Supplementary Materials

Levodopa-Reduced *Mucuna pruriens* Seed Extract shows Neuroprotective Effects against Parkinson's Disease in Murine Microglia and Human Neuroblastoma Cells, *Caenorhabditis elegans*, and *Drosophila melanogaster*

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HPLC-DAD analyses of *Mucuna pruriens* extracts

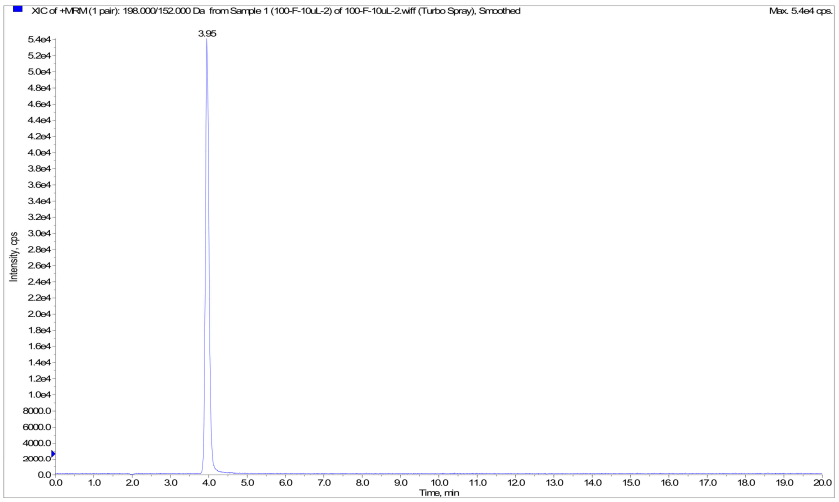
Chemical profiles of *Mucuna pruriens* extracts were performed using HPLC-DAD method. *M. pruriens* extracts including crude methanol, n-hexanes, ethyl acetate, butanol, and water extracts were prepared in 50% methanol/water (25 mg/mL). The column used was a Waters Sunfire® C18 column (250 mm × 4.6 mm i.d., 5 μm; Milford, MA, USA) at room temperature. Solvent system consisted of 0.1% trifluoroacetic acid in water (A) and methanol (B). 0-25 min 97% A, 25-66 min 50% A, 66-81 min 5% A, 81-95 min 5% A, 95-96 min 97% A, and 96-110 min 97%A at a fow rate of 0.75 mL/min. Wavelength range for DAD detection was 220-520 nm and peaks were monitored at the wavelength of 250 nm. HPLC-DAD chromatograms are shown in Figure S2.

Morphological analyses

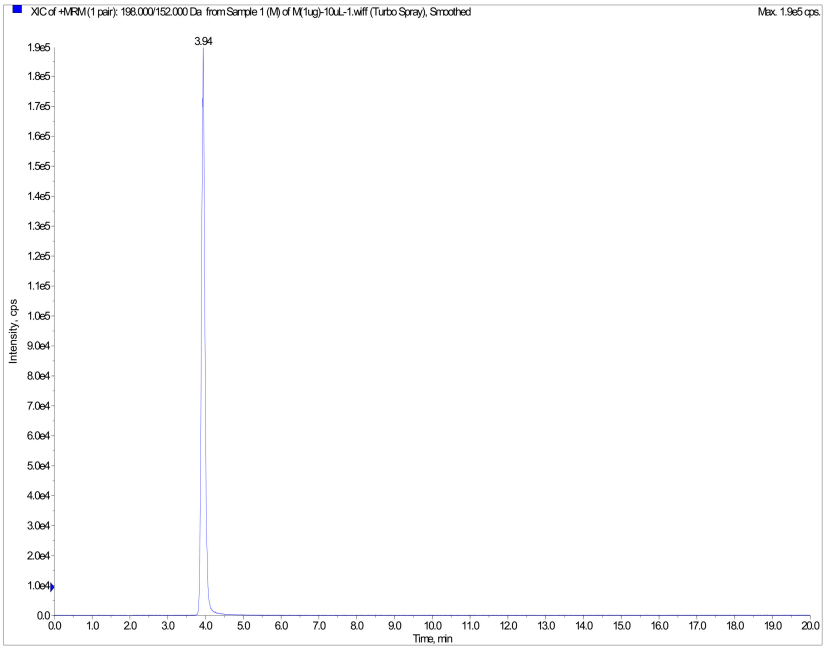
Murine BV-2 microglia cells were stained with crystal violet staining post treatments to visualize morphological changes. Cells were fixed in 70% ethanol for 5 min, then stained with 0.5% crystal violet stain (Sigma-Aldrich Chemical Co.,St. Louis, MO, USA) for 10 min. Cells were then washed in phosphate buffered saline, then imaged with EVOS ® FL Cell Imaging System (ThermoFisher Scientific, Waltham, MA, USA) in phase at 40X (Figure S4).

