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Research Article

OXIDATIVE STRESS IN BRAINS OF MALE RATS INTOXICATED WITH ALUMINIUM AND NEUROMODULATING EFFECT OF *CELASTRUS PANICULATUS* ALCOHOLIC SEED EXTRACT

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ABSTRACT

The objective of the present study was to investigate whether the alcoholic seed extract of *Celastrus paniculatus* (ASECP) could potentially prevent aluminium induced neurotoxicity in the cerebral cortex, hippocampus and cerebellum of the rat brain. Male albino rats were administered with $AlCl_3$ at a dose of 4.2mg/kg/day i.p. for 4 weeks. Experimental rats were given *Celastrus paniculatus* seed extract in two different doses of 200mg and 400mg/kg/day orally 1hr prior to the $AlCl_3$ administration for 4 weeks. At the end of the experiments, aluminium administration significantly decreased the level of GSH and the activities of SOD, CAT, GPx, GR, Na^+/K^+ ATPase, Ca^{2+} ATPase and Mg^{2+} ATPase and increased the level of LPO and the activities of ALP, ACP, ALT and AST in all the brain regions when compared with control rats. Pre-treatment with ASECP at a dose of 200mg/kg b.w increased the antioxidant status and activities of membrane bound enzymes and also decreased the level of LPO and the activities of marker enzymes significantly, when compared with aluminium induced rats. Al treatment also revealed an increase in DNA fragmentation as evidenced by an increase in number of comets. Interestingly, ASECP pretreatment reduced the damage inflicted on DNA by aluminium. Aluminium induction also caused histopathological changes in the cerebral cortex, cerebellum and hippocampus of rat brain which was reverted by pretreatment with ASECP. The present study clearly indicates the potential of seed extract of *Celastrus paniculatus* in counteracting the damage inflicted by Al on rat brain regions.

Keywords: Aluminium, Neurotoxicity, *Celastrus paniculatus,* Cerebral Cortex, Hippocampus, Cerebellum.

INTRODUCTION

The blooming industrialized society increases aluminium bioavailability as a result of continued acidification of the environment. Aluminium salts are used in the pharmaceutical industry and they are generally used as flocculants in treating drinking water. Nowadays, aluminium is recognized as a neurotoxic agent [1, 2]. During the last 30 years, the emergence of extensive evidence on aluminium poisoning has demonstrated the adverse effect of aluminium in inducing memory impairment, personality changes and dementia in humans [3, 4, 5, 6]. Additionally, bioavailable aluminium seems to be associated with neurological deterioration during aging [7]. Aluminium (Al) is one of the most abundant elements in the biosphere and causes adverse effects on various organs [8, 9]. Human population is constantly exposed to Al through various sources such as Al cooking utensils, certain beverages and drinking water [10, 11]. High levels of Al have been linked with an increased risk of a number of pathogenic disorders such as microcytic anemia, osteomalacia as well as neurodegenerative disorders [12, 13, 14]. Al induced neurotoxicity is caused by various factors which include induction of nitric oxide, free radical induced damage and neurotransmission alterations [15, 16]. Both apoptosis and necrosis are suggested to be the mechanisms involved in cellular death resulting from Al toxicity [17]. Sua´rez-Ferna´ndez et al. [18] have reported DNA fragmentation and changes in nuclear morphology after Al exposure. Further, mutagenic effects of Al toxicity have also been observed by various researchers [19, 20]. Al-induced free radicals load may be responsible for initiating the process of apoptosis.

A variety of plant products have been used for prevention and treatment of a broad range of diseases. *Celastrus paniculatus* willd (CP) belongs to family Celastraceae was in use from time immemorial to treat brain related disorders and to enhance learning and memory [21]. It is a large, woody, climbing shrub, distributed almost all over India up to an attitude of 1800m and is known for its ability to improve memory in man [22] and improve memory process in rats [23]. The seeds and seed oil have been used in Ayurvedic medicine as a memory enhancer. The seed contain sterol, alkaloids and a bright colouring matter. Celapanin, Celapanigin, Celapagin, Celastrine and paniculatine are the some important alkaloids present in the seeds [24].

More recently extracts of CP plants have exhibited a variety of pharmacological effects. A methanolic extract of CP plant material exhibited free radical scavenging effects [25], and CP seed aqueous extract was shown to have cognitive-enhancing properties in rats and antioxidant effects in rat brain [26]. The CP seed water soluble extracts could be potentially useful for stimulatory antioxidant defense mechanism, thereby preventing neuronal damage resulting from a variety of oxidative stress events. A characteristic of some of the brain disorder is that oxidative stress resulting from increased production of reactive oxygen species (ROS) increases the rate at which brain disease progresses [27]. The plant *Celastrus paniculatus* also exhibits potent anti-inflammatory [24], antioxidant [25], hypolipidemic [28], anti-arthritic [29], antimalarial [30], antibacterial [31] and antifungal [32] properties.

Various drugs/compounds have been examined for their role against aluminium-induced neurotoxicity in rat and mice models [33, 34, 35, 36].The present study was designed to investigate the effect of alcoholic extract of *Celastrus paniculatus* [ASECP] in counteracting aluminium toxicity in various brain regions.

Materials and Methods

Chemicals

Glutathione reductase, glutathione (GSH) reduced form, glutathione oxidized form (GSSG) tert-butyl hydroperoxide, 5, 5' dithiobis-2

nitrobenzoic acid (DTNB) were purchased from SRL. Nicotinamide adenine dinucleotide phosphate reduced (NADPH) was purchased CDH. All the other chemicals used were of the analytical grade.

Preparation of plant extract

The seeds of *Celastrus paniculatus* were purchased at Chennai, Tamil Nadu and will be authenticated by, Dr.A.Sasikala, Captain Srinivasa Murthi Drug Research Institute for Ayurveda, Arumbakkam, Chennai, Tamil Nadu. The shade dried seeds were crushed and extracted with ethyl alcohol in the ratio of 1:3 for 30 days and all the traces of the solvent were consequently removed. Thick brown extract obtained from the turf and used for the treatment of the rats [37].The above brown extract was dissolved in water and given to the rats as aqueous suspension.

Animals

Male Albino rats weighing 200-250g were obtained from Central Animal House, DR.ALMPGIBMS, University of Madras, Taramani campus, Chennai-113, Tamil Nadu, India. Rats were housed separately in polypropylene cages and fed standard pellet diet kept under hygienic conditions. Rats were kept on a 12hr light and dark cycle with free access to water ad *libitum*. All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of DR.ALMPGIBMS, University of Madras, Taramani campus, Chennai-113, Tamil Nadu, India.

