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Ameliorative Effects Of Vortioxetine In 3-NPA Induced Huntington's Disease

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ABSTRACT:

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease caused by a mutation in the Huntingtin (HTT) gene. Clinically progressive motor dysfunction with cognitive decline, neuropsychiatric disorders like mood and behavioural abnormalities, and choreiform movements are its hallmarks. To lessen 3-nitropropionic acid (3-NPA)-induced HD-like pathology in a rodent model, this study sought to assess the neuroprotective pharmacological effects of vortioxetine, a primarily antidepressant drug that exhibits agonistic activity on the 5-HT1A receptor and antagonizes the serotonin transporter (SERT). The mitochondrial complex II inhibitor 3-NPA disrupts aerobic metabolism of cellular energy, induces oxidative stress, and causes neuronal damage like the pathophysiology of HD. To induce HD-like symptoms, 3-NPA (10 mg/kg intraperitoneally) was administered to male Wistar rats every other day for 28 days. Vortioxetine was subsequently administered orally at 5 and 10 mg/kg every day. Cognitive function was measured using the Morris Water Maze and Elevated Plus Maze, while the Rota rod test was used to measure motor coordination, and the Actophotometer was used to measure locomotor activity. Biochemical analyses measured striatal neurotransmitters, mitochondrial enzyme activity, and oxidative/nitrosative stress-related markers. Histopathological analysis of the cortex and hippocampal regions was performed to determine its impact on neuronal integrity. Vortioxetine treatment reduced oxidative stress, preserved the expression of mitochondrial electron transport complexes I, II, and IV, restored neurotransmitter equilibrium, and significantly ameliorated motor and cognitive deficits. Histopathology results showed a dose-dependent reduction in the evidence of neuronal degeneration, suggesting a neuroprotective effect. Thus, our findings suggest that Vortioxetine may exert its therapeutic effects in Huntington's disease by improving mitochondrial function, reducing oxidative stress, and acting on the serotonin pathways. Thereby, vortioxetine, in addition to its known antidepressant properties, may emerge as possible treatment options

in HD management. One might note limitations such as the need for validation in genetic HD models and long-term studies. Future studies should explore its therapeutic potential and molecular targets. The study emphasizes how Vortioxetine, including its role as a pharmacological agent, can be used for the treatment of neurodegenerative diseases, particularly in Huntington's disease (HD), alleviating neuropsychiatric disorders. Additional clinical trials should determine the long-term safety and efficacy of vortioxetine for humans.

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

Introduction

Huntington's Disease (HD) is a deadly neurological disorder characterized by gradual motor impairment, emotional disturbance, and mental deterioration, emaciation, and depression (**Adhikari et al., 2020**). In this study, rats were administered the drug 3-NPA that causes HD. Vortioxetine is a selective serotonin reuptake transporter (SERT) inhibitor and 5-HT1A receptor agonist (**Afzal et al., 2023**). Through inhibition of mitochondrial complex II, 3-nitropropionic acid (3-NPA) alters energy metabolism and leads to damage of both the striatum and cortex. Effects of the neurotoxin, which include diminished gait, hypokinesia, increased anxiety and/or depression, and memory issues, emulate some of the histological and neuropathology changes that accompany HD (**Albert & Vahid, 2021**). The number of symptoms identified is extensive, and most of them are associated with a change in behaviour, a decline in motor or cognitive function, or a combination of these symptoms (**Brouillet et al., 2005; Carmo et al., 2018**).

Using a complex II inhibitor of the electron transport chain called 3-NPA, it has been successfully employed, resulting in specific behavioural deficits and, in a rat model, lesions in the striatal region consistent with those observed in Huntington's disease. 3-NPA, despite its ability to target multiple areas of the brain, selectively damages the striatum, leading to an animal model of Huntington's disease (**Bhateja et al., 2022**).

3-NPA inhibits mitochondrial complex II, which contributes to damage of the striatum and cortex, leading to energy metabolism disturbance (**Binawade & Jagtap, 2021**). The neurotoxin, given the characteristics of gait and anxiety-like phenotypes, provides an appropriate experimental model to study Huntington's disease because the neurotoxin causes many of the histological and neuropathological changes seen in HD, such as gait impairments, hypokinesia, and increased anxiety and/or depression, and memory (**Albert & Vahid, 2021**). Arthrinium sp. is the fungus that produces the mycotoxin 3-NPA, a suicide inhibitor of the succinate dehydrogenase enzyme (SDH) of the Krebs cycle as well as the respiratory chain. Besides inducing striatal lesions and inducing proliferation of spiny neuron dendrites, 3-NPA inhibits ATP production in the brain's mitochondria, causing mitochondrial dysfunction. These effects lead to basal ganglia neuronal loss and movement dysfunction in HD patients (**Beck et al., 2020**). Within the brain, 3-NPA decreases levels of antioxidant enzymes and increases concentrations of reactive oxygen/nitrogen species, resulting in oxidative/nitrosative injury (**Carter et al., 2019**).

In particular, the mechanisms of this neuroprotective action, though shown in many in vitro and in vivo model systems, remain poorly understood, yet stimulation of the 5-HT1A receptor seems to be neuroprotective (**Emmanouil et al., 2018; Havey et al., 2019; Zanos and Gould, 2018**). Upon activation of the 5-HT1A receptor, levels of SOD and catalase, antiapoptotic members of the pro-survival BCL family (e.g., Bcl-XL, Bcl-2), and inhibitors of apoptosis proteins are elevated (e.g., Bax, XIAP) (**Chaudhary et al., 2020**).

The serotonergic system is important in regulatory events of numerous physiological and behavioural systems, where changes affect mood and anxiety levels, cognition, food consumption, and more. Thus, much research has been devoted to 5-HT1A receptors. These 5-HT1A heteroreceptors are found in areas of the brain, such as the thalamus, hypothalamus, and basal ganglia, as well as the hippocampus, prefrontal cortex, lateral septum, and amygdala of limbic systems. 5-HT1A receptor activation improves cognitive deficits. Moreover, 5-HT1A

agonists are expected to mitigate psychiatric symptoms such as depression and anxiety (**Gil-Mohapel et al., 2014; Panossian et al., 2010**).

Selective serotonin reuptake inhibitors inhibit the reuptake of serotonin in the presynaptic cell, increasing extracellular serotonin levels. Numerous studies have demonstrated that antidepressants possess antioxidant and neuroprotective properties by increasing the levels of antioxidant enzymes and thereby reducing oxidative damage (**Ertaş et al., 2019**).

Vortioxetine acts as an antagonist of the 5-HT receptors but also binds with partial agonism of the 5-HT1A & 5-HT1B receptors, as well as inhibiting the SERT. Vortioxetine can act on multiple distinct neurotransmitter systems, manipulating signal transmission through glutamatergic, GABAergic, noradrenergic, dopaminergic, cholinergic (ACh), and histaminergic systems in an indirect manner. The cognitive effect of vortioxetine is likely mediated, to a large degree, by indirect modulation of gamma-aminobutyric acid and glutamate neurotransmission, ACh, or histamine neurotransmission, according to our hypothesis. Many of these neurotransmitter systems are implicated in cognitive function. This is not to say these concepts are always mutually exclusive (**Ferguson et al., 2020**), but it is vital that empirical research be conducted on the relationship between the neuroplastic effects of vortioxetine across diverse neurotransmitter systems and cognition.

Vortioxetine elevated the levels of the mood-enhancing neurotransmitters serotonin, dopamine, noradrenaline, histamine, and acetylcholine in the rat prefrontal cortex (PFC) and ventral hippocampus, regions of the rat brain known to be involved in depression. In the rat nucleus accumbens, Vortioxetine produced increases in serotonin but not dopamine or noradrenaline. Further rat investigations indicate that Vortioxetine may also possess a more rapid attenuation of 5-HT neuronal activity in the dorsal raphe nucleus relative to previously identified antidepressants. Neurogenic action of vortioxetine in rodents was identified in their hippocampus, a neurogenic characteristic that may be related to the action of an antidepressant (**Fontaine et al., 2021**).

5-HT1AR may also play a role in motor coordination and fine-tuning. Multiple 5-HT1AR agonists have previously been shown to confer robust neuroprotection in a variety of cell injury models by down-regulating glutamate release, regulating anti-apoptotic functions, or up-regulating the expression of protective proteins (**Garcia-Garcia et al., 2020**).

The current study focuses on assessing the neuroprotective effect of Vortioxetine in a 3-NPA-induced animal model of Huntington's disease.

