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# Calcium Channel Blockers Attenuates Alcl3 Induced Neurotoxicity

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**ABSTRACT**: Usefulness of CCBs against AD pathology are controversial. Observational studies indicate that CCBs preclude or tardy the rate of progression of AD. The calcium channel blockers are the category of drugs commonly used to treat hypertension and its influence on dementia is controversial, has lead us investigate the effect of Ca+ blockers uponaluminumchloride-triggered dementia in Albino rats.Administration of $AlCl_3$ to rats led to a decrease in reduced glutathione (GSH) levels in the brain tisuue indicating generation of oxidative stress. Our results were consistent with these data as injection of $AlCl_3$developed a significant rise in lipid peroxide level indicated as MDA, oxidized protein,TBARS,AChE, SOD, GPx and CAT effect in rat brains compared to vehicle control rats.The oxidative damage induced by the aluminum chloride was well antagonized by CCBS.In our studies we observed that in EPM test decrease in the tranceferlataency in all the CCBS (calcium channel blockers) treated animals.,thedistinction index in novel object recognition test also decreased with all the calcium channel blockers. Studies also demonstrated that verapamil was good in prophylactic studies and diltiazem in curative studies.

**KEYWORDS:** alcl3, alzheimer"s disease, oxidative stress, ca+ channel blockers, learning and memory

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## I. INTRODUCTION

The most common form of dementia in the old agealzheimer's disease (AD) that slowly destroys neurons.AD is a progressive neurodegenerative disorder[1] .Currently,AD affects nearly 4% of people 63-year above and over 29% of those 82-year old, affecting more than 27 million people in the developed world [2,3,]. AD is characterized by atrophy of cerebral cortex and selective neuronal damage in the hippocampus brain tissues. Oxidative damage and the formation of free radicals may occur for several reasons such as exposure to chemicals,metals, irradiation and toxins causing to lipid peroxidation,which in turn affects the effecayof protective enzymatic antioxidants that are greatly sensitive signed of rised oxidation reactions[4,5]. When lipids affeceted by free radicals,the lipid peroxidation chain reaction proceed [6]. This lipid degradation reaction leads to cleavage chemical bonds, crosslinkages,and conformational changes of many bio molecular compounds.. Aluminum can bind to different metal binding proteins such as Ca, Fe, Cu and Zn that accordingly influences homeostasis of other metals.Aluminum may exert its neurotoxicity via free radical production and peroxidation damage to lipids and proteins [7].. There are also reports indicating that Al exposure is associated with impairment of mitochondrial functions, in vivo and in vitro, as well as the antioxidant defense system [8,9] . Al decreases the antioxidant enzyme status can cause also a disturbance in the enzyme activity involved in acetylcholine metabolism and leads to cognitive dysfunction[10],[11,].A calcium channel is an ion channel which displays selective permeability to calcium ions. It is sometimes synonymous as voltage-dependent calcium channel,[12]although there are also ligand-gated calcium channels.

Basic research into Alzheimer's disease (AD) more than two decades ago demonstrated early and profound loss of cholinergic neurons, a finding that led to the first therapeutic advance with the development and licensing of the first specific treatments: the acetyl cholinesterase inhibitors. Calcium channel blockers are the drugs used in treatment of CVS diseases such as hypertension and angina pectoris[13]. Usefulness of CCBs against AD pathology is controversial. Epidemiological studies suggest that CCBs prevent[14] or slow the rate of progression of AD[15]. A large clinical trial with nimodipine did not show significant benefits from the primary outcome measures but has shown moderate benefits for treatment of AD in the secondary outcome measures[16]. In a recent study, however, nimodipine selectively stimulated the secretion of Aβ1–42 in vitro and in the plasma of Tg2576 mouse model of AD, questioning the

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usefulness of this CCB for AD[17,18]. So this concept became controversial and planned to carry the effectiveness of CCB on dementia against aluminium chloride induced dementia in Albino rats.

**Methods,**

**Experimental animals**

Female wistar albino rats weighing 250±10 gm were procured from Venkateshwara enterprises Bengaluru. The animals were maintained under standard husbandry conditions (temp 23±2oC, relative humidity 55±10% and 12 hour light dark cycle). Animals were fed with standard laboratory food and ad libitum during the study period. All animals were used for the study as per CPCSEA guidelines. The whole experiment was approved by IAEC (SKVCP/IAEC/PGCOL/14/06 Dated 19-03-2014)

**Dose fixation**

The maximum human therapeutic dose all the drugs usedin studies was extrapolated to rats based on the bodysurface area (BSA)[21.]

Materials and methods: Dementia was induced by $Alcl_3$. The behavioural changes in the studyanimals were assessed by behavioural paradigms such aselevated plus maze, and novel object recognition testsand biochemical parameters such as catalase, superoxidedismutase, glutathione peroxidase, malonylaldehyde,protein and AChE.

**Drugs, chemicals and its preparation**

**DRUGS, CHEMICALS AND ITS PREPARATION:**

**Piracetam** (50mg/kg)(p.o)[19]

**$AlCl_3$** (45mg/kg) (i.p.)[20]

**CCB**:Diltiazem(10.8mg/kg),verapamil.(21.6mg/kg) nimodipine (0.9mg/kg) p.o[21].$AlCl_{3,,}$ Piracetam, diltiazem, verapamil, nimodipine dissolved 0.1% CMC[33] and used for the administration of the drugs to the animals.

Animals were divided into ten groups of containing sixanimals in each group. Inprophylactic studiesanimals were treated with piracetam (50mg/kg/day) (p.o),diltiazem (10.8 mg/kg/day) (p.o), verapamil (21.6mg/kg/day) (p.o), and nimodipine (0.9 mg/kg/day) (p.o),half an hour before the administration of$Alcl_3$. In curative studies the animals were injectedwith $Alcl_3$ half an hour later animals were treatedwith piracetam (50 mg/kg/day) (p.o), diltiazem (10.8mg/kg/day) (p.o), verapamil (21.6 mg/kg/day) (p.o), andnimodipine (0.9 mg/kg/day) (p.o), respectively.

