Int. J. Adv. Res. Sci. Technol. Volume 10, Issue 1, 2022, pp.835-844.

IJARST

International Journal of Advanced Research in Science and Technology

journal homepage: www.ijarst.com

ISSN 2319 – 1783 (Print)

ISSN 2320 – 1126 (Online)

Evaluation of neuroprotective activity of *Caesalpinia sappan* Linn. against Aluminum chloride induced neurotoxicity in rats

Mohammed Zubair A S. [1], S. Babitha[2], Pooja R C[3], M S Naveeda Khanum[4], Rekha Y V[5]

Department of Pharmacology, Sree Siddaganga College of Pharmacy, B H Road, Tumkuru, Karnataka, India.

ARTICLEINFO

Article history:

Received : 23 Dec 2021

Accepted : 05 Jan 2022

Available online : 12 Jan 2022

Keywords:

Neuroprotective,
Aluminumchloride,
Monosodiumglutamate,
Donepezilhydrochloride,
Dextromethorphan,
Caesalpiniasappan.

Objective: The present study was designed to evaluate the neuroprotective activity of hydroalcoholic extract of Caesalpinia sappan Linn. (HAECS) against aluminum chloride (AlCl3) induced neurotoxicity in rats.

Materials and methods: The neuroprotective activity of HAECS was assessed by using AlCl3 induced neurotoxicity models in rats. In this study, neurotoxicity was induced by the administration of AlCl3 (100 mg/kg, p.o.) for 42 days. In this model both the test doses of HAECS (200 and 400mg/kg) were administered 1 hour after AlCl3 administration. The reference standard was Donepezil hydrochloride (1mg/kg p.o.). The degree of protection was assessed by various behavioral, antioxidant parameters and Acetylcholinesterase (AChE) estimation. Further, neuronal damage was assessed by brain histopathological observations.

Results: Treatment with HAECS significantly attenuated AlCl3 alteration in behavioral and physiological responses. It prevented the elevation of AchE and LPO. An increase in GSH, SOD, and CAT was observed in groups treated with HAECS and it also reduced the histopathological changes induced by AlCl3 administration.

Conclusion: The present study suggests that the hydroalcoholic extract of Caesalpinia sappan Linn. possesses significant neuroprotective activity against AlCl3 induced neurotoxicity in rats. The observed effect could be possibly attributed to its potential antioxidant activity and anti-inflammatory property due to the presence of various neuroprotective constituents.

PAPER-QR CODE

Citation: *Mohammed Zubair et al., Evaluation of neuroprotective activity of Caesalpinia sappan Linn. against Aluminum chloride induced neurotoxicity in rats. Volume 10, Issue 1, 2022 January, pp.835-844.*

Introduction

An organism's nervous system is a complex network that allows it to communicate with its surroundings. Signaling within these circuits permits thinking, language, feeling, learning, memory, and all other functions and sensations in the nervous system[1].

Neurodegeneration is the death and loss of neurons in the brain over time, a process that begins with molecular dysregulation and progresses to gross regional dysfunction and, eventually, clinical impairment[2].

According to WHO data, neurological and mental illnesses constitute a significant and growing source of morbidity. Mental, neurological, and behavioral diseases have a major impact on the world's population, impacting more than 450 million individuals. According to the Global Burden of Disease Report, neurological and mental illnesses account for four out of the six primary causes of years lived with disability, accounting for 33 percent of years lived with disability and 13 percent of disability-adjusted life years[3].

Neurodegenerative illnesses are defined by the gradual loss of neurons in the central nervous system (CNS),

resulting in impairments in specific brain processes (e.g., memory, mobility, and cognition) performed by the affected CNS region. These neurodegenerative diseases include Alzheimer's disease (AD), multiplesclerosis (MS), Parkinson's disease (PD), Huntington's disease (HD)amyotrophic lateral sclerosis (ALS)[4].

Neurodegenerative diseases are characterized by the loss of neurons in the central nervous system (CNS)[5], resulting in deficits in specific brain functions (e.g., memory, movement, and cognition) performed by the affected CNS region. Dementia in Alzheimer's disease is linked to neurodegeneration, which begins with synaptic damage and progresses to neuronal death[6].

NDDs are caused by a variety of pathogenic processes which include; Abnormal protein dynamics with misfolding, faulty degradation, proteasomal malfunction, and Oxidative stress and the formation of free radicals/reactive oxygen species (ROS) are typically generated by the activities and mutations of molecular chaperones, mitochondrial dysfunctions, and other factors. Bioenergetic dysfunctions and, DNA damage, Fragmentation of neuronal Golgi apparatus, cellular/axonal transport disruption Neurotrophins dysfunction and neuroinflammatory/neuroimmune processes[7].Infections, ischemia, environmental and dietary poisons are the leading causes of neurodegeneration[8]. Metal exposure raises the chance of Alzheimer's disease. Aluminum (Al) is a heavy element that primarily affects brain development and has been linked to neurodegenerative disorders like Alzheimer's disease[9].

Al is a poisonous substance that is widely dispersed and abundant in the environment and industry, as well as a component of many foods[10]. Al is a potent neurotoxin that has been linked to a variety of mental illnesses. Long-term Al exposure causes oxidative stress and degenerative changes in many brain regions of neonatal rats. Chronic exposure to Al has been shown to generate neurologic indications that mimic progressive dementia, as well as neurofilaments changes in the hippocampus and cerebral cortex, as well as metabolic abnormalities. Both human and animal investigations show that AlCl3 exposure decreases learning, memory, and cognition function[11].

