

Original Research Article

Tanshinone IIA protects against dopaminergic neuron degeneration via regulation of DJ-1 and Nrf2/HO-1 pathways in a rodent model of Parkinson's disease

Jingzhou Zhang¹, Yahong Wang², Xingwang Ji³, Zunhua Shu^{4*}

¹The Center of Preventive Treatment of Disease, ²Department of Liver, Spleen and Stomach Diseases, Affiliated Hospital of Changchun University, ³Department of Emergency, The First Clinical Hospital of Jilin Academy of Traditional Chinese Medicine, ⁴Department of Outpatient, Affiliated Hospital of Changchun University of Traditional Chinese Medicine, Changchun, Jilin 130021, China

*For correspondence: **Email:** SSophiatsco@yahoo.com; **Tel/Fax:** 0086-15204307345

Sent for review: 10 October 2018

Revised accepted: 18 April 2019

Abstract

Purpose: To study the potential neuroprotective effects of tanshinone IIA, a diterpene quinone, in an experimental model of 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced Parkinson disease (PD).

Methods: Mice (C57BL/6) were administered freshly-prepared MPTP at a dose of 20 mg/kg body weight intraperitoneally, 4 times at 2-h intervals, to induce PD. Doses of 12.5, 25 and 50 mg/kg tanshinone IIA were administered to the mice as treatments for PD. Pole and Rota-rod tests were carried out to assess muscular coordination and bradykinesia. Protein expressions, reactive oxygen species (ROS) and malonaldehyde and other parameters were evaluated.

Results: Tanshinone IIA at doses of 12.5, 25 and 50 mg/kg reduced deficits in muscular coordination and improved learning ability of MPTP-treated mice. It also reduced loss of tyrosine hydroxylase (TH)-positive neurons following MPTP-induction. Tanshinone IIA regulated apoptotic pathway proteins, i.e., Bax and Bcl-2, and inhibited the translocation of Cyt C to the mitochondria. Oxidative stress induced by MPTP was significantly inhibited by tanshinone IIA via up-regulation of DJ-1/Nrf2/HO-1 expression and reduction of ROS and MDA levels. Brain tissue total glutathione content was increased by tanshinone IIA treatment.

Conclusion: Tanshinone IIA effectively improves antioxidant status and reduces neuronal loss following MPTP treatment. These results indicate that tanshinone IIA exerts protective effects in MPTP-induced PD in mice. Thus, tanshinone IIA has a good potential for use as a therapy for PD.

Keywords: Tanshinone IIA, Dopamine, DJ-1, Heme oxygenase 1, Nrf2, Parkinson's disease

This is an Open Access article that uses a fund-ing model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder

characterized by postural instability, tremors, muscular stiffness and bradykinesia [1]. The disease is prevalent in the aged population (60 years and above) [2]. The etiology of PD is

complex and yet to be unravelled completely. Pathological observations in PD include the presence of Lewy bodies, degeneration of dopaminergic neurons, gliosis in the substantia nigra pars compacta (SNpc), and proteinaceous cytoplasmic inclusions [1]. Oxidative stress is critically associated with neurodegeneration in PD [3]. Data from autopsy studies in brain tissues of PD patients reveal oxidative modifications and lipid peroxidation of DNA, and protein oxidation [4]. Oxidative stress due to loss of redox balance of the cell leads to excessive generation of reactive oxygen species (ROS). The brain is highly susceptible to oxidative damage by ROS owing to high oxygen consumption, low antioxidant levels and higher levels of oxidizable polyunsaturated fatty acids. Aging brains are more affected by oxidative stress than younger brains, resulting in neuronal damage [5].

Protein deglycase DJ-1 or Parkinson's disease protein 7 (PARK7) is one of the key proteins involved in the cell survival process by regulating antioxidant defence mechanisms [6]. The protein prevents cellular apoptosis under oxidative stress conditions, regulates the synthesis of key antioxidant molecule i.e. reduced glutathione (GSH) [6], and enhances the transcription of MnSOD which scavenges superoxide radicals and prevents cell damage [7]. Nuclear factor erythroid 2-related factor (Nrf2), a transcription factor is also upregulated, thereby modulating the Nrf2-dependent detoxification pathways [8]. Nuclear factor erythroid 2-related factor (Nrf2) regulates the stress-inducible enzyme heme oxygenase 1 (HO-1) [9]. It also regulates the expression of nicotinamide adenine dinucleotide phosphate (NADP)H quinone oxidoreductase-1 (NQO1), an antioxidant enzyme which detoxifies protein-bound quinone, and aids in maintenance of reduced forms of α -tocopherol and coenzyme Q10.

Cellular apoptotic dysfunction is regarded as a major event that leads to neuronal cell death in PD. Elevated expressions of caspase 3 and caspase 8 were noticed in brain tissues of PD patients, suggesting activation of the caspase pathway [10]. Therefore, identification of compounds capable of activating DJ-1/Nrf2 and down-regulating apoptotic cascades would be potentially beneficial in PD treatment.

Recent studies have demonstrated the potential benefits of plant-derived compounds in the management of PD [11]. Tanshinone II A is a diterpene quinone isolated from the roots of *Salvia miltiorrhiza*. It possesses several beneficial properties such as neuroprotective

[12], anti-cancer and antioxidant effects [13]. In this study, the protective effect of tanshinone IIA against MPTP-induced PD was investigated in a mouse model of PD.