**Table: Table: 1 Experimental models of dementia: $AlCl_3$induced dementia.**

**Table: 8- Aluminium chloride induced dementia**

| Group No. | Treatment | No. of animal |
|---|---|---|
| Group-I | NC -G(vehicle 0.1% CMC for 28 days) | 6 |
| Group-II | Negative control ( $AlCl_3$ 4.2mg/kg/day for 28 days) | 6 |
| Group-III | Prophylactic study (Piracetam 50mg/kg/day & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ injected half an hour after the administering with piracetam for 28 days | 6 |
| Group-VI | Prophylactic study (diltiazem 10.8mg/kg/day for & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ injected half an hour after the administering with diltiazem for 28 days | 6 |
| Group-IV | Prophylactic study (verapamil 21.6mg/kg/day & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ injected half an hour after the administering with verapamil for 28 days | 6 |
| Group-V | Prophylactic study (nimodipine 0.9mg/kg/day & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ injected half an hour after the administering with nimodipine for 28 days | 6 |
| Group-VII | Curative study (piracetam 50mg/kg/day & $AlCl_3$ 4.2mg/kg/day) AlCl3 half an hour later treated with piracetam for 28 days | 6 |
| Group-VIII | Curative study (diltiazem 10.8mg/kg/day & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ half an hour later treated with diltiazem for 28 days | 6 |

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| | | |
|---|---|---|
| Group-IX | Curative study (verapamil (21.6mg/kg/day & $AlCl_3$ 4.2mg/kg/day) $AlCl_3$ half an hour later treated with verapamil for 28 days | 6 |
| Group-X | Curative study (nimodipine 0.9mg/kg/day & $AlCl_3$ 4.2mg/kg/day)$AlCl_3$ half an hour later treated with nimodipine for 28 days | 6 |
| Total | | 60 |

**EPM- test**

The elevated plus maze recently been extended to measure the cognitive performance, otably to evaluate the spatial long term memory in rats. The elevated plus maze, which was introduced by Pellow with rats, consists of two open and two enclosed arms, and is based on the apparent natural aversion of rodents to open and high spaces and is used to measure the anxiety state in animals[23,24]. Animals spend more time in enclosed arms than the open arms because they dislike open arms. The aversive quality of the open arm is not apparent until the animals enter them. Based on this parameter it could be demonstrated that transfer latency (the time in which the animal moves from the open arms to the closed arms) will be markedly shortened if the animal had previously experienced entering the open arms, and the increased transfer latency has been shown in cognitive declined condition. Rats were placedindividually at end of an open arm facing away from central platform and the time took to move from the end of open arm to either of closed arm (Transfer latency, TL) was recorded. If the animal did not enter into one of the enclosed arms within 90 sec, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 sec. The rat was allowed to explore the maze for another 10 sec and then returned to its home cage. Retention of this earned-task was examined 24 hoursafter the first day trial. Transfer latency after 24 hours was expressed as "inflexion ratio, IR"" using the formula IR = (L1 - L0)/ L0. Where L0 is the transfer latency after 24 hours and L1 is the initial transfer latency in seconds.Rats of either sex, weighing around 150-200 gm, were divided into ten groups consisting of 6 animals each, and the drugs were administered as mentioned in Table 1.Orally for 7 days, scopolamine was given intraperitoneally.After 90 min of administration on 7th day transfer latency was recorded. Retention of learned taskwas examined after 24 hours and inflexion ratio was calculated using the following formula.

IR = (L1/L0)/L0.s**p Treatment No. of animals**

**Novel object recognition (NOR)**

To examine the factors driving exploration, curiosity andreaction to novel stimuli, is an experimental paradigmcommonly known as novel object recognition (NOR) ornovel object preference (NOP). The novel objectrecognition test can be evaluated by the differences in theexploration time of novel and familiar objects. Itsapplication is not limited to a field of research andenables that various issues can be studied, such as thememory and learning, the preference for novelty, theinfluence of different brain regions in the process ofrecognition, and even the study of different drugs andtheir effect[25].The studies have employed NOR to examine differencesin memory and reaction to novelty, in various remindertreatments[40]. Curiosity and basic exploratory responsesare key components to the behavioural repertoires of themost animal species, and thus a thorough and completeunderstanding of these issues is vital. Simply put, I amcurious about curiosity. The essential role it plays in theeveryday lives of both humans and non-human animals isundeniable.

**Novel object recognition procedures[26]**

The open field apparatus consisting of colored plywood(70 x 60 x 30 cm). The objects to be discriminated wereplaced at diagonally opposite corners of the box. On the day of test in the first trial (T1), two identical objectswere presented in two opposite corners of the box and theamount of time taken by each rat to complete 20 secexplorations was measured. Exploration meant directingthe nose at a distance less than 2cm to the object and/ortouching with the nose . During the second trial (T2,90 min after T1) a new object replaced one of the objectpresented during T1 and time spent for exploring new (N)and familiar (F) objects was recorded. This procedurecarried on animals before administering the drugs (pretrail)as well after completing drug administration (posttrail).The Discrimination index (DI) = (N-F)/ (N+F).After completing behavioural paradigms, the animalswere fasted for 18 hours. After 24 hours of

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drugtreatment the animals were scarified by cervicaldislocation, brains were removed. Each brain wasseparately put on ice and rinsed with ice-cold isotonicsaline. A (10% w/v) homogenate was prepared in 0.1 Mphosphate buffer (pH 7.4). The homogenate wascentrifuged at 3000 rpm for 15 minutes and aliquots ofsupernatant were separated and used for biochemicalparameters estimation.

**Biochemical parameters**

An antioxidant is a molecule capable of inhibiting theoxidation of other molecules. Oxidation is a chemicalreaction that transfers electrons from a substance to anoxidizing agent. Oxidation reactions can produce freeradicals. In turn, these radicals can start chain reactionsthat damage cells. Antioxidants terminate these chainreactions by removing free radical intermediates andinhibit other oxidation reactions.Although oxidation reactions are crucial for life, they canalso be damaging; hence, plants and animals maintaincomplex systems of multiple types of antioxidants, suchas glutathione, vitamin C, and vitamin E as well asenzymes such as catalase, superoxide dismutaseglutathione peroxidase. In the present study thebiochemical parameters such as SOD, CAT, GPx andMDA, oxidized protein are estimated as an index ofantioxidant status and oxidative stress respectively.AChE was estimated to evaluate cholinergic function.

**Malonylaldehyde (MDA)[27]**

Oxygen derived free radicals react with membrane lipidsto form lipid peroxides and malondialdehyde (MDA)which is the end product of lipid peroxidation. Reactive oxygen species degrade polyunsaturated lipids, formingmalondialdehyde. This compound is a reactive aldehydeand is one of the many reactive electrophile species thatcause toxic stress in cells and form covalent proteinadducts referred to as advanced lipoxidation end-products (ALE), in analogy to advanced glycation end-products(AGE).30 The production of this aldehyde is used as abiomarker to measure the level of oxidative stress in anorganism.

**Thiobarbituric acid reactive substances (TBARS) assay[28]**

This assay is used to determine the lipid peroxidation.Aliquots of 0.5 mL distilled water were added with 1 mLof 10% trichloroacetic acid and were added with 0.5 mLof brain tissue homogenate.

This was centrifuged at 3000 rpm for 10 min. To the0.2 mL supernatant, 0.1 mL hiobarbituric acid (0.375%)was added.Total solution is placed in water bath at 80°C for 40 minand cooled at room temperature. Absorbance was read at532 nm.

**Catalase[29,29.]**

Catalase is a common enzyme found in living organisms.Its function includes catalysing the decomposition ofhydrogen peroxide to water and oxygen.Removal of the H2O2 from the cell by catalase providesprotection against oxidative damage to the cell. It‟s rolein oxidative stress related diseases has been widelystudied.

