

# *Polygonum multiflorum* Extracts Protect the SH-SY5Y Cells against the Oxidative Stress Injury Induced by MPP<sup>+</sup>

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## Abstract

**Objective:** To investigate the protective effect of *Polygonum multiflorum* extracts (EPM) on SH-SY5Y cells treated with MPP<sup>+</sup>.

**Methods:** SH-SY5Y cells were first exposed to various doses of EPM, and then treated with MPP<sup>+</sup>. The cell viability was detected with MTT assay, cell morphology was observed with microscope by Hoechst33258 staining, the level of glutathione (GSH), Malondialdehyde (MDA) and the activity of Lactate dehydrogenase (LDH) were measured with UV spectrophotometer. Reactive oxygen species (ROS) and the mitochondrial membrane potential were observed with fluorescence microscope. The expression of p-JNK and Caspase 3 was analyzed by Western Blot.

**Results:** After 0.5 mmol/L MPP<sup>+</sup> treatment for 48 h, the viability of SH-SY5Y cells was decreased to 44.7% (VS. control group), the shrunk cell body and nuclear condensation were observed. Compared with control group, the activity of lactate dehydrogenase (LDH), the level of malondialdehyde (MDA) and reactive oxygen species (ROS) and the expression of p-JNK and Caspase 3 were increased significantly in MPP<sup>+</sup> treated SH-SY5Y cells, whereas the level of GSH and the mitochondrial membrane potential was reduced significantly in MPP<sup>+</sup> treated group. However, pretreatment with EPM (at the concentration of 5, 25 and 100 mg/L) 4 h prior to being exposed to MPP<sup>+</sup> rescued the cell viability of SH-SY5Y cells, and restored the cell morphological features in a dose-dependent manner. The increase of lactate dehydrogenase (LDH), malondialdehyde (MDA) and reactive oxygen species (ROS) and the expression of p-JNK and Caspase 3 induced by MPP<sup>+</sup> were also inhibited significantly by pretreatment with EPM. Exposure to EPM also blocked the reduction of the GSH level and the mitochondrial membrane potential induced by MPP<sup>+</sup> in SH-SY5Y cells.

**Conclusion:** Our results suggested that the EPM was able to protect the SH-SY5Y cells against the damage induced by MPP<sup>+</sup>, with the mechanism involved in resisting the oxidative stress.

**Keywords:** *Polygonum multiflorum* extracts (EPM); 1-methyl-4-phenyl-pyridine (MPP); SH-SY5Y cell; Oxidative stress; Neuroprotection

**Abbreviations:** EPM: *Polygonum multiflorum* Extracts; GSH: Glutathione; LDH: Lactate Dehydrogenase; MPP: 1-methyl-4-phenyl-pyridine; PD: Parkinson's Disease; MDA: Methane Dicarboxylic Aldehyde; MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide; ROS: Reactive Oxygen Species; TCM: Traditional Chinese Medicine

## Introduction

Parkinson's disease (PD) is a common neurodegenerative disease. The main pathological features of PD are the changes of nigra dense area and the lack of dopamine neurons [1]. It's clinically manifested as resting tremor, muscle rigidity, bradykinesia, and postural reflex disorder. The mechanism of PD is not clear so far, though mitochondrial dysfunction, oxidative stress, the endoplasmic reticulum stress and nerve inflammation are generally considered as the major causes of PD [2]. The current clinical drugs for the treatment of PD are only for one or several targets in the pathogenesis and their side effects cannot be ignored [3].

Traditional Chinese medicine (TCM) in the prevention and treatment of PD and other age-related diseases have rich practical experience with fewer and lower toxicity, getting more and more attention of medicinal and pharmaceutical industry [4].

Fleece-flower root, Traditional Chinese medicine Heshouwu (*Polygonum multiflorum*), is often used as its form of raw *radix polygoni multiflori* or *radix polygoni multiflori preparata* in prescription. After

processing, the raw *radix polygoni multiflori* can become *polygoni multiflora preparata*, which tastes bitter, sweet, acerbity, tepid, belonging to the liver, heart, kidney, kidney, shows benefiting JingXie, strengthening bones and muscles, blacking beard, turbidity, lipid-lowering effects [5].

Studies have shown that fleece-flower root has certain nerve protective effects.

Using a neurotoxin MPP<sup>+</sup> (1-methyl-4-phenyl-pyridine (ion)) to establish a PD model on SH-SY5Y cells, we attempted to explore the protective effects of fleece-flower root extract on this model and its possible mechanism, to provide experimental basis for the exploitation of safe and effectivity of the TCM fleece-flower root.