Herbal therapies have made a huge difference in people's health and wellbeing[12]. Ayurveda, Unani, and other alternative medicine systems use a variety of herbs and herbal products to treat various neurological illnesses[13]. *Caesalpinia sappan*(Fabaceae) Species have been utilized in traditional medicine for a long time and offer a wide range of medicinal characteristics. The heart wood contains various neuroprotective chemical constituents such asflavonoids (quercetin), triterpenoids, polyphenols, and steroids. Caesalpinia sappan heartwood extract has been shown to have high antioxidant activity[14]. The chemicals, which have antioxidant and anti-inflammatory characteristics, have been shown helpful in animal models of various neurodegenerative diseases[15]. Therefore, the present study was designed to evaluate the neuroprotective activity of *Caesalpinia sappan* Linn. against Aluminum induced neurotoxicity in experimental animals.

Materials and methods:

Collection and authentication of plant[16]

The heartwood of C. sappan L. was purchased from an online medicinal materials seller.

Dr. R Nandeesh M.Pharm, Ph.D., Head, Department of Pharmacognosy, Sree Siddaganga College of Pharmacy, Tumkur, identified and authenticated the sample.

Preparation of plant extract:

Heartwood of C. Sappan was collected; shade dried and coarsely powdered. The Hydroalcoholic extract was prepared by taking the powder in 80% (v/v) ethanol and this condition was maintained for 72 hours Then the residues were separated using a vacuum filter and the filtrate is dried under reduced pressure, yielding dark reddish-brown extract which was stored in the refrigerator for study.

Drugs and chemicals:

Aluminum chloride (AlCl3) purchased from S.D. Fine Chemicals Ltd, Mumbai and donepezil hydrochloride as Donecept-5 from Cipla. The rest of the chemicals and reagents used in the experiment were of analytical quality.

Animals:

Male Albino Wistar Rats weighing 180-220 g were used for the present study. The animals were collected from Sree Siddaganga College of Pharmacy animal house, Tumakuru. The animals were kept in a controlled environment with temperature (22° C), humidity (50%), and 12 hour light-dark cycles. Before the study, all of the animals were given a seven-day acclimatization period. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) (Approval No. SSCPT/IAEC. Clear/ 193/ 2019-20).

Experimental design[17]:

The animals were placed into six groups, with six animals in each group. Group I was kept as normal control and received vehicle. Group II was kept as inducer control; Group III was as HAECS extract alone. Group IV and V received HAECS at doses 100 and 200 mg/kg orally respectively, and Group VI served as standard received Donepezil hydrochloride (1 mg/kg). The plant extract (HAECS) and Donepezil hydrochloride were given after aluminum chloride administration for a period of 42 days. On the 42nd day, the rats were evaluated for ambulatory behavior, muscle coordination, and strength activity. 24 hours after the

administration of the last dose, rats were subjected to scarification, and their brains were isolated. hippocampal, septum, and frontal cortex was dissected out on a Petri dish chilled on crushed ice and was used for AchE estimation. The remaining sections of the brain were used to calculate GSH, LPO, catalase, and SOD, as well as conduct a histopathological examination.

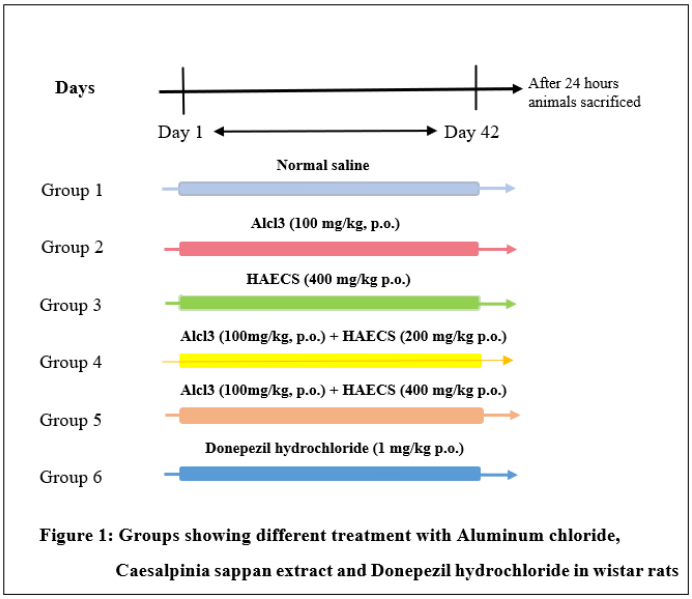

Figure 1: Groups showing different treatment with Aluminum chloride, Caesalpinia sappan extract and Donepezil hydrochloride in wistar rats

Behavioural Parameters

All the behavioral tests were conducted during the light portion between 8:00 am-12:00 pm to avoid circadian influences.

Elevated plus maze test[18]

To evaluate memory dysfunction elevated plus-maze is used. It is made up of two open arms (50 cm 10 cm) on opposite sides, A 40 cm high wall is bridged by two closed arms of equal proportions. Central Square (10 cm x 10 cm) is used to link the arms. The animals were placed in the maze's middle, with their heads towards the open arm, and the stopwatch was started to record the following parameters (the cut-off time was 5 minutes). Animals entered in the open and closed arms to be counted as an entry if all the four paws have to be in the arm and the duration of time animal spent in the open and closed arms to be counted.

Locomotor activity (ambulatory behaviour)[19]

Actophotometer was used to track spontaneous locomotor activity consisting of photocells that are sensitive to infrared light. The experiment was taking place in a dark room. Turn on the equipment (verify that all photocells are functional for accurate recording) and place the mice in the activity cage one at a time for 5 minutes. Take note of the score. Animals were individually placed in the activity meter for 1 minute prior to the locomotor challenge for habituation. Following that, locomotor activity was monitored for 5 minutes.

Rota-rod (muscle co-ordination test)[20]

A Rota-rod treadmill was used to assess the motor coordination of the rats. A plastic rod of 3 cm diameter, 30 cm long with a non-slippery surface, and 15 cm over the base was employed. This rod is divided into five equal portions by six discs, allowing five mice to walk on it at once, the speed employed is 24 r.p.m. The performance time was measured between the time the animal was mounted on the rod and when it fell off.

