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Ameliorative Effects of Vortioxetine in 3-NPA induced Huntington's Disease

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Abstract. Huntington disease (HD) occurs by development of mutant huntingtin protein which primarily affects the CNS and peripheral organs. In addition to the loss of motor coordination it also causes mental and behavioural changes, weight loss and bioenergetics deficiency. The study examined the neuroprotective effect of Vortioxetine against 3-Nitropropionic acid (3-NPA) induced HD in rats. Various parameters including, motor coordination (Rota rod), locomotor activity (Actophotometer), and learning-memory were evaluated. Brain striatum oxidative stress (Lipid per oxidation-MDA, SOD, Catalase), nitrosative stress (Nitrate/nitrite level), acetyl cholinesterase level, inflammatory markers- TNF-α & IL-6 and BDNF levels were also assessed along with the mitochondrial enzyme complex-I, II and IV. Treatment with Vortioxetine considerably reduced the weight loss brought on by 3-NPA, increased movement, motor coordination, and cognition, and improved metabolic parameters. It can be concluded that Vortioxetine showed a neuroprotective effect in 3-NPA induced HD animal model. It can be a promising drug for various neurodegenerative disorders.

Keywords: Vortioxetine, Huntington's Disease, Antioxidant, 3-NPA, Oxidative stress

Introduction

A deadly neurological disorder called HD is described by gradual motor impairment, emotional disturbance, mental deterioration, emaciated and depression [1]. In the present work, rats were given the drug 3-NPA, which causes HD. Vortioxetine is an inhibitor of the SERT transporter and agonist of the 5-HT1A receptor [2]. By inhibiting mitochondrial complex II, 3-NPA disrupts energy metabolism and damages the striatum and cortex. The neurotoxin's side effects, which can include significant gait impairment, hypokinesia, increased anxiety and/or depression, and memory problems, resemble some of the histological and neuropathological alterations seen in HD [3]. There have been many symptoms identified, the majority of which are accompanied by a change in behavior, a deterioration in motor or cognitive function, or a combination of these symptoms [4, 5].

It has been effectively used to induce specific behavioral impairments and selective striatal lesions that match those in Huntington's disease in rats using 3-NPA, a complex II inhibitor of the electron transport chain. Despite affecting various areas of the brain, 3-NPA selectively harms the striatum, which was used to create an experimental Huntington's disease model [6].

Damage to the striatum and cortex result from 3-NPA inhibition of the mitochondrial complex II, which disrupts energy metabolism [7]. This is an appropriate experimental model to investigate Huntington's disease since the neurotoxin's effects replicate some of the histological and neuropathological abnormalities identified in HD, such as significant gait impairment, hypokinesia, and increased anxiety and/or depression, and memory problems [3].

The mycotoxin 3-NPA, a suicide inhibitor of the Succinate dehydrogenate enzyme (SDH) of both the respiratory chain and Krebs cycle is produced by the fungus Arthrinium sp. In addition to causing particular striatal lesions and causing spiny neuron dendrites to proliferate, 3-NPA prevents the brain's mitochondria from making ATP, which leads to mitochondrial malfunction. As shown in HD patients, these consequences result in basal ganglia neuronal loss and movement impairment [5]. In the brain, 3-NPA reduces the level of antioxidant enzymes and raises amounts of reactive oxygen/nitrogen species, causing oxidative/nitrosative damage [8].

Although stimulation of the 5-HT1A receptor is neuroprotective in many in vitro and in vivo model systems, the underlying processes are not fully understood.

It has been shown that the amount of SOD and catalase, anti-apoptotic proteins from the prosurvival BCL family (including Bcl-XL and Bcl-2), and inhibitors of apoptosis proteins rises in response to 5-HT1A receptor activation (e.g., Bax, XIAP) [9].

The serotonergic system is crucial for many physiological and behavioral processes, including those that affect mood and anxiety, cognition, food intake, and other areas. The 5-HT1A receptors have therefore received a lot of research. The thalamus, hypothalamus, and basal ganglia all express 5-HT1A heteroreceptors, as do the hippocampus, prefrontal cortex, lateral septum, and amygdala in the limbic systems. Cognitive deficiencies are improved by activating 5-HT1A receptors. Additionally, 5-HT1A agonists are anticipated to lessen psychiatric symptoms like despair and anxiety [11, 12].

Selective serotonin reuptake inhibitors increase the level of extracellular serotonin by blocking serotonin reuptake in the presynaptic cell. Antidepressants have been shown in several studies to offer antioxidant and neuroprotective advantages by elevating the production of antioxidant enzymes and thus reduce oxidative damage [13].

In addition to being an antagonist of the 5-HT receptors, vortioxetine binds to the 5-HT1A & 5-HT1B receptors and inhibits the Serotonin transporter protein (SERT). Interestingly, vortioxetine can indirectly modify signaling across several distinct neurotransmitter systems, including glutamate, GABA, norepinephrine, dopamine, acetylcholine (ACh), and histamine. Our hypothesis suggests that the cognitive effects of vortioxetine may be particularly attributed to the indirect regulation of g-aminobutyric acid and glutamate neurotransmission, ACh, or histamine neurotransmission. Some of these neurotransmitter systems are known to be related to cognitive function. It is crucial to do empirical research on the connection between vortioxetine's effects on multiple neurotransmitter systems and cognition, even though these ideas aren't always mutually incompatible [14].

Vortioxetine increased the extracellular levels of the mood-regulating neurotransmitters serotonin, dopamine, and noradrenaline as well as the neurotransmitters histamine and acetylcholine in the prefrontal cortex (PFC) and ventral hippocampus of rats, regions of the brain connected to depression. In the rat nucleus accumbens, Vortioxetine also caused increases in serotonin but not dopamine or noradrenaline. Additional rat investigations suggest that Vortioxetine may have a faster reversal of 5-HTneuronal activity in the dorsal raphe nucleus than other antidepressants. In the hippocampus of rodents, vortioxetine exhibited neurogenic effects, a characteristic that may be related to an antidepressant's action [15].

Additionally, 5-HT1AR may be involved in motor coordination and fine-tuning. It has been demonstrated that several 5-HT1AR agonists exhibit strong neuroprotective effects in cell injury models by reducing glutamate release, controlling anti-apoptotic activities, and upregulating the expression of protective proteins [16].

This study deals with the examination of the Neuroprotective ability of Vortioxetine in an animal model of Huntington's disease induced by 3-NPA administration.

Material and methods

Drugs and reagents

The source of vortioxetine hydrobromide was (Metrochem API Pvt. Ltd., Hyderabad). Sigma Aldrich, India provided with the Lowry's reagent, 3-nitropropionic acid (3-NPA), 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), N- naphthyl ethylene diamine, and Folin-Ciocalteu reagent. The following products were bought from Mumbai, India's SISCO Research Laboratory Pvt. Limited: Niacinamide adenine dinucleotide (NADH), mannitol, glycyl glycine buffer, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES), and nitro blue tetrazole.

Animals

Male albino Wistar rats weighing 180–200g of body weight were used in this study. A conventional laboratory pellet diet with water *ad libitum* was used to feed the animals. To keep the animals' circadian rhythms, a 12-hour natural cycle of light and dark was provided for them. Between the hours of 9:00 and 5:00, the tests were conducted in a mostly soundproof lab. The animals were housed in the lab for the duration of the laboratory investigation; they had become acclimatized to it five days before the behavioral test.

3-Nitropropionic acid (3-NPA) induced animal model

3-NPA was given intraperitoneally in a dose of 10 mg/kg at a rate of 0.5 ml/100g on an alternate day for 28 days. 3-NPA was dissolved in a 0.9% saline solution, and sodium hydroxide was used to adjust the pH to 7.4 [1, 17]. It was found that daily administration of 3-NPA (10 mg/kg) for two weeks had a greater death rate because they lose significant weight, but intraperitoneal administration of 3-NPA (10-20 mg/ kg) for four days showed no noticeable changes in behavior or biochemical data. So, on alternate day's administration of the 3-NPA schedule results in substantial behavioral and biological changes. On day 1 before the 3-NPA treatment and day 30 following the trial's end, body weight, motor functions, and locomotor activity were evaluated (day 28).

Drug administration

Before use, each medication solution was freshly prepared. Based on previous data from other laboratories, the dosing schedule and dose choice were made. Systemic administration of 3-NPA (10 mg/kg alternatively for 28 days) has been shown to result in striatal lesions that are consistent with HD symptoms, considerable body weight loss, decreased locomotor activity, and irregular and abnormal movement patterns [18]. Treatments in all groups of the animal were started from 0 to the 28th day followed by 3-NPA administration to evaluate the neuroprotective effect.

