



HUPERZINE A FROM *HUPERZIA SERRATA* - A SYSTEMATIC REVIEW

Sangita A. Kumbhar*, Chitra B. Hangargekar, Amol A. Joshi

ASPM'S K. T. Patil College of Pharmacy, Siddhartha Nagar, Osmanabad, Maharashtra, India.

*Corresponding author: sangitakumbhar25@gmail.com

ABSTRACT

Huperzine A is an alkaloid isolated originally from a traditional Chinese plant of *Huperzia serrata*. *Huperzia serrata* belongs to family Huperziaceae. Several members of the Huperziaceae (*Huperzia* and *Phlegmariurus* species) have been used as medicines in China for thousands of years for the treatment of contusions, strains, swellings, schizophrenia and myasthenia gravis. Huperzine A has attracted intense attention since its marked anticholinesterase activity was discovered by Chinese scientists. Huperzine A has been marketed in China as a new drug for Alzheimer's disease (AD). This review attempts to bring together all aspects related to Huperzine A with respect to its source, discovery, chemistry, pharmacology and toxicology.

Keywords: Huperzine A, Alkaloid, *Huperzia serrata*, Alzheimer's disease

1. INTRODUCTION

Huperzine A (Hup A) is a sesquiterpene alkaloid which was isolated from traditional Chinese herbal plant *Huperzia serrata* in 1986. Huperzine A is a potent, selective, reversible acetylcholine esterase inhibitor. It is used for the treatment of Alzheimer's disease and in the case of organophosphate poisoning in China. In USA it is used as a dietary supplement to enhance cognition and for neuroprotection. *Huperzia serrata* has been used in China for treatment of various ailments such as fever, cold, swelling, rheumatism, myasthenia gravis for over one thousand years. Later on pharmacological studies carried out in the late 1980's in China proved that Hup A is very potent inhibitor of the enzyme acetylcholine esterase [1].

2. ALZHEIMERS DISEASE (AD)

To understand the mechanism of action of Hup A, a brief discussion of AD is necessary. AD is a progressive, degenerative disease of the brain. The disease is the most common form of dementia affecting elderly people. It leads to progressive loss in cognitive abilities, performance of routine task, communication skills, abstract thinking and personality [2]. From diagnosis of AD until death, individual's median life expectancy is about eight years. The incidence of AD increases with age. In USA alone it affected 4,000,000 people. Worldwide Some 12 million persons have AD, and by 2025, that number is expected to increase to 22 million [3].

Neuropathologically, AD is characterized by (1) parenchymal amyloid deposits or neuritic plaques; (2) intraneuronal deposits of neurofibrillary tangles; (3) cerebral amyloid angiopathy, and (4) synaptic dysfunctioning. Hup A works by improving synaptic dysfunctioning. One of the problem associated with AD is cholinergic dysfunction, resulting from deficiency in the neurotransmitter acetylcholine, which play functional role in memory. While deficiencies in other neurotransmitter system have been found, the cholinergic connection seems to be most important. Inhibition of acetylcholine esterase (AChE) enzyme (this enzyme causes hydrolysis of acetylcholine) will allow small amount of acetylcholine that are still being synthesized will release and persist longer within synaptic cleft and improve cell to cell communication [4].

Current treatment for AD in most countries consists in the administration of acetylcholine esterase inhibitors (AChEI) to increase the amount of acetylcholine (ACh) at the neuronal synaptic cleft by inhibiting AChE. To date, four AChEIs, Cognex (tacrine), Aricept (donepezil or E2020), Exelon (rivastigmine), and Reminyl (galanthamine hydrobromide) currently are approved as prescription drugs by the United States to treat the symptoms of mild-to-moderate AD. However, the clinical usefulness of AChEIs has been limited because of their side effects. Short half-lives, excessive side effects caused by activation of peripheral cholinergic systems and hepatotoxicity are major side effects of AChEI.

Hepatotoxicity is the most frequent and important side effect of tacrine therapy. Hence there is need of alternative therapy which most effective, cheaper as well as safe [5, 6].

