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Protective effect of cyclooxygenase (COX)-inhibitors against drug-induced catatonia and MPTP-induced striatal lesions in rats

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ABSTRACT

The present study explored the involvement of cyclooxygenase (COX) in the pathophysiology of Parkinson's disease (PD). Further, the protective effect of COX-inhibitors against perphenazine-induced catatonia and 1-methyl-4-phenyl-1, 2, 3, 6-tertahydropyridine (MPTP)-induced striatal lesions in rats was evaluated. Administration of perphenazine (5 mg/kg., i.p.) produced severe catatonia (rigid behavior) in rats; the maximum score reached at 4 h (estimated as 100% AUC) and declined within 24 h. An intrastriatal injection of MPTP produced hypolocomotor activity in rats. Both perphenazine and MPTP produced oxidative stress as demonstrated by increased levels of lipid peroxides, nitrite and decreased antioxidant defense system in the whole brain and striatal region, in particular. Pretreatment with various COX-inhibitors *viz.* rofecoxib, celecoxib, nimesulide or naproxen offered protection against perphenazine-induced catatonia, the effect was more pronounced with rofecoxib. Rofecoxib and celecoxib (both selective COX-2 inhibitors) also reversed the perphenazine-induced oxidative stress. Further, prior treatment with rofecoxib (8 mg/kg, p.o.) reversed both the behavioral and biochemical changes induced by MPTP. These results suggest that COX-inhibitors particularly, rofecoxib offers protection against drug-induced catatonia and MPTP-induced striatal lesions possibly by modulating dopaminergic neurotransmission and/or oxidative stress.

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder, characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) region of the brain and typified by four cardinal features *viz.* bradykinesia (slowness of movement), resting tremor, increased muscular rigidity and impaired postural balance (Montastruc et al., 1996). The most common therapeutic strategy in PD includes combination of levodopa (L-DOPA; dopamine precursor) and carbidopa (a peripheral DOPA-decarboxylase inhibitor). However, the chronic use of this therapy is limited due to the development of "on–off" phenomenon which results in decreased efficacy (Sweet et al., 1975; Fahn, 2005). Other promising drug therapies include treatment with anticholinergics, dopamine receptor (D2/D3) agonists, monoamine oxidase (MAO) inhibitors and catechol-o-methyl transferase (COMT) inhibitors. However, despite the availability of large number of drugs, the relapse rate in PD patients is extremely high which has led the researchers to focus on the search of some alternative therapeutic approaches by which the progressive loss of dopaminergic neurons can be halted.

Perphenazine, a phenothiazine is known to block dopamine D_2 receptors and produces motor disturbance in the form of catatonia (rigidity) (Albin et al., 1989). This exemplifies a very simple and preliminary model for the evaluation of antiparkinson activity of drugs (Khanna and Madan, 1975; Kulkarni et al., 1980; Singh and Kulkarni, 2002; Arzi and Rezaei, 2003; Singh et al., 2003). Although, perphenazine-induced catatonia does not resemble the actual pathophysiological basis of PD; however, it is known to produce symptoms that mimics the disease phenotype, for example, rigidity measured in the form of catatonia (Kulkarni et al., 1980). This model reflects some of the early symptoms of Parkinson disease and can be employed for the preliminary screening of new molecules proposed to be useful for the treatment of the disease (Singh and Kulkarni, 2002; Singh et al., 2003). It is important to mention here that various dopamine D_2 receptor agonists possess antiparkinson properties (Neusch et al., 2000) which justify the importance of this model in antiparkinson drug discovery.

It is hypothesized that neuroinflammation plays an active role in the progression of PD (Hernan et al., 2006; Bartels and Leenders, 2007). Studies have revealed an increase in the expression of various inflammatory molecules within the neurons of PD patients (Teismann et al., 2003; Kim and Joh, 2006). Cyclooxygenase (COX) is a rate-limiting enzyme involved in the production of various prostaglandins and thromboxanes and is known to exist in two isoforms, COX-1 and

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COX-2. COX isoenzymes which are generally expressed only in the peripheral organs (kidneys, stomach, uterus etc.) have been recently found to be up-regulated in the brain following any neuronal insult. Out of these two isoforms, evidences have revealed the involvement of COX-2 isoform in neuropathological conditions (Teismann et al., 2003; Minghetti, 2004). Studies from our laboratory have demonstrated the protective action of COX-inhibitors in various neurological disorders including epilepsy, drug addiction, depression and stress related pathologies (Naidu and Kulkarni, 2002; Dhir et al., 2007; Akula et al., 2008).

Ongoing research using various animal models has demonstrated the neuroprotective potential of COX-2 inhibitors in this disease (Aubin et al., 1998; Reksidler et al., 2007; Sanchez-Pernaute et al., 2004). In one of the experimental studies, parecoxib, a selective COX-2 inhibitor exhibited neuroprotection against 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP)-induced Parkinson-like symptoms in rats (Reksidler et al., 2007). Similarly, aspirin, a non-selective COX-inhibitor has demonstrated neuroprotective effect against MPTP-induced dopamine depletion in mice (Aubin et al., 1998). The mechanism of antiparkinson-like effect of these COX-inhibitors is not clear. Some of the proposed hypothesis includes: i) inhibition of nitric oxide free radicals formation, ii) agonistic action for peroxisome proliferator-activated receptor gamma, and/or iii) possible suppressive effects against dopamine quinone formation (Asanuma et al., 2003). Contrary to this, one of the clinical studies has demonstrated the ineffectiveness of COX-inhibitors in the treatment of PD (Bornebroek et al., 2007). Although the experimental and epidemiological studies suggest the beneficial role of COX-inhibitors in the treatment of PD (Aubin et al., 1998; Esposito et al., 2007; Etminan et al., 2008), still the exact mechanism of their protective action has not been properly explored.