On days 0 through 7, 14, 21, and 28, behavioral tests were performed on all animal groups. The same animals underwent biochemical estimations after behavioral and cognitive evaluations.

Experimental protocol

A total of five groups with six animals in each of the groups were divided (n=6).

Group I — Normal control group

Animals were administered with normal saline (1 ml/100 g b.w.) through the oral route by oral gavage.

Group II — 3-NPA treated group

Animals were administered with normal saline (1ml/100g b.w.) through the i.p. route followed by 3-NPA (10 mg/kg) through the i.p. route on an alternate day till the 28th day.

Groups III: Standard Drug Imipramine and 3-NPA

Animals were administered Imipramine (15 mg/kg) orally daily followed by 3-NPA (10 mg/kg) through the i.p. route on an alternate day till the 28th day.

Groups IV: Vortioxetine and 3-NPA (5 mg kg^{-1} orally)

Animals were administered Vortioxetine (5 mg/kg) orally daily, followed by 3-NPA (10 mg/ kg) through the i.p. route on an alternate day till the 28th day.

Groups V: Vortioxetine and 3-NPA (10 mg kg^{-1} orally)

Animals were administered Vortioxetine (10 mg/kg) orally daily, followed by 3-NPA (10 mg/ kg) through the i.p. route on an alternate day till the 28th day.

Pharmacological Screening

Body weight

The body weight measurements recorded on days 1 and 28 were used to compute the percentage change in weight [19].

Behavioral assessment

Assessment of locomotor activity by Actophotometer

An Actophotometer was equipped to measure the amount of locomotor activity (INCO, Ambala, India). During the evaluation, the equipment was put in a room that was ventilated, sound-attenuated, and darkened. Before starting the real locomotor activity task for the following 10 minutes, for three minutes, each animal was individually placed in the exercise cage to help them become used to it. The animals' baseline activity levels were counted. The vertical and horizontal combined activity were recorded as counts per 10 minutes. The locomotor activity index is measured in counts/10 minutes. All locomotor activity trials were conducted between 9:00 to 5:00 pm on the 0th, 7th, 14th, 21st, and 28th days [20, 21].

Assessment of motor coordination by Rota rod

Motor coordination between the forelimbs and the hindlimbs was assessed using rota rod tests [22, 23]. Individual animals were put on the 7 cm diameter spinning rod rotating at a speed of 25rpm. Rats were taught to utilize the Rota rod device for a 2-minute trial, the day before the first day of testing (25 rpm). Three different trials, each with a 180-second cutoff period, were performed by each rat at 5-minute intervals. After each trial, there was a five-minute pause to ease stress and fatigue. Every trial was concluded on the first and 28th days between the hours of 9:00 and 17:00. Unaware of the experiment's methodology, a trained observer noted each rat's declining time delay. [24].

Cognitive Assessment

Assessment of learning and memory by Morris water maze (MWM)

The MWM was a massive, 150-cm-diameter, 45-cm-high pool that was filled to a depth of 30 cm with 28°C water. The water was made white to make it opaque. Two threads that were attached at an angle to one another on the pool's rim separated the tank into four equal quadrants. Within the pool's target quadrants, a white-painted submerged platform (10 cm^2) was positioned at a 1 cm depth. Throughout the training, the platform stayed in the same spot. The rat was gently lowered into the water between each pool quadrant, facing the pool wall, and allowed 120 seconds to find a submerged platform. It was then permitted to remain standing on the platform for 20 seconds. It was gently directed to the platform after 120 seconds if it couldn't find it on its own and given

another 20 seconds to stay there. Quadrant 4 (Q4) was the target quadrant for each acquisition trial and the daily starting locations varied from day to day. The platform was taken away on the sixth day, allowing the rats 120 seconds of unrestricted access to the pool. Four of these trials, each of which started in a different quadrant, were performed on each rat. By calculating the typical amount of time spent in each of the four quadrants, the retrieval index was produced—Q1, Q2, Q3, and Q4—as well as the time spent looking for them in Q4 on a single platform. At all times, the experimenter stayed still and in the same location. To guarantee that the water maze's position did not vary during the experiment, it was closely watched concerning other lab items acting as clear visual cues. All of the trials were finished between 9:00 and 17:00, or the light cycle [25, 26].

Assessment of learning and memory by Elevated Plus Maze

For the purpose of evaluating spatial long-term memory, the Elevated Plus maze was used. The equipment comprised two open arms and two closed arms as per protocol. The central platform of the maze stood 50 cm above the ground, and its arms were spread out in all directions. Every animal was placed at the end of an open arm on the first day. Calculating transfer latency (TL) was done by measuring how long it took the rat to enter one of the surrounding arms. The animal was gently coaxed into one closed arm, and if it didn't enter one within 90 seconds, the TL delay was set to 90 seconds. The rats had twenty seconds to explore the maze before being put back in their cage. On days 0 and 28 of the trial, TL was recorded [27].

Tissue Preparation

The animals were decapitated after the assessment of behavioral and cognitive measures. Each animal's brain was dissected, the striatum placed on ice, and then each one's weight was recorded. 0.1 M PBS was used to homogenize 10% (w/v) of the tissues (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 minutes at 4 °C. Supernatants were divided into aliquots, which were used for biochemical studies [18].

Biochemical Estimations

Assessment of Oxidative and Nitrosative stress

The chemical mechanisms in HD that lead to neuronal death include oxidative stress, which also includes nitrosative stress. Additionally revealed in 3-NPA-induced neurotoxicity are mechanisms for oxidative stress and nitric oxide production [28].

Assessment of striatum lipid peroxidation

TBARS, a marker of lipid peroxidation, was measured by the technique developed by Ohkawa et al (1979). The TBARS readings were given as nanomoles per mg of protein [29].

Assessment of striatum non-proteic thiol (glutathione-GSH) level

90-95% GSH level in the striatum was measured using a spectrophotometer (Shimadzu Corporation in Japan) [1]. Trichloroacetic acid (10% w/v) was combined in a 1:1 ratio with the homogenate's supernatant. The tubes were centrifuged at 1000 g for 10 minutes at 4 °C. It was combined with 2 ml of 0.3 M di sodium hydrogen phosphate to create the supernatant (0.5 ml). After adding 0.25 ml of DTNB solution at 0.001 M concentration, spectrophotometrically measure the absorbance at 412 nm. The findings were reported in terms of moles of reduced glutathione per mg of protein on a conventional graph using reduced glutathione concentrations ranging from 10 to 100 M. [30].

Assessment of striatum superoxide dismutase (SOD) activity

According to Beauchamp and Fridovich's recommendations, the striatum SOD was measured spectrophotometrically (Shimadzu Corporation, Japan) (1971). The rate of increase in absorbance units (A) per minute for the control and the test sample were calculated and then the % inhibition for the test sample(s) was calculated [31].

Assessment of striatum catalase activity

A spectrophotometer (Shimadzu Corporation, Japan) was used to assess the striatum catalase activity spectrophotometrically at 240 nm using the Aebi approach (1984). The catalase activity was evaluated based on the units per mg of proteins [32].

Assessment of striatum nitrate/nitrite level

Utilizing a UV-1800 ENG240 V from Shimadzu Corporation in Japan, we used spectrophotometry to determine the quantity of striatum nitrite. As per the protocol, 100 µl of striatal or reference sample was mixed, and then 0.15 g of the copper-cadmium alloy and 400 µl of carbonate buffer (pH 9.0) were added. Nitrate was changed into nitrite by incubating the tubes at room temperature for an hour. Adding 100 µl of NaOH at 0.35 M halted the process. The samples were then treated with 400 µl of a $ZnSO_4$ solution (120 mM) to deproteinize them. After 10 minutes, the samples

were allowed to be centrifuged at 4000g for 10 min. Greiss reagent was used to measure the nitrite content of the brain striatum spectrophotometrically at 545 nm in aliquots (500 µl) of clear supernatant. Greiss reagent is made up of 250 µl of 1.0% sulfanilamide and 250 µl of 0.1% N-naphthyl ethylene diamine synthesized in 3N HCl (made with water). Plotting the sodium nitrite standard curve allowed researchers to quantify the amount of nitrite present in the brain's striatum (5 to 50 M) [31].

Assessment of striatum acetylcholinesterase (AChE) activity

Spectrophotometry (Shimadzu Corporation, Japan) was used to assess the striatum AChE activity at 412 nm [33-36]. The yellow color that is produced when thiocholine interacts with dithiobisnitro benzoate ions was ultimately used to determine this. The rate of thiocholine synthesis from acetyl thiocholine iodide in presence of cholinesterase enzyme was determined using a spectrophotometer. The brain homogenate's supernatant liquid were diluted with freshly prepared DTNB solution by pipetting 0.5 ml of it into a 25 ml volumetric flask (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). Two 4 ml portions from the volumetric flask were pipetted into two test tubes. Add 2 drops of eserine solution in one of the test tubes. Add 1 ml of the substrate solution (50 ml of distilled water and 75 mg of acetylcholine iodide) to each test tube. Eserine-containing test tube served as a blank. At 420 nm, the test sample's change in absorbance was time-stamped for the spectrophotometric measurement.

