JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2024/43482
RESEARCH ARTICLE

# Evaluation of *Saussurea lappa* on Oxidative Stress and Cognition in Aluminium-induced Alzheimer's Disease Rats

**Humera Sadia and Govindu Sumalatha***

Chaitanya Deemed to be University, Hanamkonda, Warangal - 506001, Telangana, India; sumalatha2k@gmail.com

## Abstract

**Background**: There is a strong correlation between oxidative stress and neurodegenerative diseases like Alzheimer's Disease (AD). There has been a lot of thought put into finding medicinal plants with nootropic properties to slow the onset and course of AD. **Aim:** The study aimed to evaluate the Methanolic Extract of *Saussurea lappa* Clarke (MESC) on oxidative stress and cognitive ability induced by aluminium exposure. **Methods**: Wistar albino rats were chosen for the study. About 30 animals were selected and grouped into 5 of six animals each, group I served as the control, group II was disease induced (aluminium-induced), group III, IV and V were administered standard drug – Donepezil HCl, and MESC at two doses – 200 and 400 mg/kg. The behavioural studies were examined by using certain apparatus like the Passive Avoidance (PA) test, the elevated plus maze, the Y-maze and the actophotometer. Determination of antioxidant enzymes – Catalase (CAT) and Thiobarbituric Acid Reactive Substances (TBARS) along with acetylcholinesterase (AChE) levels was done in rat's brain homogenate. **Results:** In the PA test, MESC at doses of 200 and 400 mg/kg significantly (**p < 0.01) lengthened Step-Through Latency (STL) in rats on day 30 as compared to positive control animals. Animals at MESC (200 and 400 mg/kg) showed noticeably higher Memory Retention (MR) rates as compared to the disease-induced rats. Additionally, MESC (200 and 400 mg/kg) significantly (**p < 0.01) raised CAT and decreased the concentration of TBARS. AChE concentration was significantly (**p < 0.01) reduced at the dose of MESC at 200 and 400 mg/kg as compared to the disease animals. **Conclusion:** The present study showed that MESC had a strong nootropic effect on antioxidant indicators and cognitive ability in rats exposed to chemical-induced oxidative stress. These findings may be investigated in the treatment of neurodegenerative diseases, including AD.

**Keywords:** Acetylcholinesterase (AChE), Catalase, Learning, Memory, Oxidative Stress, *Saussurea lappa*

## 1. Introduction

Alzheimer's Disease (AD) is a progressive neurological disorder that impairs memory and cognitive abilities. In elderly people, it remains the most common cause of dementia. Alzheimer's disease is assumed to be caused by a combination of lifestyle, environmental, and genetic factors, while its exact cause is unknown[1]. The buildup of aberrant protein deposits in the brain, which impair normal brain function and cause nerve cell death, is one of the main characteristics of AD. It begins with minor disorientation and memory loss, gets worse over time. Additional typical symptoms include changes in attitude and behaviour, difficulties with language, and impaired reasoning and judgment. Although there is not a cure for Alzheimer's disease at present, there are therapies that can help some people manage their symptoms and live better[2].

In addition to the non-pharmacological therapies including cognitive stimulation therapy and caregiver support, treatments may use prescription drugs to help with behavioural symptoms and cognitive performance. The goal of current research on Alzheimer's disease is to comprehend its fundamental causes, create novel treatments, and eventually find a cure. For those with Alzheimer's disease, early detection and management

*Author for correspondence

Article Received on: 03.05.2024 Revised on: 10.07.2024 Accepted on: 05.08.2024

are crucial to optimizing treatment efficacy and enhancing outcomes[3].

The generation of free radicals and the deterioration of the body's capacity to eliminate them or repair causes to oxidative stress[4]. Reactive Oxygen Species (ROS) are very reactive oxygen-containing compounds, including hydrogen peroxide, hydroxyl radicals, and superoxide radicals[4]. These substances can harm DNA, proteins, and lipids found in cells. Oxidative stress can cause brain damage to neurons in the context of Alzheimer's disease. Because of their high metabolic activity, comparatively low amounts of antioxidant enzymes, and high concentrations of readily oxidizable lipids, neurons are especially susceptible to oxidative damage[5]. Accumulation of oxidative damage in neurons can impair their function and contribute to neuronal death, which is a hallmark of Alzheimer's pathology[6].

Moreover, the aggregation of abnormal protein, such as beta-amyloid plaques and tau tangles, exacerbates the oxidative stress which forms the characteristic feature of Alzheimer's disease[7]. These protein aggregates can further induce oxidative stress and neuronal damage, creating a vicious cycle that contributes to disease progression. Several studies have provided evidence in support of the involvement of oxidative stress in Alzheimer's disease[8,9]. For example, markers of oxidative damage, such as lipid peroxidation products and protein carbonyls, have been found to be elevated in the brains of individuals with Alzheimer's compared to healthy controls. Moreover, a higher risk of Alzheimer's disease has been connected to genetic variations linked to antioxidant defence mechanisms[10].

Although additional research is required to fully understand the intricate interplay between oxidative stress and Alzheimer's pathology, targeting oxidative stress pathways may provide a possible effective management and prevention of Alzheimer's disease[11]. According to several studies, people with AD have more protein carbonyls in various parts of their brains. In Alzheimer's patients, glutamine synthetase and creatine kinase levels in the brain are low and more susceptible to oxidative modification[12]. The brain is known to have higher amounts of lipid peroxidation in AD, where degenerative alterations are clearer. Because of their double bonds, Polyunsaturated Fatty Acids (PUFAs) in the brain are especially vulnerable to damage by free radicals. The oxidation of arachidonic and docosahexaenoic acid, specifically, causes lipid peroxidation in AD by the generation of aldehydes. Oxidation of DNA can lead to base alterations, strand breakage, sister chromatid swaps, and cross linking of DNA-protein[13].

