

Neuroprotective potential of escitalopram against behavioral, mitochondrial and oxidative dysfunction induced by 3-nitropropionic acid

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KEY WORDS

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ABSTRACT

Background: Huntington's disease (HD) is a neurodegenerative syndrome that leads to marked decline in cognitive functioning along with uncharacteristic body movements called chorea. There exists no therapeutic agent to address the disease. 3-Nitropropionic acid (3-NP) which is a suicide inhibitor of succinate dehydrogenase and a well-known experimental model to study Huntington's disease, causes substantial impairment in gait and memory through oxidative and neuronal damage.

Purpose: In the present study protective effect of escitalopram against 3-NP induced neurotoxicity was explored.

Methods: Adult female Wistar rats were subjected to per oral administration of 2 different doses of escitalopram (10 and 20 mg/kg) for 12 days followed by intraperitoneal injection of 3-NP (20 mg/kg) on the last four days.

Results: Intraperitoneal injection of 3-NP led to significant induction of HD like symptoms in rats such as impaired memory, reduced locomotor activity, hind limb impairment, decreased body weight, oxidative damage and mitochondrial dysfunction. Treatment with 2 different doses of escitalopram helped reverse the mitochondrial enzyme dysfunction along with reversal of behavioural and biochemical anomaly induced by 3-NP. Further, histopathological examination confirmed the neuroprotective potential of escitalopram against 3-NP induced pathological lesions.

Conclusion: The results obtained thus substantiate the claim that escitalopram might play an antioxidant and neuroprotective role against 3-NP induced alterations in rats and can prove to be a promising candidate for the management of HD.

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Introduction

Huntington's disease (HD) is a genetic neurodegenerative disorder that is pathologically characterised by the loss of striatal neurons, leading to behavioural symptoms like psychiatric instabilities, dementia, abnormal body movements (chorea) and unintended weight loss.¹ The exact pathogenesis leading to Huntington's is ambiguous. Abnormalities in IT15 gene gives rise to a mutant huntingtin protein with an abnormal expansion of CAG repeat. This mutated protein aggregates in striatal neurons as well as neurons in the cortex, thalamus, hypothalamus, and substantia nigra thereby leading to a sequence of events that includes increased oxidative injury, transcriptional dysregulation, glutamate excitotoxicity, apoptosis and mitochondrial dysfunction. Also studies carried out on HD post-mortem tissue have revealed selective dysfunction of components of the mitochondrial electron transport chain² which involves alteration in the functions of the complex II (*Succinate dehydrogenase*) and complex IV (*Cytochrome C*) of the electron transport chain.³

3-Nitropropionic acid (3-NP) induces neurotoxicity by irreversibly inhibiting succinate dehydrogenase enzyme that forms the complex-II of the electron transport chain, thereby producing chorea and dementia that mirrors the symptoms seen in patients with HD.⁴ Manipulating the time course of 3-NP injections leads to sustained hyperactivity and hypoactivity seen in early and late stages of HD can also be produced by means of manipulation of time course of 3-NP injections. Since intoxication with 3-NP

produces selective striatal lesions, 3-NP is exploited to produce an experimental model of Huntington's disease.⁵

Escitalopram, the S-enantiomer of citalopram, is a selective serotonin reuptake inhibitor (SSRI) that has demonstrated antidepressant and anxiolytic activities in preclinical and clinical studies. Selective Serotonin reuptake inhibitors (SSRI's) selectively block serotonin (5-hydroxytryptamine, 5-HT) reuptake in the pre synaptic cell thereby increasing extracellular serotonin levels. The antioxidant and neuroprotective effects of anti-depressants have been well documented in various studies that have demonstrated the potential of anti-depressants to attenuate the oxidative stress and restore the antioxidant enzyme levels.⁶ SSRI's are also known to decrease the excitotoxicity in the cell through suppression of the voltage gated sodium and calcium channels.⁷ They increase Brain derived neurotrophic factor (BDNF) levels.⁸ Hypothalamic-pituitary-adrenal axis (HPA-axis) hyperactivity is seen in HD patients which is attributed to the dysregulation of the 5-HT neurotransmission can be reversed by escitalopram.⁹ Thus, in the present study, the possible neuroprotective effect of Escitalopram against 3-nitropropionic acid induced behavioural, biochemical, and mitochondrial dysfunctions in the rat brain was explored

Methods

Chemicals

The drugs used in the study were 3-Nitro Propionic acid (3-NP) and Escitalopram. 3-NP was purchased from sigma chemicals whereas escitalopram was obtained as a gift sample from lupin lab. All analytical grade chemicals were used respectively.

