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Original Article

Therapeutic Potential of Lacidipine against 3-Nitropropionic Acid Induced Huntington's Disease: Based on Molecular Docking, Anti-Inflammatory and Neuroprotective Effects

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ABSTRACT

Background: Huntington's Disease (HD), a neurological ailment that leads to motor impairment, cognitive decline and psychiatric disorders, has limited treatment options. Hence, there is a pressing demand for new therapeutics to address the complex pathophysiology of HD. Lacidipine, a calcium channel blocker with antioxidative and anti-inflammatory properties, is a promising treatment option. **Aims:** This work aimed to assess the therapeutic potential of lacidipine against 3-Nitropropionic Acid (3-NPA)-induced neurodegeneration in a rodent model. **Materials and Methods:** There were 24 male Wistar rats (*n*=6) were categorized into four groups: the control group, the 3-NPA (10 mg/kg/day) intraperitoneally injected group and two lacidipine groups with dosages of 0.5 mg/kg and 1.0 mg/kg. These treatment animal clusters were exposed to a combination of lacidipine and 3-NPA for 14 days. On the 14th day, behavioral assessments were conducted. Subsequently, on the 15th day of the experiment, all animals underwent anesthesia, followed by the extraction of brain tissue for the evaluation of biochemical parameters, including Lactate Dehydrogenase (LDH), Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione (GSH). Additionally, the activity of neurotransmitters, such as Acetylcholinesterase (AChE), Gamma-Aminobutyric acid (GABA), glutamate, Dopamine (DA) and inflammatory mediators, including Interleukin-6 (IL-6), Interleukin-1β (IL-1β), Tumor Necrosis Factor-α (TNF-α) and Nuclear Factor kappa-B (NF-kB) were performed. A docking study was conducted to investigate potential interactions between lacidipine and relevant molecular targets AChE (7XN1), D3 receptor (3PBL), GABA (B) receptor (4MQF) and GluA2 receptor (2XHD). **Results:** Our research showed that treatment of rats with lacidipine after exposure to 3-NPA improved their motor coordination, sensorimotor function and muscular strength. We also observed the restoration of neurotransmitter imbalances, reduced oxidative stress and increased antioxidant defenses in treated rats. Furthermore, lacidipine prevented the upregulation of neuroinflammatory biomarkers induced by 3-NPA. Lacidipine showed a negative binding energy to the target molecules (glutamate: -7.855 kcal/mol, DA: -7.607 kcal/mol, GABA -7.523 kcal/mol and AChE: -6.949 kcal/mol). **Conclusion:** The research presented in this study offers valuable information regarding the potential of lacidipine to prevent neurodegeneration linked to HD, indicating a promising path for future investigations and possible therapeutic applications to treat this debilitating disease.

Keywords: Antioxidants, Huntington's disease, Inflammation, Oxidative Stress, Neurotransmitters.

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INTRODUCTION

Huntington's Disease (HD) is an inexorable neurological condition triggered by the amplification of CAG triplet repeats in exon 1 of the *Htt* gene, which encodes the 350 kDa huntingtin protein.[1] These mutagenesis changes generate a mutant huntingtin protein that impairs corticosteroid function and ultimately results in cell death. These include pronounced behavioral abnormalities, poor transcriptional control, excitotoxicity, oxidative damage, inflammation, mitochondrial malfunction and apoptosis, all of which are linked to the pathophysiology of the disorder.[2] The prominent manifestations of HD include motor dysfunction, cognitive difficulties and psychiatric imbalances.[3] Moreover, the loss of Medium Spiny Neurons (MSNs), which use Gamma-Aminobutyric Acid (GABA) as their principal

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neurotransmitter, is a hallmark of HD.[4] Despite exhaustive research, viable therapeutic interventions to arrest or reverse HD progression remain elusive and existing measures merely provide symptomatic relief.[5] HD prevalence varies by more than ten times between different geographical areas globally. Moreover, the incidence of HD varies dramatically between different racial and ethnic groups, with Caucasians having the highest incidence (9.71 per 10,000 people) and Asians having the lowest prevalence (0.42 per 100,000 people).[6] HD manifests typically between the ages of 30 and 40 years and death usually occurs 15 to 20 years after the onset of symptoms. In a few instances, the disease may begin earlier in young people, around the age of 20 and proceed over a period of approximately five years.[7]

3-Nitropropionic Acid (3-NPA) is naturally available in sugarcane and is produced by *Arthrinium* sp. In rodents, 3-NPA administration causes motor dysfunction and sustained morphological damage to the striatum.[8] 3-NPA has been documented to induce brain lesions in experimental animals and to disrupt GABAergic medium spiny neurons in the striatum of individuals with HD.[9] The toxicity of 3-NPA involves the inhibition of succinate dehydrogenase within mitochondrial complex II and lowers ATP production, leading to neuronal dysfunction, particularly in energy-demanding regions like the striatum.[10] Furthermore, 3-NPA can penetrate the blood-brain barrier, potentially causing adverse effects on the central nervous system.[11] 3-NPA triggers disruption of the mitochondrial electron transport chain and excessive glutamate release, which leads to excitotoxicity, a process in which excessive glutamate damages neurons.[12] Furthermore, 3-NPA-induced oxidative damage and excitotoxicity triggered inflammatory responses by releasing pro-inflammatory cytokines, with activated microglia exacerbating neuronal damage.[13,14] Inflammatory cytokines, when synthesized in large quantities and stimulating microglia for extended periods, contribute to neurodegeneration induced by inflammatory mediators. Nitric Oxide (NO), a critical inflammatory agent that leads to neuronal death demise of neurons, is produced by microglia via the enzyme NO Synthase (iNOS).[13,14] Because of the similarities in the mechanisms and pathology between 3-NPA lesions and HD, there has been a proposal to consider 3-NPA as a potential model for studying HD.[15] This is supported by a previous study that provided comprehensive insights into the utility of 3-NPA in mimicking the pathological features of HD.[16]

Lacidipine is a calcium channel blocker taken orally with a slow onset and long duration of action, with high vascular selectivity. Its antioxidant activity surpasses those of other dihydropyridine calcium antagonists.[17] Previous studies have demonstrated that lacidipine is efficacious in regulating abnormal blood pressure and exhibits therapeutic potential in addressing oxidative and inflammatory damage.[18-20] Moreover, lacidipine has been reported to attenuate apoptosis triggered by inflammatory molecules.[21] Previous research indicates that lacidipine possesses antiviral properties,[22] acts as an antibacterial agent,[23] is effective in managing neuropathic conditions,[24] functions as an antihypertensive treatment[25] and provides hepatoprotective benefits.[26]

Molecular Docking (MD) is an *in silico* structure-based method used for drug discovery. MD enables the identification of novel compounds with therapeutic potential, prediction of ligand-target interactions at the molecular level and interpretation of structure-activity relationships without prior knowledge of the chemical structures of other target modulators.[27]

Considering the severe difficulties posed by HD, which is characterized by detrimental neurodegenerative effects and limited treatment options, it is imperative to explore unconventional therapeutic avenues. This research aims to investigate the lacidipine effect against the 3-NPA-induced HD model, specifically focusing on its ability to mitigate oxidative stress, inflammation and neurodegeneration. By evaluating lacidipine's antioxidative, anti-inflammatory and neuroprotective mechanisms, we aim to provide insights into its potential as a novel therapeutic agent for HD. Furthermore, MD was used to determine the binding interactions between lacidipine and molecules that contribute to the pathophysiology of HD. By interpreting these interactions, we aimed to understand better the basic mechanisms underlying lacidipine's therapeutic potential in managing neurodegenerative processes.

MATERIALS AND METHODS

Chemicals

The 3-NP (MSWPA-001) and lacidipine (≥98% by HPLC MSWLP-01) were acquired from MSW Pharma., India. Enzyme-Linked Immunosorbent Assay (ELISA) kits to estimate cytokine levels of Interleukins-1β (IL-1β, KLR0119), Nuclear Factor-κB (NF-κB, KLR0287), IL-6 (KB3068) and Tumor Necrosis Factor-alpha (TNF-α, KB3145) were provided by Krishgen Biosystems, Mumbai, Maharashtra, India.

Animals

A total of 24 male Wistar rats (10-12 weeks old) with body weights ranging from 150 to 200g were included in this study(n=6) and were distributed into four distinct polypropylenes in individually ventilated cages. Only male rats were exclusively utilized to mitigate gender-based variability in outcomes. The conditions in the laboratory for keeping the experimental subjects consist of I) A 12 hr cycle of light followed by a 12 hr cycle of darkness, II) an optimum temperature (22-25°C), humidity (45-55%) and III) free access to both food and water. The research procedure was endorsed by the following protocols sanctioned by the Institutional Ethics Committee (LNCP/IAEC/2023/005) and in accordance with the ARRIVE guidelines for research.[28]

Experimental design

Throughout this study, we meticulously administered a comprehensive treatment regimen over a span of 14 days. Rats were systematically allocated by simple randomization to four distinct clusters, each containing six rats (*n*=6). The details of experiments are structured as follows:

Group I: The control group was orally administered saline solution.

Group II: The disease control group, conversely, received 3-NPA (10 mg/kg/day) intraperitoneally.

Group III: In this group, the therapeutic intervention involved the simultaneous application of lacidipine (0.5 mg/kg/day) via oral dose and 3-NPA (10 mg/kg/day) via intraperitoneal injection.

Group IV: Similarly, within this group, the therapeutic measures encompassed the oral administration of lacidipine (1.0 mg/kg/day) coupled with intraperitoneal injection of 3-NPA (10 mg/kg/day).

Group I (normal control) was given an oral administration of 3 mL/kg saline throughout the 14-day protocol. To induce symptoms mimicking those of HD, a dose of 10 mg/kg 3-NPA was mixed with saline intraperitoneally administered to animals in Group II (3-NPA control) over a period of 14 days. The saline solution (pH 7.4) contains 5% Dimethyl Sulfoxide (DMSO) solution (pH 7.4). For Groups III and IV (treatment groups), doses of 0.5 mg/kg and 1.0 mg/kg of lacidipine (soluble in DMSO) were administered orally for 14 days.[24,30] Additionally, both groups received an intraperitoneal dose of 10 mg/kg 3-NPA. Behavioral parameters were assessed in experimental animals throughout the protocol. On the 15th day, the body weights of all experimental subjects were carefully documented. After the end of the experiment, rats were anesthetized with ketamine/xylazine (75/10 mg/kg) intraperitoneally.[31] All rats were ethically sacrificed by cervical dislocation and their brains (striatum) were preserved for subsequent biochemical analyses (Figure 1).

Behavioural assessments

All behavioral tests were conducted during the light phase, specifically between 10:30 am and 4:30 pm, to mitigate potential circadian influences. Rotarod and beam walk evaluations were conducted on the 14th day of the experiment, whereas the grip strength test was performed on the 15th day before the animals were euthanized.

Rotarod test

The rotarod test was performed following the methodology outlined in a previous study.[32] The rotarod assessment's objective was to ensure that the animal ran continuously on a rotating rod while maintaining balance and preventing falls. Before the actual test, the animals underwent three training trials during which the rod was rotated (5 rpm). For the actual test, the rodents were kept on the rotarod device and the rotation was initiated at 3 rpm, gradually increasing to a maximum of 30 rpm. The duration until they fell from the rod was measured as the falling latency and was used for comparative analysis.