Numerous investigations have shown that the brains of AD have more DNA damage. 8-hydroxy-2-deoxyguanosine (8-OHdG) is the most highly marked DNA adduct known. Whenever proteins undergo post translational changes, they lead to the formation of advanced glycation end products, that attribute to AD through oxidative alterations of tau and A-peptides. Additionally, the brain's high oxygen consumption rate, quick transitional metal metabolism, and significant amount of quickly oxidized lipids make it extremely vulnerable to oxidative damage and weaken its antioxidant defences[14].

Aluminium is a neurotoxic that alters the concentration of oxygen in the blood, drifts the Blood-Brain Barrier (BBB), and damages the Central Nervous System (CNS). Aluminium causes the accumulation of insoluble a beta and hyper phosphorylated tau protein aggregates that contain Neurofibrillary Tangles (NFTs) and induces detrimental changes to cholinergic neurotransmission[15].

In the ancient Indian system of medicine, *S. lappa* (family Asteraceae) was popular for its medicinal uses. Also known as *Kuthroot* or *Costus* and is used for ulcers, convulsions, cancers, and possesses hepatoprotective, anti-arthritic, anti-viral activities[16]. The bioactive compounds in this plant were costunolide, isodihydrocostunolide, and cynaropicr in which were shown to have the potential to be a source for the creation of novel compounds to treat AD[17].

Therefore, the current study aimed to prospect the nootropic effect of *S. lappa* in aluminium-induced Alzheimer's model and investigate the impact on mental ability, antioxidant enzymes in the brain, and acetylcholinesterase activity in experimental rats.

## 2. Materials and Methods

### 2.1 Collection and Identification of Plant Materials

Authentication of *S. lappa* was done by an eminent botanist, Department of Botany, S V University, Tirupati and voucher specimen was (Pt 0823 and Pt 0754) preserved in the herbarium.

## 2.2 Extraction of Plant Material

Soxhlet extraction was chosen to extract the plant *S. lappa* using methanol as a solvent. Five kilograms of fresh leaves were gathered, shade-dried, and then ground into a powder using a mixer. By means of the Soxhlet apparatus, the dried plant powder was extracted with methanol to produce a proper extract. The filtrate was then filtered and dried[18].

## 2.3 Animals

Six wistar rats (25–30 g) were included in each group (n=6), with seven groups in total. To get acclimatized, animals were housed for seven days in an air-conditioning system room, 22±1°C temperature, and 55±1% humidity, free access to water, and a standard diet. The protocol bearing with the number 1447/PO/Re/S/11/CPCSEA-74/A was approved by the Institutional Animal Ethics Committee (IAEC).

## 2.4 Acute Oral Toxicity Studies

Animals of six rats (n=6) in each group were divided. Administration of doses of 100, 200, 500, 1000, 1500, and 2000 mg/kg via intragastric tube to rats with a prior fasting of 3-4 hours. The food was withheld for 1-2 hours afterwards, but supplied with water. The rats were examined for behavioural or neurological toxicity and mortality for a day[19].

## 2.4 Grouping of Animals

Rats were divided into five groups of six rats each (n=6). Group1was considered the control group. Group 2, 3, 4 and 5 animals were administered with aluminium maltolate (10 mg/kg, p.o.), Group 3, 4, and 5 were given donepezil hydrochloride (1 mg/kg, p.o.), MESC (200 and 400 mg/kg, p.o.).

## 2.5 Behavioural Study in Animals

Animals were trained from $10^{O}$ clock in the morning to $3^{O}$ clock in an insusceptible to sound area in the apparatus prior to the administration of the test extract.

### *2.5.1 Passive Avoidance (PA) Test*

The passive avoidance test is a commonly used behavioural assay in animal research, particularly in the fields of neuroscience and pharmacology. It is employed to evaluate memory and learning, particularly aversive memory, in rodents like mice and rats. The apparatus has a two-chambered box or apparatus. One chamber is illuminated and considered the "safe" chamber, while the other chamber is darkened and associated with an aversive stimulus. There were two compartments in the apparatus, each 270 mm deep and (360X370 mm) in area, with a 90 mm-diameter sliding door. An independent grid floor set measuring 0.9 cm was linked to a shock generator that could produce shocks at a rate of up to 0.5 mA. A fluorescent lamp lit the light compartment. Common aversive stimuli used in the test include mild foot shocks or a brief exposure to an unpleasant odour. There were two phases named training and testing phase, in which each animal had to undergo for the evaluation of the test drugs in both the phases. During the training phase, in an illuminated chamber, an animal was placed, as soon as the animal was comfortable, the entrance to the dark cell is opened. As soon as the animal entered the dark area, it received a mild electrical foot shock for 3 seconds. The door is then closed, preventing the animal from returning to the illuminated chamber. The testing phase was conducted after 24 hours of training phase, the animal was placed in the illuminated chamber, and the latency period (called Initial Transfer Latency (ITL)) to enter the dark cell was recorded. After the training period, up to 300 seconds, the number of latencies to re-enter the dark cell was identified as Step-Through Latency (STL). Animals with intact fear memory tend to show longer latencies to enter the dark cell compared to animals with impaired fear memory[20].

On $29^{th}$ and $30^{th}$ days ITL and STL were determined. The formula was used to determine the percentage of Memory Retention (MR) was as follows -

$$\% \text{ MR} = (\text{STL} - \text{ITL})/\text{ITL} \times 100$$

The passive avoidance test is valuable for studying various aspects of learning and memory, as well as for evaluating potential therapeutic interventions for conditions involving memory deficits in Alzheimer's disease.

### *2.5.2 Elevated Plus Maze Method*

This is a valuable tool for studying anxiety-related behaviour and assessing the effects of pharmacological or genetic manipulations on anxiety levels in rodents. In the description of the apparatus, it consisted of four

arms arranged in a plus-shaped configuration. The two arms that formed a "plus" configuration was enclosed by walls, while the other two, which were in opposition to one another, were open and free of barriers. The maze was elevated above the ground for the induction of aversion to open spaces, thereby triggering with anxiety – like behaviours. Before testing, animals are usually acclimatized to the testing room for a period to reduce the influence of novel environmental factors. The animal was positioned in the centre at the intersection of the open and closed arms, facing one of the closed arms. A 5-minute reading was recorded. The open and closed arm entries were recorded[21].

