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Flavonoids from *Herba epimedii* Protect against MPP+-induced Toxicity in MES23.5 Cells

Na Li[1], Ming-Chun Jiang[2], Wen-fang Chen[1]

[1]Department of Physiology, State Key Disciplines and Shandong Provincial Collaborative Innovation Center for Neurodegenerative Disorders, Medical College of Qingdao University, Qingdao 266071, China

[2]Department of Physiology, Medical College of Taishan, Taian 271016, People's Republic of China

Abstract: Flavonoids, the active components of *Herba epimedii* (HEP), exerts many pharmacological effects, such as improvement of neurological function and sexual dysfunction, anti-osteoporosis and treatment of cardiovascular diseases in China over the centuries. According to the Chinese Pharmacopoeia, icariin, epimedin B and baohuoside-1 are three major flavonoid compounds isolated from HEP. The present study aims to characterize the neuroprotective effects of total flavonoid fraction of HEP and its three major flavonoid compounds against 1-methyl-4-phenylpyridinium ion (MPP^+)-induced neurotoxicity in MES23.5 cells. MTT assay showed that treatment with MPP^+ significantly suppressed the cell viability. The effect could be respectively reversed by HEP, icariin, epimedin B and baohuoside-1 treatment in a dose-dependent manner. The present results provided the evidence that HEP and its main active compounds could protect against MPP^+-induced neurotoxicity in dopaminergic MES23.5 cells.

Keywords: *Herba epimedii*; Icariin; Epimedin B; baohuoside-1; 1-methyl-4-phenylpyridinium ion; MES23.5 Cells

1.Introduction

Herba epimedii (HEP), a traditional Chinese herb medicine, is well-known for its various biological functions including anti-osteoporosis, anti-inflammation and cardiovascular system protective activities[1-3]. Icariin, epimedin B and baohuoside-1, the major flavonoid compounds isolated from HEP, account for its biological effects both in vivo and in vitro. Recently, many studies have focused on the neuroprotective effects of HEP and its flavonoid compounds, especially icariin. In vitro, studies showed that icariin could protect PC12 cells by weakening β-amyloid-induced neurotoxicity[4]. Moreover, icariin also could suppressed Tau phosphorylation in SH-SY5Y cells[5]. In vivo study, icariin could regulate the memory deficits and improve the learning and memory abilities[6]. Our previous study also demonstrated the neuroprotective properties of icariin in MPTP-induced mouse model[7]. In recent study, we also indicated that total flavonoid fraction of HEP had protective effects on dopaminergic neurons both in vivo and in vitro study[8]. These studies further indicated that flavonoid compounds of HEP might be account for its neuroprotective effects. However, at present, no data have evaluated the neuroprotective effects of epimedin B and baohuoside-1 on dopaminergic MES23.5 cells.

In the present study, we aim to study the neuroprotective effects of total flavonoid fraction of HEP, icariin, epimedin B and baohuoside-1 using MPP^+-induced neurotoxicity in the MES23.5 cells.

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Wenfang Chen (Correspondence)
chenwenfangqd @ 163.com
+(86-532) 83780051 Fax: (86-532) 83801449

2.Materials and methods

2.1. Preparation of flavonoid fraction from HEP

The origin, extraction and classic chromatographic profile of flavonoid have been reported in our previous research[8, 9]. Fig. 1 shows a typical high performance liquid chromatography (HPLC) profile of the 50% and 95% ethanol eluate of HEP total extract. The peaks of epimedin B, icariin and baohuoside-1 in the profile were identified by the authentic markers with the same retention time. The purity of the three isolated compounds were more than 98%.

2.2 Culture of MES23.5 cells

The MES23.5 cell line provided by Dr. Weidong Le (Baylor College of Medicine, Houston, USA) were routinely cultured in DMEM/F12 medium, which contains 5% fetal bovine serum (FBS), Sato's components growth medium, penicillin 100 IU/ml and streptomycin 100 μg/ml (Invitrogen, Carlsbad, CA, USA) at 37◦C in a humidified atmosphere of 95% air and 5% CO_2 as previously described[10].