3. DISCOVERY OF HUP A

During the early 1980s, Chinese investigators screened *Lycopodium* species for new drugs for the treatment of myasthenia gravis. The period of 1986-1990 was a highlight in *Lycopodium* alkaloid research. *In vitro* and *in vivo* pharmacological studies demonstrated that *Lycopodium* alkaloids such as Hup A, Hup B, N-methyl-huperzine B, Huperzine, Lycoporine A and Carinatamine A and B produced AchE enzyme inhibition activity and exhibited significant improvement in learning and memory impairment in AD and vascular dementia patients (VD) in China. Of these, Hup A which was isolated from the Chinese folk medicinal plant of *Huperzia serrata* by Chinese scientist Liu and co-workers appeared to be the most potent and selective one against AchE enzyme. Hup A has attracted world attention after the revelation of its acetylcholine esterase activity. In 1996 Shuangyiping (Huperzine A Tablet) was approved in China and has been marketed in China since 1996, later on it is also marketed in USA as a dietary supplement [7,8].

4. SOURCE OF HUP A

Original source of Hup A is *Huperzia serrata*. Hup A was first time isolated from *Huperzia serrata* in 1986. Phytochemical studies indicate that Huperzine A is mainly present in Huperziaceae family. This family consists of genus *Huperzia* and genus *Phlegmariurus*. Huperziaceae family comprises 150 species worldwide, of this; 48 species, 2 varieties and 2 forma were reported in china and 29 species were endemic species of China. The distribution of this plant family is global, but these plants are found in relatively in greater abundance in tropical habitats of America. In china they are distributed mainly in the area along the Yangtze River and throughout the southern parts of China [9]. In India it is mainly found in sub-tropical to temperate forests at an altitude of 900 to 3500m in North-Eastern region of India like, West Bengal (Darjeeling), Sikkim, Arunachal Pradesh, Assam, Meghalaya and Manipur, and also in Nilgiri hills of Tamilnadu [8]. Nevertheless, these plants are not abundant and are only found in very specialized habitats, and original source of plant *Huperzia serrata* possess very low content of Huperzine A (0.007 %).

Table 1 and Table 2 indicates distribution of *Huperzia* species in china and India

Table 1 *Huperzia* species in China with their reported Hup A content

<i>Huperzia</i> species	Hup A content (mg/g$\pm$SD)
<i>H. cancellata</i>	358.44 $\pm$ 0.16
<i>H. carinata</i>	560.46 $\pm$ 0.21
<i>H. fordii</i>	376.18 $\pm$ 0.23
<i>H. herteriana</i>	254.58 $\pm$ 0.22
<i>H. phlegmaria</i>	345.23 $\pm$ 0.18
<i>H. pulcherrima</i>	342.57 $\pm$ 0.20
<i>H. selago</i>	114.58 $\pm$ 0.24
<i>H. serrata</i>	80.16 $\pm$ 0.17
<i>H. squarrosa</i>	378.83 $\pm$ 0.33

Table 2: *Huperzia* species in India with their distribution

Species	Distribution
<i>Huperzia aloifolia</i>	Eastern India
<i>H. cancellata</i>	Arunachal Pradesh
<i>H. carinata</i>	Tamilnadu, Andaman & Nicobar Islands
<i>H. ceylanica</i>	Meghalaya , Tamilnadu
<i>H. cryptomeriana</i>	Arunachal Pradesh
<i>H. fordii</i>	Sikkim
<i>H. hamiltonii</i>	Throughout in hilly regions
<i>H. herteriana</i>	Sikkim, West Bengal & Manipur
<i>H. nilgirica</i>	Tamilnadu, Kerala
<i>H. nummulariifolia</i>	Nicoabar islands
<i>H. petiolata</i>	Meghalaya
<i>H. phlegmaria</i>	West Bengal, Sikkim, Assam, Tripura, Arunachal Pradesh, Kerala, Andaman & Nicobar Islands
<i>H. phyllantha</i>	Kerala, Karnataka, Tamilnadu, Andaman & Nicobar Islands
<i>H. pulcherrima</i>	Uttarakhand, North-East Himalaya
<i>H. selago</i>	Sikkim
<i>H. Serrata</i>	West Bengal, Sikkim, Arunachal Pradesh, Assam, Meghalaya, Manipur, Tamilnadu
<i>H. squarrosa</i>	West Bengal, Sikkim, Meghalaya, Assam, Kerala
<i>H. subulifolia</i>	West Bengal, Sikkim, Meghalaya, Uttarakhand
<i>H. vernicosa</i>	Tamilnadu, Kerala
<i>H. vorwerkii</i>	Himalayas

