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Original Research Article

Multifaceted Neuroprotection by Magnolol in a 3-Nitropropionic Acid-Induced Rat Model of Huntington's Disease: Restoring Behavior, Redox Balance, and Neurotrophic Signaling

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Abstract: Huntington's disease (HD) is an insidious neurodegenerative pathology characterized by motor deficits, progressive cognitive impairment, and neurodegeneration of the striatum, and the existing disease-modifying treatment options are still limited. The 3-nitropropionic acid (3-NP) is a mitochondrial toxin that causes neuropathology that reproduces the prominent signs of HD in rodents. Magnolol powder is imported from Xi'an Chen Lang Bio Tech Co., Ltd, China. It possesses antioxidant, anti-inflammatory, and neurotrophic functions, but little has been done to investigate its effects in HD models. Premise: This research investigated whether or not magnolol may have neuroprotective effects against 3-NP induced HD-like neurotoxicity in a rat model. ***Methods:*** Adult Wistar rats were assigned randomly in six groups: vehicle control; magnolol alone *per se*; 3-NP disease control (10mg/kg/i.p); tetrabenazine (2mg/kg) plus 3-NP, and two treatment groups treated with magnolol (20 or 40 mg/kg/p.o.) plus 3-NP. The treatments were done on a daily basis for 21 days. It was the behavioral testing, which consisted of rotarod performance, grip strength, locomotor activity, and Morris water maze. After euthanasia, striatal tissues were collected to measure the markers of oxidative stress (MDA, GSH, SOD, CAT, nitrite), pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) and neurotransmitter levels (glutamate, GABA) and BDNF levels (qRT-PCR and western blot), Apoptotic signaling (cleaved caspase-3), and histopathologic assessment. ***Findings:*** The administration of 3-NP caused significant motor incoordination, muscle weakness, a decrease in spontaneous activity, and spatial memory deficits. Biochemical studies demonstrated high oxidative stress and pro-inflammatory cytokine level, increased glutamate, reduced antioxidant defenses, reduced GABA, and repressed BDNF gene expression; caspase-3 activity and neuronal degeneration of the striatum were also observed. All behavioural deficits were dose-dependently ameliorated by pre-treatment with the higher doses of 40 mg/kg of magnolol. It restored oxidative/antioxidative balance, reduced neuroinflammation, re-established the balance of glutamate/GABA, BDNF upregulation, and prevented apoptosis. The striatal neuronal architecture was supported by histopathology. The neuroprotective effect of high-dose magnolol was comparable to or higher than that of the reference tetrabenazine drug. ***Conclusion:*** Magnolol offers global neuroprotection of the 3NP rat model of Huntington disease through amelioration of behavioural pathology, oxidative stress, neuroinflammation, excitotoxicity, and apoptosis, and improvements in neurotrophic support. These results support the multi-target action mechanism and present magnolol as a phytotherapeutic candidate of HD treatment.

Keywords: Huntington's disease; 3-nitropropionic acid; Magnolol; Neuroprotection; Oxidative stress; Striatum.

INTRODUCTION

Huntingtin (Htt) is, essentially, ubiquitous in the body. It is a giant protein consisting of more than 3,100 amino acids and made by the HTT gene located on the short arm of chromosome 4 [1]. Huntington's disease (HD) is an inherited neurodegenerative disease that is a rather ugly one. It is caused by an unstable expansion of CAG repeats in the exon protein 1 in the HTT gene - over 36 repeats in mutant protein Htt (mHtt) results in a polyglutamine (polyQ) tract [2]. A clinical description dates back to 1872 with George Huntington publishing his treatise, On Chorea, and HD is presented as a ruthless trifecta of chorea, cognitive problems, and psychiatric problems that entirely devastate people with their lives in shambles [3]. The genetics of the disease were discovered in 1993, yet the symptoms remain largely different due to the variations in CAG sequences, modifier genes, and social influences that may delay a diagnosis [4]. The earliest confirmed case was in Malaysia in 1994, when a 40-year-old man reported two-year memory loss and had motor problems for a decade; this was followed by a national registry at the University of Malaya Medical Centre in 1995 [5]. The meta-analyses demonstrate that HD is not uniform worldwide: the total world prevalence is 5-10 per 100,000 in the West, whereas it is 0.70 per 100,000 (95 percent confidence interval of 0.44-1.0) in populations in Asia, likely due to less expansive CAG in C-haplogroup families and non-reporting in low-resource environments [6,7]. Nevertheless, in Asia and Africa, big data information is lacking, and we are relying mostly on isolated case reports rather than systematic research [8].

HD typically begins at the age of 30–50, and individuals live 15-20 years following the diagnosis; cases in children (below 21) are hyper-aggressive, and in the elderly (above 60), the disease is less aggressive [9]. The key symptoms are a slow set of psychiatric changes, including irritability, apathy, depression, psychosis, and a decrease in executive (planning, flexibility, abstract thinking) and memory impairment, whereas the language remains relatively intact [10, 11]. The motor symptoms are between hyperkinetic chorea and hypokinetic rigidity, which later come to leave them incapable of walking, difficulties of swallowing, and they may talk but only without utterances, which fundamentally deprives them of the independence aspect, and there remains a probability of pushing them to institutions [12]. Besides that, suicide risk is increased 5-10 times in HD, hence the need for robust psychosocial support [13]. In the end, there is extreme atrophy of striatal or cortical neurons (previously, most of the medium spiny neurons), which causes significant debilitation [14]. Still, we can only treat the disease through palliative measures: vesicular monoamine transporter (deuteretrabenazine) hitchhikers, which adjust the dopamine, help with chorea, but they do not prevent the disease from progressing [15]. New studies are attempting to target the mutant Htt aggregates, transcription issues, excitotoxicity, mitochondrial energy dysregulations, and inflammatory mechanisms, and stem cell transplants and caspase blockers are under study [16]. AAV5-miHTT gene therapy showed a 75% reduction of disease progression in early clinical tests [17, 18]. Another method is pridopidine, which demonstrates a small improvement in the motor in certain groups in the phase 3 [18]. Despite these developments, multi-target neuroprotectants are still required in the case of oxidative, inflammatory, and metabolic issues.

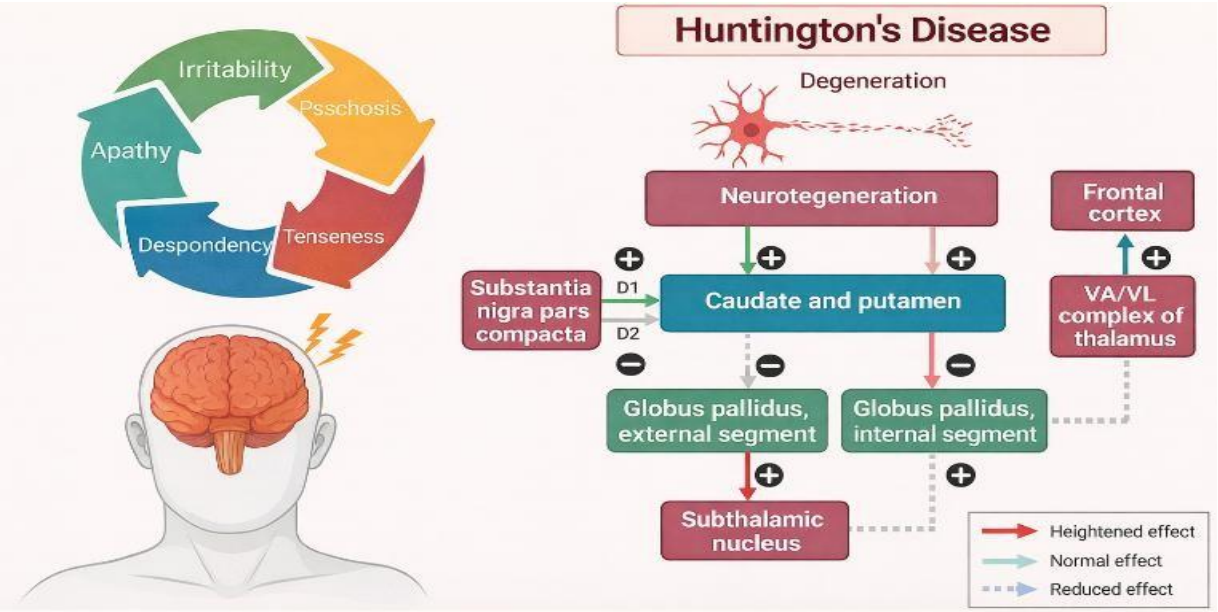

Fig. 1: Huntington's Disease: Neurobehavioral Symptoms and Abnormal Circuitry of the Basal Ganglia.

In essence, the figure is an additive representation of the key psychiatric symptoms, illustrating how the basal ganglia pathways become disorganized as neurons in Huntington's disease degenerate. These are arrows coded in colour, indicating whether the activity is up, normal, or down at main locations such as the cortex, caudate-putamen, globus pallidus, subthalamic nucleus, and thalamus.

Pathways involved in HD. Mutant Htt produces atrophy of the striatal medium spiny neurons, which results in diminished metabolism of the caudate-putamen and globus pallidus and downstream disinhibition of subthalamic outputs and augmented thalamic drive to the motor cortex- causing chorea. Additional extra-striatal damage depletes hypothalamic, thalamic, and nigral integrity, distorting substance P, enkephalin, and dynorphin loops and intensifying excitotoxic cascades and cell death. VA = ventral anterior nucleus; VL = ventral lateral nucleus.

