

CHAPTER I

INTRODUCTION

Neurodegenerative diseases can be defined as hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. Alzheimer's disease (AD) is one of the major neurodegenerative diseases. According to a report from Alzheimer's Disease International (ADI), it is estimated that there are currently about 18 million people with AD worldwide. According to Alzheimer's Disease Foundation Malaysia (ADFM), approximately 50,000 Malaysians are currently diagnosed with the illness. The production of reactive oxygen species during oxidative stress is speculated to be pathologically important in neurodegenerative diseases. Degeneration of cholinergic neurons and concomitant impairment of cortical and hippocampal neurotransmission lead to cognitive and memory deficits (Schorderet, 1995). Therefore, the characterization of neurite formation, maturation and collapse/ resorption is an area of interest because these cellular processes are essential for the interconnection of neuronal cell bodies.

Choline supplementation (lecithins) and/ or acetylcholinesterase inhibitors (Tacrine) have been used to attenuate the cognitive and memory deficits. However, these agents have showed several side effects such as gastrointestinal troubles, hepatitis and reversible hepatotoxicity. The use of neurotrophin NGF (nerve growth factor) has been initiated to treat neurodegenerative diseases. However, NGF cannot pass through the blood-brain barrier. Therefore, it needs to be injected directly into the brain to be effective (Kawagishi *et al.*, 2002). If a substance can permeate the membrane and stimulates the NGF synthesis in brain, this may result in the repair of the damaged nervous functions.

Mushrooms, belonging to the kingdom fungi, are well-known for their medicinal and therapeutic values for centuries, since every culture has a written or oral tradition of using mushroom for their healing powers (Hobbs, 1995). There are over 1.5 million species of fungi on earth, but mushrooms only constitute 14,000 species (Hawksworth, 2001). However, the well investigated known species of mushrooms are still very low. Only 700 species are eaten as food and 50 species are poisonous (Halpern, 2007).

In recent years, studies on the medicinal values of the edible mushrooms have gained a great deal of interests from researchers, as there is demand for more natural remedies for life's ailments. Mushrooms are valuable health food - low in calories, high in vegetable proteins, chitin, iron, zinc, fiber, essential amino acids, vitamins and minerals. Besides that, mushrooms have been used as bioengineering resources in the development of food materials (functional foods) as well as starting materials in the production of drugs. For example, the hot water extracts from dried fruitbodies of *H. erinaceus* are used as health drink (Yang and Jong, 1989). It has been pickled in brewed wine to give a health drink (Mizuno, 1999).

Mushrooms possess many medicinal properties, pharmacological effects and physiological properties such as bioregulation, maintenance of homeostasis, regulation of biorhythm, prevention and improvement in cancer, cerebral stroke and heart diseases, decreasing blood cholesterol, antifungal, anti-inflammatory, antiviral, antibacterial and antiseptic, antidiabetic, serve as kidney and nerve tonic, hepatoprotective and sexual potentiator (Wasser and Weis,1999).

Hericium erinaceus, belonging to the Basidiomycetes class, is an edible mushroom occurring widely in Japan and China. These mushrooms grow on dead or dying wood. *Hericium erinaceus*, known as Yamabushitake (mountain hidden mushroom), Jokotake (drinker fungus), Usagitake (rabbit fungus) and Harisenbon

(balloon fish) in Japan; Houtou (monkey head mushroom) and Hedgehog mushroom in China and cauliflower mushroom (cendawan ‘bunga kobis’) in Malaysia. As a culinary delicacy, *H. erinaceus* is one of the few mushrooms imparting the flavor of lobster and shrimp when cooked.

Hericium erinaceus has served as traditional medicines in many regions. In China, it is prescribed for stomach disorders, ulcers and gastrointestinal ailments. In North American, native Americans used *H. erinaceus* as a styptic, applied as a dried powder to cuts and scratches to stop them from bleeding.

Some compounds have been successfully isolated from the fruiting bodies and mycelia of *H. erinaceus* which showed NGF stimulation. Hericenones isolated from the fruiting bodies of *H. erinaceus* have been shown to promote NGF synthesis (Kawagishi *et al.*, 1991). Erinacines isolated from mycelium of *H. erinaceus* have been identified as stimulators of nerve growth factor (NGF) synthesis (Kawagishi *et al.*, 1996; Shimbo *et al.*, 2005). Dilinoleoyl-phosphatidylethanolamine (DLPE) isolated from the fruiting bodies of *H. erinaceus* may reduce the risk of neurodegenerative diseases by reducing the endoplasmic reticulum (ER) stress (Nagai *et al.*, 2006).

