Interpretation of mushroom as a common therapeutic agent for Alzheimer’s disease and cardiovascular diseases

Mohammad Azizur Rahman, Noorlidah Abdullah & Norhaniza Aminudin

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Interpretation of mushroom as a common therapeutic agent for Alzheimer’s disease and cardiovascular diseases

Mohammad Azizur Rahman¹,², Noorlidah Abdullah¹, and Norhaniza Aminudin¹

¹Mushroom Research Centre, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia and ²Department of Biochemistry and Molecular Biology, Jahangirnagar University, Savar, Dhaka Bangladesh

Abstract
Alzheimer’s disease (AD) and cardiovascular diseases (CVD) share common etiology and preventive strategies. As the population of old-aged people is increasing worldwide, AD complications tend to afflict global healthcare budget and economy heavily. CVD is the prime cause of global mortality and remains a grave threat to both the developed and the developing nations. Mushroom bio-components may be promising in controlling both diseases. Based mainly on in vitro, ex vivo, cell line and animal studies, this review interprets the polypharmaceutic role of mushrooms treating AD and CVD.

Keywords
Amyloid beta, anti-oxidants, bio-active components, neuro-degeneration, vitamins

Introduction
The unprecedented global rate of increased life span has posed an alarming threat towards the age-onset complications such as Alzheimer’s disease (AD), the most common form of dementia. Epidemiological studies indicate global dementia prevalence to be higher than 34 million and this figure would rise to 100 million by 2050 (Reitz et al., 2011). One in every eight males and one in every four females have been predicted to develop AD during their life time. The fastest growing number of the oldest old people (those aged 90 years and above) live mainly in developed countries like the USA, Canada, Australia, Japan and Europe (Reitz et al., 2011). In every year, about 4.6 million new cases of dementia are added, with the highest rate in China and South Asia. If the current growth rate proceeds in the same pattern, by 2040, 71% of 81.1 million dementia cases will be in the developing world (Bullain & Corrada, 2013). Worldwide, cardiovascular diseases (CVD) are the prime cause of mortality (http://www.who.int/cardiovascular_diseases/en/). CVD causes a global annual death toll of about 17 million and according to WHO projection, this figure may rise to 23 million by 2030 (http://www.who.int/cardiovascular_diseases/en/). Modern dietary pattern and life style have been incurred for provoking CVD.

Among the common linking factors between AD and CVD are hypertension, hypercholesterolemia, inflammation, oxidative stress and diabetes. Hypertension affects AD pathogenesis with increased cerebro-vascular oxidative stress, increased amyloid beta (Aβ) production and its decreased clearance (Girouard & Iadecola, 2006; Harrison & Gongora, 2009). Hypertension in mid-life has been linked with reduced cognitive function and 24% increased AD risk in late life. Increased brain cholesterol has been found during AD progression. In the cellular model, decreased cholesterol level has been reported to be inhibitory towards Aβ formation (Buxbaum et al., 2001; Wolozin, 2001). Experimentally induced hypercholesterolemic animals had exhibited significantly increased AD neuropathology (Shie et al., 2002). Autopsy studies of young patients (40–55 years old) showed a correlation between hypercholesterolemia with the presence of neuritic plaques (Pappolla et al., 2003). Hypercholesterolemia is among the major modifiable risk factors of CVD (Backer et al., 2003). Cholesterol lowering drugs, statins or 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors have been reported to lower the risk of death or cardiovascular events in patients with or without CVD as well as being protective against the risk of developing AD (De Kosky, 2005). Statins have been found effective in reducing the risk of AD from 60% even up to 74% (Wolozin et al., 2000). Statins can directly withstand the production of Aβ and also facilitate their removal from the brain (Fassbender et al., 2001; Hyman et al., 2000). Blood inflammatory markers such as IL-6 and α1-antichymotrypsin have been found at higher levels in AD patients than their controlled counterparts (Federico et al., 2000). Astrocytes in the brain produce pro-inflammatory factors and matrix proteins. Neurons synthesize several mediators of inflammation...
such as amyloid P, complements and C-reactive proteins whose rate of synthesis increases in AD pathology and they co-localise with the senile plaques (Casserly & Topol, 2004; Yasojima et al., 2000).

Oxidative stress has been implicated in being involved in multiple diseases including AD and CVD. Long-term (more than 15 years) usage of antioxidant vitamins C and E has been recommended for optimal neuroprotective effects (Praticò, 2008; Zandi et al., 2004). Vitamin D deficiency has been associated as an independent risk factor of CVD complications (Kendrick et al., 2009). A seven-year long follow-up study has reported lowered risk of AD in women taking vitamin D2 (Annweiler et al., 2012). Saturated fatty acid and trans-unsaturated fatty acids are among the dietary risk factors of CVD (Mozaffarian et al., 2006). However, polyunsaturated fatty acids (PUFA) have been suggested to be involved in reduced CVD complications (Virtanen et al., 2014). Similarly, ω-3 fatty acids have been found protective against AD (Freund-Levi et al., 2006).

