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Peroxisome proliferator-activated receptor-α activation attenuates 3-nitropropionic acid induced behavioral and biochemical alterations in rats: Possible neuroprotective mechanisms

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ABSTRACT

Peroxisome proliferators activated receptor is regarded as potential therapeutic targets to control various neurodegenerative disorders. However, none of the study has elucidated its effect in the treatment of Huntington's disease. We explored whether peroxisome proliferators activated receptor-α agonist may attenuate various behavioral and biochemical alterations induced by systemic administration of 3-nitropropionic acid (3-NP), an accepted experimental animal model of Huntington's disease phenotype. Intraperitoneal administration of 3-NP (20 mg/kg., i.p.) for 4 days in rats produced hypolocomotion, muscle incoordination, and cognitive dysfunction. Daily treatment with fenofibrate (100 or 200 mg/kg., p.o.), 30 min prior to 3-NP administration for a total of 4 days, significantly improved the 3-NP induced motor and cognitive impairment. Biochemical analysis revealed that systemic 3-NP administration significantly increased oxidative and nitrosative stress (increase lipid peroxidation, protein carbonyls and nitrite level), lactate dehydrogenase activity whereas, decreased the activities of catalase, superoxide dismutase, reduced glutathione, and succinate dehydrogenase. Fenofibrate treatment significantly attenuated oxidative damage, cytokines and improved mitochondrial complexes enzyme activity in brain. In the present study, MK886, a selective inhibitor of peroxisome proliferators activated receptor-α was employed to elucidate the beneficial effect through either receptor dependent or receptor independent neuroprotective mechanisms. Administration of MK886 (1 mg/kg, i.p.) prior to fenofibrate (200 mg/kg, p.o.) abolished the effect of fenofibrate. The results showed that receptor dependent neuroprotective effects of fenofibrate in 3-NP administered rats provide a new evidence for a role of PPAR-α activation in neuroprotection that is attributed by modulating oxidative stress and inflammation.

1. Introduction

Huntington's disease is an autosomal dominant, progressive neurodegenerative disorder characterized by uncontrolled choreiform movements, cognitive impairment and severe degeneration of basal ganglia neurons within the neostriatum (Beal et al., 1993). 3-NP is an irreversible inhibitor of succinate dehydrogenase, an enzyme located in the mitochondrial inner membrane responsible for inhibiting both Kreb's cycle and complex II and III of the respiratory chain. Thus, a 3-NP induced features of Huntington's disease in rodents is a well-studied phenotypic model of Huntington's disease (Brouillet et al., 2005). It has been demonstrated that systemic administration of 3-NP produced behavioral, biochemical, histological, and neurochemical features of Huntington's disease (Beal et al., 1993; Kumar et al., 2006). In focus to biochemical alterations oxidative stress and excitotoxicity play a role in pathogenesis of 3-NP induced experimental Huntington disease (Kumar et al., 2007b,c). A growing body of evidence indicates that microglia and astroglia are also activated in Huntington disease patients and 3-NP-induced neurotoxicity (Pavese et al., 2006; Ryu et al., 2003). In 3-NP experimental model, activated microglia release proinflammatory cytokines, such as interleukin (IL)-1β, and tumor necrosis factor (TNF)-α, excitatory amino acid, increase expression of enzymes, such as cyclooxygenase-2 and inducible nitric oxide synthase results in increased production of prostaglandins and nitric oxide, respectively, which collectively promote neurodegeneration (Kumar et al., 2007b; Ryu et al., 2003). Findings from various studies on deactivation of proinflammatory enzymes (i.e. cyclooxygenase and nitric oxide synthase), inhibition of metabolism of these enzymes in microglia, and reduction in oxidative stress with neuronal inflammation could be novel target sites to attenuate neurotoxicity and neuronal death observed in experimental Huntington's disease.

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors, which belong to the superfamily of nuclear hormone receptors (Heneka and Landreth, 2007). The

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subtype of PPAR receptor includes PPAR-α, PPAR-β/δ, and PPAR-γ, which regulate metabolism, cell differentiation, immune, and inflammatory response (Heneka and Landreth, 2007). PPAR-α is expressed in brown adipose tissue, liver, kidney, heart, skeletal muscles and T-cells. Previously, it has been reported that PPAR-α is also present in brain region like striatum and CA1 pyramidal cells, granular, polymorphic layer of dentate gyrus and non-neuronal cells, particularly microglia and astroglia (Kainu et al., 1994; Moreno et al., 2004). Moreover, it has also been reported that activation of PPAR-α could reduce the inflammation by decreasing cytokines and proinflammatory enzymes (Collino et al., 2006; D'Agostino et al., 2007; Gelinas and McLaurin, 2005). Furthermore, PPAR-α activators have also shown to reduce oxidative stress and increasing endogenous antioxidant enzymes (Collino et al., 2006; Deplanque et al., 2003). Thus, PPAR-α activators act as neuroprotectant in various CNS disorders like Alzheimer's disease, Parkinson's disease, multiple sclerosis, and cerebral ischemia (Besson et al., 2005; Deplanque et al., 2003; Heneka and Landreth, 2007). Therefore, the present study has been designed to explore the possible role of PPAR-α in 3-NP-induced neurotoxicity, an animal model of Huntington's disease.

2. Material and methods

2.1. Experimental animals

Male Wistar rats, bred in the Central Animal House of the facility of ISF College of Pharmacy, Punjab, India, and weighing between 180 and 220 g were used. Animals were acclimatized to laboratory conditions before experimentation. The animals were kept in groups of three, in plastic cages with soft bedding, under standard conditions of light and dark cycle, with free access to food and water. All the experiments were carried out between 08:00 am and 04:00 pm. The protocol was approved by the Institutional Animal Ethics Committee and carried out in accordance with the Indian National Science Academy guidelines for the use and care of animals, efforts were made throughout to minimized animal discomfort and to use the minimum number of animals (n = 6 to 8 per group) need for statistical significance.

2.2. Drugs and chemicals

In the present study, all chemicals and biochemical reagents of analytical grade and highest purity were used. 3-NP (Sigma-Aldrich Chemical, USA) was diluted with saline (adjusted pH 7.4 with NaOH) and administered intraperitoneally (*i.p.*), Fenofibrate hydrochloride (USV Chemicals, India) was freshly prepared by suspending in 0.5% sodium carboxy methyl cellulose and was administered orally (*p.o.*). 3-[1-(4 chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2dimethylpropanoic acid (MK886; Tocris, UK) was diluted in 10% dimethylsulfooxide and administered intraperitoneally (*i.p.*) in rats. Rat IL-1β and TNF-α ELISA kits (R&D systems, MN, USA) were used to quantify proinflammatory cytokines.

