Current Research in Pharmacology and Drug Discovery 3 (2022) 100130

Contents lists available at ScienceDirect

Current Research in Pharmacology and Drug Discovery

journal homepage: www.journals.elsevier.com/current-research-in-pharmacology-and-drug-discovery

Neuroprotective effect of silymarin against 3-Nitropropionic acid-induced neurotoxicity in rats

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ARTICLE INFO

Keywords:
Oxidative stress markers
3-NPA- 3-Nitropropionic acid
Mitochondrial dysfunction
Silymarin
Glutamate
HD- Huntington's disease

ABSTRACT

(HD) Huntington's disease is a severe hereditary catastrophic neurological disease with an autosomal dominant heritable changes manifested by cognitive, behavioural, and motor progression deficits, resulting in death. Several mechanisms are involved in the pathogenesis of this complex and rare disease, including excitotoxicity, mitochondrial dysfunction, neurotransmitters imbalance, and oxidative stress. Silymarin was selected as an investigational drug, due to its numerous activities in current research, it possesses substantial antioxidant and neuroprotective functionalities. The present research attempts, i.p. injections of 3-NPA (10 mg/kg) were given for 21 days to trigger Huntington-like symptoms in rats. The percentage fluctuations in body weight, the footfall counts, and the time required to transverse the beam and motor functions were analyzed at multiple time points. Oxidative stress markers like MDA/LPO, GSH, protein, nitrite, catalase, and superoxide dismutase levels were examined in the striatum region. The current study results conclusively demonstrate that chronic 3-NPA administration significantly decreased the body weight and showed marked abnormalities in motor coordination, locomotion, and increased striatal generation of free radicals. Furthermore, treatment with silymarin (100 & 200 mg/kg/*p.o.*), mitigated 3-NPA triggered behavioural and biochemical alterations. Our study results could conclude that Silymarin may be advantageous and might develop an adjuvant treatment for the management of Huntington's disease.

1. Introduction

(HD) Huntington disease is a severe hereditary catastrophic neurological disease with an autosomal dominant heritable changes manifest as abnormal body movements, i.e. chorea, psychiatric disorders, and cognitive impairment in the brain region, including the basal ganglia, cerebral cortex, and hippocampus (Jamwal and Kumar, 2018), (Kumar et al., 2010). Alteration of neurotransmitters level in cortex and neostriatum (caudate and putamen) leads to neurological symptoms due to neuronal and axonal loss (Wang et al., 2017), (Suganya and Sumathi, 2016). Although the exact mechanisms underlying the neuropathology of HD-induced striatal neurotoxicity are still not fully comprehended, various conceptions and assumptions have been presented as a means of explaining the mechanism and pathways of HD progression, including the interaction of expanded polyglutamine repeat protein with other proteins in the brain (CNS) (Xiang et al., 2018), apoptosis (Wang et al., 2015a), excitotoxicity (Domise and Vingtdeux, 2016), oxidative stress (Kim et al., 2015), neuroinflammation (Fisher and Hayden, 2014), (Evans et al., 2013), abnormal mitochondrial bioenergetics and impairment of axonal trafficking (Jimenez-Sanchez et al., 2017). However, the precise processes underlying HD neuropathology is not thoroughly comprehended.

The mitochondrial toxin 3-NPA (mycotoxin) permanently inhibits (SDH) succinate dehydrogenase enzymes (mitochondrial complex II) with massive deterioration of medium spiny neurons with GABAergic input (MSNs) (Huang et al., 2006), (Kumar et al., 2012a). Chronic usage of 3-NPA causes mitochondrial dysfunction and reactive oxygen species formation, and changes in various intercellular processes (Garcia et al., 2002). Cell death event results from the 3-NPA induced deregulations of multiple downstream processes that mimic the HD brain's pathophysiology (Gonchar et al., 2021). Therefore, due to the resemblance of 3-NPA in the pathophysiological processes of HD, this model is enlisted as the most reliable animal model to study HD.

A limited number of approaches are available to treat such disorders. Therefore, quality of life is improved by delaying the beginning or retarding the development of neurodegenerative diseases (Durães et al.,

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https://doi.org/10.1016/j.crphar.2022.100130
Received 27 June 2022; Received in revised form 7 September 2022; Accepted 19 September 2022

Table 1
Experimental protocol.

Sr. No.	Groups
1.	Vehicle control
2.	3-NPA treated groups (10 mg/kg/i.p./day) for 21 days
3.	Silymarin (50mg/kg/p.o./day) + 3-NPA (10mg/kg/i.p./day) for 21 days
4.	Silymarin (100mg/kg/p.o./day)+3-NPA (10mg/kg/i.p./day) for 21 days
5.	Silymarin (200mg/kg/p.o./day)+3-NPA (10mg/kg/i.p./day) for 21 days
6.	Silymarin*Perse* (200mg/kg/p.o./day) for 21 days

2018). Neurodegenerative disorders are usually managed using various therapeutic strategies. Recent evidence suggests that antioxidants and anti-inflammatory agents of natural origin can prevent/delay damage and cell death when used concomitantly with several neurotoxins. Silymarin, a flavonolignan originating from the' milk thorn' plant seeds (Valková et al., 2020), Apart from ability to cross the blood brain barrier (BBB), silymarin have proven antioxidant, anti-apoptotic, anti-inflammatory and enzyme inhibitory activities and represent its potential by providing protection of neurons against oxidative insults in the brain under distress. Its mechanism of action makes this molecule different form other neuronal moieties. Its diverse mechanism of action includes general antioxidant nature to specific anti-amyloidogenic, pro--estrogenic, anti-inflammatory properties (Borah et al., 2013). Previous data represents good neuroprotective features and has a broad spectrum of pharmacological actions, including upregulating glutamate transporter (GLUT-transporter), antioxidant activity, anti-inflammatory, and proven to exhibit neuroprotective properties around the series of neurological conditions including AD, PD and CI (Borah et al., 2013).

The empirical study investigate the impacts of Silymarin against 3-NPA induced Huntington disease in experimental wistar rats (Chandolia, 2021).