With this background, the present study was designed to evaluate the effect of various selective and non-selective COX-inhibitors *viz.* rofecoxib, celecoxib (both selective COX-2 inhibitors), nimesulide (preferential COX-2 inhibitor) and naproxen (non-selective COX-inhibitor) against perphenazine-induced catatonia in rats. Further, the protective effect of rofecoxib, a selective COX-2-inhibitor against MPTP-induced striatal lesions was studied in rats. The possible role of oxidative stress and its reversal by COX-2 inhibitors in its neuroprotective action was also explored.

2. Materials and methods

2.1. 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. 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.

2.2. Drug treatment and schedule

Perphenazine (PPZ) (5 mg/kg, i.p.) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (32 μmol in 2 μl) (Sigma, St. Louis, MO, USA), rofecoxib (ROF) (2–8 mg/kg, p.o.), celecoxib (CEL) (10–40 mg/kg, p.o.), nimesulide (NIM) (2.5–10 mg/kg, p.o.), naproxen (NPX) (7–20 mg/kg, p.o.) (Panacea Biotec Ltd., Lalru, India) were used in the present study. All the drugs except perphenazine or MPTP were suspended in 0.25% w/v carboxymethyl cellulose (CMC). Perphenazine was dissolved with the aid of diluted hydrochloric acid, pH adjusted to neutral and volume was made up with distilled water. MPTP was dissolved in normal saline.

All the drugs except MPTP were administered in a constant volume of 0.5 ml/100 g body weight of rat. MPTP was administered by intrastriatal injection (32 μmol in 2 μl). Perphenazine was administered by intraperitoneal injection. The present study was carried out in two stages:

Study 1 — Perphenazine-induced catatonia: COX-inhibitors were administered per orally 60 min before challenging with perphenazine. All the doses were selected based on the previous studies reported from our laboratory (Naidu and Kulkarni, 2002; Dhir et al., 2007; Akula et al., 2008).

Study 2 — MPTP-induced striatal lesions: Rofecoxib treatment was started 7 days before the administration of MPTP. On the 7th day, 60 min after the rofecoxib treatment, MPTP was administered by intrastriatal injection (32 μmol in 2 μl) and rofecoxib treatment was continued further for 5 days.

2.3. Sterotaxic surgery

Animals were anesthetized with thiopental sodium (45 mg/kg, i.p.), MPTP was infused as a single intrastriatal (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 (Paxinos et al., 1985).

2.4. Experimental protocols and procedures

2.4.1. Study 1 — perphenazine-induced catatonia

Two tests were employed in the present study to assess the severity of catatonia following the perphenazine administration (5 mg/kg, i.p.) and the assessment of catatonic response was done at 0, 0.25, 0.5, 1, 1.5, 2, 4, 6, 12 and 24 h in both the tests as per the protocols described below. Six to eight animals were included in each group.

2.4.1.1. Bar test. This test was conducted as per the procedure previously validated in our laboratory (Singh and Kulkarni, 2002; Singh et al., 2003). In brief, front paws of the rat were gently placed on a horizontal metal bar with 5–6 mm diameter and placed 10 cm above ground level and the length of time, the rats maintained in this abnormal posture with at least one paw was recorded. The test was terminated when the animal withdrew its paw and attained the normal posture or 180 s had passed. The total time till which animals stayed on the bar was recorded. If the animal did not hold on to the bar after three attempts, it received zero score (Singh et al., 2003). Area under the curve (AUC) (0 to 24 h) was calculated graphically using trapezoidal rule.

2.4.1.2. Block test. In block test, the development and severity of the four stages of catatonia were observed and scored as follows: Stage 1, rat moves when placed on the table, score = 0; Stage 2, rat moves only when touched or pushed, score = 0.5; Stage 3, rat placed on the table with front paws set alternately on a 3 cm high block fails to correct the posture in 10 s, score = 0.5 for each paw with a total of 1 for this stage; State 4, rat fails to move when the front paws are placed alternately on a 9 cm high block, score = 1 for each paw with a total score 2 for this stage. Thus, the maximum possible score would be 3.5 reflecting full catatonia. Lesser score would mean an apparently lesser degree of catatonia (Kulkarni et al., 1980). Area under the curve (AUC) (0 to 24 h) was calculated graphically using trapezoidal rule.

2.4.2. Study 2 — MPTP-induced striatal lesions

Seven groups were employed in the present study, each comprising of 6–8 animals. Group I comprised of control group and

received equivalent volume of vehicle only (0.25% w/v CMC) for 12 days. Group II animals served as sham control and received equivalent volume of vehicle (0.25% w/v CMC) of drug for 7 days and on the 7th day received an intrastriatal injection of normal saline, followed by oral administration of drug vehicle for 5 days. Group III animals were received equivalent volume of vehicle (0.25% w/v CMC) for 7 days and challenged with intrastriatal injection of MPTP (32 µmol in 2 µl) on the 7th day followed by vehicle treatment for further 5 days. Group IV and Group V animals comprised of rofecoxib pretreated group (4 or 8 mg/kg, p.o.) for 7 days and on the 7th day received an intrastriatal injection of normal saline, followed by rofecoxib treatment for 5 days respectively. Group VI and VII comprised of rofecoxib treated group, where animals received rofecoxib in dose of 4 or 8 mg/kg, p.o. for 7 days followed by intrastriatal injection of MPTP, 60 min after last dose and treatment continued for 5 days.