2.3. Study design

All animals were acclimatized to laboratory environment for at least 2 h before testing. Animals were randomly divided into seven groups and each group consisted of eight animals. Group 1, the vehicle-administered control group, received vehicle for fenofibrate (*p.o.*) and also physiological saline (*i.p.*). Group 2, received 3-NP (20 mg/kg, *i.p.*) for 4 consecutive days. Group 3 (per se), received fenofibrate (200 mg/kg, *p.o.*) once daily for a period of 4 days. Group 4, received fenofibrate (100 mg/kg, *p.o.*) once daily for a period of 4 days, 30 min before 3-NP administration. Group 5, received fenofibrate (200 mg/kg, *p.o.*) once daily for a period of 4 days 30 min before 3-NP administration. Group 6, received MK886 (1 mg/kg, *i.p.*) once daily for a period of 4 days 1 h before 3-NP administration. Group 7 received MK886 (1 mg/kg, *i.p.*), 30 min before fenofibrate only at high dose (200 mg/kg, *p.o.*) once daily for a period of 4 days 30 min before 3-NP administration.

2.4. Induction of Huntington's disease-like symptoms

3-NP (20 mg/kg) was repeatedly administered intraperitoneally (*i.p.*) for a period of 4 days to induce the symptoms of Huntington's disease. All the behavioral parameters were observed before drug administration and 24 h after the first dose and then 24 h after the last dose of 3-NP administration.

2.5. Measurement of body weight

Body weight was noted on the first and last days of the experimentation. Percentage change in body weight was calculated in comparison with the initial body weight of rats on the first day of experimentation.

2.6. Behavioral test paradigm

2.6.1. Assessment of motor activity

2.6.1.1. Locomotor activity. Gross behavioral activity was assessed on days 1 (before 3-NP), day 2 (24 h after the first dose of 3-NP) and day 5 (24 h after the last dose of 3-NP). Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells, using a digital photoactometer. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room. The values are expressed as counts per 5 min (Kumar et al., 2006).

2.6.1.2. Movement analysis. 3-NP administration was associated with severe behavioral defects that prevent animals from walking. Severity of the 3-NP-induced motor abnormalities in all groups were evaluated using a quantitative neurological scale adapted from Ludolph et al. (1991); normal behavior: score 1, general slowness of displacement resulting from mild hind limb impairment: score 2, incoordination and marked gait abnormalities: score 3, hind limb paralysis: score 4, incapacity to move resulting from forelimb and hind limb impairment: score 5, recumbency. A neurological score was determined for each animal on a daily basis, immediately before the 3-NP injection.

2.6.1.3. String test for grip strength. The rat was allowed to hold with the forepaws a steel wire (2 mm in diameter and 35 cm in length), placed at a height of 50 cm over a cushion support. The length/duration of time that the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength (Shear et al., 1998).

2.6.1.4. Limb withdrawal test. In this behavioral test, the animal was placed on a 20 cm height, 30 × 30 cm Perspex platform containing four holes, two holes of 5 cm diameter for the hind limbs and two holes with a diameter of 4 cm for the forelimbs. The rat was placed on the platform in such a position that first the hind limbs and then the forelimbs were placed into the holes. The times taken by the animal to retract its first hind limb and the contralateral hind limb were recorded. The difference between the retraction times (s) of both hind limbs was determined. This is considered to be an important parameter to measure functional abnormalities of the hind limbs, which are indicative for the extent of striatal degeneration (Vis et al., 1999). The test was performed three times with a 45 min interval and the average value was recorded.

2.6.2. Assessment of cognitive function

The acquisition and retention of a spatial navigation task was examined using a Morris water maze (Duckworth et al., 1999). The

water maze apparatus consisted of a cylindrical pool 180 cm in diameter and 60 cm, filled with water (approximately at 25 °C) to a depth of 40 cm. The pool was divided into four quadrants. In the center of north quadrant a 9 cm in diameter platform submerged 1 cm beneath the surface. Testing was performed from 9 to 11 am daily.

2.6.2.1. Acquisition. All the rats underwent training over 5 consecutive days, consisting of six swimming trials per day, during which each rat could escape by finding the submerged platform, and subsequently, was allowed to rest for 60 s on the platform before starting the next trial. The trials lasted for a maximum of 90 s after which the rats that had not found the platform were placed on it and allowed to rest there for 60 s to find out the exact location. Each rat was randomly started in the east, west or south quadrant and started twice in each of these quadrants during the 1st day session. The rats were started by being placed at the edge of pool in the center of the appropriate quadrant, facing the wall. For each trial, latency to finding the platform, entries into each quadrant, and time spend in target quadrant were noted. Following the training, the expert rats were selected and randomly divided into different groups before starting experimental protocol.

2.6.2.2. Retention trial. On day 5, a retention trial was conducted, in which the platform was removed and rats were allowed to swim for 2 min. All the rats were started in the south quadrant and were tested for only one trial, during which they were assessed for memory of platform location. Latency to entering platform area, entries into that area (where platform was placed), and time spent in that area was used as indices of retention.

2.7. Assessment of biochemical parameters

Biochemical tests were carried out immediately after behavioral observations on day 5 following 3-NP administrations.

2.7.1. Brain homogenate preparation

Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice-cold 0.1 mol/L phosphate buffer (pH 7.4) 10 times (w/v) and divided into two portions. One part of the homogenate was centrifuged for at 2000×*g* for 15 min and aliquots of supernatant were separated and used for biochemical estimation and another part of homogenate was mixed 4 µl/ml protease inhibitor cocktail. These samples were cold centrifuged at 14,000×*g* at 4 °C for 15 min and the supernatant was used for estimation of pro-inflammatory cytokines.

2.7.2. Estimation of lipid peroxidation

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills (1966). In this 0.1 ml of supernatant was incubated with 0.5 ml tris hydrochloric acid (0.1 M, pH 7.4) for 2 h. To this, 1 ml of trichloroacetic acid (10% w/v) was added and centrifuged at 1000×*g* for 10 min. To 1 ml supernatant, 1 ml (0.67% w/v) thiobarbituric acid was added and kept in the boiling water bath for 10 min, cooled and then 1 ml distilled water was added. The amount of lipid peroxidation products was measured by reaction with thiobarbituric acid at 532 nm using the spectrophotometer (UV-1700 Shimadzu, Japan). The values were calculated using molar extinction coefficient of chromophore ($1.56\times10^5\ M^{-1}\ cm^{-1}$) and expressed as µmol per mg protein.

2.7.3. Determination of protein carbonyl

Protein carbonyl content was determined by the most common and reliable method based on the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine to form 2, 4-dinitrophenylhydrazone (Levine et al., 1990). In this, 100 µl of supernatant from brain homogenate was incubated with 0.5 ml 2,4-dinitrophenylhydrazine for 60 min. Subsequently, the protein was precipitated from the solution using 20% trichloroacetic acid. The pellet was washed after centrifugation (3400×*g*) with ethyl acetate: ethanol (1:1 v/v) mixture, to remove excess of 2,4-dinitrophenylhydrazine. The final protein pellet was dissolved in 2.5 ml of 6 M guanidine hydrochloride. The carbonyl content was evaluated in a spectrophotometer at 370 nm, the values were calculated using molar extinction coefficient ($22{,}000\ M^{-1}\ cm^{-1}$) and expressed as nmol per mg protein.

