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# Development of AD like symptoms following co-administration of AlCl3 and D-gal in rats: A neurochemical, biochemical and behavioural study

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# Development of AD like symptoms following co-administration of $AlCl_3$ and D-gal in rats: A neurochemical, biochemical and behavioural study

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**Abstract**: Alzheimer's disease (AD) is an age-related neurodegenerative disorder associated with neurochemical and neurobehavioural alterations. Aluminium (Al) is considered as a contributing factor in the etiology of several neurodegenerative disorders like AD. D-galactose (D-gal) is a physiological nutrient but over supply induces some neurochemical and biochemical changes that exacerbate natural aging process. In this study, we aimed to develop AD animal model by co-administration of Al and D-gal in rats. Male albino Wistar rats were intraperitoneally injected with $AlCl_3$ and D-gal at a dose of 150mg/kg and 300mg/kg respectively for one week. After one week rats were subjected to behavioural analysis. After behavioural analysis rats were decapitated to remove their brain. Biochemical and neurochemical analysis were conducted in whole brain. $AlCl_3$+D-gal significantly induced depressive and anxious behaviour in rats. Rats cognitive abilities were also significantly impaired following $AlCl_3$ and D-gal co-administration. $AlCl_3$+D-gal significantly altered antioxidant enzyme activities and biogenic amine levels in whole brain. A marked increase in brain lipid peroxidation and acetylcholinesterase activity was found in test rats. These findings suggest that co-administration of $AlCl_3$ and D-gal for one week could induce AD like symptoms and may be used to develop AD animal model.

**Keywords***:* Alzheimer's disease, aluminium chloride, D-galactose, oxidative stress, learning and memory.

## INTRODUCTION

Alzheimer's disease (AD) is the most common and gradually progressing neurodegenerative disorder characterized by alterations in brain structure and functions, starting from mild cognitive dysfunctions and ending with severe brain damage (Mahdi *et al*., 2006). The characteristic symptoms observed in AD include a progressive decline in cognitive functions and behavioural alterations such as difficulties in learning and memory, confusion and agitation (Parsons *et al*., 2013). Many environmental, genetic and physiological factors have been implicated in the pathology of AD (Zatta *et al*., 2002). Aluminium (Al) is considered as a contributing factor in the progression of several neurodegenerative disorders like AD (Bitra *et al*., 2014). Along with neuritic deposits, plaques and neurofibrillary tangles elevated levels of Al have also been observed in AD brain (McLachlan *et al*., 1991). Acidification of environment including usage of chemical fertilizers in agriculture areas and water pollution due to discharge of industrial wastage increases Al bioavailability (Dave *et al*., 2002). A variety of human and animal studies suggested that Al accumulation increases with age and retained in the brain (Good *et al*., 1992) which may be due to either excess of exposure or decreased ability to remove Al from the brain (Dave *et al*., 2002). A number of evidence have indicated that increased Al accumulation in brain may lead to free radical generation and thereby result in the pathology of several neurodegenerative disorders such as AD (Abu-taweel *et al*., 2012). Al causes promotion and accumulation of insoluble amyloid-β (Aβ) protein and aggregation of hyperphosphorylated tau protein which is a characteristic indication of neurodegeneration associated with AD (Kumar *et al*., 2009). D-galactose (D-gal) is a physiological nutrient and a normal reducing sugar in body. D-galactokinase and galactose-1-phosphate uridyl transferase normally metabolize galactose, but long term exposure or over supply results in its abnormal metabolism (Haider *et al*., 2015). Increased D-gal undergoes oxidation processes eventually resulting in production of aldose and hyperoxide radicals through the action of galactose oxidase. Oxidation of D-gal leads to the formation of super oxide anions and other oxygen-derived free radicals that cannot be further metabolized and may get accumulated in the cell resulting in production of reactive oxygen species (ROS) (Wu *et al*., 2008). ROS induced neurotoxicity can exacerbates natural aging process and produces cognitive impairment (Dufour and Larsson, 2004). Brain aging is a threat for many neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (Cui *et al*., 2006). We have

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previously shown that D-gal at a dose of 300mg/ml/kg for one week could induce alterations in biochemical, neurochemical and neurobehavioral parameters, simulating natural aging process and could be used to develop an aging animal model (Haider *et al*., 2015). D-gal mimic natural aging process while Al promotes neurodegeneration by acting as a neurotoxin so their co-administration may result in pathological changes that are associated with AD. With the aim of gaining better understanding of neurodegenerative mechanism of AD, our purpose of study was to evaluate the short term effects of co-administration of $AlCl_3$ and D-gal at a dose of 150mg/kg and 300mg/kg respectively in rats in order to develop an AD animal model that could be further utilized for studying neurodegenerative processes associated with AD.