Figure S1. LC-ESI-MS/MS spectra of L-dopa (**A**) and L-dopa in the *Mucuna pruriens* extracts including crude methanol (**B**), n-hexanes (**C**), ethyl acetate (**D**), butanol (**E**), and water (**F**) extracts. The presence of L-dopa in the Mucuna extracts was identified as a peak with a retention time of 3.95 min with an ion transition of 198/152.

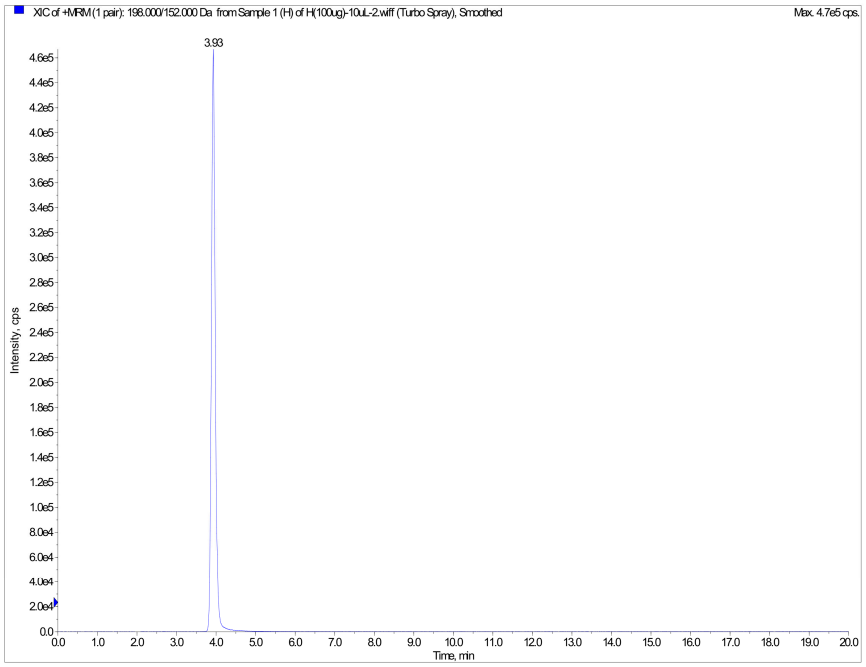
(A)



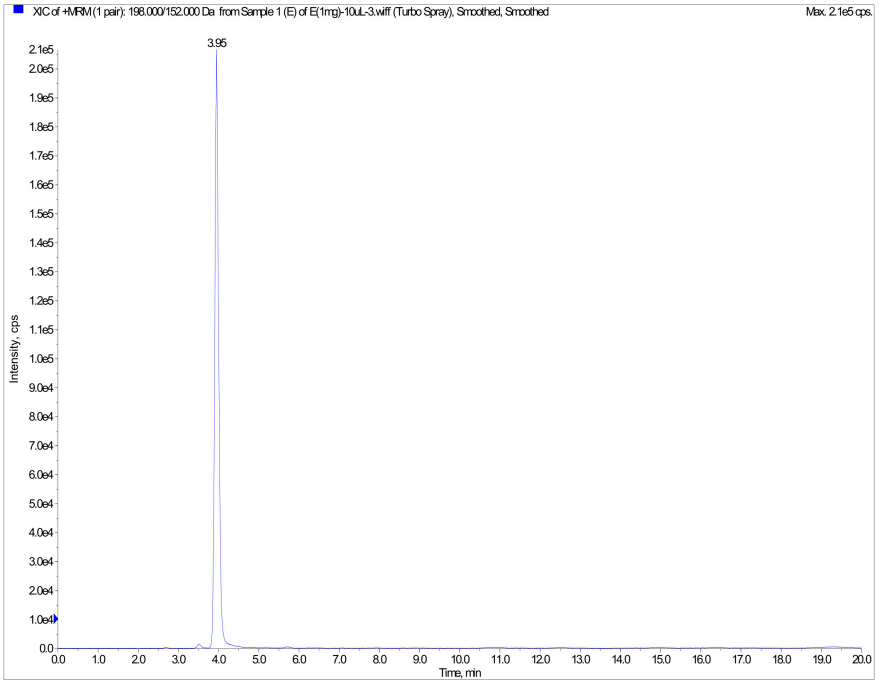
(B)



