Effect of preferential cyclooxygenase-2 (COX-2) inhibitor against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced striatal lesions in rats: Behavioral, biochemical and histological evidences

Amit Gupta, Ashish Dhir, Anil Kumar & S K Kulkarni*
Pharmacology Division, University Institute of Pharmaceutical Sciences
Panjab University, Chandigarh 160 014, India

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Cyclooxygenase (COX) isoenzyme is known to play an important role in the pathophysiology of Parkinson’s disease. The present study evaluated the neuroprotective effect of nimesulide, a preferential COX-2-inhibitor against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-model of Parkinson’s disease. Intrastriatal administration of MPTP (32 µmol in 2 µl) produced a significant decrease in the locomotor activity. Biochemical investigation of striatal region revealed a significant enhancement in the oxidative stress as evidenced by increased lipid peroxidation levels, nitrite levels and myeloperoxidase activity along with depleted antioxidant pool (reduced glutathione and superoxide dismutase levels) and reduced redox (GSH/GSSG) ratio. MPTP administration also showed significant mitochondrial complex-I inhibition and reduction in the mitochondrial viability. Histological examination of the MPTP-treated brain sections revealed alteration in the histo-architecture as well as undifferentiated bodies of varying contour and lesions. Chronic administration of nimesulide (5 or 10 mg/kg, po) for 12 days, significantly reversed the behavioral, biochemical, mitochondrial and histological alterations induced by MPTP. In conclusion, the findings of the present study implicate the possible neuroprotective potential of nimesulide in MPTP-treated rats and thus highlight the therapeutic potential of COX-inhibitors in treatment of Parkinson’s disease.

Keywords: Cyclooxygenase, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Nimesulide, Neuroprotection, Oxidative stress, Parkinson’s disease

Parkinson’s disease (PD) is one of the major neurodegenerative disorders, caused by degeneration of dopaminergic neurons in the nigro-striatal pathway and impairs motor coordination1. The complexity of this disease progression poses a great hindrance in the treatment and management of PD. Growing number of evidences from experimental and clinical studies strongly points towards the active role of neuroinflammation in the etiopathogenesis of PD2-4.

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is a potent neurotoxin which results in selective degeneration of dopaminergic neurons projecting from substantia nigra pars compacta (SNpc) into striatum and mimics PD-like symptoms in experimental models5. MPTP causes degeneration of dopaminergic cell in the striatum by influencing the mitochondrial function as one of the mechanisms6. Challenge with MPTP stimulates certain neuroinflammatory cascade and activates glial cells which up-regulates cyclooxygenase-2 (COX-2) expression (the rate-limiting enzyme involved in the production of prostaglandins from arachidonic acid) and enhanced expression of various pro-inflammatory mediators causing the neuronal death7,8. MPTP has also been demonstrated to induce reactive oxygen and nitrogen species in animals that further worsen neuropathological condition and promote neuronal death6,9. Besides this, enhanced oxidative stress activates various cellular mediators that cause secondary neuronal damage via glial-mediated neuroinflammation10. Clinical investigations on Parkinson patients during postmortem studies have also demonstrated the presence of activated microglia as well as enhanced levels of COX-2 expression in substantia nigra pars compacta region of brain8,11. COX is expressed in different parts of brain and therefore, it can be speculated that it is involved in the pathophysiology of various central nervous system related disorders3,12. The neuroprotective effect of non-steroidal anti-inflammatory agents (NSAIDs)
against MPTP-induced neuro-degeneration\textsuperscript{13,14} has been revealed. NSAIDs mainly exert their anti-inflammatory properties by inhibiting COX isoenzyme. The beneficial effects of COX-inhibitors in various neurodegenerative diseases\textsuperscript{15,16} have been reported. Since MPTP produced less dopaminergic neuronal degeneration in COX-2 deficient transgenic mice as compared to normal wild type mice, it seems that certain genes coding for inflammation present in the dopaminergic neurons become inactive in COX-2 deficient mice and thus exhibits neuroprotection\textsuperscript{17}. Therefore, it is speculated that inhibiting COX-isoenzyme may be a useful tool/target in preventing the neuronal loss in Parkinson’s disease. This speculation has been substantiated by the fact that inhibition of either COX-1 or COX-2 isoenzyme has neuroprotective potential in various models of neuronal injury\textsuperscript{3,14}.