Diabetes has multiple physiological complications and AD has been dubbed as “type 3 diabetes” as its pathophysiology strongly correlates with diabetes, especially type 2 diabetes (Suzanne, 2014). In this aspect, AD has been described as a manifestation of metabolic disorder emanating from brain insulin resistance and associated signaling derangements (Suzanne, 2014). The 15 years long Hisayama study (2011) found significantly higher rates of AD in diabetic subjects than their normal counterparts (Ohara et al., 2011). The entire brain, especially the hippocampus, the site of memory and learning, contains ample insulin receptors (Zhao & Alkon, 2001). Insulin affects neurotransmission and causes micro- and macrovascular damage to the neurons that link AD with diabetes (Hirvonen et al., 2011). Besides, brain mitochondrial dysfunction generates oxidative stress to the neurons that act as the common etiology of both AD and CVD (Moreira et al., 2007). Adults with diabetes are 4–6 times more prone to suffer from CVD complications and about 65% of diabetics die from heart diseases (Schnell, 2005). Diabetes remains as an independent risk factor for atherosclerosis and CVD (Dokken, 2008).

Mushrooms possess immense nutritional and medicinal bio-components that substantiate their usage in maintaining global public health (Cheung, 2010; Wasser, 2011). However, there is a scarcity of information regarding the common usage of mushrooms for ameliorating AD and CVD pathogenesis. In this review, mushrooms’ cardio-protective and AD ameliorating effects have been reviewed with special emphasis upon the common etiological and preventive approaches of both the diseases. To our knowledge, this is the first review in this aspect of the subject.

Interpretation of mushroom as the common therapeutic agent

Mushrooms against CVD

Antihypertensive mushroom bioactives

Anti-hypertensive treatments have been successful in reducing the risk of CVD from 55% to as low as 18% (Mancia, 2007). Both edible and medicinal mushrooms contain anti-hypertensive bio-components (Yahaya et al., 2014). Most of the mushroom bio-components exert their anti-hypertensive effects by inhibiting the angiotensin-converting enzyme (ACE) that modulates the activity of the renin-angiotensin-aldosterone system (RAAS) and lowers blood pressure (Yahaya et al., 2014). Among the Ganoderma, bioactives are the triterpenoids and polysaccharides including ganoderic acids, ganoderic aldehydes (ganoderals) and ganoderic alcohols (ganoderol). Recently, Ansor and colleagues indicated the effect of Ganoderma lucidum ACE inhibitory peptides in lowering hypertension (Ansor et al., 2013). An anti-hypertensive peptide from Tricholoma giganteum has been found to be more potent than the commercially available anti-hypertensive drug, captopril (Lee et al., 2004) (Table 1). ACE inhibitory anti-hypertensive peptides from other mushrooms have also been reported among which the most notable species are Pleurotus species, Grifola frondosa, Pholiota adipose, Hericium erinaceus, Hypsizigus marmoreus, Agaricus bisporus, Flammulina velutipes and G. lucidum (Yahaya et al., 2014). Other anti-hypertensive mushroom bioactives, worth mentioning, include lentinan, chitin and K+ from Lentinula edodes, L-pipeolic acid from Sarcodon spratus, 3,3,5,5-tetramethyl 4-piperidone (TMP) from Marasmius androsaceus and t-mannitol from Pleurotus cornucopiae (Yahaya et al., 2014) (Table 1).

Hypocholesterolemic effect

Both edible and medicinal mushrooms lower blood total cholesterol (TC), very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in rats (Hossain et al., 2003). Investigations involving the potentiality of mushrooms as the inhibitor of cholesterol biosynthetic key enzyme 3-hydroxy 3-methyl glutaryl co-enzyme A reductase (HMGCo-AR) has yielded a promising outcome. Among others, Pleurrotus spp. has undergone extensive studies in this aspect and mevinolin (lovastatin), the HMGCo-AR inhibitor has been extracted from Pleurrotus ostreatus agent (Gunde-Cimerman et al., 1993a, b; Gunde-Cimerman & Cimerman, 1995). The content of lovastatin varies between fruiting bodies and the mycelium. For fruiting bodies, the highest amount of lovastatin has been reported to be 606.5 mg/kg of P. ostreatus and 565.4 mg/kg dry weight of Agaricus bisporus (Park et al., 2002 2009). In case of mycelia, the highest lovastatin content has been found to be 995.7 mg/kg dry weight of Cyathus striatus, followed by 254.9 mg/kg, 220.5 mg/kg and 200.4 mg/kg for Cordyceps cicadae, Clonorchis sinensis and Agaricus blazei, respectively (Chen et al., 2012; Lo et al., 2012). The mushroom cultivating environment also influences the content of lovastatin. For fruiting bodies of wild P. ostreatus, a lovastatin content up to 2.80% dry weight and for those cultures cultivated on wheat straw, up to 2.07% dry weight and lovastatin concentration in liquid culture up to 30 mg/l had been found (Alarcon et al., 2003).