2.7.4. Determination of catalase

Catalase was assayed as described by Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 mol/l phosphate buffer (pH 7), 0.1 ml of brain homogenate supernatant and 0.4 ml of 2 mol/l hydrogen peroxide. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The absorbance was measured at 620 nm and expressed as µmol of hydrogen peroxide consumed per min per mg protein.

2.7.5. Determination of superoxide dismutase

Superoxide dismutase (SOD) activity is measured according to a method described by Misra and Fridovich (1972). Autooxidation of epinephrine at pH 10.4 was spectrophotometrically measured. In this method, supernatant of the tissue was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4 and the reaction was started by the addition of 0.02 ml (−)-epinephrine. After 5 min the absorbance was measured at 480 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). The activity of SOD was expressed as % activity of vehicle-treated control.

2.7.6. Estimation of reduced glutathione

Reduced glutathione (GSH) in the brain was estimated according to the method described by Ellman (1959). 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4 °C for 1 h. The samples were centrifuged at 1200×*g* for 15 min at 4 °C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 mol/l, pH 8) and 0.2 ml of 5, 5-dithio-bis (2-nitrobenzoic acid) were added. The color developed was measured immediately at 412 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). Results were calculated using molar extinction coefficient of chromophore ($1.36\times10^4\ M^{-1}\ cm^{-1}$) and expressed as nmol per mg protein.

2.7.7. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al., 1982. Equal volumes of supernatant and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature in the dark and the absorbance was noted at 540 nm using UV-1700 Spectrophotometer (Shimadzu, Japan). The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as µmol per mg protein.

2.7.8. Succinate dehydrogenase activity

Succinate dehydrogenase (SDH) is a marker of impaired mitochondrial metabolism in the brain. The quantitative measurement of SDH level in the brain was performed according to the method described previously by Kumar et al., 2006. 0.3 ml of sodium succinate solution was mixed with the 50 µl of gradient fraction of homogenate. The mixture was incubated at 37 °C for 10 to 20 min and then 0.1 ml of solution of p-iodonitrotetrazolium (INT) violet was added and again incubated further for 10 min. The reaction was stopped by adding 1 ml of a mixture of ethyl acetate:ethanol:trichloroacetic acid, (5:5:1, v/v/w) and centrifuged at 15,000 rpm for 1 min and the absorbance was measured at 490 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). Results

Table 1
Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on % change in body weight in 3-NP treated rats. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. Data are presented as means ± S.E.M. of at least eight determinations.

Treatment (mg/kg)	% change in body weight
VC	4.24 ± 0.98
3-NP	−26.76 ± 2.38[a]
F 200	0.43 ± 1.62
F 100 + 3-NP	−11.96 ± 2.34[b]
F 200 + 3-NP	−2.47 ± 0.90[b,c]
M + 3-NP	−25.75 ± 1.19
M + F 200 + 3-NP	−24.95 ± 1.48[d]

[a] $P<0.05$ vs VC.
[b] $P<0.05$ vs 3-NP.
[c] $P<0.05$ vs F 100 + 3-NP.
[d] $P<0.05$ vs F 200 + 3-NP.

were calculated using molar extinction coefficient of chromophore ($1.36\times10^4\ M^{-1}\ cm^{-1}$) and expressed as INT reduced μmol per mg protein.

2.7.9. Lactate dehydrogenase activity

Lactate dehydrogenase activity (LDH) catalyzes the interconversion of lactate and pyruvate. The amount of this enzyme may be used as a marker of tissue breakdown. LDH in brain was assayed using the commercially available kit (Vital Diagnostics [P] Ltd., Mumbai, India). The absorbance was measured at 340 nm (UV-1700 Spectrophotometer, Shimadzu, Japan) and the activity of LDH is expressed as units per liter (U/L) (Kumar et al., 2007b).

2.7.10. Measurement of pro-inflammatory cytokines

The levels of IL-1β and TNF-α were quantified in brain of rats. The brains were isolated immediately after behavioral studies on day 5 and weighed sections were homogenized in homogenization buffer containing a protease inhibitor. The samples were cold centrifuged and the supernatant was used for estimation of IL-1β and TNF-α protein concentrations using the quantitative sandwich enzyme immunoassay according to manufacturer's instructions (R&D systems, MN, USA). The cytokine level was determined from the standard curve generated from the respective kits at 450 nm and was expressed as pg per mg protein.

2.7.11. Protein estimation

The protein content was measured by the biuret method using bovine serum albumin as standard.

2.8. Statistical analysis

All values were expressed as mean ± S.E.M. Statistical analysis was performed using SigmaStat Statistical Software. Data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. In all tests, $P<0.05$ was considered statistically significant.

3. Result

3.1. Effect of fenofibrate on body weight in 3-NP treated rats

There was no change in the initial and final body weight of vehicle treated animals, while 3-NP treatment caused significant decrease in body weight (% change in body weight) on day 5 when compared with vehicle treated group. Fenofibrate per se (200 mg/kg, *p.o.*) treatment had no effect on body weight, whereas fenofibrate (100 or 200 mg/kg, *p.o.*) treatment in 3-NP treated rats significantly attenuated the decrease in body weight with marked effect observed at the high dose as compared to 3-NP treated rats (Table 1). MK886 alone had no effect on decrease in body weight in 3-NP treated rats, however, administration of MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) abolished the effect of fenofibrate (Table 1).

3.2. Effect of fenofibrate on locomotor activity in 3-NP treated rats

Repeated treatment with 3-NP significantly decreased the total activity counts compared to vehicle treatment. Pretreatment with fenofibrate (100 or 200 mg/kg, *p.o.*) significantly reversed the 3-NP induced decrease in motor activity. Fenofibrate per se (200 mg/kg, *p.o.*) had no effect on the gross behavioral activity as compared with vehicle treatment in the control group (Fig. 1). MK886 (1 mg/kg, *i.p.*) alone did not alter motor activity in 3-NP treated rats. In contrast, MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly antagonized the effect of fenofibrate on locomotor activity (Fig. 1).

3.3. Effect of fenofibrate on movement analysis in 3-NP treated rats

After 2 to 3 days of repeated treatment, most of the rats showed a general decrease in movement and coordination of their hind limbs. These symptoms often evolved towards severe dystonic posturing as the 3-NP treatment progressed. By the end of the experiment, the 3-NP treated rats were almost unable to move. Fenofibrate (200 mg/kg, *p.o.*) alone did not alter normal movement behavior, however fenofibrate (100 or 200 mg/kg, *p.o.*) significantly prevented the

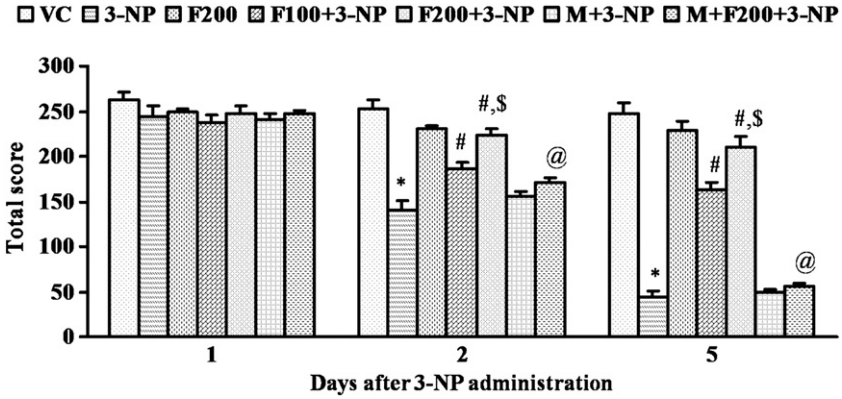

Fig. 1. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on locomotor activity in 3-NP treated rats. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. *$P<0.05$ vs VC; #$P<0.05$ vs 3-NP; $$P<0.05$ vs F 100 + 3-NP; @$P<0.05$ vs F 200 + 3-NP.

