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Nootropic Activity of *Asteracantha longifolia* in Streptozotocin Induced Amnesia

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ABSTRACT

Male Swiss albino mice weighing about 25-30g were taken and amnesia was induced by injecting Streptozotocin (STZ) on 1 and 3 days (3mg/kg) bilaterally. After 5 days of injection the animals were treated with 100mg/kg and 200mg/kg (p.o) of *Asteracantha longifolia* (AL) extract for 2 weeks. Various behavioural parameters like Elevated plus Maze, Passive shock avoidance, Y-Maze tests were performed. *Asteracantha longifolia* improved learning and memory of mice as indicated by decreased transfer latency, increased step-down latency and increase in percentage alteration. *Asteracantha longifolia* extract has shown decreased levels of Acetylcholine esterase enzyme. The methanolic extract of the plant possessed anti-amnesic property at a dose level.

KEY WORDS: Nootropic, *Asteracantha longifolia* and Streptozotocin.

1. INTRODUCTION

Ayurveda is India's traditional, natural system of medicine that has been practiced more than 5,000 years. Ayurveda is a Sanskrit word that literally means "Science of life". Unlike chemically synthesized drugs that may produce many side effects, herbs can effectively realign the body's defence [1]. Alzheimer's disease (AD) is a progressive neuro-degenerative brain disorder that occurs gradually and results in memory loss, unusual behavior, personality changes and finally death (cognitive performance) [2]. The neurotransmitter acetylcholine mainly modulates learning and memory process. In recent years, there has been a rise in the interest of scientific community to explore the pharmacological actions of herbs. In traditional Practices of medicine, numerous plants have been used to alleviate memory impairment both in healthy individuals and those with disease states which are now recognized as a specific cognitive disorder [3]. *Asteracantha longifolia* plant is used for treating many ailments such as the roots, leaves and seeds have been used in Indian systems of medicine as diuretics and also employed to cure jaundice, dropsy, rheumatism, nootropic and diseases of the urinogenital tract [4].

2. MATERIALS AND METHODS

2.1. Plant material

The roots are shade dried and coarsely powdered and then subjected to defating using petroleum ether and then it is dried to remove the solvent. Later the plant mater is extracted with methanol using Soxhlet apparatus.

2.2. Animals

The Nootropic activity was evaluated by Streptozotocin induced amnesia in male Swiss albino mice weighing between 25-30g. The animals were housed in standard isolation cages under environmentally controlled conditions with a 12-h light/12-h dark cycle *ad libitum*.

2.3. Drugs

Drugs for Oral administration were freshly prepared as a homogenized suspension of *Asteracantha longifolia* in doses of 100mg/kg and 200mg/kg in 2% w/v of tween 80 and administered orally. All other chemicals and reagents were purchased locally and were of the highest analytical grade.

2.4. Lesioning procedure

The general procedures for surgery were same for all groups, except that artificial cerebrospinal fluid (CSF) was injected into the control group, whereas Streptozotocin was microinjected into the animal at a dose of 3mg/kg body weight. The anesthetized animals were (50mg/kg pentobarbital, i.p.) was placed. The skin over the mice's skull was shaved and then incision was made through the skin and muscle to expose the skull. Two holes were drilled in the skull at the location with stereo-toxic coordinates on brain atlas. A 22-guage syringe filled with either artificial CSF, Streptozotocin was infused at

0.2µl/min using a microinjection pump for 5 min and then 1 µl injection [5].

2.5. Experimental Protocol

Each group contains 6 animals each. Male swiss albino mice group I (control) was treated with CSF used as vehicle. Group II (Negative control) was treated with Streptozotocin and Group III, Group IV were treated with Streptozotocin + 100mg/kg and Streptozotocin+200mg/kg respectively [6]. After 14days behavioral studies were carried out. The results are expressed as the Mean ± S.E.M. Mean and the statistical significance of differences between groups was analyzed by analysis of variance (ANOVA) followed by Dunnets "T" test. $P < 0.05$ was considered as significant.

2.6. Behavioural models

2.6.1. Elevated plus-maze

Elevated plus-maze served as the ear that receives and responds to stimuli originating from outside the body behavioral model to evaluate memory in mice. The procedure, technique and endpoint for testing memory were followed as per the parameters described by the investigators working in the area of psychopharmacology. The elevated plus-maze for mice consisted of two open arms and two covered arms extend from a central platform, and the maze was elevated to a height of 25cm from the floor. On the first day (i.e. eighth day of drug treatment), each mouse was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was defined as the time (in seconds) taken by the animal to move from the open arm into one of the covered arm with all its four legs. TL was recorded on the first day (training session) for each animal. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned task (memory) was examined 24 hours after the first day trial (i.e. ninth day, 24 hours after last dose). Significant reduction in the TL value of retention indicated improvement in memory [7].

