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The potential therapeutic role of Lisinopril in augmenting the striatal neuroplasticity via the striatal ACE2/Ang1-7/MAS receptor axis in 3-nitropropionic acid-induced Huntington's disease in rats: shifting paradigms in Huntington's disease treatment

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Abstract

Background The exact pathogenesis of Huntington's disease (HD) remains unclear. However, mitochondrial dysfunction and oxidative stress are supposed to play a significant role. The objective of this study was to examine the possible neuroprotective effect of Lisinopril (Lisino) in a 3-nitropropionic acid-produced HD in rats.

Methods Sixty-four rats were divided into four groups (16/group): Group (1): Normal control group, Group (2): Lisinopril control group, Group (3): 3-NP non-treated group, and Group (4): (3-NP + Lisinopril) group. Behavior assessments (open field test, rotarod test, grip strength test) were performed along with different histological and biochemical parameters.

Results Lisinopril upregulated the expression of the ACE2/Ang1-7/MAS receptor (MasR) axis of RAS, which triggered the PI3K/Akt pathway and prompted the CREB/BDNF neurogenesis signal. Furthermore, Lisinopril remarkably downregulated the inflammatory cytokines (NF-κB, TNF-α, IFN-γ and IL-6), decreased apoptotic markers (p53, BAX/Bcl2 ratio, Cyt-c and caspase-3) and upgraded the mitochondrial TFAM content and SDH activity along with restoration of the redox mechanism by recovering SOD, catalase, GSH and Nrf2.

Conclusion Notably, the outcomes of this study disclosed that Lisinopril could be a future neuroprotective therapeutic candidate against HD.

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Research highlights

- Lisinopril alleviated the mitochondrial dysfunction and restored redox balance via Nrf2/TFAM signaling.
- Lisinopril downregulated the inflammatory cytokines (NF-κB, TNF-α, IFN-γ and IL-6).
- Lisinopril upregulated the expression of ACE2/Ang1-7/MAS receptor axis of RAS.
- Lisinopril activated PI3K/Akt/CREB pathway and evoked the neurogenesis via its downstream product BDNF.
- Lisinopril could be a future neuroprotective treatment against Huntington disease.

Keywords Huntington's disease, Renin–angiotensin system, Lisinopril, Mitochondrial dysfunction, 3-nitropropionic acid

Background

Huntington's disease (HD) is a rare, inherited neurodegenerative disorder affecting approximately 3 to 7 per 100,000 people worldwide. It typically manifests in mid-adulthood, though symptoms can appear earlier or later. HD is a highly neurodegenerative condition arising from the mutant Huntington protein (mHTT), caused by a CAG (cytosine, adenine, guanine) trinucleotide repeat at one end of the Huntington gene. The striatal basal ganglia are the target locus of neuronal loss, but neurodegeneration has similarly been reported in other brain areas [1–4].

It is unclear exactly how mHTT contributes to HD pathophysiology; however, it has been connected to dangerous gain of function in a number of biological systems. Mutant HTT causes cell death through mitochondrial dysfunction, leading to bio-energetic failure and oxidative stress in the brain [1, 5, 6].

Although HD is a low-prevalence disease, it has significant morbidity and mortality. It is characterized by unusual involuntary movements and cognitive deterioration, leading to uncontrolled cognitive and motor deficits and neuropsychiatric manifestations. Once symptoms manifest, the median survival time is 18 years. Studies illustrate peripheral alterations in HD pathology, including musculoskeletal disorders represented by unintentional loss of body weight, muscle atrophy, insufficient insulin production, osteoporosis and heart failure [1, 7–11]. Most of the current therapeutic candidates are symptomatic like tetrabenazine for chorea, olanzapine and citalopram for psychotic and depressive behavior. Despite the tremendous advance in exploring newer agents that target mHTT protein like particularly antisense oligonucleotides, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) therapies and stem cell therapy [12], there are still under investigation for their safety profile and efficacy. Therefore, drug repurposing is an efficient strategy to address unmet clinical needs in a more timely and cost-effective manner.

Noteworthy, brain-derived neurotrophic factor (BDNF) has acritical role in regulating neuronal plasticity and abating the apoptosis. It was found its level dramatically dropped in patient with HD [13]. Additionally, accumulating evidence has pointed to the positive correlation between BDNF and nuclear factor erythroid 2-related factor 2 (Nrf2). It is importantly to mention that Nrf2 augments the scavenging capacity by upregulating the redox genes in line with its significant downregulation of NF-κB [14, 15] and its downstream inflammatory cytokines.

Notably, the renin–angiotensin system (RAS) is a complicated hormonal regulatory system involving multiple organs that regulate various body functions. It initially focused on cardiovascular function and related pathologies. Recent findings suggest that the RAS is more complex, consisting of two arms: the classic RAS, which comprises renin/angiotensin II and its AT1 receptor, and the other RAS, composed of elements that counter-regulate its actions [16, 17]. The classic RAS involves inflammatory, hypertrophic and fibrotic pathologies, leading to chronic diseases in different body systems [18]. The ACE2/Ang1-7/MasR axis is an alternative regulatory arm that triggers the downstream signaling of the MasR/PI3KAKT/CREB. Ang1-9, Ang1-5 and Ang1-7 are the downstream molecules produced by the action of ACE2, and angiotensin-converting enzyme 2 (ACE2) Ang1-7 is the last molecule in this counterregulatory arm [19, 20]. It is interesting to note that cAMP-response element-binding protein (CREB) upregulates the target gene BDNF [21].

Prior research has demonstrated potential interaction of systemic RAS with RAS in the brain, particularly in circumventricular organs that lacks the blood–brain barrier (BBB) [22, 23]. The RAS has been associated with neuropsychiatric and neurodegenerative disorders, including Parkinson's disease (PD), stroke, and Alzheimer's disease (AD). Neurodegeneration in brain diseases results from oxidative stress, apoptosis, and neuroinflammation induced by its traditional axis [23, 24]. Furthermore, Hariharan and his coworkers unveiled

the positive outcomes of trandolapril, ACE inhibitor, in experimentally induced HD model in rats [25]

It is worth to mention that ACEIs have been associated with reduced rates of cognitive deterioration in AD patients and in numerous non-clinical in vitro and in vivo models. [26–30]. Numerous hypotheses have been advanced despite a lack of knowledge regarding the cellular and molecular processes underlying these antihypertensive medications [31].

According to a review of the relevant literature, the implicated function of RAS in HD pathology remains unexplored and requires further investigation. However, changes in RAS within the central nervous system (CNS) are observed in this disorder, indicating that this system may be involved in the pathogenesis of HD [26]. Consequently, therapeutic modification of the RAS components might constitute a potentially therapeutic approach to managing HD.

In light of these relevant studies, the present investigation was focused to examine the potential neuroprotective consequences of Lisinopril against 3-NP-induced HD and to explore some of the potential molecular pathways that may be implicated in its therapeutic effect.

Methods

Determination of sample size

The size of the sample was determined utilizing the G*Power program (Version 3.1.9.2, developed by Franz Faul, Kiel, Germany) and adjusted appropriately, according to a study by Sayed et al. [32]. The primary outcome (effect size) was hypothesized to be the level of striatal CREB expression (upward stream regulator of BDNF) and attrition rate of 20%, setting the alpha level at 5% and power at 80% using a one-way ANOVA test within the four groups. According to that proposal, the sample size was 16 rats per group. The right striata were subdivided into two subsets, including 8 per set for ELISA and histopathological examination, and the left striata were subdivided into two subsets, including 8 per set for western blot and gene expression analysis.

Ethical declaration

The Institutional Animal Care and Use Committee reviewed and authorized the revised investigational protocol following the protocols outlined in the Guide for the Care and Use of Laboratory Animals (NIH publication) and ARRIVE guidelines. Every possible endeavor has been employed to minimize animal suffering through the research.

Animals

In the current investigation, sixty-four adults male Wistar rats ranging in weight from 180 to 220 g were procured from the Egyptian Drug Authority in Giza, Egypt. They were housed in standard conditions, including a temperature of 25 ± 2°C, humidity of 60 ± 10%, and a 12/12-h light/dark cycle (with lights on at 6:00 am). Before starting any method of investigation, animals were housed in the animal facility for one week for acclimatization. Chow morsels and water were available indefinitely to the rats.

Experimental methodology

As displayed in Scheme1, rats were divided at random into four distinct groups by the animal house assistant (n = 16 per group) as follows: Saline was administered to the initial two groups: (1 ml/kg; p.o.) or Lisino (10 mg/kg; p.o.) [33] to be designated as control and the Lisino groups, respectively. The remaining two groups were administered 3-NP intraperitoneally (10 mg/kg/day; i.p.; Sigma-Aldrich, MO, USA) [34], with one group remaining without treatment designated to represent the 3-NP group (3rd group), and the other group received Lisino (10 mg/kg; p.o.), two hours after the 3-NP injection, to serve as the 3-NP + Lisino group (4th group). Lisinopril and 3-NP were freshly prepared by dissolving in normal saline throughout the experimental period. Each treatment regimen was scheduled for a duration of 14 days.

Behavioral test

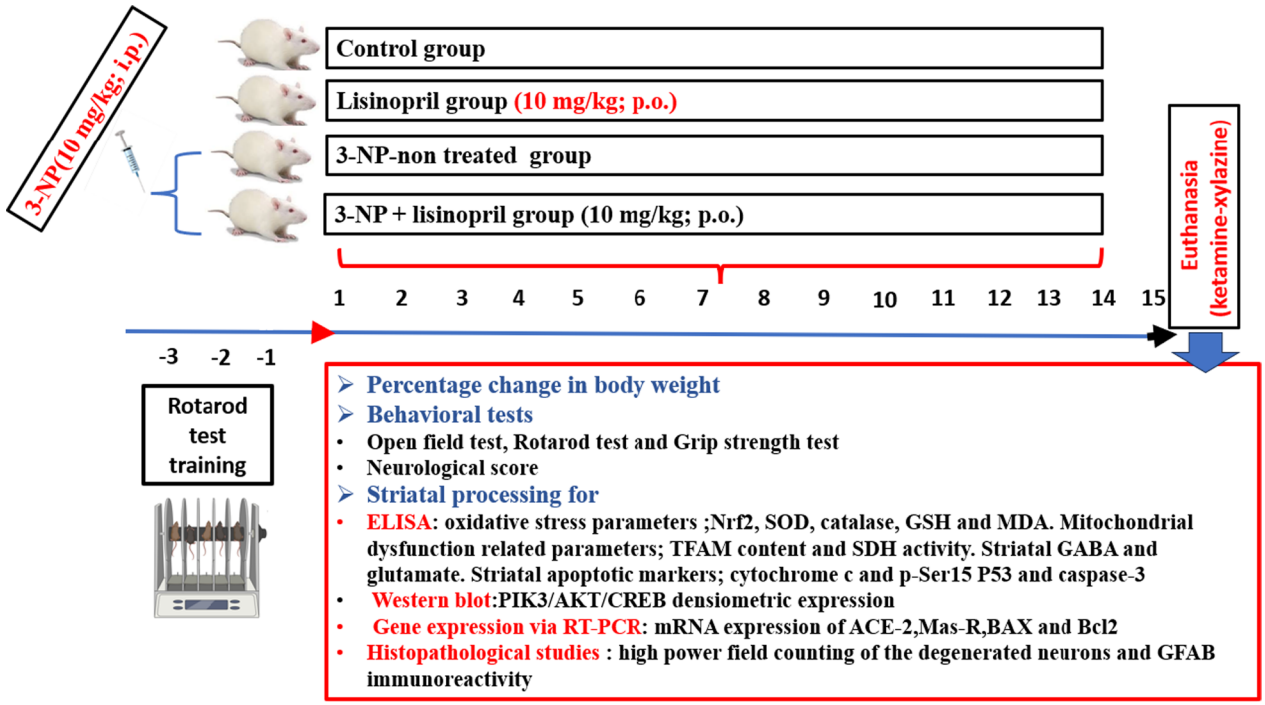
All the behavioral tests were performed by a blinded investigator to the experimental group identity and repeated 3 times per animal, and the data were averaged.

