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Neurotonic Thai plants reduce reactive oxygen species production in SH-SY5Y neuroblastoma cells

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Abstract

Oxidative stress is considered an important causative factor in several neurodegenerative diseases. This study was aimed to determine the antioxidant properties of seven neurotonic Thai plants with possible neuroprotective effect in humans. Antioxidant power was evaluated by ferric reduction, lipid peroxidation inhibition and intracellular reactive oxygen species (ROS) suppression. The results showed that the extracts from *Terminalia bellirica* (Gaertn.) Roxb. and *Albizia procera* (Roxb.) Benth. could act as ferric reducing agents, whereas those of *Cassia fistula* L. and *Stephania suberosa* Forman seemed to be potent inhibitors of lipid peroxidation. These plant extracts could also effectively suppress the formation of intracellular ROS in differentiated SH-SY5Y neuroblastoma cells. In the conclusion, most of the selected plants demonstrated strong antioxidant activity by acting as metal reducing agents, lipid peroxidation inhibitors, and/or intracellular ROS suppressants. This study provides the potential mechanisms of Thai neurotonic plants as neuroprotective agents which could be beneficial in the prevention or delay of neurodegenerative processes.

Keywords: Antioxidant, reactive oxygen species, neuroprotection, Thai plant, herbal medicine

Introduction

There is substantial evidence showing the relationships of ROS production and the induction of cell death and pathogenesis of neurological disorders including Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (1). Thus, there are large numbers of experiments published showing the neuroprotective effect of antioxidants including vitamins and natural substances in *in vitro* and in animal models for neurodegeneration (2). Although the efficacy of these antioxidants for treatment of neurodegenerative disorders is still unclear, their potentials as alternative therapy or nutritional supplement to slow down the progression of those neuronal diseases have received much attention. In Thailand, there exist a number of herbal medicines that are believed to possess rejuvenating and neurotonic effects. For some of these plants, their beneficial effects for Alzheimer's disease by inhibiting acetylcholinesterase (AChE) activity were previously demonstrated (3). This study was aimed to test the ability of these plant extracts to suppress the oxidative stress in test tube and cell culture models.

Methods

Preparation of plant extracts

The specific parts of plants were collected, cut into small pieces and dried in a hot-air oven at 50 ^{o}C. The dried plant materials were coarsely powdered and macerated with 95% ethanol for 3 days. The extracts were filtrated, dried under reduced pressure, and kept at -20 °C until use.

Cell culture preparation

Human neuroblastoma SH-SY5Y cells were grown in DMEM/Ham's F-12 containing 10% fetal bovine serum and 1% penicillin–streptomycin. Cells were maintained at 37 °C in a CO_2 incubator containing 5% CO_2. The medium was then changed to DMEM supplemented 1% FBS and 10 μM retinoic acid and the cells were allowed to differentiate for 6 days

Lipid peroxidation determination

Each plant extract was added to the brain homogenate before induction of lipid peroxidation by 400 μM $FeCl_2$ and 200 μM ascorbic acid. TBAR solution (10% trichloroacetic acid, 7% thiobarbituric acid, and 4% HCl final) was added to the mixtures, which were then heated to 95°C for 1 h. After spinning, the clear supernatant was read out on a plate reader at 532 nm.

Ferric reducing antioxidant power (FRAP assay)

The FRAP reagent was freshly prepared by mixing 3 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ (10:1:1) together. To be tested, plant extracts were added to FRAP reagent. The absorbance was read out at 593 nm after 1 h of reaction.

Determination of reactive oxygen species (ROS)

A fluorescent DCFH-DA (10 μM) probe were added to the medium and incubated at 37°C for 30 min. The differentiated SH-SY5Y cells were incubated with the extract for 30 min before adding the free radical generator APPH. The fluorescent product 2',7'-dichlorofluorescein (DCF) was monitored spectrofluorometrically (Ex 485 nm and Em 530 nM).

Results

Our result showed the increasing amount of Fe^{2+} ion in the presence of increasing concentrations of all plant extracts in a dose-dependent manner (Figure 1). The extract from *T. bellirica* exhibited the highest reducing activity, followed by *A. procera*, *C. rotundus*, *C. fistula*, *T. divaricata*, *S. suberosa*, and *B. superba*. All selected neurotonic plant extracts also inhibited the lipid peroxidation reaction of brain homogenate in a dose-dependent manner. The IC_{50} values of lipid peroxidation inhibitory activity of all plant extracts were calculated and are shown in table 1. The production of ROS inside SH-SY5Y cells was decreased after incubating with the tested plant extracts in a dose-dependent manner (Figure 2). The decrease in the ROS level was not the result of the decrease of cultured cell numbers because the extracts at all tested concentrations did not have an effect on the SH-SY5Y cell viability (data not shown).

