Protective role of sertraline against 3-nitropropionic acid-induced cognitive dysfunction and redox ratio in striatum, cortex and hippocampus of rat brain

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Huntington’s disease (HD) is an inherited progressive neurodegenerative disorder in human characterized by progressive loss of movement and cognitive disturbances. 3-nitropropionic acid (3-NP; a mitochondrial toxin) produces age-dependent oxidative linked striatal damage, responsible for HD like symptoms. In the present study protective effect of sertraline in 3-NP induced HD like symptoms was evaluated in rats. Systemic administration of 3-NP (10 mg/kg for 14 days) resulted in impairment of memory as assessed in Morris water maze and elevated plus paradigm tasks. Biochemical analysis revealed that systemic 3-NP administration significantly impaired reduced glutathione, total glutathione, oxidized glutathione and glutathione–S-transferase levels, whereas the level of acetylcholinesterase enzyme increased in striatum, cortex and hippocampus regions of rat brain. Sertraline (5 and 10 mg/kg po) treatment once daily for 14 days significantly improved cognitive performance tasks and glutathione levels in 3-NP treated group. However, combination of yohimbine (2 mg/kg) (non selective serotonin receptors antagonist) with the higher dose of sertraline (10 mg/kg) did not influence the protective action of sertraline. Result shows that neuroprotective and antioxidant like effect of sertraline is independent of its conventional action on 5-HT receptor.

Keywords: Glutathione, Huntington’s disease, Memory, Oxidative, Sertraline, Stress

Huntington’s disease (HD) is a neurodegenerative disorder predominantly affecting striatum, cerebral cortex and other areas of brain responsible for motor control and memory storage. Cognitive deficits are core neuropsychiatric problem among HD patients, while impaired attention and visuospatial processing are also observed during early stages of this illness. With progress of disease the HD patients often exhibit deficits in executive tasks requiring planning, problem solving, and cognitive flexibility. The complexity and unpredictability of the disease poses challenges for health and social-care professionals.

3-Nitropropionic acid (3-NP) is a natural environmental toxin obtained from various plants, fungi and induces HD like symptom both in humans and experimental animals. In human, 3-NP intoxication occurred first time in China via ingestion of fungal contaminated sugarcane. Through the exact mechanism is still not clearly understood, prime mechanism is due to irreversible, covalent binding of 3-NP with subsequent inhibition of succinate dehydrogenase (SDH), an enzyme of the citric acid cycle that transfers electrons to the electron transport chain via its complex II function. Therefore, 3-NP has been proposed to cause both cellular and mitochondrial stress. Reports suggest the involvement of oxidative stress initially as a prime candidate mediating behavioural impairment and memory deficits in age related neurodegenerative disorders. 3-NP produces selective lesions in basal ganglia (striatum), cortex, hippocampus and produce dystonia in humans. Further, it produces free radicals and consequently disturbs glutathione redox cycle.

Glutathione (GSH) system is body’s own antioxidant defense system. GSH is a small protein produced naturally in the cells from three amino acids namely glycine, glutamine, and cysteine.

Sertraline is a well known selective serotonin reuptake inhibitor (SSRI). The antidepressant effect of sertraline is presumed to be linked to its ability to inhibit the neuronal reuptake of serotonin. Its neuroprotective and antioxidant actions have also been reported in neurodegenerative diseases. Various reports suggest the beneficial effect of sertraline in transgenic model of HD.

Based on the above finding, present study has been designed to explore the possible role of sertraline in 3-NP induced cognitive dysfunction and redox ratio in rats.
Materials and Methods

Animals—Adult Male Wistar rats (weighing between 250-300 g) bred in Central Animal House facility of the Panjab University, Chandigarh were used. Animals were acclimatized to laboratory conditions prior to experimentation. The animals were kept under standard conditions of light and dark cycle with food and water *ad libitum* in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 09:00 and 15:00 hrs. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy Guidelines for the use and care of animals.