### 2.5.3 Actophotometer test (Locomotor Count)

The Actophotometer test provides a quantitative assessment of locomotor activity and is valuable for studying various physiological and pharmacological factors that influence motor function and behaviour in rats and mice. This device typically consists of a small chamber equipped with sensors that detect movement. The sensors may be infrared beams or photocells positioned around the chamber. Allow the animals to acclimate to the testing room for a period to reduce the influence of novel environmental factors, the animal was placed in the chamber and the movement of the rat that passed the light beam through the photocell was interrupted, and the locomotor count was then recorded[22].

## 2.6 Biochemical Study

Isolating the brain from rats for biochemical analysis involves several steps to ensure the integrity of the tissue and accurate measurement of biochemical parameters. At the end of the experiment, by means of cervical dislocation, the animals were sacrificed. After dividing the brain region free of the cerebellum, 10% of the brain was homogenized using ice-cold 30 mM phosphate buffer (pH 7.6) and cleaned with ice-cold saline. Then the homogenate was allowed to be centrifuged at 4ºC for half-an hour at 20,000 RPM. Antioxidant enzymes and AchE enzymes were estimated using standard methods[23].

### 2.6.1 Catalase (CAT) Assay

In 10% of the brain tissue homogenate, 3 ml of the test extract, 0.4 ml of 5.9 mM $H_2O_2$, and 2.5 ml of 50 mM phosphate buffer (pH 5.0) were dissolved. The absorbance at 240 nm was then measured after the reaction mixture was incubated for one minute[24].

### 2.6.2 Lipid Peroxidation (TBARS) Assay

In a test tube, add 0.2 ml of the 10% tissue homogenate, 0.02 ml of 100 mM $FeCl_3$, 0.02 ml of 100 mM ascorbic acid, and 0.58 ml of 0.1 M phosphate buffer (pH 7.4), then 1.0 ml of the extract. Following the incubation for an hour at 37 °C, the reaction was stopped with the addition of 1.0 ml of 10% TCA in a water bath shaker after it had been. Each test tube was brought to a boil in a water bath for 20 minutes after 1.0 cc of 0.67% TBA had been added. After that, the test tubes were placed in a bath of crushed ice and centrifuged for ten minutes at 2500 g. At 535 nm in UV light, the amount of TBARS produced in each sample was measured and represented as nM/min/mg protein, with a value of $1.56 \times 105\ M^{-1}\ cm^{-1}$ as a molar extinction coefficient[25].

### 2.6.3 Acetylcholinesterase (AChE) Assay

With the modifications to the ellman method, AChE activity was determined. In a 96 well plate, 25µl of 15 mM ATCI, 75µl of 3 mM DTNB and 75µl of 50 mM Tris-HCl (pH 8.0) and 0.1% of BSA were added, and at 25°C it was incubated for 5 min. The absorbance was measured at 405 nm after the addition of 25µl of brain tissue homogenate that was incubated at 25°C for five minutes. The AChE activity was represented as M/min/g protein[26].

## 2.7 Histopathological Examination of Brain Hippocampus

The brain tissues that were extracted underwent standard procedures for light microscopy and were conserved in a 10% neutral buffered formal saline solution. Neurons within the CA1 region of multiple hippocampal areas were quantified utilizing a morphometric lens, followed by the computation of the average count[27,28].

## 2.8 Statistical Analysis

The data was represented as mean ± SEM. One-way ANOVA followed by post hoc Dunnett's test, a p value <0.05 was considered statistically significant.

# 3. Results

Preliminary phytochemical screening revealed the presence of carbohydrates, amino acids, proteins,

alkaloids, cardiac glycosides, triterpenoids, saponins, flavonoids, phenolic compounds, tannins, and steroids. GC-MS analysis carried on methanolic fraction of *S. lappa* showed the existence of bio active compounds like 2-nonynoic acid, lupeol and dodecanal[29].

## 3.1 Determination of Acute Toxicity

Throughout the course of 14 days of observation, there were no detrimental effects on the behavioural, motor, or neural reactions of the experimental rats with MESC up to a dosage level of 2000 mg/kg, thus the extracts were assumed to be safe.

## 3.2 Assessment of Behavioural Paradigm

In the passive avoidance test procedure, the ITL, STL and percentage of memory retention was recorded on the 29$^{th}$ and 30$^{th}$ days. In test drug treated animals, there was an increase in STL with both doses significantly (200 and 400 mg/kg), the values were found to be significant as represented in Table 1a and 1b respectively.

In the assessment of the locomotor activity of rats, the locomotor score on days 3, 5 and 7 was recorded. It was observed that the locomotor score increased subsequently from day 3 to 5, showed a remarkable and a significant (**p<0.01) effect with the treatment of the test extracts (Table 2 and Figure 1).

**Table 1a.** Effect of MESC on learning and memory in PA test on day 29

| Groups | Latency in passive shock avoidance (seconds) | | % of Memory retention |
|---|---|---|---|
| | ITL | STL | |
| Normal control | 91.12 ± 2.10 | 165.60±2.42 | 22.50±1.9 |
| Positive control | 78.31±1.22 | 120.43±1.81 | 53.8±1.52 |
| Standard | 113.54±4.21** | 205.67±3.65** | 81.41±1.71** |
| MESC 200mg/kg | 119.11±2.32** | 194.87±3.24** | 63.02±1.09** |
| MESC 400mg/kg | 109.91±2.41** | 189.42±3.52** | 72.34 ±1.76** |