In the laboratory, transgenic mouse models such as R6/2 and zQ175 knock-ins are used to recapitulate polyQ accumulation and synaptic loss, and the electron transport chain is halting with 3-nitropropionic acid (3-NP), a fungal toxin that crosses the blood-brain barrier and indefinitely inhibits succinate dehydrogenase in complex II to induce a reduction in ATP, halt the electron transport chain stalling, and release waves of reactive oxygen and nitrogen ions onto the brain [19]. In laboratory mice that received 10-20 mg/kg/i.p, short-term exposure induces dose-dependent impaired movement, rotarod performance, and memory loss, as well as striatum gliosis, hippocampal vulnerability, and cortical atrophy, which resembles the changes of the brain in HD [20, 21]. This condition ignites NF 2 kβ-mediated inflammasomes, NLRP3, and RIPK1/3 MLKL necroptosis, increasing calcium overload via glutamate and synapse loss [22, 23]. Recent research involving conifer aldehyde inhibition of JAK2/STAT3 and barbigerone - an inducer of Nrf2 indicates that 3 - NP is a godsend to test neuroprotectants of plant-based origin [24, 25]. Essentially, the 3-NP model is a fantastic laboratory instrument that can be used to investigate the energy-inflammation relationship of HD.

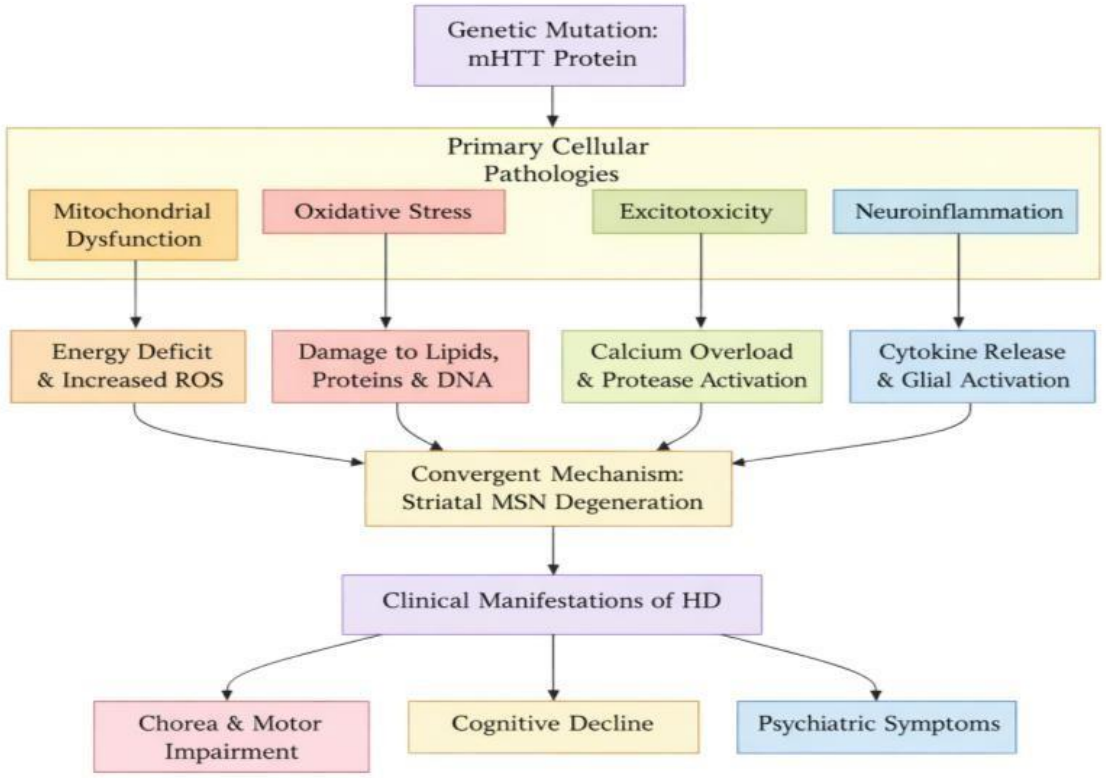

Fig. 2 Schematic representation of cellular mechanisms leading to striatal degeneration in Huntington's disease

A popular example of a natural compound commonly utilized in traditional East Asian medicine is magnolol $C_{18}H_{18}O_2$; 266.33 g/mol; systematically 5,5′ -diallyl 2,2 -biphenyl diol) (Fig. 3) a compound of a Magnolia officinalis tree that is found in its bark. It exists in 28 plant families, and since it is lipophilic, it may enter the brain through paracellular transport and not through some special carriers [26]. It enhances Nrf2/HO-1, reduces peroxynitrite, restricts NF-kβ motility, and supports BDNF-Trkβ signalling to preserve brain function in models of Alzheimer's, dopaminergic cell death in Parkinson and reduces brain injury in post-stroke models [27, 28, 29].

Magnolol can improve the TLR4-induced microgliosis and rescue the SIRT1-p53-dimerization in traumatic brain injury mice to alleviate memory impairment [30]. Although there is limited HD research (the majority are investigations into the Nrf2/Keap1 activity of mangiferin in 3-NP rats), magnolol would fit the hierarchy of HD disorders (due to its ability to match HD mitochondrial and inflammatory issues) [31, 32]. The absence of direct evidence inspires this project: I am interested in checking whether or not magnolol can prevent the symptoms of HD-like induced by 3-NP in rats based on the impacts of the agent on behaviour, redox balance, cytokines, and brain morphology.

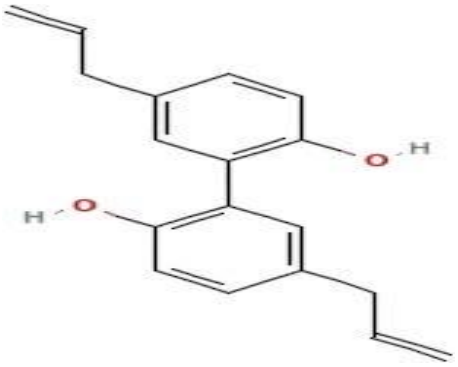

Fig. 3. Chemical structure of magnolol.

MATERIALS AND METHODS:

2.1 Experimental Animals

The Wistar albino rats used as male subjects were put in Wistar cages containing 220 g of bedding wood, and the defined weight of food was 40 g at each feeding. There were two chambers in a Wistar cage, each consisting of a mesh and a bedding dish. The rats were housed under standard laboratory conditions in polyacrylic cages not exceeding four rats per cage at a temperature of 22 o C 50 °C and a 12-hour light/12-hour dark cycle, and during this period, lights were switched on at 12:00 o'clock. Rodents were given a standard pellet diet, and purified water was available. They permitted all the animals to adjust to the experimental room within a period of seven days prior to the beginning of the study. The protocol (IIRT/IAEC/111/2024/029) was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of IIRT following CPCSEA guidelines.

2.2 Experimental design and treatment schedule

Thirty-six Wistar albino rats were randomly allocated into six groups (n = 6 per group) using a computer-generated random number table (GraphPad Quick Calcs). All behavioural assessments, biochemical estimations, and histopathological evaluations were performed by an investigator blinded to the group allocation.

- **Group 1 – Vehicle Control**: Received normal saline (0.9% NaCl, 2 mL/kg, i.p.) + 1% DMSO in saline (10 mL/kg, p.o.) daily for 21 days.
- **Group 2 – Magnolol *per se***: Received magnolol (40 mg/kg, p.o.) alone daily for 21 days.
- **Group 3 – 3-NP Disease Control**: Received 3-nitropropionic acid (3-NP, 10 mg/kg, i.p.) once daily from day 1 to day 21.
- **Group 4 – Standard Treatment**: Received tetrabenazine (2.5 mg/kg, i.p.) 1 h prior to each 3-NP injection from day 1 to day 21.
- **Group 5 – Magnolol Low Dose + 3-NP**: Received magnolol (20 mg/kg, p.o.) 1 h before each 3-NP injection from day 1 to day 21.
- **Group 6 – Magnolol High Dose + 3-NP**: Received magnolol (40 mg/kg, p.o.) 1 h before each 3-NP injection from day 1 to day 21.

2.3 Behavioral Assessment

Behavioral assessment was performed on days 1, 7, 14, and 21 of the experiment. Rotarod test: Motor coordination and balance were evaluated by recording the latency to fall from an accelerating rotating rod (10–25 rpm over 300 s) [33, 34]. Locomotor activity: Spontaneous activity was measured using a digital actophotometer over a 10-minute session, and total beam breaks were recorded as an index of ambulatory movement [35]. Grip strength test: Forelimb muscular strength was assessed using a digital grip strength meter, and the peak force (g) exerted before release was recorded [36].

Animals underwent a four-day acquisition phase (four trials/day) to locate a submerged platform, and escape latency was recorded. On day five, a probe trial was conducted after platform removal, and time spent in the target quadrant was analyzed as an index of memory retention [37, 38].

2.4 Biochemical Estimations

On day 22, animals were euthanized, and the striatum was rapidly dissected and homogenized (10% w/v) in ice-cold phosphate buffer. Oxidative stress parameters: Lipid peroxidation was assessed by estimating malondialdehyde (MDA) and TBARS levels [39, 40]. Endogenous antioxidant status was determined by measuring reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activities [41, 42, 43]. Nitrite concentration was estimated as an indicator of nitrosative stress using the Griess reaction [44].

Neuroinflammatory markers: Levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) were quantified in striatal homogenates using ELISA kits according to the manufacturer's instructions [45].

Neurotransmitters: Striatal glutamate and GABA levels were analyzed using HPLC coupled with fluorescence detection [46].

2.5 Histopathological Analysis

Brains were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, and sectioned coronally (5 µm). Sections were stained with hematoxylin and eosin (H&E), and examined under a light microscope for neuronal degeneration, vacuolization, and nuclear pyknosis. All slides were evaluated by a pathologist blinded to treatment groups [47].