The screening for neurite outgrowth activity by *H. erinaceus* in an *in vitro* model provides important preliminary data to select mushroom extracts for isolation purposes. The neural hybrid clone, NG108-15 cell line is most widely used as an *in vitro* model of neuronal differentiation because of its high proliferative activity and rapid elaboration of neurites (Smalheiser, 1991). The advantages of this bioassay is that it uses a continuous cell line, thus avoiding the need for dissection.

Hericium erinaceus is a temperate mushroom reported to fruit in cool temperature. Currently, it is successfully cultivated in Malaysia. The mushroom now grown in tropical climate, may have bioactive profiles different from temperate grown

H. erinacius. However, Wong *et al.* (2007, 2009) have shown that the cultivation temperature did not affect this. Both the ethanol and water extract of *H. erinaceus* grown locally displayed stimulation of the neurite outgrowth using an *in vitro* model. Further, antioxidant and antimicrobial activities have been reported (Wong *et al.*, 2009). It was reported that extracts of *H. erinaceus* enhanced nerve regeneration (Wong *et al.*, 2009; 2011). It was therefore of interest to identify the chemical constituents in the mushroom extract which may be responsible for stimulating neurite outgrowth.

Objectives of study

The objectives of the study were to:

- (a) evaluate the crude and fractionated extracts of *H. erinaceus* for their effects in stimulating the neurite outgrowth using the neural cell line NG 108-15.
- (b) identify the most active fraction.
- (c) identify the components present in the most active fraction .

CHAPTER II

LITERATURE REVIEW

2.1 MEDICINAL MUSHROOMS AND ITS USAGES

Fleshy mushrooms (members of the class basidiomycetes) have long been used for their medicinal and therapeutic values. The term 'medicinal mushroom' is now increasingly gaining worldwide recognition due to its value in the prevention and treatment of diseases. Furthermore, it can be easily obtained from the natural environment.

Medicinal properties of mushrooms have been widely studied. It was recorded that mushrooms can exert a number of beneficial physiological effects. *Auricularia auricula-judae* has been identified as a mushroom with reducing effect on the risk factors of cardiovascular diseases. It has been reported to lower down the total cholesterol and low density lipoprotein (LDL) level in hypercholesterolemic rats (Cheung, 1996; Chen *et al.*, 2008) and reduced blood platelet binding which will cause arterial thromboses (Fan *et al.*, 1989). *Cordyceps sinensis*, *Grifola frondosa* and *Lentinus edodes* were effective in reduce the triglyceride level (Francia *et al.*, 1999).

Besides that, *Ganoderma lucidum* and *G. frondosa* reduced blood pressure in spontaneously hypertensive rats (Kabir *et al.*, 1988; 1989). There were few species of mushrooms which possessed hypoglycemic action such as *Agaricus bisporus* (Swanston-Flatt *et al.*, 1989), *Agrocybe aegerita* (Kiho *et al.*, 1994), *C. sinensis* (Kiho *et al.*, 1996) and *G. frondosa* (Kubo *et al.*, 1994). Ergosterol, an antitumor compound which has been isolated out from the mushroom *Agaricus blazei*, reduced the tumor growth with no side effects (Takaku *et al.*, 2001).

Mushrooms are also good candidates for promoting neuronal differentiation and survival. For example, polysaccharides in aqueous extract of *G. lucidum* induce

neuronal differentiation of rat pheochromocytoma PC12 cells and prevent NGF-dependent PC12 neurons from undergoing apoptosis (Cheung *et al.*, 2000; Silva, 2004). Cyathane diterpenoid, termed scabronines, have been isolated from *Sarcodon scabrosus*, a bitter mushroom (Ohta *et al.*, 1998), and have been reported to stimulate neurite outgrowth in rat pheochromocytoma cells (PC12) cultivated with the conditioned medium of human astrocytoma cells (1221N1) (Obara *et al.*, 1999). Water extract of *Tremella fuciformis* induced neurite outgrowth in PC12 cells and improved the memory deficit in rats by increasing the central cholinergic activity (Kim *et al.*, 2007).

Wu Ri, a famous Chinese physician from the Ming Dynasty (A.D. 1368- 1644), claimed that *L. edodes* contain the ability to increase energy, cure colds, eliminate worms and improve blood circulation. In “Shen Nong Ben Cao Jing”, *G. lucidum*, is ranked under the superior medicine reported to be effective for multiple diseases and mostly responsible for maintaining and restoring the body balance with no unfavorable side effects. In the Taoist tradition, *G. lucidum* is said to enhance spiritual receptivity and it was used by monks to calm the spirit and mind. It is also considered a symbol of feminine sexuality as it refines the beauty and complexion.

2.2 *HERICIUM ERINACEUS*

2.2.1 Origin

Hericium erinaceus (Bull.: Fr.) Pers. (Figure 2.1), a member of the basidiomycetous fungus, is well known as a traditional medicine or food in Japan and China. In Japan, *H. erinaceus* is called Yamabushitake because it resembles the ornamental cloth worn by Yamabushi. It is also called Jokotake (drinker fungus), Usagitake (rabbit fungus), Harisenbon (balloon fish) due to its shape.



