ORIGINAL ARTICLE

# Rosiglitazone Synergizes the Neuroprotective Effects of Valproic Acid Against Quinolinic Acid-Induced Neurotoxicity in Rats: Targeting PPARγ and HDAC Pathways

Jitendriya Mishra · Tanya Chaudhary · Anil Kumar

**Abstract** Huntington's disease (HD) is an autosomal dominant progressive neurodegenerative disorder which affects medium spiny GABAergic neurons mainly in the striatum. Oxidative damage, neuro-inflammation, apoptosis, protein aggregation, and signaling of neurotrophic factors are some of the common cellular pathways involved in HD. Quinolinic acid (QA) causes excitotoxicity by stimulating $N$-methyl-D-aspartate receptors via calcium overload leading to neurodegeneration. Neuroprotective potential of peroxisome proliferator activated receptor-γ (PPARγ) agonists and histone deacetylase (HDAC) inhibitors have been well documented in experimental models of neurodegenerative disorders; however, their exact mechanisms are not clear. Therefore, present study has been designed to explore possible neuroprotective mechanism of valproic acid (VPA) and its interaction with rosiglitazone against QA induced HD-like symptoms in rats. Single bilateral intrastriatal QA (200 nmol/2 µl saline) administration significantly caused motor incoordination, memory impairment, oxidative damage, mitochondrial dysfunction (complex I, II, II and IV), cellular alterations [tumor necrosis factor-alpha (TNF-α), caspase-3, brain derived neurotrophic factor, acetylcholinesterase], and striatal neurodegeneration as compared to sham group. Treatment with rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) for 21 days significantly attenuated these behavioral, biochemical, and cellular alterations as compared to control (QA 200 nmol) group. However, VPA (100 mg/kg) treatment in combination with rosiglitazone (5 mg/kg) for 21 days synergized their neuroprotective effect, which was significant as compared to their effects per se in QA-treated animals. The present study provides an evidence of possible interplay of PPARγ agonists and HDAC inhibitors as a novel therapeutic strategy in the management of HD.

**Keywords** Acetylcholinesterase · BDNF · Caspase-3 · TNF-α · Mitochondrial dysfunction

## Introduction

Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disorder manifested by motor dysfunction, cognitive, and psychiatric complications in middle-aged people. HD is caused due to expansion of the cytosine–adenine–guanine (CAG) repeats in the gene coding for the N-terminal region of the huntingtin protein, leading to the formation of a polyglutamine stretch physiological HD alleles which comprise 6–35 CAGs, whereas above 39 CAGs, there is obligatory disease development; greater the expansion of CAGs, earlier is the disease manifestation (Gardian and Vecsei 2004; Zadori et al. 2009). Although HD usually involves chorea and other abnormal movements, the progressive cognitive impairment and behavioral problems are perhaps even more disabling and remains as a challenge.

Quinolinic acid (QA) is one of the reliable experimental models used to reproduce HD-like symptoms as observed clinically in HD patients (Schwarcz et al. 1983, 2010; Ferrante et al. 1993; Kalonia et al. 2012). The first step in QA-induced neuronal damage involves hyper-stimulation of $N$-methyl-D-aspartate (NMDA) receptors (Choi 1992) leading to a massive $Ca^{2+}$ influx and activation of $Ca^{2+}$ dependent phospholipases A2 activity, which later converts

J. Mishra · T. Chaudhary · A. Kumar (✉)
Pharmacology Division, UGC Centre of Advanced Study (UGC-CAS), University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh 160014, India
e-mail: kumaruips@yahoo.com

into prostaglandin G2 (PGG$_2$) by cyclo-oxygenases and subsequently reduces to PGH$_2$ with the production of free radical intermediates (Kukreja et al. 1986). In addition, QA-induced neurotoxicity involves an increased expression of the pro-inflammatory cytokines such as interleukins, tumor necrosis factors (Kalonia et al. 2012).

Increasing evidences suggest that an altered balance between histone acetyl transferases and histone deacetylase (HDAC) activity may accompany the development of chronic neurodegenerative disorders, including HD (Langley et al. 2005; Sadri-Vakili and Cha 2006). The HDACs remove acetyl groups which lead to reduction in transcription processes. Valproic acid (VPA), a HDAC inhibitor has been reported to exert its neuroprotective effects in several neurodegenerative conditions (Jeong et al. 2003; Mark et al. 1995; Hashimoto et al. 2002). Besides, different authors suggest different neuroprotective mechanisms of VPA, which include inhibition of glycogen synthase kinase-3β (GSK-3β), attenuation of glutamate-induced excitotoxicity, blockade of sodium and calcium channels, elevating GABA and reducing aspartate levels within the brain, prevention of mitochondrial dysfunction, reduction of apoptosis, and promoting neuronal survival (Valvassori et al. 2010; Phiel et al. 2001; Kwan and Brodie 2001). However, its exact mechanism of action is not clear yet.

Rosiglitazone, selective peroxisome proliferator activated receptor-γ (PPARγ) agonist, has been demonstrated to regulate lipid and carbohydrate homeostasis, and exert anti-inflammatory actions in nervous system (Heneka et al. 2007; Bernardo and Minghetti 2008). Moreover, PPARγ has been detected in both neuronal and glial cells (Cristiano et al. 2001), which regulates the expression of COX, iNOS, and inflammatory cytokines (Bernardo and Minghetti 2008; Cristiano et al. 2001). Above all, neuroprotective effects of various PPARγ agonists in animal models of neurodegenerative disorders are mainly attributed to their anti-oxidant and anti-inflammatory properties (Kiaei et al. 2005; Drew et al. 2006; Kalonia et al. 2010a). However, exact neuroprotective mechanism of PPARγ agonists in HD and related conditions are yet to be explored.

Till date the neuroprotective potential of PPARγ agonists (rosiglitazone) has been attributed to their anti-oxidant, anti-inflammatory (Lee et al. 2012; Kalonia et al. 2010a; Quintanilla et al. 2008; Park et al. 2007), and microglial modulation (McGeer and McGeer 2008) properties. HDAC inhibition also has been reported to ameliorate motor deficits in several movement disorder conditions including HD, and the role of VPA has been well documented in these conditions (Sadri-Vakili and Cha 2006; Langley et al. 2005; Ferrante et al. 2003). However, none of the hypotheses have adequately explained the exact role of HDAC and PPARγ in the amelioration of this multifactorial disease (HD). Hence, the present study has been undertaken to investigate the possible interplay between VPA and rosiglitazone against QA-induced neurobehavioral, biochemical, mitochondrial, and cellular alterations in rats.

## Materials and Methods

### Animals

Male Wistar rats (250–300 g), bred in Central Animal House (CAH) of Panjab University, Chandigarh were used in the study. The animals were kept under standard laboratory conditions, maintained on 12-h light/dark cycle, with food and water ad libitum. Experiment was carried out between 9.00 and 17.00 h. The experimental protocol was approved by the Institutional Animal Ethics Committee, Panjab University, Chandigarh (IAEC/170-175/UIPS-17) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

### Intrastriatal Administration of QA

Animals were anesthetized with intraperitoneal administration of thiopental sodium (45 mg/kg), and surface of the skull was exposed by making an incision on the scalp. Small holes (l–2 mm diameter) were made in the skull at anterior +1.7 mm; lateral ±2.7 mm; ventral −4.8 mm on both sides of bregma and dura as described by Paxinos and Watson (2007). QA (200 nmol/2 µl saline) (Sigma-Aldrich, St Louis, MO, USA) was administered bilaterally over a period of 2 min in the striatum by means of a 28-gauge stainless steel needle attached to a Hamilton syringe. The injection needle was left in place for another 2 min to prevent back diffusion of the injected drug solution.

### Drug and Treatment Schedule

The present study protocol (Fig. 1) includes fifteen treatment groups ($n = 12$) (Table 1). Rosiglitazone (Ranbaxy Laboratories, Gurgaon, India) suspended in 0.25 % w/v sodium carboxy methyl cellulose (CMC) and VPA (Sigma Chemicals, St. Louis, MO, USA) dissolved in normal saline were administered intraperitoneally (i.p.) as per body weight (not exceeding 5 ml/kg b.w.) to all the treatment groups daily in the morning 10: 00 h, for 21 days starting from the day after QA administration. Doses were selected on the basis of our preliminary laboratory results as well as published reports (Zadori et al. 2009; Shear et al. 1998; Jin et al. 2013; Carta et al. 2011; Escribano et al. 2010; Verma et al. 2011).
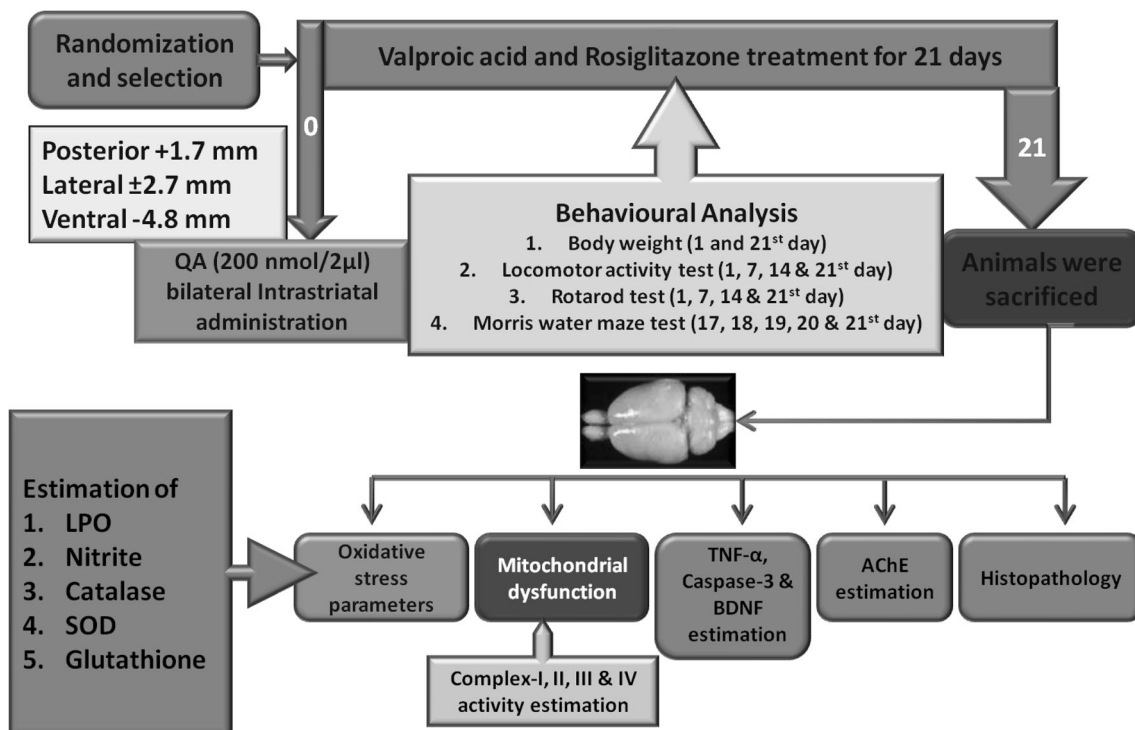
Fig. 1 Experimental protocol

Behavioral Assessments

*Body Weight*

The body weight of animals was recorded just before QA administration and on the last day of the study (21st day). The change in body weight was calculated according to the following formula:

$$\text{Change in body weight} = \text{Body weight on day 21} \\ - \text{body weight on day 1}$$

*Assessment of Total Locomotor Activity*

The locomotor activity was assessed using actophotometer (IMCORP, Ambala, India) on weekly intervals. Animals were placed individually in the activity chamber for 3 min as a habituation period before recording actual motor activity for next 5 min. The instrument consisted of a closed arena equipped with 12 infrared light-sensitive photocells in two rows (six in each row), at a distance of 3 and 9 cm, respectively, and values expressed as counts per 5 min (Kumar et al. 2012b).

