

**Research Article****Neuroprotective effect of *Gracilaria corticata* against aluminium-induced neurotoxicity in the hippocampus and cerebral cortex of rat brain: Biochemical and histological approach**Asha Chacko<sup>a\*</sup>, Sibi P. Ittiyavirah<sup>b</sup><sup>a</sup>Department of Pharmaceutical Sciences, Centre of Professional and Advanced Studies, Cheruvandoor Campus, Ettumanoor- 686631, Kottayam, Kerala, India<sup>b</sup>Department of Pharmaceutical Sciences, Centre of Professional and Advanced Studies, RIMSR, Thalappady- 686009, Kottayam, Kerala, India

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**Abstract**

**Objective:** The present study investigated the effect of ethanolic extract of *Gracilaria corticata* on Aluminium induced neurotoxicity. **Materials and methods:** Wistar rats were divided into six experimental groups. The duration of treatment was for 60 days. Aluminium chloride at a dose of 50mg/kg; p.o was administered to all animal group except animals in control group. The extract at different concentration (50, 100, 200 mg/kg; p.o) and rivastigmine at 0.3mg/kg were administered along with aluminium chloride. At the end of the study, animals were sacrificed and brain processed for the evaluation of biochemical and histological analysis. **Results:** The ethanolic extract of *Gracilaria corticata* significantly ameliorated the toxic effect induced by aluminium chloride in rat hippocampus and cerebral cortex. It reduced the oxidative stress, improved acetyl cholinesterase level and morphological changes in the rat brain. **Conclusion:** The observed results indicated that the marine red algae, *Gracilaria corticata* can be used for its antioxidant and neuroprotective properties.

**Keywords:** Marine algae, Al-induced, Acetyl cholinesterase, neuroprotective, oxidative stress

**Introduction**

Aluminium (Al) is the most abundant metal on the earth crust. The main target for the accumulation of aluminium is the brain thereby leading to neurotoxicity (Yokel and O'Callaghan, 1998). It mainly gets deposited in the hippocampal regions (Roskams and Connor, 1990) and high levels of Al leads to neurofibrillary degeneration (Trapp et al., 1978). Exposure to Al in humans reported to cause cognitive impairment and neurological toxicity (Wills and Savory, 1985; Rifat et al., 1990; Hewitt et al., 1990). Chronic exposure to Al leads to memory impairment and locomotor dysfunction (Kaizer et al., 2008). As aluminium is a non-redox metal, it possess the potential to increase the cellular oxidative damage by increasing the pro-oxidant properties of metals like iron and copper (Bjertness et

al., 1996). This indicates its oxidative potential in producing reactive oxygen species (Amar Jyoti et al., 2006). Al exposure leads to disturbance in the acetyl cholinesterase (AChE) activity (Moshtaghi, 1999), damage to lipids and proteins (Julka and Gill, 1996). Al accumulation in the brain is implicated in the pathophysiology of neurodegenerative disorders including Alzheimer's disease (AD), Amyotrophic Lateral Sclerosis (ALS), Dementia in Parkinson's disease (PD) etc (Kurland, 1988). Thus multiple actions of Al leads to the consideration that Al exposed animals can be used as animal model for AD (Gulya et al., 1990).

Among world's plant and animal species, 80% are covered with marine organism with novel biologically active compounds (Jha and Zi-rong, 2004). Among them, marine algae provide the most promising bioactive compounds (Faulkner, 2002; Almeida et al., 2011). Marine red algae, *Gracilaria corticata* belongs to the family *Rhodophyceae*. Of 300 species of this genus, 160 were taxonomically identified (Guiry, 1996; Skriptsova et al., 2001). Compounds isolated from *Gracilaria* species have exhibited various biological properties such as antibacterial, antioxidant, anti-

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inflammatory, anticoagulant, anti-viral and apoptotic activity (Almeida et al., 2011). *G.corticata* is found in tropical and subtropical seas. It is commercially used as animal and human food (Shynuet al., 2014). The phytoconstituents reported in *G. corticata* include flavonoids, phenolics, alkaloids, steroids, tannins, proteins, polysaccharides etc (Krishnaveni and Johnson, 2012). It is reported to possess antioxidant (Taheri, 2016), anti-inflammatory (Deepa et al., 2017), anti-cancer, antibacterial (Krishnaveni and Johnson, 2012), anti-viral (Edhaya and Vinod, 2015), anti-pyretic (John, 2014) properties. Its antioxidant and anti-cholinesterase activity (Ghannadi, 2013) leads to the selection of this alga for studying its neuroprotective potential in aluminium chloride induced neurotoxicity.