Figure 2.1: *Hericium erinaceus* (Bull.: Fr.) Pers.

This mushroom is called Houtou (monkey head mushroom) in China due to the close resemblance of fruiting body to the head of a baby monkey. It also known as Hedgehog mushroom according to its shape. A Chinese traditional drug prepared by drying this mushroom is also called Houtou. The hot water extracts from dried fruitbodies are used as health drink (Yang and Jong, 1989). It can be pickled in brewed wine to give a health drink (Mizuno, 1999).

Hericium erinaceus is a wood destroying fungus and grows in standing and decayed broadleaf trees such as oak, beech, and walnut. The cultivation of *H. erinaceus*

has been established using artificial logs made with agricultural residues in either bottles or polypropylene bags (Mizuno, 1999; Chang and Miles, 2004).

2.2.2 Classification

Kingdom	:	Fungi
Phylum	:	Basidiomycota
Class	:	Basidiomycetes
Order	:	Russulales
Family	:	Hericiaceae
Genus	:	<i>Hericium</i>
Species	:	<i>erinaceus</i>

2.2.3 Medicinal properties, nutritional and bioactive components derived from *Hericium erinaceus*

Medicinal properties of *H. erinaceus* have been widely studied. Both the fruiting bodies and mycelia of *H. erinaceus* contain bioactive polysaccharides which exhibit various pharmacological activities including immunomodulatory effect, as well as anti-tumor, hypoglycemic and anti-aging properties (Zhang *et al.*, 2007). Fifteen polysaccharides have been successively extracted out with hot water. Five types of polysaccharides which showed relatively strong antitumor activity and a good life prolongation effect were glucoxylan, xylan, heteroxyloglucan, glucoxylan protein complex and galactoxyloglucan protein complex (Zhang *et al.*, 2007).

Besides the polysaccharides, an ergosterol derivative was also isolated from *H. erinaceus*. This compound showed cytotoxic effects on the cervical carcinoma HeLa cells (Mizuno, 1999), antitumor activity against Walker carcinosarcoma and human mammary adenocarcinoma cell lines *in vitro* (Jong and Donovan, 1989), human gastric

tumor cell line, human hepatoma cell line, human colorectal tumor cell line and murine sarcoma-180. It also showed antivenom, anti-inflammatory (Keyzers and Davies-Coleman, 2005) and antimicrobial activity (Lu *et al.*, 2000).

Hericenones, erinacines, hericerin and hericenes, the aromatic compounds that identified in *H. erinaceus*, showed a wide range of *in vitro* and *in vivo* bioactivities (Shang *et al.*, 2012). The novel oxyketo acid, Y-A-2, cytotoxic phenols, hericenone A and hericenone B (Kawagishi *et al.*, 1990), two novel γ -pyrones, erinapyrone A and erinapyrone B (Figure 2.2) (Kawagishi *et al.*, 1992) extracted from the fruiting body of *H. erinaceus* using ethanol or acetone, showed inhibition against the proliferative activity of HeLa cells.

Besides that, various acidic phenol-like and neutral fatty acid-like compounds such as hericenones and hericerins found in *H. erinaceus* (Kim *et al.*, 2000) were effective against pathogenic microorganisms and showed antibacterial activity at low concentrations against *S. aureus*, *B. subtilis* and *E. coli* respectively. Two novel and a known chlorinated orcinol derivatives were also isolated from the mycelium of *H. erinaceus*. These three compounds exhibited antimicrobial activities against *Bacillus subtilis*, *Saccharomyces cerevisiae*, *Veticillium dahlia* and *Aspergillus niger* (Okamoto *et al.*, 1993).

Ethanol extract of mycelia or fruitbodies promoted better antimutagenic effects than water extract examined with the Ames test (Wang *et al.*, 2001). On the other hand, methanol extract of fruitbodies was found to have hypoglycemic effect and reduce elevation rates of serum triglyceride and total cholesterol levels when administered to streptozotocin-included diabetic rats (Wang *et al.*, 2005). Yang *et al.*, (2003) investigated the hypolipidemic effect of an exo-biopolymer produced from a submerged culture of *H. erinaceus* in dietary-included hyperlipidemic rats. The exo-biopolymer reduced the level of plasma total cholesterol, low density lipoprotein cholesterol,

triglyceride, phospholipids, atherogenic index and hepatic HMG-CoA reductase activity; and preserving the high density lipoprotein at relatively high level. These effects would help to reduce the risk of atherosclerosis.