Narrow Beam Walk Test

The narrow-beam walk test is a behavioural evaluation used to assess sensorimotor function, balance and coordination in experimental animals, as described in previous works.[33,34] The subjects underwent training to navigate a wooden beam of length 150 cm. The beam was divided into three sections, each spanning 50 cm and the animal travelled from a platform at one end to its home cage positioned at the opposite end. The beam was positioned horizontally situated horizontally at a height of 60 cm above the floor. Beneath the beam, a container containing sawdust was positioned to provide a protective cushion in the event of a falling rat. The rat's adaptation process involved a 5 min period during which it was permitted to familiarize itself with the beam. The elapsed crossing time was meticulously documented upon the rat traversal of the beam.

Grip strength test

The grip strength test was employed to assess the motor function and muscle strength of rats as described by Ibrahim WW *et al*.[35] Animals from each experimental group were allowed to suspend themselves using their front paws on a horizontally stretched steel wire measuring 2 mm in diameter and 35 cm in length. This wire was positioned at a height of 50 cm above the cushioned support. The time to grasp the horizontal wire was regarded as an indirect indicator of the grip strength.

Neurochemical assessments

Acetylcholinesterase (AChE) estimation

Acetylcholinesterase (AChE) activity was assessed per Ellman *et al*.'s protocol.[36] A solution was created in a test tube by mixing 3 mL of 0.2 M phosphate buffer (pH 8), 0.05 mL of brain homogenate, 0.1 mL of acetylthiocholine iodide and 100 µL of 5,5′-Dithiobis-(2-Nitrobenzoic acid) (DTNB). In a test tube, a solution was prepared in a test tube by sequentially combining 3 mL of pH 8, 0.2 M phosphate buffer, followed by 0.05 mL of brain homogenate, 0.1 mL of acetylthiocholine iodide and finally, 100 µL of DTNB. The absorbance of the resultant solution was subjected to spectrophotometric analysis at a wavelength of 412 nm, extending over a period of 120 sec, with measurements taken at 30 sec intervals.

GABA, DA and glutamate estimation

Neurotransmitter concentrations, including GABA, DA and glutamate, were precisely quantified by high-performance liquid chromatography (HPLC Agilent 1100 VWD Detector) according to a previously described methodology.[37] Before derivatization,

the samples were deproteinized using methyl alcohol. The specimens were combined with 2,4-Dinitrofluorobenzene (DNFB) and allowed to react for a duration of 1 hr at 60°C. Subsequently, 50 mM potassium dihydrogen phosphate was added to terminate the reaction. The obtained compounds were analyzed using a UV detector operating at a wavelength of 360 nm. We used ChemStation software to control and analyze data.

Biochemical assessments

Lactate Dehydrogenase (LDH) estimation

LDH was assessed using a previously described method.[38] The assay mixture comprised 0.5 mL of buffered substrate formulated in 0.1 M glycine buffer (pH 7.9) and 0.1 mL of the prepared tissue homogenate. Incubation was performed out for 30 (37.8°C). Subsequently, colour enhancement was achieved by adding 5.0 mL of 0.4N NaOH. Absorbance at 440 nm was measured using a spectrophotometer. Enzyme activity was quantified in terms of units per Liter.

Malondialdehyde (MDA) estimation

Oxidative stress was evaluated by estimating the expression of Malondialdehyde (MDA) in rat brain tissue using the methodology outlined in a previous study.[39] Tissue homogenates were meticulously prepared and the protein content was determined. MDA, a lipid peroxidation product, was quantified by reaction with thiobarbituric acid. The resultant MDA-thiobarbituric acid adduct was recorded spectrophotometrically at 532 nm. Standard MDA solutions aided in the calibration of the measurements and the output was represented by nanomoles of MDA per mg of protein.

Superoxide Dismutase (SOD) estimation

The antioxidant defense was evaluated by measuring the expression of SOD in rat brain tissue as per the method described in previous study.[39] Tissue homogenates were prepared and their protein content was determined. The SOD level was assessed based on its capacity to prevent the auto-oxidation of epinephrine and reaction kinetics was monitored spectrophotometrically at a wavelength of 480 nm. Enzyme activity was quantified in units per milligram.

Reduced Glutathione (GSH) estimation

The quantification of reduced glutathione in brain homogenates was performed following the procedure described and outlined by Ellman in a previous study.[40] 1 mL of supernatant was mixed with an equal volume of 4% sulfosalicylic acid for precipitation, followed by cold digestion at 4°C for 1 hr and centrifuged at 1200×g for 15 min. A reaction mixture of 10 mL was prepared, comprising 2.7 mL of 0.1 M phosphate buffer solution (pH 8), 0.2 mL DTNB and 0.1 mL of the supernatant. Spectrophotometric estimation was conducted at 412 nm and the quantified GSH level was presented as units per milligram.

Neuroinflammatory markers assessments

Neuroinflammatory responses were evaluated by quantifying IL-1β, IL-6, NF-κB and TNF-α levels in the brain tissue of rats. Homogenized tissue samples were meticulously prepared and the protein concentration was subsequently determined. Quantification of IL-1β, IL-6, NF-κB and TNF-α concentration was accomplished using commercial ELISA kits, meticulously following the guidelines prescribed by the manufacturer to ensure precision and consistency in measurements.

Molecular Docking

Target protein retrieval and preparation

To predict the strength of the bonds between various ligands and target molecules, including AChE, DA, GABA and glutamate, we obtained the corresponding FASTA sequences from the

Table 1: Comparison between standard values and retrieved protein for validation of selected protein.

Parameters	Details				Standards
Target	ACHE	Dopamine (D3 receptor)	Gamma-Aminobutyric Acid GABA (B) receptor	Glutamate (GluA2 receptor)	-
Protein Id and	7XN1	3PBL	4MQF	2XHD	-
Method of experiment	X-RAY Diffraction	X-RAY Diffraction	X-RAY Diffraction	X-RAY Diffraction	X-RAY Diffraction
Mutation	No	No	No	No	No
Resolution	2.85 Å	2.89 Å	2.22 Å	1.80 Å	Near about 3.00 Å
wwPDB Validation	Better	Better	Better	Better	Better
Co-Crystal Ligand	Tacrine	ETQ	2BQ	7T9	-
Ramchandran Plot (by PROCHECK server) Residues in favoured+Allowed regions	89.8 %	91.5 %	92.2 %	94.2 %	>88 %

National Center for Biotechnology Information (NCBI). We then utilized the Basic Local Alignment Search Tool (BLAST) to locate similar sequences within the Protein Data Bank (PDB). The 5-10 sequences selected for further analysis were carefully evaluated based on several factors, including query coverage, percentage identity and E-value. Three-dimensional X-ray crystallographic structures of AChE (7XN1), D3 receptor (3PBL), GABA (B) receptor (4MQF) and GluA2 receptor (2XHD) were retrieved from the PDB) using their respective accession numbers. Thorough evaluations were conducted to ensure the integrity of these structures, including examination of resolution, mutations, World Wide Protein Data Bank validation, compatibility with co-crystal ligands and analysis of the Ramachandran plot.

The PDB sum server was used to select the binding pockets of the target proteins to prepare molecules for docking. Missing residues were added to the protein structure and side chains were generated using CHIMERA v1.16. To ensure structural integrity and the protein's readiness for docking, 1000 steepest descent steps were performed with a descent size of 0.1 Å, followed by 100 conjugate gradient steps with a conjugate gradient size of 0.1 Å. Hydrogen was added to simulate the behavior of proteins in an aqueous environment and the protonation states were adjusted. Standard and nonstandard residues were treated using AMBER ff14SB and AM1-BCC force fields, respectively. Nonstandard residues were removed from the protein structure using the Biovia Discovery Studio visualizer V21.1.0.20298.

Grid generation

To identify the docking regions of target proteins, including AChE (7XN1), D3 receptor (3PBL), GABA (B) receptor (4MQF) and GluA2 receptor (2XHD), AutoDockTools, Chimera and Maestro software were utilized. To ensure high precision in the docking process, the precise volume for the docking grid was calculated by determining the dimensions of the protein pockets based on data retrieved from the CASTp (Computed Atlas of Surface Topography of proteins) server.

Ligands preparation

The 2D and 3D structures of the ligands were refined with precision in the ligand preparation process using the MarvinSketch software, version 21.13. This was performed to ensure accuracy and consistency and the refined structures were saved in the 3D mol2 file format.

Molecular docking of target protein with ligands

All biomolecules were converted to pdbqt format to perform docking using AutoDock 1.5.6 and ADFRsuit for ligands and proteins, respectively. Rotatable bonds were allowed to rotate freely for ligands, whereas the receptor proteins remained rigid. Autodock Vina 1.2.3 was used for docking studies with a specified grid spacing of 0.375 Å. The grid box was positioned to facilitate the identification of potential interaction sites between the ligands and the target protein's active site (Table 2), thereby allowing the program to conduct a thorough search. The XYZ coordinates, as specified in (Table 3), CPU set at 23, exhaustiveness of 32, nine modes and energy range of 3, were carefully configured. To validate the results, re-docking was performed using the same configuration as the initial docking. Autodock Vina analysis results in generated molecular complexes in the Biovia Discovery Studio visualizer. Visualization of these complexes, which included both 2D and 3D representations, was accomplished using Maestro 12.3 (academic edition).

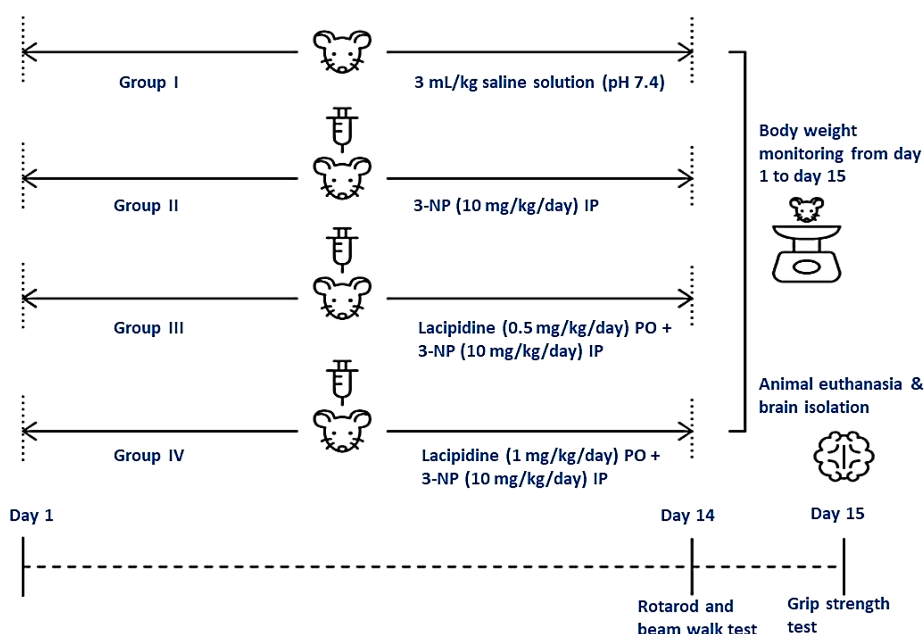

Figure 1: Summary of experimental layout outlining research methods and processes.