2. Methods & materials

2.1. Experimental rodants

The empirical research attempts, wistar rodants (either sex) weighed 200–250g, aged-matched (to avoid variability) was acquired from the MRSPTU, major animal house in Punjab (Bathinda) India. These rodants were housed in polyacrylic containers and were subjected to regular laboratory procedures, including 12 h light/dark cycle, 22-degree Celsius room temperature as well as humidity levels maintained up to 55 to 60 percent. RO filtered water and food stuffs were available at all times. All behaviour examinations occurred between 9:15 a.m. to 6:00 p.m. The research was authorised under two separate protocols with unique reference no.MRSPTU/IAEC/2019/05. The protocol was performed in accordance with the criteria of CPCSEA and approved by the IAEC.

2.2. Groups of experimentalist

36 animals were assigned, and six animals were randomly entrusted to each different treatment group. The experimental groups (Table 1).

2.3. Drugs and chemicals

Sigma Aldrich Chemicals Pvt. Ltd, Bangalore (India) supplied the Silymarin and 3-NPA. Biochemicals and other chemicals and employed in this investigation had higher analytical scores.

2.4. Treatment schedule

To induce HD-like symptoms, 3-NPA was disseminated in buffered saline at an appropriate 7.4 pH level (given once a day by *i.p.* route during 21 days at a dosage of 10 mg/kg). Silymarin (50,100, and 200 mg/kg) was diluted in saline and given orally half an hour before treatment with 3-NPA. Bodyweight and behavioural parameters like rotarod, locomotor activity, open field activity, and narrow beam walk were assessed weekly for 21 days. Terminally on the 22nd day, to estimate biochemical parameters, the striatum was isolated after the animals were sacrificed, and biochemical parameters such as catalase, peroxidation of lipids, nitrite, glutathione reduction, estimation of proteins, and superoxide dismutase were estimated.

2.5. Determination of rat's body weight

Body weight of wistar rats was determined before and after 3-NPA administration on day 1st and then on day 22nd of the current study. The % weight change was computed by utilizing the given formula below (Kumar et al., 2012b), (Kumar et al., 2011).

$$\text{Body weight change} = \text{Body wght. (1st day-21st day)} / \text{1st day body wght.} \times 100$$

2.6. Evaluation of behavioural action

2.6.1. Rotarod workout

At weekly intervals, motor coordination, grip strength, and integrity of animals were evaluated (IMCORP, Ambala, India) with the help of rotarod as described in the literature (Khan et al., 2015), (Kumar et al., 2012c). The rotarod apparatus consists of a non-slippery rod divided into four equal sections. A prior training session was used to acclimatise the rat to the equipment. In both studies, the rats were placed on a 7-cm-diameter spinning rod (speed 25 rotation per minute). Time limit was set at 180 s to measured fall-off latency (Bishnoi et al., 2008), (Datta et al., 2016).

2.6.2. Open field test

VJ instrument was used to record the behaviour of all animals at the end of the investigation framed above the open field square. To monitor locomotor activity, all rats were quietly put on a black wooden apparatus (OFT) comprising 36 squares. The experimentation area was entirely dark, with only an artificial illumination to assist the video monitoring system. Before behavioral evaluations, animals were given 5 min to acclimate and investigate the surroundings. A video monitoring system monitored the open field investigations, including the amount of squares walked traversed distance (with all paws), traveled, time allocated with movement (active time), time spent not moving (passive time), and average speed through (VJ instruments, India) (Aswar et al., 2017). The final value was calculated by averaging the 3 times monitored activity throughout the experiment, with a cutoff duration of 10 min. After exposure to the For 10 min, rats were distress in places (a rectangular shaped wooden enclosure) had been sanitized by using ethanol at a previous rate of 70% conducting this event.

2.6.3. Narrow beam walking test

A narrow beam (8 cm in diameter) was employed in this work, which was connected by a cylindrical steel rod (thickness of 0.5 mm, width of 2.0 cm, with a 120 cm length). The beam was raised 1 m above floor level. To cushion the rat's landing, under the beam sawdust-filled box was levelled. Before training, the rats were given 5 min to adjust to the new environment. In each trial, the count of slips and how long it would take a rat to traversed beam from one end to the other were recorded. The final value was calculated by averaging the three recorded transfer latencies, and the inter-trial period was conducted for 2 min (Khan et al., 2015), (Kumar et al., 2012c).

2.6.4. Striatal tissue homogenate preparation

On day 22nd, rats were euthanized, and their brains were taken and preserved in a refrigerator at −20 °C until they could be analyzed. Deeply

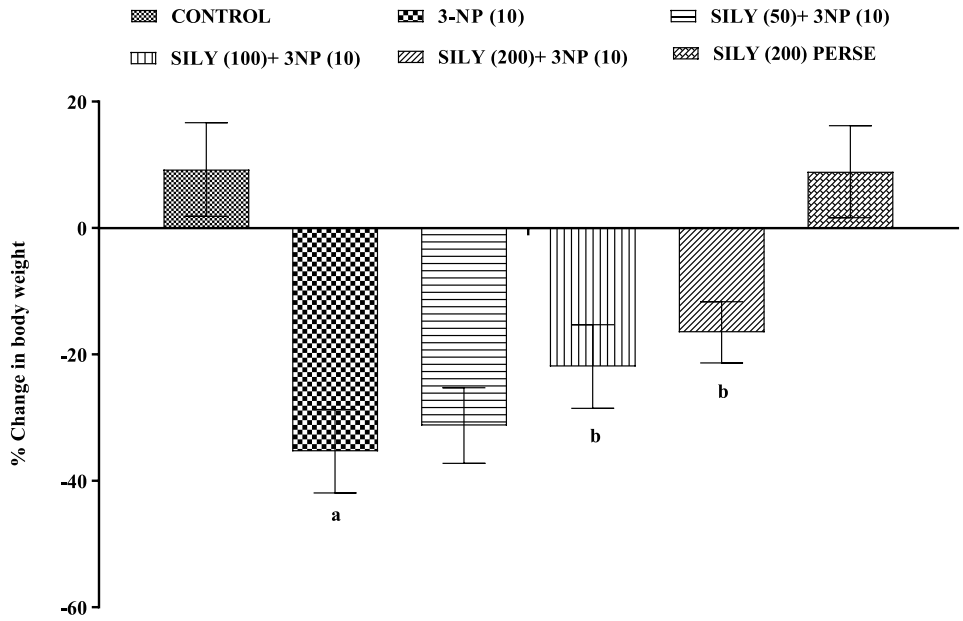

Fig. 1. Effect of silymarin on body weight in 3-NP treated rats. [a]p<0.01 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

frozened brains were evacuated on the day of the experiment, in addition, the striatum was separated by positioning them on ice bags, weighing them, and homogenising them with phosphate buffer (0.1 Molar) with the pH 7.4. After centrifuging homogenization was done for 15 min at 10,000 g, all aliquots of the supernatant were separated and used for biochemical examination.

