



# Postnatal zinc or paraquat administration increases paraquat or zinc-induced loss of dopaminergic neurons: insight into augmented neurodegeneration

Namrata Mitra<sup>1,2</sup> · Amit Kumar Chauhan<sup>1,2</sup> · Garima Singh<sup>1,2</sup> · Devendra Kumar Patel<sup>3</sup> · Chetna Singh<sup>1,2</sup>

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## Abstract

Epidemiological evidences have shown an association of exposure to pesticides or heavy metals with increased incidences of Parkinson's disease (PD) in humans. Exposure to pesticides or metals during the decisive period of the brain development increases the susceptibility of dopaminergic neurons upon re-exposure in adult rodents. However, the effect of early life exposure to pesticide on the heavy metal-induced neurodegeneration or heavy metal on pesticide-induced neurodegeneration is not yet explored. The current study explored the effect of developmental exposure to zinc (Zn), a metal or paraquat (PQ), a pesticide on the nigrostriatal dopaminergic neurons of rats challenged to Zn or PQ during adulthood. Exposure of Zn or PQ during adulthood alone exhibited marked reduction in motor activities, striatal dopamine and metabolites, glutathione content and number of dopaminergic neurons. However, the levels of lipid peroxidation, protein carbonyls, superoxide dismutase activity, pro-inflammatory cytokines and 4-hydroxynonenal-protein adducts were increased. While the expression of vesicular monoamine transporter-2 and tyrosine hydroxylase were attenuated, dopamine transporter and microglial marker Iba-1 expression, activated microglia, nuclear factor-kappa B activation, mitochondrial cytochrome c release and caspase-3/9 activation were augmented following Zn or PQ exposure. Albeit postnatal alone exposure did not alter any of the studied parameters, the developmental administration of Zn/PQ in re-challenged adult rats produced more pronounced changes in the aforementioned variables as compared with adulthood Zn or PQ alone intoxicated animals. The results demonstrate that postnatal Zn/PQ intoxication dents the oxidative stress, inflammation, cell death and dopamine metabolism and storage regulating machineries, which speed up the toxicant-induced degeneration during adulthood.

**Keywords** Zinc · Paraquat · Neurodegeneration · Oxidative stress · Inflammation

## Introduction

Parkinson's disease (PD) is an age-related debilitating neurological disorder characterized by symptoms depicting motor dysfunction due to marked striatal dopamine deficit, which

is caused by selective loss of dopamine synthesizing neurons in the substantia nigra pars compacta (SNpc) region of the mid brain [1, 2]. PD exhibits a multi-factorial etiology resulting from a composite interplay between age, genetic predisposition and environmental factors [2, 3]. Epidemiological data have recognized exposure to pesticides and heavy metals as putative risk factors for increased incidences of PD in humans [3–6].

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride; PQ), a widely used bipyridyl herbicide, is a well established environmental toxin associated with dopaminergic neurodegeneration leading to PD in animal models and humans [7, 8]. PQ is a free radical generator, which itself undergoes redox cycling leading to ROS generation and subsequently oxidative stress [9]. Increased oxidative stress, mitochondrial dysfunction, microglial activation and neuroinflammation are recognized as main contributors of PQ-induced

✉ Chetna Singh  
chetna@iitr.res.in; singhchetnaitrc@rediffmail.com

<sup>1</sup> Developmental Toxicology Laboratory, Systems Toxicology and Health Risk Assessment Group, CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Vishvgyan Bhawan, 31, Mahatma Gandhi Marg, Post Box No. 80, Lucknow, Uttar Pradesh 226 001, India

<sup>2</sup> Academy of Scientific and Innovative Research (AcSIR), CSIR-IITR Campus, Lucknow, Uttar Pradesh 226 001, India

<sup>3</sup> Analytical Toxicology Laboratory, Regulatory Toxicology Group, CSIR-IITR, Lucknow, Uttar Pradesh 226 001, India

dopaminergic neurodegeneration [8, 10]. PQ impairs the dopamine reuptake and storage by modulating the expression of monoamine transporters (dopamine transporter/DAT and vesicular monoamine transporter-2/VMAT-2) and inhibiting dopamine binding to DAT resulting in dopamine dyshomeostasis [9, 11] thereby further facilitating loss of dopaminergic neurons.

Zinc (Zn), the second most abundant trace elements in biological systems, plays an imperative role in structural, catalytic and regulatory functions in cellular biology [12]. Presence of high levels of Zn in the brain of PD patients suggested its contribution in disease pathogenesis [13], which was later experimentally supported by Zn-induced PD model in rodents [9, 14–16]. Despite Zn being redox inert, experimental evidences have documented involvement of oxidative stress-mediated dopaminergic neuronal loss in Zn-induced Parkinsonism [9, 17]. Similar to PQ-induced neurotoxicity, microgliosis and neuroinflammation are implicated in Zn-induced neurodegeneration [14, 15, 17, 18]. Zinc salts are widely employed in day-to-day life making human exposure inevitable viz., used in anticorrosive coating for water supply pipes, cosmetics, plastics and pharmaceuticals and zinc sulfate is commonly used as weed killer/fertilizer in agricultural sector. People working/living near zinc mining area, smelting/galvanizing industries and paint industries also get exposed to high levels of zinc due to environment and anthropogenic activities. Additionally, zinc supplements are given to pregnant females and infants, which also result in postnatal exposure of zinc in humans.

Real life situations warrant for simultaneous or sequential exposure to different compounds rather than exposure to single environmental toxin substantiated by experimental evidences illustrating increased propensity to neuronal loss following combinatorial exposure to pesticides and/or metals [9, 19]. Maneb enhances susceptibility to PQ-induced neurodegeneration in humans and PD model [7, 20, 21]. Iron potentiates PQ-induced neurodegeneration via increased oxidative stress-mediated damage [22] and Zn increases neurotoxicity induced by 4-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), dopamine and PQ [9, 23, 24]. Furthermore, early life exposures (perinatal and postnatal) to toxins result in increased vulnerability of nigrostriatal dopaminergic system upon re-exposure in later life [25–28]. Neonatal exposure to inflammatory agent lipopolysaccharide (LPS) and hepatochlor results in increased neurotoxicity upon re-challenge with rotenone and MPTP, respectively during adulthood [29, 30]. Similarly, postnatal exposure to pesticides viz., maneb + paraquat, rotenone and cypermethrin increases the extent of dopaminergic neuronal degeneration in re-challenged rodents suggesting that exposure to non-toxic doses during critical developmental window causes changes at gene levels, which results in exacerbated response upon re-exposure during adulthood [20, 27, 31].

Although many studies have documented increased susceptibility of dopaminergic neurons by developmental exposure to pesticides/metals, no study has explored the effect of early life exposure to metals on pesticide-induced neurodegeneration during adulthood re-exposure or vice versa. The present study was therefore undertaken to investigate the effect of developmental exposure of Zn or PQ on dopaminergic neurodegeneration in rats re-challenged during adulthood. To evaluate the effects of developmental exposure on Zn/PQ-induced neurodegeneration in adults, the neurodegenerative indices were analyzed viz., neurobehavioral parameters, striatal dopamine and its metabolites, oxidative stress indices, levels of monoamine transporters, microglial activation along with neuronal apoptosis in unexposed and exposed animals.

## Materials and methods

### Chemicals

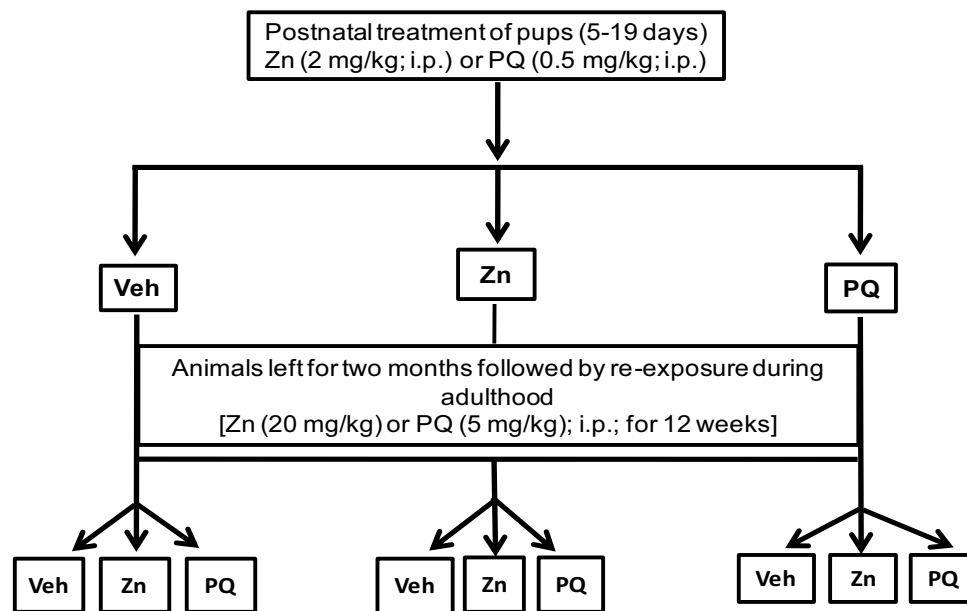
Mouse monoclonal antibodies against beta-actin ( $\beta$ -actin), DAT, VMAT-2, microglial marker Iba-1, pro-caspase-3, tyrosine hydroxylase (TH), tumor necrosis factor-alpha (TNF- $\alpha$ ), lamin A, p65 subunit of nuclear factor-kappaB (NF- $\kappa$ B) and cytochrome c (cyt c); goat polyclonal antibodies for detection of interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), mitochondrial protein -Tim 44 and rabbit polyclonal antibody for pro-caspase-9 along with alkaline phosphatase (AP)-conjugated goat anti-mouse, rabbit anti-goat and bovine anti-rabbit secondary antibodies were supplied from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Acetic acid, dibutyl phthalate xylene (DPX), nicotinamide adenine dinucleotide phosphate reduced form (NADPH), disodium hydrogen phosphate, sodium dihydrogen phosphate, heptane sulfonic acid, phenazine methosulfate, sodium fluoride, potassium chloride and xylene were obtained from Sisco Research Laboratories (SRL, Mumbai, India). Bovine serum albumin (BSA), biotinylated anti-mouse secondary antibody, 5-bromo-4-chloro-3'-indolylphosphate disodium salt (BCIP), normal goat serum, 5,5'-dithiobis(2-nitrobenzoic acid) [DTNB], nitro tetrazolium blue chloride (NBT), paraquat dichloride hydrate, phenylmethyl sulphonyl fluoride (PMSF), protease inhibitor (PI) cocktail, protein carbonyl content assay kit, streptavidin peroxidase, diaminobenzidine tetrahydrochloride (DAB) and zinc sulfate ( $\text{ZnSO}_4$ ) were procured from Sigma-Aldrich (St. Louis, MO, USA). Millipore Corporation (MA, USA) supplied polyvinylidene difluoride (PVDF) membrane and anti-NeuN antibody. Anti-4-hydroxy nonenal (4-HNE) antibody and Neg-50 were ordered from Abcam (Cambridge, UK) and Richard Allen Scientific (Kalamazoo, MI), respectively. Alexa Fluor 488 goat anti-mouse antibody and Prolong gold antifade reagent

with DAPI were supplied by Invitrogen, Thermo Fischer Scientific Corporation, USA. Remaining chemicals (analytical grade) used in the study were procured from Sigma-Aldrich (St. Louis, MO, USA).

