https://doi.org/10.1016/j.phymed.2016.12.017

Neuroprotective properties of icariin in MPTP-induced mouse model of Parkinson's disease: involvement of PI3K/Akt and MEK/ERK signaling pathways

Wen-Fang Chen[a, 1*], Lin Wu[a,b, 1], Zhong-Rui Du[a], Lei Chen[a], Ai-Li Xu[a,c], Xiao-Han Chen[a], Ji-Jun Teng[d], Man-Sau Wong[e]

[a]Department of Physiology, Shandong Provincial Collaborative Innovation Center for Neurodegenerative Disorders, Medical College of Qingdao University, Qingdao 266071, China.

[b]Department of Physiology, Heze Medical College, Heze 274000, China.

[c]Department of Physiology, Binzhou Medical University, Yantai 264003 , China.

[d]Department of Neurology, Affiliated Hospital of Qingdao University, Qingdao 266071, China

[e]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hung Hom, Kowloon, Hong Kong SAR, PR China.

* Correspondence: Dr. Wen-Fang Chen

Department of Physiology, Shandong Provincial Key Laboratory of Pathogenesis and Prevention of Neurological Disorders, Shandong Provincial Collaborative Innovation Center for Neurodegenerative Disorders, Medical College of Qingdao University, Qingdao 266071, China

Tel: (86-532) 83780051 Fax: (86-532) 83801449

E-mail: chenwenfangqd@163.com (W.F. Chen)

[1] Wen-Fang Chen and Lin Wu contributed equally to this work.

Abstract

Background: *Epimedium sagittatum* is a traditional Chinese herb normally which is used to treat the osteoporosis, cardiovascular dysfunction, and to improve neurological and sexual function in China, Korea and Japan. Icariin is the major active ingredient in *Epimedium sagittatum*. In the present research, we examined the neuroprotective effects of icariin on dopaminergic neurons and the possible mechanisms in a mouse model of Parkinson's disease (PD).

Methods: Ovariectomized PD mice were treated with vehicle or icariin (3 days before MPTP injections) with or without the phosphatidylinositol 3-kinase (PI3K) inhibitor LY294002 or mitogen-activated protein kinase kinase (MEK) inhibitor PD98059. The dopamine (DA) content in the striatum was studied by HPLC. Western blot was used to determine the protein expressions of Bcl-2, Bax and Caspase 3 in the striatum. The numbers of tyrosine hydroxylase-immunoreactive (TH-IR) neurons in the substantial nigra pars compacta (SNpc) were assessed by immunohistochemistry. The activation of Akt and ERK by icariin were detected in doparminergic MES23.5 cells.

Results: Icariin pretreatment could ameliorate the decreased striatum DA content and the loss of TH-IR neurons in the SNpc induced by MPTP. The MPTP-induced changes of Bcl-2, Bax and caspase 3 protein expressions in the striatum could be reversed by icariin pretreatment. Blockade of PI3K/Akt or MEK/ERK signaling pathway by LY294002 or PD98059 could attenuate the increase of DA content in the striatum and TH-IR in the SNpc induced by icariin in PD mice model. Additionally,

icariin treatment alone significantly induced the phosphorylation of Akt and ERK in a time dependent pattern in dopaminergic MES 23.5 cells. These effects were abolished by co-treatment with LY294002 or PD98059.

Conclusion: These data demonstrated that icariin has neuroprotective effect on dopaminergic neurons in PD mice model and the potential mechanisms might be related to PI3K/Akt and MEK/ERK pathways.

Keywords : icariin; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; phosphatidylinositol 3-kinase; mitogen-activated protein kinase kinase; dopamine; Parkinson’s disease

Abbreviations:

DA, dopamine; IGF-1R, insulin-like growth factor-1 receptor; IRS-1, insulin receptor substrate-1; MAPK, mitogen activated protein kinase; MEK, mitogen-activated protein kinase kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MTT, 3-[4, 5-dimethylthiazol 2-yl] 2, 5-diphenyltetrazolium bromide; PD, Parkinson's disease; PI3K, phosphatidylinositol 3-kinase; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase-immunoreactive

Introduction

Parkinson’s disease (PD) is a chronic neurodegenerative disease which is related to the dysfunctions of nigrostriatal dopaminergic systems (Dauer & Przedborski, 2003). Clinical studies support that the incidence of PD in male is higher than that in women, suggesting the potential neuroprotective effects of estrogen on nigrostriatal system (Miller & Cronin-Golomb, 2010; Smith & Dahodwala, 2014). In animal models of PD, estrogen treatment could protect against toxic exerted apparent neuroprotective effects on the dopaminergic neurons when administered prior to or coinciding with a toxic insult (Campos et al, 2012; Cordellini et al, 2011). Even though, the undesirable side effects of hormone replacement therapy limited the use of estrogen in clinic. Many women turn to natural products due to the few side effects.

Epimedium sagittatum is a traditional Chinese herb commonly which is used to treat the kidney-yang or yin deficiency, rheumatoid arthritis, osteoporosis and cardiovascular diseases (Campos et al, 2012; Chen et al, 2011; Zhang et al, 2014). Icariin, the major bioactive compound of *Epimedium sagittatum*, is considered to be a potential drug to treat major age-related diseases (Li et al, 2015). Our previous study showed that icariin could stimulate bone formation and suppress bone resorption in ovariectomy-induced osteoporosis (Mok et al, 2010). Zhu's research group indicated that icariin could protect against brain injury in experimental stroke (Zhu et al, 2010). Using the mice model of senescence-accelerated mouse prone 8, researchers have demonstrated that orally administered icariin could prevent learning and memory impairment. Furthermore, several lines of evidence suggest that icariin could improve

the learning and memory deficits in aging rats (Wu et al, 2012) and APP transgenic Alzheimer's disease mice (Zhang et al, 2014). However, there are no previous studies referring to the neuroprotective effect of icariin on dopaminergic neurons.