Hanging wire test[21]

This test was used to assess the rats' gripping and forelimb strength. Animals were suspended from a wire by their forelimbs (60 cm long and 0.3 cm diameter) extended 40 cm above a foam pillow between two posts. The ability of the rat to hold the wire was recorded. Cut off time was the 90s.

Balancebeamwalk task[22]

The beam walking task for motor coordination was used to measure the ability of rats to traverse the horizontal narrow beam (2.3 cm × 120 cm) suspended 50 cm above a foam-padded cushion. During testing, the rats were given 2 mins to traverse the beam. If they did not complete the task or if they fall off the beam, the trial was ended.

Estimations

Acetylcholinesterase estimation[23]

The tissue was homogenated using 0.1 M Phosphate buffer (PH 8). Totally, 128 µl of tissue homogenate (without centrifugation) was added to the cuvette containing 0.83 ml of buffer and 32 µl DTNB (0.82 mg/ml). The contents were thoroughly mixed and absorbencies were measured at 412 nm using UV Spectrophotometer. When the reading reaches the stable value, it is recorded as the basal reading, then 6 µl of the substrate i.e., Acetylthiocholine was added and

the change in absorbance is recorded for 5 mins. Finally, the change in absorbance was thus determined.

Estimation of GSH[24]

Ellman's method was used to determine glutathione reduction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5- thio-2- nitrobenzoic acid (TNB).The absorbance of TNB at 412 nm can be used to calculate the amount of glutathione in a sample. 0.5 ml of homogenate is added to Eppendorf containing 0.1 ml 25% TCA. Then centrifuge the reaction mixture at 4000 rpm for 5 min. Take 0.3 ml of the supernatant solution and mix it with 0.5 ml of 0.1 M phosphate buffer and 0.2 ml of 10 mM DTNB in an Eppendorf tube. Incubate the reaction mixture for 10 min and read at 412 nm.

Estimation of LPO[25]

The assay is based on the reaction of Thiobarbituric acid (TBA) with Malondialdehyde (MDA), a breakdown product derived from many oxidized lipid molecules. The resulting chromophore of TBA - MDA complex formation, which was measured at its absorbance maximum of 532 nm. To 0.1 ml of tissue, homogenate add 2 ml of TCA-TBA-HCL reagent and heat at 90˚C for one hour at the water bath. Then cool the solution at ice-cold water, then centrifuge the mixture at 5500 rpm for 5 min and read the absorbance of clear supernatant at 535 nm.

Estimation of Catalase[26]

Catalase is the anti-oxidant enzyme found in all living organisms, which catalyzes the deterioration of hydrogen peroxide (H2O2) to water. This enzyme will protect the cell from reactive oxygen free radicals or Reactive Oxygen Species (ROS). This conversion can be measured at 240 nm wavelength. 0.95 ml of 10mM H202 in 60mM phosphate buffer (pH 7.0) was mixed with 50µl of tissue homogenate. The amount of rate of degradation of H202 was measured spectrophotometrically at 240nm per minute.

Superoxide dismutase[27, 28]

(Cu-Zn) SOD activity was determined by using a simple and rapid method, based on the ability of the enzyme to inhibit the autoxidation of Pyrogallol. Pyrogallol undergoes 50% autoxidation in the presence of EDTA at pH 8.2. The principle of this approach is based on the struggle between O2 • autoxidation of pyrogallol and the SOD dismutation of this radical. 0.1 ml of homogenate was added to the test tube containing 1 ml of Tris buffer and 1 ml of pyrogallol reagent. The absorption of the reaction mixture was read at the wavelength of 420 nm against Tris-EDTA buffer at zero time. The control reading was taken by vomiting the homogenate and adding distilled water. The readings were taken at 0, 1, 2, and 3 minutes.

Histopathological study

A piece of the brain was fixed in 10% formalin and embedded in paraffin wax before being cut into 5m thick sections. For histological examinations, the slices were stained with hematoxylin and eosin dye. Morphological changes in the hippocampus or striatal neurons were discovered depending on the model.

Result

Table 1: Percentage yield of Hydro alcoholic extract of *Caesalpinia sappan Linn*.(HAECS)

Extract	Method of Extraction	Physical nature of extract	Color of extract	%Yield
HAECS	Soxhlation	Crystalline powder	Reddish brown	8.07 %

Thepercentageyieldofheartwoodofhydroalcoholicextractof*CaesalpiniasappanLinn.* was foundto be8.07 %

Effect of treatment on Behavioural parameters

In the Elevated plus maze test, the AlCl3 alone treated group showed a significant ($p<0.001$) increase in the anxiogenic behavior by increase in time spent in the closed arm (229.80 ± 1.76 sec) than the open arm (21.42 ± 1.71 sec) when compared to the normal group animals which spent more time in open arm (275.20 ± 3.80 sec) than in closed arm (20.69 ± 1.19 s). This indicates the effect of AlCl3 on mood elevation in rats. The HAECS (400 mg/kg) alone treated group showed the comparatively same effect as that of normal group animals. Group treated with HAECS (200 and 400 mg/kg) and Donepezil showed a significant ($p<0.001$) reduction in anxiogenic behavior as the time spent in the open arm (175.3 ± 2.19, 274.5 ± 3.01 and 264.4±3.5 sec) was more than that of time spent in the closed arm (29.69 ± 1.67, 29.49 ± 3.7 and 24.93±1.61 sec) when compared to AlCl3 alone treated group. (Fig 2)

In the Rotarod test, AlCl3 alone treated group showed a significant ($p<0.001$) reduction in motor coordination by a decrease in the fall of time (11.00 ± 3.17 sec) when compared to the normal group (67.17 ± 2.19 sec). the HAECS (400 mg/kg) alone treated group showed similar results (62.50 3.43 sec) in the time fall to the normal control group. The group treated with HAECS showed a significant ($p<0.05$ and $p<0.001$) increase in motor coordination by an increase in fall of time. Both doses of HACES 200 and 400 mg/kg increased the fall of time to 39.17 ± 10.97 sec and 49.33 ± 2.94 sec respectively when compared to AlCl3 alone treated group.