Separate groups were used for biochemical estimations.

2.5. Measurement of locomotor activity

Animals were individually placed in actophotometer for studying the effect of drug treatment on locomotor activity. After the first 2 min of acclimatization, the locomotor activity was recorded for a period of 5 min (Dhir et al., 2005).

2.6. Biochemical estimations

2.6.1. Preparation of brain homogenate

After sacrificing the animals, their brain was quickly removed; striatum was dissected out (in study 2), 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 $\times g$ for 20 min, 4 °C to obtain the post mitochondrial supernatant (PMS), which was used for further enzymatic analysis.

2.6.2. Estimation of lipid peroxidation

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) (Ohkawa et al., 1979). 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 homogenate. 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) was added, shaken well and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. The MDA levels are expressed as nanomoles of MDA per milligram protein and as % of control.

2.6.3. Estimation of reduced glutathione

The reduced glutathione (GSH) was measured by the method of Ellman (1959). In brief, 1.0 ml of PMS (10% w/v) was precipitated with 1.0 ml of sulphosalicylic acid (4% w/v). The samples were then kept at 4 °C for at least 1 h and then subjected to centrifugation at 1200 rpm for 15 min at 4 °C. The assay mixture contained 0.1 ml of filtered aliquot and 2.7 ml of phosphate buffer (0.1 M, pH 7.4) and 0.2 ml of DTNB (40 mg/10 ml of 0.1 M phosphate buffer, pH 7.4) in a total volume of 3.0 ml. 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 ($1.36\times10^3\ M^{-1}\ cm^{-1}$). The results are expressed as micromoles of GSH/mg protein and as % of control.

2.6.4. Estimation SOD activity

The superoxide dismutase (SOD) activity was assessed by the method of Kono (1978). 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 hydroxylamine and 0.05 ml brain homogenate were taken and the auto-oxidation of hydroxylamine was observed by measuring the absorbance at 560 nm.

2.6.5. Estimation of brain nitrite concentration

Nitrite levels were estimated using Griess reagent that served as an indicator of nitric oxide production (Raghavendra et al., 2000). Briefly, 1.0 ml of Griess reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthylethylenediamine dihydrochloride in water) was added to 1.0 ml of brain homogenate and absorbance was measured at 546 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite and nitrite levels were expressed as percentage of control.

2.6.6. Protein estimation

The protein content was measured according to the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.6.7. Estimation of brain myeloperoxidase activity

Myeloperoxidase (MPO) activity was determined by modified technique of Bird et al. (1988). After sacrificing the animals, the brain was removed; striatum was dissected out 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_2O_2 was added to initiate the reaction. Absorbance was measured at 492 nM at an interval of 30 s for 2 min. Change in optical density/minute was calculated and results were expressed as % myeloperoxidase activity considering 100% myeloperoxidase activity in the sham group.

2.7. Statistical analyses

Values are expressed as mean ± SEM. One Way Analysis of Variance (ANOVA) followed by Dunnett's test or Tukey's test were employed to calculate the statistical significance between various groups. A value of $p<0.05$ was considered to be statistically significant. Area under the curve (AUC) (0 to 24 h) was calculated graphically using trapezoidal rule.

3. Results

3.1. Study 1

3.1.1. Effect of various COX-inhibitors against perphenazine-induced catatonia

Perphenazine (5 mg/kg, i.p.) induced a severe state of catatonia in both bar and block tests. The peak response was observed at 4 h ($p<0.05$) which remained constant for 12 h and diminished over a period of 24 h (Figs. 1 and 2). Area under the curve (AUC) was calculated starting from 0 h to 24 h and was considered to be 100%. For all the further experiments, the level of significance was calculated at 4 h.

Pretreatment with rofecoxib (2–8 mg/kg, p.o.) (Table 1) dose-dependently decreased the catatonic score in bar test (Fig. 1A) ($p<0.05$) as well as block test (Fig. 2A) ($p<0.05$), respectively. Celecoxib (10–40 mg/kg, p.o.) significantly decreased the catatonic score in both bar (Fig. 1B) ($p<0.05$) and block test (Fig. 2B) ($p<0.05$). The AUC values showed no significant difference in the anti-catatonic effect at a dose of 10 and 20 mg/kg., p.o. However, celecoxib at 40 mg/kg, p.o. showed significant decrease in catatonic score as compared to other two doses ($p<0.05$) (Table 1). Nimesulide (2.5–10 mg/kg, p.o.) (Table 1) also produced a significant decrease in the degree of

