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Elucidation of neuroprotective potential of Basella alba extracts in chronic alcohol and aluminium chloride induced neurodegeneration

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ABSTRACT

Basella alba is an edible herb which is widely using as dietary supplement in southern part of India. It is also known as Malabar Spinach, the present study designed to evaluate the neuroprotective effect of Basella alba in chronic alcohol and Aluminium chloride induced neuro degeneration rat model. Two types of extraction have used in the present study, aqueous extraction and ether extraction both were shown potential anti-oxidant property and exhibited neuroprotective effect. The biochemical and behavioral parameters were evaluated and it has shown significant improvement as compared with disease control group at a concentration of 200 mg/kg in both aqueous and ether extracts. An enzyme Acetylcholine esterase (AchE) was notably down regulated and enhanced cognitive functioning in treatment groups. The behavioral parameters such as locomotor activity ($p < 0.001$), Morris water maze ($p<0.001$) and elevated plus maze ($p<0.001$) results ware proved that Basella Alba extract is neuroprotective.

Keywords: *Basella Alba, Aluminium chloride, Morris water maze, Neuroprotection*

INTRODUCTION

The traditional medicinal practice comprises natural herbs, animal parts, minerals, and it has been widely in usage from the ancient times to till date in India, China and some other counties of the world. These herbs are cheap in cost and easily available as compared with synthetic marketed drugs, and also medicinal herbs represents a great deal of untapped reservoir of molecules and the structural diversity of their active constituents which is a valuable source of novel lead compounds[1]. In many areas of the world relies on the use of a wide variety of plant species in phytotherapy and traditional medicine became an option for the people due to its efficacy, cost effectiveness and less in causing adverse drug reactions[2]. Herbal medicines contain an active ingredient which will be collected from aerial or underground parts of plants such as leaf, bark, fruit, seeds, petal and roots or combinations thereof, whether in the crude state or as herbal formulation. The alternate system of medicine like Ayurveda, Siddha, Unani, and other tribal folklore medicines are not only complementary but also competitive in the treatment of various deadly diseases and these traditional medicinal practices have significantly

contributed to the health care system in order to heal acute and chronic diseases with their potent efficacy[3]. The neurodegenerative disorders (NDDs) are the most challenging and life threatening conditions and the prevalence of recovery from NDDs is low as compared with other systemic acute and chronic diseases because of rapid damage caused by the oxidative stress, excitotoxicity and cytokine storm, apart from that regeneration of nervous tissue is unattainable, that's why most of the NDDs are not curable but maintainable. Basella Alba is an edible leafy vegetable of Indian subcontinent which has been proven for its anti-oxidant and anti-inflammatory effect. The present study is designed to elucidate the neuroprotective property of Basella Alba using chronic alcohol and aluminium chloride induced neurodegeneration in rat model.

MATERIALS AND METHODS

Chemicals and reagents

Aluminium chloride was obtained from Lobachemie Pvt. Ltd, Mumbai; Anaesthetic ether was obtained from the Merck specialties Pvt Ltd, Mumbai. Chloroform was obtained from RFCL Ltd, New Delhi; Dithiobisnitrobenzoate was procured from the Hi-media Laboratories Pvt. Ltd, Mumbai; Ethanol was procured from NICE Chemicals Pvt. Ltd, Cochin and Sodium chloride from Fischer scientific Ind. Pvt ltd, Mumbai.

Experimental animals

Swiss albino male mice (20- 25g) were used for acute toxicity studies and albino male wistar rats (150-200g) were taken for evaluation of neuroprotective activity. All the animals were housed in a separate polypropylene cage in a good ventilated room and were maintained at 25 ± 2 °C temperature and 55% relative humidity (RH) conditions with a 12 h light/ dark cycle the animals were acclimatized by keeping them in animal house Nalanda College of Pharmacy for a week. All the experimental procedures carried out according to the "guide for the care and use of laboratory animals" Institutional Animal Ethical Committee (IAEC) No.3185/PO/Re/S/2001/CPCEA-107,India approved the study protocol.