H. crispata, *H. austrosinica*, *H. herteriana*, *H. emeiensis*, *H. delavay*, *H. sutchueniana*, *H. chinensis*, *H. bucaawangensis*, *H. selago* var. *appressa*, *H. ovatifolia*, *H. whangshanensis*, *H. tibetica*, *H. liangshanica*, *H. kunmingensis*, *H. laipoensis*, *H. nanchuanensis* and *H. obscure-denticulata* are the species from *Huperzia* genus shows the presence of Huperzine A [10].

4.1. *Huperzia serrata*-Original source of Hup A

4.1.1. History of *Huperzia serrata*

Huperzia serrata is club moss found in deep forest of Southeast Asia including China and India. It was first recorded as a traditional Chinese medicine (TCM) where it was listed in Chinese pharmacopoeia *Ben cao shi Yi* as *Shi song* which was used to treat rheumatism and cold and improve blood circulation and relaxes smooth muscle. Because *shi song* can also refer to multiple medicinal herbs from the genus *Lycopodium*, *Huperzia serrata* more specifically known as *Qian Ceng Ta*, which means thousand layer pagoda, referring to plants shape. In Traditional Chinese Medicine (TCM) it is usually employed in the form of tea to relieve pain and to treat pain, fever, strain, contrusion, swelling and inflammation [11].

4.1.2. Morphology of *Huperzia Serrata*

Huperzia serrata is a club moss fern. Club mosses are plants of phylum Lycopodiophyta. They reproduce by spore formation. These are evergreen herbs having erect or creeping stems. It grows in the forest, shrubbery, and roadside in the altitude of 300-2700m. *Huperzia serrata* grows very slowly requiring 15-20 years to grow from spore germination to maturity. It grows in very specialized habitat and also contain very low amount of Huperzine A that is 0.007%.



Fig. 1: Plant of *Huperzia Serrata*

- These plants are over 15 years old still reach height of less than 10 cm
- Small herb up to 30cm in height, thick flaccid and up to 6 cm in diameter
- Stems are clustered, erect or pendent or decumbent
- Plant decumbent at base-lying or growing on ground and erect above
- Two to six times branched at intervals from base
- Leaves are lanceolate
- Margin coarsely and irregularly serrate more prominent towards apical part
- Midrib is distinct and prominent throughout
- Spores are in the axils of leaves all down the stem and branches, reniform, bivalvate, on short, slender stalks [8].