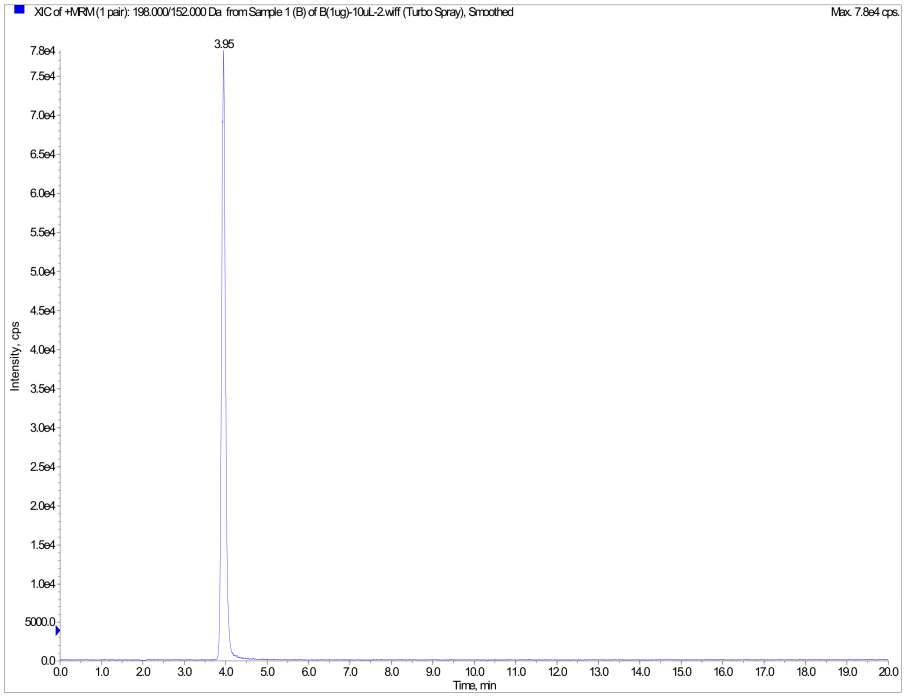
(C)


XIC of +MRM (1 pair): 198.000/152.000 Da from Sample 1 (H) of H(100ug)-10uL-2.wiff (Turbo Spray), Smoothed
Max. 4.7e5 cps.
3.93
Intensity, cps
Time, min

(D)


XIC of +MRM (1 pair): 198.000/152.000 Da from Sample 1 (E) of E(1mg)-10uL-3.wiff (Turbo Spray), Smoothed, Smoothed
Max. 2.1e5 cps.
3.95
Intensity, cps
Time, min

(E)


XIC of +MRM (1 pair): 198.000/152.000 Da from Sample 1 (B) of B(1ug)-10uL-2.wiff (Turbo Spray), Smoothed
Max. 7.8e4 cps.
3.95
Intensity, cps
Time, min

(F)