On day 15, rats were evaluated for locomotor activity as well as motor function 24 h following the final dose of 3-NP and Lisinopril. This was accomplished through open field, rotarod, and grip strength tests, with a 2-h interval between each test [32]. Behavior testing was conducted in a sound-isolated research facility.

Open field test (OFT)

The OFT was carried out in a box of wood (80cm × 80cm × 40cm) with a red wall and black floor partitioned into sixteen squares (4 × 4) to assess the locomotor activity.

Each rat was positioned in the box center and allowed to investigate the field for 5 min. The box was cleansed with 70% alcohol after each tested animal to remove odor [35]. Total distance traveled, mean velocity, duration of immobility, along with frequency of rearing were documented and assessed using ANY-MAZE video monitoring software (version 7.1, Stoelting Co., IL, USA) [36].



Scheme 1 The timeline of the experimental design

Rotarod test

A rotarod measuring 120 cm in length, 3 cm in diameter, and revolving at 20 rpm was used to test the motor coordination and balance of rats. Rats were trained six times over the course of three days prior to the experiment, and the test was conducted on the rats that remained on the wire for five minutes. After completing OFT, the experiment was carried out, and the rats were watched for five minutes in order to quantify the fall-off latency [37].

Test of grip strength

Grip strength measurement equipment from Ugo Basile, Italy, was utilized to evaluate the forelimb strength of the rat. In this test, every rat seized a trapeze-shaped steel rod attached to a force gauge with its forepaws. The animal was then gently pulled by its tail until the clasp came off. The maximum power was assessed in gf, and the mean of three repeated values was estimated for every animal [38].

Neurological scoring

Locomotive scoring was used to assess the extent of motor impairment provoked by the 3-NP neurotoxin. The scoring was according to a prior study of Danduga et al. [39] as follows: normal, 0; slowness of movement due to mild hindlimb handicap, 1; loss of coordination with obvious gait abnormality, 2; hindlimb palsy, 3; inability to move due to impairment in both forelimbs and hindlimbs, 4; and recumbency, 5

Striatal processing

Following the completion of the behavioral evaluations, rats were anesthetized and sacrificed via cervical dislocation; the brains were quickly separated, and the striata from every brain were dissected directly and rapidly frozen in liquid nitrogen. Subsequently, the collected striata were grouped into four sets; the first right set (n = 8) was utilized to determine parameters using the ELISA method following homogenization in phosphate-buffered saline (PBS, pH = 7.4).

The other right set (n = 8) was utilized for H&E analysis and evaluation of striatal glial fibrillary acidic protein (GFAP) immunoreactivity. The left 8-striatal set was dipped in RNA lysis buffer and utilized for qRT-PCR assessment. Meanwhile, the remaining striatal set was submerged in a RIPA buffer and supplied with a cocktail of proteases and phosphatase inhibitors for western blot testing.

Enzyme-linked immunosorbent assay (ELISA)

Rat MyBioSource ELISA kits (CA, USA) were intended for the assessment of SDH (cat#: MBS760721), BDNF (cat#: MBS 355345), TNF-α (cat. #: MBS2507393), pS536-NFκB-p65 (cat#: MBS9511033), IFN-γ (cat#: MBS766197), TFAM (cat#: MBS1600609), Ang 1–9 (cat#:

Table 1 The primer sequence of the studied genes

mRNA	Gene accession number	Primer sequence 5'-3'
ACE2	NM_001012006	F: TCAGAGCTGGGATGCAGAAA
		R: GGCTCAGTCAGCATGGAGTTT
MasR	NM_012757.2	F: ACTGCCGGGCGGTCATCATC
		R: GGTGGAGAAAAGCAAGGAGA
β-Actin (housekeeping gene)	NM_031144.3	F: TATCCTGGCCTCACTGTCCA
		R: AACGCAGCTCAGTAACAGTC
BAX	(NM_007527.2)	F: GGCCGGGTTGTCGCCCTTTT
		R: CCGCTCCCGGAGGAAGTCCA
Bcl2	NM_016993.1	F: CTGGTGGACAACATCGCTCTG
		R: GGTCTGCTGACCTCACTTGTG
GAPDH (housekeeping gene)	(NM_017008)	F: TGGCATTGTGGAAGGGCTCA
		R: TGGATGCAGGGATGATGTTCT

ACE2: Angiotensin-converting enzyme 2, MasR: Mas receptor, BAX: Bcl2-associated x protein, Bcl2: B cell lymphoma 2, F: forward, R: reverse

MBS2700688), Ang1-7 (cat#: MBS2022279), and Nrf2 (cat#: MBS752046). In parallel, the levels of malondialdehyde (MDA) and superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) were evaluated by colorimetry using specific ELISA kits obtained from Eagle Biosciences Inc. (MA, USA), BioVision (CA, USA), Bio-Diagnostic (El-Dokki, Egypt), respectively. ELISA kits were also purchased from RayBiotech, Georgia, USA (Cat#: PEL-P53-S15-T, Phospho-P53 (Ser15), to measure striatal contents of P53. ELISA quantitative assay for the striatal glutamate and GABA was performed using (cat#: KA1909 Abnova, Taipei, Taiwan) and (cat#: E0900r, Elabscience, Wuhan, China), respectively. For the activity level of caspase-3 (Casp-3) and cytochrome c, ELISA assay kits were obtained from CUSBIO BIOTEC CO., Wuhan, China (Cat#CSB-E08857r), and Cat. #. CSB-EL006328RA, respectively. All experimental protocols were performed according to the manufacturer's guidelines.

Quantitative RT-PCR

Striata were homogenized in lysate buffer to quantify ACE2 and MasR, BAX and Bcl2 mRNA expression. The RNeasy Mini kit (Qiagen, Venlo, Netherlands) was utilized to extract total RNA from the striatum. To ensure the integrity of the extracted RNA, spectrophotometric analysis was performed at an OD of 260/280 nm. cDNA was derived from equivalent quantities of the RNA extracted using an RT-PCR reagent (Promega, Leiden, Netherlands) following the manufacturer's procedure. SYBR Green Master Mix (Applied Biosystems, CA, USA) performed qRT-PCR per the manufacturer's specifications. To summarize, in a 25-µl reaction volume, 5µl of cDNA was added to 12.5µl of the SYBR Green mixture, 5.5µl of RNase-free water, and 2µl of the primer (Table 1). Forty cycles of PCR were performed, consisting of denaturation (95°C for 15 s), annealing (60°C for 60 s), and extension (72 °C for 60 s). The $2^{-\Delta\Delta CT}$ formula was employed to normalize the relative expression of required genes to β-actin for ACE2 and Mas receptor and normalized to GAPDH for BAX2 and Bcl2 [40].

Western blot analysis

To preserve protein integrity, the striata in the third grouping were homogenized in radioimmunoprecipitation assay (RIPA) buffer, which had a combination of phosphatase and protease inhibitors and contained 50 mM Tris HCl pH 8, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, and 0.1% SDS. After protein measurement using the Bradford assay [41], 10 µg of proteins from each sample were introduced and subjected to SDS-PAGE separation prior to being transferred to a PVDF membrane and blocked with 5% bovine serum albumin (BSA). Following that, the membrane was incubated overnight at 4°C using a roller agitator with either anti-p85/p55 (pY458/199)-PI3K (1:1000; cat#: PA5-99,367), anti-pS473-Akt (1:1000; cat#: PA5-85729), anti-pS133-CREB (1:1000; cat#: PA1-4619), or anti-β-actin (1:5000; cat# MA1-140) antibodies (ThermoFisher Scientific, MA, USA). Next, membranes were washed and cultured at room temperature for one hour with HRP-conjugated goat anti-rabbit immunoglobulin (1:1000; Dianova, HH, Germany). Subsequently, the analysis of band intensity was done by ChemiDoc™ imaging system with Image Lab™ software version 5.1 (Bio-Rad Laboratories Inc., Hercules, CA, USA). Upon standardization for GAPDH protein expression, the outcomes are displayed in arbitrary units (AU).

Analytical histopathology

A blinded pathologist to the experimental group identity performed the analytical histopathology.

Histopathological examination by hematoxylin and eosin

Brain specimens (n = 8) were submerged in 10% formalin-filtered saline for a full 72 h after scarification. Following manual evaporation with progressively increasing ethanol concentrations, the specimens were purified in xylene and encapsulated in paraplast. Hematoxylin and eosin (H&E) were utilized to stain 4μm sagittal brain sections to detect histological irregularities in the striatum with quantification of the darkly stained degenerated neuron from six randomly selected areas for each section. For histological analysis, photomicrographs were acquired utilizing a Leica application module-controlled full HD microscope camera (Leica Microsystems GmbH, Wetzlar, Germany) [42].

Immunohistochemical detection of GFAP

Deparaffinized sections (5 μm) were cut to assess striatal GFAP as a marker for astroglia activation. Samples experienced incubation with animal antibody against GFAP (Cat: # ab7260; 1:200; Abcam; MA; USA) at 37°C for 60 min, then washed with PBS, and cultured for 20 min with the secondary antibody HRP Envision reagent (DAKO, CA, USA). The reaction was then visualized using 3,3′-diaminobenzidine tetrahydrochloride (DAB Substrate Kit, CA, USA) after the slices were rinsed with PBS. Subsequently, samples were dehydrated and clarified in xylene before being counterstained with hematoxylin, and the cover was slipped in preparation for microscopic examination. Leica Microsystems GmbH, Wetzlar, Germany, utilized a full HD microscopic camera equipped with an application module for histological analysis to scan six non-overlapping fields to calculate the area percentage of striatal GFAP immunoreactivity [43]. All histopathological evaluations were performed by an investigator who was not privy to the identities of the samples to mitigate any potential bias.

Statistical analysis

Data were analyzed using GraphPad prism Version 8. 4.3 Software (GraphPad Software; San Diego, CA, USA). Normal distribution was assessed using the Shapiro–Wilk test. Results were blotted as mean ± S.D. Statistical analysis was evaluated using a one-way analysis of variance (ANOVA) test followed by Tukey's multiple comparison test for data with a normal distribution. The nonparametric neurological scoring data set was analyzed by Kruskal–Wallis test with Dunn's multiple comparison test. Correlations between quantitative variables (striatal content of BDNF and mRNA expression of ACE2) were done using Pearson correlation coefficient. At $p < 0.05$, the degree of statistical significance was established.

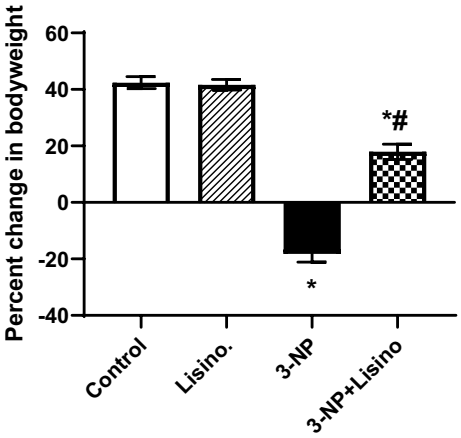


Fig. 1 The effect of Lisinopril on the percentage change in body weight. Data are displayed as mean ± S.D. (n = 16/group) and subjected to one-way ANOVA and Tukey's multiple comparison test for analysis. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group; Lisino.: Lisinopril; 3-NP: 3-nitropropionic acid

Results

As determined by one-way ANOVA, there was no statistically noteworthy distinction in any measured parameters between the normal control and Lisino-treated groups; herein, all comparison was performed relative to the normal control group (NC).

