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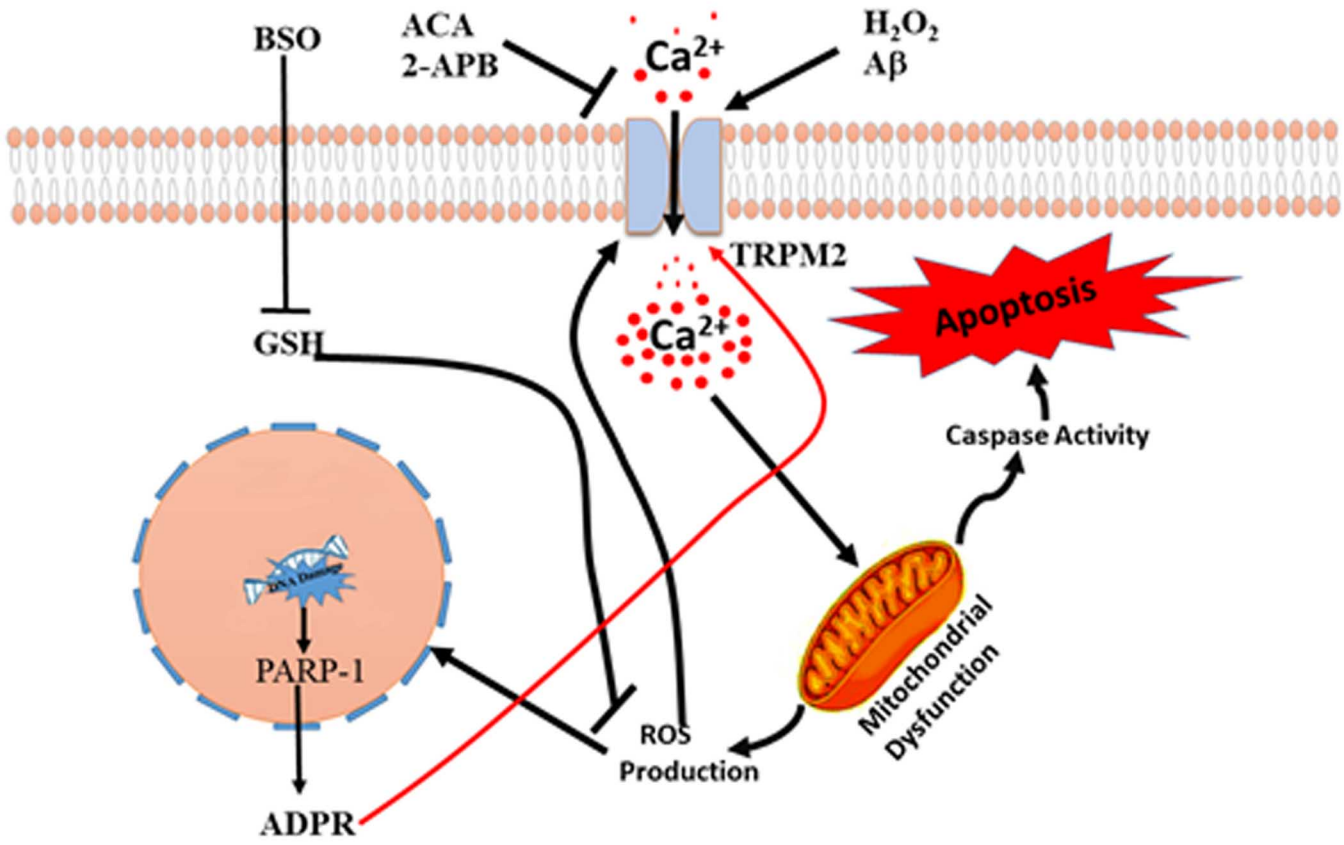

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AIM AND SCOPES
Journal of Cellular Neuroscience and Oxidative Stress is an online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+- K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals)

C- Interaction Between Oxidative Stress and Ion Channels in Neuroscience
(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels in neurodegenerative diseases such Parkinson's and Alzheimer's diseases)

D- Gene and Oxidative Stress
(Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics	Biochemistry
Biology	Biomedical Engineering
Pharmacology	PhysiologyGenetics
Cardiology	Neurology
Oncology	Psychiatry
Neuroscience	Neuropharmacology

Keywords
Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide, ageing, antioxidants, neuropathy, traumatic brain injury, pain, spinal cord injury, Alzheimer's Disease, Parkinson's Disease.

RESEARCH ARTICLE

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The beneficial effect of Pluchea lanceolata on aluminum chloride-induced Alzheimer's disease in rats

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List of Abbreviations;

***Ach**, Acetylcholine; **AlCl$_3$**, Aluminum chloride; **AD**, Alzheimer's disease; **AFR**, Ayurvedic Formulation of Rasna; **Aβ**, β-amyloid; **CAT**, Catalase; **CNS**, Central nervous system; **CS**, Conditional stimuli; **DA**, Dopamine; **EPM**, Elevated plus maze; **H & E**, Haematoxylin and Eosin; **HMEPL**, Hydro methanolic extract of Pluchea lanceolata; **MDA,** Malondialdehyde; **NA**, Norepinephrine; **NFTs**, neurofibrillary tangles; **ANOVA**, One Way Analysis Of Variance; **PASS**, Prediction of Biological Activity Spectra for Substance; **PL**, Pluchea lanceolata; **5HT**, 5 Hydroxy Tryptamine (serotonin); **GSH**, Reduced glutathione; **ROS**, Reactive Oxygen Species; **SEM**, Standard Error Mean; **SOD**, Superoxide dismutase; **TP**, Total protein;*

Abstract

Aluminum chloride ($AlCl_3$) causes neuroinflammation in rats, which leads to the development of Alzheimer's disease (AD). The current study focused on the anti-Alzheimer and antioxidant potential of hydromethanolic extracts of *Pluchea lanceolata* (PL), a well-known Rasna source. Phytoconstituents such as pluchine and moretenol acetate are selected for the Prediction of biological activity spectra for substance (PASS) online and molecular docking (in silico) experimental model. A total of 36 Wistar rats were divided into 6 groups, each with six rats, as the negative control, disease control, rivastigmine (0.3 mg/kg, p.o), hydromethanolic extract of PL (HMEPL, 200 mg/kg, 400 mg/kg, p.o), and Ayurvedic Formulation of Rasna (AFR) (1ml/kg, p.o). Except for negative control, all of the animals were given $AlCl_3$ (300 mg/kg, p.o). $AlCl_3$ and plant extracts were given for a 20day treatment. On the 0th, 7th, 14th, and 20th days, the behavioral changes were evaluated. Rats were sacrificed on the 21st day, their brains were separated, and antioxidant enzyme levels, protein levels, and neurotransmitter levels were measured. The

number of entries, as well as time spent in the closed arm and time taken to ascend the pole, were all increased in disease control animals, but this was reversed in groups treated with 200 mg/kg, 400 mg/kg, and 1 ml/kg dosages of HMEPL and AFR. In the disease control group, $AlCl_3$ (300 mg/kg, p.o.) caused an increase in protein content as well as malondialdehyde (MDA), similarly, reduction in body weight, superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) level were observed and were corrected and restored in groups treated with HMEPL and AFR. Furthermore, the histopathology findings revealed that HMEPL and AFR provided the cellular-level protection. In conclusion, the active components of HMEPL were found to have anti-Alzheimer and antioxidant potential and were confirmed in an *insilico* investigation. HMEPL > AFR was the order of anti-Alzheimer and antioxidant effectiveness.

Keywords: Aluminum Chloride, Alzheimer's disease, *insilico*, neurotransmitters, *Pluchea lanceolata*, Rasna.

