

## Protective Effect of *Centella asiatica* against Aluminium-Induced Neurotoxicity in Cerebral Cortex, Striatum, Hypothalamus and Hippocampus of Rat Brain- Histopathological, and Biochemical Approach

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### Abstract

Aluminum (Al) is a potent environmental neurotoxin, which is involved in the progression of neurodegenerative processes. Remarkable biological and medicinal properties of *Centella asiatica* (CA) are well known in last few decades. Therefore, the present study has been designed to explore the neuroprotective effect of CA on chronic aluminium chloride (AlCl<sub>3</sub>) exposure induced neurotoxicity in rat brain regions (cerebral cortex, striatum, hypothalamus and hippocampus). Wistar albino rats were segregated into four groups: group 1-control rats, group 2-rats received AlCl<sub>3</sub> (300 mg/kg body weight, every day orally) for 60 days, rats in group 3-received CA (500 mg/kg body weight, orally) and group 4 rats were initiated with both AlCl<sub>3</sub> and CA treatment. Administration of AlCl<sub>3</sub> developed behavioral deficits, triggered lipid peroxidation (LPO), compromised acetylcholine esterase (AChE) activity, and reduced the levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH) and glutathione-S-transferase (GST) and caused histologic aberrations. AlCl<sub>3</sub>-induced alterations in the activities of SOD, CAT, GSH and GST, levels of LPO, activity of AChE, behavioral deficits and histologic aberrations were attenuated on treatment with CA. Results of the study demonstrate neuroprotective potential of CA against AlCl<sub>3</sub>-induced oxidative damage and cognitive dysfunction.

**Keywords:** Aluminum chloride; Oxidative stress; *Centella asiatica*; Neuroprotection

### Introduction

Aluminum (Al) is a powerful neurotoxin and has been associated with various cognitive disorders. Prolonged Al exposure induces oxidative stress and pathological alterations in diverse areas of brain of neonatal rats [1]. It has been demonstrated that chronic exposure to Al not only causes neurologic signs, which mimic progressive neurodegeneration, but also results in neurofilamentous changes in the hippocampus, cerebral cortex and biochemical changes. Al (or) AlCl<sub>3</sub> role in exacerbating neurodegenerative disorders like Alzheimer's disease (AD) and Parkinsonism dementia are under debate [2]. Medicines, food additives, water purification process and cosmetics remain the major routes that clout humans for Al toxicity [3]. AlCl<sub>3</sub> exposure leads to impairment in learning, memory and cognition function as observed both from clinical and from animal experiments [4].

One possible mechanism of AlCl<sub>3</sub> induced neurotoxicity is by oxidative stress. AlCl<sub>3</sub> is a non-redox active metal capable of increasing the cellular oxidative milieu by potentiating the pro-oxidant properties [5]. Chronic AlCl<sub>3</sub> exposure generates reactive oxygen species (ROS) that cause lipid peroxidation (LPO) and oxidative damage to proteins, DNA, and decreasing intracellular antioxidants [6]. Furthermore, AlCl<sub>3</sub> compounds can reach systemic circulation in different ways and ultimately increasing its concentration in brain. Upon entering the brain it affects the slow and fast axonal transports, induces inflammatory responses, inhibits long-term potentiation,

causes synaptic structural abnormalities which results profound neurodegeneration. AlCl<sub>3</sub> cause degeneration of cholinergic terminals in the cortical areas and cell depletion in the brain regions to induce learning deficits [4].

Natural antioxidants, which alleviate the oxidative stress or induce the cellular antioxidant milieu would most probably treat and/or protect against Al poisoning. *Centella asiatica* (CA) (L.) Urban, also known as gotu kola and Indian pennywort is Apiaceae family. It is highly regarded as a rejuvenating herb and is reputed to increase intelligence and memory. Numerous active constituents of CA are found to be potent antioxidant in different animal models [7,8]. It possesses numerous pharmacological effects such as anti-inflammatory [9], memory improvement [7], anticancer [10] and antioxidant activities [11,12]. Recently CA has been reported as a powerful neuroprotectant against different neurodegenerative disorders [13]. Based on this background, the present study was carried out to investigate the possible neuroprotective efficacy of CA against Al-induced neurotoxicity in terms of oxidative stress, behavioral, biochemical and histologic aspects of cerebral cortex, striatum, hypothalamus and hippocampus of rat brain regions.

### Materials and Methods

#### Chemicals

Aluminum chloride hexahydrate (AlCl<sub>3</sub>. 6H<sub>2</sub>O) was purchased from Sd fine-chem, Mumbai, India. All other chemicals and reagents used were of analytical grade.