Gil-Ramirez et al. (2011, 2013a) screened 26 species of edible and medicinal mushrooms and reported that their aqueous and methanolic extracts have in vitro HMGCo-AR inhibitory effects. At 50 mg/ml concentration, the HMGCo-AR inhibitory effects were 54.4% for P. ostreatus, 60% for...
Table 1. Mushroom bio-components conferring protection against Alzheimer's Disease (AD) and Cardiovascular Diseases (CVD).

<table>
<thead>
<tr>
<th>Biocomponent</th>
<th>Structure</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tripeptide</td>
<td>Gly-Glu-Pro</td>
<td>Tricholoma giganteum</td>
</tr>
<tr>
<td>Hexapeptide</td>
<td>Val-Ile-Glu-Lys-Tyr-Pro</td>
<td>Grifola frondosa</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td></td>
<td>Pleurotus cornucopiae</td>
</tr>
<tr>
<td>Oligo peptides</td>
<td>Arg-Leu-Pro-Ser-Glu-Phe-Asp-Leu-Ser-Ala-Phe-Leu-Arg-Ala</td>
<td>P. cornucopiae</td>
</tr>
<tr>
<td>Potassium</td>
<td>K⁺</td>
<td>Lentinula edodes</td>
</tr>
<tr>
<td>Lentinan</td>
<td></td>
<td>L. edodes</td>
</tr>
<tr>
<td>Chitin</td>
<td></td>
<td>L. edodes</td>
</tr>
<tr>
<td>Beta-β-glucan</td>
<td></td>
<td>Pleurotus spp., Ganoderma spp., Agaricus spp.</td>
</tr>
<tr>
<td>Ergosterol</td>
<td></td>
<td>Hericium erinaceus, Flammulina velutipes, G. lucidum</td>
</tr>
<tr>
<td>Tocopherol</td>
<td></td>
<td>H. erinaceus, F. velutipes, G. lucidum</td>
</tr>
</tbody>
</table>

DOI: 10.3109/07388551.2015.1100585
Agaricus bisporus and 76% for L. edodes (Gil-Ramirez et al., 2011, 2013a). The responsible bio-components were β-D glucans and ergosterol (Gil-Ramirez et al., 2011, 2013b). In case of the 70% methanolic extracts of Pholiota spp. fruiting bodies, the in vitro HMGCo-AR inhibitory effects were 55.8% for Pholiota adiposa, 28.1% for Pholiota squarrosa, 51.3% for Pholiota nameko, 36.5% for Pholiota malicola and 11.3% for Pholiota squarrosides, respectively (Yu et al., 2007). The HMG Co-AR inhibitory component identified was stigmasterol (Yu et al., 2007).

Antioxidative effects
Mushroom-based antioxidative bio-components include polysaccharides, phenolics, triterpenes, nicotinic acid, sterol (ergosterol) and vitamin E (tocopherol) (Barros et al., 2007, 2008; Ferreira et al., 2009). Ganoderma polysaccharides are noteworthy in relation to this mushroom’s anti-oxidative and other medicinal properties (Ferreira et al., 2014; Paterson, 2006; Zhou et al., 2007). In rats’ thoracic aorta endothelial cells, L. edodes polysaccharide increased anti-oxidative enzymatic activity along with reduced expression of
VCAM-1 mRNA that corresponds towards its anti-oxidative and anti-atherosclerotic performance (Xu et al., 2008). Phenolic compounds abound in both edible and medicinal mushrooms (Cheung et al., 2003; Kim et al., 2008; Palacios et al., 2011). Ergosterol, present in mushroom, has also been reported to provide an anti-oxidative and anti-atherosclerotic defense (Kim et al., 1999) (Table 1).

Ergothioneine (ERT) is another anti-oxidative mushroom bioactive that has been demonstrated to reduce the expression of pro-inflammatory mediators VCAM-1, ICAM-1, E-selectin and binding of monocytes to the endothelial cells, thus contributing to anti-atherosclerotic and CVD preventing effects (Martin, 2010; Xu et al. 2008).

Rajasekaran and Kalaimagal (2012) reported a cardioprotective role of G. lucidum as they noted increased levels of antioxidative enzymes and reduced glutathione levels in rats fed with G. lucidum extract.

