidation of protein contents of the neuronal tissue in the brain homogenate was determined by 2, 4-dinitrophenyl hydrazine (DNPH) method<sup>16</sup>. 4 ml of 10 mM 2, 4-dinitrophenylhydrazine in 2.5 M HCl was added to 0.5 ml brain homogenates, and mixed thoroughly. The tubes were incubated for 1 h at room temperature in the dark and shaken intermittently. The mixtures were sequentially treated with 5 ml of 20 % and 10% TCA. After addition of initial 20 % TCA the mixture was incubated in ice for 10 min and then centrifuged at 1000 X g. The supernatant after centrifugation was carefully aspirated and discarded. The protein pellet which remained at the bottom was washed with 10 % TCA. Further centrifugation was carried out at 3500 rpm for 20 min. The pellets were washed three times with 4 ml of ethanol/ethyl acetate mixture (1:1 v/v). The final pellets were dissolved in 2 ml of 6 M guanidine HCl and incubated for 15 min at 37°C in an incubator with occasional mixing. It was further centrifuged at 2000 g for 10 min.

The blank was performed without DNPH. DNPH was substituted with 4 ml of 2.5 M HCl. Absorbance of the yellow colored supernatants was measured at 370 nm and protein carbonyls were quantified using the molar extinction coefficient of  $2.2 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ . The results were expressed as nanomoles of oxidized protein per mg of protein.

##### **2. Estimation of endogenous antioxidant systems**

**a. Reduced glutathione (GSH):** The reduced glutathione in the brain was determined according to the method by Jollow et al with slight modification<sup>17, 18</sup>. In brief, 0.5 ml of the brain homogenate was precipitated with 0.5 ml of Sulfosalicylic acid (SSA, 4%). The samples were then incubated at 4°C for 1 h. To this, 0.5 ml of DTNB and 2.3 ml 0.1 M phosphate buffer (pH 7.4)

was added, mixed thoroughly and incubated for 5 min. It was further centrifuged at 1500 X g for 5 min. The yellow color developed was read immediately at 412 nm. The results were expressed as  $\mu$ moles per mg protein.

**b. Superoxide dismutase (SOD):** 50  $\mu$ l of test brain homogenate was added to 500  $\mu$ l of tris HCl buffer 75 mM, pH 8.2, 50  $\mu$ l of 30 mM EDTA, 2 Na. Then 400  $\mu$ l of 2 mM pyrogallol was added. The OD value was detected every 30 s at 325 nm. The pyrogallol autoxidation velocity was measured in terms of change in absorbance/30 s to ensure that OD remained approximately at 0.07/min. Similarly, the influence of the autoxidation velocity was measured using the solvent as the blank.

Then, the  $O_2^{\cdot -}$  scavenging ratio (% inhibition) was calculated as:

$$I = \frac{[1 - (\Delta OD_{\text{test}})]}{[\Delta OD_{\text{blank}}]} \times 100$$

where  $\Delta OD_{\text{test}}$  is the test optical density and  $\Delta OD_{\text{blank}}$  is the blank optical density. Units of SOD was calculated per ml of brain homogenate and then expressed as units per mg protein<sup>19</sup>.

**c. Catalase (CAT):** Catalase is determined according to the method of Aebi et.al, 1974 by the depletion rate of  $H_2O_2$  at 240 nm in a reaction buffer<sup>20</sup>. Briefly, diluted hydrogen peroxide (0.2 ml of 20-fold dilution, 30% w/v) and 2.5 ml of 50 mM phosphate buffer (pH 8.2) was added in a cuvette. To the resultant, 0.02 ml of brain homogenate was added and mixed thoroughly. This was read against a control cuvette containing enzyme solution (0-150 units/ml of CAT) without  $H_2O_2$  phosphate buffer at 240 nm.  $\Delta t$  was noted for a decrease in the optical density i.e from 0.450 to 0.400. The decrease in the absorbance of reaction mixture due to catabolism of hydrogen peroxide was recorded at 240 nm after every 30 s by spectrophotometer. CAT activity was expressed in terms of nmol  $H_2O_2$  consumed per min per mg protein.

**Measurement of nitrosative stresses in terms of nitric oxide (NO) level:** Nitric oxide production was estimated as nitrite released from tissue homogenate. Concentrations of nitrite in tissue homogenates were measured by using the

diazotization method based on the Griess reaction, which is an indirect assay for NO production.

To measure the nitrite content, 100  $\mu$ l of brain homogenate was mixed with equal volume of Griess reagent in a 96 well plate. It was incubated at room temperature in dark for 10 min. The absorption at 540 nm was determined using a microplate reader. The results were expressed as  $\mu$ M of nitrite per g of brain tissue<sup>21, 22</sup>.