2.3. Cell viability assa*y*

The MES23.5 cells were cultured in 96-well plates (1×10^4 cells/well) using culture medium. For analysis of the neuroprotective function, the MES23.5 cells were treated with HEP (0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) or vehicle for 24 h after which it was replaced with medium which containing MPP^+ (100 μM) and HEP (0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) for another 24 h. The same process in MES23.5 cells was treated with different dosages of icariin (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M), epimedin B (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) and baohuoside-1 (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M). Cell viability was measured by the 3-[4, 5-dimethylthiazol 2-yl] 2, 5-diphenyltetrazolium bromide (MTT) assay. Briefly, 20 μl of tetrazolium (MTT, 5 mg/ml, Sigma, St. Louis, MO, USA) in phosphate-buffered saline was added into each well. Then, the plates were incubated at 37°C for 4 h. After that, 100μl dimethyl sulfoxide was added in and the plates were shaken for 15 s. Signal detection was implemented by microplate reader at the wavelength of 595 nm.

2.4 Statistical analysis

Data were presented as the mean ± SEM. The data were performed by One-way analysis of variance (ANOVA) with Tukey's multiple comparison tests. Values of $P<0.05$ were considered significant.

3. Results

3.1 Neuroprotective properties of different dosage of HEP against MPP^+-induced MES23.5 cell death

Our result showed that pretreatment with different dosage of HEP (0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) could protect against MPP^+-induced cell death and the significant rescue occurred at 0.25 μg/ml (Fig. 2A). While HEP (0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) treatment alone did not affect cell viability (Fig. 2B).

3.2 Neuroprotective properties of different dosage of icariin against MPP^+-induced MES23.5 cell death

Pretreatment with icariin (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} mol/L) 24h before and during MPP^+ exposure resulted in an enhancement of survival and the most effective concentrations were 10^{-9} and 10^{-8} mol/L (Fig. 3).

3.3 Neuroprotective properties of different dosage of *epimedin* B against MPP^+-induced MES23.5 cell death

As shown in Fig. 4, epimedin B increased cell viability of MES23.5 cells in a dose-dependent manner. The maximal rescue of MES23.5 cells at concentrations of epimedin B (from 10^{-10} to 10^{-5} mol/L) occurred at 10^{-7} mol/L.

3.4 Neuroprotective properties of different dosage of baohuoside-1 against MPP^+-induced MES23.5 cell death

Fig. 5 also clearly shows that baohuoside-1 (from 10^{-10} to 10^{-5} mol/L) also increased cell viability of MES23.5 cells in a dose-dependent manner; the

significant increase was found at 10^{-6} mol/L.

4. DISCUSSION

Icariin, epimedin B and baohuoside-1 were identified in the HPLC profile of total flavonoid fraction of HEP using authentic markers. Accumulating evidences have indicated that HEP has the potential activity against osteoporosis[11-13]. Recently, more and more researchers focused on the neuroprotective properties of HEP and its flavonoid compounds, especially for icariin. Zhu's reported that icariin could protect against brain injury in experimental stroke[14]. Other researchers findings pointed out that icariin orally administration could extenuate learning and memory impairment[15]. Moreover, data proofed that icariin could ameliorate the learning and memory deficits in aging rats[16] and APP transgenic Alzheimer's disease mice[17]. While, there are few studies focusing on the neuroprotective properties of HEP and its three flavonoid compounds in MES23.5 cells.

The present data systematically evaluated the effects of HEP and three flavonoids on cell viability in MES23.5 cells. The results indicated that three tested flavonoids and HEP could significantly enhance the cell viability. The protective effects of icariin on MES23.5 cell viability have been indicated by our previous research[18]. As for epimedin B and baohuoside-1, We first time reported the protective effects on MES23.5 cell viability. It should be noted that the protective dosages for the enhancement of cell viability by the three flavonoid compounds were different. For example, the most effective concentrations for icariin was 10^{-9} and 10^{-8} mol/L; epimedin B was most active at 10^{-7} mol/L in promoting cell viability; baohuside-1 was most active at 10^{-6} mol/L. The above results indicated the neuroprotective effects of HEP and its three major flavonoids in MES23.5 cells.