Nimesulide is an anti-inflammatory drug belonging to the class of NSAIDs. The anti-inflammatory activity of nimesulide is presumed to be linked to its ability to inhibit COX-2 enzyme preferentially\textsuperscript{16}. Nimesulide has been withdrawn from market in many countries due to its certain side effects\textsuperscript{18}. Recent experimental studies have highlighted the potent neuroprotective potential of nimesulide, where it produced a long lasting neuroprotection rather than delaying the cellular death\textsuperscript{16,19}. However, the neuroprotective potential of nimesulide has not been investigated in MPTP model of Parkinson’s disease in animals. Also, studies have not explored the exact mechanism by which COX-2 inhibition exerts neuroprotection of dopaminergic neurons.

Based on the above observations, the present study is an attempt to unravel the possible cellular mechanism and to gain more insight into the neuroprotective effect of COX-2 preferential inhibitor against MPTP-induced striatal lesion in rats.

**Materials and Methods**

*Animals* — Male Wistar rats (250-300 g), bred in Central Animal House (CAH) facility of the Panjab University, Chandigarh, India were used. The animals were housed under standard laboratory conditions and maintained on natural light and dark cycle, and had free access to food and water. Animals were acclimatized to laboratory conditions before the experiment. Each animal was used only once. All the experiments were carried out between 0900 and 1500 hrs. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the Indian National Science Academy (INSA) Guidelines for the use and care of experimental animals.

**Drugs treatment and administration** — MPTP was purchased from Sigma (St.Louis, MO, USA). Nimesulide was a gift sample from Panacea Biotec Ltd., New Delhi, India. Nimesulide was suspended in 0.25% (w/v) carboxymethyl cellulose (CMC) solution and administered orally in a constant volume of 0.5 ml/100 g body weight of rat. MPTP was dissolved in normal saline and administered by intrastrital injection. Nimesulide was started 7 days before the surgery and on the 7\textsuperscript{th} day, 60 min after the nimesulide treatment, MPTP (32 µmol in 2 µl) was administered by a single intrastrital injection followed by nimesulide treatment for further 5 days.

**Sterotaxic surgery** — Animals were anesthetized with thiopental sodium (45 mg/kg, ip), MPTP was infused as a single intrastrital\textsuperscript{20} (coordinates: anterior+1.7 mm; lateral±2.7 mm; ventral-4.8 mm from bregma and dura) injection (32 µmol in 2 µl) using the Hamilton microsyringe.

**Experimental protocol and procedure** — Seven groups were employed in the present study, each comprising of 6-8 animals. Group I comprised of control and received equivalent volume of vehicle of drug (0.25% w/v CMC) for 12 days. Group II animals were sham control and received equivalent volume of vehicle (0.25% w/v CMC) of drug for 7 days and on the 7\textsuperscript{th} day, they received an intrastrital injection of normal saline, followed by CMC administration again for 5 days. Group III animals received equivalent volume of vehicle (0.25% w/v CMC) for 7 days and challenged with intrastrital injection of MPTP (32 µmol in 2 µl) on the 7\textsuperscript{th} day followed by vehicle treatment for further 5 days. Group IV and V animals comprised of nimesulide pretreated groups (5 or 10 mg/kg, po) for 7 days and on the 7\textsuperscript{th} day received an intrastrital injection of normal saline, followed by nimesulide treatment for 5 days respectively. Group VI and VII comprised of nimesulide treated group, and they received nimesulide in dose of 5 or 10 mg/kg, po for 7 days followed by intrastrital injection of MPTP, 60 min after last dose and treatment continued for 5 days. Separate groups were used for biochemical estimations and histological examination.