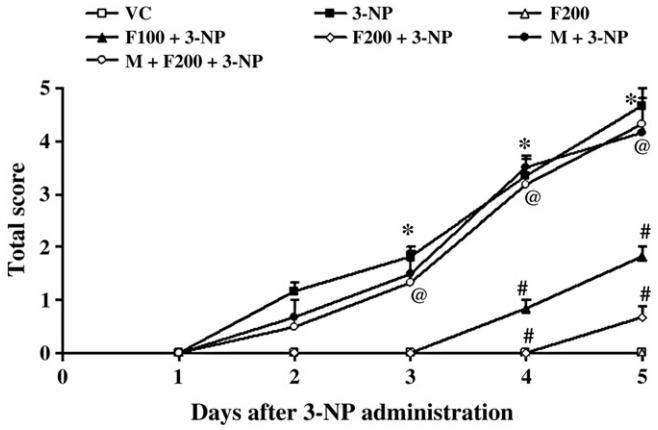

Fig. 2. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on movement analysis in 3-NP treated rats. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. *$P<0.05$ vs VC; #$P<0.05$ vs 3-NP; @$P<0.05$ vs F 200 + 3-NP.

movement abnormalities caused by 3-NP treatment with marked effect observed at the high dose as compared to 3-NP treated rats (Fig. 2). MK886 (1 mg/kg, *i.p.*) abolished the effects of fenofibrate on movement abnormalities in 3-NP treated rats (Fig. 2).

3.4. Effect of fenofibrate on the limb withdrawal test in 3-NP treated rats

The difference between the retraction times of both the two hind limbs was significantly higher in 3-NP treated rats as compared to vehicle-treated control rats that were able to quickly retract their both hind limbs. Fenofibrate per se (200 mg/kg, *p.o.*) had no effect on the limb retraction time. However, the performance of 3-NP treated rats in the limb withdrawal test was significantly improved by fenofibrate (100 or 200 mg/kg, *p.o.*) as compared to 3-NP treated rats. In this, a high dose of fenofibrate has shown marked effect as compared to the low dose. MK886 (1 mg/kg, *i.p.*) alone had no effect in this test whereas MK886 (1 mg/kg, *i.p.*) prior to fenofibrate blocked effect of fenofibrate in 3-NP treated rats (Table 2).

3.5. Effect of fenofibrate on string test in 3-NP treated rats

Mean length of time that the rat was able to hold the wire was recorded. 3-NP has shown a significantly lower latency to the grip loss as compared with vehicle treated groups. Fenofibrate per se (200 mg/kg, *p.o.*) had no effect on latency to grip loss as compared with vehicle treated group. MK886 alone had no effect on the lower latency to grip loss, however, administration of fenofibrate (100 or 200 mg/kg, *p.o.*) significantly increased the latency time in 3-NP treated animals as compared to 3-NP treated group. Administration of MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) abolished the effect of fenofibrate in 3-NP treated rats (Fig. 3).

Table 2
Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on limb withdrawl test in 3-NP treated rats. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. Data are presented as means ± S.E.M. of at least eight determinations.

Treatment (mg/kg)	Day 1	Day 2	Day 3
	(in seconds)		
VC	1.17 ± 0.17	0.83 ± 0.17	1.17 ± 0.17
3-NP	1.17 ± 0.17	7.50 ± 0.96[a]	94.50 ± 4.94[a]
F 200	1.00 ± 0.00	1.17 ± 0.17	1.17 ± 0.17
F 100 + 3-NP	1.00 ± 0.26	3.33 ± 0.76[b]	7.00 ± 1.37[b]
F 200 + 3-NP	1.00 ± 0.26	1.00 ± 0.26[b]	2.83 ± 0.31[b]
M + 3-NP	1.00 ± 0.26	6.67 ± 1.05	92.00 ± 5.43
M + F 200 + 3-NP	1.00 ± 0.00	6.33 ± 0.67[c]	89.50 ± 6.36[c]

[a] $P<0.05$ vs VC.
[b] $P<0.05$ vs 3-NP.
[c] $P<0.05$ vs F 200 + 3-NP.

3.6. Effect of fenofibrate on Morris water maze task in 3-NP treated rats

3.6.1. Acquisition

Prior to 3-NP administration, there were no significant differences in the ability of all groups to find the submerged platform during learning phase of the study.

3.6.2. Retention

There was a significant decrease in the performance of 3-NP lesioned rats as compared with controls. The relative values for the three parameters used to assess memory in the Morris water maze task: latency to entering the platform area (Fig. 4), entries into platform area (Fig. 5), and time spent in the target quadrant (Fig. 6). Mean escape latency to platform in fenofibrate per se (200 mg/kg, *p.o.*) group was not altered when compare with vehicle treated animals. 3-NP administration significantly showed the higher mean retention latencies when compared with vehicle control. The results suggest that 3-NP caused significant cognitive impairment. Fenofibrate (100 or 200 mg/kg, *p.o.*), starting before 3-NP administration, caused a significant decline mean latency to enter platform area on day 5 and improved retention of spatial navigation task as compared to 3-NP treated rats. MK886 alone had no effect in 3-NP-treated rats. MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly antagonized the effect of fenofibrate on transfer latency (Fig. 4).

Further, fenofibrate per se (200 mg/kg, *p.o.*) did not alter entries into platform area and time spent in target quadrant when compared with vehicle treated rats. Chronic administration of 3-NP caused a significant decrease in entries into platform area (Fig. 5) and time spent in the target quadrant (Fig. 6) when compared with vehicle control. The results suggest that 3-NP caused significant cognitive impairment. Fenofibrate (100 or 200 mg/kg, *p.o.*), starting before 3-NP administration, caused significant higher entries into platform area and time spent in target quadrant on day 5 and improved retention of spatial navigation task. MK886 alone had no effect on entries into platform area, time spent in target quadrant. On the contrary, MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) abolished the effects of fenofibrate on entries into platform area (Fig. 5), time spent in target quadrant (Fig. 6).