Icariin exerts its biological effects via several relevant pathways including insulin-like growth factor (IGF), mitogen activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K), transforming growth factor-β (TGF-β), nitric oxide synthase (NOS) and others. The IGF signaling system plays an important role in mediating growth and development. Binding of IGFs to IGF-1 receptor (IGF-1R) will induce IGF-1R autophosphorylation, insulin receptor substrate (IRS)-1 phosphorylation, and subsequent activation of PI3-K/Akt pathway and MAPKs pathway (Valentinis & Baserga, 2001). MAPK pathway and PI3K pathway play important roles in cell proliferation, survival, differentiation and adaptation. It was reported that icariin could stimulate angiogenesis by activating the PI3K/Akt/eNOS and MEK-ERK-dependent signal pathways (Chung et al, 2008). Icariin was also shown to inhibit amyloid β-induced apoptosis in PC12 cells and corticosterone-induced apoptosis in hypothalamice neurons by the Akt pathway (Zhang et al, 2015; Zhang et al, 2012). It is unclear if the neuroprotective actions of icariin on dopaminergic neurons are mediated through PI3K/Akt and MEK/ERK signaling pathways.

In the present study, we aim to characterize the protective effects of icariin on 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-damaged nigrostriatal system in the mice as well as to elucidate the mechanism of actions involved in mediating its

neuroprotective effects in vivo and in vitro.

Materials and Methods

Materials

Icariin was purchased from Shanghai Tauto Biotech Co., Ltd. (Shanghai, China). The purity of icariin (>98%) was determined by HPLC. Figure 1 shows the formular structure of icariin. It was dissolved in DMSO and dilute with saline (saline containing 1% DMSO) before intragastric administration. LY294002 was purchased from cell signaling Technology Inc (Danvers, MA, USA). PD98058 was purchased from Calbiochem (La Jolla, CA, USA). Primary antibody of tyrosine hydroxylase (TH) was purchased from Millipore (Bedford, MA, USA). Primary antibodies of Bcl-2, Bax and secondary antibody were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Monoclonal mouse anti-β-actin was supplied by Cell Signaling Technology, Inc. (Hertfordshire, England). All other chemicals were obtained from commercial sources.

Animals and treatment

10-12-week-old female C57BL/6 mice (18-22g) were obtained from Vital River Experimental Animal Center of Beijing (Beijing, China). Ovariectomy was performed as described previously (Khajuria et al, 2012) and housed for 14 days. All surgery was performed under chloral hydrate anesthesia and conducted according to the "Guide for the Care and Use of Laboratory Animals" (NIH publications No. 80-23, revised 1996). The experiment was approved by the Animal Ethic Committee of Qingdao University.

Exp 1: *dose-dependent effects of icariin in ovariectomized mice*

The 30 mice were randomly divided into five groups: control, MPTP, icariin 50mg/kg (ICA50) , icariin 100mg/kg (ICA100), icariin 200mg/kg (ICA200). Three days after the icariin pretreatment, MPTP (15mg/kg, i.p) injection (four times with intervals of 2h) was performed at 2h after the intragastric administration. Oral administration of icariin lasted 8 consecutive days. The mice were sacrificed after the behavioral test and the brains were removed for HPLC analysis, immunohistochemistry and western blot analysis.

Exp 2: *the blocking effects of LY294002 and PD98059 on the protective effect of icariin in ovariectomized mice*

The 42 mice were classified into seven groups: (1) Control group: mice were microinjected with 1μl 0.1% ethanol in saline into lateral ventricle. Ten min later, saline containing 1% DMSO (10ml/kg) were orally administrated. The treatment lasted 8 consecutive days. (2) MPTP group: Three days after the vehicle treatment as described for control group, the mice were treated with MPTP as mentioned in Exp 1. (3) Icariin+MPTP group: Same as in MPTP group except that icariin (100mg/kg) instead of vehicle was by intragastric administration. (4) Icariin+LY294002+MPTP group: Same as in icariin group except 1μl LY294002 (1μg/μl) microinjection into lateral ventricle. (5) Icariin+PD98059+MPTP group: Same as in icariin group except 1μl PD98059 (1μg/μl) microinjection into lateral ventricle. (6) LY294002 group: Same as in control group except 1μl LY294002 (1μg/μl) microinjection into lateral ventricle. (7) PD98059 group: Same as in control group except 1μl PD98059 (1μg/μl) microinjection into lateral ventricle.

153 On Day 9 two sides of the striatum were rapidly removed and used for HPLC
154 analysis and western blot, respectively. The substantia nigra was used for
155 immunohistochemistry studies.
156 Rotarod test
157 Motor skill performance was evaluated on the rotarod equipment. Before icariin
158 treatment, mice were pre-trained for three consecutive days with 2 training sessions
159 of 2 min at 4 rpm and followed by 5 min on the automated accelerating rotarod
160 apparatus (from 4 to 40 rpm) per day. The movement coordination function was
161 calculated at day 9 after icariin administration. Evaluation method is the time of
162 residence on the automated accelerating rotarod apparatus (from 4 to 40 rpm). Each
163 trial lasted 5 min with a 60 min inter-trial interval. The results are expressed as the
164 average time of 3 trials.
165 Determination of dopamine content
166 HPLC was used to determine the dopamine (DA) content as previously describe
167 (Liu et al, 2008). Briefly, the striatum of left side was homogenized in 0.3 ml liquid
168 A (0.4 M perchloric acid) immediately after weighing, followed by centrifugation at
169 12,000 rpm for 20min at 4°C. 80 μl of the supernatant was mixed with 40 μl liquid B
170 (20 mM citromalic acid-potassium, 300 mM dipotassium phosphate, 2 mM
171 EDTA-2Na). The supernatants were collected to detect the DA content.
172 TH immunohistochemistry staining
173 Immunohistochemistry was performed as described previously (Liu et al, 2008).
174 Briefly, the midbrains containing the SN were fixed in 4% paraformaldehyde for 6h,
175 after that, transferred into 30% sucrose for 48h. Frozen coronal sections (16 μm)

were serially cut through the SN with a freezing cryostat (Leica, German). TH-IR neurons in the SNpc were visualized by DAB. The counting on 12 sections of each mouse was performed blind on an Olympus microscope.

