Neuroprotective potential of ethanolic extract of *Pseudarthria viscida* (L) Wight and Arn against β-amyloid(25-35)-induced amnesia in mice

J C Hanish Singh¹, V Alagarsamy², P Parthiban², P Selvakumar³ and Y Narsimha Reddy⁴*

¹Department of Pharmacology, Lalitha College of Pharmacy, Ghatkesar, Gr. Hyderabad 501301, Andhra Pradesh
²Medicinal Chemistry Research Lab., MNR College of Pharmacy, Sangareddy, Gr. Hyderabad 502294, Andhra Pradesh
³Organic Chemistry Division, Central Leather Research Institute, Adyar, Chennai 600020, Tamil Nadu, India
⁴Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506 009, Andhra Pradesh, India

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The neuroprotective potential of ethanolic extract of roots of *Pseudarthria viscida* (L) Wight and Arn (EEPV) was investigated against β-amyloid(25-35)-induced amnesia in mice which is a suitable animal model for Alzheimer’s disease (AD). The senile plaques of β-amyloid (Aβ) are major constituents accumulated during the progression of AD as a potent neurotoxicant. In our investigation, intracerebroventricular injection of Aβ(25-35) in mice induced the neurodegeneration, exhibited the increased time of escape latency in behavioral pattern using water maze and decreased the levels of antioxidants namely superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and vitamin C with elevated level of acetylcholinesterase enzyme (AChE). The neuroprotective potential of EEPV was determined by behavioral pattern using water maze and biochemical parameters such as SOD, CAT and GPx and vitamin C content as well as AChE. Mice were treated with EEPV at 200 and 400 mg/kg doses for 21 days. Except control, all animals received a single injection of neurotoxicant Aβ(25-35) on 14th day. In behavioural assessment, treatment with ethanolic extract improved the cognitive function in the water maze and attenuated the elevated levels of AChE with increase in antioxidant enzymes, indicating the neuroprotection with increased levels of vitamin C. These findings suggest that ethanolic extract of *P. viscida* exerts anti-amnesiac effects and enhances cognitive function.

**Keywords:** Alzheimer’s disease, β-Amyloid, Acetylcholinesterase, Water maze, Oxidative stress, *Pseudarthria viscida* (L) Wight and Arn, Antioxidant enzymes, Vitamin C.

Cognitive impairment represents the initial stage of memory loss leading to Alzheimer’s type of dementia. Alzheimer’s disease (AD) is a typical neurodegenerative disease develops substantially in the aging brain. It is associated with neuropathological changes by accumulation of β amyloid peptide (Aβ) fragments. Aβ(25-35) fragment is more toxic to neurons and forms the neurofibrillary tangles, causing the increased vulnerability. Amyloid plaques appear during prime phase of AD in temporal neocortex, entorhinal cortex and hippocampus.

The pathological change of AD includes cholinergic dysfunction, increased levels of acetylcholinesterase (AChE), monoamine oxidase (MAO) and oxidative damage. The cholinergic alternation is evidenced with reduction of muscarinic and nicotinic acetylcholine receptors located on the presynaptic nerve terminals. AChE activity is increased around amyloid plaques which further promote the amyloid fibrils and complexes with Aβ. The complex thus formed exerts potent toxicity than Aβ and was suggested to disturb the calcium homeostasis causing excitotoxicity. Free radicals are enhanced by the process of aggregation of Aβ, leading to oxidative stress and inflammation.

In AD, brain is under extensive oxidative stress manifested by lipid peroxidation (LPO), increased protein oxidation in the hippocampus and frontal cortex. DNA oxidation is denoted by increased levels of 8-hydroxyl-2-deoxyguanosine and 8-hydroxyguanosine associated with deficiency of DNA repairing. Protein carbonyl levels are elevated and increased nitration of tyrosine residues indicates the oxidative modification of proteins. MAO-A and B are implicated in AD. On biogenic amine metabolism, oxidation and deamination produce NH₃ and H₂O₂ with potential toxicity. Moreover, on accompanying with cholinergic hypofunction, MAO is thought to increase secondarily in the hippocampus.

