



# INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)

Platform for Pharmaceutical Researches & Innovative Ideas  
www.ijprd.com

## EVALUATION OF NOOTROPIC POTENTIAL AND *IN-VITRO* ANTIOXIDANT ACTIVITY AQUEOUS EXTRACT OF ROOTS OF *ASPARAGUS RACEMOSUS* IN RATS

Sumalatha Gindi\*<sup>1</sup>,

Baburao Chandu<sup>2</sup>, Mukkanti Khagga<sup>3</sup>, Siva R Challa<sup>4</sup>, Varun Dasari<sup>1</sup>

<sup>1</sup>Hindu College of Pharmacy, Amaravathi road, Guntur, Andhra Pradesh, India

<sup>2</sup>Donbosco PG college of Pharmacy, 5th Mile, Pulladigunta, Guntur, Andhra Pradesh, India

<sup>3</sup>Centre for Pharmacy, JNT University, Hyderabad, Andhrapradesh, India

<sup>4</sup>KVSR Siddhartha College of Pharmaceutical Sciences, Vijayawada, Andhra Pradesh, India

### ABSTRACT

The objective of the study was to investigate the influence of aqueous extract of *Asparagus racemosus* (AR) on learning, memory and antioxidant properties of the roots of *Asparagus racemosus*. The animal models selected for the present study were a model of Scopolamine induced cognitive deficits and Aluminium induced cognitive deficits in rats for evaluating Nootropic activity. The antioxidant activity of *Asparagus racemosus* was determined by estimating DPPH radical scavenging activity, nitric oxide radical scavenging activity using spectrophotometric methods. The reversal effect of *Asparagus racemosus* on Transfer latency measured on 1<sup>st</sup> and 2<sup>nd</sup> day was significant in scopolamine treated rats and it was found that *Asparagus racemosus* prevented the cognitive deficits produced by sub-chronic aluminium administration in rats in Elevated plus maze technique. In addition, moderate anti-oxidant activity in terms of DDPH radical scavenging activity and NO radical scavenging activity was observed. It was concluded that aqueous extract of *Asparagus racemosus* improves the memory in two animal models of cognitive deficits.

### Correspondence to Author



Mrs. G. Sumalatha

Assistant Professor, Department of Pharmacology, Hindu College of Pharmacy, Amaravathi road, Guntur, Andhrapradesh, India

### Email

sumacology@gmail.com

### Key Words

*Asparagus recemosus*, cognitive function, memory, Aqueous extract.

## INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder, which according to World Health Organization (WHO) affects 22 million people world wide, out of which, over 3 millions are in India. Its prevalence rises sharply from about 5% at the age of 95 years<sup>1</sup>. Though there are drugs available that aim at slow progression of disease, an affirmative cure to Alzheimer's disease still eludes researchers.

Principally memory process consists of registration, consolidation and retrieval. Registration is the process of sensory perception and ability to act on the information perceived (behavioral response), which involves a change in brain activity (electrical or electrochemical) and referred to as short term memory. Consolidation is the process of conversation of registered short term information to a long term memory trace, which involves physicochemical changes in neuronal networks. The stored information is made accessible by the process known as retrieval.

Recent behavioral, pharmacological and neurobiological studies have provided evidence for a cholinergic involvement in learning and memory. The cholinergic hypothesis claims that the decline in cognitive function in dementia is predominantly related to a decrease in cholinergic neurotransmission. The cholinergic muscarinic antagonist scopolamine is the drug most widely used to induce amnesia in experimental animals<sup>2,3</sup>.

Since times immemorial, natural herbs have been recognized to possess important medicinal activities. The nature provides a new opportunity to regain one's full mental capacity. A number of herbs have been extensively studied and reported to have memory enhancing property. There is a lack of scientific data regarding the effect of aqueous root extract of *Asparagus racemosus* on learning, memory and antioxidant properties. The main objective of investigation was to evaluate nootropic and antioxidant activities of *Asparagus racemosus*.