## Preparation of the plant extract

Fresh leaves of CA were collected from regular vendor and species identification was done from Center for Advanced Studies in Botany, University of Madras, Chennai, India. The leaves were cleaned shade dried and coarsely ground with grinder. The coarse powder of plant was extracted with 8 parts of distilled water under boiling for 5 h and was filtered through a 400  $\mu$ M mesh cloth to collect the extract. The extract was concentrated and finally spray dried to yield a greenish brown powder. The percentage w/w yield of the extract was 41%.

## Dosage selection and drug treatment

AlCl<sub>3</sub> was dissolved in double distilled water, and adjusted to pH 7.0 was used for inducing neurotoxicity in rat brain (60 days). For the induction, rats (n = 6) were orally administered with AlCl<sub>3</sub> at a dosage (300 mg/kg b.wt. daily for 8 weeks) to promote neurotoxicity [14]. The dose of CA is set based on the effective dosage fixation studies. To fix the effective optimum dosage, a initial pilot study was carried out with five different dosages of CA (100, 300, 500, 700 and 900 mg/kg b.wt.), administered orally, simultaneously, animals were also exposed to AlCl<sub>3</sub> induction for a period of 60 days. It was noted that CA treatment at a concentration of 500 mg/kg b.wt significantly (p<0.05) altered behavioral deficits, the levels of AChE and oxidative stress markers (data not shown). Thus, 500 mg/kg b.wt. was chosen as the optimum dosage for subsequent studies.

## Experimental animals and design

Wistar albino rats of either sex (120-150g) were procured from King Institute and Central Animal House Facility (CAHF) of University of Madras, Chennai, India. Animals were fed with commercial pellet diet and water ad libitum and maintained at an ambient temperature of 25  $\pm$  2°C with a 12-hr light: dark cycle. The experiments were conducted in accordance with guidelines of Institutional animal ethical committee (IAEC) governed by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines, Government of India.

The animals were divided into four groups (n=6). Group 1 control rats. Group 2 rats administered with AlCl<sub>3</sub> (300 mg/kg b.wt. orally) for 60 days. Group 3 rats received CA alone (dissolved in water, 500 mg/kg b.wt.). Group 4 rats co-treated with CA and induced with AlCl<sub>3</sub> simultaneously for 60 days. After the experimental period, the rats were anaesthetized by diethyl ether and killed by cervical decapitation after an overnight fast. Brains were dissected out by making midline incision to view the skull. Then, a small incision from the caudal part of parietal bone and a firm cut in the anterior part of the frontal bone were made to remove the brain more easily. Isolated brains were dissected on ice (using large and small curved serrated forceps) to obtain cerebral cortex, striatum, hypothalamus and hippocampus according to the method of Glowinski and Iversen [15]. The isolated brain parts were homogenized using 0.1% Triton X-100 buffer (pH 7.4), in a Potter-Elvehjem homogenizer and then centrifuged at 12,000 rpm (4°C) for 30 min. The supernatant was stored at -80°C and used for further biochemical assays.

## Behavioral assessment

**Hebb-William's maze task:** The acquisition of learning and memory was evaluated using Hebb-William's maze [16]. The test was carried out by placing the overnight fasted animal at one end of maze and recording the transit time (in seconds) to reach the other end of the

maze where food pellets were kept. Assessment was made in control and experimental groups before starting of treatment (0 day) and after 7, 15, 30, 45 and 60 days of treatment.

**Rota-rod test:** Effect on motor function was assessed by Rota-rod [17]. Animals were placed on the rotating rod for 3 min to test initially their ability to maintain on the rod. Motor integrity and coordination were assessed by the time latency from placement of the animal on the rotating rod until it fell. Assessment was made in control and experimental groups before starting of treatment (0 day) and after 7, 15, 30, 45 and 60 days of treatment.

**Actophotometer:** Based on the technique described by Robert and Lim [18], motor activity was measured by means of photocells and counters activated by the movement of rat across the projected light beams inside the actophotometer, thus registering the walking and running activity. The activity during the stay period of three minutes in the actophotometer was recorded as counts. Assessment was made in control and experimental groups before starting of treatment (0 day) and after 30 and 60 days of treatment.

## Biochemical studies

Total protein was estimated by the method of Lowry et al. [19]. AChE enzymatic activity was assayed using standard method Ellman et al. [20]. The activities of enzymic antioxidants superoxide dismutase (SOD) (Marklund and Marklund) [21], catalase (CAT) [22], reduced glutathione (GSH) Ellman [23] and glutathione-S-transferase (GST) Habiget al. [24] were assayed in brain regions. LPO was measured by formation of thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa et al. [25].

