ISSN: 2395-0536 Impact Factor- 1.90*

INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

# IN-VIVO NEUROBEHAVIORAL AND BIOCHEMICAL EVALUATION OF *TINOSPORA CORDIFOLIA* ON RATS

**K.Sivaji*, M.Sneha, K.Sireesha, CH.Satya Surya, N.Naga Lakshmi, V.Vidya, K.Asha, Y.Ganga Sireesha**

**Department of Pharmacology, JITS College of Pharmacy, Kalagampudi, Palakol, Andhra Pradesh, India**

## ABSTRACT

Main objective of evaluating the alcoholic leaf extract of *Tinospora cordifolia*. For anti Alzheimer's activity using aluminum chloride induced neurotoxicity model in rats as an experimental animal. Chronic administration of aluminium chloride significantly raised MDA and nitrite concentration, depleted reduced GSH, and superoxide dismutase, and catalase activities in the whole brain compared to naive rats ($p < 0.05$). However, chronic ELTC(250 and 500 mg/kg administration to the rats significantly attenuated oxidative damage (as indicated by reductions in MDA, nitrite concentration and reduced GSH, and decreased superoxide dismutase, and catalase activities) as compared to control rats.

**Key Words:** *Tinospora cordifolia,* anti Alzheimer's activity, aluminium chloride induced neurotoxicity

**Author for correspondence**
**K.Sivaji,**
Department of Pharmacology,
JITS College of Pharmacy,Kalagampudi,Palakol,
Andhra Pradesh, India.
Email id: siva.bpharm09@gmail.com.

## INTRODUCTION

Alzheimer's disease (AD), also known in medical literature as Alzheimer disease, is the most common form of dementia. There is no cure for the disease, which worsens as it progresses, and eventually leads to death. It was first described by German psychiatrist and neuropathologist Alois Alzheimer in 1906 and was named after him [1]. Most often, AD is diagnosed in people over 65 years of age, although the less-prevalent early-onset Alzheimer's can occur much earlier. In 2006, there were26.6 million people worldwide with AD. Alzheimer's is predicted to affect 1 in 85 people globally by 2050 [2].Although Symptoms are often mistakenly thought to be 'age-related' concerns, or manifestations of stress. In the early stages, the most common symptom is difficulty in remembering recent events, known as short term memory loss. When AD is suspected, the diagnosis is usually confirmed with tests that evaluate behavior and thinking abilities, often followed by a brain scan if available however, examination of brain tissue is required for a definitive diagnosis. As the disease advances, symptoms can include confusion, irritability, aggression, mood swings, trouble with language, and long-term memory loss. As the person declines they often withdraw from family and society. Gradually, bodily functions are lost, ultimately leading to death. Since the disease is different for each individual, predicting how it will affectthe person is difficult. AD develops for an unknown and variable amount of time before becoming fully apparent, and it can progress

undiagnosed for years. On average, the life expectancy following diagnosis is approximately seven years [3]. Fewer than three percent of

Individuals live more than fourteen years after diagnosis. The cause and progression of Alzheimer's disease are not well understood. Research indicates that the disease is associated with plaques and tangles in the brain. Current treatments only help with the symptoms of the disease. There are no available treatments that stop or reverse the progression of the disease. As of 2012, more than 1,000 clinical trials have been or are being conducted to test various compounds in AD. Mental stimulation, exercise, and a balanced diet have been suggested as ways to delay cognitive symptoms (though not brain pathology) in healthy older individuals, but there is no conclusive evidence supporting an effect. It is classified as a neurodegenerative disorder.Because AD cannot be cured and is degenerative the person increasingly relies on others for assistance. The role of the main caregiver is often taken by the spouse or a close relative. Alzheimer's disease is known for placing a great burden on caregivers; the pressures can be wide-ranging, involving social, psychological, physical, and economic elements of the caregiver's life [4-6].