Material and Methods

Drugs and reagents

The source of vortioxetine hydrobromide and Imipramine Hydrochloride was (Indus Pharma and Getz Pharma, Pakistan respectively. Rest were procured from Sigma -Aldrich, Lowry's reagent, 3-nitropropionic acid (3NPA), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB), bovine serum albumin (BSA), N- naphthyl ethylene diamine, and Folin-Ciocalteu reagent,Niacinamide adenine dinucleotide (NADH), mannitol, glycyl glycine buffer, 4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES), and nitro blue tetrazole.

Animals

In this study, male albino Wistar rats (180--200g of body weight) were utilized. Animals were fed a standard laboratory pellet diet with ad libitum access to water. To maintain the animals' circadian rhythms, they were given a natural light/dark cycle of 12 hours each. The tests were performed in a largely soundproof lab between 9:00 AM and 5:00 PM. The animals were maintained in the lab throughout the laboratory investigation, and on the day of experimentation were acclimatized to the laboratory setting 5 days prior to the behavioural trial. All experimental techniques involving research and animal handling adhered to the Ethical Committee's approved protocols for Use of Animals (ECUA), Bashir Institute of Medical Sciences, Islamabad Pakistan. Ethical approval was provided by ECUA before the commencement of research with reference no. Dir/BIMS/ECUA-8-203/02, 24-01-2024.

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

3-NPA was administered i.p. at the dose of 10 mg/kg (0.5 ml/100 g) on alternate days for 28 days. 3-NPA was dissolved in a 0.9% saline solution, and sodium hydroxide was added to bring the pH to 7.4 (**Brouillet et al., 2005; Sandhir et al., 2010**). It was found that there was a higher rate of deaths when administered daily (for 2 ensuing weeks with the dosage of 3-NPA being 10 mg/kg) as they lose much weight; however, intraperitoneal administration of 3-NPA (10-20 mg/kg) for 4 days didn't result in any evident changes in their behavioral or biochemical data (**Kumar et al., 2012**). Therefore, the 3-NPA schedule delivered every other day produces profound behavioral and biological effects. Body weight, motor functions, and locomotor activity were assessed on day 1 prior to 3-NPA dosing and on day 30 after the conclusion of the trial (day 28).

Drug administration

Each solution of medication was prepared fresh for use. The dosing schedule and the dose were determined according to previous data from other laboratories. Systemic administration of 3-NPA (10 mg/kg alternatively for 28 days) has resulted in striatal lesions that are consistent with HD symptoms, considerable body weight loss, decreased locomotor activity, and irregular and abnormal movement patterns (**Gupta & Sharma, 2021**). Neuroprotective effect was evaluated on the 0--28 days followed by the administration of 3-NPA in all groups of the animal treatments.

Behavioural testing of all animal groups was performed on days 0--7, 14, 21, and 28. Biochemical estimations were carried out on those same animals after behavioural and cognitive assessments.

Experimental protocol

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

Group I - Normal control group

Normal saline (1 ml/100 g b.w.) was orally (through oral gavage, by mouth) given to animals. **Group II - 3-NPA control group**

Animals were injected with normal saline (1ml/100g b.w.) via i.p. route and 3-NPA (10 mg/kg) via i.p. route on alternate days till the 28th day.

Group III - Imipramine (15 mg/kg, p.o.) and 3-NPA

Imipramine (15 mg/kg, p.o.) was given once daily for 28 days followed by injection of 3-NPA (10 mg/kg, i.p.) on alternate days.

Group IV: Vortioxetine and 3-NPA (5 mg/ kg orally)

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

Group V: Vortioxetine (10 mg/kg orally) and 3-NPA

Vortioxetine (10 mg/kg) was given once daily via oral route to the animals followed by 3-NPA (10 mg/kg) through the i.p. route on alternate days till the 28th day.

Pharmacological Screening

Body weight

We measured the body weight on day 1 and day 28 and calculated the percent change in weight (**Han et al., 2020**).

Behavioural assessment

Locomotor Activity Analysis Using Digital Actophotometer

Digital Actophotometer (JAPSON**)**was used to measure the spontaneous locomotor activity**.** All the equipment during evaluation was placed in a ventilated, sound-attenuated, darkened room. Three minutes before the real locomotor activity task (10 min), each animal was isolated in the exercise cage to allow for acclimation. They counted the animals' baseline activity levels. Vertical and horizontal combined activity was recorded in counts

per 10 min. Locomotor activity is expressed in counts/10 minutes. Locomotor activity tests were performed during the 0, 7, 14, 21, and 28 days between 9:00 to 5:00 pm (**Kumar et al., 2012; Sandhir et al., 2010**).

Motor coordination tested by Rota rod

Rota rod tests were performed to assess motor coordination between the forelimbs and the hindlimbs (**Kumar et al., 2012; Sandhir et al., 2010**). Adherently, these individuals were added to the rotating spinning rod (7 cm diameter; 25rpm). The day before the first day of testing (25 rpm), rats were taught to use the Rota rod device for 2 minutes. Each rat conducted three separate trials per session separated by 5 minutes, where each trial terminated after the 180-second time limit. Each trial was followed by a five-minute break, to alleviate stress and fatigue. All trials ended on days 1 and 28 between 09.00--17.00. Unfamiliar with the method of the experiment, a trained observer recorded the decreasing time delay of each rat (**King et al., 1976**).

Cognitive Assessment

Morris water maze (MWM) test to assess learning and memory

The MWM was a large (150-cm diameter, 45-cm height) pool filled to a height of 30 cm with 28°C water. Gold was added to make the water opaque. Two threads stretched at an angle across the rim of the pool divided the tank into four equal quadrants. A submerged platform (10 cm2) was painted white and was 1 cm submerged in the pool's target quadrants. The platform remains in a fixed position throughout training. The rat was carefully placed into the water facing the pool wall in between each pool quadrant and given 120 seconds to reach an area with (hidden) platform below the water surface. It was then allowed to stand on the platform for 20 seconds. It was gently steered to the platform after 120 seconds if it couldn't find it on its own and given another 20 seconds to linger there. All acquisition trials targeted Quadrant 4 (Q4) as the puzzle quadrant, and the starting locations varied daily. The platform took them on the sixth day and allowed the rats 120 seconds of access to the pool without restrictions. Each rat was subjected to four of these trials, one starting in each quadrant. The retrieval index---and Q1, Q2, Q3, and Q4, as well as the time employed searching for them in Q4 on just one platform---was generated by computing the average amount of time spent in each of these four quadrants. The experimenter remained stationary in one Position at all times. To ensure that the water maze position remained constant throughout the experiment, it was closely monitored in relation to other laboratory materials acting as conspicuous visual references. All trials were concluded between 9:00--17:00, or the light cycle (**Kumar et al., 2012; Sandhir et al., 2010; Zuccato, C. et al.,2007**).

Elevated Plus Maze: Assessment of learning and memory

The Elevated Plus maze was used to assess spatial long-term memory. According to protocol, the equipment consisted of two open arms and two closed arms. The main platform of the maze was 50 cm above the ground, and its arms expanded in all directions. On Day 1 of testing, each animal was positioned at the end of an open arm. TOC was measured by the amount of time it took the rat to enter one of the surrounding arms for calculating transfer latency (TL). If it was outside, the TL was represented in the first open arm to enter in, and if the animal didn't enter, the TL delay was set appropriately (90 seconds). The rats were then given 20 seconds to explore the maze, before being returned to their cage. TL was recorded on days 0 and 28 of the trial (**Kumar et al., 2012; Yoshikawa, M et al.,2008**).

Tissue Preparation

The animals were decapitated following the assessment of behavioral and cognitive metrics. The brains of each animal were dissected, and the striatum was placed in ice before the weight of each was recorded. Homogenization of the tissues (10% (w/v)) was performed in 0.1 M PBS (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min at 4 °C, and supernatants were divided into aliquots and used for biochemical studies (**Gupta & Sharma, 2021**).

Biochemical Estimations

Evaluation of Oxidative and Nitrosative stress

Neurodegeneration in HD is primarily mediated by chemical mechanisms such as reactive oxygen species (ROS) and nitrative stress. In addition, the 3-NPA-induced neurotoxicity reveals mechanisms for oxidative stress and nitric oxide production (**Kumar et al., 2012**).