## Histological studies

Excised brain tissues were isolated and post-fixed with 10% neutral buffered formalin (NBF). After overnight at 4°C tissues were processed following standard protocol [26] and embedded in paraffin wax. Sagittal sections of 8 $\mu$ m thickness were cut using microtome, and used for hematoxylin and eosin staining. Nissl's staining was performed to assess the extent of neuronal damage in rats brain regions. Brain sections were used for Nissl's staining using 0.25% thionine (Sigma, USA). The appearance of Nissl-stained dark neurons (NDN) was analyzed in the cortex, striatum, hypothalamus and hippocampus of control and experimental groups of rats. All slides were then evaluated under light microscope (Olympus BX 5.1, Japan).

## Statistical analysis

All the data were evaluated using SPSS/10.0 software. One-way analysis of variance (ANOVA) followed by least significant difference test. P<0.05 was considered to indicate statistical significance. Data represent as mean  $\pm$  standard deviation (SD) (n=6)

## Results

### CA enhances AlCl<sub>3</sub>-induced behavioral changes in rats

Transit time of animals trained to receive the reward after overnight fast has been found to be increased in AlCl<sub>3</sub> treated group, which clearly indicates decreased spatial memory and learning (significant increase in time latency to end the task). Impairment in this group of rats was seen during daily exposure to AlCl<sub>3</sub>. In contrast, rats treated with CA significantly decreased transit time to achieve the task by

suppressing the effect of AlCl<sub>3</sub> (Figure 1A) as compared to AlCl<sub>3</sub>-induced rats. Control and CA alone treated group of rats shows similar pattern for Hebb-William's maze task.

Rota-rod test is an index of neuromuscular strength. The climbing attempts made by AlCl<sub>3</sub>-induced rats were significantly ( $p < 0.05$ ) low as compared with control rats. However, CA treated rats (group 4) significantly increased clung than AlCl<sub>3</sub>-induced groups (Figure 1B). Normal behavior was observed in control and CA alone groups of rats. Care was taken to avoid the bias due to the effect of body weight in making differences in the results.

Actophotometer is an index of locomotor function. AlCl<sub>3</sub> decreased locomotor activity as compared to control rats indicating locomotor deficits. CA treatment improved locomotor function significantly ( $p < 0.05$ ) and the effect of AlCl<sub>3</sub> has been found to be suppressed by co-administration with CA (Figure 1C). No significant difference was observed between control and CA control groups. These results suggest that AlCl<sub>3</sub> causes behavioral impairments, which then attenuated with CA treatment.

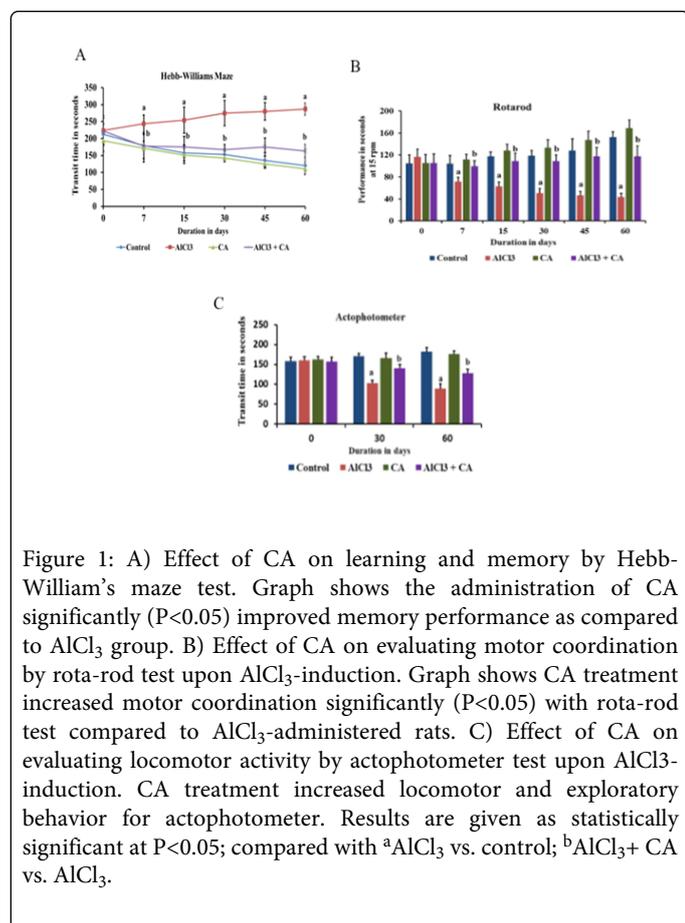


Figure 1: A) Effect of CA on learning and memory by Hebb-William's maze test. Graph shows the administration of CA significantly ( $P < 0.05$ ) improved memory performance as compared to AlCl<sub>3</sub> group. B) Effect of CA on evaluating motor coordination by rota-rod test upon AlCl<sub>3</sub>-induction. Graph shows CA treatment increased motor coordination significantly ( $P < 0.05$ ) with rota-rod test compared to AlCl<sub>3</sub>-administered rats. C) Effect of CA on evaluating locomotor activity by actophotometer test upon AlCl<sub>3</sub>-induction. CA treatment increased locomotor and exploratory behavior for actophotometer. Results are given as statistically significant at  $P < 0.05$ ; compared with <sup>a</sup>AlCl<sub>3</sub> vs. control; <sup>b</sup>AlCl<sub>3</sub>+ CA vs. AlCl<sub>3</sub>.