The impact of Lisinopril on the body weight change percentage

Regarding the body weight change percentage, the 3-NP-injected group showed a significant reduction in final body weight compared to the NC ($p < 0.0001$). Conversely, treatment with Lisinopril raised the final body weight ($p < 0.0001$) in contrast to the 3-NP group, as shown in Fig. 1.

The impact of Lisinopril on locomotor activity in 3-NP rats using the open field test and rotarod test, as well as the hand grip strength test

As shown in Fig. 2, the 3-NP group exhibited a considerable reduction ($p < 0.0001$) in the overall distance traveled, mean velocity, and rearing frequency by about 71.35%, 96%, and 70.73%, respectively, in the open field test. Additionally, 3-NP group demonstrated significant hypoactivity, as evidenced by extending the immobility time ($p < 0.0001$), reaching approximately 1.35-fold

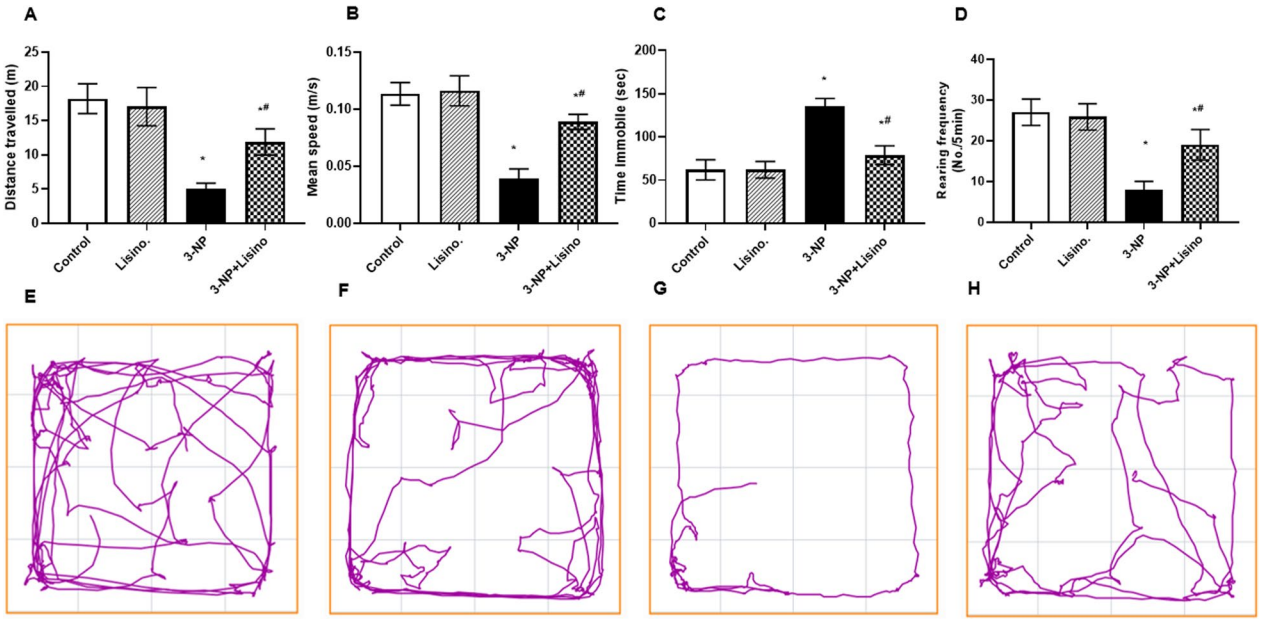


Fig. 2 The impact of Lisinopril on locomotor activity using open field test (OFT). Track plots for the Lisino, 3-NP, 3-NP + Lisino, and normal control groups are represented by the letters A, B, C, D, E, F, G, and H, respectively, representing total distance traveled, average speed, immobility time, and rearing frequency. Tukey's multiple comparison test is performed after one-way ANOVA, and the data are reported as mean ± S.D. (n = 16/group). Any value of $p \sim < 0.05$ indicates a significant difference. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group; Lisino.: Lisinopril;3-NP: 3-nitropropionic acid

the corresponding values in the NC. In contrast, the 3-NP + Lisino group displayed a notable increment ($p < 0.0001$) in the distance traveled, mean speed, and rearing frequency by about 215.64%, 123%, and 137.5%, respectively, compared to the 3-NP group. Furthermore, Lisino treatment demonstrated a considerable decline in the immobility time by about 77.89% ($p < 0.0001$) in comparison with the 3-NP3 group.

3-NP3 group exhibited significant drawdown ($p < 0.0001$) in fall-off latency and maximal force by about 83.11% and 53.24% in rotarod and grip strength tests. Alternatively, the Lisino treatment significantly ($p < 0.0001$) extended the fall-off latency time and increased the peak force by about 230% and 73%, respectively, in comparison with the 3-NP group (Fig. 3).

Assessment of the effect of Lisinopril on the neurological score 3-NP rats

As displayed in Fig. 4, the 3-NP group exhibited a considerable threefold increase in the neurological score when compared to the normal control group ($p < 0.0001$) and this was evidenced by the significant motor dysfunction that was obvious in slowing of the movement, gait incoordination, impairment of forelimb and hindlimb, hindlimb paralysis, and recumbency. Conversely, Lisinopril in 3-NP + Lisino group remarkably decreased the neurological score by about 66% in contrast to the 3-NP group ($p = 0.0364$).

Assessment of Lisinopril on striatal GABAergic and glutaminergic content 3-NP rats

Herein, 3-NP group showed a significant decline in the striatal content of GABA by about 70% with a 1.54-fold upleveling of glutamate by about in regard to normal

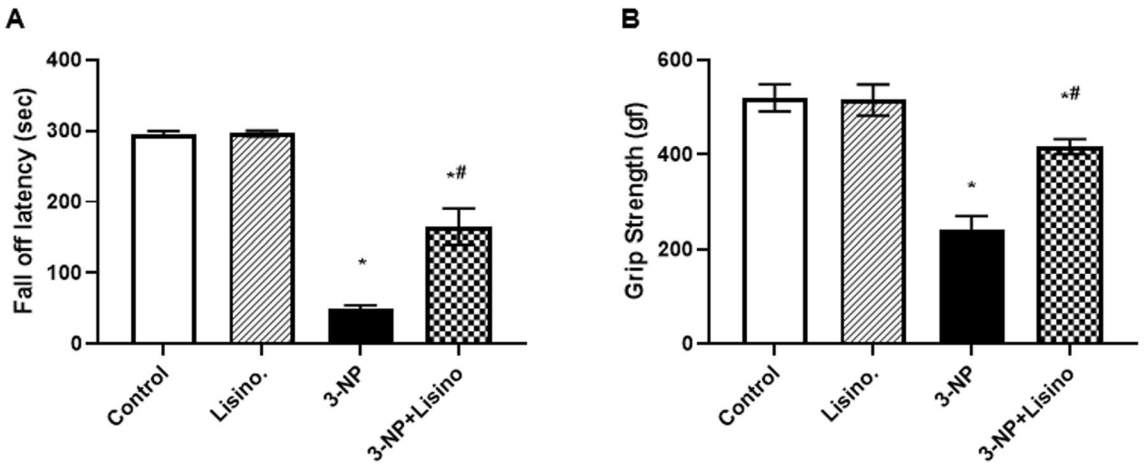


Fig. 3 The Lisinopril's impact on fall-off latency and peak force using rotarod and hand grip strength test. **A** Fall-off latency, **B** Grip strength. Data are displayed as mean ± S.D. (n = 16/group) and subjected to one-way ANOVA and Tukey's multiple comparison test for analysis. When $p \sim < 0.05$, a significant difference is noted. Comparisons that are statistically significant (*) when compared to the normal control group and (#) when compared to the 3-NP group

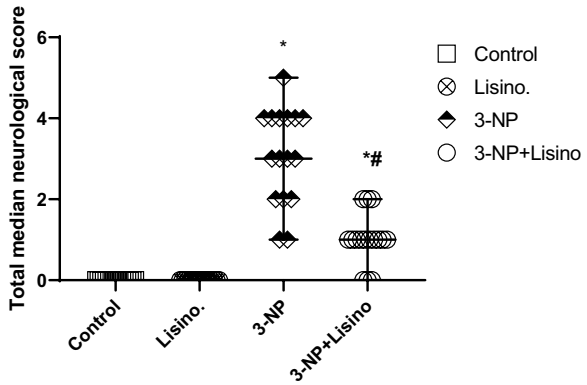


Fig. 4 The Lisinopril's impact on neurological score. Data are displayed as median and range (Min. to max.). (n = 16/group) and analyzed via Kruskal–Wallis test with Dunn's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

control group ($p < 0.0001$). On the other hand, Lisinopril promisingly amended the neurochemical perturbation by suppressing the upleveling of glutamate by about 41% ($p < 0.0001$) with a 1.14 fold increase the GABA level ($p = 0.0043$) as compared to the control group as displayed in Fig. 5.

Assessment of Lisinopril on striatal histopathological perturbations in 3-NP rats

As depicted in Fig. 6a–h, a photomicrograph of the control and Lisino groups revealed normal neuronal architecture in the striatum with well-organized intact neurons, glial cells, capillaries, and neuropil. In contrast, 3-NP-intoxicated rats exhibited noticeable structural alteration, as evidenced by degenerated, pyknotic, and disfigured neurons' glial cell proliferation, with a fried egg appearance (Fig. 7). The blood capillaries displayed marked congestion with dilatation of the peri-vascular Virchow–Robin space. Lisino markedly improved the structural degenerative changes in almost intact neurons with vesicular and prominent nucleoli. In contrast to the NC, the 3-NP group exhibited a substantial escalation in the quantity of degenerated neurons ($p < 0.0001$). Conversely, the Lisino-treated group demonstrated a remarkable decline in the degenerated neurons ($p < 0.0001$) in contrast to the 3-NP group, and mild vascular congestion could be detected as displayed in Fig. 8a.

Assessment of Lisinopril on striatal GFAP immunoreactivity in 3-NP rats

GFAP is critical in astrocyte activation, intimately supporting neuronal functions [44]. Herein, the NC exhibited homogenous GFAP immunoexpression. On the other hand, the 3-NP insult caused a substantial surge ($p < 0.0001$) in GFAP distribution by about 15.56% compared to their counterparts in the NC. The Lisino-treated group exhibited a remarkable decline ($p < 0.0001$) in GFAP immunoexpression by about 40.80% compared to 3-NP-intoxicated rats with an overall GFAP distribution of 9.33%. These results signify the potential neuroprotective effect of Lisinopril against the 3-NP insult (Fig. 7a–h) and (Fig. 8b).