Anti-platelet aggregation and anti-thrombotic effect
In rabbit aorta and coronary arteries, Pleurotus spp. have shown inhibitory effects on atheromatous plaque and atherosclerotic lesion formation (Bobek & Galbavy, 1999; Percario et al., 2008). Dissolution of the thrombus or fibrinolysis in blood vessels is an important strategy for maintaining normal vasculature. Intravenous injection of commercial fibrinolytic enzymes very often develop uncontrolled fibrinolysis and hemorrhage. Fibrinolytic enzymes from several edible and medicinal mushrooms have been found to lower the plausibility of thrombus formation without increasing the risk of hemorrhage. Artificial fibrin plate assay showed a Schizophyllum commune fibrinolytic enzyme possessing more potent fibrinolytic activity than the commercial fibrinolytic agent, plasmin (Lu & Chen, 2012). Only 0.5 μg of S. commune fibrinolytic enzyme was capable of causing the same amount of clot degradation (degradation zone 9 mm) as plasmin did at 1 μg concentration (Lu & Chen, 2012). Other mushrooms, from which fibrinolytic enzymes have been isolated and purified, include Armillaria mellea, P. ostreatus, Cordyceps militaris, C. sinensis, F. velutipes, Fusarium sp. and G. lucidum (Lu & Chen, 2012). They are the reservoirs of the fibrinolytic enzymes, and mushrooms function as thrombolytic and cardio-protective agents.

Anti-inflammatory effects
Mushrooms have been reported to be a potential source of anti-inflammatory molecules. They lower the expression of adhesion molecules (VCAM-1, ICAM-1, E-selectin-1) and decrease the rate of monocyte binding to the endothelium in vitro (Martin, 2010). Anti-inflammatory substances present in common mushrooms showed inhibitory effects towards nitric oxide (NO) and TNF-α production and they were heat labile (Gunawardena et al., 2014; O’Callaghan, 2014). Their anti-inflammatory led immunomodulatory effects have also been documented (Lull et al., 2005). Jedinak et al. (2011), through murine macrophage RAW264.7 cell line and murine splenocytes-based studies, elucidated that the anti-inflammatory effects of oyster mushrooms is mediated by the inhibition of NF-κB and AP-1 signaling. An ethanolic extract of Lentinus polycephus has been implicated in the decreased production of pro-inflammatory markers NO, iNOS, IL-1β, IL-6, TNF-α, COX-2 in lipopolysaccharide-induced RAW264.7 cell line (Junlatat et al., 2013). Suppressive effects upon carageenan-induced rats’ paw edema was also observed for this mushroom (Junlatat et al., 2013). Cell line-based studies have identified mushroom anti-inflammatory molecules to be belonging to polysaccharides, triterpenes, peptides and phenolics group of substances (Table 1).

Vitamin content of mushrooms
Animal studies have suggested vitamin D to act as a hypotensive agent by inhibiting the RAAS (Li et al., 2002). Both in vitro and in vivo studies have indicated the ameliorating effects of vitamin D on CVD through improvement of vascular injury (Rahman et al., 2007). Mushrooms produce ergosterol, the precursor of vitamin D (Mattila et al., 2001). Mushrooms are amongst the rare natural sources of vitamin D2 and by sunlight or UV exposure, the rate of conversion of ergosterol to vitamin D2 is high (Phillips et al., 2011). From 100 g of A. bisporus, UV exposure produces about 11.2 μg of vitamin D2 as compared to only 0.2 μg in non-UV condition (Koyyalamudi et al., 2009). Table 2 describes the content of ergosterol (mg/100 g) and vitamin D2 (μg/100 g fresh weight) in different mushroom species (Phillips et al., 2011). Ergosterol has been identified as a novel inhibitor of 3-hydroxy 3-methyl glutaryl co-enzyme A reductase (HMGC-Co-AR), and thus a unique inhibitory mode of cholesterol biosynthesis has been ascribed to mushrooms (Gil-Ramirez et al., 2011). In humans, high absorption of vitamin D from mushrooms and its bioavailability have been reported (Outila et al., 1999). Vitamin D, obtained from mushrooms exposed to ultra violet light, has also been found to be safe for consumption and of high bioavailability (Calvo et al., 2013; Jasinghe et al., 2005).

For some wild species, the total tocopherol level has been detected up to 250.35 ng/g fresh weight, α tocopherol up to 27.13 ng/g fresh weight and β tocopherol up to 40.46 ng/g and γ tocopherol up to 25.69 ng/g fresh weight. In another study, Heleno et al. (2010) detected the level of total tocopherol to be 8.04 μg/g fresh weight in the wild mushroom, Laccaria laccata.