3.7. Effect of fenofibrate on oxidative stress in 3-NP treated rats

3.7.1. Effect of fenofibrate on lipid peroxidation

3-NP administered rats had significantly increased level of thiobarbituric acid reacting substances (TBARS) in brain in comparison to vehicle treated control animals (Table 3). In this study, treatment with fenofibrate (200 mg/kg, *p.o.*) in normal control animals did not alter brain TBARS level as compared to vehicle treated control group. On the contrary, similar administration of fenofibrate (100 or 200 mg/kg, *p.o.*) significantly reduced elevated TBARS level in the brains of 3-NP administered rats in comparison to the levels observed in 3-NP treated rats. MK886 (1 mg/kg, *i.p.*) alone was without any effect on TBARS level, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly antagonized the effect of fenofibrate on TBARS levels in the tissues of 3-NP administered rats (Table 3).

3.7.2. Effect of fenofibrate on protein carbonyl levels in 3-NP treated rats

There was a significant increase in protein carbonyl level in brains of 3-NP administered rats, in comparison to vehicle treated control animals, indicating oxidative stress (Table 3). In this study, treatment

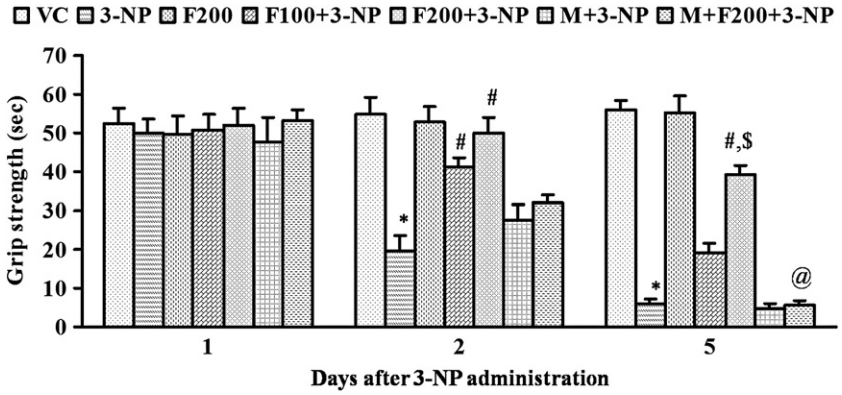

Fig. 3. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on grip strength in 3-NP treated rats. Each point represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{\$}P<0.05$ vs F 100 + 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

with fenofibrate (200 mg/kg, *p.o.*) in normal animals had no effect on brain protein carbonyl level as compared to vehicle treated control group. Conversely, similar administration of fenofibrate (100 or 200 mg/kg, *p.o.*) dose dependently reduced elevated brain protein carbonyl level in 3-NP administered rats as compared to the level observed in 3-NP treated rats (Table 3). MK886 alone (1 mg/kg, *i.p.*) had no effect on brain protein carbonyl level, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly blocked the effect of fenofibrate on protein carbonyl level in 3-NP administered rats (Table 3).

3.7.3. Effect of fenofibrate on reduced glutathione in 3-NP treated rats

3-NP administered rats showed significantly decreased level of GSH in the brain. In this study, administration of fenofibrate (200 mg/kg, *p.o.*) had no effect on GSH level in normal animals as compared to vehicle treated control group. On the other hand, fenofibrate (100 or 200 mg/kg, *p.o.*) improved GSH level in brain (Table 3) as compared to 3-NP treated rats. MK886 alone (1 mg/kg, *i.p.*) was without any effect on GSH level, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly blocked the effect of fenofibrate on GSH level in 3-NP administered rats (Table 3).

3.7.4. Effect of fenofibrate on catalase activity in 3-NP treated rats

Systemic 3-NP administration has shown significantly decreased activity of catalase in brain comparison to vehicle-treated control animals (Table 4). In this study, a four day treatment with fenofibrate (200 mg/kg, *p.o.*) in normal animals did not alter brain catalase activity as compared to vehicle-treated control group. On the contrary, similar administration of fenofibrate (100 or 200 mg/kg, *p.o.*) significantly restored the reduced activity of catalase in the brain of 3-NP administered rats in comparison to that observed in 3-NP treated rats (Table 4). MK886 alone (1 mg/kg, *i.p.*) was without any effect on catalase activity, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly abolished the effect of fenofibrate (100 or 200 mg/kg, *p.o.*) on catalase activity in brain of 3-NP administered rats (Table 4).

3.7.5. Effect of fenofibrate on superoxide dismutase activity in 3-NP treated rats

3-NP administered animals showed significantly decreased activity of SOD in the brain in comparison to vehicle treated control animals (Table 4). In this study, administration of fenofibrate (200 mg/kg, *p.o.*) had no effect on SOD activity in 3-NP administered animals as compared to vehicle treated control group. On the other hand, fenofibrate (100 or 200 mg/kg, *p.o.*) improved SOD activity in brain of 3-NP administered rats (Table 4) as compared to 3-NP-treated rats. MK886 alone (1 mg/kg, *i.p.*) was without any effect on SOD activity, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly blocked the effect of fenofibrate on SOD activity in 3-NP administered rats (Table 4).

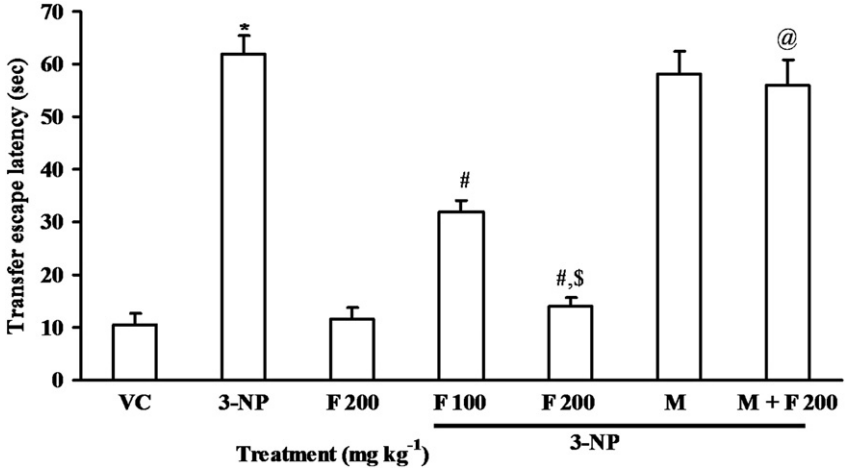

Fig. 4. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on transfer latency in Morris water maze test in 3-NP treated rats. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{\$}P<0.05$ vs F 100 + 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

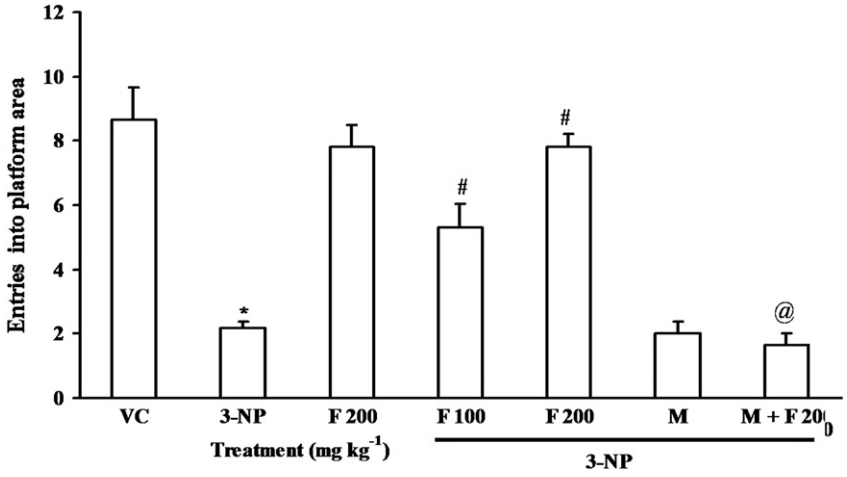

Fig. 5. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on number of entries in platform area in Morris water maze test in 3-NP treated rats. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