In indigenous preparations of traditional medicine, herbs are the common element which makes healthy life-span. The leads of CNS active plants are vastly explored, due to their rejuvenating and neurotonic potential. In Indian traditional medicine, the whole plant and the roots of *Pseudarthria viscida* (L) Wight and Arn, a shrub belonging to the family Fabaceae are used as nervine tonic, astringent, thermogenic, digestive, anthelmintic, anti-inflammatory, diuretic,
aphrodisiac, cardiotonic and for rejuvenation. The plant is also useful in conditions of cough, bronchitis, asthma, tuberculosis and general debility. Earlier, antifungal activity in various extracts obtained from callus of leaves, stem and roots, in vitro angiotensin-converting enzyme inhibitory activity in ethanolic extract of roots and antioxidant activity due to the presence of phenolic compounds present in it have been reported from the plant.

In the present study, we have investigated the neuroprotective potential of roots extract in AD type of amnesia induced by Aβ(25-35) in mice by determining behavioral pattern using escape latency in water maze and biochemical parameters such as antioxidant enzymes namely SOD, GPx and CAT and vitamin C content, as well as AChE.

Materials and Methods

Chemicals

Acethyliothiocholine iodide, β-amyloid, reduced glutathione, oxidized glutathione and dithiobisnitro benzoic acid were purchased from Sigma-Aldrich, USA. All other reagents were of analytical grade and from SD Fine Chemicals, Mumbai, India.

Plant material and extraction

Pseudarthria viscida (L) Wight and Arn was collected from Trivandrum, Kerala, India during the month of August. The roots were authenticated by plant biotechnologist Dr. Girija Kumari, Head, Department of Plant Biology, Sree Ayappa College, Chunkankadai, Kanyakumari district, Tamilnadu (India). A voucher specimen was deposited in the Pharmacognosy department, MNR College of Pharmacy, Hyderabad, Andhra Pradesh (India). A voucher specimen was deposited in the Pharmacognosy department, MNR College of Pharmacy, Hyderabad, Andhra Pradesh (India) for future reference. The roots were thoroughly washed on running water, shade-dried, coarsely powdered and extracted with absolute ethanol in soxhlet apparatus for 72 h. The extract obtained was concentrated and dried under vacuum condition and the yield was noted in terms of w/w of dry material as 16.8%. The thick consistent extract was stored in desiccators and suspension of the extract made in 2.5% acacia was used for the experimental investigations.

Preliminary phytochemical analysis

The preliminary screening carried out with standard procedures indicated the presence of alkaloids, flavonoids, tannins, phenolic compounds and saponins.

Experimental animals

Male Swiss albino mice weighing 22-25 g and with 5-6 weeks of age were used for the pharmacological screening. Standard conditions were maintained, 25 ± 3°C; 35 to 60% humidity and 12 h light/dark cycle, and animals were given standard pellet diet (Hindustan Lever Ltd, Bangalore) and water ad libitum. Prior to pharmacological investigation, the animals were acclimatized to the laboratory conditions for a week. The animals were randomly divided into four groups of each six animals. The experimental protocol complied with the Institutional Animal Ethical Committee of CPCSEA and duly approved by C L. Baid Metha College of Pharmacy, Chennai, Tamilnadu (India). (IAEC/XXIX/15/CLBMCP/2010).

Acute oral toxicity study

Acute oral toxicity was performed as per the OECD guidelines. The animals were kept fasting for overnight providing only water, and after which the extract (500 mg/kg) was administered orally and observed for 14 days. The animals were observed continuously for 3 h and then for each h during 24 h after administration of the extract for any change in general behavior or other physiological activities. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic and if mortality was observed in 1 animal, the dose administered was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 2000 mg/kg.

