# EFFECT OF EXTRACTS FROM *HERICIUM ERINACEUS* SOLID-STATE FERMENTED CEREALS ON NERVE CELLS GROWTH

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## Abstract

Nerve growth factor (NGF) is one of neurotrophic factors, which is responsible for neuronal development and prevents of neurodegeneration. *Hericium erinaceums* (*H. erinaceus*) can produce biological metabolites such as erinacines and hericenones, which can NGF production, and increase the neurite outgrowth. This study was to produce 28-days *H. erinaceus* solid-state fermented cereals (wheat, soybean, barley, brown rice, oat), and to feed the rat pheocromocytoma PC12 cell with ethanol extracts from the different fermented cereals. The effect of different concentration (1, 10 and 100 $\mu$g/mL) ethanol extracts from different 0 and 28-days fermented cereals toxicity on cultured PC12 cells were no significant cytotoxicity. The NGF contents and neurite were significantly increased by different 10 $\mu$g/mL 28-days fermented cereals and the maximum NGF secreted into the 3 mL medium was significant different with blank after PC12 cell 72 h cultivation by ELISA measurement.

## Introduction

*Hericium erinaceus* is a mushroom has been known as Chinese medicine or food in China and Japan without harmful effects. Recently, the *Hericium erinaceus* be cultivated by solid-state fermented, submerged fermentation or wood. Hericenone and erinacine have been isolated from the fruiting body and mycelia of *H. erinaceum*, respectively, and hericenone C-H and erinacine A-I have been shown to stimulate NGF synthesis in cultured astrocytes. And the different type of erinacine can increase nerve cell neurite outgrowth. The neuroprotective effects of *H. erinaceum* in neurodegenerative diseases, including dementia, motor dysfunction, neurasthenia, Alzheimer's disease and Parkinson's disease. The objective of this study was to produce 28-days *H. erinaceus* solid-state fermented cereals (wheat, soybean, barley, brown rice, oat), and to feed the rat pheocromocytoma PC12 cell with ethanol extracts from the different fermented cereals. Observe the growth of neurite and measure the content of NGF.

## Materials and method

*Hericium erinaceus* (BCRC 36470)
↓
shake-cultured for 7 days (25℃ 、150 rpm)
↓
inoculate *Hericium erinaceus* to different solid-state (w.b. 50%)
(soybean, wheat, barley, brown rice, oat)
↓
inoculated bags culture at 25℃ 28-day
↓
85% ethanol extraction by ultrasonic bath 2h
↓
centrifuged and sampled supernatants
↓
evaporate to dryness → extraction rate
↓
MTT assay
(concentration 1, 10, 100 $\mu$g/mL)
↓
10 $\mu$g/mL ethanol extracts on PC12 cell at 72 h cultivation
↓
observe the neurite (inverted microscope) | measure NGF (ELISA reader)

## Results and discussion

Fresh fruiting bodies, *H. erinaceus* fermented and unfermented cereal products dry powder was extracted with 85% ethanol for 2 h, and fruiting bodies ethanol extraction ratio (21.76%), *H. erinaceus* fermented soybean (16.66%)(Table 1). By MTT assay, the different concentration (1, 10 and 100 $\mu$g/mL) ethanol extracts from different 0 and 28-days fermented cereals toxicity on cultured PC12 cells were no significant cytotoxicity, and 28-days fermented cereals extracts increased the viability (Table 2). The synapse growth instance of adding 10 $\mu$g/mL different ethanol extracts from *H. erinaceus* 28-days solid-state fermented with PC12 cell 72 h cultivation (Fig. 1). The neurite were significantly increased by fermented soybean (b), fermented brown rice (d), fruiting body (g). The NGF level in the culture medium 3 mL was also significant increased by the ethanol extracts of different *H. erinaceus* 28-days solid-state fermented (Fig. 2), and the NGF content in 3 mL medium were 557.4 pg by ethanol extract of fermented brown rice.

Table 1. Extraction ratio of different *H. erinaceus* fermented and unfermented cereal products extract by 85% ethanol

| | Extraction ratio (%) | |
|---|---|---|
| | Unfermented | Fermented |
| Wheat | $3.90 \pm 0.65^{bc}$ | $12.44 \pm 0.31^{bc*}$ |
| Brown rice | $2.20 \pm 0.21^{a}$ | $8.52 \pm 0.22^{a*}$ |
| Barley | $4.45 \pm 0.26^{c}$ | $10.65 \pm 0.92^{ab*}$ |
| Oat | $3.41 \pm 0.12^{b}$ | $13.08 \pm 0.47^{c*}$ |
| Soybean | $7.08 \pm 0.17^{d}$ | $16.66 \pm 0.84^{d*}$ |
| Fruiting body | | $21.76 \pm 2.72^{e}$ |

[a–d] Means in unfermented group with different letters are significantly different ($p < 0.05$).
[a–e] Means in fermented group with different letters are significantly different ($p < 0.05$).
* Mean in fermented with * are significantly different from unfermented ($p < 0.05$).