3.8. Effect of fenofibrate on nitrite level in 3-NP treated rats

3-NP rats significantly increased brain nitrite level in comparison to vehicle treated control animals (Table 4). In this study, treatment with fenofibrate (200 mg/kg, *p.o.*) in 3-NP administered animals had no effect on brain nitrite level as compared to vehicle treated control group. Conversely, similar administration of fenofibrate (100 or 200 mg/kg, *p.o.*) dose dependently reduced elevated brain nitrite level in 3-NP administered rats as compared to the level observed in 3-NP treated rats (Table 4). MK886 alone (1 mg/kg, *i.p.*) had no effect on brain nitrite level, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *i.p.*) significantly blocked the effect of fenofibrate on nitrite level in the 3-NP administered rats (Table 4).

3.9. Effect of fenofibrate on brain succinate dehydrogenase activity in 3-NP treated rats

Systemic 3-NP administration showed significant decline in brain SDH activity when compared with vehicle treated rats. Fenofibrate (200 mg/kg, *i.p.*) alone did not cause any change in brain SDH activity. In contrast, repeated administration of fenofibrate (100, 200 mg/kg, *i.p.*) in 3-NP-treated rats significantly attenuated the reduction in SDH activity compared with 3-NP treated rats. (Table 5). MK886 alone (1 mg/kg, *i.p.*) had no effect on brain SDH levels, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *i.p.*) significantly abolished the effect of fenofibrate on SDH level in the 3-NP administered rats (Table 5).

3.10. Effect of fenofibrate on brain lactate dehydrogenase in 3-NP treated rats

The LDH activity was used as cell death marker. Systemic 3-NP administration showed significant increase in brain LDH when compared with vehicle treated rats. Fenofibrate (200 mg/kg, *i.p.*) per se treatment did not cause any change in the brain LDH activity as compared to vehicle-treated rats. However, treatment of fenofibrate (100, 200 mg/kg, *i.p.*) in 3-NP treated rats significantly reduced the increased LDH activity when compared with 3-NP treated group (Table 5). MK886 alone (1 mg/kg, *i.p.*) was without any effect on LDH activity, whereas, pretreatment with MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *p.o.*) significantly blocked the effect of fenofibrate on LDH activity in 3-NP administered rats (Table 5).

3.11. Effect of fenofibrate on cytokine production

The concentration of brain IL-1β (Fig. 7) and TNF-α (Fig. 8) was significantly elevated in 3-NP treated rats when compared to that of

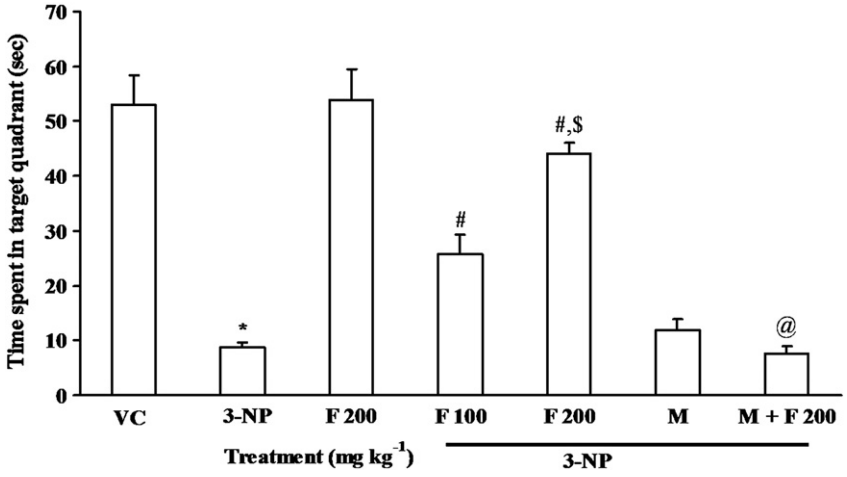

Fig. 6. Effect of fenofibrate (FEN; 100 or 200 mg/kg, *p.o.*) on time spent in quadrant in Morris water maze test in 3-NP treated rats. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{\$}P<0.05$ vs F 100 + 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

Table 3
Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on TBARS, protein carbonyl, and GSH in 3-NP treated rat's brain.
GSH, reduced glutathione; M, MK886 (1 mg/kg, *i.p.*); TBARS, thiobarbituric acid reacting substances; VC, vehicle control. Data are presented as means ± S.E.M. of at least eight determinations.

Treatment (mg/kg)	TBARS (nmol/mg protein)	Protein carbonyl (nmol/mg protein)	GSH (nmol/mg protein)
VC	0.46 ± 0.04	14.04 ± 1.09	80.17 ± 3.83
3-NP	0.96 ± 0.04[a]	55.40 ± 1.72[a]	9.41 ± 1.62[a]
F 200	0.43 ± 0.02	15.84 ± 2.12	83.10 ± 2.82
F 100 + 3-NP	0.78 ± 0.01[b]	36.89 ± 1.90[b]	29.87 ± 3.98[b]
F 200 + 3-NP	0.64 ± 0.01[b,c]	28.65 ± 0.43[b,c]	64.29 ± 2.84[b,c]
M + 3-NP	0.92 ± 0.03	54.03 ± 1.68	8.15 ± 0.58
M + F 200 + 3-NP	0.87 ± 0.03[d]	52.50 ± 0.97[d]	11.73 ± 1.22[d]

[a] $P<0.05$ vs VC.
[b] $P<0.05$ vs 3-NP.
[c] $P<0.05$ vs F 100 + 3-NP.
[d] $P<0.05$ vs F 200 + 3-NP.

Table 5
Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on SDH and LDH in 3-NP treated rat's brain. SDH activity is expressed in μmol of INT reduced per mg protein. LDH, lactate dehydrogenase; M, MK886 (1 mg/kg, *i.p.*); SDH, succinate dehydrogenase; VC, vehicle control. Data are presented as means ± S.E.M. of at least eight determinations.

