P03-028

Increased dopamine TH and apoptosis inhibition in MPP+ induced Parkinson's disease cell model in *Gastrodia elata* Blume with ρ-cresol removed

Myoung Jin Kim[1], Yong Rae Cho[1], Min Hyung Seo[2], Sujung Yeo[2], Gwi Yeong Jang[1], Su Ji Choi[1], Jehun Choi[1*]

[1] Industrial Crop Utilization Division, NIHHS, RDA, Eumseong, Korea.
[2] Department of Meridian and Acupoint, College of Korean Medicine, Sangji University, Wonju, Korea.

농촌진흥청
국립원예특작과학원

Abstract

Background : *Gastrodia elata* Blume (GE) has been used for a long time because it is effective in neurological diseases such as headache, stroke, insomnia, and high blood pressure. However, GE is very inconvenient to drink due to its unique odor. The ρ-cresol contained by GE is the cause of the odor. Parkinson's disease (PD) is a degenerative disease of the central nervous system. Drugs used as anti-Parkinson treatments slow the progression of PD, but may also be accompanied by complications. Therefore, natural materials with anti-Parkinson effects are attracting attention.

Methods and Results : In this study, the effect of protecting neuronal cells and inhibiting neuronal cell apoptosis were compared in the Parkinson's disease cell model that treated MPP+ on neuroanl cell line PC-12 by securing the GE extract and the para-cresol-removed GE extract (GP-). Cell viability of GE and GP- was analyzed, and dopamine tyrosine hydroxylase (TH) and apoptosis were analyzed using western blotting. Dopamine TH and apoptosis (Bax / Bcl-2) were analyzed by immunostaining and western blotting. Treatment of GE and GP- reduced apoptosis and was not cytotoxic. In addition, TH expression and apoptosis protein were inhibited.

Conclusion : Pre-treatment of GE and GP- promoted the expression of dopamine TH and inhibited the apoptosis protein in cells of MPP+ induced PC-12. These results suggest that GP- can help improve apoptosis in neurons over normal GE, and improve MPP+ induced PD.

Materials and Mehtods

■ *Gastrodia elata* extraction and ρ-cresol removed

- The same amount of water was added to the frozen Gastrodia elata and extracted by reflux cooling at 100℃ for 12 hours, and then filtered and lyophilized to obtain the extract (GE).
- After Gastrodia elata extraction at 12 hours, the extract was passed through MN102 (a polystyrene adsorbent resin) to remove ρ-cresol. And freeze-dried to obtain dried extract (GP-).

■ Parkinson's Neurocellular Model

- 1 mM of 1-Methyl-4-phenylpyridinium (MPP+) was treated on PC-12 to induce neuronal cell death.

Results

Fig. 1. Roots of *Gastrodia elata* Blume, 천마 덩이뿌리

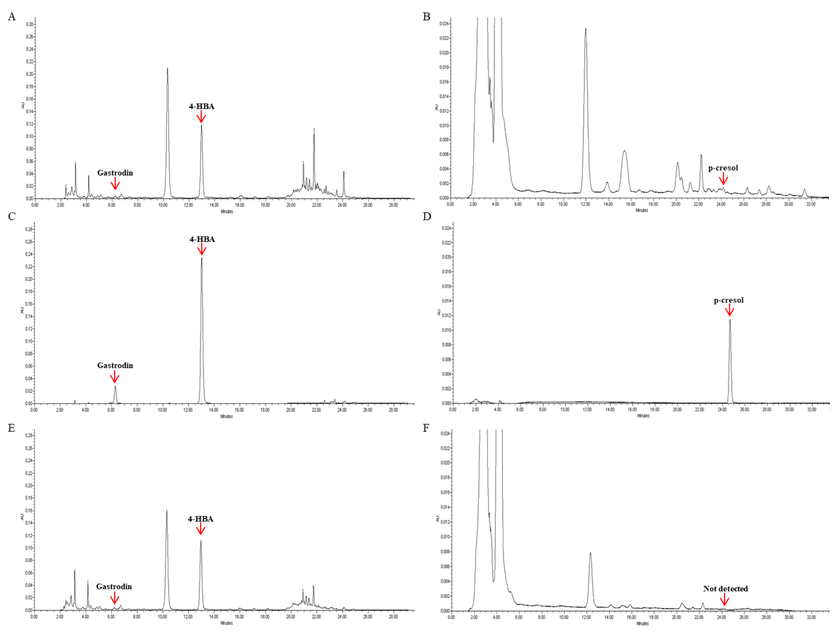

Fig. 2. HPLC chromatograms of *Gastrodia elata* extract and para-cresol component removed *Gastrodia elata* extract.

(A and B) standard mixture of gastrodin, 4-HBA and p-cresol; (C and D) extract of gastrodia elata blume; (E and F) gastrodia elata blume extract with p-cresol component removed

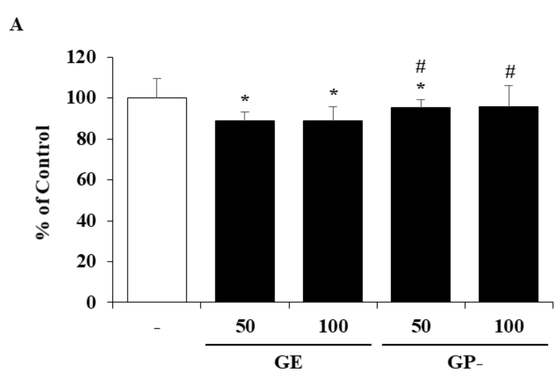

Fig. 3. Effects of GE and GE p- and MPP+ on cell viability in PC12.