*Rotarod Activity*

The rotarod activity task was performed in the animals individually on weekly intervals for the evaluation of motor

coordination and grip strength after QA administration. Animals were given a prior training session before rotarod performance task. Animals were placed on the rotating rod (speed 25 rpm) with a diameter of 7 cm (IMCORP, Ambala, India) individually for a fixed cut-off time (90 s), and three separate trials were performed at 5 min interval for each rat as described by Gaur and Kumar (2011).

*Morris Water Maze Task*

Morris water-maze (MWM) test is most commonly used to test memory (Morris 1984; Tuzcu and Baydas 2006; Kumar et al. 2012a, 2013) consisting of a large circular pool (150 cm in diameter, 45 cm in height, filled to a depth of 30 cm with water at $28 \pm 1$ °C). The tank was divided into four equal quadrants and a submerged platform ($10 \times 10$ cm$^2$), placed 1 cm below the surface of water in the middle of the target quadrant. The position of platform was kept unaltered throughout the training session. The tank was located in a large room where there were several brightly colored cues external to the maze, which were visible from the pool and could be used by the rats for spatial orientation and kept unaltered throughout the study. The water maze task was carried out for four consecutive days (seventeenth to twentieth day) where animals received four consecutive daily training trials, each at an interval of 30 min approximately. For each trial, individual rat was gently put into the water at one of four starting

**Table 1** Grouping of the experimental animals

| Sl. no. | Group | Treatment |
|---|---|---|
| 1 | Naive | Healthy animals (vehicle administered) |
| 2 | Sham | Surgery performed, vehicle administered |
| 3 | Control (QA) | Single intrastriatal bilateral QA (200 nmol/2 µl) administration |
| 4 | Rosi (5) per se | Rosiglitazone (5 mg/kg, i.p.) administered to sham animals |
| 5 | Rosi (10) per se | Rosiglitazone (10 mg/kg, i.p.) administered to sham animals |
| 6 | Rosi (5) | Rosiglitazone (5 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |
| 7 | Rosi (10) | Rosiglitazone (10 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |
| 8 | VPA (100) per se | Valproic acid (100 mg/kg, i.p.) administered to sham animals |
| 9 | VPA (200) per se | Valproic acid (200 mg/kg, i.p.) administered to sham animals |
| 10 | VPA (100) | Valproic acid (100 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |
| 11 | VPA (200) | Valproic acid (200 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |
| 12 | Rosi (5) + VPA (100) per se | Rosiglitazone (5 mg/kg, i.p.) + valproic acid (100 mg/kg, i.p.) administered to sham animals |
| 13 | Rosi (10) + VPA (200) per se | Rosiglitazone (10 mg/kg, i.p.) + valproic acid (200 mg/kg, i.p.) administered to sham animals |
| 14 | Rosi (5) + VPA (100) | Rosiglitazone (5 mg/kg, i.p.) + valproic acid (100 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |
| 15 | Rosi (10) + VPA (200) | Rosiglitazone (10 mg/kg, i.p.) + valproic acid (200 mg/kg, i.p.) administered to QA (200 nmol/2 µl) administered animals |

positions (the sequence of which being selected randomly) to locate the submerged platform (maintained at Q4) and allowed to stay on the platform for 20 s. If animal failed to find the platform within 90 s, same was gently guided onto the platform and allowed to remain there for 20 s. Escape latency time (ELT) to locate the hidden platform in water maze was noted as index of acquisition or learning using a computer tracking system with EthoVision software (Noldus Information Technology, Wageningen, The Netherlands).

### Memory Consolidation Test

A probe trial was performed (Tuzcu and Baydas 2006) 24 h after the last training session (on 21st day) to assess the extent of memory consolidation wherein the animal was placed into the pool for a total duration of 90 s as in the training trial, except that the hidden platform was removed from the pool. Various parameters such as time spent in target quadrant, frequency of appearance in target quadrant, and percentage of total path length traversed in target quadrant were measured using computer tracking system with EthoVision software (Noldus Information Technology, Wageningen, The Netherlands). All these parameters indicate the degree of memory consolidation that has taken place after learning.

### Biochemical Assessments

#### Dissection and Homogenization

Following behavioral assessments, the animals were divided into three groups; first group was used for the biochemical assays, second one used for hematoxylin and eosin staining, and the third one was used for the estimation striatal lesion volume. For biochemical analysis, animals were sacrificed by decapitation, and the brains were divided into two equal halves. One half was used for anti-oxidant enzyme activity estimation and the second one was used for estimation of mitochondrial complex analysis. Striatum, cortex, and hippocampus were dissected out on ice and weighed. For anti-oxidant enzyme activity estimation, a 10 % (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at $10,000 \times g$ at 4 °C for 15 min. Aliquots of supernatants were separated and used for biochemical estimations. Double beam UV–Vis Spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)] was used for the estimation of biochemical and mitochondrial parameters throughout the study.

#### Measurement of Lipid Peroxidation

The quantitative measurement of lipid peroxidation (LPO) was carried out according to the method as described by Wills (1966). The amount of malondialdehyde (MDA), a measure of LPO was measured by the reaction with thiobarbituric acid at 532 nm using UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. The values were calculated using molar extinction coefficient of chromophore ($1.56 \times 10^5$ M$^{-1}$/cm).

#### Estimation of Nitrite

Accumulation of nitrite, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent as described by Green et al. (1982). Equal volumes of supernatant and Greiss reagent

were mixed, and the mixture was incubated in dark for 10 min at room temperature. Absorbance was recorded at 540 nm with a UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve.

### Catalase Estimation

Catalase activity was assayed by the method of Luck, where in the breakdown of hydrogen peroxides ($H_2O_2$) is measured at 240 nm. In brief, 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 with a UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. The results were obtained as micromole $H_2O_2$ decomposed per milligram of protein/min (Luck 1965).

### Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was assayed according to the method of Kono, wherein the reduction of nitro blue tetrazolium (NBT) was inhibited by the SOD and measured at 560 nm using a UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. In brief, the reaction was initiated by the addition of the hydroxylamine hydrochloride to the assay mixture containing NBT and sample (Kono 1978). 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 %.

### Glutathione (GSH) Assay

Different glutathione contents in striatum were estimated according to the different methods. Total glutathione analysis was done by the method of Zahler and Cleland (1968) which is based on the reduction with dithioerythritol and determination of the resulting mono thiols with DTNB in the presence of arsenite. Reduced glutathione in the brain was estimated according to the method described by Ellman (1959). A 1.0 ml of homogenate was precipitated with 1.0 ml of 4 % sulfosalicylic acid by keeping the mixture at 4 °C for 1 h, and the samples were immediately centrifuged at 1,200×g for 15 min at 4 °C. The assay mixture contains 0.1 ml of supernatant, 2.7 ml of phosphate buffer of pH 8.0, and 0.2 ml of 0.01 M dithiobisnitro benzoic acid (DTNB). The yellow color developed was read immediately at 412 nm using a UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. Results were calculated using molar extinction coefficient of chromophore ($1.36 \times 10^4$ $M^{-1}$/cm) nano mole GSH per milligram protein. Oxidized glutathione (GSSG) was quantified by

subtracting the value of glutathione reduced from total glutathione. Redox ratio (reduced glutathione/oxidized glutathione) was determined for all the groups by taking the ratio of reduced glutathione/oxidized glutathione.

### Protein Estimation

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

### Estimation of Acetyl Cholinesterase (AChE) Activity

AChE activity (marker of cholinergic neurons in the brain) was assessed in the hippocampal region by the method of Ellman et al. (1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s interval at 412 nm using a UV–Vis spectrophotometer [UV-Pharmaspec 1700 Shimadzu (Japan)]. Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg of protein (percentage of sham).

### Mitochondrial Complex Enzyme Estimation

### Isolation of Rat Brain Mitochondria

Rat brain mitochondria were isolated by the method as described by Berman and Hastings. The striatal regions were homogenized in isolation buffer with EGTA (215 mM Mannitol, 75 mM sucrose, 0.1 % BSA, 20 mM HEPES, 1 mM EGTA, and pH 7.2). Homogenates were centrifuged at 13,000×g for 5 min at 4 °C. Pellets were resuspended in isolation buffer with EGTA and spun again at 13,000×g for 5 min. The resulting supernatants were transferred 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 (Berman and Hastings 1999).

### Complex I (NADH Dehydrogenase) Activity

NADH dehydrogenase activity was measured spectrophotometrically according to the method of King and Howard, which involves catalytic oxidation of NADH to $NAD^+$ with subsequent reduction of cytochrome C. In brief, the reaction mixture contained 0.2 M glycyl glycine buffer pH 8.5, 6 mM NADH in 2 mM glycyl glycine buffer, and 10.5 mM cytochrome C. The reaction was initiated by the addition of requisite amount of solubilized mitochondrial sample and the absorbance change at 550 nm was recorded

over the period of 2 min, and the results were expressed as percentage of sham (King and Howard 1967).

### Complex II (Succinate Dehydrogenase) Activity

Succinate dehydrogenase (SDH) activity was measured spectrophotometrically according to the previously reported method of King which involves oxidation of succinate by an artificial electron acceptor (potassium ferricyanide). The reaction mixture consists of 0.2 M phosphate buffer pH 7.8, 1 % BSA, 0.6 M succinic acid, and 0.03 M potassium ferricyanide and the reaction was initiated by the addition of mitochondrial sample (King 1967). The absorbance change was followed at 420 nm for 2 min, and the results were expressed as percentage of sham.

### Complex IV (Cytochrome Oxidase Activity)

Cytochrome oxidase activity was assayed in striatal mitochondria according to the method of Sottocasa et al. The assay mixture contained 0.3 mM reduced cytochrome C in 75 mM phosphate buffer, and the reaction was started by the addition of solubilized mitochondrial sample. The absorbance change was recorded at 550 nm over the period of 2 min, and the results were expressed as percentage of sham (Sottocasa et al. 1967).

### Mitochondrial Redox Activity (Complex III) Assay

The method (MTT assay) employed 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 as described by Liu et al. The absorbance of the resulting medium was measured by an ELISA reader (Biorad-Xmerk) at a wavelength of 580 nm, and the results were expressed as percentage of sham (Liu et al. 1997).