Drugs and treatment schedule—The following drugs were used in the present study: 3-NP (Sigma Chemicals, USA) was diluted with saline (adjust pH 7.4) and administered ip whereas sertraline (Sigma Chemicals, USA) was given per oral (po) route. Animals were randomly divided into following 7 groups of 6 animals each: Group-1 vehicle treated, control group; Group-2 received 3-NP (10 mg/kg, ip) for 14 days, Groups- 3 and 4 received (5, 10 mg/kg) *per se*, Group-5 received sertraline (5) + 3-NP (10 mg/kg) for 14 days, Group-6 animals received sertraline (10 mg/kg)+ 3-NP (10 mg/kg) for 14 days. Animals of group- 7 received yohimbine (2 mg/kg) + sertraline (10 mg/kg) + 3-NP (10 mg/kg, ip) for 14 days. In groups 5 to 7, sertraline was administered 1 h prior to 3-NP treatment.

Morris water maze test—The acquisition and retention of a spatial navigation task was examined using Morris water maze. Animals were trained to swim to a platform in a circular pool (180 cm diameter × 60 cm height) located in a test room. The pool was filled with water (28° ± 2°C) to a depth of 40 cm. A movable circular platform 9 cm in diameter, mounted on a column, was placed in the pool 1 cm above the water level for maze acquisition test. The platform was fixed in the center of one of the 4 quadrants and remained at same position throughout the experiment. Animals received a training session consisting of 4 trials in a day for 4 days, starting from first day of 3-NP administration. The latency to find the platform was recorded up to 2 min. The latency time taken by the animal to reach the platform on 5th day was recorded as initial acquisition latency (IAL). On 15th day, the platform was removed and retention of the memory was assessed by releasing the animal at any one of the edges (N, S, E, W) facing the wall of the pool. The time taken to reach the quadrant where platform was placed was recorded. On the 15th day the time spent by the animal in the target quadrant was also measured.

Elevated Plus Maze test for spatial memory—Elevated plus maze consists of two opposite open arms (50 × 10 cm), crossed with two closed arms of same dimensions with 40 cm high walls. The arms are connected with central square (10 × 10 cm). Acquisition of memory was assessed on day 14th after initiating 3-NP treatment. Rat was placed individually at one end of an open arm facing away from the central square. The time taken by animal to move from open arm and enter into one of the closed arms was recorded as initial transfer latency (ITL). Rat was allowed to explore the maze for 30 sec after recording ITL and returned to its home cage. Retention latency was noted again on 15th day. Percent retention of memory was calculated by the formula

\[
\text{Memory retention (\%) = } \frac{\text{Transfer latency (day 14) - Transfer latency (day 15)}}{\text{Transfer latency (day 14)}} \times 100
\]

Dissection and homogenization—On day 15, after behavioural assessments, animals (n=6) were sacrificed by decapitation. The brains were removed, forebrain was dissected out on ice. Striatum, cortex and hippocampus were separated and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min at 4°C and aliquots of supernatant was separated and used for biochemical estimation.

Biochemical estimations

Estimation of glutathione levels—Reduced glutathione (GSH) in striatum, cortex and hippocampus was estimated as per Ellman. Results were calculated using molar extinction coefficient of chromophore (1.36 × 10^4 M^-1 cm^-1) and expressed as percentage of control. Total glutathione analysis was done by the method of Zahler and Cleland. The method is based on the reduction with dithioerythritol and determination of the resulting monothiols with DTNB in presence of arsenite. Oxidized glutathione was quantiated by subtracting the value of glutathione reduced from total glutathione. Glutathione –S-transferase (GST) was assayed by the method of...
Habig\textsuperscript{16}. GST catalyses the formation of the glutathione conjugates of CDNB which absorb maximum at 340 nm and have an extinction coefficient of 9.6 $M\text{m}^{-1}\text{cm}^{-1}$. Redox ratio was determined for all the groups by taking the ratio of reduced glutathione / oxidized glutathione.

Estimation of acetylcholinesterase levels—The quantitative measurement of acetylcholinesterase level in brain was performed according to the method of Ellman \textit{et al}\textsuperscript{17}. The assay mixture contained 0.05 ml supernatant, 3 ml of 0.01$M$ sodium phosphate buffer ($pH$ 8), 0.10 ml acetylthiocholine iodide and 0.10 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412 nm using Perkin Ellman lambda 20 spectrophotometer. Results were calculated using molar extinction coefficient of chromophore ($1.36 \times 10^4 M\text{m}^{-1}\text{cm}^{-1}$) and expressed as percentage of control. Protein estimation was done by biuret method using bovine serum albumin as standard\textsuperscript{18}.

