IJPSR (2012), Vol. 3, Issue 11 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 July, 2012; received in revised form 10 August, 2012; accepted 29 October, 2012

EVALUATION OF THE ANTI-AMYLOIDOGENIC POTENTIAL OF NOOTROPIC HERBAL EXTRACTS *IN VITRO*

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Keywords:
Alzheimer disease, Amyloid ß, thioflavin T, Medicinal plants

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QUICK RESPONSE CODE

IJPSR:
ICV (2011)- 5.07

Website:
www.ijpsr.com

ABSTRACT

Alzheimer's disease (AD) is characterized by the abnormal aggregation of amyloid ß (Aß) peptide into insoluble fibrils called amyloid plaques. Preventing this process of fibrillization may offer effective therapy for treatment of AD. The aim of the present study is to investigate the effect of methanolic extracts of 13 plant species which are known to be brain boosters in Ayurvedic system of medicine. Our study addressed the influence of these extracts on (i) prevention of aggregation of Aß and (ii) dissociation of preformed Aß fibrils. The aggregation status was monitored by thioflavin T fluorescence assay. The results showed that extracts from *Bacopa monneria, Centella asiatica, Convolvulus pluricaulis, Withania sominfera, Nardostachys jatamansi* and *Glycirrhiza glabra* exhibited promising activity by preventing Aß fibril formation/ retention thus identifying Aß as the molecular target for their action. These findings prompt further studies to isolate the active ingredients from these extracts to ultimately determine their therapeutic potential in AD treatment.

INTRODUCTION: Alzheimer's disease (AD), the leading cause of dementia in the elderly, is an irreversible, progressive neurodegenerative disorder. AD brain has two characteristic neuropathological features i.e., extracellular amyloid ß (Aß) plaques and intra-neuronal neurofibrillary tangles [1].

Abnormal processing and extracellular deposition of Aβ (1-42), a proteolytic derivative of the larger amyloid precursor protein (APP) is a key step in the pathogenesis of Alzheimer's disease [2]. The current therapeutic efforts are directed towards developing drugs that reduce Aβ burden or toxicity by inhibiting its production, aggregation or misfolding, toxicity, Aβ-metal interactions or by promoting Aβ clearance by neutralizing or removing the toxic aggregate or misfolded forms of these proteins [3, 4].

Also immunization with Aβ (1-42) has been shown to decrease brain Aβ deposition and improve cognitive performance in the transgenic mouse models of AD [5].

India has the traditional Ayurvedic system of medicine in which a number of plants are used for the treatment of variety of diseases. Over the last 4000 years, Ayurvedic medical practitioners across India have been using some of the plants which are classified as "medhya rasayanas" or nootropic cerebral activators for treating disorders of the central nervous system (CNS) and also to improve the memory and intellect. While pharmaceutical companies continue to invest enormous funding and time on identifying agents that could be useful for treating AD, these Ayurvedic phytochemicals would appear to have significant benefits without causing any side effects that have yet to be fully exploited.

Experimenting with novel therapeutic molecules becomes relevant and this study is an attempt to shortlist a few plant based compounds which might interfere with the disease pathology either by preventing or slowing the progress of AD. Plants included in the present study are: *Bacopa monniera*, *Centella asiatica, Withania Somnifera*, *Acorus calamus*, *Nardostachys jatamansi*, *Convolvulus pluricalis, Glycirrhiza glabra, Emblica officinalis, Punica granatum, Terminalia chebula, Coriandrum sativum, Saussurea lappa* and *Tinispora cordifolia.* These plants have been shown to have anti-anxiety activity, anti-fatigue and memory enhancing effects [6, 7].

Among these, bacosides from B*acopa monniera* extract have been shown to reduce the amyloid plaque levels in PSAPP mice [8]. Asiaticoside from *Centella asiatica* has been reported to have protective effects from Aβ induced neurotoxicity [9]. Extracts from *Withania somnifera* have reversed AD pathology in APP/PS1 transgenic mice by enhancing low density lipoprotein related receptor levels in the liver [10].

Our current study focused on identifying whether Aß is the molecular target of these phytocompounds in exerting their beneficial effects. This was achieved by evaluating the ability of the methanolic extracts of the useful parts from the above 13 nootropic plants on;

(i) Prevention of aggregation of Aß and;

(ii) Dissociation of preformed Aß fibrils.

MATERIALS AND METHODS:

Materials: Dimethylsulfoxide and thioflavin T were purchased from Sigma Aldrich (Bangalore). The desiccated plant materials were obtained from Arya Vaidya Sala (Kottakkal). The native Aß used in the present study was recombinantly expressed and purified as described earlier [11]. All other reagents used were of analytical grade and obtained locally.

