MedLife Clinics

ISSN: 2689-5943

Research Article

Effects of the Extracts of *Cistanche Harba* on the Viability, Differentiation and the Aβ-Induced Damage of PC-12 Cells

Qi Jin Feng[1], Takuji Yamaguchi[1], Masahiro Tabuchi[1], Ai Ling Hu[1] and Hiroyuki Kobayashi[1,2]*

[1]*Department of Personalized Kampo Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan*

[2]*Department of Hospital Administration, Juntendo University Graduate School of Medicine, Tokyo, Japan*

Abstract

Objectives: The Amyloid β peptide (Aβ) has been proposed to play an important role in the pathogenesis of Alzheimer's disease. The purpose of this experiment is to compare the effect of the two traditional Chinese medicines, Cistanche deserticola (C. deserticola) and Cistanche tubulosa (C. tubulosa), on the viability, the neural differentiation and the Aβ-induced damage on PC12 cells. The antioxidant activity of both drugs was also examined.

Methods: PC12 cells were cultured and then administrated Aβ and the crude extracts of C. deserticola and C. tubulosa. The cells were analyzed the viability, the neurite length, the dopamine contents. Antioxidant activities of crude extracts were also examined.

Results: The crud extract of C. deserticola increased the viability of PC 12 cells at a limited concentration, and those of C. tubulosa showed no influence. As to the neural differentiation, both extracts of drugs shows the elongation activity of neurites and the decrease of dopamine contents of PC 12 cells. The pre-administration of the extract of C. deserticola to PC 12 cells demonstrated the protective effect from the Aβ-induced damage of viability. On the other hand, the extract of C. tubulosa did not showed such effect. The antioxidant activities of both extracts were higher than those of vitamin C.

Discussion: From the experimental results, it can be concluded that the crude extracts of C. deserticola promotes the viability and the neural differentiation of PC 12 cells, and further showed the protective effects for the Aβ-induced damage of PC 12 cells and the high activity of antioxidant.

Keywords: Alzheimer's disease; PC 12 cells; Traditional chinese medicines; Amyloid β peptide

Introduction

The Traditional Chinese Medicines (TCM) is often used mainly in alleviating symptoms such as BPSD (Behavioral and Psychological Symptoms of Dementia) of Alzheimer's disease, and the effect as a therapeutic agent was also mixed [1-3]. In recent years, substances showing NGF activity have been extracted and discovered one after another from several crude drugs [4]. In contrast with poor effects of Western medicine, the expectation for prevention of dementia with TCM with relatively few side effects is increasing.

Cistanches Herba has been prescribed as one of the prevention and amelioration drugs for dementia since the ancient times in China [5]. There are mainly two kinds of crude drugs that are known as *Cistanche tubulosa (C. tubulosa)* and *Cistanche deserticola* Y.C.Ma (*C. deserticola*), but currently what is being studied mainly is *C. tubulosa* which is rich in wild resources and easy to cultivate. However, the *Cistanches Herba* that was initially listed by the Chinese pharmacists only refers to the *C. deserticola* which is living at desert area, and the active ingredient and medicinal value of the *C. deserticola* differ so much with those of *C. tubulosa*.

The Amyloid β peptide (Aβ) has been proposed to play a role in the pathogenesis of Alzheimer's disease [6]. The purpose of this experiment is to examine the effects of the crud extracts of *C. deserticola* and *C. tubulosa* on the viability, the neural differentiation and the Aβ-induced damage on PC12 cells. Moreover, the antioxidant activity of both crude drugs was also examined.

Citation: Feng QJ, Yamaguchi T, Tabuchi M, Ling Hu A, Kobayashi H. Effects of the Extracts of *Cistanche Harba* on the Viability, Differentiation and the Aβ-Induced Damage of PC-12 Cells. Med Life Clin. 2021; 3(1): 1025.

Publisher Name: Medtext Publications LLC

Manuscript compiled: Jan 05th, 2021

***Corresponding author:** Hiroyuki Kobayashi, Department of Personalized Kampo Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, 113-8421, Tokyo, Japan, E-mail: koba@juntendo.ac.jp

Methods

Extracts preparation from herbal drugs

Two herbal drugs of *Cistanches Herba* (*C. deserticola* Y.C.Ma and *C. tubulosa*) were cut into small pieces individually and pulverized with a mill machine, referring to the extraction method in the literature [7,8]. In briefly one gram of the powder was added with 50 mL distilled water contained 50% methanol and stir for 2 hours with ultrasound while heating at 60 degrees. The liquid was centrifuged and the supernatant was concentrated under the reduced pressure to evaporate the water. The remaining solid material was weigh and regarded as crude extract of herbal drug. The extract was dissolved in the serum-free culture medium at all cell assays.