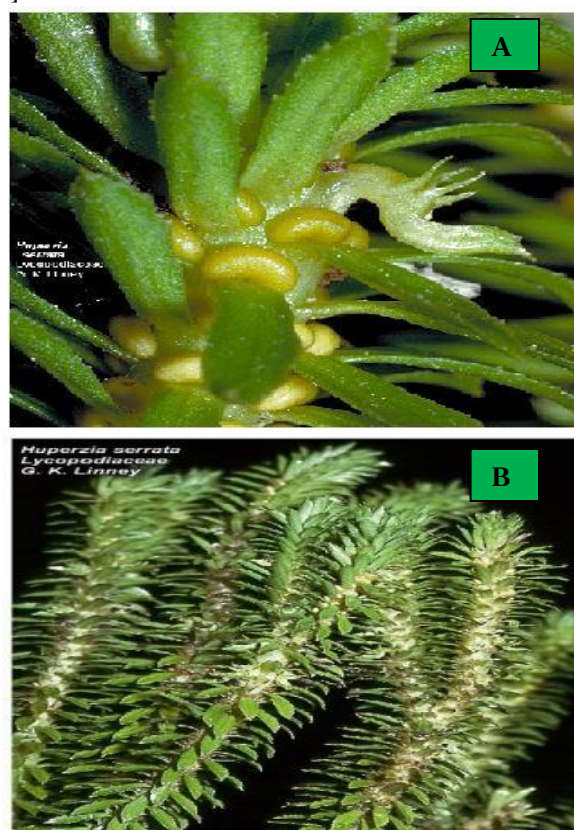


Fig. 2: Parts of *Huperzia Serrata*
(A) Spores (B) Leaves

4.1.3. Genus *Phlegmariurus* (Ph)-Source of Huperzine A

Ph. Squarrosus, *Ph. Fordii*, *Ph. Phlegmaria*, *Ph. Guangdongensis*, *Ph. Cancellatus*, *Ph. Yunnanensis*, *Ph. Carinatus*, *Ph. Henryi*, *Ph mincheensis* are the species from genus *Phlegmariurus* reported to contain Hup A [10]. Plants from Genus *Phlegmariurus* mostly epiphytic, pendent to erect; gemmae absent; fertile leaves in strobili

of smaller leaves. Nineteen species are recorded in natural resources and used as a traditional medicinal in China. Fig 3 indicates *Phlegmariurus mincheensis* species form Genus *Phlegmariurus* growing epiphytically [9].



Fig. 3: Plant *Phlegmariurus mincheensis*

5. CHEMISTRY OF HUP A

- Hup -A is a three ring system molecule. It consists of Tetrahydroquinolinone, three Carbon Bridge, exocyclic ethylidene and a primary amino group.
- IUPAC Name of Hup A- ([5R-(5-, 9-, 11E)]-5-amino-11-ethylidene-5, 6, 9, 10- tetrahydron-7-methyl-5, 9-methanocycloocta[b]pyridine-2-(1H)-one)
- Empirical Formula is $C_{15}H_{18}N_2O$
- Molecular Weight 242 g/mol
- Compound is optically active and in plant it is present only in its (-) enantiomer
- It is soluble in methanol, ethanol but insoluble in water [10].

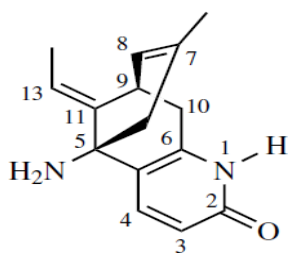


Fig. 4: Structure of Huperzine A

5.1. Structure activity relation of Hup A

The computer-generated superposition of Hup A and acetylcholine (ACh) suggested that Hup A possessed the basic structural features of ACh.

Structural similarity found between the nitrogen, oxygen and carbonyl in ACh with the corresponding amino nitrogen, nitrogen and carbonyl in Hup A. Therefore,

the 5-aminomethyl-2-(1H)-pyridone part of Hup A may constitute a pharmacophoric moiety.

Three-carbon bridge ring and its double bond, the methyl of the bridge, and the exocyclic double bond are required for high anti-AChE activities. Elimination or substitution of these structural features by other groups causes the activity to drop dramatically.

Alterations that used a benzyl ring, pyramine ketone ring, hydroxybenzene, benzcatechin, or similar structures instead of the pyridone ring (ring A) of Hup A significantly lowered the bioactivity.

Activity was almost completely lost when aminomethyl, hydroxymethyl, azide or similar groups were introduced to the amido position of HupA.

With respect to stereoselectivity (-)-Hup A was the more potent enantiomer. (+) Hup A inhibited the enzyme 38-fold less potently.

Racemic Hup A was about two-fold less potent than the more active isomer, (-) Hup A [7].

5.2. Hup A Lycopodium alkaloid

Hup A is a Lycopodium alkaloid; a structurally related yet diverse group of compounds. Lycopodium alkaloids are isolated from plants of family Huperziaceae and lycopodiaceae. They have unique ring system consist 3 or 4 rings having structural formula $C_{16}N$ or $C_{16}N_2$. Lycopodium alkaloids are divided into four major classes; Lycopodine class, Lycodine class, Fawcettimine class, Miscellaneous group. Hup A comes under lycodine class. This class has four rings, C ring cleavage and elimination of Carbon-9 gives rise to $C_{15}N_2$ skeleton of Hup A.