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## Materials and Methods

SH-SY5Y cell line was a gift from the cell bank of Sun Yat-sen University in Guangzhou; Fleece-flower root were purchased from Guangzhou Zisun pharmaceutical co., LTD; which were identified as TCM *radix polygoni multiflori preparata* by professor Zeng Lingjie from the School of Chinese Medicine, Guangdong Pharmaceutical University; the rest of the reagent, respectively bought from the following company: trypsin, fetal bovine serum and L-Glutamine from Gibco of Thermo Fisher Scientific; Penicillin streptomycin mixture from Solarbio Co. Ltd., Beijing, China; phosphate buffer solution from Hyclone of GE Healthcare; 1-methyl-4-phenylpyridinium ion and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) from Sigma-Aldrich Co.; Hoechst33258 from F. Hoffmann-La Roche Ltd. Assay Kit of glutathione (GSH), Methane Dicarboxylic Aldehyde (MDA) and lactate dehydrogenase (LDH) from Jiancheng Biotech. Nanjing, China; Assay Kit for mitochondrial membrane potential (JC-1) and superoxide dismutase from Beyotime Biotech. Antibodies for p-JNK and Caspase 3 from Abcam.

### The preparation of EPM and composition analysis

1 kg of *radix polygoni multiflori preparata* herbs were precisely weighted, smashed and added 10 times the amount of 50% (v/v) ethanol, heating reflux extraction for 3 times, 1 h per time. The extracted liquid was merged, and filtered with 300 mesh sieve, then concentrated to about 1 ml/g. The concentrated liquid was put through macroporous resin for adsorption, then washed with 6 times column volume of 70% ethanol. The eluent were collected and concentrated under 60°C and lower air pressure, and then dried at 60°C in vacuum for 48 h. High performance liquid chromatography was used to assay the extract of *radix polygoni multiflori preparata* (EPM), EPM was dissolved in DMSO (final concentration < 0.01%), used at definite concentrations with DMEM culture medium in the following experiment.

### Cell culture and experimental grouping

$1 \times 10^5$  SH-SY5Y cells were seeded in the culture plates and inoculated in DMEM complete medium, which contains 10% FBS, 1% penicillin-streptomycin, and 1% L-Glutamine, and cultured in 5% CO<sub>2</sub>, 37°C incubator. Cell growth state was observed under the microscope every day, the medium was refreshed every 3 days. Cells in logarithmic growth phase were used in this experiment. Cells were divided to control group with serum-free DMEM culture, model group induced by MPP<sup>+</sup>, and treatment groups of 3 different doses of *Polygonum multiflorum* extracts (EPM) at the concentration of 5 mg/L, 25 mg/L, 100 mg/L.

### Cell morphology observation

After digested into cell suspension, SH-SY5Y cells were seeded in  $2 \times 10^5$  cells/well and then inoculated in 6-well culture plates and in 24-well culture plates with a glass (Poly-L-lysine coated overnight) respectively at cells density of  $2 \times 10^5$  per well and  $5 \times 10^4$  per well. Cultured with different concentrations of EPM (5 mg/L, 25 mg/L, 100 mg/L) for 4 h, SH-SY5Y cells were cultured with 0.5 mM MPP<sup>+</sup> administration for 48 h. 6-well plate cells were observed under the inverted microscope with white light to take photos. Cell culture medium were removed and discarded in 24-well plate and the cell layer was briefly rinse with PBS to remove all traces of medium. Then fresh 4% paraformaldehyde were added to fix the cells for 10 min. 5 mg/L Hoechst33258 staining solution was then added to react with the cells for 10 min, mounting, observing, picturing under inversed fluorescent microscope.

### MTT assay for the detection of cell activity

SH-SY5Y cell layer was digested into cells suspension, seeded into 96-well culture plates at the density of  $1 \times 10^4$  cells per well, each set 5 parallel holes and cultured 24 h. Each well was given 0.5 mM MPP<sup>+</sup> and cultured in an incubator with 5% CO<sub>2</sub>, at 37°C for 24 h and 48 h, respectively. The best concentration of MPP<sup>+</sup> and time were determined with MTT test. In order to detect the effect of EPM on SH-SY5Y cell survival rate and determine the range of nontoxic concentrations of EPM, 8 different gradient concentrations were selected between 1 ~ 500 mg/L for 48 h on SH-SY5Y cells. 5 mg/L MTT was placed into the plates in incubator for the last 4 h of the train. And then the medium was removed and discarded, each well was joint 150  $\mu$ L dimethyl sulfoxide. 10 min shock at the shaker (45 rpm), making crystals dissolve completely. Enzyme-linked immunosorbent assay was conducted to determine the absorbance value at the wavelength of 570 nm. These values were compared and analyzed to determine the best MPP<sup>+</sup> drug concentration and the optimal concentration range of EPM. Assuming a growth rate in the control group of 100%, the cell survival rate of model groups and the intervention group could be calculated using the following formula: Cell viability=(absorbance value of experimental group/absorbance value of control group)\*100%.

### Colorimetric detection of LDH in cell supernatant and GSH and MDA contents in cells

SH-SY5Y cell layer was seeded into 6-well culture plates at the density of  $2 \times 10^5$  cells per well. Each well was given definite concentrations of EPM and cultured in an incubator with 5% CO<sub>2</sub>, at 37°C for 4 h, then cultured with 0.5 mM MPP<sup>+</sup> for 48 h. Cell content of GSH and MDA and cell supernatant fluid LDH levels were detected by colorimetric method according to GSH, MDA, and LDH assay Kit manual operation.

