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Neuroprotective effects of total flavonoid fraction of the *Epimedium koreanum Nakai* extract on dopaminergic neurons：*in vivo* and *in vitro*

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Abstract Flavonoids, the active components of *Epimedii Genus*, have been demonstrated to protect against osteoporosis, cardiovascular diseases and rheumatoid arthritis. The present study aimed to investigate the neuroprotective effects of total flavonoid (TF) fraction of *Epimedium koreanum Nakai* on dopaminergic neurons in the cellular and mice models of Parkinson's disease (PD). TF pretreatment could ameliorate the decrease of striatal dopamine (DA) content and the loss of tyrosine hydroxylase (TH) -immunoreactive neurons in the substantia nigra pars compacta (SNpc) induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). TF treatment could reverse the changes of Bcl-2 and Bax protein expressions in the striatum of PD mice. 1-Methyl-4-phenylpyridinium ion (MPP^+) significantly decreased the cell viability and mitochondrial membrane potential in MES23.5 cells. These effects could be reversed by TF treatment. In addition, MPP^+-induced changes of Bcl-2 and Bax mRNA and protein expressions were also reversed by TF pretreatment. These data demonstrated that TF of *Epimedium koreanum Nakai* could protect against MPTP-induced dopaminergic neuronal death in mice and MPP^+-induced neurotoxicity in dopaminergic MES23.5 cells. Anti-apoptosis might be involved in this process.

Keywords： *Epimedium koreanum Nakai*; total flavonoids; dopaminergic neuron; neuroprotection; Parkinson's disease

Abbreviations

DA, dopamine; ERE, estrogen response element; MES23.5, dopaminergic hybridoma cell line; MPP^+, 1-Methyl-4-phenylpyridinium ion; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; SN, substantial nigra; SNpc, substantia nigra pars compacta; TF, total flavonoid; TH, tyrosine hydroxylase

1. **Introduction**

Accumulated clinical studies support that the incidence of Parkinson's disease (PD) in male is higher than that in women, suggesting the potential neuroprotective effects of estrogen [1]. The association between hormone replacement therapy and the risk of development of PD remains controversial in clinic at present [2]. Animal and cellular researches provide strong evidence for the neuroprotective effects of estrogen on dopaminergic neurons [3, 4]. Even though, flavonoids that display estrogenic properties might be one of the potential alternatives of estrogens due to the few side effects comparing with estrogens [5]**.**

Epimedium Genus is a traditional Chinese herb used for the treatment of osteoporosis, cardiovascular diseases and rheumatoid arthriti [6-8]. Flavonoids are the active components of *Herba Epimedii* extract, which account for its biological effects both *in vivo* and *in vitro*. Our previous study showed that total flavonoids (TF) of *Epimedium koreanum Nakai* could promote the differentiation of primary osteoblasts and stimulate bone formation and suppress bone resorption in ovariectomized mice [9, 10]. In the following study, we demonstrated that four compounds isolated from TF, including epimedin B, icariin, sagittatoside A and baohuoside I, had estrogenic effects on UMR106 cells [11]. Sun's research group found that *Herba Epimedii* decoction could regulate the expression of transcription factors of in the hypothalamus of aged rats, indicating the neuro-endocrine regulatory effect of *Herba Epimedii* [7]. Water extract of *Herba Epimedii* could regulate lipid metabolism by decreasing the total cholesterol and triglyceride and elevating estrogen level in postmenopausal women [12]. Icariin, the major ingredient of TF of *Herba Epimedii*, has been demonstrated the neuroprotective effects including the improvement of learning and memory of on the aged and transgenic mice [13]. Guo et al. has reported that icariin could protect against lipopolysaccharide-induced deficits of spatial learning and memory in rat [14]. While, there is no previous report about the anti-neurotoxic effects of TF of *Epimedium koreanum Nakai* on DA neurons both *in vivo* and *in vitro*.

In the present study, we aim to study the neuroprotective effects and the potential mechanisms of TF using MPTP-mediated nigrostriatal injury in the mice and MPP^+-induced neurotoxicity in the dopaminergic MES23.5 cells.

2. Materials and Methods

2.1 Materials

Antibody against tyrosine hydroxylase (TH) was purchased from Millipore (Bedford, MA, USA). Antibodies against Bcl-2, Bax and secondary antibody were supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Antibody against β-actin was supplied by Cell Signaling Technology, Inc. (Hertfordshire, England). QuantiFast™ SYBR Green PCR Kit was provided by Qiagen (Germantown, MD, USA). All other chemicals were obtained from commercial sources.