In conclusion, our findings provide new insights for the protective effect of HEP and its flavonoid compounds on MES23.5 cell viability. Further study will be performed on the detailed neuroprotective mechanism continually.

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CONFLICTS OF INTERESTS

Declaration that the manuscript is original, has not been submitted to or is not under consideration by another publication and has not been previously published in any language or any form, including electronic. All of the authors declare that there have no conflicts of interests.

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Figure legends

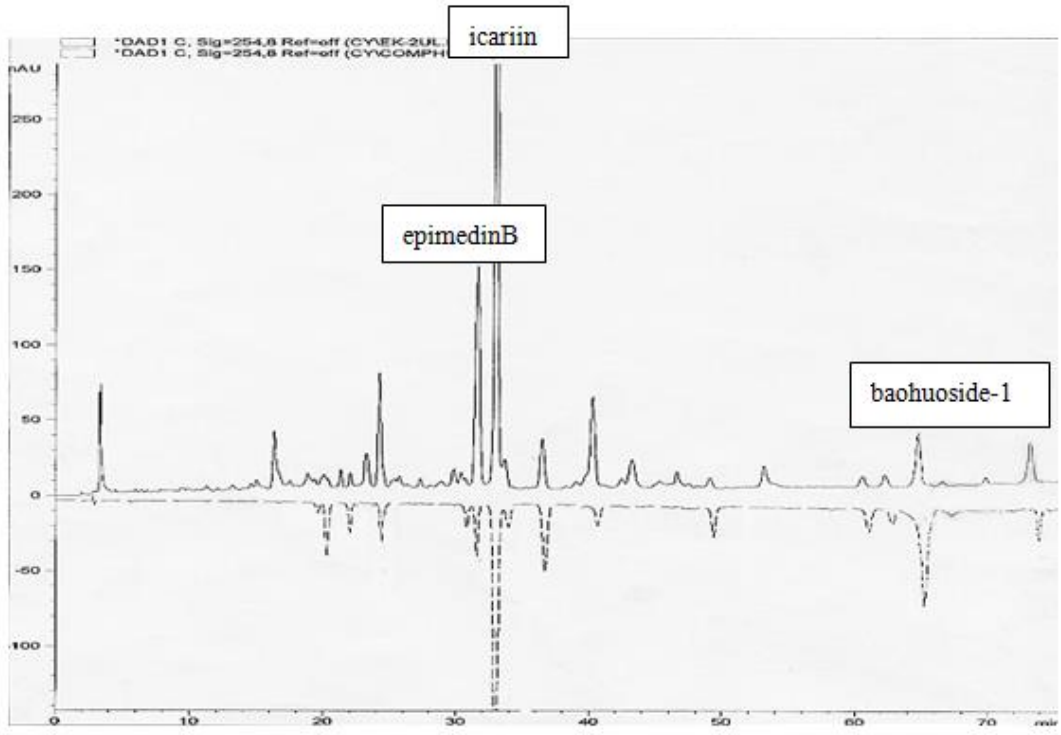

Fig1. Reverse-phase HPLC profile of the 50% ethanol eluate of HEP. HPLC analysis of standard compounds has been performed using the same elution procedure as that for the 50% ethanol eluate. The peaks of icariin, epimedin B and baohuoside-1 in the profile of 50% and 95% ethanol eluate were identified by the authentic markers with the same retention time.

