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SHORT COMMUNICATION

Neuroprotective and Anti-Inflammatory Effects of Diphenylheptanes from the Fruits of *Amomum tsaoko*, a Chinese Spice

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Abstract Two novel diphenylheptanes, 2,3- dihydro-2 - (4′ - hydroxy-phenylethyl) - 6 - [(3″,4″ - dihydroxy-5“ - methoxy) phenyl] -4 - pyrone (CG-A) and 4 - dihydro-2 - (4’ - hydroxy-phenylmethyl) -6 - [(3”,4″ - dihydroxy-5″ - methoxyphenyl) methylene]-pyran-3, 5 - dione (CG-B), were isolated from the dried fruits of *Amomum tsaoko*, a commercially important spice. This study was designed to investigate their protective effects against H_2O_2-induced nerve injury, using PC-12 cells to determine the cell cytotoxicity and cell viability. The inhibitory effect on (nitric oxide) NO production was also determined in (lipopolysaccharide) LPS-stimulated macrophage RAW 264.7 cells. The results showed that CG-A and CG-B displayed significant neuroprotective effect and exhibited anti-inflammatory activity in a dose-dependent manner. These findings suggest that CG-A and CG-B are very important nutritional ingredients responsible for the neuroprotective and anti-inflammatory health benefits of *A. tsaoko*.

Keywords *Amomum tsaoko* · Diphenylheptanes · Neuroprotective effect · Anti-inflammatory

Introduction

Reactive oxygen species play a major role in the development of oxidative stress that can lead to many diseases [1]. Many compounds exhibiting antioxidant activity act as excellent anti-inflammatory agents [1]. *Amomum tsaoko* is widely distributed in the south-west of China [2]. The dried fruit is a well-known and commercially important spice. Some constituents from essential oil of *A. tsaoko* exhibit various biological activities such as antitumor, and antioxidant activities [3]. But relatively few studies have been performed on the detailed chemical composition and the biological studies were restricted to its crude extracts.

Diphenyl heptanes compounds, a class of compounds with a special structure (heptane skeleton with a 1,7 - disubstituted phenyl), are mainly found in the family of Zingiberaceae. We have isolated two new diphenyl heptanes compounds (Fig. 1, the purity >96 %), 2,3- dihydro-2 - (4′ - hydroxy-phenylethyl) - 6 - [(3″,4″ - dihydroxy-5“ - methoxy) phenyl] -4 - pyrone (CG-A) and 4 - dihydro-2 - (4’ - hydroxy-phenylmethyl) -6 - [(3”,4″ - dihydroxy-5″ - methoxyphenyl) methylene]-pyran-3, 5 - dione (CG-B) from the dried fruits of *A. tsaoko* and demonstrated that they possess excellent antioxidant activities [2]. It is well known that the neuroprotective effect and anti-inflammatory action have a good correlation with the antioxidant activity [4, 5]. Therefore, in order to find the neuroprotective potential of CG-A and CG-B, an investigation was undertaken with the aim of evaluating the protective effects of CG-A and CG-B against H_2O_2-induced oxidative injury in PC-12 cells, and their anti-inflammatory activity were also determined.

Materials and Methods

PC-12 Cell Treatment and Morphology Observation The effect of H_2O_2 treatment on the proliferation of PC-12 cells was measured by MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide) assay and cell

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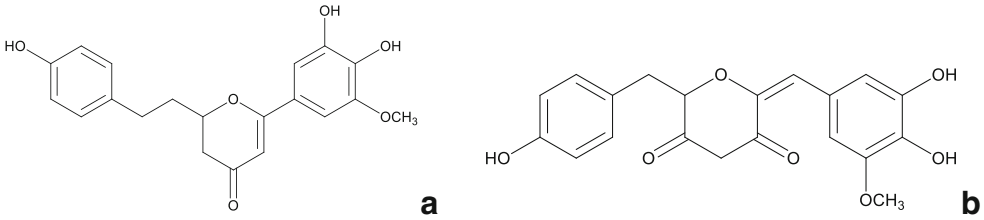

Fig. 1 Chemical structures of (**a**) CG-A and (**b**) CG-B

morphology [6, 7]. Dried samples were dissolved in dimethyl sulfoxide (DMSO) and diluted with culture medium into different concentrations. Moreover, the final concentration of DMSO in the culture medium was less than 0.1 % (*v*/v) to avoid solvent toxicity.

Nitric Oxide Assay The cells were pre-incubated in medium with various concentrations of compounds for 1 h, and stimulated with LPS (final concentration 1 μg/mL) at 37 °C for 24 h. The nitrite, accumulated in the culture medium, was measured using the Griess reagent system [8].