Statistical analysis—The data were analyzed by using analysis of variance (ANOVA) followed by Tukeys’ test. All the values are expressed as mean ± SE. In all tests, the criterion for statistical significance was $P < 0.05$.

Results

Effect of sertraline on spatial navigation task in 3-NP treated rats—The cognitive function was assessed in Morris water maze paradigm. All the animals in each group quickly learned to swim directly to the platform during training period i.e. from 1\textsuperscript{st} to 5\textsuperscript{th} day. However, when the animals were tested for retention of memory on 10\textsuperscript{th} and 15\textsuperscript{th} day, 3-NP treated rats showed higher latency as compared to the control group ($P < 0.05$). Chronic sertraline treatment (5 and 10 mg/kg, po) showed a significant improvement in memory performance on day 10\textsuperscript{th} and 15\textsuperscript{th} ($P < 0.05$ vs. 3-NP-treated rats) (Fig. 1). However, pretreatment of yohimbine (2 mg/kg) (non selective serotonin antagonist) with the sertraline (10 mg/kg) did not influence the transfer latency in elevated plus maze as compare to sertraline (10 mg/kg) + 3-NP.

Effect of sertraline on redox ratio in 3-NP treated rats—Chronic 3-NP treatment significantly decreased redox ratio (reduced glutathione/oxidized glutathione) (Fig. 4). Total glutathione and reduced glutathione...
glutathione levels were also reduced in striatum, cortex and hippocampus regions of brain as compared to control ($P<0.05$) (Table 1). However the oxidized glutathione level was not influenced significantly in 3-NP treated groups (Table 1). Further, sertraline treatment significantly restored total glutathione (Table 1), reduced glutathione levels (Table 1) and redox ratio (Fig. 4). Yohimbine (2 mg/kg) (non selective serotonin antagonist) pretreatment with sertraline (10 mg/kg) did not influence the action of

![Table 1](https://via.placeholder.com/150)

Table 1: Effect of sertraline and yohimbine (YOM) on 3-Nitropropionic acid -induced glutathione changes in striatum, cortex and hippocampus of rat brain

<table>
<thead>
<tr>
<th>Groups</th>
<th>Brain parts</th>
<th>Total glutathione (µ mole of GSH/mg protein) (% of control)</th>
<th>Reduced glutathione (µ mole of GSH/mg protein) (% of control)</th>
<th>Oxidized glutathione (µ mole of GSH/mg protein) (% of control)</th>
<th>Glutathione-S-transferase (µ mole of CDNB formed/min/mg protein) (% of control)</th>
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<tbody>
<tr>
<td>Control</td>
<td>Striatum</td>
<td>100±3.5</td>
<td>100±3.8</td>
<td>100±3.6</td>
<td>100±4.2</td>
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<td></td>
<td>Cortex</td>
<td>100±4.8</td>
<td>100±3.5</td>
<td>100±2.8</td>
<td>100±3.2</td>
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<tr>
<td></td>
<td>Hippocampus</td>
<td>100±4.0</td>
<td>100±4.6</td>
<td>100±4.7</td>
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<td>3-NP (10 mg)</td>
<td>Striatum</td>
<td>52±4.5</td>
<td>42±4.7</td>
<td>72±3.6</td>
<td>32±4.3</td>
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<tr>
<td></td>
<td>Cortex</td>
<td>70±3.4</td>
<td>68±4.7</td>
<td>81±4.3</td>
<td>52±5.0</td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>75±4.7</td>
<td>59±2.7</td>
<td>88±3.3</td>
<td>70±2.4</td>
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<td>Sertraline (5 mg)</td>
<td>Striatum</td>
<td>102±3.2</td>
<td>106±4.7</td>
<td>94±3.2</td>
<td>99±4.9</td>
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<td>106±3.0</td>
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<tr>
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<td>107±1.5</td>
<td>87±3.1</td>
<td>99±3.9</td>
</tr>
<tr>
<td>(10 mg)</td>
<td>Cortex</td>
<td>100±3.8</td>
<td>114±3.8</td>
<td>80±2.2</td>
<td>100±4.5</td>
</tr>
<tr>
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<td>Hippocampus</td>
<td>101±2.4</td>
<td>101±3.9</td>
<td>103±3.4</td>
<td>100±5.4</td>
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<tr>
<td>Sertraline (5 mg)</td>
<td>Striatum</td>
<td>65±3.3</td>
<td>68±4.5</td>
<td>58±4.1</td>
<td>53±3.8</td>
</tr>
<tr>
<td>(10 mg)+ 3-NP (10 mg)</td>
<td>Striatum</td>
<td>76±3.5</td>
<td>77±3.2</td>
<td>75±3</td>
<td>64±5.4</td>
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<td></td>
<td>Hippocampus</td>
<td>84±2.4</td>
<td>76±4.9</td>
<td>101±4.6</td>
<td>80±5.2</td>
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<td>Sertraline (10 mg)+ 3-NP (10 mg)</td>
<td>Striatum</td>
<td>85±3.6</td>
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<td>79±3.9</td>
<td>66±3.3</td>
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<td>90±4.8</td>
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<td>84±3.5</td>
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<td>YOM (2 mg)</td>
<td>Striatum</td>
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<td>80±3.6</td>
<td>76±4.9</td>
<td>64±3.9</td>
</tr>
<tr>
<td></td>
<td>Cortex</td>
<td>81±4.9</td>
<td>81±4.9</td>
<td>80±5.9</td>
<td>76±4.8</td>
</tr>
<tr>
<td>Sertraline (10 mg)+ 3-NP (10 mg)</td>
<td>Hippocampus</td>
<td>84±3.2</td>
<td>85±5.3</td>
<td>82±2.8</td>
<td>82±3.8</td>
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</tbody>
</table>