Statistical Analysis

The experiment data was statistically analyzed using GraphPad PRISM (version 8.0.2). Unless otherwise stated, all values are presented as Mean±Standard Deviation (SD). Shapiro-Wilk tests were used to determine the normality of numerical variables. Behavioural and biochemical assessments involved one-way Analysis of Variance (ANOVA) in assessing overall differences, followed by Tukey's *posthoc* test to compare the control and treatment groups. The threshold for statistical significance was established at $p<0.05$.

RESULTS

Body weight measurement

During the experiment, it was observed that the group administered with 3-NPA (Disease Group) showed a notable depletion in body weight compared to the control group. However, both doses of lacidipine led to a notable increase in body weight among the experimental subjects of both treatment groups [F (3, 20)=5.261, (P=0.0167)] (Figure 2).

Behavioural assessments

Rotarod test

The rotarod test provided insight into the motor coordination deficits caused by 3-NPA. The disease control group showed a significant decrease in the time taken to fall from the rotating rod compared with the normal control group, indicating impaired motor coordination due to 3-NPA exposure. Administration of both doses of lacidipine (0.5 mg/kg and 1 mg/kg) resulted in improved rotarod performance [F (3, 20)=21.76, ($p<0.0001$)] (Figure 3A).

Narrow Beam Walk Test

The narrow-beam walk test revealed impairments caused by 3-NPA, with the disease control group exhibiting extended traversal times across the narrow beam. Conversely, animals treated with both doses of lacidipine (0.5 mg/kg and 1 mg/kg) displayed reduced traversal times, indicating improved sensorimotor coordination [F (3, 20)=13.94, ($p<0.0001$)] (Figure 3B).

Grip strength test

The grip test was used to indicate muscular strength and neuromuscular efficiency. Animals in the disease control group showed reduced grip strength due to 3 NPA administration. However, treatment with both doses of lacidipine resulted in a significant regain of grip strength. The higher dose (1.0 mg/kg) of lacidipine demonstrated more pronounced improvement in grip strength than the lower dose (0.5 mg/kg) [F (3, 20)=31.96, ($p<0.0001$)] (Figure 3C).

Neurochemical assessmentsAChE estimation

As a result of 3-NPA exposure, increased AChE activity was evident in the disease group collated with the control group. Both doses of lacidipine significantly reversed the effect of 3-NPA, which corresponded to diminished AChE activity. However, when comparing the two lacidipine treatment groups, it became

Table 2: The active sites amino acids.

Protein ID	The active sites amino acids
7XN1	ASP74, TRP86, GLY121, GLU202, TYR337, TYR341, HIS447, GLY448
BPBL	ASP110, VAL111, CYS114, ILE183, VAL189, SER192, PHE345, PHE346, HIS349, THR369, TYR373
4MQF	PRO105, GLY106, THR109, SER131, THR134, LEU135, GLU138, ALA139, ALA156, ASN159, ARG162, GLN196, GLN197, THR198, THR199, GLU200, THR203, SER204, ASP207, ARG223, GLN224, SER225, PHE226, PHE227, SER228, ASP229, ALA231, VAL232, PRO233, ASN236, ARG239, GLN240, ALA69, LYS70, SER72, ILE73, ASN110, ALA111, LEU114, VAL134, PRO136, SER137, SER140, ILE141, GLU144, ALA157, ALA158, THR159, THR160, PRO161, VAL162, ALA164, ASP165, LYS166, LYS167, LYS168, TYR169, ARG174, VAL176, PRO177, SER178, ASP179, ASN180, ALA181, VAL182, ASN183, PRO184, ILE186, THR200, THR202, ASP204, VAL205, GLN206, ARG207, PHE208, SER209, GLU210, VAL211, ARG212, ASN213, ASP214, LEU215, THR216, VAL218, TYR220, SER227, THR229, GLU230, SER231, SER233, SER239, LYS242, ASN246, LEU252, GLY253, GLN254, GLN257, ILE280, ILE281, PRO282, GLY283, TRP284, TYR285, GLU286, PRO287, VAL317, ASP318, PHE319, PRO321, LEU322, GLU339, TYR342, ASN343, GLY350, PRO351, SER352, LYS353, PHE354, HIS355, ARG418, GLU421, ARG422, MET423, GLY424, ILE426, LYS427, TYR442, ALA444
2XHD	TYR35, GLY62, ALA63, ARG64, ASP65, ALA66, TRP71, ASN72, THR91, ILE92, THR93, LEU94, VAL95, ARG96, GLU98, VAL99, LYS104, PRO105, PHE106, MET107, SER108, ASP139, SER140, GLY141, SER142, LYS144, GLU145, PHE146, ARG148, ARG149, SER150, LYS151, ILE152, PHE155, GLN202, LEU215, ASP216, SER217, LYS218, GLY219, VAL238, LEU239, SER242, GLU243, GLN244, GLY245, LEU247, ASP248, LYS251, ASN252, TYR256, GLY259, CYS261, GLY262, ALA263, ILE113, GLU122, SER123, ALA124, GLU125, ASP126, VAL154, VAL211, GLY212, GLY213, ASN214

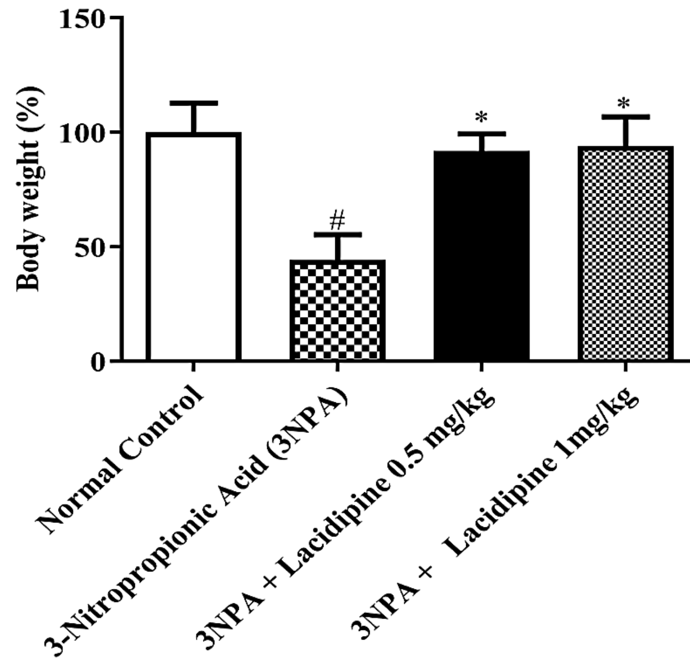

Figure 2: Impact of lacidipine on body weight in 3-nitropropionic acid-induced Huntington's Disease in rats. One ANOVA followed by Tukey's test *post hoc* test (n=6). #p<0.001 vs. normal control, *p<0.05 *Vs* 3-nitropropionic acid group.

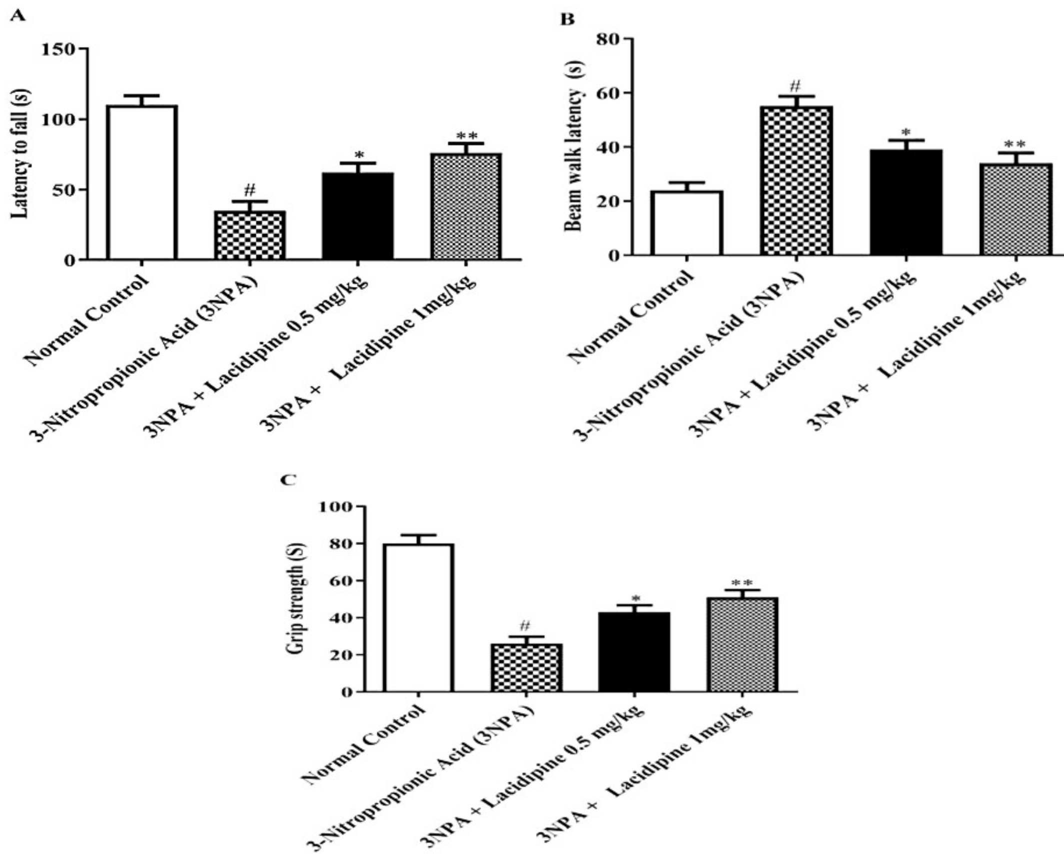

Figure 3: Impact of lacidipine on A) Rotarod test, B) Narrow-beam walk test, C) Grip test of animals mimicking Huntington's Disease triggered by 3-nitropropionic acid administration. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, *p<0.05, ** p<0.001 *Vs* 3-nitropropionic acid group.

apparent that the higher dose (1.0 mg/kg) exerted a more pronounced effect in reducing AChE activity than the dose of 0.5 mg/kg [F (3, 20)=17.41, (p<0.0001)] (Figure 4A).

GABA, DA and glutamate estimation

The altered levels of GABA, DA and glutamate revealed neurotransmitter imbalances due to 3-NPA exposure. In the disease group, elevated levels of glutamate and DA were evident, whereas decreased GABA activity was observed compared to the control group. Both lacidipine doses (0.5 mg/kg and 1 mg/kg) effectively restored the expected levels of these neurotransmitters i.e., GABA [F (3, 20)=8.839, (p<0.0001)], DA [F (3, 20)=24.63, (p<0.0001)] and glutamate [F (3, 20)=25.84, (p<0.0001)] (Figures 4 B-D).

Biochemical assessments

LDH estimation

An increase in LDH activity was observed in the disease control group due to 3-NPA exposure, compared to that in the control group. Treatment with both doses of lacidipine (0.5 mg/kg and 1 mg/kg) led to a significant decline in LDH levels [F (3, 20)=14.57, (p<0.0001)] (Figure 5A).

MDA estimation

Elevated MDA levels were observed in the 3-NPA-exposed subjects, in contrast to the control group. Animals treated with both high and low doses of lacidipine (0.5 and 1 mg/kg) exhibited diminished MDA levels, thereby reversing the impact of 3-NPA exposure significantly [F (3, 20)=38.00, (p<0.0001)] (Figure 5B).