**Locomotor activity** — Animals were individually placed in actophotometer for studying the effect of drug treatment on locomotor activity\textsuperscript{21}. After 2 min
acclimatization, the locomotor activity was recorded for a period of 5 min.

**Biochemical estimations**

*Post-mitochondrial supernatant preparation (PMS)* — After sacrificing the animals, their brains were quickly removed; striatum was dissected out, perfused immediately with ice-cold normal saline and weighed. A 10% (w/v) tissue homogenate was prepared in chilled 0.1 M phosphate buffer (pH 7.4) using a Potter Elvenhjem homogenizer. The homogenate was centrifuged at 12000 g for 20 min, 4°C to obtain the post mitochondrial supernatant (PMS), which was used for further enzymatic analysis.

*Estimation of lipid peroxidation* — The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS)\(^2\). In brief, the reaction mixture consisted of 0.2 ml of 8.1% (w/v) sodium lauryl sulfate, 1.5 ml of 20% (v/v) acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% (w/v) aqueous solution of thiobarbituric acid was added to 0.2 ml of 10% (w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95 °C for 1 h. After cooling with tap water, 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) were added, shaken well and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. TBARS were quantified using extinction coefficient of 1.56 \(\times\) 10\(^5\) M\(^{-1}\) cm\(^{-1}\) and expressed as nanomoles of MDA/mg protein and as % of control.

*Estimation of antioxidant pool* — The reduced glutathione (GSH) was measured by the method of Ellman\(^23\). The yellow color developed by the reduction of Ellman’s reagent by –SH group of GSH was read at 412 nm. The –SH group was calculated on the molar extinction coefficient of yellow colored anion, 2-nitro mercaptobenzoic acid (1.36 \(\times\) 10\(^3\) M\(^{-1}\) cm\(^{-1}\)). The results are expressed as µmol of GSH/mg protein and as % of control. Redox ratio (GSH/GSSG), the primary determinant of cellular redox state was calculated by estimating the total glutathione (TSH) levels\(^2\). Once the values for total glutathione (TSH) and reduced glutathione (GSH) were obtained, levels of oxidized glutathione (GSSG) were calculated by taking the difference of total glutathione and reduced glutathione. Further, the ratio of GSH/GSSG was calculated to determine the redox status\(^2\).

The superoxide dismutase (SOD) activity was assessed by the method of Kono\(^2\). The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture, 0.05 ml of hydroxyamine and 0.05 ml PMS were taken and the auto-oxidation of hydroxyamine was observed by measuring the absorbance at 560 nm.

*Estimation of brain myeloperoxidase activity* — Myeloperoxidase activity was determined by modified technique of Bird et al\(^2\). After sacrificing the animals, the brain was removed; striatum was collected and homogenized in 5 ml of phosphate buffer (0.01 M). Homogenized tissue was centrifuged and supernatant collected was mixed with O-phenylenediamine (660 µg/ml in phosphate buffer) and 300 mM H\(_2\)O\(_2\) was added to initiate the reaction. Absorbance was measured at 492 nM at an interval of 30 sec for a total period of 2 min. Change in optical density/min was calculated and results were expressed as % myeloperoxidase activity considering 100% myeloperoxidase activity in the sham control group.

*Estimation of nitrite concentration* — Nitrite levels were estimated using Griess reagent, which served as an indicator of nitric oxide production\(^2\). Briefly, 1.0 ml of Griess reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water) was added to 1 ml of homogenate and absorbance was read at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite and nitrite levels were expressed as the % of control.

*Estimation of protein* — The protein content was measured according to the method of Lowry et al\(^2\) using bovine serum albumin as standard.