*P <0.05* versus control; *b*versus 3-NP; *c*versus 3-NP + Sertraline (5)
sertraline (10 mg/kg) on glutathione levels and redox ratio as compare to their effect per se in 3-NP treated groups.

Effect of sertraline on glutathione-S-transferase (GST) enzyme activity in 3-NP treated rats—Chronic 3-NP treatment significantly decreased glutathione-S-transferase activity as compared to the control group \((P<0.05)\). Sertraline (5 and 10 mg/kg, po) treatment significantly attenuated glutathione-S-transferase in striatum, cortex and hippocampus regions of 3-NP treated rats \((P<0.05)\) (Table 1) Yohimbine (non selective serotonin antagonist) (2 mg/kg) pretreatment with the sertraline (10 mg/kg) did not influence sertraline effect on GST.

Effect of sertraline on acetylcholinesterase levels in 3-NP treated rats—Further, chronic 3-NP treatment significantly increased acetylcholinesterase enzyme level in striatum, cortex and hippocampus regions of brain as compared to control group and sertraline per se \((P<0.05)\). Sertraline treatment significantly attenuated acetylcholinesterase enzyme activity in striatum, cortex and hippocampus regions on 3-NP treated animals. Yohimbine (2 mg/kg) pretreatment with the sertraline (10 mg/kg) did not influence significantly the protective effect of sertraline on acetylcholinesterase enzyme activity as compare to their effect per se.

Discussion
Sertraline, a selective serotonin reuptake inhibitor (SSRIs) is used to treat depression and many other neurodegenerative disorders. HD is an autosomal dominant inherited neurodegenerative disease, characterized by progressive motor and cognitive deterioration. In the present study, the protective effect of sertraline against 3-NP induced memory dysfunction and disturbed redox ratio was explored.