Western blotting

The striatum of right side or MES23.5 cells were lysed in Nonidet P-40 lysis buffer containing protease inhibitors as previously described (Chen et al, 2013). Protein electrophoresis were performed by 10% SDS–PAGE and transblotted onto PVDF membranes. The blots were probed with primary antibodies against Bcl-2, Bax and caspase 3 (1:1000). After washing, the membranes were incubated with secondary antibody (1:2000) for 2h at room temperature. The enhanced chemiluminescence reagent was used to detect the antigen–antibody complexes and the density of each band was measured by Imager (UVP Biospectrum 810, USA).

Cell culture and treatment

MES23.5 dopaminergic cell line was obtained from Dr. W.D. Le (Baylor College of Medicine, Houston, USA) (Crawford et al, 1992). As previously described (Ge et al, 2010), MES23.5 cells were cultured in DMEM/F12 supplemented with Sato's components, 5% fetal bovine serum (FBS), penicillin 100 IU/ml and streptomycin 100μg/ml. Cells were treated with icariin (0.01μM) for 5, 10, 20min in the presence or absence of LY294002 (5μM) or PD98059 (10μM). The activation of Akt and ERK were determined using western blot analysis. The dosage of icariin, LY294002 and PD98059 were chosen based on the previous reports (Chen et al, 2015; Di Santo et al, 2014; Ge et al, 2010).

198 Statistical analysis

199 Data are expressed as mean ± SEM. Statistical analysis was performed by
200 Kruskal-Wallis test followed by Dunn's multiple comparison test for the animal study.
201 The in vitro data was analyzed by One-way analysis of variance (ANOVA) followed
202 by Tukey's multiple comparison test. $P<0.05$ was considered to indicate statistic
203 significance.

Results

Rotarod test

Motor skill performance was evaluated on the rotarod equipment. The mean residence time of control is 252.2±19.4 sec. MPTP treatment significantly increased this value to 298.9±0.8 sec. No significant difference was found in 50mg/kg, 100mg/kg and 200mg/kg icariin treatment group (275.7±10.7, 271.6±4.4, 289.5±8.1 sec).

Dose dependent effects of icariin on striatal DA

Comparing with the control group, MPTP significantly decreased the DA content by 71.5% in the striatum of mice. Pretreatment with 50mg/kg, 100mg/kg and 200mg/kg icariin increased the DA content by 6%, 57% and 21% respectively, when compared with the MPTP group (Fig 3). So, 100mg/kg icariin was chosen for subsequent experiments.

Effects of icariin on TH-IR neuronal death in the SNpc

Excepting the deficit of DA in the striatum, dopaminergic neuronal damage is associated with the deficiency of TH-IR neurons in the SNpc. MPTP treatment resulted in only 45% survival of the dopaminergic neurons. In contrast, 50mg/kg, 100mg/kg and 200mg/kg icariin treatment protected against the neurotoxicity of MPTP and the survival ratio was 49%, 73% and 54% respectively, when compared to the control group (Fig 4).

Effects of icariin on Bcl-2, Bax and caspase 3 protein expressions in the striatum

Studies have demonstrated that MPTP can induce dopaminergic neuronal

apoptosis in the SNpc (Xu et al, 2014). The present study showed that the protein expression of Bcl-2 in the striatum was significantly decreased in MPTP group (Fig 5). Icariin treatment attenuated the MPTP-induced decrease in Bcl-2 protein expression. On the contrary, Bax and caspase 3 protein expression significantly increased in MPTP group (Fig 5). Icariin (100mg/kg) treatment significantly attenuated the increase of Bax and caspase 3 protein levels induced by MPTP (Fig 5).

LY294002 and PD98059 block the neuroprotective effects of icariin on nigrostriatal system

In order to demonstrate that the PI3K/Akt and MEK/ERK pathways are involved in the protective actions of icariin on nigrostriatal system, PI3K inhibitor LY294002 or MEK inhibitor PD98059 were administrated by microinjection to lateral cerebral ventricle. The HPLC results showed that the neuroprotective effect of icariin against MPTP-induced decrease of DA content could be blocked by LY294002 or PD98059 (Fig 6). The immunohistochemistry results also showed that the blocking effect of LY294002 or PD98059 on icariin-induced increase of TH-IR neurons in SNpc (Fig 7). LY294002 or PD98059 treatment alone had no effect on striatum DA content and the survival of TH-IR neurons in SNpc (data not shown).

Effects of icariin on the phosphorlyation of Akt and ERK and the blocking effect of LY294002 or PD98059 in MES23.5 cells

In order to further confirm the direct effect of icariin on Akt and ERK signaling pathways, we assessed the effects of icariin on the phosphorlyation of Akt and ERK

and the blocking effects of LY294002 or PD98059 in dopaminergic MES23.5 cells. Exposure to 0.01μM icariin for 5, 10 and 20min, the phosphorylation levels of Akt and ERK significantly increased in a time dependent pattern (Fig 8A&8B). The maximum occurred at 5min and these effects could be completely blocked by LY294002 or PD98059 (Fig 8C&8D).

Discussion

Accumulating evidence has shown that icariin possess various biological functions including anti-inflammations, anti-osteoporosis, antioxidants and cardiovascular system protective activities. Several lines of evidence have indicated the beneficial actions of icariin on learning and memory (He et al, 2010; Nie et al, 2010; Wu et al, 2012). Until now, neuroprotective effects of icariin on nigrostriatal system in PD mice model remains unexplored. The present study clearly demonstrated that icariin could ameliorate the decrease of striatum DA content and loss of doparminergic neurons in SNpc induced by MPTP. Mechanism study revealed that modulation of apoptosis-related protein expressions as well as PI3-K/Akt and MEK/ERK pathways is involved in the neuroprotective actions of icariin.

