Pharmacology, Biochemistry and Behavior 96 (2010) 115–124

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Pioglitazone ameliorates behavioral, biochemical and cellular alterations in quinolinic acid induced neurotoxicity: Possible role of peroxisome proliferator activated receptor-ϒ (PPARϒ) in Huntington's disease

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ARTICLE INFO

Article history:
Received 7 October 2009
Received in revised form 13 April 2010
Accepted 25 April 2010
Available online 5 May 2010

Keywords:
Antioxidant
Mitochondrial dysfunction
Pioglitazone
Neurotoxicity
Oxidative stress

ABSTRACT

Emerging evidence indicates that PPARϒ activators attenuate neurodegeneration and related complications. Therefore, the present study focused on the neuroprotective potential of pioglitazone against quinolinic acid (QUIN) induced neurotoxicity. Intrastriatal (unilaterally) administration of QUIN significantly altered body weight and motor function (locomotor activity, rotarod and beam walk performance). Further, QUIN treatment significantly caused oxidative damage (increased lipid peroxidation, nitrite concentration and depleted endogenous antioxidant defense enzymes), altered mitochondrial enzyme complex (I, II and IV) activities and TNF-α level as compared to sham treated animals. Pioglitazone (10, 20 and 40 mg/kg, p.o.) treatment significantly improved body weight and motor functions, oxidative defense. Further, pioglitazone treatment restored mitochondrial enzyme complex activity as well as TNF-α level as compared to QUIN treated group. While Bisphenol A diglycidyl ether (BADGE) (15 mg/kg), PPARϒ antagonist significantly reversed the protective effect of the pioglitazone (40 mg/kg) in the QUIN treated animals. Further, pioglitazone treatment significantly attenuated the striatal lesion volume in QUIN treated animals, suggesting a role for the PPARϒ pathway in QUIN induced neurotoxicity. Altogether, this evidence indicates that PPARϒ activation by pioglitazone attenuated QUIN induced neurotoxicity in animals and which could be an important therapeutic avenue to ameliorate Huntington like symptoms.

1. Introduction

Huntington's disease (HD) is one of the movement disorders having complex pathophysiology and only symptomatic treatment is available. Loss of striatal neurons in initial stages (Graham et al., 2009) and subsequently cortex and other brain regions are manifested HD pathogenesis (Brandt et al., 1996). Transcriptional deregulation (Cui et al., 2006) and proteasome dysfunction (Díaz-Hernández et al., 2003) have been suggested as significant contributors to the pathogenesis of HD. Additionally, calcium homeostasis deregulation (Panov et al., 2002) and mitochondrial dysfunction (Panov et al., 2002; Milakovic and Johnson, 2005; Tabrizi et al., 1999; Stahl and Swanson, 1974; Vonsattel et al., 1985) also have been strongly suggested to be involved pathogenesis of HD. Earlier findings have provided compelling evidence that mitochondrial dysfunction is central to the HD pathogenesis (Sanberg et al., 1989; Panov et al., 2002; Ganzella et al., 2006). In addition, mitochondrial respiration and ATP production are impaired significantly in striatal cells expressing mutant huntingtin (Milakovic and Johnson, 2005). Further, higher serum levels of IgA, soluble TNFα, soluble IL-2 receptor, neopterin C3 and lower serum tryptophan have been reported in HD patients (Leblhuber et al., 1998; Wang et al., 2003) suggesting the role of neuroinflammatory mediators in HD pathogenesis.

Quinolinic acid (QUIN) is an endogenous metabolite of tryptophan at the kynurenine pathway (Amori et al., 2009), causes early low-grade neuroinflammation (Braidy et al., 2009) and reported to mimick the clinical symptoms of HD in experimental animals. Of note, several reports documented significant behavioral/biochemical alterations against intrastriatal QUIN infusion (Tzeng et al., 2005; Maragos et al., 2004; Scattoni et al., 2007). Further, intrastriatal injection of QUIN has been reported to cause significant oxidative damage, activation of PKC, NF-kβ that further leads to DNA fragmentation and neuronal apoptosis (Wang et al., 2003; Ballerini et al., 2005).

Peroxisome proliferator activated receptors (PPARs) are members of the nuclear hormone receptor family of ligand-activated transcription factors (Rosen and Spiegelman, 2001). To date, three mammalian PPAR subtypes have been isolated and termed PPARα, PPARβ, and PPARϒ. PPARα is highly expressed in brain and PPARβ is an APC-regulated target of non-steroidal anti-inflammatory drugs (He et al., 1999). PPARϒ is a ligand-activated nuclear receptor implicated in several human pathologies, including cancer, atherosclerosis and inflammation (Berger and Moller, 2002). Previous studies suggest the

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doi:10.1016/j.pbb.2010.04.018

therapeutic potential of the PPARϒ agonist in animal model of Alzheimer disease (AD) and transgenic mouse model of amyotrophic lateral sclerosis (Watson and Craft, 2003; Watson et al., 2005; Kiaei et al., 2005). Thiazolidinediones (TZDs) have been proposed as potential therapeutic agents for both AD and multiple sclerosis (Watson and Craft, 2006), and their neuroprotective effects have been ascribed to either improved insulin sensitivity, or to their anti-inflammatory action through PPARϒ activation in glial cells (Bernardo and Minghetti, 2006; Heneka and Landreth, 2007). However, activation of PPARϒ by three different TZDs protected rat hippocampal neurons against β-amyloid (Aβ)-induced damage (Inestrosa et al., 2005), and rosiglitazone protects human neuroblastoma SH-SY5Y cells against acetaldehyde induced cytotoxicity (Jung et al., 2006). In addition, PPARϒ activation by rosiglitazone up-regulates Bcl-2 and prevents neuronal degeneration induced by both oxidative stress and Aβ fibrils, with a concomitant increase in mitochondrial viability (Fuenzalida et al., 2007).