Acute toxicity studies (LD_{50})

The acute oral toxicity study[4] of aqueous and petroleum ether extracts of Basella Alba was determined using male albino mice. Animals were well maintained under standard husbandry conditions and kept for 4 h before the experiment. OECD Guidelines No. 425 as well as CPCSEA guideline lines were adopted for acute toxicity studies. Animals were administered with single dose of each extract and observed for their mortality for the period of 48 h (Short term toxicity). Based on the short term profile of extracts, the doses for the next animals were determined. All the animals were observed for long term toxicity (7 days). The LD_{50} studies of the test extracts were conducted up to the maximum dose level of 2000 mg/kg body weight.

Experimental design

Aluminium chloride and Alcohol induced neurotoxicity

Aluminium chloride solution was prepared in distilled water at a concentration of 175mg/kg and given by oral route. Male albino mice (18–22 g) were randomized based on their body weight and were divided in to nine groups with six mice in each group and treatment was planned for 120 days daily administration. The group I was kept as normal control and received 5% Tween 80-orally. Group II was received with only Alcohol (Al) 5mg/kg, Group-III was administered with Alcohol and Basella alba Aqueous Extract (BaAE) 200 mg/kg and Group-IV administered Alcohol and Basella alba Petroleum ether Extract (BaPE) 200 mg/kg. Group V administered only Aluminium chloride (Al.ch) 100mg/kg, Group VI administered Aluminium chloride and Basella alba Aqueous Extract (BaAE) 200 mg/kg Group-VII administered in combination of Aluminium chloride and Basella alba Petroleum ether Extract (BaPE) 200 mg/kg. Group VIII was administered with Aluminium chloride + Alcohol + Basella alba Aqueous Extract (BaAE) and Group VIII was administered with Aluminium chloride + Alcohol + Basella alba Petroleum ether Extract (BaPE).

In-vitro Anti-oxidant studies/free radical scavenging activity

DPPH free radical scavenging activity

DPPH assay was performed to assess the free radical scavenging capability as described

earlier[5,6]. Briefly, 0.1mM DPPH solution was prepared in methanol and the Basella Aalba was dissolved in methanol to get the required stock solution of concentration 50 g /1ml. From the stock solution 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5 ml and 0.6ml was taken in test tubes and made up the volume equal to 1ml by adding 0.9ml, 0.8ml, 0.7ml, 0.6ml, 0.5ml and 0.4ml of solvent methanol to above tubes. The final concentration was 05, 10, 20, 40, 80 and 160 µg/ml respectively. Ascorbic acid was used as a reference standard. It is dissolved in methanol to make stock solution with same dilution to get the same concentration of extract. Control sample was prepared without extract and the reference is ascorbic acid. 1ml of methanol was used as blank, 3ml of DPPH solution was added to all the above tubes of test, standard, control and shaken vigorously and then kept in a dark chamber for 30 min. read the absorbance of all the tubes with UV spectrophotometer at 517 nm. The percentage of the standard and extract and percentage of inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where, A0 is the absorbance of control; A1 is the absorbance of test.

Hydrogen peroxide scavenging assay

The ability of the extract to scavenge hydrogen peroxide (H_2O_2) was determined according to the method of Ruchet al[7, 8]. Briefly, 0.1 mL of extracts (25–400 µg/mL) was transferred into the Eppendorf tubes and their volume was made up to 0.4 mL with 50 mM phosphate buffer (pH 7.4) followed by 0.6 mL of H_2O_2 solution (2 mM) was added. The reaction mixture was vortexed after 10 min of reaction time, vitamin-C was used as the positive control and the absorbance was measured at 230 nm. The percentage of the standard and extract and percentage of inhibition was calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where, A0 is the absorbance of control; A1 is the absorbance of test.

Behavioral Assessment

Locomotor Activity

Locomotor activity was performed on 21st and 42nd day of aluminum chloride administration. Each animal was kept in digital actophotometer and observed for 10 min, the apparatus equipped with infrared light sensitive photocells. When the beam of light falling on the photo cell is cut off by the animal that considered as one count and was recorded, values were expressed as number of counts per 5 min[9].

