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# *Hericium erinaceus*: A possible future therapeutic treatment for the prevention and delayed progression of Alzheimer's disease? – A narrative review

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**Review Article**

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**Abstract**

At present, the treatment of Alzheimer's disease involves only symptomatic medications which have continually demonstrated little efficacy, primarily due to the presence of biological barriers. Despite efforts, researchers have yet to discover a therapeutic treatment that delays neurodegenerative progression or restores associated Alzheimer neuropathological processes. For centuries, *Hericium erinaceus* (HE) has been used predominantly in Asian countries for its culinary and medicinal purposes; however, this mushroom has not yet been utilised in western pharmacology. This review systematically investigates evidence pertaining to the use of HE as a potential future therapeutic treatment for the prevention and delayed progression of Alzheimer's disease, by highlighting any fundamental neurotrophic and neuroprotective properties. In total, three human clinical trials and thirteen animal-model studies were included in review. The use of HE demonstrated positive significant differences in results obtained from behavioural, histological and biochemical assessments from both human clinical trials and animal model studies accentuating its utility for the improvement of cognitive function. In addition, erinacine-A-enriched HE appears to demonstrate the highest bioactive potency of all HE extracted compounds, providing the greatest effects while also showing transportability ease across biological barriers. In conclusion, evidence suggests that intake of HE may be an appropriate and relevant future therapeutic treatment for the prevention and delayed progression of Alzheimer's disease; however, continued research is necessary to provide further significant evidence of this relationship, through an increased quantity of human clinical trials.

## Chapter 1: Introduction

### *1.1 Introduction*

Alzheimer's disease (AD), also referred to as senile dementia, is the most common progressive central nervous system neurodegenerative disease globally and demonstrates an increased risk in those >65 years[(1)]. In 2022, the worldwide estimation of those living with AD was 55 million, with >60% originating from low- or middle-income countries[(2)]. With figures expecting to double every 20 years, it is estimated that 139 million people will suffer from AD by 2050[(3)] with the economic burden surpassing £2·8 trillion[(4)], demonstrating a prominent public health crisis.

It is speculated that AD is a normal and expected sign of ageing due to its close association with older generations; despite this, an increasing incidence of AD is now being recorded among younger individuals[(5)]. Externally, AD is characterised by a decline in cognitive function with memory deficit, ultimately affecting motor skills, speech, visuospatial orientation and behaviour[(6)]. Internally there is a triad of pathological processes that characteristically represent AD development including (1) 'positive' β-amyloid (Aβ) plaque lesions and cerebral amyloid angiopathy, (2) tau neurofibrillary tangles and glial responses and (3) 'negative' lesions such as synaptic and neuronal loss[(7)]. The initiation and progression of AD neuropathology determined by hallmark lesions and neuronal and synaptic loss presumably takes place after an extended initial compromise in neurogenesis[(8)].

*Hericium erinaceus* (HE), commonly known as lion's mane or Yamabushitake, is a rare mushroom that can be found on dead or decaying broadleaf trees. For centuries, it has been frequently consumed in Asia owing to its well-known culinary and medicinal properties. To date, studies investigating the potential medicinal properties of HE have found that neuroprotective effects are exerted when consumed in the diet[(9)]. Hericenones C–H and erinacines A–I are compounds isolated from the fruiting body and mycelium of HE, respectively. Erinacines have demonstrated their capability of stimulating nerve growth factor (NGF) synthesis and promoting NGF-induced neurite outgrowth and regeneration in nerve cells both *in vitro*[(10)] and *in vivo*[(11)]. Moreover, the neuroprotective properties of erinacine-A-enriched HE mycelia have been demonstrated in various studies investigating mood disorders,

depression and anxiety, Parkinson's disease, stroke and ageing[12]. This is, therefore, highly suggestive that erinacines are significant candidates for further exploration of neurodegenerative diseases. Hericenones, however, have failed to promote NGF activity in 1321N1 human astrocytoma cells[10] or cross the blood–brain barrier, suggesting that they are not the key compounds responsible for the neuroprotective effects of this mushroom[12].

The aim of this review was to systematically investigate the neuroprotective pathways impacted by dietary supplementation of *Hericium erinaceus,* and to highlight the importance of continued research to determine the potential relevance of this therapeutic treatment for the prevention and delayed progression of Alzheimer's disease. The research question was formulated using the PICOT framework, a mnemonic used for the formulation of questions in evidence-based practice[13].

### 1.2 Objectives:

- Use evidence-based PRISMA and CASP checklists to critically appraise and collate the best peer-reviewed evidence investigating the relationship between HE intake and neurodegenerative health, specifically AD.
- Use evidence-based 'study quality assessment' tools to validate the quality of the studies in according to the inclusion/exclusion criteria and limit risk of bias; Cochrane's 'RoB 2' tool for randomised control trials and 'SYRCLE's risk of bias' tool for animal studies.
- Collate data from key assessments undertaken on human participants following HE intake and understand their relevance to AD.
- Outline key AD-related mechanisms influenced by HE intake and highlight its specific neuroprotective properties from animal model study assessments.
- Provide a summary exposing the true association between HE intake and its influence on AD pathogenesis to decipher the true potential of this possible future therapeutic treatment.

Due to the growing expansion of lifespan and, therefore, an increasing incidence and morbidity rate of AD, neuroscience researchers are increasingly focusing on discovering true AD causes and identifying possible disease-modifying therapies[14]. Currently, only symptomatic dementia medications and therapies exist, all of which have displayed limited efficacy[15]. So far, there are no medicines or treatments for the prevention of AD, although numerous treatments are currently undergoing investigation in various extensive animal models and clinical trials[16]. The promising findings pertaining to HE and its neuroprotective effects from both clinical and animal trials strengthen the perception that this mushroom may have beneficial mechanistic applications for the reduction of various neurodegenerative diseases, including AD. This, in turn, may highlight the importance of these therapeutic benefits to aid in the future development of new drugs and treatments for the prevention and delayed progression of AD.

### 1.3 Study design and rationale

This study design is that of a narrative review. With the aim of this study taken into consideration, other study designs could have been used. For example, a randomised control trial (RCT) could have been conducted to collect new data; however, this would have been difficult to complete due to the extensive data collection involved with RCT. In addition, evident ethical issues would have been present while working with elderly populations demonstrating AD related pathologies. Concerns as to whether the participants would have been *compos mentis* or whether the authors are appropriate conductors for this research must be considered. Due to the given time frame or the potential for a small sample size, an observational study or meta-analysis would not have been appropriate. It must be noted that, although HE and its associated neurohealth properties have been explored, a narrative review of all current literature has yet to be conducted to highlight the therapeutic potential of HE for the preventative treatment and delayed progression of AD. Therefore, it was established that a narrative review would be the most appropriate design to synthesise all the existing data and decipher the appropriateness of HE as a potential therapeutic treatment for AD.

## Chapter 2: Methods

### 2.1 Search strategy

A systematic search of PubMed, Cochrane library and Google Scholar was carried out by two independent researchers and according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) protocols. The MESH terms '*Hericium erinaceus*', 'Alzheimer's disease', 'erinacines', 'hericenones', 'cognitive function', 'cognitive decline', 'lions mane mushroom', 'hedgehog mushroom', 'yamabushitake', 'brain function', 'neurodegenerative disease' and 'neuropathy' were searched in conjunction in each database with the use of Boolean operators 'AND' and 'NOT', resulting in more focused and productive search results. No publication date limits were imposed; however, the date of each study was considered when assessing the relevance of the data.