### Animal treatment

The experiments were initiated after the approval by the Institutional Animal Ethics Committee of CSIR-IITR, Lucknow. Male Wistar rats procured from the animal colony of CSIR-Indian Institute of Toxicology Research (CSIR-IITR), Lucknow (India), were housed under standard conditions

of temperature and humidity and fed standard pellet diet and water. The male pups (10–15 g) were divided into three groups—control (C), zinc (Zn) and paraquat (PQ)-treated groups. The pups were treated intraperitoneally during postnatal days (5–19) with zinc sulfate (Zn; 2 mg/kg b.w.) or paraquat (PQ; 0.5 mg/kg b.w.) twice a week along with saline controls. The animals were then left untreated for 2 months and each group was further divided into three groups—subsequently treated with vehicle (Veh), Zn (20 mg/kg) or PQ (5 mg/kg, i.p.) twice weekly for 12 weeks. Treatment schedule is represented below by a flowchart.



[Total nine groups: Control group; groups exposed to Zn or PQ during adulthood only; groups exposed to Zn or PQ during postnatal days only; groups exposed to Zn or PQ during postnatal days and re-challenged with PQ in adulthood or vice versa and groups exposed to Zn or PQ during postnatal days and re-exposed to same agent in adulthood].

The substantia nigra and striatum were removed from rat brain under ice cold conditions, immediately frozen using liquid nitrogen and stored at  $-80^{\circ}\text{C}$  till further use. The nigrostriatal tissues (pooled substantia nigra and striatal tissues) were utilized for biochemical estimations and Western blotting experiments except for neurotransmitter measurement where striatal tissues were used while frozen brain sections were employed for immunohistochemical and immunofluorescence studies.

### Neurobehavioral studies

Motor functions were assessed through spontaneous locomotor activity (SLA) and rotarod performance tests using standard procedures [17]. SLA was determined by measuring the locomotor activity for 5 min in Opto-Varimex chamber as described previously [17], while the rotarod performance test was carried out in different groups using Omnirator (Omnitech Electronics Inc., Columbus, OH, USA) by recording the time spent on the rotating rod as described earlier [17]. The results are expressed in terms of percent of control.

## Estimation of neurotransmitters

The level of striatal dopamine and its metabolites i.e., 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were estimated using high performance liquid chromatography as described previously [16]. The known standards for the respective metabolites were used to calculate the experimental values of neurotransmitters. Final results are expressed as % of control.

## Immunohistochemistry

Degeneration of TH-positive dopaminergic neurons is the hallmark of PD therefore; TH/NeuN immunohistochemical (IHC) staining was performed to assess the neurotoxic potential of Zn/PQ in exposed and unexposed groups. IHC staining was carried out in brain sections (20  $\mu$ m) obtained using cryostat as described earlier [14]. The slides were coded and number of TH-positive neurons was counted bilaterally in each section to ascertain unbiased counting. A minimum of 4 animals were used per group for TH/NeuN immunoreactivity. The data were calculated as mean  $\pm$  standard error of means (SEM). The results are expressed as percent of control.

## Immunofluorescence (IF) analysis of microglial activation

The brain sections (14  $\mu$ m) were used for IF studies to assess the microglial activation in control and treated groups as described earlier [15]. The sections were incubated with blocking solution followed by incubation with anti iba-1 (1:300) antibody. Subsequently sections were washed, incubated with Alexa fluor 488-labeled secondary antibody. The anti-fade mounting medium containing DAPI was used for mounting the sections and finally images were captured by fluorescence microscope at  $\times$  40 magnification. The number of iba-1 positive cells was counted bilaterally in each section and results are expressed in terms of % of control.

## Oxidative stress indexes

### Lipid peroxidation (LPO) and protein carbonyl content

LPO is an established oxidative stress marker therefore LPO levels were determined by standard thiobarbituric acid method in terms of n moles Malondialdehyde (MDA)/mg tissue as described previously [17] and the results are expressed as percent of control.

Protein carbonylation, another indicator of oxidative damage, was estimated in control and treated groups using commercial kit for measuring protein carbonyl content (Sigma-Aldrich) as per manufacturer's protocol. The carbonyl

content was calculated in terms of n moles carbonyl/mg protein and the results are expressed as percent of control.

### Superoxide dismutase (SOD) and Glutathione (GSH) content

SOD activity and GSH content were determined in the nigrostriatal tissue of control and treated groups using a standard NBT and DTNB methods, respectively as described elsewhere [14, 17]. The results are expressed as % of control.

### Protein estimation

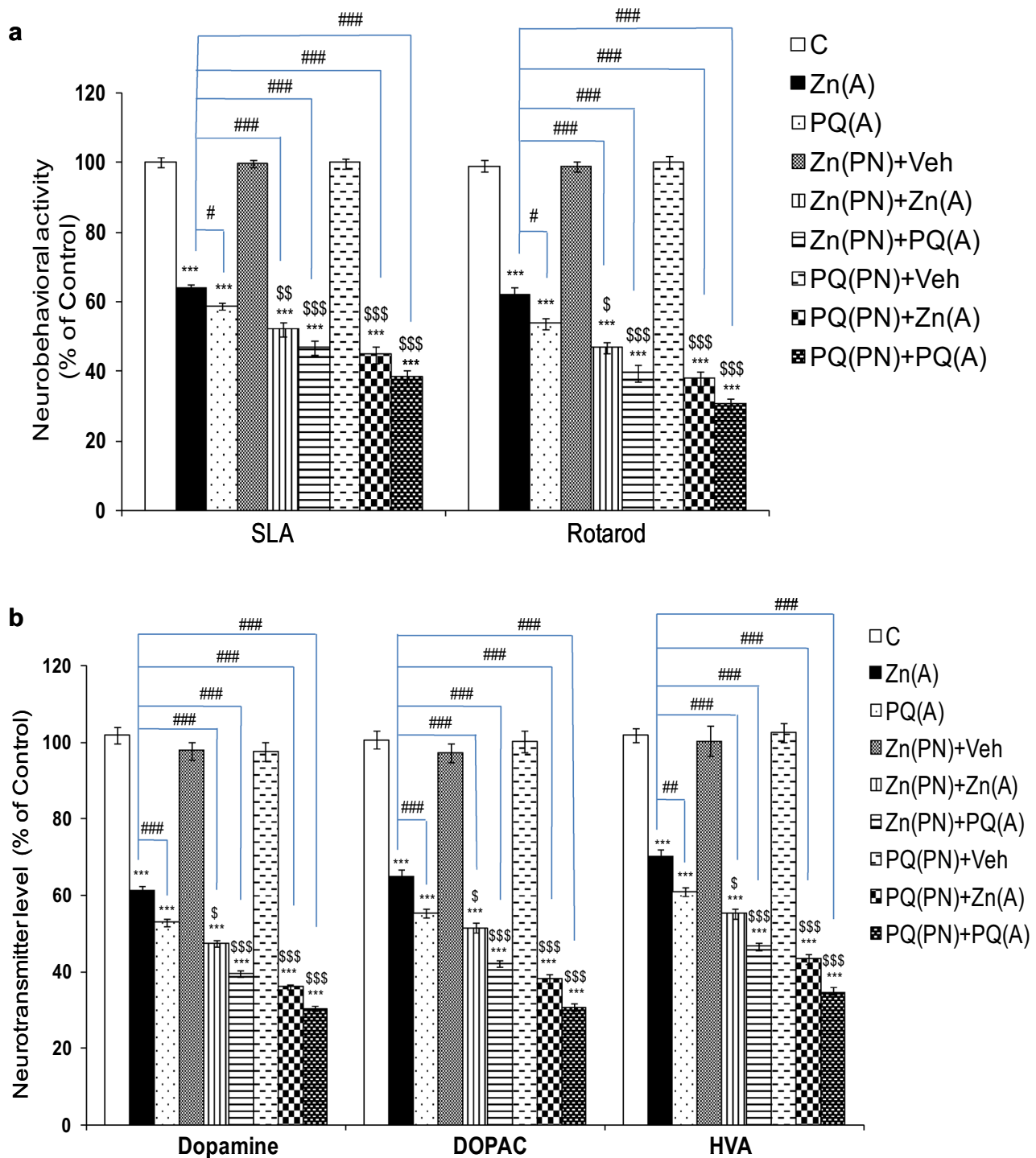
Protein content was estimated in tissue lysate and different sub-cellular fractions by Lowry method using BSA as a standard as described elsewhere [18].

### Western blotting

4-HNE, a by-product of lipid peroxidation, forms adducts with proteins, which are established as a marker for oxidative stress-mediated damage. The levels of HNE-protein adducts were analyzed by Western blotting of tissue homogenate from control and treated groups employing anti-HNE antibody. For remaining protein expression analysis, the tissue homogenate was fractionated into nuclear, mitochondrial and cytosolic fractions as previously [18]. The protein expression of TH, Iba-1, VMAT-2, pro-inflammatory mediators (TNF- $\alpha$ , IL-1 $\beta$ , and IL-6), pro-caspase-9 and pro-caspase-3 was assessed in the cytosolic fraction of the nigrostriatal tissue homogenate while DAT level was analyzed in membrane fraction. Mitochondrial cyt c release was evaluated by analyzing its relative levels in the cytosolic and mitochondrial fractions while NF- $\kappa$ B activation was investigated by assessing relative protein levels of its p65 subunit in the nuclear and cytosolic fractions. The denatured proteins were resolved on SDS-polyacrylamide gel (8–15%) and transferred onto PVDF membrane. The blots were processed using specific primary antibodies and respective AP-conjugated secondary antibodies as reported previously [18] and finally BCIP/NBT substrate system was used to develop the blots. Lamin, Tim-44 and  $\beta$ -actin were taken as the reference for nuclear, mitochondrial and cytosolic fractions, respectively to calculate the relative band density ratio of concerned protein and the data are expressed in terms of mean band density ratio  $\pm$  SEM.