Morris Water Maze (MWM)

MWM test was used to assess spatial memory task in experimental animals. It was performed as per earlier method described[10] with slight modifications. Briefly, a large circular swimming tank (150 cm diameter, 45 cm height) consisted of four equal quadrants (NW, NE, SE, and SW) containing water (25 ± 1°C) was used. Visual cues in the form of red and blue colored tapes were placed around the water tank for facilitation of the spatial orientation in experimental animals and positions of the cues were kept unchanged throughout the experiment. The submerged platform (10 × 10 cm) was kept 1 cm above the surface of water in the acquisition phase. During the acquisition phase, the animal was placed in the tank, facing toward the wall of tank and allowed for 120 s to locate the platform. The animal was guided to reach the platform, if failed within 120 s and placed for 30 s on the platform. Daily four trials were given to each animal for four consecutive days (17th to 20th day) at the interval of 10 min maintained between each trial. Animal was gently placed in different quadrants of swimming tank during each trial. During the retention phase, water surface made opaque by using milk powder in order to hide the platform and kept 1 cm below the level of water in the tank. The animal was placed in the quadrant of tank facing toward the wall of the tank and retention of memory of the animal was evaluated on 21st and 42nd day. Escape latency was calculated by measuring time taken to locate the hidden platform by the animal in water maze.

Elevated Plus Maze (EPM)

EPM test was performed to assess anxiety in rodents and was performed as per earlier method described[11]. Briefly, the elevated plus maze apparatus consisted of two closed walls (50 × 10 cm), transverse with two open arms with same dimensions each and having 40 cm high walls. Both the arms of EPM were connected with Central Square (10 × 10 cm) and maintained 50

cm height above the ground level. During the acquisition phase, the animal was placed at one end of arm, facing away from central square area. Initial transfer latency (ITL) was recorded as the time required to the animal to move from open arm to closed arm. After recording ITL, each animal was allowed to explore for 20s inside the maze and after that returned to the home cage. Retention of memory was evaluated after 24h by placing the animal in an open arm and the 1st transfer latency and 2nd transfer latency was recorded on day 21st and 42nd, respectively.

Biochemical Assessment

Collection of Brain Tissues

At the end of the behavioral studies, animals were sacrificed with excessive anesthesia and brain tissues were collected, rinsed with ice-cold isotonic saline solution and stored at −80°C for neuro-biochemical estimation.

Estimation of Oxidative Stress Parameters

Hippocampus and cortex part of the brain were excised and homogenized in ice-cold 0.1M phosphate buffer solution (pH 7.4) using probe homogenizer (Polytron PT 2500E, Kinematica, Switzerland). Total protein content was determined in as such homogenate as the method described by Lowry et al.[12], MDA level in hippocampus and cortex were determined using the method described by Ohkawa et al.[13], SOD assay was measured in post-mitochondrial supernatant according to the method described by Paoletti et al.[14], post-nuclear supernatant obtained from homogenate was used to perform catalase assay as method. Reduced glutathione level in hippocampus and cortex was measured according to method of Ellman[15].

Acetylcholinesterase Activity (AChE)

The acetylcholinesterase activity in brain tissue was performed according to the method described by Ellman et al. [16] with slight modifications. Briefly, the assay mixture contained 0.1 ml of supernatant, 2 ml of sodium phosphate buffer (0.1M, pH 8.0) containing 0.1% BSA, 0.1 ml of dithio-bis-nitrobenzoic acid (DTNB) and 0.05 ml of Acetylthiocholine Iodide (AChI) were mixed thoroughly. The change in absorbance was measured for 2 min at 1 min interval at OD 412 nm using UV-VIS Spectrophotometer, (Perkin Elmer Lambda 20, USA). Acetylcholinesterase activity was expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein.

RESULTS AND DISCUSSION

In-vitro antioxidant activity

DPPH Method

DPPH radical scavenging activity of aqueous and petroleum ether extract of Basella Alba ware compared with Ascorbic acid. It was observed that the plant extract had higher scavenging activity at a concentration of 160 µg/ml, the scavenging activity of aqueous extract of Basella Alba have shown 66.21 %, while at the same concentration of the petroleum ether extract have observed that 71.24 % (**Figure 1**). The reductive capabilities of the Basella Alba plant extracts 160 µg/ml were compared to ascorbic acid. However, ascorbic acid which was used as a positive control showed better radical scavenging effect (86.19 % at the concentration of 160 µg/ml).