When compared to the normal group (153.8 ±6.9), the locomotor count in the AlCl3 alone treated group (20.83± 5.46) was considerably (p<0.001) lower. The HAECS 200mg/kg treatment group had a substantial (p<0.05) increase in the locomotor count of (86.12± 6.40). The locomotor count increased to (59.64± 4.48) after receiving a 200mg/kg dosage of HAECS. However, this effect was statistically negligible. When compared to the inducer control group, the standard donepezil-treated group had a substantial (p<0.01) increase in locomotor count (98.83± 17.15). (Fig 2)

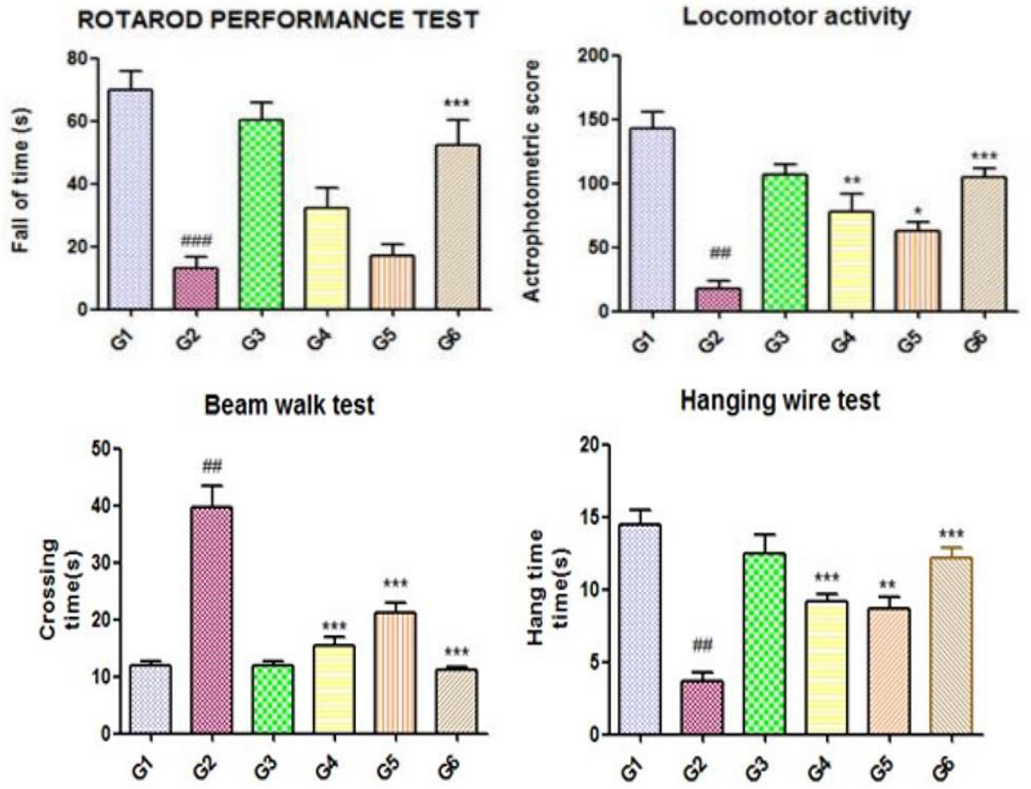

Figure 2: Effect of HAECS treatment on behavioral parameters in $AlCl_3$ induced neurotoxicity. All values are expressed as mean ± SEM, ### p<0.001, ## p<0.01 and # p<0.05 compared To Normal control; ***p<0.001, **p<0.01, *p<0.05,when compared to$AlCl_3$alone treated rats. One-way ANOVA followed by Tukey's multiple comparison tests.

In the balance beam walk test, Body balance and motor coordination were significantly (P<0.01) affected by the treatment of Alcl3 alone, when compared to normal group animals. As the normal group animals crossed the beam in 11.83 ± 0.29 s, whereas AlCl3 treated animals took more time to cross (39.67 ±3.87 s). Even the HAECS alone treated groups crossed the beam in 11.93 ± 0.76 s when compared to the normal group. The HAECS 200 and 400 mg/kg treated groups and the standard showed a significant (p<0.001) effect against AlCl3 induced impairment in muscle coordination. The animals crossed the beam at 15.50 ± 1.52 s and 21.17 ± 1.72 s respectively. (Fig 2)

Rats administered with AlCl3 alone for 42 days exhibited a significant p<0.01 reduction in grip strength (3.66 ± 0.55 s) when compared to the normal group (14.50 ± 0.99 s). The HAECS alone (400 mg/kg) treated group showed comparable results as that of the normal group (12.50 ± 1.23 s). However, The HAECS 200 and 400 mg/kg treated groups showed significant (p<0.001 and 0.01) increase (9.16 ± 0.42 s and 8.66 ± 0.80 s respectively) in muscle grip strength when compared to AlCl3 treated groups.

