

**EVALUATION OF NEURITE OUTGROWTH STIMULATION
AND NEUROPROTECTIVE ACTIVITIES OF *HERICIUM*
ERINACEUS EXTRACTS ON CELL LINE NG108-15**

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ABSTRACT

Neurotrophic factors play an indispensable role in the functionality and development of the nervous system. One of the well-studied neurotrophic factors is nerve growth factor (NGF), which is required for the growth and differentiation of dorsal root ganglion and sympathetic ganglion neurons. Studies have shown that the bioactive compounds - hericenones and erinacines of *Hericium erinaceus* (Bull.: Fr.) Pers., a medicinal mushroom widely used in Japan, Korea and China, can induce NGF synthesis in nerve cells. This temperate mushroom has been successfully domesticated in Malaysia and cultivated in lowland conditions. The aim of this study was to evaluate the synergistic interaction, if any, between locally grown *H.erinaceus* aqueous extract and commercially available NGF on neurite outgrowth stimulation activity in the neuroblastoma-glioma hybrid cell line NG108-15. The study also aimed to evaluate the neuroprotective effect of *H.erinaceus* aqueous extract in neuronal cells subjected to H₂O₂-induced oxidative stress. Aqueous extract of *H.erinaceus* was shown to be non-cytotoxic to NG108-15 cells and human lung fibroblast MRC-5. The combination of 10 ng/mL NGF with 1 µg/mL *H.erinaceus* aqueous extract gave the highest percentage increase of 60.6% neurite outgrowth compared to untreated neuronal cells. The effect of neurite outgrowth stimulation was enhanced in this combined mixture when compared to the concentration of extract or NGF applied individually. The *H.erinaceus* extract contained neuroactive compound which induced the secretion of extracellular NGF, thereby enhancing neurite outgrowth stimulation activity. The neuritic processes stained positive for neurofilament-200 antibody indicating the extensions in the differentiated cells were of neuronal origin. On the contrary, the *H.erinaceus* aqueous extract failed to protect NG108-15 cells against H₂O₂-induced oxidative stress. There was no significant improvement in the cellular viability of NG108-15 when pre-treated with *H.erinaceus* extract prior to being subjected to H₂O₂ or when the cells were co-treated with

H.erinaceus extract and H₂O₂. The neuroprotective effect of *H.erinaceus* aqueous extract was not observed even with extended hours of extract incubation in the pre-treatment mode. In conclusion, the aqueous extract of locally grown *H.erinaceus* was not cytotoxic and contained neuroactive compounds which induced the secretion of extracellular NGF. The combined treatment of *H.erinaceus* extract and suboptimal concentration of NGF showed additive response in the neurite outgrowth stimulation activity of NG108-15 cells. However, the neuroprotective activity was absent in the aqueous preparation of *H.erinaceus* used in this study.