EXPERIMENTAL

Chemicals and antibodies

Dimethyl sulfoxide (DMSO), 1-Methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP), 3, 3'-diaminobenzidine (DAB), and Tanshinone IIA were procured from Sigma-Aldrich (St. Louis, MO, USA). Primary antibodies against Bax, Bcl-2, cleaved-caspase-3, HO-1, DJ-1, Nrf-2, NAD(P)H dehydrogenase: quinone 1/(NQO1) and cytochrome-c were products of Santa Cruz Biotechnology, Inc. Santa Cruz, CA, USA. Tyrosine hydroxylase (TH) was obtained from Millipore (Billerica, MA), while β -actin and SOD-1 were purchased from Cell Signalling Technology (Beverly, MA, USA). Avidin-biotin complex was procured from Vector Labs (Burlingame, CA, USA). All other chemicals were obtained from Sigma-Aldrich, unless otherwise stated.

Animals

For this study, male C57BL/6 mice (n = 80, 7- 8 weeks, 25 - 30 g) procured from Changchun University animal centre were used. The mice were held under standard in-house conditions of 12-h day /12-h night cycle, temperature between 22 - 24 °C, and relative humidity of 55 \pm 10 %. They were permitted unrestricted access to animal feed and water. The mice were kept in sterile acrylic cages (6 per cage), and were acclimatised to laboratory environment for 5 days prior commencement of the study. They were maintained with care in accordance with the Animal Care and Use Guidelines of the Institution/Hospital, and in line with National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals [14]. This study was approved by the ethics committee of Affiliated Hospital of Changchun University of Traditional Chinese Medicine (no. TY20160311).

Experimental design and drug administration

The animals were divided into 6 treatment groups (12 per group). Separate groups of mice were administered tanshinone IIA at doses of 12.5, 25 and 50 mg/kg/day via oral gavage for 10 days. Freshly-prepared MPTP in saline was injected intraperitoneally at a dose of 20 mg/kg body weight, 4 times at 2-h intervals on the 6th day of tanshinone treatment [15]. The control mice received MPTP only. The control group received equivalent volume of saline in place of

MPTP. The tanshinone IIA alone-treated group of mice were given 50 mg per day for 10 days, but were not administered MPTP.

Statistical analysis

The results are presented as mean \pm standard deviation (SD, $n = 6$). The analyses were performed using SPSS software (version 22.0, SPSS Inc., Chicago, IL). The results obtained were compared with one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) *post-hoc* analysis. Values of $p < 0.05$ were considered statistically significant.

RESULTS

Tanshinone IIA improved the behaviour of PD mice

The effect of tanshinone IIA on MPTP-induced impairment of muscular coordination and bradykinesia was assessed by pole test and Rota-rod test. Experimental PD induction significantly ($p < 0.05$) reduced retention time in mice to 30.15 % of normal control (Figure 1 a - c). Tanshinone IIA administration markedly ($p < 0.05$) increased the retention time, when compared with MPTP control. The retention time increased to 57, 69.9 and 86.2 % of normal control on tanshinone IIA treatment at dose of 12.5, 25 and 50 mg, respectively. In the pole test, T-turn and T-LA were significantly ($p < 0.05$) longer than those in normal control (Figure 1b). Interestingly, tanshinone IIA treatment at the three tested doses significantly ($p < 0.05$) shortened T-turn and T-LA. At 50 mg, tanshinone IIA decreased T-turn and T-LA to 98.2 and 99.10 %, respectively, almost to the same levels in the normal control group. Tanshinone IIA alone-treated animals exhibited retention time and T-turn and T-LA time similar to normal control.

Tanshinone IIA reduced MPTP-induced dopaminergic neuronal cell loss

Immunohistochemical analysis was done to determine the effects of tanshinone IIA on dopaminergic neuronal loss following MPTP injections, and TH-immunohistochemistry (TH-IHC) was conducted in the ST and SNpc tissues. Mice that were treated with MPTP exhibited markedly reduced number of TH-immunopositive neurons (Figure 2). In the MPTP-treated mice, TH-immunopositive neurons were 35.05 %, relative to the control. However, administration of tanshinone IIA significantly ($p < 0.05$) prevented MPTP-induced neuronal loss. The TH-positive counts increased to 90.20 % with 50 mg tanshinone IIA. When administered alone,

tanshinone IIA at 50 mg did not cause any neuronal loss in mice.