MPTP is a neurotoxic compound which could induce apoptosis and generate the mice model of PD (Yeo et al, 2015). In the present study, we found that icariin significantly increased the striatum DA content in a dose-dependent manner and the most effective dosage of icariin is 100 mg/kg. Moreover, Immunohistochemistry results indicated that icariin (100 mg/kg) could partially reversed the decreases of TH-IR in the SN induced by MPTP treatment. Furthermore, in order to elucidate the possible mechanism of the neuroprotective effect of icariin, the apoptosis-related protein expressions in striatum were determined. Bcl-2, as an anti-apoptotic protein, is modulated by the PI3K/Akt. Activated Akt could increase the transcription and post-transcription of Bcl-2 which played an important role in anti-apoptosis in many

cell types, including hippocampal neurons, PC12 cell and dopaminergic neuron MES23.5 cells (Ge et al, 2010; Pugazhenthi et al, 2000). Bax and caspase 3 play an important role in the execution of apoptosis (Salakou et al, 2007). The western blot results demonstrated that MPTP-induced decrease in Bcl-2 and increase in Bax and caspase 3 protein expressions could be reversed by icariin pretreatment, indicating the anti-apoptosis effect of icariin.

Rotarod test results showed that MPTP treatment significantly increased the residence time, while no significant difference was found in 50mg/kg, 100mg/kg and 200mg/kg icariin treatment group. Numerous studies have measured the changes of behavioral activity in MPTP mice, but conflicted results show decreased, increased and neutral phenomenon on behavioral activity. The reason might be due to different intoxication protocols or different gender and sources of mice. Several lines of evidence show that acute MPTP treated mice can exhibit a remarkable capacity of functional recovery within several days, so that MPTP treated mice look relatively unimpaired soon after cessation of treatment (Rousselet et al, 2003; Sedelis et al, 2001).

Research literature have demonstrated that several signaling pathways, such as IGF-1, TGF-β, PI3K, MAPK, NOS et al., are involved in the biological effects of icariin. Icariin enhances the osteogenic differentiation by increasing IGF-1 and TGF-β mRNA expressions (Chen et al, 2007). Chung (2008) reported that MEK/ERK and PI3K/Akt/eNOS signaling pathways are involved in the angiogenesis mediated by icariin.

Studies have shown that the PI3K pathway plays a significant role to protect against many apoptotic stimuli (D'Astous et al, 2006). The Akt protein kinase is a key downstream effector of PI3K signaling pathway and Akt phosphorylation is required for PI3K/Akt signaling transduction (Abeyrathna & Su, 2015; Franke et al, 1995). A number of studies demonstrate the involvement of Akt in various brain disorders such as Alzhermer's disease (Onyango et al, 2005), ischemia (Brywe et al, 2005), autism (Sheikh et al, 2010), seizures (Cote et al, 2005). In the present study, our results clearly showed that PI3K inhibitor LY294002 attenuated the increase of DA content in the striatum and TH-IR in the SNpc induced by icariin in PD mice model. These data suggest that Akt activation is involved in the protective effect of icariin on nigrostriatal system.

The MEK/ERK signaling pathway is known to stimulate cell growth and inhibit cell apoptosis (Smith & Dahodwala, 2014). The ERK is a serine/threonine kinase which could be phosphorylated by MEK. In the present study, we consistently found that MEK/ERK-mediated signaling pathway was involved in the neuroprotective effects of icariin against MPTP-induced neurotoxicity. Using MEK inhibitor PD98059 attenuated the increase of DA content in the striatum and TH-IR in the SNpc induced by icariin.

Dopaminergic MES23.5 cells are derived from somatic cell fusion characterized by TH expression and DA synthesis (Crawford et al, 1992). We reported here for the first time that icariin treatment alone induced the phosphorylation of Akt and ERK. These effects could be attenuated by LY294002 or PD98059, further suggesting the

involvement of Akt and ERK signaling pathways in the neuroprotective effect of icariin. The study by Song et al. showed that icariin could induce osteoblast proliferation, differentiation and mineralization through estrogen receptor-mediated ERK and JNK signal activation (Song et al, 2013). A recent study reported that icariin could induce estrogen receptor α phosphorylation at serine 118 residue without exhibiting binding affinities toward estrogen receptor α and β in UMR-106 cells (Xiao et al, 2014). Our recent data indicated that icaritin, a natural derivative of icariin, could protect against MPP+-induced toxicity in MES235 cells via IGF-1 receptor pathway (Jiang et al, 2016). Therefore, our future study will focus on the upstream pathway of Akt and ERK activation by icariin.

In conclusion, the present study provided new evidence for the neuroprotective effects of icariin on nigrostriatal system against MPTP-induced dopaminergic neuronal death in mice. The mechanism of its actions might be related to the PI3K/Akt and MEK/ERK signaling pathways. Icariin may be an alternative strategy to protect against neurotoxin damage in nigrostriatal system.

Conflict of interest

The authors have no conflict of interests to declare.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (30971004, 31271150), the State Key Program of National Natural Science of China (81430024).

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Figure Legends

Fig 1. Formular structure of icariin.

Fig 2. Rotarod test

8 days after icariin treatment, the time of residence on the automated accelerating rotarod apparatus (from 4 to 40 rpm) was evaluated. Each trial lasted 5 min with a 60 min inter-trial interval. The results are expressed as the average time of 3 trials. Data represent the mean ± SEM. n=5. $^{*}p<0.05$ vs the control group.

Fig 3. Dose-dependent effects of icariin on the striatum DA content of PD model mice

OVX mice were orally administered with vehicle or three doses of icariin (ICA50, 50mg/kg per d; ICA100, 100mg/kg per d; ICA200, 200mg/kg per d) for 8 days. Three days after the icariin pretreatment, MPTP injection (four times with intervals of 2h, i.p) was performed at 2h after the intragastric administration of icariin or vehicle. 8 days after icariin treatment, the mice were sacrificed and the striatum were removed for HPLC assay. Data represent the mean ± SEM. n=9. $^{**}p<0.01$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPTP group.

Fig 4. Effects of icariin on MPTP-induced TH-IR neuronal death in the SNpc

(A) Immunohistochemistry of TH-IR neurons in sections of the SN in the control group, MPTP group, MPTP+ICA50 group, MPTP+ICA100 group and MPTP+ICA200 group. Bar=100μm. (B) Numbers of TH-IR neurons in the SNpc. Data represent the mean ± SEM. n=6-7. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPTP group.