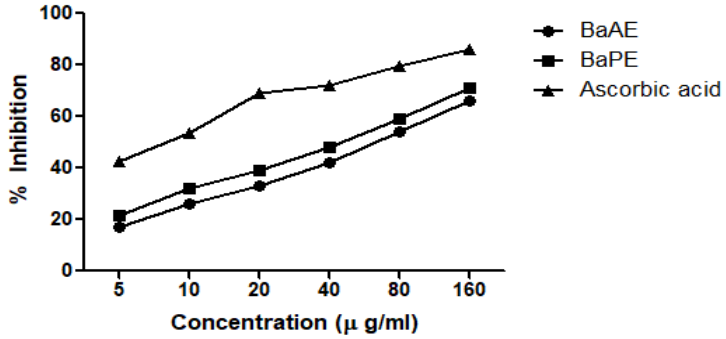

FIGURE 1: Graph of DPPH free radical scavenging activity of Basella Alba compared to that of Ascorbic acid. (Basella alba Aqueous Extract (BaAE), Basella alba Petroleum ether Extract (BaPE).

Hydrogen peroxide (H_2O_2) Method

The free radical scavenging activity of Basella Alba was evaluated for by hydrogen peroxide (H_2O_2) scavenging method. From the results Basella Alba shown that concentration dependent activity and the H_2O_2 scavenging effect and it was found that 63.36 % foe aqueous extract (**Figure 2**) at a concentration of 160 µg/ml, and 69.14% for petroleum ether extract has been noted. The standard Vitamin-C has shown 88.29%.

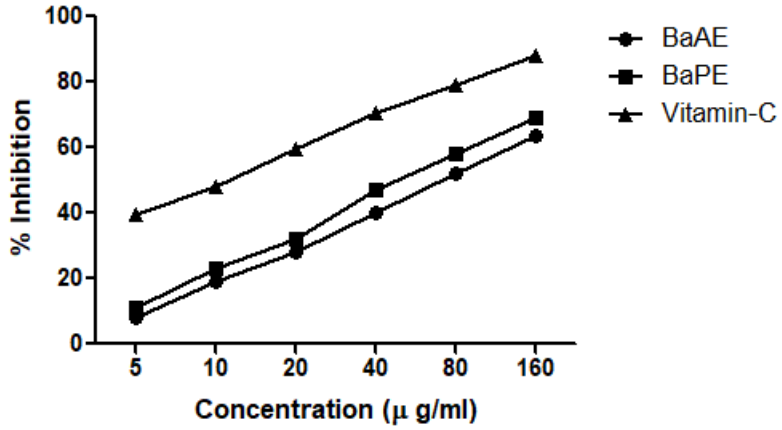

FIGURE 2: Graph of Hydrogen peroxide scavenging activity of Basella Alba compared to that of Vitamin-C.(Basella alba Aqueous Extract (BaAE), Basella alba Petroleum ether Extract (BaPE).

Locomotor Activity

The locomotor activity was significantly decreased in the aluminum chloride treated group when compared to the normal control group. Treatment with Aluminium chloride combined with alcohol plus both the extracts combination significantly improved locomotor activity as compared with disease control group on day 21st and 42nd ($p < 0.001$) when compared with the disease control group indicating improvement in aluminum chloride induced impaired locomotion. (**Figure 3.**).

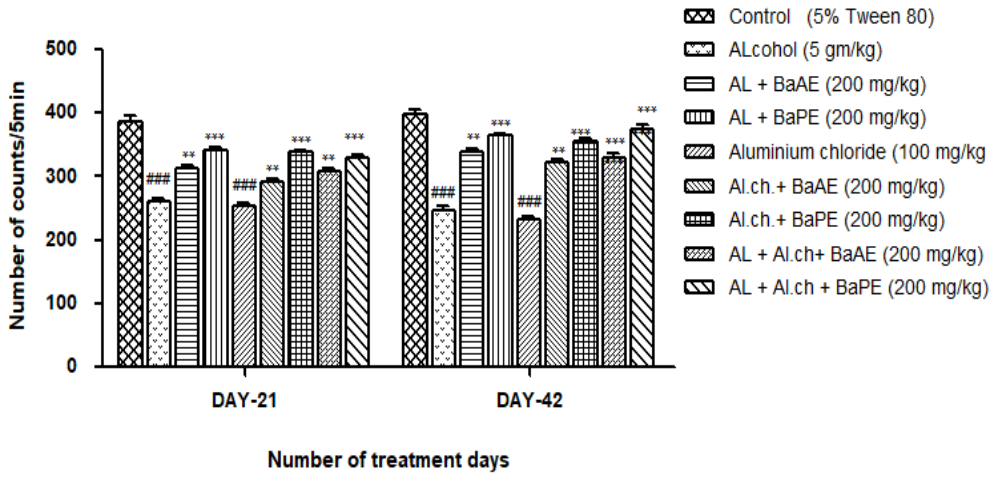

FIGURE 3: The Effect of Basella alba Extracts on Behavioral Assessment in alcohol and aluminium chloride induced neurotoxicity. Locomotor activity by using Data are expressed as Mean±SEM (n = 6), ###p <0.001 when compared with normal control, ***p <0.001, **p <0.01, *p <0.05 when compared with disease control group.