SOD estimation

Administration of 3-NPA caused a marked decrease (p<0.001) in brain SOD activity in the disease group compared with that in the control group. On the contrary, treatment with lacidipine (at doses of 0.5 and 1 mg/kg) in rats subjected to 3-NPA noticeably (p<0.001) increased the levels of SOD compared to rats that were only administered 3-NPA [F (3, 20)=13.31, (p<0.0001)] (Figure 5C).

GSH estimation

A notable depletion in GSH levels was observed in the diseased group (3-NPA) compared to the control group. However, both doses of lacidipine effectively counteracted the adverse effects of 3-NPA, resulting in a significant increase in GSH levels. Notably, when comparing the two lacidipine treatment groups, the higher dose (1.0 mg/kg) resulted in a more noticeable impact in elevating GSH levels than the lower dose (0.5 mg/kg) [F (3, 20)=13.16, (p<0.0001)] (Figure 5D).

Neuroinflammatory markers assessments

A notable elevation in the concentration of neuroinflammatory biomarkers, specifically IL-6, IL-1β and TNF-α was conspicuously discerned in rats subjected to 3-NPA treatment, in contrast to the control group. Lacidipine at both dose concentrations (0.5 and 1 mg/kg) exhibited a robust and statistically significant attenuation of the 3-NPA-induced upregulation of IL-6 [F (3, 20)=29.52, (p<0.0001)], IL-1β [F (3, 20)=30.91, (p<0.0001)] and TNF-α [F (3, 20)=28.18, (p<0.0001)] inflammatory mediators (Figures 6A-C). Additionally, a noteworthy hike in NF-κB expression levels was observed in the disease group exposed to 3-NPA relative to the control group. In contrast, the lacidipine treatment groups (0.5 and 1 mg/kg) demonstrated a significant reduction in NF-κB activity [F (3, 20)=10.58, (p<0.0001)] (Figure 6D).

Molecular Docking

The docking analysis of lacidipine with the target proteins yielded valuable information for drug discovery and bioactivity assessments. The results of the molecular docking study indicated that lacidipine has a significant binding affinity with all selected targets, suggesting a multitargeted approach (Tables 1-4). We evaluated the validity of these structures using resolution, mutation presence, wwPDB validation, co-crystal ligand compatibility and Ramachandran plot analysis (Figures 7 A-D). Lacidipine shows a favourable negative binding energy to the GluA2 receptor with docking scores of -7.855 kcal/mol, which suggests that the drug has the strongest affinity for the glutamate receptor. Target GluA2 receptor (2XHD) exhibits hydrophobic interactions with LEU247B at 3.87 Å, ASP248B at 3.96 Å and LYS251B at 3.94 Å. Hydrogen bonds are observed with SER108B at 1.64 Å, ASN214A at 2.51 Å and ASP248B at 2.29 Å. This suggests that the affinity of lacidipine is primarily maintained through hydrogen bonding, while the other targets are held together through hydrophobic interactions.

Lacidipine demonstrates a binding energy of -6.949 kcal/mol for AChE (7XN1). The intermolecular interactions primarily involve hydrophobic interactions with several amino acid residues:

Table 3: Grid parameters for docking study.

Sl. No.	Protein Id	Centre Coordinates			Size Coordinates		
		x	y	z	x	y	z
1	7XN1	48.32	-40.0	-30.0	40	40	40
2	BPBL	0.09	-14.83	10.43	25	25	25
3	4MQF	-48.64	21.41	-26.17	20	20	20
4	2XHD	30.6	44.4	18.81	20	20	20

Table 4: Docking Score and intermolecular interactions of ligands against AChE (7XN1), D3 receptor (3PBL), GABA (B) receptor (4MQF) and GluA2 receptor (2XHD).

Sl. No.	Molecule	Binding Energy	Type of Interaction	Residue ID	Distance
1	7XN1_LACIDIPINE	-6.949	Hydrophobic Interactions	TYR72A	3.9
				TYR124A	3.99
				TRP286A	3.83
				TRP286A	3.93
				TRP286A	3.68
				TRP286A	3.67
				LEU289A	3.83
				PHE297A	3.52
				TYR337A	3.62
				PHE338A	3.87
				TYR341A	3.83
				TYR341A	3.72
				TYR341A	3.79
			Hydrogen Bonds	TYR124A	2
2	3PBL_LACIDIPINE	-7.607	Hydrophobic Interactions	VAL86A	3.91
				LEU89A	3.52
				GLU90A	3.63
				PHE106A	3.96
				VAL180A	3.46
				PRO362A	3.72
				TYR365A	3.64
				TYR365A	4
				TYR365A	3.55
			Hydrogen Bonds	ASN352A	2.55
			Salt Bridges	HIS349A	5.49
3	4MQF_LACIDIPINE	-7.523	Hydrophobic Interactions	TRP65A	3.79
				VAL201A	3.67
				PHE202A	3.45
				TYR250A	3.75
				TRP278A	3.8
				TRP278A	3.72
			Hydrogen Bonds	TRP278A	2.7
			π-Stacking	TRP65A	4.87
4	2XHD_LACIDIPINE	-7.855	Hydrophobic Interactions	LEU247B	3.87
				ASP248B	3.96
				LYS251B	3.94
			Hydrogen Bonds	SER108B	1.64
				ASN214A	2.51
				ASP248B	2.29

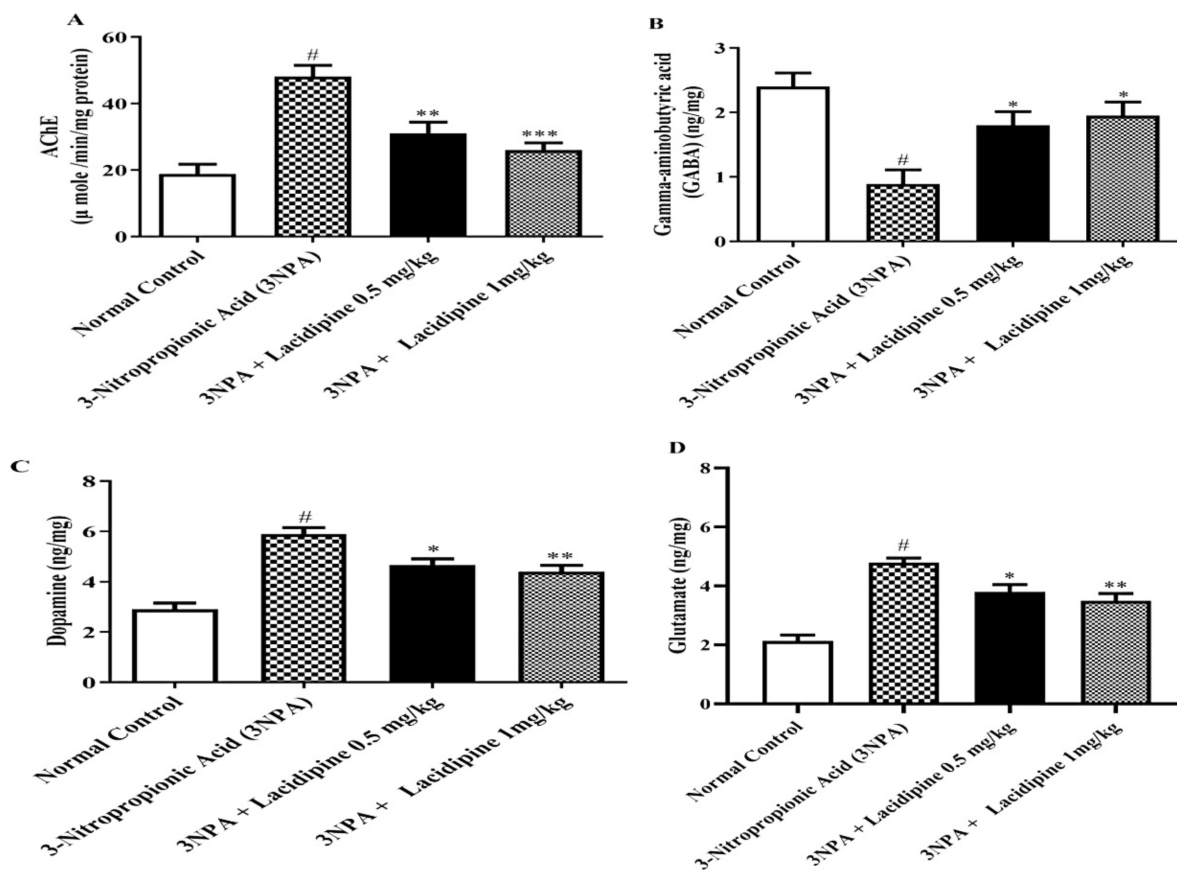

Figure 4: Impact of lacidipine on the activity of A) Acetylcholinesterase, B) GABA, C) Dopamine and D) Glutamate of animals mimicking Huntington's Disease triggered by 3-nitropropionic acid administration. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, *p<0.05, **p<0.001, ***p<0.0001 *Vs* 3-nitropropionic acid group.

TYR72A at 3.9 Å, TYR124A at 3.99 Å and TRP286A at multiple distances (3.83, 3.93, 3.68, 3.67 Å), LEU289A at 3.83 Å, PHE297A at 3.52 Å, TYR337A at 3.62 Å, PHE338A at 3.87 Å and TYR341A at various distances (3.83, 3.72, 3.79 Å). Additionally, a hydrogen bond is formed with TYR124A at 2.0 Å.

Lacidipine shows a binding energy of -7.607 kcal/mol for the D3 receptor (3PBL). Hydrophobic interactions are observed with VAL86A at 3.91 Å, LEU89A at 3.52 Å, GLU90A at 3.63 Å, PHE106A at 3.96 Å, VAL180A at 3.46 Å, PRO362A at 3.72 Å and TYR365A at various distances (3.64, 4.00, 3.55 Å). A hydrogen bond is formed with ASN352A at 2.55 Å and a salt bridge is present with HIS349A at 5.49 Å. Furthermore, lacidipine displays a binding energy of -7.523 kcal/mol for the GABA (B) receptor (4MQF). Hydrophobic interactions occur with TRP65A at 3.79 Å, VAL201A at 3.67 Å, PHE202A at 3.45 Å, TYR250A at 3.75 Å and TRP278A at multiple distances (3.80, 3.72 Å). There is also a hydrogen bond with TRP278A at 2.70 Å and a π-stacking interaction with TRP65A at 4.87 Å (Figures 8 A-D).