2.7. Oxidative stress parameters prediction

2.7.1. Calibration of malon-di-aldehyde

To quantify the MDA-end product, a quantitative assessment of LPO levels in the striatum of the brain, and cortical regions was performed applying (Wills methodology), which was introduced in 1966 (Wills, 1966). By accessing a Shimadzu spectrophotometer at 532 nm, optical density was measured.

2.7.2. Quantification of nitrite

The Griess reagent (0.1 percent N-(1-naphthyl) ethylenediamine dihydrochloride, 2.5 percent phosphoric acid, and 1 percent sulfanilamide was used to estimate nitrite deposition in the striatum supernatant homogenate (Green et al., 1982). A UV spectrophotometer at 540 nm, was utilized to identify absorbance.

2.7.3. Quantification of the depletion of glutathione levels

Ellman's method was used to estimate the depletion of glutathione (GSH) in the brain's striatum and cortical areas (Ellman, 1959).

2.7.4. Protein quantification

The Lowry procedure, which uses Folin phenol as a reagent, was used to test protein levels in the brain's striatum and cortical areas (Gornall et al., 1949).

2.7.5. Measurement of catalase

Activity of catalase was determined with techniques outlined by Luck et al., where the distension of H_2O_2 was measured at 230 nm (Luck, 1963) only for 2 min intervals by using a Shimadzu UV/visible spectrophotometer.

2.7.6. Assessment of super-oxide dismutase content

Super-oxide dismutase content was examined by using approach given by Kono et al., (1978) (Kono, 1978).

2.8. Statistic validation

The assembled data is represented as Standard Mean Deviation. Two-way (ANOVA) had been used to investigate the behavioral data, proceeded by Bonferroni's post hoc analysis for a couple of correlations. A one-way ANOVA is used to make the correlations of biochemical parameters, proceeded by Tukey's post hoc analysis.

3. Results

3.1. SILY's effect on body weight in rats injected 3-NPA

The first and last body weights of the controlled animals did not differ significantly. 3-NPA treatment via intraperitoneal route with dose (10 mg/killogram/day) results ($P < 0.001$) steady weight decrease in correlation to control. Pretreatment with silymarin via orally route with dose (100 & 200 mg/killogram) substantially ($p < 0.001$) greatly lowered the decline in body weight in comparison to 3-NPA treated group whereas low dose of silymarin via orally route with dose (50 mg/kg/) and higher dose (200 mg/kg/) *perse* demonstrated no substantial difference between the 3-NP and control-treated groups, respectively (Fig. 1).

3.2. SILY's effect on the time to cross and the number of foot slips on a narrow beam walking device in 3-NPA-treated rats

In compared to the control group, 3-NPA delivered via intraperitoneal route with dose (10 mg/killogram) systemically increased the time it takes to cross and the number of footfalls on the narrow beam considerably (P0<001). Silymarin as a pre-treatment via orally route at various two dosage (100 & 200 mg/killogram) substantially (P < 0.05) reduces the counts of footfalls and time it takes to transverse the beam as compared to the 3-NPA group. Whereas smaller doses of silymarin via orally routes with dose (50 mg/killogram) and higher dose (200 mg/killogram) *perse* showed no substantial effect as correlation to 3-NPA and

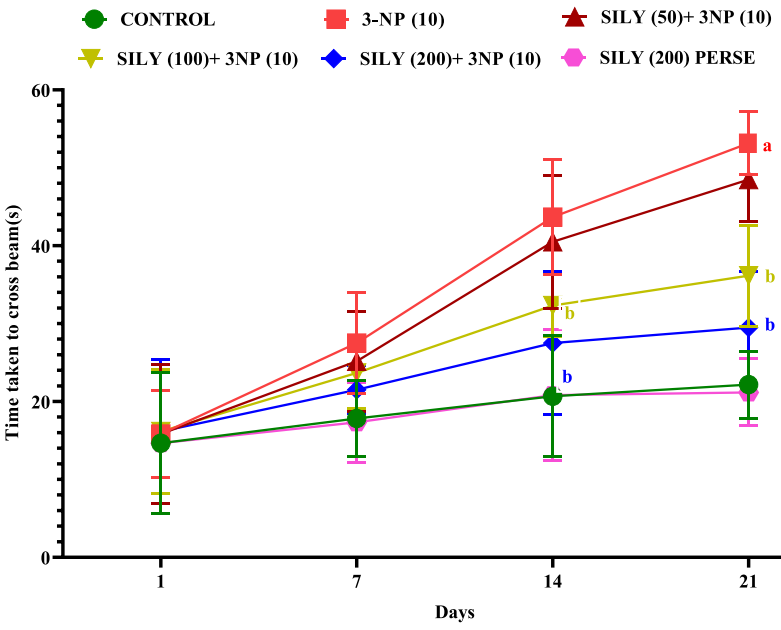

Fig. 2A. Effect of silymarin on time taken to cross beam (s) in 3-NP treated rats (n = 6). [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP. 3-NP = 3-Nitropropinic acid, SILY= Silymarin.