ABSTRAK

Faktor-faktor neurotropik memainkan peranan yang penting dalam fungsi dan perkembangan sistem saraf. Salah satu faktor neurotropik yang dikaji dengan terperinci ialah faktor pertumbuhan saraf (nerve growth factor – NGF). NGF memainkan peranan yang penting dalam pertumbuhan dan proses pembezaan ganglion akar dorsal dan sel-sel ganglion simpati. Kajian-kajian menunjukkan bahawa kompaun-kompaun bioaktif dalam cendawan *Hericium erinaceus* (Bull.: Fr.) Pers. iaitu hericenone dan erinacine, boleh merangsangkan sintesis NGF dalam sel-sel saraf. *Hericium erinaceus* merupakan sejenis cendawan yang mempunyai nilai perubatan dan sering digunakan di negara-negara seperti Jepun, Korea dan Cina. Cendawan ini biasanya ditanam di kawasan yang beriklim sederhana tetapi kini ditanam di Malaysia yang beriklim tropika. Matlamat kajian ini adalah untuk menilai kesan tindakan bersama antara ekstrak akueus *H.erinaceus* dan NGF yang didapati di pasaran dalam aktiviti rangsangan pertumbuhan saraf terhadap sel hibrid neuroblastoma-glioma NG108-15. Kajian ini juga bermatlamat untuk menilai kesan perlindungan ekstrak akueus *H.erinaceus* terhadap sel-sel saraf yang didedahkan kepada pengoksidaan H₂O₂. Ekstrak akueus *H.erinaceus* didapati tidak bertoksik terhadap sel NG108-15 dan sel fibroblast peparu manusia MRC-5. Kombinasi antara 10 ng/mL NGF dan 1 µg/mL ekstrak akueus *H.erinaceus* mencatatkan peningkatan peratusan yang tertinggi dalam pertumbuhan saraf, iaitu sebanyak 60.6% dibandingkan dengan sel saraf tanpa ekstrak (kawalan). Tambahan pula, kombinasi ekstrak cendawan dan NGF ini menghasilkan tindakan paduan yang lebih berkesan dalam aktiviti rangsangan pertumbuhan saraf daripada aplikasi ekstrak dan NGF secara berasingan. Ekstrak *H.erinaceus* mengandungi kompaun neuroaktif yang merangsangkan penghasilan NGF secara ekstraselular yang dapat mempertingkatkan lagi aktiviti rangsangan pertumbuhan saraf pada sel-sel saraf. Dalam teknik pewarnaan imunohistokimiawi, tonjolan-tonjolan pada sel saraf menunjukkan reaksi positif

terhadap pewarnaan antibodi neurofilamen-200. Ini mengesahkan bahawa tonjolan-tonjolan yang dilihat merupakan serabut saraf. Walaubagaimanapun, ekstrak akueus *H.erinaceus* gagal melindungi sel-sel NG108-15 daripada pengoksidaan H₂O₂. Jangka hayat sel NG108-15 yang terdedah kepada pengoksidaan H₂O₂ tidak dapat dipertingkatkan dengan pra-rawatan ekstrak akueus *H.erinaceus* mahupun rawatan gabungan ekstrak dan H₂O₂. Ekstrak akueus *H.erinaceus* tidak memberi kesan perlindungan yang baik walaupun tempoh rawatan ekstrak telah dipanjangkan dalam mod pra-rawatan. Kesimpulannya, ekstrak akueus *H.erinaceus* mengandungi kompaun neuroaktif yang meningkatkan aktiviti rangsangan pertumbuhan saraf pada sel NG108-15 melalui penghasilan NGF ekstraselular. Namun demikian, ekstrak akueus *H.erinaceus* yang diguna dalam kajian ini tidak menunjukkan kesan perlindungan terhadap sel-sel saraf yang didedahkan kepada pengoksidaan H₂O₂.

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TABLE OF CONTENTS

ABSTRACT.....	ii
ABSTRAK.....	iv
ACKNOWLEDGEMENTS.....	vi
TABLE OF CONTENTS.....	vii
LIST OF FIGURES.....	x
LIST OF TABLES.....	xii
LIST OF SYMBOLS & ABBREVIATIONS.....	xiii

CHAPTER 1

INTRODUCTION.....	1
1.1 Objectives.....	5

CHAPTER 2

LITERATURE REVIEW.....	6
2.1 Medicinal Mushrooms.....	6
2.1.1 <i>Hericium erinaceus</i> – The monkey head mushroom.....	8
2.1.2 Medicinal and therapeutic properties of <i>H.erinaceus</i>	11
2.2 Neurotrophic Factors.....	14
2.2.1 Nerve growth factor (NGF).....	18
2.2.2 Herbs and mushrooms with neurotrophic activity.....	24
2.2.3 Neurotrophic activity of <i>H.erinaceus</i>	27
2.2.4 Neurotrophic factors in combination.....	29
2.2.5 Enhancement of neuritogenic activity of neurotrophic factors by plants and herbs.....	31
2.3 Neuroprotection.....	33
2.3.1 Oxidative stress.....	33
2.3.2 Apoptosis.....	36
2.3.3 Neuroprotection by neurotrophic factors.....	38
2.3.4 Plants, herbs and mushrooms with neuroprotective activity.....	40