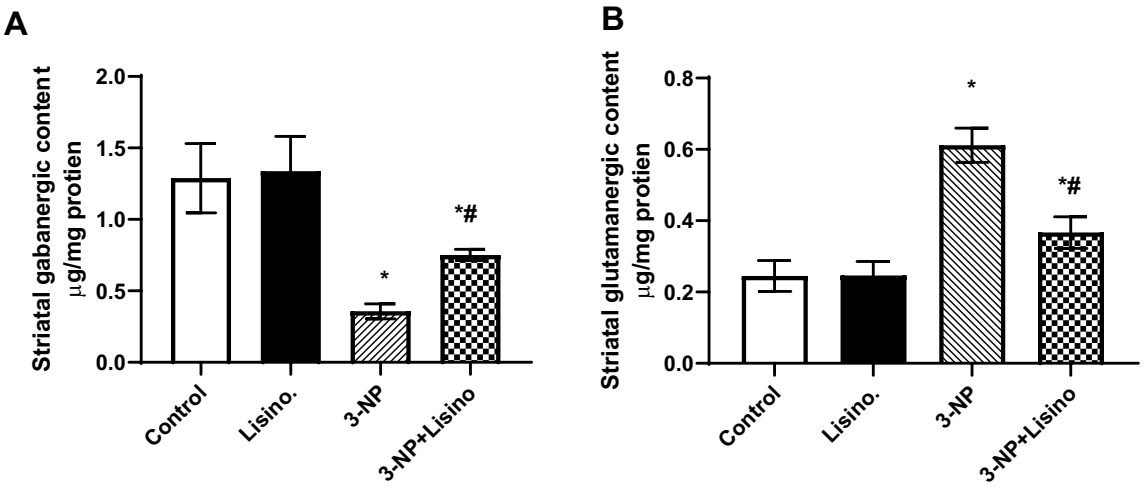


Fig. 5 The effect of Lisinopril on striatal GABAergic and glutaminergic content. **A** striatal GABAergic content, **B** striatal glutaminergic content. Data are displayed as mean ± S.D. (n = 16/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant when compared to normal control group and (#) statistically significant when compared to 3-NP group

The impact of Lisinopril on striatal redox balance in 3-NP rats

The 3-NP toxin-induced reactive oxygen species (ROS) with subsequent mitochondrial dysfunction that was evidenced by a 7.7-fold increase in the lipid peroxidation marker, MDA, with a noticeable decrease remarkable reduction ($p < 0.0001$) in the level of striatal antioxidants; GSH activity, SOD, catalase, and Nrf2 content by about 72.70%, 71%, 74%, and 83%, respectively, in comparison with the corresponding values in the standard control group. In contrast, the mitochondrial redox state was significantly augmented in the 3-NP + Lisino group. This was evidenced by a remarkable decline in MDA activity by about 75%, with a considerable surge in Moreover, the ROS production was dampened by a significant increase in the redox capacity of GSH activity, SOD, catalase as well as Nrf2 content by 187%, 139%, 119%, and 43.34%, respectively ($p < 0.0001$) as shown in Fig. 9.

The effect of Lisinopril on the striatal mitochondrial dysfunction

Our results revealed a noticeable decrease ($p < 0.0001$) SDH (complex II) activity and TFAM content by about 216% and 56%, respectively, signifying the mitochondrial respiratory chain dysfunction and ATP depletion. On the other hand, Lisinopril succeeded in mitigating the mitochondrial dysfunction that was obvious with the considerable surge in SDH activity and TFAM content by about 38% and 77.89%, respectively ($p < 0.0001$), and these findings might provide insight into the promising benefit of Lisinopril in constraining the energy depletion and subsequently improving the oxidative insult as presented in Fig. 10.

The impact of Lisinopril on striatal pro-inflammatory cytokines in 3-NP rats

The 3-NP group demonstrated significant elevation ($p < 0.0001$) of inflammatory cytokines by about 220%, 75.11%, and 95.83% in comparison with the NC regarding NF-κB, TNF-α, and IL-6 content, respectively. Moreover, there was a considerable 4.84-fold rise ($p < 0.0001$) in the level of IFN-γ in comparison with the NC. In contrast, Lisino treatment succeeded in significant damping ($p < 0.0001$) of inflammatory cytokines by 46.94%, 26.63%, and 44.23% when compared to 3-NP-intoxicated animals concerning NF-κB, TNF-α, and IL-6 content, respectively. The immunomodulatory effect of Lisino was evidenced by a significant decrease ($p < 0.0001$) in IFN-γ content by 34.34% in contrast with 3-NP group (Fig. 11).

The effect of Lisinopril on the striatal apoptotic markers

Our data revealed that 3-NP group demonstrated a significant 5.39-, 10.25-, 1.64-, and 6.16-fold increase in the striatal level of p-serP53, BAX/Bcl2 ratio, cytosolic cytochrome c, and caspase-3, respectively ($p < 0.0001$), when compared to the normal control group. On the other hand, Lisinopril-treated group revealed a striking amelioration in the striatal level of p-serP53, BAX/Bcl2 ratio, cytosolic cytochrome c, and caspase-3 by about 43.5%, 79.7%, 28%, and 24.4%, respectively ($p < 0.0001$), in contrast to 3-NP group. Notably, Lisinopril remarkably restored BAX/Bcl2 ratio back to reach approximately

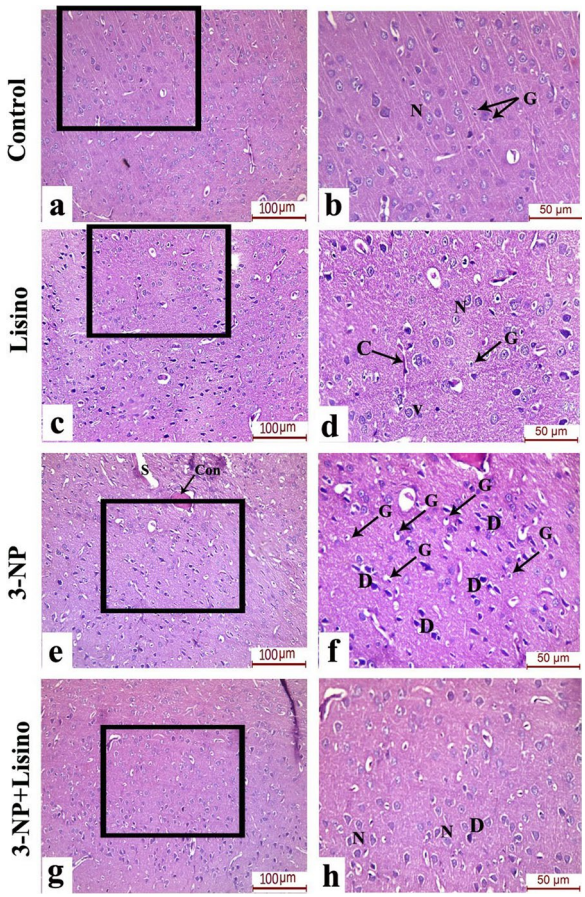


Fig. 6 Effect of Lisinopril on histopathological changes in 3-NP rats. Photomicrographs illustrating H&E staining of the striatum from **a**, **b** control group, **c**, **d** Lisinopril group, **e**, **f**: 3-NP group, **g**, **h** 3-NP + Lisino group. N: intact neurons, G: glial cells, D: degenerated neurons, C: Capillary, Con: Congestion, S: Peri-vascular space. low magnification × 200 (scale bar 100µm) in (a-g), higher magnification × 400 (scale bar 50µm) in (b-h)

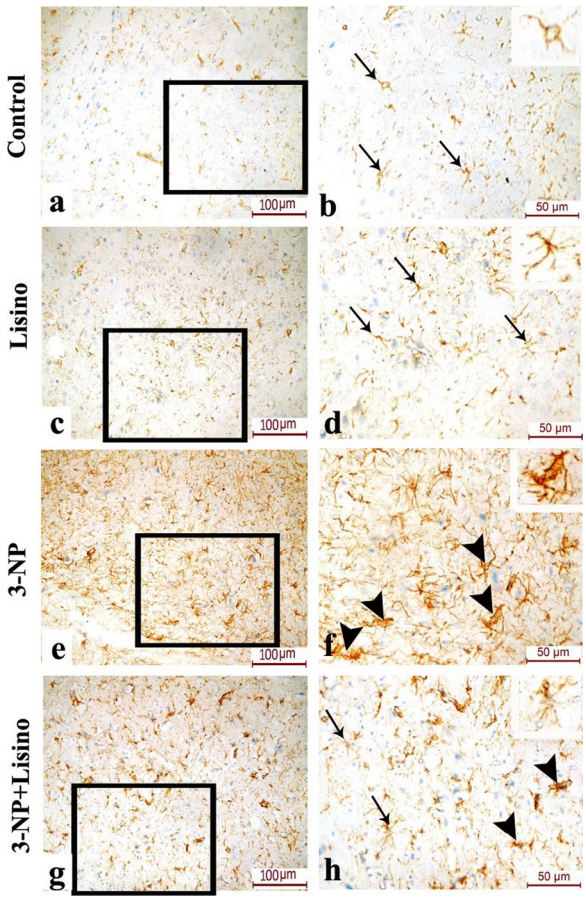


Fig. 7 The impact of Lisinopril on rat's striatal GFAP staining. Sample photomicrographs showing immunohistochemical staining of GFAP in striatum. **a**, **b**: control group, **c**, **d**: Lisinopril group, **e**, **f** 3-NP group, **g**, **h** 3-NP + Lisino. Magnifications: × 200 original Magnification (**a**–**g**) and × 400 original Magnification (**b**–**h**). Data are presented as median with interquartile range (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to NC group, (#) statistically significant as compared to 3-NP group. 3-NP; 3-nitropropionic acid, NC; normal control, Lisino; Lisinopril

its corresponding values in the normal control group (p = 0.33) positioning Lisinopril a promising candidate that can dampen the apoptotic drive in HD as presented in Fig. 12.

The influence of Lisino on the striatal counterregulatory axis of ACE2/Ang1-9/Ang1-7 protein/MasR expression

The current study demonstrated a notable down-regulation ($p < 0.0001$) of the striatal ACE2 expression by about 46.68%, along with a significant decline ($p < 0.0001$) in its downstream products, Ang1-9, and Ang1-7, by about 68.80% and 68.06, respectively. Similarly, MasR gene expression was significantly down-regulated ($p < 0.0001$) by about 64%. Conversely, Lisino administration to 3-NP-intoxicated rats remarkably upregulated striatal gene expression of ACE2 to reach approximately 1.28-fold rise compared to the 3-NP group, with subsequently 1.45 and 1.12-fold rise ($p < 0.0001$) in Ang1-9 and Ang1-7, respectively. Furthermore, Lisino meaningfully amended the decline in MasR gene expression and elevated it ($p < 0.0001$) by 100% (Fig. 13).

The impact of Lisinopril on striatal neuroplasticity markers via Pi3K/AKT/CREB/BDNF signaling in 3-NP rats

Notably, 3-NP mitigated the protein expressions of p-Pi3K, p-AKT, p-CREB, and BDNF to reach approximately 67.28%, 88.57, 62.5, 67.30, and 64.57%,

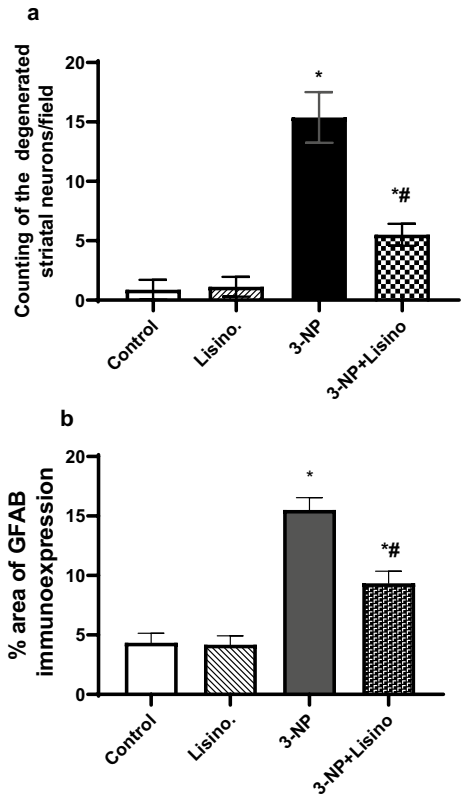


Fig. 8 The impact of Lisinopril on **a** counting of the degenerated striatal neurons per field and **b** percentage area of GFAP immunoexpression in striatal sections of the four studied groups. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

respectively, in contrast to 3-NP group. At the same time, Lisino treatment elicited a considerable increment ($p < 0.0001$) of the protein expressions of p-Pi3K, p-AKT, p-CREB, and BDNF by 88.57, 74.35, 123, and 115%, respectively (Fig. 14).