## MATERIALS AND METHODS

### *Experimental protocol*

Sixteen locally bred young adult male Albino Wistar rats weighing 100-150g were used in the present study. Rats were randomly divided into two groups (n=8): group 1: assigned as control where as group 2: assigned as test. $AlCl_3$ and D-gal were dissolved in physiological saline (0.9%) and were freshly prepared at the beginning of each experiment. Rats in test group were intraperitoneally (i.p) injected with $AlCl_3$ and D-gal at a dose of 150 mg/kg and 300 mg/kg respectively for one week, while rats of the control group received equivalent volume of 0.9% saline. 24h after the last injection rats were subjected to behavioural analysis and 24h after the last behaviour rats were decapitated to collect their brain for further biochemical and neurochemical analysis. Each experiment was conducted in a balance designed with proper conditions and responses were evaluated in a quiet fixed schedule to avoid time and order effect.

### *Behavioural analysis*

Anxiety of rats was assessed with the help of light dark transition (LDT) test, number of entries and time spent in light compartment was monitored. Forced swim test (FST) was used to assess depression like symptoms in rats by monitoring immobility time. Working memory of rats was assessed by Morris water maze (MWM) test, passive avoidance test (PAT) and elevated plus maze (EPM) test. In MWM escape latency was monitored. Step through latency was monitored in PAT. In EPM transfer latency was monitored. The assessment of exploratory activity was performed by using open field test (OFT) in which number of squares crossed by rats were monitored, as described previously (Haider *et al*., 2015).

### *Biochemical estimations*

Estimation of lipid peroxidation (LPO) was performed as described by Chow and Tappel (1972) with slight modifications (Haider *et al*., 2015) in terms of malondialdehyde (MDA) levels and was presented as µmoles of MDA/g of brain. Brain superoxide dismutase (SOD) activity was estimated by the method of Chidambara *et al* (2002). Activity of SOD was presented as U/g of brain. Catalase (CAT) activity was assessed as described by Sinha (1972). CAT activity was presented as consumption of $H_2O_2$ µmol/min/g of brain. Glutathione peroxidase (GPx) activity was determined by the procedure of Flohe and Gunzler (1984). Activity of GPx was presented as µmol/min/g of brain.

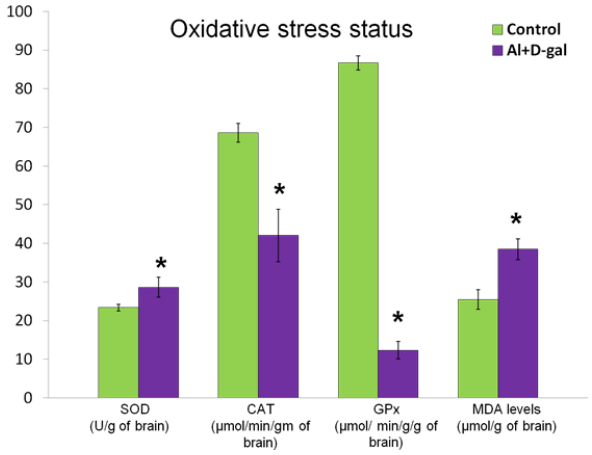


**Fig. 1**: Oxidative stress status of $AlCl_3$+D-gal treated and control group rats. Effect of $AlCl_3$+D-gal administration on antioxidant enzymes activities including superoxide dismutase (SOD) (U/g of brain tissue), catalase (CAT) (µmol/min/g of brain tissue), glutathione peroxidase (GPx) (µmol/min/g of brain tissue) and lipid peroxidation in terms of malondialdehyde (MDA) levels (µmol/g of brain tissue). Values are mean ± SEM (n=8). Mean differences for each group were evaluated by an independent sample t-test. Statistical difference is represented as **P*<0.01.