### Mitochondrial membrane potential detection

SH-SY5Y cell was seeded into 24-well culture plates with glass coated with Poly-L-lysine overnight, at the density of  $2 \times 10^5$  cells per well. Each well was given definite concentrations of EPM and cultured in an incubator with 5% CO<sub>2</sub>, at 37°C for 4 h, then with 0.5 mM MPP<sup>+</sup> for 48 h. Then cell culture medium was removed and discarded, and rinsed with PBS for 3 times, 0.5 mL of serum-free culture medium and 0.5 mL JC-1 fluid were added and blended, incubated for 20 min in incubator 37°C. Cell layer was washed with JC-1 buffer 2 times before the fluorescence intensity detected by fluorescence microscope photograph. The red and green fluorescence ratio was calculated then.

### Western Blot Assay for p-JNK and caspase-3 expression

To evaluate the expression of p-JNK and caspase-3, SH-SY5Y cells were homogenized in 80/100  $\mu$ l reaction buffer (1% NP-40(w/v), 20 mM Tris-HCl (pH 7.5), 137 mM NaCl and 10% glycerol (v/v)) containing 10  $\mu$ l caspase-3 substrate (Ac-DEVD-pNA) (2 mM) after all treatments. Lysates were incubated at 37°C for 2 h. Samples were measured with WB. The detail analysis procedure is described in the manufacturer's protocol. The expression of p-JNK and caspase-3, normalized for total proteins of cell lysates, was then expressed as fold of the expression of p-JNK and caspase-3 of control cells.

### Statistical Analysis

The data were processed from two to three independent experiments with five to six cultures per experiment, with SPSS22.0 and expressed as the mean  $\bar{x} \pm SD$ . Raw data were analyzed with GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, CA). Statistical

significance was determined by using one-way analysis of variance (ANOVA), followed by post hoc test least significant difference (LSD). Differences were considered to be significant for  $p < 0.05$ .

## Results

### Components analysis of polygonum multiflorum extract

95 g of polygonum multiflorum extracts (EPM) were obtained from 1000 g of fleece-flower root herbs through the process above. 29.5% styrene glycosides, 2.1% free anthraquinone and 5.2% combined anthraquinone were detected in extracts sample by the method of HPLC. HPLC diagrams were showed in Figure 1.

### Cell morphological observation

Control cells were concentrated with even diameter in size and shuttle or oval in shape. Some cells processed distinct axons and some axons connected network form 24 h later. The cells shrunk with their volume decreased obviously and showed small pale gray spots. Compared with the model group, cell morphology has been markedly improved in the EPM treated groups in a dose-dependent manner. By comparing with Hoechst33258 dyeing cells, the control group cells were showing a low intensity of blue fluorescence in uniform dispersion, the margin of nucleus was neat in the control cells. Shrinkage, condensation and fragmentation was found in cell nucleus of model group exposure to 0.5 mM MPP<sup>+</sup>, which presented typical apoptotic bodies in bright blue. Compared with the model group, Pyknosis and bright blue apoptotic body were markedly reduced in the cell nucleus EPM treated cells at low 5 mg/L, 25 mg/L (Figure 2).

### SH-SY5Y cells survival rate by MTT test

To determine the concentration and time required for SH-SY5Y cell injury induced by MPP<sup>+</sup>, definite concentrations of MPP<sup>+</sup> were given to the cells. With the increase of MPP<sup>+</sup> concentration and exposure time, growth inhibition of SH-SY5Y cells increased correspondingly (Table 1). Cell survival rate fell to 44.7% in the control group with the administration of 0.5 mM MPP<sup>+</sup> for 48 h. This concentration and exposure time of MPP<sup>+</sup> were used as a condition of model group in the following experiment (Figure 2).