The correlation analysis between the striatal BDNF content and mRNA expression of ACE2

Pearson correlation analysis revealed a strong positive correlation between the striatal BDNF content and mRNA expression of ACE2 (r = 0.973, p value < 0.0001). The positive correlation provides a striking insight about the modulatory effect of Lisinopril on the striatal counterregulatory axis of ACE2/Ang1-9/Ang1-7 on striatal BDNF expression (Fig. 15).

Discussion

The current study sheds light on the potential neuroprotective benefit of Lisino in a 3-NP-induced HD model. Neurodegeneration induced by 3-NP in the striatum results in loss of grasp strength and movement dysfunction, analogous to the symptoms observed in late-stage HD patients [45].

Systemic 3-NP treatment replicates the late-stage symptoms of HD [46]. The current investigation confirms prior research [47, 48], demonstrating that when 3-NP is administered systemically to animals, their motor performance is significantly impaired compared to the control group of healthy rats. The motor impairment observed in the neurotoxic rats induced with 3-NP may have been caused by specific striatal lesions and neuronal damage in the cortex and hippocampus [49].

The findings revealed that Lisinopril remarkably regressed the 3-NP-induced motor deficits. The OFT findings showed shorter immobility times and a significant increase in speed, distance traveled, and rearing frequency, indicating improved functional testing. Lisinopril significantly improved grip strength, whereas it deteriorated in the 3-NP group. Moreover, Lisinopril notably improved motor balance and coordination, which have been echoed here using the rotarod test, where Lisinopril-treated rats exhibited longer fall-off latency.

Our study found that rats challenged with 3-NP showed increased neurological score indicating serious motor dysfunction. This result is in line with earlier research showing the neurotoxic consequences of 3-NP, which include paralysis of the hindlimb, poor mobility, and gait incoordination [38]. Treatment with Lisino led to a noteworthy decrease in the neurological score in the 3-NP + Lisino group, suggesting the enhanced motor function.

The observed improvement in motor performance was prominently reflected in the histopathological findings. Administration of Lisinopril ameliorated the 3-NP-induced histopathological perturbation; the neurorestorative effect was demonstrated by better morphology and structure, along with decreased degeneration, gliosis, and local inflammation.

The result presented herein, illustrated that the diffusely detected gliosis in the striatum of the 3-NP-induced HD model was further supported by the augmentation of GFAP, which can be used as an index of gliosis during neurodegeneration, being the chief intermediary filament of astrocytes and upgraded as consequence of astrocyte activation and inflammation [44]. Interestingly, Lisinopril markedly downregulated GFAP, as demonstrated in the results of IHC studies. The present results align with previous reports that revealed a significant accumulation and activation of microglial cells in the regions of the

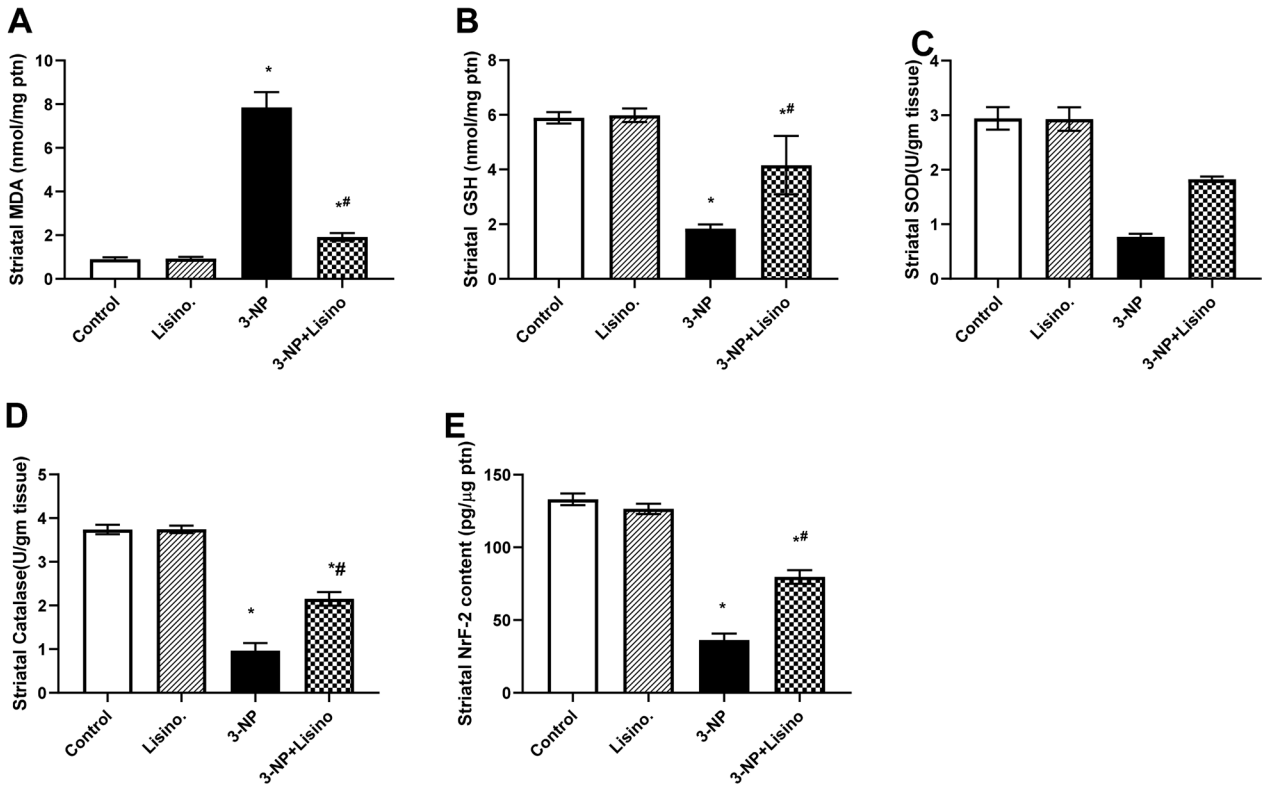


Fig. 9 The impact of Lisinopril on the striatal oxidative stress markers. **A** MDA, **B** GSH, **C** SOD activity, **D** catalase, and **E** Nrf2 content. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

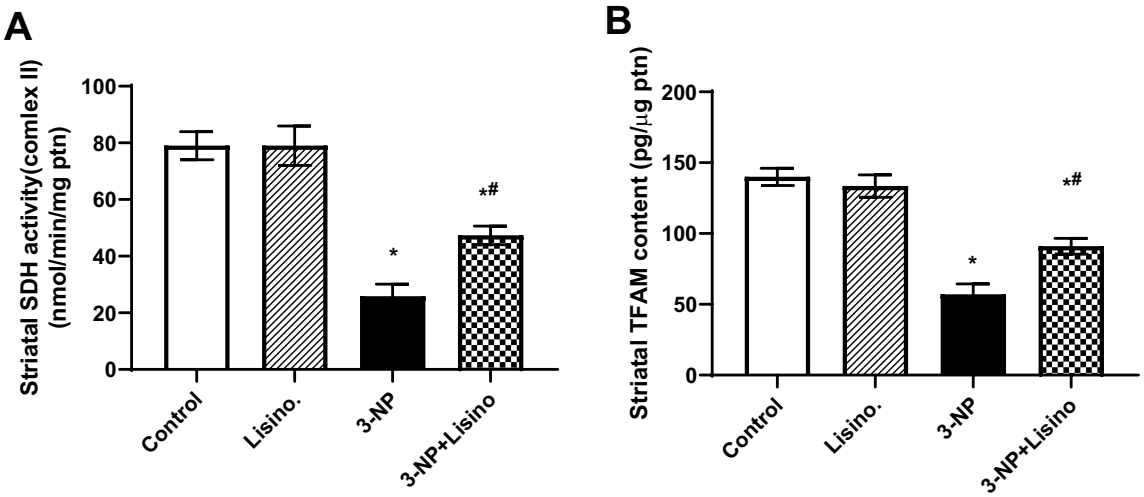


Fig. 10 The effect of Lisinopril on the mitochondrial dysfunction. **A** SDH activity and **B** TFAM content. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

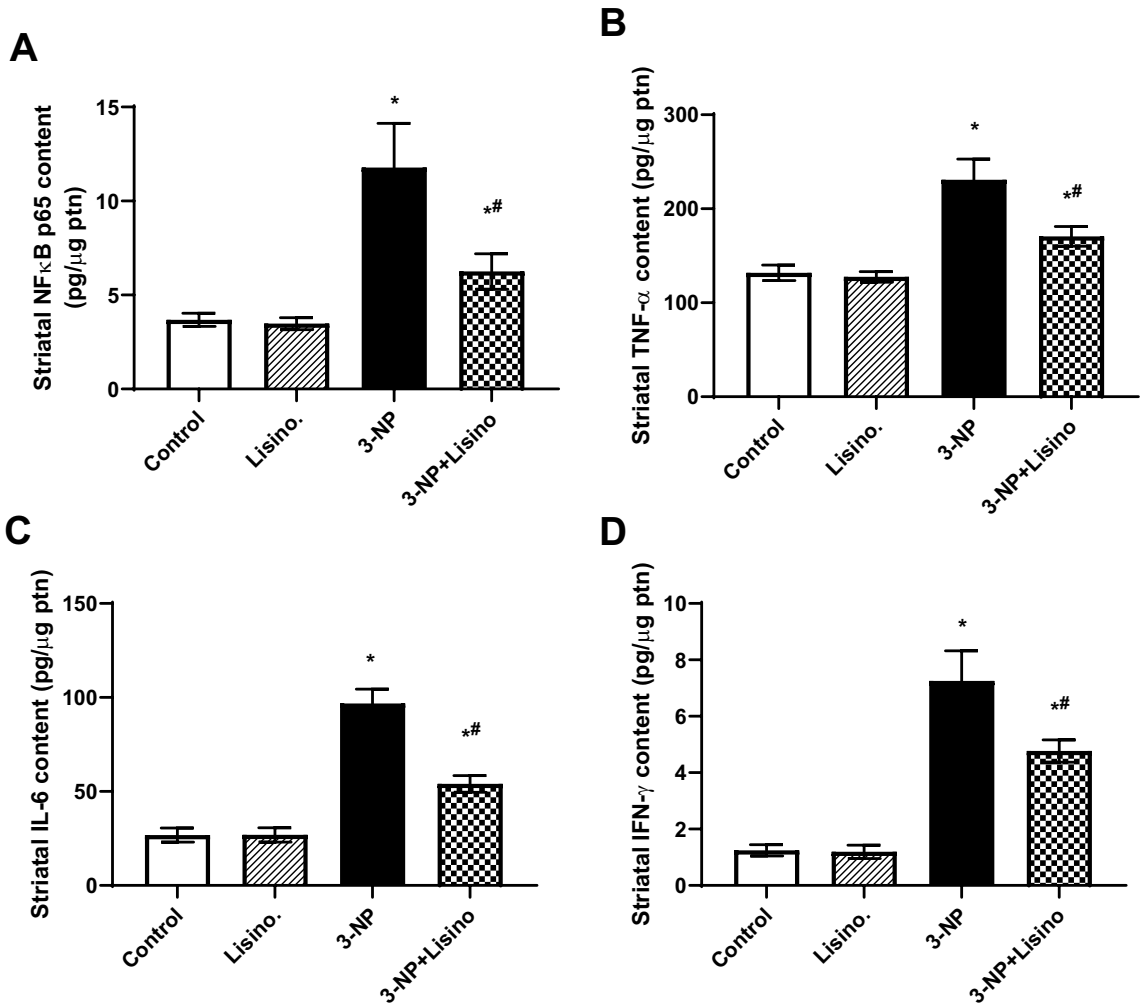


Fig. 11 The effect of Lisinopril on the pro-inflammatory cytokines. **A** NF-κB p65 content, **B** TNF-α content, **C** IL-6 content, **D** IFN-γ content. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

brain affected by HD, which correlates with the degree of neuronal loss [45].