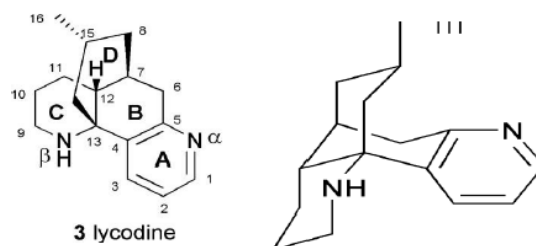


Fig. 5: Huperzine A-Lycopodium Alkaloid

5.3. Analogues of Hup A

To prepare drug candidates against AD, that are even more effective, Scientist have prepared synthetic and semisynthetic analogue of Hup A. Among these, only a very few compounds have obvious AChEI activity. Those that do have this activity include H-1, H-2, and ZT-1.

5.4.H1 synthetic analogue of Hup A

H1 is a substituted analog with two methyl groups in the C-10 position of Huperzine A. Introduction of an axial methyl group into the C-10 position of Huperzine A increased the potency for AChE inhibition by a factor of 8. The introduction of substituents larger than methyl resulted in a drop in activity. Ethyl analog was found to be about 100-fold less active than Huperzine A. X ray modeling studies indicate that AChE enzyme has a small cavity that can just fit a methyl group.

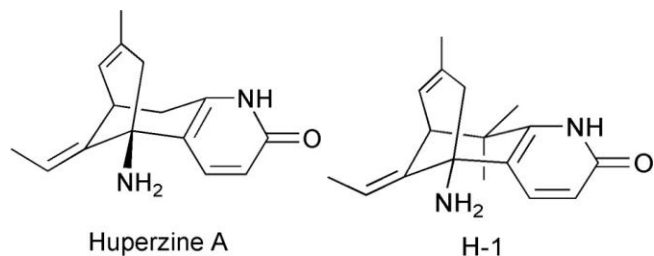


Fig. 6: H1-Synthetic analogue of Huperzine A

5.5.H2 synthetic analogue of Hup A

H2 analogue has a cyclopropane group at C-10. Its activity *in vitro* was similar to that of natural Hup A.

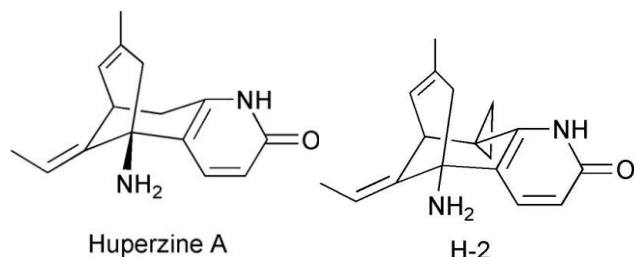


Fig. 7: H2-Synthetic analogue of Huperzine A

5.6.ZT1 semisynthetic analogue of Hup A

ZT-1 is most effective semisynthetic analogue of Hup A. ZT-1 is a Schiff base made by a condensation reaction between Huperzine A and 5-Cl-O-vanillin. This semi-synthesis pathway only requires two steps and the materials are readily available, inexpensive, and easily prepared. Experimental data demonstrated that ZT-1 possesses AchE activity similar to Huperzine A. However, it has more selective inhibition on Acetylcholine esterase as well as less toxicity in mice than Hup A. ZT-1 has similar properties to Hup A, regarding the ability to cross the blood-brain barrier, its oral bioavailability, and its longevity of action. ZT-1 is a promising candidate for clinical development as a symptomatic treatment for AD [7, 12].

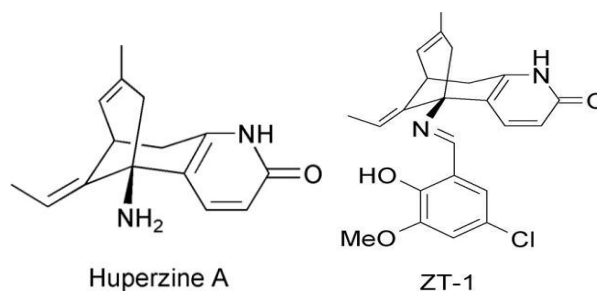


Fig. 8: ZT-1-Semisynthetic analogue of Huperzine

6. BIOACTIVITIES OF HUP A

6.1.Acetylcholine-Esterase inhibition (AChEI)

Hup A acts as very potent AChEI, thereby alleviating many of the symptoms and in potentially slowing the progression of AD. Key symptoms associated with AD is caused by cholinergic dysfunctioning in the brain, acetylcholine level are markedly decreased in Alzimers disease. Hence AChE (enzyme which hydrolyses acetylcholine) inhibitors (AChEI) are the first group of compounds to be effective in the treatment of AD. Hup A produces marked effect by inhibiting acetylcholine-esterase, delaying hydrolysis of acetylcholine, and enhancing the level of acetylcholine in synaptic cleft. Structural biology investigations (particularly by X-ray crystallography and computational modeling) have found that Hup A acts against AChE by directly binding to the opening of the active site in this enzyme, thus preventing access to the active site by the normal substrate.