### 2.2 Study selection including inclusion/exclusion criteria

Studies were appropriately screened by two independent researchers (Figure 1) and selected using the following inclusion and exclusion criteria:

#### 2.2.1 Inclusion criteria

(1) Clinical trials (double-blind, placebo-controlled and fixed-dose intervention); (2) adult participants >30 years; (3) general population with or without AD-related pathologies (including apparently healthy, with mild cognitive impairment, probably AD or memory deficits); (4) animal studies (mouse, rabbit, fish or any other non-human animal) investigating direct intervention of HE on AD-related pathologies; (5) studies that conduct behavioural and biochemical assessments; (6) studies that outline specific AD-related pathways or mechanisms altered, or expected to be altered, by HE.

#### 2.2.2 Exclusion criteria

(1) Observational studies (including case–control studies, cohort studies and retrospective/prospective studies); (2) clinical trials that had an existing intervention (that is, AD medication consumption, post-surgery, dietary intervention such as Mediterranean diet or vitamin supplementation); (3) adults <30 years; (4) conducted on participants exclusively selected for having an unrelated condition to AD (for example, ischaemic brain injury, Parkinson's disease, stroke, presbycusis or mood disorders such as anxiety, depression, post-traumatic stress disorder (PTSD), schizophrenia, bipolar disorder); (5) studies that reported use of acetylcholine enzyme inhibitors or *N*-methyl-D-aspartate receptor antagonists (including tacrine,


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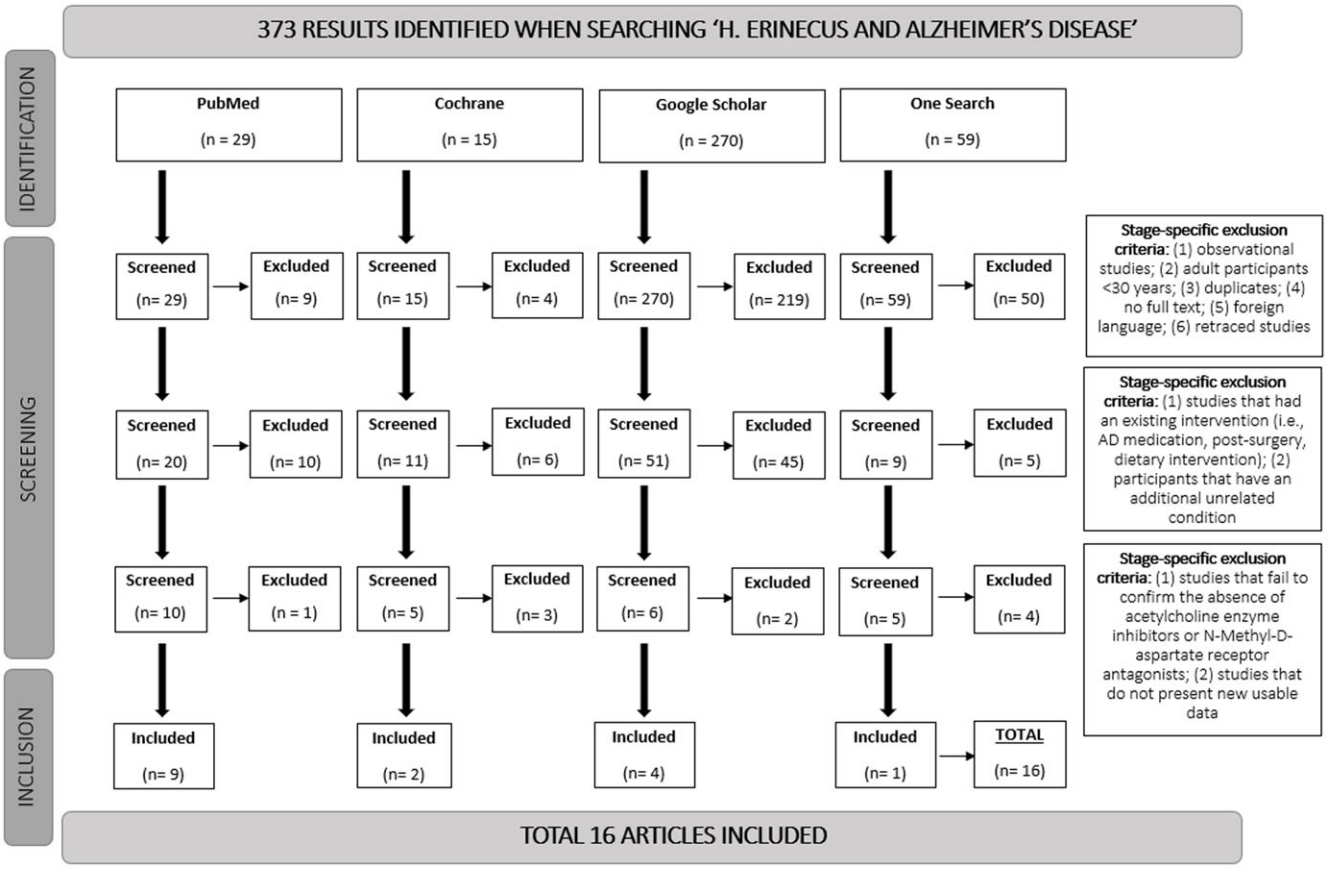


**Figure 1.** PRISMA flowchart demonstrating identified studies for narrative review consideration. Includes the screening process with the applied exclusion criteria at each stage indicated.

donepezil, rivastigmine, galantamine or memantine) or failed confirmation of the absence of such medications; (6) if search results consisted in literature or mechanistic reviews and did not supply new usable data.

Due to the limited number of studies, no automation tools were required, and the screening and selection process was completed manually by two independent researchers. The researchers independently deciphered the publications appropriate for use in the narrative review and cross-referenced to conclude a final sixteen studies appropriate for review. Among the sixteen studies, there were three randomised control trials selected that involved the use of HE for Alzheimer's disease and associated cognitive decline. The remaining thirteen animal-model studies were considered appropriate for evidence synthesis owing to the associations revealed between HE supplementation, various neurodegenerative mechanisms and associated cognitive function tests.

### 2.3 Study quality assessment

Following study selection, the quality, scientific rigour and bias potential of all studies screened appropriate for the narrative review at the study screening stage were assessed using appropriate tools by two independent researchers. For human trials, the Cochrane quality assessment tool for RCT, RoB 2 tool[17], was used to establish assessment consistency and avoid discrepancies. This, in turn, prevents discussions and conclusions being made on the basis of low-quality studies that are unable to provide clarity regarding methodology, data handling and bias potential. Table 1 presents the scores acquired by each individual human study.

Cochrane adapted their RoB 2 tool for animal model studies to create SYRCLE's risk of bias tool[18]. This tool has been adjusted for specific aspects present in animal model studies, and signalling questions are formulated to facilitate judgement to enhance the applicability and transparency of the study methodologies[18]. Many variations seen between the RoB 2 tool and SYRCLE tool are due to the differences in study design between human and animal studies. Table 2 presents the scores acquired by each individual animal model study. Following the study quality assessment, the researchers cross-referenced their individual assessments to mitigate any discrepancies.

### 2.4 Ethical considerations

The study protocol was approved by the Liverpool Hope University ethics committee.