Dysfunction of both GABAergic and glutamatergic neurons plays a pivotal role in the pathogenesis of HD. mHTT induces changes in GABAergic inhibitory networks, which occur before overt symptoms in HD manifest. mHTT disrupts transcriptional regulation by inhibiting key transcriptional activators and repressors, leading to gene expression dysregulation. mHTT interacts with Huntingtin-associated protein 1 (HAP1), disrupting $GABA_AR$ trafficking and lowering synaptic inhibition. mHTT modifies GABA receptor signaling, impacting neuron integrity, synaptic transmission, and receptor subunit composition [50].

Furthermore, earlier research has demonstrated the function of inflammatory mediators in HD brains, such as TNF-a, IL-1b, and NF-kB, and how they impact GABAergic neurotransmission and neuronal excitability. Neuroinflammation and reactive gliosis worsen neuronal dysfunction; mHTT intensifies these conditions. GABA receptors are adversely affected by neuroinflammation, which further reduces the strength of inhibitory synapses [51, 52]

Additionally, prior research has demonstrated a particular degeneration of striatal medium spiny neurons (MSNs) as a result of high levels of glutamatergic excitotoxicity and metabolic stress, which may be a factor in HD's motor deficiencies. Motor deficiencies in HD are further exacerbated by changes in the striatal

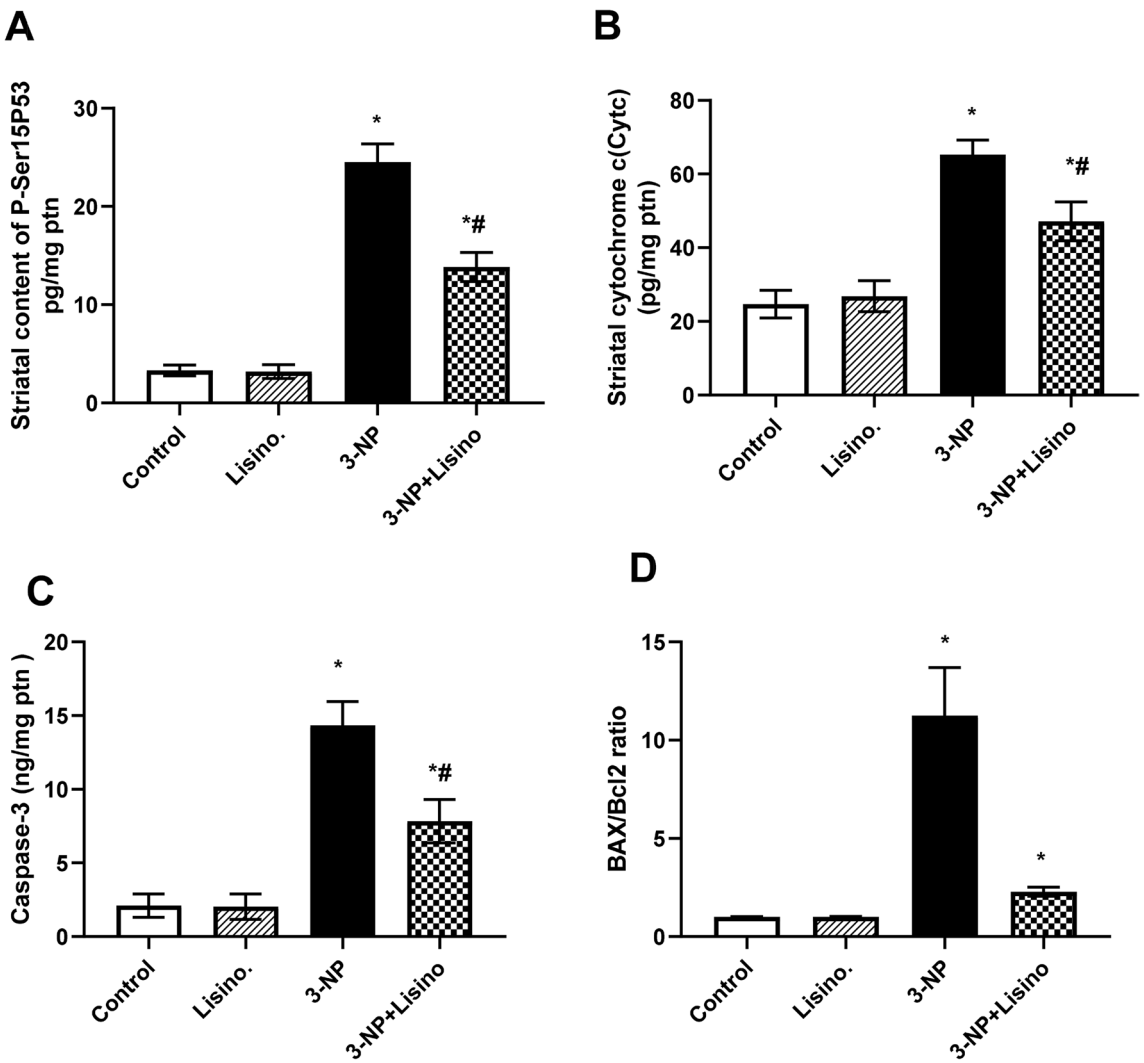


Fig. 12 The effect of Lisinopril on apoptotic parameters. **A** p-Ser-15P53 content, **B** Cyt c content, **C** caspase-3 content, **D** Bax/Bcl2 ratio. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to normal control group and (#) statistically significant as compared to 3-NP group

glutamatergic transmission, such as lower spine densities and increased glutamate release [50, 53, 54].

The results of our study revealed a significant decline in striatal GABA content and a concomitant upregulation of glutamate levels in 3-NP-intoxicated rats compared to the normal control group, reflecting disrupted inhibitory-excitatory balance in the striatum, a hallmark feature of HD pathophysiology. Various studies have shown that 3-NP selectively targets GABAergic neurons, disrupting the balance between excitatory and inhibitory signaling, leading to neuronal hyperexcitability and excitotoxicity. Additionally, it alters glutamatergic neurotransmission, exacerbating excitotoxicity and neuronal damage [55, 56]. On the other hand, our results showed that Lisino treatment exhibited encouraging neuroprotective effects, reducing the 3-NP-induced neurochemical disruption. Compared to the 3-NP group, Lisinopril significantly reduced the elevation of glutamate levels and increased GABA levels, suggesting that it may be able to regulate neurotransmitters and lessen the excitotoxicity linked to HD.

Moreover, the current research evaluates the effect of Lisinopril on striatal oxidative stress. 3-NP, a non-competitive inhibitor of complex II of mitochondrial SDH, causes mitochondrial dysfunction, oxidative phosphorylation disruption in the tricarboxylic acid cycle, loss of membrane potential, and mitochondrial dysfunction, all contributing to cellular energy deficit and oxidative

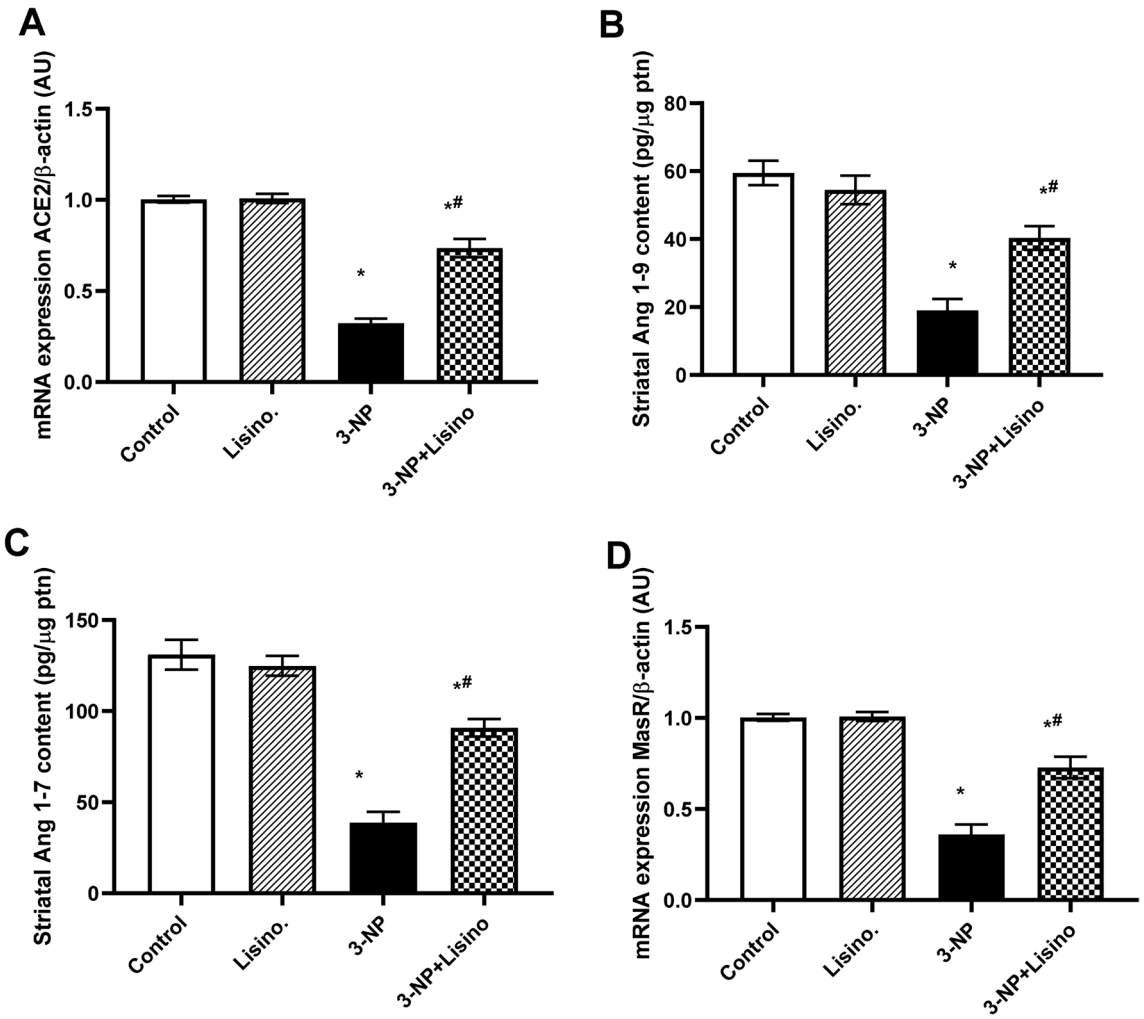


Fig. 13 The effect of Lisinopril on striatal counterregulatory axis of ACE2/Ang1-9/Ang1-7 protein expression. **A** ACE2 gene expression, **B** Ang 1–9 content, **C** Ang 1–7 content, **D** MasR expression. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to NC group and (#) statistically significant as compared to 3-NP group

stress. Ultimately, these events cause mitochondrial dysfunction and ATP depletion and cause an increased metabolism of fat and stored glycogen, resulting in weight loss. Additionally, anorexia and reduced food consumption connected with motor deficits and bradykinesia can contribute to the observed weight loss [57, 58]. Consequently, Lisino treatment effectively halted the progression of weight loss in the animals. This effect may be related to improving motor deficits with the Lisino treatment.