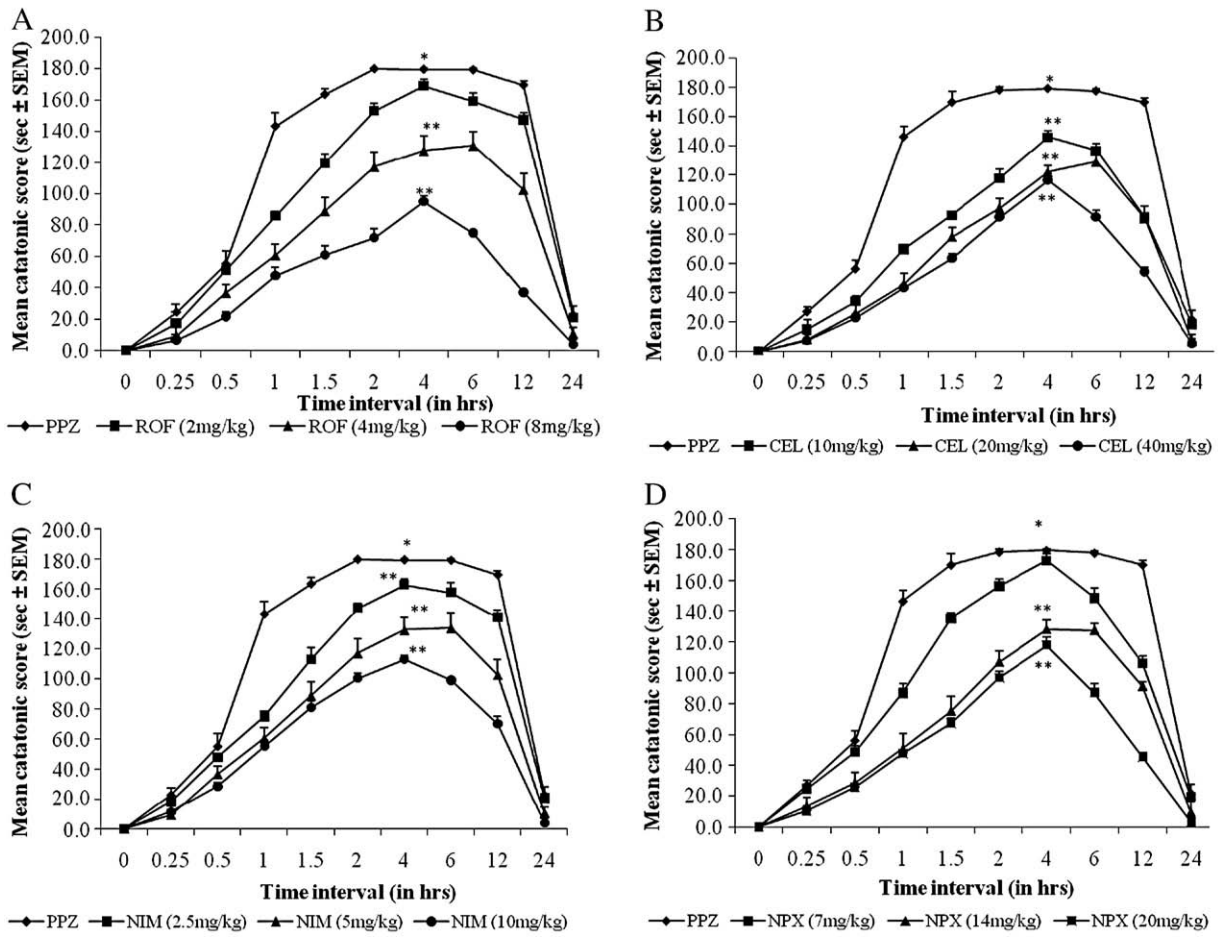

Fig. 1. Effect of various COX-inhibitors in perphenazine-induced catatonia in bar test in rats. All values are expressed as mean ± SEM. *$p<0.05$ as compared to 0 min; **$p<0.05$ as compared to perphenazine treated group at 4 h (one-way ANOVA followed by Dunnett's test) ($n=6$ to 8) PPZ — perphenazine; ROF — rofecoxib; CEL — celecoxib; NPX — naproxen; NIM — nimesulide.

catatonia in both bar test (Fig. 1C) ($p<0.05$) as well as in block test (Fig. 2C) ($p<0.05$). Similarly, as shown in Table 1, naproxen (7–20 mg/kg, p.o.), significantly decreased the catatonic response in both bar test (Fig. 1D) ($p<0.05$) as well as block test (Fig. 2D) ($p<0.05$).

When the AUC values at the highest doses of each COX-inhibitor were compared, rofecoxib (8 mg/kg) exhibited significantly more ability to decrease the degree of catatonia as compared to other COX-inhibitors.

3.1.2. Effect of selective COX-2 inhibitors, rofecoxib or celecoxib on oxidative stress induced by perphenazine

When the animals were sacrificed 4 h after perphenazine administration (the time when catatonic score was maximum), a significant increase in oxidative stress parameters was observed ($p<0.05$). As shown in Table 2, the brain levels of lipid peroxide and nitrite were increased and there was a decrease in the antioxidant pool (reduced glutathione and SOD levels) as compared to control. Pretreatment with selective COX-2 inhibitors, rofecoxib or celecoxib significantly attenuated the oxidative stress by decreasing the increased levels of lipid peroxides ($p<0.05$) and brain nitrite ($p<0.05$). Furthermore, it also restored the brain antioxidant pool as compared to perphenazine treated group (Table 2) ($p<0.05$).