Treatment Schedule

Rats were divided into five experimental groups of 6 animals each. Group I: Control, Group II: $AlCl_3$ (4.2 mg/kg b.w) intraperitoneally for 28 days [38], Group III: $AlCl_3$+ASECP (200 mg/kg b.w) orally for 28 days, Group IV: $AlCl_3$+ASECP (400 mg/kg b.w) orally for 28 days, Group V: ASECP alone (200 mg/kg b.w) orally for 28 days.

Tissue Preparation

After treatment period, experimental animals and control animals were sacrificed by cervical dislocation. Brains were immediately taken out and washed with ice cold saline to remove blood and kept at -80°C. Various regions of the brain viz., cerebral cortex, hippocampus and cerebellum were rapidly dissected from the intact brain carefully on ice plate according to the stereotaxic atlas of Paxinos and Watson [39]. The right and the left cerebral cortices and hippocampus were pooled to make one sample of the tissue. The cerebral cortex, hippocampus and cerebellum were homogenized individually in Tris buffer (pH 7.4). The tissue homogenate (10%) was made (w/v), which was centrifuged at 3000g for 10min. The resulting pellet (P_1) consisting of nuclear and cellular material was discarded. The supernatant (S_1) containing mitochondria, synaptosomes, microsomes and cytosol was further ultracentrifuged at 25,000xg for 1hr. Pellet had membrane fraction, while the supernatant had cytosol fraction. In this study, all biochemical estimations were performed in the cytosol fraction. Homogenates were kept at -80°C and thawed just before the start of biochemical estimation. All processes were carried out in cold conditions.

Biochemical Estimation

Lipid peroxidation products were determined according to the method of Devasagayam and Tarachand [40], as the quantity of malondialdehyde (MDA) produced. The reaction product was measured spectrophotometrically at 540 nm. Reduced glutathione (GSH) was assayed by the method of Moron *et al*., [41] on the basis of the reaction of 5-5, Dithiobis-2-nitrobenzoic acid which is readily reduced by sulfhydryls forming a yellow substance which is measured at 412nm. The enzyme Glutathione peroxidase (GPx) was assayed according to the method of Rotruck *et al*., [42]. The assay takes advantage of concomitant oxidation of NADPH by GR, which is measured at 340nm. Enzyme activity was expressed as units/mg protein. GR activity was assayed by the method of Carlberg and Mannervik [43]. The enzyme activity was quantitated at room temperature by measuring the disappearance of NADPH at 340nm and was calculated as nmol NADPH oxidized/min/mg protein. Superoxide dismutase activity was measured according to the method by Marklund and Marklund [44]. Enzyme activity was expressed as units/mg protein. One unit is equivalent to the amount of SOD required to inhibit 50% of pyrogallol auto oxidation. Catalase (CAT) activity was determined by following the decrease in 240 nm absorption of hydrogen peroxide (H_2O_2). It was expressed as nano moles of H_2O_2 reduced per minute per milligram of protein [45]. Na^+/K^+ ATPase was assayed by the method of Bonting [46]. The reaction mixture consists of Tris HCl buffer (pH 7.5), 50mM $MgSO_4$, 50mM KCl, 600mm NaCl, 1mm EDTA, 40mM ATP, 10% TCA, 2.5% Ammonium molybdate and desired reagent in 1ml. Na^+/K^+ ATPase activity was expressed as µmoles of phosphorous liberated/min/mg protein under incubation conditions. The activity of Mg^{2+} ATPase was assayed by the method of Ohinishi *et al.,* [47]. The enzyme activity was expressed as µmoles of phosphorous liberated/min/mg protein. Ca^{2+} - ATPase was estimated as described by the method of Hjerten and Pan [48]. Alkaline phosphatase (ALP) and acid phosphatase (ACP) activities were measured using p-nitrophenyl phosphate as substrate, according to the method described by Dasgupta and Ghosh [49]. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) of brain were measured through determination of 2, 4 –dinitrophenyl hydrazone of pyruvate [50]. The protein content in the rat brain homogenates (cerebral cortex, hippocampus and cerebellum) was measured by Lowry's method [51].

DNA fragmentation studies

Tissue slices were placed in the digestion buffer (10 mM Tris–HCl with pH 8.0; 0.1 M EDTA with pH 8.0; 1% SDS and proteinase K with concentration of 1 mg/10 ml) and were incubated (14–18 h, 55°C) in a shaking water bath. The DNA contents were extracted, precipitated, and stored as described below for the analysis by agarose gel electrophoresis. After digestion, samples were extracted three times with 10% saturated phenol/chloroform/isoamyl alcohol (24:24:1) and were precipitated using ethanol. The precipitates were rinsed two times with 70% ethanol, air dried, and resuspended in Tris EDTA buffer. DNA contents were measured using a spectrophotometer (A260/A280), and only samples with 1.8 ratios were used. Agarose gel electrophoresis was then carried out to analyze the fragmentation.

Histopathology

After sacrifice of all animals the brain was dissected out and fixed in 10% neutral formalin. The tissues were processed in the usual way for paraffin embedding and sections were mainly stained with Hematoxylin and Eosin (HE) for histopathology (20x).

Statistical analysis

Data represents mean ± SD. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by student't' test using SPSS 10 version. If ANOVA analysis indicated significant differences, Tukey's post-hoc test was performed to compare mean values between treatment groups and control. A value of $p<0.05$ was considered as statistically significant.

RESULTS

The observations made on the different groups of experimental rats indicate that continuous administration of $AlCl_3$ induced alterations in the biochemical parameters as well as the histopathology in various brain regions that were analyzed at the end of the experimental period (28 days). Oral administration of alcoholic seed extract of *Celastrus paniculatus* (ASECP) reverted back all the alterations to near normal when compared with $AlCl_3$ induced rats. However, rats treated with ASECP alone did not show any alterations and resembled that of control rats.

Fig 1 shows the changes in the LPO levels in cerebral cortex, hippocampus and cerebellum of control and experimental rats. The level of LPO was found to be increased ($p<0.01$) in all the above brain regions of $AlCl_3$ induced group when compared with control group. Pretreatment with 200mg/kg b.w. of ASECP decreased the level of LPO in all the brain regions significantly than 400mg/kg b.w dosage.