*Isolation of mitochondria* — Isolation of mitochondria from the rat brain was done by the method of Berman and Hasting\(^2\). Briefly, brain homogenate was prepared in isolation buffer (consisting of 215 mM mannitol, 75 mM sucrose, 0.1% w/v bovine serum albumin, 20 mM HEPES buffer and 1 mM of EGTA in 100 ml of distilled water and pH adjusted to 7.2 with potassium hydroxide) and centrifuged at 13000 g for 5 min at 4°C. Pellet was resuspended in isolation buffer with EGTA and centrifuged at 13000 g for 5 min at 4°C. Supernatant was transferred to microcentrifuge tubes and tapped off with isolation buffer with EGTA and centrifuged at 13000 g for 10 min at 4°C. After centrifugation, supernatant was discarded and pellet
was resuspended in 1 ml of isolation buffer without EGTA and further centrifuged at 10000 g for 10 min at 4°C. Finally, pellet free of contamination was dissolved in 200 µl of isolation buffer without EGTA and used for further analysis.

**Mitochondrial complex-I activity** — Mitochondrial complex-I activity was estimated by the spectrophotometric method. Briefly, the reaction mixture consists of 350 µl of 0.2 M glycyl glycine buffer, 100 µl of 1.05 mM cytochrome-c, 100 µl of 6 mM NADH (in glycyl glycine buffer), 2.4 ml of distilled water, 10 µl of sample and 20 µl of 0.02 M sodium bicarbonate. The change in optical density at 550 nm was recorded and activity was expressed as % of control.

**MTT assay** — MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a measure of viable cells. In this assay, yellow colored MTT was reduced to purple colored formazan by mitochondrial reductase enzymes present in living cells and a solubilising agent, usually dimethylsulfoxide was added to dissolve insoluble formazan product into a colored solution. Briefly, to 100 µl of sample, 10 µl of MTT (10 mg/ml in 0.1 M phosphate buffer) was added and incubated for 3 h at 37°C. After incubation, 200 µl of dimethyl sulphoxide was added to stop the reaction. The absorbance of this colored solution was quantified at 580 nm and the results in terms of ‘viability’ are expressed as % of control.

**Histological examination** — Histological examination of the mid brain sections was done with hematoxylin and eosin staining method. Brain was isolated immediately after sacrificing the animals and washed with ice-cold saline. It was then fixed with 10% v/v neutral buffered formalin solution. After fixing the tissue, sections were thoroughly washed and dehydrated in graded ethanol. After dehydration was complete, tissue was cleared in benzene and finally embedded in paraffin (52-55°C). Sections of 5 µm thicknesses were cut, deparaffinized in xylene, downgraded (hydrated) in decreasing % of alcohol and brought to water and stained with hematoxylin, upgraded (dehydrated) in alcohol till 70 % (v/v) and stained with 1 % (v/v) alcoholic eosin for appropriate time, differentiated in 90 % (v/v) alcohol, cleared in xylene and finally mounted in DPX.

**Statistical analysis** — Values were expressed as mean±SE. One-way analysis of variance (ANOVA) followed by post-hoc Tukey’s test was applied to calculate the statistical significance between various groups. A value of P<0.05 was considered to be statistically significant.

**Results**

**Effect of nimesulide on locomotor activity in MPTP-treated rats** — There was no significant difference between the locomotor activity of animals of control group (group II) and MPTP-treated group (group III) when assessed on day 1 or day 5. When group III animals challenged with intrastriatal injection of MPTP on the 7th day and evaluated for their locomotor activity on the 8th day, there was a significant decrease in the locomotor activity as compared to control group (group II) which was further decreased to 12th day (P<0.05) (Fig. 1). Pretreatment with nimesulide 5 or 10 mg/kg, po did not modify the locomotor activity when activity was assessed on day 1 or day 5. However, daily treatment with nimesulide (group VI and VII treated with MPTP) significantly reversed the decrease in locomotor activity as assessed on day 8 and day 12 (P<0.05) (Fig 1).