Recent *in-vivo* and *in-vitro* studies have evidenced that expression of PGC-1α (a potent coactivator of PPARϒ), is repressed by mutant huntingtin expression. When PGC-1α knock-out (KO) mice are crossed with HD knockin mice, this resulted in an increased neurodegeneration of striatal neurons and motor abnormalities in the HD mice (Quintanilla et al., 2008) and therapeutic potential of PPARϒ agonists (Park et al., 2007). In another set of findings PPARϒ antagonist Bisphenol A diglycidyl ether (BADGE) have been reported to antagonize the protective effect of rosiglitazone (Harold et al., 2000). However, effects of PPARϒ agonist in animal model of HD have not been well understood so far. Present study has been designed to explore the therapeutic potential of pioglitazone against QUIN induced behavioral, biochemical and mitochondrial dysfunction in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (250–300 g) bred in Central Animal House, Panjab University, Chandigarh were used. The animals were kept under standard laboratory conditions, maintained on 12-h light/dark cycle, free access to food and water. Animals were acclimatized to laboratory conditions before start of the experimental study. Experiment was performed between 9:00 and 17:00 h. The experimental protocol was approved by Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

2.2. Intrastriatal administration of QUIN

Animals were anesthetized with thiopental sodium (45 mg/kg, ip). The surface of the skull was exposed by making incision on the scalp. QUIN (300 nmol/4 µl) (Sigma-Aldrich, St Louis, Mo) was dissolved in normal saline and administered unilaterally in right striatum (4 µl) 28-gauge stainless steel needle attached to a Hamilton syringe. Injections were made via a l–2 mm diameter hole made in the skull using a small hand drill at anterior +1.7 mm; lateral ±2.7 mm; ventral −4.8 mm from bregma and dura as described by Paxinos and Watson (Paxinos and Watson, 2007). 4 µl of QUIN was delivered over a period of 2 min, and injection needle was left in place for another 2 min to prevent back diffusion of the injected drug solution.

2.3. Drug and treatment schedule

Pioglitazone (Ind-Swift Laboratories, Chandigarh) and BADGE (Sigma Chemicals, St Louis, Mo USA) were suspended in 0.25% w/v sodium carboxy methyl cellulose (CMC) and administered orally as per body weight (5 ml/kg). Study includes various treatment groups, consisting of 16 animals in each group.

Group-1: Naïve (without treatment), Group-2: sham (surgery performed, vehicle administered), Group-3: Intrastriatal QUIN 300 nmol (single injection), Group-4: QUIN 300 nmol + pioglitazone (10 mg/kg), Group-5: QUIN 300 nmol + pioglitazone (20 mg/kg), Group-6: QUIN 300 nmol + pioglitazone (40 mg/kg). Group-7: QUIN 300 nmol + BADGE (15 mg/kg). Group-8: QUIN 300 nmol + pioglitazone (40 mg/kg) + BADGE (15 mg/kg). Group-9: Pioglitazone (40 mg/kg) *per se* (alone) treatment.

Each group received treatment daily in the morning 10:00 h, for 21 days starting from day 1 after recovery from anesthesia. Doses selection for QUIN and pioglitazone was made on the basis earlier studies (Pathan et al., 2006; Kumar et al., 2009).

2.4. Behavioral assessments

2.4.1. Body weight

The body weight of animals was recorded before the start of drug treatment (before QUIN administration) and on last day of the study.

2.4.2. Assessment of gross behavioral activity (locomotor activity)

The locomotor activity was assessed by using actophotometer (IMCORP, Ambala, India) on weekly intervals. Animals were placed individually in the activity chamber for 3 min as a habituation period before making actual motor activity tasks for next 5 min. Total activity (horizontal and vertical) was expressed as counts per 5 min as described by Kumar (Kumar et al., 2007). The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room during the assessment.

2.4.3. Rotarod activity

All animals were evaluated for motor coordination and balance on rotarod test on weekly intervals after QUIN injection. The animals were given a prior training session before actual recording on rotarod apparatus (IMCORP, Ambala, India). Animals were placed individually on the rotating rod with a diameter of 7 cm (speed 25 rpm). The cut off time (90 s) was fixed and each rat performed three separate trials at 5 min interval as described by Kulkarni (Kulkarni, 1999).

2.4.4. Beam-crossing task

This task requires an animal to walk on across a narrow wooden beam, measuring its motor coordination ability. The beam consisted of two platforms (8 cm in diameter) connected by a wooden beam (0.5 mm in thickness, 2.0 cm in width, and 120 cm in length). The beam was elevated 65 cm above ground. A box filled with sawdust was placed below the beam, serving as protection for a falling rat. In order to adapt to the elevated beam, a rat was allowed to explore it for 5 min before training. A training trial started by placing a rat on the platform at one end. When a rat walked across the beam from one end to the other end, slipping of its feet occurred. Number of slips in each trial was recorded as done by Wang (Wang et al., 2006), and additionally motor performance of rats was scored on a scale ranging from 0 to 4. A score of 0 was assigned to animal that could readily traverse the beam. Score 1, 2 and 3 were given to animals demonstrating mild, moderate and severe impairment, respectively. Score 4 was assigned to the animals completely unable to walk on the beam.

2.5. Biochemical assessments

2.5.1. Dissection and homogenization

Animals were sacrificed by decapitation immediately after behavioral assessments on day 21st. Striatum of each animal was isolated by putting on ice and then weighed separately. A 10% (WV^{-1}) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 10,000 *g* at 4 °C for 15 min.

Aliquots of supernatants were separated and used for biochemical estimations.

2.5.2. Measurement of lipid peroxidation

The quantitative measurement of lipid peroxidation was performed according to the method of Wills (Wills 1966). The amount of malondialdehyde (MDA), a measure of lipid peroxidation was measured by reaction with thiobarbituric acid at 532 nm using Perkin Elmer lambda 20 Spectrophotometer (Norwalk, CT, USA). The values were calculated using molar extinction coefficient of chromophore ($1.56\times10^5\ M^{-1}\ cm^{-1}$) and expressed as percentage of sham.

2.5.3. Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediame dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green (Green et al. 1982). Equal volumes of supernatant and Greiss reagent were mixed, and then the mixture was incubated for 10 min at room temperature in the dark. The absorbance was determined at 540 nm with Perkin Elmer lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as percentage of sham.

2.5.4. Catalase estimation

Catalase activity was assayed by the method of Luck (Luck, 1971), wherein the breakdown of hydrogen peroxides (H_2O_2) is measured at 240 nm. Briefly, assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and change in absorbance was recorded at 240 nm. The results were expressed as micromole H_2O_2 decomposed per milligram of protein/min.

2.5.5. Superoxide dismutase activity (SOD)

Superoxide dismutase activity was assayed according to the method of Kono (Kono, 1978) wherein the reduction of nitrazobluetetrazolium (NBT) was inhibited by the superoxide dismutase and measured at 560 nm using spectrophotometer. Briefly, the reaction was initiated by the addition of the hydroxylamine hydrochloride to the mixture containing NBT and sample. The results were expressed as unit/mg protein, where one unit of enzyme is defined as the amount of enzyme inhibiting the rate of reaction by 100%.

