

The synergetic effect of edaravone and scutellarin in the MPP(+)-induced cell model of Parkinson's disease

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Summary. Parkinson's disease (PD) is a limb movement disorder caused by the degeneration of brain neurons and seriously affects the quality of life of the elderly. However, the current drugs are symptomatic treatments that cannot prevent or delay the development of the disease. Targeted therapy for pathogenesis may be the direction of development in the future. Oxidative stress and the inflammatory response are involved in the pathogenesis of PD. Edaravone and scutellarin have been studied in antioxidant and anti-inflammatory reactions. The focus of this study is whether there is synergy between the two and its mechanism. Through research, we found that edaravone and scutellarin at different dose combinations have synergistic effects in 1-methyl-4-phenylpyridinium (MPP+)-induced PC12 cells using the Chou-Talalay joint index method. According to the CompuSyn software calculation, the results showed that the combination index (CI) of the combined application of 15 μ M edaravone and 15 μ M scutellarin was the lowest, indicating that the synergistic effect was the best. Compared with the single drug, the synergy increased superoxide dismutase (SOD) and reduced glutathione (GSH) levels, reduced the levels of tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β), and enhanced the anti-apoptosis ability in the MPP(+) induced cell model of PD.

Key words: Edaravone, Scutellarin, Parkinson's disease, Synergetic, Oxidative stress

Introduction

Parkinson's disease (PD) is common in the elderly; with the aging of the global population, it is expected to double in the next 30 years (Tolosa et al., 2021). Except for a few genetic factors, most PD cases are sporadic, and its pathogenesis is complex, including oxidative stress and the inflammatory response (Bloem et al., 2021). Although the treatment of PD has made great progress in recent years (Tarazi et al., 2014), the currently available therapeutic drugs can only alleviate symptoms and cannot control or prevent disease progression. Furthermore, the surgical treatment of PD is only considered under the condition of poor drug treatment, so the compliance and satisfaction of patients are low (Kabra et al., 2018). Thus, actively looking for effective PD treatment strategies is still a long-term task. The combination of traditional Chinese and Western medicine is a unique method of treating diseases in China. The purpose of this study is to explore the combined effect of edaravone and scutellarin.

Edaravone is the first known free radical scavenger and has shown cytoprotective properties in animals and humans. Due to its antioxidant activity, edaravone can regulate oxidative damage in various diseases, especially neurodegenerative (Kapoor, 2017). Edaravone was approved in Japan for the treatment of amyotrophic lateral sclerosis in 2015 (Cha and Kim, 2022). Edaravone has a potent capacity to inhibit A β aggregation and attenuate A β -induced oxidation *in vitro* (Jiao et al., 2015). Edaravone protects neurons in the substantia nigra from oxidative stress induced by 6-hydroxydopamine (Liu et al., 2014).

Scutellarin is the main effective component of *Erigeron breviscapus*, a characteristic Chinese medicine, which is widely used to treat various cardiovascular and cerebrovascular diseases (Wu et al., 2021b). Because of its minimal side effects and definite curative effect, it has been widely studied worldwide (Wang and Ma, 2018). Scutellarin has anti-oxidant stress, anti-apoptosis,

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anti-inflammatory, and other effects (Liu et al., 2019). Scutellarin can inhibit the aggregation of α -synaptic nuclear proteins and the inflammatory response induced by microglia, exerting a protective effect on PD (Yao et al., 2020; Zaidi and Deep, 2020).

The pharmacokinetic study showed that the bioavailability of the active ingredient of scutellarin was low and the removal rate *in vivo* was high. Its effect can be enhanced by combining with other drugs (Wang et al., 2021). However, the combination of the two drugs is not a simple superposition of effects and often produces synergistic or antagonistic effects (Yan et al., 2018). To further understand whether the combination of edaravone and scutellarin has a synergistic effect, this study used a PD model constructed with PC12 cells treated with 1-methyl-4-phenylpyridinium (MPP(+)) as the research object. We used the Chou-Talalay joint index method to evaluate the interaction between drugs, observing the combination of edaravone and scutellarin, the effect on oxidative stress and the inflammatory response, and exploring its mechanism (Chou, 2006).

Materials and methods

Cells and drugs

PC12 cells were kindly provided by Stem Cell Bank, Chinese Academy of Sciences. MPP(+) ($\geq 98\%$ HPLC) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Edaravone was produced by Nanjing Xiansheng Dongyuan Pharmaceutical Co., Ltd. (Nanjing, China). Scutellarin ($\geq 97\%$ HPLC) was produced and supplied by Jiangsu Yongjian Pharmaceutical Co., Ltd. (Taizhou, China).

Main reagents

A cell counting kit-8 (CCK-8) was purchased from Biosharp Biotechnology Co., Ltd. (Hefei, China). An Annexin V/7-amino-actinomycin D (7-AAD) apoptosis kit was purchased from Becton Dickinson and Company (Becton Dickinson, USA). Superoxide dismutase (SOD) detection kits and reduced glutathione (GSH) detection kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). An interleukin-1 β assay kit (IL-1 β) and tumor necrosis factor- α (TNF- α) assay kit were purchased from Nanjing Boyan Biotechnology Co., Ltd. (Nanjing, China).