CHAPTER 3

MATERIALS & METHODS.....	43
3.1 Preparation of Aqueous Extract of <i>H.erinaceus</i>	43

3.2	Cell Culture.....	43
3.3	Assessment of Cytotoxic Activity in <i>H.erinaceus</i> Extract.....	44
3.4	Assessment of Neurite Outgrowth Stimulation in NG108-15 Cell Line.....	45
3.4.1	Quantification of neurite-bearing cells in culture.....	45
3.4.2	Measurement of extracellular NGF levels in cell culture.....	45
3.4.3	Immunofluorescence staining of neurofilament 200 kDa subunit.....	46
3.5	Assessment of Neuroprotective Activity of <i>H.erinaceus</i> Extract on NG108-15 Cell Line.....	47
3.5.1	Assessment of cellular viability using MTT assay.....	47
3.5.2	Assessment of cellular viability using trypan blue exclusion assay.....	48
3.5.3	Assessment of apoptosis using TUNEL assay.....	48
3.6	Statistical Analysis.....	49

CHAPTER 4

RESULTS	50
4.1 Assessment of Cytotoxic Activity of <i>H.erinaceus</i> Extract.....	50
4.1.1 Evaluation of optimum cell density for MTT Assay.....	50
4.1.2 Cytotoxic activity of <i>H.erinaceus</i> extract in NG108-15 and MRC-5 cell lines.....	52
4.2 Assessment of Neurite Outgrowth Stimulation in NG108-15 Cell Line.....	54
4.2.1 Quantification of neurite-bearing cells in culture.....	54
4.2.1.1 The effect of varying concentrations of NGF on neurite outgrowth stimulation.....	54
4.2.1.2 The effect of varying concentrations of <i>H.erinaceus</i> aqueous extract on neurite outgrowth stimulation.....	54
4.2.1.3 The effect of varying concentrations of <i>H.erinaceus</i> aqueous extract combined with 5, 10 and 20 ng/mL NGF on neurite outgrowth stimulation.....	56
4.2.1.4 Morphology of NG108-15 cells under different treatment conditions.	60
4.2.2 Measurement of extracellular NGF levels in cell culture.....	64
4.2.2.1 The extracellular NGF levels in NG108-15 cell line treated with varying concentrations of <i>H.erinaceus</i> aqueous extract.....	64
4.2.2.2 The extracellular NGF levels in NG108-15 cell line treated with varying concentrations of <i>H.erinaceus</i> aqueous extract combined with 10 ng/mL NGF.....	65

4.2.3	Immunofluorescence staining of neurofilament 200 kDa subunit.....	67
4.3	Assessment of Neuroprotective Activity of <i>H.erinaceus</i> Extract on NG108-15 Cell Line.....	69
4.3.1	Assessment of cellular viability using MTT assay.....	69
4.3.1.1	Evaluation of cytotoxic activity of H ₂ O ₂ in NG108-15 cells.....	69
4.3.1.2	Cellular viability of NG108-15 cells pre-treated with <i>H.erinaceus</i> extract prior to H ₂ O ₂ -induced oxidative stress.....	71
4.3.1.3	Cellular viability of NG108-15 cells co-treated with <i>H.erinaceus</i> extract and H ₂ O ₂	72
4.3.2	Assessment of cellular viability using trypan blue exclusion assay.....	73
4.3.2.1	Cellular viability of NG108-15 cells pre-treated with <i>H.erinaceus</i> extract prior to H ₂ O ₂ -induced oxidative stress.....	73
4.3.2.2	Cellular viability of NG108-15 cells co-treated with <i>H.erinaceus</i> extract and H ₂ O ₂	75
4.3.3	Assessment of apoptosis using TUNEL assay.....	76
4.3.3.1	Evaluation of apoptotic cells in NG108-15 cells pre-treated with <i>H.erinaceus</i> aqueous extract.....	76
CHAPTER 5		
DISCUSSION & CONCLUSION.....		
5.1	Cytotoxicity of <i>H.erinaceus</i> Aqueous Extract.....	79
5.2	The Neurite Outgrowth Stimulation Activity of <i>H.erinaceus</i> Aqueous Extract.....	81
5.3	The Neuroprotective Effect of <i>H.erinaceus</i> Aqueous Extract.....	90
5.4	Properties of <i>H.erinaceus</i> Aqueous Extract.....	96
5.5	Conclusion.....	100
REFERENCES.....		
APPENDIX.....		
Appendix A	– Cell Culture Techniques.....	121
Appendix B	– Buffers, Reagents & Solutions.....	126
Appendix C	– Analytical Techniques.....	129
Appendix D	– Data & Statistical Tables.....	130