2.5.6. Glutathione (GSH) assay

Different glutathione content in striatum was estimated according to the different methods such as reduced glutathione (Ellman, 1959), Total glutathione (Zahler and Cleland, 1968), Oxidized glutathione (GSSG) was quantified by subtracting the value of glutathione reduced from total glutathione. Redox ratio of reduced glutathione/oxidized glutathione (GSH/GSSG) was also calculated.

2.5.7. Protein estimation

Protein estimation was done by Gornall method (Gornall et al., 1949) using bovine serum albumin as standard.

2.6. Mitochondrial complex estimation

2.6.1. Isolation of rat brain mitochondria

Rat brain mitochondria were isolated by the method of Berman and Hastings (Berman and Hastings, 1999). The striatum regions were homogenized in isolation buffer with EGTA (215 mM Mannitol, 75 mM sucrose, 0.1% BSA, 20 mM HEPES, 1 mM EGTA, pH 7.2). Homogenates were centrifuged at 13,000*g* for 5 min at 4 °C. Pellet was resuspended in isolation buffer with EGTA and spun again at 13,000*g* for 5 min. The resulting supernatants were transferred to new tubes and topped off with isolation buffer with EGTA and again spun at 13,000*g* for 10 min. Pellets containing pure mitochondria were resuspended in isolation buffer without EGTA.

2.6.2. NADH dehydrogenase activity

NADH Dehydrogenase activity was measured spectrophotometrically (UV-Pharmaspec 1700 Shimadzu, Japan) by the method of King and Howard (King and Howard, 1967). The method involves catalytic oxidation of NADH to NAD^+ with subsequent reduction of cytochrome C.

2.6.3. Succinate dehydrogenase (SDH) activity

Succinate Dehydrogenase (SDH) activity was measured spectrophotometrically (UV-Pharmaspec 1700 Shimadzu, Japan) according to King (King, 1967). The method involves oxidation of succinate by an artificial electron acceptor, potassium ferricyanide.

2.6.4. MTT (mitochondrial redox activity) assay

The method employed in the present study is based on the *in-vitro* studies to evaluate mitochondrial redox activity through the conversion of MTT tetrazolium salt to formazan crystals by mitochondrial respiratory chain reactions in isolated mitochondria by the method of Liu (Liu et al., 1997). The absorbance of the resulting medium was measured by an ELISA reader at 580 nm wavelength.

2.6.5. Cytochrome oxidase activity

Cytochrome oxidase activity was assayed according to the method of Sotocassa in striatal mitochondria (Sottocasa et al., 1967).

2.7. Estimation of tumor necrosis factor-alpha (TNF-α)

Tumor necrosis factor-alpha (TNF-α) was estimated by using rat TNF-α kit (R&D Systems, Minneapolis, MN, USA). It is a solid phase sandwich enzyme linked immuno-sorbent assay (ELISA) using a microtitre plate reader at 450 nm. Concentrations of TNF-α were calculated from plotted standard curves.

2.8. TTC staining, striatal lesion volume measurement

At the end of drug administration, animals were sacrificed for 2,3,5-triphenyltetrazolium chloride (TTC) staining. Brains were quickly removed and placed in icecold saline solution. Brains were sectioned at 2-mm intervals using rat brain matrix. Slices were then subjected in 2% TTC for 5 min at 37 °C in the dark and removed and placed in 4% formaldehyde, pH 7.4 in 0.1 M phosphate buffer. For measurement of lesion volumes, serial, coronal sections (25 mm) were cut throughout the entire striatum using a cryostat as explained by Maragos (Maragos et al., 1998). Using computer-based image analysis (Image J 1.42q, NIH, USA), lesion volumes of different treatment groups were calculated from each section and multiplying this value by the distance between the sections (Kim et al., 2005).

2.9. Statistical analysis

One specific group of sixteen ($n=16$) animals was assigned to a specific drug treatment. All the values were expressed as means ± SEM. The data was analyzed using one way analysis of variance (ANOVA) and two way ANOVA followed by Tukey's test. In all the tests, criterion for statistical significance was $P<0.05$.

3. Results

3.1. Effect of pioglitazone and BADGE on body weight, locomotor activity and rotarod performance in QUIN treated animals

There were no significant changes in the body weight, locomotor activity and fall off time (rotarod performance) of sham as compared to

Table 1
Effect of pioglitazone and BADGE against QUIN induced reduction in body weight, mitochondrial complex enzymes dysfunction and TNF-α level in striatum.

Treatment (mg/kg)	% Reduction in body weight	Complex-I *n* mole NADH oxidized/min/mg protein (% of sham)	Complex-II *n* mole/min/mg protein (% of sham)	MTT assay (% of sham)	Complex-IV *n* mole Cyto-c oxidized/min/mg protein (% of sham)	TNF-α (pg/ml) (% of sham)
Sham	1.83	100 ± 6.58 (84.61)	100 ± 4.69 (83.13)	100 ± 2.65 (0.69)	100 ± 5.23 (68.34)	100 ± 3.06 (102.00)
QUIN 300	−14.71[*]	67.31 ± 2.94[*]	47.17 ± 5.6[*]	67.20 ± 2.79[*]	52.50 ± 4.42[*]	242.55 ± 2.57[*]
PGZ 10 + QUIN 300	−11.37[b]	77.16 ± 4.67[b]	58.32 ± 4.3[b]	76.94 ± 3.07[b]	66.73 ± 5.01[b]	198.06 ± 2.80[b]
PGZ 20 + QUIN 300	−6.82[b,c]	90.74 ± 5.71[b,c]	78.18 ± 4.3[b,c]	85.90 ± 2.16[b,c]	83.00 ± 4.59[b,c]	161.07 ± 4.23[b,c]
PGZ 40 + QUIN 300	−1.40[b,c,d]	102.40 ± 4.79[b,c,d]	100.52 ± 6.44[b,c,d]	102.76 ± 2.57[b,c,d]	99.79 ± 4.72[b,c,d]	109.41 ± 4.28[b,c,d]
BADGE 15 + QUIN 300	−2.20[NS]	65.32 ± 6.1[NS]	52.56 ± 5.0[NS]	66.32 ± 3.9[NS]	58 ± 3.12[NS]	246.32 ± 3.12[NS]
BADGE 15 + PGZ 40 + QUIN 300	−4.32[b,e,f]	76 ± 7.3[b,e,f]	75.56 ± 5.8[b,e,f]	82 ± 3.03[b,e,f]	84 ± 4.3[b,e,f]	154 ± 4.3[b,e,f]