Estimation of Serotonin [37]

Procedure

On the 28th day of the experiment, immediately after MWM, animals were decapitated, their brains were removed, and the striatum including the subcortical area, was separated. Tissue slices were weighed and homogenized in 5 ml of HCl-butanol solution for 5 min. Then centrifuge the homogenate at 2000 rpm for 10 min. Now 1 ml of supernatant was collected in a test tube and then add 2.5 ml of heptanes and 0.31 ml of 0.1M HCl were mixed thoroughly for one minute and then centrifuged to separate the phases. The organic layer was discarded. Then 0.2 cc of the aqueous phase was collected for the 5HT test. Everything was done at 0°C. (Note: The amount of tissue used was between 50 and 75 mg per 5 ml of HCl-butanol, as opposed to the 1.5 to 5 mg per 0.1 ml of HCl-butanol used by Schlumpf M et al in 1974). This is carried out to acquire sufficient supernatant liquid for analysis. 0.25 ml of OPT reagent was added to 0.2 ml of aqueous extract. It took 10 minutes of heating to 100°C to produce the fluorophore. Readings in the spectrofluorimeter between 360 and 470 nm were acquired once the samples had attained thermal equilibrium with the surroundings. On a tissue blank, 0.25 cc of conc. HCl without OPT was applied. Internal benchmark: In distilled water, 500 g/ml of a 1:2 combination of HCl and butanol was created.

Measurement of 5-HIAA

The quantities of 5-HIAA in the supernatant of tissue that had been homogenized in $HClO_4$ after spinning for 10 min at 5000g in a microcentrifuge were measured using a modified version of the procedure reported by Beck et al (Fisher Scientific) [38]. In a test tube containing 1.9 mL of 0.01 M, acetate buffer (pH 5.5), an aliquot of the $HClO_4$ supernatant was added. After the mixture was incubated at room temperature for 5 minutes in total darkness, the samples were read using a spectrofluorometer with 333 nm emission and 296 nm excitation lengths. Values are reported in nMoles/g of moist tissue and were extrapolated from a standard curve [39].

Assessment of striatum total protein

The total protein was measured spectrophotometrically at 750 nm. To quantify the striatum's total protein concentration, BSA was used as a reference. 0.15 ml of homogenate supernatant was diluted up to 1 ml with water followed by the addition of 5 ml of Lowry's reagent. All the ingredients were thoroughly mixed, and allow standing at room temperature for 15 minutes. After adding 0.5 ml of Folin-Ciocalteu reagent, the mixture was allowed to sit for 30 minutes at room temperature. The standard curve of BSA was plotted between 0.2 and 2.4 mg/ml concentration range [33].

Isolation of rat brain striatum mitochondria and mitochondrial Complex estimation

This protocol includes modifications to previously described procedures [40, 41]. Slices of the brain's striatum were homogenized in an isolation buffer containing EGTA (75 mM sucrose, 215 mM Mannitol, 20 mM HEPES, 0.1% BSA, 1 mM EGTA, and pH 7.2). After that, the homogenate was centrifuged for five minutes at 10,000g at room temperature. The pellets were obtained which were again resuspended in the isolation solution containing EGTA and centrifuged again at 10,000g for the next 5 min. After that clear supernatant was collected in other tubes and filled with isolation buffer containing EGTA after being spun at 10,000g for 10 min. The isolated buffer without EGTA was used to resuspend the pellets containing pure mitochondria. Consequently, rat brain mitochondria were isolated [41].

Assessment of complex I (NADH dehydrogenase) activity

Using UV-Spectrophotometer from Shimadzu Corporation in Japan, NADH dehydrogenase activity was determined spectrophotometrically following King and Howard's technique [42]. It took two minutes to see the change in absorbance at 550 nm.

Assessment of complex II (succinate dehydrogenase-SDH) activity

According to King [43], SDH was determined using a spectrophotometer (Shimadzu Corporation, Japan). At 420 nm, the absorbance change was captured for two minutes.

Assessment of complex IV (cytochrome oxidase) activity

At 420 nm, the absorbance change was captured for two minutes [44] technique. Using a spectrophotometer, the change in absorbance was measured spectrophotometrically for two minutes at 550 nm. (Shimadzu Corporation, Japan).

Histopathological examination

After being preserved in 10% formalin saline for 24 hours, samples of brain tissue were rinsed with tap water. Then, tissues were sequentially diluted to alcohol to cause dehydration. Before being embedded in paraffin, samples were cleaned in xylene and then heated to 56°C for 24 hours in a hot air furnace. A rotary microtome was used to slice tissue blocks made of paraffin bee wax at a thickness of 4 µ for coronal sectioning. For routine inspection by a light electric microscope, the acquired tissue slices were collected on glass slides, deparaffinized, and stained with haematoxylin (H) and eosin (E).

Statistical analysis

The statistical analysis was conducted using Graph Pad Prism 7. Each result was displayed as Mean±SEM. For statistically analysing the data, One-way analysis of variance (ANOVA) was used, and Tukey's test was used as a post-test. At $P< 0.05$, statistics were deemed significant.

Results

Body Weight:

On day 28, 3-NPA-treated animals' body weight significantly decreased in comparison to the vehicle-treated group ($P< 0.05$). As opposed to the negative control group, treatment with the vortioxetine (5 and 10 mg/kg) and standard drugs Imipramine (15 mg/kg) reduced the body weight loss in the 3-NPA-treated rats ($P<0.05$).

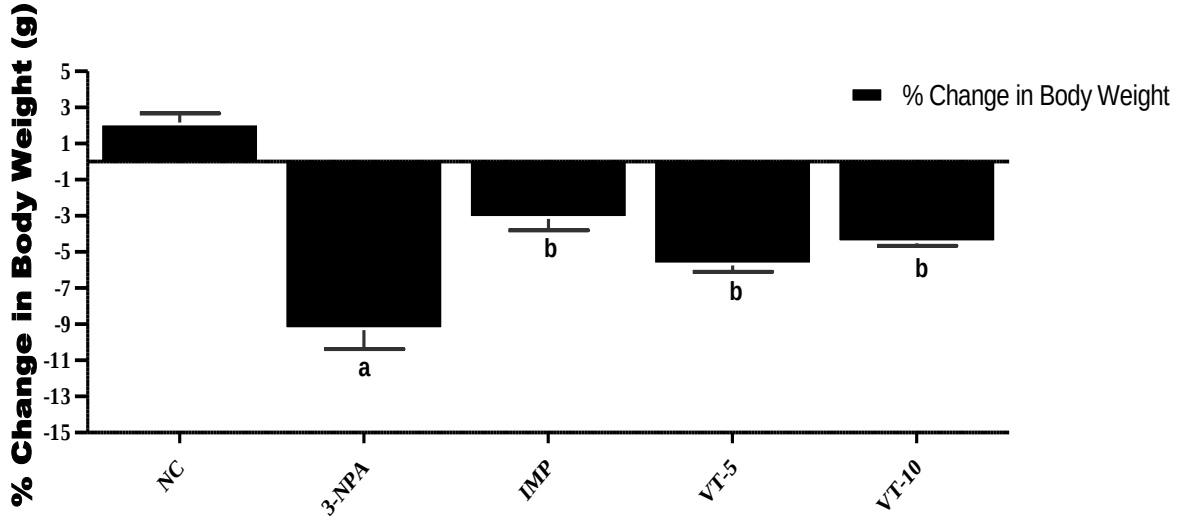


Figure 1. Effect of Vortioxetine on Body Weight in different experimental groups. [a]Statistically significant from the Normal control group (NC); [b]Statistically significant from the Negative control group (NGC).

Assessment of Behavioural Activity:

Figure 2 demonstrates the impact of vortioxetine on the Actophotometer's evaluation of locomotor activity in 3-NPA-induced HD in rats. Systemic 3-NPA (10 mg/kg) administration significantly ($P< 0.001$) decreased the locomotor activity in an open field as compared to vehicle control, as shown by a two-way ANOVA followed by a Bonferroni post-test. When compared to the negative

control group, vortioxetine treatment (5 mg/kg and 10 mg/kg) resulted in statistically significant changes in the length of locomotor activity on days 21 and 28 (P<0.05 and P<0.001, respectively). On days 14, 21, and 28, respectively, IMP (Positive control group) and 3-NPA treatment (Negative control group) showed a significant difference (P< 0.05, P< 0.001, and P< 0.001).