Amnesia induction and grouping

Amnesia was induced by intracerebroventricular (i.c.v.) injection of Aβ(25-35) peptide (Sigma: A4559-250UG) through bregma point in skull; 10 µl containing 10 µg of Aβ peptide was administered for each animal. In brief, bregma was identified by rubbing the point of needle over the skull (approximately 1-3 mm rostral to the line drawn through anterior base of ears). Then at 45° angle, the needle was inserted 2 mm lateral to midline and Aβ peptide was injected into the cortex.

Four groups, each with six animals were maintained, such as Group I: vehicle control received with i.c.v. injection of phosphate-buffered saline (PBS), Group II: negative control received only with i.c.v. injection of neurotoxic Aβ, groups III and IV animals injected with Aβ and treated with 200 and
400 mg/kg (p.o) ethanolic extract of *P. viscida* (EEPV), respectively. Except control group, all the animals received Aβ injection on the 15th day of EEPV pretreatment and continued up to 21 days. The negative control animals received only the neurotoxic Aβ.

**Behavioral study (Morris water maze test)**

The Morris water test was performed as described. The experimental apparatus consisted of a circular water tank (diameter = 100 cm; height = 35 cm) containing water at 28°C to a depth of 15 cm and rendered opaque by adding powdered milk. A platform (diameter 4.5 cm; height 14.5 cm) was submerged 0.5 cm below the water surface and placed at the midpoint of one quadrant. Training sessions were conducted before the injection of Aβ for their spatial recognition. After several trials, the test was conducted on the 5th day after injection of Aβ and the time required to escape on to the platform was recorded in sec (s).

**Biochemical analysis**

AChE activity was measured in 20% of brain homogenate in phosphate buffer (0.1 M; pH 8) by the rate of formation of thiocline from acetylcholine iodide in the presence of brain cholinesterase on basis of formation of yellow colour, due to the reaction of acetylthiocholine with 5,5'-dithiobis-(2-nitrobenzoic acid). The change in optical density (OD) was monitored using spectrophotometer (Shimadzu) at 412 nm.

Superoxide dismutase (SOD) activity was assayed by pyrogallol oxidation. The assay mixture contained 1 ml of pyrogallol-Tris-DETPA, 0.2 ml of suitably diluted tissue homogenate and 0.8 ml of water. The rate of pyrogallol oxidation was taken by pyrogallol oxidation degraded/min/mg of protein.

Catalase (CAT) activity was determined by H₂O₂ degradation method. Briefly, 50 µl of sample was mixed with 50 µl of substrate (6.5 µM H₂O₂ in phosphate buffer) for 60 s, then 100 µl of 32.4 mM ammonium molybdate solution were added and absorbance change was measured at 405 nm. One unit of the enzyme was defined as mm of H₂O₂ degraded/min/mg of protein.

For the determination of vitamin C, to 0.5 ml of homogenate, 0.5 ml of water and 1 ml of trichloroacetic acid was mixed thoroughly and centrifuged. To the supernatant, 2, 4-dinitrophenyl hydrazine reagent was added and incubated at 37°C for 3 h. Then sulfuric acid was added, mixed well and the solutions were allowed to stand at room temperature for another 30 min. The color developed was read at 520 nm. The level of ascorbic acid was expressed as µg/mg protein.

**Statistical analysis**

The data were expressed as mean ± SEM and statistically analyzed using one-way ANOVA, followed by Tukey’s multiple comparison test. *P* < 0.05 was considered to be statistically significant. The statistical analysis was carried out with Graph Pad prism 4.0 software.

**Results**

The hippocampal learning and memory activity assessed by water maze and the activities of AChE determined in brain tissue and of various enzymes of free radical scavenging system and vitamin-C are depicted in Table 1. The changes in escape latency after the induction of amnesia by β-amyloid was significantly (*P* < 0.001) reduced in group II, when compared with control animals. EEPV 200 mg/kg and 400 mg/kg treated groups showed significant (*P* < 0.05, *P* < 0.01) decrease in escape latency in a dose-dependent manner.

Microinjection of β-amyloid significantly increased the AChE activity (*P* < 0.001), when compared with the control group treated with PBS. In the treatment groups, both low and high doses of EEPV showed significant (*P* < 0.01, *P* < 0.001) decrease in levels of AChE in dose-dependent manner, when compared with negative control.