Cell viability assay

PC12 cells were seeded in 48-well culture plates with 3×10^4 cell/well using RPMI-1640 contained 10% FBS. The medium was changed

to the serum-free culture medium 24 hours before dosing drugs in all assays. Then the culture medium was changed to the medium containing crude extract of herbal drug at the concentrations of 100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L. Control wells were added by the serum-free culture medium only. Subsequently the viability of PC 12 cells was determined after 24 hours, 48 hours and 72 hours of dosing using a Cell Counting Kit-8 (CCK-8 kit, Dojindo Laboratories, Japan) which measures the reaction products of NADH in cells referring to the metabolic activity or the cell viability, which measurement sample processing was performed according to the manufacturer's instructions of the kit. The absorbance was measured at 450 nm on Multiskan FC (Thermo Fisher Scientific Inc., Japan).

Measurements of Neurite Length and Dopamine Content

The potential of the herbal drug extracts for the neural differentiation of PC 12 cells was assessed by measuring the neurite outgrowth and the dopamine contents in cells. 24 hours after dosing herbal drug extracts at the concentration of 100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L, the length of the longest neurite of PC 12 cells in each 4 random field for each well was measured with an image processor software (Image J, NIH). After calculating the average neurite-length of each well, average of the 6 wells was obtained as the value of each concentration, according to previous reports [4,9].

Dopamine contents in the PC 12 cells suspension solution collected at 3^{-}, 24^{-}, 48^{-} and 72^{-} h after extracts administration (500 μg/L) was measured using Dopamine Research ELISA Kit (ImmuSmol Inc., France) according to the manufacturer's instructions.

Assay for the Aβ-induced damage of PC12 cells

For examining the effect of drug extracts on the Aβ-induced damage of PC 12 cells, the damage induced by Aβ on PC12 cells was preliminary examined in the viability of 24 hours after administration of Aβ at concentrations of 0.5 20 μM, according to previous report [9]. On the basis of the results obtained above, the final assay concentrations were decided 20 μg/L for Aβ and 500 μg/L for each drug extract. Using these concentrations, the order of administration of Aβ and drug extracts was adopted next two kinds. One was that Aβ was added 24 hours after dosing drug extract and a further 24 hours passed, and the other was that drug extract was added 24 hours after dosing Aβ *vice versa*. and a further 24 hours passed. Thereafter the cell viability of both experiments was determined using by CCK-8 kit.

Antioxidant activity of drug extracts

FREE Carrio Duo (DI-601M, Diacron International, Italy) was used to measure the antioxidant activity of extracts of two drug extracts (100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L) as presented by the production of HClO. The antioxidant activity of vitamin C with the same concentration was compared with the above results. The crude extracts of herbal drugs and vitamin C were dissolved in the serum-free culture medium according to the manufacturer's instructions and control groups were added the serum-free culture medium only.

Statistical analysis

All statistical analysis was performed using the GraphPad Prism (ver. 8.4.2) software (GraphPad Inc., San Diego, CA, USA). Data are presented as the mean ± SEM and analyzed by ANOVA followed by Bonferroni's test $p<0.05$ was considered significant.

Results and Discussion

Effects of extracts of two *Cistanches Herba* on the viability

The viability of PC 12 cells dosed by the extract of *C. deserticola* at the concentration of 250 and 500 μg/L was significantly increased compared with control group after 24 hours ($p<0.05$) as shown in (Figure 1A). This increasing effect was diminished at 48 hours and 72 hours (data not shown). On the other hand, those of *C. tubulosa* does not show any effects compared with control group (Figure 1B).

Effects of two *Cistanches Herba* on the neurite elongation

The neurite length of PC 12 cells dosed by the extract of *C. deserticola* at the concentration of 100 μg/L, 250 μg/L, 750 μg/L and 1000 μg/L significantly elongates compared with control group after 24 hours ($p<0.01$ and $p<0.001$, Figure 2A). This effect of elongation was diminished at 48 hours and 72 hours (data not shown). Those of *C. tubulosa* also significantly elongates at the concentration of 500 μg/L compared with control group ($p<0.05$, Figure 2B).