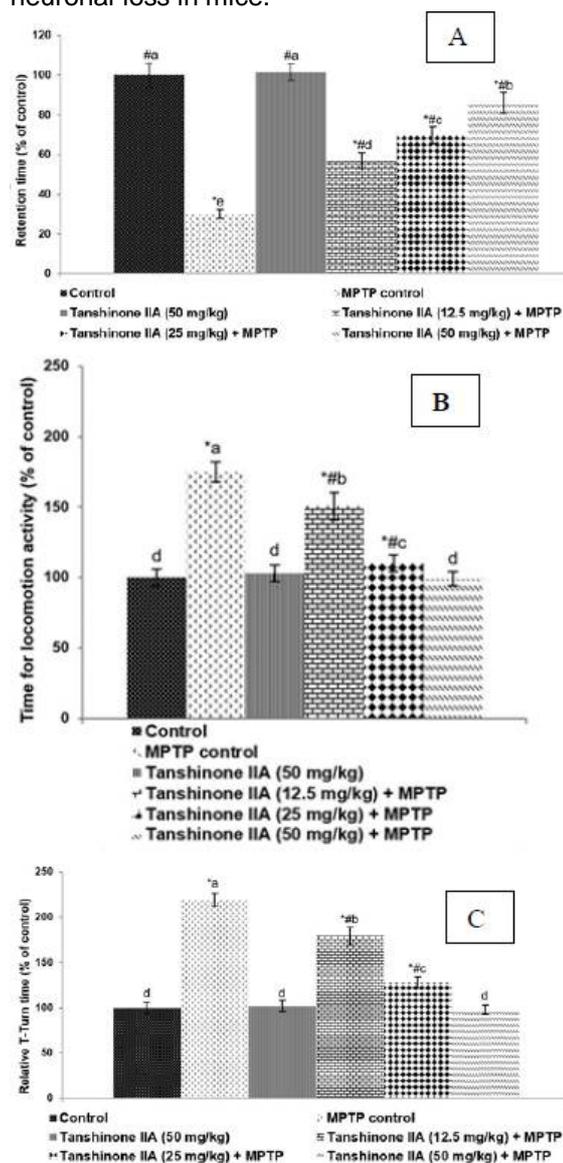


Figure 1: Tanshinone IIA improved the motor coordination and behaviour of animals. (a) Retention time as determined by Rota-rod test; (b and c) T-turn and T-LA values as determined from Pole test. Results are expressed as mean \pm SD ($n = 6$); * $p < 0.05$, compared to control; # $p < 0.05$, compared to MPTP control; a-d represent means from unlike experimental groups that differ at $p < 0.05$ as determined by one-way ANOVA and DMRT

Protective effects of tanshinone IIA against MPTP-induced apoptotic pathway dysfunction

In the present study, MPTP caused a significant ($p < 0.05$) increase in cleaved caspase-3 levels. Interestingly, tanshinone IIA dose-dependently suppressed caspase-3 expressions (Figure 3 a-c). The expressions were brought down to 107 % by treatment with 50 mg tanshinone IIA, as against 188% in MPTP control. The mitochondrial Bax

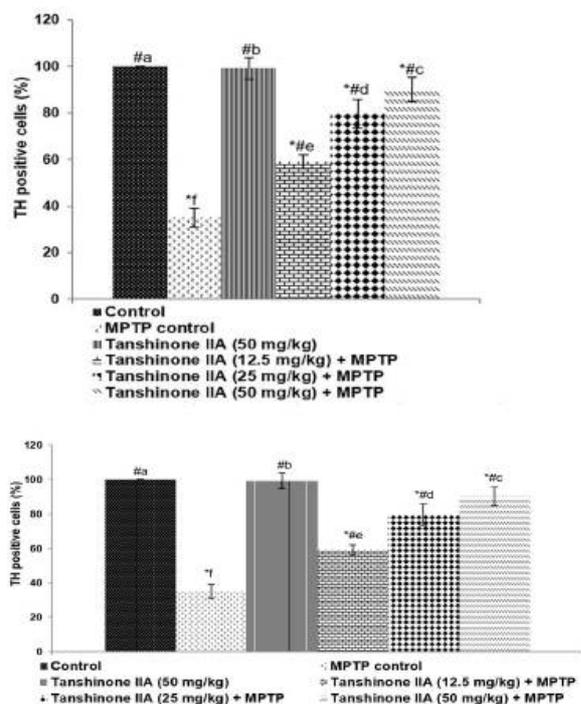


Figure 2: Tanshinone IIA improved TH-positive cells. Results are presented as mean \pm SD (n = 6). * p < 0.05, relative to control; # p < 0.05, compared to MPTP control; a - f represent means from different experimental groups that differ at p < 0.05 as determined by one-way ANOVA and DMRT

levels were markedly increased (p < 0.05), while Bcl-2 levels were decreased by MPTP exposure, when compared to control group (Figures 3 a - 3c). However, tanshinone treatment markedly (p < 0.05) reduced Bax and improved Bcl-2 levels, thereby restoring the Bcl-2/Bax ratio. Furthermore, the MPTP-induced toxicity significantly increased (p < 0.05) cytosolic Cyt c level to 167.8 %, relative to normal control, indicating translocation. Tanshinone IIA significantly (p < 0.05) suppressed the translocation of Cyt c from mitochondria to cytosol (Figure 3c). These results indicate that tanshinone II A effectively inhibited the apoptotic cascade, thereby exerting protective effects.

Tanshinone IIA reduced oxidative stress in PD mice

Reactive oxygen species (ROS) and MDA levels were determined in the SNpc tissues as indicators of oxidative stress. Significant elevations in ROS and MDA were produced by MPTP treatment (Figures 4 a and b). Tanshinone II A treatment prior to MPTP and post-MPTP exposure significantly (p < 0.05) decreased ROS and MDA levels (Figure 4 a - d). The ROS levels were reduced to 138.67, 55.81 and 11.44 % from 215.60 % on treatment with 12.5, 25 and 50 mg tanshinone IIA, respectively. Furthermore, tanshinone IIA administration (50 mg) markedly

raised levels glutathione to 0.0564 μ M/mg protein from 0.0295 μ M/mg protein. Tanshinone