## Materials and methods

### Chemicals

AlCl<sub>3</sub> (Spectrochem Pvt. Limited, India), Rivastigmine (Dr. Reddy's Laboratories, Hyderabad, India), Acetylthiocholine iodide, reduced glutathione, thiobarbituric acid, and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) were obtained from Sigma-Aldrich Co. LLC (St. Louis, MO, USA). All other chemicals used in the study were of analytical grade.

### Collection and Extraction of *G. corticata*

*G.corticata* was collected from Thirumullavaram coastal area of Kollam district, Kerala, India. It was identified and authenticated in the Department of Botany, Mar Thoma College, Thiruvalla, Kerala (Herbarium Specimen MTCHT-60). Algal samples were washed with sea water and then it was washed in tap water to remove dirt and extraneous matter. Washed seaweeds were spread out in room temperature under shade. Shade dried materials were grounded to fine powder. The algal powder (500g) was soaked in 1 litre of ethanol for a week with intermittent shaking. The whole material was filtered through Whatman filter paper (No.1). The filtrate was concentrated under rotary evaporator at 40°C. The residue was stored at 4°C until use.

### Experimental animals

Male wistar albino rats weighing 150-180g were obtained from Central Animal Facility of Department of Pharmaceutical Sciences, Centre for Professional and Advance Studies, Cheruvandoor, Kerala. The animals were acclimatized to the laboratory conditions such as 25±2°C, humidity of 50±5% and natural 12:12 h light and dark cycle throughout the experimentation. Rats were housed in polypropylene cages with free access to food and water. The experimental protocol was approved by Institutional Animal Ethics Committee (Approval Number IAEC/PhD/UCP/2017-04) and in accordance with the guidelines set by CPCSEA, India. Acute

toxicity study was accomplished as per guidelines of the Organization for Economic Cooperation and Development (OECD) 425.

### Experimental design

Aluminium was administered by gavage for 60 days. Gavage was performed using a syringe with a modified steel point to introduce the solution into the rat's oesophagus without injuring the tissue. Animals were randomly assigned to six different groups of six animals each. Group 1 (CON) received standard food and water. In group 2 (ALU) rats received aluminium chloride (AlCl<sub>3</sub>) 50mg/kg/body weight diluted in drinking water. Group 3 (AL+RIV) rats were treated with aluminium chloride (50mg/kg/body weight) and Rivastigmine tartrate (0.3mg/kg/body weight) diluted in drinking water. Group 4 (AL+EGC50), group 5 (AL+ EGC100) and group 6 (AL+EGC200) received 50, 100, 200 mg/kg/body weight along with aluminium chloride respectively. Rivastigmine solution, aluminium chloride solution and suspension of EGC was freshly prepared and given once daily. The dose of aluminium chloride (Bihaqi et al., 2009) and rivastigmine (Carageorgious et al., 2008) was selected based on previous research report. The dose of AlCl<sub>3</sub> was found to induce neuroinflammation with low incidence of mortality. Body weight of the animals was recorded for first 2 weeks on daily basis; afterwards monitored once in a week till the end of the study.

### Preparation of hippocampal and frontal cortex homogenates

After treatment period, half of animals from each animal group were sacrificed by cervical dislocation; brain was immediately removed and washed thoroughly with ice-cold saline to remove blood and microdissected into hippocampus and cerebral cortex. The hippocampus (left and right) and cortex (left and right) were separately pooled to make one sample of the tissue. Isolated brain parts were stored at -80°C. A 10% w/v of tissue homogenate was prepared in ice-cold phosphate buffer pH 7.4 which was centrifuged at 2000g for 30 min at 4°C to yield the supernatant. The supernatant was used for the estimation of superoxide dismutase (SOD), reduced glutathione (GSH), catalase (CAT), lipid peroxidation (LPO) and acetylcholinesterase (AChE) inhibition. Total protein was estimated by Lowry's method (Lowry et al., 1951).

### Determination of aluminium concentration in rat brain

The aluminium was analyzed by wet acid digestion method (Zumkley et al., 1979) in hippocampus and cortex of rat brain. A mixture of 2.5 ml of per chloric acid/nitric acid

(1:4 in volume) was added to brain parts. Then, mixture with brain sample was placed in sand bath for 44 h until the point of a white ash or residue was obtained. Then residues were dissolved in 2.5 ml of 10mM nitric acid. Then this sample (in liquid form) was placed in the sample holder of atomic absorption spectrophotometer (Perkin Elmer, India). The total concentration of aluminium was calculated in  $\mu\text{g/gm}$  of tissue or ppm.

