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The effect of Puerarin on 3-Nitropropionic acid-induced neurotoxicity.

A thesis submitted in partial fulfillment of the requirements for Doctor of Philosophy Degree in Pharmaceutical Sciences
(Pharmacology & Toxicology)

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ORIGINAL PAPER

Puerarin Ameliorates 3-Nitropropionic Acid-Induced Neurotoxicity in Rats: Possible Neuromodulation and Antioxidant Mechanisms

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Abstract Puerarin (daidzein-8-C-glucoside), a major isoflavone glycoside purified from *Pueraria lobata*, is well reported to have a neuroprotective effect primarily by the antioxidant mechanisms. This investigation was designed to evaluate the efficacy of Puerarin (Pur) to offset 3-nitropropionic acid (3-NP) induced neurotoxicity. Male Wistar strain rats were given 3-NP (20 mg/kg, s.c.) over five consecutive days, whereas Pur (200 mg/kg, i.p.) was administrated 30 min before 3-NP. Rats treated with 3-NP exhibited significant weight loss, reduction of the prepulse inhibition, locomotor hypoactivity and hypothermia. The striata, hippocampi and cortices of the 3-NP treated rats showed abnormal levels of neurotransmitters, oxidative damage and characteristic histopathological lesions. Treatment with Pur ahead of 3-NP, significantly prevented weight loss, PPI deficit, locomotor hypoactivity and hypothermia. Pur treatment blocked the 3-NP-induced neurotransmitters abnormalities (GABA, DA, 5-HT and NE), and normalized the oxidative stress biomarkers (lipid peroxidation, reduced glutathione, glutathione peroxidase). Histopathological examination further affirmed Pur's neuroprotective effect against 3-NP-induced neurotoxicity. In conclusion, Pur protected the brain tissues from 3-NP induced neurotoxicity primarily by its neuromodulation and antioxidant effect.

Keywords Huntington's disease · Puerarin · 3-Nitropropionic acid · Prepulse inhibition · Antioxidant

Abbreviations

3-NP	3-Nitropropionic acid
5-HT	Serotonin
6-OHDA	6-Hydroxydopamine
BSA	Bovine serum albumin
DA	Dopamine
dB	Decibel
GPx	Glutathione peroxidase
GSH	Glutathione
HD	Huntington's disease
HPLC–ED	High performance liquid chromatography with electrochemical detector
MDA	Malondialdehyde
NE	Norepinephrine
PEG	Polyethylene glycol
PPI	Prepulse inhibition
Pur	Puerarin
ROS	Reactive oxygen species
SDH	Succinate dehydrogenase
TBA	Thiobarbaturic acid
TBARS	Thiobarbaturic acid reactive substances

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Introduction

3-Nitropropionic acid (3-NP) is a natural neurotoxin produced by fungus Arthrinium on moldy crops, e.g. sugarcane or peanuts [1]. The main mechanism of 3-NP-induced neurotoxicity involves the irreversible inhibition of succinate dehydrogenase (SDH), a key enzyme in kreb's cycle, which results in energy impairment and oxidative stress [2]. The administration of 3-NP in rodents and non-human primates has been proposed as a useful experimental model of Huntington disease (HD); both biochemical and behavioral characteristics observed in HD patients are reproduced in this model [3–7].

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder, characterized by behavioral and psychiatric problems. Many reports suggest that oxidative stress plays a key role in the pathogenesis of HD [8–10].

Radiax Puerariae, the dried root of *Pueraria lobata* Ohwi, is one of earliest and most important edible crude herbs used for various medical purposes in Oriental medicine [11]. In China, Radiax puerariae is known as Ge Gen, and has been used as a traditional medicine for treating various diseases including cardiovascular disorders such as angina and myocardial infarction [12]. Puerarin (Pur; daidzein-8-C-glucoside) is the major isoflavonoid derived from Radix puerariae (kudzu root). Puerarin is present in the greatest amount of around 1.88–2.55 % (w/w). Such a high content of active compound in natural plant is rather rare. Some clinical proofs indicated that Puerarin could be used for the treatment of diabetes mellitus, hypertension and other heart related diseases including cardiac arrhythmia or pulmonary heart [13].

In the past decade, the neuroprotective effect of Pur was proven against 6-hydroxydopamine-induced neurotoxicity via its antioxidant and antiapoptotic mechanisms [14–16]. Furthermore, a recent study showed that, Puerarin (200 mg/kg, i.p) significantly reduce traumatic brain injury-induced neuronal degeneration, by its antioxidant properties [17]. Therefore, Puerarin attracts a lot of attention globally due to its high content as well as various bioactivities [13]. Based on this background, the present study has been designed to explore the effect of Pur against 3-NP induced psychopharmacological, neurochemical and histopathological abnormalities in the striata, hippocampi and cortices of rats' brain.

Materials and Methods

Animals

Adult male albino Wistar rats, 5 months of age and weighing 250–300 g, were housed four per cage in a room maintained at constant temperature (24 ± 2 °C) with alternating 12 h light/dark cycles where animal chow and water were provided ad libitum. On the day of the experiment, animals were brought to the experimental room and allowed to habituate to the environmental conditions for a period of approximately 60 min before the beginning of the experiment. All animal treatments adhered strictly to institutional and international ethical guidelines for the care and use of laboratory animals. The experimental protocol was approved by Ain Shams University Faculty of Pharmacy Review Committee for the use of animal subjects.