Table 3: Anticholinesterase effects of Choline-ester inhibitor *in vitro*

ChEI	(IC ₅₀) μM AChE (rat cortex)	(IC ₅₀) μM BuChE (rat serum)
HuPA	0.082	74.43
Galanthamine	1.995	12.59
Donepezil	0.010	5.01
Tacrine	0.093	0.074

Comparison studies with respect to *in vitro* and *in vivo* experiments on AChE inhibition showed that potency of Hup A was similar or superior to inhibitors currently being used in AD. Hup A causes distinct concentration dependent inhibition *in vitro* of AchE and butyrylcholinesteras (BuChE). The AchEI activity of Huperzine A relative to other AchEI is: Donepezil>HuperzineA>Tacrine>physiostigmine>galan tamine. Hup A is found to be potent and selective one. IC₅₀ (concentration of the inhibitor yielding 50%

inhibition of enzyme activity) values of inhibitors were compared in these *in vitro* experiments. These values revealed that that Hup A was more potent than tacrine and galanthamine, but about two fold less potent than donepezil [13].

6.2. Effect on learning and memory function

Based on investigations with animal models (particularly with mice), HupA has been shown to enhance learning and memory. This was found to be true for adult mice, aged mice, and mice with cognition damage. Injecting Hup A into the abdominal cavity inhibits memory damage in mice treated with cycloheximide, erinitrit, and scopolamine. This treatment also promotes memory retention in aged mice. When mice treated with Hup A were evaluated by the escaping reflection test, learning and memory were both enhanced by abdominal cavity injection and by oral administration. Hup A can also improve performance speed in the reaction of moving avoidance test.

Hup A has also been evaluated in with other animal models for effects on cognitive function. Cognitive impairment induced in monkeys via an adrenergic mechanism. Hup A improved the spatial working memory in aged monkeys and in young adult monkeys. Hup A attenuated cognitive deficits and brain injury in neonatal rats after hypoxia-ischemia and cognitive dysfunction and neuronal degeneration in rat caused by b-amyloid protein. Hup A reverses scopolamine and muscimol-induced memory deficits in chicks.

6.3. Neural cell protection

Studies showed that Hup A has also role in Alzheimers disease by non cholinergic mechanism. This role include antagonist effect on the NMDA (N-methyl-D-aspartate) receptor and the protection of neuronal cells against Ab (bamyloid peptide), These roles could also play importance in Alzheimers disease treatment [7, 12-14].

7. CLINICAL TRIALS ON HUP A

Clinical trials performed with have Hup A demonstrated that Hup A produces significant improvements in memory deficiencies in aged and AD patients. Most of these studies have been performed in China; where an estimated 100000 people have been treated by Hup A. Results of these studies indicate that Hup A is an effective and safe drug that improves cognitive function. A short summary of major findings of Huperzine A clinical trials in China and USA follows. Zhang *et al.* conducted a

randomized, placebo controlled, multicenter study with 202 patients diagnosed with possible or probable Alzmiers disease. One group of 100 patients was administered 400 µg days Hup A for 12 weeks and 102 patients received placebo. The treatment group displayed improvements in cognition measured on the AD Assessment Scale (ADASCog) as well as an increase in the ability to do activities of daily living and improvement in behavior and mood [15].

In addition, Hup A may have application for younger people as well. Sun and co-workers reported that Hup A enhanced the Memory and learning performance of adolescent students. With a double blind and matched-pair method, 34 pairs of junior middle school students complaining of memory inadequacy were divided into two groups. The memory quotient of the students receiving HupA was higher than those of the placebo group, and the scores on Chinese language lessons in the treated group were also markedly elevated [16].