Evaluation of lipid peroxidation in the striatum

TBARS, a marker of lipid peroxidation, was determined using the method of Ohkawa et al. (1979). TBARS readings were expressed as nanomoles per mg of protein (**Laura & Mahin, 2020**).

Levels of striatum non-protein thiol (glutathione-GSH)

GSH level in the striatum was measured with a spectrophotometer (Shimadzu Corporation in Japan) (**Adhikari et al., 2020**), and trichloroacetic acid (10% w/v) was mixed with the homogenate's supernatant (1: 1 ratio). The tubes were centrifuged at 1000 g for 10 min at 4 °C, and the supernatant (0.5 ml) was mixed with 2 ml of 0.3 M di-sodium hydrogen phosphate. Afterwards, add 0.25 ml DTNB solution (0.001 M), measure the absorbance at 412 nm spectrophotometrically. The authors reported results in moles of reduced glutathione per mg of protein on a conventional graph using reduced glutathione concentrations from 10 to 100 M (**Luchowska et al., 2019**).

Evaluation of SOD activity in striatum

Striatum SOD was also detected spectrophotometrically (Shimadzu Corporation, Japan) based on Beauchamp and Fridovich recommendations (1971). The increase in absorbance units (A)min-1 for both control and test sample calculated and corresponding % inhibition calculated for test sample(s) (**Mamatha et al., 2020**).

Striatum catalase activity assessment

The striatum catalase activity was evaluated spectrophotometrically at 240 nm according to the Aebi method (1984) using a spectrophotometer (Shimadzu Corporation, Japan). Maurer & Williams based approach was taken for the assessment of catalase activity based on units per mg of proteins.

Striatum nitrate/nitrite level assessment

A UV-1800 ENG240 V from Shimadzu Corporation in Japan was used for its spectrophotometric measurement of striatum nitrite. Following the protocol, 100 µl of the striatal or reference sample was combined; then, 0.15 g of the copper-cadmium alloy and 400 µl of carbonate buffer (pH 9.0) were added. The nitrate was converted into nitrite by incubating the tubes at 22 °C for 1 h. Substitution with 100 µl of 0.35 M NaOH stopped the process. The samples were then treated with 400 µl of a ZnSO4 solution (120 mM) for deproteinization. The samples were then centrifuged at 4000g for 10 min after 10 min incubation. The nitrite level was measured as brain striatum using the Greiss reagent. Aliquots (500 µl) of the transparent supernatant were spectrophotometrically quality controlled at 545 nm. Greiss reagent: 250 µl sulfanilamide (1.0%); 250 µl Nnaphthyl ethylene diamine (0.1%) synthesized in 3N HCl (water used). Using the sodium nitrite standard curve to quantify the nitrite level in the striatum of the brain (range from 5 to 50 M) (**Sastry et al., 2002**).

Determination of striatum acetylcholinesterase (AChE) activity

AChE measurement was performed at 412 nm using spectrophotometry (Shimadzu Corporation, Japan) (**Pagel et al., 2000**). This was ultimately discovered by either measuring the reaction or color change from thiocholine8 along with dithiobisnitro benzoate ions, which when combined create a yellow color tablet that appears when thiols are present. Spectrophotometer was used to determine the rate of TH synthesis from acetyl thiocholine iodide in the presence of the cholinesterase enzyme. The supernatant liquid of brain homogenate was diluted with freshly prepared DTNB solution by transferring 0.5 ml of it into a 25 ml volumetric flask (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0). 4 ml of the filtration was pipetted from the volumetric flask into two different test tubes. Take one test tube with the solution in it, and add 2 drops of eserine solution. The substrate solution is made by mixing 50 ml of distilled water and 75 mg of acetylcholine iodide, and this

solution is added in 1 ml each to every test tube. Blank was a test tube containing eserine. The absorbance of the test sample was time-stamped in the spectrophotometric method at 420 nm.

Estimation of Serotonin Procedure

On the 28th day of the experiment, immediately following MWM, animals were decapitated, their brains were extracted, and the striatum that included the subcortical region was preserved. Tissue slices were weighed and subsequently 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 taken in a test tube, add 2.5 ml of heptanes and 0.31 ml of 0.1M HCl were thoroughly mixed for one minute and then centrifuged to separate the phases. The organic layer was removed. Subsequently, 0.2 cc of the aqueous phase was isolated for the 5HT test. All was carried out at 0 °C. (This was a very high amount of tissue: If Schlumpf M et al. had an amount in the range 1.5 → 5 mg per 0.1 ml HCl-butanol, The amount used was over 50, likely up to 75, mg tissue per 5 ml HCl-butanol). This is done in order to obtain so much supernatant liquid for the analysis. A 0.2 ml portion of the aqueous extract was mixed with 0.25 ml of OPT reagent. The fluorophore was obtained by heating up to 100 °C for 10 minutes. After the samples reached thermal equilibrium with their surroundings, readings in the spectrofluorometer were obtained between 360 and 470 nm. Squinting, 1-0-K of conc. HCl without OPT was applied. Internal standard: A 1:2 mixture of HCl and butanol (500 g/ml) was created in distilled water.

Measurement of 5-HIAA

5-HIAA levels in the supernatant of HClO4 homogenized tissues (10 min at 5000g in a microcentrifuge) were determined using a modified method described by Beck et al. (Fisher Scientific) (**Paul & Borah, 2020**). An aliquot of the HClO4 supernatant was added in a test tube with 1.9 mL of 0.01 M, acetate buffer (pH 5.5). To read the samples, they were incubated for a total time of 5 minutes in the dark at room temperature before reading in a spectrofluorometer with emission and excitation lengths of 333 and 296 nm, respectively. Values are presented as nMoles/g of moist tissue and were extrapolated from a standard curve (**Peña et al., 2021**).

Evaluation of total protein in striatum

The total protein was measured at 750 nm using a spectrophotometer. BSA was used as a standard to determine the total protein concentration in the striatum. Dilution of 0.15 ml of homogenate supernatant was made to 1 ml with water followed by addition of 5 ml of Lowry's reagent. Mix all the ingredients well and let rest for 15 minutes at room temperature. 0.5 ml of Folin-Ciocalteu reagent was added to the samples and allowed to stand for 30 min at room temperature. BSA standard curve was plotted from concentration range between 0.2 to 2.4 mg/ml (**Menze et al., 2020**).

Isolation of mitochondria from rat brain striatum and estimation of mitochondrial Complexes.

This protocol is a revision of previously described methods (**Wollenman et al., 2017; Ross et al., 2011**). Brain striatum slices were homogenized in an isolation buffer (75 mM sucrose: 215 mM Mannitol: 20 mM HEPES: 0.1% BSA: 1 mM EGTA, pH 7.2). Subsequently, the homogenate was centrifuged for 5 minutes at 10,000g, at room temperature. The pellets resuspended again in the isolation solution containing EGTA, and centrifuged again for 5 min, at 10,000g. Thereafter, the clear supernatant was obtained in other tubes and after being centrifuged at 10,000g for 10 min, it was depositioned in isolation buffer containing EGTA. Pure mitochondria were resuspended in the buffer that had no EGTA. As a result, rat brain mitochondria were isolated (**Pubill et al., 2020**).

AU Assessment of complex I (NADH dehydrogenase) activity

Spectrophotometric determination of NADH dehydrogenase action was performed on UV-Spectrophotometer due to Shimadzu Corporation Japan according to the method of Ransome & Hannan King and Howard, respectively. The absorbance at 550 nm was monitored for two minutes.

Measurement of complex II (succinate dehydrogenase-SDH) activity

SDH was measured with (Shimadzu Corporation, Japan), according to King Riga et al. The absorbance shift was measured at 420 nm for 2 min.

CI (NADH dehydrogenase) activity was assessed in a similar manner.

Absorbance changes at 420 nm was recorded for two minutes Rubin & Anglister technique. Using a spectrophotometer, the change in absorbance was spectrophotometrically measured over two minutes at 550 nm. (Shimadzu Corporation, Japan) in October 2023.

Histopathological examination

The brain tissue specimens were fixed in 10% formalin saline for 24 hr, after which the brain tissues were washed with tap water. Tissues were then mounted in sequential dilutions of alcohol for dehydration. Samples were cleared with xylene and afterward heated in a hot air chamber at 56°C for 24 hours before being embedded in paraffin. Tissue blocks were sectioned at 4 µ for coronal sectioning with a rotary microtome using paraffin bee wax. For routine light electric microscope examination, the tissue slices obtained were mounted on glass slides, deparaffinized, and stained with haematoxylin (H) and eosin (E).