Fatty acid content of mushrooms
The content of saturated, unsaturated and essential fatty acids in mushrooms has been reported (Guç Ergönül, 2013; Kavishree et al., 2008; Reis et al., 2012). Their content of cis fatty acids might mediate an increased ratio of HDL to TC

Table 2. Vitamin D and ergosterol content of mushrooms.

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>Ergosterol (mg/100 g)</th>
<th>Vitamin D2 (μg/100 g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>56.3</td>
<td>0.11</td>
</tr>
<tr>
<td>Flammulina velutipes</td>
<td>35.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Lentinula edodes</td>
<td>84.9</td>
<td>0.44</td>
</tr>
<tr>
<td>Grifola frondosa</td>
<td>79.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Pleurotus ostreatus</td>
<td>68.0</td>
<td>0.72</td>
</tr>
<tr>
<td>Lentinus polycephus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
level that is beneficiary for cardiovascular health. Table 3 represents fatty acid content of some edible and medicinal mushrooms (Hossain et al., 2007).

Table 3. Fatty acid content (μg/g dried fruiting body) of some edible and medicinal mushrooms (Hossain et al., 2007).

<table>
<thead>
<tr>
<th>Mushroom species</th>
<th>Palmitic acid (C16:0)</th>
<th>Stearic acid (C18:0)</th>
<th>Oleic acid (C18:1)</th>
<th>Linoleic acid (C18:2)</th>
<th>Linolenic acid (C18:3)</th>
<th>Arachidonic acid (C20:4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agaricus bisporus</td>
<td>783</td>
<td>100</td>
<td>141</td>
<td>370</td>
<td>5.20</td>
<td>64.0</td>
</tr>
<tr>
<td>Pleurotus ostreatus</td>
<td>510</td>
<td>184</td>
<td>364</td>
<td>533</td>
<td>11.6</td>
<td>10.8</td>
</tr>
<tr>
<td>Ganoderma lucidum</td>
<td>153</td>
<td>89.0</td>
<td>173</td>
<td>161</td>
<td>1.6</td>
<td>13.0</td>
</tr>
</tbody>
</table>

**Dietary fiber in mushroom**

Mushrooms are a rich source of dietary fibers of which water-insoluble part (IDF) is about 90% and water-soluble one is 10% of mushroom dry weight (Table 4) (Cheung, 2010, 2013). Mushrooms alone are capable of fulfilling the 25% of the recommended dietary intake of dietary fiber (Cheung, 2010, 2013).

IDF, present in mushrooms, aids in reduced absorption of TC and triacylglycerol through the intestine and lowers CVD risks (Bajaj et al., 1997). Besides, the increased excretion of short chain fatty acids, like propionate, withstands incorporation of acetate for further fatty acid and sterol biosynthesis (Cheung, 2010, 2013).

**Mushrooms as an anti-diabetic agent**

According to International Diabetes Federation’s 2014 statistics, 387 million (1 in every 12) people are living with diabetes, one in every 7 s, one person died of diabetes that caused a death toll of 4.9 million (http://www.idf.org/worlddiabetesday/toolkit/gp/facts-figures). Mushrooms’ hypoglycemic and diabetes controlling effects have been reviewed by several reports (Lo & Wasser, 2011; Perera & Li, 2011). Ingestion of *G. lucidum* polysaccharide (ganopoly) lowered blood glucose levels in human subjects (Chang et al., 2007; Gao et al., 2004). *Agaricus bisporus* and *Pleurotus eryngii* have been documented as having hypoglycemic and hypolipidemic effects that might be implicated in its anti-diabetic and anti-CVD roles (Jeong et al., 2010; Kim et al., 2010). Enhanced adiponectin levels mediated amelioration of insulin resistance in type 2 diabetic patients have also been reported for *A. blazei* (Hsu et al., 2007). Insulin releasing and content of insulin-like components have been reported in the case of *Agaricus campestris* (Gray & Flatt, 1998).

**Mushrooms against AD**

**Reduced rate of synaptic degeneration**

Synaptic degeneration (loss of synaptic connection)-driven neurodegeneration is an important step in AD pathogenesis. Loss of synaptic density proteins such as synaptophysin, synaptotagmin and PSD-95 parallel Aβ-induced synaptotoxicity during AD progression (Reddy et al., 2005), consequently, neurotransmission became severely weakened.
An aqueous extract of *G. lucidum* at 500 µg/ml concentration *in vitro*, contributed significantly to the restoration of synaptic density proteins, synaptophysin and thus attenuated Aβ-induced synaptotoxicity in rat cortical neurons (Lai et al., 2008). Mechanisms involved attenuation of phosphorylation of c-Jun N-terminal kinase (JNK), c-Jun, and p38 MAP kinase (Lai et al., 2008).