### CA reduces LPO levels during AlCl<sub>3</sub>-induction

LPO is one of the main manifestations of oxidative damage, which implicates free radical oxidative cell injury in mediating the toxicity of Al. Effect of CA on the levels of malondialdehyde (MDA) during AlCl<sub>3</sub>-induced neurotoxicity in cerebral cortex, striatum, hypothalamus and hippocampus is depicted in Figure 2. MDA levels were found to be significantly increased ( $P < 0.05$ ) in AlCl<sub>3</sub>-induced rats, whereas it was significantly ( $P < 0.05$ ) reduced in CA-treated groups. However, no

significant difference was observed between control and CA control groups.

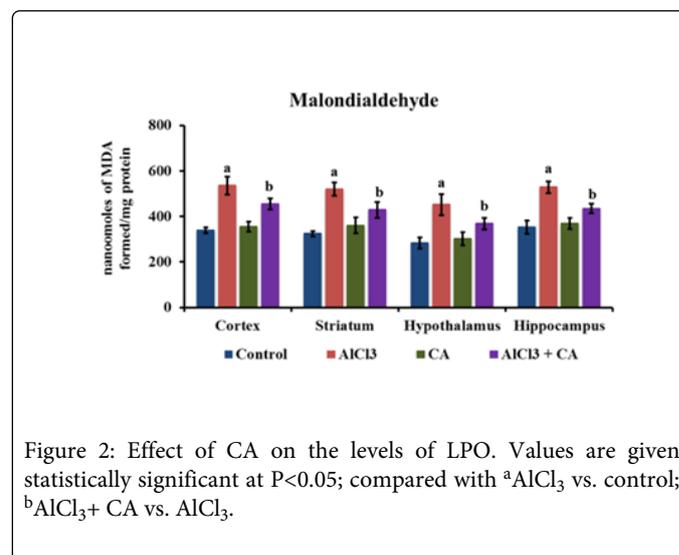


Figure 2: Effect of CA on the levels of LPO. Values are given statistically significant at  $P < 0.05$ ; compared with <sup>a</sup>AlCl<sub>3</sub> vs. control; <sup>b</sup>AlCl<sub>3</sub>+ CA vs. AlCl<sub>3</sub>.

### CA enhances endogenous antioxidants levels

As oxidative damage is mediated by free radicals, it was necessary to investigate Table 1 shows the levels of SOD, CAT, GST, and GSH in the brain regions of control and experimental groups. Chronic administration of AlCl<sub>3</sub> caused marked oxidative stress, which led to decrease ( $P < 0.05$ ) in the antioxidant enzyme activities compared to control animals. The combination group showed that CA extract was capable of increasing the activities of SOD, CAT, GST and GSH of brain regions to the normal values compared to control. However, there was no significant change ( $P < 0.05$ ) in control and rats treated with CA alone.

### CA regulates cholinergic function

AChE is a key enzyme involved in cholinergic neurotransmission and is a marker of extensive loss of cholinergic neurons in the brain. Figure 3 depicts the effect of CA on the level of neurotransmitter AChE in rat brain regions of control and experimental groups. A significant increase ( $P < 0.05$ ) in the level of AChE was observed in the animals induced with AlCl<sub>3</sub>. CA treatment significantly attenuated AChE levels. No change was observed in control and CA alone treated groups.

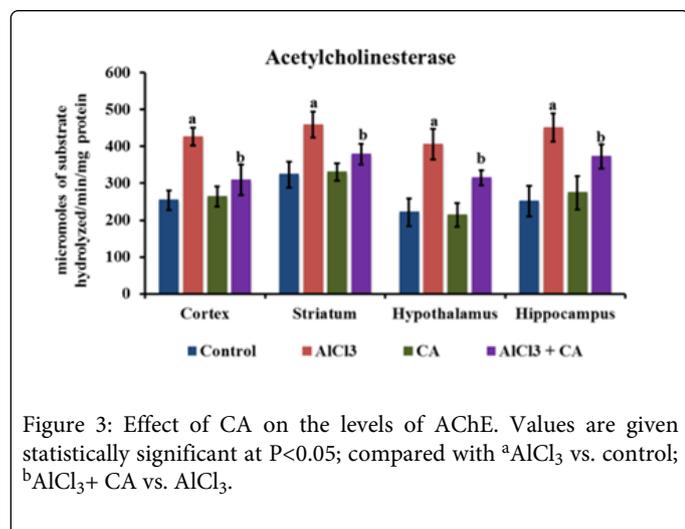
### CA ameliorates morphologic abnormalities during AlCl<sub>3</sub>-induced neurotoxicity