Cell culture

PC12 cells were grown in RPMI 1640 medium (BOSTER, Wuhan, China) containing 10% heat-inactivated fetal bovine serum (ExCell Bio, Shanghai, China) and 1% penicillin/streptomycin. They were incubated in a humidified incubator at a constant temperature of 37°C in a mixture of 5% CO₂ and 95% air. Every two to three days, we changed the culture

medium of the cells.

PD cell model construction

PC12 cells were seeded at a density of 5×10^4 cells/mL in 96-well plates. After the exponential growth period, the cells were treated with different concentrations of MPP(+) (0 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM) for 24 hours. The cell survival rate was detected by the CCK8 method, and the half maximal inhibitory concentration (IC₅₀) was calculated according to the cell survival rate by SPSS 21.0 software. PC12 cells were seeded in the same way and treated with IC₅₀ for different time periods (0h, 12h, 24h, 48h). Then, the cell survival rate was detected by the CCK8 method. Each group contained five replicate wells. Each experiment was repeated at least 3 times.

Cell Viability detected

The CCK-8 assay was used to detect cell viability. Cells with tested cell viability were seeded in 96-well plates; after the intervention, 10 μ L of CCK-8 solution was added to each well and incubated at 37°C in the dark for one to two hours. The absorbance was detected by using a spectrophotometer (Bio-Rad, Hercules, CA, USA). The cell survival rate was calculated at a wavelength of 450 nm.

Calculation of the dosage combination of edaravone and scutellarin in the PD cell model

First, PC12 cells were treated with different concentrations of edaravone (10 μ M, 15 μ M, 20 μ M, 30 μ M, 40 μ M, 50 μ M, 60 μ M, 70 μ M) and scutellarin (10 μ M, 15 μ M, 20 μ M, 40 μ M, 60 μ M, 80 μ M, 100 μ M) for 1 hour, known as pretreatment. Then, 4.6 mM MPP(+) (concentration was determined from the IC₅₀ obtained in the previous step of constructing the PD cell model) was added to each well for 24 hours. The effects of edaravone and scutellarin on the survival rate of the PD cell model were detected by CCK-8 assay.

The interaction between the two drugs was analyzed by the Chou-Talalay joint index method, which was suitable for the case of single drug use or in combination with two drugs in proportion. The combination index (CI) was calculated according to the following formula, and the drug interaction was assessed: $CI = D_1/D_{X,1} + D_2/D_{X,2}$, where D_1 and D_2 represent the concentrations corresponding to the two drugs when their combination produces the x effect; $D_{X,1}$ and $D_{X,2}$ represent the concentrations corresponding to the X effect produced by the individual use of the two drugs. Data obtained from the experiments described were analyzed using CompuSyn software (www.combosyn.com). The resulting CI represents antagonism ($CI > 1$), additive effects ($CI = 1$), and synergism ($CI < 1$). According to the results of the modeling experiment, sixteen groups were

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established as follows: MPP(+) model group (M), edaravone group (E) comprising three groups (10 μ M, 15 μ M, 20 μ M), scutellarin group (S) comprising three groups (10 μ M, 15 μ M, 20 μ M), nine groups were arranged and combined with different doses of edaravone and scutellarin. Cell survival in all groups was detected by CCK-8 assay. The CI values of all groups were calculated, and the group with the lowest CI was selected as the follow-up experimental group (E+S group).

General morphological observation

The following experiments were divided into five groups: control (C), M, E, S, and E+S groups. The cell morphology of the five groups was observed by inverted phase-contrast microscopy.

SOD detection

First, the cells were collected and centrifuged at 1000 rpm for 10 minutes after digestion with trypsin, and resuspended in 500 μ L PBS and sonicated for later use. Then, a 20 μ L sample was added to a 96-well plate, and 20 μ L enzyme dilution solution and 200 μ L substrate application solution were added and incubated at 37°C for 20 minutes. Finally, the absorbance value was measured at 450 nm, and the SOD activity value was calculated according to the formula.

GSH detection

According to the instructions of the kit, the cells were collected, and then reagent 4 was added, sonicated, and centrifuged at 3500 rpm for 10 minutes. The supernatant was extracted to determine GSH. First, a 10 μ L sample was added to a 96-well plate, then 100 μ L sample 1, 10 μ L sample 2, and 50 μ L sample 3 were added in sequence. The time was recorded at the time of adding reagent 3. The 96-well plate was gently shaken to ensure thorough mixing of the reagents. The absorbance value (A1) at 405 nm was measured at 30 seconds, and then the reagent was allowed to stand at room temperature (25°C) for 10 minutes. At 10 minutes and 30 seconds, the absorbance value (A2) was measured on

time, and finally $\Delta A = A2 - A1$ was calculated.

IL-1 β and TNF- α measurements

The IL-1 β and TNF- α activities in the cells of the five groups were evaluated by IL-1 β and TNF- α ELISA kits according to the manufacturer's instructions. In brief, after the binding reaction between the sample reagent and biotinylated antibody in all wells of the plate, 100 μ L of chromogenic substrate was added to each well, incubated at 37°C in the dark for 15 minutes, and then 50 μ L of termination solution was added to each well. Finally, the absorbance was detected by using a spectrophotometer.