Morris Water Maze

In the Morris water maze test, the aluminum chloride treated group has shown a notable increase in escape latency when compared with normal control group. However, Treatment with Aluminium chloride combined with alcohol plus both the extracts combination significantly improved and prevented the increase in escape latency produced by aluminum chloride treatment on 42nd day ($p < 0.001$) when compared with disease control group and improved the retention performance of the spatial navigation task (Figure 4.).

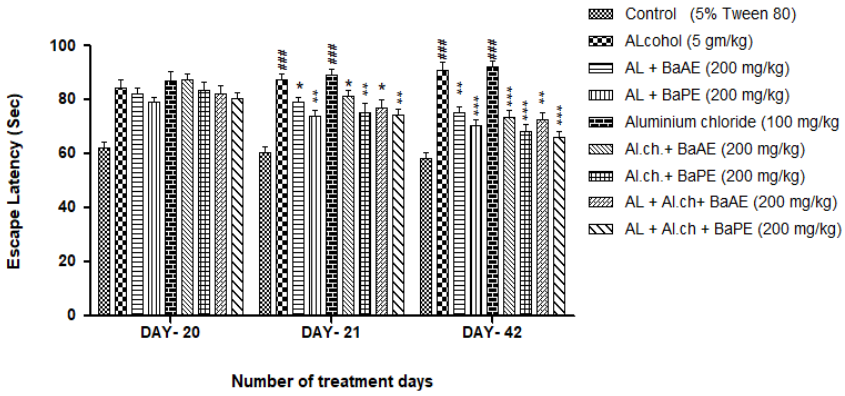

FIGURE 4: The Effect of Basella alba Extracts on Behavioral Assessment in alcohol and aluminium chloride induced neurotoxicity. Morris water maze test. Data are expressed as Mean±SEM (n = 6), ###p <0.001 when compared with normal control, ***p <0.001, **p <0.01, *p <0.05 when compared with disease control group.

Elevated Plus Maze

In elevated plus maze test, the aluminum chloride treated group showed significant increase in 1st transfer latency and 2nd transfer latency at day 21st and 42nd days respectively, with respect to initial transfer latency (ITL) at day 20th, when compared with normal control group. Treatment with Aluminium chloride combined with alcohol plus both the extracts combination significantly improved as compared with disease control group and treatment group. It prevented aluminum chloride induced increase in 1st transfer latency and 2nd transfer latency on day 21st ($p < 0.01$) and 42 ($p < 0.001$) when compared with disease control group and improved the memory performance in animals (Figure 5).

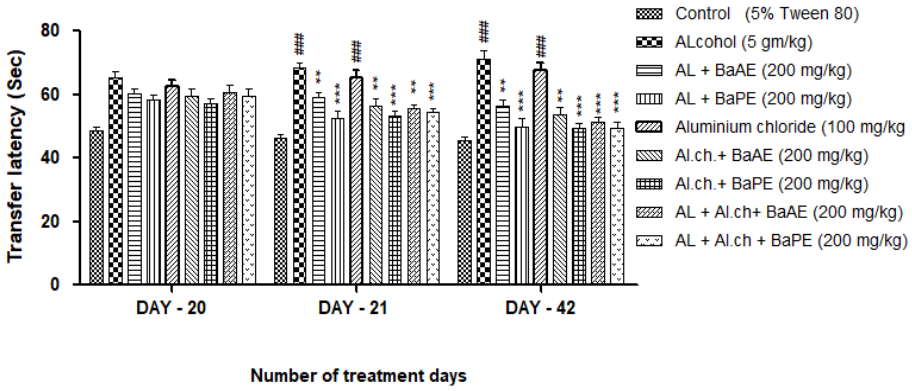

FIGURE 5: The effect of Basella alba Extracts on Behavioral Assessment in alcohol and aluminium chloride induced neurotoxicity. Elevated plus maze test. Data are expressed as Mean±SEM (n = 6), ###p <0.001 when compared with normal control, ***p <0.001, **p <0.01, *p <0.05 when compared with disease control group.