## MATERIALS AND METHODS

### Animals

Adult Sprague-Dawley rats of either sex weighing 250 to 280 g were used in the present study. Animals were acclimatized to the laboratory conditions for five days. Six animals were kept in one cage and maintained under standard housing conditions (temperature 24-27 °C and humidity 60-65 %) with 12:12 h light dark cycle. All experiments and protocols described in present study were approved by the Institutional Animal Ethical Committee (IAEC) of JITS College of pharmacy and with permission from Committee for the purpose of Control and Supervision of Experiments on Animals, Ministry of Social Justice and Empowerment, Government of India.

### Preparation of plant extract

Leavesof*Tinospora cordifolia* Collected locally in and around the Guntur. They were identified and authentified. The aerial parts were shade dried and then powdered.The Powdered material was defatted with petroleum ether (60-80 °C). The marc was dried and extracted with methanol. The methanolic leaf extract of *Tinospora cordifolia*was concentrated under Vacuum. The dried extract was stored in amber glass bottle at 4°C and used as and when required. The extract was resuspended in 0.3% Carboxymethyl Cellulose (CMC) & the Suspension was used for in vivo experiments.

### Elevated plus maze

The elevated plus maze consisted of two opposite open arms (50cm×10 cm), crossed with two closed walls of the same dimensions with 40cmhighwalls. The open arms were connected with a central square of dimensions 10cm×10cm the entire mazewas placed 50cmhigh above the ground. Animals are tested for Acquisition of memory on day 20 from the start of Aluminum chloride administration. Rats were placed away from the central square individually at one end of the open arm. The time taken by the animal on maze to move from the open arm to the closed arm within specified time was recorded as the initial transfer latency (ITL). After recording the ITL animals were allowed to explore the maze for 20 sec and were then returned to the home cages. It was pushed on the back into one of the enclosed arm, if the animal did not enter the enclosed arm within 90 sec, and the ITL was recorded as 90 sec. By placing the rat in an open arm, retention of memory was assessed and the retention latency was noted on day 21 and day 42 of the ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL) respectively [7, 8].

### 8 arm radial maze

Olton and co-workers have developed a spatial discrimination task for rodents that has been extensively used in learning and memory studies, and that has served as the basic task for one of the most important theories on the role of the hippocampus [185,186]. The rat uses spatial information provided by the distal cues in the room to efficiently locate the baited arms. The radial arm-maze allows the study of spatial reference and working memory processes in the rat. In reference memory procedures, information

is useful for many sessions/days and may usually be needed during the entire experiment. On the contrary, working memory procedures have a major temporal

component as the information presented in the maze (arms baited) is useful for one session but not for subsequent ones; the rat has to remember the information during a delay interval (min to h). Correct choices in the radial arm-maze are rewarded by food.
The apparatus is a wooden elevated eight-arm radial maze with the arms extending from a central platform 26 cm in diameter. Each arm is 56 cm long and 5 cm wide with 2 cm high rails along the length of the arm. The maze is well illuminated and numerous cues are present. Food pellets (reward) are placed at the end of the arms. During the test, rats are fed once a day and their body weights maintained at 85% of their free feeding weight to motivate the rat to run the maze.
Animals are trained on a daily basis in the maze to collect the food pellets. The session is terminated after 8 choices and the rat has to obtain the maximum number of rewards with a minimum number of errors. The numbers of errors (entries to non-baited arms) are counted during the session.

**Locomotor Activity**

Mice were tested in acrylic cages (45 x 25 cm) divided into 16 equal squares. The number ofcrossed squares was recorded for each mouse per time of5 min for 20 min investigated.

## RESULTS AND DISCUSSION

The effects of Aluminum chloride on exploratory behavior in rat are shown in table-1. The locomotor activity of rat was assessed by the number of crossing squares noted as scores per time of 5min investigated. Result showed that the activity of intoxicated rat was lesser than controls it was significant at the first 5min. As shown in Table-1, Aluminum did significantly modify head-dipping behaviors.