Effect of HAECS treatment on Biochemical parameters

Acetylcholinesterase activity

The administration of $Alcl_3$ alone caused significant (p<0.001) increase in rat brain tissue AchE levels (6.092 ± 0.66 µm/min/mg) when compared to normal control group (2.520 ± 0.11µm/min/mg). The administration of HAECS (400 mg/kg) showed a significant (p<0.001)reduction in level of AchE (3.55 ± 0.24 µm/min/mg) whereas the low dose of HAECS (200mg/kg) and Donepezil (1 mg/kg p.o,) alsoshowedasignificant(p<0.01)reductioninAchElevels(3.86±0.34µm/min/mg) when compared to the inducer control group. (Table 2) (Fig 3)

Table 2. Effect of HAECS treatment on tissue AchE levels and oxidative stressparameters in$AlCl_3$inducedneurotoxicity

Treatment	Tissue AChE (µmoles/min/mg tissue)	Tissue GSH (nm/g of tissue)	Tissue LPO (nm/g of tissue)	Tissue catalase (U/mg of tissue)	Tissue SOD (U/ml)
Normal control	2.52±0.11	52.29±0.90	12.41±0.78	0.26±0.02	21.03±0.47
$AlCl_3$ (100 mg/kg, p.o.)	6.09±0.66###	19.89±1.72###	26.72±1.82##	0.14±0.03###	10.92±1.57###
HAECS (400 mg/kg p.o.)	2.98±0.28	44.48±2.64	11.79±0.86	0.25±0.01	19.61±0.46
$AlCl_3$+ HAECS (200 mg/kg p.o.)	3.86±0.34**	40.93±1.84***	10.97±0.61***	0.28±0.02***	17.43±0.86***
$AlCl_3$ + HAECS (400 mg/kg p.o.)	3.55±0.24***	31.38±2.11**	14.09±1.30	0.20±0.013	13.81±1.36
$AlCl_3$ + Donepezil hydrochloride (1 mg/kg p.o.)	1.75±0.19***	49.19±2.93***	12.32±0.49***	0.28±0.05***	21.69±0.31***

All values are expressed as mean ± SEM, ### p<0.001, ## p<0.01 and # p<0.05 compared to Normal control; ***p<0.001, **p<0.01, *p<0.05,when compared to$AlCl_3$alone treated rats.

One-way ANOVA followed by Tukey's multiple comparison tests

Effect of HAECS treatmenton GSH, LPO, CAT, SOD activity

A significant (p<0.001) depletion in tissue GSH level was observed in AlCl3 alone treated animals (19.89 ± 1.72 nm/g of tissue) when compared with the normal control group (52.29 ± 0.90 nm/g). The GSH levels in HAECS 200 and 400 mg/kg dose was significantly (p<0.001 and p<0.01) increased (40.93 ± 1.84 nm/g and 31.38 ± 2.10 nm/g respectively) when compared to the inducer control group

The (Fig 4) indicates that LPO, CAT, and SOD enzyme levels in the hippocampus was significantly decreased (p<0.001) in the inducer control group when compared to normal control and significant (p<0.001) increase in HAECS 200 and 400 mg/kg and Donepezil (1 mg/kg p.o,) when compared to the inducer control group. The administration of HAECS (400 mg/kg)showed no significant change in GSH, CAT and SOD enzymes level when compared to the normal control group. (Table 2)

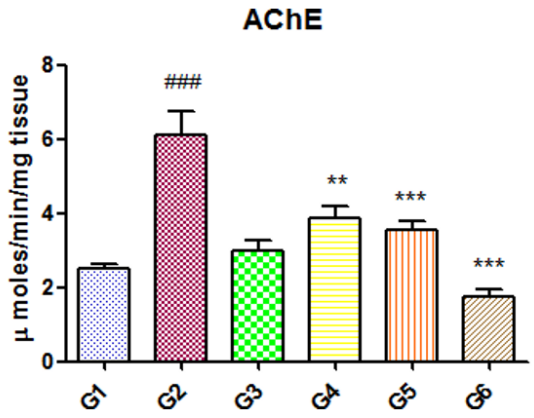

Figure 3: EffectofHAECStreatmentonratbrain tissueAChElevelsin$AlCl_3$ induced neurotoxicity. All values are expressed as mean ± SEM, ###<0.001, P## p<0.01 and # p<0.05comparedtoNormalcontrol; ***p<0.001,**p<0.01, *p<0.05,whencomparedto$AlCl_3$alonetreatedrats.One-wayANOVA Followed by Tukey's multiple comparison tests.