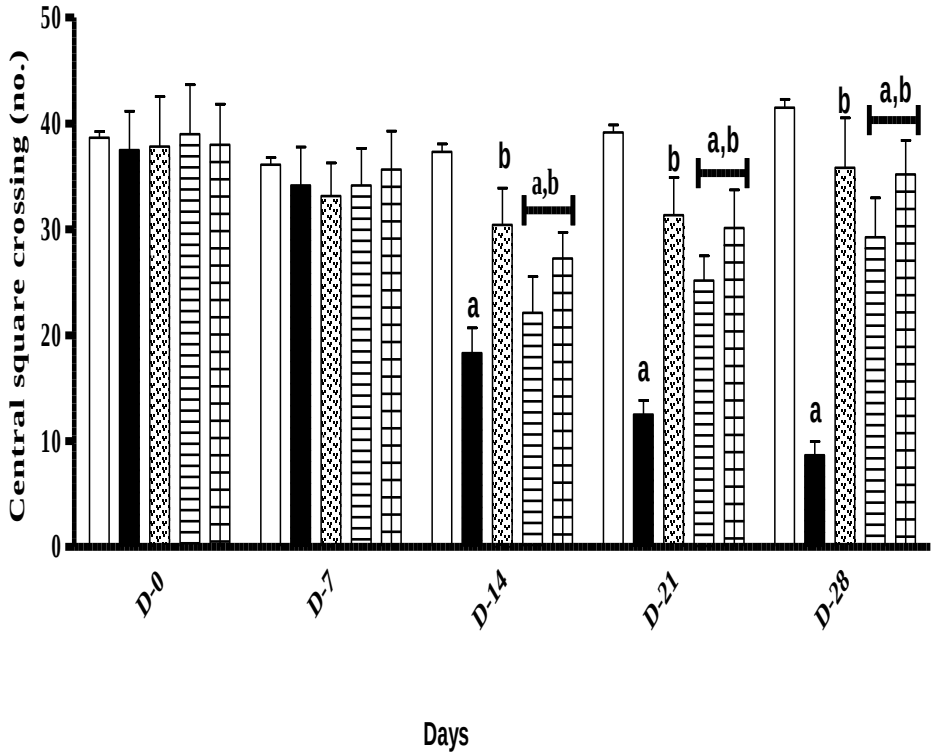


Figure 2. Effect of Vortioxetine on locomotor activity in 3-NPA induced Huntington's disease by using Actophotometer.

Rota rod:

Figure 3 demonstrates how Vortioxetine affected the length of time in 3-NPA-treated rats on the Rota rod. When compared to the Normal control group, 3-NPA (10 mg/kg) substantially (P<0.001) reduces the duration of muscular grip strength, according to a two-way ANOVA and Bonferroni post-test. When compared to the 3-NPA-treated group, treatment with vortioxetine (5 mg/kg) significantly (P<0.05 and P<0.001) lengthens the time of muscle grip strength on the 21st and 28th day, while vortioxetine (10 mg/kg) lengthens the time of muscle grip strength on the 14th, 21st, and 28th day, respectively (Negative control group).

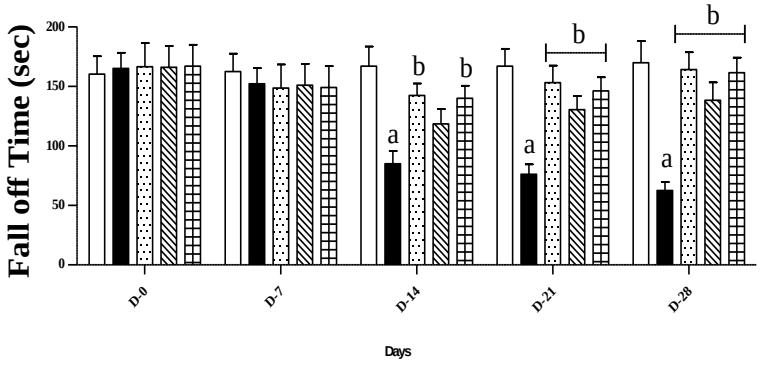


Figure 3. Effect of Vortioxetine on Muscle grip strength in 3-NPA treated rats on Rota rod.

Cognitive Impairment:

Elevated Plus Maze

Figure 4 shows the transfer latency (time in seconds) in an elevated plus maze to assess improvement in memory dysfunction.

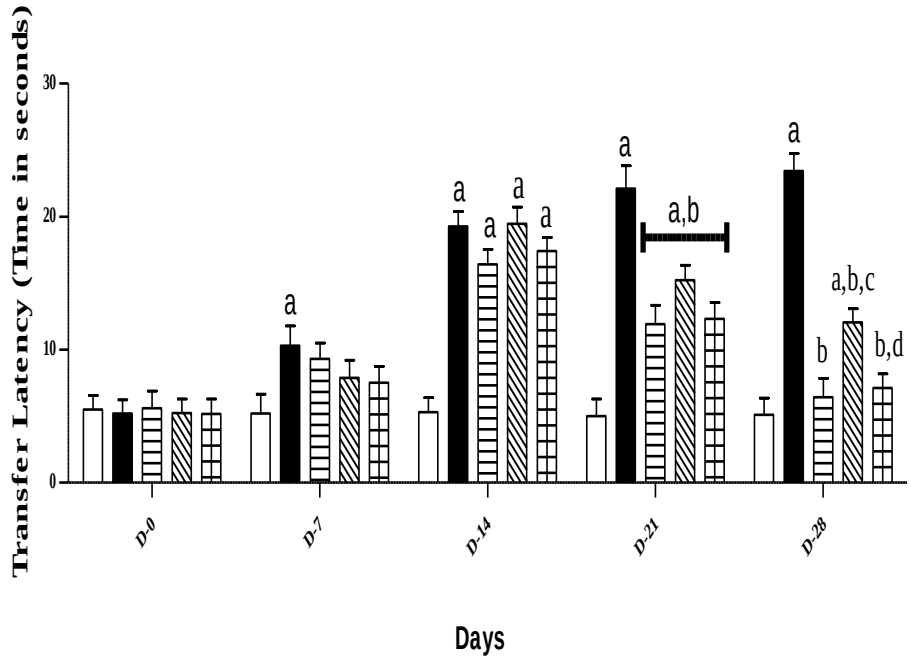


Figure 4. Effect of Vortioxetine on Time Spent and number of entries in open and closed arm in 3-NPA induced animals during EPM. [a]Statistically significant from the Normal control group (NC); [b]Statistically significant from the 3-NPA treated group (Negative control group); [c]Statistically significant from the IMP (Positive control group); [d]Statistically significant from the VT-5 (Vortioxetine-5 mg/kg)

Morris Water Maze

Prophylactic effect of Vortioxetine on Time spent in Target Quadrant in Morris water Maze Activity

Figure 5 demonstrates the effect of Vortioxetine on time spent in the target quadrant during the MWM test in 3-NPA-induced rats. When compared to the NC group, treatment with 3-NPA (10 mg/kg) considerably ($P<0.05$) reduces the amount of time that the animal spends in the target quadrant. When the treatment group VT-5 (Vortioxetine-5 mg/kg) was compared to the 3-NPA-treated (Negative control group), no significant differences were discovered; however, on days 0, 7, and 21, there was a significant difference ($P<0.01$) between the VT-10 (Vortioxetine-10 mg/kg) group and the NC group.

