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Modulating Neurodegeneration: Protective Role of Palmatine Against Aluminium-Induced Oxidative Stress

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Abstract

This study examined the effects of palmatine, on neurotoxicity induced by aluminum chloride in rats. The rats were administered with palmatine orally at doses of 10 and 20 mg/kg for 42 days alongside aluminum chloride to induce neurotoxicity. Oxidative stress marker measurements, brain levels of aluminum, and immunohistochemistry for determining the expression of BDNF and beta- amyloid plaques were performed. Palmatine treatment significantly improved cognitive function and behavior, reduced oxidative stress markers, decreased brain aluminum levels, inhibited β- Amyloid expression, and reduced neuronal damage and apoptosis. The study's findings suggest that palmatine has therapeutic potential against aluminum-induced neurotoxicity, highlighting its relevance for Aluminium induced neurodegeneration and Alzheimer's disease. Further investigations are necessary to inspect the underlying mechanisms and their translational potential in Alzheimer's disease treatment.

Keywords: Reactive oxygen species; Oxidative stress; Aluminium; Palmatine; β-Amyloid; NF- KB

.Introduction

Aluminium is a pervasive metal and a known neurotoxin that has been linked to the budding and advancement of neuro degeneration as it promotes reactive oxygen species generation and neuroinflammation. It also induces derangement of neurotransmitter balance, disruption of the expression of neurotrophic factors, and triggering accretion of β-amyloid plaques in the brain. Reactive oxygen species are highly reactive moieties that initiate cellular oxidative stress that aberrates cellular makeup and induces damage even at the genetic level. Aluminium gains entry to the system through common sources including occupational sources, food intoxicants, the water we drink, aluminium utensils, and other contaminants[1]. Aluminum exposure leads to biochemical alterations, deranged neuro filament structure, and bereavement in the hippocampus, cerebral cortex, etc, resulting in cognitive decline, impaired neuronal communication, maladaptive neuroplasticity, and neuronal loss. As a consequence of aluminium exposure, impaired antioxidant status, altered brain neurochemistry, and abnormal protein expression occur in the brain [2].

Degenerative modifications occur in the brain due to oxidative stress, and underlying inflammatory processes which prompt apoptosis of neurons and associated structures[3]. Aluminium is a key activator of oxidative stress and triggers an inflammatory cascade and the dysregulated process culminates in detrimental effects observed in neuro degeneration. Reactive oxygen species play a pivotal role in triggering the activation of several ensuing signaling molecules, including MAPKs. Few of them are responsive to various apprehensive stimuli, such as the ones initiated by inflammatory mediators. All of the above would inculcate the initiation and continuance of neurodegenerative disorders[4]. The underlying neuro inflammatory process, reactive oxygen species-induced damage, and amyloidogenesis in the brain bring about increased activation of NF-κB, over expression of B-amyloid and its accumulation, and degradation of acetylcholine (ACh), thereby harmfully affecting cognition and memory [5] .Aluminium negatively impacts BDNF signaling pathways which are consequential for the survival of neurons, amelioration of synaptic plasticity, and memory formation thereby reducing neurotrophic support and neuronal survival. All of the above exacerbates the neurotoxicity as perceived in neurodegenerative diseases[6]. The

neuropathology of Alzheimer's disease comprises various components, but it is commonly

identified by two main types of lesions, the neuro fibrillary tangles and plaques, which are extracellular accumulations of aggregated B-amyloid protein. Accumulations of aggregated B-amyloid deposits, both extracellular and occasionally intracellular, can be identified through immunochemistry techniques utilizing antibodies specifically designed to target B-amyloid[7] .

In recent years, natural compounds with potential therapeutic properties have gained significant attention as alternative strategies for treating and preventing neurodegenerative disorders. The study targets exploration of the impact of palmatine administration on Aluminium-mediated oxidative stress, and its role in managing aluminium load, BDNF expression, and β-Amyloid levels in the brains of rats with aluminium-induced neurotoxicity. By elucidating the potential neuroprotective properties of palmatine, this research seeks to illuminate the therapeutic potency of isolated palmatine from the said plant as a neuroprotectant against aluminium-induced neurotoxicity.

MATERIALS AND METHODS

Neuroprotective study using isolated palmatine against aluminium chloride-induced oxidative stress:

For the current study, we utilized Palmatine, which had been isolated from the stem bark extract of Tinospora Cordifolia using a previously established method[8].