Animal and Treatment Schedule

Adult female Wistar rats (200–230 g) were used in the present study. Prior to experimentation the animals were allowed to acclimatize to the laboratory conditions. The animals were housed under standard conditions of 12 h light/dark cycles and were provided with a standard rat feed and water ad libitum with experiments being strictly conducted between 09:00 and 17:00 h. The study was carried out in accordance with the guidelines of committee for the Purpose of Control and Supervision of Experiments on Animals for use and care of laboratory animals and the protocol was approved by the Institutional Animal Ethics Committee. Animals were randomly divided into 5 groups each containing 6 animals. 3-NP was diluted with saline (pH 7.4) and injected intraperitoneally, whereas escitalopram was dissolved in water and administered per orally. Animals from group I and II received vehicle (oral) and saline (i.p) respectively for 12 days. Group III & IV received two different doses of Escitalopram (p.o) (10 mg/kg & 20 mg/kg) for 12 days. Animals from group II, III & IV were challenged using 3-NP (20 mg/kg) (i.p) on last four consecutive days of treatment (9th to 12th day from the administration of the drug) wherein 3-NP was administered 1 hr. after saline (for group II) and escitalopram (groups II and IV) treatment. Group V animals were administered with a higher dose of Escitalopram (20 mg/kg) for 12 days and saline (i.p) for last four days (9th–12th).

Body Weight Measurement

Animal body weights were recorded on day zero and day 12th respectively. The percent change in body weight was recorded.

Behavioural Assessments

All animals were subjected to a training period of 4 days prior to the start of drug treatment. All behavioural parameters were assessed 1 hr after 3-NP administration and an interval of 30 mins was given between each test.

Elevated Plus Maze Test

Assessment of spatial long term memory was carried out using elevated plus maze apparatus.¹⁰ The maze comprised of two open arms crossed with two closed arms and elevated at a height of 50 cm from the ground with the arms connected with central square (10 × 10 cm). Animal was placed at one end of the open arm facing away from the central square and the initial transfer latency was recorded by measuring the time taken by the animal to move into one of the enclosed arms. The animal was allowed to explore the maze for another 30 secs before returning to the home cage. A gentle push was given in case the animal failed to enter into one of the enclosed arms within 90 secs, with the transfer latency (TL) being assigned as 90. TL recordings were carried out on day 0, 8 and 12.

Morris Water Maze test

Cognitive performance was evaluated using Morris water maze apparatus.¹¹ The maze comprised of a circular tank (diameter 160 cm & height 35 cm). The tank was filled with water to a depth of 20 cm and was made opaque by adding milk. The tank was divided into 4 fixed points on its perimeter assigned as 4 quadrants. The tank also comprised of a square platform (escape platform) attached at one end along the circumference of the tank placed at about 2 cm below the water surface and which was of the same colour as that of the rest of the

basin to avoid any false positive results. Animals were trained to swim to the platform by placing them gently at a start point in the middle of the rim of a quadrant not containing the escape area with their face to the wall. In case the rat failed to find the platform within 90 s it was guided to the platform and allowed to stay on it for 20 s. Training sessions comprising of four trials in a day for 4 days was provided. In all four trials, starting positions were different (acquisition trial). After the acquisition trials, the time taken by the animals to reach the platform (escape latency) was recorded on day 0, 8 and 12 respectively.

Narrow Beam test

It was carried out to measure the hind limb impairment.¹² The narrow beam comprising of a wooden beam with dimensions of 105 cm length and 4 cm width and suspended at a height of 80 cm from the ground with adequate wooden supports (Fig 1). A 20 cm line was drawn at the one end of the beam. Recording was done by placing the animal within the 20 cm starting zone, facing its home cage. The stopwatch was started immediately on releasing the animal and the time taken by the animal to traverse the beam was determined. The maximum time allotted for the task was 2 mins. Falls, if any, were also recorded. The test was performed on day 0, 8 and 12 respectively.

Assessment of gross behavioural activity

It involves assessment of locomotor and rearing activity using a photoactometer on the last day of study.¹³ An actophotometer consists of infrared sensors and a digital counter. The animal's movement interrupts the infrared beam, which gets recorded and displayed digitally. This principle is used to count total locomotor activity of an animal. A habituation period of 3 min was given by placing the animal in the cage followed by activity recordings for next 5 mins. The motor activity was detected by infrared photocells. Locomotor activity (horizontal and vertical) was estimated as counts per 5 min.