Drugs and Chemicals

3-Nitropropionic acid was purchased from Sigma-Aldrich, USA, thiobarbituric acid (TBA) and 1, 1′, 3, 3′-tetramethoxypropane, reduced glutathione (GSH), Elman's reagent [5,5-dithio-bis (2-nitrobenzoic acid); DTNB], HPLC chemicals were all purchased from Sigma Chemical Co., St Louis, MO, USA. Puerarin (99 %) was purchased from Shaanxi Jintai Biological Engineering Co., Ltd., China. A glutathione peroxidase activity detection kit was purchased from RANDOX Laboratories Ltd., UK. The rest of the chemicals were all of the highest commercial grade.

Puerarin dose selection was done depending on our preliminary experiments taking guide by previous reports [18–20]. Searching literature, there was no definite conclusion about Pur dose in animal experiments. However, the therapeutic dose of Pur according to the description, is 200–400 mg per day [21]. Pur was dissolved in PEG 400: distilled water (1:1) [22, 23], and given intraperitoneally (i.p.) in a dose of 200 mg/kg body weight, 30 min before 3-NP administration, for five consecutive days. The 3-NP was dissolved in saline, pH adjusted to 7.4 using 1 M NaOH and was administered subcutaneously (s.c.) in a dose of 20 mg/kg body weight daily for five consecutive days [24–26]. Drugs or vehicles were administered in a volume of 1 ml/kg body weight. Control animals received respective solvent injections, and were run concurrently with drug-treated groups.

Experimental Groups

Rats were divided into four groups (for each group, n = 6). The control group received PEG400: distilled water (1:1) i.p. then normal saline (s.c.), 30 min later for 5 days. The 3-NP treated group received PEG400: distilled water (1:1) i.p then 3-NP (20 mg/kg, s.c.), 30 min later. The Pur and 3-NP treated group received Pur (200 mg/kg, i.p.) then 3-NP (20 mg/kg, s.c.) 30 min later. The Pur alone treated group received Pur (200 mg/kg, i.p.) then normal saline (s.c.), 30 min later. Locomotor activity was determined for all groups a day before the experiment (day 0) and 3.5 h after drug treatments at days 1–5 of the experiment [27].

Percentage PPI of acoustic startle response was assessed 215 min after treatments at day 2 [27]. On day 5, animals were sacrificed by decapitation and skulls were split on an ice and salt mixture. The cortices, striata and hippocampi were dissected out, rapidly stored at −80 °C. In another series of experiments, rats were subjected to the same treatment conditions as previously described; sacrificed and whole brains were fixed in 4 % formol saline for the preparation of sections for histopathological analysis.

$$\%\,\mathrm{PPI} = \left(\frac{\text{Average startle amplitude on pulse alone sessions} - \text{average startle amplitude on prepulse/pulse sessions}}{\text{Average startle amplitude on pulse alone sessions}}\right) \times 100$$

Measurement of the Body Weights

Animals' body weights were recorded on the first and last day of experimentation [28]. Percent change in the body weights were calculated according to the following equation:

$$\%\,\text{change in body weight} = \frac{\text{Body weight on the last day} - \text{body weight on the first day}}{\text{body weight on the first day}} \times 100$$

Measurement of the Rectal Temperature

Rectal temperature was monitored 3 h after the second 3-NP injection [29, 30].

Prepulse Inhibition (PPI) of Acoustic Startle Response Measurement

Startle responses were measured using Responder X apparatus (Columbus, Ohio, USA) which consists of Plexiglas cages and force platforms that are equipped with precise load cells to be used as sensors. Animal movement on the platform develops a transient force, which can be transduced by an accelerometer into a voltage that is proportional to the displacement velocity, which is measured at its peak (negative or positive). These signals were amplified, digested, and fed into a data-acquisition board in a computer for further analysis. For acoustic startle measurements, animal cages were housed in a sound-attenuating chamber with a high frequency speaker located on the side of each cage. The high frequency speaker delivered the acoustic stimulus in a background noise level of 70 dB. After a 5 min acclimatization period, during which time there was no stimulus, each rat received 36 sessions of either pulse alone or prepulse/pulse sessions presented in a random order with an inter-stimulus interval of 29 s. In the pulse alone sessions, animals received 120 dB stimuli for 40 ms. In the prepulse/pulse sessions, animals received 76 dB prepulse for 20 ms followed 100 ms later by the stimulus [26, 27, 31]. PPI response was calculated according to Van den Buuse and Eikelis [32] where

Locomotor Activity Measurement

Activity monitor (Opto-Varimex-Mini Model B, Columbus Instruments, Ohio, USA) was used to measure the locomotor activity of animals. The total locomotor activity of the animals was expressed as counts/5 min. Locomotor activity was determined for all groups a day before the experiment and 3.5 h after drug treatments at days 1–5 of the experiment [27].

Neurotransmitter Level Measurements: Gamma-Aminobutyric Acid (GABA), Dopamine (DA), Serotonin (5-HT) and Norepinephrine (NE)

The concentrations of GABA, DA, 5-HT and NE were determined in striata, hippocampi and cortices, using high-performance liquid chromatography with electrochemical detection (HPLC–ED) [33]. Briefly brain samples were homogenized in 0.1 M perchloric acid. Samples were then centrifuged at 24,000×*g* at 4 °C for 15 min. The supernatants were further filtered through 0.25-μm nylon filters before injecting in the HPLC (GBC System, Australia; pump LC 1150, electrochemical detector LC 1260) fitted with glassy carbon working electrode. The voltage setting was +0.65 V for monoamines, and +0.50 V for GABA, vs. an Ag/AgCl reference electrode. The detector response was plotted and measured using a chromatointegrator. The concentrations of monoamines and GABA in each sample were calculated from the integrated chromatographic peak area.