## Chapter 3: Results

### 3.1 Included results

Results obtained were collected from both human clinical trials and animal model studies and have been ordered respectively. Animal model studies were included due to the limited number of human clinical trials for the reported research question. It is understood that data collected from animal models cannot directly correlate with human outcomes; however, the results have been carefully


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**Table 1.** Quality assessment for the three human clinical trials using the Cochrane RoB 2 quality assessment tool for randomised control trials (RCT) (adapted). Risk of bias assessment using the Cochrane RoB 2 tool. "MAX" indicates the maximum possible rating for each domain (* = 1, ** = 2, *** = 3). The overall risk of bias is scored out of 14.

| | Cochrane risk of bias (RoB 2) tool | | | | | |
|---|---|---|---|---|---|---|
| Author and year | Randomisation process (MAX ***) | Deviations from intended interventions (MAX ***) | Missing outcome data (MAX **) | Measurement of outcome (MAX ***) | Selection of the reported result (MAX ***) | Overall risk of bias (/14) |
| Mori *et al.*, 2009[22] | *** | *** | ** | ** | *** | 13/14 low |
| Li *et al.*, 2020[60] | *** | *** | ** | *** | *** | 14/14 low |
| Saitsu *et al.*, 2019[41] | *** | *** | ** | ** | *** | 13/14 low |

**Table 2.** Quality assessment for the thirteen animal model trials using the SYRCLEs RoB quality assessment tool for animal studies (adapted). Risk of bias assessment using the Cochrane RoB 2 tool. "MAX" indicates the maximum possible rating for each domain (* = 1, ** = 2, *** = 3). The overall risk of bias is scored out of 14.

| | Cochrane SYRCLE risk of bias (RoB 2) tool | | | | | |
|---|---|---|---|---|---|---|
| Author and year | Randomisation process (MAX ***) | Deviations from intended interventions (MAX ***) | Missing outcome data (MAX **) | Measurement of outcome (MAX ***) | Selection of the reported result (MAX ***) | Overall risk of bias (/14) |
| Tsai-Teng *et al.*, 2016[11] | *** | *** | ** | *** | *** | 14/14 low |
| Zhang *et al.*, 2016[61] | *** | *** | ** | *** | *** | 14/14 low |
| Cordaro *et al.*, 2021[62] | *** | *** | ** | *** | *** | 14/14 low |
| Tzeng *et al.*, 2018[63] | *** | *** | ** | ** | *** | 13/14 low |
| Mori *et al.*, 2011[64] | *** | *** | ** | ** | ** | 12/14 low |
| Shimbo *et al.*, 2005[65] | *** | ** | ** | ** | ** | 11/14 low |
| Diling *et al.*, 2017[66] | *** | *** | ** | *** | *** | 14/14 low |
| Chiu *et al.*, 2018[27] | *** | *** | ** | *** | *** | 14/14 low |
| Hu *et al.*, 2021[49] | *** | *** | ** | *** | *** | 14/14 low |
| Brandalise *et al.*, 2017[67] | *** | *** | ** | *** | *** | 14/14 low |
| Valu *et al.*, 2021[68] | *** | *** | ** | *** | ** | 13/14 low |
| Rodriguez & Lippi., 2022[69] | *** | *** | ** | *** | *** | 14/14 low |
| Lee *et al.*, 2021[70] | *** | *** | ** | *** | ** | 13/14 low |

reviewed and reported only if indicated to have some potential associations between the mechanisms and behavioural alterations of a human.

Results gathered from the three human clinical trials include behavioural, biochemical, ophthalmic and neuroimaging assessments that have demonstrated to be directly influenced by HE intake, including results that have shown positive but not always statistical differences.

Results from the thirteen animal model studies include behavioural, biochemical and histological assessments highlighting any main mechanisms previously associated with neurohealth promotion or neuropathological decline.

## 3.2 Human clinical trials: Results

For ease, the three human trials are named trials 1, 2 and 3. Table 3 specifies the characteristics of each trial, including the health status of the participant at the beginning of the trial as this may need consideration when assessing the overall specificity of any results collected. Each trial randomised the participants into two groups: a *H. erinaceus* (HE) group and a placebo group. Participants from all trials were >50 years, and average ages are specified for trials 1 and 2. The trial length and prescribed daily dose of HE are included, highlighting the variable differences between trials. The assessment methods including the intervals at which they were conducted are also noted for each trial with the main outcomes presented.

## 3.3 *Effects of* Hericium erinaceus *treatment on behavioural, biochemical, ophthalmic and neuroimaging alterations*

### 3.3.1 Behavioural assessment

*Cognitive function scale.* Trial 3 performed a cognitive function scale (CFS) assessment which is based on the Revised Hasegawa Dementia Scale (HDS-R), a scale that measures the presence of reduced cognitive function and is used across the entire course of dementia[19]. The HDS-R is a comprehensive assessment that measures twenty different cognitive functions using a scoring questionnaire (0–30). A score <20 is considered an indicator for reduced cognitive function[20].

The CFS was undertaken at each assessment interval of the trial for both the HE and placebo groups. The HE group demonstrated increased scores with duration of intake compared with the


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**Table 3.** Clinical trial characteristics including trial title, authors, publication year, participant characteristics (health status and age), *H. erinaceus* intervention dose, assessment methods used and main outcomes. ND, no data

| Trial number | Author and trial date | Trial title | Trial length (weeks) | Participant characteristics | Daily dose of *H. erinaceus* (mg) | Assessment methods and intervals | Main outcomes |
|---|---|---|---|---|---|---|---|
| Trial 1 | Y. Saitsu *et al.*, (2019)[41] | Improvement of cognitive functions by oral intake of *Hericium erinaceus* | 12 | **Health status:** Healthy **Average age:** 61.3 years | 1050 mg | **Methods:** Behavioural (MMSE and S-PA) and ophthalmologic assessments. **Intervals:** Baseline and weeks 6 and 12. | **Behavioural:** MMSE score – significant differences ($p = 0{\cdot}029$) between HE and placebo groups. |
| Trial 2 | I. Chen Li *et al.*, (2020)[60] | Prevention of early Alzheimer's disease by erinacine-A-enriched *Hericium erinaceus* mycelia pilot double-blind placebo-controlled study | 52 | **Health status:** Diagnosis of probable Alzheimer's disease **Average Age:** 75·7 years | 3200 mg | **Methods:** Behavioural (MMSE, IADL, CASI, NPI) biochemical, ophthalmologic and neuroimaging assessments. **Intervals:** Baseline and weeks 13, 25 and 49. | **Behavioural:** MMSE score – significant differences ($p = 0{\cdot}035$) between HE and placebo groups. IADL: pairwise comparison undertaken at week 49 was statistically significant ($p < 0{\cdot}012$) NPI: HE group had lower mean NPI value at week 49 than placebo group. **Biochemical: S**ignificant improvements of Hcy parameters for HE group at weeks 25 and 49 ($p = 0{\cdot}007$ and $0{\cdot}012$, respectively). **Neuroimaging:** Following 49 weeks of HE intervention, total fibre numbers in the HE group were significantly less decreased than in the placebo group ($p = 0{\cdot}001$) |
| Trial 3 | K. Mori *et al.*, (2008)[22] | Improving effects of the mushroom Yamabushitake (*Hericium erinaceus)* on mild cognitive impairment: a double-blind placebo-controlled clinical trial | 16 | **Health status:** Diagnosed with mild cognitive impairment **Average age:** ND | 1000 mg | **Methods:** Behavioural (CFS) and biochemical assessments. **Intervals:** Baseline and weeks 8, 12 and 16 | **Behavioural:** CFS: HE group scores at week 16 revealed that 71·4% were judged 'notably improved' (>3-point increase), 21·4% judged 'improved' (2-point increase), and the remaining judged 'unchanged' (score shift of 1, 0 or −1). Neither group had cases that were judged worsened by a >2-point decrease. **Biochemical:** Significant inter-group variations in BP, HDL-c and LDH parameters; however, all variations were within normal ranges. |

placebo group, with significant between group differences ($p < 0{\cdot}01$ at weeks 8 and 12, $p < 0{\cdot}001$ at week 16 and $p < 0{\cdot}05$ at week 20). Scores decreased for the HE group following a 4-week post-trial follow-up, suggesting that HE intake must be continuous to receive the benefits. The placebo group demonstrated increased scores at weeks 8 and 16 compared with scores attained at the beginning of the trial, possibly due to the placebo effect or habituation of the subjects with the CFS.

