Neurite outgrowth stimulatory effects of culinary-medicinal mushrooms and their toxicity assessment using differentiating Neuro-2a and embryonic fibroblast BALB/3T3

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Abstract

Background: Mushrooms are not only regarded as gourmet cuisine but also as therapeutic agent to promote cognition health. However, little toxicological information is available regarding their safety. Therefore, the aim of this study was to screen selected ethno-pharmacologically important mushrooms for stimulatory effects on neurite outgrowth and to test for any cytotoxicity.

Methods: The stimulatory effect of mushrooms on neurite outgrowth was assessed in differentiating mouse neuroblastoma (N2a) cells. Neurite length was measured using Image-Pro Insight processor system. Neuritogenesis activity was further validated by fluorescence immunocytochemical staining of neurofilaments. In vitro cytotoxicity was investigated by using mouse embryonic fibroblast (BALB/3T3) and N2a cells for any embryo- and neuro-toxic effects, respectively.

Results: Aqueous extracts of Ganoderma lucidum, Lignosus rhinoceros, Pleurotus giganteus and Grifola frondosa; as well as an ethanol extract of Cordyceps militaris significantly (p < 0.05) promoted the neurite outgrowth in N2a cells by 38.4 ± 2.2%, 38.1 ± 2.6%, 33.4 ± 4.6%, 33.7 ± 1.5%, and 35.8 ± 3.4%, respectively. The IC50 values obtained from tetrazolium (MTT), neutral red uptake (NRU) and lactate dehydrogenase (LDH) release assays showed no toxic effects following 24 h exposure of N2a and 3T3 cells to mushroom extracts.

Conclusion: Our results indicate that G. lucidum, L. rhinoceros, P. giganteus, G. frondosa and C. militaris may be developed as safe and healthy dietary supplements for brain and cognitive health.

Keywords: Culinary-medicinal mushrooms, Neurite outgrowth, Cytotoxicity, Mouse neuroblastoma N2a cell, Mouse 3T3 embryonic fibroblast, Neurofilament

Background

Neurite outgrowth is an important event in neuronal path finding and the establishment of synaptic connections during development [1,2]. It is also essential in neuronal plasticity, neuronal regeneration after injury [3,4] and neurodegenerative conditions such as Alzheimer's and Parkinson's diseases [5]. Therefore, treatments aiming at promoting neurite outgrowth and preserving the neurite network and synaptic connections are needed.

The potential use of culinary-medicinal mushrooms in neurodegenerative diseases is being explored [6]. On-going research in our laboratory shows that Hericium erinaceus (Bull.: Fr) Pers. (monkey's head mushroom, lion's mane mushroom and Yamabushitake) [7], Lignosus rhinoceros (Cooke) Ryvarden (tiger milk mushroom) [8,9], and Pleurotus giganteus (Berk.) Karunarathna & K.D. Hyde (morning glory mushroom, cow's stomach mushroom) [10] exhibit neurite outgrowth stimulatory effects in NG108-15 and PC12 cell lines. This observation raised a question with respect to the neurodevelopmental effects,

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