Chemicals and reagents:

Aluminium chloride (Thermo fisher Scientific), Palmatine, Hydroxylamine hydrochloride , EDTA, Nitro Blue Tetrazolium, Hydrogen peroxide, Sodium Azide, Thiobarbituric acid, 5, 5'-dithio-bis (2-nitrobenzoic acid), Nitric acid.

Experimental animals: 32 Wistar albino rats weighing 200-250g were acquired from the animal house facility of Krupanidhi College of Pharmacy, Bangalore. They were accommodated and conditioned in a well-ventilated animal house. Laboratory setups were maintained 10 days before

initiation of the experiment under regulated temperature (25±4°C) and humidity (50-60) % providing 12 hours each of dark and light environments provided with a balanced nutritious diet and clean drinking water according to the recommendation of CPCSEA. The treatment consisted of four groups, Normal control administered with normal drinking water, Aluminium chloride induced toxic control group administered with $AlCl_3$ (100mg/kg p.o), Palmatine treatment group administered with Palmatine10mg/kgp.o +$AlCl_3$ (100mg/kg p.o) and Palmatine20mg/kg group administered with Palmatine20mg/kg p.o[9] +$AlCl_3$ (100mg/kg p.o)[10].The IAEC (Institutional Ethical Committee) authorized the experimental design under reference number KCP/IAEC /PCOL/61/2020.

The treatment was executed for six weeks and at the desist of therapy, animals were sacrificed followed by dissection and amassment of the brain tissue. Two rat brains each from each group were used for immunohistochemistry studies for estimation of beta amyloid plaque accretion in brain. The brain areas the study primarily emphasized were the hippocampal and cortical areas which were segregated, sectioned out and cleansed withice-cold isotonic saline (-80^0 C). The rest of rat brain from each group were seperately homogenized with 10 parts of (0.1M) phosphate buffer chilled to icy temperatures (pH 7.4) followed by centrifugation of the same at 4000 rpm for about 20 min maintaining a low temperature. The soluble fractions of the collected supernatant were utilized to conduct biochemical estimations.

The toxic control group was compared with the normal control. The palmatine treatment groups were compared with the normal control and aluminium chloride-induced toxic control group.

Effect of Palmatine on oxidative stress related parameters in rat brain

Evaluation of SOD activity:

SOD estimation was performed by detecting the generation of superoxide radicals (O_2-) through their oxidation by hydroxylamine hydrochloride, which produces nitrite. The nitrite was measured using colorimetry at 560 nm. Additionally, the auto-oxidation of hydroxylamine at pH 10.2 also generates O_2-. In the presence of EDTA, Nitro Blue Tetrazolium (NBT) is reduced, leading to nitrite production, which can be detected using colorimetry. The units of Superoxide Dismutase (SOD) were interpreted as the quantity of enzyme that impedes the reduction of NBT (Nitro Blue Tetrazolium) by half its fraction. The specific activity of SOD was conveyed in units per milligram (mg) of protein. A parallel control in the absence of tissue homogenate is prepared using the same procedure. SOD activity was evinced as units/mg of protein[11,12].

Evaluation of Catalase activity:

The specific activity of Catalase is measured by means of a spectrophotometric method. The test measures the rate of breakdown of hydrogen peroxide (H_2O_2) by the enzyme catalase, liberating water and oxygen. The rate of this reaction was decided based on the extent of the decrease in absorbance measured at a specific wavelength, usually 240 nm, as H_2O_2 was devoured. The alteration in absorbance as time progresses yields the corresponding measure of catalase activity.

The test made use of a reaction mixture consisting of the catalase-containing sample, hydrogen peroxide, and a preferred buffer, incubated at 25°C, for a specific time period. The mixture was incubated, and cessation of the reaction was brought about by the incorporation of a stop solution of sodium azide. The reaction mixture was checked and its absorbance was measured at 240nm.The variation observed in absorbance is used to determine catalase activity. A catalase activity unit is defined as the amount of enzyme that catalyzes the decomposition of 1 μmol of H_2O_2 per minute under the specified assay conditions [13].