2.6 Molecular Analysis

RNA isolation and qRT-PCR: Total RNA was extracted from striatal tissue using TRIzol reagent, and cDNA was synthesized. Quantitative real-time PCR was performed using SYBR Green chemistry. Relative BDNF mRNA expression was normalized to Gapdh and calculated using the 2^−ΔΔCt method [48].

Western blot analysis: Striatal tissues were homogenized in RIPA buffer, and equal amounts of protein (30 µg) were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were incubated with antibodies against BDNF, cleaved caspase-3, and β-actin. Bands were visualized using enhanced chemiluminescence and quantified using Image software [49].

2.7 Statistical Analysis

Data are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism (version 10.4). Normality was assessed using the Shapiro–Wilk test [50]. Behavioural data across time were analyzed using two-way repeated-measures ANOVA followed by Tukey's post-hoc test, while other parameters were analyzed using one-way ANOVA with Tukey's multiple comparison test. A value of $p < .05$ was considered statistically significant [51].

RESULT

3.1. Effect of magnolol on the rotarod performance in 3-NP-treated rats was determined as below.

As illustrated in Fig. 4, a marked and a gradual deterioration in motor coordination was observed as indicated by a statistically significant difference in latency to fall on the rotarod following the repeated administration of 3-nitropropionic acid (3-NP) in comparison with the normal control group (ϑϑϑ, $p < 0.001$). Rodents of the normal control and magnolol per se groups were stable in rotarod performance over the course of the experiment, which demonstrates that magnolol was not associated with the modulation of the basic motor activity on its own. The motor deficits caused by 3-NP were significantly reduced through pre-treatment with the reference drug tetrabenazine (£, $p < 0.05$; ££, $p < 0.01$ versus disease control). In this way, the protective effect of magnolol with regard to 3-NP toxicity was considered to have a definite dose dependence. Low dose (20 mg/kg) showed a moderate increase in the motor performance, and high dose (40 mg/kg) showed greater improvement of fall latency (££, $p < 0.01$) comparable to the effect of tetrabenazine by day 21.

3.2 Effect of magnolol on forelimb grip strength of 3-NP-treated rats.

Fig. 5 shows that all groups were initially similar in forelimb grip strength on day 1, which is an indication that there were no differences in the grip strength before exposure to toxins. Chronic induction of 3-NP resulted in a significant decrease in grip strength on day 21 relative to control (ϑϑϑ, $p < 0.001$**)** and is indicative of severe neuromuscular injury. The grip force value in rats in the magnolol per se group was not different than the controls, indicating magnolol alone did not affect basal muscle strength. To a large extent, pre-treatment with the standard drug tetrabenazine resulted in better grip strength when compared to the 3-NP disease control group (£, $p < 0.05$; ££, $p < 0.01$). Similarly, the administration of magnolol shows a dose-dependent action to counter 3-NP-induced weakness. The low dose (20mg/kg) had a moderate effect (££, $p < 0.01$), and the high dose (40mg/kg) had a significant effect (£££, $p < 0.001$), and the values of grip force approached normal control values at the end of day 21.

3.3 Effect of magnolol on transfer latency in 3-NP-treated rats was studied.

According to Fig. 6, rats after 3-NP exposure had a severe learning and memory deficit at the acquisition and retention stages, as indicated by a significant increase in the transfer latency in most of the acquisition and retention trials compared to the normal control group (ϑϑϑ, $p < 0.001$)**.** Normal control and magnolol *per se* animals alone did not negatively affect memory. Tetrabenazine pre-treatment also resulted in less transfer latency compared to the indicating

of partial reversibility of cognitive impairments induced by 3-NP. Similarly, the cognitive performance was also improved by magnolol in a dose-dependent manner. At low dose (20mg/kg) the latency was significantly reduced compared to the disease control group (£, $p < 0.05$), but at high dose (40mg/kg) the commonality of greater effect was found (££, $p < 0.01$), close to the values in the normal control group, especially in the trial of retention

3.4 Effect of magnolol on the spontaneous locomotor activity of rats treated with 3-NP.

Fig. 7 shows that there was no significant difference between experimental groups in terms of baseline locomotor activity on day 0, which suggests that there was the same degree of exploratory behaviour before treatment. The over 3-day-long administration of 3 -NP resulted in pronounced impairment of spontaneous locomotor activity by day 21 as indicated by a significant decline in crossings relative to the normal control group (ǂǂǂ, $p < 0.001$). The magnolol *per se* group of rats had their locomotor activity similar to normal controls, which implied that the use of magnolol did not produce any effects on basal ambulation alone. Tetrabenazine pre-treatment markedly relieved the 3-NP- caused hypoactivity (£, $p < 0.05$; ££, $p < 0.01$ versus disease control). Similarly, locomotor performance was restored in a dose-dependent manner after the administration of magnolol. At low dose (20mg/kg), the number of crossings significantly increased compared to the 3 -NP group (£, $p < 0.05$), and at high dose (40mg/kg), a greater recovery was seen (££, $p < 0.01$), nearing the control levels of day 21.

3.5 Effect of magnolol and spatial learning and memory in 3 -NP -treated rats.

Fig. 8A shows that the ability of rats subjected to 3-NP in spatial learning was significantly diminished, as the escape latency in the training days was remarkably longer than that of the normal control rats (ǂǂǂ, $p < 0.001$). Conversely, animals in the normal control group and the magnolol per se group were found to exhibit a progressive decrease in the escape latency as the number of repetitions of the trial increased, which were considered to have learned the task normally and proved the claim that magnolol did not affect learning ability. The 3NP-induced increase in pre-acquisition escape latency was significantly reduced by pre-treatment with tetrabenazine (£, $p < 0.05$; ££, $p < 0.01$ versus disease control). Similarly, the dose-dependent effect of magnolol was an improvement in spatial learning. It is also noteworthy that the low dose (20 mg kg) only reduced escape latency significantly compared to the 3 -NP group (£, $p < 0.05$) whereas the high dose (40mg/kg) had a more intense effect (££, $p < 0.01$), until the end of the training day it approached the performance of the normal control group. Consistent with the acquisition data, the probe trial (Fig. 8B) revealed that 3-NP–treated rats spent significantly less time in the target quadrant than normal controls (ǂǂǂ, $p < 0.001$), indicating impaired memory retention. Rats treated with tetrabenazine showed a significant increase in time spent in the target quadrant compared with the disease control group (£, $p < 0.05$). Similarly, magnolol significantly improved memory retention in a dose-dependent manner, with the high dose (40 mg/kg) eliciting a greater restoration of target quadrant exploration than the low dose (20 mg/kg) (££, $p < 0.01$). These findings demonstrate that magnolol effectively reverses 3-NP–induced deficits in spatial learning and memory, supporting its ethnopharmacological relevance as a neuroprotective phytoconstituent.

3.6 Effect of magnolol on oxidative stress and antioxidant defence in the striatum of rats that received 3 -NP.

A pervasive effect of applying 3-nitropropionic acid (3-NP) in the chronic administration produced a series of profound oxidative stress in the striatal tissue exhibited by a substantial increase in indicators of lipid peroxidation malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS), as well as an increase in the nitrite levels compared to the normal control group (ǂǂǂ, $p < 0.001$). At the same time, the effect of 3-NP on endogenous antioxidant defences was significant, as shown by the decrease in the activity of the SOD enzyme (superoxide dismutase) and catalase (CAT), along with the reduction of reduced glutathione (GSH) (ǂǂǂ, $p < 0.001$). The magnitude of the value of rats in the magnolol per se group was similar to normal controls and so, it suggests that the use of magnolol alone did not found to disrupt redox homeostasis. Pre-treatment with the reference drug tetrabenazine significantly inhibited oxidative damage in 3-NP-induced oxidative stress as evidenced by lower MDA, TBARS, and nitrite levels and partial recovery of SOD, CAT, and GSH (£, $p < 0.05$; ££, $p < 0.01$; £££, $p < 0.001$ versus disease control). Significantly, administration of magnolol induced a dose-dependent normalization of the parameters. The low dose (20 mg/kg) had a substantial pro-oxidant and antioxidant index relative to the control (40mg/kg) and high dose ($p < 0.05$–0.001 versus tetrabenazine). All in all, the marks on lipid peroxidation and nitrosative stress in the striatum and a recovery in endogenous antioxidant potential of magnolol in the 3-NP model have shown strong antioxidative and neuroprotective potential of the folate.

3.7 Effect of magnolol in neuroinflammation and excitatory inhibitory neurotransmitter balance in striatum.

As shown in fig. 10A, the chronic use of 3-NP induced a strong neuroinflammatory response in the striatum, which was seen by a significant increase in the levels of pro-inflammatory cytokines, namely, tumor necrosis factor-alpha (TNF-α),

interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β) compared with the normal control group (ϑϑϑ, $p < 0.001$). Conversely, the levels of cytokines of rats within the magnolol *per se* group were similar to those of the normal controls, and this showed that magnolol alone did not cause inflammatory changes in the rats. Tetrabenazine pre-treatment remarkably reduced the cytokine burst induced by 3 -NP (£, $p < 0.05$; ££, $p < 0.01$ versus disease control). Similarly, the effect of magnolol was a dose-dependent inhibition of neuroinflammation. The low dose (20mg/kg) markedly brought down the level of TNF-α, as well as IL-6 and IL-1β in comparison with the 3-NP group (£, $p < 0.05$; ££, $p < 0.01$) but the high dose (40 mg/kg) caused a stronger effect (£££, $p < 0.001$), and it is greater than the anti-inflammatory action of tetrabenazine ($p < 0.05$). Fig. 10B shows that 3-NP had a significant effect by increasing the levels of glutamate in the striatum, reducing the content of 7-aminobutyric acid (GABA) significantly over the normal controls (ϑϑϑ, $p < 0.001$), which caused an imbalanced excitatory-inhibitory neurotransmitter regulation. The pre-treatment with tetrabenazine partially restored such changes (£, $p < 0.05$). Magnolol also dose-dependently restored the neurotransmitter homeostasis; the high dose (40mg/kg) produced a higher reduction of glutamate and substantial increase in GABA levels than the low dose (£££, $p < 0.001$), indicating its better ability than tetrabenazine ($p < 0.05$).

