# Neuroprotective Effects of a standardized flavonoid extract of safflower against Neurotoxin-Induced Cellular and Animal Models of Parkinson's Disease

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Table S1 | The important parameters for evaluating ECS

| Symbol | Name | Unit | Definition |
|---|---|---|---|
| α | Volume fraction | Dimensionless | Ratio of the ECS occupies of the total brain volume |
| D* | Effective diffusion coeffcient | $mm^2/s$ | $D^* = D\backslash\lambda^2$, Diffusion rate of a substance in a diffusion-limited medium, such as the brain |
| λ | Tortuosity | Dimensionless | Pathway of a substance diffusing from one point to another in the brain ECS, the length is equal to or longer than the straight line distance between these two points |
| $k'$ | Clearance | Mmol/L | Loss or clearance of a substance from the ECS, into cells, across the blood brain barrier (BBB), or by degradation through enzymatic attack or other processes |

D is a free diffusion coefficient ($mm^2$ /s) and defined as diffusion rate of a substance in a free medium, such as the diluted agarose hydrogel.

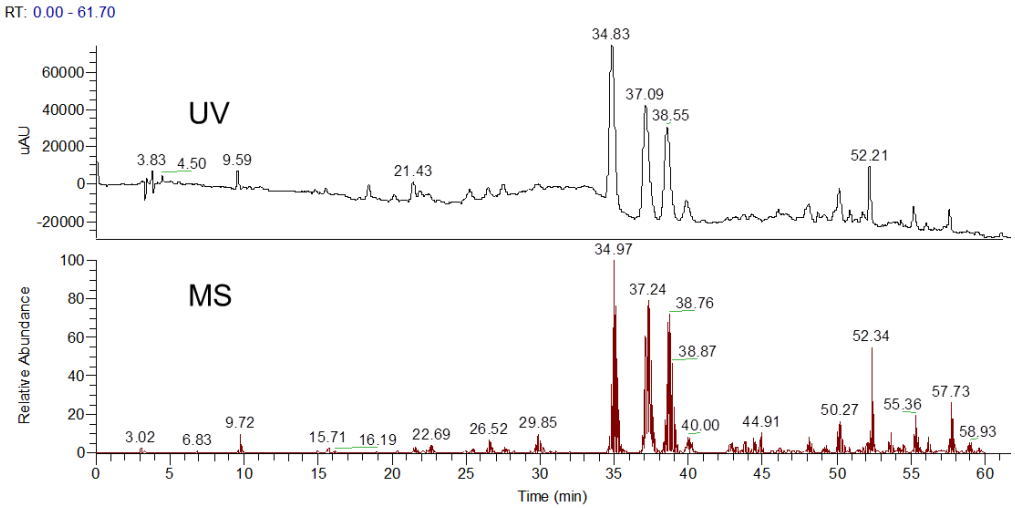


Figure S1 | The chromatogram and mass spectrum of SAFE.

Table S2 | The identified compounds in the SAFE with LC/MS analysis

| No. | RT(min) | [M-H]-(m/z) | MSn(m/z) | Identification |
|---|---|---|---|---|
| 1 | 9.72 | 611 | $MS^2$[611]:491 ,473,325 $MS^3$[491]:473,283 | hydroxysafflor yellow A (HSYA) |
| 2 | 14.93 | 771 | $MS^2$[771]:609,463,301$MS^3$[609]:301 | 6-hydroxykaempferol 3-O-rutinoside-7-O-glucoside (6H3R7G) or 6-hydroxykaempferol 3-O-β-D-rutinoside-6-O-β-D-glucoside (6H3R6G) |
| 3 | 15.71 | 625 | $MS^2$[625]:463, $MS^3$[463]:301 | 6-hydroxykaempferol 3,7-di-O-glucoside (6H3G) or 6-hydroxykaempferol 6,7-di-O-β-D-glucoside (6H6G) |
| 4 | 20.4 | 609 | $MS^2$[609]:301 | 6-hydroxykaempferol 3-O-β-D- rutinoside (6H3R) or quercetin 3-O-rutinoside (rutin) |
| 5 | 21.55 | 463 1087 | $MS^2$[463]:301; $MS^3$[301]:255,271 $MS^2$[1087]:625,461 $MS^3$[625]:463,301 $MS^4$[463]:301 | 6-hydroxykaempferol 3-O-β-D-glucoside (6HK3G) |
| 6 | 22.08 | 609 | $MS^2$[609]:429,284,339,447 $MS^3$[284]:338 | kaempferol 3-O-β-D-sophoroside (K3S) |
| 7 | 22.69 | 1043 | $MS^2$[1043]:1025,923,1007,449 $MS^3$[1025]:1007,863,671 $MS^2$[923]:449,527,759 | isomer of anhydrosafflor yellow B (AYB) |
| 8 | 21.34 | 609 | $MS^2$[609]:301,489,549,591 $MS^3$[301]:273,255,179 | quercetin 3-O-rutinoside (rutin) |
| 9 | 26.52 | 1043 | $MS^2$[1043]:1025,923,1007,449 $MS^3$[1025]:1007,863,671 $MS^2$[923]:449,527,759 | isomer of anhydrosafflor yellow B (AYB) |
| 10 | 27.59 | 463 | $MS^2$[463]:301,180,393 $MS^3$[301]: 179,254,271 | qucertin-3-O-glucoside (Q3G) |