#### Determination of Acetylcholinesterase inhibitory activity

The activity of acetylcholinesterase activity in rat hippocampus and frontal cortex was determined according to the Ellman's method (Ellman et al., 1961). The reaction mixture (250  $\mu\text{l}$  final volume) contained 0.26M sodium phosphate buffer (pH 7.8), 50  $\mu\text{l}$  of DTNB (3mM pH 7.8) and 20  $\mu\text{l}$  of 0.28 U/ml AChE and 10  $\mu\text{l}$  of acetylthiocholine iodide. The reaction was initiated by adding homogenate and following the formation of yellow color (5-thio-2-nitobenzoate anion) as a result of the reaction of DTNB with thiocholine released by the enzymatic hydrolysis of acetylthiocholine at 412 nm. The AChE activity was expressed as  $\mu\text{moles}$  of acetylthiocholine iodide hydrolyzed/min/mg of protein.

#### Estimation of lipid peroxidation (LPO)

MDA, a marker of LPO, was determined by Ohakwa et al. (1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 15 ml of 20% acetic acid (pH 3.8), 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (TBA), and 0.2 ml of brain homogenate. The mixture was made up to 4 ml with distilled water and heated at 95 °C for 60 minutes. After cooling with tap water, 5 ml of n-butanol and pyridine (15:1 v/v) and 1 ml of distilled water were added and centrifuged. The organic layer was separated and absorbance was measured at 532 nm and MDA content was expressed as  $\mu\text{moles/mg}$  protein.

#### Estimation of superoxide dismutase (SOD)

SOD activity was measured by Kono's method (Kono, 1978). The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture, 0.05 ml supernatant of brain homogenate and 0.05 ml of hydroxylamine were added, and the auto-oxidation of hydroxylamine was measured for 2 min at the 30s intervals by measuring the absorbance at 560 nm. The results were expressed as Units/mg protein.

#### Estimation of catalase activity (CAT)

One milliliter of brain tissue homogenate was mixed with 5 ml of phosphate buffer, 4 ml of 0.2M hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) was added and time was noted. Exactly 180s after adding  $\text{H}_2\text{O}_2$ , 1 ml of the reaction mixture was added to 2 ml of dichromate acetic acid, kept in boiling water bath for 10 min, cooled down under running tap water and absorbance was measured at 570 nm against blank. Catalase activity in the hippocampus and frontal

cortex region were expressed as  $\mu\text{moles}$  of  $\text{H}_2\text{O}_2$  consumed/mg protein/min (Sinha, 1972).

#### Estimation of reduced glutathione (GSH)

Reduced glutathione (GSH) was measured according to the method of Moron et al. (1979). An equal quantity of homogenate was mixed with 10% trichloro acetic acid and centrifuged to separate the protein. To 0.1 ml of this supernatant, 2 ml of phosphate buffer (pH 8.4), 0.5 ml of 5-5-dithiobis (2-nitrobenzoic acid) and 0.4 ml of double distilled water was added, then the mixture was vortexed and absorbance was read at 412 nm within 15 minutes. The concentration of GSH was expressed as  $\mu\text{moles/g}$  tissue.

#### Histopathological study

The brains were dissected out and immersed in the 4% paraformaldehyde in 0.1M phosphate buffer and embedded in paraffin wax. Sections were cut in a coronal plane of 4 $\mu\text{m}$  thick using microtome. The sections were stained with hematoxylin and eosin. The stained sections of the rat brain were observed under the light microscope and photographed for studying the morphological changes.

#### Statistical analysis

The experimental results were expressed as the mean  $\pm$ SEM. The intergroup variation between various groups was analysed statistically using one-way analysis of variance using the GraphPad Prism, Version 7 (GraphPad Software, Inc., San Diego, CA, USA) followed by Dunnett's multiple comparison test. A p value less than 0.05 was considered to be significant.

#### Results

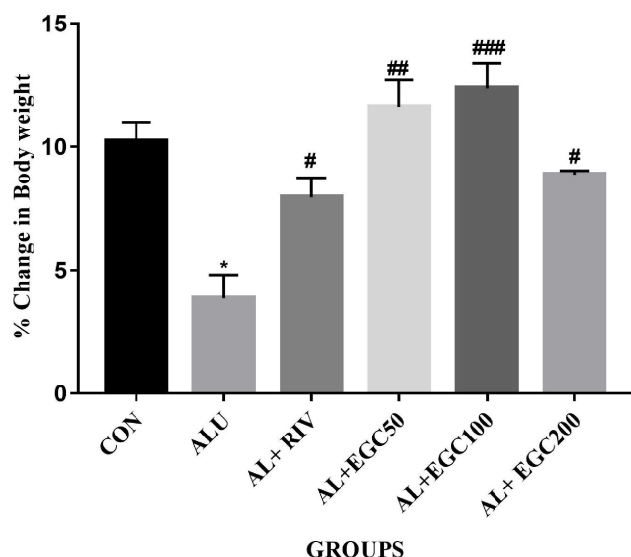
Ethanollic extract of *G. corticata* was used to study the AL induced toxicity. During the experimental study, no mortality was recorded during the experimental study. The observations made on the six different experimental groups indicated that continuous administration of  $\text{AlCl}_3$  for 60 days induced alterations in the biochemical parameters and histopathology in hippocampus and cerebral cortex of rat brain.