### Effects of EPM on SH-SY5Y cell activity

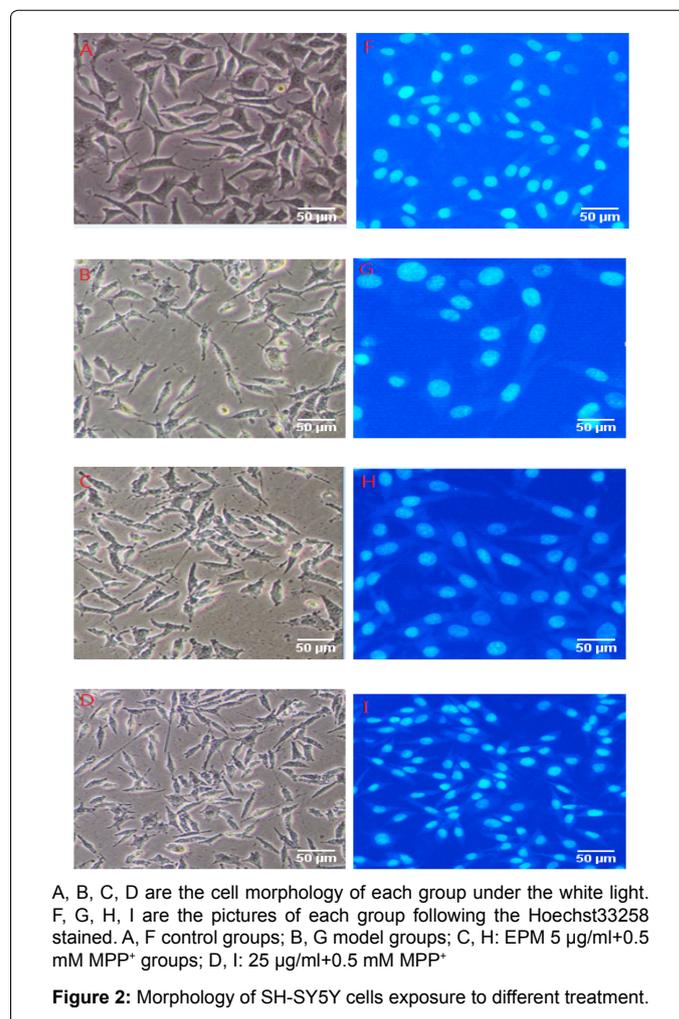
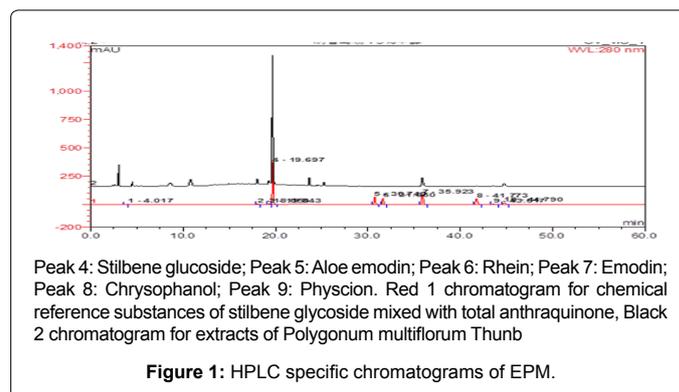
In the detection of EPM's effects on SH-SY5Y cells viability, EPM within 0 ~ 500 mg/L concentration were exposure to SH-SY5Y cells, had activity, indicated that less than 100 mg/L of EPM were found safe concentration to the cells with no obvious influence on cell viability. However, the cell survival rate dropped to about 65% after 200, 500 mg/L EPM was expose to the cell culture for 24 h (Figure 3). So 5, 25 mg/L of EPM were chosen to detect the protective effects on SH-SY5Y cells in the following experiment.

### Protective effects of EPM on the SH-SY5Y cell injury induced by MPP<sup>+</sup>

Cell survival rate of SH-SY5Y cells fell to 44.7% after exposure to 0.5 mM MPP<sup>+</sup> for 24 h. Compared with the model group, SH-SY5Y cell survival rates had been significantly improved, to 63.8%, 76.1%, respectively, in EPM treated cells at concentration of 5, 25 mg/L, suggesting EPM might dose-relatedly improve cell viability of the injured SH-SY5Y induced by MPP<sup>+</sup> (Figure 4).

### GSH level increase of SH-SY5Y cells by EPM treatment

GSH assay Kit was used to determinate the GSH level on each group of SH-SY5Y cells. Compared with control group, intracellular GSH level was significantly decreased in SH-SY5Y cells after treated with 0.5 mM



MPP<sup>+</sup> ( $P < 0.01$ ), which is about 0.3 times of the level in control group. The level of GSH in SH-SY5Y cells are significantly increased when treated with EPM, GSH levels of 5, 25 mg/l EPM group were namely equivalent to 1.5 times, 2.6 times of the model group cells respectively ( $p < 0.01 \sim 0.05$ ) (Figure 5A). These results suggested that EPM might inhibit cytotoxicity of MPP<sup>+</sup> on SH-SY5Y cells by increasing GSH levels in a concentration - dependent manner.

### MDA level reduction of SH-SY5Y cells by EPM treatment

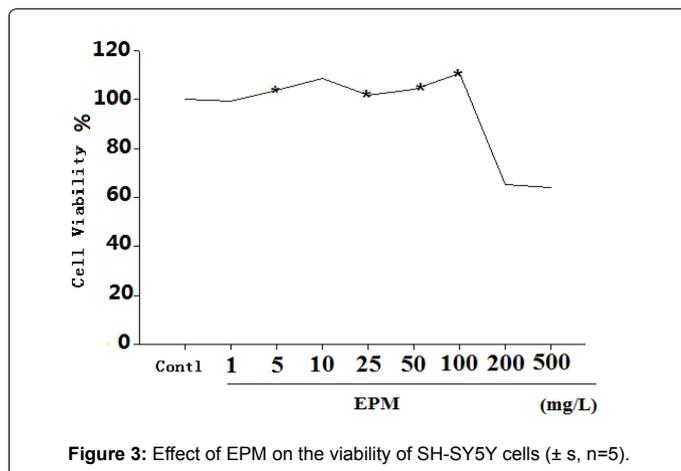
MDA assay Kit was used in the determination of MDA level on each

group of SH-SY5Y cells. Compared with control group, intracellular MDA level was significantly increased in SH SY5Y cells after treated with 0.5 mM MPP<sup>+</sup> (P<0.01), which is about 2.2 times of the level in control group. The level of MDA in SH-SY5Y cells are significantly reduced when treated with EPM, MDA levels of low, medium and high dose groups (5, 25 mg/L) were namely equivalent to 85.0%, 75.0% (p<0.05) of the model group cells respectively (p<0.01) (Figure 5B). These results suggested that EPM might inhibit cytotoxicity of MPP<sup>+</sup> on SH-SY5Y cells by reducing MDA levels in a concentration-dependent manner.