LIST OF FIGURES

Figure 2.1	Fruiting bodies of <i>H.erinaceus</i>	10
Figure 2.2	Early morphological events in neurite outgrowth.....	16
Figure 2.3	The structures of Trk receptors and p75 neurotrophin receptor (p75 ^{NTR}).....	21
Figure 4.1	Relation between absorbance value and cell number in NG108-15 cells.....	51
Figure 4.2	Relation between absorbance value and cell number in MRC-5 cells.....	51
Figure 4.3	Cytotoxic effect of <i>H.erinaceus</i> extract <i>in vitro</i>	53
Figure 4.4	Percentage of neurite-bearing cells in NG108-15 cells treated with NGF.....	55
Figure 4.5	Percentage of neurite-bearing cells in NG108-15 cells treated with <i>H.erinaceus</i> aqueous extract.....	55
Figure 4.6	Percentage of neurite-bearing cells in NG108-15 cells treated with 5 ng/mL NGF and <i>H.erinaceus</i> aqueous extract.....	57
Figure 4.7	Percentage of neurite-bearing cells in NG108-15 cells treated with 10 ng/mL NGF and <i>H.erinaceus</i> aqueous extract.....	58
Figure 4.8	Percentage of neurite-bearing cells in NG108-15 cells treated with 20 ng/mL NGF and <i>H.erinaceus</i> aqueous extract.....	60
Figure 4.9	The prototypical slow-onset and rapid-onset neurite patterns observed in NG108-15 cells.....	62
Figure 4.10	Representative images showing the morphology of NG108-15 cells treated with NGF, <i>H.erinaceus</i> aqueous extract or both.....	63
Figure 4.11	The extracellular NGF levels in NG108-15 cells treated with <i>H.erinaceus</i> aqueous extract	64
Figure 4.12	The extracellular NGF levels in NG108-15 cells treated with <i>H.erinaceus</i> aqueous extract combined with 10 ng/mL NGF	67
Figure 4.13	Immunocytochemical staining of NG108-15 cells for neurofilament 200 kDa subunit.....	68

Figure 4.14	Cellular viability of NG108-15 cells pre-treated with <i>H.erinaceus</i> aqueous extract prior to H ₂ O ₂ -induced oxidative stress.....	72
Figure 4.15	Cellular viability of NG108-15 cells co-treated with <i>H.erinaceus</i> aqueous extract and H ₂ O ₂	73
Figure 4.16	Cellular viability of NG108-15 cells pre-treated with <i>H.erinaceus</i> aqueous extract prior to H ₂ O ₂ -induced oxidative stress.....	75
Figure 4.17	Cellular viability of NG108-15 cells co-treated with <i>H.erinaceus</i> aqueous extract and H ₂ O ₂	76
Figure 4.18	Morphology of apoptotic cells stained using TUNEL assay.....	78

