

University of Macau

Abstract

**Protective effects of, 2-methoxy-6-acetyl-7-methyljuglone, a naphthoquinone in
Polygonum cuspidatum (Hu-Zhang), against PC12 apoptosis induced by *tert*-
butyl hydroperoxide**

By Lin Zhuqing

Thesis Supervisor: Prof. Wang Yitao

Dr. Maggie Pui Man Hoi

Chinese Medicinal Science

With ageing populations in most developed countries, the problem of neurodegenerative diseases presents a big challenge and potential economic burden. It is postulated that oxidative stress might induce excessive apoptosis of neuronal cells and cause aged-related neurodegeneration diseases. Here we used *tert*-butyl hydroperoxide (*t*-BHP), a commonly used oxidative agent, to induce lethal injury in rat pheochromocytoma PC12 cells as *in vitro* model for neurodegenerative diseases. The anti-oxidizing activities of several single compounds isolated from the dried rhizome of *Polygonum cuspidatum* (Chinese named Hu-Zhang) were explored.

Interestingly, the potent anti-oxidative property of 2-methoxy-6-acetyl-7-methyljuglone (MAM), was reported for the first time in the present study. Pretreatments (24 h) of MAM (0.5, 1, 2.5, 5 μ M) significantly attenuated PC12 cell death induced by *t*-BHP. MAM were able to reduce cell death induced by *t*-BHP-oxidative stress at much lower concentrations than resveratrol and lipoid acid, indicating that the protective effect of MAM was much stronger than those of resveratrol and lipoid acid, with cell viability measured by both MTT and LDH assays.

The protective effects produced by MAM was thought to be through the inhibition of the apoptotic pathways, evidenced by the reduction of the formation of apoptotic bodies inside the cell induced by *t*-BHP, which was visualized with DNA staining using Hoechst 33342 fluorescent dye. The protective effects of MAM on *t*-BHP-induced apoptotic cell death was also investigated by TUNEL labeling assay, which measures the level of DNA fragmentation and is a commonly accepted marker of apoptosis. It was shown that MAM could also reduced DNA fragmentation, providing additional evidence of the anti-apoptotic property of MAM.

The underlying mechanisms were further studied and the results suggested that MAM protected PC12 cells against oxidative damage through the inhibition of the MAPK pathways ERK 1/2, JNK and p38 MAPK. In conclusion, MAM is a potential anti-oxidative and anti-apoptotic agent useful for the prevention and the treatment of age-related neurodegenerative diseases.

Keywords:

2-methoxy-6-acetyl-7-methyljuglone; MAM; anti-oxidative; anti-apoptosis; *tert*-butyl hydroperoxide; neurodegenerative; MAPK.