## MATERIALS AND METHODS

### Drugs and Chemicals:

Scopolamine and Piracetam were procured from Sigma-Aldrich pvt.ltd, India, and all other chemicals and reagents used were of analytical grade, procured from SD Fine chemicals Ltd, India.

### Plant Extract:

*Asparagus racemosus* was collected in medicinal garden of Hindu College of Pharmacy and authenticated by V. Jaya, Professor of Botany, Hindu College, Guntur. Roots were separated, washed in water, chopped into pieces and then shade dried. The coarsely powdered root was extracted with water on water bath for one hour. The extract was concentrated and yield was found to be 145.6g/kg.

### Animals:

Sprague Dawley rats (150-200g) body weight was obtained from National Institute of Nutrition (NIN), Hyderabad and they were housed under standard husbandry conditions. The experimental protocol was approved by the Institutional Animal Ethical Committee of Hindu College of Pharmacy (1263/bc/09/CPCSEA).

### Acute toxicity Studies:

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). SD rats of either sex were selected randomly and divided in to five groups (n=2). The animals were fasted overnight and aqueous extract of *Asparagus racemosus* in dose of 500, 1000, 2000 and 4000mg/Kg body weight, were administered orally to II and V groups. Group I which received water served as control. The animals were observed continuously for 2hrs, and then intermittently for 6hrs and at the end of 24hrs, the number of deaths was noted to determine LD<sub>50</sub> of the extract. Animals were also observed for behavioral, neurological and autonomic profiles simultaneously<sup>4</sup>.

### Experimental design for Scopolamine-induced cognitive deficits in rats on Elevated plus-maze:

SD rats weighing 150-200g were used in this study. They were kept in cages provided with standard uniform diet and free access to water. They were randomly distributed in to 5 groups each containing 6 animals. Group I served as control and received only distilled water, group II was treated with scopolamine

0.3mg/Kg, group III received scopolamine 0.3mg/Kg along with Piracetam 50mg/kg serves as standard and group IV and V received *Asparagus racemosus* 200mg/kg and 400mg/Kg body weight respectively along with scopolamine. *Asparagus racemosus* was administered orally while scopolamine and Piracetam were given intraperitoneally.

The rats were placed individually at the end of one arm facing away from the center of the maze and the time the rat took to move from open arm to either of the enclosed arms (Transfer latency, TL) was recorded. On the first day, the rats were allowed to explore the plus maze for 20sec after the measurement of TL. Rats were returned to their home cages after the first trial. Twenty four hours later, the rats were placed on the elevated plus-maze individually as before and TL was recorded again. TL measured on 1<sup>st</sup> and 2<sup>nd</sup> day served as parameters for acquisition and retrieval respectively. All the drugs were administered 30min to the first trial.

#### **Experimental design for Aluminium-induced cognitive deficits in rats on Elevated plus-maze:**

Rats were randomly distributed in to 5 groups each containing 6 animals. Group I served as control and received only distilled water, group II was treated with Aluminium chloride (1000mg/10ml/Kg), group III received Aluminium chloride along with Piracetam 50mg/Kg serves as standard and group IV and V received *Asparagus racemosus* 200mg/Kg and 400mg/Kg body weight respectively along with Aluminium chloride. Rats were administered Aluminium chloride dissolved in distilled water (1000mg/10ml/kg) once daily orally for a period of 40days. From day 21 of aluminium treatment, the drugs were administered once daily to different groups. *Asparagus racemosus* was administered orally while and Piracetam was given intraperitoneally. At the end of the 40days treatment schedule, the rats were subjected to elevated plus maze task<sup>5</sup>.

#### **In vitro antioxidant activity:**

The antioxidant activities of *Asparagus racemosus* was determined by using Diphenyl picryl hydrazyl (DPPH) radical scavenging, Nitric oxide (NO) radical scavenging methods.