**Effect of nimesulide on oxidative stress-induced alterations in MPTP-treated rats** — A significant increase in the malondialdehyde levels was seen in the MPTP-treated group (group III) as compared to sham (group II) (P<0.05). However, daily treatment with nimesulide 5 or 10 mg/kg, po significantly attenuated the increased levels of lipid peroxides in group VI- and VII- treated with MPTP, respectively (P<0.05) (Table 1).

![Fig. 1 — Effect of nimesulide (5 or 10 mg/kg) on locomotor activity in MPTP-treated rats. [Locomotor activity is expressed as mean ± S.E. P<0.05 as compared to 'sham', 'MPTP-treated group', 'Nim(5)+MPTP treated group (one-way ANOVA followed by Tukey’s test)']](image-url)
Intrastriatal administration of MPTP in group III animals produced a significant decrease in the antioxidant pool (Table 1) as evident by decreased levels of reduced glutathione and superoxide dismutase (SOD) compared to sham (group II) \((P < 0.05)\). Also, the redox ratio (GSH/GSSG) was significantly decreased in MPTP challenged rats (group III) \((P < 0.05)\) (Table 2). Pretreatment with nimesulide (5 or 10 mg/kg, po) in group VI or group VII respectively, significantly restored the depleted levels of reduced glutathione and SOD as well as redox ratio to normal \((P < 0.05)\).

Significantly increased nitrite levels were observed in the animals challenged with MPTP (group III) as compared to sham (group II) \((P < 0.05)\) (Table 1). Daily treatment with nimesulide at a dose of 5 or 10 mg/kg, po in group VI or group VII respectively, significantly decreased the nitrite levels as compared to MPTP-treated animals in group III \((P < 0.05)\).

Myeloperoxidase (MPO) activity was found to increase significantly in the animals challenged with MPTP (group III) as compared to sham (group II) \((P < 0.05)\) (Fig. 2). Daily nimesulide (5 or 10 mg/kg, po) pretreatment in group VI or group VII, significantly decreased the MPO activity in as compared to MPTP-treated group (group III) \((P < 0.05)\) (Fig. 2).

**Effect of nimesulide on mitochondrial complex-I activity in MPTP-treated rats** — Mitochondrial complex-I activity was significantly decreased in the animals challenged with MPTP (group III) as compared to sham (group II) \((P < 0.05)\) (Fig. 3). Chronic nimesulide (5 or 10 mg/kg, po) pretreatment in group VI or group VII respectively, significantly restored the complex-I activity in rats as compared to MPTP-treated group (group III) \((P < 0.05)\) (Fig. 3).

**Effect of nimesulide on mitochondrial viability**

**MTT assay** — MTT assay indicated significant reduction in the viability by administration of MPTP (group III) as compared to sham (group II) \((P < 0.05)\). However, daily nimesulide administration at a dose of 5 or 10 mg/kg, po in group VI or group VII respectively, significantly

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### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Lipid peroxidation (nmol of MDA/mg protein)</th>
<th>MDA levels (% of control)</th>
<th>Reduced Glutathione levels (GSH) (µmol/mg protein)</th>
<th>GSH levels (% of control)</th>
<th>SOD levels (% of control)</th>
<th>Nitrite levels (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>1.02±0.003</td>
<td>100.0</td>
<td>0.05±0.003</td>
<td>100.0</td>
<td>100.0±4.01</td>
<td>100.0±9.20</td>
</tr>
<tr>
<td>Sham</td>
<td>1.10±0.001</td>
<td>107.6</td>
<td>0.052±0.001</td>
<td>102.6</td>
<td>99±2.94</td>
<td>98±7.45</td>
</tr>
<tr>
<td>MPTP</td>
<td>2.90±0.002 (^a)</td>
<td>285.5 (^a)</td>
<td>0.028±0.002 (^a)</td>
<td>53.9 (^a)</td>
<td>41±3.28 (^a)</td>
<td>181±7.23 (^a)</td>
</tr>
<tr>
<td>Nim (5)</td>
<td>1.00±0.003</td>
<td>98.7</td>
<td>0.048±0.003</td>
<td>94.8</td>
<td>99±1.17</td>
<td>99±8.55</td>
</tr>
<tr>
<td>Nim (10)</td>
<td>1.05±0.003</td>
<td>103.8</td>
<td>0.050±0.003</td>
<td>98.0</td>
<td>101±4.06</td>
<td>102±7.66</td>
</tr>
<tr>
<td>Nim (5)+MPTP</td>
<td>1.89±0.002 (^b)</td>
<td>186.5 (^b)</td>
<td>0.046±0.002 (^b)</td>
<td>90.2 (^b)</td>
<td>72±2.45 (^b)</td>
<td>144±7.44 (^b)</td>
</tr>
<tr>
<td>Nim (10)+MPTP</td>
<td>1.58±0.002 (^b,c)</td>
<td>155.6 (^b,c)</td>
<td>0.049±0.002 (^b)</td>
<td>96.1 (^b)</td>
<td>93±2.96 (^b)</td>
<td>109±6.75 (^b,c)</td>
</tr>
</tbody>
</table>