2.2 Preparation of TF fraction from *Epimedium koreanum Nakai* total extract

The source, extraction and typical chromatographic profile of TF have been described in our previous report [10]. Briefly, *Epimedium koreanum Nakai* were collected in June-July 2003 in Xinbin (Liaoning Province, China). They were authenticated according to the method listed in Chinese Pharmacopoeia [15] with the help of Professor Qishi Sun (Shenyang Pharmaceutical University, China). It was deposited as a voucher specimen (No. 19980816-1) in the herbarium of the Shenzhen Research Center of Traditional Chinese Medicine and Natural Products. The dried and powdered aerial parts of *Epimedium koreanum Nakai* (55 kg) were refluxed with 550 liters of water two times, for 2 h each time. After filtration, the filtrate was concentrated under reduced pressure, and then was applied to a D-101 macroporous adsorptive resin column and eluted with water, 30%, 50% and 95% ethanol (v/v) gradient to obtain four fractions with the yield of 5.5%, 1.7%, 1.2% and 0.2%, respectively. The TF fraction (770 g) was composed of the fractions of 50% and 95% ethanol. Epimedin B and icariin were selected as the authentic markers as showing in the HPLC profile of TF fraction (Fig. 1). The quantities of the two markers were about 0.392 mg/ml (epimedin B) and 1.053 mg/ml (icariin). The HPLC profiles of TF and the markers were established using Agilent series 1100 HPLC system; PR-HPLC column (Agilent Eclipse XDB-C18, 4.6 × 150 mm) with gradient methanol-water as mobile phase (0-10 min, 40%-50%; 10-30 min, 50%-60%; 30-40 min, 60%-100%; 40-50 min, 100%; 50-60 min, 40%), at a flow rate of 1.00 ml/min. Detection wavelength was at 254 nm and column temperature was at 30 ^{o}C.

2.3 Animal study

Thirty 10-12-week-old adult female C57BL/6 mice weighing 19-22 g were provided by Vital River Experimental Animal Center of Beijing (Beijing, China). All surgery was performed under chloral hydrate anesthesia and all animal procedures were followed the National Institutes of Health guide for

the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978) and approved by the Animal Ethic Committee of Qingdao University. Mice were ovariectomized as previously described [16]. Two weeks after ovariectomy, mice were divided randomly into 5 groups including control, MPTP, MPTP+TF25 (TF, 25 mg/kg), MPTP+TF50 (TF, 50 mg/kg) and MPTP+TF100 (TF, 100 mg/kg). The mice were orally administered with vehicle or three doses of TF. The dosage of TF was chosen in accordance with our previous study [9]. The treatment lasted 8 days. On the fourth day of TF pretreatment, the mice were treated with MPTP 15 mg/kg, i.p (four times with intervals of two hours) or saline. Twenty-four hours after the last orally administration, the mice were sacrificed and both sides of striatum were harvested for HPLC and immunoblotting assay. The midbrain containing substantial nigra (SN) was removed for immunohistochemistry.

2.4 Determination of dopamine content in striatum

HPLC was used to determine the dopamine (DA) content as previously described [16]. In brief, the striatum of left side was homogenized in 0.3 ml liquid A (0.4 M perchloric acid) and followed by centrifugation (12,000 rpm × 20 min). 80 μl of the supernatant was mixed with 40 μl liquid B (20mM citromalic acid-potassium, 300 mM dipotassium phosphate, 2mM EDTA-2Na). The content of DA was detected by HPLC (Waters Corp., Milford, MA, USA).

2.5 TH immunohistochemistry staining

The midbrains containing the SN were fixed in 4% paraformaldehyde for 6h which followed by transferring into 30% sucrose for 48 h. Serial coronal sections (16 μm) were cut through substantia nigra pars compacta (SNpc) on a freezing cryostat (Leica, German). Immunohistochemistry was performed as described previously [16]. Briefly, after blocking with 10% of goat serum for 30 min, the sections were incubated with polyclonal antibody against TH (1: 3,000) overnight at 4°C, followed by incubation with biotinylated goat anti-rabbit IgG for 2h at room temperature. TH-immunoreactive neurons were visualized by DAB. The counting on 12 sections of each mouse was performed blind on an Olympus microscope. The aim of the present study was to evaluate changes in TH-immunoreactive neurons between different groups. So, the survival rate of TH-immunoreactive neurons is calculated by the number of TH neuron in different treatment group relative to the number of TH neurons in control group.

2.6 Cell culture

The MES23.5 dopaminergic cell line was obtained from Dr. Wei-dong Le (Baylor College of Medicine, Houston, USA) [17]. MES23.5 cells were cultured in DMEM/F12 with Sato's components, 5% fetal bovine serum, penicillin 100 IU/ml and streptomycin 100 μg/ml.

2.7 Cell viability assay

MES23.5 cells were treated with TF (0.125, 0.25, 0.5 μg/ml) for 24h, followed by co-treatment with MPP^+ (100 μM) and TF for another 24h. The 3-[4, 5-dimethylthiazol 2-yl] 2, 5-diphenyltetrazolium bromide (MTT) assay was used to measure the cell viability as previously described [18] by the microplate reader at 595 nm.

2.8 Real time quantitative RT-PCR analysis

Real time PCR were performed as previously described [18]. Briefly, total RNA (2 μg) was used for reverse transcription. Real time PCR was performed by a QuantiFast™ SYBR Green PCR Kit. The gene-specific primers for Bcl-2, Bax and GAPDH were 5'-CCTGTGGATGACTGAGTACC-3' (Bcl-2 forward) and 5'-CCCACTCGTAGCCCCTCT-3' (Bcl-2 reverse), 5'-GGCGAATTGGAGATGAAC-3' (Bax forward) and 5'-CCGAAGTAGGAGAGGAGG-3' (Bax reverse) and 5'-ACCCAGAAGACTGTGGATGG-3' (GAPDH forward) and 5'-CCCTGTTGCTGTAGCCGTAT-3' (GAPDH reverse).

2.9 Flow cytometry

MES23.5 cells were treated with TF (0.125, 0.25, 0.5 μg/ml) for 24h, followed by the co-treatment with MPP^+ (100 μM) and TF (0.25 μg/ml) for another 24 h. As previously described [18], MES23.5 cells were exposed to Rhodamine 123 (5 μM) for 15 min at room temperature. After washing with PBS, 10000 cells were used to detect the signals by the flow cytometry (BD, USA).