Histopathological observations of brain regions in control and experimental groups of rats are shown in Figure 4. Control rats brain sections showed normal cellular architecture with intact cell membrane (Figure 4A). AlCl<sub>3</sub> exposed brain showed degenerated cerebral cortex region with vacuolation and disruption of cell membrane were noticed in cerebral cortex, striatum, hypothalamus and hippocampus (Figure 4B). CA co-treated rats showed marked reduction in number of damaged cells together with spongiform-like appearance and a large number of intact neuronal cells were observed in all brain regions denoted the protective efficacy of CA (Figure 4D). CA alone group showed well-preserved histoarchitecture (Figure 4C).

Experimental Groups	Regions	SOD	CAT	GSH	GST
Group 1	Cortex	0.927 ± 0.04	382.00 ± 48.06	3.49 ± 0.33	0.387 ± 0.03
	Striatum	1.496 ± 0.06	402.67 ± 46.89	5.86 ± 0.30	0.336 ± 0.02
	Hypothalamus	1.201 ± 0.07	893.57 ± 57.67	2.99 ± 0.14	0.356 ± 0.01
	Hippocampus	1.300 ± 0.05	360.67 ± 30.01	8.69 ± 0.51	0.427 ± 0.03
Group 2	Cortex	0.546 ± 0.03 <sup>a</sup>	208.67 ± 13.71 <sup>a</sup>	1.43 ± 0.21 <sup>a</sup>	0.189 ± 0.05 <sup>a</sup>
	Striatum	0.718 ± 0.04 <sup>a</sup>	191.50 ± 51.51 <sup>a</sup>	2.05 ± 0.14 <sup>a</sup>	0.186 ± 0.05 <sup>a</sup>
	Hypothalamus	0.748 ± 0.04 <sup>a</sup>	497.67 ± 31.93 <sup>a</sup>	1.89 ± 0.18 <sup>a</sup>	0.203 ± 0.02 <sup>a</sup>
	Hippocampus	0.786 ± 0.04 <sup>a</sup>	193.83 ± 23.46 <sup>a</sup>	5.39 ± 1.23 <sup>a</sup>	0.283 ± 0.02 <sup>a</sup>
Group 3	Cortex	0.896 ± 0.02	402.67 ± 13.71	3.51 ± 0.10	0.402 ± 0.01
	Striatum	1.431 ± 0.09	411.83 ± 32.17	5.66 ± 0.22	0.340 ± 0.02
	Hypothalamus	1.276 ± 0.02	901.63 ± 19.91	3.08 ± 0.17	0.368 ± 0.03
	Hippocampus	1.281 ± 0.13	372.00 ± 17.92	8.65 ± 1.01	0.416 ± 0.05
Group 4	Cortex	0.856 ± 0.02 <sup>b</sup>	356.33 ± 23.59 <sup>b</sup>	2.98 ± 0.12 <sup>b</sup>	0.355 ± 0.02 <sup>b</sup>
	Striatum	1.397 ± 0.06 <sup>b</sup>	298.67 ± 22.66 <sup>b</sup>	4.84 ± 0.99 <sup>b</sup>	0.304 ± 0.03 <sup>b</sup>
	Hypothalamus	1.179 ± 0.05 <sup>b</sup>	794.83 ± 12.95 <sup>b</sup>	2.64 ± 0.32 <sup>b</sup>	0.327 ± 0.01 <sup>b</sup>
	Hippocampus	1.230 ± 0.12 <sup>b</sup>	318.17 ± 30.21 <sup>b</sup>	7.96 ± 0.30 <sup>b</sup>	0.389 ± 0.03 <sup>b</sup>

Units: SOD units/min/mg protein; CAT μmoles of H<sub>2</sub>O<sub>2</sub> decomposed/min/mg protein; GSH μg/mg protein; GST nmol of CDNB conjugated/min/mg protein. Data represents as mean ± SD. Values are statistically significant at P<0.05; compared with <sup>a</sup>AlCl<sub>3</sub> vs. control; <sup>b</sup>AlCl<sub>3</sub>+ CA vs. AlCl<sub>3</sub>.

**Table 1:** Effect of *Centella asiatica* on antioxidant levels in control and experimental groups of rats.



**Figure 3:** Effect of CA on the levels of AChE. Values are given statistically significant at P<0.05; compared with <sup>a</sup>AlCl<sub>3</sub> vs. control; <sup>b</sup>AlCl<sub>3</sub>+ CA vs. AlCl<sub>3</sub>.