The SOD levels in the animals treated with β-amyloid showed a significant (*P* < 0.001) decrease, when compared to PBS-treated groups. The enzyme activity was significantly increased in the treatment...
groups of 200 mg/kg and 400 mg/kg (P<0.01, P<0.001 respectively), when compared to the group II animals. Similarly, the GPx levels decreased significantly (P<0.001) in the neurotoxicity-induced group when compared with control group. Significant increase in the enzyme activity was observed in both the treated (P<0.01, P<0.001 respectively). CAT activity also decreased significantly (P<0.001) in the group II animals when compared with control group. The EEPV-treated animals in 200 mg/kg showed significant increase (P<0.05) and 400 mg/kg treated animals highly significant (P<0.001) increase in CAT activity. The negative control group showed a significant (P<0.001) decrease in vitamin C levels when compared to the control PBS-treated animals. The level of ascorbic acid in EEPV-treated animals increased significantly (P<0.05, P<0.001) in 200 mg/kg and 400 mg/kg treated animals respectively, when compared to negative control group.

Discussion
The present study demonstrates the neuroprotective effect of *P. viscida* against Aβ(25-35)-induced neurodegeneration in mice. Aβ peptide is a factor for underlying pathology of AD. In all fragments of Aβ peptide, Aβ(25-35) in particular has shown selective potent neurotoxic property, as it promotes the inflammatory process and fibrillary aggregation. In this investigation, we induced the amnesia in mice by injection of Aβ(25-35), which is a suitable model for AD. Spatial learning in water maze test depicts the hippocampal learning of the animals. In our study, treatment of EEPV showed the improvement in cognition with hippocampal activity.

Earlier it has been demonstrated that impairment in learning, memory and behavior in AD is caused due to the dysfunction of cholinergic system. Studies in animal and human subjects have revealed that learning and memory can be modified by drugs affecting the central cholinergic system. Cholinergic transmission is terminated mainly by hydrolysis of acetylcholine through AChE in the synapse and is currently believed that one of the consequence for progression of AD. Currently, AChE inhibitors are used for the treatment of AD. In our study, we evaluated the AChE activity and interpreted with its anti-amnesiac activities. EEPV inhibited AChE activity significantly in the brain homogenate depicts its potential as an anti-amnesiac drug.

Oxidative stress of amnesiac rats resembles the clinical pathology observed in AD patients. In our experimental conditions, the treatment of Aβ caused a significant decrease of SOD, GPx and CAT and vitamin C levels. Administration of EEPV indicated the antioxidant property with significant increase of SOD, GPx and CAT levels and vitamin C in the brain, observed similar to normal control mice. GPx reduces toxic radicals using GSH (glutathione) as a substrate, in turn is reduced again to GSH by glutathione reductase with consumption of NADPH, forming a redox cycle. Antioxidants such as β-carotene and vitamins A, C and E may protect the cells from protein and nucleic
acid oxidation in neurons that leads to aging in the brain and tissues. Both vitamins C and E are antioxidants, which are likely to reduce oxidative stress and injury in the central nervous system, which may reduce the Aβ-plaque deposition in the neuronal cells. Ascorbic acid is useful for recycling tocopherol and recycles oxidized transition metals back to their reduced forms. The inhibition of AChE was evidently indicated by the up-regulation of cholinergic neurotransmission. Based on these findings, P. viscida may be used as a promising rejuvenating agent for the prevention or treatment of AD type of dementia.

In conclusion, present study demonstrated that P. viscida prevented the neurodegeneration and improved the cognitive function, followed by icv injection of Aβ(25-35) in mice by inhibiting the AChE activity and restored the neuronal function by scavenging the ROS. The inhibition of AChE was evidently indicated by the up-regulation of cholinergic neurotransmission. Based on these findings, P. viscida may be used as a promising rejuvenating agent for the prevention or treatment of AD type of dementia.

References