Introduction

Alzheimer's disease (AD) is a degenerative neurological disease in which neurofibrillary tangles (NFTs) and amyloid β (Aβ) plaques form and accumulate in the brain, leading to impaired cognitive function as well as memory (Borai et al., 2017). The etiology of the disease is multifactorial, with factors including genetic factors, oxidative stress, encephalitis, head trauma, and environmental factors including exposure to toxic aluminum (Zaky et al., 2017). The normal functions of neurons are disrupted when aluminum enters through specific transferrin receptors in the blood-brain barrier, leading to memory loss and the development of AD due to aluminum deposition in the thalamus visual and prefrontal cortex, where it causes a noxious structure (Singh et al., 2018). Alteration of cellular proteins in the brain to promotes NFT formation. Researchers have reported that "nervines" is an herb in Ayurveda that is used to strengthen the central nervous system and that their chemical components play an important role in mental recovery (Doungue et al., 2018). One of these Ayurvedic plants is "Rasna", a controversial medicinal plant. A total of 13 plant species are listed under Rasna including *Pluchea lanceolata* (DC.) Oliv. & Hiern, (PL) (Asteraceae) is the official name of Rasna. traditionally used to treat inflammation, cough, bronchitis, hemorrhoids, psoriasis, fever, used as a uterine dilator, nerve tonic, analgesic, laxative, and prevent swelling joints in arthritis (Asirvatham et al., 2021, Srivastava and Shanker, 2012, Palash et al., 2013). It contains quercetin, quercitrin, isorhamnetin, daidzein, Pluchiol, βsitosterol, Dglucoside, stigmasterol, β-sitosterol, Plucheasterolide and Pluchine. (Kumar and Khanum, 2012). In the traditional system of medicine, PL is employed as a primary ingredient in a variety of polyherbal formulations for the management and cure of 'Amavata' (arthritis) and other diseases. Arista, fine and coarse powder, semisolid and solid (tablets), medicated ghee, ointment, and oil are all available. However, its neural tonic properties have yet to be experimentally established in animal models (Srivastava and Shanker, 2012) Therefore, the present study focused on evaluating the anti-Alzheimer effects of hydromethanolic extraction of PL (HMEPL) was performed to demonstrate the potential effects of aluminum chloride ($AlCl_3$)-induced AD in rats.

Material and Methods

Plant material

The whole plant PL was collected from Jodhpur, Rajasthan in December 2019. Jodhpur is the second-largest city of Rajasthan State stretches between 2600' and 27037' at North Latitude and between 72 55' and 73 52' at East Longitude. This district is situated at a height between 250-300 meters above sea level. Dr. J Jameson, Plant Taxonomist, Department of Botany, University of St Albert`s (Autonomous), Ernakulam. Identification and authentication of plant specimens were also done and deposited (document number 482) at the Department of Botany, College of St Albert (Autonomous), Ernakulam, Kerala, India.

Extraction procedure

PL roots and leaves were isolated and washed with water to remove soil particles and dried at room temperature (dry shade). The plant material is ground into a coarse powder using a mechanical grinder to increase contact between the plant material and the solvent. The powdered ingredients (300 g) were extracted by cold soaking with water and methanol (70:30) at room temperature for 7 days with continuous shaking until the soluble ingredients were dissolved or finished. After 7 days, the mixture was filtered through a muslin cloth and squeezed to remove all remaining liquid, and passed

through a Whatmann filter, after which the solvent was recovered by a rotary evaporator under reduced pressure (Sarkar et al., 2012). The crude PL extract was named hydromethanolic extract of PL (HMEPL) and was stored in a refrigerator at -20 °C in an airtight container for further experimental purposes.

Preliminary phytochemical analysis

Tests for glycosides, flavonoids, saponins, alkaloids, protein, carbohydrates, coumarins, phytosterols, amino acids, fixed oil, phenolic compounds, and tannins were conducted as described by Mathew et al. 2021.

Tests for alkaloids

• Drageendorff's Test (Potassium bismuth iodide solution)

2 ml of an acidic solution of plant extract were neutralized with 10% ammonia solution. Dragendorff's reagent was added and turbidity or precipitate was observed as indicative of the presence of alkaloids.

• Wagner's Test (Potassium iodide solution)

2 ml of plant extract were boiled with 5 ml of 2% HCl in a steam bath. The mixture was filtered and a 1 ml portion of the filtrate was treated with 2 drops of Wagner's reagent. A reddish-brown precipitate indicates the presence of alkaloids.

• Mayer's Test (Potassium mercuric iodide solution)

Drops of Mayer's reagent were added to a portion of the acidic solution in a test tube and observed for an opalescence or yellowish precipitate indicative of the presence of alkaloids.

• Hager's Test (Iodine-picric acid)

For this test procedure, a few drops of Hager's reagent (saturated picric acid solution) were added to 2 ml of the respective plant extract. Bright yellow precipitate formation indicated the existence of alkaloids.

Test for carbohydrate

• Molisch Test

A few drops of Molisch's solution were added to 2 ml of the aqueous solution of the extract, thereafter a small volume of concentrated sulphuric acid was allowed to run down the side of the test tube to form a layer without shaking. The interface was observed for a purple color as indicative of positive for carbohydrates.

• Seliwanoff's Test

5 ml of Seliwanoff's reagent were added to a test tube containing 1 ml of plant extract and heated using hot water. The color of the test tube changed to red, indicating keto sugar (Fructose and Sucrose) was present in the solution.

• Benedict's Test

A mixture that contains 2 ml of plant extract and Benedict's solution (approximately 5 ml) was heated in a test tube for around two minutes and was then allowed to cool. Red-colored precipitate indicated the presence of carbohydrates.

Test for glycosides

• Legal Test

The plant extract was dissolved in 1 ml of water, with a few drops of 10% sodium hydroxide and 1 ml of 0.3% nitroprusside sodium reagent. The mixture turned a dark red color almost instantly.

• Baljet Test

Dissolved the plant extract in 3 ml of methanolic sodium picrate solution. Added 1 ml of NaOH solution and the mixture acquired at once a light wine red color.

• Borntrager's Test

5 ml of plant extract were added to 5 ml of 5% ferric chloride solution and 5 ml dil. hydrochloric acid, heated for 5 minutes in a water bath. Cooled and added 3 ml of benzene or organic solvent. Shook well. The separated organic layer added an equal volume of 10% ammonia solution. The formed rose pink/red at the ammonia layer showed the presence of glycosides.

• Keller-Killiani Test

5 ml of the plant extract were added to3 ml of concentrated acetic acid. Added 1 drop of iron (III) chloride test solution to the liquid and carefully transferred it to concentrated sulphuric acid. A reddish-brown ring formed at the interface, and the upper acetic acid layer soon turned bluish-green.

Test for phytosterol

• Liebermann-Burchard Test

The amount of 0.5 g of the extract was dissolved in 10 ml anhydrous chloroform and filtered. The solution was divided into two equal portions for the following tests. The

first portion of the solution above was mixed with 1 ml of acetic anhydride followed by the addition of 1 ml of concentrated sulphuric acid down the side of the test tube to form a layer underneath. The test tube was observed for green coloration as indicative of steroids.

• Salkowski Test

5 ml of plant extract were mixed in 2 ml of chloroform followed by the careful addition of 3 ml of concentrated sulphuric acid to form a layer. A layer of the reddish-brown coloration was formed at the interface thus indicating a positive result for the presence of terpenoids.

Test for coumarins

A volume of 1 ml of 10% NaOH solution was added to 1 ml of the plant extract. The yellow color was formed when it was placed in a water bath. It confirmed the existence of coumarins in the tested samples.

Test for flavonoids

• Shinoda Test

Pieces of magnesium ribbon and concentrated HCl were mixed with aqueous crude plant extract after a few minutes and the pink color showed the presence of flavonoid.

Test for phenolic compounds

• $FeCl_3$ test

A few drops of $FeCl_3$ solution were added to 1 ml of plant extract samples. Blackish red precipitate revealed the existence of flavonoids in the test samples

Test for tannin

• Gelatin Test

To a 1% gelatine solution, added a drop of 10% sodium chloride. If a 1% solution of tannin is added to the gelatine solution, tannins cause precipitation of gelatine from the solution.

Test for protein and amino acid

• Biuret Test

A quantity (2 ml) of the extract was put in a test tube and 5 drops of 1% hydrated copper sulfate were added. A quantity, of 2 ml of 40% sodium hydroxide, was also added and the test tube was shaken vigorously to mix the contents. A purple coloration shows the presence of proteins.

• Xanthoprotein Test

1 ml of extracts was treated with 1 ml of concentrated HNO_3. A white precipitate was formed then boiled and cooled. Then 20% of NaOH or NH_3 was subsequently added, which leads to the formation of an orange color, which revealed the presence of aromatic amino acids.

• Lead Acetate Test

A fraction of the extracts were treated with 1 ml of lead acetate. A white precipitate formed, which indicated the presence of proteins.

Test for saponins

1 ml of methanol extract was diluted with distilled water to 20 ml and shaken for 15 minutes in a graduated cylinder. The presence of saponin was confirmed by the formation of a layer of foam.

Test for fixed oils

• Spot Test

A quantity of 0.1 g of the extract was pressed between filter paper and the paper observed. The translucency of the filter paper indicated the presence of oils.

• Saponification Test

A few drops of 0.5 N alcoholic potassium hydroxide were added to 1 ml of plant extract along with a drop of phenolphthalein, the mixture was heated for 2 hours. The formation of soap or partial neutralization of alkali indicated the presence of fixed oils and fats.