Xu and co-workers also evaluated AD patients. Sixty AD patients were divided in to two groups taking Hup A (200 µg twice a day orally for 60 days) in either capsules or tablets, respectively. There were significant differences on all the psychological evaluations between “before” and “after” the 60 days trials for the two groups. No severe side effects except mild to moderate nausea were observed. No difference was observed between the two groups [17].

In the United States, the safety and efficacy of Hup A were evaluated in 26 patients meeting the DSM IV-R (Diagnostic and Statistical Manual of Mental Disorders - Fourth Revision) and the NINCDS-ADRDA (National Institute of Neurological and Communicative Disorders and Stroke) criteria for uncomplicated AD and possible or probable AD. This study (office based) lasted 3 months and was open label. Other therapies, including tacrine, donepezil and G. biloba were continued. Despite the small number of patients, the authors observed dose-related improvements with higher MMSE (Mini-Mental State Examination scores) at higher dosage, and no serious side effects. It was also noted that Hup A is more effective and safer than other drugs that affect the cholinergic system and that are currently on the market in the USA for the treatment of mild to moderate AD [18].

8. IN VITRO PRODUCTION OF HUPERZINE A

Huperzia serrata; the original source of Huperzine A actually possesses a very low content of Huperzine A

0.007% has very limited distribution, and grows very slowly. It takes at least 15 years from spore germination through the gametophyte stage to finally reach the mature sporophyte stage. While some other species in the Huperziaceae produce larger amounts of Huperzine A, these species are even more difficult to obtain and are much rarer in nature than *Huperzia serrata*, making them even less desirable candidates as natural sources for Huperzine A. Not much work on commercial cultivation has been reported for *Huperzia serrata* or other species in the Huperziaceae. Because of this, there is keen interest in developing alternative methods to produce Huperzine A.

Xiaoqiang Ma, David R. Gang succeeded propagating *Phlegmariurus squarrosus* in *in vitro* conditions. Gametophytes (prothalli) and mature sporophytes were successfully produced from *in vitro* cultures of *Ph. squarrosus* after six months to one years of culture. These cultured plants are now growing well, and will provide an invaluable resource for production of Huperzine A [19, 20].

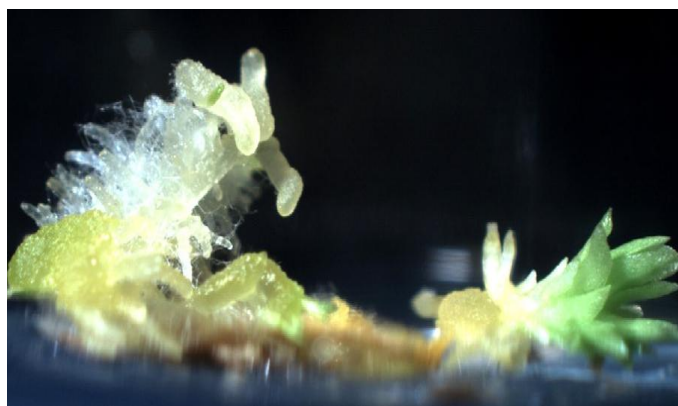


Fig 9: *Phlegmariurus squarrosus* in vitro propagated sporophyte

8.1. TOXICOLOGY

Toxicological studies conducted in different animal species indicated less severe undesirable side effects associated with cholinergic activation for Huperzine A than for other ChEIs such as physostigmine and tacrine. Histopathological examinations showed no changes in liver, kidney, heart, lung or brain after administration of Huperzine A in rats and in dogs. No mutagenicity was found in rats, and no teratogenic effect was observed in mice or rabbits [13].

9. CONCLUSION

In view of the importance of Hup A for its use in China for the treatment of Alzheimer's disease and organophosphate poisoning, and as no work has been done so far on other countries like India hence it is suggested that all the species in world of Huperzia may be investigated for their general distribution and abundance, their medicinal properties and phytochemical studies to isolate active compounds for pharmaceutical uses. Because they are the major source of Huperzine A and the only natural source, methods to propagate Huperzine A containing plants in Vitro must be developed to protect this very valuable but threatened group of natural medicinal plants.

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