Principle:Catalase measurement was carried out by the ability ofCAT to oxidize hydrogen peroxide (H2O2).Decomposition of H2O2 gives water and oxygen.The visible light absorption of hydrogen peroxidesolution can be easily measured between 420 to 600 nm.

On decomposition of hydrogen peroxide by catalase, theabsorption decreases with time. The enzyme activitycould be arrived at this decrease.

**Procedure**

The reaction mixture consisted of 150µl ph-bu(0.01 M pH 7.0), 1ml supernatant. Reaction wasstarted by adding 250 µl H2O2 0.16 M, incubated at 370C for 1 min and reaction was stopped by addition of 1ml ofdichromate: acetic acid reagent. They were immediatelykept in a boiling water bath for 15 min and the greencolour developed during the reaction was read at 570 nmon a spectrophotometer. Control tubes, devoid of enzyme,were also processed in parallel. The enzyme activity isexpressed as mol of $H2O_2$ consumed/min/mg protein.

**Assay of glutathione peroxidase[30,31]**

0.4 ml of buffer, 0.2 ml of EDTA, 0.1 ml of sodiumazide, 0.2 ml of reduced glutathione , 0.1 ml of H2O2were added to two test tubes labeled as test (T) andcontrol (C). To the test, 0.2 ml of sample and to thecontrol added 0.2 ml of water was added. The contents were mixed well and incubated at 37°C for 10 minutes.The reaction was arrested with the addition of 0.5 ml of10% TCA. To determine the glutathione content, 1.0 mlof supernatant was removed by centrifugation. To thatadded, 3.0 ml of buffer and 0.5 ml of Ellman‟s

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reagentwere added. The colour developed was read at 412nm[32,33]

**Superoxide dismutase (SOD[34,35,36]**

Principle

The method involves the generation of the superoxide radical ofriboflavin and its detection by N+ formation fromhydroxylamine hydrochloride. The nitrite reacts withsulphanilic acid to produce a diazonium compound whichsubsequently reacts with naphthylamine to produce a redazo compound whose absorbance is measured at 543 nm.

**Proteins**

Estimation of proteins by Bradford method[37,38].

**Principle**

The protein in solution can be measured quantitatively bydifferent methods. The methods described by Bradforduses a different concept-the protein,,s capacity to bind to adye, quantitatively. The assay is based on the ability ofproteins to bind to coomassie brilliant blue and form acomplex whose extinction coefficient is much greaterthan that of free dye.

**Acetylcholinesterase[39,40]**

To Assay of AChE activity in the brain.The method of AChE activity estimation is popularlyknown as Ellman‟s method named after George Ellman
who developed this method in 1961.

**Principle**

photometric procedure for determiningAchEactivity of tissue homogenates. Theenzyme activity is measured by providing an artificialsubstrate, acetylthiocholine (ATC). Thiocholine releasedbecause of the cleavage of ATC by AChE is allowed toreact with the -SH reagent 5, 5‟-dithiobis-(2-nitrobenzoicacid) (DTNB), which is reduced to thionitrobenzoic acid,a yellow coloured anion with an absorption maxima at400 to 412 nm.

**Statistical analysis**

The values were expressed as Mean±SEM. Statisticalanalysis was performed by one way analysis of variance(ANOVA) followed by Dunnett multiple comparisontests using the demo version of GraphpadInstat 3.0software. P values <0.05 were considered as significant.

## II. RESULTS

**Aluminium induced dementia**

**Behavioural paradigms**

The effect of the different types of calcium channelblockers on scopolamine induced dementia was studiedby using memory models such as elevated plus maze testand novel object recognition test.

**Table 2: The effect of CCB on inflexion ratio in elevated plus maze test against $Alcl_3$ induced dementia**

**Behavioral parameters:**

**Elevated plus maze test:** From table no 1. it was found that the inflexion ratio of animals in elevated plus maze was high in post-trail when compared to pre trail. It was observed that the IR was increased in all the CCB treated group animals when compared $AlCl_3$ treated group and was comparable to that of vehicle control group animals.

**Table no 1. Table showing theeffect of CCB on inflexion ratio in EPM test against $AlCl_3$ induced dementia model in rats**

| Groups | Treatment | INFLEXION RATIO=L1/L2 (Mean ± SEM) | |
|---|---|---|---|
| | | Pre-trail | Post -trail |
| I | Vehicle control | 1.94±0.364 | 1.58±0.4746 |
| II | Disease control | 1.37±0.121 | 0.59±0.3731 |
| III | Prophylactic-piracetam control | 1.78±0.127 * | 2.366±0.732 ns |
| IV | Prophylactic- diltiazem control | 1.51±0.186 * | 2.021±0.5346 * |
| V | Prophylactic-verapamil control | 1.85±0.463 ns | 2.06±0.626 ** |

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| | | | |
|---|---|---|---|
| VI | Prophylactic-nimodipine control | 1.33±0.343 ns | 2.268±0.352 * |
| VII | Curative- piracetam control | 1.76±0.171 * | 2.021±0.534 * |
| VIII | Curative-diltiazem control | 1.50±0.232 * | 4.37±0.387 * |
| IX | Curative-verapamil control | 1.51±0.217 * | 3.508±0.149 ** |
| X | Curative-nimodipine control | 1.37±0.289 ns | 4.703±0.242 ** |

Values are mean ± SEM (n=6) by one way ANOVA test followed by Dunnett test.Significance value = Comparison with vehicle control group animals. *p<0.05 represents significant, **p< 0.01 represents, highly significant and ***p<0.001 represents very significant. ns-no significance