3.8 Effect of Magnolol on 3 -NP induced histopathological modifications in the striatum.

The typical stains of haematoxylin and eosin are shown in the striatal sections (fig. 11). The control group (A) had intact cytoarchitecture, neurons retained morphology, nuclei were homogenous, and neuropil organization in normal limits. The group of magnolol alone (B) showed no histological deviation, thus putting in line with the absence of inherent neurotoxicity that can be linked to magnolol. Conversely, the animals under 3-NP without any other agents (C) showed severe neuropathological changes, such as massive neuronal loss, nuclear pyknosis, cytoplasmic shrinkage, and excessive vacuolization of the neuropil, which are typical of intensive neurodegeneration and tissue disorganization that occurs due to mitochondrial toxin-induced limbic injury. Pre-treatment with the reference agent tetrabenazine (D) significantly reduced the damage caused by 3-NP, as the neuronal morphology remained intact, there was less vacuolization, as well as the number of pyknotic nuclei was lower. Equally, dose-dependent histological protection was induced by magnolol pre-treatment. The moderate effect on restoring neuronal integrity was observed in the low-dose regimen 20mg/kg (E) and reduced cellular degeneration, as well as improved the organization of tissues. The 40mg/kg (F) produced a strong preservation of striatal architecture, with neuronal morphology being nearly parallel to that of the control with negligible signs of degeneration, closely resembling the normal control group.

3.9 Effect of Magnolol on BDNF and apoptotic signalling of the striatum.

As shown in fig. 12A and 12B, long-term exposure to 3-NP had a significant down-regulating effect on striatal tissue on both mRNA and protein levels of the brain-derived neurotrophic factor (BDNF) compared to the normal control group (ϑϑϑ, $p < 0.001$), indicating the inability to support neurotrophic functions. The MG *per se* group had similar BDNF expression levels as the NC, which served to rule out the effect of MG itself in negatively impacting neurotrophin homeostasis. Tetrabenazine pre-treatment had a great effect on the restoration of BDNF mRNA and protein in comparison to the 3-NP disease control (£££, $p < 0.001$). The reduction of magnolol administration led to a distinct dose-effect upregulation of BDNF expression. The low dose (20 mg/kg) effectually raised both the transcript and protein concentration compared to the disease control (££, $p < 0.01$), but the high dose (40mg/kg) had a stronger effect (£££, $p < 0.001$), almost returning the values to near-normal levels and demonstrating better activity compared with tetrabenazine (***, $p < 0.001$). Fig. 12C shows that the 3-NP treatment significantly increased cleaved caspase-3 levels, which is a major marker of apoptosis, compared to the normal controls (ϑϑϑ, $p < 0.001$). Tetrabenazine pre-treatments caused significant depressions of caspase-3 activation when compared to the disease control group (££, $p < 0.01$). Compared to the insignificant effect of the low dose (10 mg/kg), the high dose (40 mg/kg) specifically reduced the level of cleaved caspase-3, and was more effective at protection than tetrabenazine (***, $p < 0.001$).

Table 1. Oxidative stress parameters

Group	MDA (nmol/mg protein)	Nitrite (µg/ml)	GSH (µmol GSH/mg protein)	SOD (U/mg protein)
Normal Control	2.1 ± 0.3 (100%)	120 ± 10 (100%)	0.11 ± 0.02 (100%)	8.5 ± 0.5 (100%)
Magnolol 40 mg/kg	2.05 ± 0.25 (97%)	118 ± 9 (98%)	0.115 ± 0.02 (105%)	8.8 ± 0.5 (104%)
3-NP (10 mg/kg) Disease Control	7.8 ± 0.6 (365%)	250 ± 10 (210%)	0.030 ± 0.005 (27%)	3.2 ± 0.4 (38%)
Tetrabenazine	6.0 ± 0.5 (285%)	230 ± 9 (190%)	0.045 ± 0.006 (41%)	4.5 ± 0.5 (53%)
MG 20 mg/kg + 3-NP	5.8 ± 0.45 (276%)	210 ± 9 (175%)	0.048 ± 0.007 (44%)	4.8 ± 0.5 (56%)
MG 40 mg/kg + 3-NP	4.5 ± 0.4 (214%)	180 ± 7 (150%)	0.070 ± 0.008 (64%)	5.8 ± 0.4 (68%)

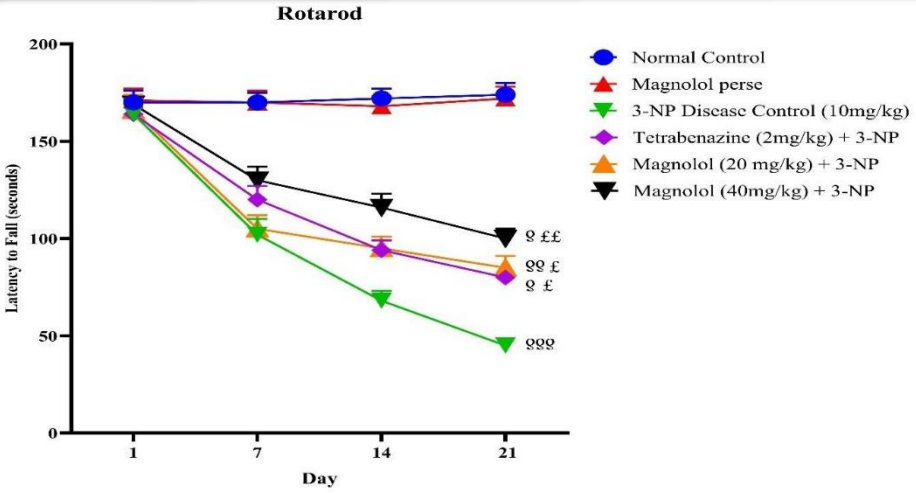

Fig. 4. Magnolol improves the deficits in motor coordination in the 3-NP-induced model. The data sets are mean ± SEM (n=6). ϱϱϱ $p < 0.001$ versus Normal Control group; ££ $p < 0.01$ and £ $p < 0.05$versus 3-NP Disease Control group.

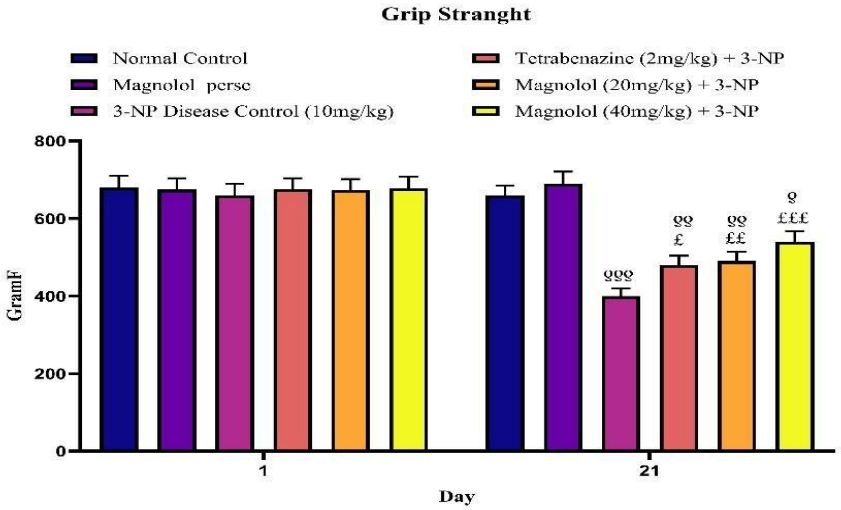

Fig. 5. Effect of Magnolol on forelimb grip strength. The data sets are mean ± SEM (n = 6). ϱϱϱ $p < 0.001$; ϱϱ < 0.01; ϱ < 0.05 vs. Normal Control; £££ $p < 0.001$; ££ $p < 0.01$, £ $p < 0.05$ vs. 3-NP Disease Control

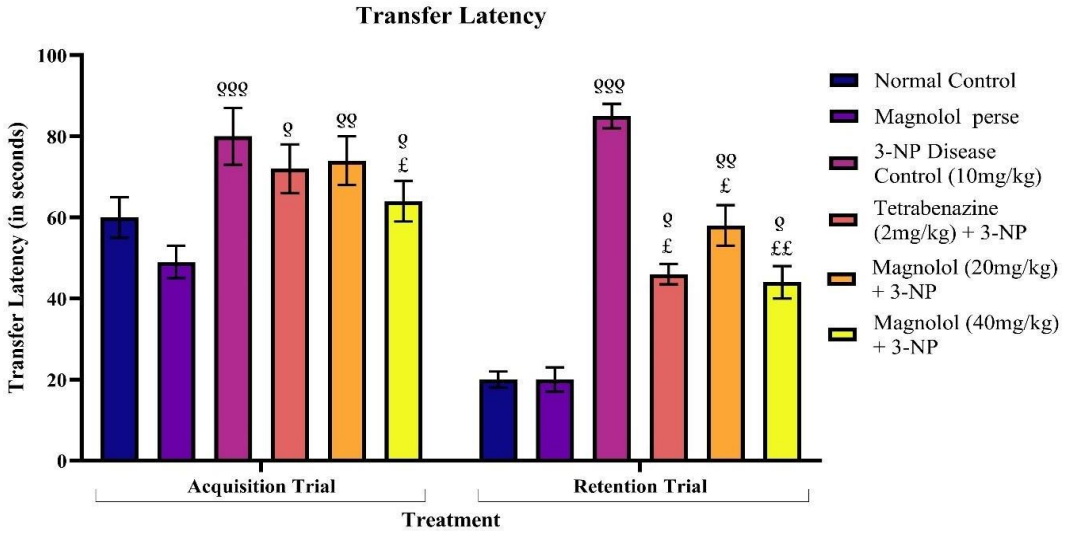

Fig. 6. Effect of Magnolol transfer latency: The data sets are mean ± SEM (n = 6). ϱϱϱ $p < 0.001$; ϱϱ < 0.01; ϱ < 0.05 vs. Normal Control; £££ $p < 0.001$; ££ $p < 0.01$, £ $p < 0.05$ vs. 3-NP Disease Control

