Antioxidative effect of Gastrodiae Rhizoma–containing herbal formula in PC12 cell model: abridged secondary publication

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KEY MESSAGES

1. The IC_{50} of hydrogen peroxide (H_2O_2) was 61.9 µM on PC12 cells.
2. Cell viability after co-treatment with Gastrodiae Rhizoma–containing herbal formula (DCXF) and H_2O_2 at all tested concentrations was similar to that with H_2O_2 treatment alone, suggesting that the antioxidative effect of DCXF is weak.
3. The antioxidant potential of DCXF was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. DCXF dose-dependently inhibited the DPPH radical at concentrations of 0.3125 to 20 mg/mL, compared with the control (0 mg/mL DCXF). However, the DPPH radical scavenging activity of DCXF was three orders of magnitude weaker than vitamin C.

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Introduction

Free radical formation and oxidative damage are main contributors to the pathophysiology of traumatic brain injury. Following the primary mechanical injury, the multiple interrelated secondary injuries cascade (including the loss of ionic homeostasis, glutamate excitotoxicity, mitochondrial dysfunction, and microvascular disruption) and result in the free radical formation such as hydrogen peroxide (H_2O_2), nitric oxide, and peroxynitrite.[1,2] The oxidative stress induced by uncontrolled free radicals may in varying degrees results in peroxidation of cellular and vascular structures, protein oxidation, cleavage of DNA, inflammatory, blood-brain barrier dysfunction, oedema formation, impairment of cerebral vascular function, apoptotic and necrotic neuronal cell death, and eventually neurologic disorders.[3,4]

The neuroprotective effects of Gastrodiae Rhizoma–containing herbal formula (DCXF) and the anti-oxidative activities of the individual herbs of DCXF and its main pharmacological active compounds (TMP, *E*-ferulic acid, *Z*-ligustilide, and gastrodin) have been extensively studied. However, the anti-oxidative effect of DCXF has not been reported. We used the H_2O_2-induced rat pheochromocytoma PC12 cell model and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical assay to evaluate the anti-oxidative effects of DCXF. The findings may be used to predict the potential therapeutic effect of DCXF on traumatic brain injury and establish groundwork for in vivo studies.

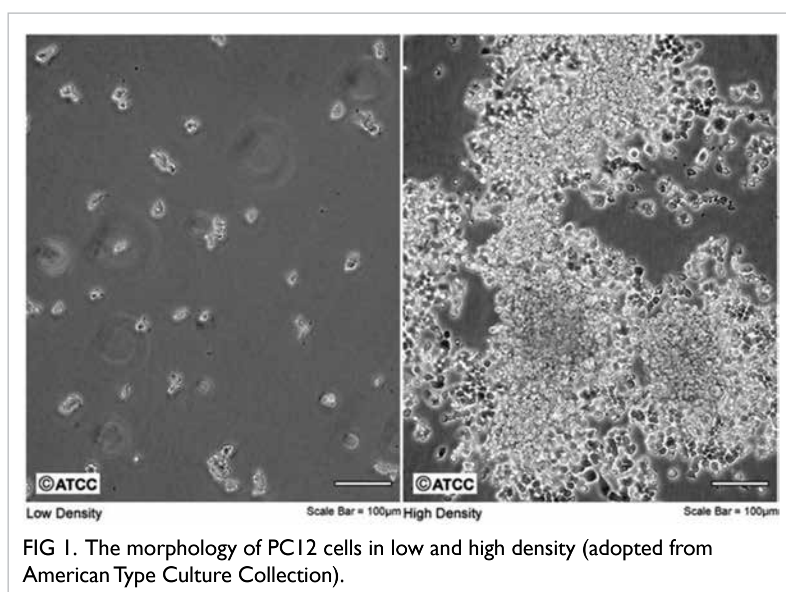

FIG 1. The morphology of PC12 cells in low and high density (adopted from American Type Culture Collection).

Materials and methods

The rat pheochromocytoma PC12 cells (Fig. 1) were obtained from American Type Culture Collection. The cells were grown in RPMI-1640 medium supplemented with 10% (v/v) heat-inactivated horse serum, 5% (v/v) FBS, and 1% (v/v) penicillin/streptomycin solution. The cell culture was maintained in poly-L-lysine-coated tissue culture flasks at 37°C in a humidified atmosphere of 5% CO_2 and 95% air.