Treatment (mg/kg)	SDH (μmol/mg protein)	LDH (IU/L)
VC	0.46 ± 0.02	38.26 ± 5.95
3-NP	0.14 ± 0.01[a]	195.81 ± 5.51[a]
F 200	0.46 ± 0.02	40.51 ± 6.58
F 100 + 3-NP	0.21 ± 0.01[b]	121.54 ± 9.28[b]
F 200 + 3-NP	0.28 ± 0.01[b,c]	61.89 ± 5.95[b,c]
M + 3-NP	0.13 ± 0.01	190.19 ± 28.24
M + F 200 + 3-NP	0.14 ± 0.01[d]	182.30 ± 14.59[d]

[a] $P<0.05$ vs VC.
[b] $P<0.05$ vs 3-NP.
[c] $P<0.05$ vs F 100 + 3-NP.
[d] $P<0.05$ vs F 200 + 3-NP.

vehicle treated control rats. Chronic administration of fenofibrate (200 mg/kg, *p.o.*) had no effect on brain IL-1β and TNF-α levels in normal control animals. However, the concentration of these cytokines was significantly lower in 3-NP administered rats that had been treated with fenofibrate (100 or 200 mg/kg, *p.o.*), but not with MK886 alone (1 mg/kg, *i.p.*). Systemic administration of MK886 (1 mg/kg, *i.p.*) prior to fenofibrate (200 mg/kg, *i.p.*,for 4 days) abolish the effect of fenofibrate on these cytokine production in 3-NP treated rats.(Figs. 7 and 8)

4. Discussion

The results of present study indicate that the pretreatment with fenofibrate significantly improved motor deficits and cognitive function, marked reduction in oxidative and nitrosative stress, restored antioxidant defense mechanisms and SDH activity and reduction in increased LDH activity and histological changes characterized by 3-NP induced neurodegeneration study. In the present study 3-NP significantly causes motor and behavioral abnormalities including bradykinesia, cognitive dysfunction, muscles weaknesses and rigidity. These finding are in agreement with earlier reports including those from our lab, which showed a variety of neurobehavioral abnormalities and motor deficits in rats following 3-NP administration (Kumar et al., 2007a,b). 3-NP produces lesions in hippocampal CA1 and CA3 pyramidal neurons, the area of brain that is associated with cognitive performance (Kumar et al., 2007a). Treatment of rat with fenofibrate produced significant dose-dependent protection of animals against 3-NP induced behavior and motor deficit. Furthermore, fenofibrate also improve memory performance in 3-NP administrated rats. It is well known that oxidative stress contributes to learning and memory deficits following oxidative brain damage (Fukui et al., 2001). Accumulating evidence indicates that PPAR-α showed significantly marked protection in various neurological disorders (Chen et al., 2007; Collino et al., 2006; Deplanque et al., 2003; Kreisler et al., 2007).

Oxidative damage and mitochondrial dysfunction are also involved in the 3-NP induced toxicity involving most importantly glutamate related excitotoxicity and generation of free radicals respectively (Beal et al., 1993; Kumar et al., 2007b). The excitotoxic mechanism of 3-NP neurodegeneration is consistent with oxidative theory, wherein activation of N-methyl-D-aspartate receptors leads to generation of free radicals, particularly superoxide radical (Lagoa et al., 2009). Inhibition of mitochondrial energy production, which is caused by the 3-NP inhibition of complex II, conceivably induces an increase in oxygen flux through the mitochondria, leading to increased generation of reactive oxygen species as well as reactive nitrogen species and are associated with oxidant antioxidant imbalance which furthermore causes increase in oxidative and nitrosative stress (Kumar et al., 2007c; Lagoa et al., 2009; Tunez et al., 2006b). Oxidative stress leads to oxidation of lipid and protein in striatum and cortex, and causes massive loss of striatal neurons (La Fontaine et al., 2000; Tunez et al., 2006b). 3-NP dramatically increases influx of calcium which results in activation of neuronal and inducible nitric oxide synthase, thus producing nitric oxide, which further reacts with superoxide to form peroxynitrite and ultimateley nitrosative stress (Kumar et al., 2006; Tunez et al., 2004).

Increase in the levels of TBARS (a marker of lipid peroxidation), protein carbonyls (a marker of protein peroxidation), nitrosative stress (characterized by increased level of total nitrite), and alteration in antioxidant defense mechanisms including endogenous antioxidant GSH, enzyme catalase and SOD have also been reported in striatum and cortex of 3-NP administrated rats (Brouillet et al., 2005; Kumar et al., 2007c; La Fontaine et al., 2000; Tunez et al., 2006a,b). These results support the oxidative stress based theory of neurotoxicity caused by 3-NP (Beal et al., 1995) In the present study, treatment with fenofibrate significantly, but receptor-dependently attenuated oxidative and nitrosative stress, along with improvement in antioxidant defenses such as catalase, GSH and SOD, in brain of 3-NP administered rats. These result are in accordance with previous findings where PPAR-α activators produced neuroprotection by decreasing oxidative and nitrosative stress via reduction in free radical generation (Beltowski et al., 2002; Chen et al., 2007; Costa et al., 2002; Deplanque et al., 2003), and increasing antioxidant enzymes such as catalase and endogenous antioxidant GSH (Chen et al., 2007; Collino et al., 2006; Deplanque et al., 2003). Moreover, MK886 (PPAR-α antagonist) pretreatment abolished the protective effects of fenofibrate

Table 4
Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on SOD, catalase, and nitrite 3-NP treated rat's brain. Catalase activity is expressed as μmol of hydrogen peroxide consumed per min per mg protein. The activity of SOD was expressed as % activity of vehicle-treated control. M, MK886 (1 mg/kg, *i.p.*); SOD, superoxide dismutase; VC, vehicle control. Data are presented as means ± S.E.M. of at least eight determinations.

Treatment (mg/kg)	% SOD activity	Catalase (μmol/mg protein)	Nitrite (μmol/mg protein)
VC	100.06 ± 5.64	11.65 ± 0.54	0.46 ± 0.04
3-NP	18.50 ± 2.91[a]	1.76 ± 0.32[a]	1.62 ± 0.06[a]
F 200	101.55 ± 12.90	12.17 ± 0.33	0.46 ± 0.07
F 100 + 3-NP	50.10 ± 3.27[b]	4.98 ± 0.29[b]	1.06 ± 0.04[b]
F 200 + 3-NP	82.10 ± 7.76[b,c]	8.14 ± 0.41[b,c]	0.89 ± 0.04[b]
M + 3-NP	20.22 ± 2.13	1.98 ± 0.41	1.65 ± 0.04
M + F 200 + 3-NP	28.08 ± 6.60[d]	2.13 ± 0.38[d]	1.52 ± 0.61[d]

[a] $P<0.05$ vs VC.
[b] $P<0.05$ vs 3-NP.
[c] $P<0.05$ vs F 100 + 3-NP.
[d] $P<0.05$ vs F 200 + 3-NP.