Insilico study

PASS Online

PASS (Prediction of Biological Activity Spectra for Substance) is an online tool for predicting biological activities such as pharmaceutical effects, biochemical mechanisms, toxic and side effects, enzyme interactions, metabolic and transporter links, gene expression influence, and so on. Important chemical ingredients of PL were discovered to be pluchine and moretenol acetate. These compounds' 2D structures were taken into an account in the activity prediction. The probability of a specific activity being revealed (Pa) or not being revealed (Na) is defined by this value (Pi) (Raju et al., 2021).

Molecular docking

PPARα was chosen as the proteins (targets) for pluchin and moretenol acetate in anti-Alzheimer research. Vina autodock PyRx is used to calculate the binding energy in Kcal/mole. Pymol visualization was used to determine the number of hydrogen bonds between the ligand and the acceptor, as well as the amino acid sequence of the ligand to the target. (Raju et al., 2021).

In-vivo study

Experimental animals

In the study, both male and female Wistar rats (adults) weighing 150-250 g were used. The animals were kept in regular circumstances (23 - 25 °C, 12-hour light/12-hour dark cycle) with a humidity of 50± 5% and given free access to rodent pellets and water ad libitum. The animals were accomplished for the behavioral investigation and acclimatized to laboratory conditions for one week before the start of the experiment. Animal research principles, treatment, and adopting procedures were presented before the ethical committee (IAEC- institutional animal ethical committee) and was approved the proposal number SJCP/IAEC/2019-13/19b to experiment on rats.

Treatment protocol

A total of 36 well-trained rats were grouped into six groups of six each. All rats in groups 2 to 6 were administered $AlCl_3$, 300 mg/kg, p.o daily for 20 days, except group 1 which served as a negative control and received normal saline (p.o). Disease control was assigned to Group 2 ($AlCl_3$, 300 mg/kg, p.o). Group 3 was treated with rivastigmine (0.3 mg/kg, p.o) as a positive control. HMEPL (200 mg/kg, p.o) was given to Group 4 animals. Group 5 animals were treated with HMEPL (400 mg/kg, p.o) and group 6 animals were treated orally with 1 ml/kg Ayurvedic Rasna formulation (AFR) for 20 days (Justin et al. (2015) and Singh et al. (2018). During the treatment period, animal behavioral studies and body weight were evaluated on days 0, 7, 14, and 20.

Memory, motor activity, and learning were all tested using the elevated plus-maze. Cook's pole climbing apparatus was utilized to evaluate cognitive function, primarily in reaction to conditioned stimuli during learning and retention, and a digital actophometer was used to evaluate motor activity. On day 21, blood was drawn from the retro-orbital plexus shortly after death to assess protein levels as well as serum antioxidant enzymes catalase (CAT), reduced glutathione (GSH), and superoxide dismutase (SOD), and malondialdehyde (MDA). Aluminum content, neurotransmitter estimate (dopamine, acetylcholine, norepinephrine, and serotonin), and histopathology of the cortex and hippocampus were all done on isolated brains.

Behavioral Studies

Before the start of the experiments, all rats were trained in the actophotometer, elevated plus maze test, and pole climbing apparatus were performed according to the literature (Cook and Weidley, 1957; Soman et al., 2004).

a) Actophotometer test

A digital actophotometer (RSPH-1 model, Aarson Scientific Works, Haryana, India) equipped with infrared light-sensitive photocells was used to measure locomotor activity. Each trained animal was placed in a digital actophotometer and had its motor activity monitored for 5 minutes. When the animal cuts off the beam of light landing on the photocell, one count is recorded, and values are reported as the number of counts every 5 minutes. Locomotor activity assessment was made in all the groups on the 0th day and after drug treatment on the 7th, 14th, and 20th day.

b) Elevated plus maze test

EPM (exteroceptive Behavior model) is widely used to assess rodent learning, memory, and anxiety. It has four arms. Two open arms and two closed arms are located opposite the central shell and lifted 50 cm above the ground floor. Each rat was placed in the center of the device under static and dark conditions. The total number of entries and the time spent on open and closed arms were recorded. Elevated plus-maze tests were performed in each group of trained rats on days 0 and days 7, 14, and 20 of post-drug treatment.

c) Pole Climbing Test

The cognitive function of a rat was assessed using Cook`s pole climbing device, where learning and memory retention were assessed under conditioned stimuli (CS). It is a wooden chamber (25 × 25 × 25 cm) with a mesh floor of stainless steel rods, a shock of 6 mA is transmitted to the floor. In the top cover, a cylinder (2.5 cm wide) is in the center of the chamber. Each mouse was placed for 45 s to explore inside the chamber. An acoustic signal is followed by an unconditioned stimulus, i.e. an electric shock, which

is delivered through the bar grating floor for 45 seconds. The trained rat learned to correlate the leg jerk following the whistle sound and tried to escape from the leg jerk by climbing the pole after the whistle signal. The escalation response time is 10 seconds. Pole climbing tests were performed on all groups of trained rats on days 0 and 7, 14, and 20 after drug treatment.

Estimation of antioxidants

Blood serum sample preparation

After collecting the blood, let it be kept at room temperature for 15 minutes to coagulate the blood then centrifuge at 1000-2000 x g for 10 minutes in a refrigerated centrifuge to remove blood clots (https://www.thermofisher.com/in). The resulting supernatant was taken for the measurement of serum antioxidant enzymes.

Antioxidant and oxidant analyses

Animals were sacrificed after the administration of ketamine (80 mg / kg, i.p.) + xylazine (10 mg / kg, i.p.) on 21st day. The brain was carefully removed and homogenized with cold phosphate buffer at pH 7.4 to prepare for brain homogenate. One part of the brain was used to prepare 10% w / v homogenate with potassium chloride (0.15 M) (homogenate I). It was centrifuged at 8000 rpm for 10 minutes and the resulting supernatant was used for estimation of total protein (TP), CAT (Whiteside et al., 1987; Aebi et al., 1974), and MDA (Ohkawa et al., 1994). Using the second portion, prepare a 0.25% w / v homogenate in phosphate buffer (5 M, pH 7.4) (homogenate II) and centrifuge at 8000 rpm for 10 minutes, the resulting supernatant was used for the measurement of SOD (Kakkar et al., 1954) and GSH (Beutler et al., 1963).

All of the above antioxidant enzyme levels from blood serum and the brain tissue homogenates were performed on a COBAS MIRA PLUS S automated analyzer (Roche, Switzerland) using the Agappe Diagnostics test kit in Kerala, India.

Estimation of brain neurotransmitters

With a homogenizer, brain tissue was homogenized with 5 mL of HCl-butanol solution for about 1 minute followed by centrifuging at 2000 rpm for 10 minutes. Under the same conditions, 1 ml of supernatant was placed in a centrifuge tube and shaken with 2.5 ml of heptane and 0.3 ml of HCl (0.1 M) for 10 minutes. Discard the organic layer; take the aqueous phase (0.2 ml) and estimate serotonin (5HT), dopamine (DA), and norepinephrine (NA). The estimation method is described by Clarlone (1978) and Schlumpf et al., 1974. This is a fluorescence analysis assay in which, the homogenate was added with the reaction mixture of alkaline sulfites and iodine solution (in the case of DA and NA) to produce fluorescence products. O-phthalaldehyde solution was added for 5HT instead of the alkaline sulfites and iodine solution. The fluorescence of DA was read at excitation 320 nm and emission 375 nm; whereas that of NA was read at excitation 380 nm and emission 480 nm. Regarding 5HT, its fluorescence was read at excitation 355 nm and emission 470 nm using Shimadzu spectrophotofluorometer (RF510, Japan). The amounts of neurotransmitters were expressed as µg/g wet tissue. This procedure was carried out at 0 °C and the estimation of acetylcholine (Ach) was performed according to the procedure of Ellman (1959).

Estimation of aluminum content

The brain (30 mg) was added to 0.05 ml nitric acid, 0.2 ml hydrogen peroxide, and 0.1 ml of polytetrafluoroethene then, incubated at 120 °C for 2 h. Estimation of aluminum was done with an atomic absorption spectrometer (Model-932B plus, GBC Scientific Equipments Pvt. Ltd. Australia) (Justin et al., 2015).