**p<0.01 was considered as significant; test and standard groups were compared with respective control groups.

**Table 1b.** Effect of MESC on learning and memory in PA test on day 30

| Groups | Latency in passive shock avoidance (seconds) | | % of Memory retention |
|---|---|---|---|
| | ITL | STL | |
| Normal control | 91.12 ± 2.10 | 165.60±2.42 | 22.50±1.9 |
| Positive control | 81.31±1.22 | 137.39±1.17 | 69.24±1.63 |
| Standard | 110.74±2.12** | 210.72±2.89** | 90.92±1.71** |
| MESC 200mg/kg | 109.11±1.54** | 194.70±1.90** | 77.98±1.09** |
| MESC 400mg/kg | 102.34±1.89** | 18.56±1.78** | 83.34 ±1.76** |

**p<0.01 was considered as significant; test and standard groups were compared with respective control groups.

**Table 2.** Effect of MESC on locomotor activity in actophotometer

| Groups | Locomotive score (sec) | | |
|---|---|---|---|
| | Day 3 | Day 5 | Day 7 |
| Normal control | 384.43±3.32 | 394.42±1.42 | 386.33±1.24 |
| Disease control | 186.42±2.65 | 162.67±2.14 | 145.33±1.45 |
| Standard | 166.33±2.61** | 217.33±4.53** | 246.33±3.17** |
| MESC 200 mg/kg | 155.33±2.48** | 184.67±3.78** | 215.67±2.52** |
| MESC 400 mg/kg | 162.57±2.55** | 198.63±2.32** | 236.67±5.85** |

**p<0.01 was considered as significant; test and standard groups were compared with respective control groups.

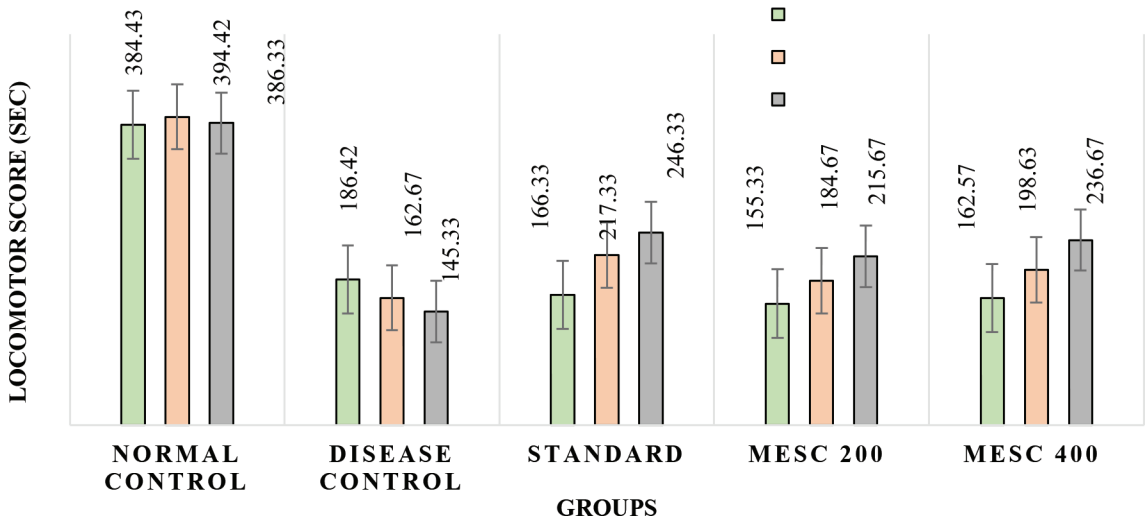


**Figure 1.** Effect of MESC on locomotor score in actophotometer.

Anxiety associated behavior was recorded and it was observed that the time spent in open arms was increased significantly with a decreased time spent in closed arms (**p<0.01) after the treatment with the test extracts at 200 and 400 mg/kg respectively (Table 3 and Figure 2).

In the estimation of antioxidant enzymes, it was noted that the treatment with MESC (i.e., 200 and 400 mg/kg) significantly (**p < 0.01) increased the amounts of CAT but reduced the concentration of TBARS as compared to the disease control animals. The standard

**Table 3.** Effect of MESC on time spent in the arms of Elevated plus maze

| Treatment | Time spent in closed arm (sec) | Time spent in open arm (sec) |
|---|---|---|
| Normal control | 10.53±4.61 | 221.36±8.52 |
| Disease control | 93.73±3.34 | 146.56±9.22 |
| Standard | 48.27±3.93** | 187.88±6.33** |
| MESC 200mg/kg | 63.73±3.34** | 156.46±9.22** |
| MESC 400mg/kg | 70.37±2.69** | 179.51±8.23** |

**p<0.01 was considered as significant; test and standard groups were compared with respective control groups.

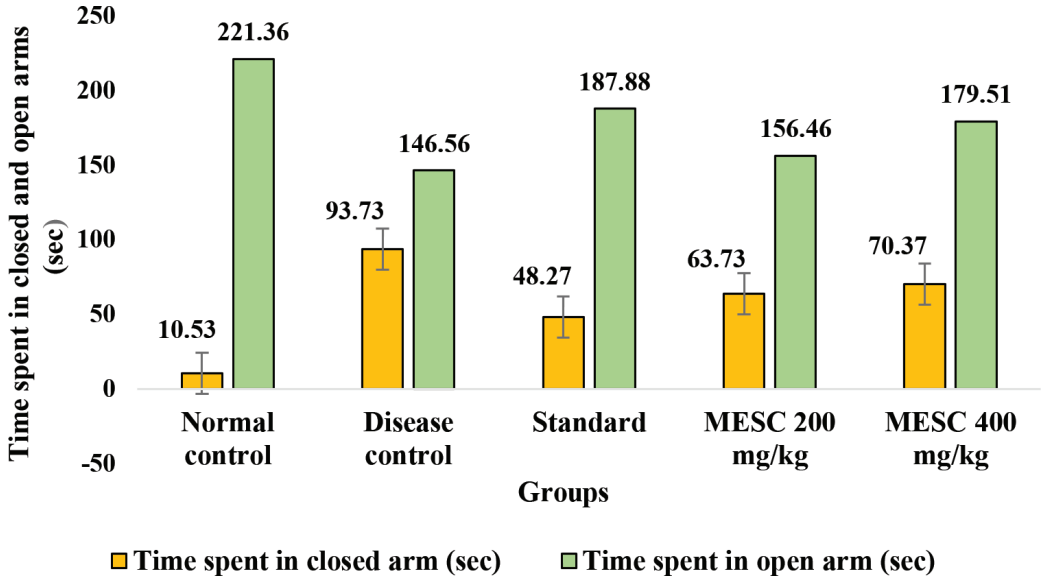


**Figure 2.** Effect of MESC on time spent in closed and open arms of elevated plus maze.

drug showed a significant decline in (**p < 0.01) the level of TBARS and a rise in catalase activity (Table 4 and Figure 3).