Cell apoptosis assay

Apoptosis was detected by the Annexin V/7-AAD apoptosis detection kit. First, the cells were resuspended with 250 μ L buffer to a concentration of 5×10^5 /mL. Then, 100 μ L cell suspension was added into a 5 mL flow tube, and 5 μ L annexin V and 5 μ L 7-AAD were added, mixed, and incubated at 37°C in the dark for 15 min. Finally, 400 μ L PBS was added to the reaction tube and analyzed by flow cytometry using a fluorescence-activated cell sorting cytometer (BD Biosciences).

Statistical analysis

Experimental data were statistically analyzed using GraphPad Prism 6.0. All data underwent normal distribution testing, had homogeneity of variance, and were expressed as the mean \pm standard deviation. All assays were performed in five independent experiments with five replicates per group. Statistical analyses were performed using the two-tailed Student's t-test with a significance level of $p < 0.05$.

Results

Construction of PD cell model

Compared with the control group, the survival rate of PC12 cells treated with MPP(+) was concentration- and time-dependent. With the increase in the

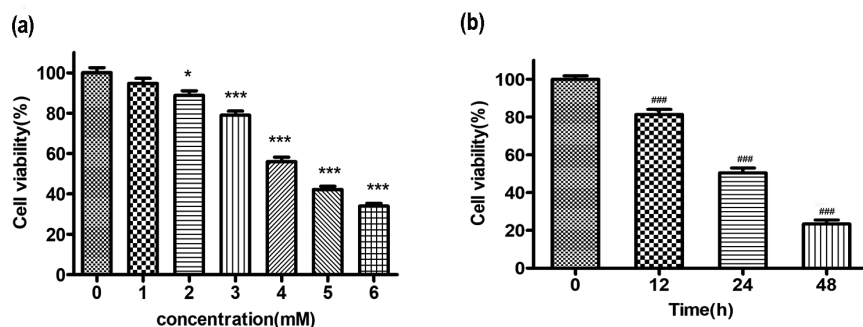


Fig. 1. Effect of MPP(+) on the viability of PC12 cells. **a.** With the increase in the concentration of MPP(+), the survival rate of PC12 cells decreased gradually. **b.** The cell survival rates of the three different action times were significantly decreased compared with those of the control group. The results represent the mean \pm SEM, $n=5$. * $p < 0.05$, and *** $p < 0.001$ vs. 0 μ M group. ### $p < 0.001$ vs. 0h group.

concentration of MPP(+), the survival rate of PC12 cells decreased gradually. When the concentration of MPP(+) was 2 mM, the difference began to be statistically significant compared with the control group (Fig. 1a). The IC_{50} of MPP(+), calculated using SPSS 21.0 software, was 4.6 mM. Using this concentration to treat PC12 cells for 12, 24, and 48 hours, the cell survival rates of the three groups were significantly decreased compared with those of the control group (Fig. 1b). According to the above results, we used 4.6 mM MPP(+) to treat PC12 cells for 24 hours as the PD model.

Effects of edaravone and scutellarin on the survival rate of the PD cell model

When different concentrations of edaravone were used to treat the PD cell model, the results showed that when the concentration of edaravone was greater than 15 μ M, the difference in cell viability was statistically significant compared with the model group, and the difference was more obvious with increasing concentration. However, when the concentration of edaravone was greater than 50 μ M, there was no difference in cell viability between the dosing groups (Fig. 2a). Similarly, when different concentrations of scutellarin were used to treat the PD cell model, the results showed that when the concentration of scutellarin was greater than 20 μ M, the difference in cell viability was statistically significant compared with the model group, and the difference was more obvious with increasing concentration. However, when the

concentration of scutellarin was greater than 60 μ M, there was no difference in cell viability between the treatment groups (Fig. 2b).

The optimal proportion of edaravone to scutellarin

Compared with the PD cell model group, there was a significant difference in cell viability between the two groups when the concentration of edaravone alone was 15 μ M but when scutellarin alone was 20 μ M (Fig. 2c). The cell viability measured by nine different dose combinations was significantly different from that of the PD cell model group (Fig. 2c). The effect of edaravone and scutellarin alone and in combination on the cell viability of the PD cell model increased with increasing concentration, and there was an obvious dose-effect relationship (Fig. 3A). The medium effect dose of combined use was higher than that of the two alone (Fig. 3B). The CI of nine dose combinations was <1 , and the CI decreased with increasing Fa (fraction of action at a dose), indicating that edaravone and scutellarin have synergistic effects (Fig. 3C). When the cell viability was 50%, 75%, and 90%, the data points of combined use fell below the line, indicating that the dosage of edaravone and scutellarin in combined use was lower than that of drugs used alone under the same effect, indicating that the combined use had a synergistic effect (Fig. 3D). According to the CompuSyn software calculation, the results showed that the CI of the combined application of 15 μ M edaravone and 15 μ M scutellarin was the lowest, indicating that the synergistic