After behavioural estimations on day 12, the animals were sacrificed using carbon dioxide chamber. A modified method of Sandhir et al¹⁴ was used to isolate the rat brain mitochondrial pellets. The whole rat brain was isolated and the cerebellum was separated. The brain was rinsed in ice cold saline and was homogenised with 10 volumes (w/v) ice cold homogenising buffer (10 mM Tris-HCl, 0.44 M sucrose, 10 mM ethylenediaminetetraacetic acid). The homogenate was subsequently centrifuged at 5000 rpm for 30 mins at 4°C to obtain the brain



Fig. 1: Narrow beam apparatus for rats.

supernatant by discarding the pellets. Estimation of lipid peroxidation (LP), glutathione reductase (GSH), total tissue proteins (TTP), Acetylcholinesterase (AChE) and Nitrite was carried out using the supernatant. The residual supernatant was re-centrifuged at 5000 rpm for 45 mins at 4°C to obtain the mitochondrial pellets. The crude pellets were washed with equal volumes of extraction buffer and centrifuged at 5000 rpm for 45 mins at 4°C. The final pellets obtained were suspended in equal volume of ice cold suspending buffer (0.44 M sucrose in 10 mM Tris-HCl, pH7.4). The pellets containing pure mitochondrial fraction were used for the estimations of mitochondrial parameters.

Mitochondrial Parameters

Complex-I (Nicotinamide adenine dinucleotide dehydrogenase (NADH) activity)

NADH activity was measured through spectrophotometry as per the method described by King and Howard.¹⁵

Complex-II (succinate dehydrogenase (SDH) activity)

SDH activity was measured through spectrophotometry as per the method described by King.¹⁶

Complex IV (Cytochrome oxidase Assay)

Cytochrome oxidase activity was determined according to protocol of Sottocasa et al.¹⁷

MTT Assay¹⁸

Mitochondrial respiratory chain activity was determined by measuring the rate of MTT reduction as per the method described by Kamboj et al.¹⁸

Anti-oxidant Parameters

Lipid peroxidation Measurement

The amount of malondialdehyde (MDA) formed by reaction with TBA was used to estimate lipid peroxidation as per the method described by Ohkawa et al.¹⁹

Reduced glutathione

Reduced glutathione (GSH) was assessed according to the method of Ellman.²⁰

Catalase activity

Catalase (CAT) activity was determined according to the method described by Luck²¹

Estimation of other parameters

Nitrite

Presence of nitrite in the supernatant was determined using Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulphanilamide and 5% phosphoric acid) through colorimetric analysis. 500 microliters of both supernatant and Greiss reagent were mixed and the²² mixture was incubated in dark at room temperature for 10 mins. The absorbance was measured at 540 nm and concentration of nitrite in the supernatant was determined from sodium nitrite standard curve and expressed as $\mu\text{mol}/\text{mg}$ protein.

Acetylcholinesterase (AChE) Estimation

The AChE activity was determined by Ellman method.²³

Total Tissue Protein Estimation

Total tissue protein in the supernatant was determined by Lowry method.²⁴

Histopathological Examination

On day 12, after behavioural estimations, the animals were sacrificed using carbon dioxide chamber and representative brain samples from each group were preserved in 10% formalin. Staining of the sections was done using hematoxylin and eosin (H&E) and were subsequently examined under the light microscope at different magnifications and photographs were taken.²⁵

Statistical Analysis

All values were expressed as mean \pm standard deviation. Statistical method employed was one way analysis of variance followed by Tukey's test. Values with $P < 0.05$ were considered as statistically significant.

Results

Effect of Escitalopram on Body Weight in 3-NP treated rats

A significant decline in body weight was observed on day 12 in 3-NP treated animals as compared to the vehicle treated group ($p < 0.05$) that showed no substantial change in body weight. On the other hand, treatment with Escitalopram (10 and 20 mg/kg) attenuated the loss in body weight of 3-NP treated ($p < 0.05$). No significant change in body weights was observed for animals receiving drug Per se (20 mg/kg) (Fig 2).

Effect of Escitalopram on Behavioural Parameters

Elevated Plus Maze Test

Significant increase in transfer latency on day 12 was observed for 3-NP treated rats ($p < 0.05$). Escitalopram pre-treatment (10 and 20 mg/kg) significantly decreased the transfer latency ($p < 0.05$). Treatment with only escitalopram (20 mg/kg) did not show any significant change in transfer latency (Fig 3).