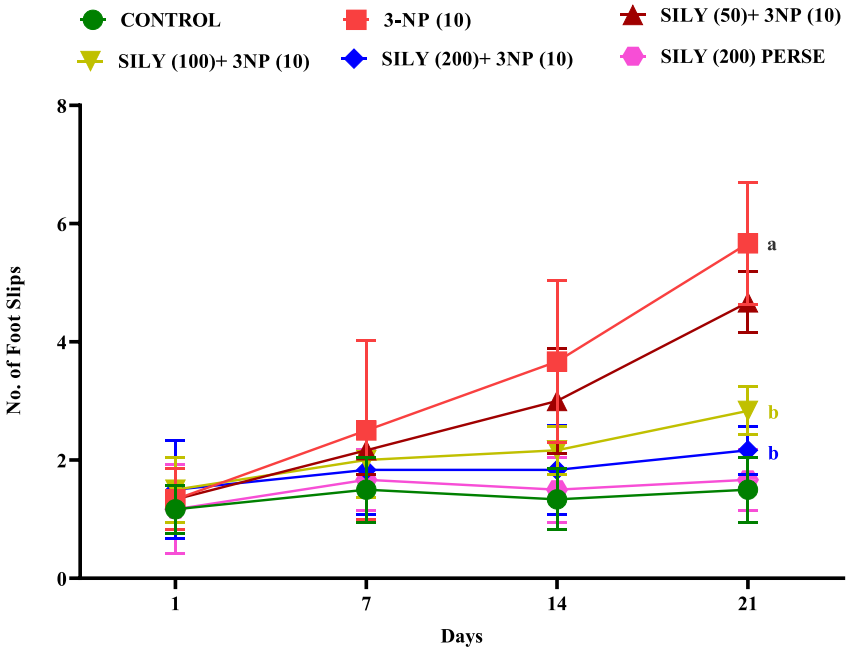

Fig. 2B. Effect of silymarin on No. of foot slips in 3-NP treated rats. Mean ± S.D. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP. 3-NP = 3-Nitropropinic acid, SILY= Silymarin.

control-treated groups, respectively (Fig 2A; 2B).

3.3. SILY's effect on 3-NPA-triggered modifications in rotarod activity

3-NPA via intraperitoneal route at a dose (10 mg/killogram) produced a substantial (P < 0.01) lower rotarod activity on the 14th and 21[st]day as correlated with control group (Fig. 3). Silymarin as a pretreatment via orally route at various two dosage (100 & 200 mg/kg) substantially (P < 0.05) averted rotarod activity deficit in correlation to 3-NPA treated rats. A smaller dose of silymarin via orally route (50 mg/kg) and a higher dose (200 mg/kg) *perse* had no substantial effect on rotarod activity comparison compared with 3-NPA and control-treated groups, respectively.

3.4. SILY's effect on open field test (OFT) for motor coordination in 3-NPA-treated rats

The (Maze Master 3.0) video monitoring software system monitored

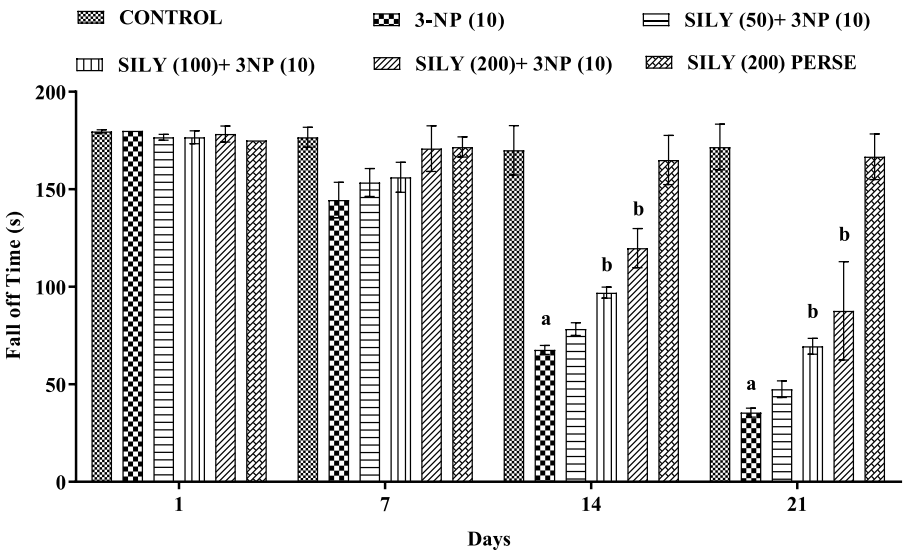

Fig. 3. Effect of silymarin on rotarod activity in 3-NP-treated rats. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

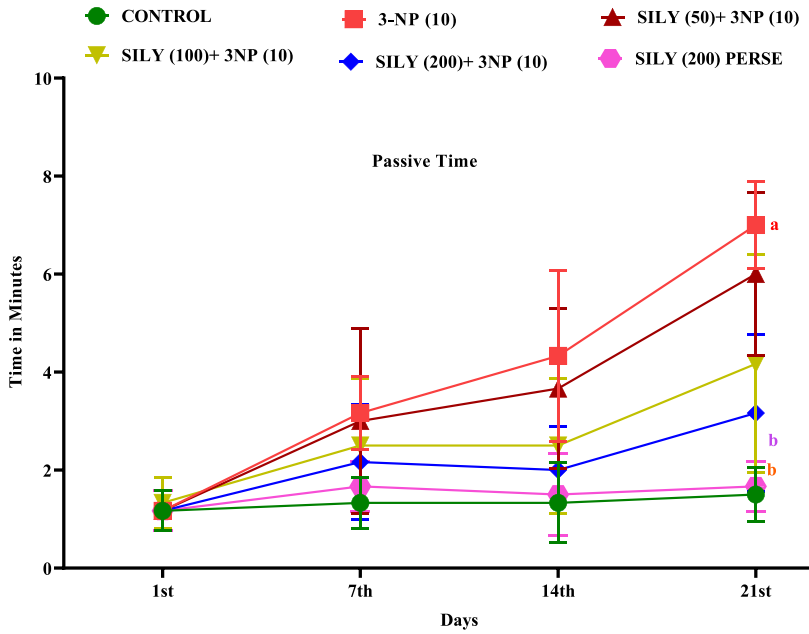

Fig. 4A. Effect of silymarin on Passive time spent in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

the action of 3-NPA animals were treated that show a remarkable reduction in crossings, total distance, average speed, active time and increased passive time on the seventh, fourteenth, and twenty-first days in comparison to control group. In contrast, pre - treatment with silymarin via orally route (100 & 200 mg/kg) substantially ($p < 0.05$) reversed the 3-NPA induced behavioural alteration. (Fig 4 A, B, C, & D).