Effects of two *Cistanches Herba* on the dopamine contents

The dopamine contents in PC 12 cells administered by the extracts of *C. deserticola* (500 μg/L) significantly decreased at 3 hours and 24 hours after dosing compared with each control group ($p<0.001$, Table 1) and these effects continued until 72 hours (data not shown). The results of *C. tubulosa* on the dopamine contents shows similar decreasing effect as those of *C. deserticola* compared with each control group significantly ($p<0.001$, Table 1). The decreasing effects of *C. tubulosa* were significantly more intensive than those of *C. deserticola* ($p<0.05$ and $p<0.001$, Table 1).

Protective effects of *C. deserticola* on the Aβ-induced damage of PC 12 cells

The results of the extracts of two *Cistanches Herba* on the Aβ-induced damage of PC 12 cells is shown in (Figure 3). The viability of cells dosed by Aβ (20 μg/L) for 48 hours is significantly decreased compared with control ($p<0.05$). The viability of cells which were firstly administrated by the extracts of *C. deserticola* (500 μg/L) during 24 hours and then induced the damage by dosing Aβ (20 μg/L) during 24 hours was significantly higher than that of Aβ dosing during 48 hours ($p<0.001$) and reaches to the similar level as control. However, the activity of cells received the reverse pattern of drugs dosing, that is, the extract of *C. deserticola* was administered after Aβ dosing, does not show any effects compared with Aβ single dosing. On the other hand, the administration of *C. tubulosa* (500 μg/L) preceding Aβ dosing on PC 12 cells does not provide any effects on the viability. The reverse pattern of drugs dosing, that is, the extract of *C. tubulosa* was administered after Aβ dosing provide the significant decrease of the cell viability ($p<0.05$).

Antioxidant activity of two *Cistanches Herba*

The antioxidant activities of the extracts of *C. deserticola* were significantly higher than each dose of vitamin C at all concentrations of 500 μg/L to 1000 μg/L ($p<0.05$ and $p<0.01$, Figure 4). The activity of the extracts of *C. tubulosa* also significantly higher at the concentration of 500 μg/L to 1000 μg/L compared with each value of vitamin C ($p<0.01$). The extracts of *C. deserticola* at 500 μg/L shows stronger activity than those of *C. tubulosa* significantly ($p<0.05$).

In the present studies, the viability of PC12 cells increased by the administration of *C. deserticola* at multiple doses compared with the control group while does not showing any effects in *C. tubulosa* group

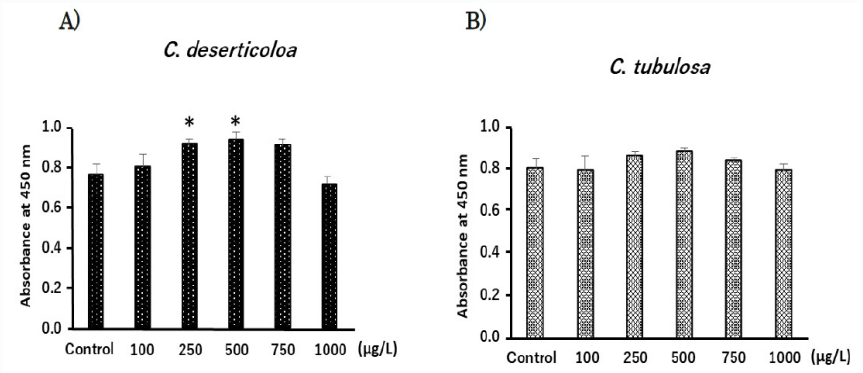

Figure 1: Effects of *Cistanches Herba* on the viability of PC 12 cells. The viability of PC 12 cells was determined at 24 hours after dosing 5 concentrations (100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L) of each drug extracts dissolved in the culture medium, using CCK-8 kit. Control wells were added by the culture medium only. A) is the results of *C. deserticola* extracts, and B) is those of *C. tubulosa* extracts. Values are presented as the mean ± SEM (n=6), **P*<0.05 *vs.* Control group.