Morris Water Maze Test

Treatment with 3-NP resulted in an increase in escape latency (time taken by the animals to locate the platform) on day 12 ($p < 0.05$). Escitalopram pre-treatment (20 mg/kg) showed improvement in the escape latency ($p < 0.05$). Treatment with

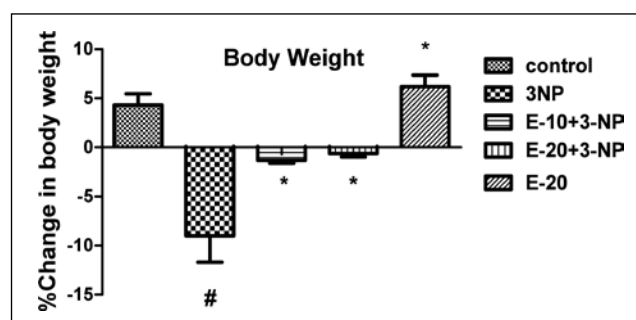


Fig. 2: Effect of Escitalopram on Body Weight.

Values are expressed as mean \pm standard deviation (SD); $n = 6$. Significance determined using one way ANOVA followed by Tukey's test. #Statistically significant from control group ($p < 0.05$). *Statistically significant from 3-NP treated group ($p < 0.05$).

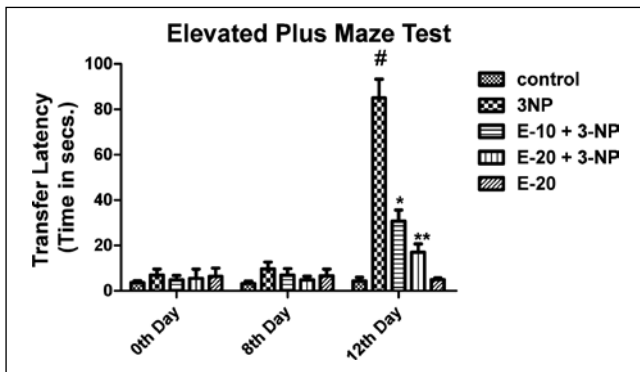


Fig. 3: Effect of Escitalopram on Transfer Latency. Values are expressed as mean \pm standard deviation (SD); $n = 6$. Significance was determined using one way ANOVA followed by Tukey's test. #Statistically significant from control group ($p < 0.05$). *Statistically significant from 3-NP treated group ($p < 0.05$).

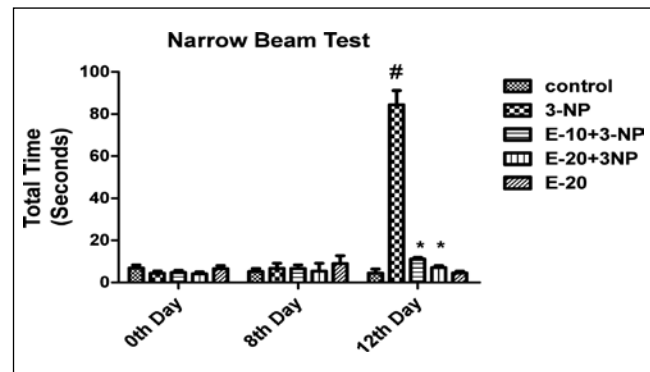


Fig. 5: Effect of Escitalopram on Narrow Beam. Values are expressed as mean \pm standard deviation (SD); $n = 6$. Significance was determined using one way ANOVA followed by Tukey's test. #Statistically significant from control group ($p < 0.05$). *Statistically significant from 3-NP treated group ($p < 0.05$).

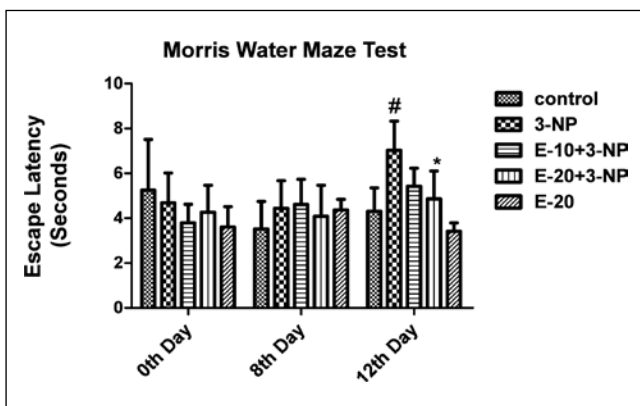


Fig. 4: Effect of Escitalopram on Cognitive Impairment. Values are expressed as mean \pm standard deviation (SD); $n = 6$. Significance was determined using one way ANOVA followed by Tukey's test. #Statistically significant from control group ($p < 0.05$). *Statistically significant from 3-NP treated group ($p < 0.05$).

vehicle and Escitalopram only (20 mg/kg) did not affect the escape latency time (Fig 4).