Fig 2 shows the alterations in the GSH levels in cerebral cortex, hippocampus and cerebellum of control and experimental rats. The

GSH level was found to be decreased in the cerebral cortex, hippocampus and cerebellum in $AlCl_3$ induced rats than in control rats. Pretreatment with ASECP at a dose of 200mg/kg b.w increased the level of GSH significantly ($p<0.01$) when compared with 400mg/kg b.w dosage.

Fig 3 and 4 shows the effect of ASECP on the activities of GPx and GR in the cerebral cortex, hippocampus and cerebellum of control and experimental rats. The activities of GPx and GR was found to be significantly decreased ($p<0.05$) in the brain regions viz., cerebral cortex, hippocampus and cerebellum of $AlCl_3$ induced group when compared with control group. Pretreatment with ASECP at 200mg/kg b.w. dosage showed a significant increase in the activities of GPx and GR than 400mg/kg b.w. dosage in all the above mentioned brain regions.

Fig 5 and 6 shows the effect of ASECP on the activities of SOD and CAT in the cerebral cortex, hippocampus and cerebellum of control and experimental rats. $AlCl_3$ induced group showed a decreased ($p<0.01$) activity of SOD and CAT in all the brain regions when compared to that of control group. Pretreatment with 200mg/kg b.w dosage increased the activities of SOD and CAT to near normal when compared with 400mg/kg b.w dosage.

Table 1 shows the protective effect of ASECP on the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in the cerebral cortex, hippocampus and cerebellum of control and experimental rats. $AlCl_3$ induced group showed a concomitant decrease in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase when compared with control group. Pretreatment with ASECP at a dose of 200mg/kg b.w significantly ($p<0.01$) prevented the decrease in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase than 400mg/kg b.w dosage.

Table 2 shows the effect of ASECP on the activities of ALP and ACP in the cerebral cortex, hippocampus and cerebellum of control and experimental rats. $AlCl_3$ induced rats showed a significant increase in the activities of ALP and ACP in all the brain regions when compared to that of control rats. Pretreatment with ASECP at a dose of 200mg/kg b.w significantly decreased the activities of ALP and ACP to near normal than 400mg/kg b.w dosage.

Table 3 shows the effect of ASECP on the activities of SGOT and SGPT in the cerebral cortex, hippocampus and cerebellum of control and experimental rats. The activities of SGOT and SGPT were found to be significantly increased in all the brain regions of $AlCl_3$ induced group when compared with control group. Pretreatment with ASECP at 200mg/kg b.w dosage reverted back the above changes to near normal than 400mg/kg b.w dosage.

Treatment with ASECP alone did not show any changes in all the above parameters and resembled control rats.

Fig 7 shows the effect of alcoholic seed extract of *Celastrus paniculatus* (ASECP) in aluminium induced histological changes in the cerebral cortex of control and experimental rats. Fig 7A (Control): Transverse section of cerebral cortex of brain showing normal histo-architecture (H&E, 20x). Fig 7B (Al induced): Transverse section of cerebral cortex of brain showing diffused gliosis and pericellular oedema (H&E, 20x). Fig 7C (Al + ASECP 200 mg/kg body weight): Transverse section of cerebral cortex of brain showing mild gliosis and mild oedema when treated with 200mg/kg body weight dosage of ASECP (H&E, 20x). Fig 7D (Al + ASECP 400 mg/kg body weight): Transverse section of cerebral cortex of brain showing more gliosis and oedema when compared with 200 mg/kg body weight (H&E, 20x). Fig 7E (ASECP 200 mg/kg body weight alone): Transverse section of cerebral cortex of brain resembles that of the control (H&E, 20x).

Fig 8 shows the effect of ASECP in Aluminium induced histological changes in the cerebellum of control and experimental rats. Fig 8A (Control) Transverse section of cerebellum showing normal histo-architecture (H&E 20x). Fig 8B (Al induced) Transverse section of cerebellum showing disruption in the Purkinjee cells layer (H&E 20x). Fig 8C (Al + ASECP 200mg/kg b.w) Transverse section of cerebellum showing the regeneration of Purkinjee cell layer (H&E 20x). Fig 8D (Al + ASECP 400mg/kg b.w) Transverse section of cerebellum showing slight disruptions of Purkinjee cell layer (H&E 20x). Fig 8E (ASECP 200mg/kg b.w alone) Transverse section of cerebellum showing resembling that of control (H&E 20x).

Fig 9 shows the effect of alcoholic extract of *Celastrus paniculatus* (ASECP) on aluminium induced histological changes in the hippocampus of control and experimental rats. Fig 9A (Control) Transverse section of hippocampus of brain shows normal histo architecture (H&E, 20x). Fig 9B (Al induced) Transverse section of hippocampus of brain shows high level of pyramidal cell degeneration with marked cell distortion (H&E, 20x). Fig 9C (Al + ASECP 200 mg/kg b.w) Transverse section of hippocampus of brain shows less pyramidal cell degeneration with less cell distortion (H&E, 20x). Fig 9D (Al + ASECP 400 mg/kg b.w) Transverse section of hippocampus of brain shows slightly more pyramidal cell degeneration and cell distortion (H&E, 20x). Fig 9E (ASECP 200 mg/kg b.w alone) Transverse section of hippocampus of brain resembling that of control (H&E, 20x).

The DNA damage caused in the cell as a result of aluminium induction was examined by agarose gel electrophoresis is shown in Fig 10. The results indicated that DNA of Al-induced group showed a comet tail indicating the DNA damage arising from the genotoxicity in the Al-induced cell when compared to the DNA of control cell. In ASECP pretreated rats, the damage to DNA was appreciably reduced when compared to Al-induced rats. ASECP alone administered group resembled that of control.