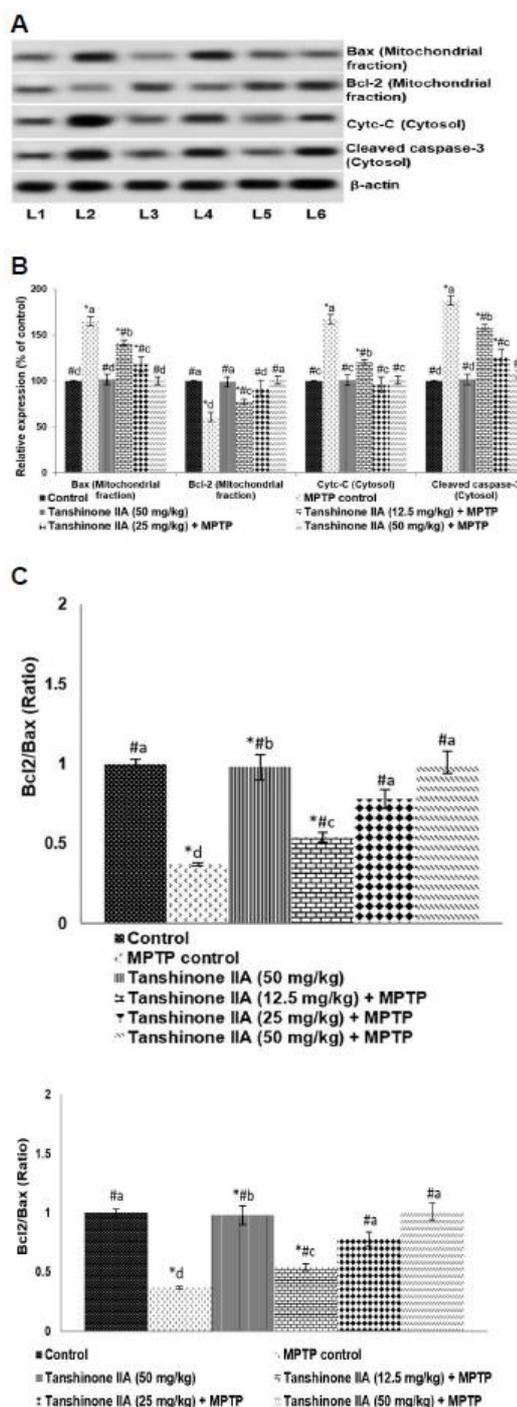


Figure 3: Effect of tanshinone IIA on apoptotic pathway protein expressions. (a - b) Bcl-2/Bax ratio; (b) relative expressions of proteins with control expressions set at 100 %; (c) Representative immunoblot. The results are presented as mean \pm SD, n = 6. * p < 0.05, compared with control; # p < 0.05, compared with MPTP control; a - d represent means of different experimental groups that differ from each other at p < 0.05. L1= Control; L2 =MPTP control; L3 = tanshinone IIA (50 mg/kg); L4 = tanshinone IIA (12.5 mg/kg) + MPTP; L5 = tanshinone IIA (25 mg/kg) + MPTP; L6 = tanshinone IIA (50 mg/kg) + MPTP

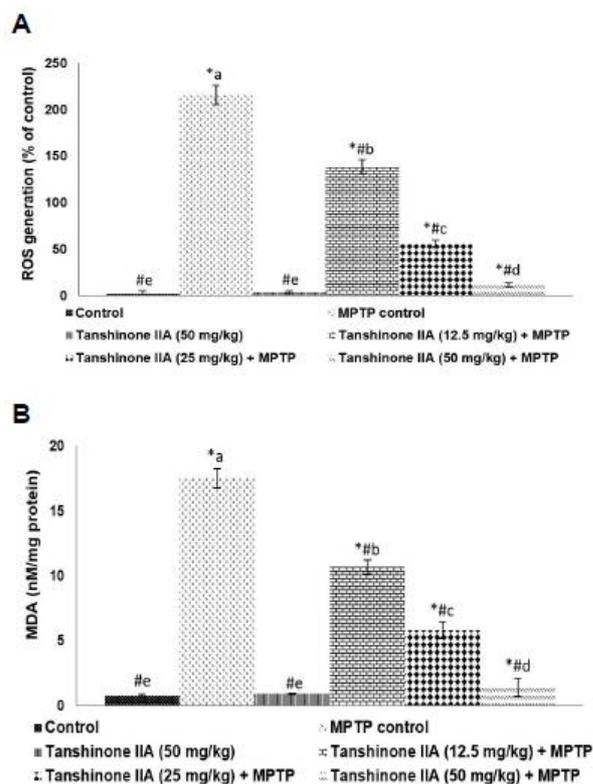


Figure 4: Tanshinone IIA effectively reduced MPTP-induced oxidative stress. (a) ROS generation (b) MDA levels. Results are presented as mean \pm SD, $n = 6$. * $p < 0.05$, compared to control; # $p < 0.05$, compared to MPTP control; a-e represent means of different experimental groups that differ at $p < 0.05$ as obtained by one-way ANOVA and DMRT

IIA at the 3 tested doses improved the PD-induced alterations in GSH: GSSG ratio (Figures 5 a and b). These observations indicate that tanshinone IIA reduced oxidative damage in PD.