Assessment of the Oxidative Stress Biomarkers

Oxidative stress was assessed in striata, hippocampi and cortices tissue lysate. The levels of malondialdehyde (MDA), as a measure of lipid peroxidation, were performed

according to the method of Mihara and Uchiyama [34] and were expressed in nanomoles per gram tissue (nmol/g tissue). Determination of the Reduced Glutathione (GSH) levels were carried out according to Ellman [35] and the results were expressed in nanomole/g tissue. The Glutathione peroxidase (GPx) enzyme activity was measured according to Paglia and Valentine [36], using Glutathione peroxidase activity detection kit from RANDOX Laboratories Ltd., UK.

Pathological Examination

For histopathological examination, Brains were immersion-fixed in 10 % buffered paraformaldehyde, embedded in paraffin wax, cut into 4 μm-thick sections. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin, then evaluated using light microscope [37].

Statistical Analysis

Statistical analysis was performed using a software program (GraphPad Prism, version 5.0, Inc. 2007). Comparisons between means of data were analyzed by one-way analysis of variance (ANOVA). If the overall F-value was found to be statistically significant, post hoc comparisons among groups were made according to Tukey–Kramer's test. The level of statistical significance was set at $P < 0.05$. All results are expressed as mean ± SD.

Results

Effect of Pur on Body Weight of the 3-NP-Treated Rats

Treatment with 3-NP caused significant reduction in rats' body weights compared to the control group ($P < 0.001$) (Fig. 1, panel a). Pur treatment ahead of 3-NP significantly prevented this weight loss compared to the 3-NP treated group ($P < 0.001$). As for the Pur alone treated group, no significant weight loss was observed compared to the control group.

Effect of Pur on Rectal Temperature of the 3-NP-Treated Rats

The 3- NP treated rats experienced significant hypothermia, which averaged a loss of 3 °C or more in core body temperature, 3 h after the second 3-NP dose (20 mg/kg body weight, s.c.), compared to the control group ($P < 0.001$) (Fig. 1, panel b). Pur treatment ahead of 3-NP significantly prevented this hypothermia ($P < 0.001$), although didn't fully restore normal body temperature compared to the control group. The Pur alone treated rats showed normal body temperature.

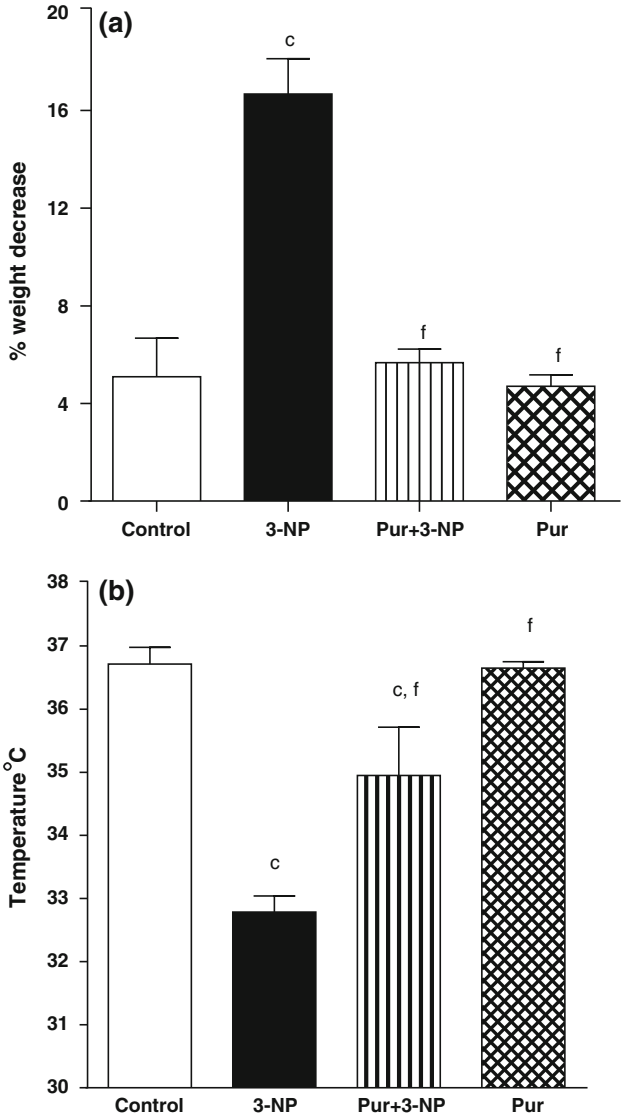

Fig. 1 **a** Effect of Pur on weight loss in the 3-NP treated rats. 3-NP was administered s.c. (20 mg/kg) for five consecutive days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. Weight measurements were done before first dose and at the end of the experiment. Data are presented as means ± SD $^{c}P < 0.001$ versus control group; $^{f}P < 0.001$ versus 3-NP group. (One-way ANOVA followed by Tukey's test). **b** Effect of Pur on body temperature in the 3-NP treated rats. 3-NP was administered s.c. (20 mg/kg) for five consecutive days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. Rectal temperature was measured 3 h after second 3-NP dose. Data are presented as means ± SD $^{c}P < 0.001$ versus control group; $^{f}P < 0.001$ versus 3-NP group. (One-way ANOVA followed by Tukey's test)