2.10 Western blotting

The striatum of right side or MES23.5 cells was lysed with Nonidet P-40 buffer containing protease and phosphatase inhibitors as previously described [18]. After protein electrophoresis and transfer film, the blots were blocked with 10% skimmed milk for 2 h and probed with primary antibody against Bcl-2, Bax or β-actin overnight at 4°C. Then, the membranes were incubated with HRP-coupled secondary antibody for 2 h at room temperature. The band density was detected with ECL reagent using Imager (UVP Biospectrum 810, USA).

2.11 Statistical analysis

Data were reported as the mean ± SEM. Statistical analyses were performed by One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. $P<0.05$ was considered significant.

3. Results

3.1 Effects of TF on the DA content in the striatum of PD mice model

In order to test if TF has protective effect on dopaminergic neurons in PD mice model, the DA content in striatum was determined by HPLC. As shown in Fig. 2, we consistently found that MPTP treatment significantly decreased the DA content in the striatum ($P<0.001$). Pretreatment with 25, 50 and 100 mg/kg TF increased the DA content in a dose dependent manner. Since the 100 mg/kg dosage was the only one providing a significant effect, it was chosen for subsequent experiments.

3.2 Effects of TF on TH-immunoreactive neurons in the SNpc of PD mice model

Damages of the nigrostriatal system are associated with the loss TH-immunoreactive neurons in the SNpc and deficit of DA in the striatum. To further confirm the neuroprotection of TF on dopaminergic neurons, the TH-immunoreactive neurons in the SNpc were examined using immunohistochemistry. The number of TH-immunoreactive neurons significantly decreased in MPTP-treated animals ($P<0.001$) (Fig. 3A & B). In contrast, TF (100 mg/kg) pretreatment significantly increased the survival of TH-immunoreactive neurons ($P<0.05$).

3.3 Effects of TF on the apoptotic-related protein expressions in the striatum of PD mice model

In vivo, the neurotoxin MPTP is converted to MPP^+ to induce mitochondrial-dependent apoptosis of dopaminergic neuron in the SNpc. To investigate the mechanism involved in the protective effects of TF, the Bcl-2 and Bax protein level in the striatum were determined by western blot. Our results clearly showed that the Bcl-2 protein level was significantly decreased in PD mice model ($P<0.05$). Pretreatment with TF attenuated the MPTP-induced down-regulation of Bcl-2 protein expression ($P<0.001$) (Fig. 4 A & B). On the contrary, MPTP treatment significantly increased Bax protein expression ($P<0.05$) which was reversed by TF pretreatment ($P<0.01$) (Fig. 4 A & C).

3.4 Neuroprotective effects of different dosage of TF against MPP^+-induced cell death on MES23.5 cells

To discern if TF could protect against the toxicity of MPP^+ in MES23.5 dopaminergic cells, firstly, the cell viability was detected. Pretreatment with TF (0.125, 0.25, 0.5 μg/ml) 24h before and co-treatment with MPP^+ for another 24 h resulted in an increase of cell viability ($P<0.05$) (Fig. 5). The most effective concentration was 0.25 μg/ml which was used in subsequent experiments. TF (0.125, 0.25,

0.5 μg/ml) treatment alone did not affect cell viability (data not shown).

3.5 TF pretreatment reversed the MPP^+-induced changes of mitochondrial membrane potential

Flow cytometry was used to detect the mitochondrial membrane potential of MES23.5 cells. As shown in Fig. 6, MPP^+ treatment (Fig. 6 A.b) significantly decreased the mitochondrial membrane potential, comparing with the vehicle-treated cells (Fig. 6 A.a) (*P<0.001*). Pretreatment with TF (Fig. 6 A.c) resulted in a significantly 22% increase comparing with MPP^+ group (Fig. 6 A.b) (*P<0.05*).

3.6 TF inhibited the MPP^+-induced changes of Bcl-2 and Bax mRNA and protein expressions

MPP^+ treatment significantly decreased the Bcl-2 expression both transcriptionally and post-transcriptionally (both *P<0.05*) (Fig. 7 A & Fig. 8 A & B). TF pretreatment not only restore the Bcl-2 mRNA and protein expressions which were significantly decreased after MPP^+ induction (*P<0.001*, *P<0.01*), but also induced a statistic significantly increase comparing with the control group (*P<0.05*). In contrast, the mRNA and protein expressions of Bax were markedly increased in MPP^+-treated cells (both *P<0.05*) (Fig. 7 B & Fig. 8 A & C). Pretreatment with TF could significantly attenuate the MPP^+-induced up-regulation of Bax mRNA and protein expressions in MES23.5 cells (both *P<0.05*).

4. Discussion

Accumulating evidence has demonstrated the bone nourishment effect of *Epimedium Genus* extracts and active ingredients both *in vivo* and *in vitro* [9, 11, 19]. However, at present, no data have shown the neuroprotective effects of TF fraction of *Epimedium koreanum Nakai* on dopaminergic neurons. Here, the data are derived from a MPTP mice model and MPP^+ cellular model of PD. *In vivo*, we found that TF of *Epimedium koreanum Nakai* could protect against MPTP-induced decrease of DA content in striatum and loss of TH-immunoreactive neurons in SNpc of mice. Pretreatment with TF could reverse the MPTP-induced dysregulation of the protein expressions of Bcl-2 and Bax in the striatum. The neuroprotective effects of TF were also found in *in vitro* cellular PD model. TF could attenuated the MPP^+-induced cell death, the decrease of mitochondrial membrane potential and dysregulation of Bcl-2 and Bax mRNA and protein expressions.