Narrow Beam Test

3-NP treatment caused prominent hind limb impairment on day 12 thereby increasing the time taken by the animals to traverse the beam ($p < 0.05$). Pre-treatment with Escitalopram (10 and 20 mg/kg) attenuated the hind limb impairment thereby showing a reduction in time taken by the animals to cross the beam as compared to the animals receiving only 3-NP ($p < 0.05$). Animals subjected to vehicle and per se treatment of Escitalopram (20 mg/kg) showed no significant change (Fig 5).

Assessment of gross behavioural activity (Locomotor activity)

A substantial decrease in locomotor as well as rearing activity was observed for 3-NP treated rats ($p < 0.05$). Escitalopram pre-treatment (10 and 20 mg/kg) significantly increased locomotor activity ($p < 0.05$). Per se treatment with Escitalopram (20 mg/kg) and vehicle treatment showed no significant change (Fig 6).

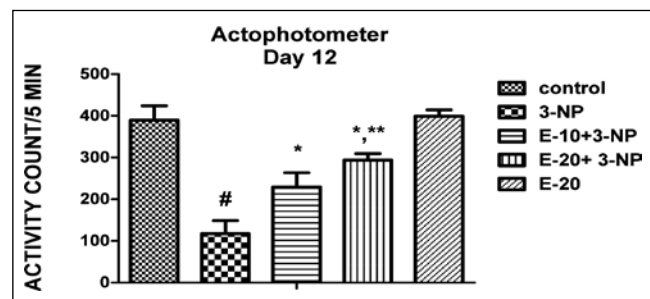


Fig. 6: Effect of Escitalopram on Locomotor. Values are expressed as mean \pm standard deviation (SD); $n = 6$. Significance was determined using one way ANOVA followed by Tukey's test. #Statistically significant from control group ($p < 0.05$). *Statistically significant from 3-NP treated group ($p < 0.05$).

Effect of Escitalopram on mitochondrial respiratory chain enzymes

3-NP administration significantly reduced all the mitochondrial enzyme complexes (I, II, IV and MTT activities) ($p < 0.05$). Pre-treatment with escitalopram (10 and 20 mg/kg) for 12 days significantly attenuated mitochondrial enzyme dysfunction in a dose dependent manner ($p < 0.05$). Treatment with Escitalopram only (20 mg/kg) did not alter the mitochondrial complexes significantly (Table 1)

Effect of Escitalopram on antioxidant parameters (Lipid peroxidation, Reduced glutathione, Catalase)

A significant increase in lipid peroxidation and decrease in catalase enzyme activity and reduced glutathione was seen with 3-NP administration (Table 2). Pre-treatment with Escitalopram (10 and 20 mg/kg) counteracted this effect by decreasing lipid peroxidation and restoring the levels of reduced glutathione and catalase enzyme activity ($p < 0.05$). Per se treatment with escitalopram (20 mg/kg) did not affect the levels of these antioxidant parameters.

Effect of Escitalopram on Nitrite and AchE enzyme levels

3-NP administration produced significant increase in AchE and nitrite levels ($p < 0.05$) (Table 3). Pre-treatment with Escitalopram

Table 1: Effect of Escitalopram on mitochondrial respiratory chain enzymes

Groups	Complex-I (μ mole NADH oxidized/ min/mg protein)	Complex-II (μ mole SDH oxidized/min/ mg protein)	Complex-IV (μ mole cytochrome oxidized/min/mg protein)	MTT reduction (% of control)
Control	9.33 \pm 0.34	93.06 \pm 8.00	7.70 \pm 0.95	100 \pm 0.029
3-NP	4.40 \pm 0.34 [#]	26.57 \pm 2.03 [#]	3.06 \pm 0.25 [#]	35.77 \pm 0.073 [#]
E-10 + 3NP	6.37 \pm 0.23 [*]	50.70 \pm 3.24 [*]	4.35 \pm 0.21 [*]	58.33 \pm 0.937 [*]
E-20 + 3NP	7.63 \pm 0.40 ^{*,**}	73.51 \pm 2.45 ^{*,**}	6.05 \pm 0.37 ^{*,**}	73.24 \pm 1.327 ^{*,**}
E-20	9.74 \pm 0.66	88.48 \pm 5.35	8.44 \pm 0.82	89.01 \pm 2.91

Values are expressed as mean \pm standard deviation (SD); n = 6.

Significance was determined using one way ANOVA followed by Tukey's test.

[#]Significantly different from control group (p<0.05).

^{*}Significantly different from 3-NP treated group (p<0.05).

^{**}Significantly different from E-10 + 3-NP-treated group (p<0.05).

SD- standard deviation; ANOVA - analysis of variance.