Tanshinone IIA effectively activated DJ-1/Nrf2 pathway following PD induction

The MPTP-induced oxidative stress resulted in a noticeable decreases in the expressions of DJ-1, Nrf2, HO-1 and NQO1 (Figures 5a - 5c). However, tanshinone IIA treatment significantly up-regulated DJ-1 expression in nuclear, mitochondrial and cytosolic fractions. In addition, Nrf2 expression was increased in the nuclear and cytosol fractions to 158 and 122 %, respectively on tanshinone treatment (50 mg/kg). Although the lower doses of 12.5 mg and 25 mg caused substantial elevation in DJ-1 and Nrf2 levels, 50 mg dose was observed to be most effective (Figures 6a - 6c). The expressions of SOD-1, HO-1 and NQO1 were significantly ($p < 0.05$) upregulated by tanshinone treatment, which could be due to enhanced DJ-1 and activation of Nrf2. Compared with the MPTP control group, the tanshinone IIA-treated groups

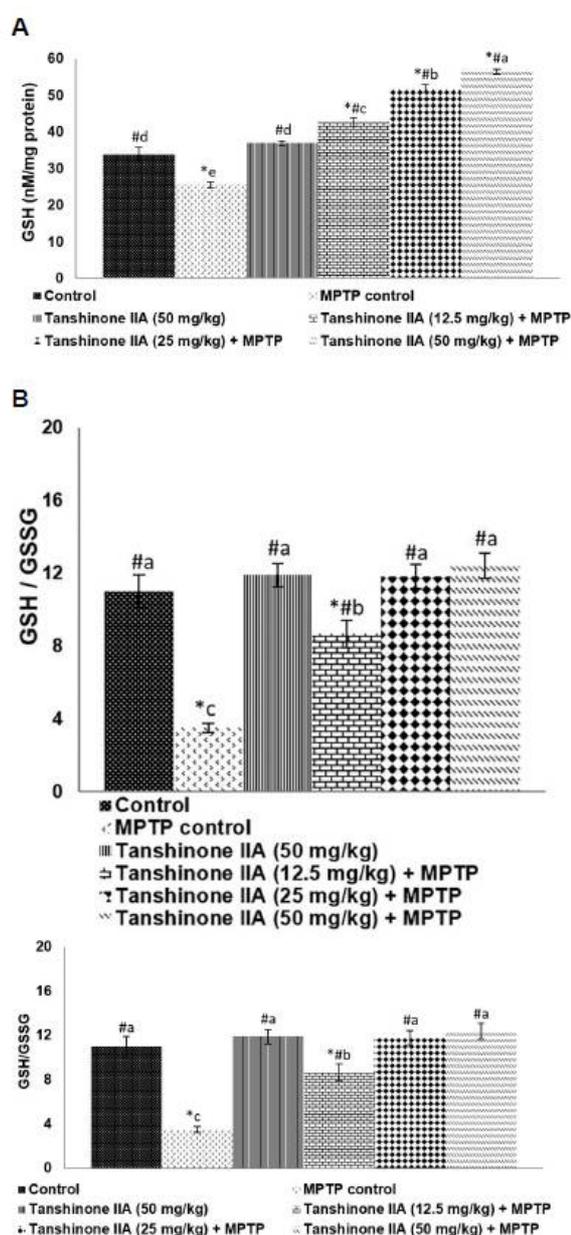


Figure 5: Tanshinone IIA improved GSH levels and (b) GSH/GSSG ratio. Results are presented as mean \pm SD ($n=6$). * $p < 0.05$, compared with control; # $p < 0.05$, compared with MPTP control; a - e represent means of different experimental groups that differ at $p < 0.05$ as determined by one-way ANOVA and DMRT

expressed HO-1 almost to near normal control levels on treatment with 50 mg dose. Similarly, tanshinone enhanced NQO1 levels in a dose-dependent manner, relative to MPTP control. Treatment with tanshinone at all 3 tested doses significantly improved antioxidant defence pathways.

DISCUSSION

Parkinson's disease affects mostly the elderly, and is characterized by postural instability,

muscular rigidity, tremors in the limbs and bradykinesia [2]. Studies have revealed evidence of elevated ROS levels, reduced glutathione (GSH) levels and raised oxidative modification of major biomolecules – nucleic acids, lipids, and proteins [16]. Thus, compounds that efficiently counter oxidative stress could be beneficial in PD treatment.