Statistical Analysis All analyses were carried out in triplicates, and the data expressed as the mean ± standard deviation. Statistical analysis was performed using SPSS software package by one-way analysis of variance. Statistical significance was considered when value of $p < 0.05$, and $p < 0.01$ indicated highly significant.

Results and Discussion

PC-12 Cell Apoptosis Induced by H_2O_2 Figure 2 displays the cell viability and the morphological evaluation of PC-12 cells after H_2O_2 treatment, showing that H_2O_2 dose-dependently decreased PC-12 cell viability (Fig. 2a). When PC-12 cells were treated with 1000 μmol/L H_2O_2 for 4 h, the cell viability was only 39.82 ± 3.50 %, suggesting that the concentration of 1000 μmol/L was not the best choice, because a good cell damage model was the cell viability of about 50 % [6]. When the treatment time was 8 h, the viability of cells treated with 800 μmol/L H_2O_2 still reached above 60 %, indicating that the treatment time could not be less than 8 h. When PC-12 cells were treated with 200 and 400 μmol/L H_2O_2 for 24 h, the cells maintained typical morphology, and cell viability was 75.32 ± 2.48 and 62.78 ± 2.95 %, respectively. There is little cell morphological change in low H_2O_2 concentration (less than 200 μmol/L), along with increasing doses of H_2O_2, the cell morphology changed significantly (Fig. 2b-f). The increasing H_2O_2 in

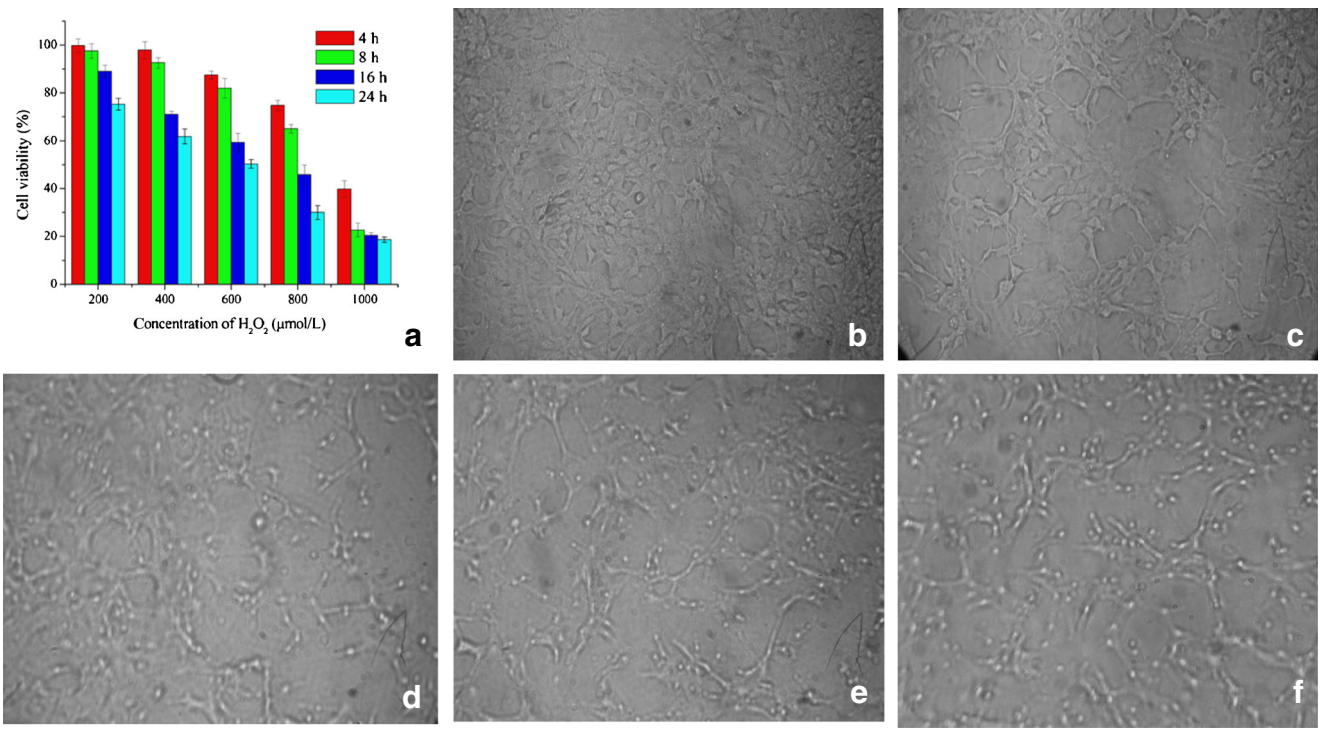

Fig. 2 Effect of H_2O_2 treatment on PC-12 cells. **a** The variation of cell viability after H_2O_2 treatment; effect of different H_2O_2 concentrations **b** 0 μmol/L, **c** 200 μmol/L, **d** 400 μmol/L, **e** 600 μmol/L, **f** 800 μmol/L on cytomorphology. Time: 24 h