Histology of brain

The cortex and hippocampus were isolated, washed with ice-cold saline, and maintained with formaldehyde (10%). Sections were stained with one or more dyes to reveal different tissue compositions under the microscope. The purpose of staining is to reveal cellular components. Reflectors are used to provide contrast. Hematoxylin/eosin (H & E) staining is used by pathologists, hematoxylin stains the cytoplasm blue, and eosin stains the cytoplasm and binding tissue pink. (Gurcan et al., 2009). Sections were examined (under10x) by a pathologist under a light microscope (Nikon, Tokyo, Japan).

Statistical analysis

All the In vivo study data were expressed as the Mean ± SEM of six values. The difference between treatment groups was compared to disease control by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test; where, $p < 0.05$ implied significance calculation.

Results

Table 1 shows the results of screening for phytoconstituents. A standard procedure was followed to test the phytochemical constituents present in the HMEPL. The extract revealed the presence of carbohydrates, terpenoids, alkaloids, glycosides, saponin, flavonoids, tannins, phytosterols, amino acids, and proteins.

Table 1 Phytochemical analysis of the HMEPL

Constituents	HMEPL
Alkaloids	+
Flavonoids	+
Tannins	+
Steroids	+
Glycosides	+
Saponins	-
Terpenoids	+
Proteins	+
Carbohydrates	+
Phenols	+

+ *Color produced (present),* - *No color produce (Absent)*

Figure 1 showed the predicted biological activity of the compound Moretenol acetate from PL. It has predicted 8 different types of CNS activity, out of which Acetylcholine neuromuscular blocking agent activity scored Pa > 0.7, which means that the ligand exhibits the CNS activity at the acetylcholine pathway was confirmed.

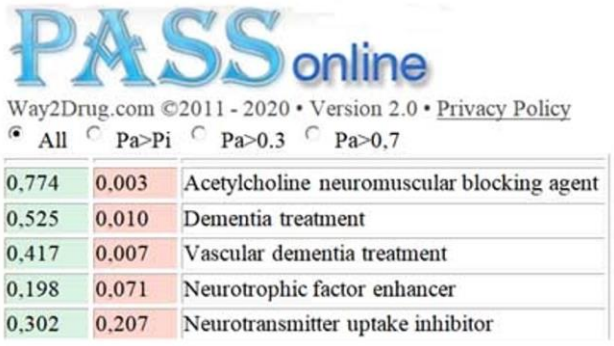


Figure 1. PASS report of Moretenol acetate from PL

Figure 2 showed the predicted biological activity of the compound Pluchine from PL. It has been predicted total of 6 different types of CNS-related biological activity, out of which nootropic activity scored Pa > 0.7, which means that the ligand revealed the CNS activity in the experiment.

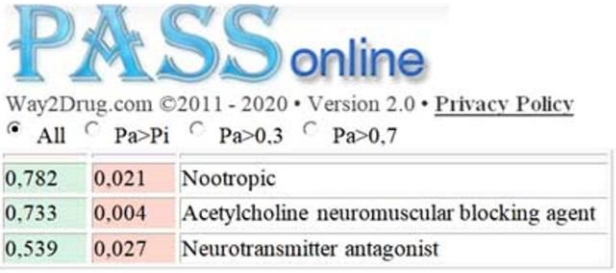


Figure 2. PASS report of Pluchine from PL

Table 2 showed the docking score, the number of hydrogen bonds, and the binding site of pluchine and moretenol acetate on the PPARα receptor. Moretenol acetate was bound with the PPARα site with the binding energy of -7.3 Kcal/mol and two hydrogen bond was identified at the position of 222 lysine and 370 leucine. Pluchine bound with PPARα site with the binding energy of -4.2 Kcal/mol and six hydrogen bonds were identified at different positions of the receptor.

Figure 3 showed docking of moretenol acetate at the active site region of PPARα, with high binding affinity, as indicated by total docking scores of -7.3, and also showed strong molecular interactions formed between 222 lysines and 370 leucine of PPARα.

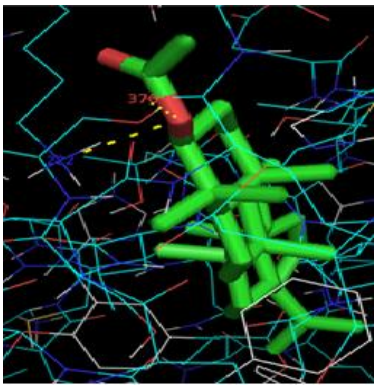


Figure 3. Visualisation of docking in pymol: PPARα with moretenol acetate

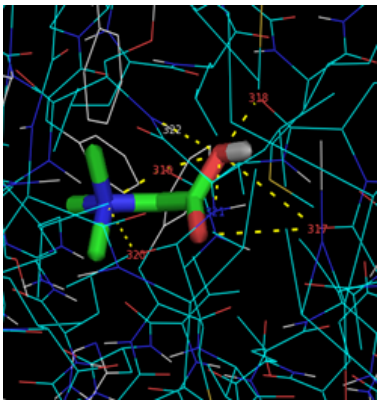


Figure 4. Visualisation of docking in pymol: PPARα with pluchine

Table 2 Docking scores of Moretenol acetate and Pluchine on PPARα receptor

Plant Name	Ligand	Receptor	Binding score (Kcal/mol)	Hydrogen bonds	Binding site	PDB
PL	Moretenol acetate	PPARα	-7.3	2	222 LYS 370 LEU	3VI8
	Pluchine		-4.2	6	320 MET 322 SER 317 ILE 319 ALA 318 PHE 321 LEU	3VI8

Table 3 Effect of HMEPL and AFR on locomotor activity of $AlCl_3$ induced AD in trained rats.

Groups	Number of counts / 5min			
	0th Day	7th Day	14th Day	20th Day
Negative Control	206.50 ± 2.29	220.00 ± 1.29	216.00 ± 0.89	223.83 ± 7.31
Disease Control ($AlCl_3$ 300 mg/kg)	201.33 ± 1.35	130.66 ± 1.78	107.17± 1.90	89.83 ± 1.42
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	204.50 ± 1.72	167.83 ± 1.66	149.33 ± 1.35	128.66 ± 1.33
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	207.50 ±0 .74^{d}	181.16 ±0 .45^{d}	159.16 ± 0.79^{c}	146.50 ±0 .66^{c}
HMEPL (400 mg/kg) + $AlCl_3$ (300 mg/kg)	207.66 ± 0.97^{d}	187.50 ± 1.83^{b}	168.00 ±0 .73^{a}	151.00 ± 1.52^{a}
AFR (1 ml/kg) + $AlCl_3$ (300 mg/kg)	209.33 ± .45^{d}	170.66 ± 2.96^{a}	150.66 ± .84^{a}	131.16 ± 1.83^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata.

Table 4 Effect of HMEPL and AFR on learning, memory and anxiety of $AlCl_3$ induced AD in trained rats.

Parameters	Number of entries in open arm & closed arm / 5min							
Days	0		7		14		20	
Treatment groups	Open	Closed	Open	Closed	Open	Closed	Open	Closed
Negative Control	4.50± 0.76	27.66± 1.35	4.33± 0.80	25.16± 2.89	5.00± 0.81	23.83± 2.89	6.50± 0.76	18.33± 2.02
Disease Control ($AlCl_3$ 300 mg/kg)	3.83± 0.60	21.50± 1.72	3.00± 0.68	27.66± 1.80	2.33± 0.49	33.16± 0.94	1.66± 0.33	37.50± 0.99
Rivastigmine (0.3 mg/kg) + $AlCl_3$(300 mg/kg)	6.33± 1.14	23.2± 1.74	17.50± 0.92	18.00± 1.84	20.33± 1.33	15.33± 1.46	23.16± 1.22	12.83± 1.51
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	4.33± 0.88^{d}	18.0± 1.73^{d}	6.50± 1.52^{c}	12.83± 1.60^{c}	9.50± 1.38^{c}	8.66± 1.64^{c}	10.66± 1.28^{c}	6.16± 1.16^{c}
HMEPL (400 mg/kg) + $AlCl_3$ 300 mg/kg)	5.16± 1.19^{d}	16.16± 1.07^{d}	10.16± 1.77^{a}	9.00± 1.75^{a}	13.66± 1.60^{a}	5.83± 1.42^{a}	16.50± 1.56^{a}	3.66± 0.88^{a}
AFR (1 ml/kg) + $AlCl_3$ (300 mg/kg)	3.5± 0.99^{d}	19.00± 2.08^{d}	14.33± 1.70^{a}	15.16± 2.02^{a}	17.50± 1.64^{a}	11.16± 1.49^{a}	20.66± 1.80^{a}	8.66± 2.24^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Figure 4 showed docking of pluchine at the active site region of PPARα, showed high binding affinity, as indicated by total docking scores of -4.2, and also showed strong molecular interactions at the positions of 320 methionine, 322 serine, 317 isoleucine, 319 alanine, 318 phenylalanine, 321 leucine of PPARα.