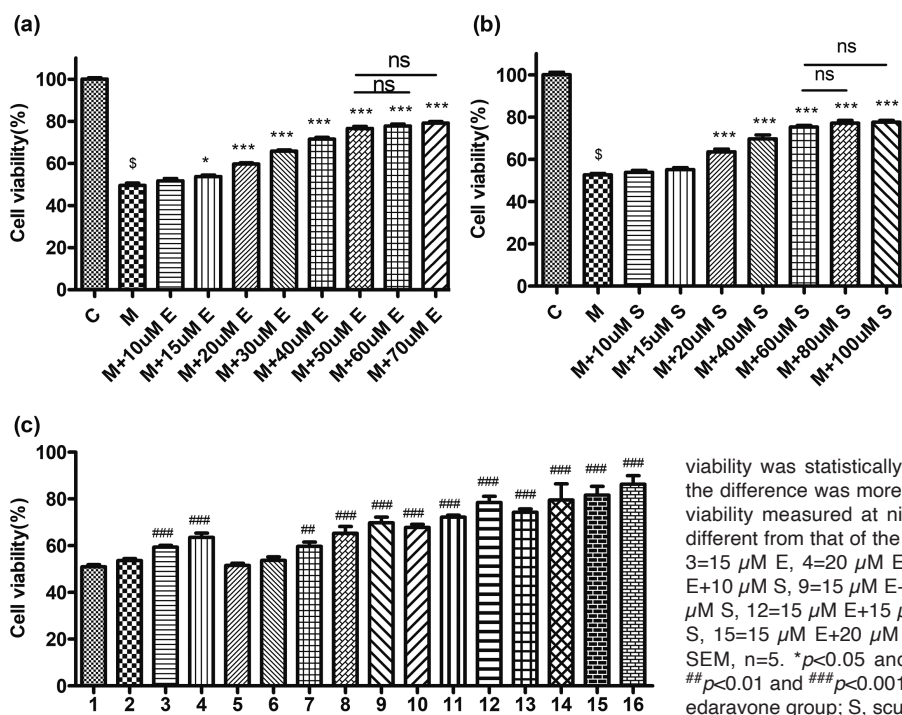


Fig. 2. Effects of edaravone and scutellarin on cells. **a.** When the concentration of edaravone was greater than 15 μ M, the difference in cell viability was statistically significant compared with the model group, and the difference was more obvious with increasing concentration. **b.** When the concentration of scutellarin was greater than 20 μ M, the difference in cell viability was statistically significant compared with the model group, and the difference was more obvious with increasing concentration. **c.** The cell viability measured at nine different dose combinations was significantly different from that of the PD cell model group. 1=model group, 2=10 μ M E, 3=15 μ M E, 4=20 μ M E, 5=10 μ M S, 6=15 μ M S, 7=20 μ M S, 8=10 μ M E+10 μ M S, 9=15 μ M E+10 μ M S, 10=20 μ M E+10 μ M S, 11=10 μ M E+15 μ M S, 12=15 μ M E+15 μ M S, 13=20 μ M E+15 μ M S, 14=10 μ M E+20 μ M S, 15=15 μ M E+20 μ M S, 16=20 μ M E+20 μ M S. Data are the mean \pm SEM, n=5. * $p<0.05$ and *** $p<0.001$ vs. M group. \$ $p<0.05$ vs. C group. ## $p<0.01$ and ### $p<0.001$ vs. Group 1. C, control group; M, model group; E, edaravone group; S, scutellarin group.

effect was the best (Table 1). This combination was used in subsequent experiments. Before constructing the PD model, PC12 cells were treated with this combination concentration for 1 hour as pretreatment.

The synergistic effect of edaravone and scutellarin on cell morphology

Under an inverted fluorescence microscope, PC12 cells were full and spindle-shaped, with obvious long axons. After MPP(+) treatment, the cell morphology changed, showing that the cells shrank, processes disappeared, and there could be granular deposition in the culture medium. After edaravone and scutellarin were pretreated separately, the degree of injury was reduced. After combined pretreatment with edaravone and scutellarin, the degree of cell injury was further reduced (Fig. 4).

The synergistic effect of edaravone and scutellarin on oxidative stress

There was a significant difference in SOD between the control and model groups ($p<0.001$). Compared with the model group, the SOD of PC12 cells pretreated with scutellarin alone did not increase significantly ($p>0.05$). After pretreatment with edaravone alone, SOD increased

significantly ($p<0.05$); when edaravone and scutellarin were pretreated together, the increase in SOD was more obvious ($p<0.01$). The SOD level of the edaravone and scutellarin combined pretreatment group was significantly higher than that of the edaravone alone group ($p<0.05$) (Fig. 5a). The same results were obtained from the GSH assay (Fig. 5b). There was a significant difference in GSH between the control and model groups ($p<0.001$). Compared with the model group, the GSH of PC12 cells pretreated with scutellarin alone did not increase significantly ($p>0.05$). After pretreatment with

Table 1. Synergistic effect of different doses of edaravone and scutellarin combinations.

Edaravone (μM)	Scutellarin (μM)	Fa	CI
10	10	0.653	0.695
15	10	0.698	0.574
20	10	0.678	0.831
10	15	0.791	0.212
15	15	0.847	0.118
20	15	0.773	0.367
10	20	0.795	0.243
15	20	0.752	0.464
20	20	0.738	0.616

Fa, fraction affected; CI, combination index.