Values are expressed mean + SEM (% age of sham).
[*] $P<0.05$ as compared to sham.
[b] $P<0.05$ as compared to QUIN 300.
[c] $P<0.05$ as compared to PGZ 10.
[d] $P<0.05$ as compared to PGZ 20.
[e] $P<0.05$ as compared to PGZ 40.
[f] $P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

naïve group. Intrastriatal QUIN treatment significantly reduced body weight, locomotor activity (on day 14th and day 21st) and fall off time (rotarod performance) as compared to sham group. Pioglitazone (10, 20 and 40 mg/kg) treatment significantly attenuated the fall in body weight (Table 1) and improved the locomotor activity and fall off time (rotarod performance) as compared to QUIN treated animals (Fig. 1A and B). BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly reversed the protective effect of pioglitazone (40 mg/kg) in QUIN treated animals [the variances were found significantly different in two way ANOVA, $P<0.001$ (DFn = 5; DFd = 126; $F=4.70$), (DFn = 8; DFd = 126; $F=17.30$)] (Fig. 1A and B). Pioglitazone (40 mg/kg) *per se* treatment did not produce any significant effect on body weight, locomotor activity and fall off time (rotarod performance) as compared to sham treated group (data not shown).

3.2. Effect of pioglitazone and BADGE on balance beam walk performance in QUIN treated animals

Sham treated animals did not show any significant effect on number of slips and distance traveled on balance beam walk performance test as compared to naive group. While intrastriatal QUIN treatment significantly increased number of slips as well as decreased the distance traveled on balance beam walk performance as compared to sham group (as indicated by neurological scoring). Pioglitazone (10, 20 and 40 mg/kg) treatment significantly decreased the number of slips as well as increased the distance traveled (Figs. 2, 3) as compared to QUIN treated group. While BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly reversed the protective effect of pioglitazone (40 mg/kg) (balance beam walk performance) in QUIN treated animals (the variances were found significantly different in one way ANOVA, $P<0.001$; DFn = 6; DFd = 28; $F=97.99$) (Figs. 2, 3). However, pioglitazone (40 mg/kg) *per se* treatment (data not shown) did not show any significant effect on balance beam walk performance as compared to sham treated group.

3.3. Effect of pioglitazone and BADGE on lipid peroxidation (MDA), nitrite concentration, catalase and SOD enzymes level in QUIN treated animals

Sham treated group did not show any significant effect on lipid peroxidation, nitrite concentration, catalase and SOD enzymes level as compared to naive treated group. However, intrastriatal QUIN (300 nmol) administration significantly raised MDA, nitrite concentration, as well as depleted catalase and SOD enzymes level in striatum as compared to the sham group. Pioglitazone (10, 20 and 40 mg/kg) significantly attenuated oxidative stress (decreased lipid peroxidation, nitrite concentration, and restored catalase and SOD enzymes level) as compared to QUIN treated animals (Table 2). BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly reversed the free radical scavenging effect of pioglitazone (40 mg/kg) in QUIN treated animals (LPO ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$), Nitrite ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$), SOD ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$), Catalase ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$)) (Table 2). However, pioglitazone (40 mg/kg) *per se* treatment did not show any significant effect on lipid peroxidation, nitrite concentration, catalase and SOD enzymes level as compared to sham treated group (data not shown).

3.4. Effect of pioglitazone and BADGE on glutathione redox status in QUIN treated animals

Intrastriatal, QUIN (300 nmol) significantly depleted reduced glutathione (GSH), redox ratio (GSH/GSSG) and increased oxidized glutathione levels in striatum as compared to sham group. While there was no significant effect on the total glutathione levels as compared to sham group. While, pioglitazone (20 and 40 mg/kg) significantly restored GSH, redox ratio and oxidized glutathione levels in the QUIN treated animals (Table 3). Pioglitazone (10 mg/kg) did not show any significant effect on reduced glutathione, oxidized glutathione and redox ratio in rodents as compared to QUIN treated animals. BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly reversed the antioxidant effect of pioglitazone (40 mg/kg) in QUIN treated animals (GSH ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$), GSSG ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$), Redox ratio ($P<0.001$; DFn = 6; DFd = 28; $F=97.99$)) (Table 3). Pioglitazone (40 mg/kg) *per se* treatment did not have any significant effect on redox ratio (data not shown).

3.5. Effect of pioglitazone and BADGE on mitochondrial enzymes complexes (I, II and IV) in QUIN treated animals

There were no significant alterations in mitochondrial enzyme complexes-I, II, IV and mitochondrial redox activity of sham treated group as compared to naïve group. Intrastriatal QUIN injection significantly impaired mitochondrial complex-I (NADH dehydrogenase), II (Succinate dehydrogenase), IV (cytochrome oxidase) enzyme activity as well as mitochondrial redox activity as compared to the sham group (Table 1). Pioglitazone (10, 20 and 40 mg/kg) treatment significantly restored the alterations in mitochondrial enzyme complex activities and mitochondrial redox activity as compared to QUIN treated groups. BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly depleted the mitochondrial enzyme complexes activity in QUIN treated animals. Pioglitazone (40 mg/kg) *per se* treatment did not show any significant effect on any of the

