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Evolvulus alsinoides Plant Extract as A Cognitive Enhancer of Spacial Memory In Ad-Induced Rat Model.

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ABSTRACT

The main objective of the present study was to evaluate the neuro-protective potential of *Evolvulous alsinoides* plant extract on special learning & memory in AD-induced rat model by employing suitable tools and techniques. Ethanolic extract of *Evolvulus alsinoides Linn* (EAE) was prepared and administered to rats orally at a dose of 200 mg/kg body weight. For assessment of spacial learning capabilities, the rats were trained for water maze and subjected to spatial memory test by measuring the time taken to reach the hidden platform. From the observation, it was obvious that simultaneous administration of EAE to AD-induced could effectively reverse the memory impairment caused by experimental induction of AD, and thus establishing the fact that EAE has the potential to attenuate the deficits in learning and memory. All these observations in the present study pave new vistas to develop safe and novel anti-Alzheimer's compounds from this selected plant extracts in future.

Keywords: *Evolvulous alsinoides* plant extract, D-Galactose, Spatial memory, Anti-AD compounds.

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INTRODUCTION

Alzheimer's disease is a neurodegenerative disorder that results in progressive and irreversible cognitive impairment, memory loss and decline in language [1]. Several diverse hallmarks, such as deposits of aberrant proteins (β-amyloid and τ-protein), oxidative stress, dyshomeostasis of biometals, and low levels of Acetylcholine (ACh) appear to play significant role in the pathophysiology of the disease [2] which gradually leads to dementia, behavioral and personality changes finally death [3]. At present, many drugs viz. such as Donepezil, Tacrine, Rivastigmine, Galantamine, Memantine and several nootropic agents such as piracetam, pramiracetam, aniracetam etc. are available to target cholinesterase, a key enzyme involved in Alzheimer's Disease. Since these drugs exerted several side effects, formulation of synthetic drugs and herbal products are moving from fringe to main stream use with a greater number of people seeking remedies and health approaches free from side effects and thus gaining popularity in the world market.

In this context, Indian traditional medical system, Ayurveda assumes lot of significance since it is believed to be safe for all chronic diseases in general. Shankhpushpi is considered as "Medhya Rasayana" in Ayurvedic texts, where the whole plant is used in various formulae as a nervine tonic recommended for nervous disorders such as stress, anxiety, mental fatigue, and insomnia [4] and also calms the nerves by regulating the body's production of the stress hormones, adrenaline, and cortisol [5].

In view of this, the present study was focused to assess the potential of *E. alsinoides* plant extract as a memory enhancer initially and later to suggest as the best compound for treating Alzheimer's Disease.

MATERIALS AND METHODS

Procurement and maintenance of experimental Rats:

Male albino Rat, *Rattusnorvegicus* of three months old, weighing 150 ± 10 grams was used as the experimental model in the present study. The rats obtained from Sri Venkateswara enterprises, Bangalore were housed in polypropylene cages under the controlled conditions of 28 ± 2°C temperature with photoperiod of 12 hours light and 12 hours dark and 75% relative humidity maintained in the animal house of the Department according to the ethical guidelines for animal protection and welfare bearing the Resolution No. 05/(i)/a/CPCSEA/ IAEC/ SVU/ KY-DV/ dt. 28/03/2011. The rats were fed with standard pellet diet and water *ad libitum.*

*EVOLVULUS ALSINOIDES (*SHANKHPUSHPI)

SCIENTIFIC CLASSIICATION:

Kingdom	:	Plantae
Phylum	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Solanales
Family	:	Convolvulaceae
Genus	:	Evolvulus
Species	:	E. alsinoide
Synonyms	:	Dwarf Morning Glory, Shankhapushpi, Vishnukranthi

Collection and preparation of plant extract

A bulk amount of fresh whole, *Evolvulus alsinoides* plant material of around 5-6 kgs was collected from in and around Tirumala Hills, washed with water and shade dried to avoid evaporation of active compounds, and made into coarse powder with motor and pestle. It was soaked in 95% ethanol for 10days at

room temperature and the solvent was filtered. This process was repeated 3 to 4 times until the extract is completely discoloured. The extract was then distilled and concentrated under reduced pressure in the Hahnvapor Rotary Evaporator HS-2005V. The resulting ethanol crude extract was air-dried and used for experimentation as given below.

Grouping of animals:

After the Rats were acclimated to the laboratory conditions for 10 days, they were randomly divided in to four main groups and all animals in each Group were administered with the following compounds only once in the morning hours between 8 to 9 AM, keeping in view the altered activity of rat during the nights compared to the day time.

Induction of Alzheimer's Disease:

In the present study, memory impairment was induced by intraperitoneal (i. p.) injection of D-Galactose (120mg/kg body weight) to rats [6] for 60 days continuously.

Administration of test substance:

E. alsinoides plant extract (200 mg/kg body weight) was dissolved in distilled water and given to the rats through a gavage tube by oral route, which is clinically accepted route for administration of any compound of plant origin. The volume of plant extract administered was kept at 0.2 ml per animal.

Group I	Control **(C)**
Group II	Rats administerd with *Evolvulus alsinoides* plant extract **(EAE)** for 60 days
Group III	AD-induced Rats (administerd with D-Galactose **(D-Gal)** for 60 days
Group IV	AD-induced Rats simultaneously treated with *Evolvulus alsinoides* plant extract **(EAE)** from 10^{th} day to 60^{th} day after induction of AD.