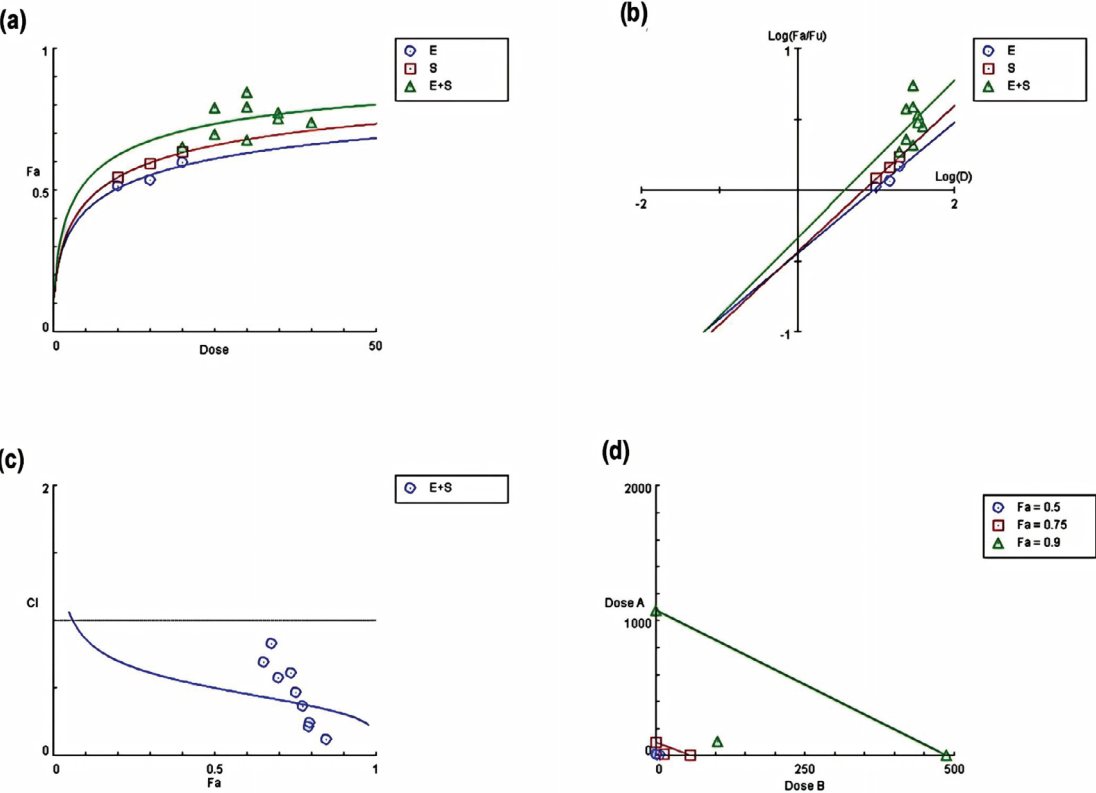


Fig. 3. Synergy results of edaravone and scutellarin. **a.** The effect of edaravone and scutellarin alone and in combination on the cell viability of the PD cell model increased with increasing concentration, and there was an obvious dose-effect relationship. **b.** The median-effect plot showed that the medium effect dose of combined use was higher than that of the two alone. **c.** Fa-CI plot showed that the CI of nine dose combinations was less than 1, and the CI decreased with increasing Fa. **d.** Isobologram showed that the data points of combined use fell below the bevel when the cell viability was 50%, 75% and 90%. Fa, fraction affected; CI, combination index; ED, effective dose.

edaravone alone, GSH increased significantly ($p<0.05$); when edaravone and scutellarin were pretreated together, the increase in GSH was more obvious ($p<0.01$). The

GSH level of the edaravone and scutellarin combined pretreatment group was significantly higher than that of the edaravone alone group ($p<0.05$).