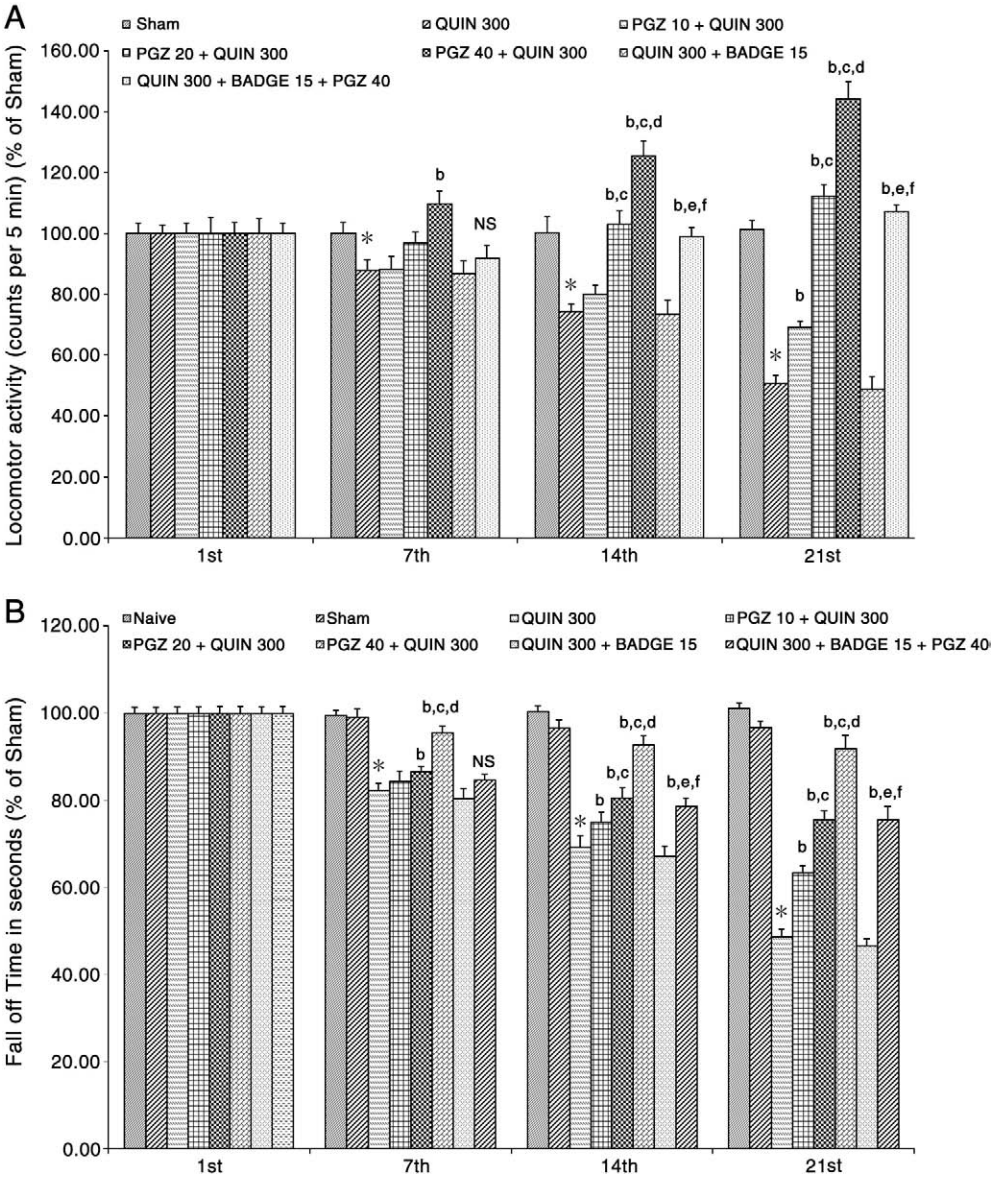

Fig. 1. Effect of pioglitazone and BADGE on locomotor and rotarod activity in QUIN treated animals. (A) Values are expressed mean + SEM (% age of sham). $^{*}P<0.05$ as compared to sham. $^{b}P<0.05$ as compared to QUIN 300. $^{c}P<0.05$ as compared to PGZ 10. $^{d}P<0.05$ as compared to PGZ 20. $^{e}P<0.05$ as compared to PGZ 40. $^{f}P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).(B) Values are expressed mean + SEM (% age of sham). $^{*}P<0.05$ as compared to sham. $^{b}P<0.05$ as compared to QUIN 300. $^{c}P<0.05$ as compared to PGZ 10. $^{d}P<0.05$ as compared to PGZ 20. $^{e}P<0.05$ as compared to PGZ 40. $^{f}P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

mitochondrial enzyme complex activities and mitochondrial redox activity as compared to sham treated group (data not shown) NADH dehydrogenase ($P<0.001$; DFn = 6; DFd = 28; $F = 97.99$), succinate dehydrogenase ($P<0.001$; DFn = 6; DFd = 28; $F = 97.99$), MTT ($P<0.001$; DFn = 6; DFd = 28; $F = 97.99$) and Cytochrome c oxidase ($P<0.001$; DFn = 6; DFd = 28; $F = 97.99$).

3.6. Effect of pioglitazone and BADGE on tumor necrosis factor-alpha (TNF-α) in QUIN treated animals

Intrastriatal QUIN treatment significantly elevated TNF-α level as compared to sham treated group (Table 1). Pioglitazone (10, 20 and 40 mg/kg, *p.o.*) treatment significantly ($P<0.05$) attenuated TNF-α levels in striatum of QUIN treated animals (Table 1). BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly increased TNF-α levels in QUIN treated animals (the variances were found significantly different in one way ANOVA, $P<0.001$; DFn = 6; DFd = 28; $F = 97.99$) (Table 1). However, pioglitazone (40 mg/kg) *per se* treatment did not show any significant effect on TNF-α level as compared to sham treated group (data not shown).

3.7. Effect of pioglitazone and BADGE on rat striatal degeneration against in QUIN treated animals

Intrastriatal QUIN injection significantly increased striatal lesion volume as compared to the sham group. Pioglitazone (10, 20 and 40 mg/kg) treatment significantly attenuated striatal lesion volume as compared to QUIN treated animals (Fig. 4). BADGE (15 mg/kg) pretreatment with pioglitazone (40 mg/kg) significantly increased striatal lesion volume in QUIN treated animals (the variances were found significantly different in one way ANOVA, $P<0.001$; DFn = 6; DFd = 28; $F = 97.99$) (Fig. 4). Pioglitazone (40 mg/kg) *per se* treatment did not produce any significant effect on striatal lesion volume as compared to sham treated group (data not shown).