### Estimation of Pro-inflammatory Cytokines [Tumor Necrosis Factor-Alpha (TNF-α)]

The quantifications of TNF-α were performed by rat TNF-α immunoassay kit (R&D Systems, Minneapolis, MN, USA) in different brain regions (striatum, cortex and hippocampus) with the help of an ELISA reader (Biorad, X-merk). The assay employs the sandwich enzyme immunoassay technique. Final concentrations of TNF-α were calculated from plotted standard curves, and results were expressed as percentage of sham.

### Estimation of Caspase-3

The quantitative measurement of caspase-3 was carried out in different brain regions (striatum, cortex, and hippocampus) using caspase-3 colorimetric kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The cleavage of the peptide by the caspase releases the chromophore pNA, which was assessed spectrophotometrically at a wavelength of 405 nm.

### Estimation of Brain Derived Neurotrophic Factor (BDNF)

BDNF acts on certain neurons of the central nervous system and the peripheral nervous system, helping to support the survival of existing neurons and encourage the growth and differentiation of new neurons and synapses. With the *ChemiKine* BDNF assay system, mouse monoclonal antibodies generated against human BDNF are coated onto a microplate and are used to capture BDNF from a sample. BDNF-specific, biotin-conjugated, mouse monoclonal antibodies detect the captured BDNF. The BDNF assay was carried out for different brain regions (striatum, cortex, and hippocampus) using *ChemiKine*[TM] BDNF Sandwich ELISA Kit (Millipore, USA) according to the supplied manufacturer's instruction with the kit.

### Striatal Lesion Volume Measurement (TTC Staining)

On the 21st day, the animals were sacrificed for 2,3,5-triphenyltetrazolium chloride (TTC) staining. Brains were quickly removed, placed in ice-cold saline, and sectioned at 2 mm intervals using rat brain matrix and stained in 2 % TTC solution in the dark at 37 °C for 30 min. After 30 min, the sections were removed and placed in 4 % phosphate buffered formalin for photography. Complete lack of TTC staining was defined as lesioned area. The lesioned regions of the hemispheres were selected, and lesion volumes were measured using computer-based image analysis software (Image J 1.42q, NIH, USA) as described by Kim et al. (2005).

### Histopathological Analysis (Hematoxylin and Eosin Staining)

Animals were sacrificed by decapitation immediately after the last behavioral test. The brains were removed and transferred to formalin (10 %, v/v). The brain tissues were cut into 3 mm thickness, and its blocks were embedded in paraffin. The brain sections (5–10 μm) thick were prepared and stained with hematoxylin and eosin.

### Statistical Analysis

Graph Pad Prism (Graph Pad Software, San Diego, CA) was used for all statistical analysis. All the values were expressed as mean ± SEM. The data were analyzed using

**Fig. 2** Effect of valproic acid, rosiglitazone, and their combination on body weight in QA-treated rats. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid
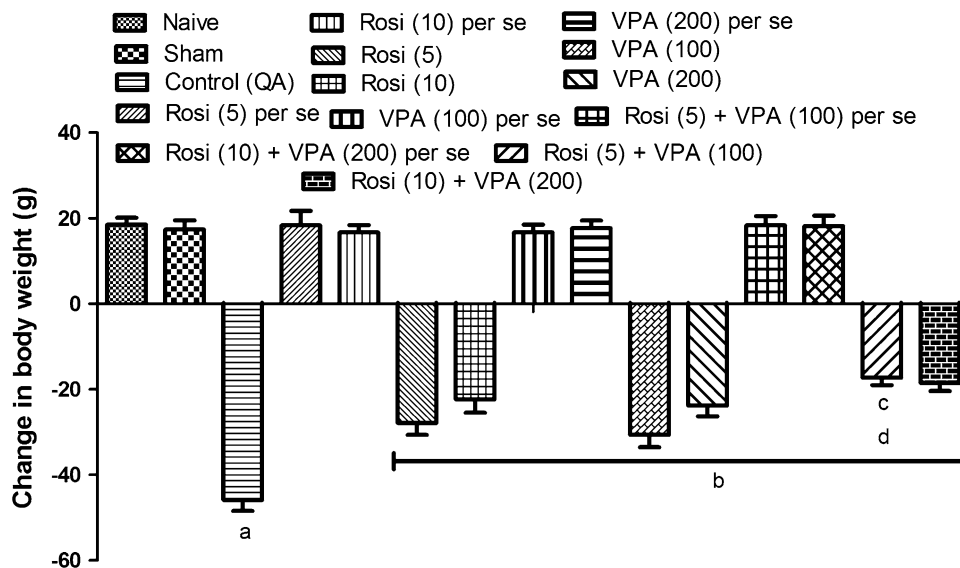


two-way analysis of variance (ANOVA) followed by Bonferroni's test. In all the tests, criterion for statistical significance was considered to be $P < 0.05$.

## Results

### Effect of VPA and Rosiglitazone on Body Weight

Single bilateral intrastriatal QA (200 nmol) administration significantly reduced body weight on day 21 as compared to sham group. Rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) treatment for 21 days significantly attenuated loss in body weight as compared to control (QA 200 nmol) group (Fig. 2). However, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) for 21 days further synergized their protective effect which was significant as compared to their effect per se in QA-treated animals ($F = 12.45$; $P < 0.0001$). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any significant improvement in body weight as compared to their effect per se in QA-treated animals which could be possibly due to the ceiling effect. Sham group did not show any significant difference on body weight as compared to naive group. Similarly, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.
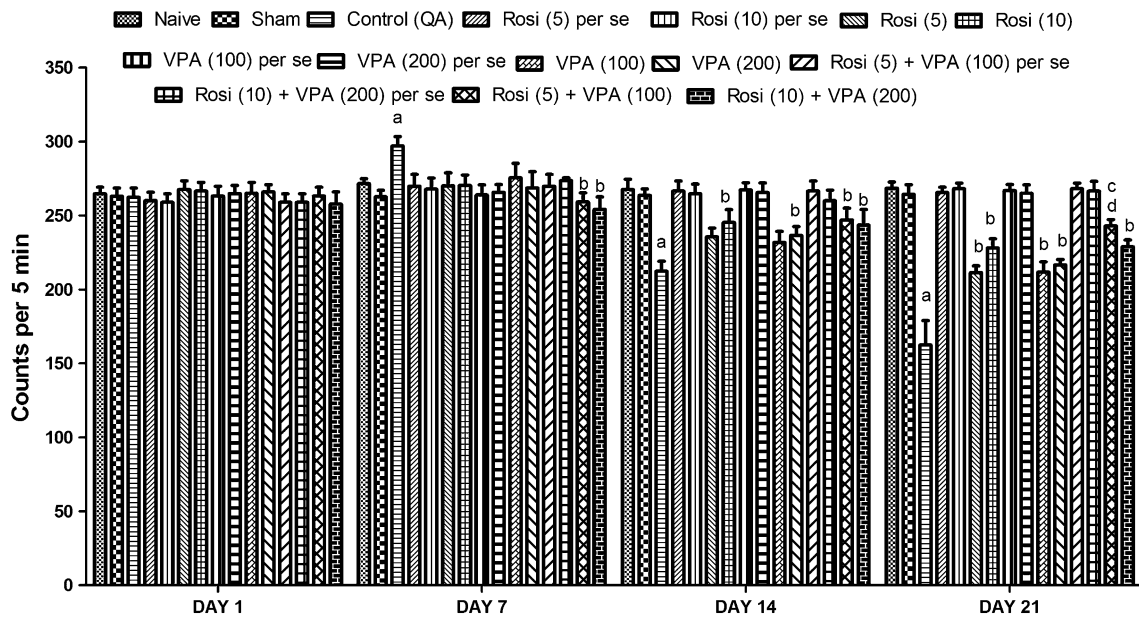
### Effect of VPA and Rosiglitazone on Locomotor Activity

Locomotor activity in all treatment groups was invariable prior to surgery. Single intrastriatal QA (200 nmol)

administration significantly increased locomotor activity on day 7; however, with disease progression, a substantial decrease in locomotor activity was observed on day 14 and 21 as compared to sham group. No significant difference in locomotor activity was observed between sham and naive group. Further, rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) treatment for 21 days showed significant improvement in locomotor activity at the end of first, second, and third week as compared to control (QA treated) group (Fig. 3). Moreover, VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) for 21 days further synergized their protective effect, which was significant only on day 21 as compared to their effects per se in QA-treated animals ($F = 6.86$; $P < 0.0001$). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked improvement in locomotor activity as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. However, per se treatments of VPA, rosiglitazone, and their combinations did not produce any significant effect on locomotor activity as compared to sham group.
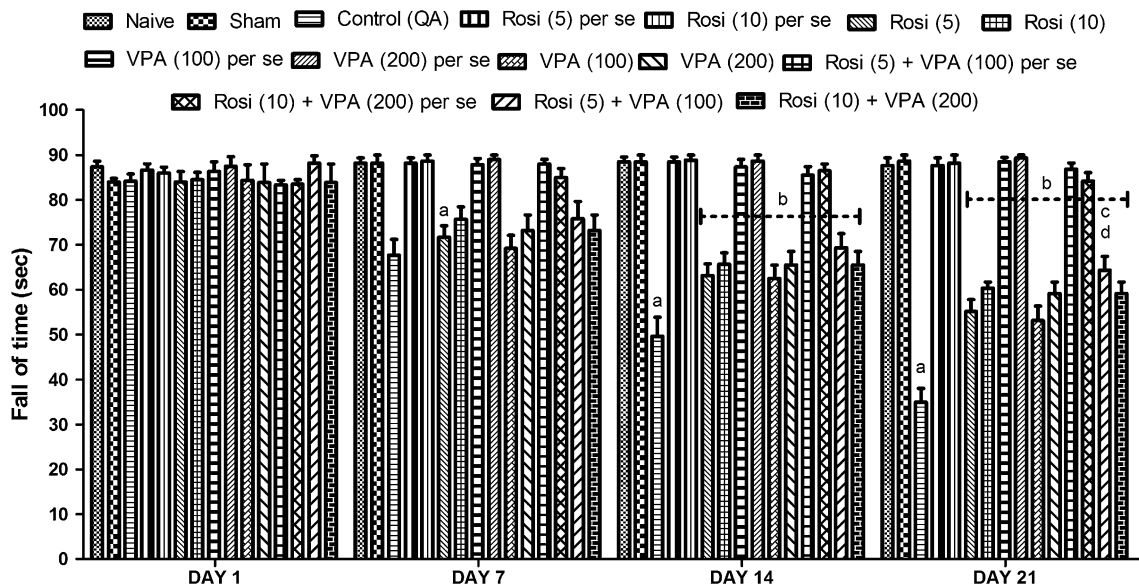
### Effect of VPA and Rosiglitazone on Rotarod Test in QA-Treated Rats

Single intrastriatal QA (200 nmol) administration significantly impaired rotarod performance and shortened fall off time of the animals as compared to sham group in rotarod test on day 7, 14, and 21, whereas sham group did not produce any significant effect as compared to naive animals. However, rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) treatment significantly attenuated fall off time on day 7, day 14, and day 21 (Fig. 4) demonstrating