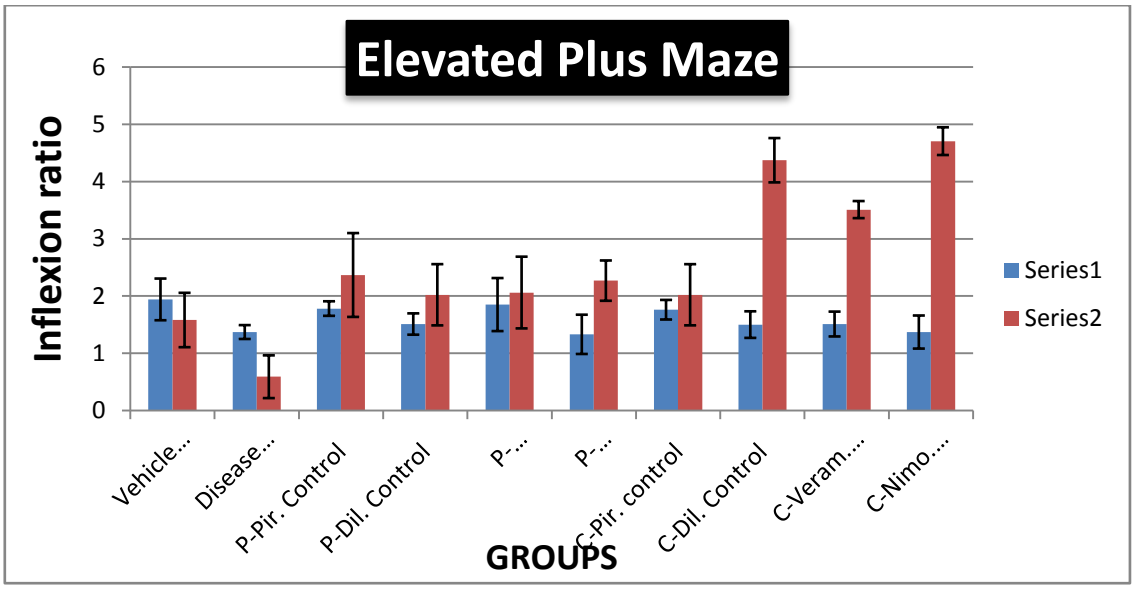


**Figure no 1. Figure showing theeffect of CCB on inflexion ratio in EPM test against $AlCl_3$ induced dementia model in rats**

**Novel Object Recognition:** From table no 14, it was found that the discrimination index(DI) was reduced in $AlCl_3$treatment group compared to vehicle control treatment. It was also observed that in both prophylactic and curative studies with CCB viz diltiazem, verapamil, nimodipine showed increased DI when compared to $AlCl_3$ treated group and was comparable to that of vehicle control group animals.

The DI of animals in novel object recognition was high in post-trail when compared to pre-trail.

**Table no 2: Table showing theeffect of CCB on discrimination index in Novel Object Recognition test against $AlCl_3$ induced dementia model in rats**

| Groups | Treatment | DISCRIMINATION INDEX (Mean ± SEM) | |
|---|---|---|---|
| | | Pre-trail | Post -trail |
| I | Vehicle control | 0.403±0.090 | 0.36 ±0.083 |
| II | Disease control | -0.405±0.098 | 0.028±0.1014 |

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| | | | |
|---|---|---|---|
| III | Prophylactic-piracetam control | 0.29±0.050 ns | 0.301±0.170 * |
| IV | Prophylactic-diltiazem control | 0.25±0.061 ns | 0.34±0.22 ** |
| V | Prophylactic-verapamil control | 0.22±0.073 ns | 0.353±0.145 ** |
| VI | Prophylactic-nimodipine control | 0.175±0.079 * | 0.28±0.148 * |
| VII | Curative-piracetam control | 0.306±0.075 ns | 0.31±0.202 ** |
| VIII | Curative-diltiazem control | 0.36±0.075 ns | 0.27±0.135 ** |
| IX | Curative-verapamil control | 0.428±0.05 ns | 0.27±0.164 ** |
| X | Curative-nimodipine control | 0.24 ±0.024 ns | 0.25±0.074 * |

Values are mean ± SEM (n=6) one way ANOVA followed by Dunnett test.Significance value = Comparison with vehicle control group animals.*p<0.05 represents significant, **p< 0.01 represents, highly significant and .***p<0.001 represents very significant.ns-no significance

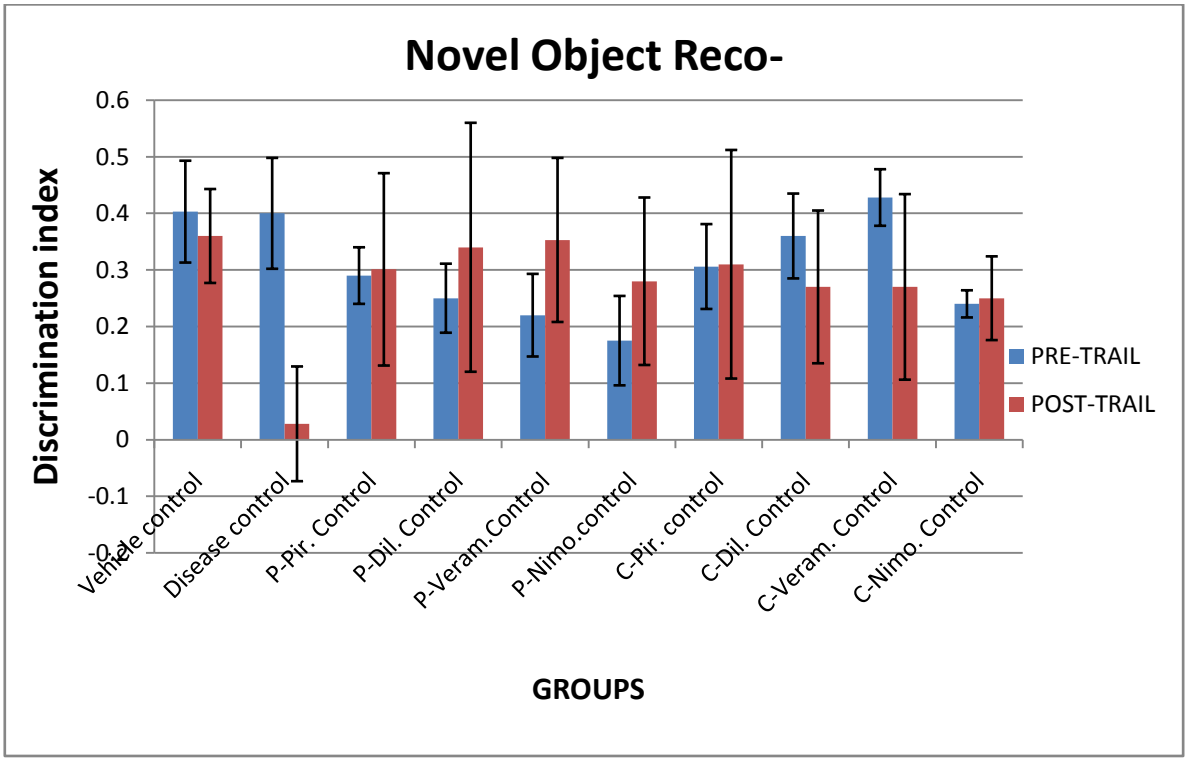


**Figure no 2: Figure showing theeffect of CCB upon discrimination index in novel object recognition test against $AlCl_3$ induced dementia in ratsGroups Treatment**

**Inflexion ratio =**

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**STATISTICAL ANALYSIS:**

The values were expressed as Mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Dunnett multiple comparison test using the GraphpadInstat 3.0 software. P values <0.05 were considered as significant.

**BIOCHEMICAL PARAMETERS**

From the table no 15 & 16, it was found the results from $AlCl_3$ induced dementia model the disease control rats showed decreased levels of antioxidant enzymes CAT, SOD, and GPx in the brain tissue indicating the damage due the generation of ROS. The lipid peroxidation end product MDA levels was elevated in $AlCl_3$ treated group when compared to vehicle control.