Overall, evaluation of the HE group scores at week 16 revealed that ten cases (71·4%) were judged 'notably improved' by a >3-point increase, three cases (21·4%) judged 'improved' by a 2-point increase and one case judged 'unchanged' by a score shift of 1, 0 or −1. Neither group had cases that were judged worsened by a >2-point decrease.

*Mini-Mental State Examination.* Mini-Mental State Examination (MMSE) is an oral screening tool widely used to provide a measure of dementia-related cognitive impairment in clinical research settings and is provided by oral examination. Recognition functions including memory, language, disorientation, calculation and spatial abilities[21] are measured to determine an individual's overall cognitive function. MMSE was originally developed to evaluate elderly psychiatric patients; however, it is now used mostly for cognitive impairment screening[22].

Trials 1 and 2 both conducted MMSE assessments following HE administration, assessing a total of seventy-two individuals. A 40-week trial difference is noted between both trials (including a 3-week screening period for trial 2), and the administered daily dose of HE in trial 2 was 2150 mg more than that of trial 1.

Nonetheless, despite the differences noted, MMSE scores for both trials were evaluated *ex-ante, interim* and *ex-post* the trial period, and *p*-values for each are presented in Table 3 based on *ex-ante* and *ex-post* score comparison. Both *p*-values determine a statistically significant difference ($p < 0{\cdot}05$) between the MMSE scores for the HE group compared with the placebo group over the time course of the trial. Due to the association between impaired cognitive function and an increase in age, the *p*-value for trial 1 includes an age amendment and time change comparison by repeated analysis of covariance (ANCOVA) to establish a clearer relation.

Trial 1 demonstrated increasingly linear MMSE trends for both groups; however, the HE group continually displayed an increased score compared with the placebo group. It must be noted that the HE group had higher baseline MMSE scores compared with the placebo group; however, this does not affect the overall score trend across the trial course.

Compared with baseline values, scores obtained from the trial 2 HE group demonstrated a significantly increasing trend, whereas the placebo groups scores only decreased.

*Instrumental activities of daily living.* The instrumental activities of daily living (IADL) assessment is used to evaluate the maintenance of independence by analysing the human functional performance of eight to twelve general day-to-day activities. Activities can include personal self-care (that is, bathing, dressing, feeding) and functional activities (that is, shopping, cleaning) and are measured on a scale of 0–8[23]. It is widely used for patients with dementia as well as individuals with disability[24].

Trial 2 assessed the IADL of both HE and placebo groups at each assessment interval and found that there was no significant difference between either group at any assessment interval except for the pairwise comparison undertaken at week 49 which was statistically significant ($p = 0{\cdot}012$).

*Cognitive abilities screening instrument.* The cognitive abilities screening instrument (CASI) is a quantitative assessment used to assess various cognitive functions including attention, concentration, orientation, long- and short-term memory, language and judgement abilities. It is commonly used for patients with dementia and is scored from 0 to 100[25]. The CASI is renowned for its reliability indicated by many test–retest studies[26].

Trial 2 performed the CASI assessment on participants with probable AD diagnosis. Comparing CASI baseline values with CASI scores at each assessment interval for the HE group revealed an increasing trend with marginal significance ($p = 0{\cdot}058$). For the placebo group, comparison of baseline values with week 49 scores showed a decreasing trend ($p = 0{\cdot}064$); however, no significant differences were noted in relation to the intra- and inter-group CASI analysis of either group. Scores are compared with the total percentage of global candidates to determine cognitive performance[27].

*Neuropsychiatric inventory.* A neuropsychiatric inventory (NPI) assessment is a retrospective interview conducted on the caregivers of patients afflicted with AD or other neurodegenerative disorders[28] and evaluates twelve neuropsychiatric symptom domains including hallucinations, delusions, aggression, disinhibition and irritability[29]. Both the frequency and the severity of symptoms are rated, making it a useful instrument for behavioural change detection[30].

Trial 2 found that NPI scores recorded at each assessment interval decreased for both the HE and placebo group compared with baseline values. The HE group had a lower mean NPI value at week 49 than the placebo group; however, there was no significant difference when both groups were compared ($p = 0{\cdot}077$ and $p = 0{\cdot}163$, respectively).

*Standard verbal paired-associate learning test.* The standard verbal paired-associate learning test (S-PA) is one of the most widely used instruments to assess associative and episodic memory performance in individuals demonstrating age-related neurological decline[31]. It involves recollection of eight word pairs that are verbally presented to the participant and scored accordingly[32]. The participants are given three attempts to recall the eight word pairs.

Trial 1 conducted the S-PA assessment and found that there were no inter- or intra-group significant differences of final S-PA scores compared with baseline values.

### 3.3.2 Biochemical assessment

Trials 2 and 3 both conducted biochemical assessments of the participants which included the measurement of homocysteine (Hcy), albumin, haemoglobin (Hb), brain-derived neurotrophic factor (BDNF), apolipoprotein E4 (APOE4), superoxide dismutase (SOD), systolic and diastolic blood pressure (BP), lactate dehydrogenase (LDH) and high-density lipoprotein cholesterol (HDL-c). Other biomarker parameters were measured but have not been included due to the insignificance of the results; however, each was still within the normal range.

Trial 2 demonstrated significant improvements of Hcy parameters for the HE group at weeks 25 and 49 ($p = 0{\cdot}007$ and 0·012, respectively) in comparison with baseline values. Significant decreases in albumin at week 49 ($p = 0{\cdot}004$), Hb at weeks 25 and 49


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($p = 0{\cdot}003$ and $p = 0{\cdot}009$, respectively) and BDNF at week 25 ($p = 0{\cdot}012$) were observed for the placebo group.

Although both the HE group and the placebo group demonstrated significant decreases in SOD and APOE4 parameters, the APOE4 parameters for the HE group showed a continually improving trend compared with the placebo group throughout the trial.

In addition, trial 3 demonstrated significant inter-group variations in BP, HDL-c and LDH parameters; however, all variations were within normal ranges.

#### 3.3.3 Ophthalmologic assessment

Trial 2 performed an ophthalmologic examination measuring visual acuity (VA) and contrast sensitivity (CS) for all participants. Apart from significant changes in CS results from baseline to weeks 49 in both the placebo group ($p = 0{\cdot}033$) and the HE group ($p = 0{\cdot}046$), no significant differences were found in inter- or intra-group results.

Trial 1 conducted a Benton visual retention test measuring various visual cognitions of all participants. No inter- or intra-group significant differences were noted over the time course of the trial.

#### 3.3.4 Neuroimaging assessment

Magnetic resonance imaging (MRI) was conducted on participants from trial 2 and performed *ex-ante* and *ex-post* the trial period. The total fibres, fractional anisotropy (FA) and apparent diffusion coefficient (ADC) from arcuate fasciculus (ARC), para-hippocampal cingulum (PHC), inferior frontal-occipital fasciculus (IFOF) and uncinate fasciculus (UNC) regions in both dominant and non-dominant brain hemispheres were measured. MRI was conducted to highlight atrophied brain regions and support a diagnosis of AD.