3.5. SILY's effect on nitrite levels in rats exposed 3-NP-induced neurotoxicity

The nitrite level in the striatum of the 3-NPA, when correlated to the controlled group, the treated group grew considerably ($p < 0.001$). Silymarin as a pre-treatment via orally route at other two doses (100 & 200 mg/kg) decreased the nitrite level correlated to the 3- NPA group (Table 2) & (Fig. 5).

3.6. SILY's effect on MDA levels in rats exposed to 3-NP-induced neurotoxicity

MDA levels inside the striatum, 3-NPA treated group increased significantly ($P < 0.001$), indicating a significant increase in lipid peroxidation. Repeated administration of silymarin via orally with dose of (100 & 200 mg/kg) significantly ($P < 0.001$) prevents the elevation in the MDA levels compared to the 3-NP group and vehicle treatment. A low

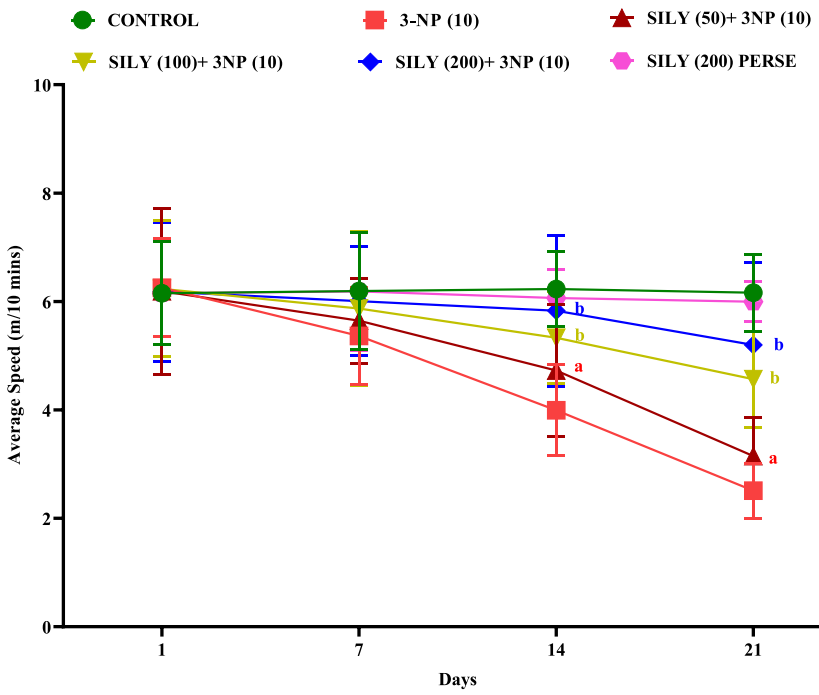

Fig. 4B. Effect of silymarin on average speed in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

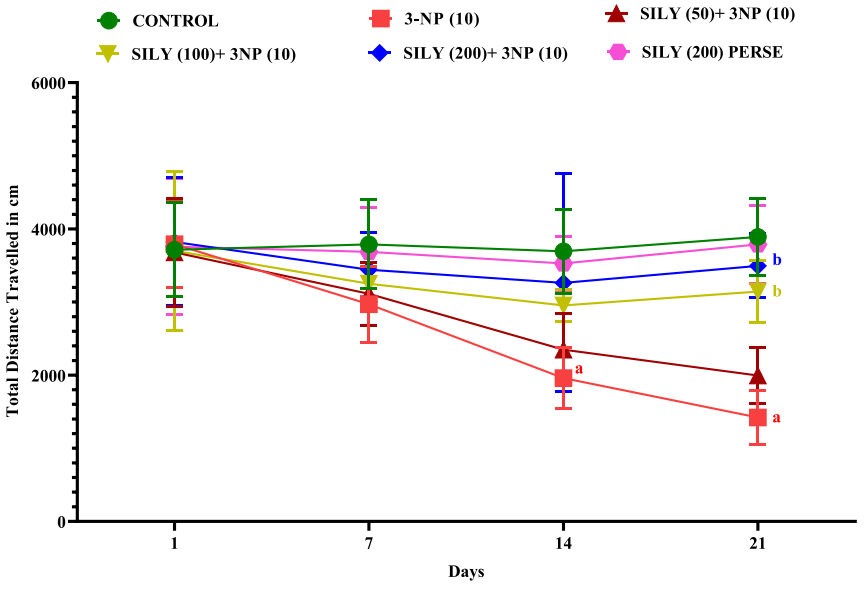

Fig. 4C. Effect of silymarin on total distance traveled in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

dose of Silymarin via orally route with dose of (50 mg/kg) did not significantly affect brain MDA levels (Table 2) & (Fig. 6).

3.7. SILY's effect on glutathione levels in rats exposed to 3-NPA-induced neurotoxicity

The GSH level inside the striatum, 3-NPA treated group was considerably lower (P < 0.001) than the controlled group on the 21st day. Silymarin as a pre-treatment via orally route at dosage of (100 & 200 mg/kg) increases the GSH level in comparison to the 3-NPA group and vehicle treatment. However, silymarin (50 mg/kg) demonstrated no statistically significant influence (Table 2) & (Fig. 7).