Preparation of methanolic extracts of the plant parts: 5g each of powdered plant parts were covered and tied in double layer muslin cloth and kept in the soxhlet extraction unit. 65ml of methanol was placed in the solvent round bottom flask and heated at 60^{o}C and the methanolic extract was collected in the upper chamber. Filtered extract was concentrated to evaporate the methanol completely in vacuum centrifuge and the dried extract was stored in -20^{o}C. Extracts were solubilised in dimethylsulfoxide and 100 µg/ µl stocks were prepared and used for aggregation studies.

Preparation and characterization of aggregates of Aß: Native Aß was dissolved in dimethylsulfoxide, followed by dilution with double distilled water and then with phosphate buffered saline to eventually prepare a 200 µM stock solution. This stock (200 µl) was incubated with 20 mM thioflavin T at 37^{o}C for 5 days with intermittent shaking during the incubation to facilitate the formation of Aß aggregates. Formation of Aß fibrils was confirmed by measuring the fluorescence intensity (Excitation: 446 nm; Emission: 490 nm) in a fluorescence spectrophotometer (Shimadzu, RF 5301 PC).

Influence of plant extracts on inhibition/ dissociation of Aß fibril formation: To evaluate the influence of extracts on dissociation of Aß fibrils, 200 µl of the above preformed Aß aggregate was incubated with 200 µg of the extract per tube for 24 h at 37^{o}C followed by fluorescence measurement studies. To assess the effect of these extracts on formation of Aß fibrils, soluble Aß (200 µM) was co-incubated at 37^{o}C along with 200 µg of the extract. After 5 days, an aliquot was taken for fluorescence measurements. Each of the extracts were incubated without Aß and processed for thioflavin T binding and those values served as the corresponding negative controls.

RESULTS AND DISCUSSION: Alzheimer's disease (AD) has a complex pathology with multifactorial mental illness, characterized by loss of memory and cognition. The etiological factors include oxidative stress, inflammation, Aß overexpression, tau hyper-phosphorylation, metal toxicity etc [12-14].

For AD, there are relatively few drugs available to treat symptoms; there is a lack of successful therapy that can modulate disease progression. Since two of the currently licensed drugs for AD are based on natural products (galantamine and rivastigmine) [15], many plants are now being investigated (**Table 1**) as a potential source of new therapy for AD [16].

TABLE 1: LIST OF SOME OF THE MEDICINAL PLANTS AND THEIR ACTIVE INGREDIENTS CLINICALLY RELEVANT TO ALZHEIMER DISEASE

Plant species	Active component	Observations
Bacopa monneria	Bacosides	Anti-anxiety activity, anti-fatigue and memory enhancing effects, decreases stress induced biomarkers in hippocampus [17, 18]
Celastrus peniculatus	Celapagine	Reverses deficits in navigational memory task in rats [19]
Ginkgo biloba	Ginkgolides	Vasoregulatory, cognition enhancing, stress alleviating and gene regulatory functions [20]
Huperzia serrata	Huperzine	AD therapy in China [21]
Ricinus communis	Ricinine	Memory enhancing property [20]
Centella asiatica	Asiaticoside	Memory enhancer [9]
Cannabis sativa	Cannabidiol	Anti-oxidant, anti-psychotic, anxiolytic and neuroprotection against Aß and hyperphosphorylated tau induced insults in neuronal cells [22]
Curcuma longa	Curcumin	Anti-oxidant, anti-inflammatory, neuroprotection against Aß [23]
Magnolia officinalis	Magnolol	Neuroprotective against Aß toxicity in vitro, prevents cholinergic deficits *in vivo* [24]
Vinca minor	Vincamine	Improves memory in animal models of AD [25]
Physostigma venenosum	Physostigmine	Improves cognition *in vivo* [26]
Withania somnifera	Withanosides	Promotes cognition and memory [27]
Convolvulus pluricaulis	Shankhapushpine	Brightens the memory and intellect [28]

In the present study, we present the results of studies designed to elucidate the molecular target of action of the extracts obtained from the following list of plants (**Table 2**) short listed with inputs from literature and traditional Indian medicinal plants originally classified as brain boosters. First, methanolic extracts of the plants were prepared from 5gm each of the dried plant parts in Soxhlet apparatus wherein the dry plant part was subjected to methanol heated at 60^{o}C. It was further filtered and vacuum centrifuged to dryness and reconstituted in dimethy sulfoxide to a final concentration of 100 µg/ µl.