Method for assessment of Spatial learning and memory:

In the present study, the efficiency of learning and memory in different groups of rats was assessed by using water maze (7) test, which is not only used as a measure of spatial memory or place navigation but also used to assess retention of that information [8]. In this test, rats were allowed to find the hidden platform below the water surface in a circular water tank. The water maze experiment was conducted for all groups of rats on selected time periods viz., 20^{th}, 40^{th} and 60^{th} days for all six animals in each group separately. For each trail, the time required (in seconds) for individual rat to find the hidden platform was recorded and used as the index of memory. The mean data from the tests was used for statistical analysis.

RESULTS AND DISCUSSION

Spacial learning and memory: Graph.1.

In the present study, the results on Morris water maze task indicated that **on 20^{th} day**, the escape latency (time taken to reach the hidden platform) in control rats was 11. 21 sec, while in rats treated with EAE, the latency was decreased to 9.49 sec. Contrary to this, AD-induced rats took more latency i.e.15.49 sec. compared to the control rats. An interesting observation in the present study was that group IV rats i.e. AD-induced but simultaneously administered with EAE, the escape latency was only a little more (12.12) than that of control rats thus revealing that EAE extract could reverse the memory loss induce by D-Galactose. A similar trend was noticed on 40^{th} and 60^{th} day of experimentation as shown in the Graph.2. Further, it was also observed that as the rat grew, its learning efficiency also enhanced in all groups of rats. However, the ethanol extract of *E. alsinoides* at a dose of 200mg/kg significantly improved the acquisition and retention of memory by reversing the memory deficits caused by D-galactose most effectively. The effect of EAE on AD-induced rat changed accordingly corresponding to the level of memory loss thus demonstrating that prolonged AD-induction can scale down the memory revival efficiency of *Evolvulus alsinoides* plant extract **(EAE)**.

D-galactose, which is used as an AD-inducer in the present study, plays a prime role in the pathogenesis of aging and also mimics some characters of cognitive dysfunction and oxidative damage. It is widely believed that the long-term injection of D-galactose (D-gal) contributes to the aging progress and slight neuronal damage and memory deficits which are the prominent changes of AD in the early stage [9]. It was further strengthened by similar reports that a low dose of D-Galactose caused mental retardation and cognitive dysfunction as measured by open field, avoidance/escape, T-maze, Y-maze and Morris maze in mice [10]. These earlier findings lend strong support to our observations in the present study wherein D-galactose senescence rat spent a longer time in finding the hidden platform during the retrieval trial in the Morris water maze test which indicates impairment of memory.

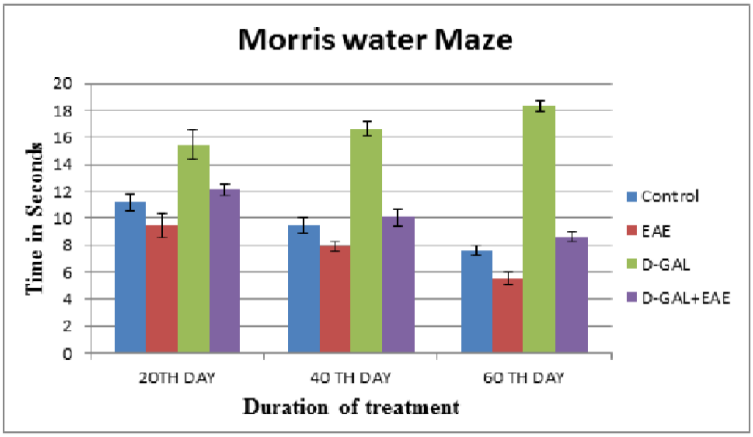
In previous studies, this d-galactose-induced aging model triggered the brain to reduce learning and memory [11], exhibited neuronal damage [12], as well as aging damage to the cardiovascular system, kidneys, and liver [13]. Research evidence shows that administration of D-galactose induces reactive oxygen species (ROS) production and inflammatory response resulting in neurodegenerative changes. D-gal, a reducing sugar which can be metabolized at normal concentration, induces the production of reactive oxygen species (ROS) and advanced glycation end products (AGEs) [14] at higher doses. Our present observations derive strong support from recent findings that ROS and AGEs induced by continuous injection of D-gal in rodent lead to decreased expression of memory-related protein, deterioration of learning and memory function, and pathological alterations of astrocytes which might be associated with the increased expression of inflammation related gene [15-17].

However, from our results, it was obvious that simultaneous administration of EAE could attenuate the impairment of memory and improved behaviour performance of AD-induced rats. As per the cholinergic hypothesis, Alzheimer's disease is directly associated with the neurotransmitter, ACh and its hydrolysing enzyme, AChE. The plant extract from *E. alsinoides* exhibited the best memory enhancing property [18] may be by suppressing acetylcholinesterase and simultaneously enhancing brain acetylcholine levels and as such holds fairly respectable anti-amnesiac effects in animal models of neurotoxicity.

Preliminary phytochemical studies on *E. alsinoides* showed the presence of flavonoids and Coumarins in the ethanol extract which have been recorded as scavengers of superoxide anions, having antioxidant and cyto protective effects and their usage in stress-induced disorders**.** The presence of Scopoletin, a furanocoumarin, in *E. alsinoides* further support the presence of memory potentiating and cognition enhancing activity as reflected by the treatment of AD-induced rat with EA extract [19].

In view of our observations supported by valid scientific data, especially as a mono therapy on learning and memory, *E. alsinoides* can be established as one of the best cognitive enhancer among a limited herbal compounds similar properties available as off today. All these observations in the present study pave new vistas in discovery of safe and novel anti-Alzheimer's compounds in future.

Graph.1. Representation of changes in special learning and memory in Control and all Experimental groups of rats on selected time intervals.



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