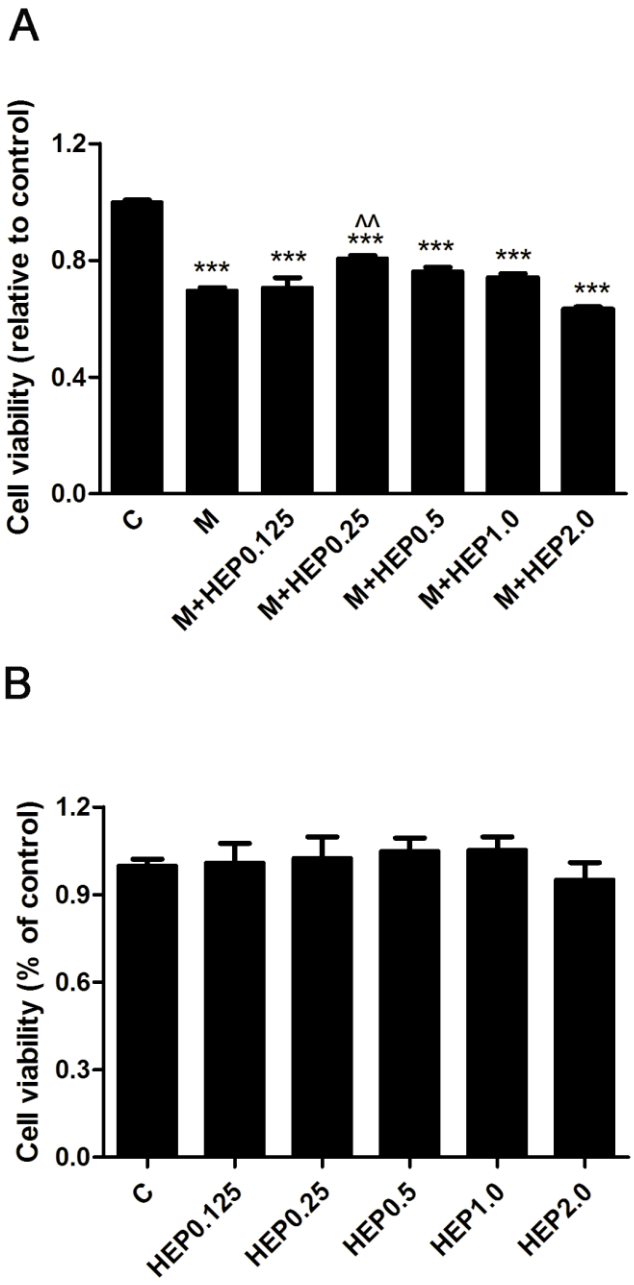

Fig 2. Effects of different dosage of HEP on MPP^+-induced neuronal death in MES23.5 cells.

A: The MES23.5 cells were treated with HEP (0.125, 0.25, 0.5, 1.0, 2.0 μg/ml) or vehicle for 24 h, then co-treatment with MPP^+ (100 μM) and HEP for another 24h. MTT assay was used to determine the cell viability. Data represent the mean ± SEM. $^{***}p<0.001$ vs the control group, $^{\wedge\wedge}p<0.01$ vs the MPP^+ group, n =3.

B: Treated with different dosage of HEP alone did not affect cell viability.

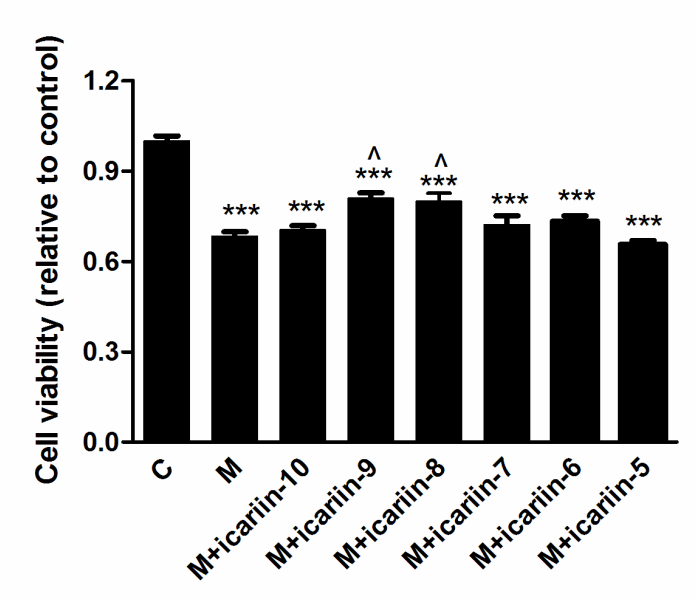

Fig 3. Neuroprotective properties of different dosage of icariin against MPP^+-induced MES23.5 cell death. The MES23.5 cells were pretreated with icariin (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) or vehicle for 24 h, then co-treatment with MPP^+ (100 μM) and icariin for another 24h. MTT assay was used to determine the cell viability. Data represent the mean ± SEM. $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPP^+ group, n =3.