Fig 5. Effects of icariin on Bcl-2, Bax and caspase 3 protein expressions in the striatum of PD model mice

Western blot analysis was used to assess the effect of icariin on the protein expressions of Bcl-2, Bax, caspase 3 and β-actin. Data represent the mean ± SEM. n=6. $^{*}p<0.05$ vs the control group, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$, vs the MPTP group.

Fig 6. The blocking effect of LY294002 or PD98059 on icariin-induced neuroprotection on the striatum DA content of PD model mice

OVX mice were treated with vehicle, icariin, icariin+LY294002 and icariin+PD98059. The treatment lasted 8 consecutive days. Three days after the icariin pretreatment in the absence or presence of LY294002 and PD98059, MPTP injection (four times with intervals of 2h, i.p) was performed at 2h after the intragastric administration of icariin or vehicle. 8 days after icariin treatment, the mice were sacrificed and the quantitative analysis of striatum DA content was performed in the control group, MPTP group, MPTP+ICA group, MPTP+ICA+LY group and MPTP+ICA+PD group. Data represent the mean ± SEM. n=6. $^{*}p<0.05$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$vs the MPTP group.

Fig 7. The blocking effect of LY294002 or PD98059 on icariin-induced neuroprotection on the TH-IR neurons in the SNpc of PD model mice

After treatment, the mice were sacrificed and the midbrain was removed for immunohistochemistry. (A) Immunohistochemistry of TH-IR neurons in sections of the SNpc in the control group, MPTP group, MPTP+ICA group, MPTP+ICA+LY group and MPTP+ICA+PD group). Bar=100μm. (B) Quantitative analysis of TH-IR neurons. Data represent the mean ± SEM. n=6. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPTP group.

Fig 8. Effects of icariin on the phosphorlyation of Akt and ERK in MES23.5 dopaminergic cells

MES23.5 cells were incubated with icariin (0.01μM) for 5, 10 and 20min, proteins were isolated and the phosphorylation of Akt (A) and ERK (B) were determined by western blot. For PI3K or MEK inhibition studies, MES23.5 cells were pretreated for 1h with LY294002 (5μM) or PD98059 (10μM) prior to icariin treatment. Five minutes after icariin treatment, the phosphorylation of Akt (C) and ERK (D) were determined by western blot. Data represent the mean ± SEM. n=3. $^{*}p<0.05$ vs the control group, $^{\wedge\wedge}p<0.01$ vs the icariin group.

Figure 1.

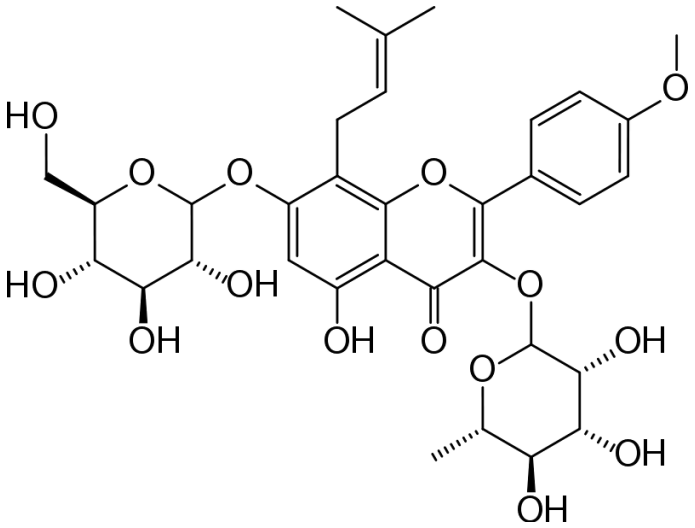
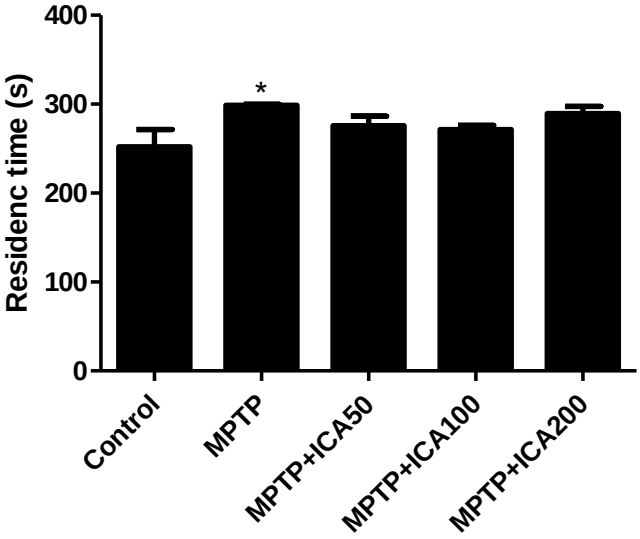


Figure 2.


400
300
200
100
0
Residenc time (s)
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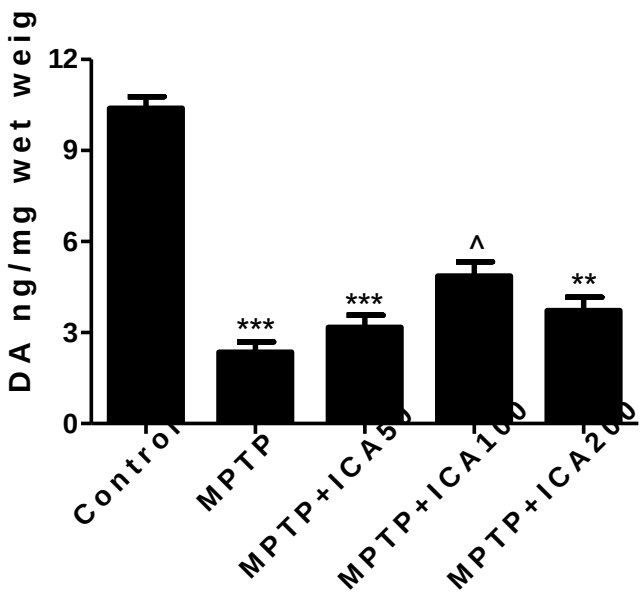
Control
MPTP
MPTP+ICA50
MPTP+ICA100
MPTP+ICA200

Figure 3.