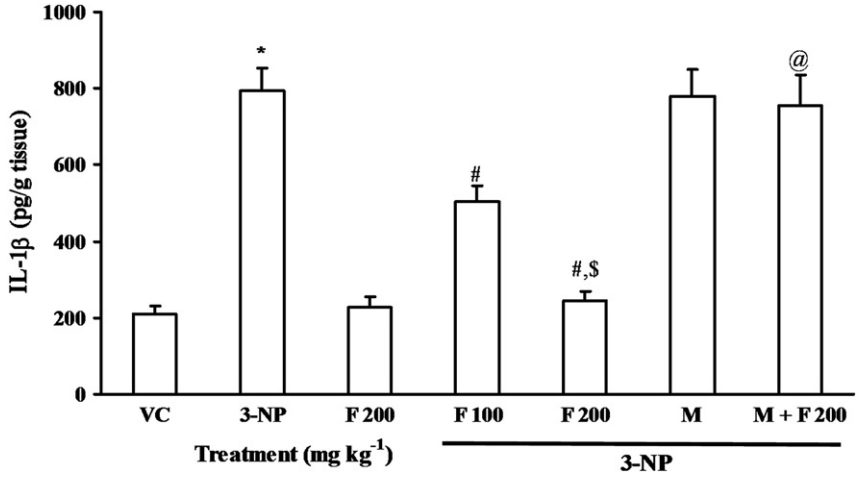

Fig. 7. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on IL-1β in 3-NP treated rat's brain. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{\$}P<0.05$ vs F 100 + 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

thus it is proposed that fenofibrate mediates its effects by activating PPAR-α receptor, supporting a receptor dependent effect.

3-NP produces a detrimental effect on glial cells and oligodendrocytes and causes oxidative stress and release of proinflammatory mediators (Ahuja et al., 2008). It is noteworthy that non-neuronal cells such as microglia and astroglia had shown to be activated in patients suffering from Huntington's disease (Pavese et al., 2006) and similar activation of glial cells is also reported in 3-NP model of Huntington's disease. (Ryu et al., 2003). It is well known that activated microglia releases proinflammatory mediators like cytokines, particularly IL-1β, IL-6, along with TNF-α, excitatory amino acid, adenosine triphosphate and increased the expression of cyclooxygenase-2 and inducible nitric oxide synthase, which further enhances the production of superoxides and nitric oxides, respectively (Pavese et al., 2006; Tai et al., 2007). It is well characterized that these mediators are also involved in neurodegeneration in Huntington's disease. A growing body of evidence shows a direct relationship between inflammation and excitotoxicity, which are two major components of brain injury and disease (Perez-De la Cruz and Santamaria, 2007). Further it has been reported that 3-NP causes oxidative stress which leads to activation of NFkB from degradation of IkB NF-κB complex (p50/p65) allowing nuclear translocation of active NF-κB dimers and thereby leading to the transcription of various inflammatory genes (Napolitano et al., 2008; Yu et al., 2000).

In the present study we observed a significant increase in IL-1β and TNF-α in brain of 3-NP administered rats. Treatment with fenofibrate dose dependently reduced the level of IL-β and TNF-α, which were blocked by pretreatment with MK886. Of relevant to this, recent studies also report the presence of PPAR-α in brain region particularly striatum (Kainu et al., 1994; Moreno et al., 2004) and CA1 pyramidal cells, granular, polymorphic layer of dentate gyrus and non-neuronal cells, particularly microglia and astroglia (Moreno et al., 2004). The presence of PPAR-α in microglia suggests that this isoform modulates central inflammation, possibly by regulation of cytokine production by microglia (Benani et al., 2003, 2004). Interestingly, the levels of pro-inflammatory cytokine are significantly higher in the absence of functional PPAR-α (Genovese et al., 2005) which indicates the anti-inflammatory role of PPAR-α. In vivo work in animals and humans also supports an anti-inflammatory action of PPAR-α agonist. Various studies showed that PPAR-α activation induced anti-inflammatory (Bougarne et al., 2009; Chen et al., 2007) and antioxidant effects in in vitro (Inoue et al., 2001; Staels et al., 1998;) and in vivo models of traumatic brain injury, cerebral ischemia (Besson et al., 2005; Chen et al., 2007; Collino et al., 2006). Moreover, PPAR-α agonists reduce levels of inflammatory markers such IL-6, TNF-α, and IL-1β (Gelinas and McLaurin, 2005). These findings, therefore, suggest that activation of PPAR-α reduce inflammation by inhibiting the activation and the subsequent release of pro-inflammatory cytokines. In

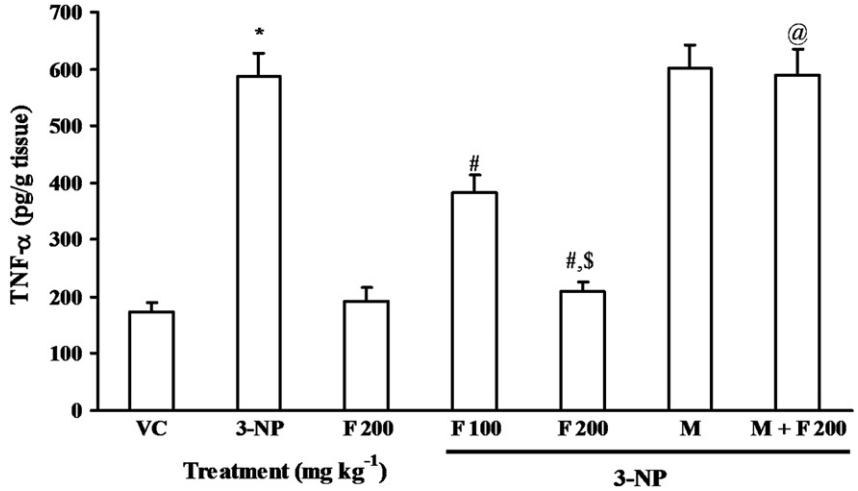

Fig. 8. Effect of fenofibrate (F; 100 or 200 mg/kg, *p.o.*) on TNF-α in 3-NP treated rat's brain. Each column represents the mean ± S.E.M. of eight animals per group. M, MK886 (1 mg/kg, *i.p.*); VC, vehicle control. $^{*}P<0.05$ vs VC; $^{\#}P<0.05$ vs 3-NP; $^{\$}P<0.05$ vs F 100 + 3-NP; $^{@}P<0.05$ vs F 200 + 3-NP.

the present study, brain LDH activity, a well-known marker of cell death and pathological changes in striatum, an area highly vulnerable to 3-NP-neurotoxicity were also done, in order to gain an insight into neuroprotective role of PPAR-α activation in experimental Huntington's disease. In cell culture medium, 3-NP caused a dose dependent neurodegeneration accompanied by an increased LDH activity (Vis et al., 2004), increased ratio of lactate to pyurvate concentration in the medium suggested that metabolic activity shifted to anaerobic energy metabolism. Consistent to this, in the present study, 3-NP treated animals showed a marked increased in LDH, which was prevented by fenofibrate treatment. It has been reported that PPAR-α modulators cause decrease in LDH and TNF-α release, which are key mediators in cell death (Yang et al., 2005).

In conclusion, the present study provides further evidence that the PPAR-α receptor dependent beneficial effects of fenofibrate are partly a reflection of the overall anti-inflammatory effects due to decreased levels of IL-1β and TNF-α in the brain and in part by the modulatory role of PPAR-α on oxidative, and nitrosative stress that might be involved in attenuating the development of behavioral abnormalities in this experimental model of Huntington's disease.

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