#### Percentage change in body weight

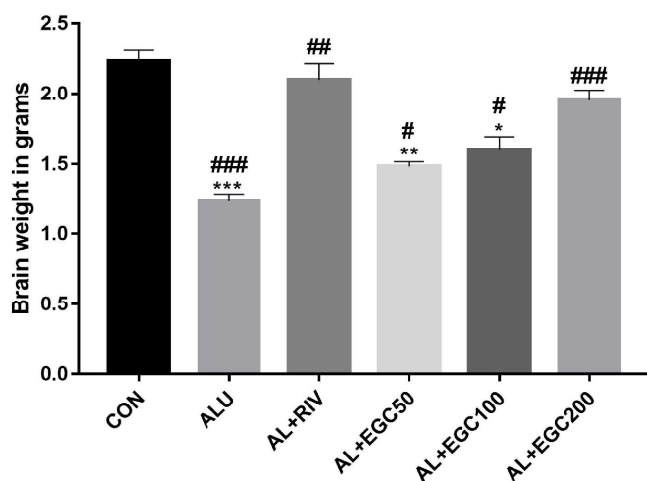
One-way ANOVA showed a significant difference in body weight among experimental animal groups. The body weights of the experimental animals were obtained on the first and last day of treatment, representing the initial and final weights respectively. The percentage change in body weight was obtained and analyzed (Figure 1). The weight of rats in CON group rose rapidly from  $164.17 \pm 3.52$  g to  $180.33 \pm 3.00$ g represented a growth rate of 10%. In ALU group, the weight of animals reduced to 3.9 % from

170.83±2.39 g to 164.17±2.39g, which is statistically significant ( $p<0.05$ ) as compared to CON. Statistical increase in body weight was seen in extract treated (50, 100, 200mg/kg body weight) when compared to ALU group. The percentage increase in body weight of group 4 and group 5 was 12% and of group 6 was found to be 8.9%.

### Brain weight



**Figure 1.** Effect of EGC on percentage change in body weight after 60 days of Al-intoxication. Data are in mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  when compared to control group. # $p<0.05$ , ## $p<0.01$ , ### $p<0.001$  when compared to Al-intoxicated group.



**Figure 2.** Effect of EGC on rat's brain weight in different experimental groups. Data are in mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  when compared to control group. # $p<0.05$ , ## $p<0.01$ , ### $p<0.001$  when compared to Al-intoxicated group.

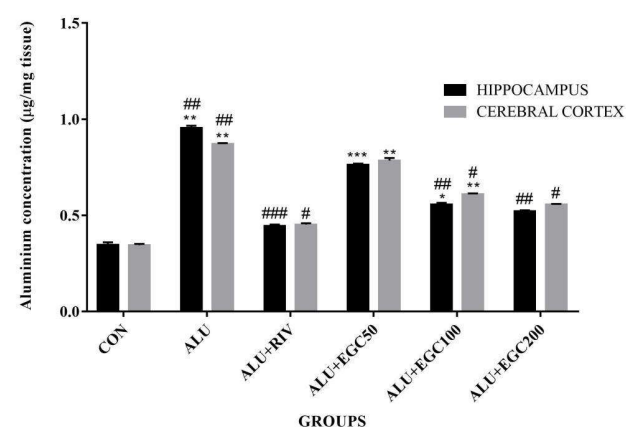
Brain weight in Al intoxicated animals was found to be significantly low ( $p<0.001$ ) when compared to CON group. No significant difference in brain weight was seen in AL+RIV and AL+EGC200 groups. Co-administration of EGC at different doses has demonstrated to increase brain weight significantly as compared to ALU group. Among three different doses, higher concentration, 200mg/kg of EGC has produced better effect ( $p<0.001$ ). The values are shown in figure 2.

### Effect of EGC on concentration of aluminium in hippocampus and cortex

Aluminium chloride treatment significantly increased ( $p<0.01$ ) the level of Al in both hippocampus and cortex areas of rat brain when compared to control rats. Figure 3 indicated that co-administration of EGC (100 and 200mg/kg, p.o) significantly attenuated the rise in aluminium concentration in hippocampus and cortex when compared to ALU group. Cerebral cortex ( $p<0.05$ ) and hippocampal ( $p<0.001$ ) regions from rats of group AL+RIV caused a significant reduction in Al concentration when compared to ALU. However, ALU+EGC50 group did not produce significant effect on brain aluminium concentration in both brain regions.