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Biochemical Assessment

Assessment of Oxidative Stress Parameters in Hippocampus

Aluminum chloride treated animals was showed a significant increase in MDA level ($p < 0.001$) and significant decrease in level of GSH ($p < 0.01$), SOD ($p < 0.01$) and catalase activity ($p < 0.01$) in hippocampus when compared with normal control animals. MDA level was significantly reduced in Basella Alba extract treatment at a dose of 200 mg/kg ($p < 0.001$) when compared with the disease control group. Basella Alba treatment showed significant improvement in GSH and SOD levels at a dose of 200 mg/kg ($p < 0.01$) as compared with disease control group. Treatment with Aluminium chloride combined with alcohol plus both the extracts combination ($p < 0.05$) significantly improved catalase activity as compared with disease control group (**Table 1**).

TABLE 1: Assessment of oxidative stress markers in Hippocampus of rat brain.

Assessment of Oxidative Stress Parameters in Hippocampus				
Treatment	**MDA (nmol mg protein $^{-1}$)**	**GSH (µmol mg protein $^{-1}$)**	**SOD (U mg protein^{-1}*100)**	**CAT (µ mol protein^{-1}**
Control group ((5% Tween 80)	4.12± 0.32	10.50± 0.51	8.17± 0.23	7.69±0.12
Alcohol (AL) only(5 gm/kg)	6.80±0.76###	5.62±0.31###	5.28±0.45##	4.76±0.3##
AL+**BaAE (**200 mg/kg)	4.32±0.45***	8.37±0.53**	6.61±0.64**	5.72±0.25*
AL+**BaPE (**200 mg/kg)	3.72±0.31***	7.56±0.51**	7.93±0.32**	6.43±0.31*
Aluminium chloride (Al.ch) (100 mg/kg)	6.61±0.73###	5.84±0.31###	5.34±0.54##	4.64±0.33##
Al.ch.+**BaAE (**200 mg/kg)	4.33±0.43***	8.34±0.52**	6.67±0.66**	5.35±0.15*
Al.ch.+**BaPE (**200 mg/kg)	3.61±0.34***	7.62±0.51**	7.93±0.34**	6.65±0.23*
AL+ Al.ch+**BaAE (**200 mg/kg)	3.94±0.22***	8.24±0.56***	7.82±0.56**	7.73±0.18**
AL+Al.ch + **BaPE (**200 mg/kg)	3.82±0.27***	8.32±0.52***	7.83±0.51**	7.42±0.28**
Data are expressed as Mean±SEM (n = 6), ###p <0.001 when compared with normal control, ***p <0.001, **p <0.01, *p <0.05 when compared with disease control group.				

Assessment of Oxidative Stress Parameters in Cortex

Cortex region showed a significant increase in MDA level ($p < 0.001$) and significant decrease in level of GSH ($p < 0.001$), SOD ($p < 0.001$), and catalase activity ($p < 0.001$) in aluminum chloride treated animals when compared with the normal control. MDA level was significantly reduced in Basella Alba at dose of 200 mg/kg ($p < 0.001$) when compared with the disease control group. Treatment with Basella Alba extract significantly ameliorated GSHlevel at a dose of and 200 mg/kg ($p < 0.001$) when compared to disease control group. SOD levels was significantly improved in 200 mg/kg ($p<0.01$) when compared to disease control group. Catalase activity was significantly improved in Basella Alba extract treated group at a dose of 200 mg/kg ($p < 0.01$) when compared with the disease control group. Treatment with Aluminium chloride combined with alcohol plus both the extracts combination ($p < 0.05$) significantly improved catalase activity as compared with disease control group (**Table 2**).

TABLE 2: Assessment of oxidative stress markers in cortex of rat brain.