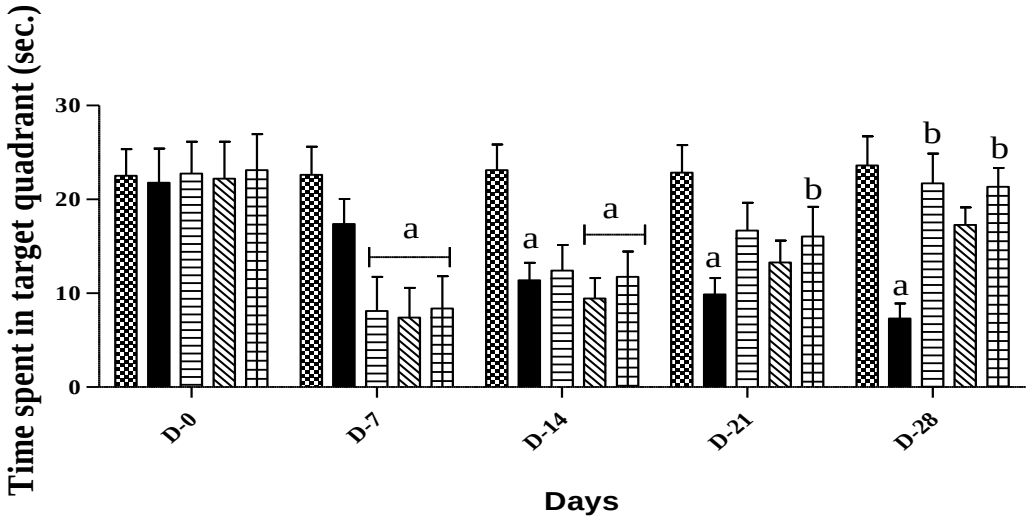
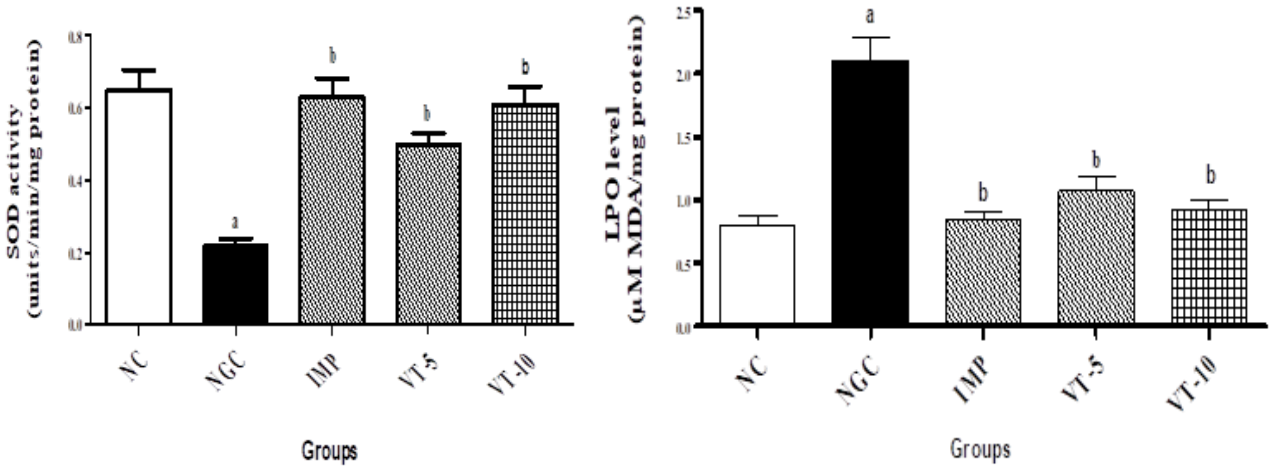


Figure 5. Effect of Vortioxetine on Time Spent in Target Quadrant in 3-NPA induced animals during MWM.

Effect of Vortioxetine on ELT in 3-NPA induced animals during Morris Water Maze (MWM) test

Figure 6 illustrates the impact of vortioxetine on the rats' escape latency time during the MWM test in 3-NPA-induced rats. The results of a two-way ANOVA showed that the groups' MWM escape latency times differed significantly from one another. The post hoc test revealed no significant variations in the escape delay time during the MWM among the groups following treatment with VT-5 (Vortioxetine 5 mg/kg) from D-7 to D-21. However, on D-28, Vortioxetine (10 mg/kg) substantially lengthened the time it took for rats to escape during the MWM compared to rats that had only received Vortioxetine (5 mg/kg) treatment or rats that had been exposed to 3-NPA.

Oxidative Stress Marker



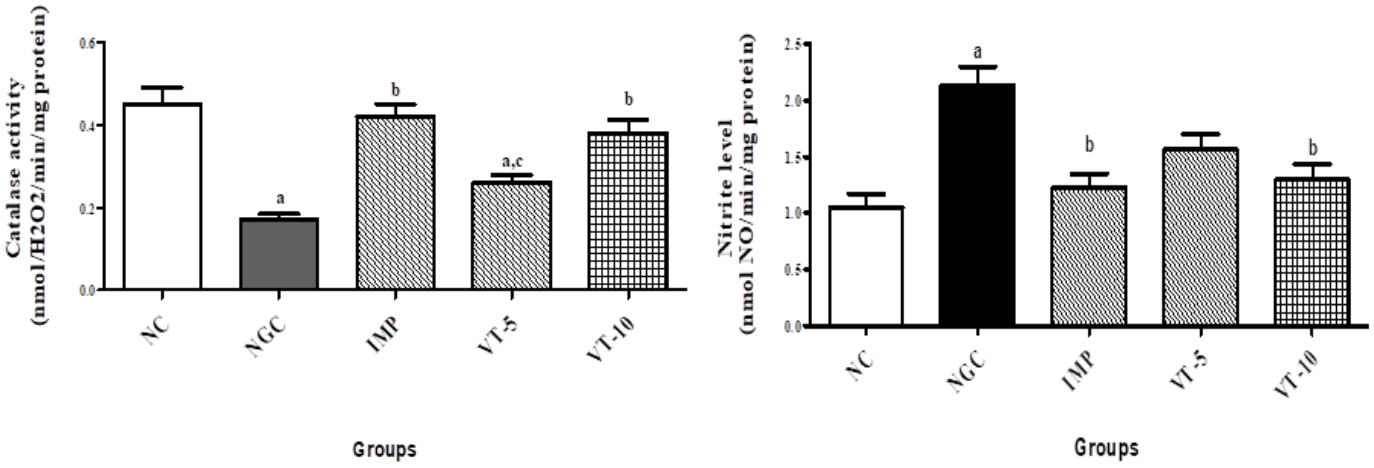


Figure 7. Show the effect of Vortioxetine on oxidative stress markers in 3-NPA-induced alterations in the levels of MDA, SOD, and Catalase in the brain tissues. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

Cognitive Impairment Marker

Figure 8 shows how 3-NPA-induced experimental rats' AchE activity is affected by vortioxetine. The results of a one-way ANOVA showed that there were significant variations in AchE activity between the groups. VT-10 considerably (P<0.001) lowered AchE levels as compared to the 3-NPA-treated Negative Control group and VT-5, according to post hoc analysis.

In Fig:-9 Vortioxetine (5 mg/kg) significantly (P<0.001) increases the Ach level as compared to 3-NPA treated Negative control group.

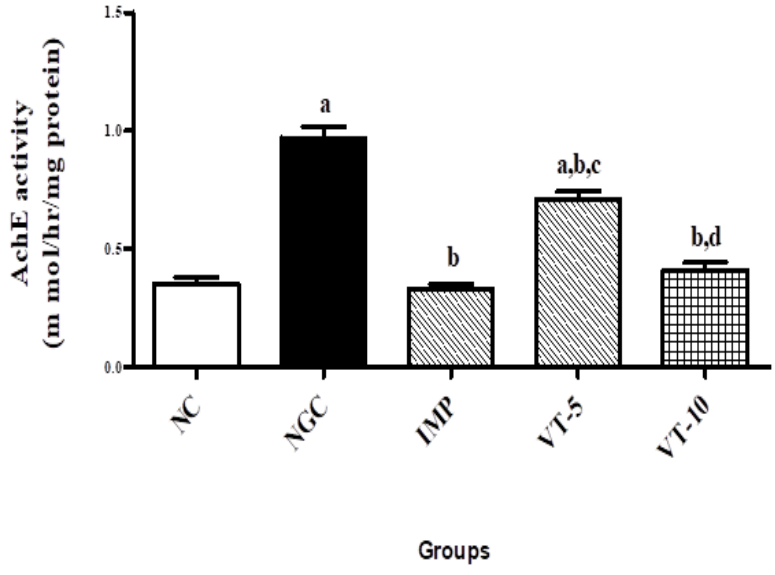


Figure 8. Effect of Vortioxetine on Acetyl

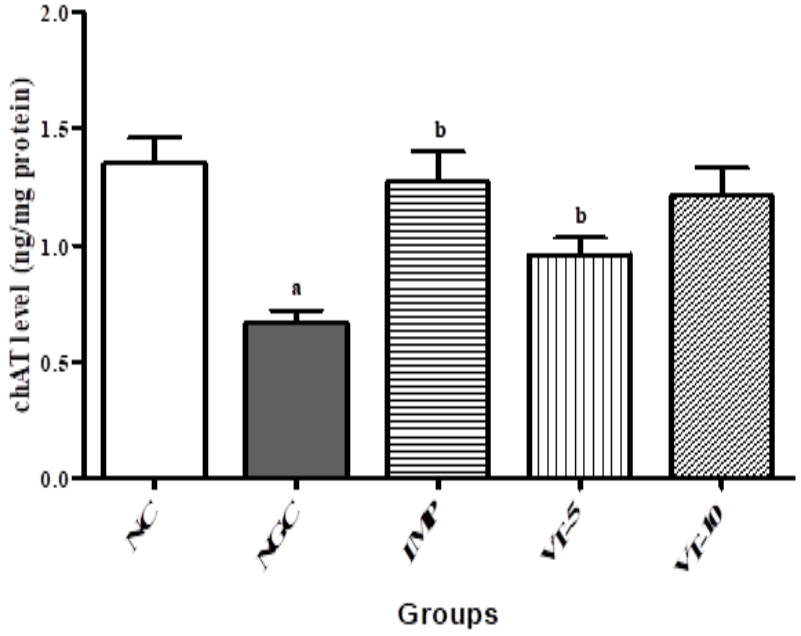


Figure 9. Effect of Vortioxetine on Choline
Cholinesterase (AchE), in 3-NPA induced animals acetyl transferase (chAT) in 3-NPA induced animals