**Statistical analysis:** Values were presented as mean  $\pm$  S.E.M. Data were statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's test for intergroup comparison using Instat software. \*P<0.05, \*\*P<0.01 and \*\*\*P<0.001 were considered statistically significant when compared with negative control group (MPTP). <sup>a</sup> P < 0.001, <sup>b</sup> P < 0.01 and <sup>c</sup> P < 0.05 were considered statistically significant when compared with the vehicle treatment group.

## RESULTS:

**Protein measurement:** Loss of protein content may be regarded as an index of cell loss or atrophy, as was observed in MPTP of PD brains (protein level of  $12.77 \pm 1.34$   $\mu$ g/mg tissue) in comparison with vehicle control ( $27.34 \pm 2.21$   $\mu$ g/mg tissue) (P<0.01). The protein content was however considerably improved at higher doses of Os HM group with  $20.11 \pm 2.44$  (P<0.05)  $\mu$ g/mg tissue as comparable to the negative control group. 100 mg/kg of *M. pruriens* HM extract and bromocriptine exhibited protein content of  $23.44 \pm 2.9$  and  $21.55 \pm 2.88$   $\mu$ g/mg tissue (P<0.01) respectively. (**Table 1**)

**Protein carbonyl content:** In the present study, the results exhibited a significant increase in protein carbonyls in the brain tissues of mice administered with MPTP ( $156.22 \pm 1.29$   $\mu$ M/mg protein, P<0.001) when compared with the vehicle control animals ( $43.4 \pm 2.35$   $\mu$ M/mg protein). This might indicate an increased oxidized protein levels in brain with the increase in neurotoxicity.

Os HM extract resulted decrease in the levels of protein carbonyls in a dose dependent manner, more significantly at a dose of 200 mg/kg ( $98.5 \pm 3.86$   $\mu$ M/mg protein, P<0.01). (**Table 1**)

**TABLE 1: THE PROTEIN LEVEL AND PROTEIN CARBONYL CONTENT IN NORMAL AND TREATED GROUPS**

Sr. No.	Groups	Protein content [ $\mu\text{g}/\text{mg}$ tissue]	Protein carbonyl content [ $\mu\text{M}/\text{mg}$ protein]
1	Vehicle control	27.34 $\pm$ 2.21	43.4 $\pm$ 2.35
2	MPTPp (Negative control)	12.77 $\pm$ 1.34 <sup>b</sup>	156.22 $\pm$ 1.29 <sup>a</sup>
3	Os HM extract, 50 mg/kg	15.39 $\pm$ 1.37	143.11 $\pm$ 2.27
4	Os HM. Extract, 100 mg/kg	18.9 $\pm$ 1.45	120.80 $\pm$ 3.23*
5	Os HM extract, 200 mg/kg	20.11 $\pm$ 2.44*	98.5 $\pm$ 3.86**
6	<i>M. pruriens</i> HM extract, 100 mg/kg	23.44 $\pm$ 2.9**	79.23 $\pm$ 2.35***
7	<i>M. pruriens</i> butanolic extract, 100 mg/kg	19.10 $\pm$ 2.31	96.24 $\pm$ 3.1**
8	Bromocriptine mesylate, 10 mg/kg	21.55 $\pm$ 2.8	89.10 $\pm$ 1.98**

Values expressed as mean  $\pm$  S.E.M (n=6). \* P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 considered statistically significant as compared with negative control group (MPTP). a P < 0.001, b P < 0.01 and c P < 0.05 were considered statistically significant when compared with the vehicle treatment group.

### Oxidative stress parameters:

#### Levels of non-enzymatic and enzymatic endogenous antioxidant pool determination:

In our present study, a significant decrease in the levels of GSH (0.015  $\mu\text{moles}/\text{mg}$  protein) was observed as compared to the vehicle control (0.055  $\mu\text{moles}$  of GSH/mg protein). Administration of Os HM significantly increased the level of glutathione in a dose dependent manner. *Mucuna pruriens* HM, 100 mg/kg showed better restoration in GSH levels (0.048  $\pm$  0.0052  $\mu\text{moles}$  GSH/mg protein) while bromocriptine group exhibited GSH levels of 0.039  $\pm$  0.0018  $\mu\text{moles}/\text{mg}$  protein.

The MPTP treatment in group II produced a significant but a compensatory increase in the levels of SOD (2.67  $\pm$  0.88 Units/mg protein,

P < 0.01) compared to vehicle control group (0.99  $\pm$  0.07 U/mg protein). Pretreatment with Os HM groups significantly reversed levels of SOD (P < 0.05).