The activity of AChE in the rat brain tissue homogenate is represented in Table 5. As compared to the disease induced rats, donepezil treated animals showed a significant (**p < 0.01) drop in AChE levels in brain tissue homogenate. Treatment with MESC at both doses -200 and 400 mg/kg demonstrated a significant (**p< 0.01) decrease in the AChE activity as compared to disease control animals.

### 3.3 Histopathological Examination

In positive control animals, there was an eosinophilic neuronal necrosis and neuronal nuclear pyknosis and a significant reduction in hippocampal Cornu Ammonis (CA1) neurons. The test drug at a dose of 200 mg/kg, decreased neuronal necrosis (Figure 4B), and at a dose of 400 mg/kg, there was moderate cell damage and increased CA1 neuron density, depicted in Figure 4.

## 4. Discussion

Herbal drugs, also known as botanical medicines, have been used since few decades in the traditional system for a various health condition, including neurological disorders. While conventional pharmaceuticals are often the first line of treatment for neurological conditions, there is a growing interest in exploring the potential benefits of herbal remedies due to their perceived safety, accessibility, and potential therapeutic effects[30]. It is important to note that the effectiveness

**Table 4.** Effect of MESC on Antioxidant enzymes in brain homogenate

| Groups | TBARS (µM/mg tissue) | Catalase (units/mg protein) |
|---|---|---|
| Normal control | 2.83±0.89 | 0.72±0.0429 |
| Disease control | 5.23±0.21 | 0.45±0.03 |
| Standard control | 2.95±0.16** | 0.67±0.02** |
| MESC 200mg/kg | 3.36±0.89** | 0.59±0.03** |
| MESC 400mg/kg | 3.59±0.88** | 0.63±0.05** |

**p<0.01 was considered as significant; test and standard groups were compared with respective control groups.

**Table 5.** Effect of MESC on enzyme activity in brain homogenate

| Treatment | AChE (moles x $10^{-6}$/min/ g of tissue) |
|---|---|
| Normal control | 30.31 ± 1.76 |
| Disease control | 43.56 ± 1.62 |
| Standard control | 14.23 ± 2.52** |
| MESC 200mg/Kg | 19.06 ±2.48** |
| MESC 400mg/Kg | 14.72 ±2.57** |

Values were expressed in Mean ± SEM. **p<0.01 was considered as significant; Test and standard groups compared with respective control groups.