MPTP is a neurotoxic compound which can induce mitochondrial-dependent apoptosis and promote dopaminergic neuron degeneration as well as declining levels of DA in the striatum in mice model of PD. The present study consistently found intraperitoneal injection of MPTP could significantly decrease the DA content in striatum. Oral administration of TF (25 mg/kg, 50 mg/kg, 100 mg/kg) attenuated the MPTP-induced deficiency of DA in a dose-dependent manner and the most effective dosage of TF is 100 mg/kg. The number of TH-immunoreactive neurons in SNpc was also markedly decreased in MPTP-treated mice which could be partly reversed by 100 mg/kg TF treatment, indicating the neuroprotective effects of TF on dopaminergic neurons.

In order to elucidate the mechanism involved in the neuroprotective effect of TF of *Epimedium koreanum Nakai*, western blot assay was used to detect the apoptosis-related protein expressions in striatum. Bcl-2 family, including Bcl-2 and Bax, plays a key role in the process of apoptosis. Bcl-2 is a classical anti-apoptotic protein which can inhibit the release of cytochrome C. On the contrary, Bax, a mitochondria-related proapoptotic protein, plays an important role in promotion of apoptosis [20]. Studies have shown that MPTP can induce dopaminergic neuron apoptosis via inhibiting complex I of the electron transport chain. In the present study, our results clearly demonstrated that MPTP treatment significantly induced down-regulation of Bcl-2 and up-regulation of Bax protein expressions. These effects could be completely overcome by TF (100 mg/kg) treatment, strongly suggesting the anti-apoptosis effect of TF.

MES23.5 dopaminergic cell line is derived from somatic cell fusion characterized by TH expression and DA synthesis [17]. Studies have demonstrated that MPP^+ treatment can promote mitochondria apoptosis which plays a key role in PD pathogenesis [21]. Consistent with previous reports, MPP^+ treatment significantly induced MES23.5 cell death after 24 h treatment. Pretreatment with different dosages of TF (0.125 μg/ml, 0.25 μg/ml, 0.5 μg/ml) could partly restore the cell viability and the most effective dosage is 0.25 μg/ml. Mechanism study provides new information about the anti-apoptosis effects of TF. TF pretreatment could partly reverse the MPP^+-induced decrease of mitochondria membrane potential. Furthermore, TF pretreatment not only restore the Bcl-2 mRNA and protein expressions which were significantly decreased after MPP^+ induction, but also induced a statistic significantly increase comparing with the control group. On the contrary, MPP^+ treatment significantly increased Bax expression transcriptionally and post-transcriptionally. TF pretreatment reversed it, further confirmed the anti-apoptosis effects of TF of *Epimedium koreanum Nakai*. The main components of TF of *Epimedium koreanum Nakai* include epimedin B, icariin, baohuoside I and sagittatoside A et al [10]. Our recent study has demonstrated the estrogen-like activity of the above four flavonoids in rat osteoblastic-like UMR-106 cells [11]. The results clearly showed that sagittatoside A significantly increased estrogen response element (ERE)-luciferase activity which can enhance the ERE-regulated gene expression. Perillo et al. has found that there are two EREs in the Bcl-2 coding region [22]. The significant increase of Bcl-2 mRNA and protein expression by TF pretreatment might be related to sagittatoside A. Icariin is the most abundant component of TF fraction in *Herba Epimedii*. It has been shown that icariin possess beneficial effects on learning and memory [23, 24]. Icariin could protect against corticosterone-induced apoptosis in hypothalamic neurons [25], suggesting that icariin might play an important role in the neuroprotective effects of TF on dopaminergic neurons. Further study should focus on the neuroprotective effects of monomer flavonoid on nigrostriatal system.

5. Conclusion

Taken together, TF of *Epimedium koreanum Nakai* could protect against MPTP-induced dopaminergic neuronal death in mice and MPP^+-induced neurotoxicity in dopaminergic MES23.5 cells. Mechanism studies suggested that anti-apoptosis might be involved in this process. This newly discovered neuroprotective effect of TF on nigrostriatal system provides novel insight to reveal the potential neuropharmacological effects of *Epimedium koreanum Nakai*.

Conflict of interest

The authors of this manuscript have declared that no competing interests exist.

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Figure Legends

Fig. 1 The typical chromatographic profile of total flavonoid (TF) extract of *Epimedium koreanum Nakai.*

Total extract of *Epimedium koreanum Nakai* was extracted with water and subjected to column chromatography. Four fractions including water, 30%, 50% and 95% ethanol were yielded. The TF was composed of the fractions of 50% and 95% ethanol. Epimedin B and icariin were selected as the authentic markers.

Fig. 2 Dose-dependent effects of TF on the striatal DA content in PD mice model.

The mice were sacrificed and the striatum were removed for HPLC assay. Data represent the mean ± SEM (n=6) ($^{***}p<0.001$ vs the control group, $^{\wedge\wedge\wedge}p<0.001$ vs the MPTP group).

Fig. 3 Effects of TF on the TH-immunoreactive neurons in the SNpc of PD mice model.