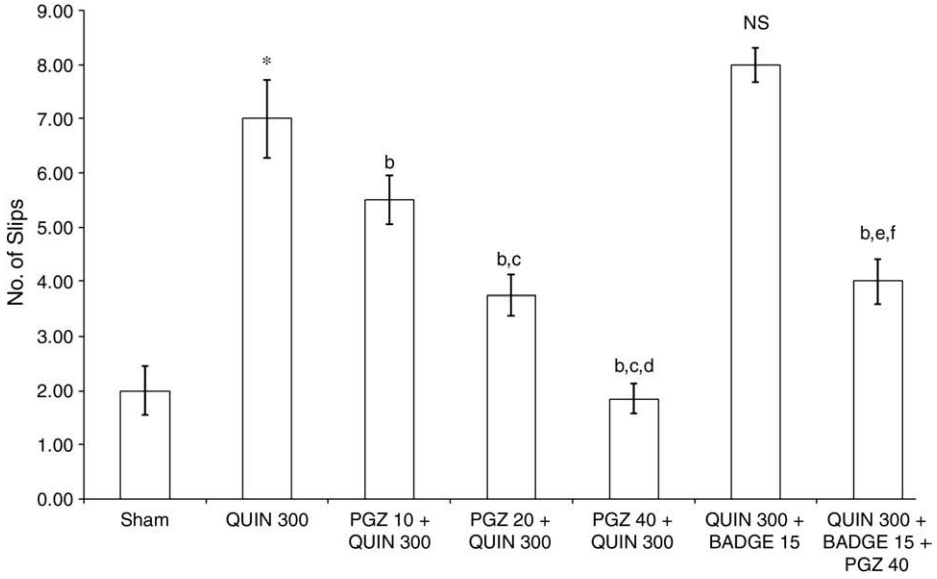

Fig. 2. Effect of pioglitazone and BADGE on no. of slips on balance beam walk performance in QUIN treated animals. Values are expressed mean + SEM (% age of sham). $^{*}P<0.05$ as compared to sham. $^{b}P<0.05$ as compared to QUIN 300. $^{c}P<0.05$ as compared to PGZ 10. $^{d}P<0.05$ as compared to PGZ 20. $^{e}P<0.05$ as compared to PGZ 40. $^{f}P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

4. Discussion

Present study highlights the protective effect of pioglitazone (a well known PPARϒ agonist) against QUIN induced neurotoxicity for the first time. Pioglitazone treatment significantly improved behavioral, biochemical, mitochondrial alterations in QUIN treated animals, while BADGE (PPARϒ antagonist) treatment with the pioglitazone significantly reversed the protective effect of the pioglitazone confirming the involvement of PPARϒ in the pathogenesis of HD like symptoms.

Evidence suggests that excitotoxicity and mitochondrial dysfunction are likely to play a central role in HD pathogenesis (Panov et al., 2002; Milakovic et al., 2006; Lim et al., 2008). Excitotoxicity has been implicated in the pathogenesis of several neurological disorders with direct neuronal damage linked to activation of glutamate receptors (Mattson, 2003). QUIN has been reported to cause N-methyl-D-aspartate receptor activation by increasing Ca^{2+} overload (Lee et al., 2002a,b), which consequently produces oxidative damage and mitochondrial dysfunction (Kalonia et al., 2009). The complex pattern of QUIN induced neurotoxicity involves increase in cytosolic Ca^{2+} concentration (Schanne et al., 1979), ATP exhaustion, GABA depletion, oxidative stress, selective GABAergic, dopaminergic and cholinergic neuronal death (Santamaría and Ríos, 1993). Reduction of Ca^{2+} influx or intracellular Ca^{2+} chelation has been proposed to prevent this process, suggesting that NMDA receptor-mediated cell death might be triggered by neuronal Ca^{2+} overloading (Tymianski et al., 1993). Further, mitochondria have been shown to take up large amounts of Ca^{2+} during excitotoxicity (Budd and Nicholls, 1996; Peng and Greenamyre, 1998). Mitochondrial Ca^{2+} overloading may trigger neuronal apoptosis via release of pro-apoptotic factors from mitochondrial intermembrane space into cytosol (Andreyev et al., 1998). Finally, Ca^{2+} induced mitochondrial dysfunction can lead to increased production of reactive oxygen species (ROS) (Dykens, 1994) and activate other processes like Ca^{2+} dependent phospholipases A2. Several studies indicate that QUIN induce oxidative stress and support

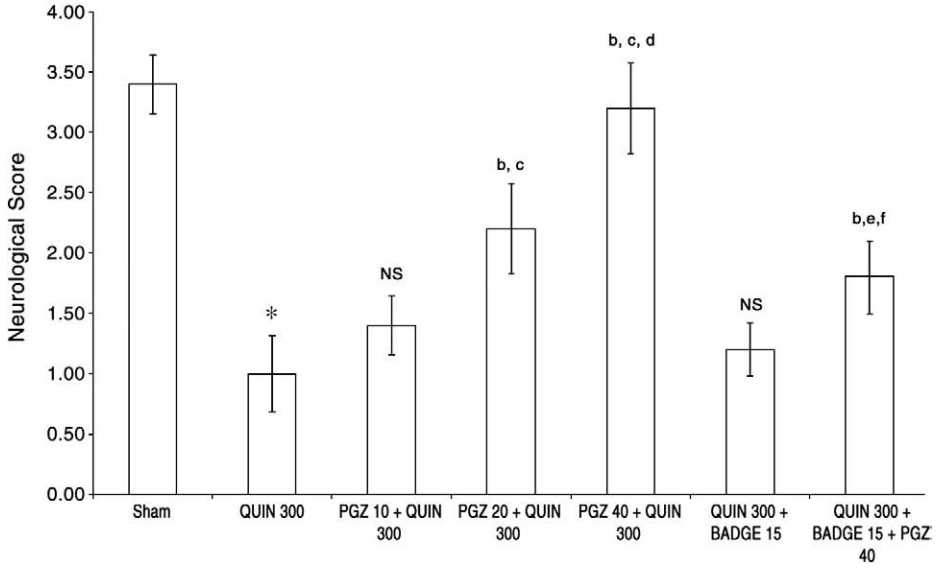

Fig. 3. Effect of pioglitazone and BADGE on neurological score in balance beam walk performance in QUIN treated animals. Values are expressed mean + SEM (% age of sham). $^{*}P<0.05$ as compared to sham. $^{b}P<0.05$ as compared to QUIN 300. $^{c}P<0.05$ as compared to PGZ 10. $^{d}P<0.05$ as compared to PGZ 20. $^{e}P<0.05$ as compared to PGZ 40. $^{f}P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

Table 2
Effect of pioglitazone and BADGE on the MDA, nitrite, catalase and SOD levels in striatum of QUIN treated animals.