DISCUSSION

Aluminium has been proposed as an environmental factor that may contribute to some neurogenerative diseases and affect some enzymes and other biomolecules relevant to Alzheimer's disease. In the present study, there were significant increases in the oxidative stress markers lipid peroxidation following aluminium exposure for 28 days in the cerebral cortex, cerebellum and hippocampus regions of rats. Such results are in harmony with those obtained by Deloncle *et al*. [52] and Johnson *et al*. [53] who reported that the neurotoxicity of aluminium may be a result of LPO. Furthermore, Nehru and Anand [54] reported a significant increase in brain thiobarbituric acid reactive substances in rats after stimulation by aluminium salts which was known to be bound by the Fe^{3+} carrying protein transferrin, thus reducing the binding of Fe^{2+} and increasing free intracellular Fe^{2+} that causes the peroxidation of membrane lipids and consequently membrane damage. Aluminum, being an inert metal, has been suggested to induce oxidative damage indirectly by potentiating the peroxidative effect of Fe^{2+}. It promotes reactive oxygen species (ROS) formation. ROS subsequently attack almost all cell components including membrane lipids thus producing lipid peroxidation [55]. The findings of the present study, also, showed that the rise in LPO in aluminium treated rats was accompanied by concomitant decrease in the activity of some antioxidant enzymes involved in the detoxification of ROS, namely SOD, CAT as well as the level of GSH in the cortex, cerebellum and hippocampus tissues comparing with the control declaring the prooxidant effect of Al. These findings agreed with the antecedent studies of Savory *et al*. [56] and Johnson *et al*. [53] whom showed that aluminium exposure enhanced the neuronal lipid peroxidative damage with concomitant alterations in the enzymatic antioxidant defense status, thus having serious bearing on the functional and structural development of the central nervous system [57]. Similar data recorded a decrease in the antioxidants such as GSH [58] and SOD activity [59] in the brain of aluminium exposed rats and human [57]. Moreover, such results are consistent with the studies indicated that aluminium intake produced an oxidative stress-related change, contributed to its neurotoxicity [60]. However, in rats, a significant relationship between aluminium exposure and the presence of oxidative stress was established also by Goó mez *et al*. [61]. This could be caused by inflicting damage to membrane lipids, proteins and antioxidative enzyme defense system [34].The elevation of LPO in the cortex, cerebellum and hippocampus in the present study and other ones [57] suggested participation of free radical- induced oxidative cell injury in mediating neurotoxicity of aluminium. Lipid peroxidation of biological membranes results in the loss of membrane fluidity, changes in membrane potential, an

increase in membrane permeability and alterations in receptor functions [54, 62]. However, the increased aluminium concentration could deleteriously affect the neurons, leading to depletion of antioxidants and metal ions [63] through the induction of free radicals, that exhausting SOD and CAT which function as blockers of free radical processes. These results are in accordance with [54] who recorded a significant decrease in the activities of SOD and CAT in brain of rats after aluminium treatment. Alternatively, the decreased enzyme activities could be related to a reduced synthesis of the enzyme proteins as a result of higher intracellular concentrations of aluminium [62]. The data obtained by the present study illustrated, further, that administration of ASECP caused a significant decrease in the level of LPO in the cerebral cortex, cerebellum and hippocampus and elevated the SOD, CAT, GPx and GR enzymes activities and GSH levels when compared with Aluminium intoxicated rats. Moreover, the plant extracts significantly, improved or restored the normal activities of the antioxidant enzymes SOD,CAT, GPx, GR and GSH in the cortex, cerebellum and hippocampus regions as compared to normal control. In fact, the glutathione peroxidase system consists of several components, including GSH that effectively remove (hydrogen peroxide) and serves as a cofactor for glutathione transferase, which helps remove certain drugs and chemicals and other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell. So, GSH is probably the most important antioxidant present in cells. ASECP had a potent increasing effect on GSH content in brain compared to Aluminium treated rats. Also, the enzymatic antioxidant defense system including SOD and CAT which can decompose superoxide and hydrogen peroxide in the cells are the main defense against oxidative injuries. The decreased level of these biomolecules may lead to increased severity of aluminium toxicities in the brain [64].

The present study illustrated that aluminium ingestion led to significant elevation in alkaline phosphatase (ALP) and acid phosphatase (ACP) activities in cerebral cortex, cerebellum and hippocampus. Alkaline phosphatase is a membrane-associated enzyme, which predominantly concentrated in the vascular endothelium in the brain. There is a more or less continuous sheath of ALP covering all internal and external surfaces of the central nervous system including the spinal cord and thus it may functionally be part in the blood-brain barrier mechanism. On the other hand, intracellular ACP is largely confined to lysosomes, which primarily respond to cellular injury. However, significant contribution by aluminium was observed to induce changes in ACP activity [49]. The increased activity of ALP and ACP enzymes in the brain of animals treated with $AlCl_3$ are in accordance with the findings of Ochmanski and Barabasz [11]. Moreover, regarding aluminium enhanced serum, cortex and hippocampus ACP activities of rats, herein, it was in agreement with the earlier observations recorded altered activities of specific lysosomal hydrolytic enzymes in neuronal tissues [65] due to aluminium administration. From these observations it can be suggested that Al induced an increase in ACP activity of the brain may be an indication of lysosomal proliferation and increasing catabolic rate. The increased ACP activity may result in phosphate accumulation within the lysosomes, and this in turn may lead to decreased plasma inorganic phosphate concentration [66]. In the present work, administration of ASECP caused marked reduction in the elevated activities of ALP and ACP in aluminium treated rats. Such decrease could be due to the antioxidant properties of CP constituents that protect cellular membranes integrity from Al-induced oxidative damage and repair the antioxidant system [67], consequently, improve brain structure and function against aluminium toxicity. The AST and ALT are important enzymes of brain; their activities are related with the maintenance of amino acid homeostasis and might be an indicator of mitochondrial injury [68]. In our present study, AST & ALT activity was found to be increased after aluminium exposure which was brought back near to normal after ASECP treatment at 200mg/kg body weight.

Exposure to aluminium may cause marked histophathological alterations in the brain tissue which were represented by focal as well as diffuse gliosis on in cerebral cortex, odema and inflammatory cell infiltration and pericellular odema in cerebral cortex with neuronal degeneration. Parallel to our findings, those recorded by Bihaqi *et al*. [69] who found that $AlCl_3$ causes histopathological lesions in cerebral cortex including neuronal degeneration as cytoplasmic vacuolization hemorrhage, ghost cell and gliosis. Our histopathological findings are correlated to those of Matyja [70] who noticed that exposure to aluminum causes marked histopathological alteration in the cerebral cortex including neuronal degeneration, perecellular odema and gliosis. According to Brodal [71], the functions of certain learning and memory have been associated with different areas of the brain like the hippocampus and cerebellum. However, aluminium treated group showed marked cell distortion groups with high level of degeneration in the cell. ASECP treatment showed less sign of degeneration and cell distortion. This supports a hypothetical statement by Yokel [72] that Aluminium exposure has neuro-degenerating effect resulting in learning deficits and also the documentation compiled by Frank [73] who stated that in human aluminium inhibits learning.