Table 2: Effect of Escitalopram on anti-oxidant parameters

Groups	Lipid peroxidation (nmole/mg protein)	Reduced glutathione (μ mole/mg protein)	Catalase (μ mole of H ₂ O ₂ decomposed/min/mg protein)
Control	4.92 \pm 0.55	27.65 \pm 3.63	8.66 \pm 0.91
3-NP	9.99 \pm 1.09 [#]	18.11 \pm 2.68 [#]	2.33 \pm 0.37 [#]
E-10 + 3NP	7.27 \pm 0.13 [*]	23.06 \pm 2.55 [*]	4.01 \pm 0.84 [*]
E-20 + 3NP	5.69 \pm 0.68 ^{**}	24.83 \pm 1.01 [*]	7.42 \pm 0.66 ^{**}
E-20	4.24 \pm 0.51	28.59 \pm 0.84	8.71 \pm 0.49

Values are expressed as mean \pm standard deviation (SD); n = 6.

Significance was determined using one way ANOVA followed by Tukey's test.

[#]Significantly different from control group (p<0.05).

^{*}Significantly different from 3-NP treated group (p<0.05).

^{**}Significantly different from E-10 + 3-NP-treated group (p<0.05).

Table 3: Effect of Escitalopram on nitrite and AchE enzyme levels

Groups	Nitrite levels (μ mole/mg protein)	Acetylcholinesterase levels (μ mole of substrate hydrolysed/mg protein)
Control	0.16 \pm 0.018	30.91 \pm 1.54
3-NP	0.27 \pm 0.0095 [#]	69.25 \pm 9.08 [#]
E-10 + 3NP	0.21 \pm 0.0041 [*]	37.85 \pm 1.47 [*]
E-20 + 3NP	0.18 \pm 0.00922 ^{*,**}	34.68 \pm 0.73 ^{*,**}
E-20	0.15 \pm 0.0076	28.67 \pm 0.73

Values are expressed as mean \pm standard deviation (SD); n = 6.

Significance was determined using one way ANOVA followed by Tukey's test.

[#]Significantly different from control group (p<0.05).

^{*}Significantly different from 3-NP treated group (p<0.05).

^{**}Significantly different from E-10 + 3-NP-treated group (p<0.05).

(10 and 20 mg/kg) in 3-NP treated animals resulted in significant decrease in AchE levels (p<0.05). *Per se* treatment with escitalopram (20 mg/kg) did not affect AchE and nitrite levels.

Effect of Escitalopram on Histopathological changes

Effect of Escitalopram on the histopathological changes in the brain striatum in 3-NP treated rats is shown in Table 4 and Fig 7.

Discussion

In the present study Escitalopram was found to be successful in overcoming the oxidative stress and mitochondrial dysfunction

induced by 3-NP which was evaluated using various biochemical, behavioural and mitochondrial parameters.

A hallmark symptom associated with Huntington's disease is weight loss. It occurs early in HD patients and is independent of calorie intake. It is reported mitochondrial energy production is inversely proportion to the length of the CAG repeats²⁶ The above claim is bolstered in our study wherein administration of 3-NP resulted in substantial decrease in body weight in rats as opposed to those receiving only saline. Escitalopram pre-treatment for 12 days helped to subdue this decrease in body weight indicating its therapeutic usefulness.

Table 4: Effect of Escitalopram on the histopathological changes in the brain striatum in 3-NP treated rats

Groups	Remarks
Control	NAD. No evidence of degeneration
3-NP	Moderate to severe neurofibrillary degeneration in striatum and hippocampus region. (+++)
E-10 + 3-NP	Mild-Moderate foci of degeneration. (++)
E-20 + 3-NP	Patchy areas of degeneration are evident in the striatum and hippocampus region. (+)
E-20	NAD. No foci of degeneration.

NAD: No abnormality detected; +: Mild; ++: Moderate; +++: Severe.

Note: Areas with degeneration are marked with arrows.