3.2. Study 2

3.2.1. Effect of rofecoxib on locomotor activity in MPTP-treated rats

There was no significant difference between the locomotor activity of animals of group II and group III when assessed on day 1 or day 5 of the treatment. When group III animals were challenged with intrastriatal injection of MPTP on the day 7 and were evaluated for their locomotor activity on the day 8, significant decrease in the locomotor activity ($p<0.05$) as compared to sham (group II) was observed, the activity further decreased till day 12 (Fig. 3) ($p<0.05$). Pretreatment with rofecoxib 4 or 8 mg/kg, p.o did not modify the locomotor activity when the activity was assessed on day 1 or day 5. However, daily treatment with rofecoxib (group VI and VII-treated with MPTP) significantly reversed the decrease in locomotor activity as assessed on day 8 ($p<0.05$) and day 12 ($p<0.05$), respectively (Fig. 3).

3.2.2. Effect of rofecoxib on biochemical alterations in MPTP-treated rats

A significant increase in MDA level was observed in the MPTP-treated (group III) animals ($p<0.05$) as compared to sham group (group II). However, daily treatment with rofecoxib (4 or 8 mg/kg, p.o) significantly attenuated the increased levels of MDA due to MPTP challenge in group VI ($p<0.05$) and group VIII ($p<0.05$), respectively (Table 3).

Intrastriatal administration of MPTP in group III animals produced a significant decrease in the antioxidant pool (Table 3) as evident by decreased levels of reduced glutathione ($p<0.05$) and superoxide dismutase (SOD) ($p<0.05$) compared to group II. Pretreatment with rofecoxib (4 or 8 mg/kg, p.o.) in group VI or group VII significantly restored the depleted levels of reduced glutathione ($p<0.05$) and SOD ($p<0.05$) to normal when compared to MPTP-treated group.

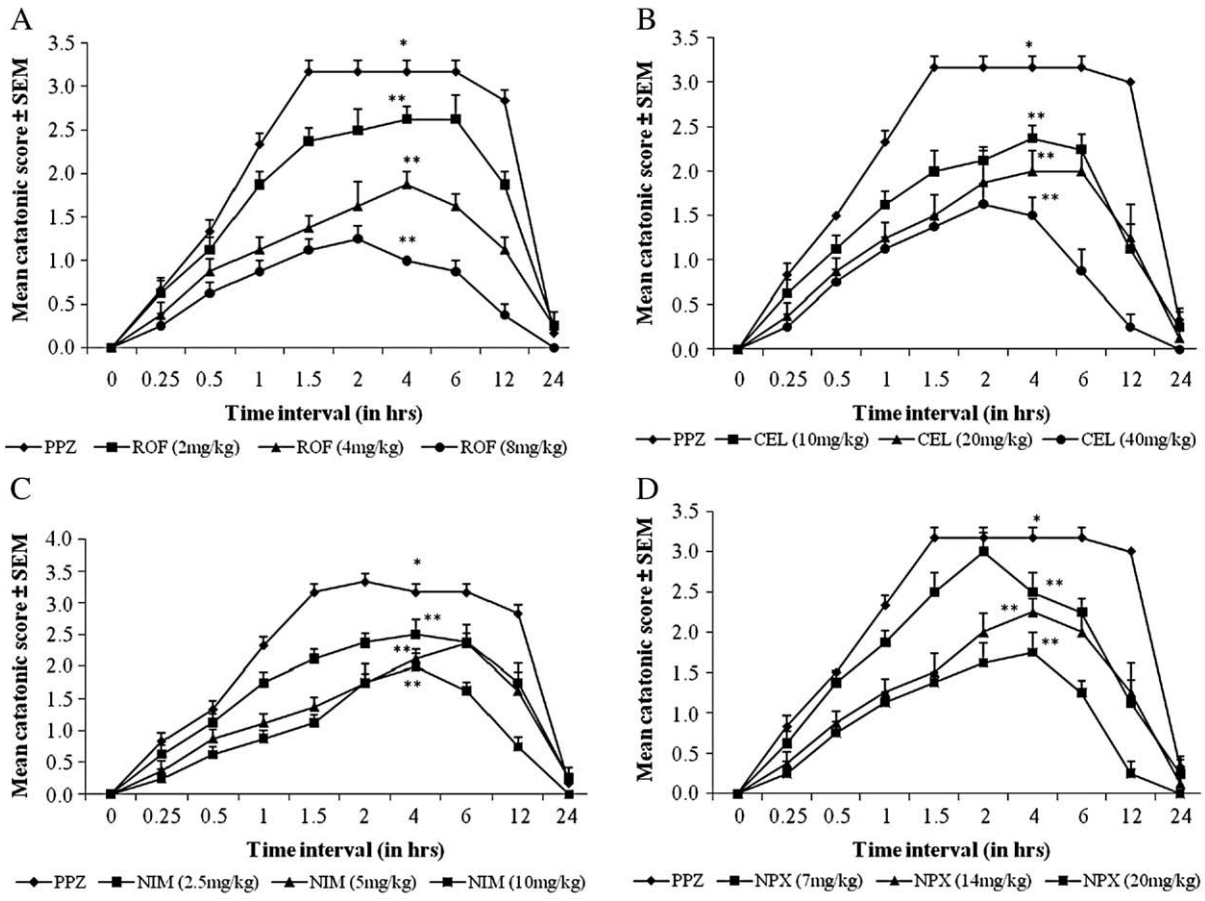

Fig. 2. Effect of various COX-inhibitors in perphenazine-induced catatonia in block test in rats. All values are expressed as mean ± SEM. *$p<0.05$ as compared to 0 min; **$p<0.05$ as compared to perphenazine treated group at 4 h (one-way ANOVA followed by Dunnett's test) ($n=6$ to 8) PPZ — perphenazine; ROF — rofecoxib; CEL — celecoxib; NPX — naproxen; NIM — nimesulide.