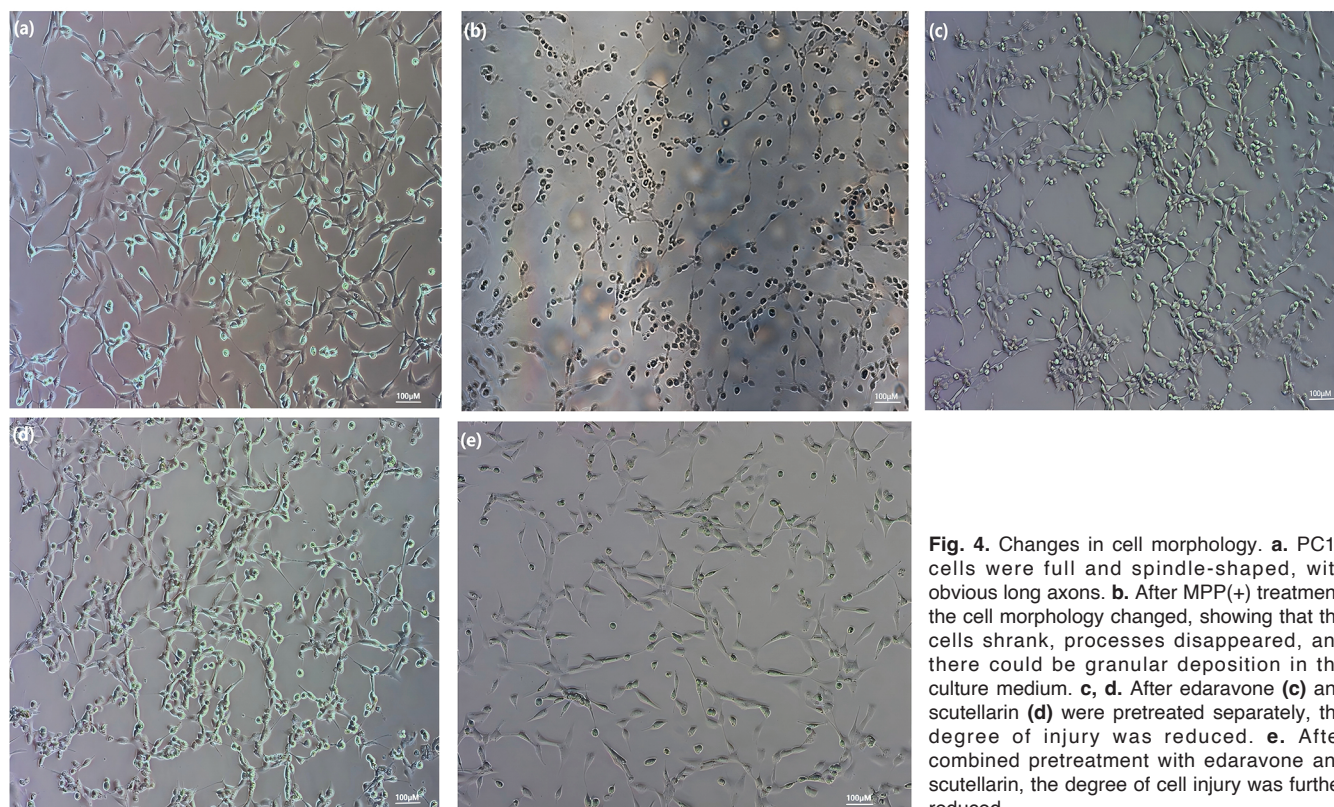


Fig. 4. Changes in cell morphology. **a.** PC12 cells were full and spindle-shaped, with obvious long axons. **b.** After MPP(+) treatment, the cell morphology changed, showing that the cells shrank, processes disappeared, and there could be granular deposition in the culture medium. **c, d.** After edaravone (**c**) and scutellarin (**d**) were pretreated separately, the degree of injury was reduced. **e.** After combined pretreatment with edaravone and scutellarin, the degree of cell injury was further reduced.

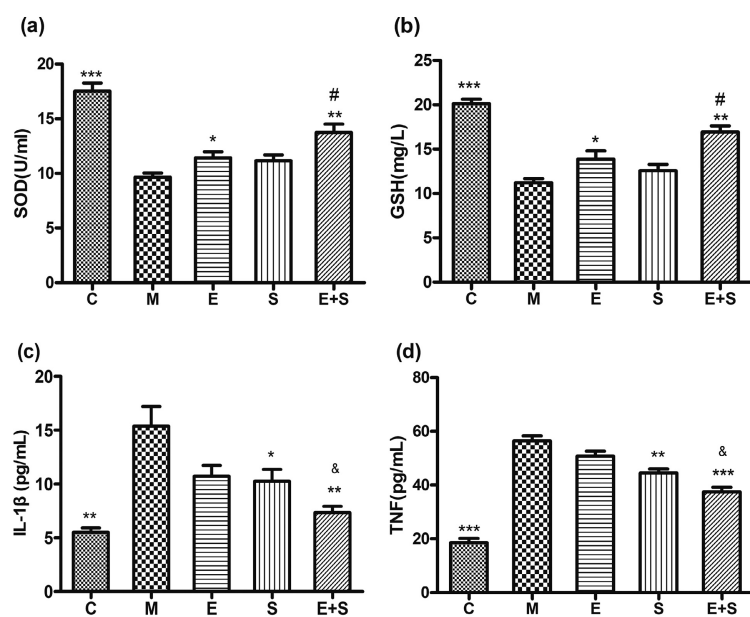


Fig. 5. Changes in oxidative stress and inflammatory cytokine levels. **a.** The SOD level of the edaravone and scutellarin combined pretreatment group was significantly higher than that of the edaravone alone group. **b.** The GSH level of the edaravone and scutellarin combined pretreatment group was significantly higher than that of the edaravone alone group. **c.** The IL-1 β level of the edaravone and scutellarin combined pretreatment group was significantly lower than that of the edaravone alone group. **d.** The TNF- α level of the edaravone and scutellarin combined pretreatment group was significantly lower than that of the edaravone alone group. The results represent the mean \pm SEM, $n=5$. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus M group. # $p<0.05$ vs. E group. & $p<0.05$ vs. S group. C, control group; M, model group; E, edaravone group; S, scutellarin group; SOD, superoxide dismutase; GSH, reduced glutathione; IL-1 β , Interleukin-1 β ; TNF- α , tumor necrosis factor- α .

The synergistic effect of edaravone and scutellarin on the inflammatory response

There was a significant difference in IL-1 β between the control and model groups ($p<0.01$). Compared with the model group, the IL-1 β value of PC12 cells pretreated with edaravone alone did not decrease significantly ($p>0.05$). IL-1 β decreased significantly after pretreatment with scutellarin alone ($p<0.05$); when edaravone and scutellarin were combined for pretreatment, IL-1 β decreased more significantly ($p<0.01$). The IL-1 β value of the edaravone and scutellarin combined pretreatment group was significantly lower than that of the scutellarin alone group ($p<0.05$) (Fig. 5c). Similar results were obtained from the TNF- α assay (Fig. 5d). There was a significant difference in TNF- α between the control and model groups ($p<0.01$). Compared with the model group, the TNF- α value of PC12 cells pretreated with edaravone alone did not decrease significantly ($p>0.05$). TNF- α decreased significantly after pretreatment with scutellarin alone ($p<0.05$); when edaravone and scutellarin were combined for pretreatment, TNF- α decreased more significantly ($p<0.01$). The TNF- α value of the edaravone and scutellarin combined pretreatment group was significantly lower than that of the scutellarin alone group ($p<0.05$).