Effect of Pur on PPI of Acoustic Startle Response in the 3-NP-Treated Rats

Systemic 3-NP treatment significantly impaired PPI, 215 min after second dose (20 mg/kg body weight, s.c.) as compared to the control group ($P < 0.001$) (Fig. 2). Pur treatment (200 mg/kg body weight, i.p.) ahead of 3-NP

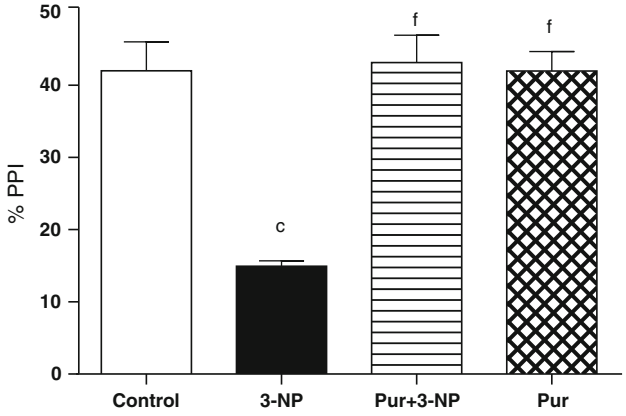

Fig. 2 Effect of Pur on PPI of acoustic startle response in the 3-NP treated rats. 3-NP was administered s.c. (20 mg/kg) for five consecutive days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. PPI was evaluated 215 min after the second 3-NP dose. Data are presented as means ± SD $^{c}P < 0.001$ versus control group; $^{f}P < 0.001$ versus 3-NP group. (One-way ANOVA followed by Tukey's test)

significantly prevented this PPI deficit as compared to 3-NP treated group ($P < 0.001$). Pur alone treated rats showed normal PPI response as compared to the control group.

Effect of Pur on Locomotor Activity in the 3-NP-Treated Rats

Systemic 3-NP treatment (20 mg/kg body weight, s.c.) significantly impaired rats' locomotor activity at days 2, 3, 4, and 5, as compared to the control group ($P < 0.001$) (Fig. 3). Notably, Pur treatment (200 mg/kg body weight, i.p.) ahead of 3-NP significantly improved rats' locomotor activity as compared to the corresponding 3-NP treated group ($P < 0.001$). Treatment with Pur alone did not produce any significant effect on rats' locomotor activity as compared to the control group.

Effect of Pur on the Neurotransmitters' Levels: GABA, DA, 5-HT and NE

Systemic 3-NP (20 mg/kg body weight, s.c. for 5 consecutive days) administration significantly decreased GABA, DA, 5-HT and NE in striata, hippocampi and cortices (Fig. 4, panels a-d). These reductions were significantly reversed by Pur (200 mg/kg body weight, i.p for 5 days) treatment ahead of 3-NP as compared to the 3-NP treated group. Treatment with Pur alone did not alter the levels of GABA or NE, as compared to control group (Fig. 4, panels a and d, respectively). However, dopamine (DA), and serotonin (5-HT) levels were significantly elevated by Pur (200 mg/kg for 5 consecutive days) as compared to control group (Fig. 4, panels b & c, respectively).

Effect of Pur on Oxidative Stress Biomarkers in the 3-NP-Treated Rats

Treatment of rats with 3-NP caused intense oxidative stress in the striata, hippocampi and cortices, characterized by a significant increase in lipid peroxidation products (MDA) ($P < 0.001$), a significant depletion of reduced glutathione (GSH) ($P < 0.001$) and a significant reduction of glutathione peroxidase (GPx) activity ($P < 0.001$) as compared to the control group (Fig. 5). Pur treatment ahead of 3-NP significantly blocked this oxidative damage and normalized the oxidative parameters as compared to the 3-NP treated group ($P < 0.001$). Meanwhile, Pur alone treatment

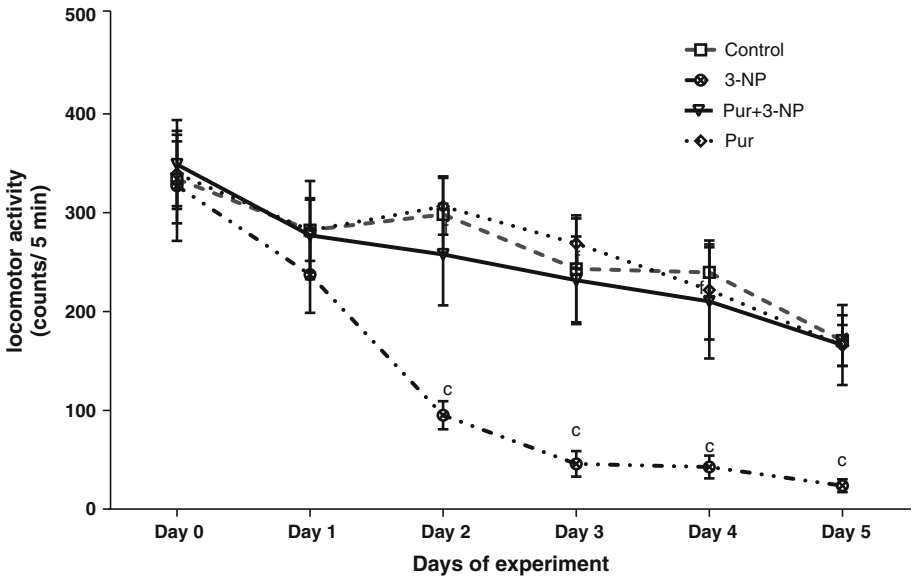

Fig. 3 Effect of Pur on locomotor activity in the 3-NP treated rats. 3-NP was administered s.c. (20 mg/kg) for five consecutive days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. Total locomotor count was assessed 1 day before start of the experiment and 3.5 h after each 3-NP administration. Data are presented as means ± SD $^{c}P < 0.001$ versus control group; $^{f}P < 0.001$ versus 3-NP group. (One-way ANOVA followed by Tukey's test)