Aβ causes decreased synaptophysin immunoreactivity along with its increased accumulation and aggregation in AD neuritis (Stagi et al., 2005). Thus, axonal transport of synaptic vesicles becomes blocked. The stress signaling pathway JNK participates in this mechanism where NO exacerbates the situation. Inhibition of the phosphorylation of the JNK can overcome the blockage of synaptophysin transport (Stagi et al., 2005). Aqueous extracts of *G. lucidum* (500 µg/ml, 14 days treatment *in vitro*) significantly inhibited the phosphorylation of JNK in Aβ-stressed rat cortical neurons that potentiated it to overcome the Aβ-induced blockage of synaptophysin transport (Lai et al., 2008).

**Reversion of neuronal apoptosis**

Neuronal apoptosis is another phenomenon in AD pathogenesis where Aβ stimulates the activity of caspase-3 (Harada & Sugimoto, 1999). The apoptotic signaling pathway involves the protein kinase pathways including the JNK, c-Jun and p38 MAP kinase (Harada & Sugimoto, 1999). An aqueous extract of *G. lucidum* reverted back Aβ-induced neuronal apoptosis by inhibiting these pathways (Lai et al., 2008). Specifically, *G. lucidum* inhibited phosphorylation at serine 67 and serine 73 of c-Jun, the substrate for JNK (Lai et al., 2008). Orally administered, *G. lucidum* polysaccharide (GLPS) at 100, 200 and 400 mg/kg body weight significantly lowered neuronal apoptosis in rats (Zhou et al., 2010). In cultured rat cortical neurons, (GLPS) at 0.1, 1.0 and 10 µg/ml, reduced neuronal apoptosis in a dose dependent manner. GLPS induced a neuro-protective mechanism that involves downregulation of the expression of caspases-3, -8 and -9 and Bax, and inhibition of the reduction of Bcl-2 expression resulting in altered Bcl-2/Bax ratio (Zhou et al., 2010). Lysoosphatidylethanolamine (LPE), derived from *G. frondosa* stimulated the activation of MAP kinase of rat pheochromocytoma PC12 cells and showed anti-apoptotic effects such as suppressed cell condensation and DNA ladder generation (Nishina et al., 2006). LPE also upregulated the expression of neurofilament M and thus promoted neuronal differentiation of PC 12 cells (Nishina et al., 2006).

**Decreased Aβ deposition**

A mouse diet supplemented with 0.3%, 0.6% and 1.8% of *G. lucidum* powder had been found to significantly lower Aβ deposition in their brain along with increased anti-oxidative enzymatic levels and improved memory-related learning abilities (Wang et al., 2004). Studies involving SH-SY5Y neuroblastoma cell lines identified enhanced non-amyloidogenic protein secretion (sAPPα) activity of *G. lucidum*. In this case, *G. lucidum* mycelial extract mimicked the nerve growth factor (NGF) activity and it stimulated the phosphorylation of ERK 1/2 and PKC and involved the signaling cascades of PI3K and ERK (Pinweha et al., 2008).

**Stimulatory effects towards neurite outgrowth and neurogeneration**

Normal maintenance and differentiation of the neurons require supportive assistance from the neurotrophic factors (neurotrophins) such as brain-derived neurotrophic factor (BDNF), glia-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and neurotrophin 3 (NT-3). The NGFs are polypeptides and too large to cross the BBB. Cyreneines A and B isolated from *Sarcodon cyrneus* have shown a stimulatory effect towards neurite outgrowth in both PC12 and NG108-15 cells and NGF generation in 1321N1 cells (Marcottuillio et al., 2007). Glaucopine C from *Sarcodon glaucus* also stimulated NGF production and the structural features of these components have been implicated to be responsible for different extents of neurite outgrowth and NGF generation in different cell lines (Marcottuillio et al., 2007). Similarly, the position of the aldehyde group has been identified as the crucial factor in promoting neurite outgrowth and NGF generation for the cyathane diterpenoids scarbornines of *Sarcodon scabrosus* (Shi et al., 2011) (Table 1). Positive role of *H. erinaceus* upon neurite outgrowth and NGF generation have been reported by several studies (Moldavan et al., 2007; Mori et al., 2008; Wong et al., 2007, 2009, 2011).

In an attempt to identify the neuro-supportive component of *H. erinaceus*, Park et al. (2002) demonstrated that an exopolysaccharide from its culture broth had neurite outgrowth promotive effect in PC12 cells. As *in vitro* mechanistic approaches very often differ from *in vivo*, these polysaccharides’ mode of action could not be explained. However, for three decades studies for identifying the memory and brain function improving effects of *H. erinaceus* have identified different members of benzyl alcohol group such as hericenones (A-I), diterpenoids, eriancins (A-R) and cyathadien 14β ol, erinacol as reviewed by Phan et al. (2014).