Effects of aluminium toxicity on DNA were also investigated. Aluminium induction caused an increased DNA damage as indicated by the increased fragmentation of DNA and the number of comets observed. DNA fragmentation and increase in the appearance of comets have also been reported in other studies as a consequence of aluminium exposure [74]. Aluminium is known to increase the levels of reactive oxygen species [75, 76] which is known to cause damage to various macromolecules and also to DNA. Damage to DNA is one of the markers and typical characteristic of apoptosis [77] and the present study shows that aluminium toxicity can lead to faster apoptosis as seen in the micrographs which clearly revealed disruption of cells. ASECP, on the other hand revealed neuroprotective effects as evidenced by decreased DNA damage observed in the pretreatment group. Further, *Celastrus paniculatus* has been shown to possess antioxidative properties and hence may decrease the levels of free radicals and ultimately the damage to DNA.

By the results obtained in the present study we conclude that *Celastrus paniculatus* possess neuroprotective properties as significant neuroprotection was observed in decreasing level of free radicals and increasing the activity of antioxidant enzymes, ATPases with improvement in brain architecture.

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Table 1. Effect of AECP on the activities of Na^+/K^+ ATPase, Mg^{2+}ATP ase and Ca^{2+}ATP ase on Aluminium induced neurotoxicity in control and experimental rats.

ATPases		Group I	Group II	Group III	Group IV	Group V
	CC	2.28 ± 0.17	0.98 ± 0.14**	1.77 ± 0.16**	0.90 ± 0.09*	2.17 ± 0.14
Na^+/K^+ ATPase	**HC**	2.27 ± 0.06	1.18 ± 0.05**	2.15 ± 0.04**	1.09 ± 0.06*	2.21 ± 0.04
	CB	2.19 ± 0.09	1.99 ± 0.08**	2.14 ± 0.06**	1.26 ± 0.05*	2.12 ± 0.07
	CC	1.51 ± 0.08	0.42 ± 0.07**	0.84 ± 0.07**	0.56 ± 0.09*	1.03 ± 0.06
Mg^{2+} ATPase	**HC**	1.18 ± 0.05	0.38 ± 0.07**	0.80 ± 0.11**	0.62 ± 0.10*	1.07 ± 0.05
	CB	1.13 ± 0.04	0.44 ± 0.08**	0.79 ± 0.04**	0.51 ± 0.07*	1.03 ± 0.04
	CC	1.23 ± 0.07	0.53 ± 0.07**	0.90 ± 0.07**	0.65 ± 0.08*	1.11 ± 0.05
Ca^{2+} ATPase	**HC**	1.28 ± 0.04	0.47 ± 0.06**	0.91 ± 0.06**	0.68 ± 0.07*	1.10 ± 0.07

	CB	1.18 ± 0.06	0.48 ± 0.05**	0.89 ± 0.05**	0.71 ± 0.06*	1.12 ± 0.06

Values are expressed as mean ± S.D (n=6). CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: $AlCl_3$(4.2mg/kg b.w); Group III: $AlCl_3$+ASECP (200mg/kg b.w); Group IV: $AlCl_3$+ASECP (400mg/kg b.w); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test. Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase units expressed as µmoles of phosphorous liberated/min/mg protein.

Table 2. Effect of AECP on the activities of ALP and ACP on Aluminium induced neurotoxicity in control and experimental rats

Markers		**Group I**	**Group II**	**Group III**	**Group IV**	**Group V**
	CC	8.2 ± 0.062	9.2 ± 0.083**	8.6 ± 0.08**	8.9 ± 0.072*	8.2 ± 0.075
ALT	**HC**	8.18 ± 0.08	9.1 ± 0.075**	8.2 ± 0.068**	8.9 ± 0.084*	8.1 ± 0.059
	CB	8.1 ± 0.073	8.9 ± 0.057**	8.1 ± 0.076**	8.6 ± 0.078*	8.0 ± 0.09
	CC	3.1 ± 0.028	5.3 ± 0.022**	3.7 ± 0.019**	4.8 ± 0.023*	3.1 ± 0.027
AST	**HC**	3.1 ± 0.035	5.3 ± 0.031**	3.8 ± 0.036**	4.8 ± 0.035*	3.2 ± 0.046
	CB	3.1 ± 0.041	4.1 ± 0.039**	3.2 ± 0.040**	3.7 ± 0.029*	3.0 ± 0.031

Values are expressed as mean ± S.D (n=6). AST and ALT units expressed as µg of pyruvate produced/mg tisssue. ALT - Alanine Transaminase; AST - Aspartate Transaminase CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w); Group III: AlCl3+ASECP (200mg/kg b.w); Group IV: AlCl3+ASECP (400mg/kg b.w); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

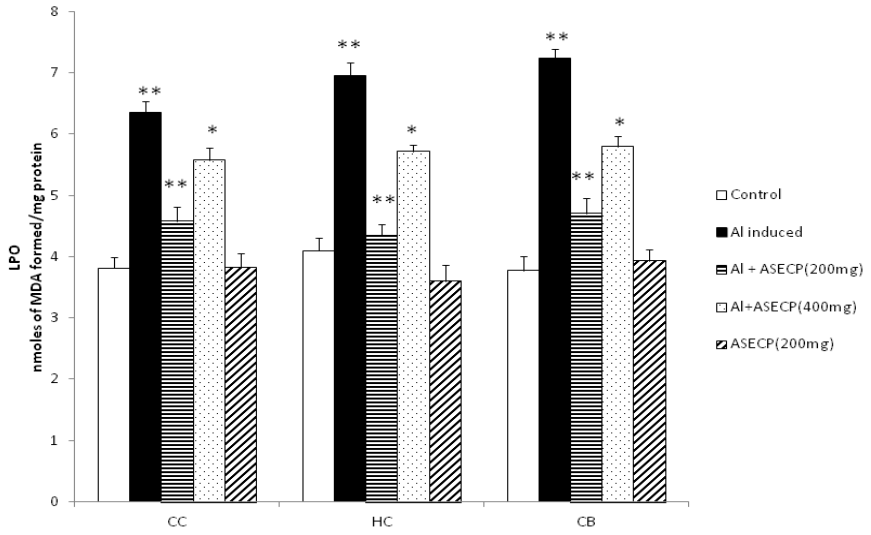

Fig 1: Effect of ASECP on the changes in the LPO levels in cerebral cortex, hippocampus and cerebellum of control and experimental rats.