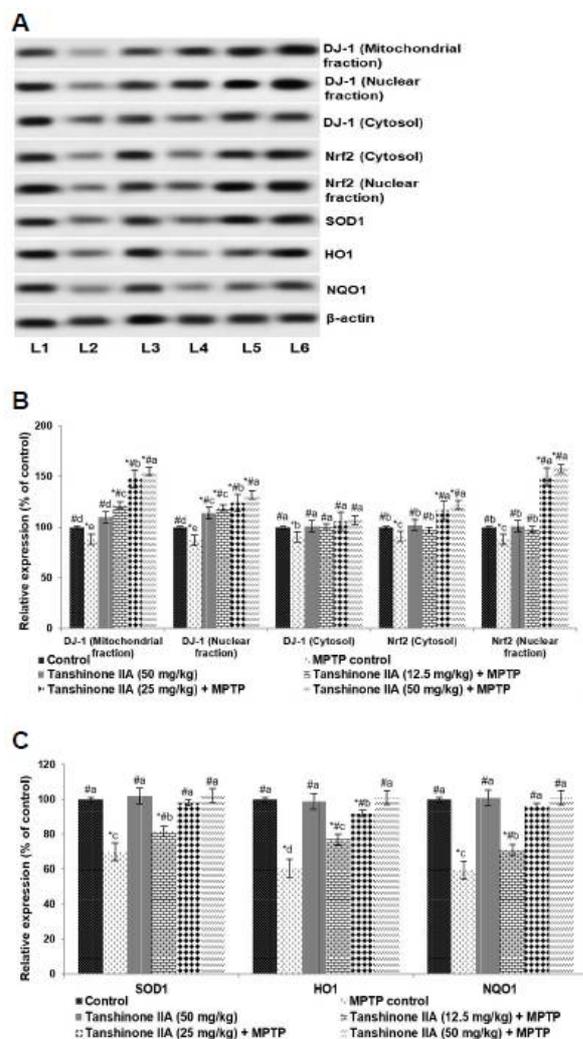


Figure 6: Tanshinone IIA activated DJ-1/Nrf2 pathway. (a) Representative immunoblot; (b, c) Relative expressions of proteins with control expression set at 100 %. Results are presented as mean \pm SD ($n = 6$); $^*p < 0.05$, compared to control; $^{\#}p < 0.05$, compared to MPTP control; a - e represent means of different experimental groups that differ at $p < 0.05$ as derived from one-way ANOVA and DMRT. L1 = Control; L2 = MPTP control; L3 = tanshinone IIA (50 mg/kg); L4 = tanshinone IIA (12.5 mg/kg) + MPTP; L5 = tanshinone IIA (25 mg/kg) + MPTP; L6 = tanshinone IIA (50 mg/kg) + MPTP

The neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) is widely employed for induction of PD in experimental animal models [17], and for evaluation of novel neuroprotective agents in PD treatment. Administration of MPTP

produces neurodegeneration and neural impairments close to those observed in PD patients [18]. Within the non-dopaminergic cells, MPTP is converted by brain monoamine oxidase B to active 1-methyl-4-phenylpyridinium (MPP⁺) which enters the dopaminergic neurons and causes neuronal intoxication [1]. The active metabolite, MPP⁺ accumulates in the substantia nigra pars compacta (SNpc) and obstructs the electron transport chain (complex I) in the mitochondria, thereby leading to ROS production [19]. Increased ROS alters the membrane permeability of the mitochondria and results in the translocation of cytochrome c from the mitochondria to the cytosol. Furthermore, ROS induce activation of caspase-3, resulting in up-regulation of apoptosis cascade [20].

In the present study, the neuroprotective effects of tanshinone IIA was assessed in experimental PD model induced by MPTP. The rota-rod test and pole tests are frequently employed for measuring bradykinesia, postural balance and motor coordination in experimental animals [15]. Mice exposed to MPTP exhibited behavioural abnormalities and deficits in motor coordination as reflected by T-turn and T-LA values. However, tanshinone IIA administration significantly improved muscular coordination and motor learning function as evidenced by changes in T-turn, T-LA and retention times, relative to mice given MPTP alone.

The dopaminergic neurons are abundant in the SNpc region. Dopamine is responsible for signal transmission between SNpc and many other areas of the brain. The association between the striatum and SNpc is vital for undisturbed, decisive movement. Irregular nerve-firing in the brain as a result of dopamine depletion causes impaired muscular coordination and movements. In the present study, dopaminergic neuronal loss was evaluated by measuring the number of TH-positive cells in SNpc. Tyrosine hydroxylase catalyses conversion of L-dopa to dopamine, the rate-limiting step in the synthesis of dopamine [21].

A substantial decrease in TH-positive cell counts following MPTP treatment was observed, indicating dopaminergic neuronal loss. It has been reported that MPTP-administered mice presented motor deficits and loss of TH-positive neurons [22]. In the present study, tanshinone IIA treatment dose-dependently improved TH-positive counts. This increase in TH immunoreactivity could have also improved behaviour and muscular coordination observed in tanshinone treated mice, which reveals the neuroprotective efficacy of tanshinone.

Loss of dopaminergic neurons involves activation of the pro-apoptotic proteins and apoptotic cascade in PD [15]. The anti-apoptotic protein Bcl-2 prevents the release of Cyt c to the cytosol, and suppresses mitochondria-mediated initiation of cell damage, while, Bax promotes Cyt c translocation to the cytosol [23]. Caspase-3 is activated by Cyt c leading to activation of the caspase cascade. In this study, MPTP increased Bax in the mitochondria and decreased Bcl-2 levels. The observed increase in Cyt c in the cytosol reflects increased Bax levels. Activation of caspase-3 is an indicator of activation of caspase pathway. Thus, level of cleaved caspase-3 is regarded as major marker of cellular apoptosis. Treatment with MPTP caused a marked increase in the activity of caspase-3 as reflected in raised cleaved caspase-3 levels in the cytosol. Enhanced activation of caspase-3 could have caused activation of the apoptotic pathway leading to neuronal loss in SNpc. Interestingly, on pre-treatment with tanshinone IIA, decreases in cleaved caspase-3 levels were observed. Moreover, tanshinone caused significant reversal of events leading to apoptosis such as improved membrane integrity, restoration of the Bcl-2/Bax ratio, and reduced release of cyt-C from mitochondria, thereby preventing loss of dopaminergic neurons.