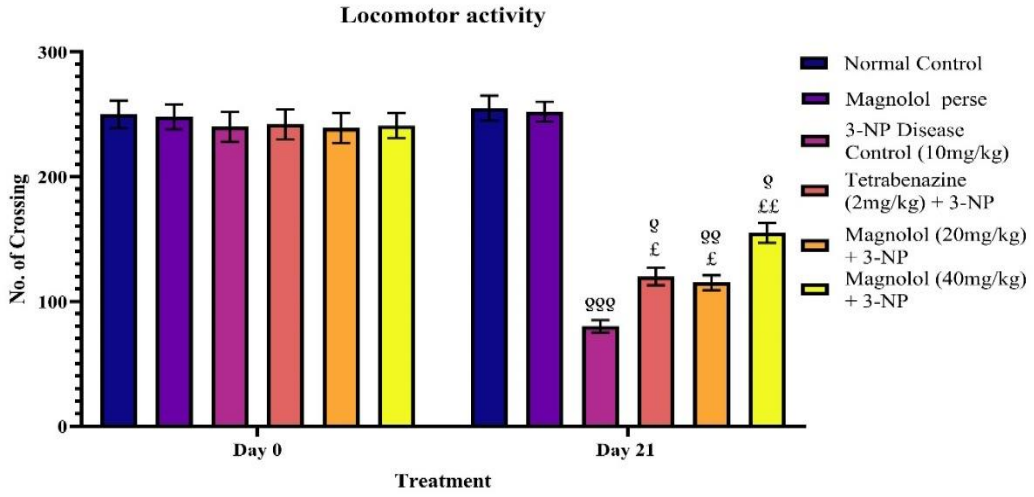

Fig.7. Magnolol restores spontaneous locomotor activity impaired by 3-NP. The data sets are mean ± SEM (n = 6/group). ϑϑϑ p < 0.001, ϑϑ p < 0.01, ϑ p < 0.05 vs. Normal Control; ££ p < 0.01, £ p < 0.05 vs. 3-NP Diseases Control.

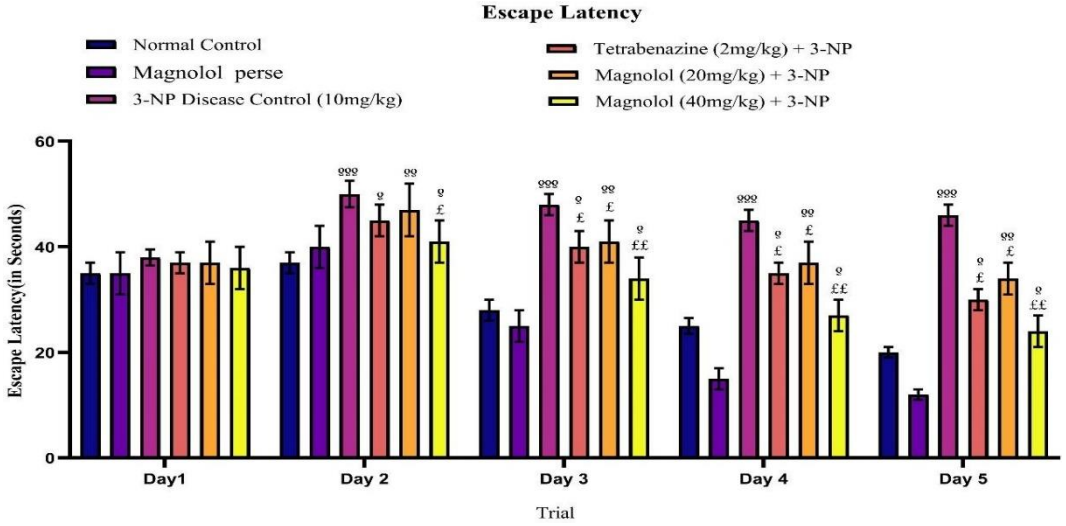

Fig. 8 A. Escape latency in acquisition trials.

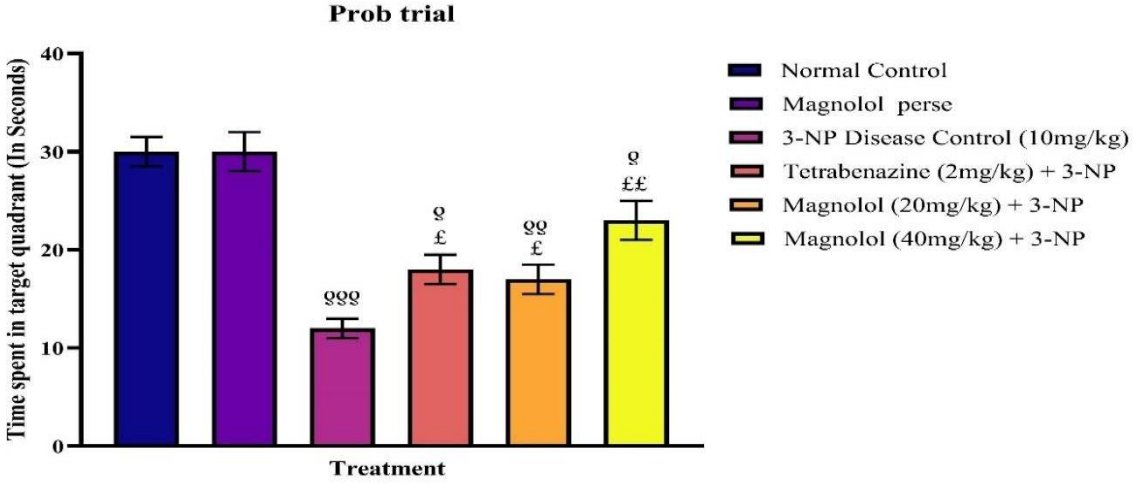

Fig. 8 B. Target quadrant time in the probe trial

Fig. 8A & B. Effect of Magnolol reverses 3-NP-induced deficits in spatial learning (A, escape latency) and memory (B, time in target quadrant). The data sets are mean ± SEM (n = 6/group). ϑϑϑ p < 0.001, ϑϑ p < 0.01, ϑ p < 0.05 vs. Normal Control; ££ p < 0.01, £ p < 0.05 vs. 3-NP Diseases Control.

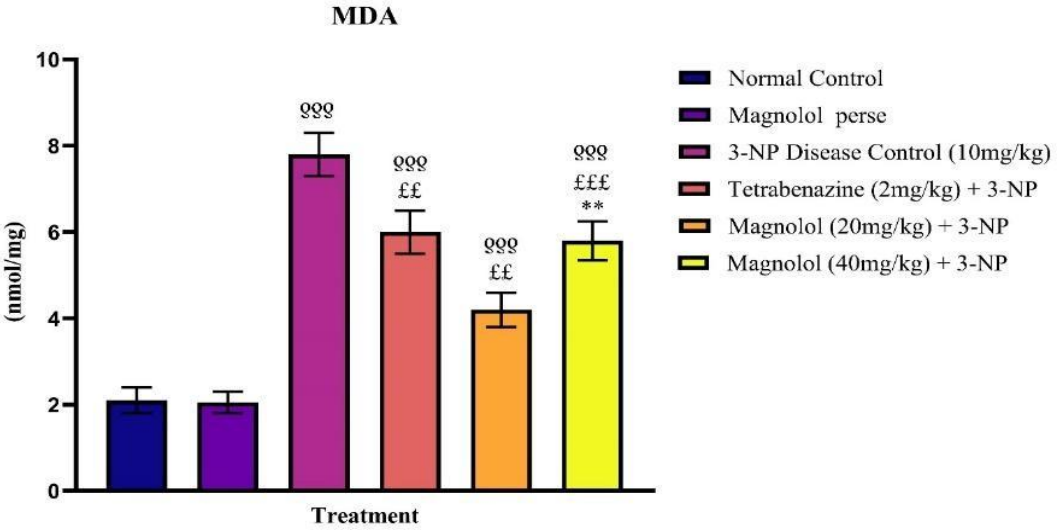

Fig. 9 A. Malondialdehyde (MDA)