After 49 weeks of HE intervention, the total fibre numbers in the HE group were significantly less decreased ($p = 0{\cdot}001$) than in the placebo group; however, inter-group differences were not significant. In addition, compared with baseline values, the ADC values at week 49 for the HE group were significantly decreased, whereas for the placebo group the values only increased. No statistical differences were noted for other parameters.

### 3.4 Animal model studies: Results

For ease, the thirteen animal models are numbered 0–13, and each is specified in Table 4. The table includes the animal species and health status as this may need consideration when assessing the overall specificity of any results collected. In some studies, specific HE compounds are isolated before administration to determine if the compounds responsible for the results are derived from the whole mushroom or only specific parts and are labelled accordingly.

### 3.5 Effects of Hericium erinaceus treatment on behavioural, biochemical and histological alterations from animal model studies

#### 3.5.1 Behavioural assessment

The Morris water maze (MWM) test is a behavioural test in which a rodent must use visual cues to locate a platform hidden under opaque water. The test is undertaken multiple times over multiple days with escape latency time recorded to assess and analyse the cognitive function and spatial memory abilities of the rodents.

The MWM test was conducted in five studies (1–5). Compared with baseline values or control models, each study noted a significant difference ($p < 0{\cdot}001$) in escape latency time of the rodents supplemented with HE as days progressed. Tau- and AD-afflicted mice demonstrated significantly longer escape latency time compared with their healthy counterparts. An electrically stimulated 5-min step-down test was conducted in conjunction with the MWM test in one study (5)and revealed that HE intervention significantly increased time on platform ($p < 0{\cdot}05$) and decreased the number of total jumps ($p < 0{\cdot}05$) compared with previous scores. In addition, a rotarod test was conducted (4) to measure the motor coordination and balance of the rodents and demonstrated a significant ($p < 0{\cdot}05$) 30% increased endurance time following HE administration compared with the control group.

The activities of daily living (ADL) test is conducted on rodents to assess non-cognitive functions of AD by the ability to perform daily activities and is, therefore, appropriate for the measurement of overall independence. The activities primarily assessed include burrowing and nesting behaviours, and the tendency of mice being able to conduct these behaviours normally represents general animal welfare.

The ADL test was conducted in two studies (2 and 3) and assessed the burrowing and nesting behaviours of the mice, and assessments were scored by two researchers blind to experimental conditions. In study 2, although burrowing behaviours did not result in significantly improved ADL measures, significant effects of genotype were revealed, showing that HE-administered wild-type (WT) and tau mice burrowed for significantly longer durations than control mice. The other study revealed that the APP/PS1 transgenic mice demonstrated decreased burrowing performance which was significantly recovered following HE administration. Nesting scores for both studies demonstrated that tau mice with HE intervention built significantly better nests than the tau mice without intervention, thus demonstrating that HE intervention improves ADL functions, albeit not always significantly.

A novel object recognition (NOR) test is used in rodent studies to assess cognitive function and recognition memory based on the spontaneous tendency that rodents will spend increased time exploring a novel object rather than a familiar one. Due to the impaired cognitive function of AD inflicted mice or fish, the time spent exploring novel objects versus familiar objects would show less differentiation.

The NOR test was conducted in four studies (1 and 6–8) and exhibited significantly increased scores ($p < 0{\cdot}001$) between the intervention group and control groups in all four studies, demonstrating improved cognitive function and recognition memory performance following HE intake. One study (8)was conducted on zebrafish, providing valuable contributions for future cross-species comparisons. In conjunction with the NOR test a Y-maze test was conducted in two studies (6 and 8). The Y-maze test measures spatial learning and memory by assessing the willingness of animals to explore new environments. Study 6 could not detect any significant inter- or intra-group differences; however, the study 8found that the fish administered HE had increased exploratory behaviour compared with their cognitively impaired counterparts who explored the novel arm less, displaying novelty response deficits.

An open field (OP) test and elevated zero maze (EZM) test was conducted in study 8 to assess the rodents' locomotor activity, emotionality and willingness to explore. The OP test revealed that tau intervention mice had significantly shortened latency times in entering the centre of the platform compared with tau control mice. The EZM test found that mice given HE made significantly more head dips than control mice; however, tau control mice performed significantly more head dips than the WT mice. Results from both tests reveal that supplementation with HE results in


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**Table 4.** Included animal model studies including trial title and health status of animals. ***H. erinaceus*, **erinacine A (HE-A) and ***erinacine S (HE-S) administered through diet or gavage. WT, wild-type mice. $AlCl_3$, SCOP and D-gal induce AD-like cognitive dysfunction. Transgenic mice mimic a range of AD-related pathologies

| Study number (*n*) | Author and study date | Trial title | Animal model and health status | Control and health tatus |
|---|---|---|---|---|
| Study 1 | (62) | Key mechanisms and potential implications of *Hericium erinaceus* in NLRP3 inflammasome activation by reactive oxygen species during Alzheimer's disease | **1.** $AlCl_3$-induced Wistar rats*<br>**2.** Wistar rats (healthy) * | **1.** $AlCl_3$-induced Wistar rats<br>**2.** Wistar rats (healthy) |
| Study 2 | (69) | Lion's mane (*Hericium erinaceus*) exerts anxiolytic effects in the rTg4510 tau mouse model | **1.** Tau transgenic mice *<br>**2.** WT mice (healthy)* | **1.** Tau transgenic mice<br>**2.** WT mice (healthy) |
| Study 3 | (63) | The cyanthin diterpenoid and sesterterpene constituents of *Hericium erinaceus* mycelium ameliorate Alzheimer's disease-related pathologies in APP/PS1 transgenic mice | **1.** APP/PS1 transgenic mice **<br>**2.** APP/PS1 transgenic mice *** | **1.** Non-transgenic WT mice<br>**2.** APP/PS1 transgenic mice |
| Study 4 | (61) | The neuroprotective properties of *Hericium erinaceus* in glutamate-damaged differentiated PC12 cells and an Alzheimer's disease mouse model | **1.** D-gal with $AlCl_3$-induced AD mice (low dose) *<br>**2.** D-gal with $AlCl_3$-induced AD mice (intermediate dose) *<br>**3.** D-gal with $AlCl_3$-induced AD mice (high dose) * | **1**. D-gal with $AlCl_3$-induced AD mice |
| Study 5 | (49) | Structural characterization of polysaccharide purified from *Hericium erinaceus* fermented mycelium and its pharmacological basis for application in Alzheimer's disease: Oxidative stress related calcium homeostasis | **1.** APP/PS1 transgenic mice (low dose) *<br>**2.** APP/PS1 transgenic mice (high dose) * | **1.** APP/PS1 transgenic mice |
| Study 6 | (64) | Effects of ***Hericium erinaceus*** on amyloid (β25-35) peptide-induced learning and memory deficits in mice | **1.** Amyloid (β25-35) peptide-induced mice *<br>**2**. Amyloid (β35-25) peptide-induced mice * | **1.** Amyloid (β35-25) peptide-induced mice<br>**2.** Amyloid (β25-35) peptide-induced mice |
| Study 7 | (67) | Dietary supplementation of *Hericium erinaceus* increases mossy fiber-CA3 hippocampal neurotransmission and recognition memory in wild-type mice | **1.** WT mice * | **1**. WT mice |
| Study 8 | (68) | *Hericium erinaceus* (Bull.) pers. ethanolic extract with antioxidant properties on scopolamine-induced memory deficits in a zebrafish model of cognitive impairment | **1.** SCOP-induced zebrafish * | **1.** SCOP-induced zebrafish |
| Study 9 | (70) | Erinacine A-enriched *Hericium erinaceus* mycelium delays progression of age-related cognitive decline in senescence accelerated mouse prone 8 (SAMP8) mice | **1**. SAMP8 transgenic mice (low dose) **<br>**2.** SAMP8 transgenic mice (intermediate dose) **<br>**3.** SAMP8 transgenic mice (high dose) ** | **1.** SAMP8 transgenic mice |
| Study 10 | (65) | **Erinacine A** increases catecholamine and nerve growth factor content in the central nervous system of rats | **1.** Wistar strain rats (healthy) ** | **1**. Wistar strain rats (healthy) |
| Study 11 | (66) | Docking studies and biological evaluation of a potential β-secretase inhibitor of 3-hydroxyhericenone F from *Hericium erinaceus* | **1.** D-gal-induced Sprague-Dawley rats (low dose) *<br>**2.** D-gal-induced Sprague-Dawley rats (high dose) * | **1.**Sprague-Dawley rats (healthy)<br>**2.** D-gal-induced Sprague-Dawley rats |
| Study 12 | (11) | Erinacine A-enriched *Hericium erinaceus* mycelium ameliorates Alzheimer's disease-related pathologies in APPswe/PS1dE9 transgenic mice. | **1.** APP/PS1 AD transgenic mice ** | **1.** APP/PS1 AD transgenic mice |
| Study 13 | (27) | Erinacine A-enriched *Hericium erinaceus* mycelium produces antidepressant-like effects through modulating BDNF/PI3K/Akt/GSK-3β signalling in mice | **1.** Restraint-stressed group (low dose) **<br>**2.** Restraint stressed group (intermediate dose) **<br>**2.** Restraint stressed group (high dose) ** | **1.** Unstressed group<br>**2.** Restraint-stressed group |