#### **Nitric oxide radical scavenging activity<sup>6</sup>:**

Nitric oxide radical scavenging activity was estimated according to the method described by Marcocci et al., (1994). Sodium nitroprusside (10 µM) in phosphate buffer pH 7.7 was incubated with 25, 50, 75, 100 and 125µM concentrations of extract dissolved in a suitable solvent (ethanol) and tubes were incubated at 25°C for 120 minutes. At intervals, 0.5ml of incubation solution was removed and diluted with 0.5ml of Griess reagent. Positive control ascorbic acid was used. The absorbance of the chromophore was measured at 546nm.

Results were expressed as means of triplicates and percentage scavenging activity was calculated as follows.

$$\% \text{ NO radical scavenging activity} = (1 - B/A) 100$$

Where B = Absorbance taken by control solution

A = Absorbance taken by different concentration of solution

#### **DPPH radical scavenging activity<sup>7</sup>:**

DPPH radical scavenging activity was estimated according to the method described by Yokozawa et al., (1998). Solutions of extract at different concentrations of 25, 50, 75, 100 and 125 µM were added to 100µM DPPH in ethanol and tubes were kept at an ambient temperature for 20 minutes and absorbance were measured at 517nm. Positive control ascorbic acid was used.

Results were expressed as means of triplicates and percentage scavenging activity was calculated as follows.

$$\% \text{ NO radical scavenging activity} = (1 - B/A) 100$$

Where B = Absorbance taken by control solution

A = Absorbance taken by different concentration of solution

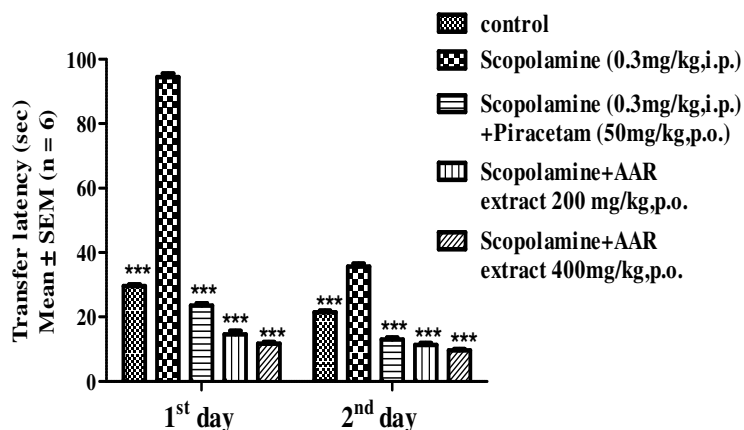
#### **Statistical Analysis:**

The data was represented as mean ± SEM. Results were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's post hoc multiple comparison test. Statistical analysis was performed using Graph Pad Prism software version 5.0.

**RESULTS:**

Data was presented in figure 1. In the present study, cholinergic muscarinic antagonist, scopolamine significantly increased the transfer latency on the first day when compared to control group animals but on the second day, scopolamine induced transfer latency was drastically decreased when compared to the 1<sup>st</sup> day. This clearly indicates the learning behavior of animals on the second day. However, the nootropic agent, Piracetam showed significant reversal of scopolamine-induced deficits. Aqueous extract of *Asparagus racemosus* significantly and dose dependently decreased the transfer latency (TL) on the first day. But even on the 2<sup>nd</sup> day, extract has shown same degree of effect on transfer latency on elevated plus maze. On 2<sup>nd</sup> day, effect of extract with two different doses was almost on par with effect of standard drug, Piracetam.

**Figure 1:** Effect of *Asparagus racemosus* on transfer latency in scopolamine induced cognitive deficits on elevated plus maze.