Statistical analysis

For all statistical analyses, data were analysed using Graph Pad Prism 7. All results were shown as Mean±SEM. One-way analysis of variance (ANOVA) was used for statistical analysis of data followed by Tukey's test as post-test. Results were considered statistically significant when $P < 0.05$.

Results

Body Weight:

Day 28: Compared to the vehicle-treated group ($P< 0.05$), body weight of 3-NPA-treated animals significantly decreased. The rats treated with the vortioxetine (5 and 10 mg/kg) and the standard drugs Imipramine (15 mg/kg) showed a lower body weight loss than the negative control group rats compared with body weight loss ($P<0.05$). 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 ($P<0.05$).

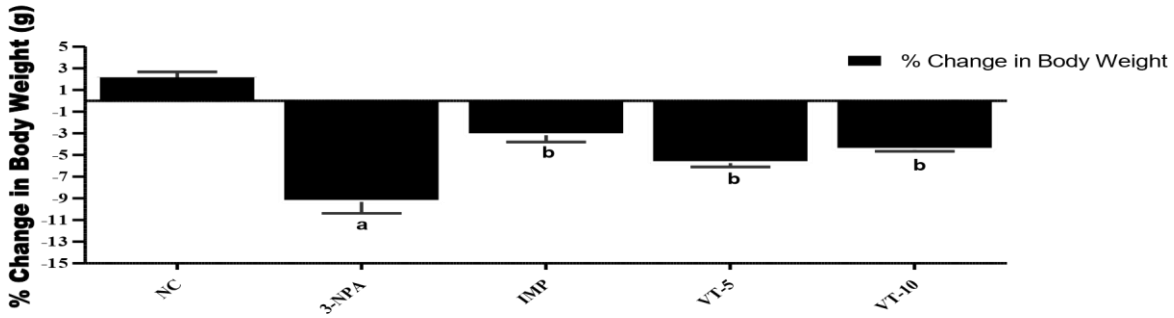


Figure 1: Effect of Vortioxetine on Body Weight in different experimental groups. [a]Statistically significant from the Normal control group (NC); [b]Statistically significant from the Negative control group (NGC).

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 3NPA treatment (Negative control group) showed a significant difference (P< 0.05, P< 0.001, and P< 0.001).

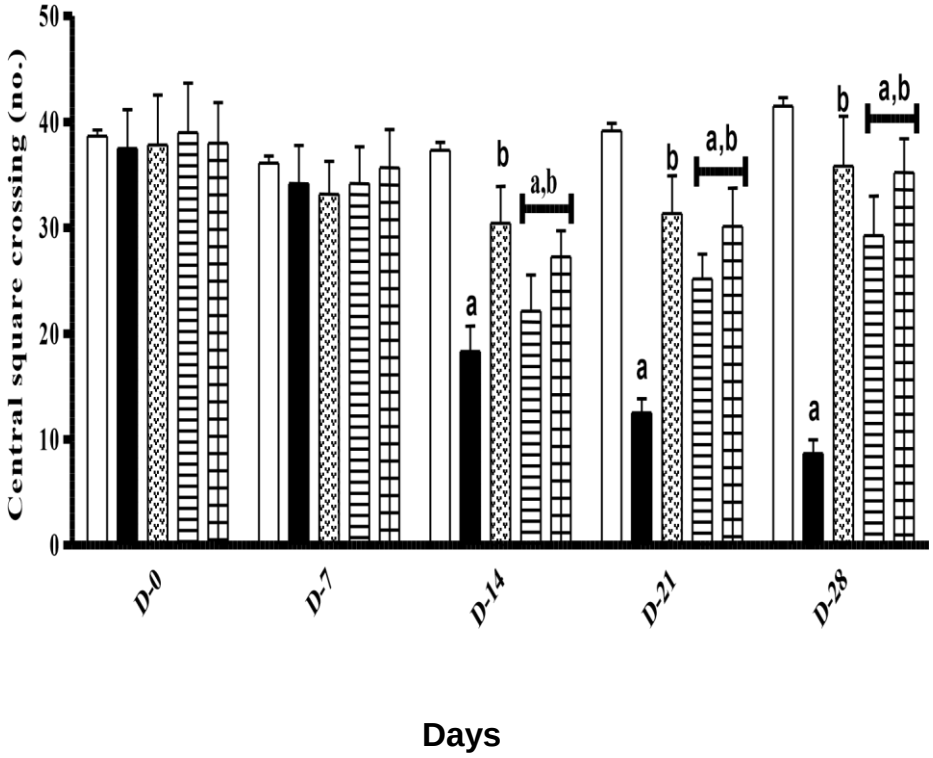


Figure 2:- Effect of Vortioxetine on locomotor activity in 3-NPA induced Huntington's disease by using Actophotometer.

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 3NPA-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).

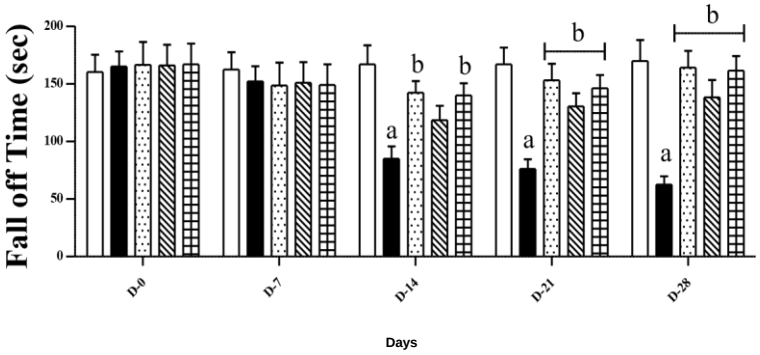


Figure 3:- Effect of Vortioxetine on Muscle grip strength in 3-NPA treated rats on Rota rod. Cognitive Impairment:

Elevated Plus Maze

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

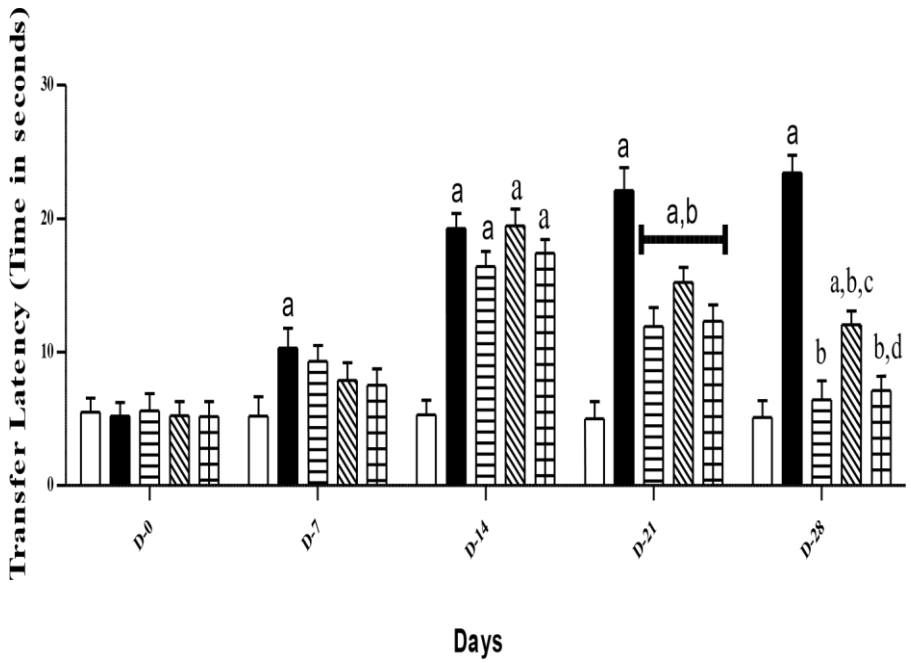


Figure 4:- Effect of Vortioxetine on Time Spent and number of entries in open and closed arm in 3-NPA induced animals during EPM. [a]Statistically significant from the Normal control group (NC); [b]Statistically significant from the 3-NPA treated group (Negative control group); [c]Statistically significant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