Assessment of Oxidative Stress Parameters in Cortex				
Treatment	**MDA (nmol mg protein −1)**	**GSH (µmol mg protein −1)**	**SOD (U mg protein−1*100)**	**CAT (µ mol protein^{-1}**
Control group ((5% Tween 80)	3.92±0.54	11.34±0.64	8.97±0.68	8.13±0.44
Alcohol (AL) only(5 gm/kg)	6.84±0.62###	4.61±0.32###	4.66±0.40###	3.24±0.23###
AL+**BaAE (**200 mg/kg)	5.24±0.48*	6.94±0.45*	7.44±0.62*	5.17±0.46*
AL+**BaPE (**200 mg/kg)	3.33±0.17*	8.22±0.54*	7.66±0.51*	6.32±0.25*

Aluminium chloride (Al.ch) (100 mg/kg)	6.85±0.62###	4.64±0.35###	4.77±0.48###	3.63±0.34###
Al.ch.+**BaAE (**200 mg/kg)	5.41±0.45*	6.88±0.48*	7.44±0.69*	5.31±0.28*
Al.ch.+**BaPE (**200 mg/kg)	3.53±0.14*	8.24±0.52*	7.67±0.50*	6.25±0.62*
AL+ Al.ch+**BaAE (**200 mg/kg)	3.16±0.13**	7.13±0.65**	7.62±0.36**	9.36±0.43**
AL+Al.ch + **BaPE (**200 mg/kg)	3.26±0.17**	7.27±0.36**	7.64±0.33**	9.28±0.82**
Data are expressed as Mean±SEM. (n = 6), ###p <0.001 when compared with normal control, ***p <0.001, **p <0.01, *p <0.05 when compared with disease control group.				

Acetylcholinesterase Assay (AChE)

Aluminum chloride treated animals showed a significant increase in AChE activity ($p < 0.001$) in the hippocampus when compared with normal control animals. Treatment with Basella alba significantly prevented the increase in AChE activity produced by aluminum chloride treatment with Aluminium chloride combined with alcohol plus both the extracts combination ($p < 0.05$) significantly improved catalase activity as compared with disease control group ($p < 0.01$) when compared with disease control animals. Cortex region showed a significant increase in AChE activity ($p < 0.01$) in aluminum chloride treated animals when compared with normal control animals. Treatment with Basella Alba extensively prevented aluminum chloride induced increase in AChE activity.

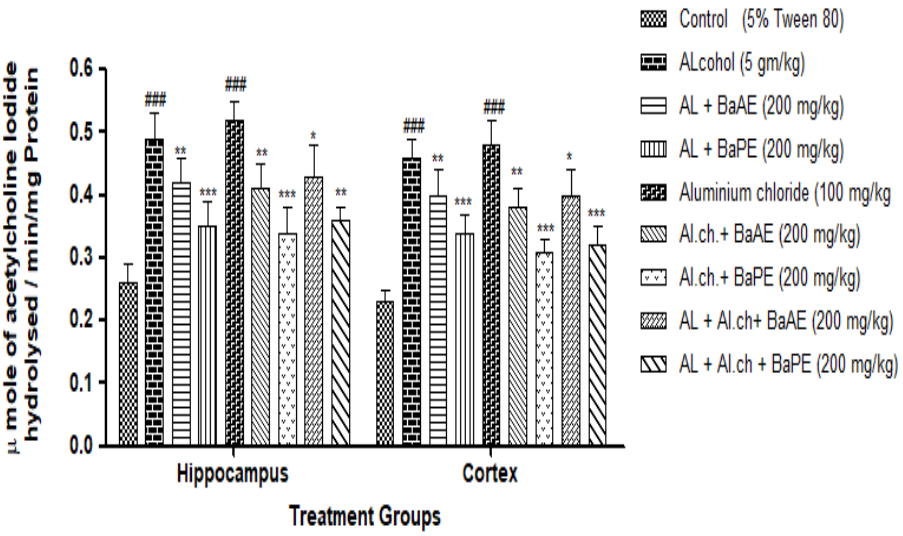

FIGURE 6: The levels of AChE in Hippocampus and Cortex regions of the rat brain with different treatment groups.

Histopathological Studies

Microscopical examination of hippocampus was observed by staining with hematoxylin and eosin. The disease control group showed various histopathological changes like multifocal moderate neuronal degeneration with pyknotic nuclei, multifocal moderate reduced layer of neuronal cell in hippocampus when compared with normal control group. Treatment with Treatment with Aluminium chloride combined with alcohol plus both the extracts combination improved catalase activity as compared with disease control group showed a decrease in neuronal degeneration and showed normal histology, normal layer of neuronal cell in when compared with disease control group.