Ctrl – control, MPTP – 1-methyl 4-phenyl 1,2,3,6 – tertahydropyridine group, Nim(5 or 10) – nimesulide (5 or 10 mg/kg, po), Nim(5)+MPTP – nimesulide (5 mg/kg, po) + MPTP; Nim(10)+MPTP – nimesulide (10 mg/kg, po)+MPTP

\(^{P<0.05}\) as compared to \(^{a}\)sham, \(^{b}\)MPTP group, \(^{c}\)Nim(5)+MPTP treated group (one-way ANOVA followed by Tukey’s test)
restored the mitochondrial viability as compared to animals challenged with MPTP (group III) \( (P < 0.05) \) (Fig. 3).

**Effect of nimesulide on MPTP-induced morphological changes in brain** — Histological examination of the MPTP-treated brain sections showed alterations in the histo-architecture and disorganization of the three layers. Undifferentiated bodies of varying contour and size and lesions were present in large number. These bodies were similar to the inclusions frequently found in the degenerating dopaminergic neurons in the Parkinson’s patients. Group I did not show any morphological changes in striatal region of the brain. However, brain of MPTP-challenged rats in group III showed significant cellular deterioration in the striatal region of the brain. Animals pretreated with

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**Fig. 3** — Effect of nimesulide (5 or 10 mg/kg) on mitochondrial function in MPTP-treated rats. [Values are expressed as % of control. \( P < 0.05 \) as compared to a sham, b MPTP-treated group, c Nim(5)+MPTP treated group (one-way ANOVA followed by Tukey’s test)]

**Fig. 4** — Hemotoxylin and eosin stained striatal sections of rat brain (400X). (A) Normal control section (B) MPTP-treated section showing histo-architectural alterations (C) & (D) Nim(5)+MPTP-treated & Nim(10)+MPTP-treated section respectively, showing a near normal morphology as compared to MPTP-treated group.
Discussion

In the present study, intrastriatal administration of MPTP significantly decreased the locomotor activity and motor function in rats coupled with a significant oxidative damage and mitochondrial dysfunction induced in the striatal region of the brain. Chronic treatment with nimesulide (5 or 10 mg/kg, po) significantly reversed these behavioral, and biochemical alterations induced by MPTP. Further, histological examination revealed that MPTP administration produced the alterations in the striatal neuronal morphology, which was attenuated by the daily administration of nimesulide. Thus, it is plausible that the administration of nimesulide produced a significant neuroprotective effect in MPTP-induced striatal lesions in rats.