**Table-1 Hole board Test & Locomotor Activity**

| Treatment | No of head dips | Time spent in head tips | Latency to first head dips | No. of rearings | No. of defaecation units | Locomotor Activity |
|---|---|---|---|---|---|---|
| **Vehicle Control** | 6±0.9487 | 10.6±0.6782 | 34.7±1.503 | 7.2±0.3742 | 2±0.4472 | 602±23.31 |
| **Disease control** 100 mg/kg | 13.4±0.2449 | 42.8±1.463 | 12.9±0.6782 | 22.8±1.114 | 0.4±0.2449 | 297.6±24.29 |
| **Test 1** 250mg/kg | 12.8±0.3742 | 23±0.8367 | 13.9±0.2449 | 19±0.4472 | 0.8±0.2 | 327.8±21.71 |
| **Test 2** 500 mg/kg | 9.4±0.5099 | 17.8±0.7348 | 24.9±1.03 | 15.8±0.8602 | 1.6±0.4 | 489.4±24 |
| **Standard** Piracetam-200 mg/kg | 14.4±1.208 | 23±1.049 | 19.8±1.208 | 16.6±0.8124 | 0.8±0.2 | 559.6±18.06 |

TL on the $42^{nd}$ day (24 hr. after the last dose) reflected the retention of information. $AlCl_3$ (100 mg/kg) impaired learning significantly ($p< 0.01$) as indicated by increase in TL as compared to control group. Administration of ELTC (250 and 500 mg/kg p.o.) showed dose dependent reduction in TL on $42^{nd}$ day when compared to $AlCl_3$ group indicating significant ($p< 0.05$) improvement in memory. Piracetam in the dose of 200 mg/kg i.p. improved memory and reversed the cognitive impairment induced by administration of $AlCl_3$, as evidenced by reduction in TL (Table-2).

**Table-2 Effect of Ethanolic leaf extract of *Tinospora cordifolia* on aluminium chloride induced behavioral alteration in rats.**

| Treatment | Entries | | Time Spent | | | Transfer latency |
|---|---|---|---|---|---|---|
| | Open Arm | Closed Arm | Open Arm | Closed Arm | Central Compartment | |
| **Vehicle Control** | 5.4±0.6782 | 16.2±0.7348 | 35±0.9487 | 190±1.612 | 73.4±0.8124 | 20.2±0.2 |
| **Disease control** 100 mg/kg | 12.2±0.2 | 5.6±0.2449 | 109.6±3.945 | 84.2±2.634 | 116±2.449 | 172.6±0.7483 |
| **Test 1** 250mg/kg | 12.8±0.5831 | 7±0.3162 | 130.4±6.12 | 117±2.47 | 52.8±1.828 | 55.6±0.4 |
| **Test 2** 500 mg/kg | 8.4±0.2449 | 15±0.3162 | 47.2±1.53 | 145.8±3.121 | 116.6±1.965 | 38.6±0.5099 |
| **Standard** Piracetam-200 mg/kg | 10.4±0.2449 | 8.4±0.4 | 113.2±2.177 | 114.4±2.482 | 73.8±2.956 | 26.2±0.3742 |

Chronic administration of aluminium chloride significantly raised MDA and nitrite concentration, depleted reduced GSH, and superoxide dismutase, and catalase activities in the whole brain compared to naive rats ($p < 0.05$). However, chronic ELTC(250 and 500 mg/kg administration to the rats significantly attenuated oxidative damage (as indicated by reductions in MDA, nitrite concentration and reduced GSH, and decreased superoxide dismutase, and catalase activities) as compared to control rats (Fig-1). Furthermore, ELTC (250 and 500 mg/kg treatment alone did not significantly influence these parameters compared to naive rats.