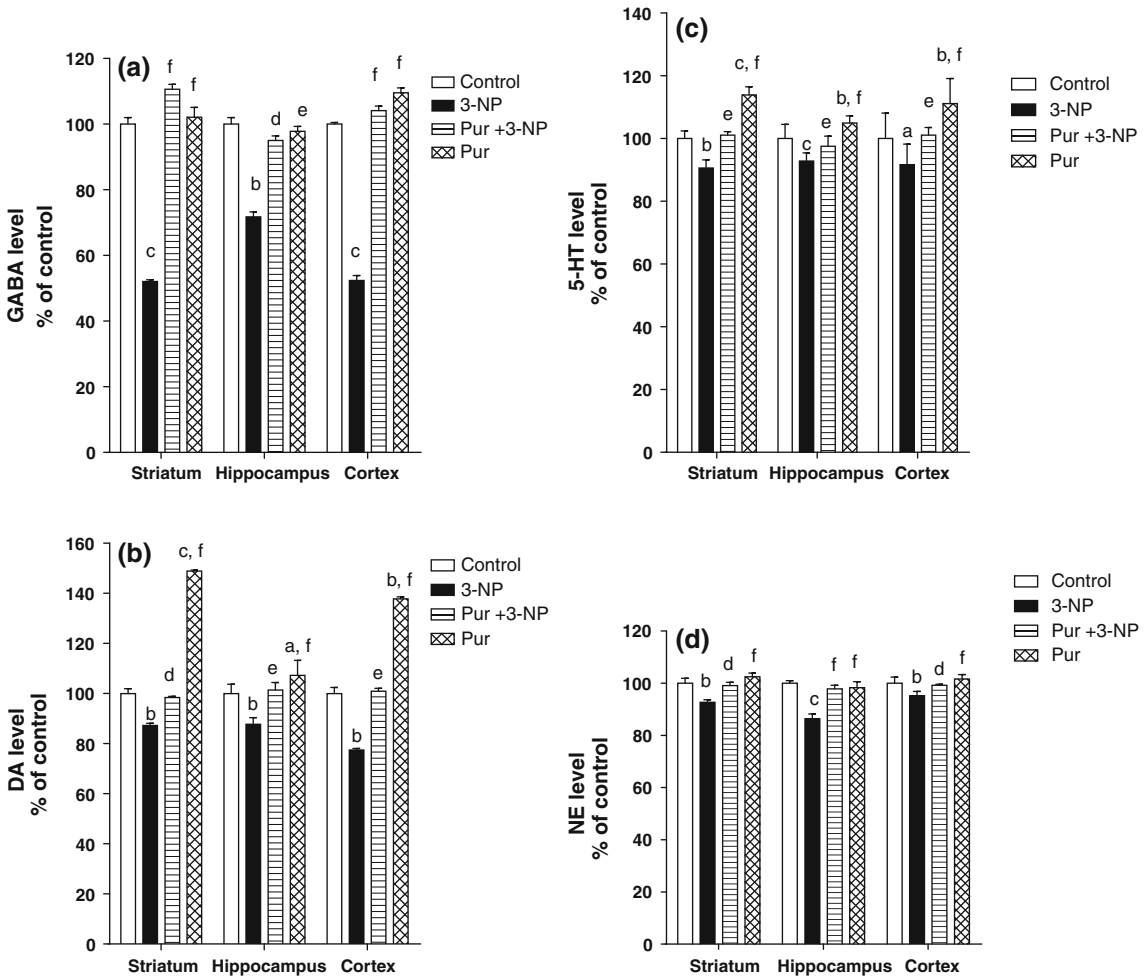

Fig. 4 Effect of Pur on GABA, dopamine (DA), serotonin (5-HT) and norepinephrine (NE) levels in the 3-NP treated rats. 3-NP was administered s.c. (20 mg/kg) for 5 consecutive days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. Data are presented as means ± SD. $^{a}P < 0.05$, $^{b}P < 0.01$ and $^{c}P < 0.001$ compared to vehicle-treated group. $^{d}P < 0.05$, $^{e}P < 0.01$ and $^{f}P < 0.001$ compared to 3-NP-treated group. (One-way ANOVA followed by Tukey's test)

showed no significant effect on the oxidative parameters as compared to the control group.

Histopathological Examination

Systemic administration of 3-NP resulted in various pathological injuries including:

Focal and diffuse gliosis in the cerebrum, striatum and hippocampus associated with cellular oedema (Fig. 6, panels 5 and 6, respectively). Hemorrhages were noticed in the fissure between the cerebrum and cerebellum, as well as in the meninges covering the cerebellum with congestion in the cerebellar blood capillaries (Fig. 6, panels 7 and 8, respectively). The medulla oblongata showed also focal hemorrhages in wide manner (Fig. 6, panel 9).

Pur treatment ahead of 3-NP prevented these pathological injuries to a large degree. As only congestion in the blood capillaries of the medulla oblongata was observed in the Pur and 3-NP treated group (Fig. 6, panel 10).

As for Pur alone treated rats, mild congestion was noticed in the cerebral blood capillaries, while the cerebellum did not show any histopathological finding (Fig. 6, panels 11 & 12).