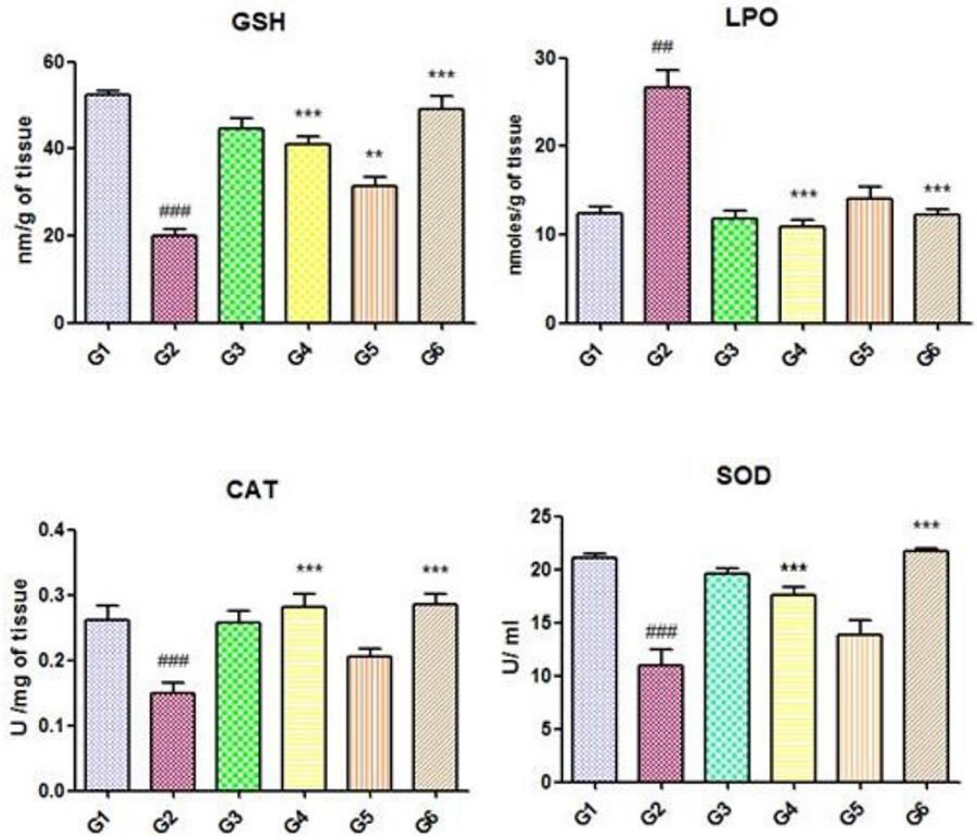

Figure 4: Effect of HAECS treatment on oxidative stress parameters in $AlCl_3$ induced neurotoxicity. All values are expressed as mean ± SEM, $^{###}$ p<0.001, $^{##}$ p<0.01 and $^{#}$p<0.05 compared to normal control;***p<0.001,**p<0.01,*p<0.05, whencomparedto$Alcl_3$ alone treated rats. One-way ANOVA followed by Tukey's multiple comparison tests.

Histopathological study

The typical laminar arrangement of pyramidal cells with normal intact neuropil fibres can be seen in the brain slice of the Normal control group in this study. The Alcl3 alone treated group's brain portion showed a disrupted laminar organization with wide intercellular spaces, dark shrunken and triangular-shaped pyramidal cells, and huge intercellular space. Treatment with 200 mg/kg HAECS resulted in a normal laminar layout with intact pyramidal cells and interconnected neuropil fibres, as well as a reduction in the number of shrunken cells. In the region of the brain treated with 400mg/kg of HAECS, a disturbing arrangement of the lamina is evident, along with a few numbers of apoptotic deteriorated shrunken pyramidal cells and degeneration in some neuronal cells. The pyramidal layer of Dextromethorphan 30 mg/kg treated rats is made up of many regular rows and a large number of dark shrunken pyramidal cells.

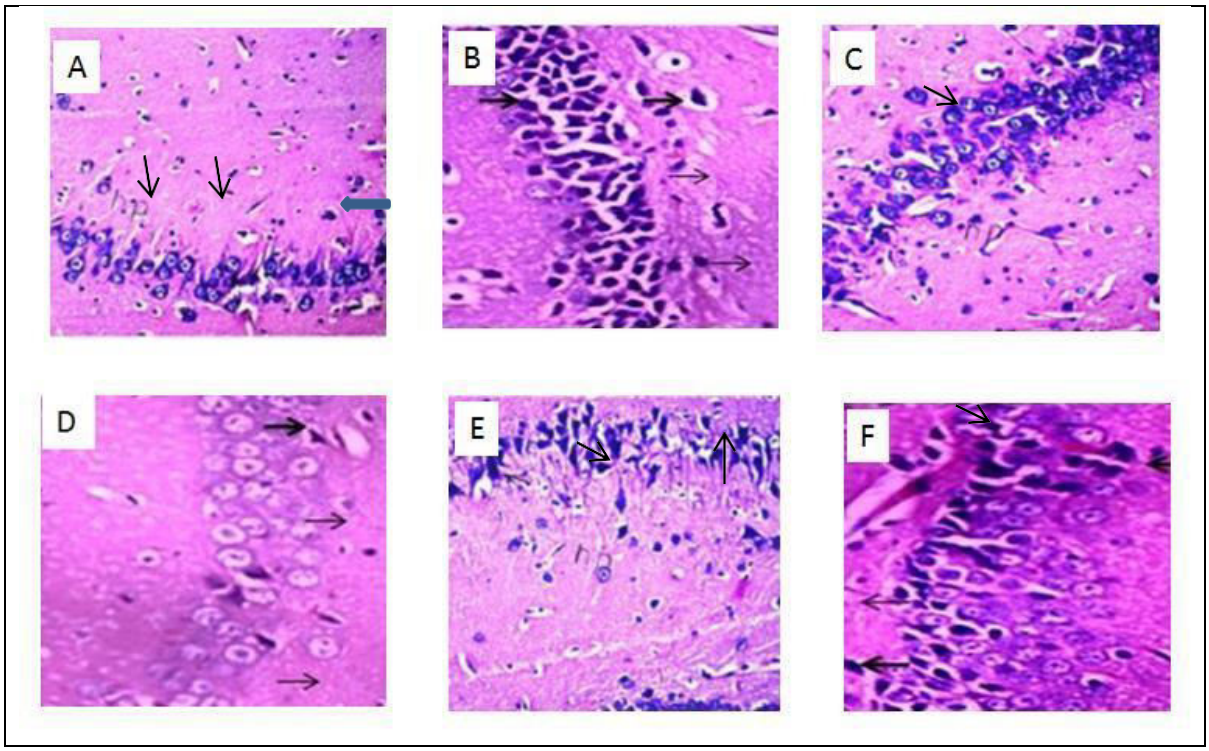

Figure 5: EffectoftreatmentofHAECSon histopathological alterations in CA1region of hippocampusinAlcl$_3$inducedneurotoxicity model. **A-**Normalcontrol, **B-**Inducercontrol:AlCl$_3$ (100mg/kg), **C-**Drugalone: HAECS(200 mg/kg), **D-**Lowdose: AlCl$_3$ +HAECS(200mg/kg), **E-**Highdose:AlCl$_3$+HAECS (400 mg/kg), **F-**Standard control: AlCl$_3$+Donepezil(1mg/kg).