Evaluation of the extent of Lipid Peroxidation in rat brain:

The magnitude of lipid peroxidation was assessed by quantifying the MDA-TBA adduct formed as a result of the interaction between malondialdehyde (MDA) and thiobarbituric acid (TBA) in samples. The adduct formed is quantified by means of colorimetry or fluorometry. TBARS assay involves a reaction between one molecule of MDA with two molecules of thiobarbituric acid yielding a chromogen. The strength of the so-formed chromogen was quantified by means of colorimetry (535nm) by means of UV spectroscopy against a blank. The MDA concentration is expressed by means of a molar extinction coefficient of 1.56×10^5 L mol^{-1} cm^{-1} and expressed in nmol g^{-1} of tissue[14].

Evaluation of Reduced Glutathione:

The test involves a reaction wherein, reduced glutathione (GSH) is oxidized by means of 5, 5'-dithio-bis (2-nitrobenzoic acid) (DTNB), a sulfhydryl reagent. This interaction yields a yellow byproduct known as 5'-thio-2-nitrobenzoic acid. The intensity of the yellow color was determined immediately by measuring the same at 412 nm. The amount of glutathione (GSH) in the reduced form present in the sample is enunciated as µg/mg protein[15].

Evaluation of Aluminium levels in rat brain:

The measure of levels of aluminium in rat brains was determined by means of atomic absorption spectroscopy (ICP OES). To determine the same, brain homogenates are digested by means of nitric acid followed by heating the mixture so as to liberate aluminum ions. A standard calibration curve is made with known concentrations of aluminum. The quantification of aluminium was made by measured the absorbance of aluminum in the brain sample by means of an ICP-OES

instrument and the concentrations were read out from the calibration curve. The ICP-OES instrument is injected with the digested brain sample solution. The emitted light emerging from excited aluminium atoms is evaluated to estimate their intensity, for determining the aluminium emission intensity or concentration for each sample. The absorbance values are cross checked with the standard calibration curve to ascertain the brain levels of aluminum [[16].

Evaluation of expression of β-Amyloid in rat brain by immunohistochemistry:

Quantification and estimation of levels of Beta-amyloid plaques in rat brains were carried out by means of immunohistochemistry technique by preparing the brain tissue in paraformaldehyde and the rotary microtome was used for thin sectioning followed by slide preparation. The slides were visualized using a photographic microscope[17] .

The Beta amyloid-positive cells or regions of interest were identified and quantified using image analysis software. By following this immunohistochemical method, the distribution and relative levels of Beta-amyloid in specific brain regions of rats can be determined, providing insights into its localization and potential roles in neurodevelopment, and plasticity.

Statistical Analysis:

The variation between the palmatine treatment and the control groups was analyzed by means of GraphPad Prism V5.0 for Windows. Test results of the Elevated plus maze were assessed by One-way ANOVA (analysis of variance) followed by Dunnett's multiple comparison test. The significance level was assessed between groups. Oxidative stress-related parameters and brain levels of aluminium were determined by means of One-way ANOVA followed by Dunnett's multiple comparison test. The data were all expressed as mean ± standard error of the mean (SEM); $p < 0.05$ was considered to be significant.

RESULTS

Effect of Palmatine on oxidative stress related parameters in rat brain:

The brain levels of antioxidant enzymes SOD (Table 1), Catalase (Table2), and reduced Glutathione (Table3) declined with aluminium administration and got regulated and elevated with palmatine treatment, especially at a dose of 20mg/kg. The extent of lipid peroxidation (Table4) and the amount of accretion of aluminium in the brain tissue (Table5) lowered with palmatine administration.

β-Amyloid expression evaluation in Rat Brain by IHC technique:

The immunohistochemistry (IHC) examinations performed on the hippocampal and cortical areas of rat brains of normal control animals (Figure 1) reveal the absence of β-Amyloid accretion. Microscopic inspection of brain tissues from the group under induced toxicity (Figure 2) demonstrates the notable presence of Amyloid-β embodiment in the hippocampal and cortical regions, ranging from moderate to marked levels. The incorporation of Palmatine, treated at a dose of 10 mg/kg (Figure 3) in the animals resulted in a mild reduction of the casting of β-Amyloid in the cortex and a moderate reduction in the hippocampal areas. Animals under Palmatine treatment at a dose of 20 mg/kg (Figure 4) led to a decline in β-Amyloid expression in the cortical areas and the hippocampal areas did not exhibit any detectable levels of β-Amyloid expression.