DA ng/mg wet weig
12
9
6
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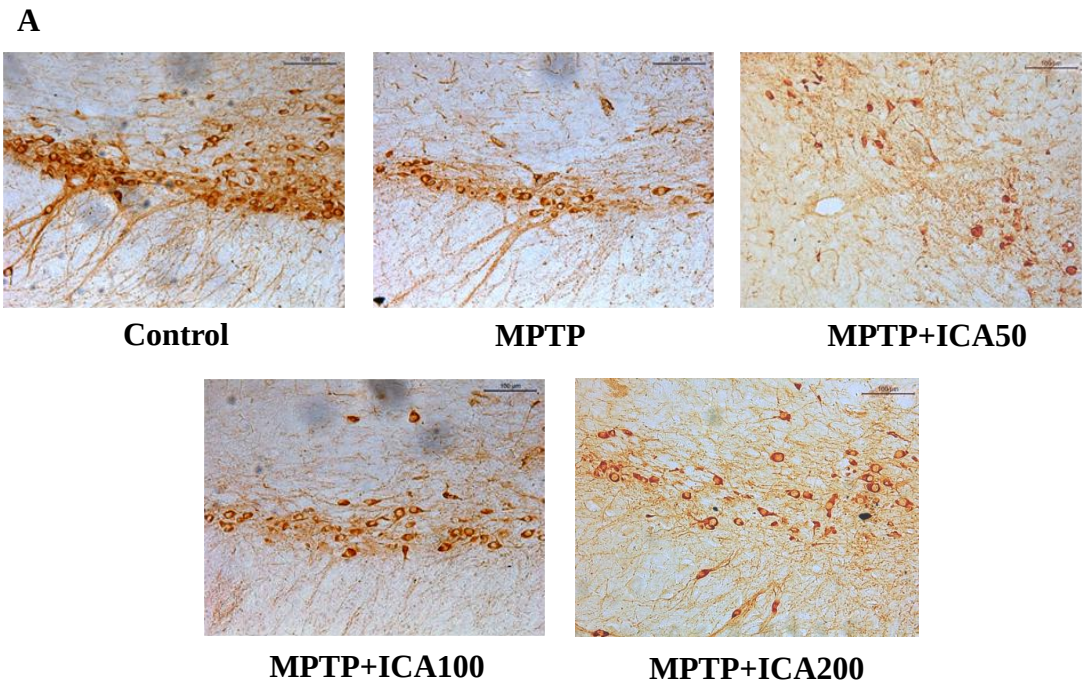
Control
MPTP
MPTP+ICA50
MPTP+ICA100
MPTP+ICA200

Figure 4.


A
Control
MPTP
MPTP+ICA50
MPTP+ICA100
MPTP+ICA200

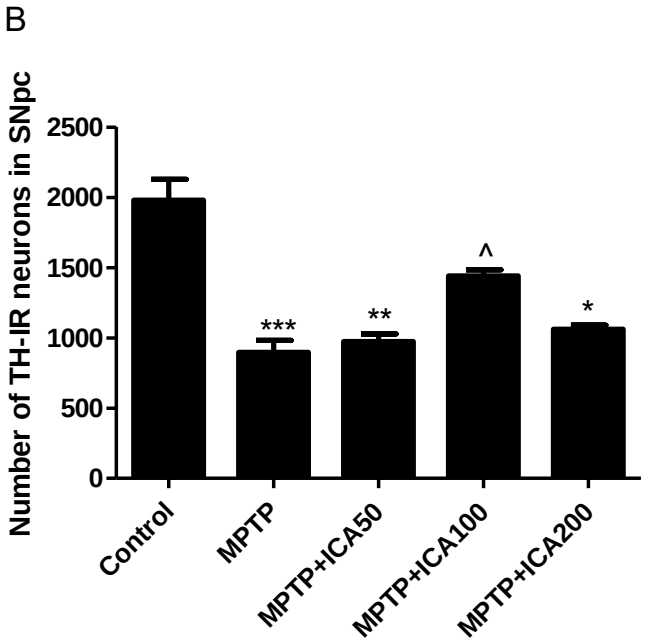

B
Number of TH-IR neurons in SNpc
2500
2000
1500
1000
500
0
***
**
^
*
Control
MPTP
MPTP+ICA50
MPTP+ICA100
MPTP+ICA200

Figure 5.