The control group showed no histopathological alteration in the meninges, cerebral cortex, hippocampus, cerebellum and medulla oblongata (Fig. 6, panels 1-4).

Discussion

3-Nitropropionic acid (3-NP) is a valuable tool to mimic HD and to help in developing new therapies [26, 38]. In the present study, 3-NP treatment significantly caused weight loss, hypothermia, PPI deficit, locomotor hypoactivity, neurotransmitters abnormality and oxidative damage, accompanied

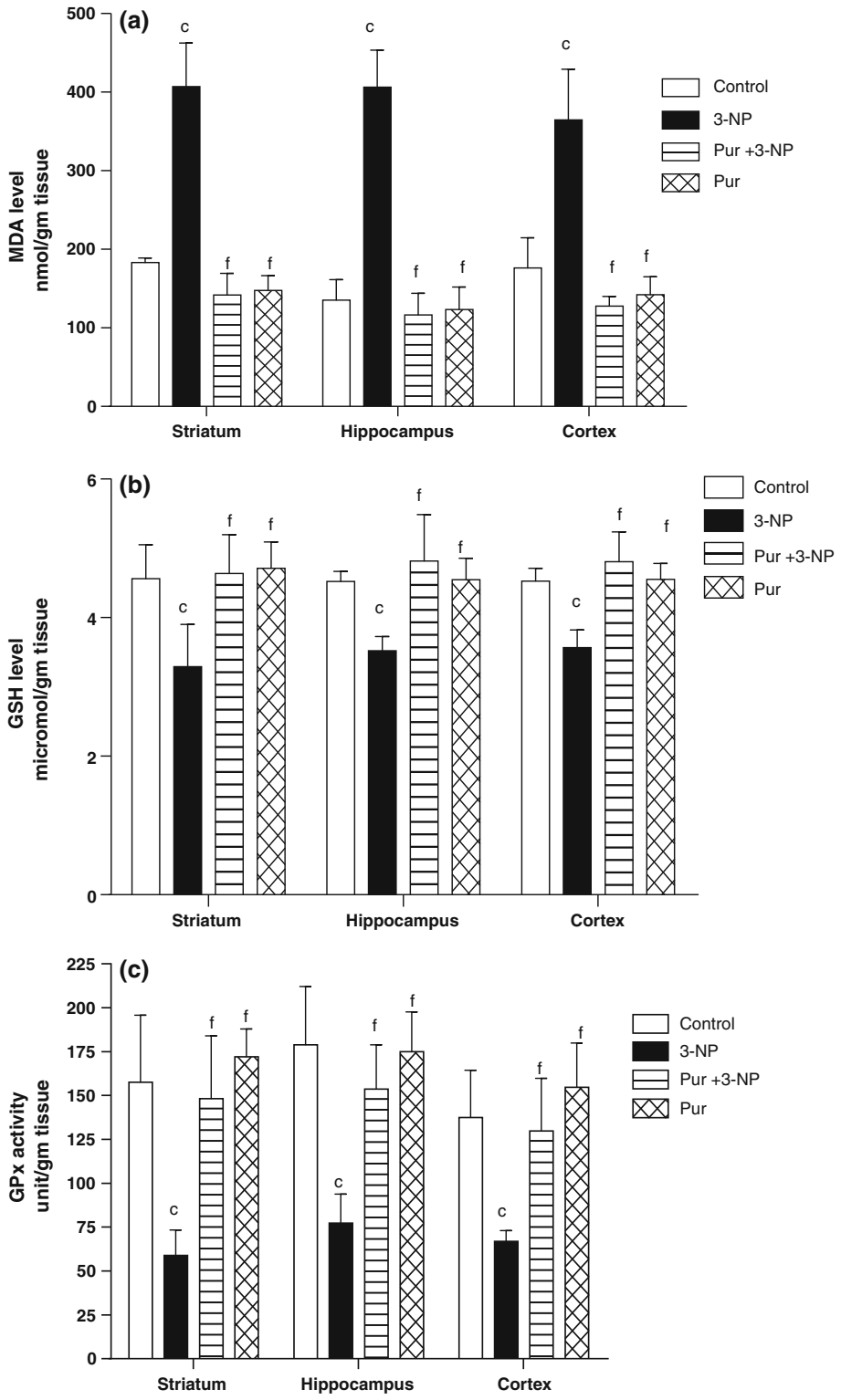

Fig. 5 Effect of Puerarin on striatal, hippocampal and cortical levels/activities of MDA, GSH and GPx in the 3-NP-treated rats. 3-NP was administered s.c. (20 mg/kg) for 5 days. Pur was administered at a dose of 200 mg/kg, i.p., 30 min before 3-NP. Data are presented as means SD. $^{c}P < 0.001$ compared to vehicle-treated group. $^{f}P < 0.001$ compared to 3-NP-treated group. (One-way ANOVA followed by Tukey's test)

with pathological injuries. Puerarin (Pur) psychopharmacological, neurochemical and antioxidant, as well as histopathological protection against 3-NP-induced neurotoxicity in the striata, hippocampi and cortices of rats' brains were all explored.