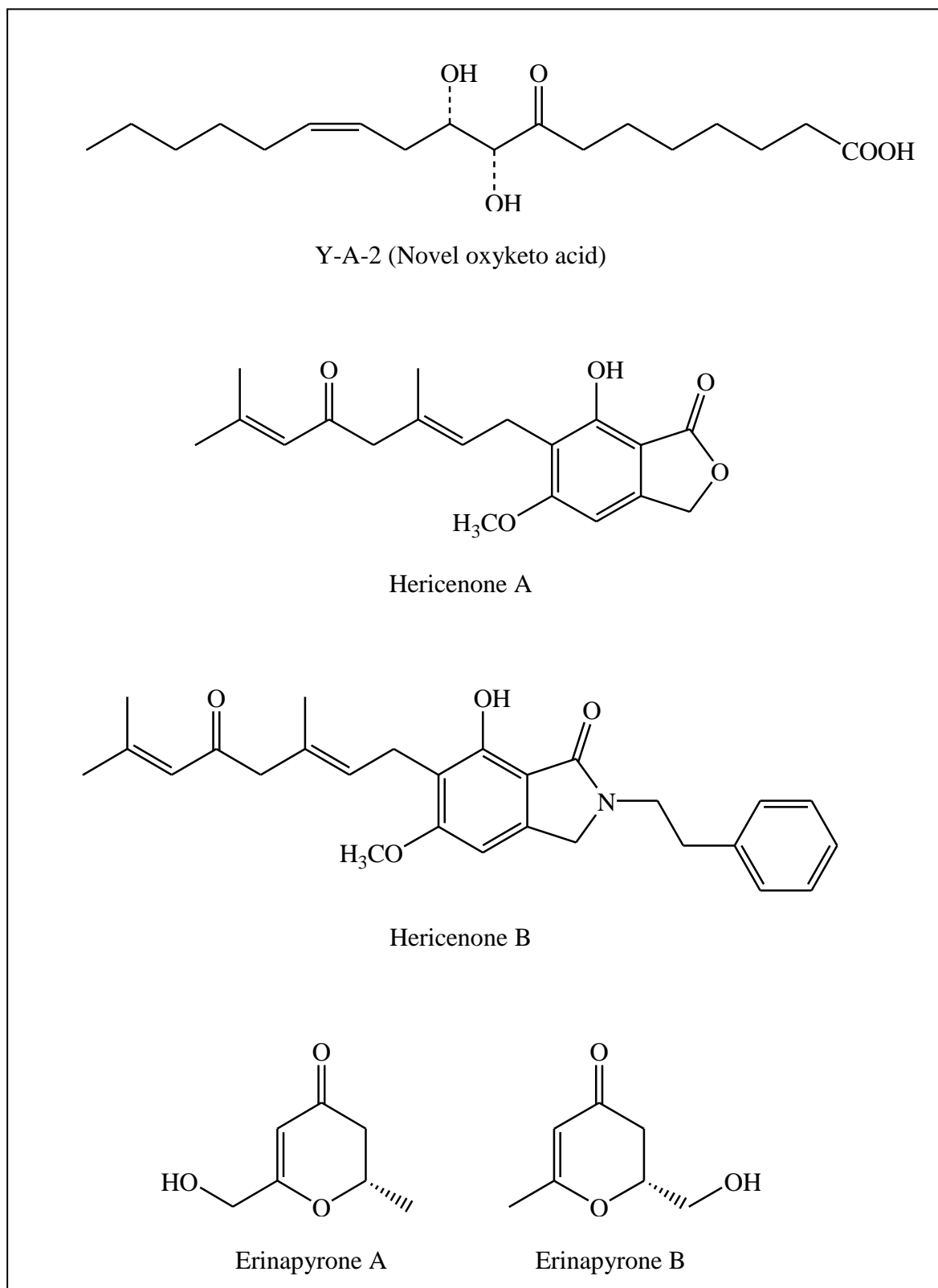


Figure 2.2: HeLa cell growth inhibitory substances isolated from *Hericium erinaceus*

This mushroom has been reported to exhibit significant antioxidant activity which might help to reduce the oxidative damage caused by the uncontrolled production of oxygen-derived free radicals (Mau *et al.*, 2001). The reduction of free radicals might lower the risk in the onset of many diseases such as cancer, rheumatoid arthritis, arteriosclerosis, degenerative processes and deterioration of physiological functions associated with aging. Besides that, total polyphenols were the major natural antioxidant components found in the methanol extract from dried *H. erinaceus* fruit bodies (Mau *et al.*, 2002).

In Chinese traditional medicine, it is used for the treatment for neurasthenic gastritis and gastroduodenal ulcer. In recent year, cultures or their extracts processed in tablets have been produced in large scale for curing gastric ulcer and chronic gastricism.