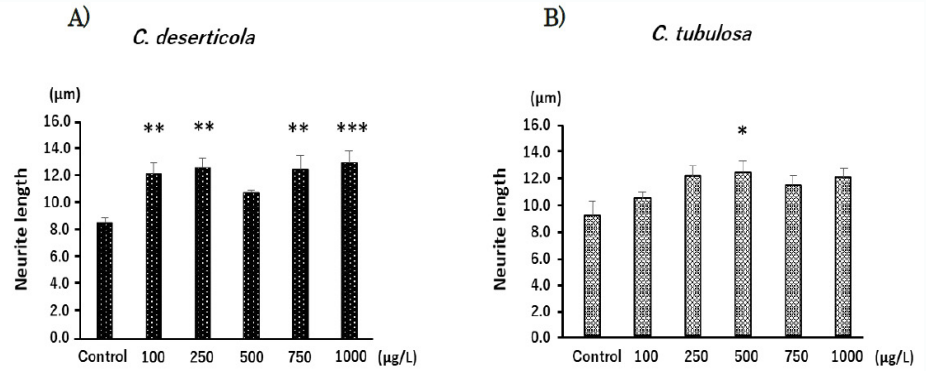

Figure 2: Effects of *Cistanches Herba* on the neurite elongation of PC 12 cells. Neurite length of PC 12 cells was measured at 24 hours after dosing 5 concentrations (100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L) of each drug extracts dissolved in the culture medium, using the image processor software. Control wells were added by the culture medium only. A) is the results of *C. deserticola* extracts, and B) is those of *C. tubulosa extracts*. Values are presented as the mean ± SEM (n=6), *,**,****P*<0.05, 0.01, 0.001 *vs.* Control group.

Table 1: Effects of *Cistanches Herba* on the dopamine contents in PC 12 cells. Dopamine contents (ng/ml) in PC 12 cells was determined at 3 hours and 24 hours after dosing *C. deserticola* extracts and *C. tubulosa* extracts at the concentration of 500 μg/L in the culture medium to PC 12 cells, using Dopamine ELISA kit. Control wells were added by the culture medium only. Values are presented as the mean ± SEM (n=3).

	Control	*C. deserticola*	*C. tubulosa*
3h	20.9 ± 1.3	$10.8 \pm 0.4^{***}$	$2.2 \pm 0.4^{***\dagger\dagger\dagger}$
24h	18.5 ± 10.7	$4.6 \pm 0.9^{***}$	$1.5 \pm 0.1^{***\dagger}$

$^{***}P$<0.001 *vs.* Control group of each time; $^{\dagger,\dagger\dagger\dagger}P$<0.05, 0.001 *vs. C. deserticola* of each time.

(Figure 1). This indicates that the reinforcing effect of *C. deserticola* on the metabolic activity of PC 12 cells is stronger than that of *C. tubulosa.*

The length of neurites has extended in both *C. tubulosa* and *C. deserticola* groups in the present study (Figure 2). This suggests that the two *Cistanches Herba* promote neurite outgrowth of PC12 cells which showed the better performance in *C. deserticola* group. Moreover, the two drugs have decrease the dopamine contents in PC12 cells, while the effect of *C. deserticola* is stronger. Previous report has shown that DA and norepinephrine levels in neurons decrease with the administration of NGF, a facilitator for the neuronal differentiation of PC12 cells [10]. Considering the result of the elongation effects on neurite outgrowth, it can be confirmed that two *Cistanches Herba* promote the neuronal differentiation of PC12 cells.

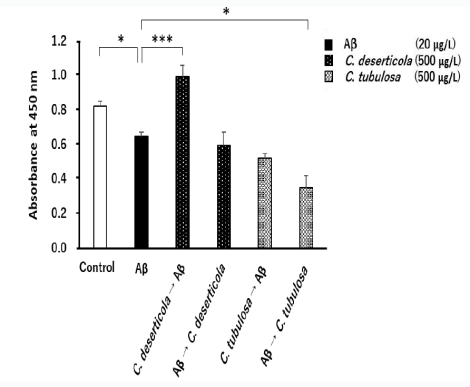

Figure 3: Protective effect of *C. deserticola* on the Aβ-induced damage of PC 12 cells. Two dosing patterns of Aβ and drug extracts was examined. One was that the extracted drug (500 μg/L) was administered to PC12 cells for 24 hours followed by the Aβ (20 μg/L) dosing for 24 hours, and the other was that the Aβ was firstly dosing followed by the extracted drug *vice versa.* Control group and Aβ group were respectively added by the culture medium and Aβ (20 μg/L) during 48 hours. Thereafter the cell viability of all experiments was determined using by CCK-8 kit. Values are presented as the mean ± SEM (n=6), *,****P*<0.05 and 0.001.