2.6.2. Passive avoidance test

Passive avoidance behavior based on negative reinforcement was used to examine the long term memory. The apparatus consist of a box having three walls of wood and one wall of Plexiglas, featuring a grid floor made up of 3mm stainless steel rods set 8-mm apart, with a wooden platform bin the centre of the grid floor. The box was illuminated with a 15W bulb during the experimental period. Electric shock (20v, AC) was delivered to the grid floor. Training (i.e. eighth day of drug treatment) was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the centre of the grid floor. When the mouse stepped- down placing all its paws on the grid floor, shocks were delivered for 15 seconds and the step down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to the grid floor with all its paws on the grid floor. Animals showing SDL in range of 12-15 seconds during the first test were used for the second test and the retention test. The second session was carried out 90 minutes after the first test. During the second session, if the animals step down before 60 seconds, electric shock was delivered again for 15 seconds. During the second test, animals were removed from the shock- free zone, if they did not step down before 60 seconds, and were subjected to the retention test. Retention (memory) was tested after 24 hours (i.e. ninth day, 24 hours after last dose) in similar manner, except that the electric shocks were not applied to the grid floor observing an upper cut-off tine of 300 seconds. Significant increase in SDL value indicated improvement in memory.

2.6.3. Y-maze task

Y-maze task is used to measure the spatial working through the spontaneous alternation of behavior. The maze is made of black painted wood. Each arm is 40 cm long, 13 cm high, 3 cm wide at the bottom, 10 cm wide at the top, and converges at an equal angle. Each mouse is placed at the end of one arm and allowed to move freely through the maze during an 8-min session. Mice tend to explore the maze systematically, entering each arm in turn. The ability to alternate requires that the mice know which arm they have already visited [8]. The series of arm entries, including possible returns into the same arm, are recorded visually. Alternation is defined as the number of successive entries into the three arms, on overlapping triplet sets. The percentage of alternation is calculated as the mice of actual alternations, defined as the total number of arm entries minus two, and multiplied by 100.

3. RESULTS AND DISCUSSION

Table 1: Effect of *AL* extract on transfer latency (TL) of mice using elevated plus-maze

Treatment	TL on first day	TL after 24 hours
CSF	23.90±3.10	25.20±2.9*
STZ	49.6±6.20*	30.15±2.89
STZ+ 100mg/kgof MEAL	22.30±1.62*	21.0±2.20*
STZ+ 200mg/kgof MEAL	21.16±4.15*	20.38±2.23*

All values are expressed of mean± SEM and n=6

Control, STZ, STZ+100mg/kg of MEAL, STZ+200mg/kg of MEAL.

Statistically significant (*p<0.05) when compared to STZ group.

Transfer latency of second day (Day 9 of drug treatment) reflected retention of learned task or memory. The mice treated with *AL* (100mg/kg and 200mg/kgp.o) showed dose dependent reduction in TL of ninth day indicating significant improvement in memory, when compared with STZ group. STZ (3mg/kg i.v) injected before training significantly increased (p<0.01) the TL of ninth day indicating impairment in memory. The mice treated with *AL* (100mg/kg and 200mg/kg p.o) for nine successive days reversed successfully the amnesia induced by STZ.

Table 2: Effect of *AL* extract on step-down latency (SDL) of mice using Passive shock avoidance test

Treatment	SDL
CSF	118±3.40*
STZ	48.6±7.03
STZ+ 100mg/kgof MEAL	282.2±4.26*
STZ+ 200mg/kgof MEAL	292.6±6.30*

All values are expressed of mean± SEM and n=6

STZ, STZ + 100mg/kg of MEAL, STZ + 200mg/kg of MEAL.

Statistically significant difference (*p<0.05) when compared to STZ group.

SDL of second day (Day 9 of drug treatment) reflected the long term memory of animals. Various concentration of *AL* (100mg/kg and 200mg/kg, p.o) administered to mice for 8 days showed dose dependent increase in SDL values as compared to respective control groups. *AL* (100mg/kg and 200mg/kg p.o) administered for 8 days reversed STZ induced amnesia.

Table 3: Effect of *AL* extract on Percentage alteration Using Y-Maze Paradigm

Groups	Percentage alteration
CSF	72.52±1.5
STZ	58.25±2.0*
100mg/kgof MEAL	61.46±1.32*
200mg/kgof MEAL	69.60±1.89*

Values are expressed as mean± SEM and n=6

Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. *P<0.05

The amnesia induced group (negative control) indicated decrease in the alternation of behavior. The results presented by the treatment groups shows significance by (p<0.01) increase in alteration of behavior in respect of 100mg/kg of MEAL and 200mg/kg Of MEAL when compared with that of the negative control.

During the process of learning and memory formation the brain undergoes a physical and chemical change which is called as synaptic plasticity. It shows involvement of various signal transduction pathways, induction of gene expression which results in formation of new synapses between nerve cells. This process undergoes a continuous remodeling with time and new experiences. The free radical theory of aging is one of the most popular, single mechanistic theories of aging, which discloses increased generation of free radical as the major cause of cellular damage. Such free radical-mediated damages are prevalent during aging which leads to age associated diseases like Alzheimer's disease (AD) and Parkinson's disease. Impairment of memory is the initial and most significant symptom. AD is associated with a decline in cognitive abilities. The most common cause of dementia in the elderly is probably AD. Despite the severity and high prevalence of this disease, the allopathic system is yet to provide a satisfactory antidote. In the present study, *AL* extract (100mg/kg and 200mg/kg) administered orally improved learning and memory of mice assessed by the behavioral models like Y-Maze, Elevated plus Maze, Passive avoidance paradigm compared to the negative control.

4. CONCLUSION

In the present investigation, the methanolic extract of the *Asteracantha longifolia* possessed anti-amnesic property at a dose level in the experimental animals.

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