DISCUSSION

The research explored the competence of palmatine as a neuroprotectant in aluminum-impelled neurodegeneration and in attenuating memory deficits. Extensive evidence suggests that aluminum exposure imparts to the pathogenesis and induction of brain-related disorders as it

promotes reactive oxygen species-mediated damage, neuronal loss, neuronal inflammation, etc. causing accretion of abnormal proteins, contributing to neurotoxicity and cognitive impairments [18,19] .

The study involved exposure of male Wistar albino rats to aluminum chloride, a known neurotoxicant [20]wherein it was orally administered at 100 mg/kg dose [21].Concurrently, palmatine is administered orally at 10 mg/kg and 20 mg/kg doses respectively for 42 days. Palmatine has been previously investigated for its beneficial effects, as it possesses potential antioxidant activity and is anti-inflammatory in nature, against various conditions[22]. The aluminium chloride administered rats exhibited impaired memory, which indicates deficits insynaptic transmission and plasticity, and also exhibited behavioral derangements and depression. Additionally, clear neuropathological indications of Alzheimer's disease were observed in rat brains, signalized by the presence of clusters of β-amyloid accretion and decline in the expression of BDNF.

Furthermore, the study examined several oxidative stress-associated parameters, a hallmark feature of neurodegenerative conditions that play a crucial part in the development and progression of such conditions[23]. Enzymes such as glutathione peroxidase, catalase, and superoxide dismutase are involved in the detoxification of reactive oxygen species. Following treatment with aluminium, there was a remarkable decline in tissue catalase, SOD, and GSH activity and an increase in lipid peroxidation. Palmatine effectively improved the antioxidant status by improving levels of SOD, catalase, and glutathione and by lowering lipid peroxidation in the brain.

Previous studies show that repeated oral administration of aluminium chloride leads to a substantial elevation in brain aluminium concentration and a similar observation is seen in the present study as well [16]. The observed outcomes of palmatine treatment may be attributed to its antioxidant properties, specifically its ability to break the chain reaction of oxidative damage.

The reactive oxygen species resulting from aluminium exposure can interact with the amyloid protein, resulting in the genesis of senile plaques, and also would down regulate the expression of protective neurotrophic factors like BDNF. This interaction can further generate oxygen radicals, which can disrupt lipid membranes and potentially lead to protein denaturation. Immunohistochemistry studies are performed on the hippocampus and cortex to evaluate the effect of palmatine on brain-derived neurotrophic factor (BDNF) expression and its contributionto protection against neuronal damage caused by β-Amyloiddeposition induced by aluminum. BDNF is a cardinal neurotrophic factor involved in the survival of neurons, synaptic plasticity, and cognitive functions [24]. Aluminum exposure has been shown to decrease BDNF assertion, leading to neuronal dysfunction and cognitive impairments[25]. Therefore, investigating the effects of palmatine on BDNF expression in the context of aluminum-induced neurotoxicity provides valuable insights into its potential neuroprotective mechanisms [26,27].

Findings of our previous study shows that, a decrease in BDNF levels is discerned in Aluminium induced group with respect to the treated group. Additionally, pronounced hyperplastic vacuolar alterations and derangements in pyramidal cells were observed in both the cortical and hippocampal regions of rat brain. A dose-dependent increase in BDNF levels following palmatine treatment suggests its ability to enhance neuronal survival, differentiation, and synaptic plasticity. Its upregulation has been associated with improved cognitive function and neuroprotection. A decline in oxidative stress, decline in beta amyloid expression coupled with the improved expression of BDNF and decline in neurodegeneration suggests the possible involvement an NF-kB-reliant pathway[8,28].

Amyloid plaques formed upset the typical signaling functionalities of neurons, ultimately accelerating neuronal apoptosis. The depletion of BDNF is thought to be enacted in the cognitive deficits observed here. Another study, wherein B-amyloid injected hippocampal areas of the brain

in rats exhibited a pronounced fall in the levels of BDNF accompanied by an increase in pro-inflammatory markers supports our finding that β-Amyloid and BDNF expression are inversely correlated [29]. NF-κB is a crucial modulator of neuronal viability, expansion, programmed cell death, migration, and progression of immune cells[30].