Nitric oxide (NO) is a pleiotropic biological molecule involved in a myriad of physiological and pathological processes such as regulation of blood pressure, neurotransmission, signal transduction, anti-microbial defense, immunomodulation, cellular redox regulation and apoptosis. The water extract of *H. erinaceus* activated the macrophages and induce NO production in peritoneal macrophages and RAW 264.7 cell line through the activation of transcription factor NF- κ B (Son *et al.*, 2006).

A 63kDa laccase, with a novel N-terminal sequence isolated from the water extract of *H. erinaceus* dried fruiting bodies showed inhibitory effect towards HIV-1 reverse transcriptase (Wang and Ng, 2004). HIV-1 reverse transcriptase was involved in HIV replication; inhibitors of this enzyme are potential therapeutic agents in the battle against HIV (Sarafianos *et al.*, 2009).

2.3 NERVOUS SYSTEM AND NEURODEGENERATIVE DISEASES

2.3.1 Neurite

The characterization of neurite formation, maturation and collapse/ resorption is an area of interest because these cellular processes are essential for the interconnection of neuronal cell bodies. Neurites are particularly interesting in relation to neuropathological disorders, neuronal injury/ regeneration and neuropharmacological research and screening (Smit *et al.*, 2003). Neurites emerging from cloned neural cell lines have been studied extensively over the past 15 years (Smalheiser & Schwartz, 1987). It was appreciated very early that some clones can express neurites spontaneously, even without inducing them to differentiate, but most neurobiologists have ignored this class of neurites in favor of studying clones such as the PC12 cell line, whose neurites are under inducible control and contain characteristics of axons in differentiated neurons. Bioassay which uses the PC12 cell line of rat pheochromocytoma was described by Greene (1977) and Greene & Tischler (1982). The matured neurite, called neuron, is responsible for receiving stimuli, producing and transmitting electrical signal called nerve impulses, or action potentials. It also synthesizes and releases neurotransmitters.

2.3.2 Neurodegenerative diseases (factors, therapies to cure and prevent)

Neurodegenerative diseases can be defined as hereditary and sporadic conditions which are characterized by progressive nervous system dysfunction. These disorders are often associated with atrophy of the affected central or peripheral nervous system structures. Neurological disorders are quite diverse, chronic, challenging to treat, and often disabling. They can be caused by many different factors, including (but not limited to): inherited genetic abnormalities, problems in the immune system, injury to

the brain or nervous system, or diabetes. Many mental illnesses are believed to be neurological disorders of the central nervous system, but they are classified separately.

The production of reactive oxygen species during oxidative stress is speculated to be pathologically important in neurodegenerative diseases which include Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease (Halliwell and Gutteridge, 1999). Alzheimer's disease is the most common form of senile dementia. Alzheimer's disease could be caused by both environmental and genetic factors. This has been proved by the genetic linkage studies on the chromosomes 14, 21 (early-onset) and 19 (late-onset). Trisomy and mutations of the β -amyloid precursor protein gene on chromosome 21 are the causes that lead to the early-onset of the Alzheimer's disease (Goate *et al.*, 1991). Early-onset familial forms of Alzheimer's disease could be caused by genetic mutations which may affect chromosome 14 (Mullan *et al.*, 1993). Mutations on chromosome 19 and the concomitant expression of variant apolipoprotein E4 (ApoE4) from ApoE gene (ϵ 4 allele) are associated with sporadic and late-onset familial forms of Alzheimer's disease (Uterman, 1994). However, less than 1% of patients who suffer from the disease are due to these genetic causes. It is probable that the majority of the cases is caused by a variety of environmental factors which may be either sufficient to trigger disease by themselves, or sufficient when acting synergistically with the patient genotype. A direct or indirect role has been attributed to normal or structurally altered amyloid β -protein (concentrated in senile plaques) and/or excessively phosphorylated tau protein (located in neurofibrillary tangles) (Schorderet, 1995). Degeneration of cholinergic neurons and concomitant impairment of cortical and hippocampal neurotransmission lead to cognitive and memory deficits (Schorderet, 1995).

Neuronal cell death is an essential feature of neurodegenerative disease. Many types of neuronal cell death, for example, these which are associated with amyloid- β ,

glutamate and nitric oxide are thought to be caused by endoplasmic reticulum stress. Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by reactive oxygen species (Lee *et al.*, 2003). Thus, it is reasonable to suspect that molecules which are able to attenuate endoplasmic reticulum stress might reduce both the risk for and the extent of the damage in neurodegenerative disease (Nagai *et al.*, 2006).

Treatments for reducing neuronal cell death are important for preventing as well treating neurodegenerative disease, including dementia and motor dysfunction. However, because neurodegenerative diseases have, typically, a long incubation period prior to diagnosis and are symptom-free; hence, there is a late diagnosis of the disease. This is a severe problem because once neurons are dead or neuronal circuits destroyed, lost of brain function associated with the neurons or neuronal circuits is almost impossible to restore.