Interestingly, the *C. tubulosa* presented lower DA levels but the *C. deserticola* group shows longer neurites than the *C. tubulosa* group. This may attribute to different bio-functions of the two drugs. As two different administration orders of herbal extracts and Aβ have

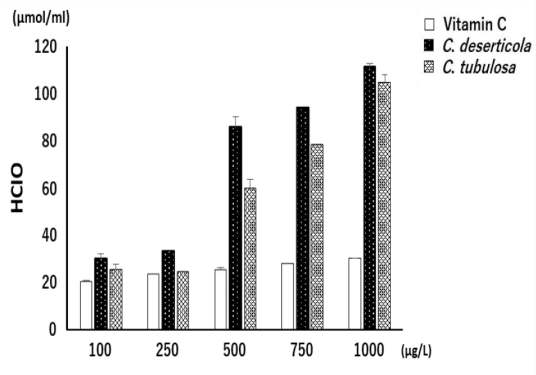

Figure 4: Antioxidant activity of *Cistanches Herba*. The antioxidant activities of extracts of *C. deserticola* and *C. tubulosa* (100 μg/L, 250 μg/L, 500 μg/L, 750 μg/L and 1000 μg/L) were measured as production of HClO. For the reference, the antioxidant activity of the same dose of vitamin C was examined. Values are presented as the mean ± SEM (n=3), *,**P< 0.05, 0.01 *vs.* each dose of Vitamin C, †P<0.05 *vs.* 500 μg/L of *C. tubulosa*.

been compared, the *C. deserticola* + Aβ group which administered the extracts of *C. deserticola* before Aβ dosing showed higher viability than the reverse order of administration (Figure 3).

It is more worthy of noting that there was no significant difference on viability between the groups with successive administrations of Aβ and *C. deserticola* and that with Aβ administration alone. These results suggest that *C. deserticola* can prevent PC12 cells against neural impairment induced by Aβ.

Oxidative stress has been proven to contribute to brain diseases including AD. In our study, the extracts of two *Cistanches Herba* demonstrated stronger antioxidant activity than vitamin C (Figure 4), which is expected to exert ameliorative effect by suppressing oxidative stress and cell damage induced by Aβ accumulation.

As the results, it can be concluded that *C. deserticola* facilitates the viability and the neuronal differentiation of PC 12 cells, featuring a superiority in anti-oxidative effects over *C. tubulosa*. In addition, *C. deserticola* could be expected to exert preventive effects on AD in clinical practices.

References

1. Li M, Heng W, Song Y, Meng Xu, Wei DP, Shunguang Hospital, et al. Effect of shenzhiling oral liquid on behavioral and psychological symptoms of dementia in patients with alzheimer's disease. Acta Universitatis Traditionis Medicalis Sinensis Pharmacologiaeque Shanghai. 2014;19(6):20-25.
2. Matsunaga S, Kishi T, Iwata N. Yokukansan in the treatment of behavioral and psychological symptoms of dementia: an updated meta-analysis of randomized controlled trials. J Alzheimers Dis. 2016;54(2):635-43.
3. Niiitsu T, Okamoto H, Iyo M. Behavioural and psychological symptoms of dementia in an Alzheimers disease case successfully treated with natural medicine: association with gonadotropins. Psychogeriatrics. 2013;13(2):124-7.
4. Chiba K, Yamazaki M, Kikuchi M, Kakuda R, Kikuchi M. New physiological function of secoiridoids: neuritogenic activity in pc 12h cells. J Nat Med. 2011;65(1):186-90.
5. Gu C, Yang X, Huang L. *Cistanches herba*: a neuropharmacology review. Front Pharmacol. 2016;7:289.
6. Li L, Li W, Jung SW, Lee YW, Kim YH. Protective effects of decursin and decursinol angelate against amyloid β-protein-induced oxidative stress in the pc12 cell line: the role of nrf2 and antioxidant enzymes. Biosci Biotechnol Biochem. 2011;75(3):434-42.
7. Lian L, Gao MB, Yin XY. Optimization of ultrasonic-assisted extraction of polysaccharides from *Cistanche herba*. Guangzhou Chem Industry. 2018;46(19):61-77.
8. Ouyang J, Zhao B, Wang XD, Chun WY. An integrated method for the extraction of effective substances from *Cistanche deserticola*. J Wuhan Botanical Res. 2003;21(6):526-30.
9. Kumaran A, Ho CC, Hwang LS. Protective effect of nelumbo nucifera extracts on beta amyloid protein induced apoptosis in pc12 cells, *in vitro* model of alzheimer`s disease. J Food Drug Anal. 2018;26(1):172-81.
10. Loyd AG, Arthur ST. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc Natl Acad Sci USA. 1976;73(7):2424-8.