| | | | | |
|---|---|---|---|---|
| 11 | 29.69 | 449 | $MS^2$[449]:287,255,167<br>$MS^3$[287]:181,259,241 | 4’,5-dihydroxyl-7-O-glucopyranosyl flavanone (4D7GF) |
| 12 | 34.97 | 593 | $MS^2$[593]:285 $MS^3$[285]:257,229,213,197 | kaempferol 3-O-rutinoside (K3R) |
| 13 | 37.24 | 623 | $MS^2$[623]:315, 300 $MS^3$[315]:300 | 6-hydroxyapigenin 6-O-β-D-glucoside-7-O-β-D-glucuronide (6H6G7G) |
| 14 | 38.76 | 1043 | $MS^2$[1043]:1025,923,449<br>$MS^3$[1025]:1007,863<br>$MS^4$[863]:845,671,771,287 | anhydrosafflor yellow B (AYB) |
| 15 | 40 | 447 | $MS^2$[447]:315, 300 $MS^3$[315]:300 | kaempferol 3-O-glucoside (K3G) |
| 16 | 43.78 | 449 | $MS^2$[449]:287, 253 $MS^3$[287]:181,167 | 4’,5-dihydroxyl-6-O-glucopyranosyl flavanone (4D6GF) or its isomer |
| 17 | 44.43 | 449 | $MS^2$[449]:287, 253 $MS^3$[287]:181,167 | 4’,5-dihydroxyl-6-O-glucopyranosyl flavanone (4D6GF) or its isomer |
| 18 | 44.91 | 836 | $MS^2$[836]:790 $MS^3$[790]:772,564,451 | isorhamnetin |
| 19 | 51.47 | 491 | $MS^2$[491]:287 ,181,431 $MS^3$[287]:181,259 | 4’,5-dihydroxyl-7-O-[6’’-acetyl(1→6)]-glucopyranosyl flavanone (4D76GF) |

Also, in the SAFE there are still some unknown compounds that still need identification