Similarly, a significant increase in Catalase (CAT) was seen after administration of MPTP (1.22  $\pm$  0.5  $\mu\text{moles}$  H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein, P < 0.01). Administration of Os HM extract was able to decrease though not significantly, the observed increase in CAT activity. In the case of positive controls, bromocriptine showed CAT activity of 0.88  $\pm$  0.033  $\mu\text{moles}$  H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein. Both the HM as well as butanolic extracts of *M. pruriens* significantly attenuated the rise in catalase activity. (Table 2)

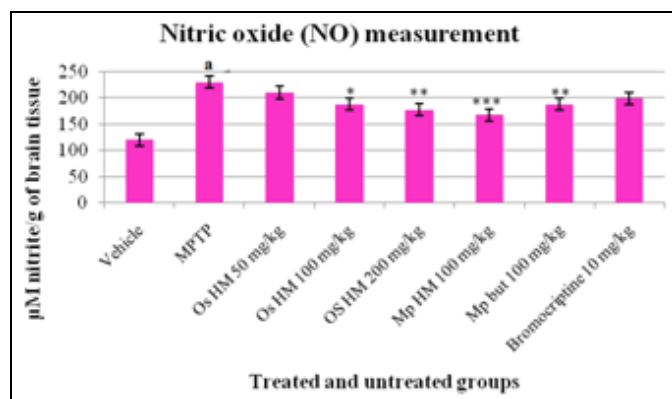
**TABLE 2: EFFECT ON ENDOGENOUS ANTIOXIDANT POOL IN THE TREATED AND UNTREATED GROUPS**

Sr. No	Groups	Reduced glutathione GSH $\mu\text{moles}/\text{mg}$ protein	Superoxide dismutase (SOD) Units / mg protein	Catalase (CAT) $\mu\text{moles}$ H <sub>2</sub> O <sub>2</sub> decomposed/min/mg protein
1	Vehicle control	0.055 $\pm$ 0.0093	0.99 $\pm$ 0.07	0.434 $\pm$ 0.078
2	MPTPp (Negative control)	0.015 $\pm$ 0.0013 <sup>a</sup>	2.67 $\pm$ 0.88 <sup>b</sup>	1.22 $\pm$ 0.5 <sup>a</sup>
3	Os HM extract, 50 mg/kg	0.029 $\pm$ 0.0043	1.88 $\pm$ 0.56	0.89 $\pm$ 0.023
4	Os HM. Extract, 100 mg/kg	0.035 $\pm$ 0.0045*	1.76 $\pm$ 0.57*	0.81 $\pm$ 0.077
5	Os HM extract, 200 mg/kg	0.041 $\pm$ 0.0044**	1.65 $\pm$ 0.49*	0.78 $\pm$ 0.048
6	<i>M. pruriens</i> HM extract, 100 mg/kg	0.048 $\pm$ 0.0052***	1.23 $\pm$ 0.8**	0.563 $\pm$ 0.083*
7	<i>M. pruriens</i> butanolic extract, 100 mg/kg	0.043 $\pm$ 0.0056**	1.61 $\pm$ 0.66*	0.64 $\pm$ 0.077*
8	Bromocriptine mesylate, 10 mg/kg	0.039 $\pm$ 0.0018*	1.94 $\pm$ 0.90*	0.88 $\pm$ 0.033

Values expressed as mean  $\pm$  S.E.M (n=6). \* P < 0.05, \*\*P < 0.01 and \*\*\*P < 0.001 considered statistically significant as compared with negative control group (MPTP). a P < 0.001, b P < 0.01 and c P < 0.05 were considered statistically significant when compared with the vehicle treatment group.

**Nitrosative stress measurement:** The nitrosative stress evaluation showed a significant increase in NO levels after MPTPp treatment as compared to the normal control. Treatment with Os HM extracts in 100 and 200 mg/kg doses significantly reduced the MPTP-induced NO levels (188  $\mu\text{M}$  nitrite/g of brain tissue, P < 0.05 and 170  $\mu\text{M}$  nitrite/g of brain

tissue, P < 0.01 respectively) (Fig. 1). Both HM and butanolic extracts of *M. pruriens* at 100 mg/kg significantly exhibited decrease in NO levels as 168 and 188  $\mu\text{M}$  nitrite/g of brain tissue respectively. However bromocriptine did not significantly decrease the levels of NO.



**FIG. 1: EFFECTS OF TREATMENT ON NITRIC OXIDE (NO) LEVELS FOR DETERMINATION OF NITROSATIVE STRESS ON WHOLE BRAIN.** Values expressed as mean  $\pm$  S.E.M (n=6). \*  $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.001$  considered statistically significant as compared with negative control group (MPTP). a  $P < 0.001$  was considered statistically significant when compared with the vehicle treatment group.