The synergistic effect of edaravone and scutellarin on apoptosis

The results of flow cytometry showed that the total

apoptosis rate of PC12 cells pretreated with edaravone and scutellarin was significantly lower than that of the model group ($p<0.01$). Compared with the model group, the total apoptosis rate of the two-drug combination group was further reduced ($p<0.001$), and the difference was also statistically significant compared with the single-drug treatment group ($p<0.01$) (Fig. 6).

Discussion

PC12 cells have some neuronal, physiological, and pathological characteristics similar to those of neuronal cells (Chua and Lim, 2021). MPP(+)-induced PC12 cells are the most commonly used PD cell model (Prasuhn et al., 2017). In our present study, it was revealed that both edaravone and scutellarin have protective effects on MPP(+)-induced PC12 cells. In addition, our results showed that edaravone and scutellarin have synergistic protective effects on MPP(+)-treated PC12 cells. The combined application can improve oxidative stress, reduce the inflammatory response, and reduce apoptosis.

There are many evaluation methods for drug interactions. The Chou-Talalay joint index method (also known as median-drug effect analysis) has become one of the most widely used methods (Jayatunga et al., 2022), with the advantages of scientific principles, complete mathematical models, and simple operation. This study used the above method and CompuSyn software to evaluate the synergistic effect of edaravone combined with scutellarin. The results showed that edaravone and scutellarin alone could improve the survival rate of the PD cell model, but the protective

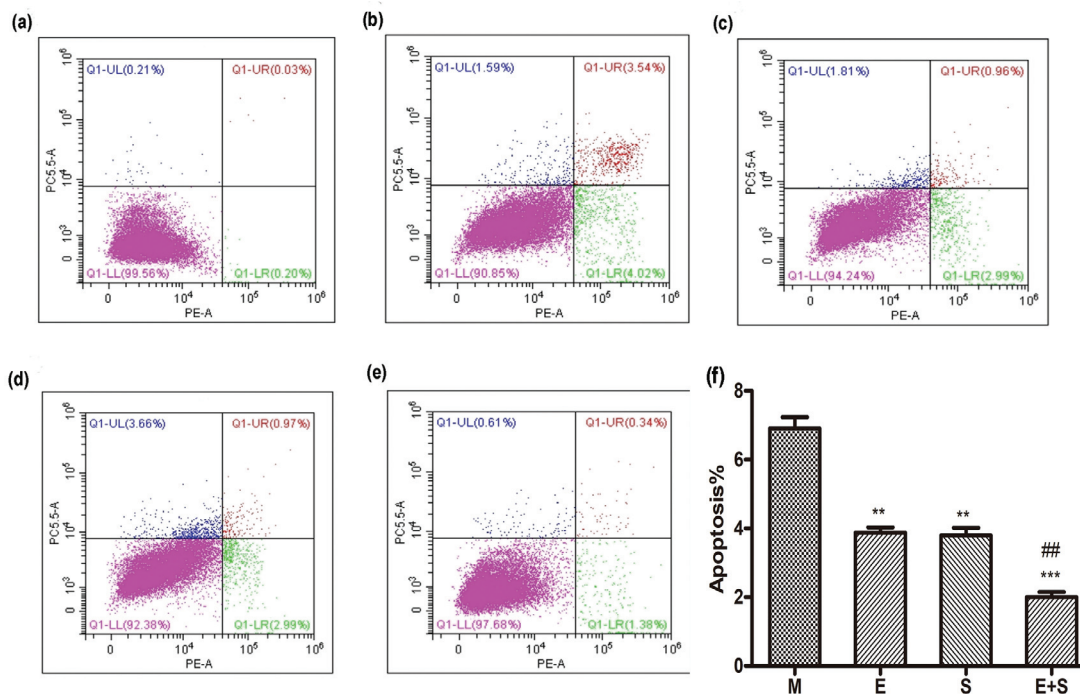


Fig. 6. Flow cytometry was performed to detect apoptosis. The total apoptosis rate of the model group was significantly lower than that of the control group (a). The total apoptosis rate of PC12 cells pretreated with edaravone (b) and scutellarin (c) was significantly lower than that of the model group. Compared with the model group, the total apoptosis rate of the two-drug combination group (d) was further reduced. (f) Comparison of total apoptosis rate. The results represent the mean \pm SEM, $n=5$. ** $p<0.01$, *** $p<0.001$ vs. the M group. ## $p<0.01$ vs. the S group and E group. C, control group; M, model group; E, edaravone group; S, scutellarin group.

effect of edaravone combined with scutellarin was significantly stronger than that of either drug alone. When the compatibility ratio of edaravone and scutellarin was 1:1 (15 μ M:15 μ M), the synergistic effect of the two drugs was the strongest. This study further studied the synergistic effect of the two drugs using this optimal combination.