Discussion

Neurotoxicity is toxicity of the nervous system that occurs when natural or manmade hazardous compounds, known as neurotoxins, affect the nervous system's normal activity in such a way that nerve tissue is damaged. This can cause neurons, which send and process impulses in the brain and other parts of the nervous system, to malfunction or even die. Exposure to compounds used in chemotherapy, radiation treatment, medication therapies, certain drug abuse, and organ transplants, as well as heavy metals, some foods, and food additives, can cause neurotoxicity[29].

Aluminum is a common metal that has been linked to the genesis of Alzheimer's disease by exacerbating oxidative damage in the brain. For many decades, AlCl3 has been regarded a possible etiological factor in a variety of neurodegenerative illnesses based on available evidence[30].

AlCl3 is a neurotoxicant that has been linked to changes in ionic, cholinergic, and dopaminergic neurotransmission in the central nervous system, which have been linked to learning ability. Following continuous exposure, it accumulates throughout the brain, with the greatest concentration in the hippocampus, which is the location of memory and learning[31].

The animals treated with AlCl3 showed fear and anxiety-like behaviour. The AlCl3 alone treated animals increased the entries to closed arm than the open arm. The animals spent more time in the closed arm than in the open arm. But the treatment of donepezil and both the test doses of HAECS reversed the effects of AlCl3 indicating its anxiolytic activity. Exposure to Al has been found to decrease locomotor activity assessed by actophotometer, which indicates a possible depressant effect on the central nervous system (CNS). Administration of HAECS was able to improve the performance of the motor activity and also improved the locomotor and exploratory behavior suggesting its potential role as a neuroprotectant against AlCl3-induced alteration in behavioral performance.

In the present study, the administration of AlCl3 showed significant functional abnormalitiesin muscle coordination and muscle grip strength. As this condition is reflected in decrease infall of time from the Rota-rod and hanging time in hanging wire test. The donepezil and HAECS administration improved muscle coordination and muscle grip strength in AlCl3treated rats. By the above observation it seems that HAECS may possess some the rapeuticpotential against such type of behavioral alteration against AlCl3 induced neurotoxicity.

Acetylcholine, a neurotransmitter associated with learning and memory, was degraded by the enzyme

acetylcholinesterase (AchE), terminating the physiological action of this neurotransmitter. In addition to its role in cholinergic transmission, cholinesterase may also play a role in morphogenesis and neurodegenerative diseases[32]. In the present study, AchE activity was significantly increased in AlCl3 alone treated rats as compared to normal control. This increase in AchE activity leads to a diminished cholinergic transmission leading to cognitive deficits. Treatment with Donepezil and HAECS treatment significantly inhibited AchE activity in AlCl3 treated rats.

Natural antioxidants protect against oxidative stress. Natural antioxidants have been reported to decrease free radical attack on bio-molecules and diminish cumulative oxidative damage[33]. In the present study, treatment with both the tested doses of HAECS and standard drug significantly attenuated the aluminum-induced oxidative stress. Administration of HAECS and Donepezil to the Alcl3 treated animals showed significantly increased levels of GSH when compared with Alcl3 alone treated rats, these findings are in accord with the earlier reports demonstrating antioxidant property of HAECS.

By raising the redox-active iron content in the brain, AlCl3 accelerates iron-mediated LPO and causes significant oxidative damage. Increased oxy-radicals and loss of cellular homeostasis cause oxidative stress that leads to neurotoxicity[32]. An imbalance in oxidant-antioxidant status is characterized by an increase in LPO and a decrease in antioxidant enzymes. In the current study, AlCl3 resulted in distinct oxidative stress as indicated by an increase in LPO. The treatment with both high and low doses of HAECS showed dose-dependent decreased LPO levels compared with Alcl3 alone treated group, which suggests a protective effect of HAECS against Alcl3 induced oxidative stress.

The SOD protects the cell against free radical injury by converting O− radical to H2O2 and prevents the formation of OH− radicals through O − driven Fenton reaction. The H2O2 formed by SOD is removed by the CAT. Hence, if the activity of CAT is not adequate to degrade H2O2, more H2O2 is converted into toxic hydroxyl radicals[33]. In the present study, Alcl3 induced oxidative stress significantly reduced the SOD and CAT activities. Significant increases in the SOD and CAT levels were found in the donepezil and HAECS treated groups at the dose of 200 and 400 mg/kg compared with Alcl3 treated group. Thus, the protective mechanism of HAECS may be due to its potent antioxidant property.

The treatment with HAECS at both doses showed comparatively less infarct lesion area indicating that HAECS could protect the neurons from Alcl3 induced neurotoxicity. The brain tissue of HAECS treated rats showed prevention of the damage caused by Alcl3. Histopathological studies, in addition to biochemical parameters, sustained and explained the neuroprotective of HAECS. However, additional research is needed to determine the exact mode of action.

Conclusion

The present study demonstrated that heartwood of Caesalpinia sappan Linn. was found to have neuroprotective properties in the current investigation. We find that Caesalpinia sappan Linn has neuroprotective properties against neurotoxicity caused by aluminum chloride and monosodium glutamate. This conclusion was drawn from behavioral assessment, brain tissue AChEestimation, antioxidant parameters and histopathological studies in their respective models. The protective effect of HAECS may attributed to its antioxidant and anti-inflammatory properties. However, additional research is needed to determine the exact mode of action.