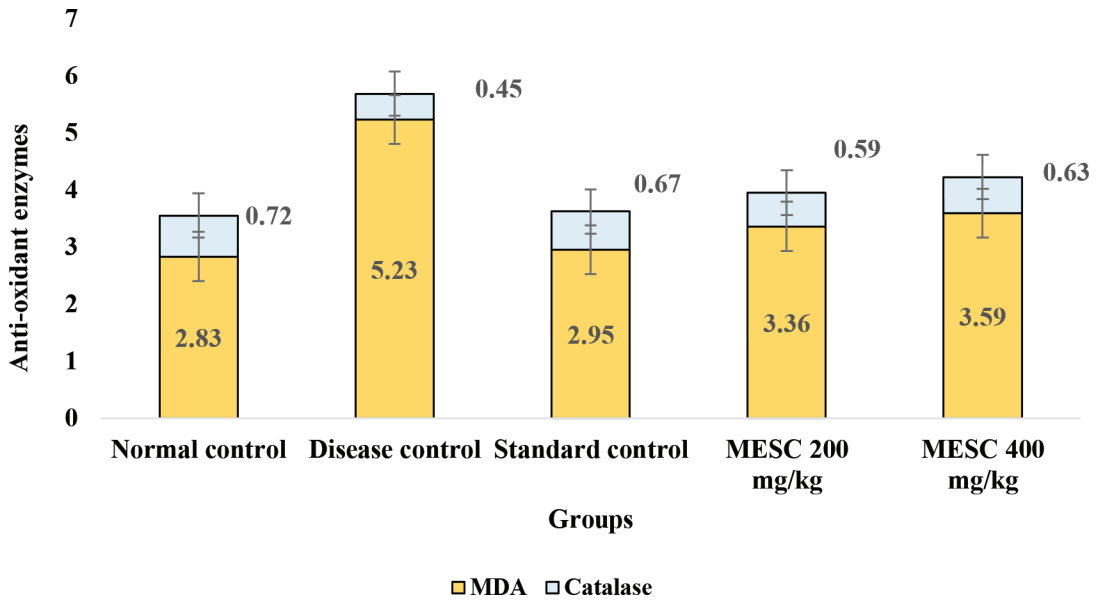


**Figure 3.** The effect of MESC on antioxidant enzymes in brain homogenate.

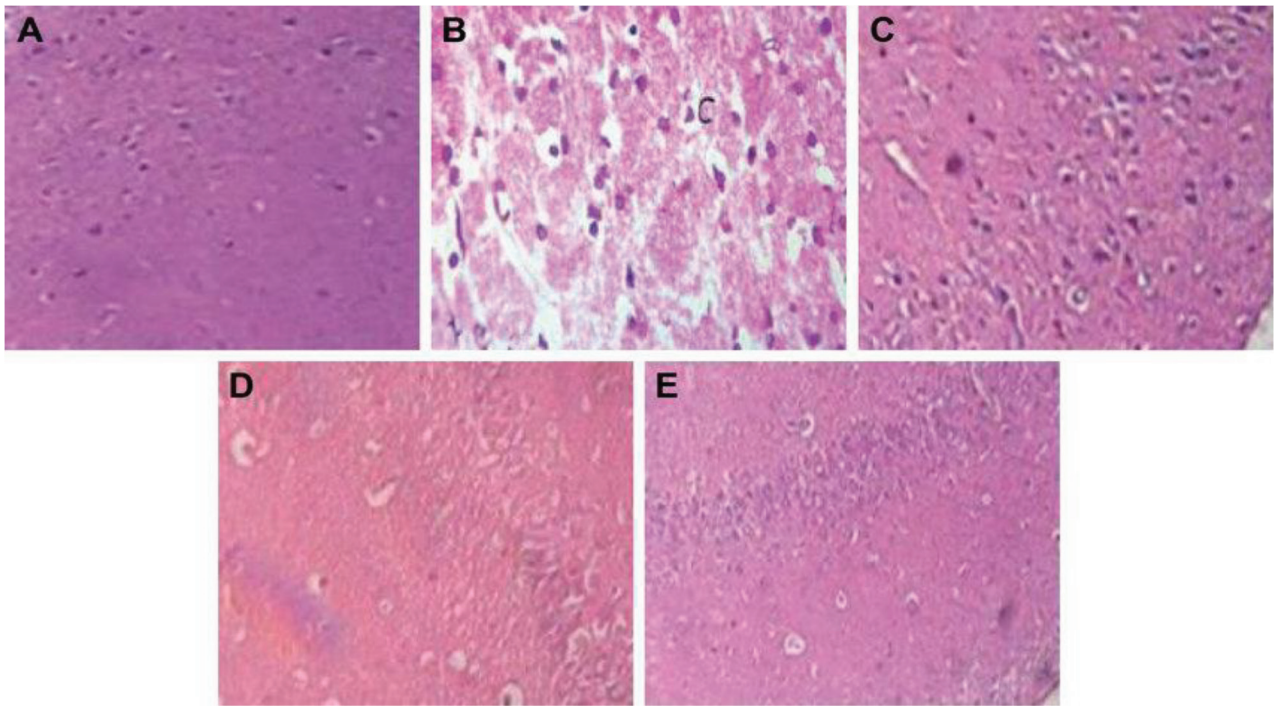


**Figure 4.** **(A).**Normal hippocampus; **(B).** An illustration of the brain region in disease-induced rats; **(C).** Standard drug donepezil HCl treated rat brain; **(D).** MESC 200mg/kg treated group; **(E).** MESC 400mg/kg treated group.

and safety of herbal drugs can vary widely, and not all herbs are suitable for every individual or condition. Additionally, scientific evidence supporting the use of herbal drugs for neurological disorders varies in terms of quantity and quality[31].

Research on medicinal plants that can improve cognitive function is highly desired right now to lessen the deleterious effects[32]. This is the first study to investigate the neuroprotective nature of the methanolic extract of *S. lappa* in rats with aluminium-induced oxidative stress and cognitive impairment using a range of behavioural and biochemical measures. An elevation of lipid peroxidation under aluminium exposure subsequently causes reduction of antioxidant enzyme activity including neurotoxicity and mitochondrial dysfunction[33]. A few consequences like the mitochondrial swelling and altered inner mitochondrial membrane along with impaired oxidative phosphorylation led to raise in the formation of reactive oxygen species[34]. As a result, aluminium induces neurons and glial cells to undergo apoptosis. There is a reduction in Long-Term Potentiation (LTP) with a chronic aluminium treatment that is widely acknowledged as a paradigm of the memory system[35].

Aluminium also inhibits a few enzymes, including those involved in the manufacture of neurotransmitters, which has an impact on the number of neurotransmitters. Aluminium also affects emotional reactivity, hinders a variety of learning and memory-related brain activities, and creates a deficiency in spatial memory[36]. In the present study, a neurotoxic effect was induced by aluminium that affected the learning and memory. The test extract of *S. lappa* was evaluated in aluminium-induced memory deficit rats using standard models.

In this study, after treatment with the test drug for 30 days, the drug exhibited a strong neuroprotective effect in rats by improving learning ability and memory, with anti-acetylcholinesterase properties. In the fear-aggravated test (PT test), rats have learned to avoid situations in which an unpleasant stimulus such as a foot shock, thus helps to assess their learning capacity[37]. As previously mentioned, the latency periods were recorded as ITL and analysed. The STL measures the degree to which a negative experience is recalled. The mean STL of the MESC-treated rats was noticeably higher than that of the other groups. Anxiety-like behaviour was investigated in an elevated

plus maze where the entry of an anxious animal into the open arms was less frequent and the animal would stay for shorter periods of time than it would in the closed arms.

This maze's capacity to examine the effects of various medications is by far its most beneficial feature. The time spent in the closed arms was less than the time spent in the open arms, after the treatment with the test extract as compared to the positive control animals. An enzyme known as catalase safeguards the cells from oxidative damage caused by Reactive Oxygen Species (ROS)[38]. Lipid peroxidation, or the oxidation of lipids, stands as a major factor responsible for several disease state in people of all ages. A consequence of exposing lipids to different ROS (hydrogen peroxide, hydroxyl radical, etc.) during their oxidative degradation is lipid peroxidation, cell damage ensues during this process[39]. Hence the antioxidant enzymes were measured in the brain homogenate, and it was noticed that there was a significant reduction in TBARS and increased levels of catalase in test drug treated rats. ACh gets inactivated by the AChE enzyme, at the neuromuscular junction and cholinergic synapse[40]. It was demonstrated that MESC-treated rats had markedly lowered AChE activity. The *S. lappa* extract boosted the levels of acetylcholine and an improved the cognitive function of rats in the present study.

In the previous study, it was proven that the GC-MS analysis revealed the presence of bioactive constituents that were responsible for the antioxidant property, anti-neurological activity, and anti-inflammatory effect. The same drug was evaluated further for learning and memory, neuroprotection, and oxidative stress. When the brain sections were examined in the test drug treated animals, there was a decrease in neuronal necrosis and cellular damage, hence the drug proved to possess protection against neuronal damage.