### *Neurochemical estimations*

Acetylcholinesterase (AChE) activity in the whole brain was estimated as described by Ellman *et al*. (1961). The activity of AChE was presented as µmol/min/g of brain. The neurochemical analysis was done to assess concentrations of brain biogenic amines as described by Haider *et al*. (2014).

## STATISTICAL ANALYSIS

Data are presented as mean ± SEM (n=8). Mean differences for each group were evaluated by an independent sample t-test via SPSS version 17.0 software and values of $p$□0.01 were defined as statistically significant. Correlation analysis was performed via SPSS by using Pearson's correlation test.

## RESULTS

### *Effect of co-administration of $AlCl_3$ and D-gal on behaviours*

Data presented in table 1 indicates the effects of co-administration of $AlCl_3$ and D-gal on behaviours. Test

**Table 1**: Effects of co-administration of $AlCl_3$ and D-gal on behaviours light dark transition (LDT), forced swim test (FST), open field test (OFT), Morris water maze (MWM) test, elevated plus maze (EPM) test, passive avoidance test (PAT). Values are mean ± SEM (n=8). Mean differences for each group were evaluated by an independent sample t-test. Statistical difference is represented as **P*<0.01.

| Behaviours | Parameters | Control | Al+D-gal | t-Values |
|---|---|---|---|---|
| LDT | Time Spent (sec) | 74.08± 5.72 s | 40.71±7.40 s* | 6.000 |
| | Number of Entries | 3.42±0.49 | 2.14±0.34* | 10.069 |
| FST | Immobility Time (sec) | 77.27±6.24 s | 97.98±8.58 s* | 5.517 |
| OFT | Number of Square Crossed | 53.35±7.75 | 67.08±8.96* | 3.276 |
| MWM | Escape Latency (sec) | 17.85±2.67 s | 49.62±5.58 s* | 14.522 |
| EPM | Transfer Latency (sec) | 38.22±8.63 s | 60.10±5.28 s* | 6.110 |
| PAT | Step through Latency (sec) | 173.14±8.54 s | 154.17±6.79 s* | 4.916 |