(A) Immunohistochemistry of TH-immunoreactive neurons in the SNpc in the control group (a. Control), MPTP group (b. MPTP), MPTP+TF (100 mg/kg) group (c. MPTP+TF100). Scale bar represents 100 μM. (B) Survival ratio of TH-immunoreactive neurons in the SNpc. Data represent mean ± SEM (n=6) ($^{*}p<0.05$, $^{***}p<0.001$ vs the control group, $^{\wedge\wedge}p<0.01$ vs the MPTP group).

Fig. 4 Effects of TF on Bcl-2 and Bax protein expressions in the striatum of PD mice model.

Total protein was isolated from the striatum and subjected to western blot analysis of Bcl-2 and Bax protein expressions. β-actin was used as loading control. Data represent mean ± SEM (n=5-6) ($^{*}p<0.05$ vs the control group, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$, vs the MPTP group).

Fig. 5 Neuroprotective effects of different dosage of TF against MPP^+-induced neurotoxicity on MES23.5 cells.

MES23.5 cells were treated with TF (0.125, 0.25, 0.5 μg/ml) for 24h, followed by co-treatment with MPP^+ (100 μM) and TF for another 24h. Cell viability was measured by MTT assay. Data represent mean ± SEM (n=6) ($^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPP^+ group).

Fig. 6 TF pretreatment reversed the MPP^+-induced changes of mitochondrial membrane potential.

Flow cytometry was used to detect the mitochondrial membrane potential. MES23.5 cells were treated with TF (0.25 μg/ml) for 24 h, followed by co-treatment with MPP^+ and TF for another 24 h. Data represent mean ± SEM (n=4) ($^{**}p<0.01$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPP^+ group).

Fig. 7 TF inhibited the MPP^+-induced changes of Bcl-2 and Bax mRNA expressions.

MES23.5 cells were treated with TF (0.25 μg/ml) for 24 h, followed by co-treatment with MPP^+ and TF

for another 24 h. The mRNA expressions of Bcl-2 (A) and Bax (B) were determined by real time RT-PCR. Data represent mean ± SEM (n=3) ($^{*}p<0.05$ vs the control group, $^{\wedge}p<0.05$, $^{\wedge\wedge\wedge}p<0.001$ vs the MPP^{+} group).

Fig. 8 TF inhibited the MPP^{+}-induced changes of Bcl-2 and Bax protein expressions.

MES23.5 cells were treated with TF (0.25 μg/ml) for 24h, followed by co-treatment with MPP^{+} and TF for another 24 h. The protein expressions of Bcl-2 and Bax were determined by western blot. Data represent mean ± SEM (n=3) ($^{*}p<0.05$ vs the control group, $^{\wedge}p<0.05$, $^{\wedge\wedge}p<0.01$ vs the MPP^{+} group).