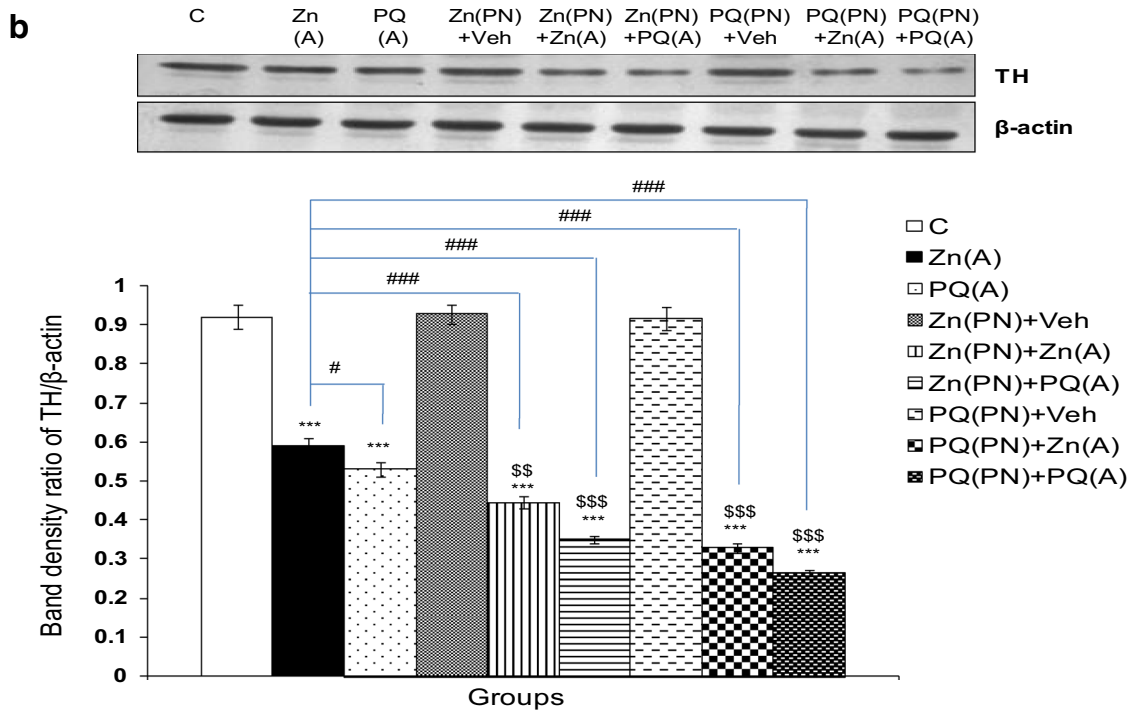
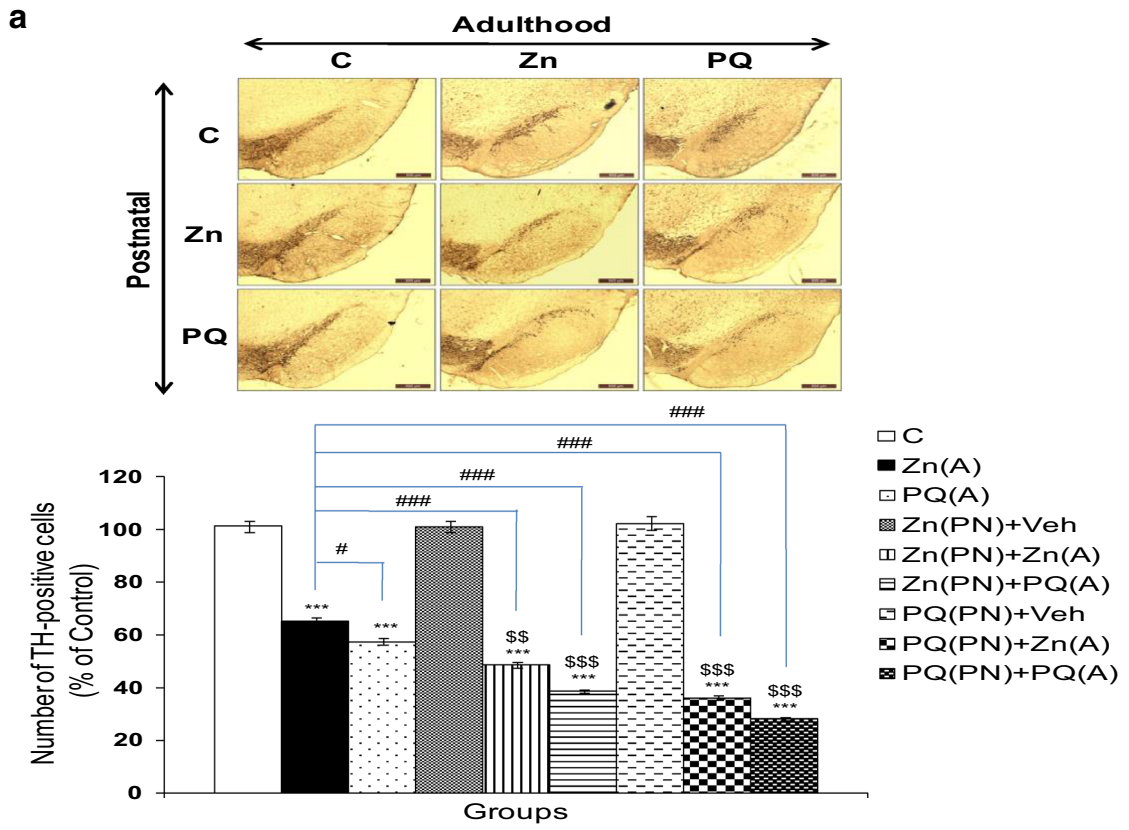
### Statistical analysis

The results are represented as mean  $\pm$  SEM of at least four independent sets of experiments and statistical analysis was performed using one-way analysis of variance (ANOVA). Multiple comparisons within different groups were carried out using Newman-Keul's post-test and only the differences



**Fig. 1** **a** Depicts the effect of Zn/PQ exposure on spontaneous locomotor activity (SLA) and rotarod performance while **b** shows striatal dopamine content and its metabolites in animals treated during postnatal days and/or during adulthood. (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehicle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days+PQ exposure during adulthood; PQ(PN)+Veh=PQ

exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days+Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*\*)= $p < 0.001$  denotes comparison with control group; ###= $p < 0.001$ , ##= $p < 0.01$  and #= $p < 0.05$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p < 0.001$ , \$\$= $p < 0.01$  and \$= $p < 0.05$  denotes comparison with group exposed to PQ during adulthood only)



**Fig. 2** Effect of Zn/PQ exposure on dopaminergic neuronal degeneration and expression of TH protein in groups exposed during postnatal period alone, adulthood only or during developmental and adult life **a** upper panel shows the representative images (scale: 500  $\mu\text{m}$ ) of TH/Neu N immunoreactivity in the substantia nigra (SN) of the brain and lower panel shows bar diagram depicting number of TH/Neu N-positive cells in control and treated groups; **b** Western blot analysis of TH protein levels in nigrostriatal tissues of animals exposed to Zn/PQ during postnatal days and/or during adulthood. Data are expressed as mean  $\pm$  SEM ( $n=4$ ). (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehicle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days + PQ exposure during adulthood; PQ(PN)+Veh=PQ exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days + Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*\*)= $p < 0.001$  denotes comparison with control group; ###= $p < 0.001$  and #= $p < 0.05$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p < 0.001$  and \$\$= $p < 0.01$  denote comparison with group exposed to PQ during adulthood only)

showing ‘ $p$ ’ value less than 0.05 were considered statistically significant.

## Results

### Neurobehavioral analysis

Zn/PQ exposure in adult rats caused significant reduction in locomotor activity and rotarod performance of exposed groups as compared with controls (Fig. 1a). Although postnatal exposure to Zn/PQ alone did not affect SLA or rotarod performance in treated animals, it markedly attenuated the motor activities in rodents re-exposed during adulthood as compared to groups exposed during adulthood only (Fig. 1a). Postnatal and adulthood exposure to Zn exhibited greater motor impairment than animals exposed to Zn or PQ during adulthood only; however, the changes were less prominent than groups exposed to different toxins during postnatal and adulthood. The most prominent motor dysfunction among all exposed groups was observed in groups exposed to PQ during postnatal days and adulthood (Fig. 1a).

### Striatal dopamine and its metabolites

Zn or PQ exposure during adulthood markedly attenuated the levels of striatal dopamine, DOPAC and HVA in the treated groups as compared with controls while postnatal treatment alone was ineffective in altering the levels of dopamine and its metabolites (Fig. 1b). Groups exposed to Zn/PQ during postnatal days followed by re-exposure during adulthood exhibited greater decline in the monoamine neurotransmitters as compared to groups exposed during

adulthood alone (Fig. 1b). Most marked decline in the level of neurotransmitters was observed in groups exposed to PQ during postnatal days and adulthood both followed by groups exposed to different toxicants in postnatal period and adulthood and subsequently in groups exposed to Zn in postnatal + adulthood period.

### Immunohistochemical analysis and level of TH protein

A significant reduction in the number of TH-positive cells was observed in the substantia nigra region of brain of Zn or PQ exposed adults as compared with controls; however, greater loss of TH-positive neurons occurred in the groups exposed to Zn/PQ during postnatal days and adulthood as compared to groups exposed during adulthood only (Fig. 2a). Postnatal exposure alone did not affect the number of dopaminergic neurons in treated groups (Fig. 2a).

Adulthood exposure with Zn/PQ abated the levels of TH protein in exposed groups as compared with controls while postnatal exposure to Zn/PQ alone did not affect the level of TH protein in treated animals (Fig. 2b). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused more pronounced decrease in the levels of TH protein as compared with groups exposed during adulthood only (Fig. 2b).

The groups exposed to PQ during postnatal + adulthood exhibited most pronounced reduction in the number of dopaminergic neurons and TH protein levels followed by groups exposed to Zn (postnatal) and PQ (adulthood) or vice versa and groups exposed to Zn during postnatal + adulthood period.

### Oxidative stress indexes

LPO levels and carbonyl content were increased in groups treated with Zn or PQ during adulthood as compared with controls (Fig. 3a). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused significant augmentation in the LPO levels and carbonyl content as compared with groups exposed during adulthood only (Fig. 3a). However, postnatal exposure to Zn/PQ did not affect LPO levels and carbonyl content in exposed animals.

SOD activity was elevated in groups treated with Zn/PQ during adulthood as compared with controls while SOD activity was unaffected in groups treated only during postnatal days (Fig. 3b). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused greater increase in the SOD activity as compared with groups exposed during adulthood alone (Fig. 3b).