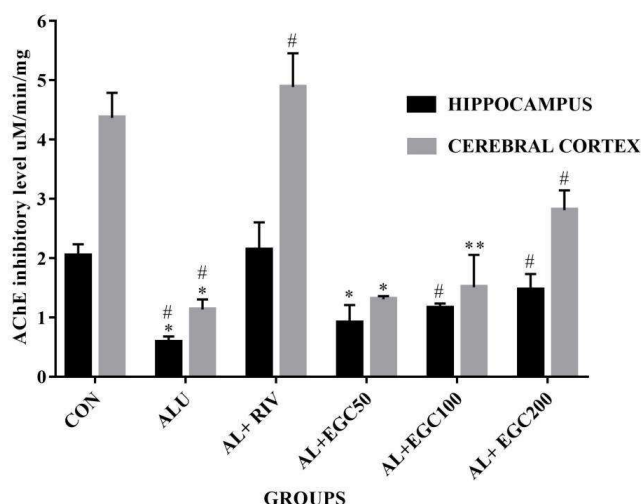
### Effect of EGC on acetyl cholinesterase enzyme activity in hippocampus and cortex

Al exposure significantly ( $p<0.05$ ) reduced the AChE level in both hippocampus and cortex regions of rat brain as indicated in figure 4. In hippocampus, no significant difference was seen in AL+EGC100 and AL+EGC200 as compared to CON group. Animals in group AL+EGC200



**Figure 3.** Effect of EGC on aluminium concentration in different experimental groups. Data are in mean ± SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  when compared to control group. # $p<0.05$ , ## $p<0.01$ , ### $p<0.001$  when compared to Al-intoxicated group.



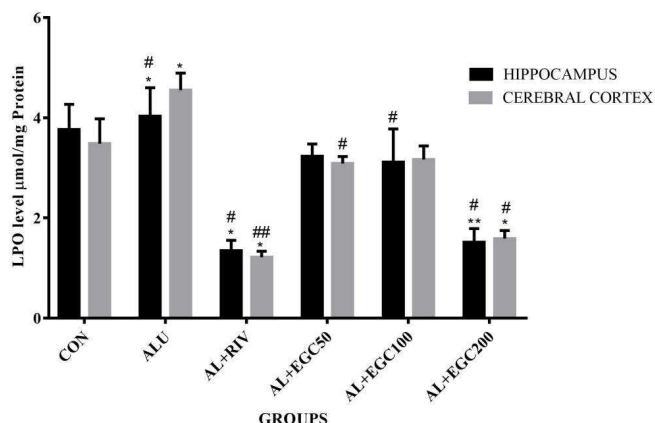


**Figure 4.** Effect of EGC on acetyl cholinesterase inhibitory activity in different experimental groups. Data are in mean  $\pm$  SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared to Al-intoxicated group.

exhibited significant ( $p < 0.05$ ) increase in enzyme activity in both regions when compared to ALU animals. A significant ( $p < 0.05$ ) increase in enzyme activity in the cerebral cortex was observed in AL+RIV group.

#### Effect of EGC on LPO level in rat brain regions

The effect of EGC on LPO was represented in figure 5. The LPO level in the hippocampus was significantly increased in Al alone treated group, when compared with control ( $p < 0.05$ ). But, there was no significant difference in the LPO level in cerebral cortex of Al treated group when compared to control animals ( $p > 0.05$ ).



**Figure 5.** Effect of EGC on lipid peroxidation in different experimental groups. Data are in mean  $\pm$  SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared to Al-intoxicated group.

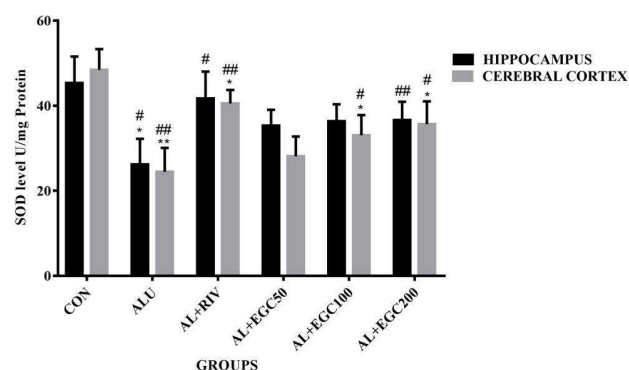
Animals in AL+RIV group showed significant decrease in the level of LPO in both hippocampus and cerebral cortex ( $p < 0.05$ ) when compared to control (CON). Also, significant decrease in LPO level was seen in AL+RIV treated animals when compared with Al treated (ALU) rats. Among three different concentration of EGC, co-treatment of higher concentration of EGC (AL+EGC200) exhibited significant decrease in LPO level in hippocampus and cerebral cortex when compared to control (CON) and Al alone (ALU) animals.