Values are expressed as mean ± S.D (n=6). LPO- Lipid peroxidation. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w); Group III: AlCl3+ASECP (200mg/kg b.w); Group IV: AlCl3+ASECP (400mg/kg b.w); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

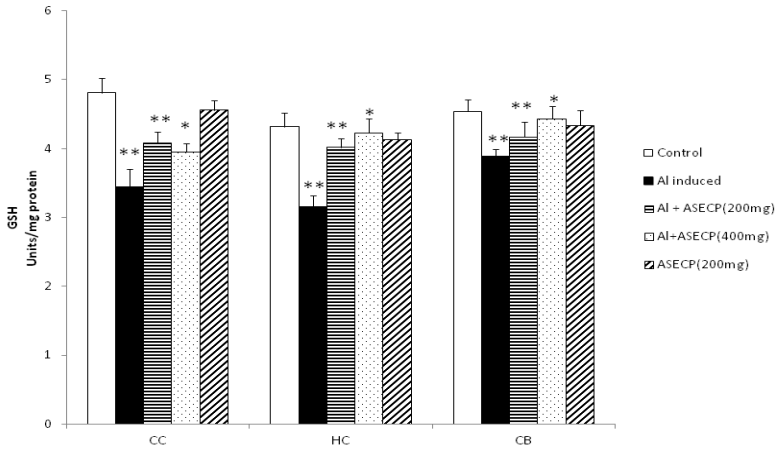

Fig 2. Effect of ASECP on the alterations in the GSH levels in cerebral cortex, hippocampus and cerebellum of control and experimental rats.

Values are expressed as mean ± S.D (n=6). GSH- Reduced glutathione. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w.); Group III: AlCl3+ASECP (200mg/kg b.w.); Group IV: AlCl3+ASECP (400mg/kg b.w.); Group V: ASECP alone

200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post- hoctest.

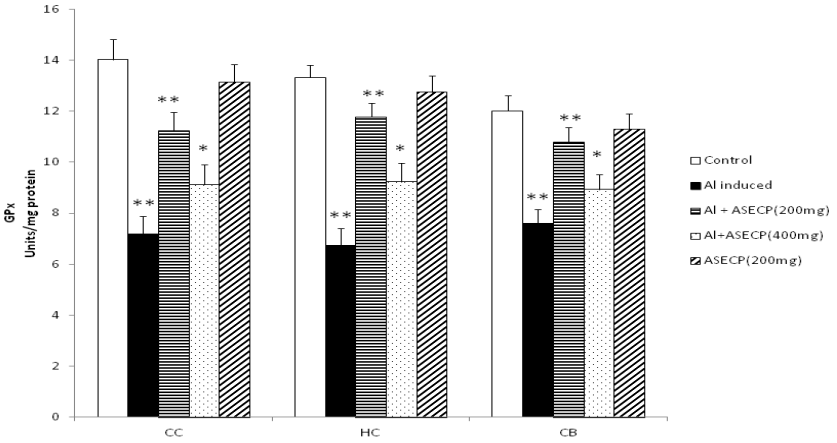

Fig 3 : Effect of ASECP on the activity of GPx in the cerebral cortex, hippocampus and cerebellum of control and experimental rats.

Values are expressed as mean ± S.D (n=6). GPx- Glutathione peroxidase. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w.); Group III: AlCl3+ASECP (200mg/kg b.w.); Group IV: AlCl3+ASECP (400mg/kg b.w.); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

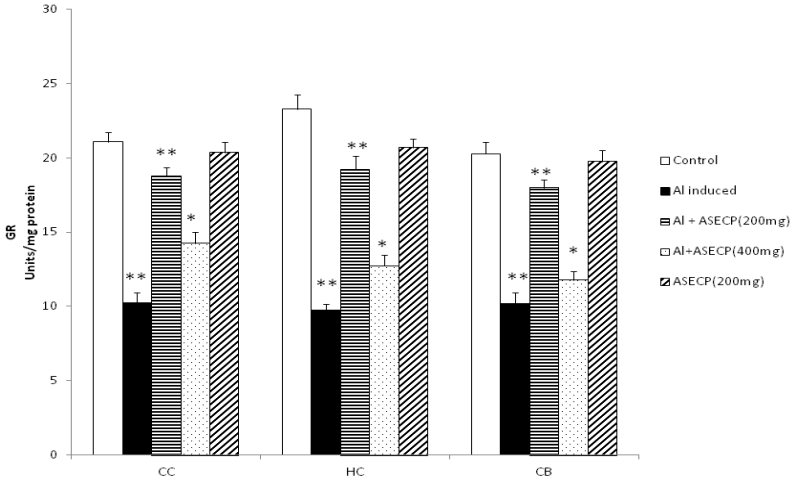

Fig 4 : Effect of ASECP on the activity of GR in the cerebral cortex, hippocampus and cerebellum of control and experimental rats.

Values are expressed as mean ± S.D (n=6). GR- Glutathione reductase. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w.); Group III: AlCl3+ASECP (200mg/kg b.w.); Group IV: AlCl3+ASECP (400mg/kg b.w.); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

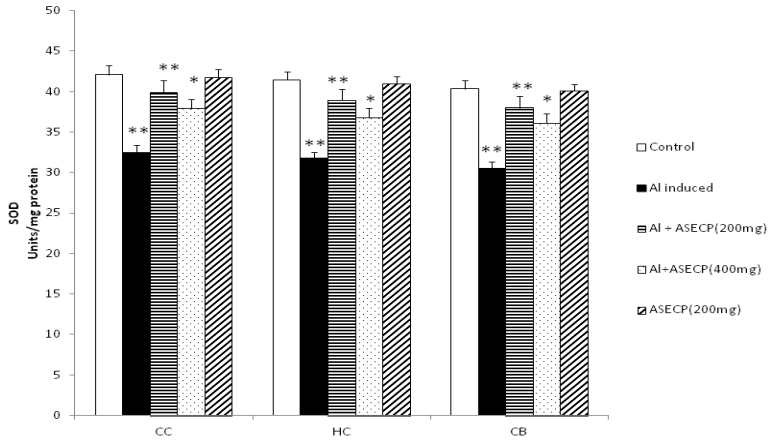

Fig 5 : Effect of ASECP on the activity of SOD in the cerebral cortex, hippocampus and cerebellum of control and experimental rats.