Attenuation of the cognitive deficits by using choline supplementation (lecithins) and/ or acetylcholinesterase inhibitors might enhance the cholinergic activity if the cognitive deficits are due to the loss of cholinergic activity. Tacrine (tetrahydroaminoacridine, Cognex®), a potent, centrally active and reversible acetylcholinesterase inhibitor, was used together with morphine to lessen respiratory depression without affecting analgesia in the mid 1940s. Tacrine has been used alone, or in combination with lecithin, to treat symptoms of the Alzheimer's disease (Chatellier *et al.*, 1990; Farlow *et al.*, 1992). It showed a slight but statistically significant improvement in the physician's score on the visual analogue scale (Chatellier *et al.*, 1990). Only a small percentage of patients, moderately affected or treated at an early stage of Alzheimer's disease, seem to benefit from the drug (Farlow *et al.*, 1992). However, it showed several side effects such as gastrointestinal troubles, hepatitis and reversible hepatotoxicity.

Investigation on the projection of neurotrophin NGF (Nerve Growth Factor) which could counter the degeneration of cholinergic neurons to the hippocampus, a recognized memory center, were recently initiated. NGF is a protein that is essential for supporting the growth and maintenance of peripheral sympathetic neurons as well as facilitating the development of some sensory neurons for a brief period during early development (Shimbo *et al.*, 2005). Infusions of NGF into the brain of a patient can improve performance in memory test and prevent or stabilize the processes of cholinergic pathway degeneration (Schorderet, 1995).

Alternatively, antioxidants, free radical scavengers and/ or non-steroidal anti-inflammatory agents such as α -tocopherol (vitamin E), ubiquinol (coenzyme Q), retinoic acid (vitamin A), and ascorbic acid (vitamin C), may be screened as potential therapies for neurodegenerative disease induced by multiple endogenous and/ or exogenous factors (Schorderet, 1995).

2.4 NEUROPROTECTIVE, NEUROTROPHIC, NEURONAL DIFFERENTIATION AND NEURITE STIMULATION EFFECTS OF *HERICIUM ERINACEUS*

Hericenone C, D, E, F, G, H have been successively isolated from *H. erinaceus* (Kawagishi *et al.*, 1991, 1993). Among them, hericenone C, D, E (Figure 2.3) have been proven to show NGF synthesis promoting activity (Kawagishi *et al.*, 1991).