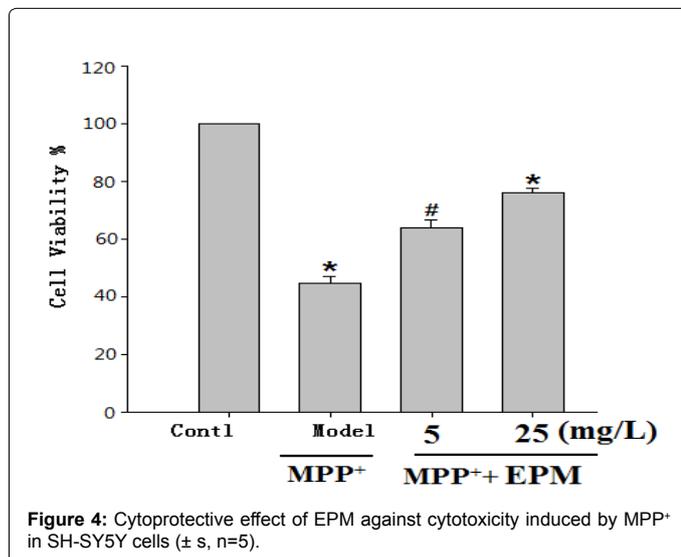
| MPP <sup>+</sup> concentration (mM) | Cell Viability (%)      |                         |
|-------------------------------------|-------------------------|-------------------------|
|                                     | 24 h                    | 48 h                    |
| control                             | 100 ± 0.5               | 100 ± 0.4               |
| 0.1                                 | 87.7 ± 0.3              | 74.6 ± 1.2 <sup>*</sup> |
| 0.25                                | 80.1 ± 0.2 <sup>*</sup> | 63.0 ± 0.8              |
| 0.5                                 | 79.7 ± 0.2              | 44.7 ± 0.6 <sup>*</sup> |
| 1                                   | 75.9 ± 0.3              | 32.1 ± 0.3 <sup>*</sup> |
| 5                                   | 47.5 ± 0.3              | 23.8 ± 0.7 <sup>*</sup> |

Compared with control group <sup>\*</sup>P<0.01 <sup>#</sup>P<0.05

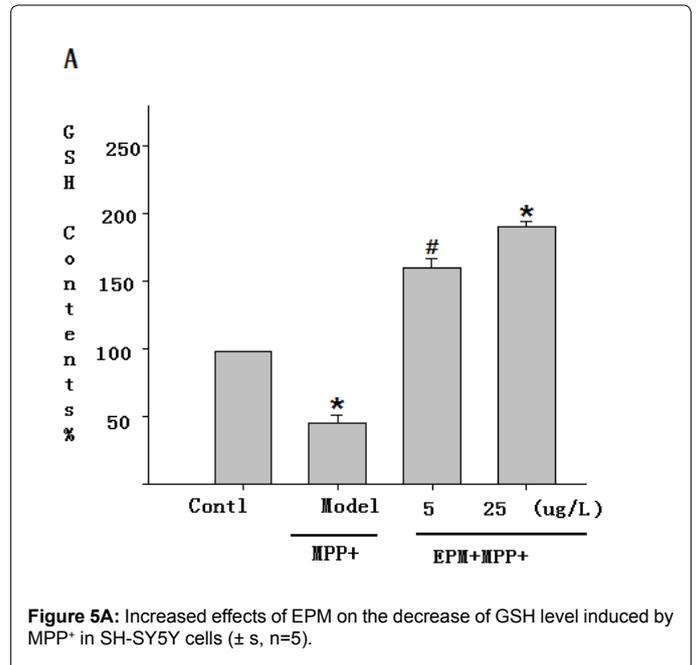
**Table 1:** Effects of MPP<sup>+</sup> on cell viability at different concentration and duration.



**Figure 3:** Effect of EPM on the viability of SH-SY5Y cells (± s, n=5).



**Figure 4:** Cytoprotective effect of EPM against cytotoxicity induced by MPP<sup>+</sup> in SH-SY5Y cells (± s, n=5).



**Figure 5A:** Increased effects of EPM on the decrease of GSH level induced by MPP<sup>+</sup> in SH-SY5Y cells (± s, n=5).