(A) GE and GE p- were cultured for 24 hours at each concentration in PC 12 cells. (B) Cells were pretreated with GE (100μg/ml) and GP- (100μg/ml) for 1 hour, and then cultured with MPP+ (1 mM) for 24 hours.

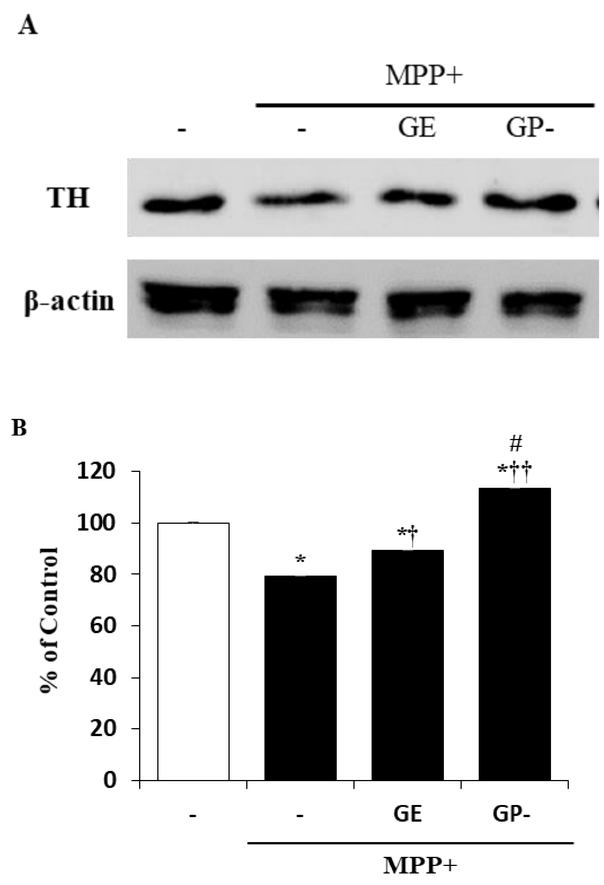

Fig. 4. Effects of GE and GE p- on tyrosine hydroxylase in PC12 induced by MPP+.

(A) In PC12 cells, GE (100μg/ml) and GP- (100μg/ml) were pretreated for 1 hour and incubated with MPP+ (1 mM) for 24 hours. The expression of tyrosine hydroxylase was performed by Western blot analysis, and beta actin was used as a loading control. (B) Relative density ratio of Bax and BCL-2/beta actin.

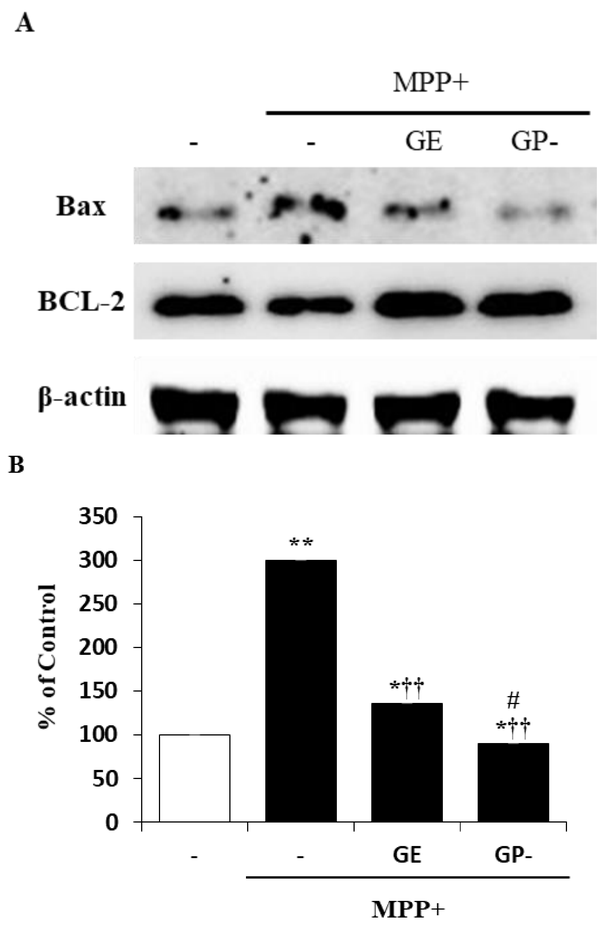

Fig. 5. Effects of GE and GE p- on apoptosis in MPP+-induced PC12.

(A) In PC12 cells, GE (100 μg/ml) and GP- (100 μg/ml) were pretreated for 1 hour and incubated with MPP+ (1 mM) for 24 hours. The expression of Bax and BCL-2 was performed by Western blot analysis, and beta actin was used as a loading control. (B) Relative density ratio of Bax and BCL-2/beta actin.

Conclusion

- Pre-treatment of GE and GP- promoted the expression of dopamine TH and inhibited the apoptosis protein in cells of MPP+ induced PC-12.
- GP- can help improve apoptosis in neuronal cells over normal GE and improve MPP+ induced PD.

***(Corresponding author) E-mail: jehun@korea.kr**