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MTT assay was used to select a suitable H_2O_2 concentration to induce oxidative stress with about 50% cell death, and to assess the toxicity and protective effect of DCXF on cell viability in the H_2O_2-induced PC12 cell model. Briefly, PC12 cells were seeded in 96-well plates pre-coated with poly-L-lysine at a density of 2×10^4 cells per well. After incubated for 24 hours, the cells were treated with H_2O_2 at concentration ranging from 0 to 200 μM or with DCXF at concentration ranging from 0 to 2000 μg/mL for 24 hours. The culture medium was then removed and MTT solution was added to each well. After further incubation for 4 hours, the incubation solution was aspirated and the dark blue formazan crystals were solubilised in DMSO. Then, the absorbance was detected at 570 nm using a microplate reader.

To determine the cytoprotective activity of DCXF on H_2O_2-induced PC12 cells, 60 μM H_2O_2 was selected as the concentration of H_2O_2-induced oxidative stress on PC12 viability. PC12 cells were seeded in 96-well plates at a density of 2×10^4 cells per well and incubated for 24 hours. Then the cells were treated with indicated concentrations of DCXF with or without 60 μM H_2O_2. The control group cells were added with medium without H_2O_2. After further incubation for 24 hours, the cell viability was then measured by MTT method. All data were expressed as a percentage of untreated groups, which were expressed as 100%.

The radical scavenging activity of the samples was determined by DPPH assay as previously described, with slight modification.[5] The chemicals' antioxidative activity can be evaluated according to the reduction of a stable DPPH radical by solution of antioxidants. The colour of DPPH in solution can be altered from deep violet to colourless or pale yellow when neutralised, which causes a decrease in absorbance and can be measured spectrophotometrically. Briefly, 100 μL of different dilutions of DCXF were mixed with 1000 μL DPPH (0.075 mM methanolic solution), followed by incubation for 30 minutes in the dark at room temperature. The absorbance of each sample was read at 517 nm using a microplate reader. Vitamin C was used as standard antioxidant compound for the comparison of IC_{50} (inhibition of 50% DPPH radical) with DCXF. All determinations were performed in triplicates. Percentage of inhibition of the DPPH radical was calculated using the following equation: inhibition of DPPH (%) = (1 - absorbance of samples (or) standard / absorbance of control) × 100.

Multiple group comparisons were made using one-way ANOVA with Dunnett post-hoc test using GraphPad Prism 6. Each experiment was repeated at least three times in triplicate or as indicated. Differences were considered statistically significant when $P<0.05$.

Results

H_2O_2 at the concentration of 37.5 to 200 μM significantly decreased the cell viability in a dose-dependent manner, compared with the untreated control group (0 μM H_2O_2) [Fig. 2a]. IC_{50} of H_2O_2 was 61.9 μM, and hence 60 μM H_2O_2 was chosen for subsequent experiments. DCXF had no cytotoxic effect on PC12 cells at all tested concentrations of 0 to 2000 μg/mL, compared with the untreated control group (0 μg/mL DCXF) [Fig. 2b]. 60 μM H_2O_2 could cause about 50% cell death, compared with the untreated control group (0 μg/mL DCXF, 0 μM H_2O_2). However, there was no significant change in the cell viability after treatment with DCXF at all tested concentrations, compared with the H_2O_2-treated alone (Fig. 2c), suggesting that the anti-oxidative effect of DCXF is weak in the H_2O_2-induced PC12 cell model.