The AChE level increased significantly in $AlCl_3$ treated group when compared to vehicle control. Even the oxidized protein levels also significantly elevated in disease group when compared to the vehicle control group.

SOD, CAT, and GPx levels were significantly elevated in CCB treated group when compared to disease control group. The brain tissue protein, AChE, and MDA levels were significantly decreased in CCB treated group when compared to disease control group, but the levels were failed to reach the drug piracetam treated group.

In prophylactic studies it was observed that verapamil offered significant protection followed by nimodipine and diltiazem.

The curative study revealed that the diltiazem offered better protection than the verapamil and nimodipine.

**Table no 3 : Effect of CCB on tissue MDA, CAT &GPx levels in $AlCl_3$ induced dementia in rats**

| Groups | Treatment | MDA (nmol/mg of tissue protein) | CAT (mol of $H_2O_2$ consumed/min/mg of tissue protein) | GPx(µg of glu.consumed/min/mg of tissue protein) |
|---|---|---|---|---|
| I | Vehicle control | 0.47±0.0180 | 0.8583±0.0040 | 0.027±0.0020 |
| II | Disease control | 0.55±0.020 | 0.308±0.054 | 0.010±0.003 |
| III | Prophylactic-piracetam control | 0.27±0.0031 * | 0.725±0.005 ** | 0.023±0.001 ** |
| IV | Prophylactic-diltiazem control | 0.23±0.020 | 0.576±0.04 ** | 0.017±0.002 * |
| V | Prophylactic-verapamil control | 0.22±0.009 * | 0.673±0.024 ns | 0.020±0.003 * |
| VI | Prophylactic-nimodipine control | 0.25±0.014 ** | 0.78±0.025 ns | 0.019±0.003 * |
| VII | Curative-piracetam control | 0.23±0.0140 ** | 0.693±0.0275** | 0.020±0.0026 * |
| VIII | Curative-diltiazem control | 0.23±0.013 ** | 0.721±0.0289 * | 0.015±0.0016 ** |
| IX | Curative-verapamil control | 0.26±0.014 ** | 0.675±0.0236 ** | 0.013±0.0016 ** |
| X | Curative-nimodipine control | 0.27±0.031 ns | 0.696±0.017 ** | 0.013±0.003 n* |

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Values are mean ± SEM (n=6) one way ANOVA followed by Dunnett test
Significance value = Comparison with vehicle control group animals

*p<0.05 represents significant; **p< 0.01 represents, highly significant and ***p<0.001 represents very significant. ns-no significance

**Table no 4: Effect of CCB on tissue SOD, Protein &AChE levels in $AlCl_3$ induced dementia in rats**

| Groups | Treatment | SOD(nmol/mg of tissue protein) | Protein (mg/g of tissue tissue) | AChE (µmol/m/mgof tissue protein) |
|---|---|---|---|---|
| I | Control group | 0.286±0.0150 | 1.091±0.012 | 0.27±0.014 |
| II | Disease control | 0.155±0.004 | 1.515±0.09 | 0.43±0.02 |
| III | Prophylactic-piracetam control | 0.576±0.006 ** | 0.95±0.003 ** | 0.19±0.01 ** |
| IV | Prophylactic-diltiazem control | 0.221±0.0017 ** | 0.83±0.0016 ** | 0.18±0.03 ** |
| V | Prophylactic-verapamil control | 0.713±0.0013 ** | 0.891±0.0065 ** | 0.18±0.008 ** |
| VI | Prophylactic-nimodipine control | 0.578±0.010 ** | 0.79±0.002 ** | 0.16±0.0012 ** |
| VII | Curative- piracetam control | 0.508±0.006 ** | 0.94±0.008 ** | 0.21±0.002 ns |
| VIII | Curative-diltiazem control | 0.565±0.005 ** | 0.833±0.0021 ** | 0.171±0.02 * |
| IX | Curative-verapamil control | 0.481±0.014 ** | 0.76±0.0036 ** | 0.17±0.012 * |
| X | Curative-nimodipine control | 0.633±0.004 ** | 0.783±0.002 ** | 0.16±0.012 ** |

The Values (n=6) by one way ANOVA followed by Dunnett test
Significance value = Comparison with vehicle control group animals; *p<0.05 represents significant; **p< 0.01 represents, highly significant and ***p<0.001 represents very significant. ns-no significance