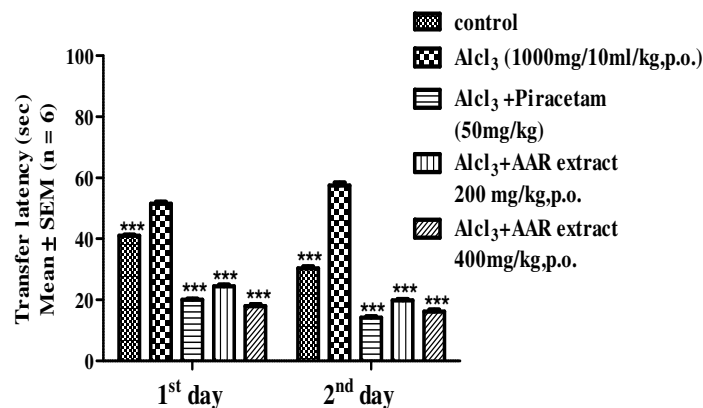


All values are represented as mean ± SEM (n = 6); \*\*\*p<0.001 when compared to scopolamine group; AAR-Aqueous extract of *Asparagus racemosus*

Data was presented in figure 2. AlCl<sub>3</sub> produced significantly increased the transfer latency on the first day but on the second day, AlCl<sub>3</sub> induced transfer latency was slightly increased. This indicates no learning behavior of animals with AlCl<sub>3</sub> induced cognitive deficits on the second day. However, the nootropic agent, Piracetam showed significant reversal of AlCl<sub>3</sub>-induced deficits. Aqueous extract of *Asparagus racemosus*

significantly and dose dependently decreased the transfer latency (TL) on the first day. Effect of extract dose of 400mg/kg was on par with the effect of standard drug. But even on the 2<sup>nd</sup> day, extract has shown more degree of effect on transfer latency on elevated plus maze when compared to the 1<sup>st</sup> day effect.

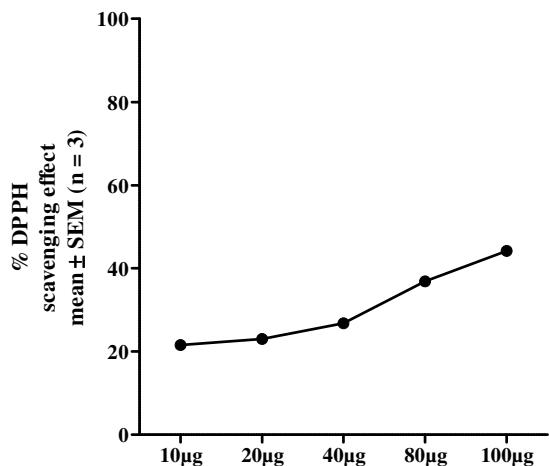
**Figure 2:** Effect of *Asparagus racemosus* on transfer latency in AlCl<sub>3</sub> induced cognitive deficits on elevated plus maze.



All values are represented as mean ± SEM (n = 6); \*\*\*p<0.001 when compared to AlCl<sub>3</sub> disease control group; AAR-Aqueous extract of *Asparagus racemosus*

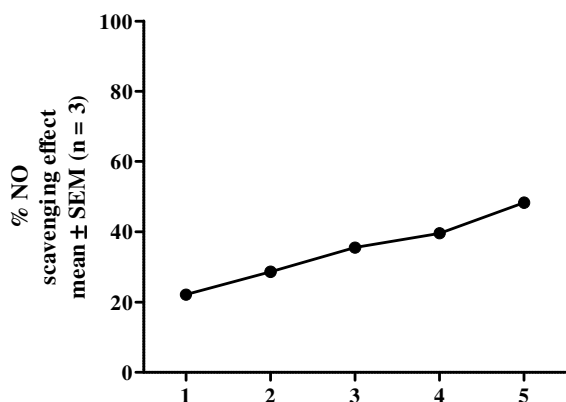
DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction of the DPPH radical is determined by its decrease in absorbance at 517nm. The extent of reduction of DPPH free radical is visualized as a discoloration from purple to yellow<sup>8</sup>. Maximum percentage inhibition of DPPH radicals by *Asparagus racemosus* was about 48% at 100µg/ml. Standard drug ascorbic acid about 78% of inhibition at 100µg. The present investigation indicated that *Asparagus racemosus* has radical scavenging capacity. In the present study, *Asparagus racemosus* was tested for *in-vitro* antioxidant activities in two different models. It was observed that *Asparagus racemosus* scavenged free radicals in a concentration dependent manner. Data was presented in figure 3.