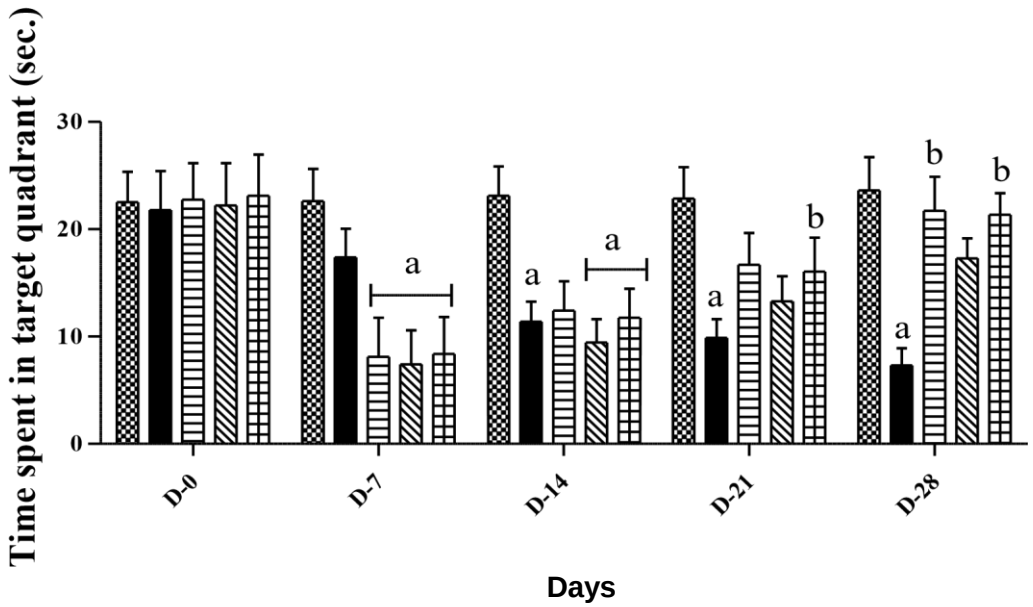


Figure 5:- Effect of Vortioxetine on Time Spent in Target Quadrant in 3-NPA induced animals during MWM.

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

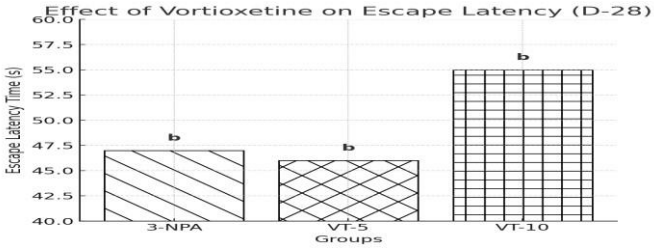
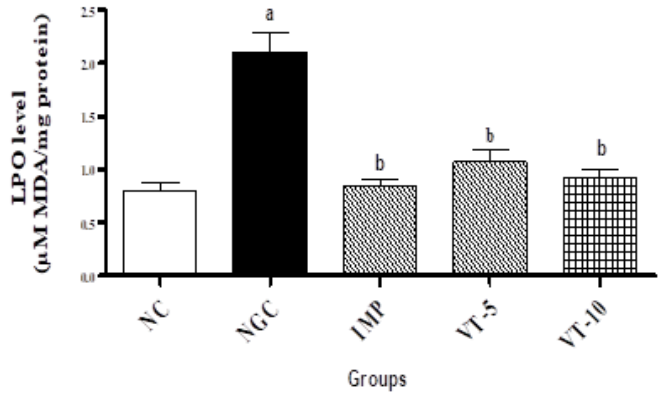


Figure 6: Impact of vortioxetine on the rats' escape latency time during the MWM test in 3-NPA-induced rats.

Figure 6 illustrates the impact of vortioxetine on the rats' escape latency time during the MWM test in 3-NPAinduced 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



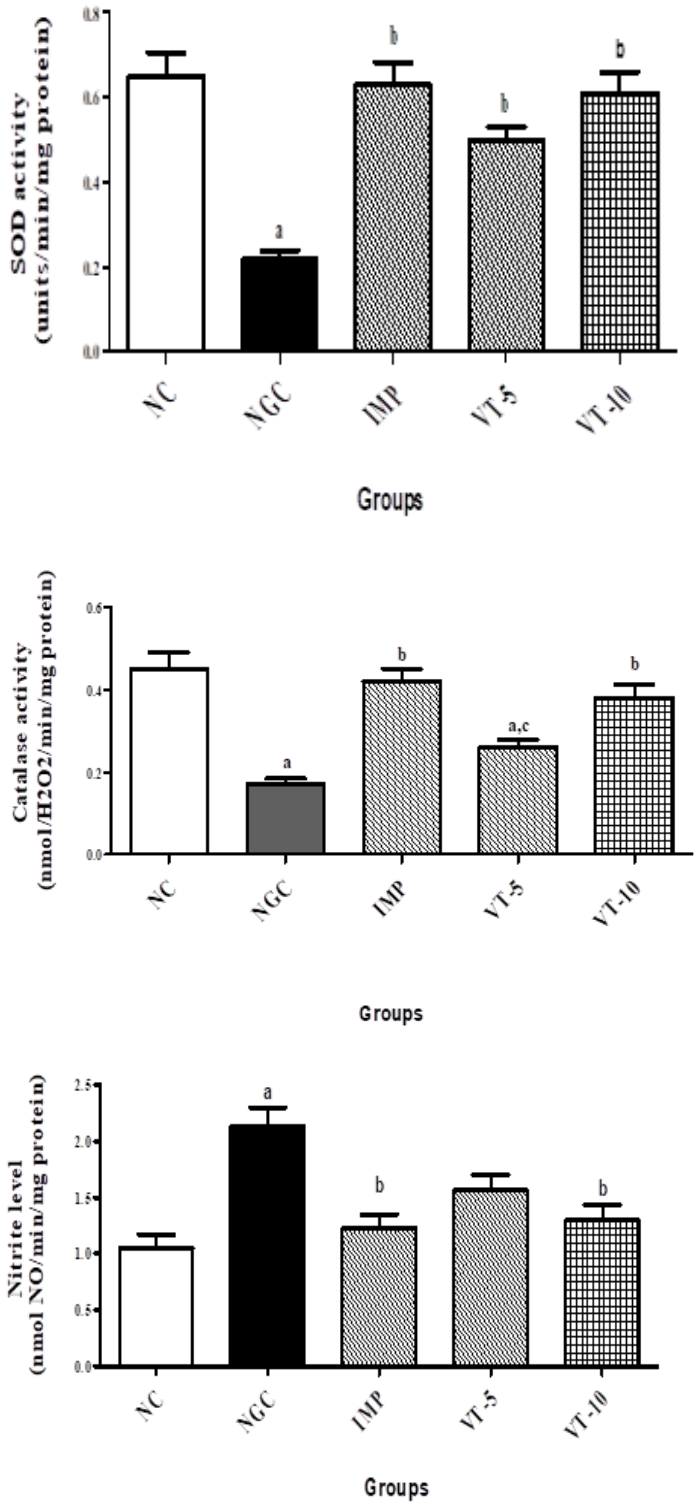


Figure 7:- Show the effect of Vortioxetine on oxidative stress markers in 3-NPA-induced alterations in the levels of MDA, SOD, and Catalase in the brain tissues. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

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

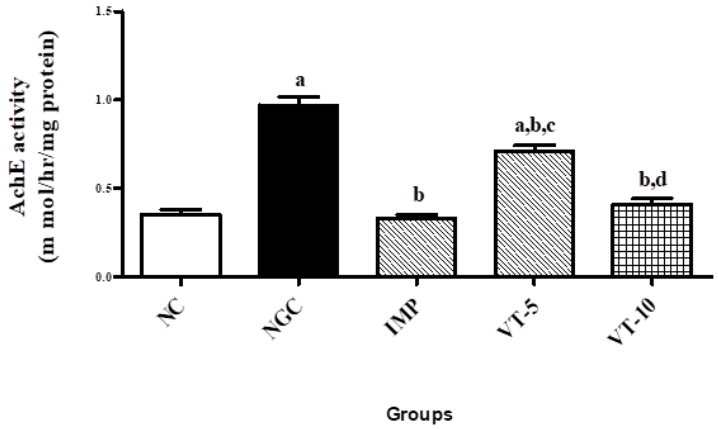


Fig 8:- Effect of Vortioxetine on Acetyl

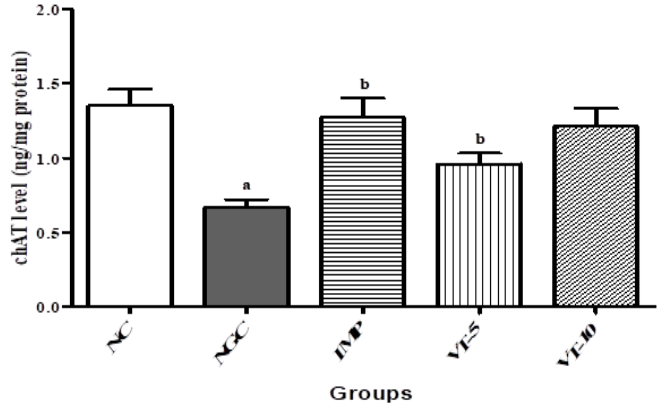


Figure 9:- Effect of Vortioxetine on Choline

Cholinesterase (AchE), in 3-NPA induced animals acetyl transferase (chAT) in 3-NPA induced animals.