GSH content was reduced in groups treated with Zn or PQ during adulthood as compared with controls while GSH content was unaltered in animals exposed during

postnatal days only (Fig. 3b). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused significantly greater decline in the GSH content as compared with groups exposed during adulthood alone (Fig. 3b).

Western blot analysis revealed marked elevation in HNE-protein adducts in Zn or PQ exposed adult animals; however, greater augmentation was exhibited in the postnatal + adulthood exposed groups (Fig. 3c). The postnatal exposure per se did not affect the levels of the HNE adducts as compared with controls (Fig. 3c).

Alterations observed in all of the above-mentioned oxidative stress indices were most noticeable in groups exposed to PQ in postnatal + adulthood followed by groups exposed to Zn during postnatal days and re-challenged with PQ in adulthood or vice versa and then in the groups exposed to Zn during postnatal days as well as adulthood.

### Expression of monoamine transporters

Adulthood exposure with Zn/PQ augmented DAT protein expression while VMAT-2 protein expression was reduced in exposed groups as compared with controls (Fig. 4). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused further elevation in the expression of DAT protein while VMAT-2 protein levels were attenuated markedly as compared with groups exposed during adulthood alone. No change was observed in DAT/VMAT-2 levels in groups treated during postnatal days alone (Fig. 4). Although developmental exposure augmented the modulations in DAT/VMAT-2 levels in all the groups re-challenged during adulthood, the most prominent modulations were observed in animals exposed to PQ at both time periods (postnatal + adulthood) followed by animals exposed to Zn (postnatal) and PQ (adulthood) or vice versa and consequently in groups exposed to Zn during both periods (postnatal + adulthood).

### Microglial activation

IF analysis exhibited increase in the number of iba-1 positive cells/activated microglia following adulthood exposure to Zn/PQ as compared with controls; however, no change was observed in the number of iba-1 positive cells in animals exposed during postnatal days alone as compared with controls (Fig. 5a, b). Adulthood re-exposure to Zn/PQ in developmentally exposed animals caused further increase in the number of activated microglia as compared with groups exposed to Zn/PQ during adulthood only (Fig. 5a, b).

Western blot analysis of iba-1 protein expression in exposed groups supported IF results. A marked elevation was exhibited in the protein expression of iba-1 in groups exposed to Zn/PQ during adulthood as compared to controls (Fig. 5c). The levels of iba-1 were augmented to a greater extent in developmentally exposed animals re-challenged

with Zn/PQ in adulthood as compared with groups exposed only during adulthood (Fig. 5c). Postnatal treatment alone was ineffective in altering the expression of the iba-1 protein in exposed groups. Among re-challenged groups, most discernible microgliosis was exhibited in groups exposed to pesticide during both time periods followed by groups exposed to combination of PQ and Zn and then in groups exposed to Zn during postnatal days and adulthood.

### Inflammatory mediators

Zn or PQ exposure in adult rats caused significant activation of NF- $\kappa$ B as evidenced by the marked reduction in the cytosolic NF- $\kappa$ B with concomitant increase of nuclear NF- $\kappa$ B levels (Fig. 5d). Adulthood re-exposure to Zn/PQ in developmentally exposed animals exhibited greater translocation of p65 subunit of NF- $\kappa$ B from cytosol to nucleus i.e., NF- $\kappa$ B activation as compared with groups exposed during adulthood only (Fig. 5d). Postnatal treatment alone did not cause any change in cytosolic and nuclear levels of p65 subunit of NF- $\kappa$ B (Fig. 5d).

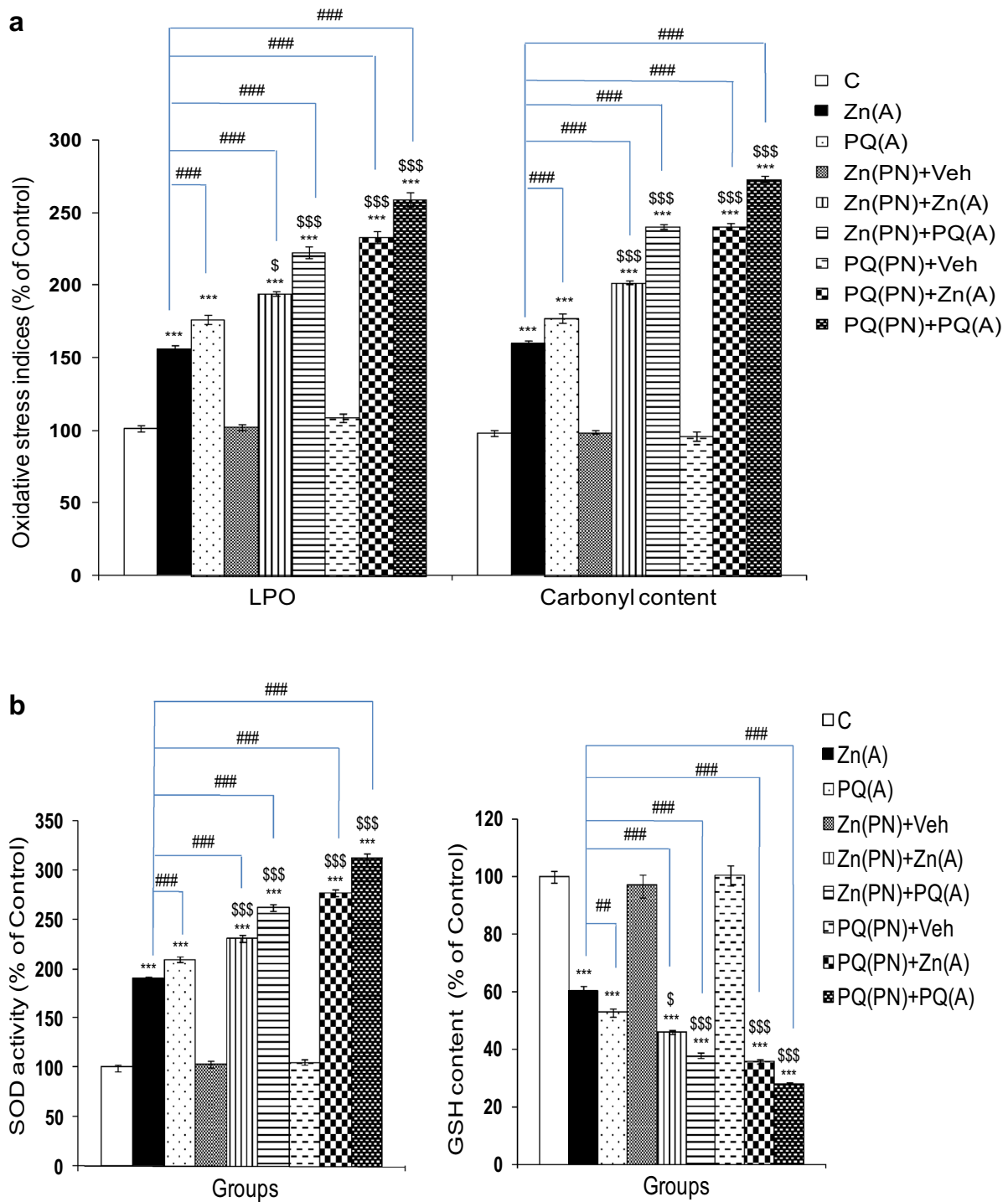
Furthermore, blot analysis of pro-inflammatory cytokines exhibited elevated levels of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  in Zn or PQ exposed adult rats as compared with controls (Fig. 5e). Adulthood re-exposure to Zn/PQ in developmentally exposed animals noticeably augmented the levels of TNF- $\alpha$ , IL-6 and IL-1 $\beta$  as compared with groups exposed during adulthood alone (Fig. 5e). Postnatal treatment per se was ineffective in altering the expression of pro-inflammatory cytokines. Among developmentally exposed re-challenged adults, groups exposed to PQ in postnatal + adulthood exhibited most pronounced augmentation followed by groups exposed to different agents in postnatal and adulthood and groups exposed to Zn in postnatal and adulthood periods.

### Cytochrome c release and caspase activation

Adulthood exposure to Zn/PQ markedly augmented mitochondrial cyt c release as observed by the marked reduction of cyt c levels in mitochondrial fraction with simultaneous increase in cytosolic cyt c expression as compared with unexposed groups (Fig. 6a). A more prominent increase in mitochondrial cyt c release was exhibited in developmentally exposed animals re-challenged during adulthood (Fig. 6a). No change was observed in mitochondrial and cytosolic cyt c levels in groups exposed during postnatal days only.

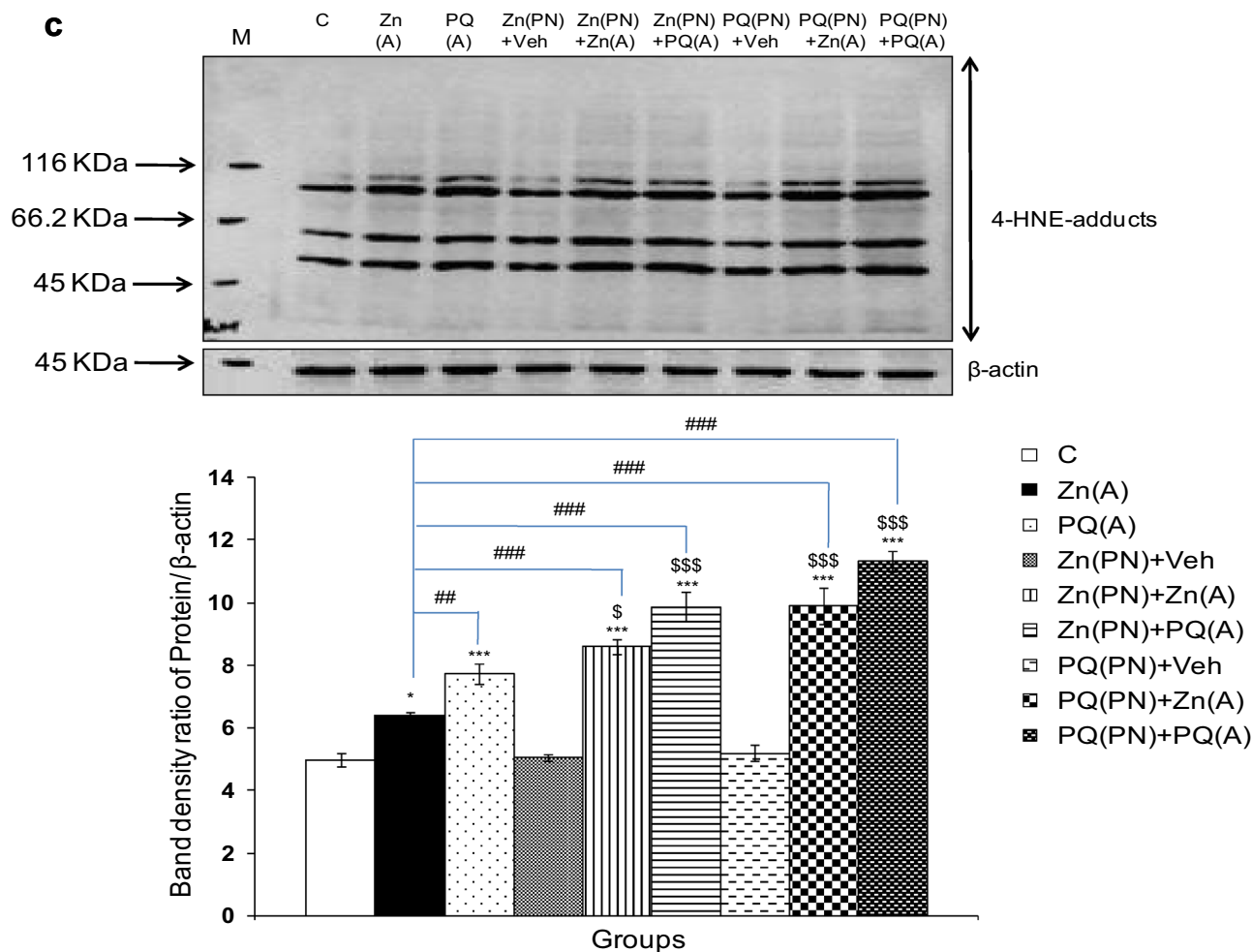
A significant reduction was obtained in the levels of pro-caspase 3 and 9 in groups exposed to Zn/PQ during adulthood as compared to control group indicating caspase cascade activation (Fig. 6b). Animals exposed both during postnatal days and adulthood illustrated considerably higher attenuation in the levels of pro-caspase 3/9 as compared with groups exposed only during adulthood (Fig. 6b). Analysis