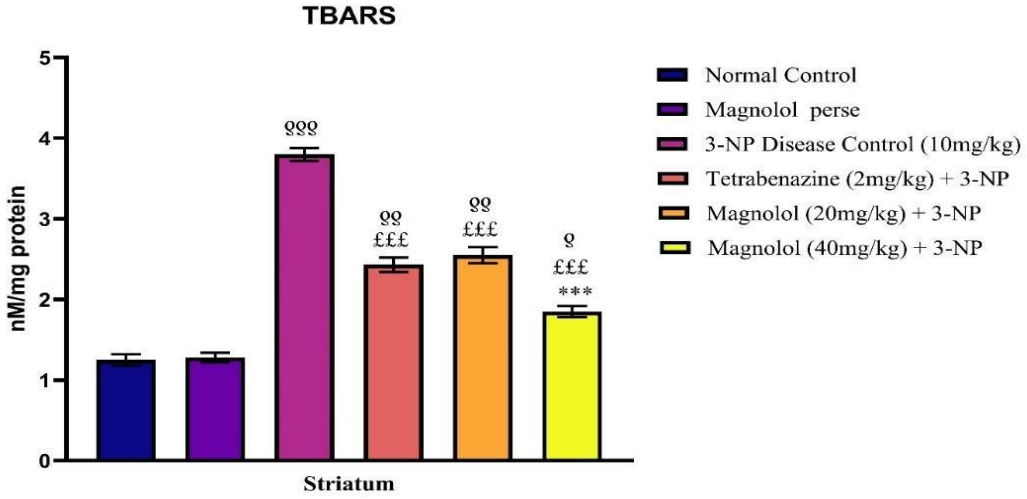

Fig. 9 B. TBARS

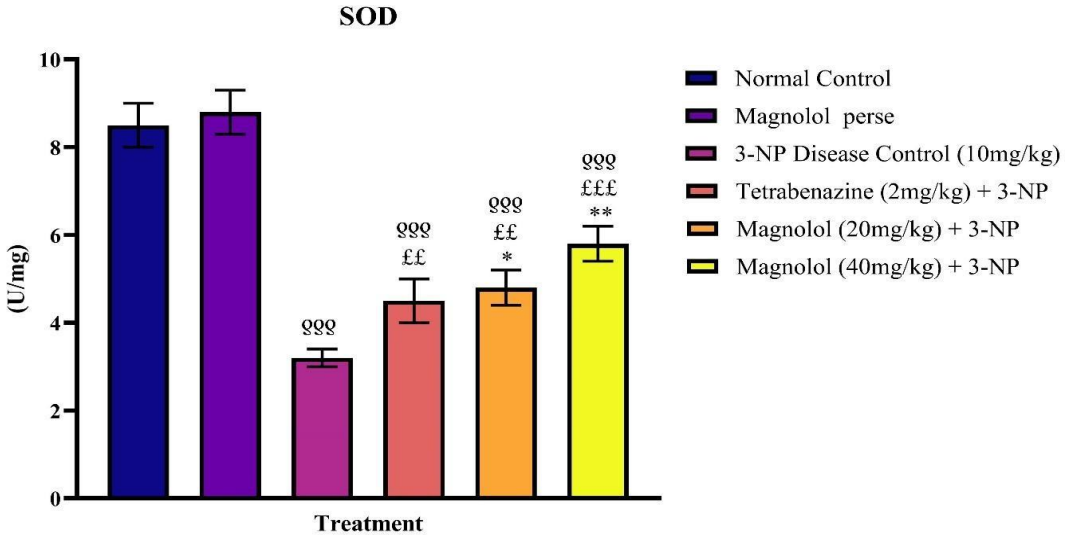

Fig. 9 C. Superoxide Dismutase (SOD)

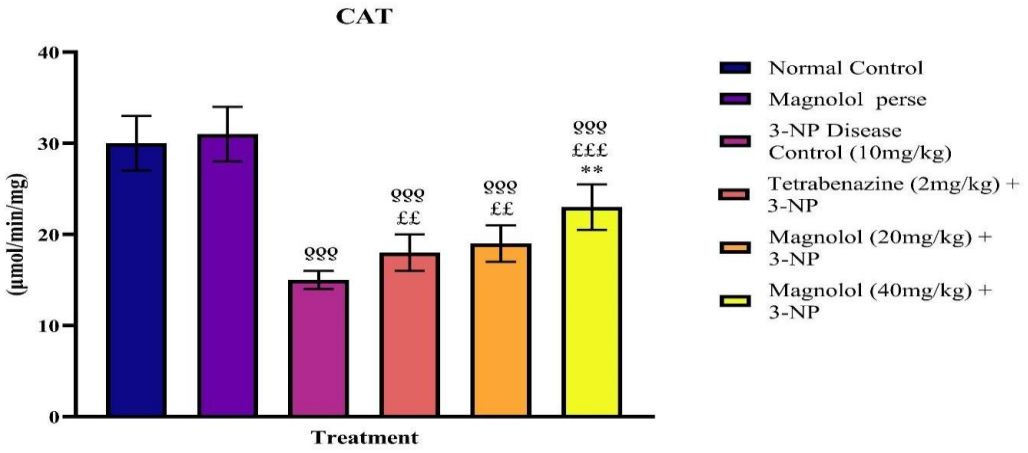

Fig. 9 D. Catalase (CAT)

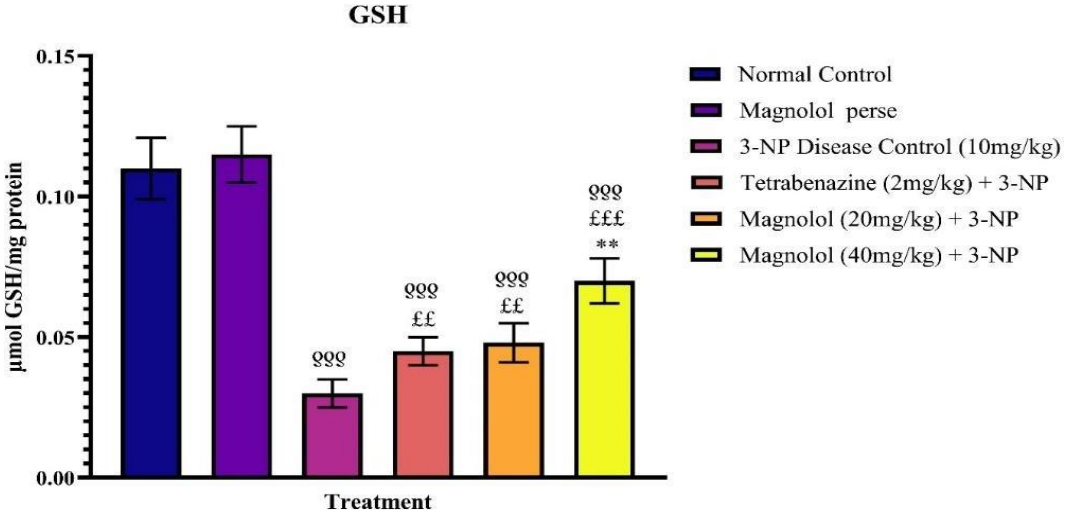

Fig. 9 E. Reduced Glutathione (GSH)

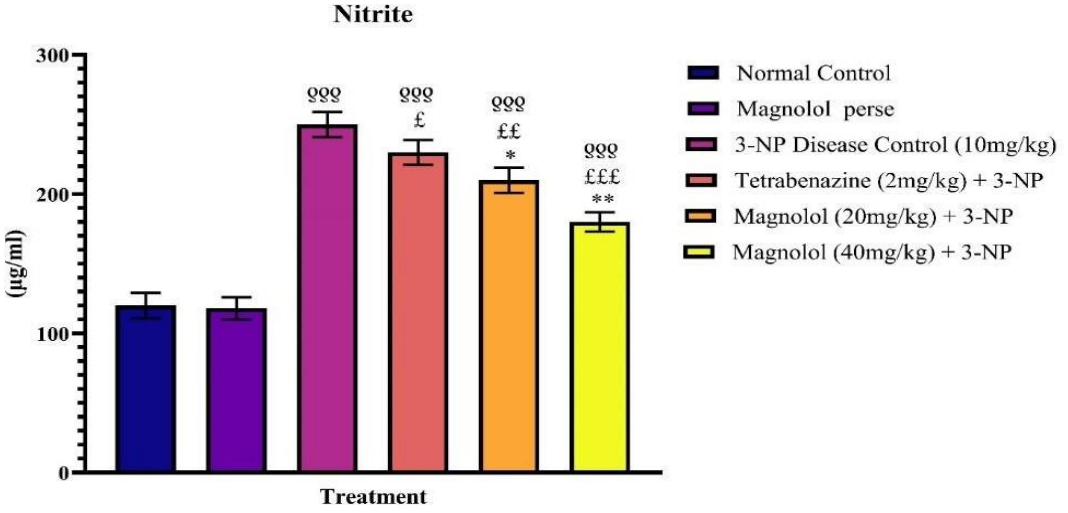

Fig. 9 F. Nitrite.

Fig. 9A-F. Antioxidant effects of Magnolol in the 3-NP-induced striatal model. The complex significantly modulated levels of (A) MDA, (B) TBARS, (C) SOD, (D) CAT, (E) GSH, and (F) Nitrite. All data sets are presented as mean ± SEM (n = 6). ϑϑϑ $p < 0.001$; ϑϑ < 0.01; ϑ < 0.05 vs. Normal Control; £££ $p < 0.001$; ££ $p < 0.01$, £ $p < 0.05$ vs. 3-NP Disease Control, and*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ vs Tetrabenazine.