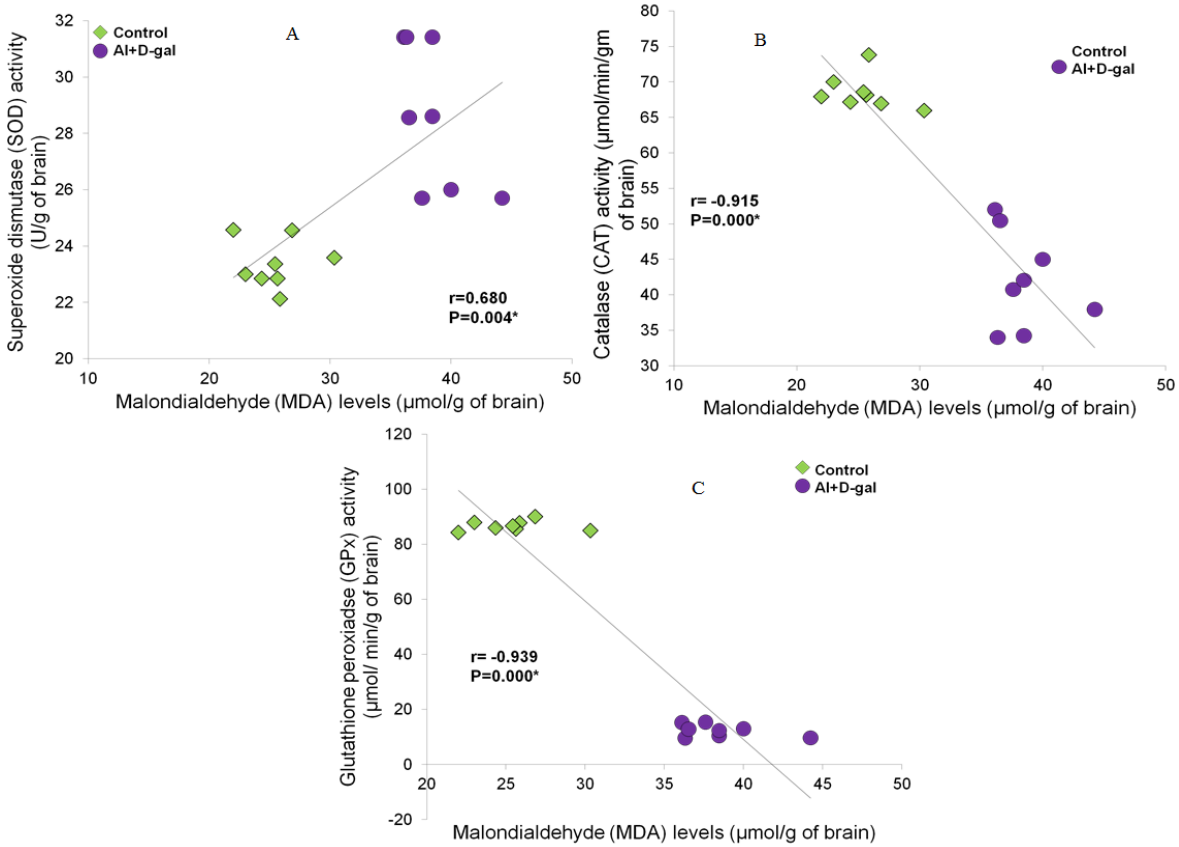


**Fig. 2**: The Pearson correlation between oxidative marker malondialdehyde and antioxidant enzymes. Correlation was performed by using Pearson's correlation test. Correlation of (a) SOD, (b) CAT and (c) GPx with MDA levels.

group rats exhibited significant ($p$□0.01) reduced numbers of entries and time spent in the light compartment as compared to control rats suggesting an anxiogenic effect of $AlCl_3$+D-gal administration. $AlCl_3$+D-gal treated rats exhibited a significant increased ($p$□0.01) immobility time in FST as compared to control rats. Increased immobility time showed depressogenic behaviour in test rats. Rats treated with $AlCl_3$+D-gal showed increased exploratory activity ($p$□0.01) as indicated by increased number of square crossed in OFT as compared to control rats. In MWM $AlCl_3$+D-gal treated rats exhibited a significant ($p$□0.01) increase in escape latency during the test session as compared to control. $AlCl_3$+D-gal treated rats showed significant ($p$□0.01) increased transfer latency as compared to control rats in EPM. The step through latency in PAT was significantly ($p$□0.01) decreased following $AlCl_3$+D-gal administration for one week than those of the control rats.

***Effect of co-administration of $AlCl_3$ and D-gal on brain lipid peroxidation (LPO) and antioxidant enzyme activities***

Fig. 1 describes oxidative stress status of control and Al+D-gal treated rats. One week co-administration of

$AlCl_3$ and D-gal significantly altered the antioxidant enzyme activities. SOD activity was significantly increased in the test group (28.60±2.60U/g) compared with control rats (23.36±0.85U/g) ($p$□0.01). CAT (42.05± 6.79µmol/min/g) and GPx (12.31±2.27µmol/ min/g) activities were significantly ($p$□0.01) decreased in the test group as compared to control rats (CAT=68.56±2.43 µmol/min/g; GPx=86.68±1.86µmol/ min/g). $AlCl_3$+D-gal treated rats (38.46±2.67 µmol/g) showed a significant increase in MDA levels ($p$□0.01) compared with control rats (25.43±2.54µmol/g). Pearson correlation analysis fig. 2(a-c) between oxidative stress marker MDA and antioxidant enzymes revealed that the activities of CAT (r=-0.915, $p$<0.01) and GPx (r=-0.939, $p$<0.01) were negatively correlated with MDA levels, while the activity of SOD (r=0.682, $p$<0.01) was positively correlated with MDA levels.

***Effect of co-administration of $AlCl_3$ and D-gal on brain acetylcholinesterase (AChE) activity and biogenic amine levels***