DISCUSSION

In this study, we analyzed the impact of lacidipine on body weight, behavioral performance (rotarod, narrow-beam walk and grip strength), neurochemical levels (AChE, GABA, DA, glutamate), biochemical markers (LDH, MDA, SOD and GSH) and inflammatory molecules (IL-6, IL-1β, TNF-α and NF-κB) in a rat model with 3-NPA neurotoxicity. 3-NP, a mycotoxin, is widely recognized as a mitochondrial complex II enzyme inhibitor. This inhibition leads to disruption of the mitochondrial electron transport chain system, resulting in energy deficiency. Consequently, it has been observed to induce a range of cognitive and motor-related abnormalities, affecting both behavioral and biochemical processes linked to memory and motor function.[39] Our study revealed that 3-NPA prompted a marked decline in the body weight of rats. Typically, individuals suffering from HD commonly encounter substantial unintentional weight loss, which is likely due to metabolic defects and disturbances in cellular energy generation.[41] The results of our study demonstrated a notable decline in body weight among subjects subjected to 3-NPA treatment. Notably, the administration of lacidipine at both concentrations led to significant restoration of body weight in the experimental animals. Symptoms of reduced motor movement are predominantly observed in the advanced stages of HD patients. These motor issues resemble the dysfunction seen in rats intoxicated by injecting them with 3-NPA.[42] Similarly, our findings of behavioral experiments revealed that 3-NPA induced significant disruptions in motor performance, gait and grip strength of rodents, which were assessed by rotarod test,

narrow beam walking test and grip strength assessment. These motor abnormalities are postulated to be caused by an excessive elevation of DA levels in the striatum, which may be attributed to the impairment of GABAergic neurons within the striatum responsible for regulating overall striatal output. In both tested treatment regimens, lacidipine resulted in a marked enhancement in behavioural abnormalities collated with animals solely treated with 3-NPA. HD is primarily linked to altered neurotransmitters, including AChE, within the cholinergic nervous mechanism, resulting in memory and psychical disruptions.[43] Elevated AChE

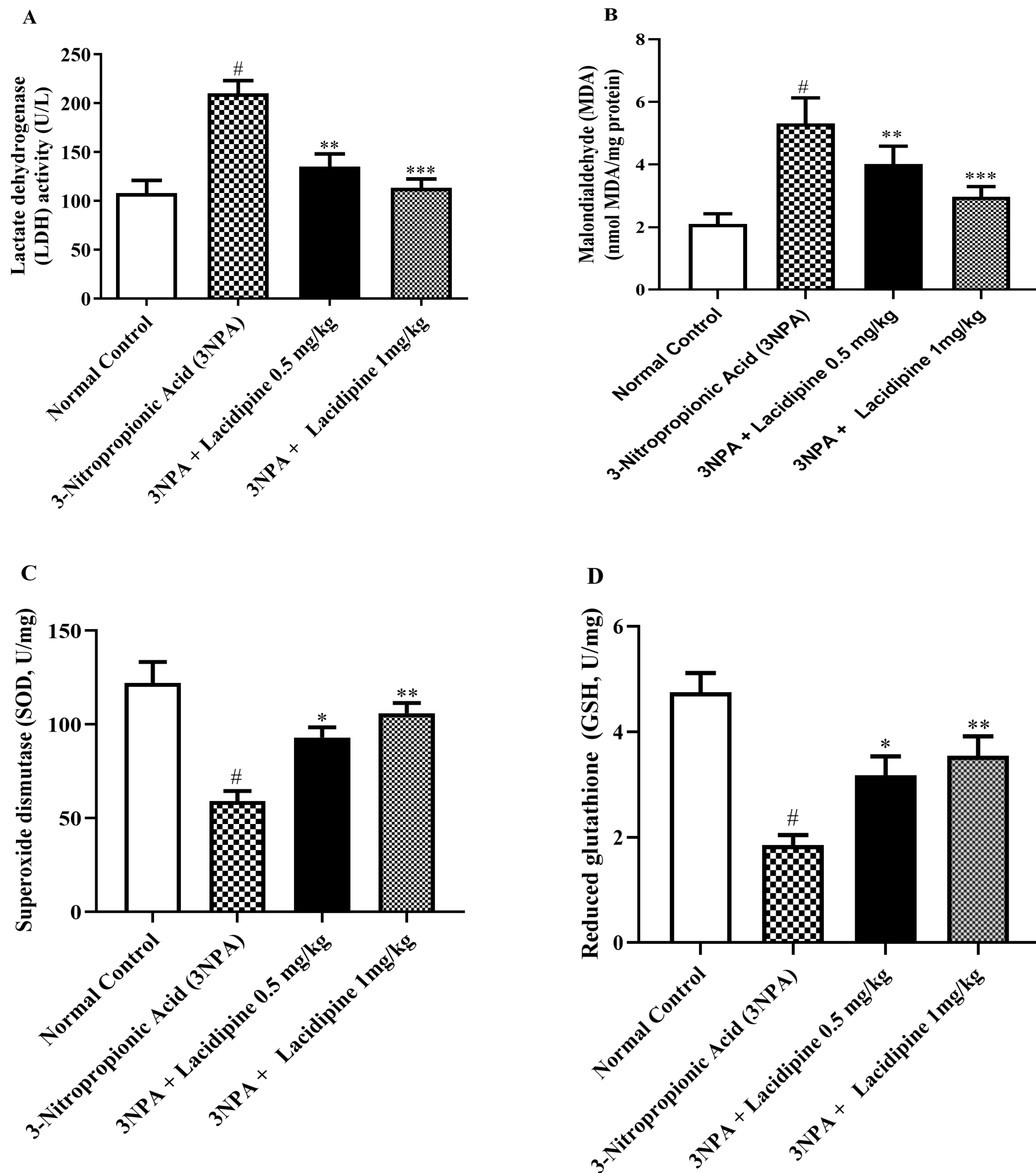

Figure 5: Impact of lacidipine on the activity of A) LDH, B) MDA, C) SOD, D) GSH of animals mimicking Huntington`s Disease triggered by 3-nitropropionic acid administration. One ANOVA followed by Tukey's test *post hoc* (n=6). #p<0.001 vs. normal control, *p<0.05, **p<0.001, ***p<0.0001 *Vs* 3-nitropropionic acid group.

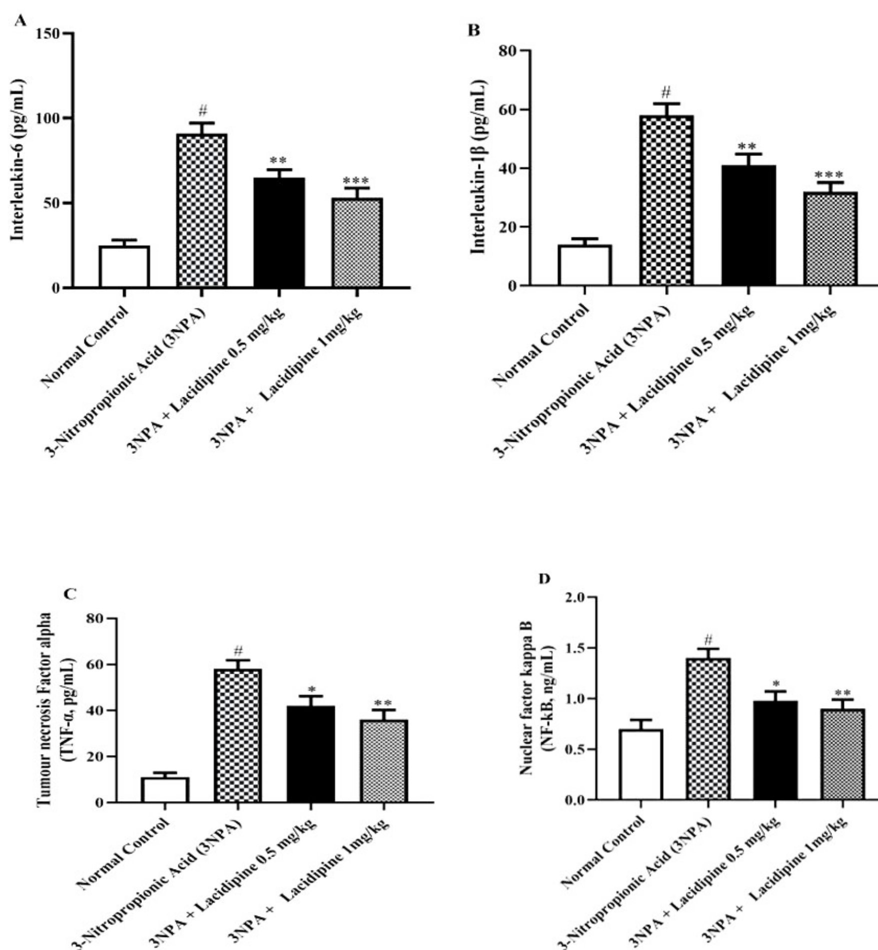

Figure 6: Impact of lacidipine on the activity of neuroinflammatory markers A) IL-6, B) IL-1β, C) TNF-α, D) NF-κB of animals mimicking Huntington's Disease triggered by 3-nitropropionic acid administration. One ANOVA followed by Tukey's test *post hoc* (*n*=6). #*p*<0.001 vs. normal control, * *p*<0.05, ** *p*<0.001, *** *p*<0.0001 Vs control; # *p*<0.001 Vs 3-nitropropionic acid group.

levels contribute to diminishing cholinergic innervation and are intricately associated with a spectrum of neurodegenerative disorders. The inhibition of AChE leads to increased acetylcholine availability, thereby promoting the enhancement of cholinergic function. AChE plays a vital role in the progression of neurodegenerative conditions, impacting diverse processes, including inflammation, apoptotic responses, oxidative stress and the accumulation of pathological substances.[44] In this research, the exposure of 3-NPA led to a marked elevation in AChE levels in experimental animals, consistent with findings from previous studies. In this investigation, both concentrations of lacidipine effectively restored AChE levels to a normal state compared to the group subjected to 3-NPA treatment. Previous studies have documented that a decrease in striatal SDH levels effectively halts the progression of HD. Nevertheless, the administration of 3-NPA increased LDH concentrations.[47,48] In our investigation, the administration of 3-NPA to the experimental animal subjects resulted in an elevation of LDH concentrations collated with the control group. However, administering low and high doses of lacidipine effectively ameliorated the LDH concentrations. A previous investigation documented that 3-NPA is associated with elevated glutamate levels and reduced GABA concentrations in striatal and cortical regions. Furthermore, modifications in glutamatergic input and GABAergic output of medium spiny neurons in the striatum have been shown to intensify their vulnerability to the detrimental effects of 3-NPA. Consequently, this exacerbates impairments in both motor and cognitive functions.[37,49] Hence, GABA and glutamate have been recognized as crucial elements in developing HD. In the current study, the application of lacidipine mitigated the excitotoxic effects induced by 3-NPA through a dual mechanism, encompassing a reduction in glutamate activity and a hike in GABA expression in the brain. Previously, it was documented that the pathomorphological changes induced by 3-NPA in the caudate nuclei result from damage to the dopaminergic pathway, which disrupts dopamine regulation. Furthermore, the toxicity of 3-NPA and the disruption of dopamine catabolism in the striatum lead to the breakdown of astrocytes. Dysregulation of the dopaminergic system in HD amplifies neuronal excitability by augmenting glutamatergic transmission, ultimately exacerbating neuronal death.[52,53] The outputs of our study demonstrated that the exposure to 3-NPA led to a depletion in dopamine activity collated in the

control group. Nonetheless, treatment with lacidipine notably counteracted the deleterious effects of 3-NPA on dopamine activity. Previous studies have established that 3-NPA toxicity induces oxidative stress through an increase in MDA levels and disrupts the antioxidant defence system by depleting the activities of SOD, GSH and CAT.[54,55] Consistently, the findings indicate that 3-NPA fosters oxidative stress, as supported by elevated MDA levels in subjects administered 3-NPA conversely to the control group. Concurrently, the expression of SOD, GSH and CAT were diminished in animals receiving 3-NPA compared with those in the control group. Notably, the test drug lacidipine effectively mitigated the toxic effects of 3-NPA, restoring normal levels of MDA, SOD, GSH and CAT activities. Aggregation of reactive microglia in the brain is associated with the development of HD. Notably, activated microglia have been identified in asymptomatic individuals before the onset of HD symptoms, along with increased levels of neuroinflammatory substances, such as IL-6. Elevated activities of pro-inflammatory mediators, notably IL-6 and TNF, have been documented in the plasma and striatal tissues of individuals affected by HD. Moreover, a prior study reported atypical NF-κB activation in astrocytes in rodents and patients with HD, indicating a potential mechanism of astrocytic inflammation in HD. Prior research has demonstrated that the administration of 3-NPA leads to an elevation in the levels of pro-inflammatory molecules, including TNF-α, IL-1β and IL-6, as well as the activation of the NF-κB pathway, within the striatal region. Consistent with previous research, our results revealed a significant increase in TNF-α, IL-1β, IL-6 and NF-κB levels in animals injected with 3-NPA collated with the control group. However, lacidipine at both concentrations significantly mitigated the toxicity and restored the inflammatory molecules to their normal levels.