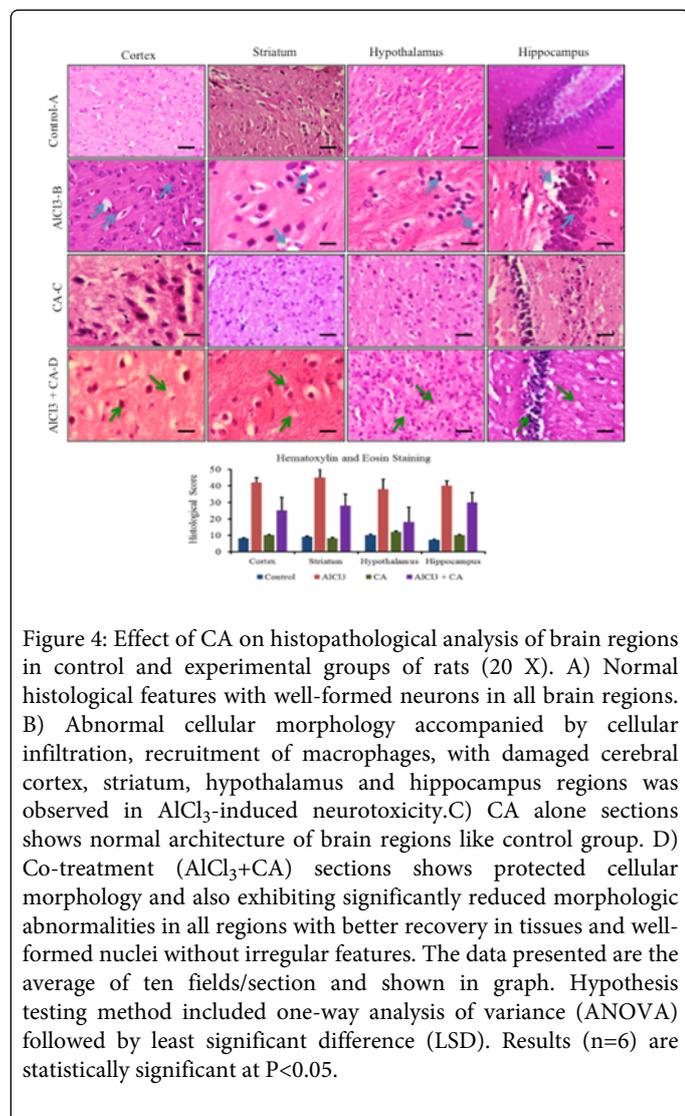
Figure 5 shows the Nissl's staining of brain regions in control and experimental rats. Control rats showed normal neuronal architecture of the cortical region and pyramidal neurons of other brain regions (Figure 5A). Nissl-stained dark neurons (NDN), a feature of extensive damage with neuronal cell loss with atrophic neurons, shrunken cytoplasm, and damaged nuclei (Figure 5B), were seen in AlCl<sub>3</sub>-induced rats. However, supplementation of CA exhibited significantly reduced nissl bodies with a marked reduction in neuronal cell loss were observed in Figure 5D. CA alone resembles the control sections.

## Discussion

Al is a ubiquitous metal and has been implicated in the etiology of Alzheimer's disease where it exacerbates brain oxidative damage. Based on the available evidences AlCl<sub>3</sub> is considered as a putative etiological factor in a range of neurodegenerative disorders for many decades [2]. Several natural compounds such as curcumin and naringin exhibit neuroprotective effect against the AlCl<sub>3</sub>-induced cell damages of different experimental model [27]. CA contains numerous flavonoids (quercetin, kaempferol, catechin, naringin), which are major contributors to the antioxidative activity of CA [13,28]. Our study explores the role of CA during AlCl<sub>3</sub>-induced neurotoxicity.

The importance of neurobehavioral studies in risk assessment lies in the fact that behavior can be regarded as the net output of the memory, motor, locomotor and cognitive functions occurring in the nervous system and can serve as potentially sensitive end points of chemically induced neurotoxicity [29]. AlCl<sub>3</sub> is a neurotoxicant potentially affecting ionic, cholinergic, and dopaminergic neurotransmission in the central nervous system and these alterations are known to be associated with learning ability. It accumulates all most all the brain following chronic exposure and the maximum being in hippocampus, which is the site of memory and learning [1]. The performance of the AlCl<sub>3</sub>-exposed rats, in contrast to control rats, did not improve with repetitive trials, which clearly reflected the AlCl<sub>3</sub>-induced impairment in learning and memory as performed by Hebb-William's maze test [30]. From maze test we observed that CA ameliorates memory impairment caused by AlCl<sub>3</sub> probably by modulating synaptic functioning and promoting neuronal survival [7]. AlCl<sub>3</sub> alters the performance of rats in rota-rod test, a test commonly used in the context of motor function, endurance and balance. Exposure to Al has been found to decrease locomotor activity assessed by actophotometer, which indicates a possible depressant effect on the

central nervous system (CNS). Administration of CA was able to improve the performance of motor activity and also improved the locomotor and exploratory behavior suggesting its potential role as a neuroprotectant against [7,13] AlCl<sub>3</sub>-induced CNS depression by improving behavioral performance.