The locomotor activity of trained rats that were treated with HMEPL and AFR on $AlCl_3$-induced AD was monitored on the 0th, 7th, 14th, and 20th days of the study and were illustrated in **Table 3**. The locomotor activity on the 0th day was not significant ($p>0.05$) between the trained rats in each group but on the 7th day, the locomotor activity of 400 mg/kg HMEPL and 1 ml/kg of AFR treated rats showed significant ($P<0.01$) effect when compared with disease control group whereas 200 mg/kg HMEPL treated rats showed non-significant ($p>0.05$) effect. After 14 days of continuous treatment, 400 mg/kg HMEPL showed a highly significant ($p<0.001$) effect than 200 mg/kg HMEPL ($P<0.05$) and 1 ml/kg of AFR ($p<0.01$) treated rats when compared with the disease control group but on 20th day of treatment the locomotor activity was highly significantly ($p<0.001$) increased rats which were simultaneously treated with 400 mg/kg HMEPL than 200 mg/kg HMEPL ($P<0.05$) and 1 ml/kg of AFR ($p<0.01$) treated rats when compared with the disease control group.

Table 4 illustrated the learning, memory ability, and anxiety of trained rats that were received with HMEPL and AFR were recorded as the number of entries in open and closed arms within 5min on $AlCl_3$ induced AD. On the 0th day, it was not significant ($p>0.05$) in extract and AFR-treated rats when compared with disease control rats. After 7 days of continuous treatment, 400 mg/kg HMEPL and 1 ml/kg of AFR treated rats showed a significant ($p<0.01$) effect on the number of entries in the open and closed arm but it was progressively improved upon continuous 20 days treatment. At the end of the study, 400 mg/kg of HMEPL and 1 ml/kg of AFR showed highly significant ($P<0.001$) whereas 200 mg/kg of HMEPL showed significant ($p<0.01$) in learning, memory ability, and anxiety than the disease control group animals.

The effect of HMEPL and AFR on change in conditioned avoidance response as the time taken to claim the pole on $AlCl_3$ induced AD was illustrated in **Table 5**. Time taken to climb the pole of trained rats was not significant ($p>0.05$) on the 0th day in rats which were simultaneously treated with both doses of HMEPL and AFR when compared with disease control trained rats. After seven days of treatment, the time taken to climb the pole was reduced highly significantly ($p<0.001$) with 400 mg/kg HMEPL and 1 ml/kg of AFR ($p<0.01$) and 200 mg/kg of HMEPL ($p<0.05$) when compared with disease control trained rats. Upon extension of continuous administration from day 14 to 20, the time taken to climb the pole outside 5 min response was significantly ($p<0.001$) decreased in rats that were treated with HMEPL (400 mg/kg), but 200 mg/kg of HMEPL showed significant ($p<0.05$) effect when compared with the disease control groups.

The effect of HMEPL and AFR on change in body weight $AlCl_3$ induced AD in rats was illustrated in **Table 6.** The body weight was not significantly ($p>0.05$) changed in rats on the 0th but with continuous treatments, it was progressively improved on day 7th, 14th, and 20th day. At the end of the study, body weight was significantly changed with 400 mg/kg HMEPL ($p<0.001$) whereas 200 mg/kg of HMEPL showed a significant ($p<0.01$) effect. Similarly, AFR also made increased body weight significantly ($p<0.01$) in $AlCl_3$-induced AD when compared with disease control rats.

Table 7 showed the effect of HMEPL and AFR on protein content as well as the aluminum concentration in the brain of $AlCl_3$-induced AD in rats. There was the increased level of protein content and aluminum concentration in the brain was found in disease control rats. Protein content in the brain was significantly ($p<0.001$) reduced after 20 days of continuous administration of 200 mg/kg and 400 mg/kg HMEPL. Administration of 1 ml/kg of AFR also showed a significant ($p<0.01$) reduction in aluminum concentration and protein content of $AlCl_3$ induced AD in rats.

Table 8 showed the effect of HMEPL and AFR on antioxidant status in the brain of $AlCl_3$ induced AD in rats. The CAT, SOD, and GSH levels were decreased in the disease control group but they were significantly increased and restored in animals treated with 400 mg/kg ($p<0.001$) and 200 mg/kg of HMEPL ($p<0.01$). Like 200 mg/kg of HMEPL, 1ml/kg of AFR also restored the antioxidant enzyme level in the brain of $AlCl_3$-induced AD in rats. There was an increased level of MDA found in the brain of disease control animals, 20 days of continuous treatment with 400 mg/kg ($p<0.001$) and 200 mg/kg of HMEPL as well as, 1 ml/kg of AFR ($p<0.01$) reduced the content significantly as that of normal in the brain of $AlCl_3$ induced AD in rats.

Table 5 Effect of HMEPL and AFR on conditioned avoidance response test of $AlCl_3$ induced AD in trained rats.

Parameters & Treatment	Time taken to climb the pole (min)			
Days	**0^{th}**	**7^{th}**	**14^{th}**	**20^{th}**
Negative Control	2.56 ± 0.05	2.48 ± 0.03	2.45 ± 0.02	2.51 ± 0.02
Disease Control ($AlCl_3$ 300 mg/kg)	2.51 ± 0.04	2.72 ± 0.07	2.92 ±0.06	3.10 ± 0.06
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	2.43 ± 0.02	2.23 ± 0.02	2.10 ± 0.03	1.90 ± 0.03
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	2.60 ±0.02^{d}	2.45± 0.03 ^{c}	2.36 ± 0.03^{c}	2.28 ± 0.03^{c}
HMEPL (400 mg/kg) + $AlCl_3$ (300 mg/kg)	2.51 ± 0.02^{d}	2.36 ± 0.03^{b}	2.21 ± 0.03^{a}	2.00 ± 0.03^{a}
AFR (1 ml/kg) + $AlCl_3$ (300 mg/kg)	2.45 ± 0.06^{d}	2.27 ± 0.06^{a}	2.14 ± 0.03^{a}	1.96 ± 0.03^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test were, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Table 6 Effect of HMEPL and AFR on change in body weight of $AlCl_3$ induced AD in rats.

Parameters Treatment	Body Weight (g)			
Days	**0^{th}**	**7^{th}**	**14^{th}**	**20^{th}**
Negative Control	160.3 ± 9.00	162.66 ± 13.72	165.16 ± 13.62	167.83 ± 13.44
Disease Control ($AlCl_3$ 300 mg/kg)	157.2 ± 6.45	140.33 ± 6.20	130.00 ± 6.10	116.66 ± 4.98
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	169.5 ± 9.73	193.66 ± 9.09	204.83 ± 7.91	221.83 ± 7.06
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	166.2 ± 8.61^{d}	172.2 ± 8.69^{d}	177.2 ± 8.67^{c}	180.5 ± 8.46^{c}
HMEPL (400 mg/kg) + $AlCl_3$ (300 mg/kg)	171.0 ± 8.72^{d}	185.5 ± 8.46^{a}	190.3 ± 9.36^{a}	196.2 ± 9.34^{a}
AFR (1 ml/kg) + $AlCl_3$ (300 mg/kg)	168.5 ± 7.64^{d}	189.0 ± 5.62^{a}	201.6 ± 5.76^{a}	216.17 ± 4.97^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test. where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Table 7 Effect of HMEPL and AFR on total protein and Aluminium content in brain of $AlCl_3$ induced AD in rats.

Parameters Treatment	Total protein content (mg/g)	Concentration of Aluminium (µgm/gm)
Negative Control	36.76 ± 0.70	-
Disease Control ($AlCl_3$ 300 mg/kg)	53.70 ± 0.74	9.1 ± 0.15
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	35.33 ± 0.68	2.3 ± 0.17
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	26.54 ± 0.70^{a}	6.3 ± 0.26^{a}
HMEPL (400 mg/kg) + $AlCl_3$ (300 mg/kg)	30.96 ± 0.71^{a}	5.7 ± 0.22^{a}
AFR (1ml/kg) + $AlCl_3$ (300 mg/kg)	32.65 ± 0.71^{a}	3.1 ± 0.16^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Table 8 Effect of HMEPL and AFR on antioxidant status in the brain of $AlCl_3$ induced AD in rats.