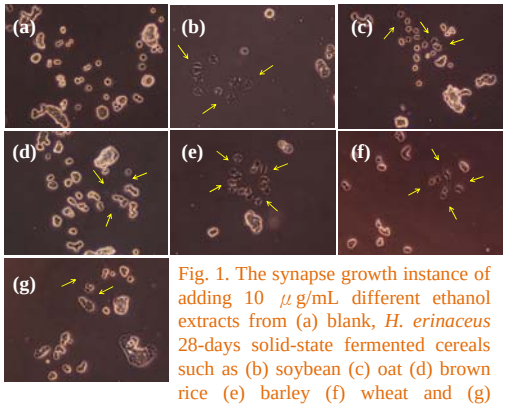


Fig. 1. The synapse growth instance of adding 10 $\mu$g/mL different ethanol extracts from (a) blank, *H. erinaceus* 28-days solid-state fermented cereals such as (b) soybean (c) oat (d) brown rice (e) barley (f) wheat and (g) fruiting body for PC12 cells after 72 h cultivation.

Table 2. Effect of adding 1、10、100 $\mu$g/mL ethanol extracts from different unfermented cereals and *H. erinaceus* fermented cereals on PC12 cell viability at 24 h cultivation

| | Cell viability (%) | | |
|---|---|---|---|
| | Concentration ($\mu g/mL$) | | |
| | 1 | 10 | 100 |
| Cereals | | | |
| Brown rice | $90.63 \pm 0.30^{a}$ | $101.36 \pm 0.36^{a}$ | $101.36 \pm 0.49^{a}$ |
| Oat | $100.00 \pm 0.41^{a}$ | $119.32 \pm 0.27^{b}$ | $102.37 \pm 0.24^{a}$ |
| Soybean | $103.39 \pm 0.46^{a}$ | $105.31 \pm 0.31^{a}$ | $100.79 \pm 0.23^{a}$ |
| Wheat | $101.02 \pm 0.86^{a}$ | $102.03 \pm 0.57^{a}$ | $109.15 \pm 0.06^{a}$ |
| Barley | $94.92 \pm 0.14^{a}$ | $98.64 \pm 0.48^{a}$ | $97.63 \pm 0.35^{a}$ |
| Fermented cereals | | | |
| Brown rice | $103.39 \pm 0.39^{a}$ | $107.46 \pm 0.41^{b}$ | $98.98 \pm 0.18^{ab}$ |
| Oat | $108.14 \pm 0.51^{ab}$ | $129.15 \pm 0.22^{b*}$ | $119.32 \pm 0.27^{b*}$ |
| Soybean | $109.83 \pm 0.60^{a}$ | $134.23 \pm 0.41^{b*}$ | $135.25 \pm 0.50^{b*}$ |
| Wheat | $127.46 \pm 0.72^{a*}$ | $122.37 \pm 0.71^{b*}$ | $128.81 \pm 1.26^{a*}$ |
| Fruiting body | $118.64 \pm 0.48^{a}$ | $117.97 \pm 0.52^{a}$ | $118.64 \pm 0.48^{a}$ |

[a-b] Means in 1-100 $\mu$g/mL groups with different letters are significantly different ($p < 0.05$).
* Mean in fermented cereals with * are significantly different from cereal ($p < 0.05$).

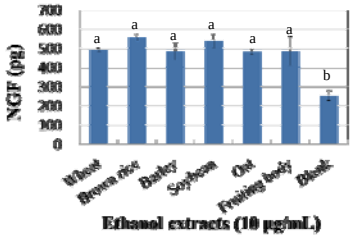


Fig. 2. Effect of adding 10 $\mu$g/mL ethanol extracts from *H. erinaceus* fruiting body and *H. erinaceus* 28-days solid-state fermented cereals on PC12 cells producing NGF after 72 h cultivation.

Data are expressed as mean ± S.D. (n = 3).
[a–b] Means in ethanol extracts 10 $\mu$g/mL group with different letters are significantly different ($p < 0.05$).

## Conclusions

It is no significant cytotoxicity on cultured PC12 cells in different concentration (1, 10 and 100 $\mu$g/mL) ethanol extracts from different 0 and 28-days fermented cereals. The 28-days fermented cereals extracts can increased the cell viability. The NGF contents and neurite were significantly increased by different 28-days fermented cereals and the maximum NGF secreted into the 3 mL medium by ethanol extract of fermented brown rice .