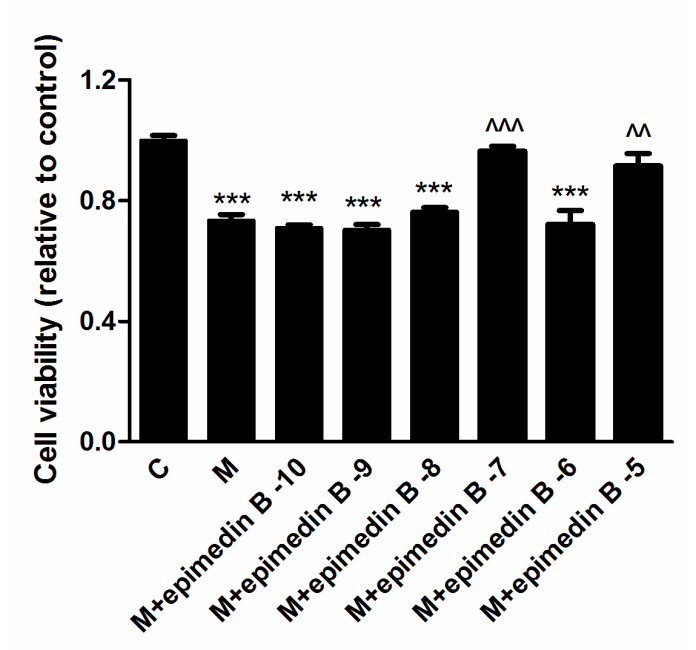

Fig 4. Neuroprotective properties of different dosage of epimedin B against MPP^+-induced MES23.5 cell death. Cells were pretreated with epimedin B (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) or vehicle for 24 h, then co-treatment with MPP^+ (100 μM) and epimedin B for another 24h. MTT assay was used to determine the cell viability. Data represent the mean ± SEM. $^{***}p<0.001$ vs the control group, $^{\wedge\wedge}p<0.01$, $^{\wedge\wedge\wedge}p<0.001$ vs the MPP^+ group, n =3.

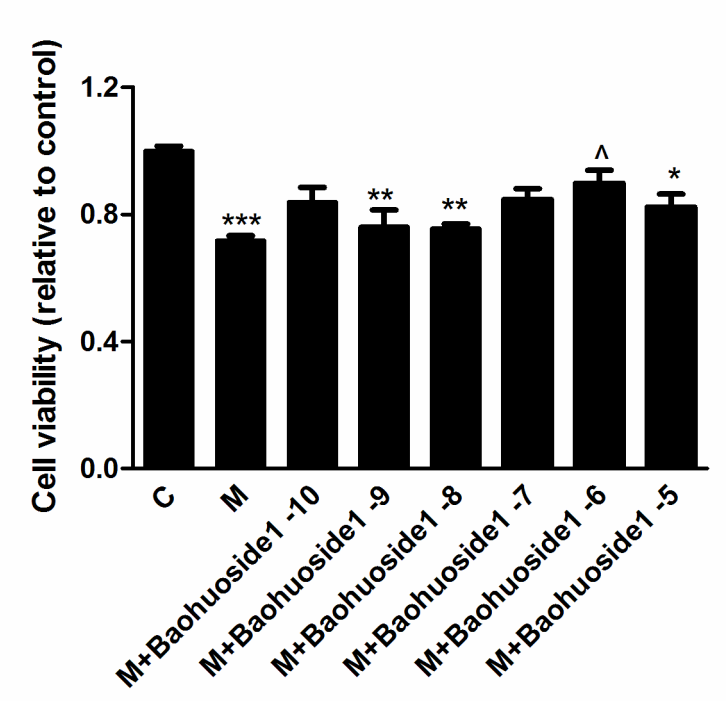

Fig 5. Neuroprotective properties of different dosage of baohuoside-1 against MPP^+-induced MES23.5 cell death. Cells were pretreated with baohuoside-1 (10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) or vehicle for 24 h, then co-treatment with MPP^+ (100 μM) and baohuoside-1 for another 24h. MTT assay was used to determine the cell viability. Data represent the mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ vs the control group, $^{\wedge}p<0.05$ vs the MPP^+ group, n =3.