decreased stress and anxiety, as well as improved incentive motivation and associative learning and memory.

Finally, using the lowest dose of HE (108 mg/kg body weight (BW)), one study (9)performed a passive avoidance (PA) test, which is a fear-aggregated test used to assess learning and memory retention of rodents. Training was provided to all rodents pre-testing. Results indicated significantly higher latency times in HE groups compared with control groups when assessed over time (3 d). Following the cessation of training, latency times of all groups consequently decreased. Overall, supplementation with HE led to


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improvements in learning and memory retention in comparison with control groups with no supplementation.

### 3.5.2 Biochemical assessment

Biochemical assessments undertaken by two studies (1 and 11) revealed that HE administration in both $AlCl_3$- and D-gal-induced rats increased overall antioxidant defences compared with control models. Measured cytokine levels of the model groups pre-HE administration displayed significantly higher pro-inflammatory cytokine levels and significantly lower anti-inflammatory cytokine levels. However, following HE intervention, cytokine levels in the model groups were reversed to control group levels, suggesting that HE can retain AD-induced deficits in the rat models. In addition, control models demonstrated significantly increased nitrite and lipid peroxidation levels; however, HE treatment considerably reduced these levels over time.

### 3.5.3 Histological assessment: Improvements in cholinergic functions

Western blot analyses were conducted on hippocampal tissues in multiple studies to investigate classic AD molecular markers and the effect HE intervention exerts, if any.

Two studies (4 and 5) measured cholinergic transmitter concentrations due to deficits having a detrimental impact on AD progression by the advancement of cognitive dysfunction and decline[33]. Acetylcholine (Ach), choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) and their concentrations in serum, hypothalamus, hippocampus and hypophysis were measured in both studies.

The primary assessment of one study (4) revealed exceedingly low Ach ($p < 0{\cdot}05$) and ChAT ($p < 0{\cdot}05$) in the serum and hypothalamus of $AlCl_3$- and D-gal-induced mice prior to HE administration, suggesting that the D-gal model successfully adopted the cholinergic dysfunction demonstrated by the $AlCl_3$ model. Following 4 weeks of HE administration, improvements in cholinergic functions of the AD mice were apparent ($p < 0{\cdot}05$), demonstrated by the dose-dependent enhancements of both Ach and ChAT concentrations.

Similarly, study 5 detected low concentrations of Ach ($p < 0{\cdot}05$) and ChAT ($p < 0{\cdot}05$), in addition to high levels of AChE ($p < 0{\cdot}05$) in the serum, hippocampus, hypophysis and hypothalamus of APP/PS1 mice. Following 6 weeks of HE administration (100 mg/kg BW), each cholinergic concentration was significantly restored ($p < 0{\cdot}05$).

### 3.5.4 Histological assessment: Decreased plaque burden

Multiple studies investigated the effect of HE on the size, number and accumulation of β-amyloid peptide (Aβ) plaques and associated cells within the hippocampus of rats and mice (studies 1, 3, 5 and 12).

In three studies (3, 5 and 12), following HE administration (>30 d) Aβ-plaques were immuno-stained by thioflavin (ThS) and AB10 antibodies. Plaque burden is presented as a percentage of the total hypothalamic area occupied by ThS or AB10 staining. In two studies (5 and 12), results revealed that the total number and size of non-compact (AB10-stained) Aβ-plaques significantly decreased ($p < 0{\cdot}05$), with a notable decrease of >55·8% being determined in one study (12) and >31·4% in another (5). However, compact (ThS-stained) Aβ-plaques demonstrated insignificant structure modulation. In study 3, compounds derived from HE were investigated separately; erinacine A (HE-A) and erinacine S (HE-S), both of which have been shown to have varied effects on AD-associated alterations. Results indicate that the number of Aβ-plaques and overall plaque burden decreased with both HE-A and HE-S administration; however, only HE-A decreased the size of the Aβ-plaques, whereas HE-S did not. This correlates with the findings in the previous studies (5 and 12) that HE administration impacts the non-compact region of Aβ-plaques but not the compact region. In addition, the level of insulin-degrading enzyme (IDE) in cortex was found to have increased (>127%) (study 12) in APP/PS1 mice compared with WT mice treated with HE, further impacting Aβ-plaque burden.

Tissues harvested from AD-induced rodents (studies 1, 5 and 11) demonstrated increased phosphorylated tau (p-tau) and amyloid precursor protein (APP) expression compared with control groups. Following HE administration, the number of hippocampal p-tau plaques was significantly supressed (1 and 5), indicating tau hyperphosphorylation alleviation in addition to a significant reduction in APP overexpression (11).

### 3.5.5 Histological assessment: Decreased microglia and astrocyte activation

Plaque-associated clusters of reactive astrocytes and activated microglia were examined by immunostaining of AB10, Iba-1 and GFAP antibodies to determine the effects of HE treatment in studies 3 and 12. Treatment with HE revealed that microglia and astrocyte clusters significantly decreased in both studies: (12) microglia and astrocyte clusters by 19·4% and 43·3%, respectively (3) ($p < 0{\cdot}01$). Furthermore, in study 3, the immunoreactivity of non-plaque-associated glial cells was also compared, revealing that the immunoreactivity of both Iba-1 and GFAP was enhanced in the HE-treated APP/PS1 mice compared with the WT mice at the beginning of the trial. However, HE treatment significantly ameliorated the levels of these inflammatory markers over time.

### 3.5.6 Histological assessment: Alterations of oxidative and pro-inflammatory parameters

Various studies demonstrated alterations in oxidative and pro-inflammatory parameters following HE administration (studies 1, 4, 5, 9, 11 and 13).

Reactive oxygen species (ROS) parameters which are often responsible for increased oxidative stress were measured using the 2′,7′-dichlorofluorescein diacetate (DCFH-DA) method (studies 1, 4 and 12). Each study noted significantly increased ROS levels in AD-induced mice compared with controls; however, ROS accumulation was significantly inhibited following HE administration ($p < 0{\cdot}001$). In study 4, results were verified using an additional flow cytometry method.