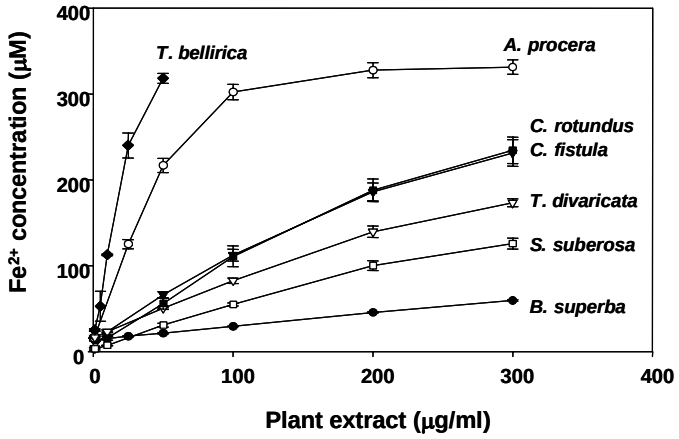

Figure 1 Ferric ion reducing activity of plant extracts. The results are mean±SEM.

Table 1 List of selected neurotonic plants and their lipid peroxidation inhibitory activities

Plant	Part	IC_{50} (μg/ml)	95% confidence interval (CI)
Stephania suberosa Forman	Rhizome	5.93	3.50-8.32
Cassia fistula L.	Root	6.47	4.15-10.09
Albizia procera (Roxb.) Benth.	Root	29.32	16.23-52.98
Tabernaemontana divaricata (L.) R. Br. ex Roem. & Schult.	Tuber	63.86	41.37-98.57
Terminalia bellirica (Gaertn.) Roxb.	Stem bark	161.7	61.27-426.9
Cyperus rotundus L.	Tuber	329.5	84.51-1285
Butea superba Roxb.	Fruit	902.9	173.2-4707

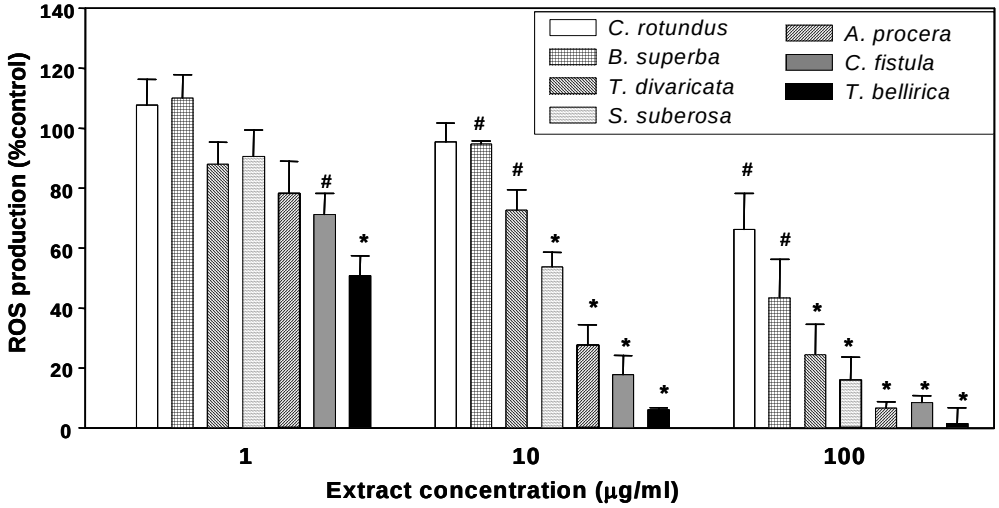

Figure 2 Effect of plant extracts on intracellular ROS production. The results are mean±SEM. (# p-value ≤ 0.05, * p-value ≤ 0.005)

Discussion

The extracellular Fe^{2+} was found to protect the intracellular space from H_2O_2, probably by initiating the Fenton reaction outside the cell (4). The increase in the Fe^{3+}/Fe^{2+} ratio was demonstrated as iron-induced oxidative stress in the blood sample of patients compared to healthy controls (5). These neurotonic plants could potentially prevent neuronal cells from the extracellular oxidative stress by suppressing the Fe^{3+}/Fe^{2+} ratio. The lipid peroxidation inhibitory effect of most extracts was proportional to their metal reducing activity (correlation analysis not shown). Metal reduction occurring in an aqueous compartment might consequently lead to the oxidation suppression of cellular lipid components. The ferric reducing activities of some of these plants seemed to be correlated with their intracellular ROS lowering effects. However, the metal-reduction ability did not seem to be the only mechanism of action for this purpose, the lipid peroxidation inhibitory effect was perhaps involved with the intracellular ROS decrement.

Taken together, some of these selected neurotonic Thai plants exhibit strong antioxidant activities which could be beneficial as neuroprotective agents in patients with certain neurodegenerative disorders or as supplement to prevent naturally degeneration of neuronal cells in risk group people.

Acknowledgements

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References

1. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44-84.
2. Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiol Aging 2002; 23:719-35.
3. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. J Ethnopharmacol 2003; 89: 261-4.
4. Hempel SL, Buettner GR, Wessels DA, Galvan GM, O'Malley YQ. Extracellular iron (II) can protect cells from hydrogen peroxide. Arch Biochem Biophys 1996; 330: 401-8.
5. Selvi R, Angayarkanni N, Bharathselvi M, Sivaramakrishna R, Anisha T, Jyotirmoy B, et al. Increase in Fe^{3+}/Fe^{2+} ratio and iron-induced oxidative stress in Eales disease and presence of ferrous iron in circulating transferrin. Curr Eye Res 2007; 32: 677-83.