The current study detected that Lisinopril treatment alleviated the striatal oxidative stress as it significantly increased the level of striatal antioxidants: GSH, SOD, catalase, and Nrf2 content, as well as ameliorated lipid peroxidation as it reduced the level of MDA in comparison with the 3-NP-only treated group. Consistent with the current findings, prior research has documented that applying 3-NP led to heightened lipid peroxidation in brain tissue, leading to elevated MDA levels and a reduction in the activity of antioxidant defense mechanisms such as GSH [39, 49, 59]. MDA, the lipid peroxidation product, could potentially serve as a biomarker to evaluate the therapeutic effectiveness of the diverse medications employed in HD [60]. Moreover, it was documented that Nrf2 induces the transcription of BDNF and hence, it shares partially in the uplevelling of BDNF [61]

The cAMP-response element-binding protein (CREB) targets nuclear factor erythroid-2-related factor (Nrf2) in its primary transcriptional pathway [62] that has

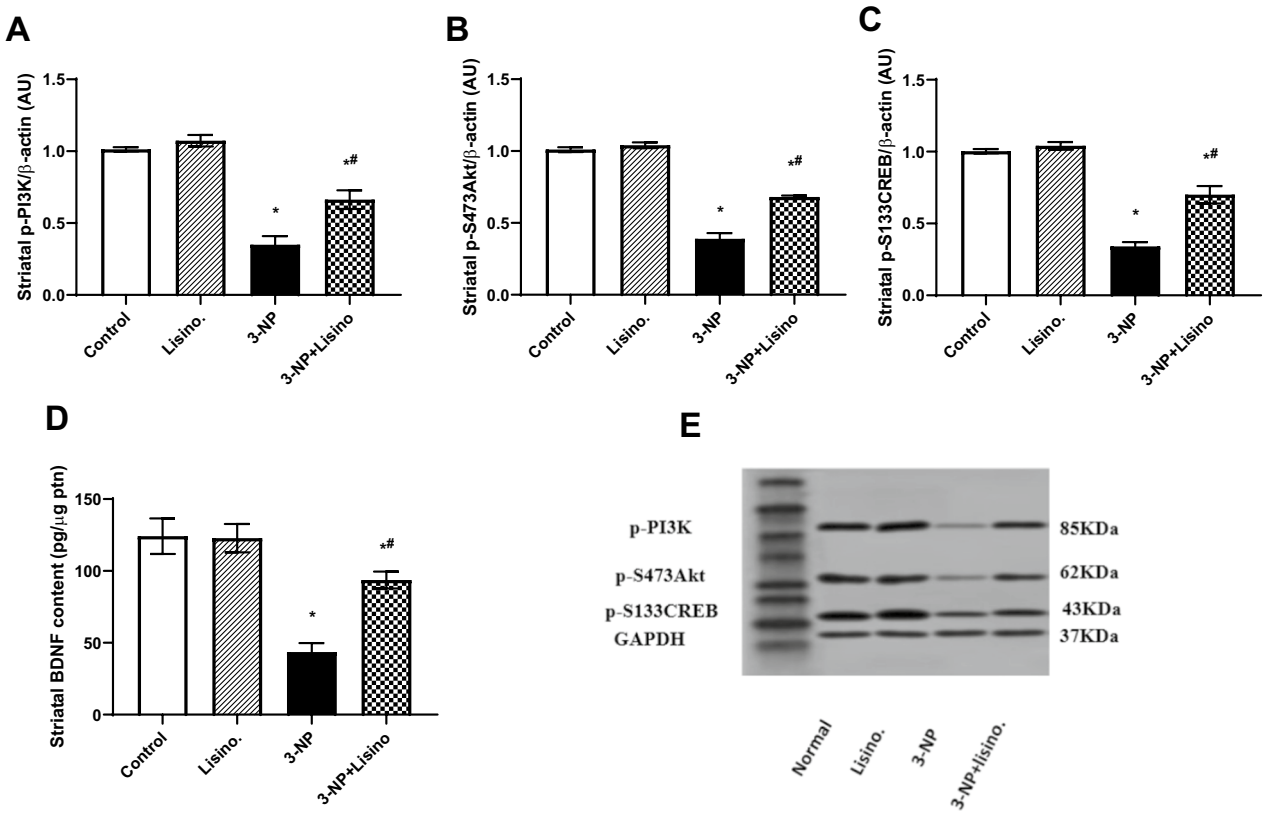


Fig. 14 The effect of Lisinopril on striatal neuroplasticity-related proteins. **A** p-PI3K protein expression, **B** p-S473Akt protein expression, **C** p-S133CREB protein expression, **D** BDNF content, **E** densiometric band expression of *Pi3K/AKT/CREB*. Data are presented as mean ± S.D. (n = 8/group) and analyzed via one-way ANOVA followed by Tukey's multiple comparison test. A significant difference is reported when $p < 0.05$. Comparison (*) statistically significant as compared to NC group and (#) statistically significant as compared to 3-NP group

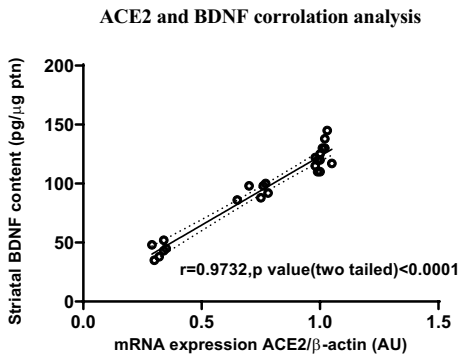


Fig. 15 Correlation analysis between the striatal BDNF content and mRNA expression of ACE2. Data analyzed by Pearson correlation coefficient

been characterized as a crucial protective mechanism against oxidative stress in the brain by increasing mitochondrial biogenesis, removing ROS and elevating antioxidant enzymes [63]. Furthermore, Nrf2 functions as an anti-inflammatory mediator within the brain, inhibiting neuroinflammation initiated in response to detrimental stimuli associated with neurodegenerative disorders [64]. The mitochondrial transcription factor A (TFAM) and Nrfs are powerful boosters of the expression of nuclear genes essential for mitochondrial respiratory function [65–67]. TFAM is a protein that binds to mitochondrial DNA (mtDNA). TFAM determines the abundance of the mitochondrial genome by fine adjusting its stability and replication. TFAM inhibits the detrimental consequences that may arise from cytosolic release and disruption of mtDNA, which initiates pro-inflammatory signals [68]. There is increasing evidence from animal, and clinical studies have revealed that TFAM levels are notably declining in HD. A decline in TFAM protein amounts correlates with a decline in the number of mitochondria, altered mitochondrial structure, and disruption of the highly delicate balance between the dynamic cycle of mitochondrial fission and fusion [69]. The study found a significant decrease in SDH (complex II) activity and TFAM content in 3-NP challenged rats, indicating mitochondrial respiratory chain dysfunction and ATP depletion. However, Lisino effectively mitigated

these issues, resulting in a surge in SDH (complex II) activity and TFAM content, suggesting its potential role in reducing energy depletion and improving oxidative insult. The findings of this study are consistent with those of Hariharan et al., who examined the impact of the ACE inhibitor trandolapril on an experimental HD model. The administration of trandolapril stopped motor and behavioral disorders and reduced oxidative stress in the brains of rats. Trandolapril additionally recovered the number of mitochondrial enzyme complexes, improved the concentration of antioxidant enzymes, and decreased the peroxidation of lipids [25].

Apoptosis plays a major role in the neurodegeneration accompanying HD. This can be ascribed to the depletion of energy, stress caused by oxidation, and an increase in mitochondrial-dependent apoptosis [70]. Apoptotic pathways are triggered when mHTT impairs mitochondrial function by changing calcium handling, increasing oxidative stress, and compromising mitochondrial dynamics. These events intensify apoptotic signaling and neuronal death by activating P53 with subsequent increasing BAX/Bcl2 ratio. The mismatch between pro-apoptotic Bax and anti-apoptotic Bcl2 proteins in HD favors mitochondrial outer membrane permeabilization, facilitating the release of apoptogenic factors like cytochrome c into the cytosol and promoting the activation caspase-3, a crucial executioner caspase [71, 72]. Recent research suggests that p53, a tumor suppressor protein, can play a role in neurodegenerative disorders. Its activity in microglia, brain's immune cells, can promote pro-inflammatory phenotypes and neurodegeneration. Studies show that p53 activity in microglia can contribute to synaptic degeneration in neurons during neuroinflammatory diseases [73]. Comparing 3-NP animals to the normal control group, our data showed a significant rise in the striatal levels of p-ser15p53, the BAX/Bcl2 ratio, cytosolic cytochrome c, and caspase-3. Lisinopril, on the other hand, dramatically attenuated these alterations, suggesting that it may be able to mitigate apoptotic changes brought on by 3-NP.