**DISCUSSIONS:** Parkinson's disease is the second most common neurodegenerative disorder characterized by loss of nigrostriatal dopaminergic neurons in the basal ganglia<sup>1</sup>. Though the pathogenesis of PD is still elusive, it is believed to be multifactorial. Oxidative and nitrosative stress is one of the important contributing factors in PD pathogenesis as dopaminergic neurons being vulnerable to the generated oxidative stress<sup>1, 23</sup>. Also, oxidative stress is interlinked with other components leading to the neurodegenerative process, which includes mitochondrial dysfunction, nitric oxide toxicity and inflammation<sup>1, 23</sup>.

A number of studies on postmortem PD brain tissues provide evidence of the implication of oxidative and nitrosative stress as a causative factor<sup>3</sup>. One of the observations includes the oxidative modification of proteins such as increased levels of oxidized protein carbonyls. These modifications lead to the structural alteration and functional inactivation of many enzyme proteins<sup>3, 16</sup>.

In the present study, MPTPp treatment lead to a significant increase in the protein carbonyl content in the brain tissues. This provides evidence for the resultant oxidative stress and the ensued damage. Oral administration of Os HM extract at doses of 50, 100 and 200 mg/kg markedly prevented the MPTP-induced carbonyl modification of the proteins. Decreased glutathione (GSH) is an earliest biochemical change seen in substantia nigra of PD patient<sup>6</sup>. Accordingly, a significant

depletion of GSH was observed in MPTPp treatment group. GSH is a major antioxidant defence against the augmented ROS and its depletion promotes generation of oxidative stress, initiating cascade of effects thereby affecting functional as well as structural integrity of cell and organelle<sup>2, 6</sup>. The potential of Os HM to significantly elevate the levels of GSH in the treatment groups protects cellular protein against oxidation through glutathione redox cycle and also to directly detoxifies ROS and/or neutralizes reactive intermediate species.

The MPTPp treatment leads to a significant increase in the activities of total SOD, CAT in the brains of mice. This increase might reflect a compensatory mechanism to counteract the effect of elevated free radicals due to the impairment of anti-oxidant defence system as compared to the vehicle treatment<sup>24</sup>. This is in lines with the earlier findings that the brains of mice when treated with moderate doses of MPTP exhibited mitochondrial dysfunction, elevated activities of SOD, CAT and glutathione peroxidase<sup>25, 26</sup>. The pretreatment with Os HM depleted the restored levels of SOD on account of its own antioxidant nature/potential.

Moreover, it is well known that both NO and peroxynitrite (ONOO<sup>-</sup>) act as main toxicants in the brain under neuropathological conditions<sup>3</sup>. It was reported that NO, as a toxic factor, could mediate the death of dopaminergic neurons through its interaction with several enzymes, proteins and lipids<sup>3, 27</sup>. Results of the study indicate that the Os HM extracts mitigate the increased free radical generation during MPTP intoxication and hence the related induced-nitrosative stress.

Thus, the results reveal a strong anti-oxidant properties of *Ocimum sanctum* extract against MPTPp induced imbalance in mice brains.

**CONCLUSIONS:** In the present study, MPTP administration decreased the redox state while pretreatment with the Os HM extract restored the altered antioxidant state in the form of GSH, attenuated the levels of oxidized protein and NO consequently inhibiting the oxidative and nitrosative stresses induced by MPTP. Medhya rasayanas include *O. sanctum* that have positive influence on the biochemical factors which was

significantly affected in MPTP-inflicted experimental animals. Thus it is plausible that the protective role of this antioxidant-enriched herb can correct the vitiated homeostasis thus modulating the impaired redox-imbalance signaling pathways primarily through its influences on stress markers. Hence this study provides an affirmation towards the therapeutic role of *Ocimum sanctum* as a “neuroprotective” agent.

**ACKNOWLEDGEMENT:** Authors are grateful towards UGC-SAP for funding the research projects. Authors would also like to thank Inga Labs, Andheri and Kamud Pharmaceuticals, Navi Mumbai for providing the gift samples.

**CONFLICT OF INTEREST:** The authors declare that there is no conflict of interests regarding the publication of this paper.

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### How to cite this article:

Redkar RG, Peshattiwar VV and Sathaye S: Neuroprotective effects of *Ocimum sanctum*, Linn extract on mptp-induced oxidative and nitrosative stress markers in male mouse brain. *Int J Pharm Sci Res* 2017; 8(4): 1694-00. doi: 10.13040/IJPSR.0975-8232.8(4).1694-00.