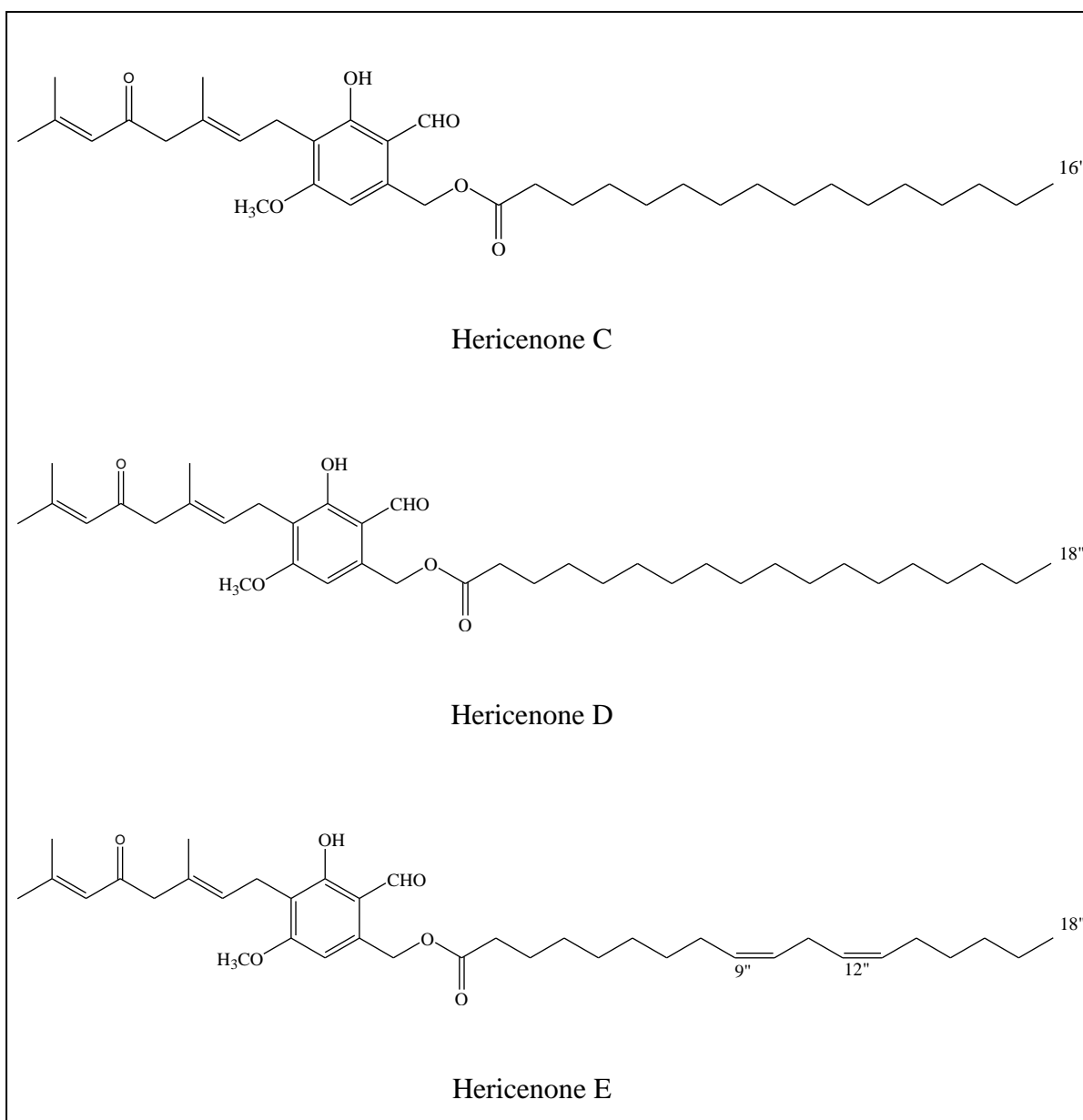


Figure 2.3: Hericenones isolated from fruiting body of *Hericium erinaceus* which showed NGF synthesis promoting activity.

This mushroom also produces erinacines A (Shimbo *et al.*, 2005), B, C (Kawagishi *et al.*, 1994), D (Kawagishi *et al.*, 1996a), E, F, G (Kawagishi *et al.*, 1996b) which have been identified as stimulators of nerve growth factor (NGF) synthesis (Figure 2.4). Stimulators of NGF synthesis have been used as medicines for degenerative neuronal disorders such as Alzheimer's disease and peripheral nerve regeneration. NGF is a protein that is essential for supporting the growth and maintenance of peripheral sympathetic neurons as well as facilitating the development of some sensory neurons for a brief period during early development (Shimbo *et al.*, 2005). NGF cannot pass through the blood-brain barrier, the semi-permeable membrane between the blood and brain. Only small and lipid soluble molecules can pass through the membrane. NGF is too large to permeate it. Therefore, it needs to be injected directly into the brain to be effective (Kawagishi *et al.*, 2002). If a substance can permeate the membrane and stimulates the NGF synthesis in brain, this may result in repairing the damaged nervous functions.

Dilinoleoyl-phosphatidylethanolamine (DLPE), an endoplasmic reticulum (ER) stress- attenuating molecule which might reduce the ER-stress, has been isolated from the fruit bodies of *H. erinaceus* and these may reduce the risk of getting neurodegenerative diseases (Nagai *et al.*, 2006). ER stress is the major cause of the neuronal cell death which leads to the neurodegenerative diseases.