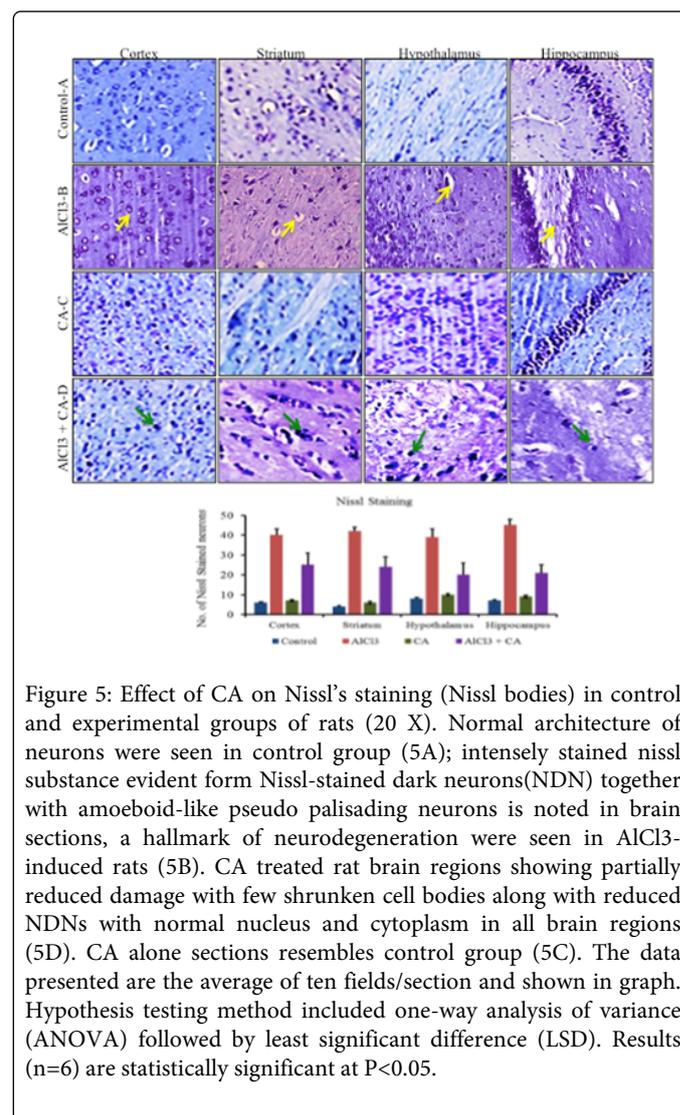


**Figure 4:** Effect of CA on histopathological analysis of brain regions in control and experimental groups of rats (20 X). A) Normal histological features with well-formed neurons in all brain regions. B) Abnormal cellular morphology accompanied by cellular infiltration, recruitment of macrophages, with damaged cerebral cortex, striatum, hypothalamus and hippocampus regions was observed in AlCl<sub>3</sub>-induced neurotoxicity. C) CA alone sections shows normal architecture of brain regions like control group. D) Co-treatment (AlCl<sub>3</sub>+CA) sections shows protected cellular morphology and also exhibiting significantly reduced morphologic abnormalities in all regions with better recovery in tissues and well-formed nuclei without irregular features. The data presented are the average of ten fields/section and shown in graph. Hypothesis testing method included one-way analysis of variance (ANOVA) followed by least significant difference (LSD). Results (n=6) are statistically significant at P<0.05.

AlCl<sub>3</sub> accelerates iron mediated LPO and causes marked oxidative damage by increasing the redox active iron concentration in the brain. Increased oxy-radicals and loss of cellular homeostasis cause oxidative stress that lead to neurotoxicity [5]. An imbalance in oxidant-antioxidant status is characterized by an increase in LPO and a decrease in antioxidant enzymes. Current study, AlCl<sub>3</sub> resulted in distinct oxidative stress as indicated by increase in LPO. MDA production is assessed commonly as an indicator of LPO [31]. Earlier studies shows that increased oxidative stress upon Al induction attack almost all cell components thereby producing LPO. Studies in the past have attributed the LPO attenuating effects of CA [11,32], which supports our present findings and proving its potential antioxidant ability [12].