Figure.1

50%+95%

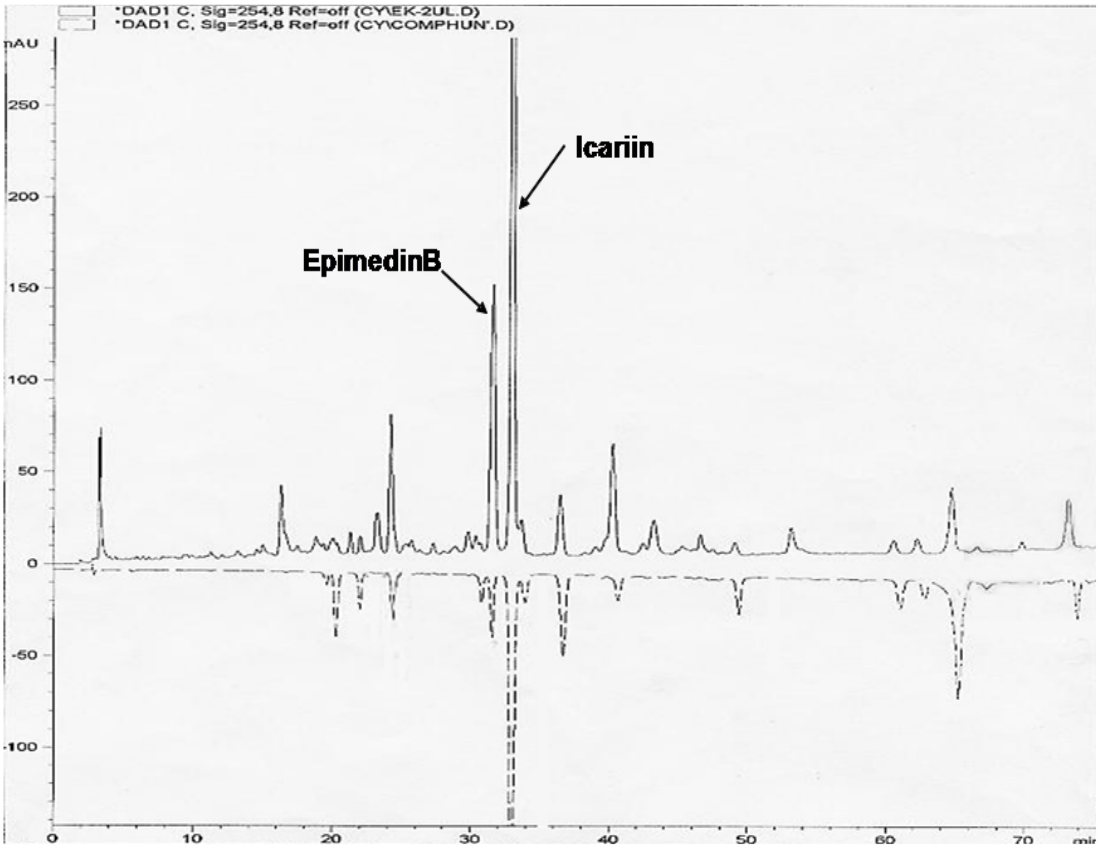


Figure.2

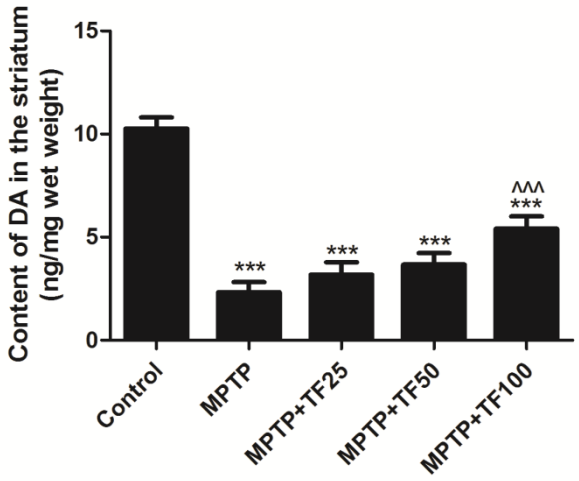
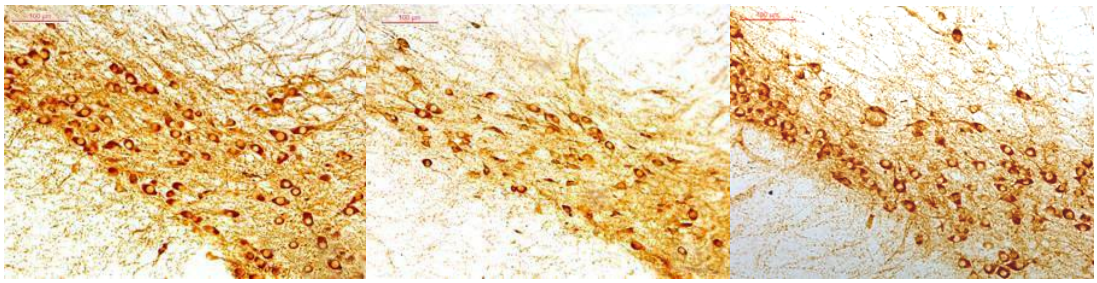

Content of DA in the striatum
(ng/mg wet weight)
15
10
5
0
***
***
***
^^^
***
Control
MPTP
MPTP+TF25
MPTP+TF50
MPTP+TF100

Figure.3

A.



a) Control **b) MPTP** **c) MPTP+TF100**

B.

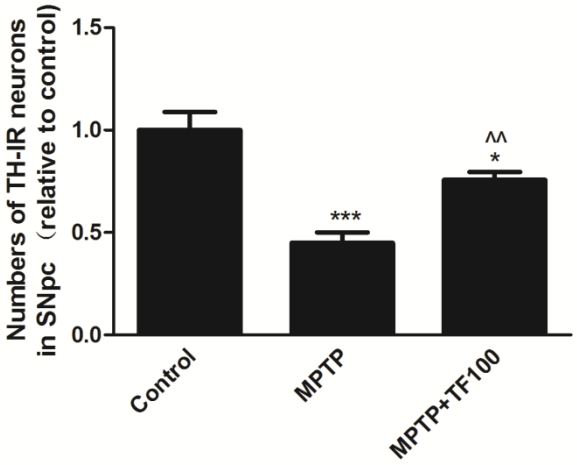
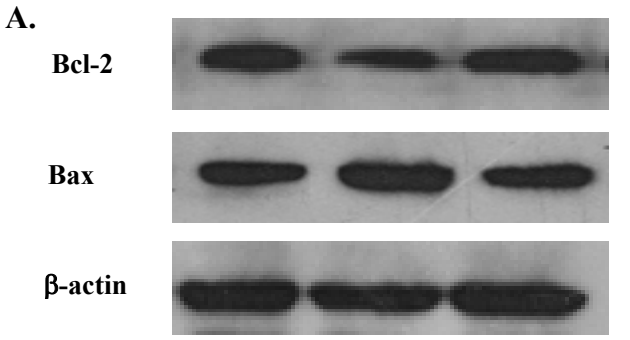
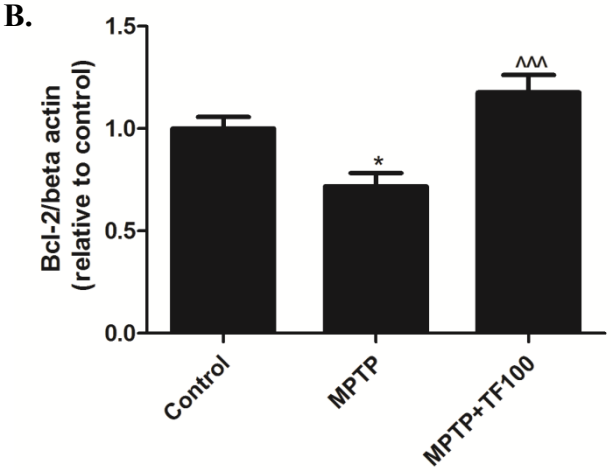