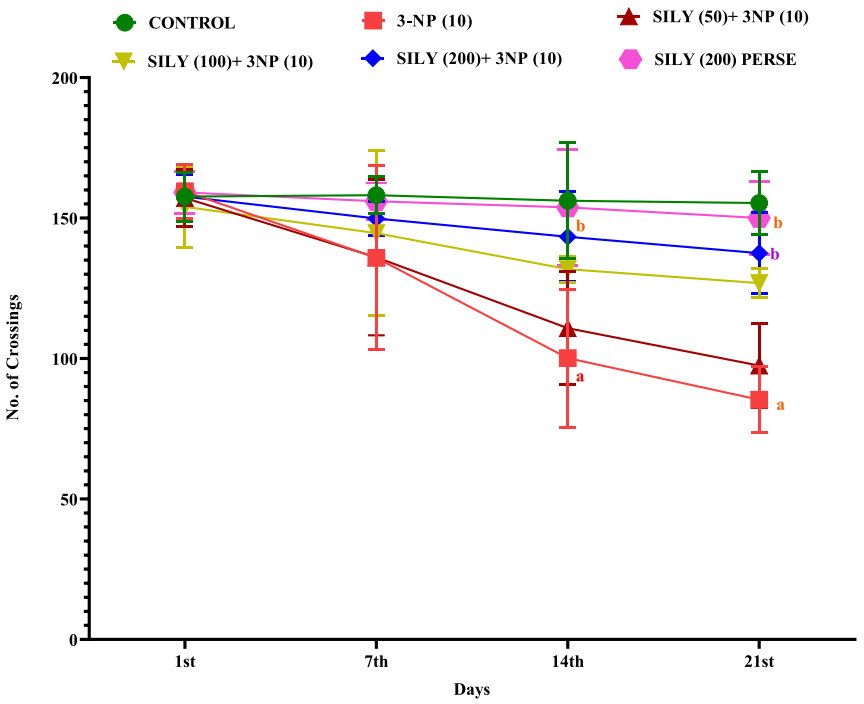

Fig. 4D. Effect of silymarin on the number of crossings in open field apparatus in 3-NP treated rats (n = 6). Data analyzed by two-way repeated-measures ANOVA followed by Bonferroni's multiple comparison. [a]p<0.001 versus control, [b]p < 0.05 versus 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

Table 2
Effect of Silymarin on 3-NPA induced biochemical changes in striatum.

Groups (mg·kg-1)	Nitritelevel, mmol mg^{-1} protein (% of vehicle)	MDA, nmol mg^{-1} protein (% of vehicle)	GSH(μmol GSH/mg protein)	Catalase, mmol of H2O2 decomposed min^{-1} mg^{-1} protein (% of vehicle)	SOD, U mg^{-1} protein (% of vehicle)
Control	126.695 ± 28.645	0.522 ± 0.110	0.016 ± 0.002	1.436 ± 0.110	2.654 ± 0.404
3-NPA (10)	473.389 ± 26272	3.906 ± 0.456	0.006 ± 0.001	0.869 ± 0.118	0.722 ± 0.188
Silymarin (50) + 3-NP A(10)	415.351 ± 32.431	3.521 ± 0.436	0.007 ± 0.002	1.049 ± 0.090	1.113 ± 0.199
Silymarin (100) + 3-NPA (10)	285.388 ± 39.374	2.126 ± 0.645	0.012 ± 0.001	1.276 ± 0.133	1.614 ± 0.329
Silymarin (200) + 3-NPA (10)	217.111 ± 40.972	1.685 ± 0.358	0.015 ± 0.002	1.328 ± 0.160	2.260 ± 0.346
Silymarin (200) Perse	124.862 ± 28.494	0.407 ± 0.107	0.015 ± 0.002	1.419 ± 0.228	2.520 ± 0.392

Values expressed as Mean ± S.D.
Data analyzed by one way ANOVA followed by Tukey's post hoc test.
[a]p< 0.001 vs. VC; [b]p < 0.05 vs. 3-NPA.

3.8. SILY's effect on catalase levels in rats exposed to 3-NPA-induced neurotoxicity

3-NPA given at a systemic dose significantly (P < 0.001) inhibited the catalase intensity as compared with the control in the striatum on the 21st day. Silymarin as a pre-treatment via orally route at two respective doses (100 & 200 mg/kg) increases the catalase intensity compared with 3-NPA group and vehicle treatment. A decreased concentration of Silymarin via oral route (50 mg/kg) demonstrated no statistically significant influence (Table 2) & (Fig. 8).

3.9. SILY's effect on SOD levels in rats exposed to 3-NPA-induced neurotoxicity

3-NPA given at a systemic dose substantially (P < 0.001) reduced the SOD intensity as correlated with the controlled in the striatum on the 21st day. Silymarin as a pre-treatment via orally route at two respective doses (100 & 200 mg/kg) increases the SOD and control group. Moreover, silymarin via orally route (50 mg/kg) demonstrated no statistically significant influence (Table 2) & (Fig. 9).

4. Discussion

The current study's findings indicates the neuroprotective potential of silymarin's against 3-NPA triggered neurotoxicity in rats. In this studies, rats were given *i.p.* injections of 3-NPA (10 mg/kg) for 21 days that showed behavioural alterations and produced chronic motor deficiency, increase in the count of footfalls as well as time took to cross the beam on narrow beam walk performance indicating stable motor impairment and striatal dysfunctioning. It was markedly overturned by the administering of silymarin in a dose-dependent manner. In comparison to the vehicle-treated group, the 3-NPA-treated group lost weight substantially. Furthermore, biochemical analysis revealed that silymarin (100 & 200 mg/kg) *p.o.*administration considerably lowered 3-NPA induced oxidative (elevation in lipid peroxidation) and nitrosative (elevated nitrite) stress in the striatum region of rats brain. Furthermore, chronic administration of 3-NPA lowered superoxide dismutase (SOD), glutathione (GSH) and catalase amounts which were significantly increased by silymarin in a dose-dependent manner.

However, the precise pathways producing neuropathology in movement disorders are uncertain, many pathways relating to

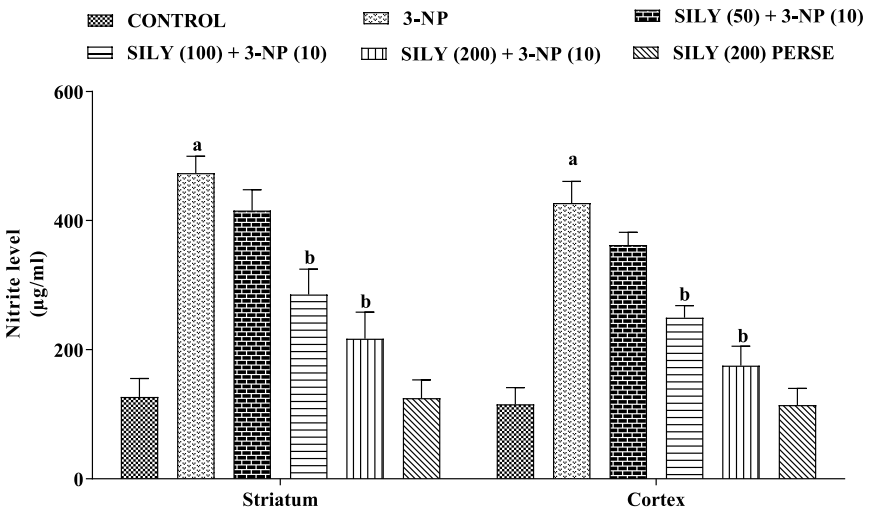

Fig. 5. Effect of silymarin on nitrite level in 3-NP treated rats. Values are expressed as mean ± S.D. $^{a}p < 0.001$ as compared to control, $^{b}p < 0.001$ as compared to 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