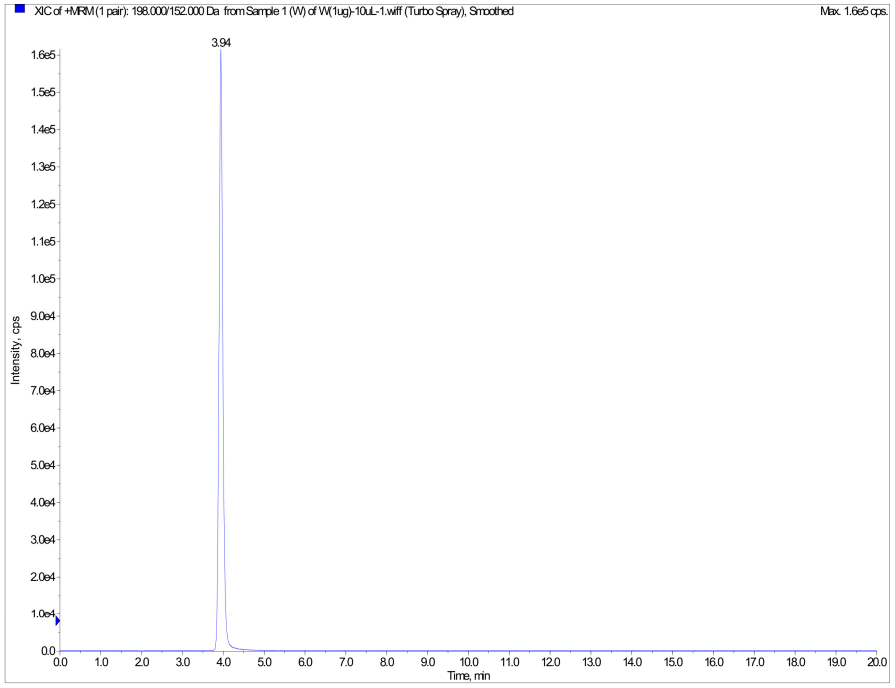

XIC of +MRM (1 pair): 198.000/152.000 Da from Sample 1 (W) of W(1ug)-10uL-1.wiff (Turbo Spray), Smoothed
Max. 1.6e5 cps.
3.94
Intensity, cps
Time, min

Figure S2. HPLC-DAD chromatograms of profiles of *Mucuna pruriens* extracts including crude methanol (**A**), n-hexanes (**B**), ethyl acetate (**C**), butanol (**D**), and water (**E**) extracts. Peaks were monitored at a wavelength of 250 nm.