LIST OF TABLES

Table 2.1	Major classes of neurotrophic factors	19
Table 4.1	IC ₅₀ values of <i>H.erinaceus</i> aqueous extract in MRC-5 and NG108-15 cell line.....	53
Table 4.2	Cellular viability of NG108-15 cells treated with varying concentrations of H ₂ O ₂ at different incubation period	70
Table 4.3	Evaluation of apoptotic cells in NG108-15 cells subjected to different treatments using TUNEL assay	78

LIST OF SYMBOLS & ABBREVIATIONS

±	Plus-minus
°C	degree Celsius
µg/mL	Microgram per millilitre
µM	Micromolar
µm	Micrometer
6-OHDA	6-hydroxydopamine
AP-1	Adaptor protein 1
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BSA	Bovine serum albumin
CAM	Complementary and Alternative Medicine
CaMK II	Ca ²⁺ /calmodulin-dependent kinase II
cAMP	Cyclic adenosine monophosphate
CNS	Central nervous system
CNTF	Ciliary neurotrophic factor
CO ₂	Carbon dioxide
CTE	Chronic traumatic encephalopathy
DAPI	4', 6-diamidino-2-phenylindole
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
DRG	Dorsal root ganglion
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinases
FBS	Foetal bovine serum
FeSO ₄	Iron (II) sulphate
FITC	Fluorescein isothiocyanate
g	Gram
g/L	Gram per litre
GAE	Gallic acid equivalents
GDNF	Glial derived neurotrophic factor
H ₂ O	Water

H ₂ O ₂	Hydrogen peroxide
HCl	Hydrochloric acid
HDL	High density lipoprotein
hr	Hour/Hours
HRP	Horseradish peroxidase
IC ₅₀	50% inhibitory concentration
ICAM-1	Intercellular adhesion molecule-1
IgG	Immunoglobulin G
IL-12	Interleukin-12
IL-1β	Interleukin-1 beta
IP ₃	Inositol 1,4,5-triphosphate
JNK	c-jun N-terminal kinase
KH ₂ PO ₄	Potassium hydrogen phosphate
L	Litre
LDL	Low density lipoprotein
M	Molarity
MAP-2	Microtubule-associated protein-2
MAPK	Mitogen activated protein kinase
MEK	Mitogen activated protein kinase kinase / MAPK kinase
mg/mL	Milligram per millilitre
min	Minute
mL	Millilitre
mM	Millimolar
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide
N	Normality
Na ₂ CO ₃	Sodium carbonate
Na ₂ HPO ₄	Sodium hydrogen phosphate
NaCl	Sodium chloride
NaH ₂ PO ₄	Sodium dihydrogen phosphate
NaHCO ₃	Sodium bicarbonate
NaOH	Sodium hydroxide
NF-κB	Nuclear factor-κB
ng/mL	Nanogram per millilitre

NGF	Nerve growth factor
nm	Nanometre
NMDA	<i>N</i> -methyl-D-aspartate
no.	Number
NOS	Nitric oxide synthase
NPY	Neuropeptide Y
NT-3	Neurotrophin-3
NT-4/5	Neurotrophin-4/5
NT-6	Neurotrophin-6
PBS	Phosphate buffered saline
pg/mL	Picogram per millilitre
PI-3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
psi	Pounds per square inch
ROS	Reactive oxygen species
rpm	Rotation per minute
SEM	Standard error of the mean
SP-1	Specificity protein 1
STAT-1	Signal transduction and transcription
SV2	Synaptic vesicle protein-2
TGF- β 1	Transforming growth factors β 1
TNF- α	Tumor necrosis factor-alpha
TUNEL	T erminal deoxynucleotidyl T ransferase B iotin-dUTP N ick E nd L abelling
U/mL	Units per millilitre
UV	Ultraviolet
v/v	Volume per volume
w/v	Weight per volume
β	Beta
γ	Gamma