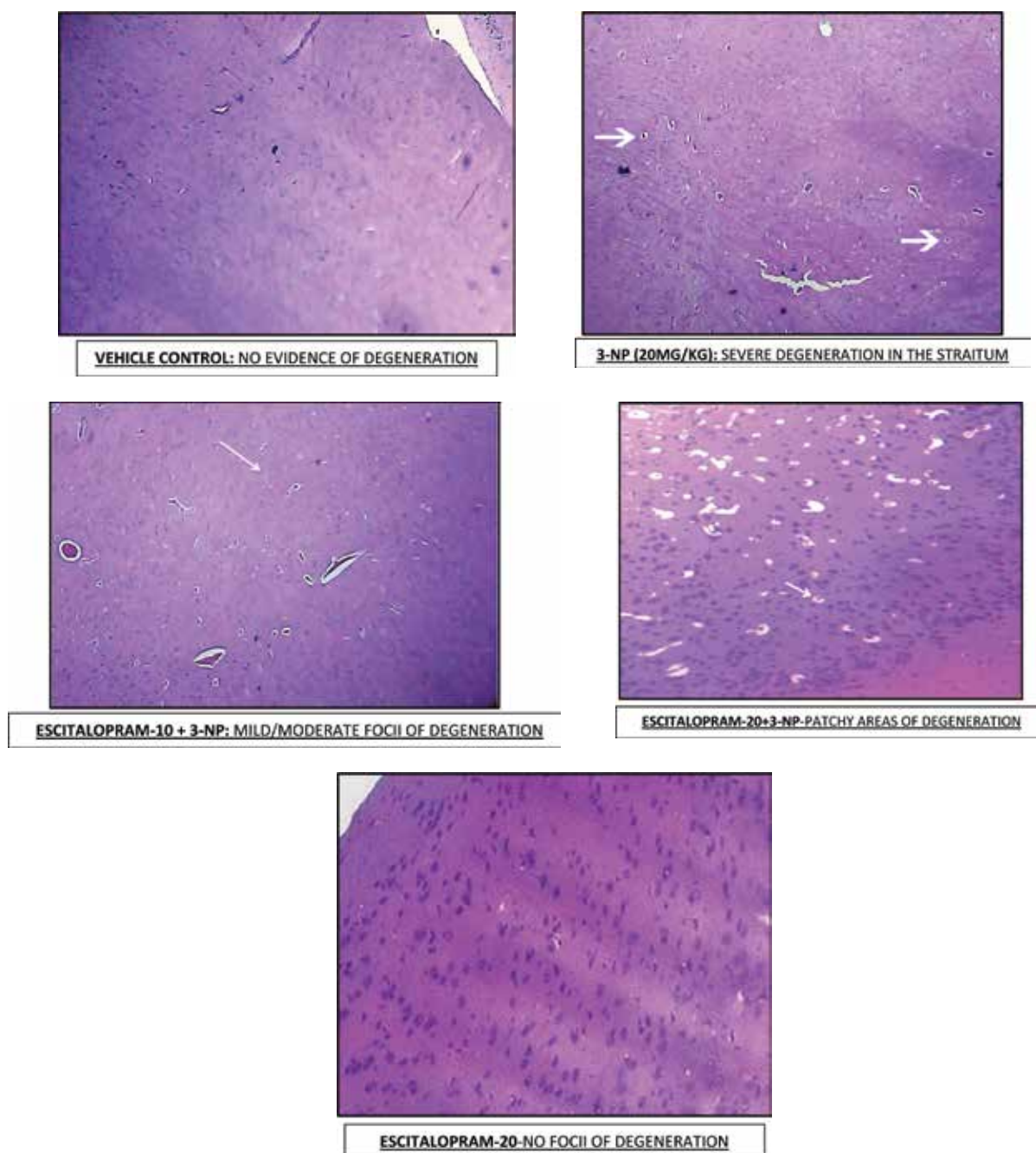


Fig. 7: Histopathological pictures from the sections through striatum of brain showing effect of Escitalopram on 3-NP induced pathological lesion.

3-NP produces locomotion defects that resemble the motor symptoms seen in HD. 3-NP causes death of striatal and leads to conspicuous decline in motor movements.⁶ Behavioural parameters assessed in the present study to examine the motor incoordination caused by 3-NP comprised of actophotometer and Narrow beam test. 3-NP administration resulted in significant hind limb impairment (narrow beam test) and decrease in locomotor and rearing activity (actophotometer). Escitalopram pre-treatment helped to attenuate this motor defects which supports the findings that SSRI's improve locomotor performance irrespective of serotonin metabolism.²⁷

Another major symptom of Huntington's disease is memory decline that occurs due to the disruption of striatal–frontal circuits in Huntington's disease patients. 3-NP produces memory impairment by causing alterations in neural processing and significantly inhibiting *Succinate Dehydrogenase* SDH.²⁸ Cognitive performance in the present study was evaluated using Elevated Plus maze test and Morris water maze test. 3-NP resulted in significant decrease in spatial long term memory and cognitive impairment. Escitalopram pre-treatment helped to subdue this cognitive decline, implying its potential to overcome memory dysfunction. The ability of SSRI's to selectively block serotonin reuptake and increase BDNF levels which in turn promotes neurogenesis might help to attenuate these behavioral anomalies induced by 3-NP in rats.²⁹