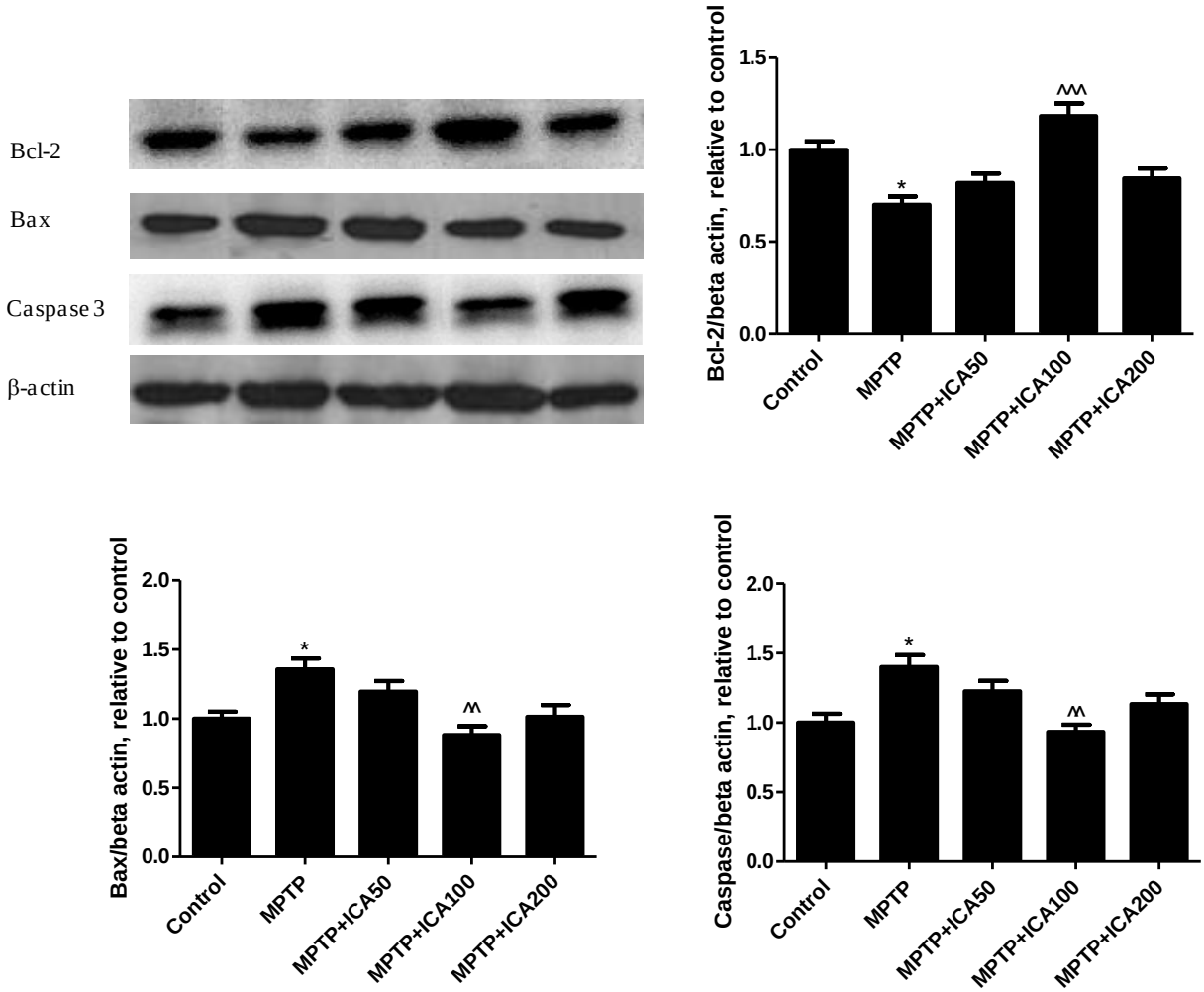
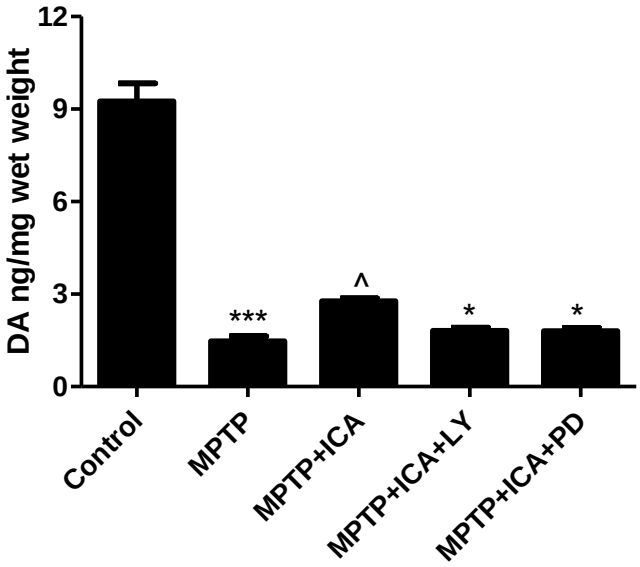

Bcl-2
Bax
Caspase 3
β-actin
Bcl-2/beta actin, relative to control
Bax/beta actin, relative to control
Caspase/beta actin, relative to control
Control
MPTP
MPTP+ICA50
MPTP+ICA100
MPTP+ICA200

Figure 6.


DA ng/mg wet weight
12
9
6
3
0
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*
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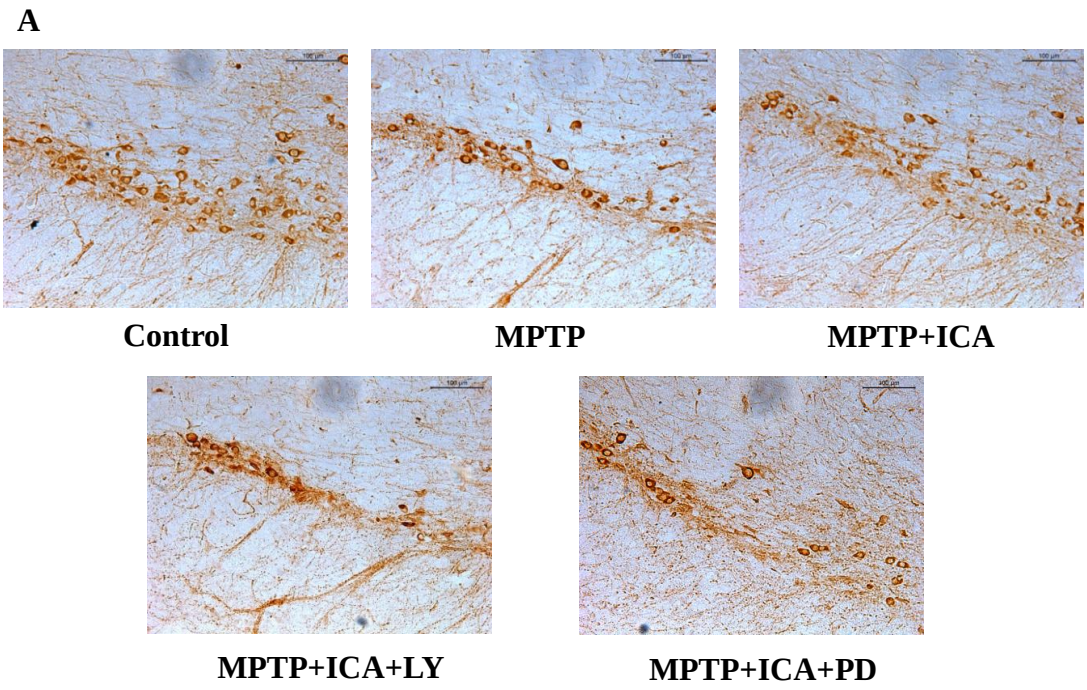
Control
MPTP
MPTP+ICA
MPTP+ICA+LY
MPTP+ICA+PD

Figure 7.