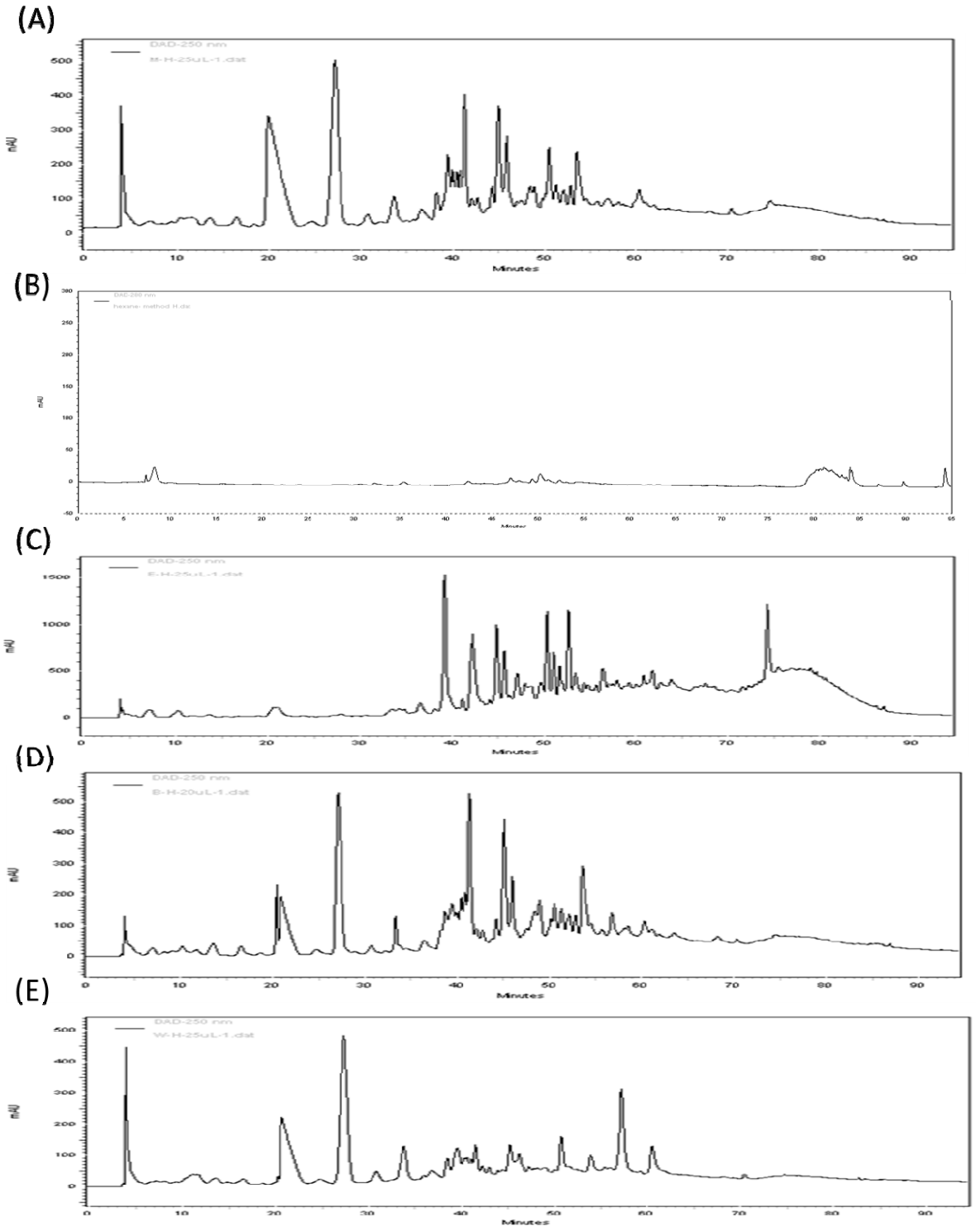


Figure S3. Effects of *Mucuna pruriens* extracts including crude methanol, n-hexanes, ethyl acetate, butanol, and water extract (at concentration of 25 μg/mL) on the cell viability (**A**) and LPS-induced NO production in murine BV-2 microglia (**B**). Significance was reported by ANOVA followed with Dunnett multiple comparison testing, as compared to control p≤0.0001 (####); as compared to toxic agent, p≤0.05 (*), p≤0.001 (***) and p≤0.0001 (****).