#### Effect of EGC on SOD level in rat brain regions

SOD activity was significantly reduced in hippocampus and cerebral cortex of aluminium intoxicated animals, when compared to control rats (Figure 6). Co-administration of Rivastigmine demonstrated a significant increase in the SOD level in the hippocampus ( $p < 0.05$ ). However, there was no significant difference in the cerebral cortex SOD level in AL+RIV group. AL+RIV treated rats increased the SOD level significantly in the hippocampus and cortex when compared with animals in the ALU group. Significant increase in hippocampal SOD level was seen in AL+EGC200 treated animals ( $p < 0.05$ ). Similarly, significant increase in SOD level was shown in the hippocampus ( $p < 0.05$ ) and cortex ( $p < 0.01$ ) of AL+EGC200 treated animals. AL+EGC50 administration exhibited no significant difference in the SOD level in both hippocampus and cortex when compared with CON and ALU groups.

#### Effect of EGC on CAT level in rat brain regions

The catalase (CAT) activity in the hippocampus and cortex of ALU treated animals was significantly reduced ( $p < 0.05$ ) (Figure 7). Rivastigmine administration along with Al significantly increase CAT activity in the hippocampus

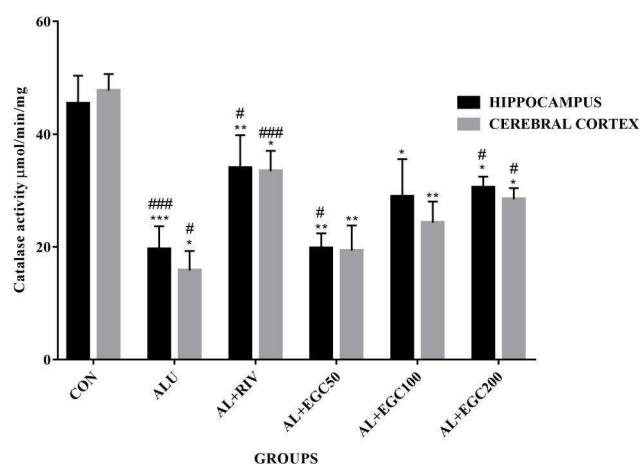


**Figure 6.** Effect of EGC on superoxide dismutase in different experimental groups. Data are in mean  $\pm$  SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared to Al-intoxicated group.

( $p < 0.01$ ) and cortex ( $p < 0.05$ ). A significant increase ( $p < 0.01$ ) in CAT activity in the cortex was seen in the AL+RIV rats, when compared with Al intoxicated rats. Significant increase in CAT activity was seen in the hippocampus and cortex of AL+EGC200 ( $p < 0.05$ ), AL+EGC100 ( $p < 0.05$ ),  $p < 0.01$ ) and AL+ EGC50 ( $p < 0.05$ ,  $p < 0.01$ ).

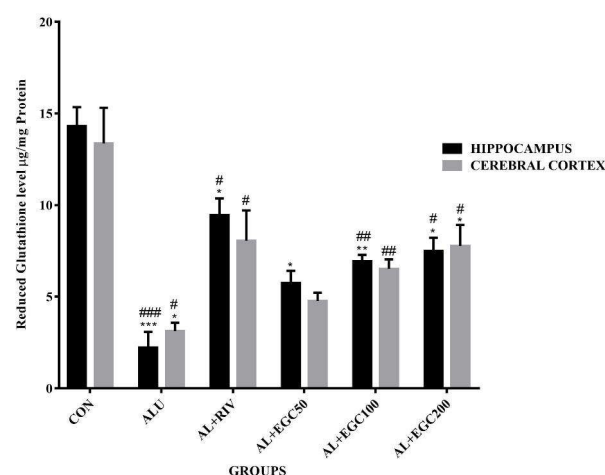
#### Effect of EGC on GSH level in rat brain regions