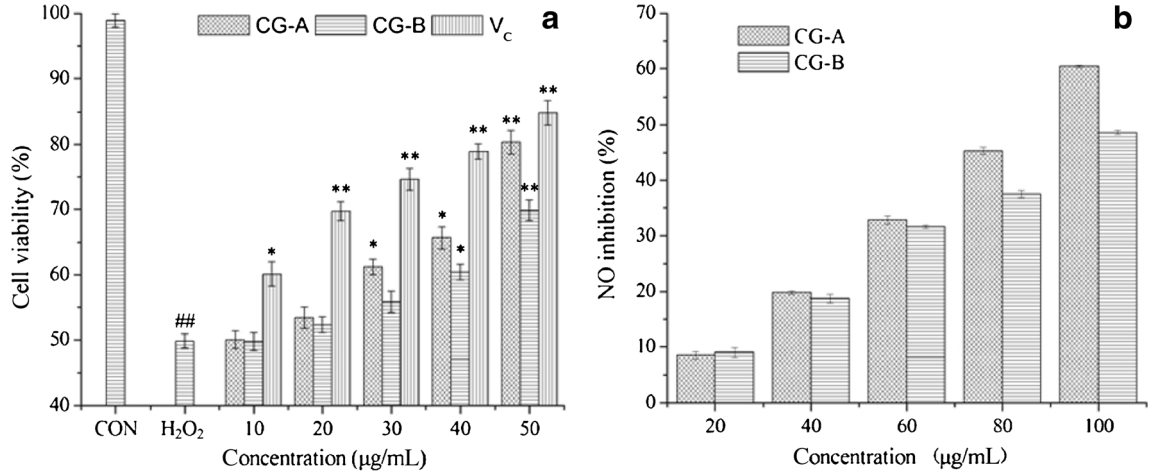

Fig. 3 The effect of CG-A and CG-B on H_2O_2-induced PC-12 cell viability decrease (**a**) and inhibitory effect on NO production in LPS-induced RAW 264.7 cells (**b**). $^{\#\#}$ $p < 0.01$ compared with control; $^{*}p < 0.05$, $^{**}p < 0.01$ compared with H_2O_2 model

the supernatant would have an impact on the subsequent detection, 600 μmol/L of H_2O_2 treating for 24 h was determined to be the most appropriate concentration which was used in the model groups.

Protective Effects of CG-A and CG-B against H_2O_2-Induced Oxidative Injury in PC-12 Cells The PC-12 cells were pretreated with different concentrations of CG-A and CG-B, and then 600 μmol/L of H_2O_2 was added. Vitamin C (Vc) was used as a reference compound. The decrease of cell viability induced by H_2O_2 was suppressed in a dose-dependent manner, indicating CG-A and CG-B effectively protected PC-12 cells from H_2O_2-induced damage (Fig. 3a). At the concentration of 50 μg/mL, the PC-12 cells treated with CG-A (80.34 ± 1.78 %) had a significant improvement in cell growth, which was very close to Vc (84.80 ± 1.86 %) at the same concentration. The cell viability reached up to 69.82 ± 1.57 % when cells treated with CG-B (50 μg/mL), indicating that its protective effect was relatively weaker than that of CG-A.

Effect of CG-A and CG-B on NO Production in RAW264.7 Macrophages The anti-inflammatory activity of CG-A and CG-B was evaluated in RAW 264.7. We first performed cytotoxicity assay to determine the appropriate concentration of these two compounds that would not affect the cell viability. Interestingly, RAW 264.7 cells were found to exhibit above 94.73 % viability up to 100 μg/mL concentration of CG-A and CG-B (data not shown).

We analyzed the effect of CG-A and CG-B on NO inhibition, a measure of anti-inflammatory response, in LPS-stimulated RAW 264.7 cells. CG-A and CG-B dose-dependently inhibited NO production with inhibition percentage values of 60.46 ± 0.23 and 48.62 ± 0.38 % at the concentration of 100 μg/mL, indicating CG-A and CG-B have evident anti-inflammatory activity at 20–100 μg/mL (Fig. 3b).

Conclusion

CG-A and CG-B could protect the PC-12 cells against H_2O_2-induced injury by reversing the H_2O_2 induced cells viability loss. CG-A displayed a higher activity in improving cell viability than CG-B, and its protective effect was close to that of V_C (50 μg/mL). In addition, these two new compounds both exhibited a certain anti-inflammatory activity in a dose-dependent manner. These findings indicated that CG-A and CG-B may have potential value in the protection of xenobiotics agents-induced nerve injury and possess significant anti-inflammatory activity for the possible application in the future.

Compliance with Ethical Standards

Conflict of Interest The authors report no conflicts of interest.

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