Figure.4


A.
Bcl-2
Bax
β-actin


B.
Bcl-2/beta actin
(relative to control)
1.5
1.0
0.5
0.0
*
^^
Control
MPTP
MPTP+TF100

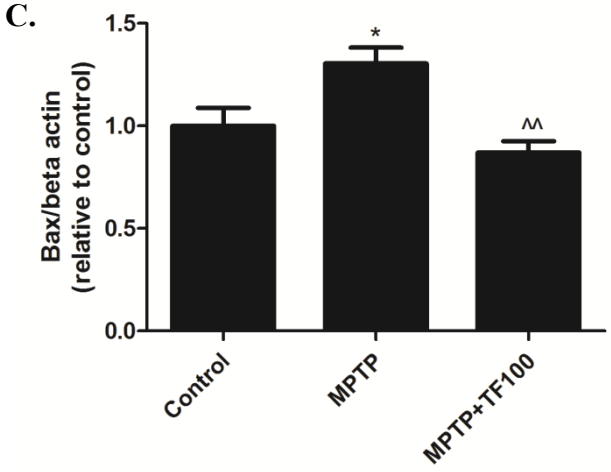

C.
Bax/beta actin
(relative to control)
1.5
1.0
0.5
0.0
*
^^
Control
MPTP
MPTP+TF100

Figure.5

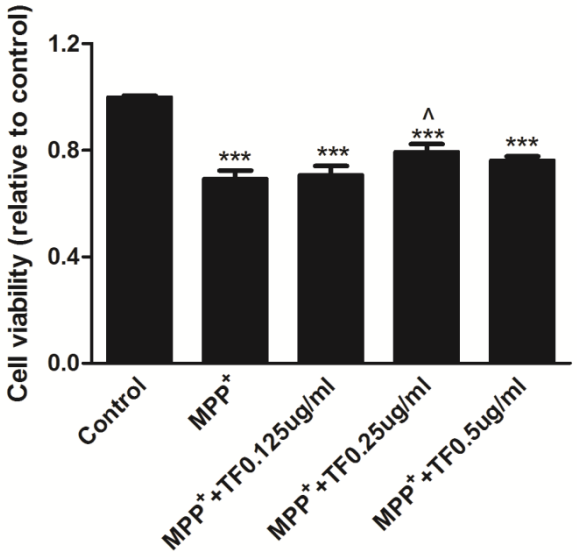
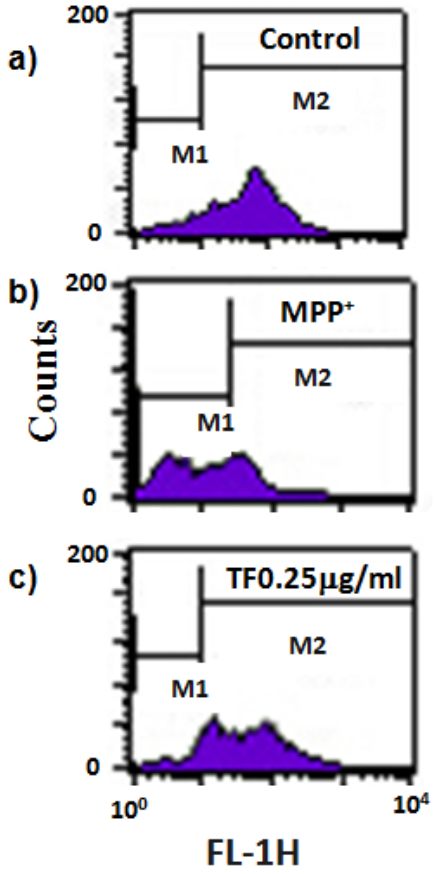

Cell viability (relative to control)
1.2
0.8
0.4
0.0
***
***
^
***
***
Control
MPP+
MPP+ +TF0.125ug/ml
MPP+ +TF0.25ug/ml
MPP+ +TF0.5ug/ml

Figure.6

A.


a)
200
Control
M2
M1
0
b)
200
MPP+
M2
M1
0
Counts
c)
200
TF0.25µg/ml
M2
M1
0
10^0
10^4
FL-1H

B.

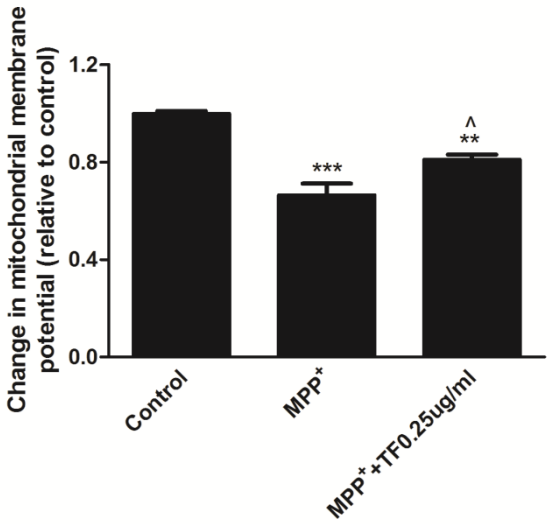

Change in mitochondrial membrane potential (relative to control)
1.2
0.8
0.4
0.0
***
^
**
Control
MPP+
MPP+ +TF0.25ug/ml