Activity of reduced glutathione (GSH) was significantly

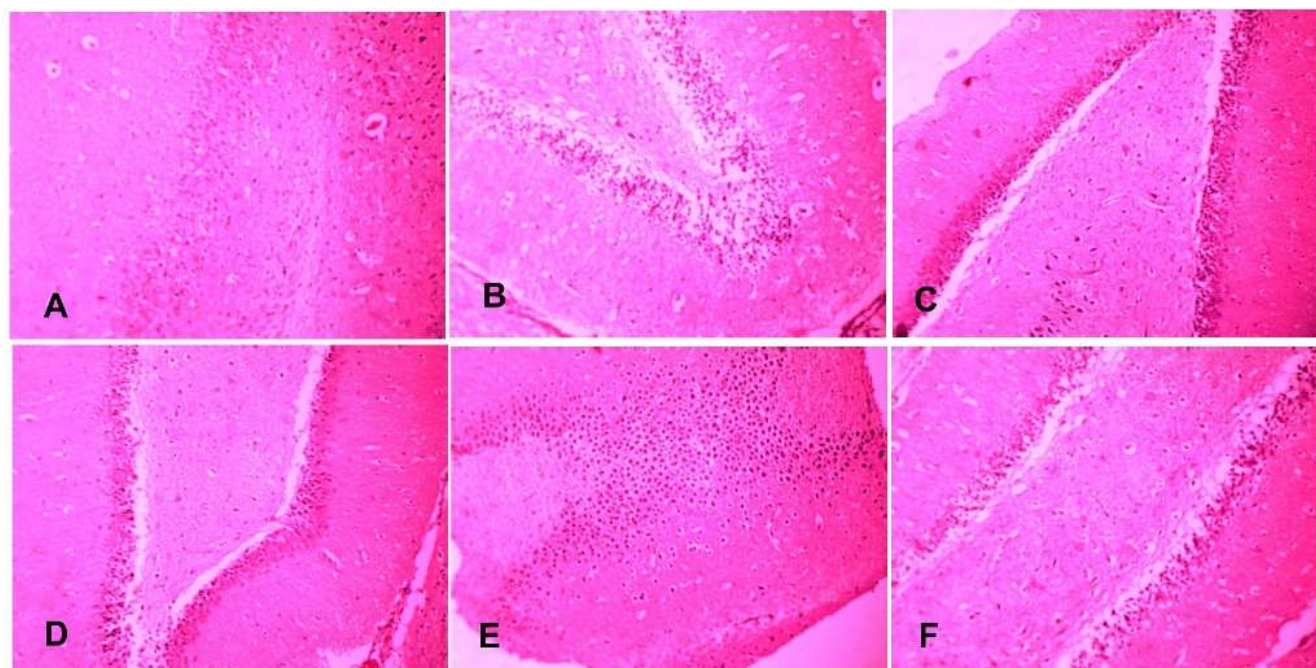


**Figure 7.** Effect of EGC on catalase activity in different experimental groups. Data are in mean  $\pm$  SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared to Al-intoxicated group.

decreased in hippocampus ( $p < 0.01$ ) and cortex ( $p < 0.05$ ) in ALU group (Figure 8). Significant increase in GSH level was seen in both hippocampus and cortex of animals in AL+RIV group when compared to Al alone treated animals ( $p < 0.05$ ). In extract treated groups, AL+EGC200 and AL+EGC100 exhibited significant increase in GSH level in hippocampus and cortex. No significant ( $p > 0.05$ ) difference was seen in the cortex of AL+EGC50 treated



**Figure 8.** Effect of EGC on reduced glutathione in different experimental groups. Data are in mean  $\pm$  SEM (n=6). Data were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  when compared to control group. # $p < 0.05$ , ## $p < 0.01$ , ### $p < 0.001$  when compared to Al-intoxicated group.



**Figure 9.** Effect of EGC on histopathological changes in rat brain regions. Photomicrographs of H & E stained brain regions (10x). A: Control animals; B: 50mg/kg Aluminium chloride treated group; C:  $\text{AlCl}_3$  + 0.3 mg/kg Rivastigmine; D:  $\text{AlCl}_3$  + 50 mg/kg EGC; E:  $\text{AlCl}_3$  + 100 mg/kg EGC; F:  $\text{AlCl}_3$  + 200 mg/kg EGC

group when compared with control and ALU.

### Histological evaluation

Gross histopathological changes, including neurodegeneration and vacuolated cytoplasm was observed in aluminium control group whereas these changes were not found in EGC extract group. In aluminium intoxicated animals, shrunken neuronal cells, massive cellular depletion and necrosis in the hippocampus and cortex region of the rat brain were observed. Such changes are improved with the high dose of co-administration of ethanolic extract of *G. corticata* (Figure 9).

### Discussion

The present study investigated the neuroprotective effect of ethanolic extract of *Gracilaria corticata* on Aluminium induced neurotoxicity for duration of 60 days. Findings from biochemical and histoarchitectural studies indicated that EGC significantly attenuated the  $AlCl_3$  intoxication. Rivastigmine was used as standardized drug as it is the only proven pharmacological agent for the symptomatic relief for AD (Mayeux and Sano, 1999).

Our extract increased the antioxidant status in a dose dependent manner. Ashwini et al. (2017) detected Camptothecin and Quercetin by HPLC analysis of ethanolic extract of *Gracilaria corticata* and another study conducted on GC-MS analyses of *G. corticata* revealed the presence of 17 secondary compounds. Among the 17 compounds, 8 compounds possessed bioactive properties (Gopalan et al., 2017). Evidence from *in vitro*, *in vivo* studies and clinical trials has demonstrated that dietary polyphenols have been associated with reduced risk of neurodegenerative disorders (Scarmeas et al., 2006; Dai et al., 2006; Ebrahimi and Schluesener, 2012; Gao et al., 2012). The mechanisms involved in neuroprotection of by EGC are mainly due to its antioxidant properties.