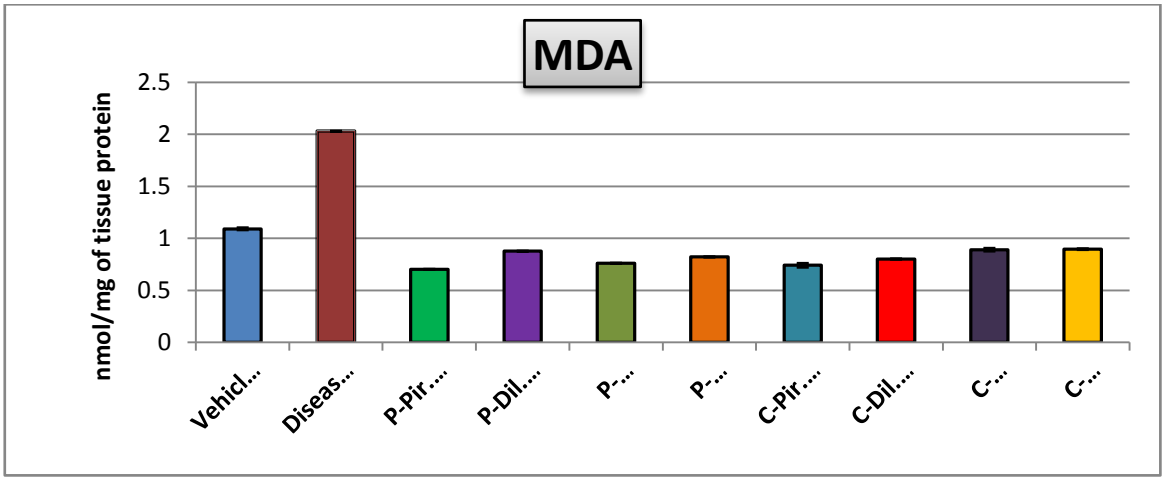


**Figure no 3 : Figure showing theeffect of CCB on tissue MDA levels in $AlCl_3$ induced dementia in rats**

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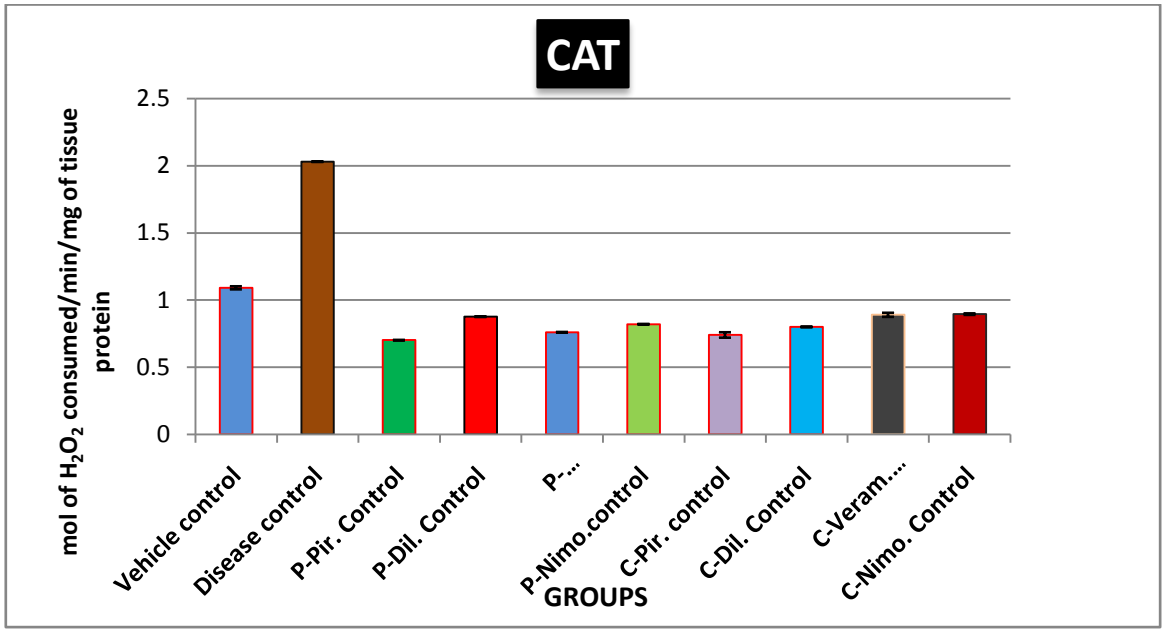


**Figure no 4 : Figure showing theeffect of CCB on tissue CAT levels in $AlCl_3$ induced dementia in rats**

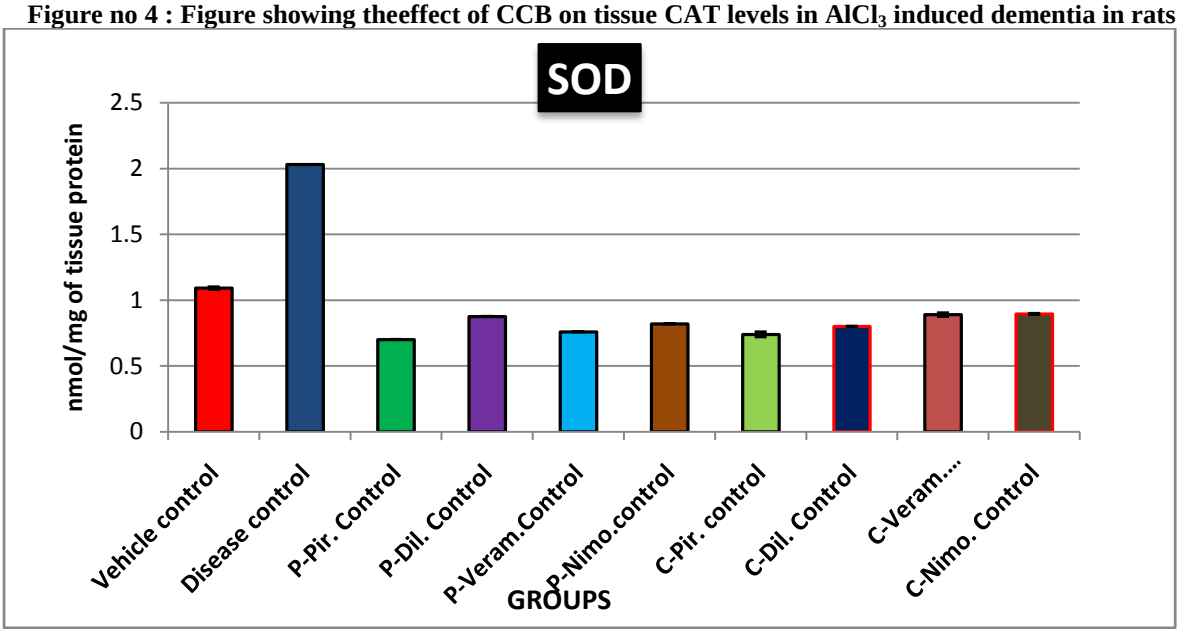


**Figure no 5 : Figure showing theeffect of CCB on tissue SOD levels in $AlCl_3$ induced dementia in rats**

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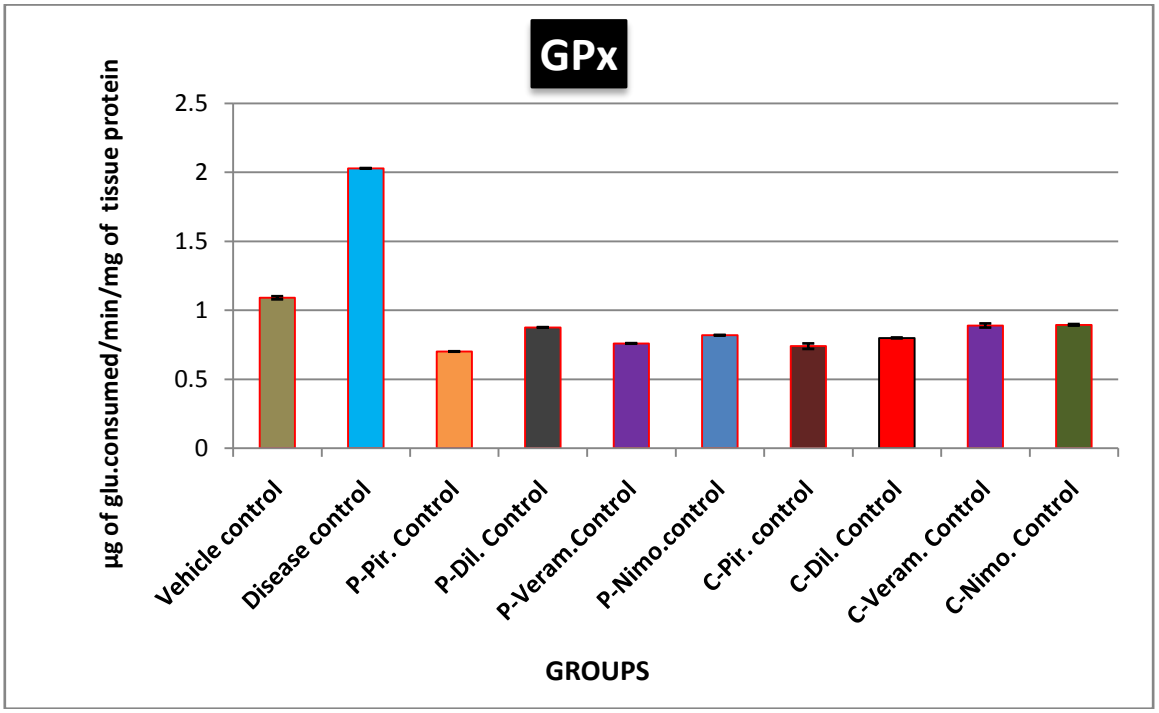