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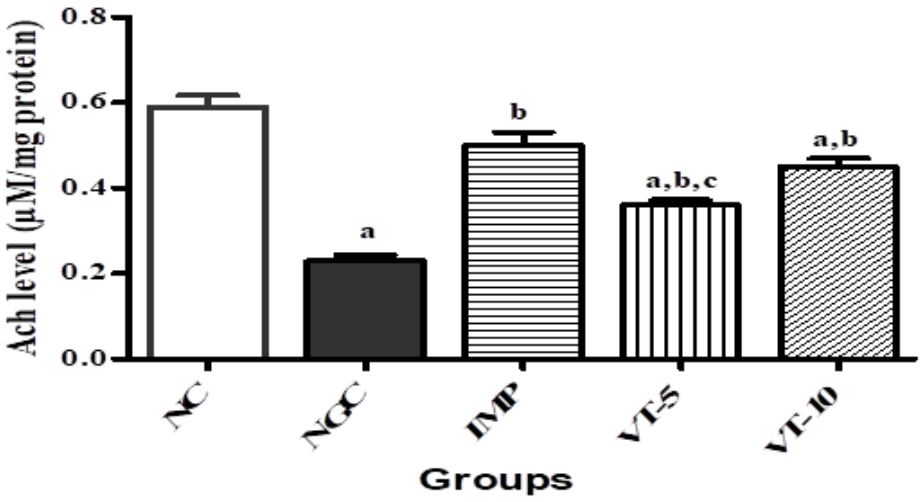


Figure 10. Effect of Vortioxetine on acetylcholine (Ach) in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference ($P < 0.05$).

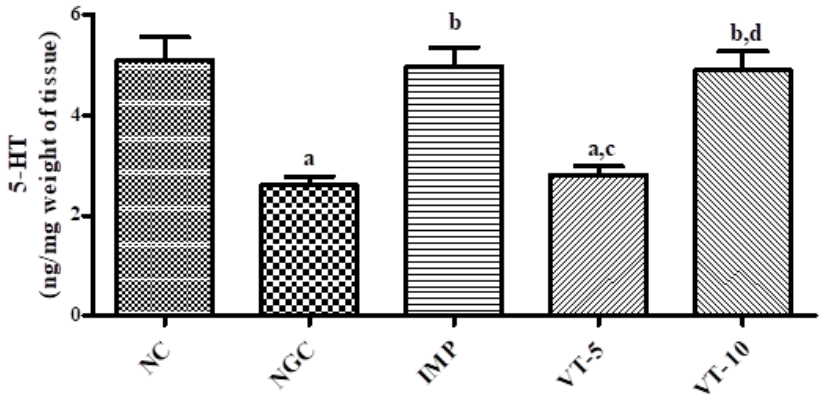


Figure 11. Effect of Vortioxetine on Serotonin (5-HT) level in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

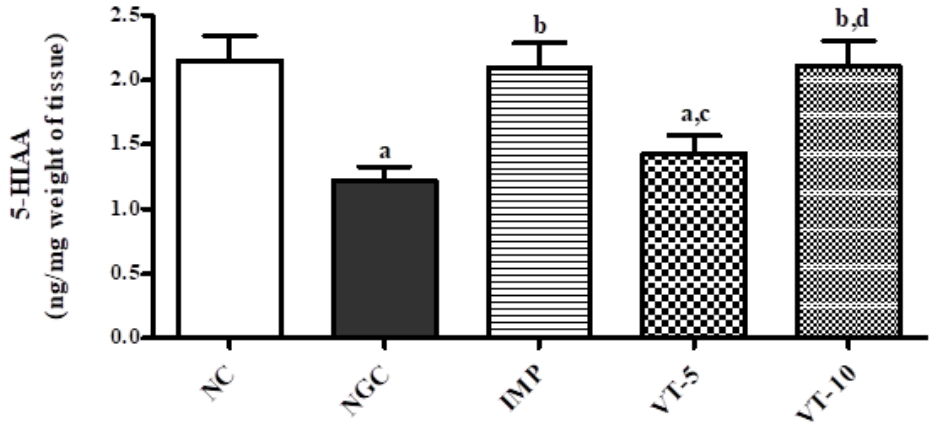
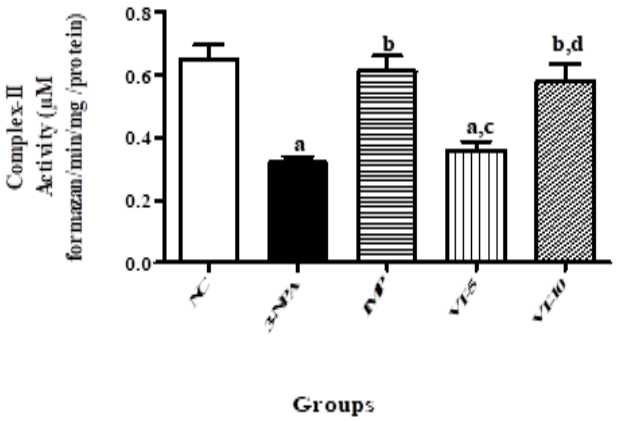


Figure 12. Effect of Vortioxetine on 5-Hydroxy Indole Acetic Acid (5-HIAA) level in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

Mitochondrial Enzyme Assay

Figure 13 demonstrate the impact of vortioxetine on mitochondrial enzyme activity in 3-NPA-induced rats. The results of a one-way ANOVA showed that the activity of the mitochondrial complex-I, II, IV, and V enzymes varied significantly depending on the group. When compared to the 3-NPA-treated Negative Control group, a post hoc analysis showed that vortioxetine (10 mg/kg) substantially (P< 0.001) lowered complex-I, II, and IV activity in brain tissues.