**Fig. 3 a** LPO levels and carbonyl content, **b** SOD activity and GSH content and **c** Western blot analysis of HNE-protein adducts in the nigrostriatal tissues of animals exposed to Zn/PQ during postnatal days and/or during adulthood. Data are expressed as mean  $\pm$  SEM ( $n=4$ ). (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehicle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days +PQ

exposure during adulthood; PQ(PN)+Veh=PQ exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days+Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*= $p < 0.01$  and \*= $p < 0.05$  denote comparison with control group; ###= $p < 0.001$  and ##= $p < 0.01$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p < 0.001$  and \$= $p < 0.05$  denote comparison with group exposed to PQ during adulthood only)



**Fig. 3** (continued)

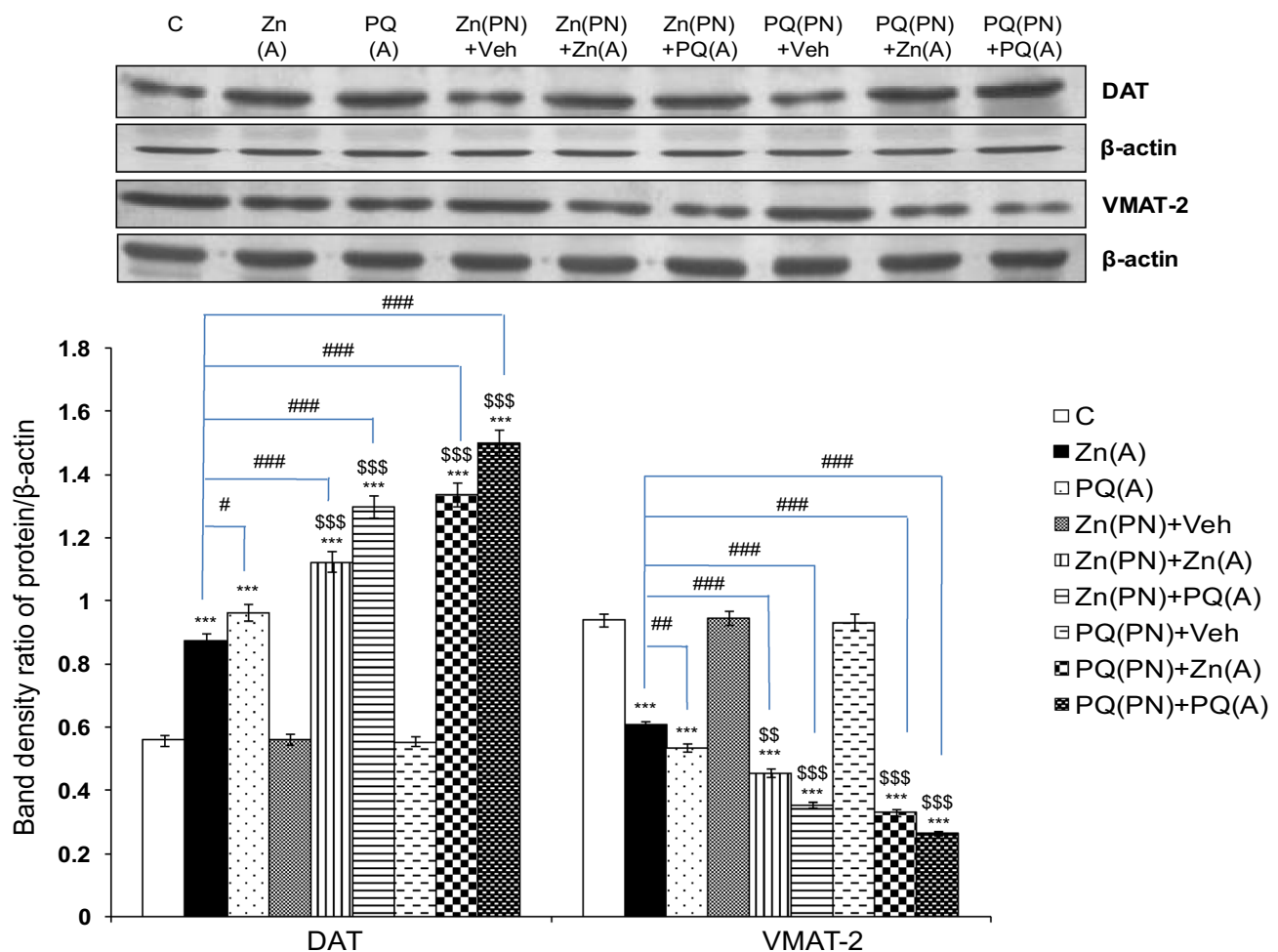
of pro-caspase 3/9 expression in groups treated with Zn or PQ during postnatal days only did not exhibit any significant alterations as compared to control group (Fig. 6b).

Apoptosis also exhibited similar trend among groups exposed during postnatal and adulthood to that observed in aforementioned parameters. In other words, developmental exposure to PQ increased apoptosis in PQ re-challenged group the most followed by groups exposed to different agents during developmental period and adult life and subsequently groups exposed to Zn in postnatal days as well as during adulthood.

## Discussion

PD is a chronic neurodegenerative disorder wherein the environmental factors also play a crucial role. While single toxin is shown to induce Parkinsonian features in experimental animals, the multiple-hit hypothesis appears to be more apt in real life situations in humans [19, 22]. Early life

exposure is found to increase the incidences of PD during the latter phase of life in humans. Besides, the developmental or early exposure to a few selected neurotoxins is found to enhance the susceptibility of dopaminergic neurons upon adulthood re-exposure [27, 32, 33]. The present study investigated whether or not the developmental exposure of Zn or PQ enhances the vulnerability of dopaminergic neurodegeneration in rats if re-challenged during adulthood. Parkinsonism in experimental rats is often assessed by measuring the reductions in motor activities. Moreover, the depletion of the striatal dopamine content is another hallmark of the Parkinson's disease phenotype in experimental animals and humans. As observed in the previous studies, Zn or PQ alone induced motor impairments and reduction in striatal dopamine [14, 18]. However, early exposure to Zn augmented the motor deficits and loss of the striatal dopamine in the animals re-challenged with Zn/PQ during the adulthood as compared with groups exposed during adulthood alone. Besides, the similar response was observed in the animals treated with PQ during postnatal days followed by adulthood



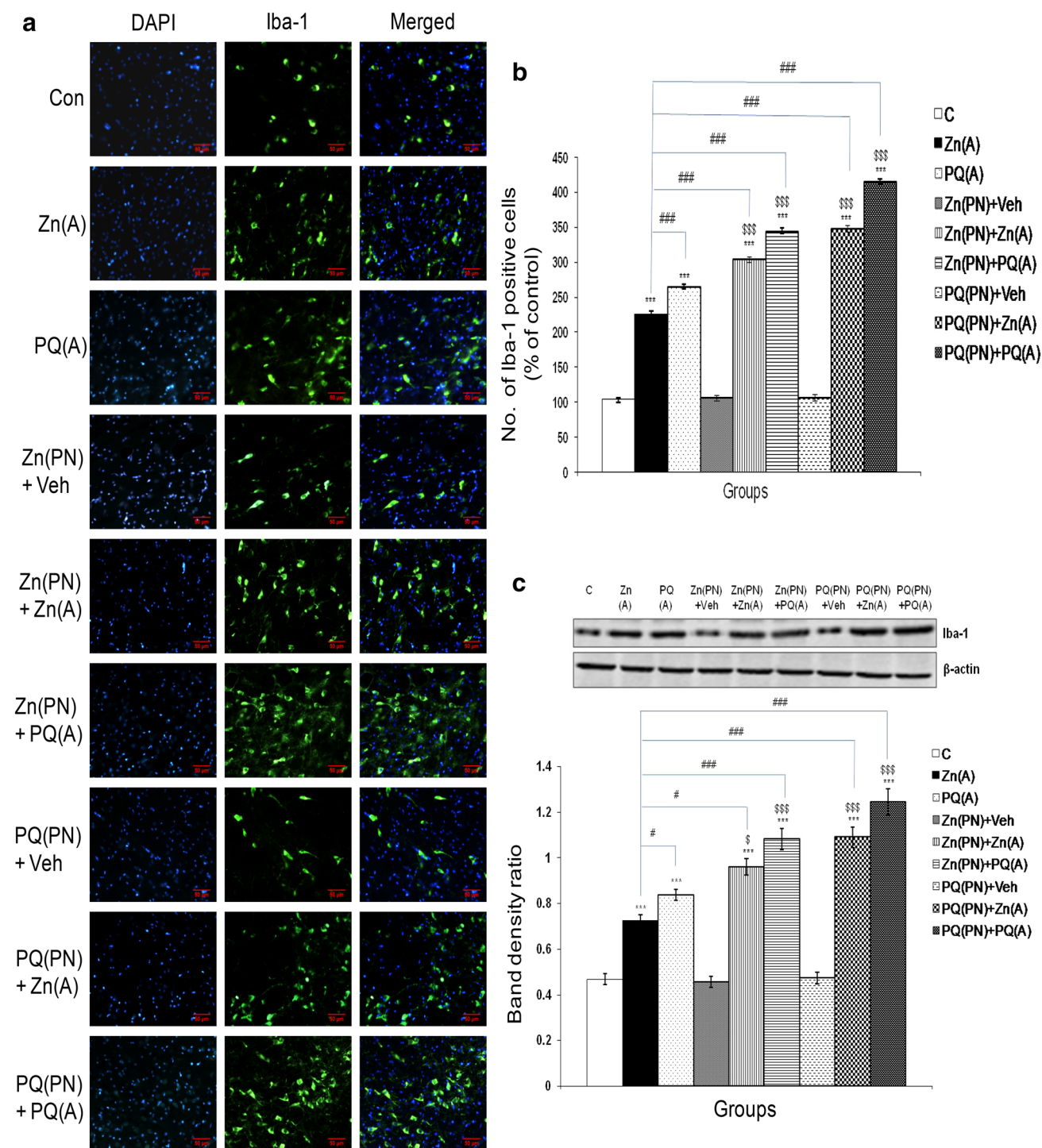
**Fig. 4** Modulation in the protein levels of monoamine transporters (DAT, VMAT-2) in nigrostriatal tissues of groups exposed to Zn/PQ during postnatal days and/or during adulthood. Data are expressed as mean  $\pm$  SEM ( $n=4$ ). (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehicle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days+PQ exposure during adulthood; PQ(PN)+Veh=PQ

exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days+Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*= $p < 0.001$  denotes comparison with control group; ###= $p < 0.001$ , ##= $p < 0.01$  and #= $p < 0.05$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p < 0.001$  and \$\$= $p < 0.01$  denote comparison with group exposed to PQ during adulthood only)

Zn/PQ exposure. The more pronounced motor dysfunction and striatal dopamine decrease in the postnatal and adulthood exposed animals than adulthood alone suggested that developmental exposure potentiates the susceptibility of animals for PD. The results are supported by the reports exhibiting increased vulnerability of dopaminergic neurons in the animals pre-challenged to low doses of pesticides followed by adulthood exposure [27, 33]. Absence of neurobehavioral changes and dopamine deficiency in the brain of only developmentally exposed animals could be due to the low/non-toxic dose used (1/10th the adult dose) or recovery of minor injury caused because of exposure in such a long time lag (~5 months after postnatal exposure). A significantly higher neurodegeneration was evident by the pronounced

reductions of TH-positive neurons and TH expression in groups exposed to Zn/PQ during postnatal days and adulthood as compared with adulthood alone suggest that though postnatal exposure itself was not toxic enough to cause neurodegenerative changes, it enhanced the neurotoxic potential of Zn/PQ upon re-exposure as documented in case of pesticides/toxin-induced neurodegeneration [20, 29, 34].

Oxidative stress is a key perpetrator in pesticide- and heavy metal-induced neurodegeneration [14, 17]. Adulthood exposure to Zn or PQ augmented the LPO, protein carbonyl content, SOD activity and HNE-protein adducts and reduced the GSH content in rats reaffirming the involvement of oxidative stress in Zn/PQ-induced neurotoxicity in accordance with earlier reports [14, 17]. Increase in the



**Fig. 5** Effect of Zn/PQ exposure during postnatal days and/or during adulthood on microglial activation and inflammatory markers. **a** Depicts immunofluorescence images of brain sections (scale: 50  $\mu$ m), and **b** shows bar diagram indicating the number of Iba-1 positive cells in the substantia nigra region of brain in control and treated groups. **c–e** illustrate Western blot analysis of protein expression of Iba-1, NF- $\kappa$ B (p65 subunit) translocation (cytosol to nuclear) and pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, IL- $\beta$ ), respectively in the nigrostriatal tissues of unexposed and exposed groups. Data are expressed as mean  $\pm$  SEM ( $n=4$ ). (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehi-

cle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days+PQ exposure during adulthood; PQ(PN)+Veh=PQ exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days+Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*= $p < 0.01$  and \*\*\*= $p < 0.001$  denote comparison with control group; ###= $p < 0.001$ , ##= $p < 0.01$  and #= $p < 0.05$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p < 0.001$ , \$\$= $p < 0.01$  and \$= $p < 0.05$  denote comparison with group exposed to PQ during adulthood only)

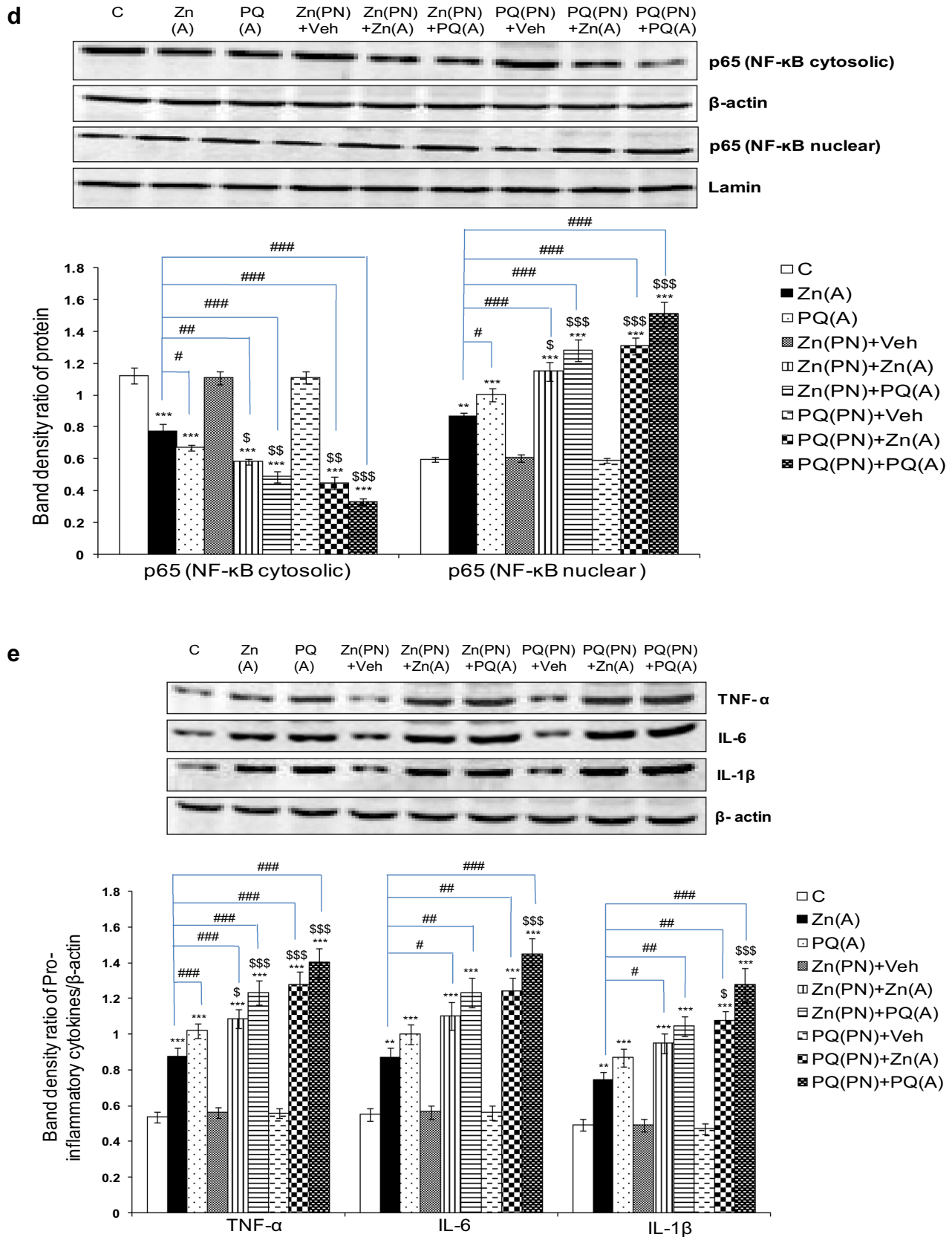
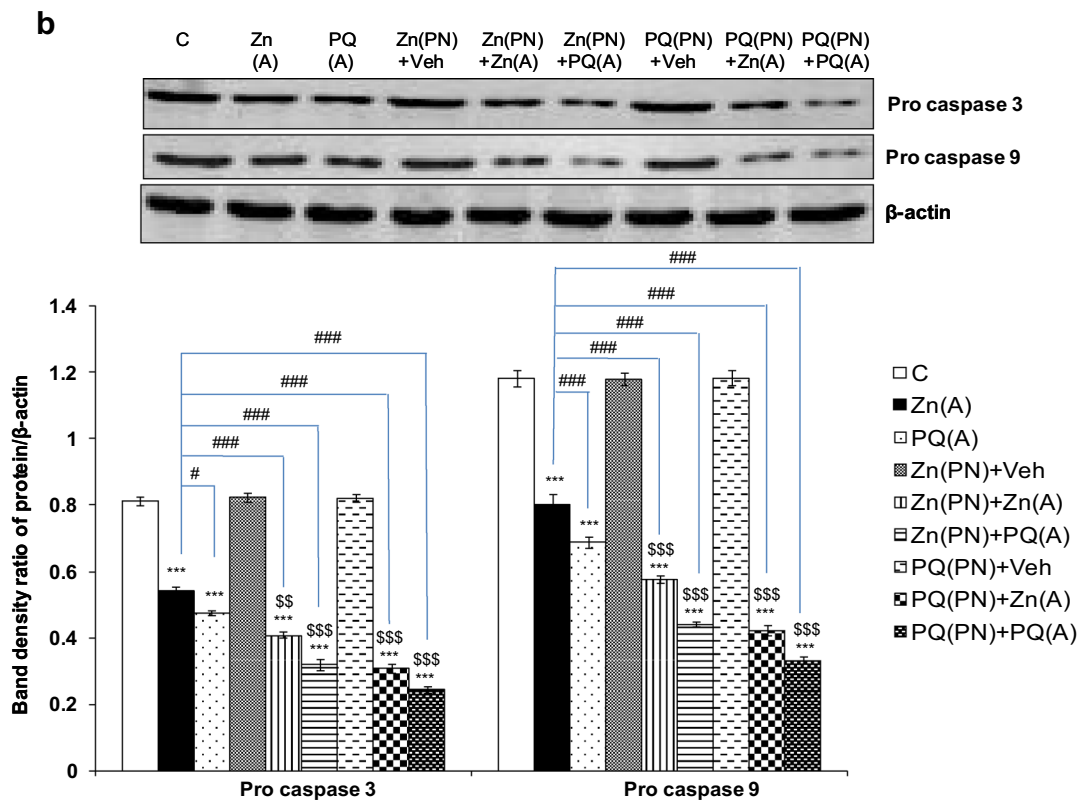
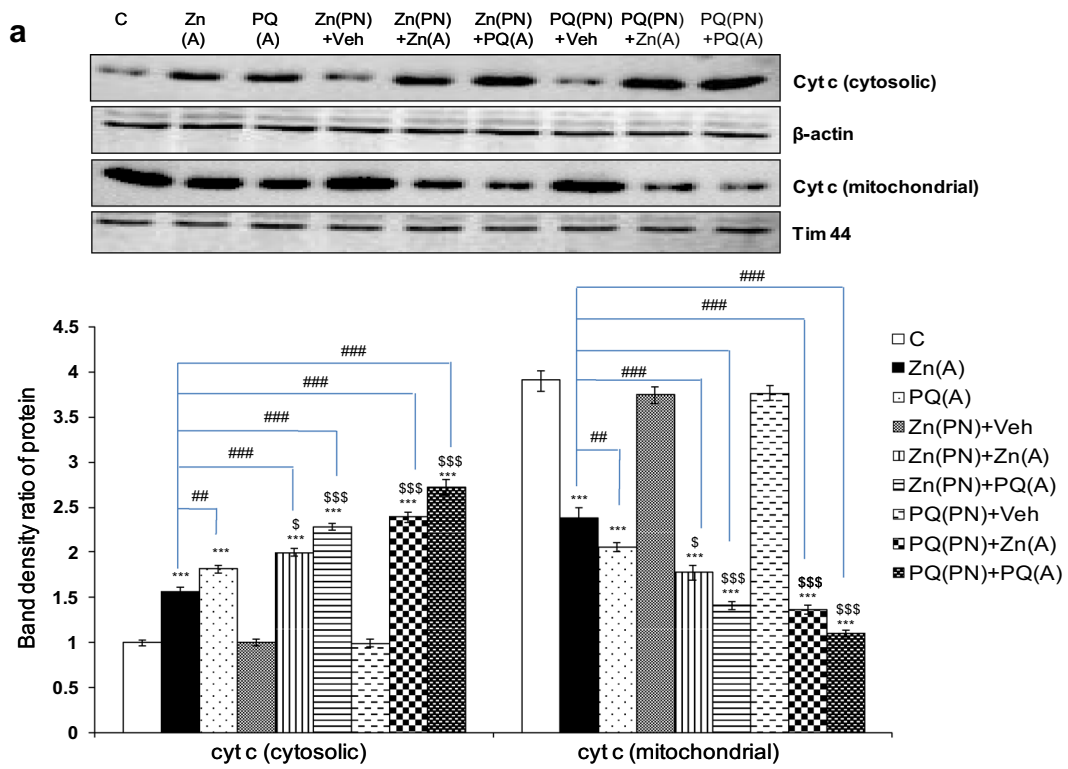