**Figure no 6 : Figure showing theeffect of CCB on tissue GPx levels in $AlCl_3$ induced dementia in rats**

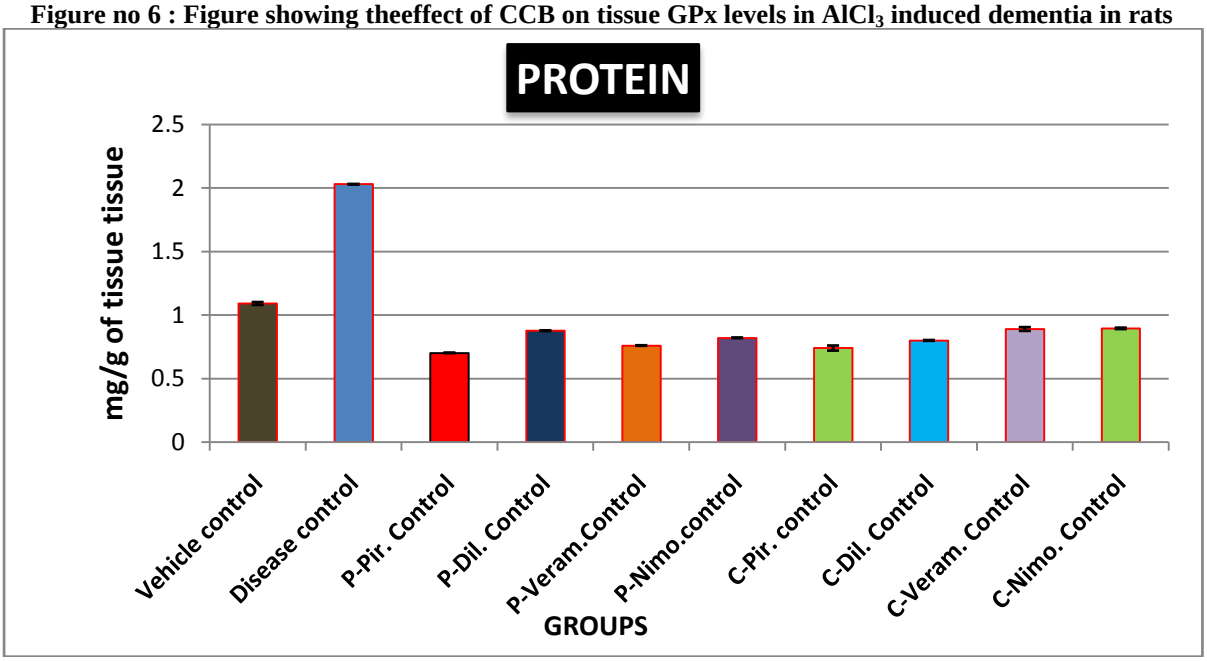


**Figure no 7: Figure showing theeffect of CCB on tissue protein levels in $AlCl_3$ induced dementia in rats**

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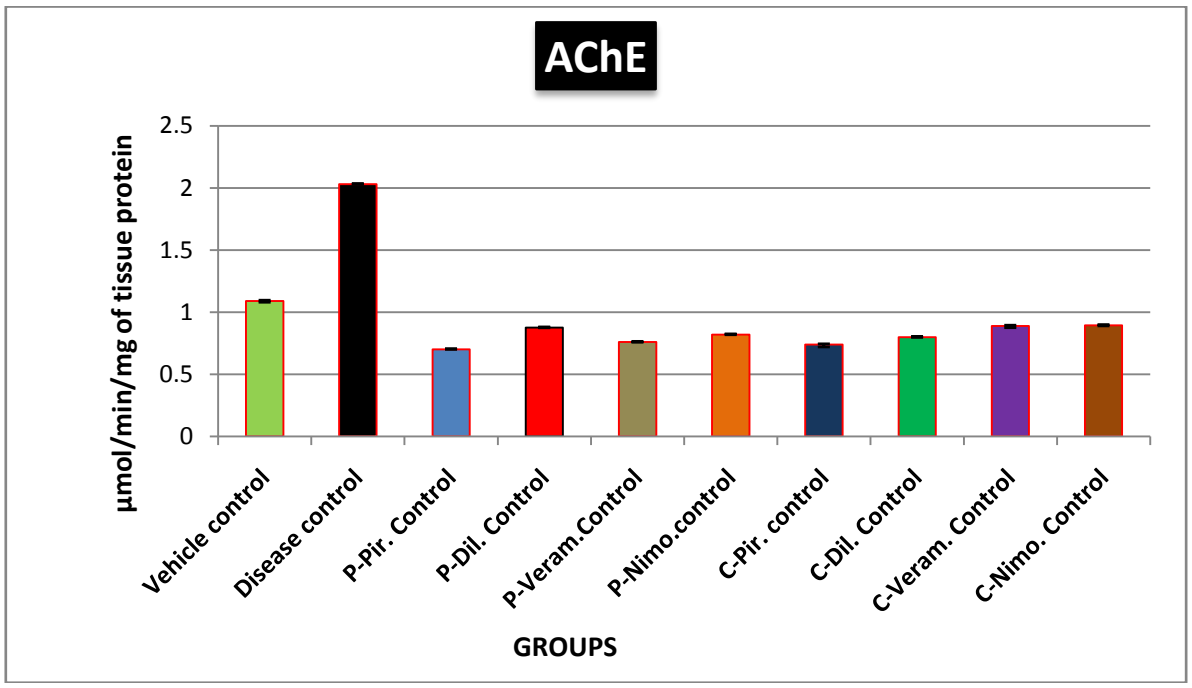


**Figure no 8 : Figure showing theeffect of CCB on tissue AChE levels in $AlCl_3$ induced dementia in rats**

## III. DISCUSSION

The mechanism of cytotoxicity and/or neurotoxicity of many compounds are thought to be mainly due to the oxidative stress involved in the production of reactive oxygen species (ROS), that include superoxide anion, hydrogen peroxide, superoxide radical and hydroxyl radical[41,42].The degree of oxidative damage depends on the balance between the oxidative stress and the efficiency of the antioxidant mechanism that found in the majority of cells[42,43]. In general, brain tissues are highly susceptible to be attacked by free radicals, which are produced because of its high rate of oxidative metabolic activity, and its low level of antioxidant enzymes. Many studies suggested that upon lipid peroxidation, due to oxidative damage and free radicals production, the concentration of polyunsaturated fatty acids decreased and theconcentration of saturated fatty acids increased. This is due to the fact that free radicals attack mainly the double bond sites of unsaturated fatty acids owing to lipid peroxidation[44].