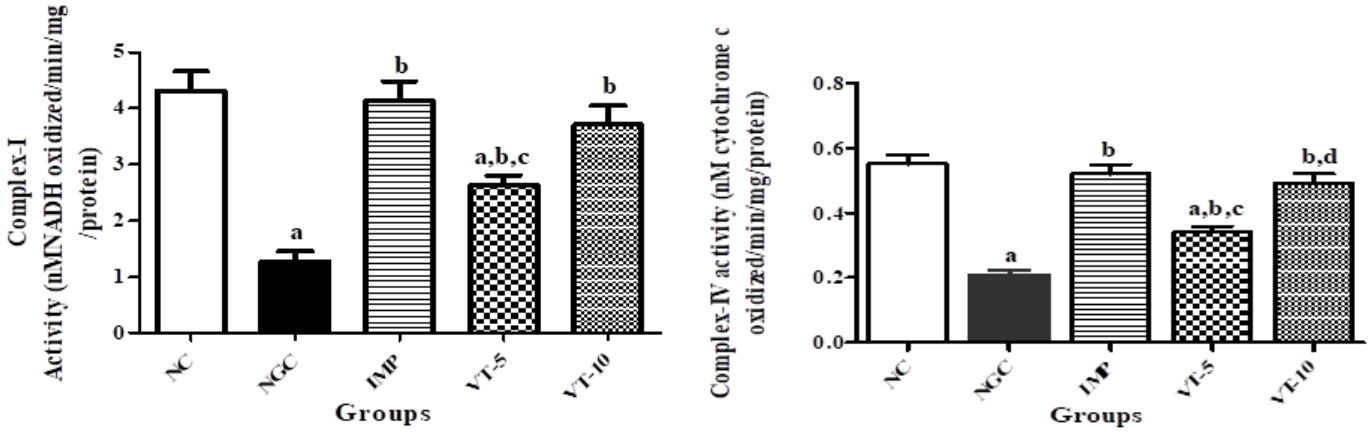
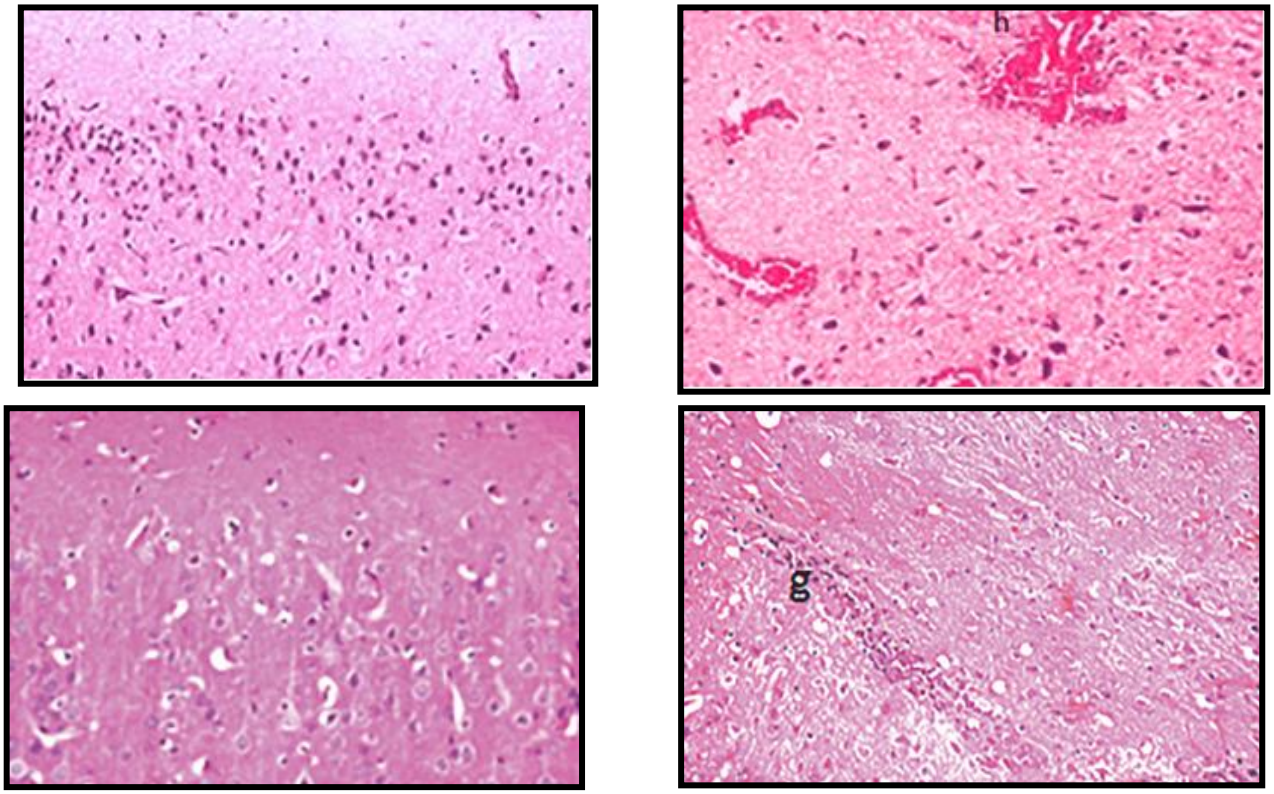


Figure 13. demonstrated the impact of vortioxetine on mitochondrial enzyme activity in 3-NPA-induced rats. [a]Statistically significant from the NC (Normal control group); [b]Statistically significant from the 3-NPA treated (Negative control group); [c]Statistically significant from the IMP (Positive control group).

Histopathological examination of the hippocampal and cortical tissues

Systemic treatment of 3-NPA (10 mg/kg) resulted in neurodegeneration and localized bleeding in the hippocampus, according to the analysis of the H & E stained brain sections. Standard Imipramine (10 mg/kg) pretreatment largely recovered the histological characteristics of the brain, but Vortioxetine (5 mg/kg and 10 mg/kg) pretreatment groups only demonstrated localized gliosis in the cortex and no change in the hippocampus, respectively. (Fig. 14 & 15).



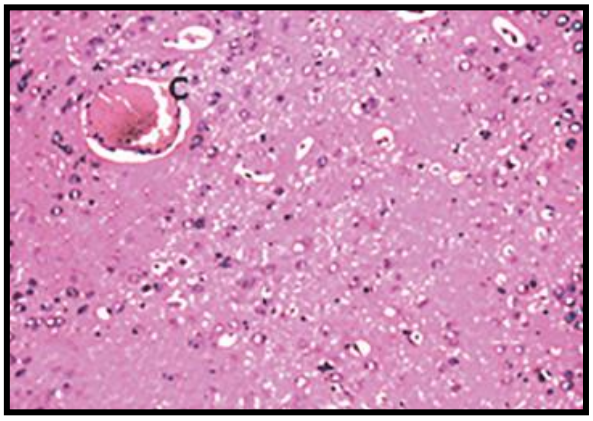


Figure 14. H & E staining of the striatal tissue of rats belonging to the Normal control group (A), Negative control group (B), Std. group treated with Imipramine and 3-NPA (C), Vortioxetine (5 mg/kg) and 3-NPA-treated group (D), Vortioxetine (10 mg/kg) and 3-NPA-treated group (E). A and C showed no histological alterations, B showed severe haemorrhage (h), D showed focal gliosis (g) and E showed congested blood vessel (C).

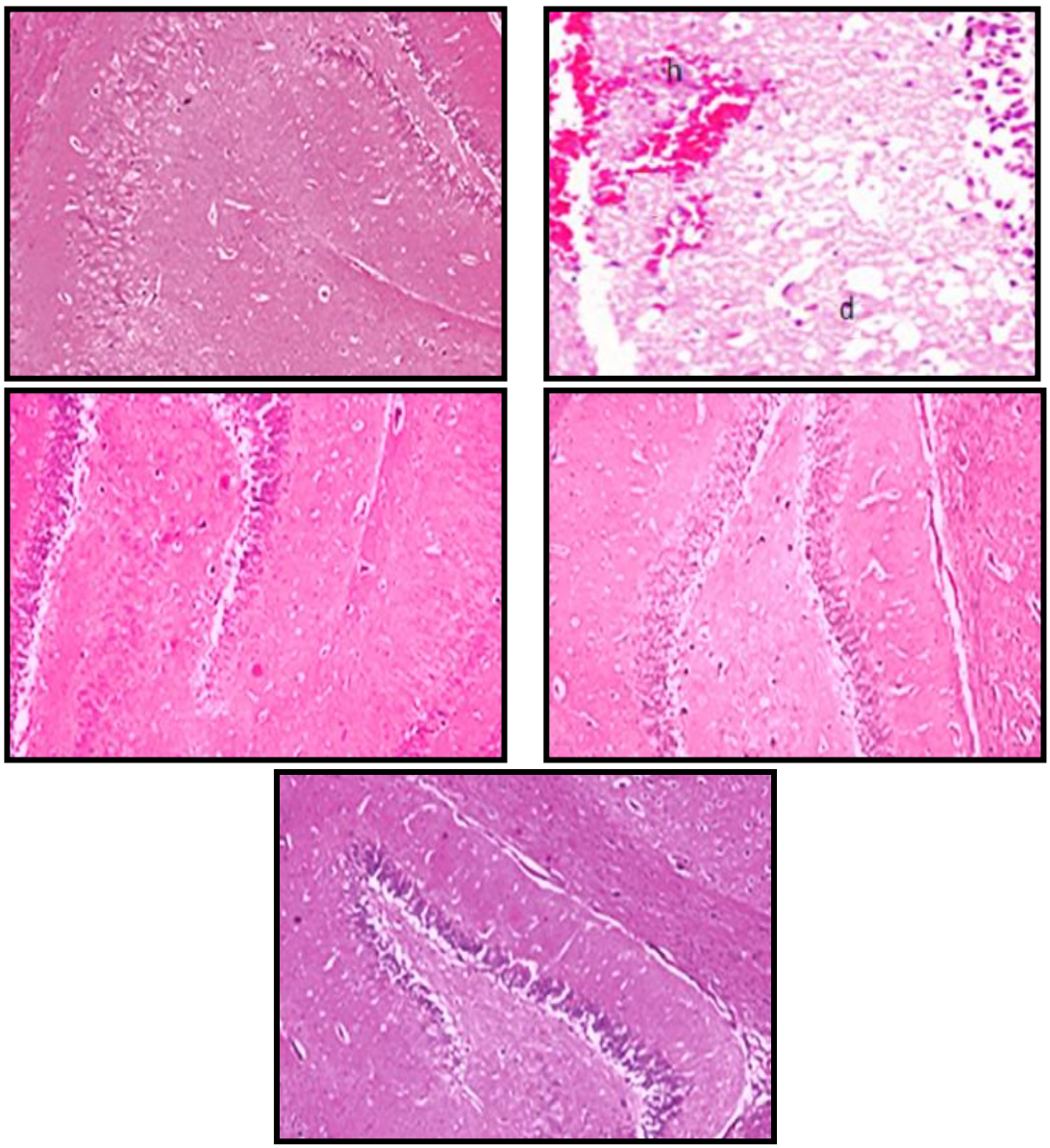


Figure 15. H & E staining of the hippocampi of rats belonging to the Normal control group (A), Negative control group (B), Std. group treated with Imipramine and 3-NPA (C), Vortioxetine (5 mg/kg)- and 3-NPA-treated group (D), Vortioxetine (10 mg/kg)

and 3-NPA-treated group (E). A, C, D, and E showed no histological alterations, B showed severe neurodegeneration (d) and haemorrhage (h)

Discussion

In this study, the neuroprotective effects of the vortioxetine were examined in 3-NPA induced HD model of rats. It is known that 3-NPA causes cognitive difficulties as seen by an increase in AchE levels in the rat brain striatum. Rats were given 3-NPA to modify the antioxidant parameters and mitochondrial complex activity in the region of the brain striatum.