Figure.7

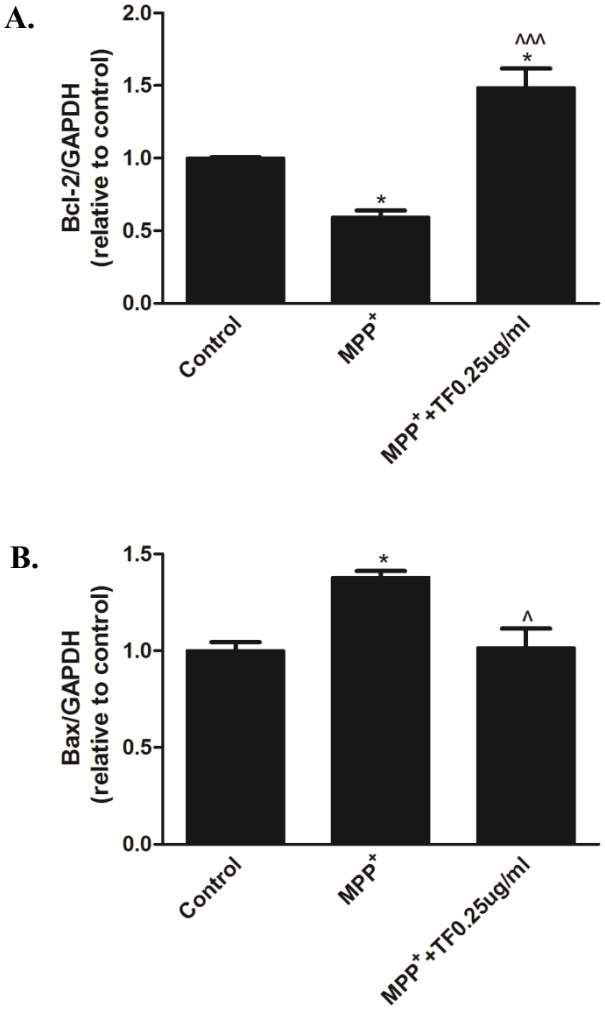
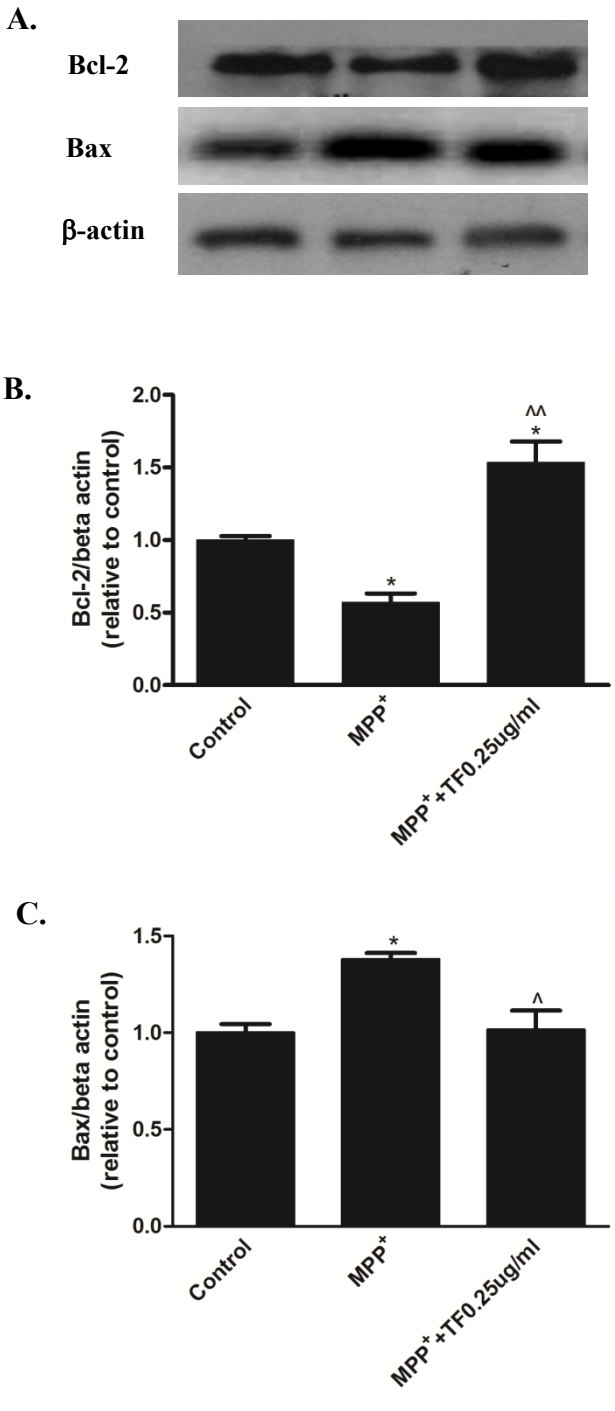

A.
Bcl-2/GAPDH
(relative to control)
2.0
1.5
1.0
0.5
0.0
*
^^^
*
Control
MPP+
MPP+ +TF0.25ug/ml
B.
Bax/GAPDH
(relative to control)
1.5
1.0
0.5
0.0
*
^
Control
MPP+
MPP+ +TF0.25ug/ml

Figure.8


A.
Bcl-2
Bax
β-actin
B.
Bcl-2/beta actin
(relative to control)
2.0
1.5
1.0
0.5
0.0
*
^^
Control
MPP+
MPP+ +TF0.25ug/ml
C.
Bax/beta actin
(relative to control)
1.5
1.0
0.5
0.0
*
^
Control
MPP+
MPP+ +TF0.25ug/ml