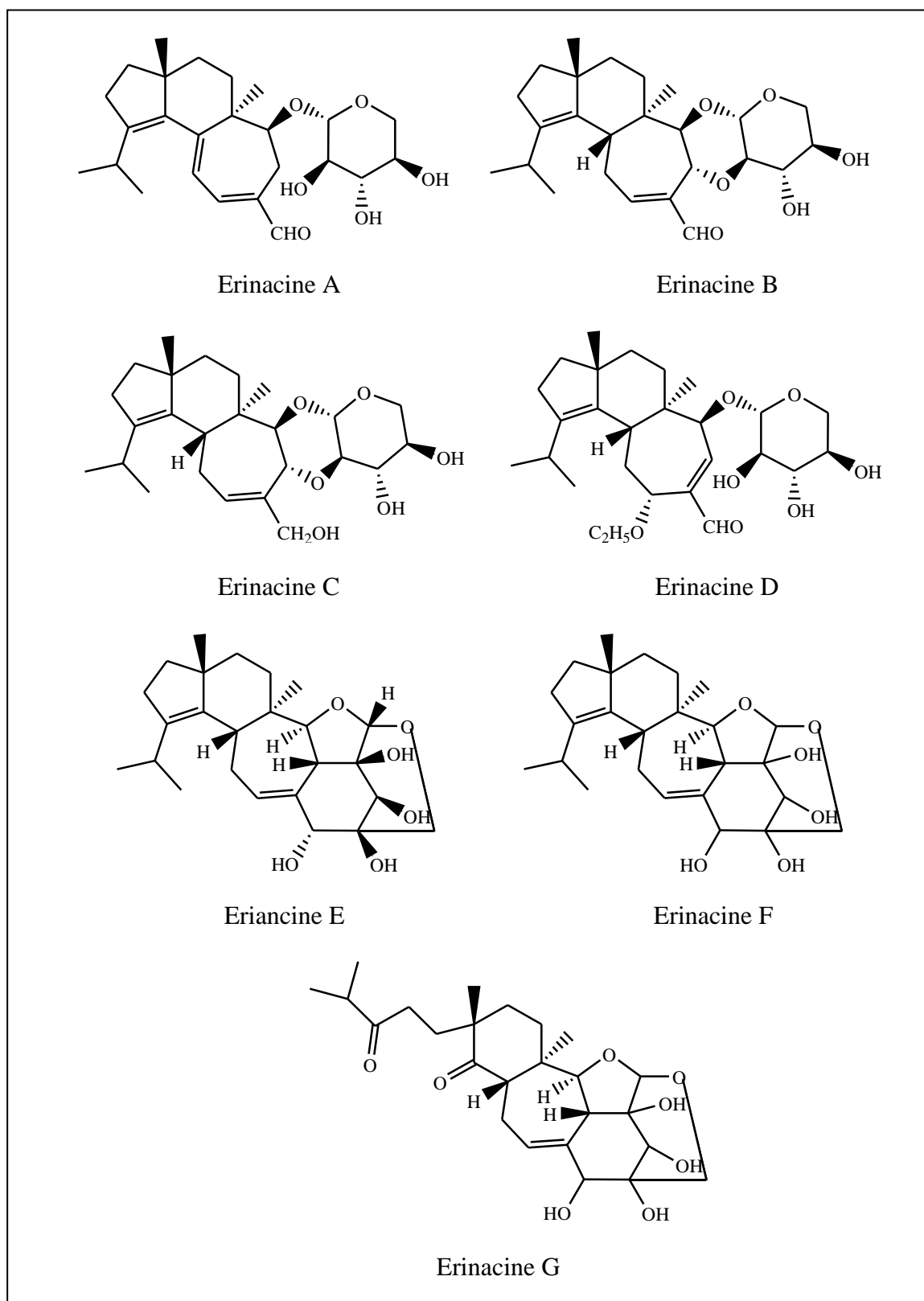


Figure 2.4: Erinacines isolated from mycelium of *Hericium erinaceus* which showed NGF synthesis promoting activity.

2.5 NEURITE OUTGROWTH BIOASSAY SYSTEM OF NEURAL HYBRID CELL LINE NG108-15

2.5.1 Formation of NG108-15 hybrid cell

6-thioguanine-resistant clonal mouse neuroblastoma cells N18TG2 and the bromodeoxyuridine-resistant rat glioma cells C6-BU-1 were fused with the aid of inactivated Sendai virus to generate the neuroblastoma x glioma hybrid cell clone, NG108-15 (Hamprecht *et al.*, 1985). Cells were grown in selective hypoxanthine-aminopterin-thymidine (HAT) medium, which was known to select for the wild-type hybrid cells and against the parental cell lines and their corresponding homokaryocytes (Littlefield, 1964).

2.5.2 Characteristics of NG108-15 hybrid cell

The hybrid cell is used as model neurons because every characteristic generally ascribed to neurons has been observed with the hybrid cell. The properties of NG108-15 are summarized in Table 2.1 (Hamprecht *et al.*, 1985). Due to the complexity of the mammalian nervous system, it is hard to assign a certain effect observed to a certain cell type. Problems that are difficult to solve with animal or tissue experiments can be tackled with the aid of cultured cells. Then, it is like having at one's disposal the numerous cell types as homogenous cell populations for studying their individual differentiated functions and their mechanisms of intercellular communication.

Table 2.1: Neuronal properties of neuroblastoma x glioma hybrid cells (NG108-15)

Neuronal Properties
Extension of long processes
Clear and dense core vesicles
Excitable membranes (inward current of action potentials carried by Na^+ or Ca^{2+})
Formation of functional synapses
Neurotransmitter enzymes <ul style="list-style-type: none">- Choline acetyltransferase- Dopamine-β-hydroxylase
Synthesis of neurohormones <ul style="list-style-type: none">- Acetylcholine- Leu- and Met- enkephalin- Dynorphine-(1-8), α-neoendorphine- β-Endorphine- Vasoactive intestinal peptide- Angiotensin- Hydra head activator- like activity
Uptake system for <ul style="list-style-type: none">- Catecholamines- Taurine
Depolarization- induced Ca^{2+} - dependent release of acetylcholine
Receptors for neurohormones <ul style="list-style-type: none">- Acetylcholine- Noradrenaline- Opioids (Morphine, enkephaline)- Prostaglandin E_1- Adenosine