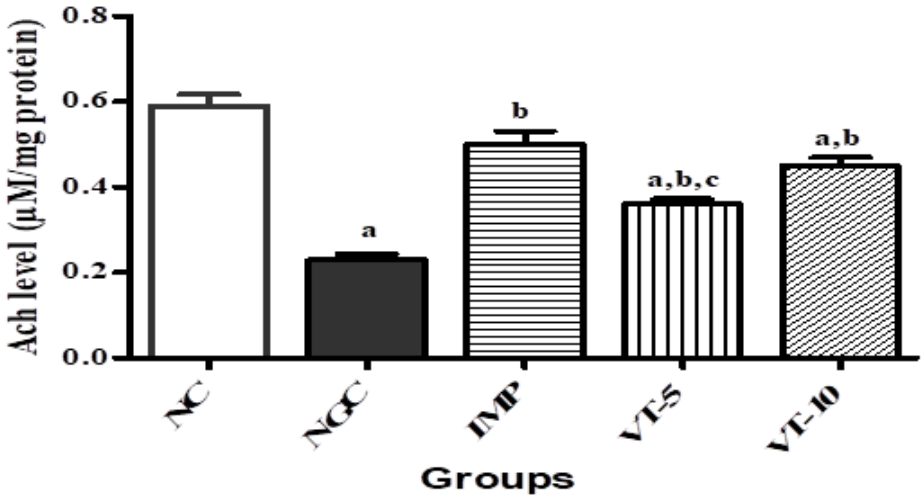


Figure 10:- Effect of Vortioxetine on acetylcholine (Ach) in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

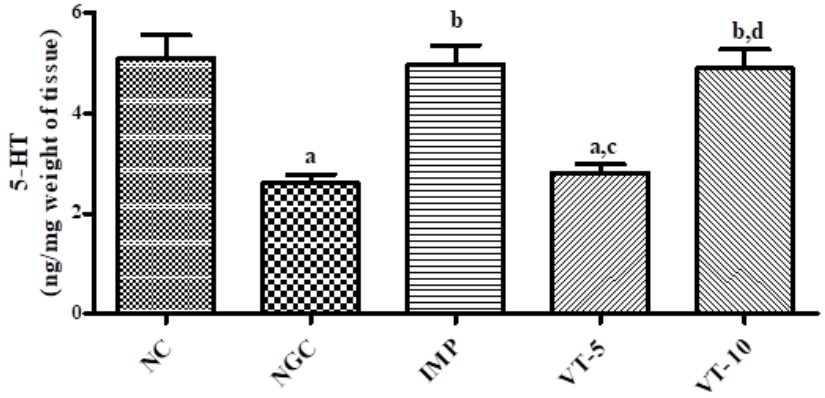


Figure 11:- Effect of Vortioxetine on Serotonin (5-HT) level in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

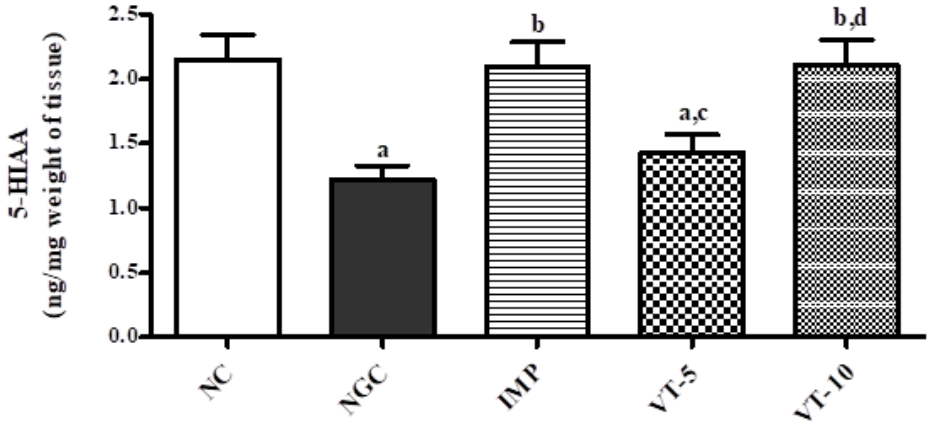
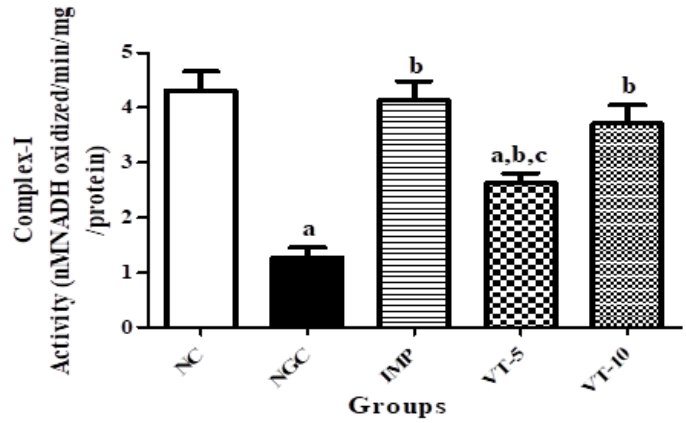


Figure 12:- Effect of Vortioxetine on 5-Hydroxy Indole Acetic Acid (5-HIAA) level in 3-NPA induced animals. One way ANOVA followed by Tukey's test as post hoc analysis showed a significant difference (P< 0.05).

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.



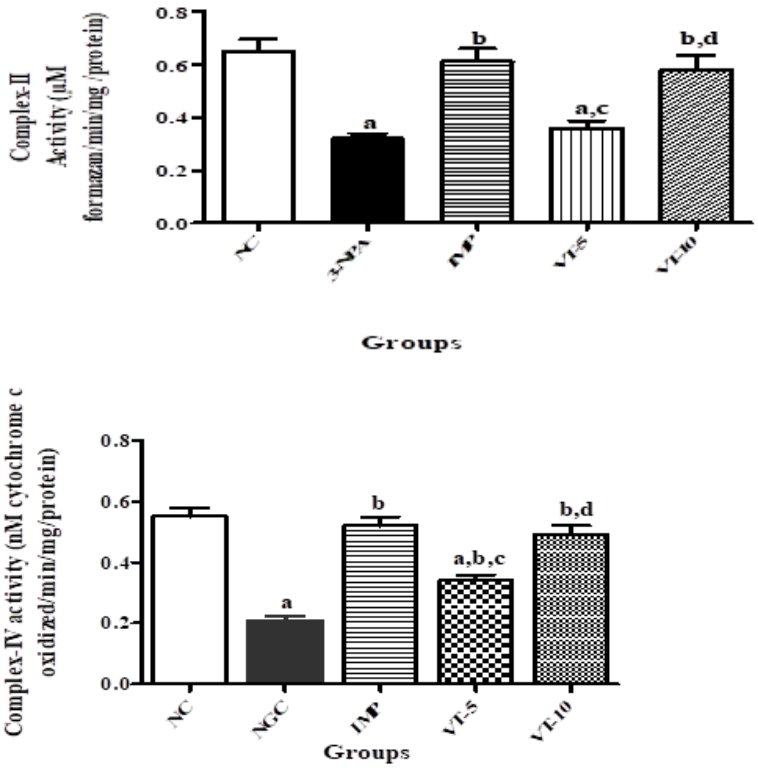
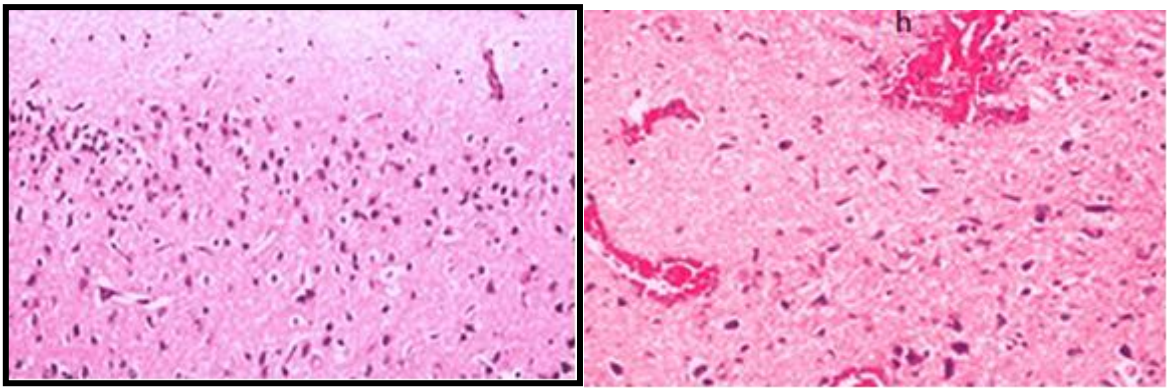