Previous molecular docking studies have suggested that a low ligand-receptor binding energy is indicative of a favourable interaction between the ligand and its target, whereas a high binding energy suggests a weaker interaction between the ligand and the target. Molecular docking analysis showed that lacidipine displayed binding to all target molecules, including AChE (7XN1),

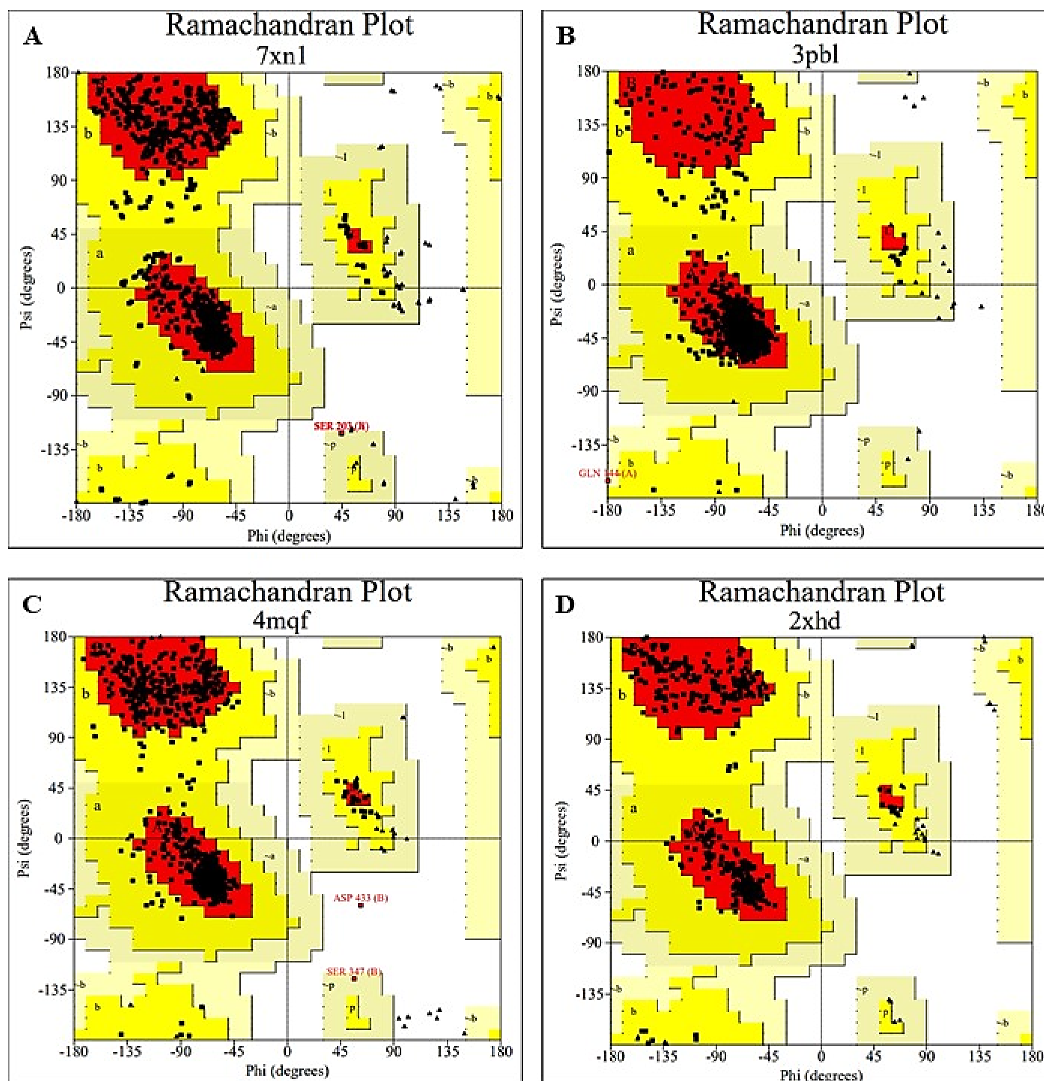

Figure 7: Ramachandran Plot obtained from PROCHECK server A) AChE, B) Dopamine, C) GABA and D) Glutamate.

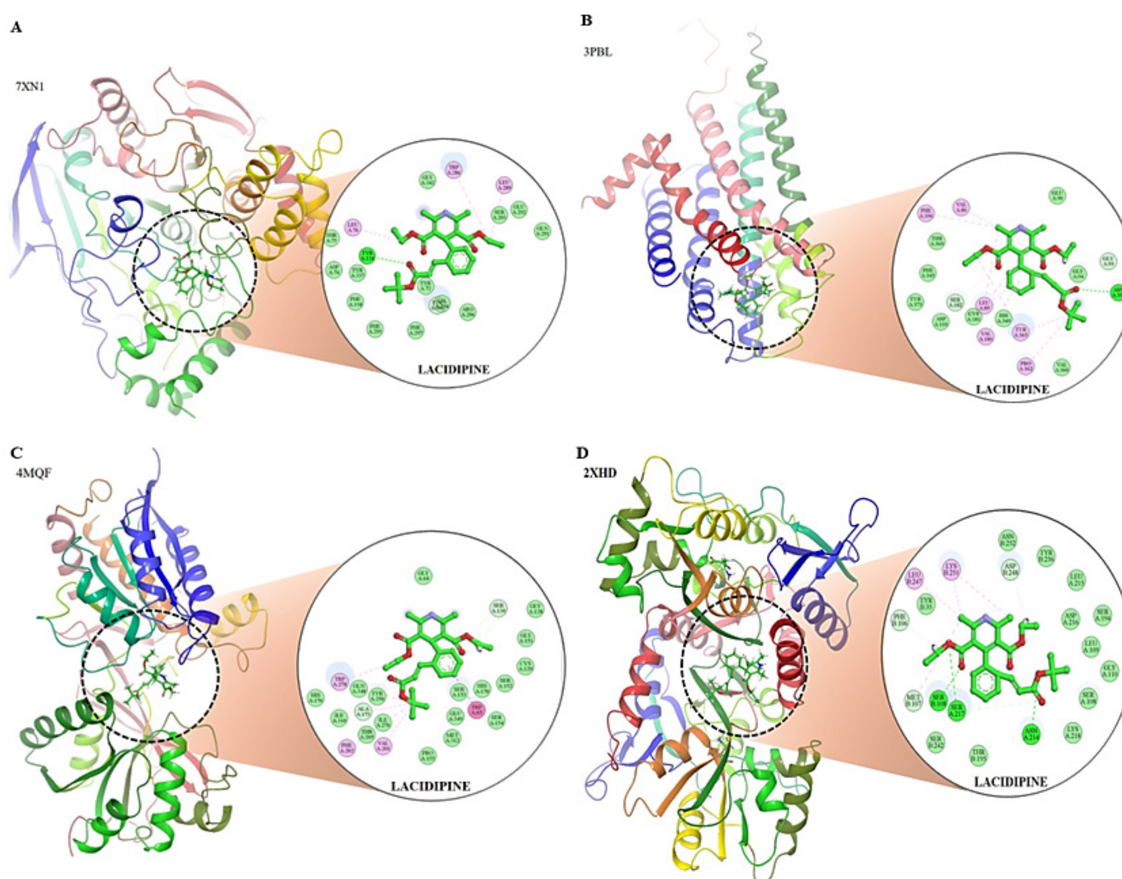

Figure 8: Docked conformation of ligand lacidipine with target proteins visualized using Maestro V12.8 and Biovia Discovery Studio A) AChE (7XN1), B) D3 receptor (3PBL), C) GABA (B) receptor (4MQF) and D) GluA2 receptor (2XHD).

D3 receptor (3PBL), GABA (B) receptor (4MQF) and GluA2 receptor (2XHD). Among these molecules, lacidipine showed a negative binding energy to the GluA2 receptor with docking scores of -7.855 kcal/mol. Previous research has emphasized the significance of hydrogen bonds in determining the strength of the interactions between molecules at their target sites. This implies that the presence of more hydrogen bonds can lead to higher binding efficiency.[61] In our research, we characterized the binding interaction between glutamate (GluA2) and lacidipine by observing the formation of three hydrogen bonds, which highlights the strong affinity between these two molecules compared to other molecules that only formed one hydrogen bond. The results of these studies suggest that lacidipine may be a potential therapeutic option for targeting glutamate.

Our study on the effect of lacidipine on 3-NPA induced HD in a murine model showed parallel results when compared with previous similar studies. The estimated oxidative stress parameters (GSH, SOD and MDA) and neuroinflammatory markers (TNF-α and IL-1β) are consistent with those previously reported. Moreover, the previous study assessed mitochondrial enzyme activities, neurobehavioral tests and oxidative stress markers and showed similar results to our study.[13] Additionally, a similar study evaluating neurotransmitters (AChE, GABA, glutamate and DA) and inflammatory mediators (IL-6, IL-1β, TNF-α and NF-κB) supports the findings of our study.[13,62]

Our research indicates that lacidipine exerts neuroprotective effects in a 3-NPA model of HD through several interrelated mechanisms. Lacidipine reduces oxidative stress by decreasing MDA levels and increasing antioxidant enzyme activity (SOD and GSH). Additionally, it exhibits anti-inflammatory effects by lowering pro-inflammatory cytokines (IL-6, IL-1β and TNF-α) and inhibiting NF-κB activation, which likely prevents further neuronal damage and supports neural repair mechanisms. Lacidipine also normalizes neurotransmitter levels by decreasing glutamate and increasing GABA concentrations while improving dopamine levels. This regulation helps counteract the excitotoxicity and neurotransmitter imbalance characteristics of HD. Molecular docking studies demonstrated a strong binding affinity of lacidipine to Glutamate (GluA2), suggesting direct inhibition of excitotoxic pathways. These combined actions of lacidipine help preserve neuronal function, reduce neuroinflammation and support overall cellular energy homeostasis.