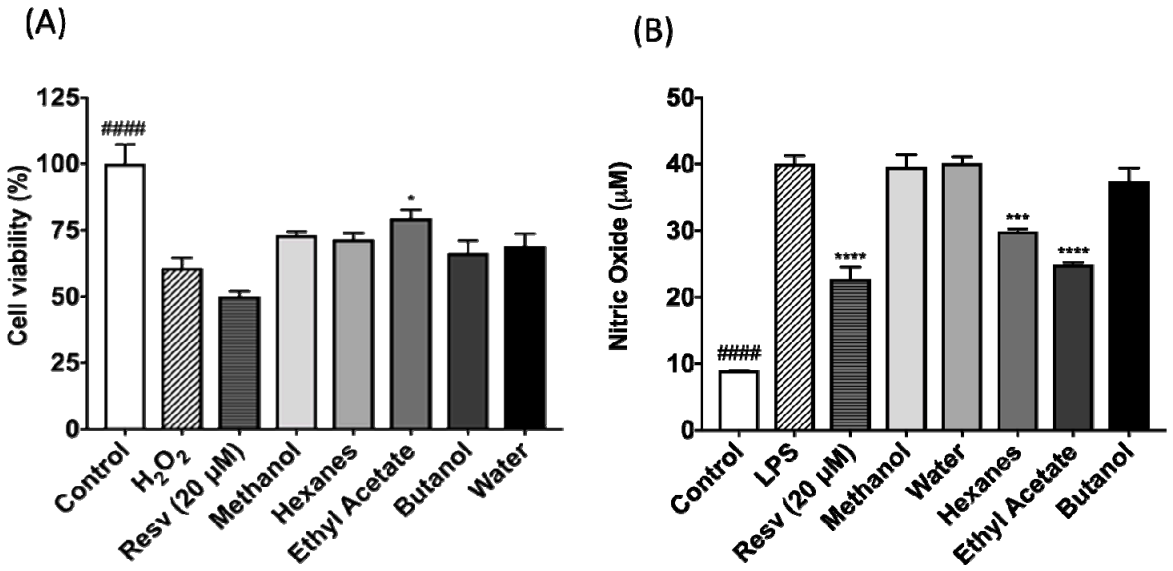


Figure S4. Morphology of murine BV-2 microglia treated with vehicle (**A**), H_2O_2 alone (**B**), H_2O_2+0.07% L-dopa (**C**), and H_2O_2+MPE (**D**); murine BV-2 microglia treated with vehicle (**E**), LPS alone (**F**), LPS+0.07% L-dopa (**G**), and LPS+MPE (**H**).