A significant increase in nitrite levels were observed in the group III animals challenged with MPTP ($p<0.05$) as compared to group II (Table 3). Daily treatment with rofecoxib (4 or 8 mg/kg, p.o.) significantly decreased the nitrite levels in group VI ($p<0.05$) or group VII ($p<0.05$) respectively, as compared to MPTP-treated animals in group III.

Table 1
Effect of various COX-inhibitors on AUC values in perphenazine-induced catatonia in rats.

Drug treatment	AUC (% of control)	
	Bar test	Block test
PPZ (5)	100 ± 1.10	100 ± 1.12
ROF (2) + PPZ (5)	76.9 ± 1.65[a]	72.6 ± 1.85[a]
ROF (4) + PPZ (5)	65.4 ± 1.55[a]	44.4 ± 1.56[a]
ROF (8) + PPZ (5)	33.2 ± 1.91[a]	23.0 ± 1.92[a]
CEL (10) + PPZ (5)	62.5 ± 1.65[a]	53.2 ± 1.33[a]
CEL (20) + PPZ (5)	60.2 ± 1.52[a]	51.8 ± 1.59[a]
CEL (40) + PPZ (5)	42.9 ± 1.43[a]	24.0 ± 1.35[a]
NIM (2.5) + PPZ (5)	73.7 ± 2.10[a]	66.9 ± 1.86[a]
NIM (5) + PPZ (5)	66.2 ± 2.30[a]	60.1 ± 1.9[a]
NIM (10) + PPZ (5)	50.2 ± 1.56[a]	40.6 ± 1.75[a]
NPX (7) + PPZ (5)	73.3 ± 2.10[a]	56.8 ± 2.13[a]
NPX (14) + PPZ (5)	60.5 ± 3.20[a]	54.1 ± 2.21[a]
NPX (20) + PPZ (5)	40.4 ± 1.91[a]	27.9 ± 1.96[a]

PPZ — perphenazine (5 mg/kg, i.p.) treated group; ROF — rofecoxib; CEL — celecoxib; NIM — nimesulide; NPX — naproxen.
AUC values are expressed as percent of control.
[a] $p<0.05$ as compared to perphenazine treated group (one-way ANOVA followed by Dunnett's test).

Myeloperoxidase (MPO) activity was significantly increased in the animals challenged with MPTP in group III ($p<0.05$) as compared to sham controls, group II. Daily rofecoxib (4 or 8 mg/kg, p.o.) pre-treatment in group VI ($p<0.05$) or group VII ($p<0.05$), significantly decreased the MPO activity as compared to MPTP-treated animals, group III (Fig. 4).

4. Discussion

PD is characterized by progressive and selective neurodegeneration of dopaminergic pathway in the nigrostriatal region of the brain (Bartels and Leenders, 2007; Mosley et al., 2006). It is known that neuroinflammatory events activate the microglial cells which results in the up-regulation of cyclooxygenase (COX) isoenzymes (Hunot et al., 1997; Watanabe et al., 2008). During neuronal damage, up-regulation of both inducible nitric oxide synthase (iNOS) and COX enzyme results in generation of free radicals and releases pro-inflammatory cytokines which may be one of the contributing factors for the oxidative stress and in turn can lead to neurodegenerative process (Ferger et al., 1998; Okuno et al., 2005; Sanchez-Pernaute et al., 2004; Vijitruth et al., 2006). Several investigators have also demonstrated the activation of striatal microglial and astroglial cells in animals subjected to MPTP-induced neurotoxicity (Kim and Joh, 2006). Further, there are some studies which support the effectiveness of non-steroidal anti-inflammatory drugs (NSAIDs) in experimental models of PD (Aubin et al., 1998; Chen et al., 2003; Hernan et al., 2006; Teismann and Ferger, 2001).

Oxidative stress and inflammatory events play a significant pathological role in the development of PD (Gu et al., 1998). Induction

Table 2
Effect of selective COX-2 inhibitors, rofecoxib or celecoxib on perphenazine-induced alterations in oxidative stress parameters in rats.

Group	Lipid peroxidation (nmol of MDA/ mg protein)	MDA levels (% of control)	Reduced glutathione levels (GSH) (μmol/mg protein)	GSH levels (% of control)	SOD levels (% of control)	Nitrite levels (% of control)
Ctrl	1.056 ± 0.005	100	0.055 ± 0.002	100	100.0 ± 2.2	100.0 ± 5.5
PPZ	3.107 ± 0.008[a]	294[a]	0.045 ± 0.001[a]	82[a]	62 ± 4.7[a]	137 ± 5.9[a]
ROF(8) + PPZ	1.570 ± 0.006[b]	149[b]	0.063 ± 0.001[b]	115[b]	97 ± 7.1[b]	88 ± 1.8[b]
CEL(40) + PPZ	1.682 ± 0.004[b]	159[b]	0.054 ± 0.001[b]	98[b]	94 ± 5.5[b]	90 ± 4.0[b]

Ctrl — control group; PPZ — perphenazine (5 mg/kg, i.p.) treated group; ROF(8) + PPZ — rofecoxib (8 mg/kg) + perphenazine group; CEL(40) + PPZ — celecoxib (40 mg/kg) + perphenazine group.
[a] $p<0.05$ as compared to control.
[b] $p<0.05$ as compared to perphenazine treated group (one-way ANOVA followed by Dunnett's test).

of inflammatory process due to activated microglia generates reactive oxygen species such as superoxide anions which may react with nitric oxide to form peroxynitrite, a potent prooxidant (Cohen and Heikkila, 1974; Lizasoain et al., 1996). It is also debated that oxidative stress may result in apoptotic death of nigrostriatal neurons (Hunot et al., 1997). This is further evident from the fact that there is generation of reactive oxygen species and consequent elevated antioxidant enzymes in the basal ganglia of rats following MPTP administration (Cassarino et al., 1997).