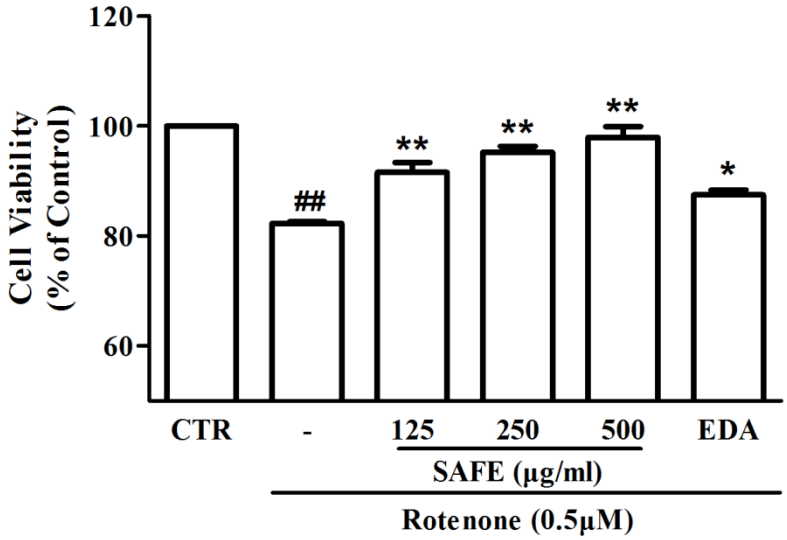


Figure S2 | Effects of SAFE on cell viability of rotenone-induced differentiated PC12 cell damage. Cells were pretreated with SAFE (125, 250 and 500 μg/ml) for 6 h before rotenone treatment for 24 h. Data are the mean ± SEM, n = 3, ##$P$ < 0.01 vs. untreated control (CTR) group; *$P$ <0.05, **$P$<0.01 vs. rotenone group.

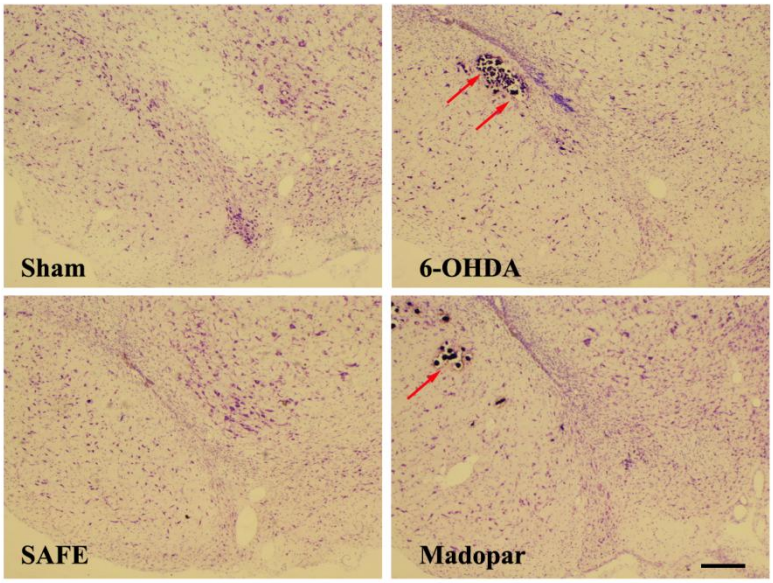


Figure S3 | Multiple foci of neuronophagia (solid arrows) as denoted by necrotic neurons surrounded or obscured by inflammatory cells. Bar = 200 μm. The sections of SN were prepared and picked up as described as immunohistochemistry and then the sections was stained with 0.5% cresyl violet acetate in the SN.

**Apparatus and chromatographic conditions for HPLC and LC/MS.** An Agilent 1100 HPLC system equipped with a quaternary solvent delivery system, an autosampler and a DAD detector was used. Separation was achieved on an Alltima-C18 column (5 μm, 250 mm × 4.6 mm, Agilent). The mobile phase consisted of A (0.3% aqueous methanoic acid) and B (Acetonitrile: methanol = 9:1), which were applied in the gradient elution as follows: 0-5 min：10-14% B；5-25 min：14-17% B；25-35 min：17-18% B；35-45 min：18-25% B；45-55 min：25-30% B; 55-60 min: 30 % B. Each run was followed by equilibration time of 5 min. The flow rate was 1.0 ml/min. The column temperature was set at 30◦C. Ultraviolet (UV) spectra were monitored at 345 nm. The injection volume was 10 μl. The data were collected and analyzed with Chemstation 10.02 software.

For MS analysis, the HPLC conditions were the same as described and the mass spectrometer was operated in (−)-ESI mode. Ultra-high purity helium and high-purity nitrogen was used as the collision gas and the nebulizing gas, respectively. The optimized parameters were as follows: ion spray voltage, 3.5 KV; sheath gas, 50 arbitrary units; auxiliary gas, 10 arbitrary units; capillary temperature, 350 ℃; capillary voltage, −49.0 KV; tube lens offset voltage, −36.0 V. Mass spectra were recorded in the range of m/z 130-1000. The collision-induced dissociation energy was adjusted to 40%, and the isolation width of precursor ions was 2.0 mass units. Data were processed by Xcalibur 1.4 software (ThermoFisher).