Vortioxetine has been shown to prevent the detrimental effects of 3-NPA on weight loss, decreased mobility, poor motor coordination, learning, and memory. Additionally, vortioxetine significantly reduced oxidative and nitrosative stress in the striatum of the brain, increased AChE activity, and prevented the degradation of mitochondrial enzyme complexes in addition to the aforementioned effects (I, II, and IV).

Weight loss has occurred as a result of the use of 3-NPA. This supported prior research [5]. Reduced food intake and higher energy expenditure may be the key factors contributing to changes in body weight [45]. Rats receiving 3-NPA lost body weight, and Vortioxetine treatment significantly increased their weight. Rats given 3-NPA showed hypolocomotion in the Actophotometer and impaired motor function on the Rota rod test, which are signs of a malfunctioning motor system. One defining aspect of the behavioral symptoms of HD is the loss of neurons, especially the medium-spiny GABAergic neurons in the caudate nucleus and putamen, which demonstrate a progressive neuropathological change [46]. When mitochondrial complex II is suppressed, It is possible to mimic this neuronal degeneration, which has been connected to motor dysfunction in 3-NPA-treated rats [47]. Deficits in behaviour and motor coordination also resemble the loss of motor abilities linked to elevated brain protein oxidation [48]. Vortioxetine therapy dramatically enhances both motor coordination and movement. 3-NPA Administration reduced memory function as seen in the MWM paradigm. The MWM has already been employed to evaluate learning and memory in HD caused by 3-NPA [1]. Increased AChE levels have been linked to cognitive abnormalities such as learning and memory impairment [49]. Performance on the MWM may be influenced by AChE activity, which has been linked to cognitive impairment [50]. Additionally, there is a loss of motility and motor coordination, which might likely affect how well MWM works. It would be inaccurate to isolate this factor alone as the cause of the loss in MWM performance in 3-NPA-treated rats; rather, oxidative stress, AChE activity in the brain, and direct effects on memory and learning are significant. The ELT of control animals in MWM is decreasing. Day 4 ELT for these animals exhibited a significant drop from day 1 ELT. The TSTQ also significantly increased on day 5 in comparison to the time spent in the other quadrants, showing normal retrieval. On the other hand, 3-NPA-treated animals showed a significant increase when compared to day 4 ELT of control mice, indicating impairment of acquisition. The day 5 TSTQ of the control animal was considerably reduced as compared to the day 5 TSTQ of the 3-NPA-treated animals, demonstrating memory impairment. Similar to this, administering Vortioxetine significantly decreased the 3-NPA-related surge in day 4 ELT, undoing the 3-NPA-related acquisition impairment. Furthermore, vortioxetine medication markedly decreased the 3-NPA-induced reduction in day 5 TSTQ, indicating a recovery from the 3-NPA-induced memory impairment. The investigation's results are in agreement with other published research [28]. The hippocampal CA1 and CA3 pyramidal neurons, which are located in the part of the brain linked with cognitive function, are damaged by 3-NPA. According to research, 3-NPA might impair the body's oxidative defenses and deplete ATP. It is generally established that learning, memory, and other cognitive processes are all impacted by hippocampus atrophy caused by mitochondrial dysfunction [51]. Treatment with 3-NPA causes striatal spiny neurons to exhibit long-term potentiation of NMDA-mediated synaptic excitation (3-NPA-LTP) by inhibiting LTD expression in the sensory-motor striatum [52, 53]. Research has shown that 3-NPA-LTP increases intracellular calcium levels and activates mitogen-activated protein kinase and extracellular signal-regulated kinase. Thus, the localized and cell type-specific neuronal death seen in HD may need 3-NPA-LTP [52]. Treatment with vortioxetine significantly decreased the 3-NPA-induced deficits in learning and memory. According to the study, crucial activities including memory, cognition, and learning depend heavily on cholinergic synapses [54]. It has been shown that the AChE enzyme regulates the levels of ACh in cholinergic synaptic clefts [55]. It has been shown that 3-NPA increases striatal AChE activity [56]. The striatal area of the brain had an increase in AChE activity in the current investigation as a result of 3-NPA. According to certain theories, forebrain cholinergic neurons are essential for the regulation of memory-related functions [57].

Evidence suggests that elevated free radical production may be a factor in the symptoms of HD and

other movement disorders [58]. A decrease in some endogenous antioxidant mechanisms, like GSH levels and the activity of antioxidant defense enzymes like catalase and SOD, may at least partially account for this impact. There is ample evidence that oxidation has a significant impact on the toxicity of 3-NPA [59].

By interfering with the mitochondrial respiratory chain, decreasing the quantity of ATP that is accessible, and inducing metabolic inhibition, 3-NPA lowers oxidative phosphorylation [60]. Additionally, the generation of free radicals appears to have a considerable impact on the pathophysiology of HD [61]. The body's depleted antioxidant system is significantly replenished by vortioxetine treatment. In HD, mitochondrial dysfunction is significant. The pathophysiology of HD is associated with oxidative phosphorylation at the complexes of the electron transport chain (ETC), which causes neuronal death, expansion of the mitochondria, flexibility of the membrane, rupture, and release of cytochrome-C [62]. Only a few membrane-based activities that these mechanisms may directly influence include the opening of the mitochondrial permeability transition pore, fission-related morphogenic alterations, and oxidative phosphorylation at the complexes of the ETC. Disruption of the action of the mitochondrial enzyme complex is associated with reactive oxygen species (ROS). 3-NPA considerably reduced the activity of the complexes I, II, and IV of mitochondrial enzymes in the striatum in the current investigation. Mitochondrial enzyme complexes that are malfunctioning significantly improve with vortioxetine treatment.

According to one theory, the substantial increase in extracellular noradrenaline brought on by oxidative stress and mitochondrial malfunction serves as a source of highly reactive free radicals, starting a self-amplifying loop that finally results in neuronal degeneration. According to this study, Vortioxetine protects the mitochondria, which may be because the mitochondria engage in anti-apoptotic pathways.

Histological analysis of the cortex and hippocampus tissue has demonstrated that Vortioxetine has a dose-dependent Neuroprotective effect against 3-NPA-induced neurodegeneration.

Although the 3-NPA model is thought to be a very excellent model that faithfully reproduces HD symptoms, more research using these chemicals in genetic models is urged. Since this is the first trial to show their effectiveness in HD, more research using complete genetic models is required to fully appreciate the potential of these medications as a possible therapy for HD patients. Another choice is to investigate the several signalling pathways that have been linked to the regulation of neurodegeneration, mitochondrial malfunction, and cognitive decline using the 3-NPA induction model.

Conclusion

According to the findings of this study, Vortioxetine may have a Neuroprotective impact on 3-NPA-induced neurotoxicity through enhanced behavioural paradigms, free radical scavenging activity, and a decrease in neuronal cell damage. The stimulation of the 5-HT1A receptor, which raises the levels of SOD and catalase as well as anti-apoptotic proteins, may be the cause of Vortioxetine Neuroprotective effects. By activating the 5-HT1a receptor, Vortioxetine also improves cognitive impairment and motor coordination. As a result, Vortioxetine may be a viable candidate for the creation of a novel therapeutic neuroprotector against 3-NP-induced neurotoxicity. To understand Vortioxetine's full potential in HD, more study is required.

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Author contribution: Dr. Vipin Kumar Garg suggested the main idea of research. Avnesh Kumar conducted the experiment. All author shared shared data analysis and interpretation and manuscript preparation.

Declaration

Conflict of Interest

The authors declare that they are not hampered by any financial or other interests.

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No financial support is provided by any organization.

Ethics of Animal Experimentation

The Institutional Animal Ethical Committee of the Meerut Institute of Engineering and Technology in Meerut duly approved the protocol on experimental animals on 15 October 2016, with approval number 711/PO/Re/S/02/CPCSEA. The CPCSEA regulations were followed in the treatment of the animals.

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