**Fig. 3** Effect of valproic acid, rosiglitazone, and their combination on locomotor activity in QA-treated rats. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid
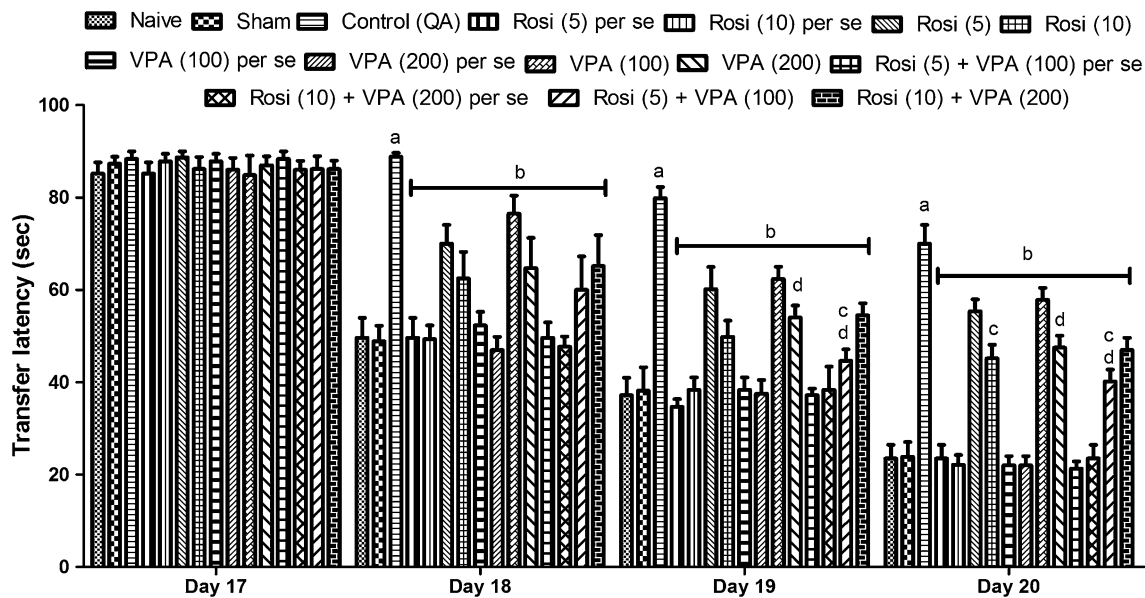


**Fig. 4** Effect of valproic acid, rosiglitazone, and their combination on rotarod test in QA-treated rats. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

improved grip strength in QA-treated animals. Moreover, combination treatment of VPA (100 mg/kg) with rosiglitazone (5 mg/kg) improved fall off time which was significant as compared to their effects per se in QA-treated animals ($F = 10.68$; $P < 0.0001$). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone

(10 mg/kg) for 21 days did not display any significant improvement in the grip strength performance as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. However, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

**Fig. 5** Effect of valproic acid, rosiglitazone, and their combination on escape latency of QA-treated rats in Morris water maze task. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid
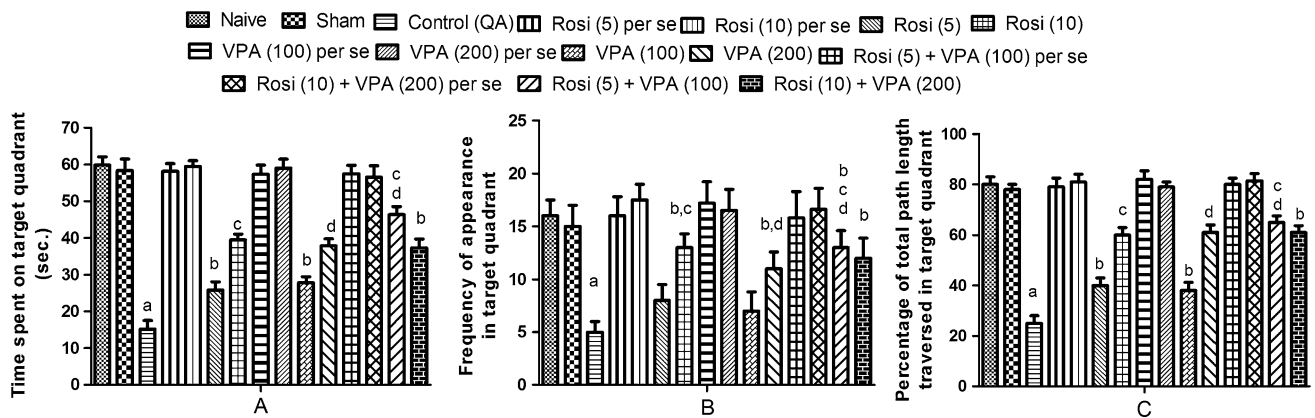
## Effect of VPA, Rosiglitazone, and Their Combination on Escape Latency of QA-Treated Rats in Morris Water Maze Task

The cognitive function was assessed in the Morris water maze test. The mean escape latency was found to be invariable between all the treatment groups on the first day of training (day 17) but from second day (day 18) onwards, significant difference in transfer latency was observed. Single intrastriatal QA (200 nmol) administration demonstrated a poor ability to find the platform and learn its location till the fourth day (twentieth day) of training (Fig. 5) which was significant as compared to the sham group. This poor performance was significantly attenuated by rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) treatment and demonstrated a decrease in latency to find the platform from second day of training (eighteenth day) onwards as compared to the control (QA 200 nmol) group. However, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) demonstrated recovery in memory performance (transfer latency) in this period (eighteenth to twentieth day), which was significant as compared to their effects per se in QA-treated animals ($F = 4.88$; $P < 0.0001$). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any synergistic effect as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. However, sham treatment did not offer any significant result as compared to

naive group animals. Similarly, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

## Effect of VPA, Rosiglitazone and Their Combination on Probe Trial/Memory Consolidation Test (Time Spent, Frequency of Appearance and Percentage of Total Path Length Traversed in Target Quadrant) in QA-Treated Rats

The probe trial reveals how well the animals were learned and consolidated the platform location during the training in Morris water maze task. All the animals showed a significant difference in the probe trial (time spent, frequency of appearance, and percentage of total path length traversed in target quadrant) on 21st day which was significantly decreased in the QA (200 nmol)-treated rats as compared to the sham group. However, treatment of VPA (200 mg/kg) and rosiglitazone (10 mg/kg) significantly improved these probe trial parameters (time spent, frequency of appearance, and percentage of total path length traversed in target quadrant) as compared to control (QA 200 nmol) group. Further, VPA (100 mg/kg) and rosiglitazone (5 mg/kg) treatment significantly improved time spent and percentage of total path length traversed in target quadrant in QA-treated animals. Meanwhile, treatment of VPA (100 mg/kg) and rosiglitazone (5 mg/kg) combination significantly augmented these parameters in QA administered animals which were significant as compared to their

**Fig. 6** Effect of valproic acid, rosiglitazone, and their combination on probe trial/memory consolidation test (time spent, frequency of appearance, and percentage of total path length traversed in target quadrant) in QA-treated rats. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

effects per se in QA-treated animals [time spent in target quadrant ($F = 41.7$); frequency in target quadrant ($F = 5.06$); percentage of path length traversed in target quadrant ($F = 41.78$); $P < 0.0001$]. Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked improvement in memory performance as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. However, sham treatment did not offer any significant effect on these parameters as compared to naive group animals (Fig. 6a–c). Further, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.
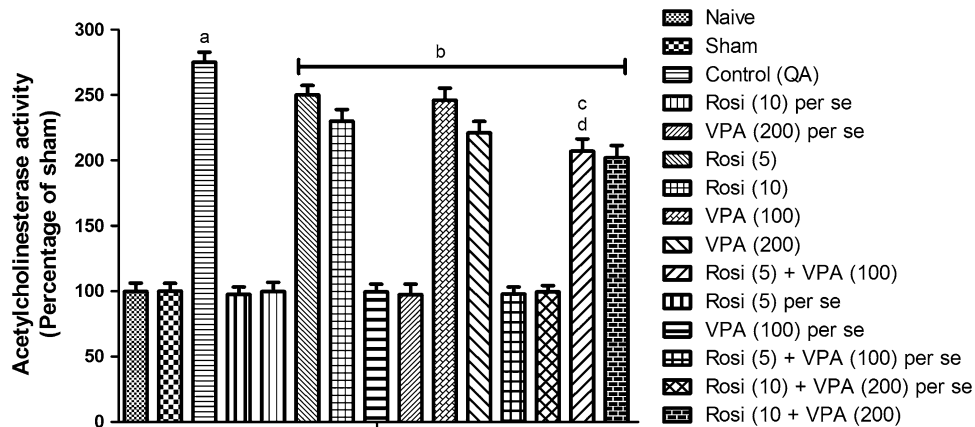
### Effect of VPA, Rosiglitazone and Their Combination on Hippocampal Acetylcholinesterase (AChE) Level in QA-Treated Rats

Sham group animals did not illustrate any significant effect on the hippocampal acetylcholinesterase enzyme (AChE) activity as compared to the naive animals, whereas single bilateral intrastriatal QA (200 nmol) administration significantly increased AChE levels as compared to the sham group. Unlike the lower dose of VPA (100 mg/kg) and rosiglitazone (5 mg/kg), 21 days pre-treatment with VPA (200 mg/kg) and rosiglitazone (10 mg/kg) significantly attenuated AChE activity as compared to control (QA 200 nmol) animals. However, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) for 21 days significantly attenuated AChE activity in the hippocampus which was significant as compared to control (QA 200 nmol) as well as their effects per se in QA-treated animals ($F = 87.28$; $P < 0.001$)(Fig. 7). Meanwhile,

treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked synergizm in the AChE activity as compared to their effect per se in QA-treated animals. Similarly, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

### Effect of VPA, Rosiglitazone and Their Combination on Brain Oxidative Damage (LPO, Nitrite, SOD, Catalase and Glutathione Levels) in QA-Treated Rats

Single intrastriatal QA administration significantly increased LPO and nitrite levels, and depleted endogenous anti-oxidant enzymes [SOD, catalase, and glutathione (GSH)] levels in the striatum as compared to the sham group. However, sham treatment did not show any significant effect as compared to naive group animals. Moreover, 21 days treatment with rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) significantly attenuated oxidative stress (decreased LPO, nitrite concentration and restored the levels of SOD, catalase, glutathione, and redox ratio) as compared to control (QA 200 nmol) group (Tables 2, 3). Meanwhile, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) for 21 days significantly synergized their protective effects which were significant as compared to their effects per se in QA-treated animals [LPO ($F = 13.21$); nitrite ($F = 21.21$); SOD ($F = 10.23$); GSH ($F = 16.36$); $P < 0.001$]. Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any improvement in the oxidative biomarkers as compared to their effect per se in QA-treated animals probably due to their ceiling effect. However, per se treatments of VPA, rosiglitazone, and

**Fig. 7** Effect of valproic acid, rosiglitazone, and their combination on hippocampal acetylcholinesterase (AChE) level in QA-treated rats. Data expressed as mean ± SEM [(percentage of sham (0.03 ± 0.007)]. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

their combinations were found to be non-significant as compared to sham group.