However, our study has limitations, first, we primarily focused on biochemical and behavioral outcomes without investigating detailed molecular mechanisms, including gene expression and protein pathways. Second, the 14-day treatment period

may not fully capture lacidipine long-term and potential side effects, warranting further studies. Third, we utilized a single animal model (Wistar rats) and a specific Neurotoxin (3-NPA) to induce HD-like symptoms, which may not fully replicate human HD complexities. Hence, further research is necessary to elucidate this mechanism, including investigations of molecular mechanisms, western blotting and histopathological studies. Moreover, the utilization of genetic models such as transgenic HD mouse models could provide more comprehensive insights into the genetic underpinnings of this neurodegenerative process. Exploring additional neurotoxin-based models in conjunction with genetic studies could validate the therapeutic potential of lacidipine in various experimental paradigms. Furthermore, examining the long-term effects through genomic, proteomic and metabolomic analyses will be crucial to fully elucidate the role of lacidipine in neuroprotection and disease modification.

CONCLUSION

Our study showed that lacidipine could alleviate memory deficits and reinstate cognitive function by normalizing antioxidant and anti-inflammatory processes. It effectively reversed the neurotransmitter imbalances induced by 3-NPA and reduced the markers of oxidative damage and neuroinflammation. These results indicate that lacidipine possesses therapeutic qualities that mitigate the neurotoxic effects of 3-NPA and enhance cognitive function. Further investigations are required to unlock its complete therapeutic potential, unveil the molecular mechanisms underlying its neuroprotective properties and optimize its application in the management of 3-NPA-induced neurotoxicity.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

3-NPA: 3-Nitropropionic acid; **AChE:** Acetylcholinesterase; **ANOVA:** Analysis of variance; **DA:** Dopamine; **ELISA:** Enzyme-linked immunosorbent assay; **GABA:** Gamma-Aminobutyric Acid; **GSH:** Glutathione; **HD:** Huntington's disease; **IL-1β:** Interleukin-1β; IL-6: Interleukin-6; **LDH:** Lactate dehydrogenase; **MDA:** Malondialdehyde; **NCBI:** National Center for Biotechnology Information; **NO:** Nitric oxide; **NF-kB:** Nuclear factor kappa-B; **PDB:** Protein Data Bank; **SD:** Standard deviation; **SOD:** Superoxide dismutase; **TNF-α:** Tumor necrosis factor-α.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study protocol received approval from the LNCP Institutional Animal Ethics Committee, India (LNCP/IAEC/2023/005) and adhered to the guidelines set by the Committee for The Control and Supervision of Experimental Animals (CCSEA).

SUMMARY

This study investigates lacidipine, a calcium channel blocker with antioxidant and anti-inflammatory properties, as a potential treatment for HD. Using a rat paradigm with 3-Nitropropionic Acid (3-NPA)-induced neurodegeneration, it was found that lacidipine improved motor function, corrected neurotransmitter imbalances, reduced oxidative stress and lowered neuroinflammation. Docking studies suggested that lacidipine binds effectively to the glutamate receptor. These results indicate that lacidipine may be a promising candidate for treating neurodegenerative conditions like HD.

REFERENCES

1. Tasset I, Pontes AJ, Hinojosa AJ, De la Torre R, Túnez I. Olive oil reduces oxidative damage in a 3-nitropropionic acid-induced Huntington's disease-like rat model. Nutr Neurosci. 2011; 14(3): 106-11. doi: 10.1179/1476830511Y.0000000005, PMID 21756531.
2. Ramachandran S, Thangarajan S. Thymoquinone loaded solid lipid nanoparticles counteracts 3-nitropropionic acid induced motor impairments and neuroinflammation in rat model of Huntington's disease. Metab Brain Dis. 2018; 33(5): 1459-70. doi: 10.1007/s11011-018-0252-0, PMID 29855977.
3. Van Raamsdonk JM, Pearson J, Slow EJ, Hossain SM, Leavitt BR, Hayden MR. Cognitive dysfunction precedes neuropathology and motor abnormalities in the YAC128 mouse model of Huntington's disease. J Neurosci. 2005; 25(16): 4169-80. doi: 10.152 3/JNEUROSCI.0590-05.2005, PMID 15843620.
4. Wenceslau CV, de Souza DM, Mambelli-Lisboa NC, Ynoue LH, Araldi RP, da Silva JM, *et al*. Restoration of BDNF, DARPP32 and D2R expression following intravenous infusion of human immature dental pulp stem cells in Huntington's disease 3-NP rat model. Cells. 2022; 11(10): 1664. doi: 10.3390/cells11101664, PMID 35626701.
5. Chakraborty J, Singh R, Dutta D, Naskar A, Rajamma U, Mohanakumar KP. Quercetin improves behavioral deficiencies, restores astrocytes and microglia and reduces serotonin metabolism in 3-nitropropionic acid-induced rat model of Huntington's disease. CNS Neurosci Ther. 2014; 20(1): 10-9. doi: 10.1111/cns.12189, PMID 24188794.
6. Rawlins MD, Wexler NS, Wexler AR, Tabrizi SJ, Douglas I, Evans SJ, *et al*. The prevalence of Huntington's disease. Neuroepidemiology. 2016; 46(2): 144-53. doi: 10.1159/0004 43738, PMID 26824438.
7. Túnez I, Tasset I, Pérez-De La Cruz V, Santamaría A. 3-nitropropionic acid as a tool to study the mechanisms involved in Huntington's disease: past, present and future. Molecules. 2010; 15(2): 878-916. doi: 10.3390/molecules15020878, PMID 20335954.
8. Geddes JW, Bondada V, Pang Z. Mechanisms of 3-nitropropionic acid neurotoxicity. In: Sanberg PR, Nishino H, Borlongan CV, editors. Mitochondrial inhibitors and neurodegenerative disorders. Totowa, NJ: Humana Press; 2000. p. 107-20. doi: 10.1 007/978-1-59259-692-8_7.
9. Khan A, Jamwal S, Bijjem KR, Prakash A, Kumar P. Neuroprotective effect of hemeoxygenase-1/glycogen synthase kinase-3β modulators in 3-nitropropionic acid-induced neurotoxicity in rats. Neuroscience. 2015; 287: 66-77. doi: 10.1016/j.n euroscience.2014.12.018, PMID 25536048.
10. Ranganayaki S, Jamshidi N, Aiyaz M, Rashmi SK, Gayathri N, Harsha PK, *et al*. Inhibition of mitochondrial complex II in neuronal cells triggers unique pathways culminating in autophagy with implications for neurodegeneration. Sci Rep. 2021; 11(1): 1483. doi: 10.1038/s41598-020-79339-2, PMID 33452321.
11. Wiprich MT, Zanandrea R, Altenhofen S, Bonan CD. Influence of 3-nitropropionic acid on physiological and behavioral responses in zebrafish larvae and adults. Comp Biochem Physiol C Toxicol Pharmacol. 2020; 234: 108772. doi: 10.1016/j.cbpc.2020.1 08772, PMID 32353558.
12. Shalaby HN, El-Tanbouly DM, Zaki HF. Topiramate mitigates 3-nitropropionic acid-induced striatal neurotoxicity via modulation of AMPA receptors. Food Chem Toxicol. 2018; 118: 227-34. doi: 10.1016/j.fct.2018.05.022, PMID 29753867.