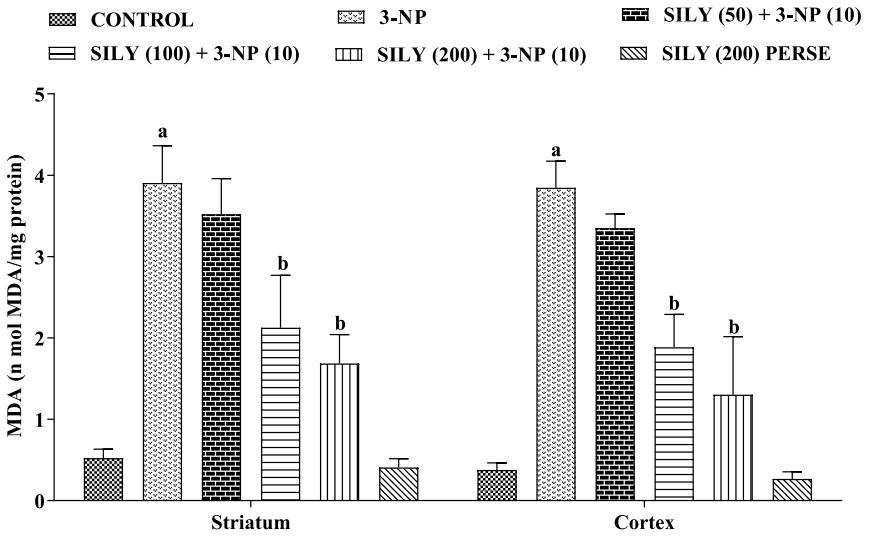

Fig. 6. Effect of silymarin on Lipid peroxidation level (LPO) in 3-NP treated rats. Values are expressed as mean ± S.D. $^{a}p < 0.001$ as compared to control, $^{b}p < 0.001$ as compared to 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

neurotransmitter changessuch as excitotoxic neuronal death, oxidative stress, mitochondrial malfunction, and neuroinflammation have been proposed in the literature (Singh et al., 2015), (Kumar and Kumar, 2009a) responsible for alteration in neuropathology. 3-NPA irreversibly blocks the enzyme SDH, which results in decreased levels of ATP, and neuronal cell death may be observed (Kumar and Kumar, 2009b), (Túnez et al., 2011). However, the specific pathophysiology of SDH silencing is yet unknown. Studies suggested that the dianion form of the toxin's carbanion more likely binds to the site of substrate to be oxidised into 3-nitroacrylic acid, that can reacted with the substrate site's thiol group to form a covalent adduct (Coles et al., 1979). It was recently discovered that 3-NPA produces a covalent adduct with the catalytic base arginine complex II after oxidation. The 3-NPA rapidly penetrates the blood-brain barrier, inactivating SDH activity within 2 h of intraperitoneal injection, and can be given systemically to rats, mice, and non-human primates. Depending on the time course of treatment, the 3-NPA model can emulate both hyperkinetic and hypokinetic HD symptoms (Wiprich et al., 2020). Thus 3-NPA toxicity results in ATP and energy levels reduction, calcium homeostasis disruption, excitotoxicity events, and neuronal death (Kumar and Kumar, 2009a). The death of excitotoxic cells has formation of oxidative stress and reactive oxygen species (ROS) has been related. Extracellular glutamate accumulation result in defected glutamate transmission and has been identified as a target for treatment in HD (Kraskovskaya and Bezprozvanny, 2021). Glutamate is the most widely known as neurotransmitter (EAA) excitatory amino acid in mammals, and open ligand-gated ion channels such NMDA, G-protein-coupled metabotropic, kainite and AMPA receptors. Due to accumulation and over-activation of EAA receptors, cell death of neurons can be found within acute and chronic neurodegenerative conditions, including ALS, HD, AD, and behavioural disorders like depression (Gegelashvili et al.,

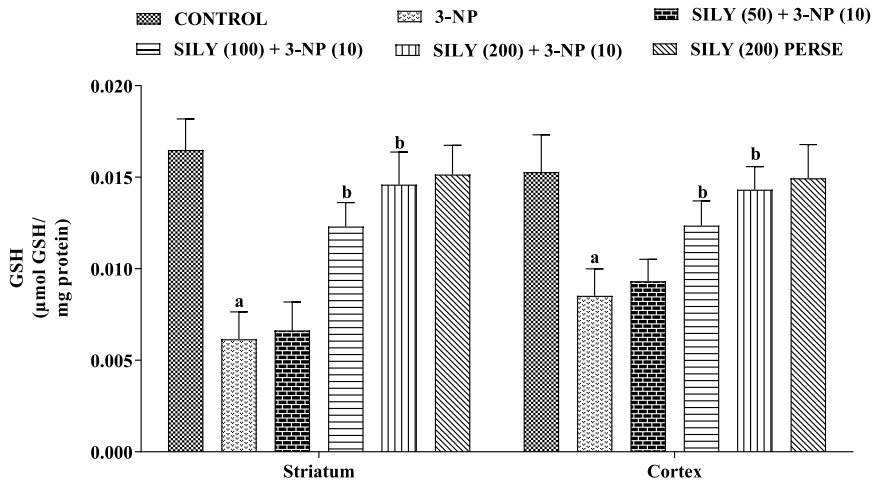

Fig. 7. Effect of silymarin on Reduced Glutathione level in 3-NP treated rats. Values are expressed as mean ± S.D. $^{a}p < 0.001$ as compared to control, $^{b}p < 0.05$ as compared to 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