Under conditions of oxidative stress, NF-κB exhibits a safeguarding role in suppressing the accumulation of reactive oxygen species. NF-κB repression leads to elevated reactive oxygen species generation, lipid peroxidative changes, and oxidation of proteins in response to TNFα. Another study demonstrates that inhibiting NF-κB activation during the recuperation phase following transient oxidative stress notably diminishes cell viability, underscoring the importance of NF-κB activation in cellular recovery [31]. In this, the involvement of NF-kB in lowering reactive oxygen species-mediated neurodegeneration and BDNF expression cannot be eluded or sidestepped [32].

Conclusion:

The results of this study demonstrate that palmatine treatment significantly improved behavioral parameters, including cognitive function, reduced brain levels of aluminum, inhibited β-Amyloid expression, reduced neuronal damage and apoptosis, and mitigated oxidative stress in the brain. The result aligns with the prior research findings highlighting the neuroprotective effects of palmatine in various neurodegenerative models. The observed improvements in behavioral parameters and the modulation of key molecular markers suggest that palmatine holds promise as a potential therapeutic intervention for neurodegenerative diseases.

Conflict of interest

The authors declare no conflict of interest.

TABLE 1 :ESTIMATION OF SOD LEVELS IN THE BRAIN

Sl.No	**Treatment group**	**SOD levels (U/mg protein)**
1	Normal Control	1.14 ± 0.059
2	$AlCl_3$ induced toxic control	0.35 ± 0.049[a]
3	Palmatine 10mg/kg	0.8 ± 0.044[b]
4	Palmatine 20mg/kg	1.02± 0.026[c]

- The findings are demonstrated as mean ± SD (n = 6), and [a,b,c]*p<0.001* in contrast to the normal group [b,c]*p<0.001* when compared to the $AlCl_3$ induced toxic control group.

TABLE 2: ESTIMATION OF CATALASE LEVELS IN THE BRAIN

Sl.No	**Treatment group**	**Catalase levels U/ml of H_2O_2**
1	Normal Control	7.15±0.241
2	$AlCl_3$ induced toxic control	4.01±0.184[a]
3	Palmatine 10mg/kg	5.1± 0.221[b]
4	Palmatine 20mg/kg	6.03± 0.282[c]

- The findings are demonstrated as mean ± SD (n = 6), [a,b,c]*p<0.001* when compared to the normal group. [b,c]*p<0.001* when *c*ompared to the $AlCl_3$induced toxic control group.

TABLE 3: EVALUATION OF THE EXTENT OF LIPID PEROXIDATION IN THE BRAIN

Sl.No	**Treatment group**	**Lipid peroxidation (nmol/min/mg protein)**
1	Normal Control	2.87 ± 0.454
2	$AlCl_3$ induced toxic control	10.82 ± 0.325[a]
3	Palmatine 10mg/kg	7.88 ± 0.553[b]
4	Palmatine 20mg/kg	6.75± 0.235[c]

- The findings are demonstrated as mean ± SD (n = 6), and [a,b,c]*p<0.001* when compared to the normal group. [b,c]*p<0.001* in contrast to the $AlCl_3$-induced toxic control group.

TABLE 4: EVALUATION OF REDUCED GLUTATHIONE LEVELS IN THE BRAIN

Sl.No	**Treatment group**	**Reduced Glutathione (nmol/min/g)**
1	Normal Control	25.28 ± 0.339
2	$AlCl_3$ induced toxic control	7.87 ±0.202[a]
3	Palmatine 10mg/kg	15.81 ± 0.198[b]
4	Palmatine 20mg/kg	19.20± 0.180[c]

- The findings are demonstrated as mean ± SD (n = 6), [a,b,c]$p<0.001$ when compared to the normal group.[b,c]$p<0.001$ in contrast to the $AlCl_3$-induced toxic control group.