### Effect of VPA, Rosiglitazone, and Their Combination on Mitochondrial Enzyme Complex (I, II, III and IV) Activities in QA-Treated Animals

Single intrastriatal QA administration significantly impaired mitochondrial enzyme complex activities (I, II, and IV) as well as mitochondrial redox activity (complex III) in the striatum as compared to the sham group, whereas the sham treatment did not substantiate any significant effect on the mitochondrial enzyme complex activities (I, II, and IV) and mitochondrial redox activity as compared to naive animals. However, rosiglitazone (5, 10 mg/kg) and VPA (100, 200 mg/kg) treatment significantly restored mitochondrial enzyme complex activities (I and II) as well as mitochondrial redox activity as compared to control (QA 200 nmol) group. Unlike the lower dose of VPA (100 mg/kg) and rosiglitazone (5 mg/kg), treatment of VPA (200 mg/kg), and rosiglitazone (10 mg/kg) for 21 days significantly restored mitochondrial enzyme complex IV activity as compared to control (QA 200 nmol) group, whereas combination of VPA (100 mg/kg) with rosiglitazone (5 mg/kg) treatment for 21 days significantly restored these QA-induced mitochondrial alterations which were significant as compared to their effects per se in QA-treated animals [I ($F = 23.8$); II ($F = 25.5$); III ($F = 28.28$); IV ($F = 33.68$); $P < 0.001$)] (Fig. 8a–d). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any significant improvement in the mitochondrial complex enzyme activities as compared to their effect per se in QA-

treated animals which might be attributed to their ceiling effect. However, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

### Effect of VPA, Rosiglitazone, and Their Combination on TNF-α, Caspase-3, and BDNF Levels in QA-Treated Animals

Single intrastriatal QA administration (200 nmol/2 μl) resulted in significant increase in the levels of apoptotic marker (caspase-3) and pro-inflammatory cytokine (TNF-α) but conversely reduced BDNF levels in the striatum, cortex, and hippocampus as compared to sham group. However, sham treatment did not produce any significant effect on these parameters (TNF-α, caspase-3 and BDNF levels) as compared to naive group. Further, treatment of VPA (100, 200 mg/kg) and rosiglitazone (5, 10 mg/kg) for 21 days significantly attenuated the increase in levels of TNF-α (Fig. 9) and capase-3 (Fig. 10), and restored BDNF levels (Fig. 11) as compared to QA-treated group. Meanwhile, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) for 21 days synergized their protective effects which were significant as compared to their effects per se in QA-treated animals [TNF-α ($F = 10.87$); caspase-3 ($F = 14.59$); BDNF ($F = 12.98$); $P < 0.001$]. Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked improvement in their protective effect as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. However, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

**Table 2** Effect of valproic acid, rosiglitazone, and their combination on brain oxidative damage (lipid peroxidation, nitrite, superoxide dismutase, catalase levels) in QA-treated rats

| Treatment (mg/kg) | MDA levels nmol MDA/mg of protein (% of sham) | Nitrite levels µmol/mg of protein (% of sham) | Superoxide dismutase Units/mg of protein (% of sham) | Catalase µmol of $H_2O_2$ decomposed/min/mg protein (% of sham) |
|---|---|---|---|---|
| Naive | 3.87 ± 0.06 (98.00) | 222.50 ± 3.2 (98.02) | 40.43 ± 0.14 (106.4) | 3.59 ± 0.26 (101.7) |
| Sham | 3.95 ± 0.08 (100) | 227.00 ± 6.46 (100) | 38.00 ± 0.19 (100) | 3.53 ± 0.22 (100) |
| QA | 9.97 ± 0.58[a] (252.5) | 416.56 ± 3.0[a] (183.51) | 13.61 ± 1.34[a] (35.82) | 1.67 ± 0.21[a] (47.3) |
| Rosi (5) per se | 3.89 ± 0.11 (98.4) | 218.6 ± 4.8 (96.29) | 41.7 ± 0.93 (109.7) | 3.58 ± 0.18 (101.4) |
| Rosi (10) per se | 3.85 ± 0.09 (97.6) | 223.6 ± 4.1 (98.5) | 39.9 ± 0.71 (105) | 3.78 ± 0.2 (107) |
| Rosi (5) | 7.74 ± 0.31[b] (195.9) | 318.7 ± 4.5[b] (140.4) | 25.27 ± 0.46[b] (66.5) | 2.37 ± 0.24[b] (67.13) |
| Rosi (10) | 5.95 ± 0.4[b,c] (150.6) | 278.77 ± 7.63[b,c] (122.81) | 27.97 ± 0.55[b,c] (73.6) | 2.67 ± 0.21[b,c] (75.6) |
| VPA (100) per se | 3.91 ± 0.13 (95.94) | 222.07 ± 3.7 (97.8) | 39.46 ± 0.6 (103.8) | 3.51 ± 0.16 (99.43) |
| VPA (200) per se | 3.83 ± 0.17 (99.0) | 217.14 ± 3.1 (97.83) | 40.2 ± 0.51 (105.8) | 3.68 ± 0.2 (104.2) |
| VPA (100) | 8.21 ± 0.57[b] (208) | 355.23 ± 5.00[b] (156.49) | 22.76 ± 0.59[b] (59.92) | 2.31 ± 0.10[b] (65.43) |
| VPA (200) | 6.62 ± 0.45[b,d] (167.74) | 296.3 ± 8.22[b,d] (130.53) | 26.93 ± 0.72[b,d] (70.89) | 2.69 ± 0.13[b,d] (76.2) |
| Rosi (5) + VPA (100) per se | 3.79 ± 0.17 (95.94) | 227.23 ± 3.1 (100.01) | 41.2 ± 0.51 (108.4) | 3.52 ± 0.22 (99.71) |
| Rosi (10) + VPA (200) per se | 3.99 ± 0.11 (101) | 219.8 ± 4.4 (96.74) | 41.05 ± 0.91 (108) | 3.55 ± 0.17 (100.5) |
| Rosi (5) + VPA (100) | 5.24 ± 0.23[c,d] (132.68) | 258.6 ± 3.27[c,d] (113.92) | 34.17 ± 4.26[c,d] (89.93) | 2.89 ± 0.2[c,d] (81.9) |
| Rosi (10) + VPA (200) | 5.97 ± 0.95[b] (151.1) | 276.8 ± 6.78[b] (121.9) | 29.93 ± 1.1[b] (78.76) | 2.71 ± 0.15[b] (76.7) |

Data expressed as mean ± SEM (percentage of sham)

*QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

[a] $P < 0.05$ compared to sham group

[b] $P < 0.05$ compared to control (QA-treated group)

[c] $P < 0.05$ compared to Rosi (5)

[d] $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test)

### Effect of VPA, Rosiglitazone, and Their Combination on Striatal Lesion Volume in QA-Treated Animals

Intra-striatal QA (200 nmol) injection significantly increased striatal lesion volume as compared to the sham group, whereas treatment with of VPA (200 mg/kg) and rosiglitazone (10 mg/kg) for 21 days significantly reduced the QA-induced striatal lesion volume (Fig. 12). Furthermore, VPA (100 mg/kg) and rosiglitazone (5 mg/kg) treatment for 21 days could not produce any significant effect as compared to control (QA 200 nmol) group, whereas their combination treatment for 21 days, significantly attenuated striatal lesion size/volume which was found to be significant as compared to control (QA 200 nmol) as well as their effects per se in QA-treated animals ($F = 9.52$; $P < 0.0001$). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked improvement in the striatal lesion volume as compared to their effect per se in QA-treated animals which might be attributed to their ceiling effect. There was no marked difference observed in sham group as compared to naive animals. Similarly, per se treatments of VPA, rosiglitazone, and their combinations were found to be non-significant as compared to sham group.

### Effect of VPA, Rosiglitazone, and Their Combination on Histopathological Analysis in QA-Treated Animals

Histopathological study was carried out on striatal and hippocampal sections of sham and drug-treated rat brains. Sham-treated striatal sections showed more number of optimum sized, undamaged, neuronal cells, and cell layers (Fig. 13a). However, neuronal inflammation and increased

**Table 3** Effect of valproic acid, rosiglitazone, and their combination on brain oxidative damage (glutathione levels) in QA-treated rats

| Treatment (mg/kg) | Reduced glutathione nmol of GSH/mg Pr (% of sham) | Total glutathione nmol of GSH/mg Pr (% of sham) | Oxidized glutathione nmol of GSH/mg Pr (% of sham) | Redox ratio (% of sham) |
|---|---|---|---|---|
| Naive | 79.51 ± 2.91 (100.87) | 112.01 ± 0.44 (100.54) | 32.50 ± 2.63 (99.72) | 2.44 ± 0.17 (100.83) |
| Sham | 78.82 ± 3.49 (100) | 111.41 ± 0.28 (100) | 32.59 ± 3.34 (100) | 2.42 ± 0.33 (100) |
| QA | 38.55 ± 2.24[a] (48.9) | 123.83 ± 1.86 (111.15) | 85.28 ± 9.84[a] (273.94) | 0.45 ± 0.08[a] (18.59) |
| Rosi (5) per se | 79.18 ± 1.40 (100.4) | 110.70 ± 5.28 (99.36) | 31.52 ± 2.16 (96.71) | 2.51 ± 0.52 (103.7) |
| Rosi (10) per se | 78.48 ± 1.67 (99.56) | 112.80 ± 6.31 (101.25) | 32.32 ± 3.27 (99.17) | 2.42 ± 0.73 (100) |
| Rosi (5) | 48.28 ± 1.64[b] (61.25) | 112.73 ± 1.87 (101.19) | 44.45 ± 0.37[b] (136.39) | 1.08 ± 0.03[b] (44.88) |
| Rosi (10) | 59.79 ± 4.36[b,c] (75.85) | 109.99 ± 3.33 (98.72) | 50.2 ± 2.74[b,c] (154.03) | 1.19 ± 0.07[b,c] (49.21) |
| VPA (100) per se | 78.27 ± 2.03 (99.30) | 109.79 ± 9.31 (98.55) | 31.52 ± 2.99 (96.71) | 2.48 ± 0.18 (102.6) |
| VPA (200) per se | 78.73 ± 2.3 (99.88) | 112.10 ± 6.31 (100.6) | 33.37 ± 3.27 (102.4) | 2.35 ± 0.73 (97.49) |
| VPA (100) | 52.12 ± 2.8[b] (66.12) | 111.55 ± 1.41 (100.12) | 59.43 ± 0.88[b] (182.35) | 0.87 ± 0.03[b] (36.23) |
| VPA (200) | 64.25 ± 3.05[b,d] (81.51) | 114.09 ± 2.32 (102.4) | 49.84 ± 1.12[b,d] (152.93) | 1.28 ± 0.04[b,d] (53.26) |
| Rosi (5) + VPA (100) per se | 79.31 ± 2.08 (100.87) | 111.07 ± 0.34 (100.54) | 31.76 ± 2.63 (99.72) | 2.49 ± 0.17 (100.83) |
| Rosi (10) + VPA (200) per se | 79.12 ± 3.14 (100.3) | 112.01 ± 0.25 (100.53) | 32.59 ± 3.34 (100) | 2.42 ± 0.33 (100) |
| Rosi (5) + VPA (100) | 70.52 ± 3.38[c,d] (89.46) | 107.03 ± 2.01 (96.06) | 36.51 ± 1.95[c,d] (112.02) | 1.93 ± 0.02[c,d] (79.81) |
| Rosi (10) + VPA (200) | 68.81 ± 4.3[b] (87.3) | 111.58 ± 2.6 (100.15) | 42.77 ± 1.03[b] (131.2) | 1.60 ± 0.07[b] (66.11) |

Data expressed as mean ± SEM (percentage of sham)

*QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

[a] $P < 0.05$ compared to sham group

[b] $P < 0.05$ compared to control (QA-treated group)

[c] $P < 0.05$ compared to Rosi (5)

[d] $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test)

number of apoptotic cells are found in the striatal sections of QA-injected rats (Fig. 13b). However, VPA (100, 200 mg/kg) and rosiglitazone (5, 10 mg/kg) treatments attenuated these parameters as compared to QA-injected rats (Fig. 13c–f). Furthermore, treatment of VPA (100 mg/kg) in combination with rosiglitazone (5 mg/kg) showed further improvement in the histopathological characteristics (neuronal inflammation and increased number of apoptotic cells) as compared to their effects per se (Fig. 13g). Meanwhile, treatment of VPA (200 mg/kg) in combination with rosiglitazone (10 mg/kg) for 21 days did not display any marked modification in the histopathological characteristics as compared to their effect per se in QA-treated animals. However, the hippocampal sections did not show any visible alterations in the histopathological parameters (data not shown).