13. Bin-Jumah MN, Gilani SJ, Alabbasi AF, Al-Abbasi FA, AlGhamdi SA, Alshehri OY, *et al.* Protective effect of Fustin against Huntington's disease in 3-nitropropionic treated rats via downregulation of oxidative stress and alteration in neurotransmitters and brain-derived neurotrophic factor activity. Biomedicines. 2022; 10(12): 3021. doi: 10.3390/biomedicines10123021, PMID 36551777.
14. Alshehri S, Al-Abbasi FA, Ghoneim MM, Imam SS, Afzal M, Alharbi KS, *et al.* Anti-Huntington's effect of Butin in 3-nitropropionic acid-treated rats: possible mechanism of action. Neurotox Res. 2022; 40(1): 66-77. doi: 10.1007/s12640-021-00462-7, PMID 34982357.
15. Borlongan CV, Koutouzis TK, Sanberg PR. 3-nitropropionic acid animal model and Huntington's disease. Neurosci Biobehav Rev. 1997; 21(3): 289-93. doi: 10.1016/s0149-7634(96)00027-9, PMID 9168265.
16. Mahdi WA, AlGhamdi SA, Alghamdi AM, Imam SS, Alshehri S, Almaniea MA, *et al.* Neuroprotectant effects of Hibiscetin in 3-nitropropionic acid-induced Huntington's disease via subsiding oxidative stress and modulating monoamine neurotransmitters in rats brain. Molecules. 2023; 28(3): 1402. doi: 10.3390/molecules28031402, PMID 36771072.
17. McCormack PL, Wagstaff AJ. Lacidipine: a review of its use in the management of hypertension. Drugs. 2003; 63(21): 2327-56. doi: 10.2165/00003495-200363210-00008, PMID 14524737.
18. Liu X, Huang Z, Zhang Y, Shui X, Liu F, Wu Z, *et al.* Lacidipine ameliorates the endothelial senescence and inflammatory injury through CXCR7/P38/C/EBP-β signaling pathway. Front Cardiovasc Med. 2021; 8: 692540. doi: 10.3389/fcvm.2021.692540, PMID 34295928.
19. Khurana K, Bansal N. Lacidipine attenuates reserpine-induced depression-like behavior and oxido-nitrosative stress in mice. Naunyn-Schmiedebergs Arch Pharmacol. 2019; 392(10): 1265-75. doi: 10.1007/s00210-019-01667-6, PMID 31187187.
20. Yu X, Li C, Tao Y, Xia T, Jia Z. Lacidipine inhibits NF-κB and notch pathways and mitigates DSS-induced colitis. Dig Dis Sci. 2024; 69(10): 3753-9. doi: 10.1007/s10620-024-08618-z, PMID 39261383.
21. Wang QL, Zhao L, Feng N, Zhou P, Wu Q, Fan R, *et al.* Lacidipine attenuates TNF-α-induced cardiomyocyte apoptosis. Cytokine. 2015; 71(1): 60-5. doi: 10.1016/j.cyto.2014.08.004, PMID 25226445.
22. Bezemer B, van Cleef KW, Overheul GJ, Miesen P, van Rij RP. The calcium channel inhibitor lacidipine inhibits Zika virus replication in neural progenitor cells. Antiviral Res. 2022; 202: 105313. doi: 10.1016/j.antiviral.2022.105313, PMID 35367280.
23. Dasgupta A, Dastidar SG. Antibacterial and antitoxic effects of the cardiovascular drug lacidipine in an animal model. Indian J Med Res. 2012; 135(6): 913-6. PMID 22825612.
24. Singh J, Aditya S, Lal H, Arora B. Effect of lacidipine pre-treatment on diabetic neuropathy in rats. Arch Physiol Biochem. 2011; 117(1): 12-7. doi: 10.3109/13813455.2010.525241, PMID 21091185.
25. Ashcheulova T, Gerasimchuk N, Kovalyova O, Honchar O. Beneficial effects of combined therapy with lacidipine and candesartan in obese hypertensive patients. Rom J Intern Med. 2018; 56(4): 257-64. doi: 10.2478/rjim-2018-0018, PMID 30521476.
26. Kamal SM. Possible hepatoprotective effects of lacidipine in irradiated DOCA-salt hypertensive albino rats. Pak J Biol Sci. 2013; 16(21): 1353-7. doi: 10.3923/pjbs.2013.1353.1357, PMID 24511746.
27. Pinzi L, Rastelli G. Molecular docking: shifting paradigms in drug discovery. Int J Mol Sci. 2019; 20(18): 4331. doi: 10.3390/ijms20184331, PMID 31487867.
28. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, *et al.* The ARRIVE guidelines 2.0: updated guidelines for reporting animal research. PLOS Biol. 2020; 18(7): e3000410. doi: 10.1371/journal.pbio.3000410, PMID 32663219.
29. Borlongan CV, Koutouzis TK, Sanberg PR. 3-nitropropionic acid animal model and Huntington's disease. Neurosci Biobehav Rev. 1997; 21(3): 289-93. doi: 10.1016/s0149-7634(96)00027-9, PMID 9168265.
30. Khurana K, Bansal N. Lacidipine attenuates caffeine-induced anxiety-like symptoms in mice: role of calcium-induced oxido-nitrosative stress. Pharmacol Rep. 2019; 71(6): 1264-72. doi: 10.1016/j.pharep.2019.07.008, PMID 31675672.
31. Azizah Salim B, Muhammad A, Auwal Ibrahim T, Fahad AA, Mustafa Z, May MA, *et al.* Malvidin improves ethanol-induced memory impairment by targeting cholinesterase, FRAP and GABA signaling: based on molecular docking study. J Biol Regul Homeost Agents. 2024; 38(3): 2165-79.
32. Wang L, Wang J, Yang L, Zhou SM, Guan SY, Yang LK, *et al.* Effect of Praeruptorin C on 3-nitropropionic acid induced Huntington's disease-like symptoms in mice. Biomed Pharmacother. 2017; 86: 81-7. doi: 10.1016/j.biopha.2016.11.111, PMID 27939523.
33. Sandhir R, Mehrotra A. Quercetin supplementation is effective in improving mitochondrial dysfunctions induced by 3-nitropropionic acid: implications in Huntington's disease. Biochim Biophys Acta. 2013; 1832(3): 421-30. doi: 10.1016/j.bbadis.2012.11.018, PMID 23220257.
34. Karandikar A, Thangarajan S. Protective activity of Esculetin against 3-nitropropionic acid induced neurotoxicity via scavenging reactive oxygen species in male Wistar rats. Int J Pharmacogn Phytochem Res. 2017; 9(5): 722-32. doi: 10.25258/phyto.v9i5.8155.
35. Ibrahim WW, Abdel Rasheed NO. Diapocynin neuroprotective effects in 3-nitropropionic acid Huntington's disease model in rats: emphasis on Sirt1/Nrf2 signaling pathway. Inflammopharmacology. 2022; 30(5): 1745-58. doi: 10.1007/s10787-022-01004-z, PMID 35639233.
36. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7(2): 88-95. doi: 10.1016/0006-2952(61)90145-9.
37. Wang L, Wang J, Yang L, Zhou SM, Guan SY, Yang LK, *et al.* Effect of Praeruptorin C on 3-nitropropionic acid induced Huntington's disease-like symptoms in mice. Biomed Pharmacother. 2017; 86: 81-7. doi: 10.1016/j.biopha.2016.11.111, PMID 27939523.
38. Kumar P, Kumar A. Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical and mitochondrial dysfunction in an animal model of Huntington's disease. J Med Food. 2009; 12(3): 591-600. doi: 10.1089/jmf.2008.0028, PMID 19627208.
39. Mehan S, Monga V, Rani M, Dudi R, Ghimire K. Neuroprotective effect of solanesol against 3-nitropropionic acid-induced Huntington's disease-like behavioral, biochemical and cellular alterations: restoration of coenzyme-Q10-mediated mitochondrial dysfunction. Indian J Pharmacol. 2018; 50(6): 309-19. doi: 10.4103/ijp.IJP_11_18, PMID 30783323.
40. Mehan S, Parveen S, Kalra S. Adenyl cyclase activator forskolin protects against Huntington's disease-like neurodegenerative disorders. Neural Regen Res. 2017; 12(2): 290-300. doi: 10.4103/1673-5374.200812, PMID 28400813.
41. Browne SE. Mitochondria and Huntington's disease pathogenesis: insight from genetic and chemical models. Ann N Y Acad Sci. 2008; 1147(1): 358-82. doi: 10.1196/annals.1427.018, PMID 19076457.
42. Puzzo D, Vitolo O, Trinchese F, Jacob JP, Palmeri A, Arancio O. Amyloid-β peptide inhibits activation of the nitric oxide/cGMP/cAMP-responsive element-binding protein pathway during hippocampal synaptic plasticity. J Neurosci. 2005; 25(29): 6887-97. doi: 10.1523/JNEUROSCI.5291-04.2005, PMID 16033898.
43. Suganya SN, Sumathi T. Effect of rutin against a mitochondrial toxin, 3-nitropropionicacid induced biochemical, behavioral and histological alterations-a pilot study on Huntington's disease model in rats. Metab Brain Dis. 2017; 32(2): 471-81. doi: 10.1007/s11011-016-9929-4, PMID 27928694.
44. Walczak-Nowicka ŁJ, Herbet M. Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis. Int J Mol Sci. 2021; 22(17): 9290. doi: 10.3390/ijms22179290, PMID 34502198.
45. Jaglan D, Singh Bindra C. Evaluating neuroprotective effects of ascorbic acid against 3-nitropropionic acid induced Huntington's disease in rats: possible involvement of gabaareceptors. Impact. International journal of research in applied, natural and social sciences (IMPACT. IJRANSS), ISSN (P). 2018: 2347-4580.
46. Bhimanwar AA, Ghaisas MM, Shete RV. Silymarin, quercetin and hesperidin combination ameliorate learning and memory deficit in 3 nitro propionic acid induced rat model of Huntington's disease. Available at SSRN 4004056. IJPI. 2022; 12(3): 363-9. doi: 10.5530/ijpi.2022.3.61.
47. Sidhu A, Diwan V, Kaur H, Bhateja D, Singh CK, Sharma S, *et al.* Nicotinamide reverses behavioral impairments and provides neuroprotection in 3-nitropropionic acid induced animal model ofHuntington's disease: implication of oxidative stress-poly(ADP- ribose) polymerase pathway. Metab Brain Dis. 2018; 33(6): 1911-21. doi: 10.1007/s11011-018-0297-0, PMID 30054774.
48. Singh P, Mishra G, Molla M, Shumet Yimer Y, Sisay W, Andargie Y *et al.* Dietary and nutraceutical-based therapeutic approaches to combat the pathogenesis of Huntington's disease. J Funct Foods. 2022; 92: 105047. doi: 10.1016/j.jff.2022.105047.
49. Danduga RC, Dondapati SR, Kola PK, Grace L, Tadigiri RV, Kanakaraju VK. Neuroprotective activity of tetramethylpyrazine against 3-nitropropionic acid induced Huntington's disease-like symptoms in rats. Biomed Pharmacother. 2018; 105: 1254-68. doi: 10.1016/j.biopha.2018.06.079, PMID 30021362.
50. Bin-Jumah MN, Gilani SJ, Alabbasi AF, Al-Abbasi FA, AlGhamdi SA, Alshehri OY, *et al.* Protective effect of Fustin against Huntingtonandrsquo;s disease in 3-nitropropionic treated rats via downregulation of oxidative stress and alteration in neurotransmitters and brain-derived neurotrophic factor activity. Biomedicines. 2022; 10(12): 3021. doi: 10.3390/biomedicines10123021, PMID 36551777.
51. Stavrovskaya AV, Voronkov DN, Yamshchikova NG, Ol'shanskiy AS, Khudoerkov RM, Illarioshkin SN. Experience of experimental modelling of Huntington's disease. Hum Physiol. 2016; 42(8): 898-904. doi: 10.1134/S0362119716080120.
52. Jamwal S, Kumar P. L-theanine, a component of green tea prevents 3-nitropropionic acid (3-NP)-induced striatal toxicity by modulating nitric oxide pathway. Mol Neurobiol. 2017; 54(3): 2327-37. doi: 10.1007/s12035-016-9822-5, PMID 26957301.
53. Abdelfattah MS, Badr SE, Lotfy SA, Attia GH, Aref AM, Abdel Moneim AE, *et al.* Rutin and selenium co-administration reverse 3-nitropropionic acid-induced neurochemical and molecular impairments in a mouse model of Huntington's disease. Neurotox Res. 2020; 37(1): 77-92. doi: 10.1007/s12640-019-00086-y, PMID 31332714.
54. Kaur N, Jamwal S, Deshmukh R, Gauttam V, Kumar P. Beneficial effect of rice bran extract against 3-nitropropionic acid induced experimental Huntington's disease in rats. Toxicol Rep. 2015; 2: 1222-32. doi: 10.1016/j.toxrep.2015.08.004, PMID 28962465.
55. Kumar P, Kumar P, Khan A, Deshmukh R, Lal Sharma P. Role of neurosteroids in experimental 3-nitropropionic acid induced neurotoxicity in rats. Eur J Pharmacol. 2014; 723: 38-45. doi: 10.1016/j.ejphar.2013.11.036, PMID 24333475.
56. Hsiao HY, Chen YC, Chen HM, Tu PH, Chern Y. A critical role of astrocyte-mediated nuclear factor-κB-dependent inflammation in Huntington's disease. Hum Mol Genet. 2013; 22(9): 1826-42. doi: 10.1093/hmg/ddt036, PMID 23372043.
57. Jamwal S, Kumar P. Spermidine ameliorates 3-nitropropionic acid (3-NP)-induced striatal toxicity: possible role of oxidative stress, neuroinflammation and

neurotransmitters. Physiol Behav. 2016; 155: 180-7. doi: 10.1016/j.physbeh.2015.12.015, PMID 26703234.

58. Singh S, Jamwal S, Kumar P. Piperine enhances the protective effect of curcumin against 3-NP induced neurotoxicity: possible neurotransmitters modulation mechanism. Neurochem Res. 2015; 40(8): 1758-66. doi: 10.1007/s11064-015-1658-2, PMID 26160706.
59. Mani V, Arfeen M, Dhaked DK, Mohammed HA, Amirthalingam P, Elsisi HA. Neuroprotective effect of methanolic Ajwa Seed extract on lipopolysaccharide-induced memory dysfunction and neuroinflammation: in vivo, molecular docking and dynamics studies. Plants (Basel). 2023; 12(4): 934. doi: 10.3390/plants12040934, PMID 36840284.
60. Dileep KV, Remya C, Tintu I, Sadasivan C. Designing of multi-target-directed ligands against the enzymes associated with neuroinflammation: an *in silico* approach. Front Life Sci. 2013; 7(3-4): 174-85. doi: 10.1080/21553769.2014.901924.
61. C S, S DK, Ragunathan V, Tiwari P, A S, P BD. CS, S DK, Ragunathan V, Tiwari P, AS, P BD. Molecular docking, validation, dynamics simulations and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. J Biomol Struct Dyn. 2022; 40(2): 585-611. doi: 10.1080/07391102.2020.1815584, PMID 32897178.
62. Alshehri S, Al-Abbasi FA, Ghoneim MM, Imam SS, Afzal M, Alharbi KS, *et al*. Anti-Huntington's effect of Butin in 3-nitropropionic acid-treated rats: possible mechanism of action. Neurotox Res. 2022; 40(1): 66-77. doi: 10.1007/s12640-021-00462-7, PMID 34982357

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