Fig. 5 (continued)



**Fig. 6** Effect of Zn/PQ on apoptotic end points i.e., mitochondrial cytochrome c release (a) and caspase cascade activation (b) in nigrostriatal tissues of animals treated during postnatal days and/or during adulthood. Data are expressed as mean  $\pm$  SEM ( $n=4$ ). (C=control group; Zn(A)=Zn exposure during adulthood; PQ(A)=PQ exposure during adulthood; Zn(PN)+Veh=Zn treatment during postnatal days and vehicle during adulthood; Zn(PN)+Zn(A)=Zn exposure during postnatal days and adulthood; Zn(PN)+PQ(A)=Zn exposure during postnatal days + PQ exposure during adulthood; PQ(PN)+Veh=PQ exposure during postnatal days and vehicle during adulthood; PQ(PN)+Zn(A)=PQ exposure during postnatal days + Zn exposure during adulthood; PQ(PN)+PQ(A)=PQ exposure during postnatal days and adulthood). (\*\*\*)= $p<0.001$  denotes comparison with control group; ###= $p<0.001$ , ##= $p<0.01$  and #= $p<0.05$  denote comparison with group exposed to Zn during adulthood only; \$\$\$= $p<0.001$ , \$\$= $p<0.01$  and \$= $p<0.05$  denote comparison with group exposed to PQ during adulthood only)

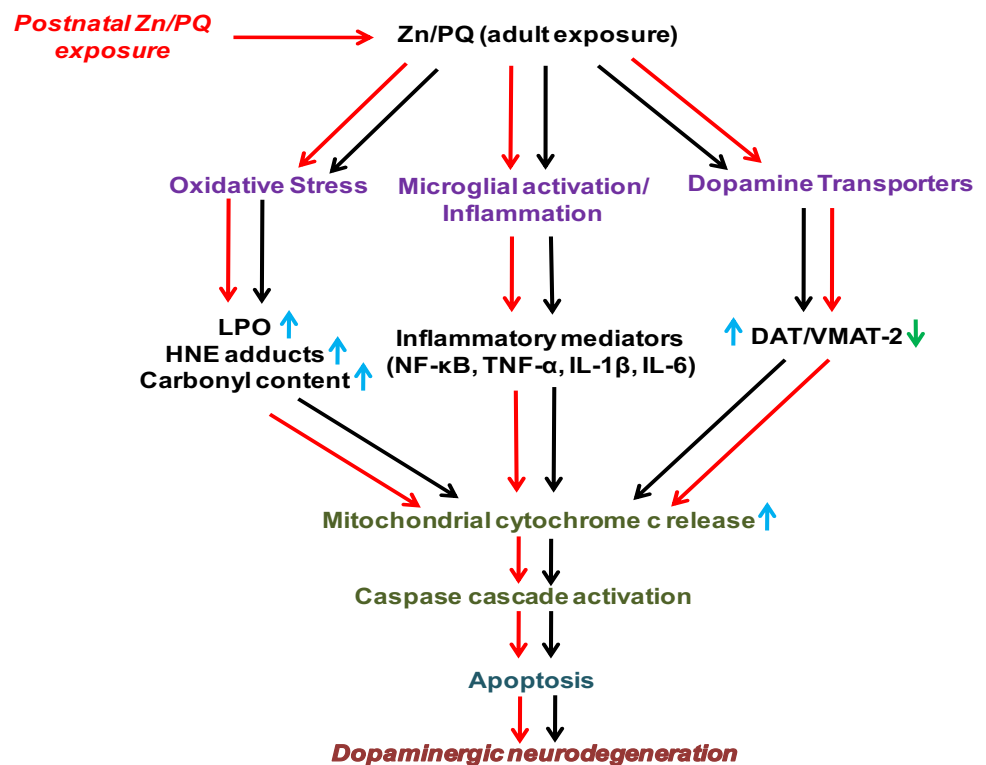
enzymatic endogenous antioxidant SOD might be an adaptive mechanism against elevated oxidative stress in the groups exposed during postnatal as well as adult life. Whilst further decline in GSH content in groups exposed both during developmental period and adulthood could plausibly be due to its increased consumption by GSH utilizing enzymes to combat elevated oxidative stress. Greater modulation in above-mentioned parameters in the groups exposed during postnatal period and adulthood suggest that developmental exposure results in irretrievable yet invisible changes, which later potentiate the neurotoxicity induced by Zn/PQ following re-exposure during adulthood [27, 29].

Microglial activation and inflammation enhance the pathophysiological aberrations in sporadic and toxicant-induced PD [34–36]. An increase in the number of activated microglia and augmented expression of microglial activation marker, *iba-1* and inflammatory mediators (NF- $\kappa$ B, TNF- $\alpha$ , IL-1 $\beta$  and IL-6) in adulthood Zn/PQ intoxicated groups indicate the role of microglia and neuroinflammation in the event as reported previously [15, 16, 18, 34]. The greater augmentation of microglial activation and inflammatory mediators in groups exposed to Zn/PQ in both developmental days and adulthood point towards their role in enhancing the Zn/PQ-induced neurotoxicity in the later life of exposed animals.

Monoamine transporters, DAT and VMAT-2, regulate dopamine content and alteration in their ratio results in dopamine dyshomeostasis, which is reported in chemical-induced PD models [25, 37]. Elevated protein expression of DAT and reduced VMAT-2 levels observed in Zn/PQ exposed adults indicated impaired dopamine homeostasis as documented previously [9, 15]. The developmental exposure amplified the Zn/PQ-induced modulations in DAT/VMAT-2 in re-challenged adult groups suggesting that postnatal exposure worsens the dopamine dyshomeostasis thereby making the nigrostriatal system more prone to neurodegeneration following adulthood re-exposure by Zn/PQ.

Apoptosis is documented to be major pathway responsible for neuronal cell death in idiopathic and chemical-induced

**Fig. 7** A flowchart depicting the effect of Zn/PQ postnatal exposure on dopaminergic neurodegeneration in rats re-challenged during adulthood. Black arrows show Zn/PQ-induced alterations following adult exposure only while red arrows indicate enhancement of Zn/PQ-induced modulations in the neurodegenerative indexes in animals pre-exposed during postnatal days. Upward blue arrows exhibit augmentation and downward green arrow depicts reduction in the given parameter



PD. The increased mitochondrial cyt c release along with diminished levels of pro-caspase-3/9 reaffirmed mitochondria-mediated apoptotic cell death in groups exposed to Zn/PQ in accordance with earlier reports [15, 16]. Developmental exposure to Zn/PQ showing greater mitochondrial cyt c release and caspase cascade activation in rats re-challenged during adulthood confirms that postnatal exposure increases the susceptibility of dopaminergic neurons to toxic insults during the latter phase of life as documented [20, 27, 33]. The results suggest that exposure during critical period of the brain development (5–19 days) causes undetectable but irreversible changes in the brain that makes it more vulnerable to toxic insults during adulthood (Fig. 7).

The developmental exposure increased the neurodegeneration via aggravation of Zn/PQ-induced modulations in aforementioned neurodegenerative indexes as compared with adult exposure alone; however, the groups exposed to PQ during both time periods exhibited the most pronounced neuronal damage followed by groups exposed to different agents during postnatal and adult life and subsequently the groups exposed to Zn during postnatal days and adult life. Several reasons could be responsible for this disparity. First of all, Zn is redox inert while PQ is a redox active molecule therefore; PQ itself converts into free radical and induces free radical generation thus causing greater oxidative stress than Zn [4]. PQ, being a xenobiotic its metabolism also results in free radical production as by-products, thereby elevating its neurotoxic potential as compared to Zn. The differential mechanisms of Zn and PQ-induced neurotoxicity could also be a plausible reason for variable neurotoxic effect [14, 38, 39]. Zn is an essential micronutrient therefore stringent regulatory mechanisms exist for controlling free intracellular  $[Zn^{2+}]$  concentration viz. metallothioneins, zinc transporters (ZnT) and zinc- and iron-like regulatory proteins (ZIPs), which guard against increasing Zn levels till certain extent thus regulating its neurotoxicity and rendering it less neurotoxic than PQ [40].