In perphenazine-induced catatonia model, the animals when challenged with perphenazine displayed Parkinson-like the symptoms such as rigidity or postural inability in the form of catatonia. The present results demonstrate that various COX-inhibitors *viz.* rofecoxib, celecoxib, nimesulide and naproxen attenuated the degree of catatonia induced by perphenazine. Out of all these COX-inhibitors, rofecoxib (8 mg/kg) exhibited pronounced protection when compared with other COX-inhibitors at their highest dose. This further demonstrates the involvement of COX-2 and prostaglandin pathway in the motor deficits produced by neuroleptics. When oxidative stress parameters were measured 4 h after perphenazine administration, there was significant increase in the brain lipid peroxidation and nitrite levels as well as diminished antioxidant pool. It was hypothesized that perphenazine administration probably induced the generation of reactive oxygen species, which further resulted in the cascade of proinflammatory events and hence the development of catatonia. However, the fact that there is reversal of perphenazine-induced catatonia after 8 h of administration in vehicle treated group implies that antioxidant defense system in the body exceeds the perphenazine-induced oxidative stress. It is also known fact that typical antipsychotics like haloperidol produce oxidative stress in brain and result in neurotoxic actions (Martins et al., 2008; Polydoro et al., 2004). The selective inhibition of COX-2 which also ameliorates oxidative stress and consequent inflammatory events appears to attenuate the behavioral deficits produced by perphenazine. Our results are in concurrence with earlier studies where neuroprotective effect of COX-2 selective inhibitors has been reported in animal studies (Aubin et al., 1998; Teismann and Ferger, 2001).

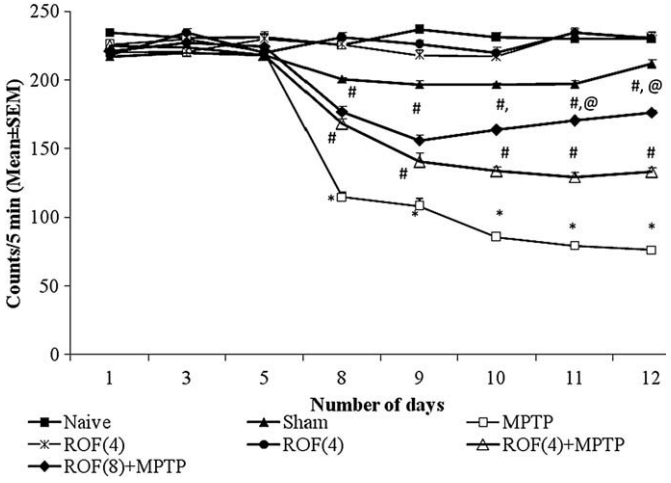

Fig. 3. Effect of rofecoxib (4 or 8 mg/kg, p.o.) on locomotor activity in MPTP-treated rats. Counts/5 min are expressed as mean ± SEM. $^{*}p<0.05$ as compared to sham; $^{\#}p<0.05$ as compared to MPTP-treated group; $^{@}p<0.05$ as compared to ROF(4) + MPTP group (one-way ANOVA followed by Tukey's test).

In study 2 of the present study, intrastriatal administration of MPTP significantly decreased the locomotor activity in rats, which was coupled with a significant oxidative damage induced in the striatal region of the brain. Chronic treatment with rofecoxib (4 or 8 mg/kg, p.o) significantly reversed the hypolocomotion and attenuated the oxidative damage. Thus, the core finding of the study is that administration of rofecoxib produced a significant neuroprotection against MPTP-induced striatal lesions leading to behavioral and biochemical changes. The study also demonstrated the antioxidant potential of rofecoxib in its neuroprotective action.

Since inflammatory process involves many pathways in the dopaminergic cell loss, COX and the subsequent formation of prostaglandins may play a crucial role and represent the potential site in attenuating the progression of PD. Therefore, the present study was an attempt to evaluate the effect of rofecoxib, a selective COX-2-inhibitor, in MPTP-induced striatal lesions in rats.

Dopamine, an important neurotransmitter involved in locomotion and reward phenomenon, is very sensitive to oxidative attack and is known to get oxidized to dopamine–quinone due to the enhanced COX-2 expression and further generation of free radicals following neuroinflammation (Hastings, 1995; Slivka and Cohen, 1985). The oxidative degradation of dopamine in the striatal neurons could have decreased the dopamine levels which resulted in the decreased motor activity in MPTP-treated rats. Pretreatment with rofecoxib (4 or 8 mg/kg, p.o.), significantly restored the motor activity which may be due to the inhibition of oxidative degradation of the neurotransmitter. Rojas et al. (2008) have earlier demonstrated that antioxidant treatment restored the striatal dopamine levels and inhibited the MPTP-induced locomotion impairment.