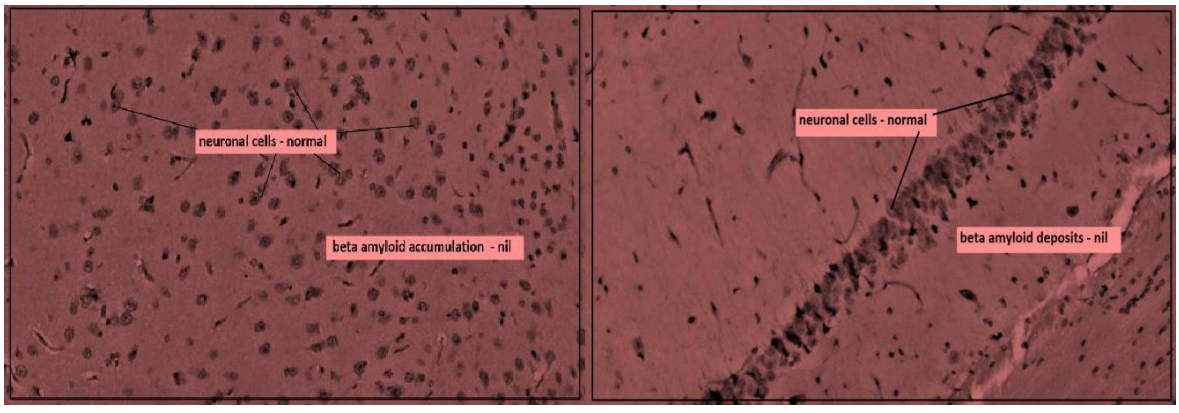
TABLE 5: EVALUATION OF BRAIN LEVELS OF ALUMINIUM

Sl.No	**Treatment group**	**Brain levels of Aluminium µg/g wet tissue**
1	Normal Control	0.43 ± 0.156
2	$AlCl_3$ induced toxic control	1.72 ± 0.106[a]
3	Palmatine 10mg/kg	1.2 ±0.120[b]
4	Palmatine 20mg/kg	1.25±0.169[c]

- The findings are demonstrated as mean ± SD (n = 6), and [a,b,c]$p<0.001$ when compared to the normal group. [b,c]$p<0.001$ in contrast to the $AlCl_3$-induced toxic control group.

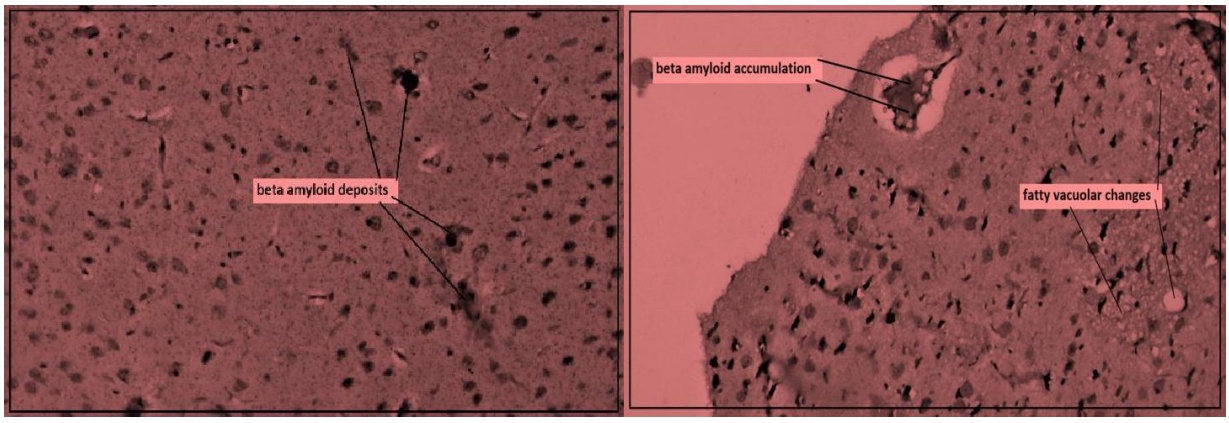
B-Amyloid expression evaluation in Rat Brain by IHC technique

(β-Amyloid stain – Dark brown deposits)