The pathogenesis of AD is multifactorial and includes degeneration of cholinergic neurons, abnormal phosphorylation of the protein tau, oxidative stress, exitotoxicity and altered protein processing resulting in abnormal β-amyloid peptide (Aβ) accumulation and increased blood glucose level which may be due to decreased pyruvate formation that affects the synthesis of acetylcholine[45]. Extracellular aggregates of amyloid-β (Aβ) peptide (amyloid plaques), neurofibrillary tangles and synaptic loss. According to the amyloid cascade hypothesis, overproduction of the hydrophobic peptide $A\beta_{1-42}$ is the basis for AD pathology. Aggregation of $A\beta_{1-42}$ is thought to occur in several steps via fibrils, which are finally deposited as amyloid plaques.

Calcium is a principal intracellular messenger mediating responses to electrical and chemical stimulation. Maintenance of the precise intracellular calcium homoeostasis is fundamental to neuronal viability and functioning. During aging, the control of the intracellular calcium concentration is impaired, leading to neuronal dysfunction.In AD, Aβ induces influx of extracellular calcium, and clinical mutations in the presenilin gene cause calcium release from the endoplasmaticreticulum.Changes in calcium flux across different cellular membranes may lead to neuropathology and cell death. Al-exposed rats showed increased blood glucose level which may be due to decreased pyruvate formation that affects the synthesis of acetylcholine[69].The important role of L-type VDCC in learning and memory is due to their involvement on the synaptic plasticity (i.e., the long lasting LTP) of hippocampal dentate CA1 field, which considers being one possible cellular mechanism underlying cognition.[46] In vivo and in vitro electrophysiological recording of

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hippocampal neuron function have demonstrated the presence of L-type VDCCs, and activity-dependent $Ca^{2+}$ entry into neurons to initiate LTP in this region has been described[49].

Administration of $AlCl_3$ to rats led to a decrease in reduced glutathione (GSH) levels in the hippocampus indicating generation of oxidative stress.(47) Our results were consistent with these data as injection of $AlCl_3$ produced a significant increase in lipid peroxide level expressed as MDA, oxidized protein and AChE and as well as attenuation of SOD, GPx and CAT activities in rat brains compared to vehicle control rats.

AChE is the ACh metabolizing enzyme estimated to evaluate cholinergic function indirectly, the AChE levels are elevated with $AlCl_3$ administration indicates reduced ACh, and decreased AChE levels with treatment of CCB indicates the ACh levels are increased.Our results showed that CCB significantly enhanced the SOD, CAT and GPxactivities that were attenuated by $AlCl_3$ administration. On the other hand, CCB markedly reduced the elevated lipid peroxide levels, MDA, oxidized protein and AChE that were augmented by $AlCl_3$ administration. The learning and memory studies with CCB indicated by a significant elevation of IR and DI in elevated plus maze test and novel object recognition test respectively. Our results are in agreement with the findings of some other studies, reported that CCB produced a marked improvement in both learning and memory, and decreased oxidative stress markers that were increased by scopolamine and $AlCl_3$ induced dementia in rats[48].

The mechanism responsible for CCB protective effect against $AlCl_3$ induced dementia in our studies might be due to relative reduction of cellular calcium influx via its effect on the slow L-type calcium channel. There is involvement of calcium in triggering oxidative damage and excitotoxicity, both of which play central role in $AlCl_3$ induced dementia and associated alterations. Accordingly, reduction of intracellular calcium concentrations by CCB may reduce free radical generating mechanisms and lipid peroxidation. A reasonable concept is that calcium overload enhances the formation of oxygen-derived free radicals and lipid peroxidation. Thus the beneficial action of CCBsmight be two reasons, first by prevention of cytosolic calcium overload and second inhibition of the toxic effects of oxygen-derived free radicals. There are reports indicating the potent antioxidant activity of CCB against lipid auto peroxidation and it was found to be nifedipine has the most potent antioxidant activity[48,49].

The neuroprotective activity of the CCBs in central nervous system was biphasic; increasing concentrations resulted in a decrement of neuroprotection. This suggests that there is a favorable balance in the amount of calcium that is required for the survival of CGCs(cultured cerebral granule cells) and that a deficiency in intracellular calcium may compromise normal function and ultimately promote cytotoxicity[50].

Another possible mechanism involved in neuroprotective property with CCB, by acting nAChRs (neuronal nicotinic cholinergic receptors). Neuronal nicotinic cholinergic receptors (nAChRs) form a heterogeneous family of ligand-gated ion channels found in the central and peripheral nervous system that regulate synaptic activity[51]. Numerous subtypes of nAChRs have been identified and many of them were recognized to be involved in specific neurological and physiological behaviors. For instance, α3β2 nAChR plays a role in dopamine release and Parkinson's disease, α3β4 regulates noradrenaline release and cardiovascular or gastrointestinal action, and α9 was found important in development of auditory functions. Moreover, the most abundant subtypes of the nAChRs in the cortex, i.e., α4β2, α4β4, and α7 are involved in memory, learning, and sensory gating functions[52].

nAChRs are activated by endogenous acetylcholine (ACh) and the group of ortosteric agonists, such as nicotine, while their activity is inhibited by a diverse group of competitive antagonists. Except for these actions, different subtypes of nAChR can be modulated allosterically by various endogenous [e.g., substance P, serotonin (5-HT), fatty acids, steroids or β-amyloid] as well as exogenous (e.g., alkaloids, venom toxins, alcohol, and other drugs) substances with different binding sites on the nAChRs. In fact, over 50 marketed drugs belonging to different therapeutic classes exert allosteric positive (noncompetitive agonists) or allosteric negative (noncompetitive antagonists) modulation on nAChRs, and many of these actions are subtype specific. CCAs can be strong noncompetitive inhibitors for the α3β4 subtype of nAChRs[53,54].

Our data extend the present knowledge about the influence of pharmacological blockade of L-type VDCC on memory and learning processes in the context of possible interactions with cholinergic transmission and oxidative stress markers.

However further studies are required to confirm the neuroprotective activity of CCBs in AD. Studies also have to be extended to find out why verapamil was good in prophylactic studies and diltiazem in curative studies.

There are different mechanisms involved in pathology of dementia includes degeneration of cholinergic neurons, abnormal phosphorylation of the protein tau, oxidative stress, excitotoxicity and altered protein processing resulting in abnormal β-amyloid peptide (Aβ) accumulation and increased blood glucose level which may be due to decreased pyruvate formation that affects the synthesis of acetylcholine, and also the CCB showed the beneficial effect in AD might be by acting as a agonist of nAChRs and reduction of intracellular calcium concentrations reduce free radical generating mechanisms and lipid peroxidation, so further studies are warranted to find out the exact mechanism through which CCB showing the protective activity in AD.

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