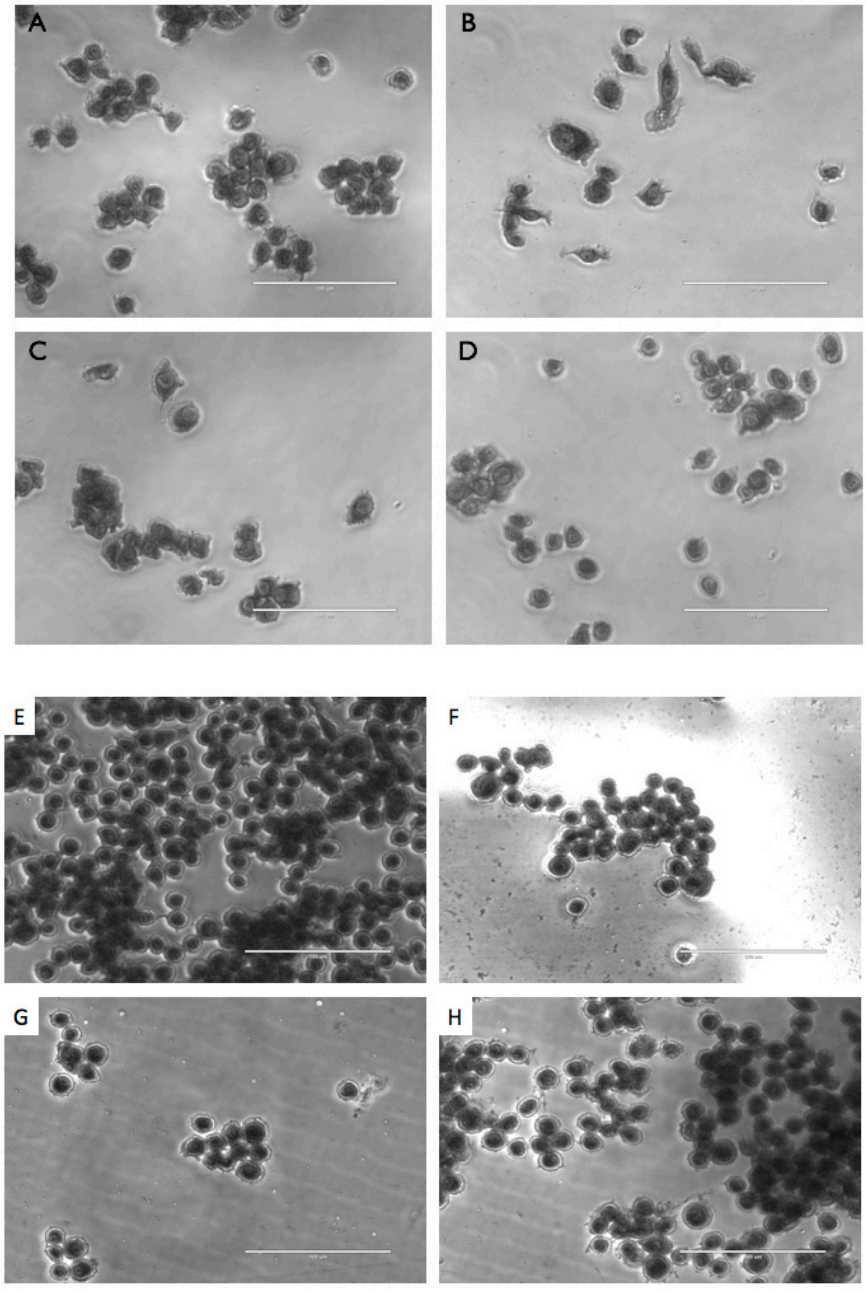


Figure S5. Effects of MPE and 0.07% L-dopa on H_2O_2-induced toxicity in murine BV-2 microglia. Significance was reported by ANOVA followed with Dunnett multiple comparison testing, as compared to control $p \leq 0.0001$ (####); as compared to toxic agent, $p \leq 0.05$ (*).

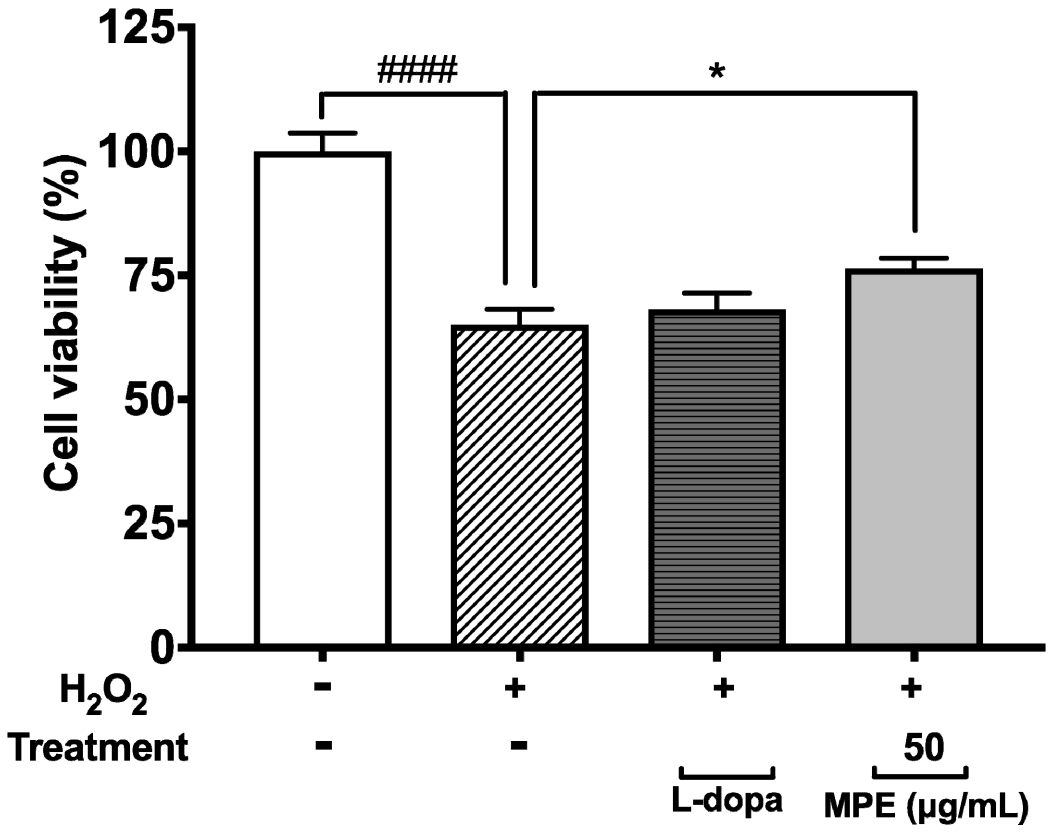


Figure S6. Effects of MPE and 0.07% L-dopa on LPS-induced NO production in murine BV-2 microglia. Significance was reported by ANOVA followed with Dunnett multiple comparison testing, as compared to control p≤0.0001 (####); as compared to toxic agent, p≤0.0001 (****).