The results from the present study indicated that Al exposure has changed the body weight brain weight, which reveal a possible detrimental effect of Al on the rats as compared to the control. These results agree with many previous research works. Julka et al. (1996) reported that sub-acute Al exposure of rats resulted in a loss of about 27.8 g in animal body weight. Tripathi et al. (2011) also noticed a reduction in body weights of Al treated animals administered for 90 days while Sharma et al. (2013) noticed a significant change in weight of about 50.44% gain in Al treated rats. The loss of brain weight after Al treatment could be a result of the spongiosis of the neuropil resulting in retarded growth of the animals (Julka et al., 1996). This agrees with our results from histology.

Under normal physiological condition, Al can alter the blood-brain barrier (BBB), thus enters the CNS and get accumulated in brain (Exley, 2001; Zatta et al., 2002). Accumulation of Aluminium in the hippocampus and cortex of rat brain was seen

after oral administration of Aluminium chloride for 60 days. Higher concentration of EGC reduced the concentration of Al in brain tissues. The study conducted by Sathiravada et al. (2012) reported higher levels of Al in hippocampus and cortex and administration of *Manasamitra vatakam* reduced the Al accumulation in the brain regions. It was found that Al gets deposited around the blood vessels in Al-based dementia patients and binds to endothelial cells of brain tissues (Perl and Brody, 1980; Wisniewski and Wen, 1985). Aluminium administration causes learning and memory deficits in rabbits (Rabe et al., 1982) and other experimental animals (Erasmus et al., 1993).

The increase in acetyl cholinesterase was indicative of disturbance in cholinergic neurotransmission (Srimal and Dhawan, 1973) and Alzheimer's disease is mainly reflected by reduced activity of this enzyme (Dai et al., 2002). In cholinergic system, the regions particularly affected in Alzheimer's disease include hippocampus and cortex (Whitehouse et al., 1981). Our study demonstrated that co-administration of EGC extract can ameliorate the altered AChE enzyme level in Al treated rats. A decrease in AChE in mouse brain was already shown experimentally (Zatta et al., 2002). Al causes a biphasic effect on AChE activity, with an initial increase in the activity during 4-14 days followed by a marked decrease (Kumar, 1998). *G. corticata* extract has been shown to elevate the AChE level in rat brain. Jope et al. (1985) showed that free radical production can also affect the activity of AChE. A decrease in hexokinase activity on Al exposure, affect the acetylcholine and AChE due to reduced pyruvate formation (Nehru et al., 2006). Similar reports has been observed by Szutowicz et al. (1998) that Al reduced the formation of Acetylcholine and hence AChE by interfering with metabolism of acetyl Co-A. The present study indicated that AChE inhibition contribute to an improvement in cholinergic transmission in both hippocampal and cortex of rat brain.

Free radicals readily reacts with large amount of lipids especially, polyunsaturated fatty acids present in the brain and undergo oxidative damage (Mahdi et al., 2008; Jesberger and Richardson, 1991). Administration of Al demonstrated to generate ROS (mediated through Fenton reaction), which in turn leads to oxidative stress (Julka and Gill, 1996; Mailloux et al., 2011). The mechanism and extent of oxidative potential of Al was explained by its binding to superoxide radical anion to form an aluminium superoxide semi-reduced radical ion (Exley, 2004). Various antioxidant enzymes are produced by the astrocytic cell to defence against accumulated reactive oxygen species. Evidence suggested that aluminium exposure in rats exhibited memory impairment and imbalance in endogenous



antioxidant system (Prema et al., 2016; Tair et al., 2016; Kasbe et al., 2015). SOD, CAT and GSH are the antioxidant enzymes that protect the brain from oxidative damage. In the current study, Al intoxicated group showed increased LPO, reduced SOD CAT and GSH level in hippocampus and cortex of rat brain. We found that simultaneous administration of EGC significantly mitigates the oxidative stress caused by Al intoxication as indicated by reduced the lipid peroxidation in a dose dependent manner. It was also found that EGC treatment could protect the brain cells from the depletion of SOD, CAT and GSH. Administration of Rivastigmine along with AlCl<sub>3</sub> significantly reduced the LPO, increased the SOD, CAT and GSH level, which was in line with other study (Bihaqi et al., 2009).

### Conclusion

In conclusion, the result of the present study indicates that ethanolic extract of *Gracilaria corticata* prevented aluminium induced neurotoxicity in the cerebral cortex and hippocampus of rat brain. EGC ameliorated oxidative stress, altered levels of acetyl cholinesterase and histopathological changes induced by aluminium. Thus our extract is found to be a promising agent for treating aluminium induced neurotoxicity. Further studies are needed to determine the active components of these extracts, its structural elucidation and also to determine the exact mechanism by which the phytochemicals exert a neuroprotective effect.

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### Conflicts of interest

None

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