Treatment & Parameters	CAT (U/mg protein)	GSH (mmol/g protein)	MDA (nmol/mg protein)	SOD (U/g protein)
Negative Control	33.05 ± 0.71	53.67 ± 0.75	43.11 ± 0.68	71.34 ± 0.61
Disease Control ($AlCl_3$ 300 mg/kg)	10.48 ± 0.84	16.87 ± 0.72	75.09 ± 0.72	31.58 ± 0.74
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	27.38 ± 0.60	50.00 ± 0.70	46.51 ± 0.76	66.91 ± 0.57
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	17.88 ± 0.70^{d}	30.88 ± 0.72^{c}	65.56 ± 0.63^{d}	36.22 ± 0.71^{d}
HMEPL (300 mg/kg) + $AlCl_3$ (400 mg/kg)	19.56 ± 0.73^{b}	37.50 ± 0.65^{a}	57.38 ± 0.69^{a}	40.82 ± 0.39^{a}
AFR (1ml/kg) + $AlCl_3$ (300 mg/kg)	24.22 ± 0.75^{a}	46.32 ± 0.91^{a}	50.64 ± 0.71^{a}	61.22 ± 0.42^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Table 9 Effect of HMEPL and AFR on serum antioxidant status of $AlCl_3$ induced AD in rats.

Treatment & Parameters	CAT U/mL serum	SOD U/mL serum	MDA nmol/mL	GSH U/L
Negative Control	14.4 ± 0.13	7.5 ± 0.67	8.65 ± 0.17	73.98 ± 0.51
Disease Control ($AlCl_3$ 300 mg/kg)	5.9 ± 0.32	3.5 ± 0.15	23.15 ± 0.26	45.08 ± 0.24
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	14.6 ± 0.42	7.35 ± 0.1	9.03 ± 0.26	74.23 ± 0.15
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	11.9 ± 0.29^{a}	6.18 ± 0.24^{a}	12.4 ± 0.13^{a}	68.08 ± 1.08^{a}
HMEPL (300 mg/kg) + $AlCl_3$ (400 mg/kg)	14.4 ± 0.3^{a}	7.3 ± 0.2^{a}	10.00 ± 0.25^{a}	75.05 ± 0.26^{a}
AFR (1ml/kg) + $AlCl_3$ (300 mg/kg)	6.7 ± 0.22^{c}	5.55 ± 0.17^{a}	19.5 ± 0.47^{a}	56.05 ± 0.95^{a}

The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, c: p<0.05. AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata

Table 10 Effect of HMEPL and AFR on brain neurotransmitter level in $AlCl_3$ induced AD in rats

Parameters & Treatment	Dopamine (ng/g tissue)	Noradrenaline (ng/g tissue)	Serotonin (ng/g tissue)	Acetylcholine (µmoles/min/mg protein)
Negative Control	0.974 ± 0.1*	0.615 ± 0.6**	0.475 ± 0.9**	7.10 ± 0.07
Disease Control ($AlCl_3$ 300 mg/kg)	0.627 ± 0.9**	0.378 ± 0.7**	0.253 ± 0.1*	16.52 ± 0.7
Rivastigmine (0.3 mg/kg) + $AlCl_3$ (300 mg/kg)	0.954 ± 0.1*	0.601 ± 0.1*	0.459 ± 0.5*	7.60 ± 0.1
HMEPL (200 mg/kg) + $AlCl_3$ (300 mg/kg)	0.763 ± 0.5**^{a}	0.538 ± 0.7**^{a}	0.403 ± 0.6**^{a}	12.70 ± 0.8^{b}
HMEPL (400 mg/kg) + $AlCl_3$ (300 mg/kg)	0.772 ± 0.6**^{a}	0.494 ± 0.9**^{c}	0.343 ± 0.9**^{b}	10.80 ± 0.7^{a}
AFR (1 ml/kg) + $AlCl_3$ (300 mg/kg)	0.758 ± 0.5**^{a}	0.543 ± 0.7**^{c}	0.397 ± 0.5**^{b}	8.80 ± 0.7^{a}

*The data were expressed as mean ± SEM, n = 6. The data were analyzed by One Way Analysis of Variance (ANOVA) followed by Newman-Keul's multiple comparison test where, a: p<0.001, b: p<0.01, c: p<0.05, d: p>0.05- not significant. SEM: *x 10^{-2}, **x 10^{-3} AFR- Ayurvedic formulation Rasna, HMEPL- Hydro methanolic extract of Pluchea lanceolata*

Table 9 showed the effect of HMEPL and AFR on antioxidant status in the serum of $AlCl_3$ induced AD in rats. The CAT, SOD, and GSH levels were decreased in the disease control group but they were significantly increased and restored in animals treated with 400 mg/kg ($p<0.001$) and 200 mg/kg of HMEPL ($p<0.01$). Like 200 mg/kg of HMEPL, 1 ml/kg of AFR also restored the antioxidant enzyme level in the brain of $AlCl_3$-induced AD in rats. There was an increased level of MDA found in the serum of disease control animals, 20 days of continuous treatment with 400 mg/kg ($p<0.001$) and 200 mg/kg of HMEPL as well as, 1 ml/kg of AFR ($p<0.01$) reduced the content significantly as that of normal in the serum of $AlCl_3$ induced AD in rats.

Table 10 showed the effect of HMEPL and AFR on brain neurotransmitter levels in $AlCl_3$-induced AD in rats. The quantity of dopamine, noradrenaline, and serotonin was reduced but acetylcholine level was increased in the disease control group, Except acetylcholine, was highly significantly ($p<0.001$) raised in rats that were treated with 400 mg/kg of HMEPL and 1 ml/kg of AFR ($p<0.01$) whereas 200 mg/kg of HMEPL was not restored the dopamine level after twenty days of treatment moreover it showed significant ($p<0.01$) effect on the normalization of serotonin and noradrenaline level.

Table 11 showed the histopathological reports of the cortex and hippocampus of the brain of $AlCl_3$-induced AD with different treatment groups. In which, the negative control rat's brain cortex and hippocampus showed all the cellular functional units were within normal limits whereas, in disease control rats, marked, multifocal, neuronal vacuolation, marked congestion/hemorrhage and diffuse hippocampus were seen in both sections. Rats that are treated with both doses of HMEPL and ARF, showed minimal neuronal vacuolation and marked congestion/hemorrhage.

Discussion

"Rasna" is one of the most significant medicinal plants in the indigenous system of medicine. It is a controversial medicinal plant and has a wide application in the health care system. 13 plants are currently being identified and used as Rasna in different parts of India. PL is an accepted source of Rasna in the Ayurvedic medicine system in India and is traditionally used for diseases of the nervous system (Palash et al., 2013). Many chemicals are used for the induction of AD including aluminum, scopolamine, colchicine, streptozotocin, sodium azide, and ethanol. Aluminum is considered one of the most frequently used heavy metals for induction of cognitive impairment (Raft et al., 2012). It also causes anemia, osteomalacia, and hepatic and neurological disorders. Oral administration of a high amount of 300 mg/kg body weight of aluminum has been reported that, induction of AD and associated oxidative stress, cholinergic deficit, and accumulation of Aβ & NFTs in the brain of rats (Mahdi et al., 2019).

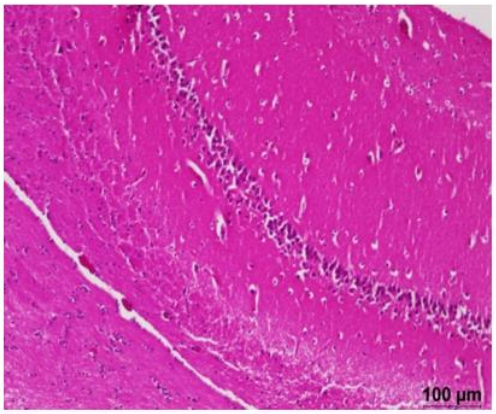
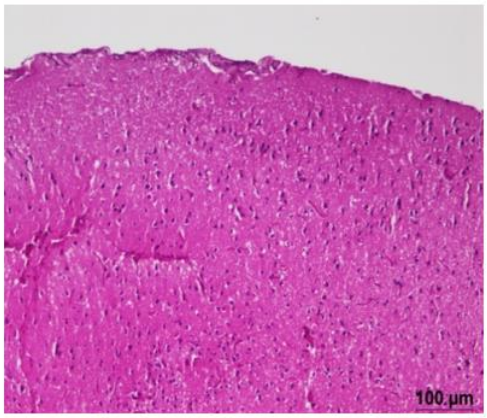
This neurotoxicity effect is due to free radical productions which make damage lipids and proteins in the brain. aluminum crosses the blood-brain barrier through a specific transferrin receptor and induces profound memory loss via disruption of various normal neuronal functions. It also causes progressive apoptotic neuronal loss, ultrastructural alterations of neurons present in the cortex and hippocampus region of the brain, protein misfolding, plaques depositions, and biochemical modifications followed by changes in gene expression in the brain (Zaky et al., 2017).