**Figure 3:** Effect of Aqueous extract of *Asparagus racemosus* on *in-vitro* DPPH scavenging activity



In nitric oxide scavenging the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25°C was reduced by *Asparagus racemosus* extract. Maximum percentage inhibition of nitric oxide radicals by *Asparagus racemosus* was about 44% at 100µg/ml. Ascorbic acid at 100µg caused about 75%. This may be due to the antioxidant principles in the *Asparagus racemosus*, which compete with oxygen to react with nitric oxide there by inhibiting the generation of nitrite indicating, the *Asparagus racemosus* has greater inhibition than ascorbic acid in scavenging NO. Data was presented in figure 4.

**Figure 4:** Effect of Aqueous extract of *Asparagus racemosus* on *in-vitro* NO scavenging activity



## DISCUSSION:

Though several models for amnesia using pharmacological drugs are available, scopolamine-induced memory deficits have been proposed to have symptomatological similarities with Alzheimer's disease and related disorders<sup>9</sup>. The present study envisages the nootropic effect of *Asparagus racemosus* on scopolamine-induced memory deficit and AlCl<sub>3</sub> induced cognitive deficits on elevated plus-maze in rats.

Scopolamine induced amnesia was well reported animal model for screening anti-amnestic molecules. In addition, this study also carried out studies on AlCl<sub>3</sub> induced cognitive deficits. Previous study describes the neurotoxic effects of intraperitoneal administration of aluminum chloride to adult rats over a two-month period and the treatment effects of GE on rats exposed to AlCl<sub>3</sub>. Aluminum exposure exerted adverse effects on learning and memory which were manifested in increases in the number of acquisition and retention errors of rats that were exposed to AlCl<sub>3</sub> for two months<sup>10</sup>.

*Asparagus Racemosus* (AR) is an Ayurvedic rasayana possessing multiple neuropharmacological activities<sup>11</sup>. Results of present study support the previous evidence of recent investigation that demonstrated that methanolic root extract of *Asparagus racemosus* enhances memory and protects against amnesia in rodent models<sup>12</sup>. However, our study demonstrates memory enhancing effect on elevated plus maze with aqueous root extract of *Asparagus racemosus*. The mechanism underlying the memory enhancement is not clear. Ojha et al., (2010) reported that methanolic extract of *Asparagus racemosus* dose-dependently inhibited acetyl cholinesterase enzyme in specific brain regions (prefrontal cortex, hippocampus and hypothalamus). This clearly indicates that the mechanism involved in nootropic action of *Asparagus racemosus* may be due to inhibition of acetyl cholinesterase enzyme and hence elevation of acetylcholine levels which maintains the normal cognitive function in the brain. Furthermore, a comparative study indicated that both *Convolvulus pluricaulis choisy* and *Asparagus racemosus* have improved the cognitive function in young and old

mice<sup>13</sup>. This provides evidence that *Asparagus racemosus* memory enhancing effect was observed in different species as well as different age group of animals. An ayurvedic formulation chyawanprash that comprises *asparagus racemosus* was found to be effective in improving the memory in mice models<sup>14</sup>.

Other possible mechanism of memory enhancing effect was attenuation of oxidative stress by *Asparagus racemosus* extract. Our study demonstrated that aqueous extract of *Asparagus racemosus* has moderate *in-vitro* anti-oxidant activity that was evident from DPPH scavenging effect and NO scavenging effect. There were few studies that reported the *Asparagus racemosus* supplementation ameliorates age-related oxidative damage in skeletal muscle lysosome of aged rats<sup>15</sup>. It is well proven that *Asparagus racemosus* ameliorates the streptozotocin-induced oxidative stress<sup>16</sup>. Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus*<sup>17</sup>. It was concluded that Aqueous extract of root of *Asparagus racemosus* promising herbal plant for the patients of Alzheimer's disease and other cognitive deficit states. However, mechanism is not clear. Our study strengthens the idea that *Asparagus racemosus* extract enhances the memory by attenuation of oxidative stress condition.