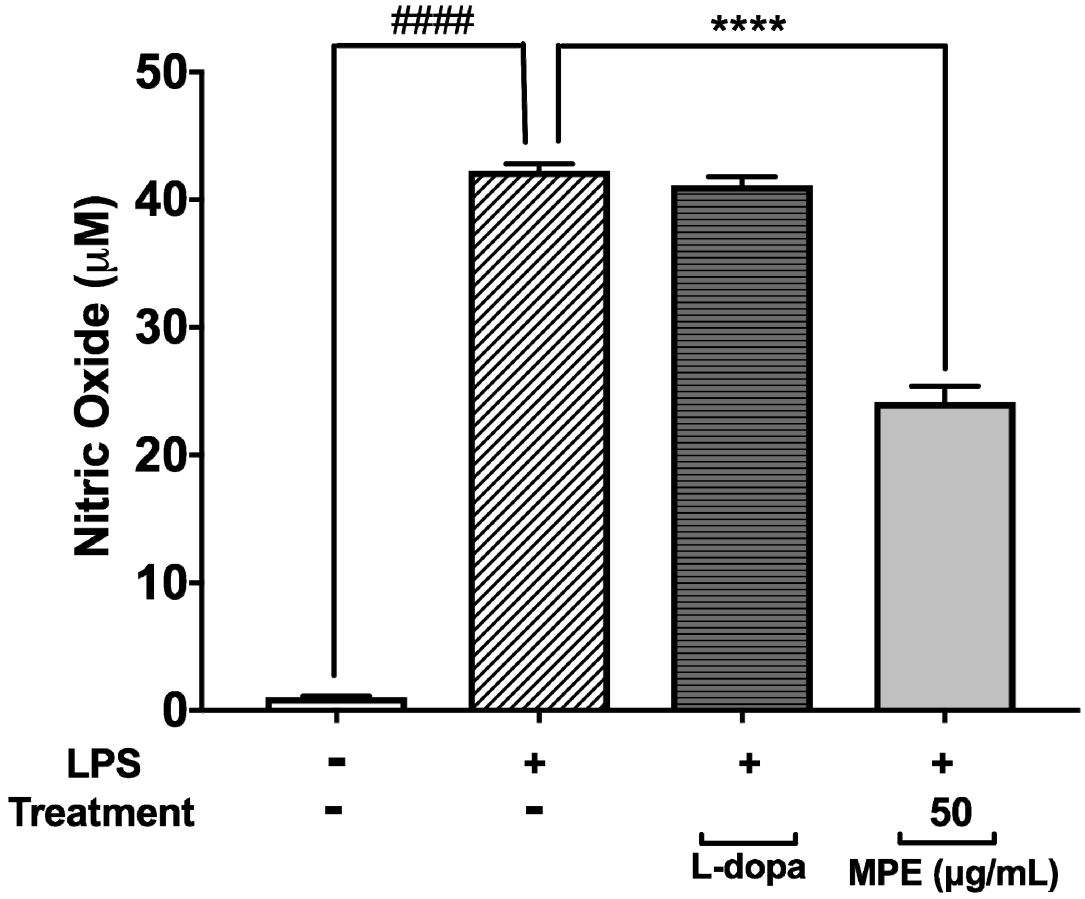


Table S1. Chemical constituents of *Mucuna pruriens*.

Type of chemicals	chemicals	References
Polyphenols	Tannins, flavonoids (e.g. genistein and daidzein), gallic acid, phenolic acids	[1][2]
Saponins		[1][2]
Terpenoids		[1][2]
Alkaloids and amino acids	β-Carboline, N,N-Dimethyl tryptamine, 5- hydroxytryptamine, bufotenine, tetrahydroisoquinoline, hydroisoquinoline, 5-oxyindole- 3- alkylamine, 6- methoxyharman, arahidicacid, arginine, glutathione, indole- 3- alkylamine	[3] [4]
Fatty acids	Linoleic acid, myristic acid, oleic acid, palmitic acid, vernolic acid, stearic acid	[5]
Carbohydrates	oligosaccharides (e.g. raffinose, stachyose, verbascose)	[6]

References:

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