While a specific biochemical mechanism underlying PD remains elusive, oxidative stress plays an undeniable role in the pathogenesis and progression of PD (Trist et al., 2019). Venkateshappa et al. proved that antioxidant function, represented by SOD and GSH, decreased significantly in patients with PD (Venkateshappa et al., 2012). Reactive oxygen species (ROS) are produced by internal dopamine metabolism, and dopaminergic neurons are very sensitive to ROS. MPP (+) induces PD-like symptoms by inhibiting mitochondrial complex I, enhancing the production of ROS (Theofanous and Kourti, 2022). Our study also confirmed this point. After inducing PC12 cells with MPP (+), the contents of SOD and GSH decreased significantly compared with the control group. Guanine in DNA and RNA is prone to oxidative damage and can be attacked by ROS and then modified. When these modified bases increase to a certain extent in the brain, PD may be induced. Therefore, it is more important to start treatment by targeting the specific pathways involved in PD (Behl et al., 2021).

Edaravone prevents neuronal death by scavenging \cdot OH and inhibiting lipid peroxidation (Wu et al., 2021a). Wu et al. found that edaravone can scavenge a variety of free radicals (\cdot OH, $\text{NO}\cdot$, $\text{ONOO}\cdot$), but it has no scavenging effect on $\text{O}_2^{\cdot-}$. They combined it with borneol to improve the clearance of free radicals (Wu et al., 2014). Similar to our study, the contents of SOD and GSH in edaravone-pretreated PC12 cells decreased at low concentrations, but scutellarin had no effect. When the two drugs were combined, they enhanced the scavenging of free radicals. Scutellarin may improve the scavenging capacity of some free radicals and the antioxidant capacity, but the specific mechanism needs to be further studied.

Neuroinflammation plays a crucial role in PD (Tansey et al., 2022). The loss of dopaminergic neurons is accompanied by changes in various inflammatory cells in PD. Neuroinflammation can protect neurons from harm, but at the same time, its neurotoxic effect aggravates neuronal injury. Thus, neuroinflammation is a therapeutic target for PD prevention (Lee et al., 2019). Recent studies have shown that scutellarin can protect the brain by inhibiting the inflammatory reaction in the brain. The protective effect may be that scutellarin inhibits $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-8 in brain tissue (Chen et al., 2013). By inhibiting the inflammatory reaction, scutellarin has shown obvious curative effects in stroke, Alzheimer's disease, depression, and other diseases (Hu et al., 2018; Lu et al., 2021; Xu et al., 2021). Our study also confirmed that a low dose of scutellarin could

produce an anti-inflammatory effect and that scutellarin reduced the contents of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ in MPP(+) PC12 cells. Edaravone also has an anti-inflammatory effect (Yang et al., 2017). However, Wu et al. found that the anti-inflammatory effect of low-dose edaravone is not obvious (Wu et al., 2014). Our study found that low-dose edaravone did not reduce the contents of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ in MPP(+) PC12 cells. Scutellarin has low bioavailability and a high elimination rate *in vivo*. Combined with other drugs, it can enhance its anti-inflammatory effect and improve bioavailability. Yuan et al. showed that the combined application of edaravone and scutellarin could reduce the infarct area and microglia-mediated inflammatory mediators, especially $\text{TNF-}\alpha$, in a mouse model of middle cerebral artery occlusion (Yuan et al., 2014). Our study also found that scutellarin combined with edaravone enhanced the anti-inflammatory effect and further reduced the contents of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$, showing an obvious synergistic effect. A possible explanation for this would be that scutellarin and edaravone inhibit different inflammatory response pathways (Sang et al., 2015).

Through morphology, we further proved the synergistic effect of edaravone and scutellarin, and the combination of the two drugs reduced the damage of MPP(+) to PC12 cells. Apoptosis is the main form of cell death; oxidative stress and inflammatory reactions are the main mechanisms of cell apoptosis (Diwanji and Bergmann, 2018).

Excessive ROS produced in oxidative stress can destroy the mitochondrial membrane, reduce membrane potential, increase membrane permeability, cause rupture of the mitochondrial membrane, and finally trigger apoptosis (Zhang et al., 2021). A study showed that $\text{IL-1}\beta$ and $\text{TNF-}\alpha$ can induce neuronal apoptosis in the brain (Koprich et al., 2008). $\text{TNF-}\alpha$ can bind to the corresponding receptors on the surface of dopaminergic neurons to initiate the $\text{NF-}\kappa\text{B}$ signaling pathway, inducing apoptosis of dopaminergic neurons (McCoy et al., 2011). Through our study, it is speculated that edaravone and scutellarin may play a protective role by reducing the oxidative stress and inflammatory response of PC12 cells induced by MPP(+), inhibiting apoptosis. Both enhance the anti-apoptosis ability of different molecular pathways.

In the present study, there were several limitations. First, PC12 cells are not neurons; they only have some neuronal characteristics. Our team will further validate them on neurons extracted from animal brain tissue. Second, we only proved that the two drugs have synergistic effects in cell experiments. Whether the two drugs also have similar synergistic anti-inflammatory effects *in vivo* remains to be further studied. Moreover, there are no guidelines for their clinical application in PD. Edaravone and scutellarin remain to be evaluated in clinical trials in PD patients. In addition, the specific molecular mechanism of the interaction between the two drugs will also be the focus of our future research.

Conclusion

In conclusion, our present study suggested that edaravone and scutellarin have synergistic protective effects on an MPP(+)-induced PD cell model, which can improve antioxidant capacity, and reduce the inflammatory response and apoptosis. Edaravone and scutellarin, as candidate combination drugs, have great application potential in the treatment of PD.

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Declaration of Competing Interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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