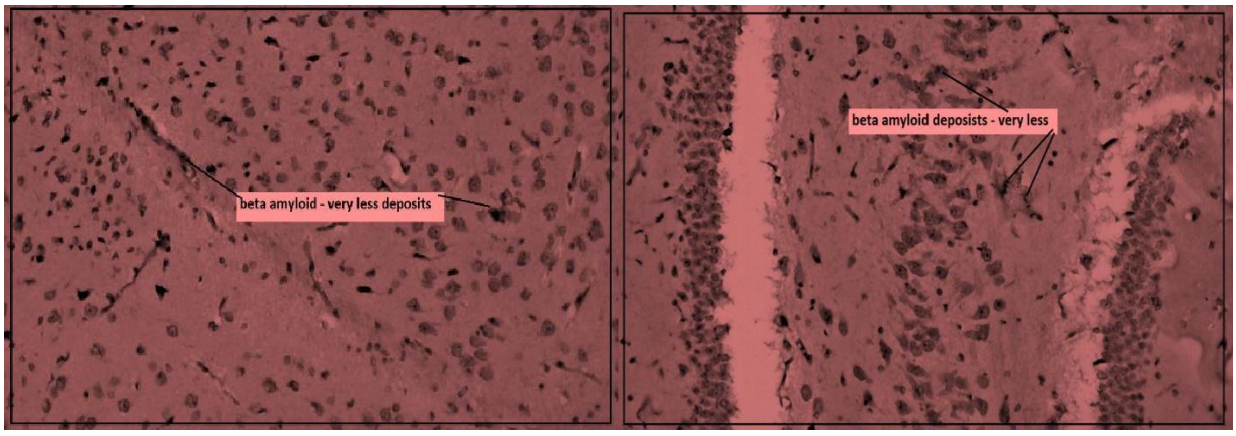
A B

Figure 1:β-amyloid expression in Brain of Normal Control animals. (A): Rat Brain Cortical region showing normal neuronal cells – (X100) (Beta amyloid - Dark brown deposits – nil) B: Rat brain hippocampus region showing nil beta-amyloid in neuronal cells (X100)



A **B**

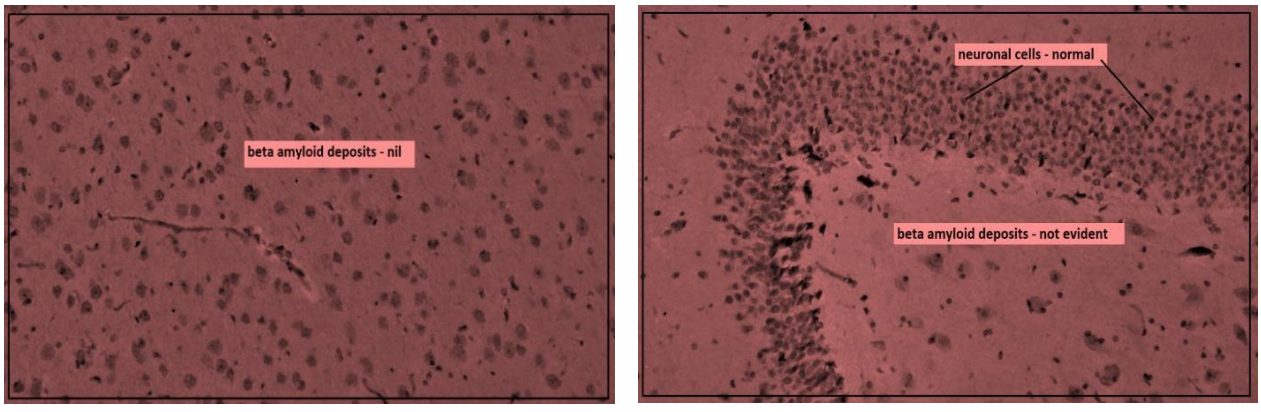
Figure 2: β-amyloid expression in Brain of AlCl3 induced toxic control animal. (A): Rat Brain Cortical region showing beta amyloid in pyramidal neuronal cells – (X100) (Note: beta amyloid in increased levels) Dark brown deposits), (B): Rat brain hippocampus region showing beta-amyloid accumulation (X100) (Fatty vacuolar changes evident)



A **B**

Figure 3: β-amyloid expression in Brain of Palmatine 10 mg/kg administered animals

(A): Palmatine 10 mg, Rat Brain: Cortical region showing BDNF in pyramidal neuronal cells – (X100) (Very less beta amyloid accumulation noticed) Dark brown deposits), (B): Rat brain hippocampus region showing beta-amyloid in pyramidal cells (Very little beta-amyloid accumulation noticed) Dark brown deposits) (X100)



A **B**

Figure 4: β-amyloid expression in Brain of Palmatine 20 mg/kg administered animals.

(A) : Rat Brain: Cortical region showing beta amyloid in pyramidal neuronal cells – (Beta amyloid levels not evident) Dark brown deposits (X100) (B): Rat Brain hippocampus region showing beta-amyloid in pyramidal neuronal cells (Beta-amyloid levels not evident) (X100).

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