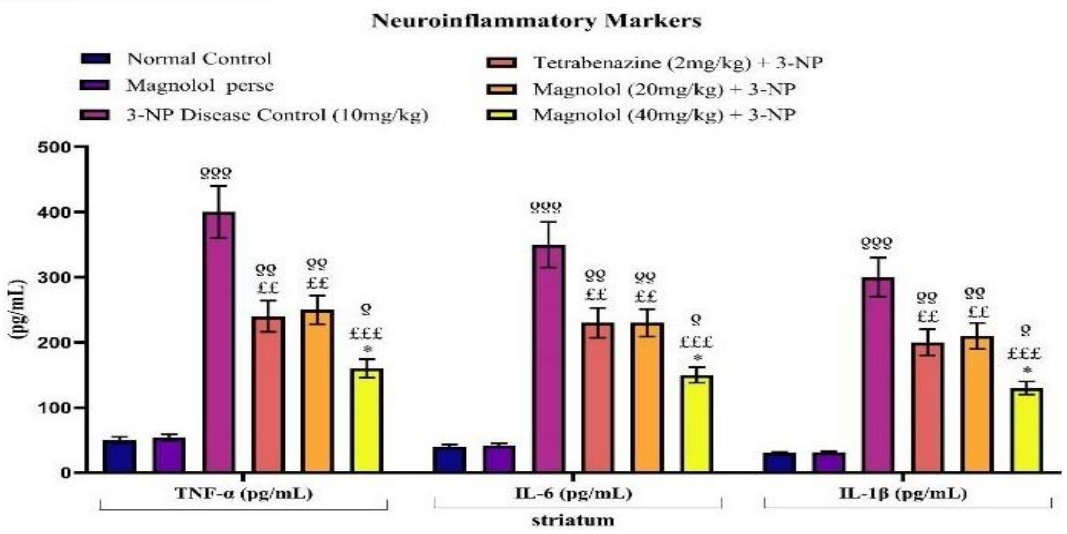

Fig. 10 A. Levels of pro-inflammatory cytokines

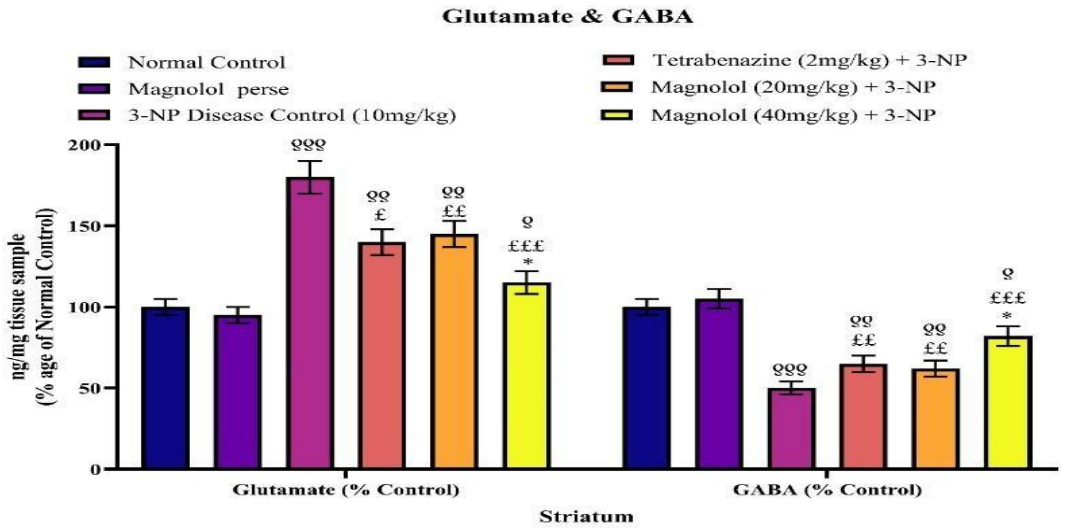

Fig. 10B. Glutamate and GABA levels

OriginalArticleFig 10A-B. Effect of Magnolol on neuroinflammation and excitotoxicity. (A) Profile of pro-inflammatory cytokines. (B) Glutamate and GABA levels. All data sets are presented as mean ± SEM (n = 6/group). ϱϱϱ $p < 0.001$; ϱϱ < 0.01; ϱ < 0.05 vs. Normal Control, £££ $p < 0.001$, ££ $p < 0.01$, £ $p < 0.05$ vs. 3-NP DC; * $p < 0.05$ vs. Tetrabenazine group.

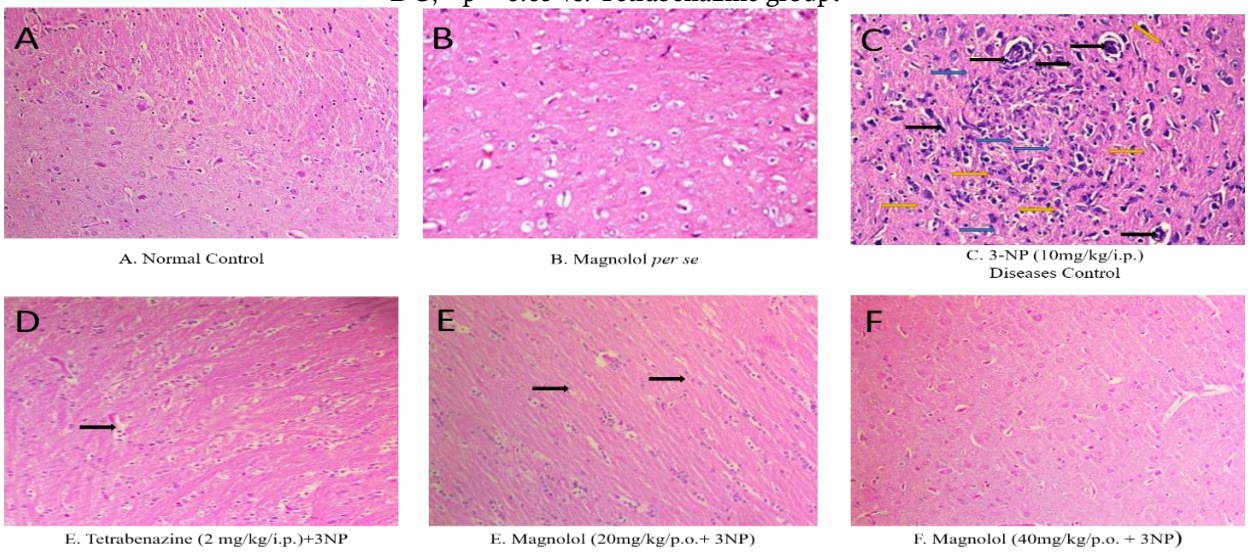

Fig. 11. Photomicrographs representative of striatal sections (H&E staining). *(A)* Normal Control. (B)

Magnolol *per se*. (C) 3-NP (10mg/kg/i.p.) Disease Control with intense neuronal loss (arrowhead), pyknosis (yellow arrow), and vacuolization (blue arrow). (D) Tetrabenazine (2mg/kg/i.p.) + 3-NP. (E) Magnolol (20 mg/kg) + 3-NP. (F) Magnolol (40 mg/kg/p.o.) + 3-NP.

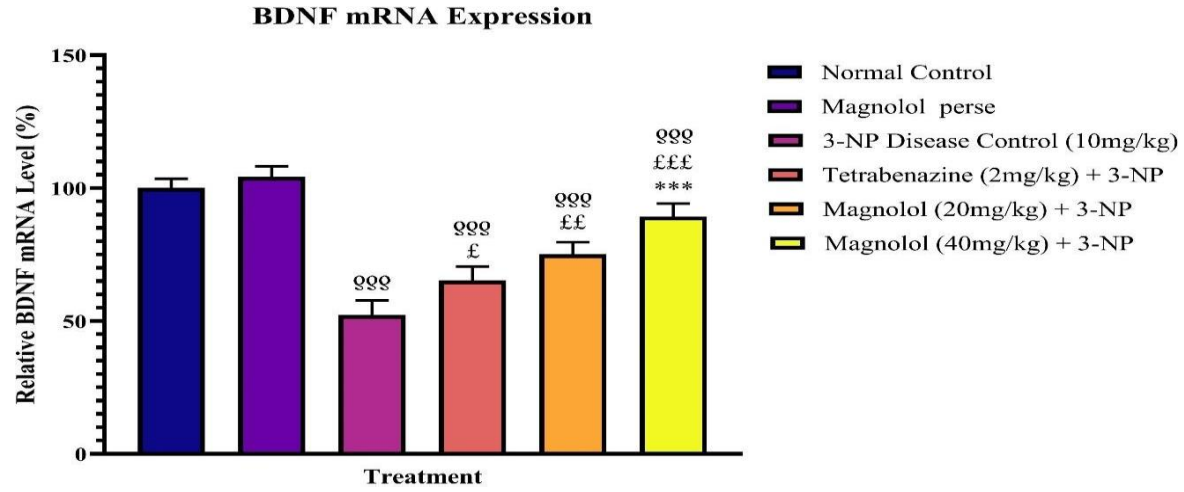

Fig. 12 A Relative BDNF mRNA expression.