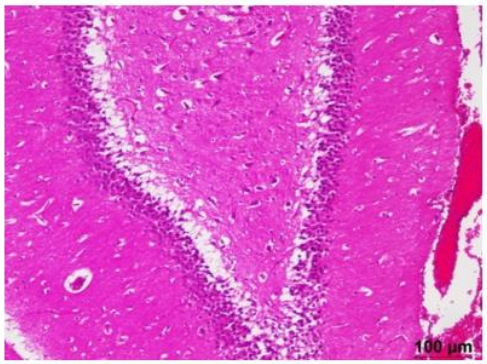
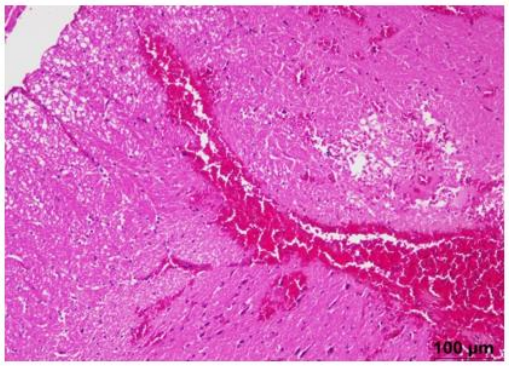
Aggressive behavior was more frequent in patients with AD. Therefore behavioral and psychological symptoms were more likely to be observed closer to the time of diagnosis of AD because it has severe dementia, neurodegeneration, and loss of functional independence which leads to early death. Different neuropsychiatric instruments were used to assess the behavioral change in AD. Long-term administration of aluminum has been reported to change behavioral patterns (Kangtao and Souravh (2018); Li et al., 2014). Hence in the present study, behavioral changes were also investigated on aluminum exposure for 20 days and the possible effect of HMEPL and AFR were assessed on the restoration of behavioral changes. Actophotometer test elevated plus maze test and pole climbing apparatus were used to monitor the behavioral changes.

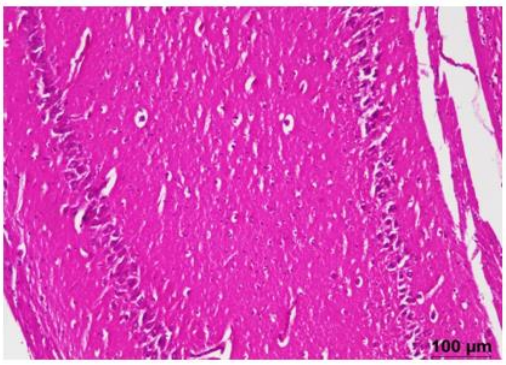
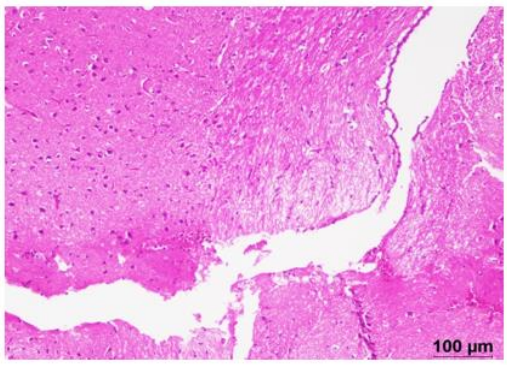
By using an actophotometer, locomotor activity was assessed to check the CNS stimulant or depressant effect on rats. Administration of aluminum for 35 days, there was a decline in locomotor activity in aluminum treated rats which indicated the CNS depressant effect on chronic Aluminium exposure (Lakshmi et al., 2015). In the present study also treatment with HMEPL and AFR corrected the locomotor incoordination caused by $AlCl_3$.

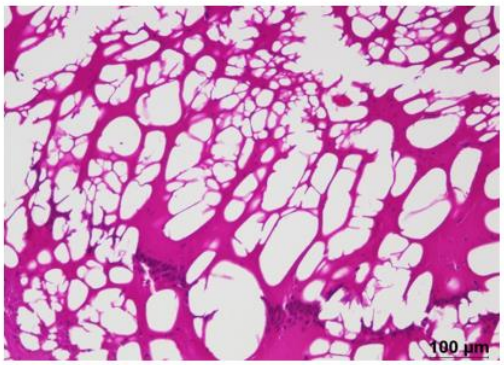
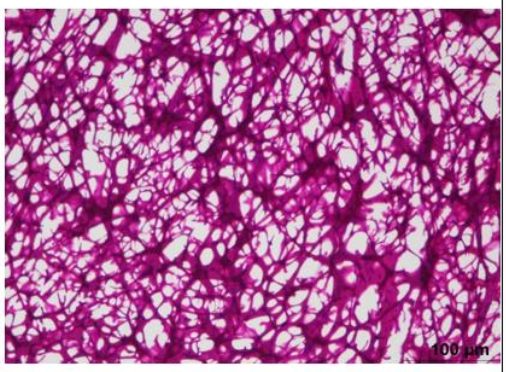
EPM is extensively employed for assessing the learning, retaining memory ability, and anxiety in rodents.

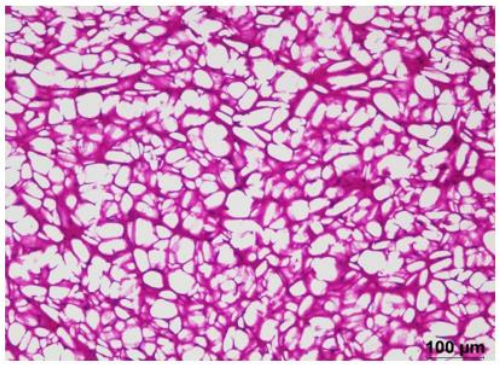
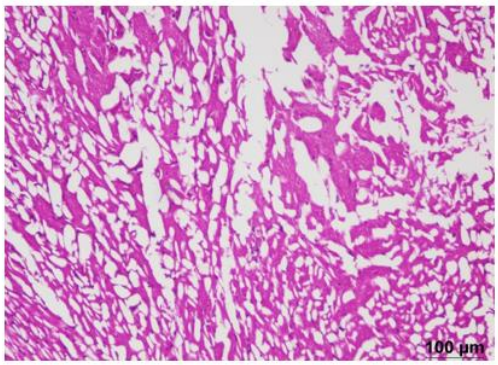
Table 11 Histopathology reports of cerebral cortex and hippocampus of brain of AD-induced rats and treated groups with HMEPL and AFR

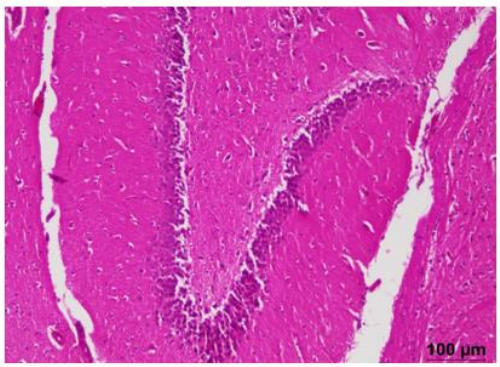
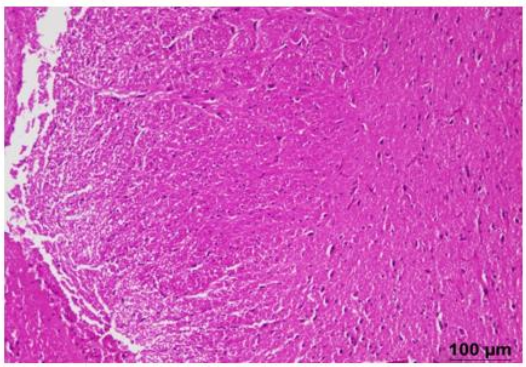
Group	**Negative control**	
Parts	**Hippocampus**	**Cortex**
Images		
Report	Section showed that all the cellular functional units were within normal limits	Section showed that all the cellular functional units were within normal limits

Group	**Disease Control ($AlCl_3$ 300 mg/kg)**	
Parts	**Hippocampus**	**Cortex**
Images		
Report	Section showed vacuolation, neuropil, multifocal, moderate congestion / haemorrhage, multifocal, moderate	Section showed vacuolation, neurophil, multifocal, marked vacuolation, neuronal, hippocampus, diffuse, marked congestion/ haemorrhage, multifocal, marked