AlCl<sub>3</sub> may react with superoxide anions forming Al-superoxide anions, which are more potent oxidants. Under the oxidative stress

conditions, SOD presents the first line of defense against superoxide as it converts the superoxide anion to H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub>. It also has an important role in detoxifying superoxide radical to H<sub>2</sub>O<sub>2</sub>, which is then converted to H<sub>2</sub>O by CAT at the expenditure of GSH. GST helps in the detoxification of ROS by maintaining a metabolic intermediate such as GSH. Therefore, the increased LPO may be interpreted here by an inhibition of SOD, CAT, GST and GSH activities in the brain leading to membrane damage. The observed decrease of these antioxidant enzymes during AlCl<sub>3</sub>-induction compared to control group is in accordance with what has been previously reported by Flora et al. [33].



**Figure 5:** Effect of CA on Nissl's staining (Nissl bodies) in control and experimental groups of rats (20 X). Normal architecture of neurons were seen in control group (5A); intensely stained nissl substance evident form Nissl-stained dark neurons (NDN) together with amoeboid-like pseudo palisading neurons is noted in brain sections, a hallmark of neurodegeneration were seen in AlCl<sub>3</sub>-induced rats (5B). CA treated rat brain regions showing partially reduced damage with few shrunken cell bodies along with reduced NDNs with normal nucleus and cytoplasm in all brain regions (5D). CA alone sections resembles control group (5C). The data presented are the average of ten fields/section and shown in graph. Hypothesis testing method included one-way analysis of variance (ANOVA) followed by least significant difference (LSD). Results (n=6) are statistically significant at P<0.05.

Taken together, our results explain that AlCl<sub>3</sub> promotes oxidative stress by decreasing the activity of free radical-scavenging enzymes, a biological effect confirmed by increasing the levels of LPO. Co-supplementation of CA significantly enhanced the tissue level of enzymatic antioxidants by reducing MDA levels compared with AlCl<sub>3</sub>-induced rats, suggests that CA modulates impaired redox homeostasis status and shows challenging role in quenching free radicals [34] as shows in previous findings [11,12]. This might indicate the usefulness of CA as an excellent source of antioxidants, in modulating AlCl<sub>3</sub> induced neurotoxicity [12].

AlCl<sub>3</sub> is cholinotoxin, which produces functional change in the cholinergic and noradrenergic neurotransmission. AChE is a specific cholinergic marker protein has received wide attention in the study of AlCl<sub>3</sub> neurotoxicity. AChE is more responsible for acetylcholine metabolism whose alterations caused neurobehavioral changes especially memory and cognitive function. AChE catalyzes the hydrolysis of acetylcholine into inactive metabolites, choline and acetate. Earlier studies have shown that augmented AChE level is a prelude to oxidative stress [35]. Consistent with the previous findings [36], a substantial increase in AChE activity was observed in AlCl<sub>3</sub> exposure rats, which denotes impaired cholinergic function. CA supplementation clearly alleviated AChE variations in rat brain regions suggesting that CA may regulate cholinergic function, thereby regulating oxidative stress and memory deficits [7,13]. This demonstrated the potent protective effect of CA with regard to ameliorate cholinergic neurotransmission, since AChE is a key enzyme involved in the synaptic connection.

Histological analyses reveal that AlCl<sub>3</sub> mediates progressive alterations (disorganization in the pyramidal cellular arrangement, dense cytosolic staining, disruption of nucleus and congestion in the blood vessels) in the rat brain regions of cerebral cortex, striatum, hippocampus and hypothalamus, which are very well corroborated with the previous reports [37,38]. Interestingly, CA supplementation noticeably reduced those changes and improved the architecture histology. In nissl's staining analysis we have observed that AlCl<sub>3</sub>-induced vacuolization, occurrence of NDN, ill-defined chromatin and faintly stained cytoplasm without clear nissl's granules (chromatolysis) [31,39] was rescued by CA. These results demonstrate that CA significantly reduced brain damage and improved functional outcome as observed in behavioral studies. The observed protection afforded by CA is probably due to its antioxidant ability [11,12].

## Conclusions

Taken together, our observations suggest that CA was found to have a challenging role in quenching free radicals/LPO and help to maintain antioxidant enzymes thereby hamper oxidative stress in rat brain regions. In addition to this, our results also showed that CA treatment attenuated the increase of AChE, which reflects that this compound may improve dysfunction of the cholinergic system. The study also highlights that CA attenuates behavioral deficit and improves learning and memory performance. It clearly demonstrates that CA has a neuroprotective effect against AlCl<sub>3</sub>-induced histological, behavioral and biochemical changes and further warrants the need for molecular studies to elucidates the mechanisms underlying the protective effects of CA.

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