## 5. Conclusion

The study revealed that treatment for 30 days developed a nootropic like effect and restored the cognitive impairment and oxidative stress that aluminium-exposure had caused in them. MESC leaves may enhance cognitive abilities, exerting control over antioxidant enzymes in the brain, modifying acetylcholine levels, and preventing cognitive dysfunction brought on by aluminium in rats' brains. Therefore, compared to other neurodegenerative conditions, AD can be managed more successfully with the use of this fruit extract. Despite these results, more investigation is required to identify and isolate any potentially useful nootropic compounds as well as to uncover any mechanisms of action.

## 6. References


1. Mehla J, Gupta P, Pahuja M, Diwan D, Diksha D. Indian medicinal herbs, and formulations for Alzheimer's disease, from traditional knowledge to scientific assessment. Brain Sci. 2020; 10(12):964. https://doi.org/10.3390/brainsci10120964 PMid:33321899 PMCid:PMC7764187
2. 2020 Alzheimer's disease facts and figures. Alzheimers Dement. 2020 Mar 10. Epub ahead of print. https://doi.org/10.1002/alz.12068 PMid:32157811
3. Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. Arch Pharm Res. 2013; 36(4):375-99. https://doi.org/10.1007/s12272-013-0036-3 PMid:23435942
4. Rao PS and Mohan GK. *In vitro* alpha-amylase inhibition and *in vivo* antioxidant potential of momordica dioica seeds in streptozotocin-induced oxidative stress in diabetic rats. Saudi Journal of Biological Sciences. 2017; 24(6):1262-7. https://doi.org/10.1016/j.sjbs.2016.01.010 PMid:28855820
5. Rahman T, Hosen I, Islam MMT, Shekhar HU. Oxidative stress and human health. Adv Biosci Bio Technol. 2012; (3):997-1019. https://doi.org/10.4236/abb.2012.327123
6. Gella A, Durany N. Oxidative stress in alzheimer disease. Cell Adh Migr. 2009; 3(1):88-93. https://doi.org/10.4161/cam.3.1.7402 PMid:19372765
7. Tamagno E, Guglielmotto M, Vasciaveo V, Tabaton M. Oxidative stress and beta amyloid in Alzheimer's disease. Which comes first: The chicken or the egg? Antioxidants (Basel). 2021; 10(9):1479. https://doi.org/10.3390/antiox10091479 PMid:34573112
8. Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. Biomed Rep. 2016; 4(5):519-22. https://doi.org/10.3892/br.2016.630 PMid:27123241
9. Sailaja Rao P, Sireesha Kalva, Aparna Yerramilli, Sadanandam Mamidi. Free radicals and tissue damage: role of antioxidants. free radicals and antioxidants. 2011; 1(4):2-7. https://doi.org/10.5530/ax.2011.4.2
10. Halliwell B. Free radicals in the brain. Springer Berlin Heidelberg; New York, NY: 1992. Reactive oxygen species and the central nervous system. 1992. p. 21-40. https://doi.org/10.1007/978-3-642-77609-0_2
11. Smith KJ, Kapoor R, Felts PA. Demyelination: the role of reactive oxygen and nitrogen species. Brain Pathol. 1999;