Values are expressed as mean ± S.D (n=6). SOD – Superoxide dismutase. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: $AlCl_3$(4.2mg/kg b.w.); Group III: $AlCl_3$+ASECP (200mg/kg b.w.); Group IV: $AlCl_3$+ASECP (400mg/kg b.w.); Group V: ASECP alone 200mg/kg b.w. $^{**}p<0.01$; $^{*}p<0.05$ - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

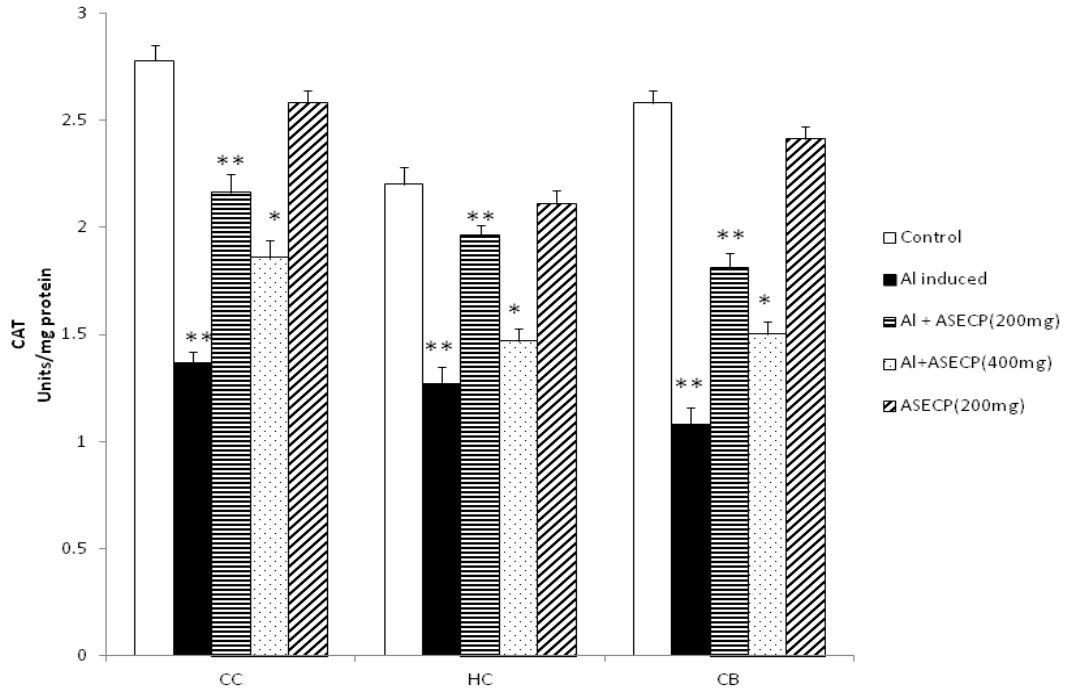

Fig 6 : Effect of ASECP on the activity of CAT in the cerebral cortex, hippocampus and cerebellum of control and experimental rats

Values are expressed as mean ± S.D (n=6). CAT - Catalase. CC - Cerebral cortex, HC - Hippocampus, CB - Cerebellum. Group I: Control; Group II: AlCl3(4.2mg/kg b.w.); Group III: AlCl3+ASECP (200mg/kg b.w.); Group IV: AlCl3+ASECP (400mg/kg b.w.); Group V: ASECP alone 200mg/kg b.w. **p<0.01; *p<0.05 - Group II compared with Group I; Group III and Group IV compared with Group II, using one way ANOVA with Tukey's post-hoc test.

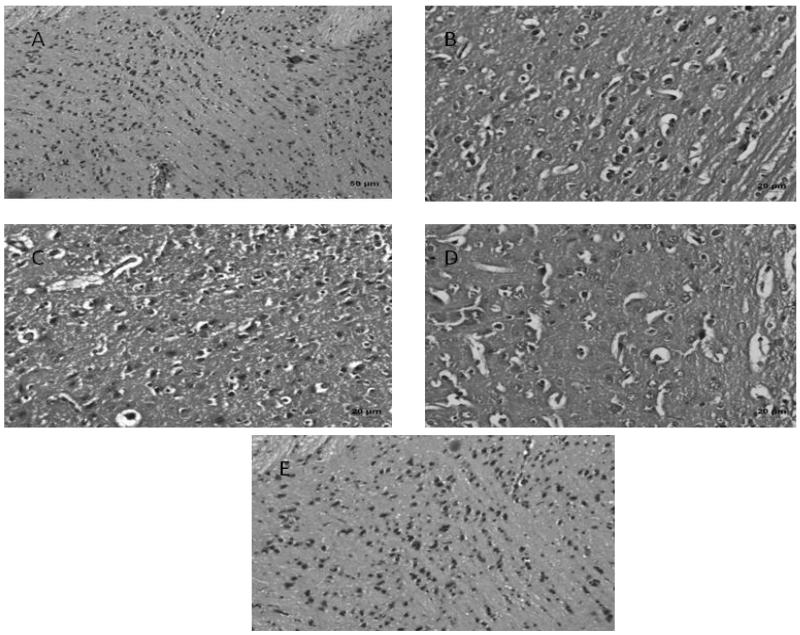

Fig 7 : Effect of alcoholic extract of *Celastrus paniculatus* in aluminium induced histological changes in the cerebral cortex of control and experimental rats

Fig 7A (Control): Transverse section of cerebral cortex of brain showing normal histo-architecture (H&E, 20x). Fig 7B (Al induced): Transverse section of cerebral cortex of brain showing diffused gliosis and pericellular oedema (H&E, 20x). Fig 7C (Al + ASECP 200 mg/kg body weight): Transverse section of cerebral cortex of brain showing mild gliosis and mild oedema when treated with 200mg/kg body weight dosage of ASECP (H&E, 20x). Fig 7D (Al + ASECP 400 mg/kg body weight): Transverse section of cerebral cortex of brain showing more gliosis and oedema when

compared with 200 mg/kg body weight (H&E, 20x). Fig 7E (ASECP 200 mg/kg body weight alone): Transverse section of cerebral cortex of brain resembles that of the control (H&E, 20x).