A
Control
MPTP
MPTP+ICA
MPTP+ICA+LY
MPTP+ICA+PD

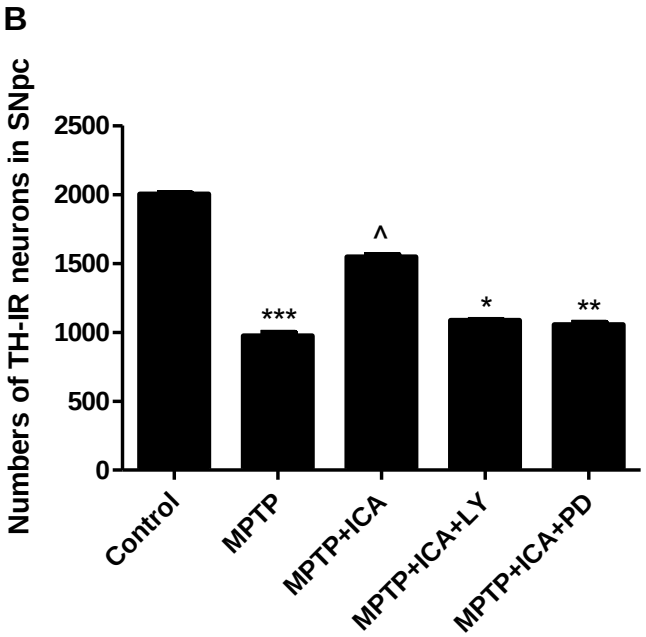
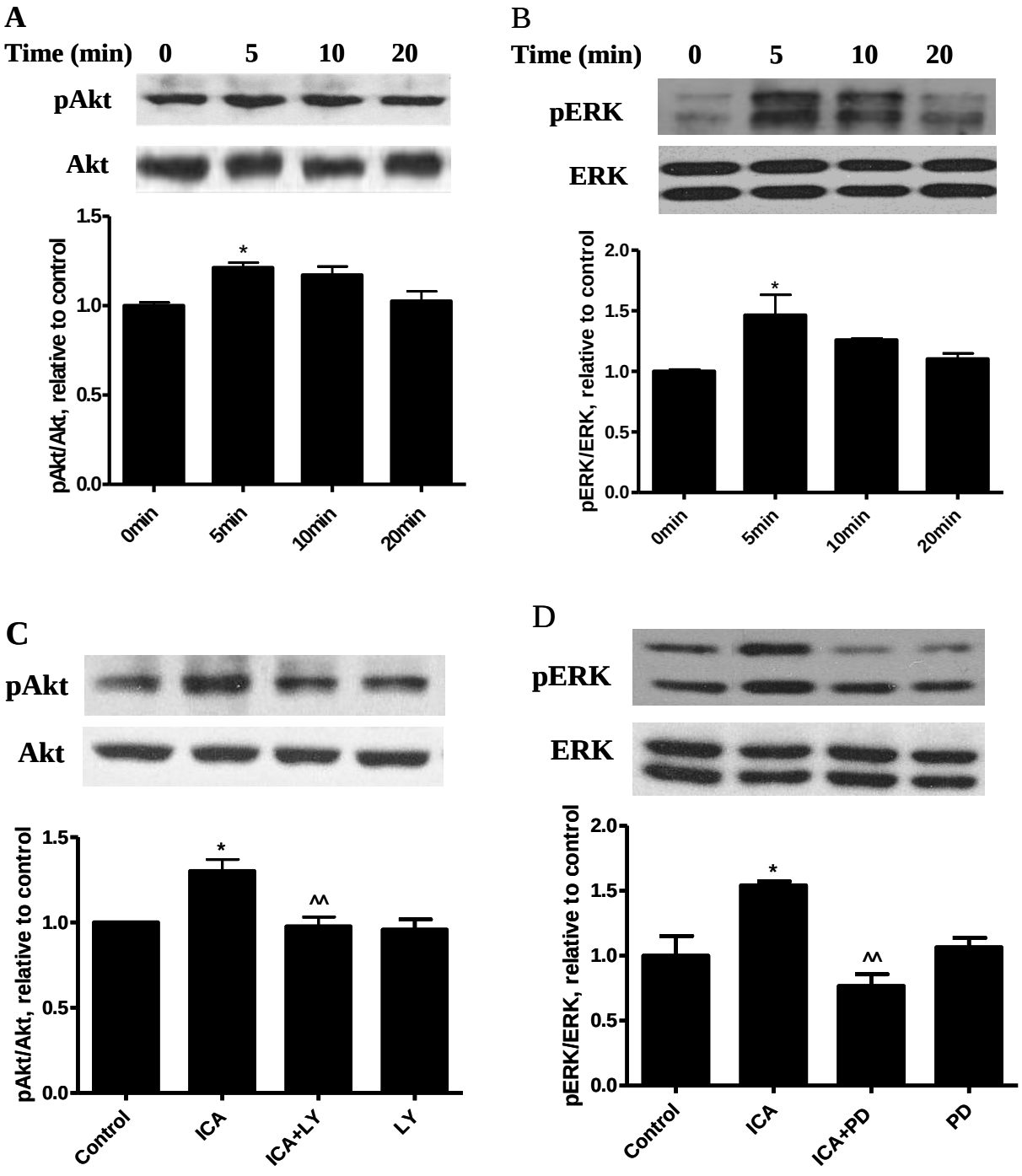

B
Numbers of TH-IR neurons in SNpc
2500
2000
1500
1000
500
0
***
^
*
**
Control
MPTP
MPTP+ICA
MPTP+ICA+LY
MPTP+ICA+PD

Figure 8.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pAkt
Akt
pAkt/Akt, relative to control
0min
5min
10min
20min
B
Time (min) 0 5 10 20
pERK
ERK
pERK/ERK, relative to control
0min
5min
10min
20min
C
pAkt
Akt
pAkt/Akt, relative to control
Control
ICA
ICA+LY
LY
D
pERK
ERK
pERK/ERK, relative to control
Control
ICA
ICA+PD
PD

e-component

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