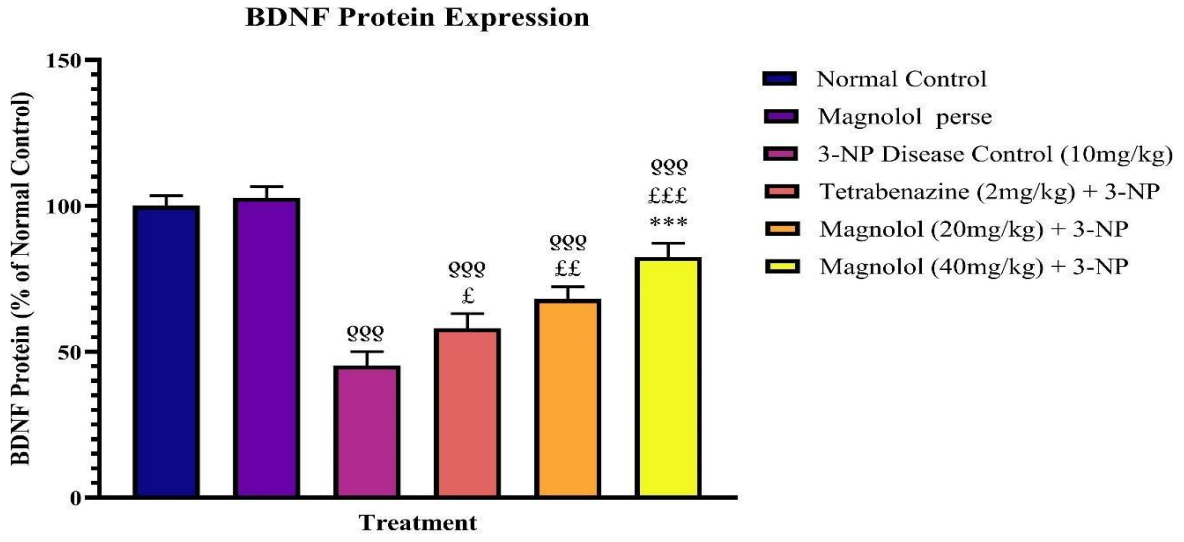

Fig. 12B. BDNF protein levels

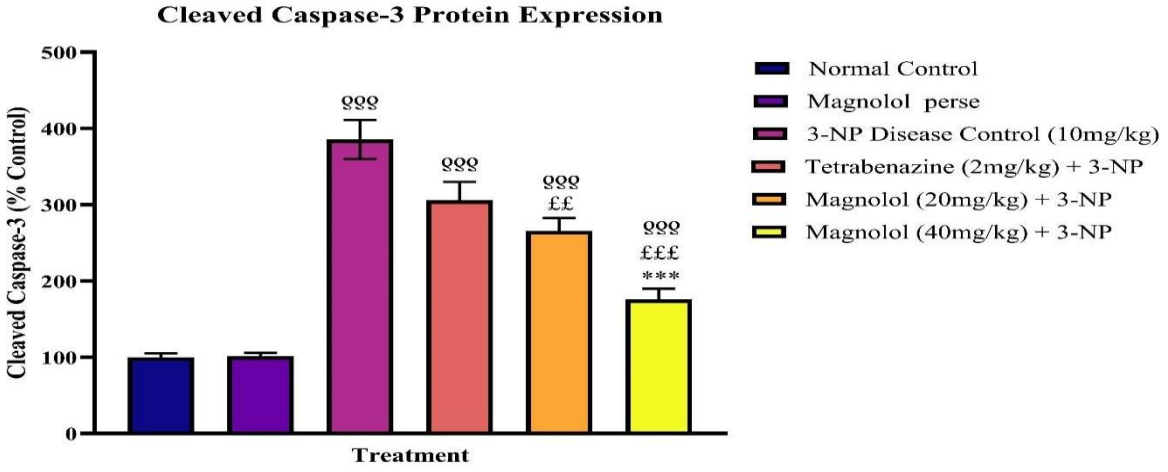

Fig. 12C. Cleaved Caspase-3 protein levels.

Fig. 12A-C. Effect of Magnolol on BDNF expression and apoptosis. (A) Relative BDNF mRNA

expression. (B) BDNF protein levels. (C) Cleaved Caspase-3 protein levels. The data sets are mean ± SEM (n = 6/group). ϑϑϑ p< 0.001 vs. Normal Control, £££ p < 0.001, ££ p < 0.01, £ p < 0.05 vs. 3-NP Diseases Control; *** p < 0.001 vs. Tetrabenazine group.

DISCUSSION

The current research establishes that magnolol has potent neuroprotective consequences against 3-nitropropionic acid (3-NP)-induced Huntington disease (HD)-like neurotoxicity in rats, which are manifested by the improvements in motor coordination, muscle strength, locomotor coordination, and cognitive functions alongside the normalization of oxidative balance, inhibition of neuroinflammatory reactions, redress of neurotransmitter imbalances, inhibition of apoptotic signaling, and preservation of striatal histoarchitecture. The above multifarious advantages highlight the turning point of magnolol as a multi-target phytotherapeutic agent in HD.

The 3-NP is an established mitochondrial toxin with reversible effects of depleting ATP, overproducing reactive oxygen species (ROS), and selectively forcing the degeneration of the striatum, making it replicate the pathology of HD in humans [19, 21]. In line with this mechanism, the disease-controlled animals were severely motor-deficient, showed memory and learning impairment, as well as massive oxidative and inflammatory destruction. Magnolol pre-treatment had significant suppression effects on these abnormalities, suggesting that the mitochondrial dysfunction-induced neurotoxicity was actually purposely mitigated.

Behaviourally, magnolol had a dose-dependent effect on the rotarod performance, grip strength, spontaneous locomotion, and Morris water maze. The impairment of the motor and cognitive functions in HD is mostly caused by the worsening of the medium spiny neurons in the striatum and dysfunction of corticosteroid circuitry [9, 10]. The observed functional recovery, therefore, is an indication of maintenance of striatal neuronal integrity as indicated by histopathological loss of neurons, vacuolization, and pyknosis, respectively, in magnolol-treated groups.

The role of oxidative stress in HD pathogenesis has been central, as demonstrated by increased lipid peroxidation and impaired antioxidant defenses in both patients and experimental models [52, 53]. The application of 3-NP in this study resulted in a significant increase in the levels of malondialdehyde (MDA), thiobarbituric acid reactive substances (TBARS), and nitrite levels, accompanied by the loss of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) levels. Magnolol greatly hindered these changes, which was consistent with the previous literature about its strong free-radical scavenging activity and activation of Nrf2/ HO-1 free radical alarm system [27, 28]. Redox balance restoration is also presumably one of the leading mechanisms of action of magnolol, which brings about neuroprotection.

Another life-threatening phenomenon that promotes HD development is neuroinflammation caused by active microglia and high concentrations of pro-inflammatory cytokines (TNF-α, interleukin-1β, interleukin-6) [54, 55]. As we have found, augmented levels of pro-inflammatory cytokines were seen in 3-NP treated rats, just as was ascertained before [23]. These cytokines were significantly suppressed by magnolol, which supports its reported ability to block the NF-Kβ activation and TLR4 activation by microglia [30, 29]. The anti-inflammatory effect of magnolol is probably the interaction of its antioxidant properties with anti-inflammatory activity to prevent secondary damage to neurons.

The striatum is vulnerable to excitotoxicity in HD due to a high level of glutamatergic transmission and low levels of GABAergic tone [56, 22]. In the current experiment, the concentrations of glutamate and decreased GABA were increased and decreased by 3-NP, respectively, and the excitatory-inhibitory balance of magnolol returned. This normalization can be an indication of indirect protection of GABAergic medium spiny neurons and inhibition of ROS-mediated synaptic dysfunction and, thus, avoid calcium overflow and apoptotic cascades.

Magnolol had substantial up-regulation effects on brain-derived neurotrophic factor (BDNF) mRNA and protein and inhibited cleaved caspase-3 expression in the brain at the molecular level. Downregulated BDNF signalling is a uniform feature of HD, and it causes loss of striatal neurons and impaired synapses [57, 9]. The reactivation of pro-survival TrkB signaling is an indication of the restoration of BDNF by Magnolol, reported in other neurodegenerative and ischemic models [28, 27]. The simultaneous inhibition of caspase 3 shows the mitochondrial apoptotic signalling to be attenuated to further support a neurotrophic-anti-apoptotic effect. The neuroprotective effect of high-dose magnolol (40 mg/kg) was equal to or even greater in relation to tetrabenazine, the reference drug used in treating the chorea in HD through symptomatic treatments [15]. In contrast, magnolol, unlike tetrabenazine, is not a modulator of dopaminergic transmission with minimal effect on disease progression but rather an upstream pathogenic event,

such as oxidative stress, inflammation, excitotoxicity, and trophic support. This multi-target profile is consistent with the newer research that requires effective HD therapeutics to target connected molecular mechanisms but not individual nodes [2, 3]. However, some weaknesses can be recognized. The 3-NP model replicates the mitochondrial and striatal toxicity but fails to replicate the genetic complexity of mutant huntingtin aggregation as seen in transgenic models [19].

The studies that should be conducted over the long term, the evaluation of the pathways of huntingtin, and the validation of them in the genetic models of HD should be justified. Further, translational relevance would also be enhanced by pharmacokinetic profiling and brain bioavailability of magnolol.

The results confirm that magnolol has a comprehensive neuroprotective effect in a toxin-based HD model, establishing its ethnopharmacological topicality and positioning it as a disease-modifying candidate.

CONCLUSION

The current research is able to show that magnolol is effective in mitigating Huntington disease-like neurodegeneration caused by 3-NP in rats. Magnolol substantially enhanced motor skills, muscle performance, locomotor functions, and cognition in addition to attentively regaining redox homeostasis, preventing neuroinflammation, rebalancing glutamate-GABA concentration, inducing BDNF synthesis, and blocking apoptotic signals. These functional as well as biochemical advantages are further supported by the preservation of striatal architecture that has been confirmed histopathologically. Taken together, these findings indicate that the mechanism of action of magnolol is a multi-target mechanism that includes anti-oxidant, anti-inflammatory, anti-excitotoxic, neurotrophic, and anti-apoptotic. Since it has a favourable safety profile and is derived from Magnolia officinalis, orchestrated Magnolol could be an ideal phytoconstituent to be advanced as a disease-modifying treatment agent in Huntington disease. Future research into its genetic HD molecular targets and its translation into the clinical realm is justified.

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Conflict of Interest section

The author has no conflict of Interest.

Author contributions

Ravinder Khatri designed the methodology, conducted the experiments, analyzed the data, and drafted the manuscript. Kamal Jeet conceived the research idea, supervised and designed it, Anjana reviewed the manuscript; Kuldeep Kumar provided advice and critical comments, and all authors read and approved the final manuscript.

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