

Supplemental Data

Batryticatus Bombyx Protects Dopaminergic Neurons Against MPTP-Induced Neurotoxicity by Inhibiting Oxidative Damage

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Materials and Methods

Brain Tissue Preparation

On day 7 after MPTP treatment, mice were anesthetized immediately and perfused transcardially with 0.05 M phosphate-buffered saline (PBS; Sigma-Aldrich), followed by addition of cold 4% paraformaldehyde (PFA; Sigma-Aldrich) in 0.1 M phosphate buffer. Brains were removed and post-fixed in 0.1 M phosphate buffer (Sigma-Aldrich) containing 4% PFA overnight at 4°C, and immersed in a solution containing 30% sucrose in 0.05 M PBS for cryoprotection. Serial 30- μ m-thick coronal sections were cut on a freezing microtome (Leica Instruments GmbH, Nussloch, Germany) and stored in a cryoprotectant (25% ethylene glycol; Sigma-Aldrich), 25% glycerol (Sigma-Aldrich), and 0.05 M phosphate buffer at 4°C until use.

Immunohistochemistry (IHC) Analysis

Brain sections were rinsed briefly in PBS, treated with 1% hydrogen peroxide (Sigma-Aldrich) for 15 min, and incubated with rabbit anti-tyrosine hydroxylase (TH) (1:1000) overnight at 4 °C in the presence of 0.3% Triton X-100 (Vector Laboratories, Burlingame, CA, USA) and normal goat serum (Vector Laboratories). After rinsing in PBS, the sections were incubated with biotinylated anti-rabbit IgG (Vector Laboratories) (1:200) for 90 min, rinsed, and incubated with ABC (Vector Laboratories) (1:100) for 1 h at room temperature. Peroxidase activity was visualized with DAB (Sigma-Aldrich) in 0.05 M Tris-buffered saline (Sigma-Aldrich). After several rinses with PBS, the specimens were mounted on gelatin-coated slides, dehydrated, and cover-slipped using histo-mount medium. Images were photographed at 40 \times and 100 \times magnification using an optical light microscope (Olympus Microscope System BX53; Olympus, Tokyo, Japan) equipped with a 20 \times objective lens.

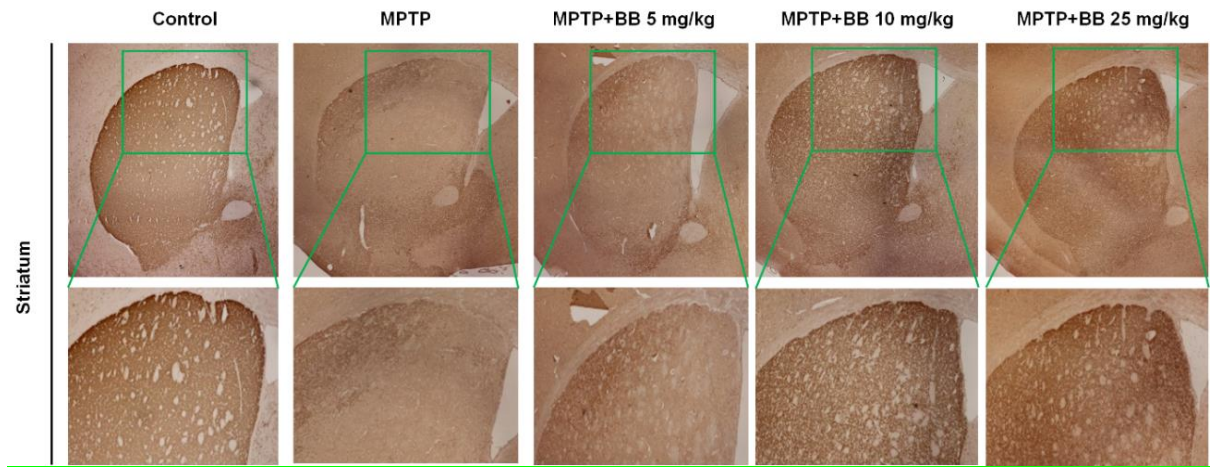


Figure S1. Effects of *Batryticatus Bombyx* (BB) on MPTP-induced dopaminergic neuronal death. Dopaminergic neurons were visualized by TH-specific immunostaining. Representative photomicrographs of the striatum (ST) were taken.