In this study, 3-NP treated rats suffered from a significant weight reduction and locomotor hypo-activity mimicking HD patients, consistent with various previous reports[31, 39–41]. Loss of body weight may be either partially due to factors outside the CNS or blockade of

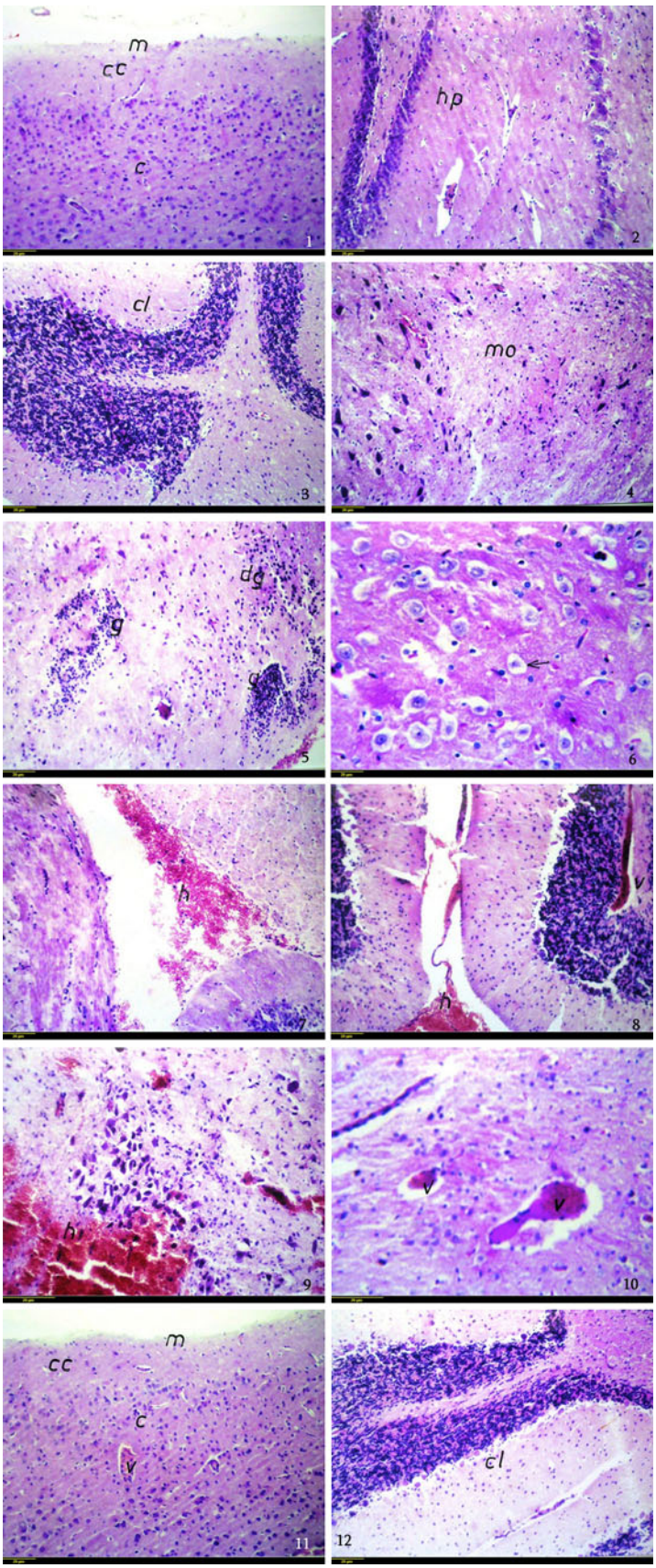

Fig. 6 Histopathological findings in rats' brains of different treatment groups. Brain sections from the control rats showing normal histological structure of (*1*) the cerebral cortex (cc) and meninges (m) (40X), (*2*) Striatum and part of the hippocampus (hp) (40X) and (*3*) Cerebellum (cl) (40X), (*4*) Medulla oblongata (mo) (40X). Brain sections from the 3-NP treated rats showing (*5*) focal (g) and diffuse gliosis (df) in the cortex and striatum (40X), (*6*) Cellular oedema in the striatum (*arrow*) (80X), (*7*) hemorrhage in the fissure between the cerebrum and cerebellum (h) (40X), (*8*) Hemorrhage in the meninges and fissure of the cerebellum (h) with congestion in the cerebellar blood vessels (v) (40X). (*9*) Hemorrhage in the medulla oblongata (h) (64X). Brain sections from the Pur and 3-NP treated rats showing (*10*) Congestion in the blood capillaries of the medulla oblongata (v) (64X). Brain sections from the Pur alone treated rats showing (*11*) Mild congestion in the cerebral capillaries (v) (40X). And (*12*) Normal histological structure of the cerebellum (40X). (*scale bar* 20 μm)

SDH enzyme in the Krebs cycle by 3-NP which ultimately cause ATP loss (the major energy source for living organisms). It has also been reported that striatal lesions and bradykinesia is responsible for reducing rat appetite and food intake [42, 43].

Progressive hypothermia, which reached a loss of 3 °C or more in core body temperature 3 h after second dose of 3-NP (20 mg/kg, s.c.) was observed. The hypothermic effect of 3-NP may reflect the acute condition of mitochondrial dysfunction and reduction of cellular energy [29, 30].

Pur treatment ahead of 3-NP prevented the weight loss and locomotor hypoactivity and significantly alleviated the hypothermia to a lesser degree. Treatment with Pur alone did not cause any significant weight loss, locomotor hypoactivity, hypothermia or abnormal behavior, compared to the control group, in consistent with previous report by Chang et al. [44].