Figure 13:- demonstrated the impact of vortioxetine on mitochondrial enzyme activity in 3-NPA-induced rats. [a]Statistically significant from the NC (Normal control group); [b]Statistically significant from the 3NPA treated (Negative control group); [c]Statistically significant from the IMP (Positive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



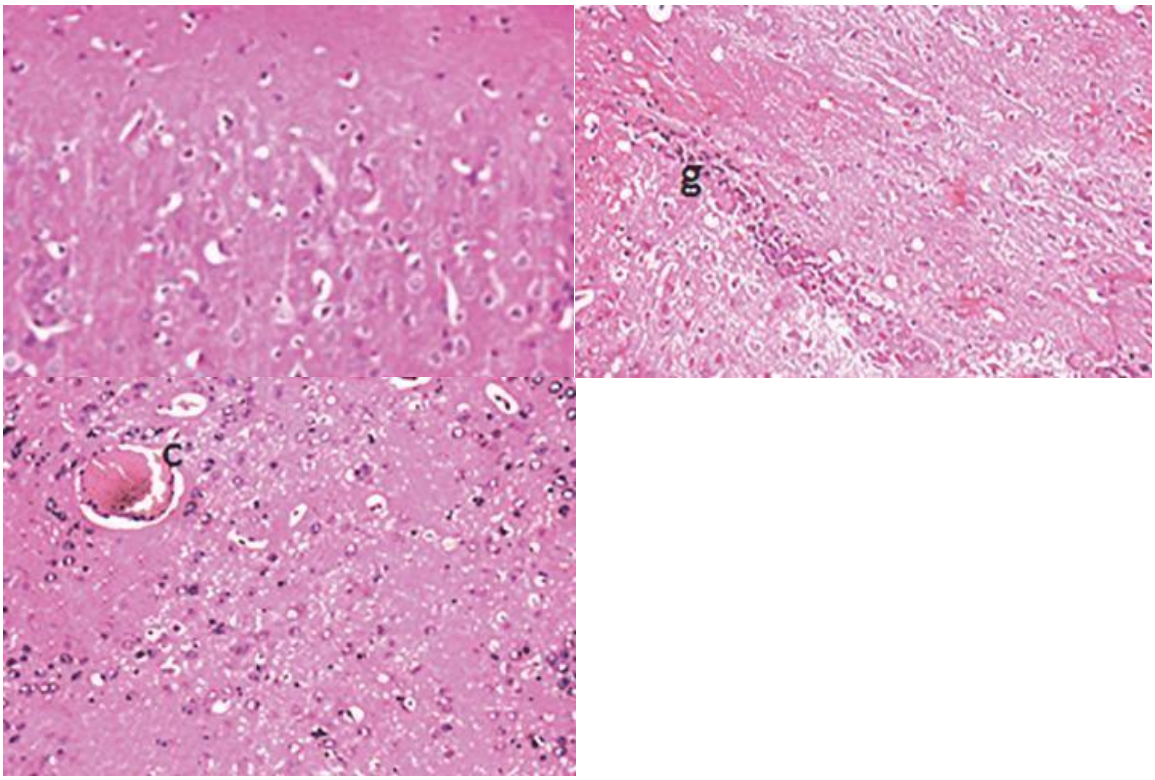
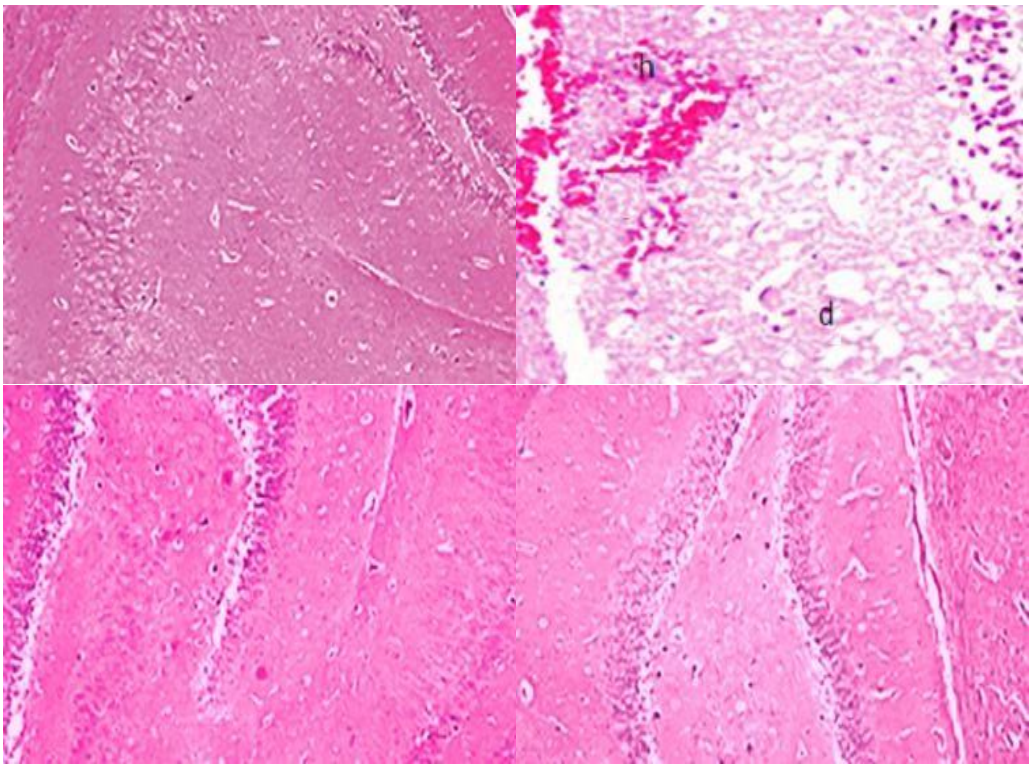


Figure 14:- H & E staining of the striatal tissue of rats belonging to the Normal control group (A), Negative control group (B), Std. group treated with Imipramine and 3-NPA (C), Vortioxetine (5 mg/kg) and 3-NPA-treated group (D), Vortioxetine (10 mg/kg) and 3-NPA-treated group (E). A and C showed no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



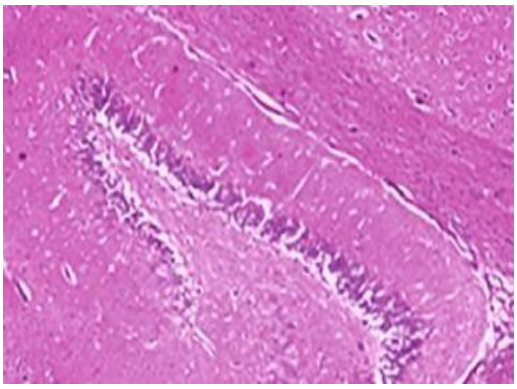


Figure 15:- H & E staining of the hippocampi of rats belonging to the Normal control group (A), Negative control group (B), Std. group treated with Imipramine and 3-NPA (C), Vortioxetine (5 mg/kg)- and 3NPA-treated group (D), Vortioxetine (10 mg/kg) and 3-NPA-treated group (E). A, C, D, and E showed no histological alterations, B showed severe neurodegeneration (d) and haemorrhage (h)

Discussion

In this study, neuroprotective effects of the vortioxetine were studied on 3-NPA model of HD in rats. It is known that 3-NPA produces cerebellar impairment by increased AchE levels in the rat brain striatum. 3-NPA was administered in the rats to change antioxidant parameters and mitochondrial complex activity in the striatum region of the brain.