## Discussion

Single bilateral intrastriatal QA (200 nmol) administration produced HD-like symptoms in rats characterized by significant loss in body weight, motor incoordination, memory impairment, oxidative damage, mitochondrial (complex I, II, III, IV) dysfunction, alteration in the levels TNF-α,

caspase-3, BDNF, acetylcholinesterase as well as striatal degeneration and histopathological alterations (Kalonia et al. 2010a, 2012; Rossato et al. 2002; Pemberton et al. 1997; Sanberg et al. 1989). Moreover, treatment with VPA, rosiglitazone, and their combination significantly attenuated these behavioral, biochemical, and cellular alterations suggesting possible synergistic interaction between HDAC and PPARγ pathways in HD-like conditions.

The use of QA in animal models of HD has been well established in our laboratory and has been shown to mimic HD-like symptoms as hyperactivity (Sanberg et al. 1989; Kalonia et al. 2010b, 2012), learning deficits (Block et al. 1993; Curtis et al. 1992; Furtado and Mazurek 1996), and loss of neurons when injected into the striatum of rats. In consistent with these reports, present study demonstrated marked motor impairment (decrease in locomotor activity and grip strength in rotarod test) following intrastriatal QA administration which are further supported by several findings of our own laboratory as well (Kalonia et al. 2010a; Shear et al. 1998). However, Jin and its co-workers reported that chronic administration of rosiglitazone (10 mg/kg/day, i.p.) significantly improved motor function in N171-82Q HD mice demonstrating the neuroprotective efficacy of rosiglitazone against motor abnormality in HD-like conditions (Jin et al. 2013). Consistent with these
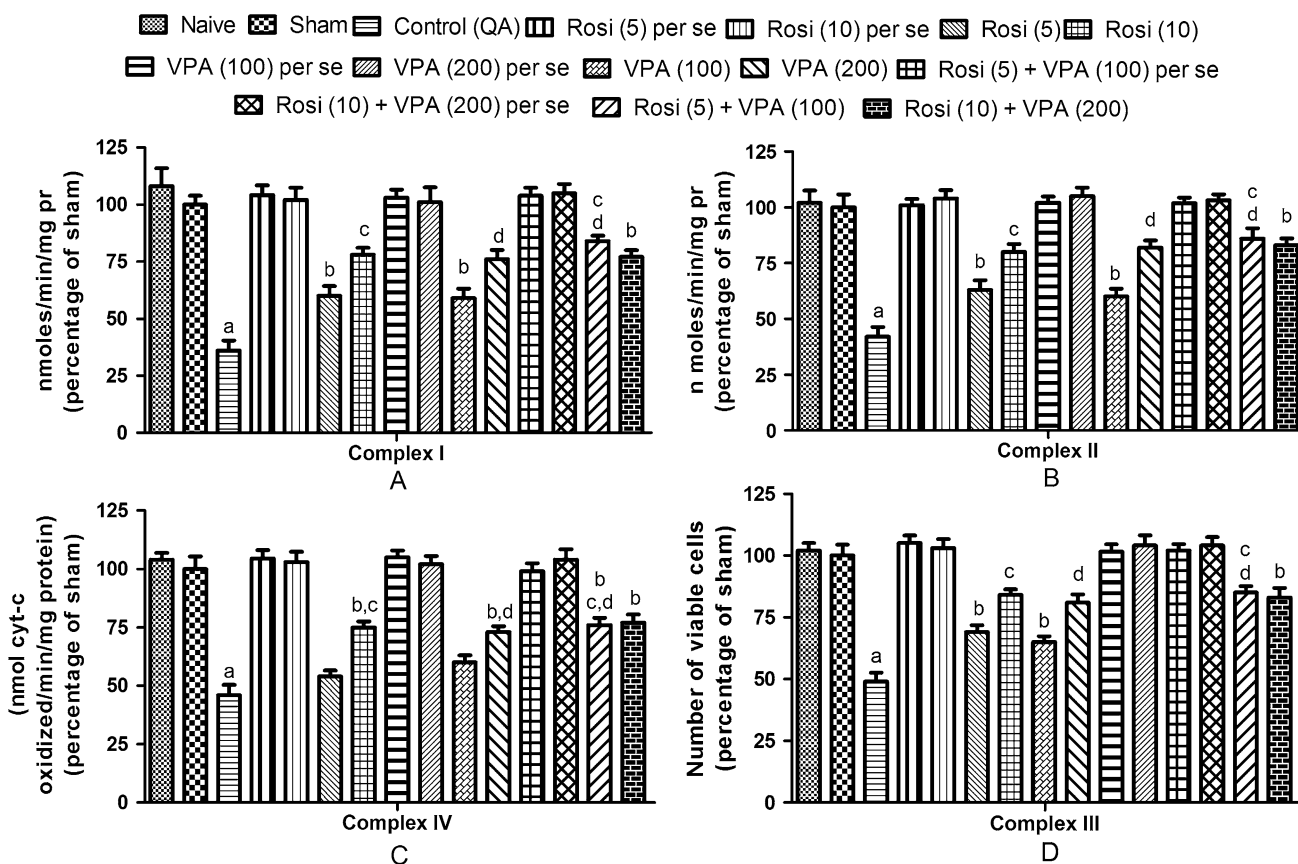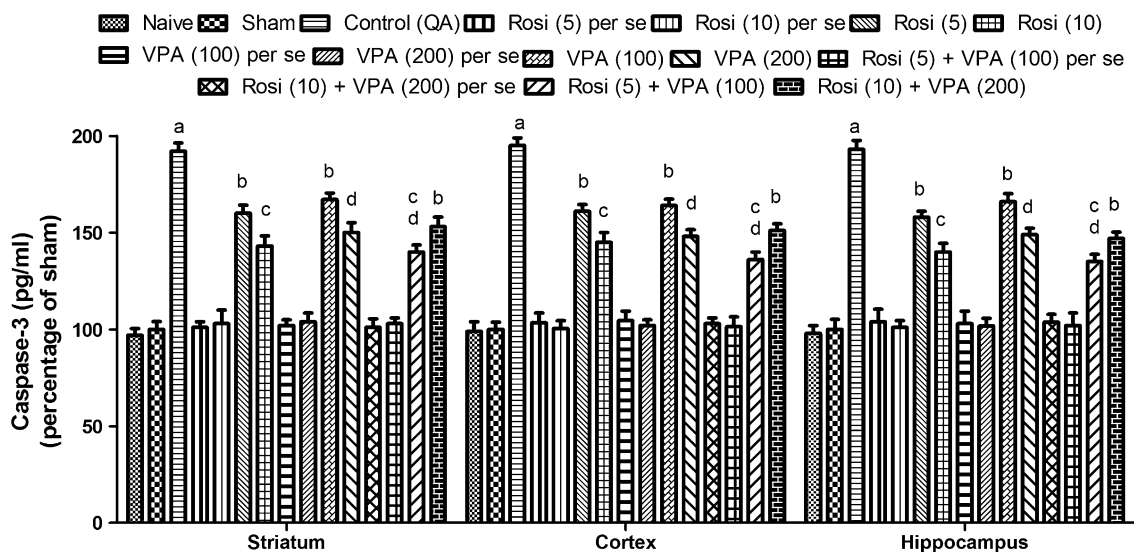
**Fig. 8** Effect of valproic acid, rosiglitazone, and their combination on mitochondrial enzyme complex (I, II, III, and IV) activities in QA-treated animals. Data expressed as mean ± SEM (percentage of sham). (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid. [Mean ± SEM (sham) *Complex I* 207 ± 12.57, *Complex II* 182 ± 8.93, *Complex III* 169 ± 8.35, and *Complex IV* 194 ± 7.68]
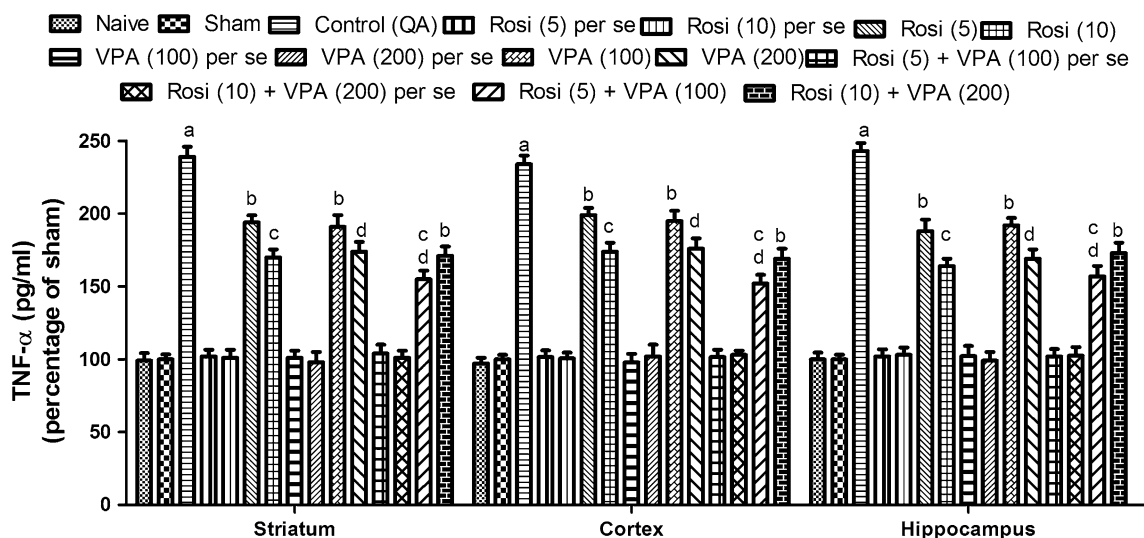