#### LDH leakage decrease of SH-SY5Y cells with EPM treatment

The supernatant LDH content of SH-SY5Y cell was detected follow the kit instructions. Compared with the control group, elevated LDH levels of SH-SY5Y cells in the model group to 1.8 times of the control group (p<0.01). Compared with the model groups, the levels of LDH in SH-SY5Y cells were significantly reduced in 5, 25 mg/L of EPM treatment group respectively dropped to 88.8%, 76.2% of model group (p<0.05) (Figure 5B). Thus, EPM shows effect of reducing the elevated LDH levels of SH-SY5Y cells supernatant caused by MPP<sup>+</sup>.

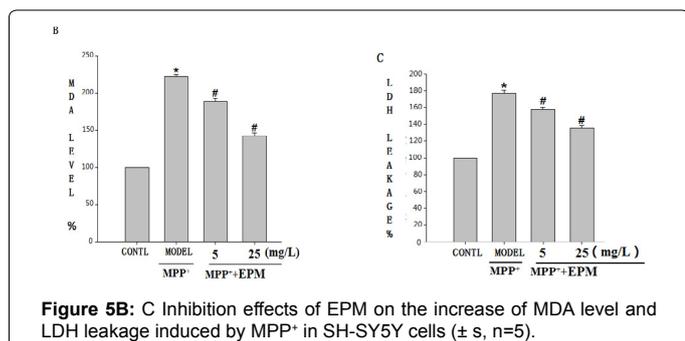
#### Inhibition of EPM on ROS within SH-SY5Y cells

Compared with the control group, when each group of cells loaded with fluorescent probe DCFH-DA, intracellular chloride fluorescein (DCF) signal was significantly enhanced in model group, levels of reactive oxygen species were greatly increased, fluorescence intensity was obviously increased. Compared with the model groups, reactive oxygen species generation induced by the damage of MPP<sup>+</sup> was markedly inhibited in EPM treated group as the fluorescence intensity decreased significantly (Figure 6). Results showed that EPM could inhibit intracellular ROS level increase induced by the cytotoxicity of MPP<sup>+</sup> in a concentration dependent way.

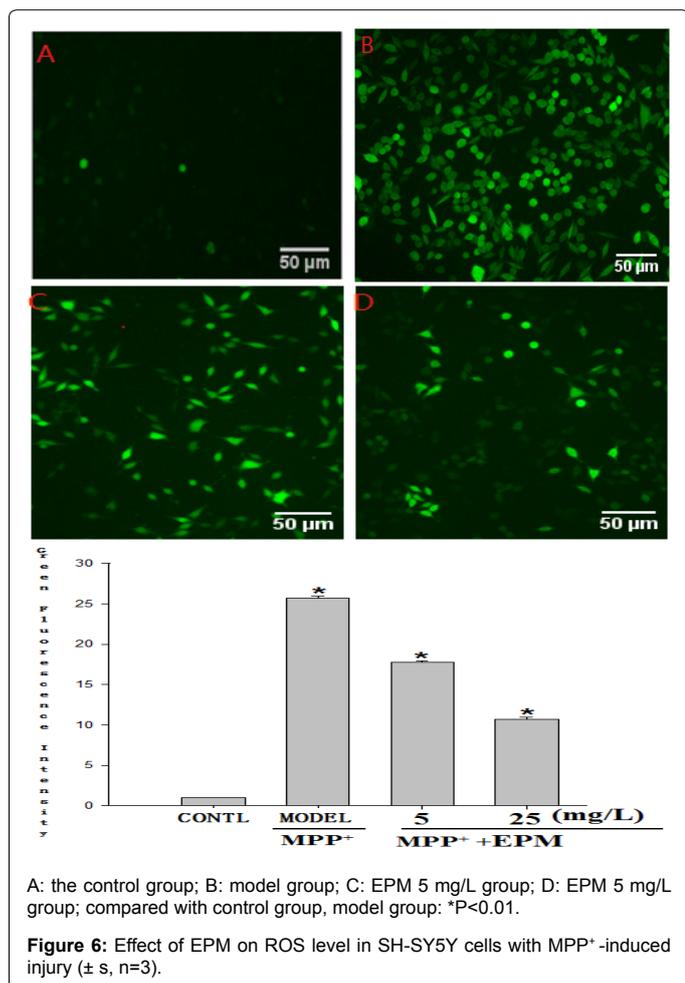
#### EPM effects on the changes of the mitochondrial membrane potential induced by MPP<sup>+</sup>

Compared with control group, model group significantly turned to green fluorescent color, which showed a decreased mitochondrial membrane potential. Compared with model group, EPM groups presented an enhanced red fluorescence and subdued green fluorescence in a dose dependent manner (Figure 7). These indicated that EPM could significantly inhibit the MPP<sup>+</sup> caused loss of mitochondrial membrane potential and then might have the ability to repair the MPP<sup>+</sup> induced damage of mitochondria (Figure 8).

Western blotting was used to analyze the protein expression of p-JNK and caspase-3 in impaired SH-SY5Y cells caused by the MPP<sup>+</sup> when EPM had been treating for 48 h. With the administration of MPP<sup>+</sup>, p-JNK, caspase-3 protein expression in the model group was



**Figure 5B:** C Inhibition effects of EPM on the increase of MDA level and LDH leakage induced by MPP<sup>+</sup> in SH-SY5Y cells (± s, n=5).