In rat pheochromocytoma (PC) 12 neuronal cell lines, stimulatory effect of *G. lucidum* towards *in vitro* neurite outgrowth and neuronal differentiation has been demonstrated (Cheung et al., 2000). The mushrooms were also effective in guarding against NGF-mediated apoptosis (Cheung et al., 2000). The probable mode of action is ras/extracellular signal-regulated kinase (Erk) and signaling cascade involving cAMP-response element binding protein (CREB) (Cheung et al., 2000). The lipophilic fraction of *G. lucidum* at 125 and 500 mg/L was found to promote neurite outgrowth, stimulate NGF activity and potentiate PC 12 neuronal differentiation (Zhang et al., 2005). Seow et al. (2013) reported the comparatively enhanced neuritogenic effect of the aqueous extract of *G. neo-japonicum* than those of the *G. lucidum* and *G. frondosa* in PC 12 cells (Seow et al., 2013). At 50 µg/ml concentration, the *G. neo-japonicum* stimulated 14.22% neuritogenesis, whereas at 75 µg/ml concentration, the effect was 12.61% for the *G. lucidum* and 12.07% for the *G. frondosa* extract (Seow et al., 2013). Bio-components present in these mushroom extracts activated the MEK/ERK1/2 and PI3K/Akt signaling pathways to promote neuritogenesis (Seow et al., 2013). At 20 µg/ml concentration, the aqueous sclerotium of *Lignosus rhinocerotis* induced PC 12 cells’ neurite outgrowth of 24.4% (Eik et al., 2012). Both aqueous and ethanolic extracts of *Pleurotus giganteus* had been found...
to induce neurite outgrowth of PC12 cells in dose- and time-dependant fashion (Phan et al., 2012). Similar patterns of neurite-outgrowth stimulatory effect, along with restoration of impaired memory in rats, have been observed for Tremella fuciformis (Park et al., 2012; Kim et al., 2007). In scopolamine-induced learning and memory deficient rats, C. militaris reverted back memory loss of the rats and promoted dose-dependent (5–20 μg/ml) neuritogenesis in Neuro2A mouse neuroblastoma cells (Lee et al., 2011).

Acetylcholine esterase inhibitory effects

Decreased availability of the cholinergic neurotransmitter acetylcholine leads towards AD consequences (Inestrosa et al., 1996; Recanatini & Valenti, 2004). The reason for this is the activity of the enzyme acetyl choline esterase (AchE) (Inestrosa et al., 1996; Recanatini & Valenti, 2004). Thus, developed treatment strategies targeted at anti-acetyl choline esterase (AchEI) activities would have an ameliorating effect on AD (Inestrosa et al., 1996; Recanatini & Valenti, 2004). Since 1970s, different AchEIs have been developed, namely donepezil, galantamine, rivastigmine and tacrine (Benzi & Moretti, 1998; Francis et al., 1999). They have been reported to improve cognitive and behavioral performance of the AD subjects (Terry & Buccafusco, 2003). Mushrooms, in parallel with the synthetic AchEIs, have been regarded for maintaining the neurotransmitter acetylcholine level up to appreciable amounts by inhibiting its degrading enzyme acetylcholine esterase. Ganoderma lucidum at 2 mg/ml concentration showed 57% inhibition of the acetylcholine esterase activity in vitro (Hasnat et al., 2013). Patocka et al. (2003) isolated AchEIs from the fruiting bodies of the agaricoid fungus Cortinarius brunneus. The AchEIs included beta-carboline alkaloids brunnine A-C and 3-(7-hydroxy-9H-beta-carboline-1-yl) propanoic acid (Patocka, 2012). AchEIs have more selectivity than the synthetic agent galantamine, and have also been isolated from the mushroom Cortinarius infractus, where the responsible compounds were infractopicrin and 10-hydroxy-infractopicrin (Geissler et al., 2010). Tel et al. (2012) reported the in vitro AchE inhibitory effect of the hexane extract of Tricholoma imbricatum (71.8% inhibition at 0.2 mg/ml concentration). The coral mushroom, Clavicipha pyxidata showed significant potent AchEI effects. At 100 μg/ml concentration, its water, ethanol and butanol extracts showed 91.7%, 93.7% and 99.3% AchEI effects, respectively (Lee et al., 2006). Restoration of impaired memory in rats by T. fuciformis has been reported to be through activation of the cholinergic system (Park et al., 2012). Thus, mushrooms possess cholinergic functions and the cholinergic deficiency that occurred in AD could be ameliorated with the mushroom.