Treatment (mg/kg)	MDA levels (μmol of MDA/mg protein) (% of sham)	Nitrite levels (μmol of nitrite/mg protein) (% of sham)	Catalase levels (μmol of catalase/mg protein) (% of sham)	SOD levels (μmol of SOD/mg protein) (% of sham)
Sham	100.00 ± 2.67 (6.80)	100.00 ± 1.99 (125.50)	100.00 ± 3.45 (9.05)	100.00 ± 5.26 (2.26)
QUIN 300	175.79 ± 3.70[*]	192.83 ± 4.13[*]	43.98 ± 4.53[*]	40.35 ± 4.35[*]
PGZ 10 + QUIN 300	153.21 ± 3.63[b]	167.73 ± 5.46[b]	59.46 ± 5.67[b]	57.89 ± 3.03[b]
PGZ 20 + QUIN 300	128.60 ± 3.27[b,c]	143.43 ± 3.33[b,c]	76.18 ± 4.21[b,c]	77.19 ± 4.55[b,c]
PGZ 40 + QUIN 300	106.72 ± 2.67[b,c,d]	108.37 ± 3.60[b,cd]	96.17 ± 4.38[b,c,d]	98.25 ± 3.57[b,c,d]
BADGE 15 + QUIN 300	181.79 ± 4.80[NS]	198.83 ± 4.03[NS]	39.98 ± 4.13[NS]	37.35 ± 3.35[NS]
BADGE 15 + PGZ 40 + QUIN 300	145.69 ± 3.70[b,e,f]	152.83 ± 4.13[b,e,f]	63.98 ± 4.53[b,e,f]	68.35 ± 4.35[b,e,f]

Values are expressed mean + SEM (% age of sham).
[*] $P<0.05$ as compared to sham.
[b] $P<0.05$ as compared to QUIN 300.
[c] $P<0.05$ as compared to PGZ 10.
[d] $P<0.05$ as compared to PGZ 20.
[e] $P<0.05$ as compared to PGZ 40.
[f] $P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

the possible relationship between excitotoxicity and oxidative process (Rossato et al., 2002; Ganzella et al., 2006).

In the present study, QUIN treatment caused significant alterations in motor function as indicated by altered locomotor activity, rotarod performance and balance beam walk test. These motor alterations can be explained on the basis of dopaminergic neuronal death, possibly due to excitotoxicity and mitochondrial dysfunction. As both, excitotoxicity and mitochondrial dysfunction leads to the generation of free radicals and selective and high vulnerability of dopaminergic neurons for free radical mediated death leads to the behavioral and motor abnormality in the QUIN treated animals (Sanberg et al., 1989). Further, this increased generation of the free radical leads to the oxidative burden (Kalonia et al., 2009). The present study also demonstrated the increased lipid peroxidation, nitrite concentration and diminished levels of endogenous antioxidants such as SOD, catalase. Beside, intrastriatal QUIN significantly increased TNF-α level and striatal lesion volume (distinct marker for the selective striatal neurodegeneration) suggesting QUIN mediated oxidative and neuroinflammatory damage. Interestingly pioglitazone treatment significantly reversed the behavioral, biochemical alterations in the QUIN treated animals, suggesting its therapeutic potential against QUIN induced neurotoxicity.

As PPARϒ plays a critical role in energy metabolism due to its direct effects on mitochondrial function and ultimately ATP production. Mitochondria can be a key player in the neuronal degeneration as observed in HD, as this organelle plays a critical role in both energy metabolism as well as neuronal apoptosis. In the diseased brain, numbers of neuronal functional mitochondria reduced significantly and remaining have very distinct morphological changes in their size and number of cristae they contain. Fuenzalida et al. 2007 have postulated that PPARϒ agonists improve mitochondrial functions and this explain possible basis of its beneficial effects on memory dysfunction in AD patients. PPARϒ activation by pioglitazone results in significant increase in mitochondrial DNA copy number as well as expression of genes involved in mitochondrial biogenesis in fat tissue (Laplante et al., 2006). According to earlier findings PPARϒ activation is reported to stimulate brain mitochondrial biogenesis and this stimulation is dependent on the ApoE isoforms. PPARϒ may elicit these changes through the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1) family of proteins. These coactivators positively regulate mitochondrial function and metabolism. (Li et al., 2005) Further, present study demonstrates that pioglitazone improved mitochondrial enzyme complexes and mitochondrial redox activity in the QUIN treated animals. Thus PPARϒ likely to play a key role in regulating and restoring mitochondrial function, and activation of this pathway could result in protection against different stressors such as mitochondrial and excitotoxins (Fuenzalida et al. 2007; Yu et al., 2008; Zhao et al., 2006). It is tantalizing to speculate that PPARϒ-induced increase in mitochondrial biogenesis in combination with the up-regulation of key mitochondrial and anti-apoptotic proteins (*e.g.* Bcl-2), increases the defense mechanisms against oxidative stress, and mitochondrial damage (Fuenzalida et al. 2007).

Besides, studies have demonstrated the involvement of mitochondrial dysfunction in various neurodegenerative processes (Minghetti et al., 2007). Several studies have suggested a cross-talk between excess NMDA receptor function (associated with excitotoxicity) and

Table 3
Effect of pioglitazone and BADGE on the total glutathione, reduced glutathione, oxidized glutathione and redox ratio in striatum of QUIN treated animals.

Treatment (mg/kg)	Total glutathione (μmol of GSH/mg protein) (% of sham)	Reduced glutathione (μmol of GSH/mg protein) (% of sham)	Oxidized glutathione (μmol of GSH/mg protein) (% of sham)	Redox ratio (GSH/GSSG) (% of sham)
Sham	100.00 ± 2.71 (135.62)	100.00 ± 2.64 (49.00)	100.00 ± 5.81 (85.83)	100.00 ± 843 (0.58)
QUIN 300	102.32 ± 2.40[NS]	44.46 ± 3.56[*]	135.88 ± 3.53[*]	32.64 ± 7.07[*]
PGZ 10 + QUIN 300	99.23 ± 2.08[NS]	59.58 ± 3.83[b]	122.22 ± 3.75[b]	48.65 ± 7.58[b]
PGZ 20 + QUIN 300	100.58 ± 2.12[NS]	72.23 ± 3.16[b,c]	117.02 ± 4.01[b,c]	61.60 ± 7.16[b,c]
PGZ 40 + QUIN 300	97.29 ± 3.32[NS]	95.78 ± 3.23[b,c,d]	98.17 ± 7.17[b,c,d]	97.84 ± 10.62[b,c,d]
BADGE 15 + QUIN 300	100.32 ± 4.10[NS]	40.67 ± 5.86[NS]	139.88 ± 3.73[NS]	28.14 ± 4.07[NS]
BADGE 15 + PGZ 40 + QUIN 300	101.12 ± 2.10[NS]	74.41 ± 3.96[b,e,f]	115.81 ± 3.13[b,e,f]	52.14 ± 6.07[b,e,f]