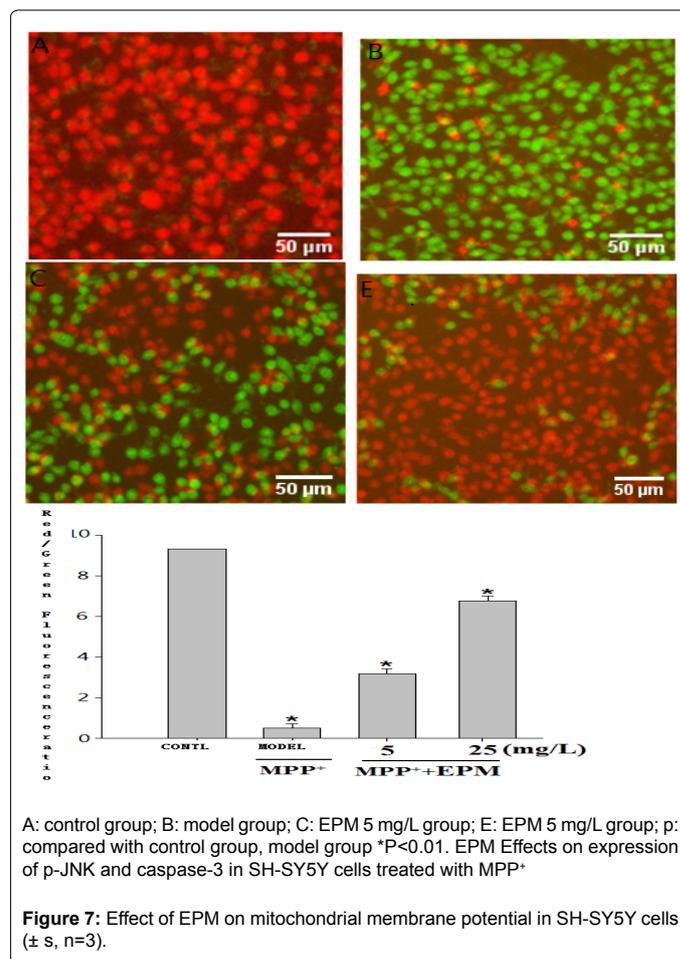


**Figure 6:** Effect of EPM on ROS level in SH-SY5Y cells with MPP<sup>+</sup>-induced injury (± s, n=3).

significantly increased. However, compare with model group, p-JNK, caspase-3 protein expressed in SH-SY5Y cell were obviously and dose-dependently inhibited by EPM exposure.

## Discussion

It is generally accepted that the degeneration of dopamine (DA) neurons in midbrain nigra compacta, which maintain the content of dopamine neurotransmitter in the striatum, causes Parkinson's disease. How to prevent dopaminergic neurons relative neurodegeneration, and to alleviate or even reverse the process of PD, has become a research focus and hotspot in the treatment of PD [6]. 1-methyl-4-phenyl pyridine ion (MPP<sup>+</sup>) is the metabolite of MPTP, has strong selectivity to dopaminergic neurons, that can lead to the similar pathological damage