References

1. Ludwig PE, Varacallo M. Neuroanatomy, Central Nervous System (CNS).
2. Wood LB, Winslow AR, Strasser SD. Systems biology of neurodegenerative diseases. Integrative Biology. 2015 Jul 6;7(7):758-75.
3. Disease control priorities related to mental, neurological, developmental and substance abuse disorders, Mental Health: Evidence and Research Department of Mental Health and Substance Abuse. World Health Organization: Geneva; 2006. 627 p.
4. Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends in immunology. 2008 Aug 1;29(8):357- 65.
5. Auti ST, Kulkarni YA. Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. Frontiers in Neurology. 2019 Apr 30; 10:399.
6. Crews L, Masliah E. Molecular mechanisms of neurodegeneration in Alzheimer's disease. Human molecular genetics. 2010 Apr 15;(19):12-20.
7. Jellinger KA. Basic mechanisms of neurodegeneration: a critical update. Journal of cellular and molecular medicine. 2010 Mar ;14(3):457-87.
8. Weil ZM, Norman GJ, DeVries AC, Nelson RJ. The injured nervous system: a Darwinian perspective. Progress in neurobiology. 2008 Sep 1;86(1):48-59.
9. Auti ST, Kulkarni YA. Neuroprotective effect of cardamom oil against aluminum induced neurotoxicity in rats. Frontiers in Neurology. 2019 Apr; 30; 10:399.

10. Fulgenzi A, Vietti D, Ferrero ME. Aluminium involvement in neurotoxicity. BioMed research international. 2014 Jan 1;2014.

11. Amjad S, Umesalma S. Protective effect of Centella asiatica against aluminium-induced neurotoxicity in cerebral cortex, striatum, hypothalamus and hippocampus of rat brain histopathological, and biochemical approach. Journal of Molecular Biomarkers & Diagnosis. 2015 Jan 1;6(1):1.

12. Igbinosa OO,Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemica lscreening of stem bark extracts from *Jatropha curcas* (Linn). African journal of pharmacyandpharmacology.2009Feb28;3(2):058-62.

13. Hardman JG, Limbird LE, Goodman Gilman A. editors. The pharmacological basis of therapeutics. 10th ed. New York: McGraw-Hill; 2001. 549-66 p.

14. Badami S, Moorkoth S, Rai SR, Kannan E, Bhojraj S. Antioxidant activity of Caesalpinia sappan heartwood. 2003 Nov;26(11):1534-7.

15. Nagakannan P, Shivasharan BD, Thippeswamy BS, Veerapur VP, Bansal P. Protective effect of hydroalcoholic extract of Mimusopselengi Linn. flowers against middle cerebral artery occlusion induced brain injury in rats. Journal of ethnopharmacology. 2012 Mar 27;140(2):247-54.

16. Chellappan D R, Purushothaman A K, Brindha P. Gastroprotective potential of hydroalcoholic extract of Pattanga (CaesalpiniasappanLinn.). Journal of ethnopharmacology. 2017; 197:294-305.

17. Auti ST, Kulkarni YA. Neuroprotective Effect of Cardamom Oil Against Aluminum Induced Neurotoxicityin Rats. Frontiers in neurology. 2019;10.

18. Pellow S, File SE. Anxiolytic and anxiogenic drug effects on exploratory activity in anelevated plus-maze: a novel test of anxiety in the rat. Pharmacology Biochemistry andBehavior.1986 Mar1; 24(3):525-9.

19. VermaA,JanaGK,SenS,ChakrabortyR,SachanS,MishraA.Pharmacologicalevaluation of Saraca indica leaves for central nervous system depressant activity in mice. JPharmSci Res. 2010 Jun1;2(6):338-43.

20. Kuribara H, Higuchi Y, Tadokoro S. Effects of central depressants on rota-rod and traction performance sinmice. The Japanese Journal of Pharmacology.1977;27(1):117-26.

21. ShivasharanBD,NagakannanP,ThippeswamyBS,VeerapurVP,BansalP,UnnikrishnanMK.Protectiveeffectof CalendulaofficinalisLinn.flowersagainst3-nitropropionic acid induced experimental Huntington's disease in rats. Drug and chemicaltoxicology.2013 Oct 1;36(4):466-73.

22. Luong TN, Carlisle HJ, Southwell A, Patterson PH. Assessment of motor balance and coordination in mice using the balance beam. JoVE (Journal of Visualized Experiments).2011Mar10(49):2376.

23. Khalil RM, Khedr NF. Curcumin protects against monosodium glutamate neurotoxicity and decreasing NMDA2B and mGluR5 expression in rathippocampus. Neurosignals.2016;24(1):81-7

24. Taïr K, Kharoubi O, Taïr OA, Hellal N, Benyettou I, Aoues A. Aluminium-induced acute neurotoxicity in rats: Treatment with aqueous extract of Arthrophytum (Hammada scoparia). Journal of acute disease. 2016 Nov 1;5(6):470-82.

25. GelvanD, Saltman P. Different cellulartargets of Cu- and Fe-catalyzedoxidation observed using a Cu-compatible thiobarbiturateacidassay. Biochem BiophysActa1990;1: 7-8

26. SinhaAK. Colourimetricassay of catalase. AnalBiochem1972; 47:389-94.

27. Magnani L, Gaydou M and Jean CH, Spectrophotometric measurement of antioxidantproperties of flavones and flavonols against superoxide anion, Anal. Chim. Acta, 2000,*411*(1-2)1: 209-216.

28. Marklund, S. and Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient as say for superoxidedismutase.Eur.J.Biochem.1974, *47*: 469-474.

29. Klaassen C D. Casarett and Doull's Toxicology: The basic science of poisons. 7thed. McGraw-Hill medical publishing Division; 2010.

30. SavoryJ, Herman M M, GhribiO (2006) Mechanisms of aluminum induced neurodegeneration in animals: Implications for Alzheimer's disease. J Alzheimers Dis 10:135-144.

31. Yuan CY, Lee YJ, Hsu GS (2012) Aluminum overload increases oxidative stress in four functional brain areas of neonatal rats. J Biomed Sci 19: 51.

32. HFerreira-VieiraT, MGuimaraesI, RSilva F, MRibeiroF. Alzheimer's disease: targeting the cholinergic system. Current neuropharmacology. 2016Jan 1;14(1):101-15.

33. Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/nitrosativestress: current state. Nutrition journal.2015Dec;15(1):1-22.