The NF-kB pathway is an essential pro-inflammatory transcription factor and induces the expression of numerous pro-inflammatory genes[34]. The activation of this pathway was measured in studies 1 and 12 using the ELISA method. Following AD induction, the NF-kB pathway in the mice and rats was upregulated, increasing inflammation by increased NF-kB nuclear localisation. In both studies, the administration of HE reduced the activation of the NF-kB pathway and restored nuclear NF-kB expression to basal levels. This, in turn, reduced the expression of induced nitric oxide synthase (iNOS), another pro-inflammatory mediator.

Intracellular calcium ($Ca^{2+}$) concentrations were measured in three studies (4, 5 and 11) owing to the association between $Ca^{2+}$ imbalance and decreased neuronal plasticity, synaptic deficits,


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mitochondrial dysfunction and the overall promotion of Aβ-plaque burden[35]. Following AD induction, an overload of $Ca^{2+}$ and a concomitant decrease in mitochondrial membrane potential was noted in the mice. Administration of HE in two studies (5 and 11)regulated the overload of oxidative-stress-mediated $Ca^{2+}$ in both the low- and high-dose AD mice restoring homeostasis. In addition, the mitochondrial membrane potential was also significantly increased, alleviating mitochondrial dysfunction.

#### *3.5.7 Histological assessment: Improved nerve growth factor/ pro-nerve growth factor ratio*

Nerve growth factor (NGF) and proNGF (NGF precursor) were measured in two studies (10 and 12) by western blot analysis, due to the association between NGF deprivation and an imbalanced NGF/proNGF ratio with numerous AD-related pathologies. Immunoassay was conducted to analyse the NGF and pro/NGF levels in the olfactory bulb (OLB), cerebral cortex (CC), hippocampus (Hip) and locus coeruleus (LC) brain regions in study 10, and in the hippocampus only in study 12. The effects of HE-A administration in the various brain regions of HE-treated mice in study 10 are as follows: in LC and Hip, NGF was significantly higher in the HE group compared with the control group; in OLB and CC, there were no significant differences between groups.

Administration of HE in study 12 significantly increased the NGF/proNGF ratio by 124·7 ± 54·1% and 109·9 ± 25·7%, respectively.

## Chapter 4: Discussion

Despite the ever-growing number of studies investigating the many key mechanisms that drive the pathology of AD, findings continue to be elusive and present considerable challenges for the development of successful AD interventions[36]. This review involved the critical evaluation of evidence to construct a clear and comprehensive paper highlighting the impact that HE supplementation has on behaviour and cognitive health and, therefore, its potential application for the treatment of AD.

The diagnosis of AD cannot be definitively confirmed until post-mortem neuropathological evaluation is conducted to confirm the presence of hallmark Aβ-plaques and tau neurofibrillary tangles[6]. Although researchers are investigating alternative methods capable of distinguishing the presence of these structures[37], ethical implications are ever-present, posing a clear challenge for the determination of AD in living humans. Behavioural, biochemical, neuroimaging and ophthalmologic assessments appear to be the most comprehensive and appropriate methods for establishing AD diagnosis. The purpose of cognitive behavioural assessments is to assess well-recognised AD behavioural symptoms to gain a further understating of the participant's overall cognitive function. No one cognitive assessment is recognised to be the best initial assessment; however, the use of multiple assessments in conjunction is the most appropriate method to reduce uncertainty in decision-making and to determine whether additional dementia evaluation is required[38].

Results obtained from the CFS, MMSE and IADL assessments from the human clinical trials revealed significantly improved inter- and intra-group scores following HE intervention. The use of the CFS assessment has continually shown a high internal and inter-rater reliability, especially in conjunction with an MMSE assessment where a strong correlation has been noted between the parameters of both assessments[39]. No human trial conducted both CFS and MMSE in conjunction; however, this would be highly recommended for future trials to obtain increased assessment reliability. Despite this, CFS assessment scores from trial 3 demonstrated significant differences between the scores for the HE group and placebo group at every assessment interval. The consistency in results indicates that a continuous intake of HE could restore cognitive function and increase overall participant independence.

The MMSE assessment is so widely used as a dementia screening tool due the reliability it possesses along with its modest sensitivity[40]. Trials 1 and 2 both revealed significant differences between the MMSE scores for the HE group in comparison with the placebo group across the course of each trial. It must be noted that the MMSE assessment in trial 1 was conducted on healthy participants demonstrating no dementia-related behavioural decline; however, it could be assumed that, due to the age of the participants and the increased possibility of suffering from cognitive decline[41], some age-related cognitive decline could be assumed present, retaining the relevance of results collected. At the beginning of trial 1, the healthy participants had a baseline score of >28, indicating an absence of dementia related cognitive decline; nevertheless, following HE administration, scores only increased. Although only slightly above the threshold, the MMSE screening assessment showed that the participants gained some restoration in cognitive function following HE intake. Overall, results from the MMSE assessments of both trials demonstrate that an intake of HE improved overall cognitive function in both healthy individuals and individuals diagnosed with probable AD, while also having the potential to restore the MMSE assessment scores for AD-afflicted individuals to pre-dementia scores.

The use of IADL assessments is often preferred over many other functionality screening assessments (such as the Barthel scale)[42] owing to its ability to assess increasingly complex functional abilities. However, the use of IADL alone as a dementia screening tool has numerous limitations[43]. Many factors in addition to dementia-related cognitive decline may influence IADL scores. For example, a physically inactive individual may generally find the assessed functional abilities more difficult, or the presence of a mood disorder such as anxiety or depression could also influence an individual's motivation to undertake such functional tasks. This could result in unspecific IADL scores being given, so the use of IADL for dementia screening alone may not be the most appropriate tool.

The CASI, NPI and S-PA cognitive assessments showed no statistically significant results; however, CASI and NPI still demonstrated decreasing trends following HE intervention in comparison with placebo groups.

While it is understood that data collected from animal models cannot directly correlate with human outcomes, any evidence pertaining to HE and its applicatory effect on brain health and cognition must be considered to gain the most comprehensive understanding of the medicinal properties granted by this mushroom. Understanding the key bioactive compounds of the mushroom and any mechanisms or physiological processes that they influence is essential to evaluating its use as a potent intervention. Fortunately, animal studies allow additional investigation by histological assessment, providing additional and potentially substantial evidence pertaining to AD and its physiological implications.

Results obtained from the MWM, IADL, rotarod, NOR, Y-maze, OP, EZM and PA behavioural assessments of each animal study revealed significant restoration in the behaviour of intervention


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models in comparison with control groups. Collectively, each behavioural assessment revealed improvements in cognitive function, recognition and spatial memory, functional performance, emotionality or locomotor activities of the AD-induced animals following HE intervention in comparison with control groups.

Results from each animal model study demonstrated that HE intervention can often positively impact neurological processes or related mechanisms. Oxidative stress mediated by the excessive generation of ROS, over-activation of the NF-kB pathway or over-accumulation of intracellular calcium ultimately leads to neuronal death and cell apoptosis[44], features that are often present in an individual afflicted with AD. Supplementary to biochemical assessments, HE intervention significantly ameliorated ROS production, reduced the activation of the NF-kB inflammatory pathway and restored intracellular calcium concentrations to homeostatic levels, alleviating associated oxidative stress. Furthermore, the recruitment and activation of astrocyte and microglial clusters were significantly prevented, and cluster sizes decreased, while the immunoreactivity of associated inflammatory markers also ameliorated over time. Normal microglia and astrocyte reactions encompass both anti- and pro-inflammatory responses in an attempt to defend neurons from damage; however, the chronic activation of these reactions subjects cells to inflammation and ultimately neuronal apoptosis[45].