Reactive oxygen species (ROS) disturb the integrity of mitochondrial membrane causing raised levels of Cyt c in the cytosol, thereby activating caspase-3 [24]. Following MPTP injection, increased levels of ROS and MDA were observed, suggesting oxidative stress in the brain tissues. Furthermore, the decreased total glutathione content and altered GSH:GSSG ratio observed indicate redox imbalance. Reduced glutathione (GSH) is a major antioxidant in the brain [25]. Reduced levels of GSH in brain tissues of MPTP-induced PD model have been reported [26]. Tanshinone IIA treatment remarkably suppressed oxidative stress status by reducing ROS production and MDA levels, and improving GSH levels and GSH:GSSG ratio. Altered redox balance has been reported in PD [27]. Thus, restoration of the redox balance with enhanced antioxidant status would aid in PD treatment.

Studies have reported the neuroprotective effects of DJ-1, a multifunctional protein in various experimental models of oxidative stress-induced cell death [22]. A direct antioxidant property of DJ-1 as a scavenger of ROS has been reported as one of the major mechanisms for combating oxidative stress [28]. It regulates the expression of glutathione synthase under oxidative stress conditions and also regulates critical transcription

factor-nuclear factor erythroid-2-related factor 2 (Nrf2) [6, 8]. The Nrf2 is a master transcription factor implicated in regulation of oxidative stress responses. In the absence of oxidative stress, Nrf2 remains localized in the cytoplasm bound to Keap1, leading to its degradation by ubiquitination. Under oxidative stress, DJ-1 sequesters Keap1, resulting in translocation of Nrf2 from cytosol to nucleus causing activation of various antioxidative stress response genes such as HO-1, NAD(P)H: quinone oxidoreductase-1 (NQO1) and enzymes involved in GSH synthesis [29]. Decline in Nrf2 activity is considered a chief risk factor for PD [30]. Moreover, Nrf2 knockout mice exposed to MPTP exhibited a greater loss of dopaminergic neurons, when compared with wild type mice [31]. In the present investigation, MPTP caused similar significant decreases in DJ-1, Nrf2, HO-1 and NQO1 expressions. Tanshinone IIA-mediated up-regulation in the expressions of DJ-1 and Nrf2 could have been responsible for the reduction in MDA and ROS levels as observed. The enhanced expressions of SOD1, HO-1 and NQO could be due to enhanced DJ-1 and Nrf2 which is likely to have aided in reduction of MPTP-induced oxidative stress. Improvement in the levels of GSH suggests the activation of GSH synthesis enzymes. Furthermore, DJ-1 is known to positively regulate the expression of the TH gene [32]. Thus, tanshinone IIA-induced DJ-1 expression possibly increased TH-positive cells and reduced dopaminergic neuron degeneration. These observations in part could be via direct antioxidant scavenging effects of tanshinone IIA, or indirectly through activation of the DJ-1/Nrf2 pathways.

CONCLUSION

Tanshinone IIA significantly reduces MPTP-mediated neuronal loss and improves muscular coordination and oxidative stress in mice. Thus, tanshinone IIA is a potential candidate drug for the treatment of PD. However, further experiments need to be conducted to explore the mechanisms involved.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this research study was executed by the author(s) - Jingzhou Zhang,

Yahong Wang, Xingwang Ji and Zunhua Shu - and all liabilities pertaining to claims relating to the content of this article will be borne by the authors". All authors equally contributed to this study. Jingzhou Zhang and Zunhua Shu designed this study, collected and analysed the data, and also prepared the manuscript. Yahong Wang and Xingwang Ji had contributed in the experimental works and also in statistical study.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

- Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron* 2003; 39: 889-909.
- Willis WA, Evanoff BA, Lian M, Criswell SR, Racette BA. Geographic and ethnic variation in Parkinson disease: a population-based study of US Medicare beneficiaries. *Neuroepidemiology* 2010; 34: 143-151.
- Chakraborty S, Bornhorst J, Nguyen TT, Aschner M. Oxidative stress mechanisms underlying Parkinson's disease-associated neurodegeneration in *C. elegans*. *Int J Mol Sci*. 2013; 14: 23103-23128.
- Banerjee R, Starkov AA, Beal MF, Thomas B. Mitochondrial dysfunction in the limelight of Parkinson's disease pathogenesis. *Biochim Biophys Acta* 2009; 1792: 651-663.
- Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. *Parkinsonism Relat Disord* 2003; 9: S59-S64.
- Zhou W, Freed CR. DJ-1 up-regulates glutathione synthesis during oxidative stress and inhibits A53T α -synuclein toxicity. *J Biol Chem* 2005; 280: 43150-43158.
- Zhong N, Xu J. Synergistic activation of the human MnSOD promoter by DJ-1 and PGC-1 α : regulation by SUMOylation and oxidation. *Hum Mol Genet* 2008; 17: 3357-3367.
- Clements CM, McNally RS, Conti BJ, Mak TW, Ting JP. DJ-1, a cancer- and Parkinson's disease associated protein, stabilizes the antioxidant transcriptional master regulator Nrf2. *Proc Natl Acad Sci USA* 2006; 103: 15091-15096.
- Yang L, Calingasan NY, Thomas B, Chaturvedi RK, Kíaei M, Wille EJ, Liby KT, Williams C, Royce D, Risingsong R, et al. Neuroprotective effects of the triterpenoid, CDDO methyl amide, a potent inducer of Nrf2-mediated transcription. *PLoS One* 2009; 4: e5757.
- Hartmann A, Troadec JD, Hunot S, Kikly K, Faucheux BA, Mouatt-Prigent A, Ruberg M, Agid Y, Hirsch EC. Caspase-8 is an effector in apoptotic death of dopaminergic neurons in Parkinson's disease, but pathway inhibition results in neuronal necrosis. *J Neurosci* 2001; 21: 2247-2255.
- Khasnavis S, Pahan K. Sodium benzoate, a metabolite of cinnamon and a food additive, upregulates neuroprotective Parkinson disease protein DJ-1 in astrocytes and neurons. *J Neuroimmune Pharmacol* 2012; 7: 424-435.
- Zhu Y, Tang Q, Wang G, Han R. Tanshinone IIA protects hippocampal neuronal cells from reactive oxygen species through changes in autophagy and activation of phosphatidylinositol 3-kinase, protein kinase B, and mechanistic target of rapamycin pathways. *Current Neurovascular Res* 2017; 14: 132-140.
- Xie J, Liu J-H, Liu H, Liao XZ, Chen Y, Lin MG, Gu YY, Liu TL, Wang DM, Ge H, et al. Tanshinone IIA combined with adriamycin inhibited malignant biological behaviors of NSCLC A549 cell line in a synergistic way. *BMC Cancer* 2016; 16: 899.
- Garber JC. Committee for the update of the guide for the care and use of laboratory animals. In: *Guide for the care and use of laboratory animals, 8th edn*, National Academy of Sciences, USA; 2011.
- Li H, Park G, Bae N, Kim J, Oh MS, Yang HO. Anti-apoptotic effect of modified Chunsimyeolda-tang, a traditional Korean herbal formula, on MPTP-induced neuronal cell death in a Parkinson's disease mouse model. *J Ethnopharmacol* 2015; 176: 336-344.
- Sanders LH, Greenamyre JT. Oxidative damage to macromolecules in human Parkinson disease and the rotenone model. *Free Radic Biol Med* 2013; 62: 111-120.
- Smeyne RJ, Jackson-Lewis V. The MPTP model of Parkinson's disease. *Mol Brain Res* 2005; 134: 57-66.
- Bergman H, Deuschl G. Pathophysiology of Parkinson's disease: from clinical neurology to basic neuroscience and back. *Mov Disord* 2002; 17(Suppl 3): S28-S40.
- Chen XC, Chen Y, Zhu YG, Fang F, Chen LM. Protective effect of ginsenoside Rg1 against MPTP-induced apoptosis in mouse substantia nigra neurons. *Acta Pharmacol Sin* 2002; 23: 829-834.
- Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. *Genes Dev* 1999; 13: 1899.
- Daubner SC, Le T, Wang S. Tyrosine hydroxylase and regulation of dopa- mine synthesis. *Arch Biochem Biophys* 2011; 508: 1-12.
- Inden M, Kitamura Y, Abe M, Tamaki A, Takata K, Taniguchi T. Parkinsonian rotenone mouse model: re-evaluation of long-term administration of rotenone in C57BL/6 mice. *Biol Pharm Bull* 2011; 34: 92-96.

23. Pak YMK. Reviews: mitochondria: the secret chamber of therapeutic targets for age-associated degenerative diseases. *Biomol Ther* 2010; 18: 235-245.
24. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. *Free Radic Biol Med* 2010; 48: 749-762.
25. Meister A, Anderson ME. Glutathione. *Annu Rev Biochem* 1983; 52: 711-760.
26. Lu C, Zhang J, Shi X, Miao S, Bi L, Zhang S, Yang Q, Zhou X, Zhang M, Xie Y, et al. Neuroprotective effects of tetramethylpyrazine against dopaminergic neuron injury in a rat model of Parkinson's disease induced by MPTP. *Int J Biol Sci* 2014; 10: 350-357.
27. Lee KW, Im JY, Woo JM, Grosso H, Kim YS, Cristovao AC, Sonsalla PK, Schuster DS, Jalbut MM, Fernandez JR, et al. Neuroprotective and anti-inflammatory properties of a coffee component in the MPTP model of Parkinson's disease. *Neurotherapeutics* 2013; 10: 143-153.
28. Andres-Mateos E, Perier C, Zhang L, Blanchard-Fillion B, Greco TM, Thomas B, Ko HS, Sasaki M, Ischiropoulos H, Przedborski S, et al. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. *Proc Natl Acad Sci USA* 2007; 104: 14807-14812.
29. Ansari N, Khodaghali F, Amini M. 2-Ethoxy-4,5-diphenyl-1,3-oxazine-6-one activates the Nrf2/HO-1 axis and protects against oxidative stress-induced neuronal death. *Eur J Pharmacol* 2011; 658: 84-90.
30. Jazwa A, Rojo AI, Innamorato NG, Hesse M, Fernández-Ruiz J, Cuadrado A. Pharmacological targeting of the transcription factor Nrf2 at the basal ganglia provides disease modifying therapy for experimental parkinsonism. *Antioxid Redox Signal* 2011; 14: 2347-2360.
31. Chen PC, Vargas MR, Pani AK, Smeyne RJ, Johnson DA, Kan YW, Johnson JA. Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte. *Proc Natl Acad Sci USA* 2009; 106: 2933-2938.
32. Zhong N, Kim CY, Rizzu P, Geula C, Porter DR, Pothos EN, Squitieri F, Heutink P, Xu J. DJ-1 transcriptionally up-regulates the human tyrosine hydroxylase by inhibiting the sumoylation of pyrimidine tract-binding protein-associated splicing factor. *J Biol Chem* 2006; 281: 20940-20948.