## Conclusion

Developmental exposure to low doses of Zn/PQ does not produce any visible effect but it can generate a signal that induces the oxidative stress, dopamine dyshomeostasis and inflammation leading to enhanced vulnerability of dopaminergic neurons upon adulthood if re-challenged with another agent in latter life.

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## Compliance with ethical standards

**Conflict of interest** The authors declare no conflicts of interest.

**Ethical approval** The study was carried out in compliance with the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA) after the approval by Institutional Animal Ethics Committee.

## References

- Dexter DT, Jenner P (2013) Parkinson disease: from pathology to molecular disease and mechanisms. *Free Radic Biol Med* 62:132–144
- Kalia LV, Lang AE (2015) Parkinson’s disease. *Lancet* 386:896–912
- Fleming SM (2017) Mechanisms of gene-environment interactions in Parkinson’s Disease. *Curr Environ Health Rep* 4:192–199
- Singh C, Ahmad I, Kumar A (2007) Pesticides and metals induced Parkinson’s disease: involvement of free radicals and oxidative stress. *Cell Mol Biol (Noisy-le-grand)* 53(5):19–28
- Goldman SM (2014) Environmental toxins and Parkinson’s disease. *Annu Rev Pharmacol Toxicol* 54:141–164
- Bjorklund G, Stejskal V, Urbina MA, Dadar M, Chirumbolo S, Mutter J (2018) Metals and Parkinson’s disease: mechanisms and biochemical processes. *Curr Med Chem* 25:2198–2214
- Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B (2009) Parkinson’s disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. *Am J Epidemiol* 169:919–926
- Bastias-Candia S, Zolezzi JM, Inestrosa NC (2019) Revisiting the paraquat-induced sporadic Parkinson’s disease-like model. *Mol Neurobiol* 56:1044–1055
- Kumar A, Ahmad I, Shukla S, Singh BK, Patel DK, Pandey HP, Singh C (2010) Effect of zinc and paraquat co-exposure on neurodegeneration: modulation of oxidative stress and expression of metallothioneins, toxicant responsive and transporter genes in rats. *Free Radic Res* 44:950–965
- Zhang XF, Thompson M, Xu YH (2016) Multifactorial theory applied to the neurotoxicity of paraquat and paraquat-induced mechanisms of developing Parkinson’s disease. *Lab Invest* 96:496–507
- Ossowska K, Wardas J, Kuter K, Nowak P, Dabrowska J, Bortel A, Labus L, Kwiecinski A, Krygowska-Wajs A, Wolfarth S (2005) Influence of paraquat on dopaminergic transporter in the rat brain. *Pharmacol Rep* 57:330–335
- Quiroga MJ, Carroll DW, Brown TM (2014) Ascorbate- and zinc responsive Parkinsonism. *Ann Pharmacother* 48(11):1515–1520
- Dexter DT, Carayon A, Javoy-Agid F, Agid Y, Wells FR, Daniel SE (1991) Alterations in the levels of iron, ferritin and other trace metals in Parkinson’s disease and other neurodegenerative diseases affecting the basal ganglia. *Brain* 114:1953–1975
- Kumar A, Singh BK, Ahmad I, Shukla S, Patel DK, Srivastava G, Kumar V, Pandey HP, Singh C (2012) Involvement of NADPH oxidase and glutathione in zinc-induced dopaminergic neurodegeneration in rats: similarity with paraquat neurotoxicity. *Brain Res* 1438:48–64



15. Kumar V, Singh BK, Chauhan AK, Singh D, Patel DK, Singh C (2016) Minocycline rescues from zinc-induced nigrostriatal dopaminergic neurodegeneration: biochemical and molecular interventions. *Mol Neurobiol* 53:2761–2777
16. Chauhan AK, Mittra N, Patel DK, Singh C (2018) Cyclooxygenase-2 directs microglial activation-mediated inflammation and oxidative stress leading to intrinsic apoptosis in Zn-induced Parkinsonism. *Mol Neurobiol* 55(3):2162–2173
17. Singh BK, Kumar A, Ahmad I, Kumar V, Patel DK, Jain SK, Singh C (2011) Oxidative stress in zinc-induced dopaminergic neurodegeneration: implications of superoxide dismutase and heme oxygenase-1. *Free Radic Res* 45(10):1207–1222
18. Chauhan AK, Mittra N, Kumar V, Patel DK, Singh C (2016) Inflammation and B-cell lymphoma-2 associated X protein regulate zinc-induced apoptotic degeneration of rat nigrostriatal dopaminergic neurons. *Mol Neurobiol* 53(8):5782–5795
19. Jomova K, Vondrakova D, Lawson M, Valko M (2010) Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* 345(1–2):91–104
20. Cory-Slechta DA, Thiruchelvam M, Richfield EK, Barlow BK, Brooks AI (2005) Developmental pesticide models of the Parkinson's disease phenotype. *Environ Health Perspect* 113:1263–1270
21. Patel S, Singh V, Kumar A, Gupta YK, Singh MP (2006) Status of antioxidant defense system and expression of toxicant responsive genes in striatum of maneb- and paraquat-induced Parkinson's disease phenotype in mouse: mechanism of neurodegeneration. *Brain Res* 1081:9–18
22. Peng J, Peng L, Stevenson FF, Doctrow SR, Andersen JK (2007) Iron and paraquat as synergistic environmental risk factors in sporadic Parkinson's disease accelerate age-related neurodegeneration. *J Neurosci* 27:6914–6922
23. Hussain S, Ali SF (2002) Zinc potentiates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine induced dopamine depletion in caudate nucleus of mice brain. *Neurosci Lett* 335:25–28
24. Lo HS, Chiang HC, Lin AMY, Chiang HY, Chu YC, Kao LS (2004) Synergistic effects of dopamine and Zn<sup>2+</sup> on the induction of PC12 cell death and dopamine depletion in the striatum: possible implication in the pathogenesis of Parkinson's disease. *Neurobiol Dis* 17:54–61
25. Richardson JR, Caudle WM, Wang M, Dean ED, Pennell KD, Miller GW (2006) Developmental exposure to the pesticide dieldrin alters the dopamine system and increases neurotoxicity in an animal model of Parkinson's disease. *FASEB J* 20:1695–1697
26. Slotkin TA, Seidler FJ (2011) Developmental exposure to organophosphates triggers transcriptional changes in genes associated with Parkinson's disease in vitro and in vivo. *Brain Res Bull* 86:340–347
27. Singh AK, Tiwari MN, Upadhyay G, Patel DK, Singh D, Prakash O, Singh MP (2012) Long term exposure to cypermethrin induces nigrostriatal dopaminergic neurodegeneration in adult rats: post-natal exposure enhances the susceptibility during adulthood. *Neurobiol Aging* 33:404–415
28. Tartaglione AM, Venerosi A, Calamandrei G (2016) Early-life toxic insults and onset of sporadic neurodegenerative diseases—an overview of experimental studies. *Curr Top Behav Neurosci* 29:231–264
29. Richardson JR, Caudle WM, Wang MZ, Dean ED, Pennell KD, Miller GW (2008) Developmental hepatochlor exposure increases susceptibility of dopamine neurons to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in a gender specific manner. *Neurotoxicology* 29:855–863
30. Fan LW, Tien LT, Lin RC, Simpson KL, Cai RPG, Cai Z (2011) Neonatal exposure to lipopolysaccharide enhances vulnerability of nigrostriatal dopaminergic neurons to rotenone neurotoxicity in later life. *Neurobiol Dis* 44:304–316
31. Tiwari MN, Singh AK, Agrawal S, Gupta SP, Jyoti A, Shanker R, Prakash O, Singh MP (2012) Cypermethrin alters the expression profile of mRNAs in the adult rat striatum: a putative mechanism of postnatal pre-exposure followed by adulthood re-exposure-enhanced neurodegeneration. *Neurotox Res* 22(4):321–334
32. Jia Z, Misra HP (2007) Developmental exposure to pesticides zineb and/or endosulfan renders the nigrostriatal dopamine system more susceptible to these environmental chemicals in later life. *Neurotoxicology* 28:727–735
33. Cai Z, Fan LW, Kaizaki A, Tien LT, Ma T, Pang Y, Lin S, Lin RC, Simpson KL (2013) Neonatal systemic exposure to lipopolysaccharide enhances susceptibility of nigrostriatal dopaminergic neurons to rotenone neurotoxicity in later life. *Dev Neurosci* 35:155–171
34. Singh AK, Tiwari MN, Dixit A, Upadhyay G, Patel DK, Singh D, Prakash O, Singh MP (2011) Nigrostriatal proteomics of cypermethrin-induced dopaminergic neurodegeneration: microglial activation-dependent and-independent regulations. *Toxicol Sci* 122:526–538
35. Bartels AL, Leenders KL (2007) Neuro-inflammation in the pathophysiology of Parkinson's disease: evidence from animal models to human in vivo studies with [11C]-PK11195 PET. *Mov Disord* 22:1852–1856
36. Yuan YH, Sun JD, Wu MM, Hu JF, Peng SY, Chen NH (2013) Rotenone could activate microglia through NF- $\kappa$ B pathway. *Neurochem Res* 38:1553–1560
37. Tiwari MN, Singh AK, Ahmad I, Upadhyay G, Singh D, Patel DK, Singh C, Prakash O, Singh MP (2010) Effects of cypermethrin on monoamine transporters, xenobiotic metabolizing enzymes and lipid peroxidation in the rat nigrostriatal system. *Free Radic Res* 44(2):1416–1424
38. Gupta SP, Patel S, Yadav S, Singh AK, Singh S, Singh MP (2010) Involvement of nitric oxide in maneb- and paraquat-induced Parkinson's disease phenotype in mouse: is there any link with lipid peroxidation? *Neurochem Res* 35(8):1206–1213
39. Singh BK, Kumar V, Chauhan AK, Dwivedi A, Singh S, Kumar A, Singh D, Patel DK, Ray RS, Jain SK, Singh C (2017) Neuronal nitric oxide negatively regulates zinc-induced nigrostriatal dopaminergic neurodegeneration. *Mol Neurobiol* 54:2685–2696
40. Portbury SD, Adlard PA (2017) Zinc signal in brain diseases. *Int J Mol Sci* 18:2506

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