Values are expressed mean + SEM (% age of sham).
[*] $P<0.05$ as compared to sham.
[b] $P<0.05$ as compared to QUIN 300.
[c] $P<0.05$ as compared to PGZ 10.
[d] $P<0.05$ as compared to PGZ 20.
[e] $P<0.05$ as compared to PGZ 40.
[f] $P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

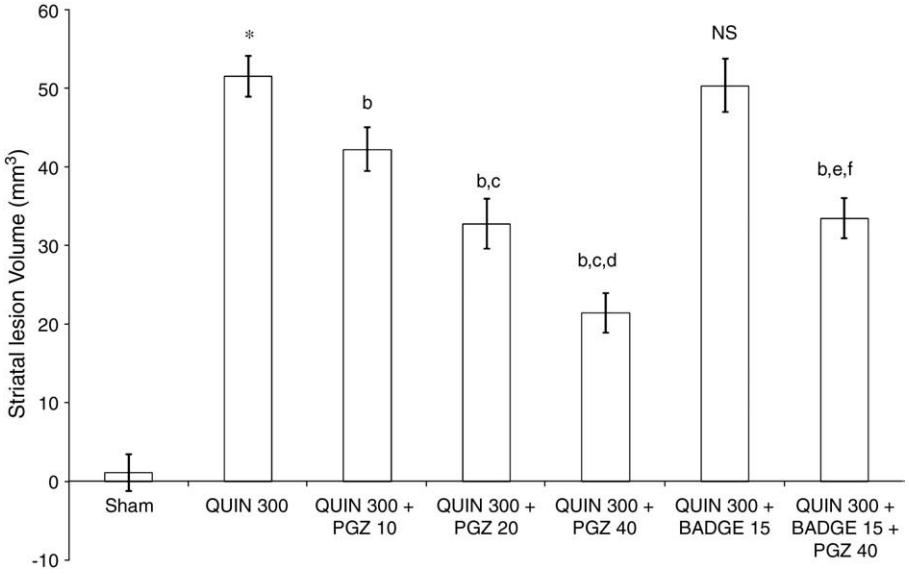

Fig. 4. Effect of pioglitazone and BADGE on rat striatal lesion volume in QUIN treated animals. Values are expressed mean + SEM (% age of sham). $^{*}P<0.05$ as compared to sham. $^{b}P<0.05$ as compared to QUIN 300. $^{c}P<0.05$ as compared to PGZ 10. $^{d}P<0.05$ as compared to PGZ 20. $^{e}P<0.05$ as compared to PGZ 40. $^{f}P<0.05$ as compared to BADGE 15 (ANOVA followed by Tukey test).

neuroinflammation (Lee et al., 2002a,b). Furthermore, NMDA receptor antagonists, memantine and MK-801 have also been reported to act against LPS-induced neuroinflammation in rats. Recent reports suggest a persistent activation of cytokines and microglia in the various neurodegenerative conditions, suggesting the role of neuroinflammatory mediators (Leblhuber et al., 1998; Wang et al., 2003; Minghetti et al., 2007). In the present study, increased level of TNF-α has been observed in QUIN treated rats in the striatum, which are in line with earlier reports (Leblhuber et al., 1998; Wang et al., 2003; Tzeng et al., 2005) demonstrating involvement of mitochondrial dysfunction induced Ca^{2+} rise in neuroinflammatory conditions. Treatment with pioglitazone significantly reduced TNF-α level in QUIN treated animals, suggesting its therapeutic potential, which can be attributed to restoration of mitochondrial function. Further in the present study BADGE (PPARϒ antagonist) treatment in combination with the pioglitazone significantly increased the TNF-α level in the QUIN treated animals, suggesting the involvement of PPARϒ in expression of inflammatory mediators in the QUIN in neurotoxicity and furthers the therapeutic potential of PPARϒ agonist for the neurodegenerative conditions. The inhibitory effects of PPARϒ activation on TNF-α action discussed above highlighted the anti-inflammatory properties of PPARϒ agonists. Monocytes and macrophages play an important part in the inflammatory process through the release of inflammatory cytokines such as TNF-α and IL-6 and the production of nitric oxide (NO) by inducible nitric oxide synthase (iNOS). Expression of PPARϒ was robustly upregulated upon the differentiation of monocytes into macrophages (Tzeng et al., 2005). Further these findings provide another indication for the linkage of mitochondrial dysfunction and neuroinflammation in the excitotoxic neuronal degenerations.

It has been reported that QUIN induces continuous neuronal depolarization that leads to a depletion of neuronal energy stores and disturbs neuronal ionic gradients of sodium and chloride (Schanne et al., 1979; Rothman, 1985). Consistent with the reports, an early morphologic finding accompanying excitotoxic damage is dendritic swelling (Schwarcz et al., 1984). Several studies report an increased striatal lesion volume (Maragos et al., 1998; Kim et al., 2005). In addition QUIN induced mitochondrial dysfunction leads to the formation of permeability transition pore that release cytochrome c into cytoplasm, accelerates apoptotic machinery. In the present study, QUIN administration significantly caused striatal lesion as evidenced by TTC staining in animals as compared to sham group (Maragos et al., 1998; Kim et al., 2005) and pioglitazone treatment significantly attenuated the striatal lesion in QUIN treated animals. Additionally the BADGE (PPARϒ antagonist) treatment in combination with the pioglitazone significantly reversed the protective effect of the pioglitazone, provide another evidences for involvement of PPARϒ in the neurodegeneration.

Taken together, the present study highlight the neuroprotective effect of pioglitazone against QUIN induced HD like symptoms in rats. Present study further highlight the possible involvement of the PPARϒ against QUIN induced HD like symptoms. However, further studies are required to confirm the exact mechanism of its involvement in HD pathogenesis.

Acknowledgements

The authors gratefully acknowledged the financial support of the University Grant Commission (U.G.C.), New Delhi for carrying out this work. The Research Fellowship in Science for Meritorious Student (Harikesh Kalonia) of the University Grant Commission (U.G.C.), New Delhi, is gratefully acknowledged.

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