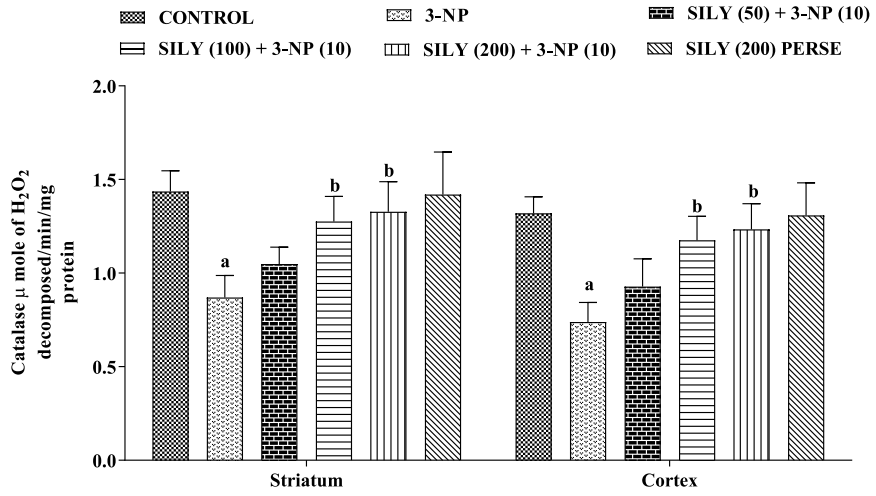

Fig. 8. Effect of silymarin on Catalase activity in 3-NP treated rats. Values are expressed as mean ± S.D. $^{a}p < 0.001$ as compared to control, $^{b}p < 0.05$ as compared to 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

2001). Hence, The gait abnormalities observed in present study are in accordance with previous researches following 3-NP administration (Rodríguez-Campuzano and Ortega, 2021).

On the other hand, Silymarin is the polyphenolic flavonoid derived from Silybum marianum dried fruit, which has been used very frequently since ancient times for hepatoprotective activities (Wang et al., 2015b), (AbouZid et al., 2016). Because of its comprehensive medicinal properties, it is one of the most commonly used flavonoids among phytochemicals (Mady et al., 2016; Pogačnik et al., 2020; Singhal et al., 2011; Baluchnejadmojarad et al., 2010). Silymarin has been reported safely profile in various pathological conditions such as kidney, liver, CNS, pancreas, and skin (Pogačnik et al., 2020). Silymarin is known to have antioxidant and anti-apoptotic properties (Singhal et al., 2011). Its antioxidant action has been demonstrated to be beneficial in animal models of PD and AD (Baluchnejadmojarad et al., 2010).

In the current research, Silymarin significantly attenuates the behavioral and biochemical alterations induced by 3-NPA. It is found that Silymarin possesses significant neuroprotective effects against 3-NPA induced biochemical and behavioral alterations. Silymarin therapy at doses of (100 & 200 mg/kg/oral) dramatically minimize lipid peroxidation and concentrated nitrite extent in striatum of the brain, while also preserving antioxidant enzyme levels indicating putative antioxidant activity. All these results are in good agreement with previous studies (Kumar and Kumar, 2009b). Furthermore, apart from antioxidant activity in association with scavenging of free radicals, its mechanism include maintenance in the production of cellular glutathione (GSH) (Singhal et al., 2011) which can be the reason behind improvement in antioxidant activity in present study.

It could be concluded that, Silymarin can attenuate 3-NPA induced motor deficits and oxidative nitric oxide stress through antioxidative mechanisms. Antioxidant activity evaluated in the present study inidicates as well as verify its scavenging free radicals mechanism. Furthermore, favourable enhancement of glutamate transporter and free radical quenching property by silymarin is an important factor observed in the study. More research must be carried out to focus exclusively on potential antioxidative mechanism.

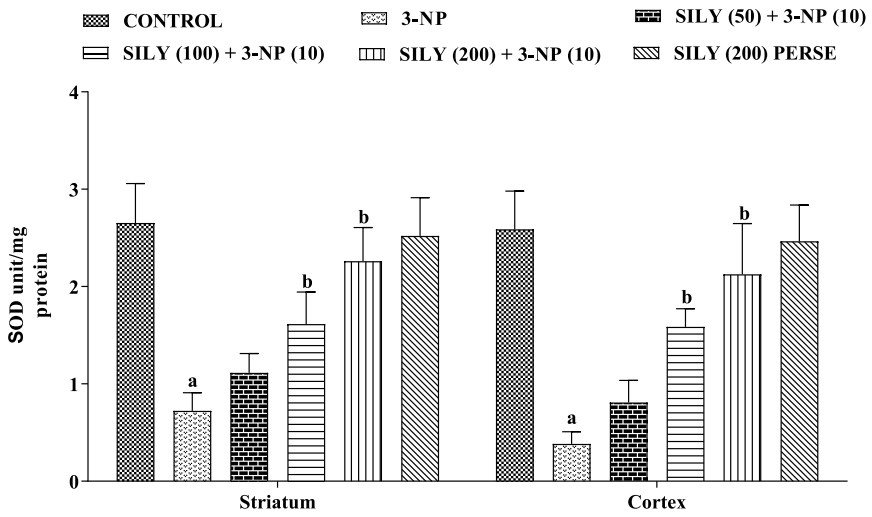

Fig. 9. Effect of silymarin on superoxide dismutase activity in 3-NP treated rats. Values are expressed as mean ± S.D. $^{a}p < 0.001$ as compared to control, $^{b}p < 0.05$ as compared to 3-NP.
3-NP = 3-Nitropropinic acid, SILY= Silymarin.

CRediT authorship contribution statement

Priyanka Chandolia: conducted the experiment, wrote the manuscript. All author read and approve the manuscript and all data were generated in-house and that no paper mill was used. **Vikrant Rahi:** wrote the manuscript. All author read and approve the manuscript and all data were generated in-house and that no paper mill was used. **Puneet Kumar:** conceived and design of research, wrote the manuscript. All author read and approve the manuscript and all data were generated in-house and that no paper mill was used.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data has been sheared in manuscript as results

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