The antioxidant potential of DCXF was evaluated by the DPPH assay. DCXF dose-

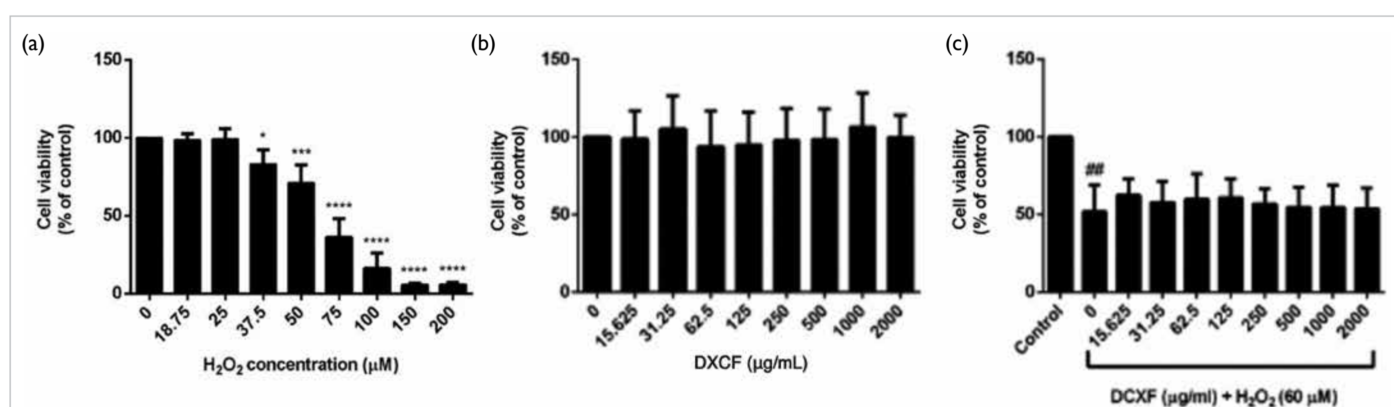

FIG 2. (a) Hydrogen peroxide (H_2O_2)–induced cytotoxicity on PC 12 cells. (b & c) Effect of Gastrodiae Rhizoma–containing herbal formula (DCXF) for 24 h with or without 60 μM H_2O_2 on viability of PC12 cells. Cell viability is determined by MTT assay.

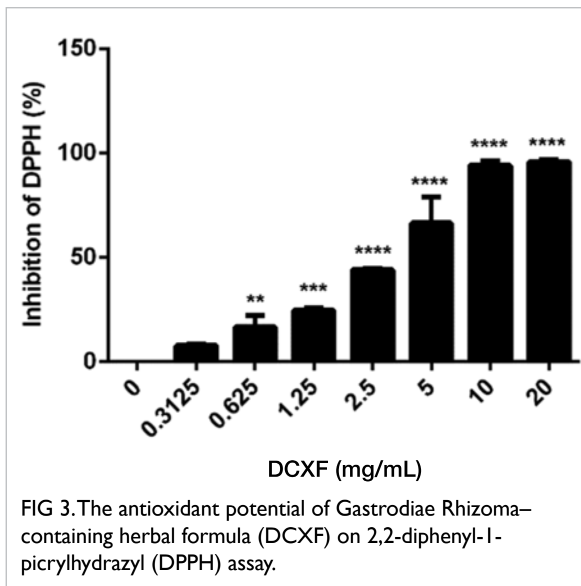

FIG 3. The antioxidant potential of Gastrodiae Rhizoma–containing herbal formula (DCXF) on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

dependently inhibited the DPPH radical at concentrations of 0.3125 to 20 mg/mL, compared with the control (0 mg/mL DCXF) [Fig. 3]. However, the DPPH radical scavenging activity of DCXF (IC_{50}=3.4 mg/mL) was three orders of magnitude weaker than vitamin C (IC_{50}=2.8 μg/mL) [data not shown], indicating that DCXF had a very weak antioxidant effect.

Discussion

PC12 cells have been extensively applied to study neurotoxicity (eg, cellular H_2O_2 toxicity), neurosecretion, neuronal differentiation, neuronal function, and neurodegeneration owing to their similarity with sympathetic neurons and their reversible differentiation response to nerve growth factor. Moreover, PC12 cells are widely used for neuroprotection studies of various neurological disorders such as traumatic brain injury, Parkinson disease, and Alzheimer disease. H_2O_2 is the major cause of oxidative stress and diffuses readily through cells and tissues, which can cause proteins oxidation, lipid peroxidation, DNA strand breakage, base modification, and eventually cell death via apoptosis or necrosis. H_2O_2 involves in the pathogenesis of various neurological disorders and has been used as an inducer of oxidative stress to elucidate the neuroprotective mechanisms of anti-oxidative therapeutics.[4] Moreover, the H_2O_2-induced PC12 cell model has been validated in analysing the neurological apoptosis and the therapeutic mechanisms of antioxidants.

The result of the DPPH chemical method demonstrated that DCXF had very weak antioxidant activity. In contrast to our previous studies, the aqueous extract of Gastrodiae Rhizoma provided neuroprotective effect on beta-amyloid-induced toxicity in PC12 cells and drosophila models, and demonstrated to attenuate locomotor deficit and reduce the inflammation on controlled cortical impact–induced traumatic brain injury rat model. The presence of Chuanxiong Rhizoma in DCXF may mitigate the antioxidative effect and reinforce the anti-inflammatory effects. Therefore, we will focus on the anti-inflammatory effects of DCXF.

Funding

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Disclosure

The results of this research have been previously published in:

1. Liu ZK, Ng CF, Shiu HT, et al. Neuroprotective effect of Da Chuanxiong Formula against cognitive and motor deficits in a rat controlled cortical impact model of traumatic brain injury. J Ethnopharmacol 2018;217:11-22.
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