3-NP is a well known neurotoxin which produces HD like symptoms in animals and human. In the present study, 3-NP administration significantly impaired memory (in Morris water maze and plus maze) with alterations in glutathione and acetylcholinesterase enzyme activity in discrete regions of rat brain. In Morris water maze test, mean transfer latency on the 5th day of training was same in all the groups. However, memory retention and time spent in target quadrant was significantly decreased on 10th and 15th day in 3-NP treated animals as compared to vehicle treated animals. Similarly, memory retention percentage was also significantly reduced in elevated plus maze performance task in 3-NP treated animals on 15th day. Results of the present study duly support the previous studies suggesting 3-NP causes memory dysfunction. Previous reports also suggested that 3-NP produced hippocampal CA1 and CA3 pyramidal neurons lesion, the areas of brain that are associated with cognitive performance. Further, 3-NP treatment significantly increased acetylcholinesterase enzyme activity in hippocampus as compared to other areas, suggesting the involvement of hippocampus in cognitive dysfunction. Some reports also suggested relation between cognitive impairment and oxidative stress. Sertraline treatment significantly improved cognitive performance in both Morris water maze and elevated plus maze performance, suggesting its therapeutic potential in this disorder. Sertraline treatment decreased acetylcholinesterase enzyme levels in all regions of brain with associated improved memory performance in 3-NP treated animals. Previous studies also reported that antidepressants have a role in improving memory performance in animals and humans. Schmitt et al also concluded that non-serotonergic mechanisms are associated in improving cognitive effects of sertraline and putative dopamine reuptake activity of sertraline. In the present study, yohimbine, a non selective serotonin antagonist administration with sertraline (10 mg/kg) did not influence the action of sertraline, indicating the involvement of antioxidant like mechanism in its protective action. It is possible that antioxidant like mechanism could be involved in the protective action of sertraline.
Oxidative stress can induce neuronal cell death in a variety of circumstances when the normal balance between oxidative events and antioxidant defence is disrupted either by loss of reducing agents/antioxidant enzymes or by increased production of oxidizing species. Emerging evidence from a number of studies suggest that oxidative stress can be a final common pathway in various forms of neuronal cell death including a wide variety of acute and chronic neurological diseases, as well as in normal aging.

Glutathione (GSH), a tripeptide composed of L-glutamate, L-cysteine and glycine and most important systems in body, plays a crucial role as free radical scavenger. The ability of GSH to scavenge non-enzymatically both singlet oxygen and OH provides the first line of antioxidant defense. Glutathione exists in both reduced (GSH) form and an oxidized dimer (GSSG) form, the former by far the largest fraction in a ratio. Reduced GSH strongly modulates redox state (ratio of oxidizing to reducing equivalents) of the cell, a role which is critical for cell survival. The ratio of GSSG/GSH serves as a sensitive index of oxidative stress.

Redox ratio or disruption of glutathione system was another hallmark of the 3-NP neurotoxicity. In the present study, 3-NP significantly decreased total glutathione, reduced glutathione and oxidized glutathione levels in the striatum, cortex and hippocampal regions of the brain, indicating weak antioxidant defense. 3-NP exposure significantly decreased the level of the glutathione–S-transferase in the same areas of brain. It seems that 3-NP induced neurotoxicity and cognitive dysfunction could be related to imbalance between reactive oxygen species and antioxidant defenses resulting in marked oxidative stress. Earlier reports have suggested that 3-NP induced toxicity significantly affect redox ratio. Therefore, glutathione imbalance could play a key role in the 3-NP induced neurotoxicity. 3-NP inhibits succinate dehydrogenase complex in the mitochondria which ultimately generates free radicals and nitrogenous species. Excessive free radical generation impairs the capacity of antioxidant defense enzyme specifically glutathione. Sertraline treatment restored glutathione as well as glutathione–S-transferase enzyme level in a dose dependent manner, suggesting its antioxidant like effect. Sertraline has been reported to enhance neurogenesis, which appeared to be responsible for its beneficial effects in HD mice. Clinical reports also suggest that sertraline improves the symptoms in HD patients. The antioxidants like actions of various antidepressants have already been reported. Present study also suggests that other mechanisms may be involved in addition its action on serotonin receptor. However, yohimbine (non selective serotonin receptor antagonist) treatment did not influence the protective effect of sertraline. This suggests that protective action of sertraline could be mediated through its antioxidant like action which is independent of its action on serotonin receptor. Muñoz-Castañeda et al reported the relationship between serotonin metabolism and oxidative stress. Chronic administration of sertraline and oxotremorine increases the expression of cAMP response element binding protein (CREB) and downstream gene BDNF in rodents. It is further possible that antioxidant like effect of sertraline could be due to increased BDNF levels, preserved chaperone protein HSP70 and Bcl-2 levels in the brain.

Taken together, these data suggest that sertraline has antioxidant activity and the propensity to modulate neurotoxicity induced oxidative impairments in the brain and can be effectively employed as a neuroprotective adjuvant to abrogate oxidative stress in vivo. The results further suggest that the protective action of sertraline could be independent of serotonin receptors. The preclinical findings obtained in the present study may provide a rationale for clinical trials of SSRIs in humans with HD.

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