It has been reported that 3-NP administration tends to alter the oxidants/antioxidant defence system by some anonymous mechanisms.⁸ Inactivation of Succinate dehydrogenase (SDH) brought about by the irreversibly binding of 3-NP leads to the impairment of electron transport chain leading to decreased energy production and depolarization of membrane potential. This is followed by release of precursors for radical species production and consequently oxidative stress. Generation of superoxide radicals can be brought about by the activation of NMDA receptors by 3-NP allowing calcium influx into the cell, which can further exacerbate oxidative damage.³⁰

GSH is a non-enzymatic antioxidant that plays a vital role in reduction of ROS in brain by interacting directly to detoxify certain ROS. Lipid peroxidation leads to the loss of cell function in conditions like oxidative stress in brain and in neurodegenerative disorders.³² Reaction of O₂ with Nitric oxide results in the production of ONOO⁻ which highly cytotoxic and induces hydroxyl radical formation which makes it critical in the pathogenesis of neurodegenerative disorders.³³ Catalase is an antioxidant enzyme that is essential for protection against oxidative damage to the cell.³⁴

3-NP treatment resulted in significant oxidative stress by decreasing GSH and catalase levels and increasing LPO and nitrite levels. These alterations were overcome by escitalopram pre-treatment. Previous studies have reported that SSRI's indeed have an antioxidant effect⁷ in addition to its action on serotonin receptor. Increased metabolism of dopamine and nor epinephrine increases the radical burden and oxidative stress. Escitalopram most likely exerts its antioxidant effect by inhibiting the reuptake of serotonin by means of selective inhibition of presynaptic 5- hydroxytryptamine (5-HT) uptake site. Suppression of immune T cells and P450 enzymes is associated with antidepressant treatment. This effect might help to overcome the oxidative stress.³⁵ However, similar studies carried out in different animal model also suggest the thera-

peutic role of interventions like bacopa monierrra in alzheimer's model of mice.³⁶

Reduced levels of acetylcholine are one of primary biochemical alterations seen in HD leading to cognitive defects. Acetylcholinesterase (AChE) is an enzyme that catalyses hydrolysis of acetylcholine thereby serving to terminate synaptic transmission.³⁷ 3-NP increased AChE levels, whereas Escitalopram administration helped to restore the levels of AChE to normal.

One of the major pathways leading to huntington's disease is dysfunction in mitochondrial energy metabolism. Experimental and clinical evidences indicate specific impairment of complex II in Huntington's disease patients. 3-NP acts by selectively causing the impairment of complex-II of mitochondrial respiratory chain thereby leading to decreased ATP levels.³⁸ In this study, 3-NP significantly decreased mitochondrial complex activity (Complexes-I, II, and IV). Complex-II enzyme activity was significantly reduced as compared to other complexes because of the specificity of 3-NP towards succinate dehydrogenase. 3-NP also declined number of viable cells in the mitochondria as estimated by MTT assay.

Escitalopram treatment significantly attenuated the dysfunctional mitochondrial enzyme complex. Inhibition of mitochondrial permeability transition and extracellular catabolism of ATP can be brought about by antidepressants. Therefore, it is possible to suggest that changes induced by antidepressants on mitochondrial complexes in this study may be due to modulating ATP levels in the mitochondria of brain.³⁹ Neuroprotective effect of escitalopram was further determined by histopathological examinations.

Conclusion

Above data in the study suggests that escitalopram has antioxidant activity and also the ability to overcome the oxidative damage induced in the brain and presents itself as a potential neuroprotective agent to ameliorate the oxidative stress and neuronal damage induced in HD. Thus, these findings may provide a guide for the clinical trials in humans with HD. Escitalopram is a well-tolerated drug and devoid of potential lethal side effects. The present study thus warrants further investigation.

Authorship Contribution

Shruthi Shetty, Ashwini Hariharan: Experimental, **Shruthi Shetty:** Writing of manuscript, **Aarti G Jagtap:** Writing of manuscript & supervision on experiment, **Trupti Shirole:** Biochemical studies

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Abbreviations

BDNF:	Brain derived neurotropic factor
	Control-Vehicle treated rats
E-10 + 3-NP:	Escitalopram (10 mg/kg) +3-Nitropropionic acid (20 mg/kg) treated rats
E-20 + 3-NP:	Escitalopram (20 mg/kg) +3-Nitropropionic acid (20 mg/kg) treated rats
E-20:	Escitalopram (20 mg/kg) treated rats

Huntington's disease: HD
5-hydroxytryptamine: 5-HT
3-Nitropropionic acid: 3-NP
Selective Serotonin
reuptake inhibitors: SSRI's

This article complies with International Committee of Medical Journal editor's uniform requirements for manuscript.

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