Activation of microglia following neuroinflammation up-regulates nitric oxide synthase (NOS) and COX expression\(^\text{14,33,34}\). During pathological conditions and toxin insult, enhanced expression of COX-2 is seen leading to induction of free radicals which oxidizes dopamine, an important neurotransmitter involved in the locomotion\(^\text{21,35,36}\). Oxidative degradation of dopamine results in the decreased levels of dopamine required for maintaining the motor function and hence the probable cause of motor dysfunction seen in MPTP-treated rats. Daily treatment with nimesulide (5 or 10 mg/kg, po), a preferential COX-2 inhibitor significantly restored the motor activity. Thus, our results indicate that antioxidant potential of nimesulide might be responsible for inhibiting dopamine oxidation and prevent motor dysfunction associated with MPTP challenge.

MPTP, a potent neurotoxin, is metabolized to its active form MPP\(^+\) by monoamine oxidase-B (MAO-B) enzyme, where it inhibits mitochondrial function by blocking the electron transport system, stimulates microglia for the induction of various proinflammatory mediators such as cytokines (TNF-\(\alpha\), IL-1\(\beta\)) and further regulates the genes required for the expression of iNOS and COX\(^\text{29,37}\). In the present study also, significant mitochondrial dysfunction (decreased complex-I activity and viability) and increased levels of nitric oxide were seen which may be due to inhibitory activity of MPTP on mitochondrial function as well as inducing expression of iNOS. Also, nitric oxide combines with the reactive oxygen species generated as a result of microglial activation to form a more toxic insult peroxynitrite\(^\text{38}\). Therefore, it may be postulated that nimesulide might have prevented the mitochondrial dysfunction and the resultant oxidative stress induced by MPTP administration and thus prevented the initiation of further cell damaging pathways.

Glutathione, a potent antioxidant, plays an important role in the dopamine turnover and pathogenesis of Parkinson’s disease\(^\text{35,36,39}\). Dopamine metabolism results in the formation of cellular oxidant, hydrogen peroxide\(^\text{40}\). During persistent oxidative stress, cellular glutathione peroxidase (responsible for scavenging hydrogen peroxide under normal conditions) is not sufficiently enough to attenuate the oxidative alterations. Thus, increased levels of oxidized glutathione (GSSG) are observed during exposure to cellular toxins, which alters the redox state of dopaminergic neurons\(^\text{41,42}\). The GSH/GSSG ratio, one of the primary determinants of the cellular redox, alters due to elevated levels of GSSG and a decline in the ability for de novo GSH biosynthesis. In the present study, redox state was significantly decreased following MPTP administration. Daily treatment with nimesulide restored the altered antioxidant enzymes and redox state. Thus, it is plausible that nimesulide preserved the normal cellular glutathione levels as well as inhibited the resultant oxidative alterations induced by MPTP.

Myeloperoxidase (MPO) activity, an important marker of tissue damage involving inflammatory cells caused by disease or environmental toxins\(^\text{21,43}\). MPTP administration increased the myeloperoxidase activity in the striatum in the present study; which indicates towards the increased expression of inflammatory pathways in the striatum following MPTP injection. Therefore, it can be speculated that daily treatment with nimesulide decreased the tissue damaging pathways by inhibiting the inflammatory mediators and reversed the myeloperoxidase activity.

Histological examination of the brain sections of MPTP-challenged rats showed significant alterations in the neuronal architecture of striatum. Also, MPTP-induced lesions were present in large number, which were similar to the clinical anatomical abnormality seen in the Parkinson’s diseases patients\(^\text{44}\).
nimesulide treatment prevented these neuro-architectural changes. Thus, it can be speculated that nimesulide offered protection to the dopaminergic neurons by preventing the histological changes induced by MPTP.

In summary, findings of the present study provide evidence that COX-2 inhibitor alleviates behavioral deficits and oxidative damage induced by MPTP in rats implicating the neuroprotective role COX-2 inhibitors play in the pathophysiology of Parkinson’s disease.

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References

30 King TE & Howard RL, Preparations and properties of soluble NADH dehydrogenases from cardiac muscle, Methods Enzymol, 10 (1967) 275.
32 Humanson GL, Animal tissue techniques (WH Freeman, San Francisco) 1962, 126.