**Fig. 9** Effect of valproic acid, rosiglitazone, and their combination on TNF-α level in QA-treated animals. Data expressed as mean ± SEM. [percentage of sham (57.62 ± 2.3)]. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

Legend: Naive, Sham, Control (QA), Rosi (5) per se, Rosi (10) per se, Rosi (5), Rosi (10), VPA (100) per se, VPA (200) per se, VPA (100), VPA (200), Rosi (5) + VPA (100) per se, Rosi (10) + VPA (200) per se, Rosi (5) + VPA (100), Rosi (10) + VPA (200)
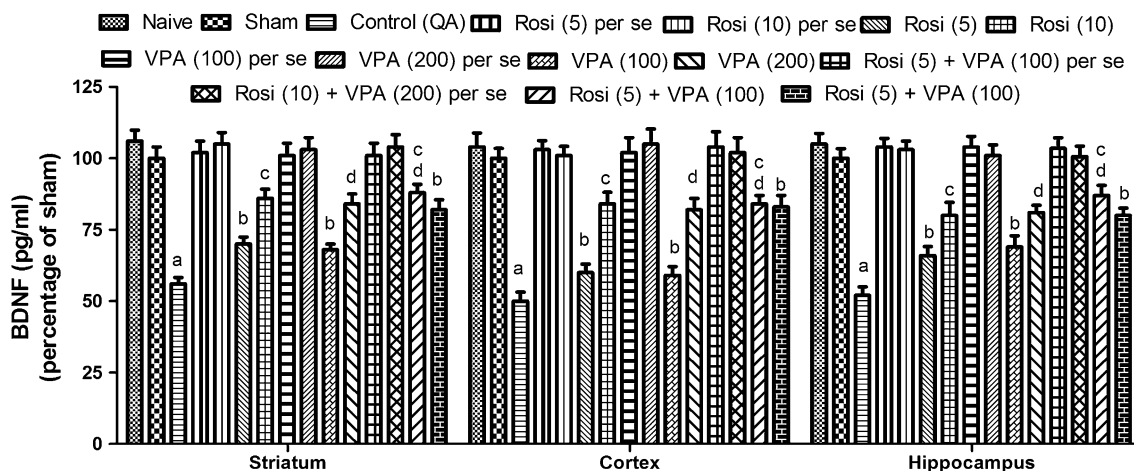
**Fig. 10** Effect of valproic acid, rosiglitazone, and their combination on caspase-3 level in QA-treated animals. Data expressed as mean ± SEM [percentage of sham (percentage above control; 137.62 ± 7.3)]. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid



Legend: Naive, Sham, Control (QA), Rosi (5) per se, Rosi (10) per se, Rosi (5), Rosi (10), VPA (100) per se, VPA (200) per se, VPA (100), VPA (200), Rosi (5) + VPA (100) per se, Rosi (10) + VPA (200) per se, Rosi (5) + VPA (100), Rosi (5) + VPA (100)
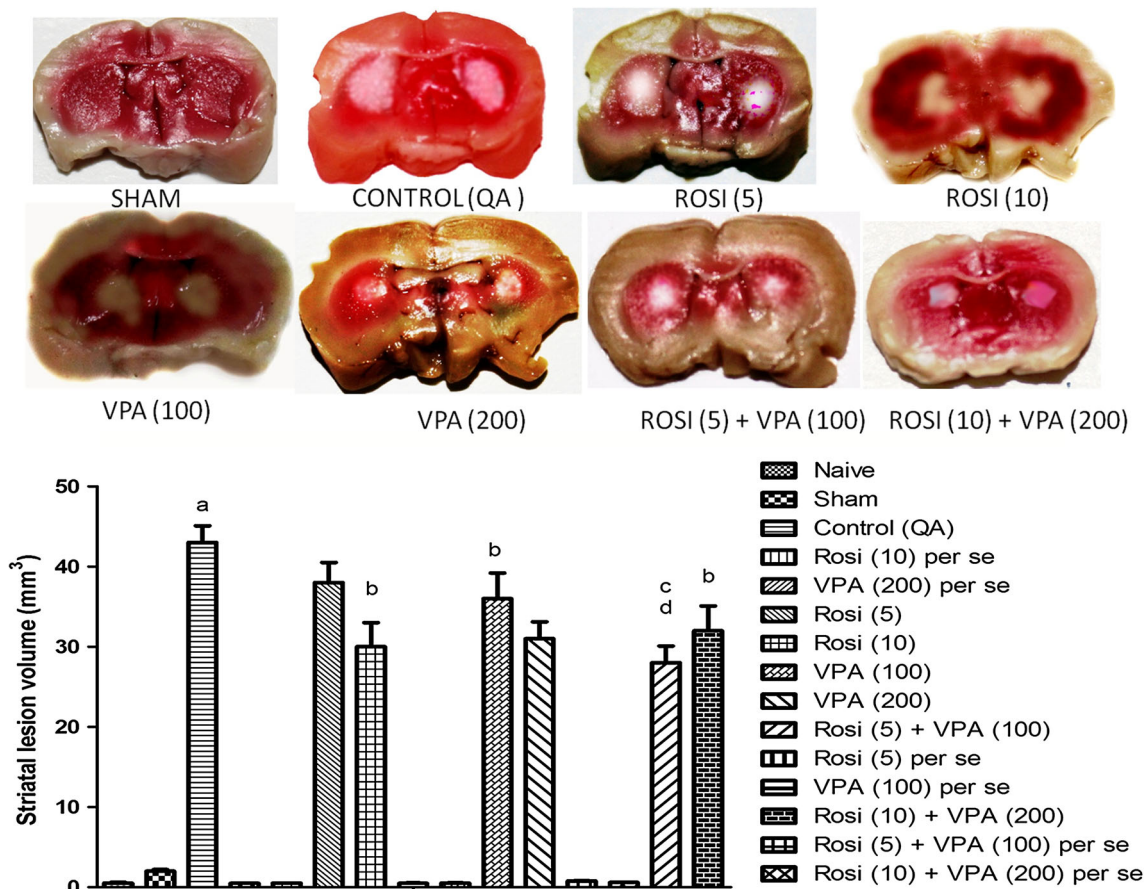
**Fig. 11** Effect of valproic acid, rosiglitazone, and their combination on BDNF level in QA-treated animals. Data expressed as mean ± SEM [percentage of sham (153.58 ± 7.3)]. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

reports, marked attenuation in these motor impairments in QA-treated animals following VPA and rosiglitazone treatment suggest their neuroprotective potential in HD-like conditions. Furthermore, the combination of VPA and rosiglitazone at their lower doses revealed further improvement in their neuroprotective potential demonstrating possible synergizm, which was further supported by several study reports (Dash et al. 2010; Lee et al. 2012).

In Morris water maze paradigm, escape latency to reach the hidden platform was significantly increased, whereas in probe trial, the time spent in target quadrant, frequency of appearance in target quadrant and percentage of total path length traversed in target quadrant was significantly decreased in QA (200 nmol) administered animals as compared to sham group suggesting cognitive impairment. Several reports demonstrated that altered AChE activity in hippocampus modulates memory performance of the animals (Sudha et al. 1995; Baitharu et al. 2013). Similarly, in the present study, memory impairment following QA administration could be attributed to the increased hippocampal AChE levels, which were significantly ameliorated by rosiglitazone and VPA treatment demonstrating marked

**Fig. 12** Effect of valproic acid, rosiglitazone, and their combination on striatal lesion volume in QA-treated animals. Data expressed as mean ± SEM. (*a*) $P < 0.05$ compared to sham group, (*b*) $P < 0.05$ compared to control (QA-treated group), (*c*) $P < 0.05$ compared to Rosi (5), (*d*) $P < 0.05$ compared to VPA (100); (two-way ANOVA followed by Bonferroni's test). *QA* Quinolinic acid, *Rosi* rosiglitazone, *VPA* valproic acid

improvement in memory performance of the QA-treated animals. Although the lower doses were unable to demonstrate significant effect on the AChE levels, still they were able to demonstrate significant improvement in memory performance of the QA-treated animals which could be attributed to their beneficial effect on oxidative stress, neuro-inflammation, apoptosis, and BDNF levels. Our results are in accordance with the previous findings (Block et al. 1993), who found significant deficits in acquisition and probe trial performance in Morris water maze task in QA administered rats. These results are further supported by several earlier study reports demonstrating improvement in memory performance following VPA and rosiglitazone administration in several neurological conditions possibly via HDAC and PPARγ pathways, respectively, (Pedersen et al. 2006; Escribano et al. 2009). However, treatment of VPA in combination with rosiglitazone, showed further improvement in memory of the QA administered animals suggesting possible synergizm.

Several studies in our laboratory as well as other laboratories indicated that QA induces oxidative stress and support the possible relationship between excitotoxicity and oxidative process (Rossato et al. 2002; Ganzella et al. 2006; Kalonia et al. 2012). Studies have confirmed that QA induces LPO (Santamaria and Rios 1993) and neuronal damage that can be inhibited by neuroprotectants and antioxidants. Further, PPARγ agonists and HDAC inhibitors have been reported to enhance the activities of several antioxidant enzymes like superoxide dismutase-1 and -2 (SOD-1 and -2), catalase (Hwang et al. 2005; Ding et al. 2007). Further, the behavioral abnormalities in HD have been attributed to the death of dopaminergic and GABAergic neurons due to QA-induced excitotoxicity (Poeggeler et al. 2007) and other related cellular cascades such as oxidative stress (Perez-De La Cruz et al. 2012), mitochondrial dysfunction (Kalonia et al. 2010a), and neuro-inflammation. Recent reports suggest that rosiglitazone protected a neuroblastoma cell line (N2A) from mHtt-evoked mitochondrial deficiency as well as normalized the reactive oxygen