Group	**Rivastigmine (0.3 mg/kg) + $AlCl_3$(300 mg/kg)**	
Parts	**Hippocampus**	**Cortex**
Images		
Report	Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion / haemorrhage, multifocal, minimal	Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion / haemorrhage, multifocal, minimal

Group	HMEPL (200 mg/kg) + $AlCl_3$(300 mg/kg)	
Parts	Hippocampus	Cortex
Images		
Report	Section showed vacuolation, neuropil, diffuse, severe, congestion / haemorrhage, multifocal, minimal	Section showed acuolation, neuropil, diffuse, severe, congestion / haemorrhage, multifocal, minimal

Group	HMEPL(400 mg/kg) + $AlCl_3$(300 mg/kg)	
Parts	Hippocampus	Cortex
Images		
Report	Section showed vacuolation, neuropil, diffuse, severe, congestion / haemorrhage, multifocal, minimal	Section showed vacuolation, neuropil, diffuse, severe, congestion / haemorrhage, multifocal, minimal

Group	Ayurvedic formulation (1 ml/kg) + $AlCl_3$ (300 mg/kg)	
Parts	Hippocampus	Cortex
Images		
Report	Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion / haemorrhage, multifocal, minimal	Section showed vacuolation, neuropil, multifocal, minimal vacuolation, neuronal, hippocampus, diffuse, minimal congestion / haemorrhage, multifocal, minimal

Animals which were received only Aluminium decreased the number of entries in the open arm, decreased the percentage of time spent in open arms, and increased the number of entries in the closed arm (Murugaiyan and Bhargavan, 2020; Rabiei et al., 2018). Researchers reported that the percentage of time spent in open arms and the number of entries to open arms are related to the anxiety indicator parameters in the EPM and are related to the $GABA_A$ receptor complex (Jafarian et al., 2019). However, the treatment with HMEPL and AFR reverse effects on $AlCl_3$- induced AD in rats.

From the Cooks pole climbing apparatus, memory retrieval capacity was determined as the ability of an animal to retain the acquired memory process. It was indicated by the increasing number of avoidance responses (Reddy et al., 2020). Ganga Raju et al., 2020 reported that the time taken to climb the pole was increased in the $AlCl_3$ exposure group and was due to dementia. In the present study, taken to climb the pole was noted that the time taken to escape from the electric shock field was reduced to that of normal trained rats after continuous 20 days of treatment of HMEPL and AFR.

Weight loss is the common problem found in AD which leads to weaker muscle mass, hard to maintaining physical balance, and more susceptibility to getting the systemic infection. The reason behind weight loss is the change in the olfactory system which contains neurotransmitters such as acetylcholine which was deficient in AD. Changes in food consumption and behavioral disturbances also occur in AD leading to decreased energy intake but increased energy expenditure is not the cause of weight loss in AD (Tamura et al., 2007). In the present study, $AlCl_3$ administration significantly diminished the body weight in the disease control group of animals. It was because of less desired water and food intake, transient diarrhea, and reduced efficacy in converting feed which leads to a reduction in body mass.(Mathiyazahan and Arokiasamy, 2019). The gain in body weight was observed among other groups treated with HMEPL and AFR.

Toxic beta-amyloid plaques proteins are formed in Alzheimer's and collect between neurons which affects the cell function. Similarly, neurofibrillary tangles (tau) are formed and get accumulated inside neurons. In the brain, one type of glial cell called microglia engulfs and removes waste and toxins from the healthy brain. In AD, microglia fails to clear the waste debris and protein including beta-amyloid plaques. Sometimes oxidatively modified proteins (carbonyl protein) are also formed in the brain (hippocampus) due to oxidative stress in AD (Aksenov, MY., 2001). The declined level of protein was found in the Aluminium treated group and was reported by (Yokel and McNamara, 1989). This was due to less intake of food, increased catabolism of proteins, and formation of reactive oxygen species (ROS) in which hydroxyl radicals are responsible for the oxidation of the side chains of some amino acids resulting in proteins hydrolysis (Doungue et al., 2018). However, in the present study also decreased level of protein content was found after the administration of HMEPL and AFR.

Crapper et al., 1973 stated that long-term exposure to $AlCl_3$ is associated with a high aluminum concentration in the brain. It enters the brain via the specific high-affinity receptors for transferrin (TfR) expressed in the blood-brain barrier (BBB) and gets accumulated in all the regions of rats with AD. In the present study also high Al concentration was found in disease control group rats, but treatment with HMEPL and AFR reduced the aluminum level in rats.

Neurotoxicity caused by aluminum is mediated mainly by increasing cellular oxidative stress which gets accumulates and enhances reactive oxygen species (ROS) formation, which depletes the normal antioxidant defense mechanism, thereby further enhancing oxidative stress and lipid peroxidation processes. It also causes changes in iron homeostasis, causing excessive free iron ions leading to oxidative damage, finally culminating in neurodegeneration (Lakshmi et al., 2015). Long-term exposure with $AlCl_3$ resulted in marked oxidative stress, which is indicated by increased lipid peroxidation resulting in the increased level of MDA as well as a decrease in reduced GSH, CAT, and SOD activity. This activity may be due to the reduced axonal mitochondria turnover, disruption of the Golgi, or reduction of synaptic vesicles induced by aluminum exposure (Prema et al., 2017). The present study also found that decreased levels of CAT, GSH, and SOD as well as an increased level of MDA content were found with disease control rats but re-established in rats that were treated with HMEPL and AFR.

Neurotransmitters play an important role in maintaining synaptic and cognitive functions by sending signals across synapses. They also have a major role in causing oxidative stress, which is known to be involved in AD pathogenesis (Reddy, 2017). The neurotoxic effect of

aluminum significantly increases AChE activity, the key enzyme which is responsible for acetylcholine hydrolysis thereby reducing acetylcholine levels found in the brain (Ramachandran et al., 2019). Similarly, $AlCl_3$ causes depletion of dopaminergic transmitters in the central nervous system and induced neurotoxicity (Zheng and Liang, 1998). Moreover, it also causes neuronal loss in the brain region. This neuronal loss and the resultant compensatory mechanisms lead to changes in the level of norepinephrine available in the brain, which consequently affect cognitive functions (Gannon et al., 2015). Reynolds et al., 1995 also reported that in AD, a significant decline of serotonin occurs which is consistent with cognitive processing. In the present study, reduced levels of acetylcholine, dopamine, noradrenaline, and serotonin levels were observed in only $AlCl_3$-treated animals. However, the treatment with HMEPL and AFR raised the level of the neurotransmitters as that of negative control rats.

Histopathology of hippocampus and cortex samples was analyzed from a neuroprotective study to know the structural changes and organ toxicity. The three main structural changes occurring in the brain include neuronal loss, formation, and accumulation of hyperphosphorylated tau protein called (NFTs) and aggregation of β-amyloid (Aβ) peptides termed senile or amyloid plaques. These changes are most prominent in the cholinergic system, particularly in the hippocampus and cortex, which are closely associated with memory loss and cognitive dysfunction in AD. So cortex and hippocampus of the brain were selected for the histopathology (Vecchio et al., 2018). Researchers found that the $AlCl_3$ induced cellular damage in organs such as the brain was analyzed by histopathology. In the present study also it was confirmed by the pathologist. It's being a comparative neuroprotective study, and the order of potency was HMEPL > AFR.

In conclusion, Al is not an essential element for living organisms continuous exposure to Al a considerable amount of Al crosses the blood-brain barrier, enters into the brain, and accumulates in a semi-permanent manner and causes more than 200 biologically important adverse effects on the mammalian central nervous system. Al exhibits only one oxidation state, Al^{3+}. It has an affinity for negatively charged, oxygen-donor ligands Al^{3+} binds to the phosphate groups of DNA and RNA, affecting various genes essential for brain functions (Kawahara et al., 2011). The antioxidant potential of the plant extracts prevents the interaction of Al^{3+} with the negatively charged phosphate group of DNA and RNA and protects brain function. Oral administration of HMEPL and AFR reverses the neurotoxic effects given by $AlCl_3$ in a dose-dependent manner. Therefore PL can be used as a remedy for the treatment of AD and neurotoxicity.

Declaration

The research protocol was approved by the institutional animal ethical committee (IAEC) of St. Joseph's College of Pharmacy, Cherthala (Proposal number: SJCP/IAEC/2019-13/19b)

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

There is no financial disclosure for the current study

Authors' contributions

SAS performed the all experiments; RA wrote the manuscript; DPA analyzed all data, and RA edited the manuscript. All authors read and approved the final manuscript.

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