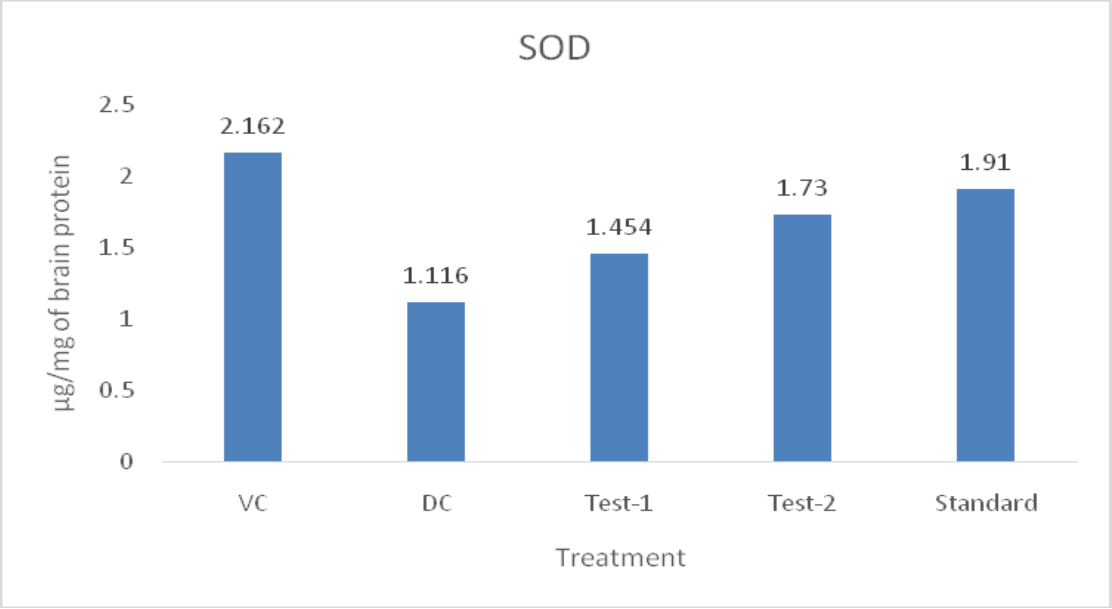


**Fig-1 SOD of treated groups**

## CONCLUSION

The present study provides further evidence for the neurotoxic action of Aluminium in the rat brain. Administration of Aluminium chloride resulted in distinct morphological alterations in the brain and behavioral results indicate cognitive impairment and enhanced anxiety of mice in an unfamiliar environment. The rat behavior in the 8 arm radial maze and elevated plus maze demonstrates that the impairment in spatial working memory of Aluminium-exposed rat was caused by inefficient use of a spatial mapping strategy, however, rat showed intact spatial reference memory. This may be induced by the deterioration of hippocampal function after Aluminium exposure.

## REFERENCES

1. Berthold NC, Cot man CW. Evolution in the Conceptualization of Dementia and Alzheimer's disease: Greco-Roman Period to the 1960s. *Neurobiol.Aging*. 1998; 19(3):173–89.
2. Brook Meyer R, Johnson E, Ziegler-Graham K, MH Arrighi. *Forecasting the global burden of Alzheimer's disease. Alzheimer's and Dementia*. 2007 [Retrieved18June2008];3(3):18691.World population prospects: the 2006 revision highlights [PDF]. 2007 [Retrieved 27 August 2008].
3. Molsa PK, Marttila RJ, Rinne UK. Survival and Cause of Death in Alzheimer's disease and Multi-Infarct Dementia. *ActaNeurolscand.* 1986; 74(2):103–7.
4. Thompson CA, Spilsbury K, Hall J, Birks Y, Barnes C, Adamson J. Systematic Review of Information and Support Interventions for Caregivers of People with Dementia. *BMCGeriatric*. 2007; 7:18.
5. Schneider J, Murray J, Banerjee S, and Mann A. EUROCARE: a cross-national study of co-resident spouse careers for people with Alzheimer's disease: I—Factors associated with career burden. *International Journal of Geriatric Psychiatry*. 1999; 14(8):651–661.
6. Murray J, Schneider J, Banerjee S, Mann A. EUROCARE: A Cross-national Study of Co-resident Spouse Careers for People with Alzheimer's disease: II—A Qualitative Analysis of the Experience of Caregiving.*International Journal of Geriatric Psychiatry*. 1999;14(8):662–667.
7. Consumer Reports; Drug Effectiveness Review Project (May 2012). "Evaluating Prescription Drugs Used to Treat: Alzheimer's Disease Comparing Effectiveness, Safety, and Price". Best Buy Drugs (Consumer Reports): 2. Retrieved 1 May 2013.Which claims Alzheimer's Association guidance as a source
8. Niggard L. Instrumental Activities of Daily Living: A Stepping-stone towards Alzheimer's disease Diagnosis in Subjects with Mild Cognitive Impairment? *ActaNeurolscand*. 2003; Supple (179):42–6.