**Fig. 13** Effect of valproic acid, rosiglitazone, and their combination on histopathological analysis (H&E staining) in QA-treated animals representative photomicrographs of striatum of rat brain sections. Sections were stained with hematoxylin and eosin. *Short arrows* indicate neuro-inflammation and *long arrows* indicate apoptotic cells. **a** sham: neurons are intact, **b** QA (200 nmol): infiltration of inflammatory cells with large number of apoptotic cells, **c** Rosi (5): moderate inflammation of neurons with less apoptotic cells, **d** Rosi (10): mild inflammation of neurons with very less apoptotic cells, **e** VPA (100): moderate inflammation of neurons with less apoptotic cells, **f** VPA (200): mild inflammation of neurons with very less apoptotic cells, **g** Rosi (5) + VPA (100): mild inflammation of neurons with very less apoptotic cells, and **h** Rosi (10) + VPA (200): mild inflammation of neurons with very less apoptotic cells
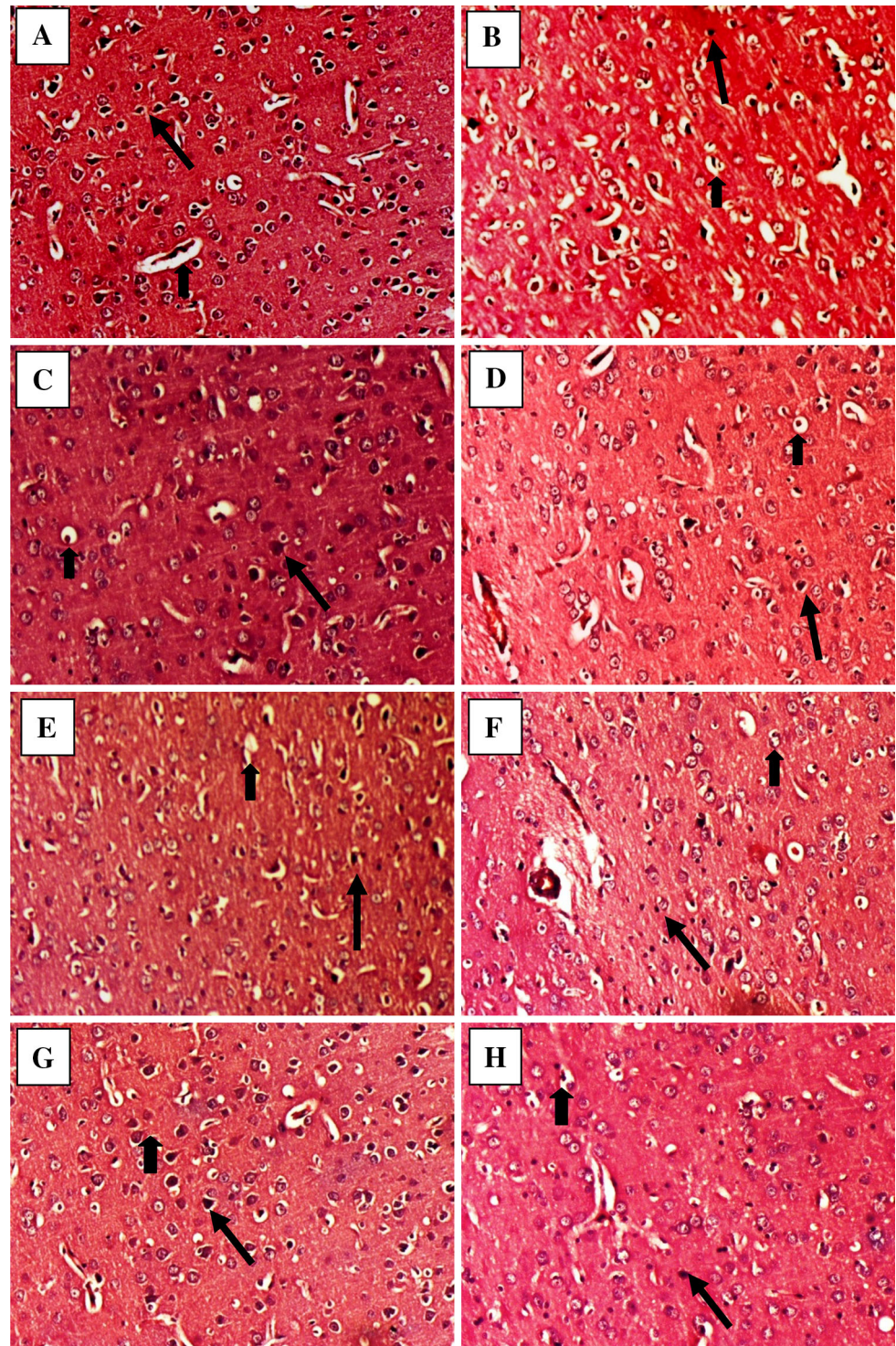


species levels (Chiang et al. 2012). In consistent with these reports, the results of the present study demonstrated impairment in anti-oxidant defense (increase in LPO, nitrite concentration and decrease in SOD, catalase as well as glutathione enzyme activities) as well as mitochondrial complex (I, II, III, and IV) activities following QA administration, suggesting increased oxidative damage. However, treatment with rosiglitazone and VPA showed increased endogenous anti-oxidant defense, suggesting their protective effects against increased oxidative and nitrosative stress. Meanwhile, combined treatment with VPA, rosiglitazone at their lower doses showed a marked

protection against QA-induced oxidative and nitrosative dysfunction which is further supported by the several reports (Valvassori et al. 2010; Kwan and Brodie 2001; Kalonia et al. 2012). As HDAC inhibition and PPARγ activation by VPA and rosiglitazone, respectively, ameliorated the QA-induced oxido-nitrosative stress and mitochondrial dysfunction, the PPARγ-HDAC interaction may be considered as a rational therapeutic target in the treatment HD-like symptoms (Quintanilla et al. 2008).

The role of HDAC in the pathogenesis of HD has recently been addressed showing a stark correlation between abnormal HDAC activity and disease progression (Langley et al. 2005; Sadri-Vakili and Cha 2006). Further, VPA treatment has been reported to decrease brain infarct volume and inhibit other inflammatory markers in the brain (Kim et al. 2007). In addition to this, VPA has also been reported to attenuate the secretion of TNF-α and interferon-γ (Chen et al. 2007; Peng et al. 2005). Recently, rosiglitazone has also been reported to attenuate dopaminergic cell loss in mouse model of Parkinson's disease (PD), an effect attributed to its anti-inflammatory properties (Lee et al. 2012). Supplementary to this, several reports from our laboratory suggest that intrastriatal QA administration results in increased level of pro-inflammatory cytokines (TNF-α and IL-6) (Kalonia et al. 2012). In consistent with the above-mentioned study report, present study demonstrated a significant rise in the TNF-α levels following QA administration. However, treatment with VPA and rosiglitazone for 21 days restored TNF-α levels in different brain regions in QA-treated animals suggesting their anti-inflammatory potential against neuroinflammatory conditions. Further, treatment of VPA in combination with rosiglitazone for 21 days restored TNF-α level providing evidence toward possible synergizm between HDAC and PPARγ activities.

It has been well reported that striatal neuronal apoptosis is preferentially enhanced by NMDA receptor activation following excitotoxin injection (Shehadeh et al. 2006). Several other reports suggest that destruction of striatal GABAergic and dopaminergic neurons following excitotoxin injection are principally mediated through the apoptotic mechanisms (Simonian et al. 1996; Kalonia et al. 2011). In consistent with the above reports, intrastriatal QA administration in the present study resulted in the increased caspase-3 enzyme levels in striatum, cortex, and hippocampus. However, treatment with VPA and rosiglitazone showed a marked protection against QA-induced apoptosis as evident from the significantly restored caspase-3 level. Moreover, treatment of VPA in combination with rosiglitazone further attenuated the caspase-3 levels in the different brain areas suggesting that the PPARγ-HDAC interaction may play a crucial role in the management of HD-like conditions.

Neurotrophic factors are signaling molecules that mediate important physiological processes in the nervous system. BDNF deficiency is a major contributor to striatal degeneration and many phenotypes in HD. Maintenance and plasticity of striatal neurons are dependent on BDNF, which are depleted in HD striatum due to reduced expression and disrupted cortico-striatal transportation (Zuccato and Cattaneo 2007; Baquet et al. 2004). Several reports suggest that levels of BDNF are decreased especially in striatum, cortex, and hippocampus of as observed in post-mortem HD brains as well as in number of mouse and cellular models for HD (Zuccato and Cattaneo 2007; Gauthier et al. 2004; Ferrer et al. 2000; Jiang et al. 2013; Baquet et al. 2004). Jin et al. (2013) demonstrated that rosiglitazone (10 mg/kg i.p.) administration rescued BDNF deficiency in the cerebral cortex and prevented loss of orexin-A-immunopositive neurons in the hypothalamus of N171-82Q HD mice. However, BDNF improves pathology and delayed neuronal dysfunction in HD mice, and overexpression of BDNF in the striatum or administration of compounds increasing BDNF levels delayed the onset of motor dysfunction (Jin et al. 2013). In agreement with the above study reports, our results demonstrated considerably reduced BDNF levels following QA administration, which confirms cortical BDNF-dependent striatal vulnerability in rats. However, treatment with rosiglitazone, VPA and their combination resulted in substantial rescue in BDNF levels of QA-treated animals, which may be correlated with the positive impact on the behavior (motor function and memory performance) of the animals. Our study results are further supported by several reports which suggest that superior BDNF levels result in reversal of motor and cognitive dysfunction in neurodegenerative disorders (Samadi et al. 2013; Reiner et al. 2012; Wang et al. 2012).

Further, intrastriatal QA administration, caused a significant increase in the striatal lesion volume, which was subsequently reduced by the 21 days treatment with rosiglitazone, VPA and their combination (Kalonia et al. 2011). Finally, the histopathological analysis confirmed neuronal inflammation and increased number of apoptotic cells in the striatal sections of QA administered animals, which are remarkably altered by the treatment of VPA, rosiglitazone suggesting neuroprotection. These findings are further supported by the previous of our laboratory suggesting striatal histological alterations following QA administration (Kalonia et al. 2009). Further, the treatment of VPA in combination with rosiglitazone additionally improved these histological alterations showing remarkable neuroprotection.

Interestingly, reports suggest that VPA interacts with the PPARs (Lampen et al. 2001). In the non-neuronal tissue, valproate treatment has been found to increase the activity of peroxisomal enzymes (Van den Branden and Roels 1985) and alter the ultrastructure of the peroxisomes (Ponchaut et al. 1991), indicating possible modulation of PPAR

signaling. It is an established fact that HDAC redistributes under oxidative stress, moving into the nucleus, and co-localizing with PPARγ in neuronal nuclei (Yang et al. 2011). Yang et al. (2011) demonstrated that transient transfection of cortical neurons with HDAC significantly reduced endogenous PPARγ transcriptional activity. Thus, HDAC inhibition results in activation of PPARγ activity in the neurons. In agreement with the published report, in the present study, the possible synergistic interaction between VPA and rosiglitazone could be attributed to the increased PPARγ activity following HDAC inhibition by VPA. However, further detailed studies are necessary before moving toward any final conclusion.

Taken altogether, the neuroprotective effect produced by VPA and rosiglitazone treatment might be attributed to the interplay of HDAC inhibition and PPARγ activation in QA-mediated oxidative stress, mitochondrial dysfunction, and neuroinflammatory-apoptotic signaling cascades. To our knowledge, this study is the first report showing the beneficial effects of the PPARγ agonist rosiglitazone, VPA, and their combination in QA-induced HD-like symptoms; the novelty of the present results is that we found synergizm between PPARγ agonist and HDAC inhibitors normalizing the behavioral, biochemical, cellular, and histopathological abnormality in QA-induced HD model.

## Conclusion

The present study suggests the possible involvement of PPARγ–HDAC interaction in the neuroprotective effect of VPA, rosiglitazone. and their combination against QA-induced behavioral, biochemical, cellular. and histological alterations in rats, and further recommends that targeting HDAC and PPARγ pathways could be a useful approach in the management of HD-like symptoms. However, detailed investigations on interplay of these pathways are warranted before approaching toward any final implications.

**Conflict of interest** The authors have no competing financial interests to declare. There is no conflict of interest between any of the authors.

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