*In vivo* studies have continually demonstrated that NGF cannot penetrate the blood–brain barrier, deeming its clinical utility useless without the aid of transferrin receptor antibody conjugate treatments or invasive neurosurgery[46]. Some studies, however, have indicated that the low molecular weight of the hericenone and erinacine compounds from the fruiting body and mycelium of HE can stimulate NGF synthesis within the brain[12], addressing the NGF penetrating issue described. One study revealed that administration of HE demonstrated a significant increase in NGF synthesis within the hippocampal and locus coeruleus brain regions, supporting the previous statement. Another study found a significant increase in the NGF/pro-NGF ratio both in mice treated with ethanol-extracted HE and those treated with water-extracted HE. The mice administered the ethanol-extracted HE demonstrated a two-fold increase compared with the mice administered water-extracted HE, suggesting that the isolated compounds from the ethanol-extracted HE were more potent and had a higher affinity for NGF expression and excretion. The differentiation, growth and maintenance of neuronal cells rely on a homeostatic ratio of NGF and pro-NGF synthesis and stimulation[47]. Deprivation of NGF or pro-NGF or a neurotrophic imbalance of them directly affect AD pathological mechanisms, specifically alterations in the concentration of cholinergic transmitters which are responsible for signal transduction associated with memory and learning abilities[48]. Cholinergic neurotransmission is the foundation of many present AD-targeting treatments; however, they have continually shown limited efficacy or success[33]. Cholinergic transmitter concentrations measured by two studies demonstrated significant dose-dependent improvements following 4 weeks of HE administration, suggesting that regulation of the cholinergic system may be influenced by HE-mediated improvements, including improved learning and memory abilities in the AD mice.

Studies demonstrated a significant decrease in the overall Aβ-plaque burden and tau hyperphosphorylation following HE intervention. The use of erinacine-A-enriched HE was found to have increased efficacy for reducing both the number and size of Aβ-plaques compared with erinacine-S-enriched HE, providing insight into the bioactive compounds that provide the most beneficial effects. Aβ-plaque burden has also been found to reduce the activities of cholinergic transmitters by cholinergic neuronal apoptosis[49]. Accumulation of insoluble Aβ-plaques causes a cascade of disruptive functions in cell communication and synaptic decline, both of which are renowned for the acceleration and progression of AD[50]. One study found that levels of IDE were significantly increased, and considering that this enzyme plays a key role in the degrading of Aβ-plaques[51], it is an additional modification corroborating the use of HE for the treatment of AD. Alongside Aβ-plaque deposits, hyperphosphorylated tau and neurofibrillary tangles are almost always present. The suppression of tau hyperphosphorylation after HE administration alongside reduced APP overexpression are additional improvements presented. Alterations in APP metabolism have been found to have numerous implications in the promotion of tau hyperphosphorylation[52], and therefore, maintaining a regulated expression of this transmembrane protein is crucial in preventing the formation of neurofibrillary tangles.

Although the measurement of biomarkers alone cannot usually provide enough evidence for AD diagnosis, it often provides clinical value for AD screening. The placebo group from trial 2 demonstrated significant decreases in albumin, Hb and BDNF by the end course of the trial. Serum albumin is the most abundant plasma protein and has a potent role in the sequestering of Aβ-proteins from blood plasma[53]. Reduced levels of serum albumin therefore decrease the capacity of Aβ excretion resulting in Aβ-plaque disposition within the brain. In addition, decreased levels of serum BDNF have been specifically associated with a decline in cognitive function[54] until eventually compensatory repair mechanisms begin to fail, further accelerating the disease. Elevated Hb levels have shown correlations with an increased long-term risk of AD[55], and following HE intake, the elevated Hb levels noted in the placebo group from trial 2 were decreased and restored to normal parameters by the end of the trial.

Elderly populations commonly encounter an increase in Hcy parameters, which is considered a normal sign of ageing[56]; however, increasingly elevated levels have shown to be a strong, independent risk factor for the development of dementia[57]. The HE group from trial 2 demonstrated significantly lowered Hcy parameters following HE administration, indicating that an intake of HE can influence Hcy plasma levels in a beneficial manner. Superoxide dismutases (SOD) are prominent antioxidant-inducing enzymes efficient for the neutralisation of ROS-mediated diseases, including AD[58]. Trial 2 found decreased SOD parameters in both groups providing little determination if HE had any influence. Finally, the strongest known risk factor gene for AD is APOE; however, possession of this gene does not always mean AD development is definite[59]. The main function of the APOE gene is to regulate lipid metabolism; however, for an individual carrying the APOE4 allele, lipid metabolism can become dysregulated and lipid build-up can occur. A lower expression of the APOE4 allele was observed for both the HE group and the placebo group from trial 2. Additional investigation would be required to determine if HE had any influence on APOE4 expression due to similar trends observed in the placebo group.

Ophthalmologic and neuroimaging assessments conducted in the human clinical trials exhibited few results and so provided no fully conclusive associations following HE intake.

There are limitations that must be recognised and considered regarding the evidence presented in this review. The limited number of human clinical trials as well as the limited number of


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participants is apparent as investigation into the medicinal properties of HE is a relatively new research interest. Therefore, the data collected from any current trials may hold relatively low reliability, accuracy and stability. Expanding the number of human clinical trials would be essential in addressing these issues while also potentially providing new and more relevant data into the research set for this topic. Although technically these trials have reproducibility, due to the age of the participants and the nature of AD, it would likely be challenging to determine whether the participants are *compos mentis*, and therefore, ethical challenges would be prominent. Nonetheless, reproducibility is most definitely doable with correct procedures and time. Results obtained from the 4-week post-trial follow-up in trial 3 suggest that HE intake must be consistent to harbour continuous benefits. Mori *et al*. found that cessation of HE supplementation decreased scores for the CFS assessment significantly, suggesting that cognitive decline and AD pathological processes are rebounded to pre-HE states, and continuous use of HE presents a challenge when the intended participants for treatment suffer from AD memory impairment.

Studies investigating the presence of any adverse or toxicological effects following HE intake have stated that, aside from occasional minor stomach discomfort or nausea, no adverse effects or toxicological issues have been noted. To achieve neurohealth benefits, the most appropriate dose of HE has been between 1000 and 2000 mg/d.

## Chapter 5: Conclusion

This review demonstrates a critical evaluation of both human trials and animal model studies to reveal sufficient evidence suggesting that HE, specifically erinacine-A-enriched HE, may be an appropriate candidate for the future therapeutic treatment of those afflicted with AD. The application of HE demonstrated numerous improvements in AD-related behaviour, biomarker parameters, histological features and physiological mechanisms while neuroprotective and neurotrophic properties were also clearly established. The true relevance and potential of HE for the therapeutic treatment and delayed progression of AD has been revealed. Nevertheless, continued research, in particular, human clinical trials, is necessary to contribute further evidence surrounding the use of HE for AD, and to determine the most appropriate constructive methods for the future targeting of the AD population, providing direction for future research.

**Authors' contribution.** The corresponding author, N.C., was responsible for the conception and design, data acquisition, analysis, interpretation and discussion of the manuscript. In addition, drafting of the manuscript was completed by the corresponding author. The co-author, M.C., was responsible for critically revising the manuscript for important and appropriate intellectual content, study supervision and administrative, technical and material support. Both authors agreed upon the final version of manuscript to be published.

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