9(1):69-92. https://doi.org/10.1111/j.1750-3639.1999.tb00212.x PMid:9989453

12. Clarke JR, Lyra E Silva NM, Figueiredo CP, Frozza RL, Ledo JH, Beckman D, Katashima CK, Razolli D, Carvalho BM, Frazão R, Silveira MA, Ribeiro FC, Bomfim TR, Neves FS, Klein WL, Medeiros R, LaFerla FM, Carvalheira JB, Saad MJ, Munoz DP, Velloso LA, Ferreira ST, De Felice FG. Alzheimer-associated Aβ oligomers impact the central nervous system to induce peripheral metabolic deregulation. EMBO Mol Med. 2015; 7(2):190-210. https://doi.org/10.15252/emmm.201404183 PMid:25617315
13. Perluigi M, Coccia R, Butterfield DA. 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neuro degenerative diseases: a toxic combination illuminated by redox proteomics studies. Antioxid Redox Signal. 2012; 17(11):1590-609. https://doi.org/10.1089/ars.2011.4406 PMid:22114878
14. AbuArrah M, Yuli Setianto B, Faisal A, Hamim Sadewa A. 8-Hydroxy-2-Deoxyguanosine as Oxidative DNA Damage biomarker of medical ionizing radiation: A scoping review. J Biomed Phys Eng. 2021; 11(3):389-402. https://doi.org/10.31661/jbpe.v0i0.2101-1258 PMid:34189127
15. Kandimalla R, Vallamkondu J, Corgiat EB, Gill KD. Understanding aspects of aluminum exposure in Alzheimer's disease development. Brain Pathol. 2016; 26(2):139-54. https://doi.org/10.1111/bpa.12333 PMid:26494454
16. Abdel-Rahman M, Rezk MM, Ahmed-Farid OA, Essam S, Abdel Moneim AE. *Saussurea lappa* root extract ameliorates the hazards effect of thorium induced oxidative stress and neuroendocrine alterations in adult male rats. Environ Sci Pollut Res Int. 2020; 12:13237-46. https://doi.org/10.1007/s11356-020-07917-y PMid:32016872
17. Islam F, Khadija JF, Harun-Or-Rashid M, *et al*. Bioactive compounds and their derivatives: an insight into prospective phytotherapeutic approach against Alzheimer's disease. Oxid Med Cell Longev. 2022; 5100904. https://doi.org/10.1155/2022/5100904 PMid:35450410
18. El-Rahman GIA, Behairy A, Elseddawy NM, *et al*. *Saussurea lappa* ethanolic extract attenuates triamcinolone acetonide-induced pulmonary and splenic tissue damage in rats via modulation of oxidative stress, inflammation, and apoptosis. Antioxidants (Basel). 2020; 9(5):396. https://doi.org/10.3390/antiox9050396 PMid:32397156
19. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Toxicol Int. 2013; 20(3):224-6. https://doi.org/10.4103/0971-6580.121674 PMid:24403732
20. Eagle AL, Wang H, Robison AJ. Sensitive assessment of hippocampal learning using temporally dissociated passive avoidance task. Bio Protoc. 2016; 6(11):1821. https://doi.org/10.21769/BioProtoc.1821 PMid:29119127
21. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc. 2007; 2(2):322-8. https://doi.org/10.1038/nprot.2007.44 PMid:17406592
22. Bhosale U, Yegnanarayan R, Prachi P, Zambare M, Somani RS. Study of CNS depressant and behavioral activity of an ethanol extract of achyranthes aspera (Chirchita) in mouse model. Ann Neurosci. 2011; 18(2):44-7. https://doi.org/10.5214/ans.0972.7531.1118204 PMid:25205920
23. Carbone L, Carbone ET, Yi EM, *et al*. Assessing cervical dislocation as a humane euthanasia method in mice. J Am Assoc Lab Anim Sci. 2012; 51(3):352-6.
24. Khan RA, Khan MR, Sahreen S. Brain antioxidant markers, cognitive performance and acetylcholinesterase activity of rats: efficiency of sonchus asper. Behav Brain Funct. 2012; 8:21. https://doi.org/10.1186/1744-9081-8-21 PMid:22591917
25. Patro G, Bhattamisra SK, Mohanty BK, Sahoo HB. *In vitro* and *in vivo* antioxidant evaluation and estimation of total phenolic, flavonoidal content of *Mimosa pudica* L. Pharmacognosy Res. 2016; 8(1):22-8. https://doi.org/10.4103/0974-8490.171099 PMid:26941532
26. Freitas AP, Santos CR, Sarcinelli PN, Silva Filho MV, Hauser-Davis RA, Lopes RM. Evaluation of a brain acetylcholinesterase extraction method and kinetic constants after methyl-paraoxon inhibition in three brazilian fish species. PLoS One. 2016; 11(9):0163317. https://doi.org/10.1371/journal.pone.0163317 PMid:27655611
27. Snyder JM, Radaelli E, Goeken A, *et al*. Perfusion with 10% neutral-buffered formalin is equivalent to 4% paraformaldehyde for histopathology and immune his to chemistry in a mouse model of experimental autoimmune encephalomyelitis. Vet Pathol. 2022; 59(3):498-505. https://doi.org/10.1177/03009858221075588 PMid:35130806
28. Nardi L, Schmeisser MJ, Schumann S. Fixation and staining methods for macroscopical investigation of the brain. Front Neuroanat. 2023: 17:1200196. https://doi.org/10.3389/fnana.2023.1200196 PMid:37426902
29. Sadia H, Sumalatha G. *In vitro* antioxidant activity and gas chromatography-mass spectrometry analysis of methanolic extracts of *Saussurea lappa* Clarke and *Premna mucronata* Rox b. Int J Pharm Sci Drug Res. 2022; 14(6):788-9. https://doi.org/10.25004/IJPSDR.2022.140617
30. Dos Santos-Neto LL, de Vilhena Toledo MA, Medeiros-Souza P, de Souza GA. The use of herbal medicine in Alzheimer's disease - A systematic review. Evid Based Complement Alternat Med. 2006; 3(4):441-5. https://doi.org/10.1093/ecam/nel071 PMid:17173107
31. Mantle D, Pickering AT, Perry E. Medical plant extracts for treatment of dementia: A review of their pharmacology, efficacy and tolerability. CNS Drugs. 2000; 13:201-13. https://doi.org/10.2165/00023210-200013030-00006
32. Cummings J, Aisen PS, DuBois B, Frölich L, Jack CR Jr, Jones RW, Morris JC, Raskin J, Dowsett SA, Scheltens P.

Drug development in Alzheimer's disease: the path to 2025. Alzheimers Res Ther. 2016; (8):39. https://doi.org/10.1186/s13195-016-0207-9 PMid:27646601

33. Skalny AV, Aschner M, Jiang Y, *et al*. Molecular mechanisms of aluminum neurotoxicity: Update on adverse effects and therapeutic strategies. Adv Neurotoxicol. 2021; 5:1-34. https://doi.org/10.1016/bs.ant.2020.12.001 PMid:34263089
34. Cheng H, Yang B, Ke T, *et al*. Mechanisms of metal-induced mitochondrial dysfunction in neurological disorders. toxics. 2021; 9(6):142. https://doi.org/10.3390/toxics9060142 PMid:34204190 PMCid:PMC8235163
35. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem. 2010; 345(1-2):91-104. https://doi.org/10.1007/s11010-010-0563-x PMid:20730621
36. Bryliński Ł, Kostelecka K, Woliński F, *et al*. Aluminium in the human brain: Routes of penetration, toxicity, and resulting complications. Int J Mol Sci. 2023; 24(8):7228. https://doi.org/10.3390/ijms24087228 PMid:37108392
37. Atallah HE, Frank MJ, O'Reilly RC. Hippocampus, cortex, and basal ganglia: insights from computational models of complementary learning systems. Neurobiol Learn Mem. 2004; 82(3):253-67. https://doi.org/10.1016/j.nlm.2004.06.004 PMid:15464408
38. Nandi A, Yan LJ, Jana CK, Das N. Role of catalase in oxidative stress- and age-associated degenerative diseases. Oxid Med Cell Longev. 2019; 9613090. https://doi.org/10.1155/2019/9613090 PMid:31827713
39. Lardinois OM. Reactions of bovine liver catalase with superoxide radicals and hydrogen peroxide. Free Radic Res. 1995; 22(3):251-74. https://doi.org/10.3109/10715769509147544 PMid:7757201
40. Anand P, Singh B. A review on cholinesterase inhibitors for Alzheimer's disease. Arch Pharm Res. 2013; 36(4):375-99. https://doi.org/10.1007/s12272-013-0036-3 PMid:23435942