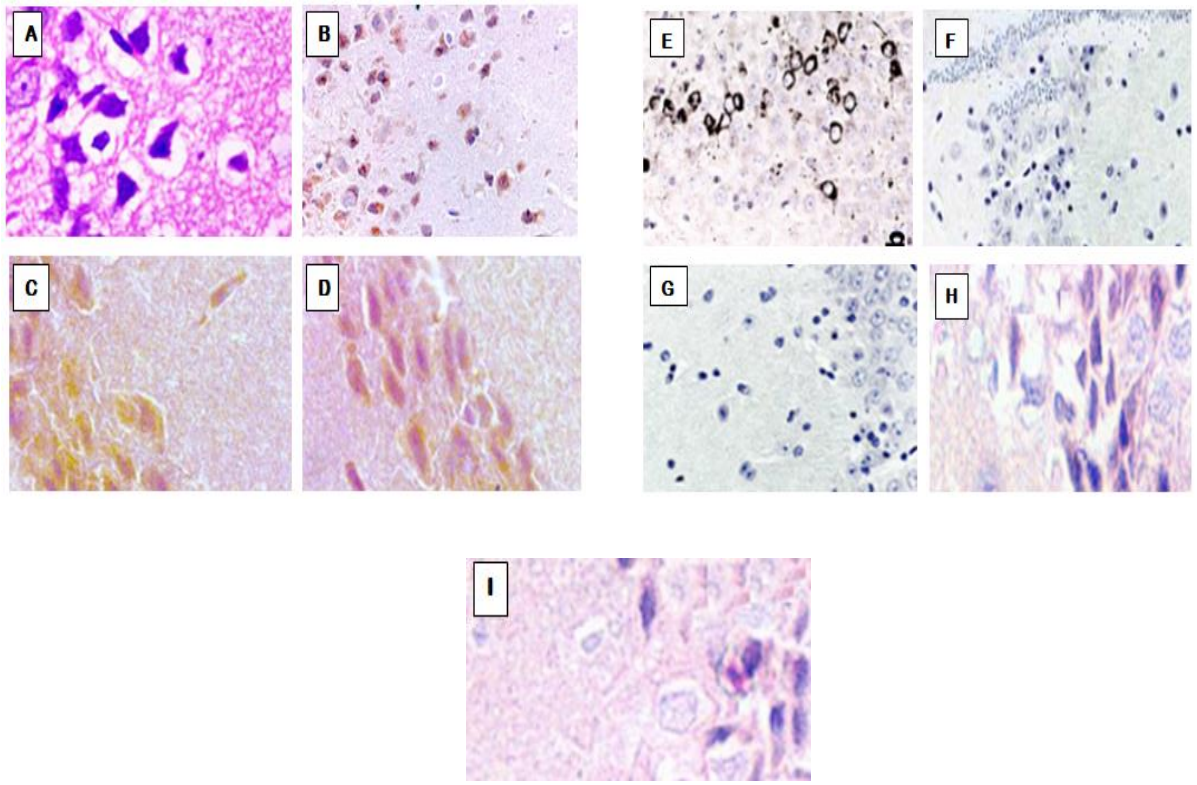

FIGURE 7: The Histopathological observations of various Basella alba extract treatment groups on Hippocampus tissue in alcohol and aluminium chloride induced neurotoxicity as neuronal degeneration with pyknotic nuclei. A) Normal control. B) Alcohol (AL) (5 gm/kg). C) AL+BaAE (200 mg/kg). D) AL+BaPE (200 mg/kg). E) Aluminium chloride (Al.ch.) (100 mg/kg). F) Al.ch.+BaAE (200 mg/kg). G) Al.ch.+BaPE (200 mg/kg). H) AL+ Al.ch+BaAE (200 mg/kg). I) AL+Al.ch + BaPE (200 mg/kg).

CONCLUSION

Basella alba extract treatment revealed its anti-oxidant property in chronic alcohol and aluminium chloride induced neurodegeneration. The extract of Basella alba drastically reduced the oxidative stress and improved the cognition in terms of restoration of behavioral changes at a concentration of 200mg/kg and also it notably regulated the AchE levels and exhibited neuroprotective effect through anti-oxidant and anti-AchE mechanisms.

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