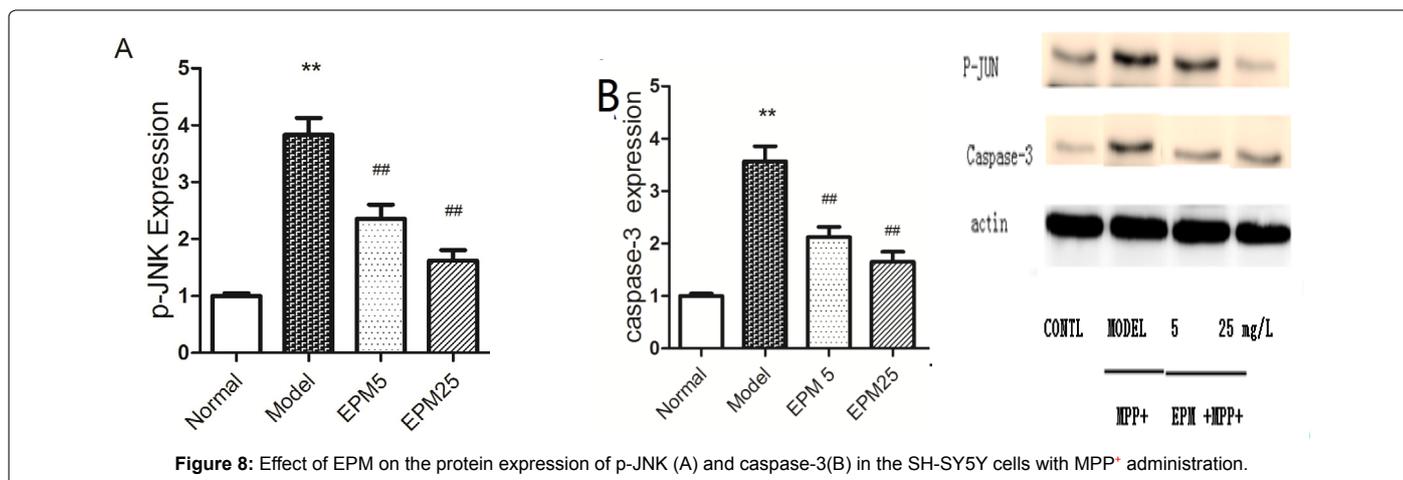


**Figure 7:** Effect of EPM on mitochondrial membrane potential in SH-SY5Y cells (± s, n=3).

of human DA neurons in the substantia nigra and other chemical changes, appearing similar to the symptoms of PD. Thus, we used the MPP<sup>+</sup> as an inducer, human neuroblastoma cells (SH-SY5Y cells) for the model carrier. It has been well proven that the MPP<sup>+</sup> induced SH-SY5Y cell model is commonly used and highly reproducible to simulate the mechanisms of PD [7,8].

Modern pharmacological studies showed that the most important components of polygonum multiflorum Thunb, stilbene glycoside, has the protective effects to alleviate neurodegenerative diseases mainly through resisting oxidation stress, scavenging free radicals [9]. Thereafter, it can reduce neuronal apoptosis induced by  $\beta$ -amyloid protein, and ameliorate symptoms of neurodegenerative diseases such as AD and PD [10-13]. However, the effects of fleece-flower root extract on PD model of SH-SY5Y cells induced by MPP<sup>+</sup> have not been reported.

Our results showed that EPM had protective effects on SH-SY5Y cells damage induced by MPP<sup>+</sup>. And these protective effects of EPM may be mediated through oxidative stress resistance to repair the damage of mitochondria. From the morphological variation, we found cell body shrinkage, disappeared axons and small spots in the cells of model group, wrinkled nuclei and large number of apoptotic body were appeared in Hoechst33258 stained cells. It showed that MPP<sup>+</sup> could obviously induce apoptosis in SH-SY5Y cells. MTT test results showed cell survival rates drop to 44.7% of control group by 0.5 mM MPP<sup>+</sup> exposure for 48 h. And Intracellular reactive oxygen species (ROS) and malondialdehyde (MDA) level were dramatically increased



by 25.6 times and 2.2 times that of control group respectively, lactate dehydrogenase (LDH) leakage rate was 1.8 times that of control group. 5, 25 mg/L of EPM can improve the cell survival rate by increasing intracellular GSH and reducing the contents of intracellular MDA level and extracellular LDH leakage.

Mitochondrial function disorders and increase in membrane permeability are the main characteristics of cell apoptosis [14]. And membrane potential decrease is a sign of early apoptotic cells, there are potentials for cell apoptosis related to oxidative stress, dramatic increase of intracellular ROS may lead to changes of mitochondrial membrane potential, injuries of mitochondrial DNA and interrupts of electronic transport chain, and then damage mitochondrial function, eventually lead to neurons degenerative death [15].

When the MPP<sup>+</sup> accumulates in the mitochondria, mitochondrial complex I activity will be inhibited, which lead to the increased ROS, consumption of the antioxidant, then oxidative stress occurs, acts an important role in the pathological process of neurodegenerative diseases.

Excessive ROS may cause mitochondrial damage through various pathways, which will decrease mitochondrial membrane potential. The mitochondria are the main place of cellular energy metabolism. The reduced activity of mitochondrial complex I damages mitochondrial respiratory chain, that the production of the ATP will decline, leading to cell death due to the depletion of energy. Our results showed that 5, 25 mg/L of EPM can significantly remove the intracellular ROS of SH-SY5Y cells induced by MPP<sup>+</sup> and increase the mitochondrial membrane potential to restore mitochondrial functions in dose-dependent way [16].

Research have shown that MAPK an important signaling pathways in the activation of ROS. C-Jun N-Terminal kinase (JNK) is one of target molecules in the MARK family, mediating a variety of extracellular stimulation (such as oxidative stress, Fas, TNF- $\alpha$ )-induced apoptosis. JNK was involved in the occurrence of apoptosis in many kinds of cells, and played an important role in the development of neurodegenerative diseases. Caspase-3 is one of the most important members of the Caspase family, is the final implementation regulator of cell apoptosis.

Our results showed that EPM has the ability to down-regulate the protein expression of p-JNK and caspase-3, which may be one of the mechanisms that EPM inhibits apoptosis of SH-SY5Y cells induced by MPP<sup>+</sup>.

Styrene glycosides is the main active constituent in EPM, made up 29.5% of total chemical components, with the effects of antioxidant and scavenging free radicals, It suggested that styrene glycosides may be the main chemical basis of EPM for its neuroprotection.

## Conclusion

Our results show that EPM has significantly protective effects on SH-SY5Y cell lesion induced by MPP<sup>+</sup>. The mechanisms may be involved in its resistance to oxidative stress, improvement of the mitochondrial functions, and down-regulation of apoptotic protein p-JNK and caspase-3.

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