MPTP is metabolized to its active form MPP^{+} by monoamine oxidase-B (MAO-B) enzyme resulting in the up-regulation of various proinflammatory mediators such as cytokines (TNF-α, IL-1β) and further, the gene expression of iNOS and COX (Smeyne and Jackson-Lewis, 2005). Due to increased expression of iNOS, there is increased level of brain nitric oxide which combines with the reactive oxygen species generated as a result of microglial activation to form a more toxic insult "peroxynitrite" (Gerlach et al., 1999). It is a well known fact that during neuronal damage, nitric oxide and COX act as key players in production of free radicals (Ferger et al., 1998; Sanchez-Pernaute et al., 2004; Vijitruth et al., 2006). Thus,

Table 3
Effect of rofecoxib on MPTP-induced alterations in oxidative stress parameters in rats.

Group	Lipid peroxidation (nmol of MDA/ mg protein)	MDA levels (% of control)	Reduced glutathione levels (GSH) (μmol/mg protein)	GSH levels (% of control)	SOD levels (% of control)	Nitrite levels (% of control)
Naïve	1.05 ± 0.004	99.06	0.055 ± 0.002	98.21	100.0 ± 2.6	100.0 ± 9.12
Sham	1.06 ± 0.002	100.0	0.056 ± 0.002	100	98 ± 2.45	100 ± 8.45
MPTP	2.89 ± 0.042[a]	272.64[a]	0.030 ± 0.002[a]	53.57[a]	35 ± 3.45[a]	185 ± 11.23[a]
ROF(4)	1.05 ± 0.002	99.06	0.055 ± 0.003	98.21	100 ± 2.16	99 ± 9.54
ROF(8)	1.05 ± 0.002	99.06	0.056 ± 0.002	100	102 ± 1.86	101 ± 9.56
ROF(4) + MPTP	1.72 ± 0.037[b]	162.26[b]	0.052 ± 0.002[b]	92.86[b]	78 ± 3.42[b]	141 ± 8.56[b]
ROF(8) + MPTP	1.32 ± 0.011[b,c]	124.53[b,c]	0.059 ± 0.002[b]	105.36[b]	91 ± 3.25[b]	107 ± 7.89[b]

Naive; Sham — sham control; MPTP-treated group; ROF(4 or 8) — rofecoxib (4 or 8 mg/kg, p.o.) group; ROF(4) + MPTP or ROF(8) + MPTP — rofecoxib (4 or 8 mg/kg, p.o.) + MPTP group.
[a] $p<0.05$ as compared to sham.
[b] $p<0.05$ as compared to MPTP-treated group.
[c] $p<0.05$ as compared to ROF (4) + MPTP-treated group (one-way ANOVA followed by Tukey's test).

it is plausible that rofecoxib by selectively inhibiting COX-2 isoenzyme, decreased the prostaglandin production which might have decreased the resultant oxidative damage produced by MPTP administration. Therefore, it may be the antioxidant property of rofecoxib which may be responsible for its neuroprotective potential in MPTP-treated rats.

MPO activity is a marker of tissue damage involving inflammatory cells caused by disease or environmental toxins (Penn, 2008). In the present study, MPTP administration increased the MPO activity in the striatum and it can be predicted that there is an increased expression of inflammatory mediators in the striatum following MPTP challenge. Thus, pretreatment with rofecoxib decreased the tissue damage by inhibiting the inflammatory pathways and by reversing the MPO activity.

The findings of the present study indicate the beneficial role of selective COX-2 inhibitors (rofecoxib, celecoxib), preferential COX-2 inhibitor (nimesulide) and non-selective COX inhibitor (naproxen) in alleviating perphenazine-induced motor deficits in rats. Further, involvement of oxidative pathway in the motor dysfunction and its reversal by COX-inhibitors are speculated. These finding also substantiate the neuroprotective potential of rofecoxib against neurotoxic damage induced by MPTP in rats.

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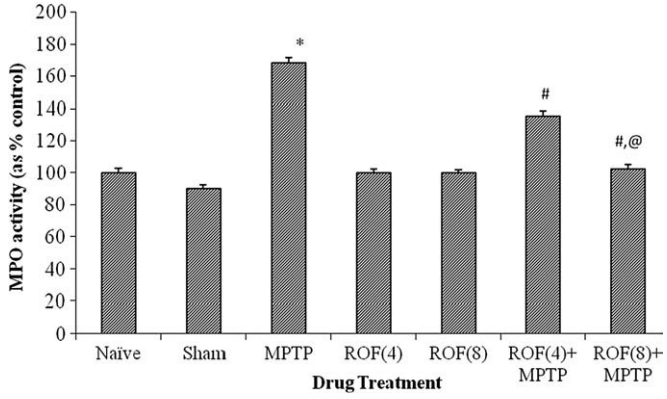

Fig. 4. Effect of rofecoxib (4 or 8 mg/kg, p.o.) on myeloperoxidase activity in MPTP-treated rats. All values are expressed as mean ± SEM. $^{*}p<0.05$ as compared to sham; $^{\#}p<0.05$ as compared to MPTP-treated group; $^{@}p<0.05$ as compared to ROF(4) + MPTP group (one-way ANOVA followed by Tukey's test).

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