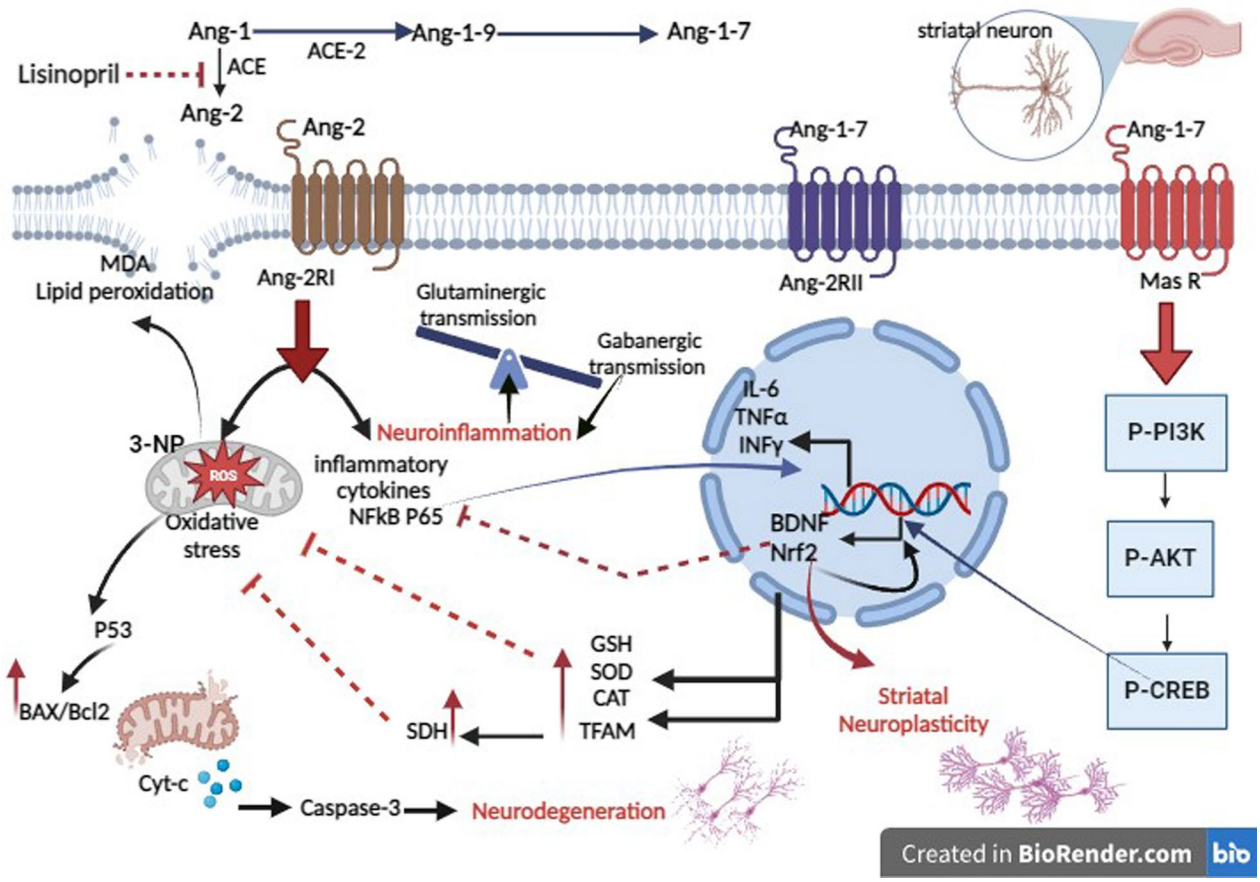
The pro-inflammatory cytokines that can induce inflammation significantly contribute to the onset of numerous neurological diseases, including HD [74]. When microglial cells notice a warning signal to the CNS's homeostasis, such as excessive ROS, they release a range of cytokines and pro-inflammatory mediators to defend against the threat. While this acute response aims to maintain CNS homeostasis, the prolonged microglial activation is linked to numerous neurodegenerative illnesses [75]. Mutant HTT-expressing microglia produce pro-inflammatory cytokines, chemokines, complement factors, nitric oxide, free radicals, and proteases, leading to neurodegeneration and brain damage. Pro-inflammatory mediators like matrix metalloproteinase can compromise the blood–brain barrier, allowing peripheral immune cells (activated T cells) to infiltrate the brain. This, in turn, triggers the release of more inflammatory molecules by neurons, astrocytes, and microglia, exacerbating neuroinflammation and neurodegeneration. Moreover, previous studies showed that increased NF-κB-p65 activation contributes to neurotoxicity and NF-κB signaling pathway dysregulation results in the overabundance of inflammatory cytokines in HD. NF-κB and nuclear factor of activated lymphocytes (NFAT) enhance the transcriptional activation of the INF-γ gene, resulting in increased INF-γ production. Also, it was documented previously that elevated INF-γ levels in HD are linked with the severity of the disease [76, 77]. By evaluating the level of the pro-inflammatory cytokines, this study detected that Lisino significantly ameliorated the synthesis of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interferon-gamma (INF-γ), and nuclear factor kappa (NF-Kβ) that were markedly elevated in the 3-NP group.

In addition to its widely recognized functions in the cardiovascular and renal systems, components of RAS have been detected in the brain [78]. This discovery has stimulated research into the potential pathophysiological involvement of RAS in neuropsychiatric diseases and neurodegenerative disorders, including AD and PD [79, 80]. Limited research has been conducted on the role of RAS components in HD [26]. In the past, elevated ACE activity in the cerebrospinal fluid of HD patients was identified [81]. Furthermore, changes in the concentrations of AT1 and AT2 receptors in brain homogenates from patients with HD have been documented in prior research [82]. This suggests that components of the RAS classical axis may be involved in the pathogenesis of HD. The molecular mechanism through which Lisinopril, an ACE inhibitor, is hypothesized to exert its neuroprotective effect was investigated considering these results.

There has been prior research on the function of RAS in inflammatory processes. The counterregulatory arm of the RAS, the ACE2/Ang-(1–7)/Mas receptor, has anti-inflammatory characteristics, whereas the conventional arm, the ACE/Ang II/AT1 receptor, promotes many inflammatory pathways [83]. A component of the RAS counterregulatory axis, MasR, is expressed in several brain regions. Through a rise in PI3K/Akt, it can counteract the negative effects of the ACE/Ang II/AT1R axis and provide neuroprotective benefits [84, 85]. PI3K activation induces phosphorylation of Akt, which in turn triggers phosphorylation and activation of cAMP-response element-binding protein (CREB). Phosphorylated CREB, through transcription of the brain-derived neurotrophic

factor (BDNF) and its receptor TrKB, is considered the cornerstone of neurotrophin-mediated neuronal survival [86]. A feed-forward loop is created as CREB restimulates the MasR and PI3K/Akt axes, promoting neurogenesis and neuronal survival. Additionally, phosphorylated TrKB re-activates CREB to maintain this cascade [87].

The findings of the present investigation demonstrated that 3-NP downregulated ACE2 and decreased the magnitude of striatal Ang1-7 and Ang1-9. Moreover, 3-NP reduced the Mas receptor's expression with the stimulation of PI3K, Akt, CREB, and BDNF proteins. Interestingly, the findings of the present investigation demonstrated that Lisinopril upregulated ACE2 and promoted the levels of striatal Ang1-7 and Ang1-9. The present research indicated that treatment with Lisinopril had a neuroprotective impact against 3-NP through the upregulation of MasR protein expression and the subsequent activation of the PI3K/Akt/CREB/BDNF cascade. Additionally, the study found a strong positive correlation between striatal BDNF content and ACE2 mRNA expression, suggesting that Lisinopril modulates the striatal counterregulatory axis of ACE2/Ang1-9/Ang1-7. These results were consistent with those obtained by Kangussu et al., where the transgenic model of HD demonstrated a notable decrease in the activity of the ACE2/Ang-(1–7)/MasR axis in crucial brain regions linked to HD, including the striatum and hippocampus [79]. Furthermore, these findings align with those of Rocha et al. regarding Alzheimer's patients, who discovered a positive correlation between elevated plasma levels of ACE2 and enhanced verbal function [88]. Consistent with the present findings, Liu et al. and Kehoe et al. [80, 89] noted that people with Alzheimer's had decreased ACE2 activity relative to controls. Steventon et al. demonstrated that undiagnosed hypertensive HD patients, irrespective of the antihypertensive agent, experienced a more rapid decline in motor functions and a more pronounced worsening of symptoms than treated patients [90]. This can



Scheme 2 Schematic representation of the proposed therapeutic role of Lisinopril in ameliorating 3-nitropropionic acid-induced Huntington's disease in rats. Lisinopril upregulated ACE2/Ang1-7/MAS receptor axis with downstream activation of PI3K/Akt pathway and evoked the neurogenesis signal CREB/BDNF. Blocking AT2R1 receptor alleviated the mitochondrial dysfunction, decreased the elevated levels of lipid peroxidation, and improved the antioxidant defense by recovering SDH, GSH, and Nrf2/TFAM signaling and downregulating the inflammatory cytokines in addition to suppressing the apoptotic cascade

support the present finding that blocking the traditional axis of RAS, ACE/Ang II/AT1R, by Lisinopril improved the locomotor deficit and the histopathological alternations induced by 3-NP. To the author's knowledge, no previous reports have assessed the impacts of the anti-RAS drugs in HD patients with normal blood pressure, apart from a study that investigated the efficacy of RAS blockade (RASB) and the frequency of mental retardation in AD [91]. Their results revealed that RASB reduces cognitive impairment and AD risk. In addition, Pelisch et al. reported that therapy with AT1 receptor inhibitors improves memory problems in a manner independent of blood pressure by decreasing the blood–brain barrier permeability [92].

The strength of that work lies in investigating and studying the neuroprotective effect of Lisinopril against a 3-NP-induced HD-like model. The results provided compelling evidence regarding the improved locomotor functions and recovery of the striatal architecture which is severely perturbed in the 3-NP group. Lisinopril enhanced the neuroprotective signaling pathways and abrogated others critical for neurodegeneration.

Limitations of the study

This study is that the experiment was conducted exclusively on a single HD model and only in male Wister rats. We did not measure the regulatory Keap1 expression and the nuclear translocation of Nrf2, as well as the phosphorylation status and nuclear localization of NF-κB subunits. Therefore, we plan to include them in future work to provide a detailed understanding of their regulatory mechanisms. Also, we did not perform TUNEL staining which detects DNA fragmentation, which is a hallmark of apoptosis (programmed cell death) As a result, the authors recommend further investigation into various HD models, species, and genders. Moreover, additional experimental works including TUNEL staining, the regulatory Keap1 expression, and the nuclear translocation of Nrf2, as well as the phosphorylation status and nuclear localization of NF-κB subunits, are needed to validate these molecular pathways.

Conclusion

The present investigation documented the neurological protective properties of Lisinopril in an HD rat model. Lisinopril significantly ameliorated the motor and histopathological alternations induced by 3-NP. The results of the present investigation revealed the potential molecular mechanisms for the neuroprotective role offered by the ACE inhibitor, Lisinopril, in the 3-NP-induced HD animal model. A neurogenesis signal, CREB/BDNF, was induced by Lisinopril, which upregulated the expression of the ACE2/Ang1-7/MasR axis and activated the PI3K/Akt/CREB/BDNF signaling pathway. Furthermore, Lisinopril dampened the inflammatory cytokines production, apoptosis, mitochondrial dysfunction indicators and decreased the increased concentrations of peroxidation of lipids with upgrading of the striatal scavenging capacity by recovering SOD, catalase, GSH and Nrf2. Notably, the outcomes of this study might position Lisinopril as a future neuroprotective therapeutic candidate against HD (Scheme 2).

Abbreviations

3-NP	3-Nitropropionic acid.
ACEIs	Angiotensin-converting enzyme inhibitors
AKT	Protein kinase B
BDNF	Brain-derived neurotrophic factor.
CAG	Cytosine, adenine, guanine.
CREB	CAMP-response element-binding protein
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats.
GFAP	Glial fibrillary acidic protein
HD	Huntington's disease.
IL-6	Interleukin 6
MasR	MAS receptor
mHTT	Mutant Huntington protein
NFκB	Nuclear factor kappa
NFAT	Nuclear factor of activated T cells
Nrf2	Nuclear factor erythroid 2-related factor 2
PD	Parkinson's disease.
PI3K	Phosphatidylinositol 3-kinase
RAS	Renin–angiotensin system.
SDH	Succinate dehydrogenase
TFAM	Mitochondrial transcription factor A
TNFα	Tumor necrosis factor-alpha

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Author contributions

Hanaa Wanas and Amira Khalifa contributed to conceptualization; Mostafa Rabie and Amira Khalifa were involved in formal analysis; Basma Aboulhoda contributed to investigation; Hanaa Wanas, Mostafa Rabie, and Amira Khalifa were involved in methodology and writing—original draft; and Nagwa Ramadan, Sahar Khedr, Sara Abdallah, Eid Musa, Leyan Nasruddeen Khayruddeen, Yasir Hassan Elhassan, and Hadel Alghabban contributed to writing—review and editing. All authors reviewed the results and approved the final version of the manuscript.

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Availability of data and materials

Data will be made available on reasonable request.

Declarations

Ethics approval and consent to participate

The investigational protocol was revised and approved by the Institutional Animal Care and Use Committee, Cairo University (CU-IACUC) (Approval number: CU-III-F-4922), in accordance with ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals protocol (NIH publication).

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

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