The other plant-based compound showed positive results, including alleviating the adverse effects of 3-NPA on weight loss, decreased movements, impaired motor coordination, learning, memory, and recognition. Thus, vortioxetine also reduced oxidative and nitrosative stress in addition to affecting (AChE) in the striatum and preventing the degradation of the mitochondrial enzyme complexes (I, II, and IV).

3-NPA is known to cause weight loss. This was in line with previous studies (**Beck et al., 2020**). Decreased food consumption and increased rate of energy expenditure may serve as the principal mechanism for the shift in body weight (**Malathi et al., 2021**). Decreased body weight was observed in rats treated with 3-NPA, and weight was significantly increased by administration of Vortioxetine. Acts of hypo locomotion were observed in 3-NPA treated rats when tested in the Actophotometer, and impaired motor function was seen in the Rota rod test, which manifested dysfunctional motor system. The loss of neurons, in particular the loss of mediumspiny GABAergic neurons in the putamen and caudate nucleus, is a hallmark of the behavioral symptoms of HD with a consequence of progressive neuropathological change (**Brouillet et al., 2005; Carmo et al., 2018**). This neuronal degeneration can be mimicked through inhibition of mitochondrial complex II and has been associated with motor dysfunction in rats treated with 3-NPA (**Kumar et al., 2012**). Similarly, behavioral and motor coordination deficits mimic loss of motor ability associated with increased protein oxidation in the brain (**Sandhir et al., 2010**). The administration of vortioxetine leads to significant improvement in both rates of motor coordination and movement. The administration of 3-NPA caused a memory impairment, demonstrated by the MWM paradigm. The MWM has been previously used to test learning and memory in HD induced by 3-NPA (**Adhikari et al., 2020**). AChE abnormalities have also been associated with cognitive abnormalities, including impaired learning and memory (**Sharma et al., 2021**). AChE may also influence performance on putting through the MWM as higher AChE activity has been associated with cognitive impairments (**She et al., 2020**). Moreover, there is loss of motility and motor coordination, that probably also affects the efficacy of MWM. Concerning overhead mentioned factors should not be isolated as the only factors related to the deterioration of MWM performance in 3-NPA on its own rats, but it also matters oxidative stress, AChE activity in the brain, and direct effects on memory and learning. The control animals in MWM shows decreasing ELT. Day 4 ELT for these animals was markedly lower than day 1 ELT. The TSTQ had also significantly increased on day 5 relative to the time spent in the other quadrants in accordance with normal retrieval. Conversely, day 4 ELT of 3-NPA-treated mice predicted significantly higher than controls, suggesting that acquisition was impaired. There was a significant reduction of the control animal day 5 TSTQ relative to the 3-NPA-treated animals, highlighting memory impairment. Administering Vortioxetine in this

way vastly attenuated the day 4 ELT elevation associated with 3-NPA, abolishing the 3-NPA-induced acquisition deficit. The decrease in day 5 TSTQ induced by 3-NPA was significantly restored with vortioxetine treatment. The findings of the investigation correlate with another study published (**Kumar et al., 2012**). Three-NPA is responsible for damaging the pyramidal neurons in the hippocampal CA1 and CA3 region, which is associated with cognitive function. Research shows that 3-NPA may inhibit the oxidative defenses of the body and lead to ATP depletion.

It is generally accepted that hippocampus atrophy resulting from mitochondrial aberrations affects learning, memory, and other cognitive functions (**Shetty et al., 2021**). Treatment with 3-NPA confers 3-NPA-Long-Term Potentiation (3-NPA-LTP), which results in long-term potentiation of postsynaptic N-Methyl-D-aspartate receptor (NMDA)-mediated synaptic excitation within the striatal spiny neurons through a mechanism that inhibits expression of long-term depression (LTP) in the sensory-motor striatum (**Shinomol & Muralidhara, 2021**). 3-NPA-LTP increases intracellular calcium levels and activates mitogen-activated protein kinase and extracellular signal-regulated kinase. Hence, the focused and cell-specific neuronal death observed in HD may require 3-NPA-LTP (**Shinomol & Muralidhara, 2021**). Vortioxetine treatment reduced the learning and memory deficits induced by 3-NPA. As per the study, cholinergic synapses play a significant role in fundamental processes like memory, cognition, and learning (**Skogvold et al., 2021**). It has been demonstrated that the AChE enzyme controls the concentration levels of ACh in cholinergic synaptic clefts (**Soni & Kumar, 2021**). 3-NPA is known to augment striatal AChE activity (**Thampi et al., 2021**). The present study revealed that 3-NPA induced upsurge of AChE activity in the striatal area of the brain. Forebrain cholinergic neurons play a crucial role in modulating memory functions, according to some theories (**Túnez et al., 2010**). What is this Impact representing about the oxidative theory of HD and the new therapeutic goals from this perspective are important questions for future research, but expert evidence is proving that the increase of free radical production may contribute to the symptoms of HD and other movement disorders (**Vanova et al., 2021**). Probably, an at least partial role in this effect is the decrease of some endogenous antioxidant mechanisms, as GSH levels, and the activity of antioxidant defense enzymes as catalase and SOD. However, there is strong evidence showing that oxidation affects the toxicity of 3-NPA (**Wang et al., 2017**).

3-NPA reduces oxidative phosphorylation by disrupting the mitochondrial respiratory chain, reducing the amount of ATP available, and promoting metabolic inhibition (**Kumar et al., 2012**). Also, the production of free radicals seem to play an important role in HD pathophysiology (**Johri & Beal, 2012**). Significantly, the body's depleted antioxidant system is restored upon treatment with vortioxetine. Mitochondrial dysfunction is prominent in HD. In relation to the pathophysiology of HD, oxidative phosphorylation occurs at the electron transport chain (ETC) complexes and contributes to neuronal death, swelling of the mitochondria, increased membrane flexibility, rupture, and release of cytochrome-C (**Carmo et al., 2018**). Some examples of membrane-based processes that these mechanisms may have a direct impact on include mitochondrial permeability transition pore opening, fission-related morphogenic changes, and oxidative phosphorylation at ETC complexes. ROS production is implicated by dysregulation of the mitochondrial enzyme complex action. In this study, 3-NPA significantly inhibited the activity of mitochondrial enzyme complexes I, II, and IV in the striatum. Malfunctioning mitochondrial enzyme complexes significantly improve in response to vortioxetine treatment.

One theory postulates that the large elevation of extracellular noradrenaline caused by oxidative stress and mitochondrial dysfunction becomes a source of highly reactive free radicals, establishing a self-reinforcing cycle that ultimately leads to neuronal degeneration. The study demonstrated that Vortioxetine showed mitochondrial protection, probably because mitochondria were involved in anti-apoptotic pathways. Vortioxetine exerted a dose-dependent neuroprotective effect against 3-NPA-induced neurodegeneration in the histochemical analysis of the cortex and hippocampus tissues.

While the 3-NPA model is considered by many to be a very good model reflecting HD symptoms, additional studies using these chemicals in genetic models are recommended. As this is the first trial to demonstrate their efficacy in HD, further studies utilizing complete genetic models are needed to fully illuminate the potential of these compounds as a novel therapeutic for HD patients. Alternatively, with the 3-NPA induction model, you can examine the various signalling pathways implicated in the regulation of neurodegeneration, mitochondrial dysfunction, and cognitive decline.

Conclusion

The findings of this study suggest that Vortioxetine possesses neuroprotective properties against 3nitropropionic acid (3-NPA)-induced neurotoxicity, as exhibited by enhanced behavioural performance, increased free radical scavenging activity, and reduced neuronal cell damage. Furthermore, the neuroprotective effects of Vortioxetine may be mediated through 5-HT1A receptor activation, reversing the depletion of antioxidant enzymes superoxide dismutase (SOD) and catalase, and that would increase the amount of antiapoptotic proteins

Additionally, Vortioxetine showed rapid improvement in cognitive deficits and motor coordination, likely attributable to its agonistic action on the 5-HT1A receptor. This study provides evidence that Vortioxetine may be beneficial as a novel therapeutic against 3-NPA-induced neurotoxicity. Further studies are warranted to elucidate the mechanisms to investigate its efficacy in genetic models of HD and long-term clinical applications.

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