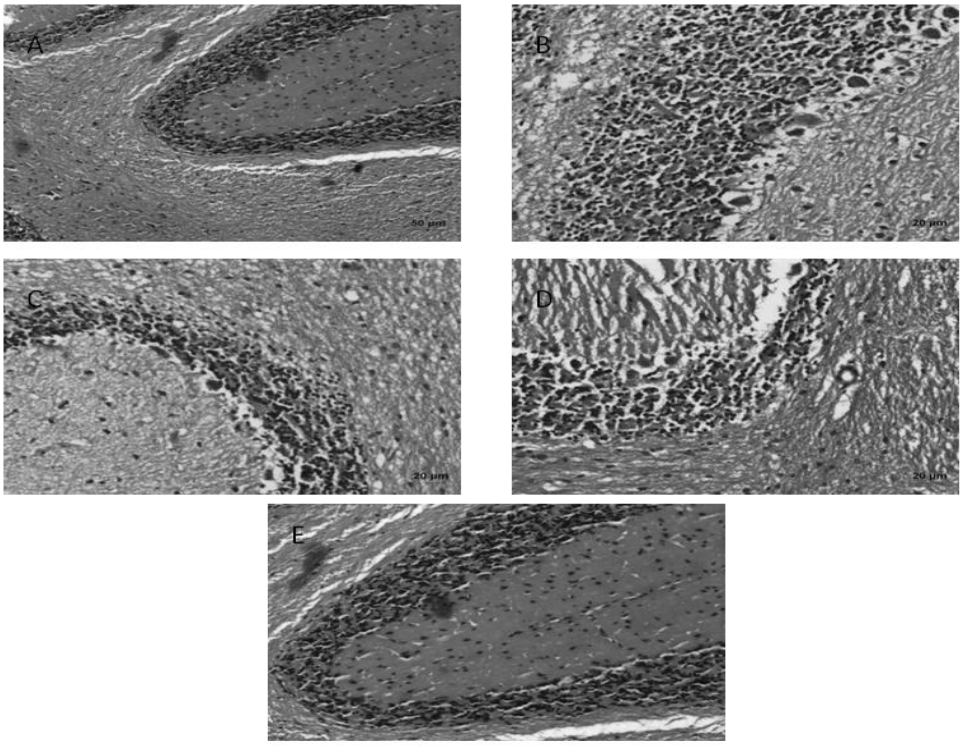

Fig 8 : Effect of ASECP in Aluminium induced histological changes in the cerebellum of control and experimental rats

Fig 8A (Control) Transverse section of cerebellum showing normal histo-architecture (H&E 20x). Fig 8B (Al induced) Transverse section of cerebellum showing disruption in the Purkinjee cells layer (H&E 20x). Fig 8C (Al + ASECP 200mg/kg b.w) Transverse section of cerebellum showing the regeneration of Purkinjee cell layer (H&E 20x). Fig 8D (Al + ASECP 400mg/kg b.w) Transverse section of cerebellum showing slight disruptions of Purkinjee cell layer (H&E 20x). Fig 8E (ASECP 200mg/kg b.w alone) Transverse section of cerebellum showing resembling that of control (H&E 20x).

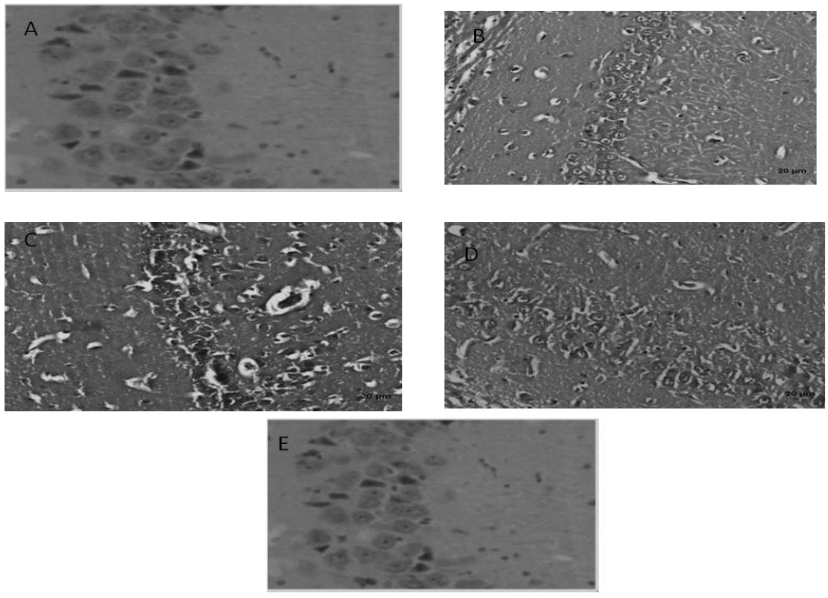

Fig 9 : Effect of alcoholic extract of Celastrus paniculatus (ASECP) on aluminium induced histological changes in the hippocampus of control and experimental rats

Fig 9A (Control) Transverse section of hippocampus of brain shows normal histo architecture (H&E, 20x). Fig 9B (Al induced) Transverse section of hippocampus of brain shows high level of pyramidal cell degeneration with marked cell distortion (H&E, 20x). Fig 9C (Al + ASECP 200 mg/kg b.w) Transverse section of hippocampus of brain shows slight pyramidal cell degeneration with slight cell distortion (H&E, 20x). Fig 9D (Al + ASECP 400 mg/kg b.w) Transverse section of hippocampus of brain shows medium level of pyramidal cell degeneration with medium level of cell distortion (H&E, 20x). Fig 9E (ASECP 200 mg/kg b.w alone) Transverse section of hippocampus of brain resembling that of control (H&E, 20x).

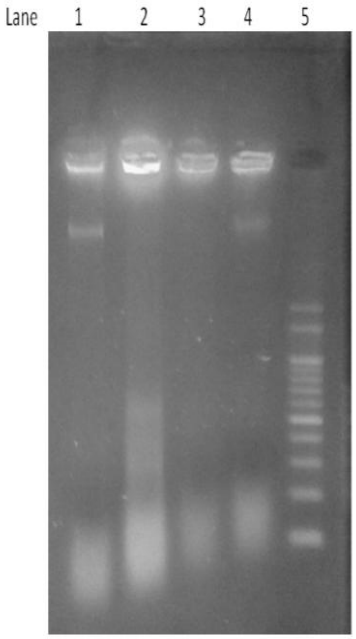

Fig 10: Agarose gel electrophoresis of genomic DNA extracted from rat brain from various treatment groups. (6µg DNA/lane).

Lane 1 DNA from the control group. Lane 2 DNA from the Al treated group. Lane 3 DNA from Al and ASECP (200mg/kg b.w) combined treated group. Lane 4 DNA from ASECP alone treated group. Lane 5 DNA Ladder.

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