In this study, a significant impairment in Prepulse inhibition (PPI) of acoustic startle response was observed in 3-NP treated rats, 215 min after the second 3-NP dose (20 mg/kg, s.c.). Prepulse inhibition of the startle reflex is an operational measure of sensorimotor gating, in which the motor response to an abrupt, intense stimulus is inhibited by a weak lead stimulus [45]. PPI deficit was evident in patients with HD as well as the 3-NP animal models [27, 46, 47]. Pur treatment ahead of 3-NP prevented the PPI deficit, providing an indication that Pur protected neural circuitry of PPI [48, 49]. GABAergic, Dopaminergic and serotonergic as well as noradrenergic activity have been demonstrated to be important substrates modulating PPI and locomotor activity in rats [48, 50, 51]. GABA and GABA receptors in different brain areas regulate the PPI as demonstrated by many previous reports [52, 53]. Systemic administration of the mitochondrial neurotoxin 3-NP induced loss of GABAergic cells that form the striatopallidal efferent projection and caused PPI deficit of both acoustic and tactile startle [25, 31, 54]. Dopamine (DA) is a main neurotransmitter in the striatum and medial prefrontal cortex, which has a major role in controlling motor coordination as well as startle response. As previously shown, the depletion of nigrostriatal DA via 6-OHDA (6-hydroxydopamine) lesions causes profound catalepsy but no significant reduction in PPI, while depletion of DA from the medial prefrontal cortex causes no generalized motor debilitation but significantly disrupts PPI [55, 56]. As for 5-HT role, Prinssen et al. [51] and Fletcher et al., [57] proved that reduction of brain 5-HT disrupts Prepulse inhibition of the acoustic startle reflex in rats. Norepinephrine (NE) has a well-known role in locomotor activity since Segal and Mandell [58] showed that intraventricular infusion of NE increased the locomotor activity of rats. Thus, Decreases in the GABA, DA, 5-HT and NE levels may be associated with PPI deficit and the locomotor hypoactivity in 3-NP neurotoxicity [50, 56, 59].

In this study, GABA, DA, NE and 5-HT assessment revealed that 3-NP treatment (20 mg/kg body weight, s.c. for 5 consecutive days) induced a significant depletion in GABA, DA, NE and 5-HT in striata, hippocampi and cortices of rats which may explain the PPI deficit and locomotor hypoactivity induced by the 3-NP. Previously, Ahuja et al. [40], reported a significant decrease in the levels of DA, NE and 5-HT in striata after Sub-chronic administration of 3-NP and suggested that this decrease may be due to increase in the metabolism of DA and NE, resulting in generation of free radicals. Kumar et al. [28] also, demonstrated reduction of DA and NE in all regions of the rats' brain treated with 3-NP.

Pur treatment ahead of 3-NP protected the neurotransmitters from depletion and maintained PPI at the normal value as compared to the control group. Treatment with Pur alone had no effect on PPI or the levels of GABA and NE, as compared to control group. However, DA, and 5-HT levels were significantly elevated by Pur which may be attributed to DA, and 5-HT metabolism inhibition by Pur as reported by Keung and Vallee [60]. These finding suggest the neuromodulation role of Pur neuroprotectrion against 3-NP induced neurotoxicity.

Mitochondrial dysfunction linked to the increased oxidative stress can be considered one of the major events underlying 3-NP neurotoxicity [5, 28, 38, 40, 41, 61, 62]. Neurochemical analysis of cortical, striatal and hippocampal lipid peroxidation products (MDA), reduced glutathione level and glutathione peroxidase (GPx) activity revealed that 3-NP administration (20 mg/kg body weight) for 5 consecutive days significantly increased lipid peroxidation (MDA level), decreased reduced glutathione (GSH) and reduced antioxidant enzyme activity (GPx activity). Lipid peroxidation has been reported to be a major contributor for the loss of cell function under oxidative stress in brain and age-related neurodegenerative disorders [63, 64]. GSH, a non-enzymic antioxidant, plays an important role in the reduction of reactive oxygen species (ROS) in brain where it interacts directly to detoxify certain ROS (e.g., hydroxyl radical). Decreased GSH level as observed in this study might be due to enhanced utilization of this antioxidant for free radicals scavenge [65, 66]. Glutathione peroxidase (GPx) is a critical intracellular enzyme involved in detoxification of hydrogen peroxide (H_2O_2) to water and is an important enzyme for the protection against 3-NP induced toxicity [67]. As shown in this study, 3-NP caused massive oxidative damage in different brain areas.

In animals treated with Pur and 3-NP, a significant prevention of 3-NP-induced oxidative damage was evident by normalizing MDA, GSH and GPx levels/activities.

Thus, Pur antioxidant activity may provide protection against 3-NP-induced toxicity. This is consistent with previous studies where Pur showed neuroprotective effect against beta-amyloid induced neurotoxicity in cultured hippocampal neurons by increasing cell survival, preventing GPx and catalase (CAT) activity reduction as well as preventing ROS production [68]. Moreover, Pur protected against cerebral ischemia–reperfusion injury by enhancing the activity of anti-oxidative enzymes, inhibiting lipid peroxidation and attenuating free radicals-mediated brain damage [18, 44, 69].

Histopathological alterations in rats' brain were obvious following systemic 3-NP adminstration, as represented in this study, and various previous studies [24, 26, 27, 70]. The promising neuroprotective effect of Pur was confirmed with milder degenerative changes in the Pur and 3-NP treated group as compared to the 3-NP group. Meanwhile, the Pur alone treated group showed only mild congestion in the cerebral blood capillaries because of the increased cerebral blood flow caused by Pur [44, 71].

In conclusion, Pur protected the brain tissues from 3-NP induced neurotoxicity primarily by its neuromodulation and antioxidant effects.

Future research may be directed towards the use of Puerarin in clinical trials to evaluate its neuroprotective effect on humans.

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Conflict of interest The authors declare that there are no conflicts of interest.

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