## REFERENCES

1. Marke Bachman L, Ahlborn A, Winbald B, *Neurology*,1991,41,1886-92.
2. Drachman D, Memory and cognitive function in man: Does the cholinergic system have a specific role? *Neurology*, 1977, 27, 783-90.
3. Bartus R, Evidence for direct cholinergic involvement in scopolamine induced amnesia in monkeys: effect of concurrent administration of physostigmine and methyphenidate with scopolamine. *Pharmacol Biochem Behav*,1978, 9, 833-6.
4. Sheth UK, Dadkar NK, and Usha GK, Selected topics in experimental pharmacology,1972, 124-125.
5. Sharma AC, Kulkarni SK, *Drug Dev Res*, 1991; 22:251.

6. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L .The nitric oxide-scavenging properties of Ginkgo bilobaextract EGb 761. *Biochem Biophys Res Commun*. 1994; 15: 748–755.
7. Yokozawa T, Chen C P, Dong E, Tanaka T, Nonaka G I. and Nishioka I. Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radical. *Biochemical Pharmacology*. 1998; **56**: 213-222.
8. Hseu YC, Chang WH, Chen CS, Liao JW, Huang CJ, Lu FJ, Chia YC, Hsu HK, Wu JJ, Yang HL: *Food and Chem Toxicol* 2008; 46: 105–114.
9. Smith G (1988). Animal models of Alzheimer's disease, experimental cholinergic denervation. *Brain Res Rev* 13:103-18.
10. He Shuchang, Niu Qiao, Niu Piye, He Mingwei, Sun Xiaoshu, Shao Feng, Wang Sheng, Mark Opler (2008). Protective effects of gastrodia elata on aluminium-chlorideinduced learning impairments and alterations of amino acid neurotransmitter release in adult rats. *Restor Neurol Neurosci* 26(6): 467–470.
11. Saxena G, Mamta S, Meena P, Barber S, Sharma D, Shukla S, Bhatnagar M (2007). Neuroprotective Effects of *Asparagus Racemosus* Linn Root Extract: An Experimental and Clinical Evidence. *Annals of Neurosciences* 14(3).
12. Ojha R, Sahu AN, Muruganandam AV, Singh GK, Krishnamurthy S (2010). *Asparagus racemosus* enhances memory and protects against amnesia in rodent models. *Brain Cogn* 74 (1):1-9.
13. Sharma K, Bhatnagar M, Kulkarni SK (2010). Effect of *Convolvulus pluricaulis* Choisy and *Asparagus racemosus* Wild on learning and memory in young and old mice: a comparative evaluation. *Indian J Exp Biol* 48(5):479-485.
14. Milind P, Nitin B (2010). Anti-amnesic activity of an ayurvedic formulation chyawanprash in mice *eCAM* 30: 1-10.
15. Sivanandham V, Vava MHB (2008). *Asparagus Racemosus* Supplementation Ameliorate Age-related Oxidative Damage in Skeletal Muscle Lysosome of Aged Rats. *International Journal of*

*Pharmaceutical Sciences and Nanotechnology*  
1(1): 37-44.

16. Govindaajan R, Vijayakumar M, Rao ChV, Kumar V, Rawat AKS & Pushpangadan P (2004). Action of *Asparagus racemosus* against streptozotocin-induced oxidative stress, *Natural Prod Sci* 10: 177.

17. Parihar MS, Hemnani T (2004). Experimental excitotoxicity provokes oxidative damage in mice brain and attenuation by extract of *Asparagus racemosus*. *J Neural Transm* 111: 1.

\*\*\*\*\*