TABLE 2: LIST OF THE PLANTS USED IN THE PRESENT STUDY AND THE PERCENTAGE YIELD OF THE METHANOLIC EXTRACT

Botanical name	Common name	Part used	% Yield of the methanolic extract
Bacopa monniera (BM)	Brahmi	Whole plant	7.32
Centella asiatica (CA)	Gotukola	Whole plant	6.35
Acorus calamus (AC)	Sweet flag	root	11.33
Convolvulus pluricaulis (CP)	Shankhapushpi	Whole plant	4.39
Withania somnifera (WS)	Ashwagandha	root	3.94
Nardostachys jatamansi (NJ)	Jatamansi	root	3.38
Glycyrrhiza glabra (GG)	Licorice	root	5.66
Emblica officinalis (EO)	Gooseberry	fruit	7.6
Punica granatum (PG)	Pomegranate	fruit	26.97
Terminalia chebula (TC)	Harithaki	fruit	25.58
Coriandrum sativum (COR)	Coriander	leaves	10.06
Saussurea lappa (SL)	Costus	root	20.11
Tinospora cordifolia (TC)	Guduchi	stem	2.02

Figure 1 shows the results on anti-amyloidogenic potential of the above listed plant extracts. All the extracts showed varying degrees of inhibition and dissociation of the preformed Aß aggregates. Some of the plant extracts those which have effectively interfered with the aggregation of Aß did not have any effect on dissociation of preformed Aß fibrils.

Almost complete inhibition of aggregation was obtained by extracts of *Nardostachys jatamansi, Coriandrum sativum*, *Glycyrrhiza glabra, Convolvulus pluricaulis, Bacopa monniera, Centella asiatica, Withania somnifera* and *Tinospora cordifolia* whereas moderate inhibition was exhibited by *Terminalia chebula, Saussauria lappa* and *Punica granatum.*

Similarly *Glycyrrhiza glabra, Convolvulus pluricaulis, Bacopa monniera, Centella asiatica, Nardostachys jatamansi* and *Emblica officinalis* showed considerable effect on the dissociation of aggregates among which *Glycyrrhiza glabra* was the most effective.

Extracts of *Glycyrrhiza glabra, Convolvulus pluricaulis, Bacopa monniera, Centella Asiatica and Nardostachys jatamansi* inhibited aggregation as well as dissociated the preformed aggregates to a considerable way.

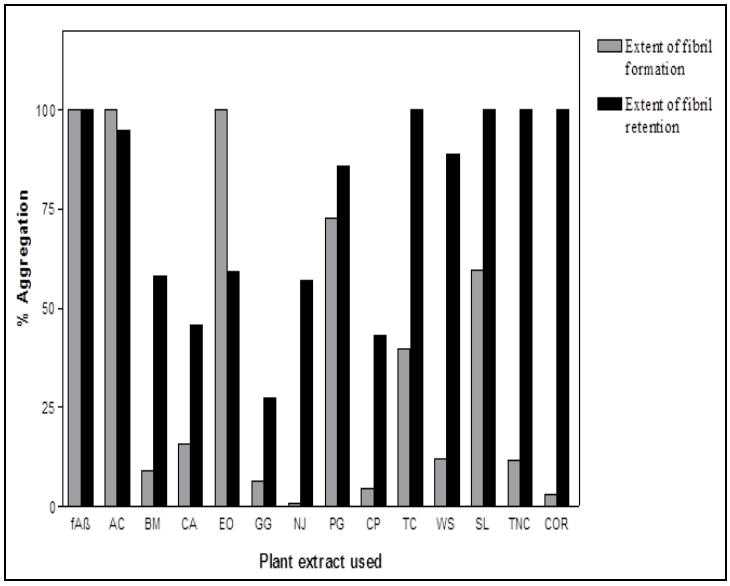

FIGURE 1: EFFECT OF PLANT EXTRACTS ON INHIBITION OF AGGREGATION AND DISSOCIATION OF PREFORMED AGGREGATES. FLUORESCENCE INTENSITY OBTAINED WITH FIBRILLAR Aß IN THE ABSENCE OF ANY PLANT EXTRACT WAS TAKEN AS 100%.

In summary, herbal extracts which are widely used in Ayurveda for improving the CNS functions were evaluated for their anti-amyloidogenic properties using *in vitro* Aβ aggregation studies. Among the plants tested, results obtained with *Bacopa monniera, Centella asiatica* and *Withania somnifera* are in agreement with the reports from literature in terms of their influence on destabilization of Aß fibrils.

In addition, the current findings offer direct evidence on the influence of the extracts from *Glycyrrhiza glabra, Convolvulus pluricaulis* and *Nardostachys jatamansi* in favour of Aß-centric therapy for AD. Further studies are in progress regarding the isolation of active phytocompounds from these extracts.

However, since this study provides only results obtained from *in vitro* studies, further studies must be carried out in animals, to assess the activity and also the bioavailability of the isolated compounds or extracts, which is especially critical for their action in the central nervous system.

ACKNOWLEDGEMENTS: Financial assistance to SS from Department of Science & Technology, New Delhi is gratefully acknowledged. MM is a fellow of WOS-A scheme funded by Department of Science & Technology, New Delhi, India.

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How to cite this article:
Mathew M and Subramanian S: Evaluation of the Anti-Amyloidogenic Potential of Nootropic Herbal Extracts *in-vitro*. *Int J Pharm Sci Res*. 3(11); 4276-4280.