Dopaminergic activity

Shielding effects against dopaminergic neuronal loss through reduced production of microglial proinflammatory factors have been associated with G. lucidum (Zhang et al., 2011). A methanolic extract of G. lucidum at 400 μg/ml, significantly inhibited the dopaminergic neurodegeneration of MES 23.5 cell membranes (Zhang et al., 2011). In the cerebral cortex of rats fed with diet containing Mycoleptodonoides atchisonii, a 1.5-fold increased level of dopamine was detected (Okuyama et al., 2004). Aqueous extracts of M. atchisonii also increased dopamine release from rat striatum (Okuyama et al., 2004). Its mode of action involved antioxidative enzymatic defense through activation of the signaling factor Nrf2 (Kokubo et al., 2002).

BACE1 inhibitory effect

Aß is generated from the amyloid precursor protein (APP) by the sequential proteolytic cleavage of aspartyl protease beta-site APP-cleaving enzyme 1 (BACE1) and gamma secretase. BACE1 is an aspartyl protease of the pepsin family and a type-1 transmembrane protein (Yang et al., 2003). BACE1 catalyzes the regulatory step for the production of toxic Aß, whose level increases in AD subjects and in aged individuals (Fukumoto et al., 2002).

Modification of the BACE1-mediated APP-cleavage results in altered AD pathology (Jonsson et al., 2012). Thus, it is the enzyme of choice for AD drug development. Unfortunately, the active site of BACE1 is comparatively large and compounds withstanding its effects are also larger in size that impedes their crossing of the BBB. This poses a threat to development of BACE1 inhibitors. Interestingly, some mushrooms have been found to inhibit BACE1 activity. For instance, different extracts of the coral mushroom C. pyxidata inhibited BACE1 activity to different extents (ethanolic 28.7%, hexane 20.8%, butanol 20.2% inhibition at 100 μg/ml) (Lee et al., 2006). A methanolic extract of L. edodes was found to inhibit BACE1 activity (by 40.1%) followed by P. eryngii (33.7%), F. velutipes (22.3%) and A. bisporus (18.3%) (Seo et al., 2008). Through characterization of the cell-free extracts of different bacteria, fungi and yeast, Lee et al. (2007) identified the BACE1 inhibitory effects (percentage scale) of mushrooms second only to Saccharomyces cerevisiae (Lee et al., 2007). Mushroom species having anti-BACE1 effects were F. velutipes, P. ostreatus, G. frondosa, Dictyophora echinovolvata, P. adspose, Fomitella fraxinea and I. obliquus (Lee et al., 2007).

BACE1 inhibitory components have been identified as heat stable through further studies with Auricularia polytricha (Bennett et al., 2013). Hispidin, isolated from Phellinus linteus, had been found to non-competitively inhibit the BACE1 (Park et al., 2004). It also inhibited the activity of ß-secretase, the non-pathological enzyme in Aß generation.

The therapeutic potentiality of the AD drug warrants a molecular weight lower than 700 Da, specifically in order to cross the blood brain barrier (BBB). Peptide-based agents having a large size and relatively high molecular weight fail to fulfill this criterion very often and thus non-peptidyl agents like hispidine seems promising in this aspect. Hispidine is a polyphenolic compound found in abundance in the mushroom P. linteus. It non-competitively inhibits BACE1 and scavenges free radicals (Park et al., 2004). BACE1 inhibitory effect of A. polytricha has also been indicated to be hispidine mediated (Bennett et al., 2013).

Antioxidative and memory enhancing effect of mushrooms

An aqueous extract of G. lucidum, grown on germinated brown rice was shown to possess both in vitro and in vivo anti-oxidant activities (Hasnat et al., 2013). In mice sera, liver
and brain homogenates, this mushroom increased the level of anti-oxidative enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Hasnat et al., 2013). In senescent accelerated SAMP8 mice, *G. lucidum* powder at 0.3%, 0.6% and 1.8% of diet, significantly increased the activities of SOD, GPx, glutathione reductase (GSH-Rd) of RBC, brain and liver (Wang et al., 2004). The same dose lowered the level of mice brain Aβ level and the *G. lucidum* fed to mice performed better in the active shuttle avoidance test. Thus, free-radical scavenging and anti-oxidative mode of action of *G. lucidum* might impart their protective role against Aβ deposition that represents this mushroom’s memory enhancing effect (Wang et al., 2004).

**Conclusion**

AD and CVD have common etiology and preventive strategy. Mushrooms stand at the linking point of both of the diseases especially due to their bio-active components capable of withstanding the pathogenesis of both. Thus, mushrooms function as excellent food-based alternative therapeutic agents for both of these diseases. However, up to the present time, most of these findings are based on *in vitro*, *ex vivo*, cell line and animal models based rather than on human subjects. Large scale clinical studies involving humans and efficacy and safety of individual mushrooms is now essential to further their usage in AD and CVD therapeutics.

**Declaration of interest**

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**Mushroom for treating AD and CVD**