AChE activity was significantly ($p$<0.01) increased in the brain of $AlCl_3$+D-gal treated rats (3678.04±252.40 µmol/min/g) as compared to control rats (2627.40± 162.79µmol/min/g) as shown in fig. 3. Data in fig. 4(a) shows the chromatogram of standard solution containing biogenic amines noradrenaline (NA), dihydroxyphenyl acetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT) whereas fig. 4(b) shows the chromatogram of biogenic amines in the whole brain of control rats and $AlCl_3$+D-gal treated rats. The results of neurochemical estimation in fig. 4(c) shows the changes in whole brain biogenic amine levels. $AlCl_3$+D-gal administration also reduced 5-HT (176.28± 31.56ng/g) and 5-HIAA (126.46±25.36ng/g) ($p$<0.01) levels in whole brain of the test rats when compared to control rats 5-HT (274.28±26.561ng/g), 5-HIAA (312.26±58.56ng/g). The DA (124.65±13.0ng/g) and DOPAC (153.75±15.90ng/g) levels in the test rats showed a significant decline ($p$<0.01) as compared to control rats DA (413.11±60.162ng/g), DOPAC (423.58± 46.79ng/g).

## DISCUSSION

The results of present study demonstrate that co-administration of $AlCl_3$ and D-gal results in marked impairment of working memory as indicated by increase in retention latencies as compared to the control group in both MWM and EPM. Cognitive dysfunction and behavioural alteration by Al have been reported in a number of studies (Gong *et al*., 2006; Abu-taweel *et al*., 2012). The results of the present study indicated that co-administration of $AlCl_3$ and D-gal to rats significantly deteriorates working memory in MWM. This memory deterioration was also observed in PAT and EPM. $AlCl_3$+D-gal treatment showed depression like symptoms as indicated by increased immobility time in test rats compared to control rats in FST. $AlCl_3$+D-gal injected rats also exhibited anxiogenic behaviour in LDT. Anxiety and depression are the most common neuropsychiatric conditions observed in AD patients (Teri *et al*., 1999) these observations are consistent with our results. 5-HT is known to be involved in many psychological conditions such as depression, anxiety, aggression or psychosis. Previous reports have shown extensive serotonergic denervation in AD and reduced serotonergic functions have been associated with aggressive behaviour (Lancot *et al*., 2001). Consistent with the previous reports in our study following $AlCl_3$+D-gal treatment 5-HT and its metabolite 5-HIAA levels were altered in whole brain of test rats as compared to control rats, possible cause of depressive and anxiogenic behaviour in rats. Decrease 5-HT levels in brain may result in up regulation of serotonergic receptors leading to anxiogenic like behaviour (Nutt *et al*., 1999). $AlCl_3$+D-gal injected rats also showed hyperactivity in OFT. Hyperactivity is associated with stress conditions as rats feel anxious in an unfamiliar or novel environment (Rebai and Djebli, 2008).

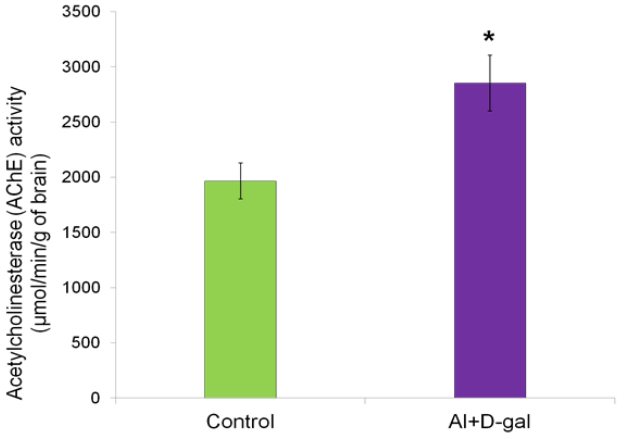


**Fig. 3**: Brain acetylcholinesterase (AChE) activity (µmol/min/g of brain tissue) was measured in control and test rats. Values are mean ± SEM (n=8). Mean differences for each group were evaluated by an independent sample t-test. Statistical difference is represented as *$P$<0.01.

Short term memory is generally associated with the levels of acetylcholine (ACh) in brain, as depletion in ACh levels is closely related to dementia (Amberla *et al*., 1993). The determination of key enzymes of cholinergic system such as AChE is used to indirectly reflect ACh levels (Gong *et al*., 2006). In our study $AlCl_3$+D-gal treatment resulted in marked increase in AChE activity. This increase in AChE activity may be due to the genetic over expression of AChE by oxidative stress produced by D-gal (Zhong *et al*., 2009) while increased AChE activity following $AlCl_3$ administration may be due to allosteric interaction between the cation and peripheric anionic site of the enzyme may further results in enhanced AChE

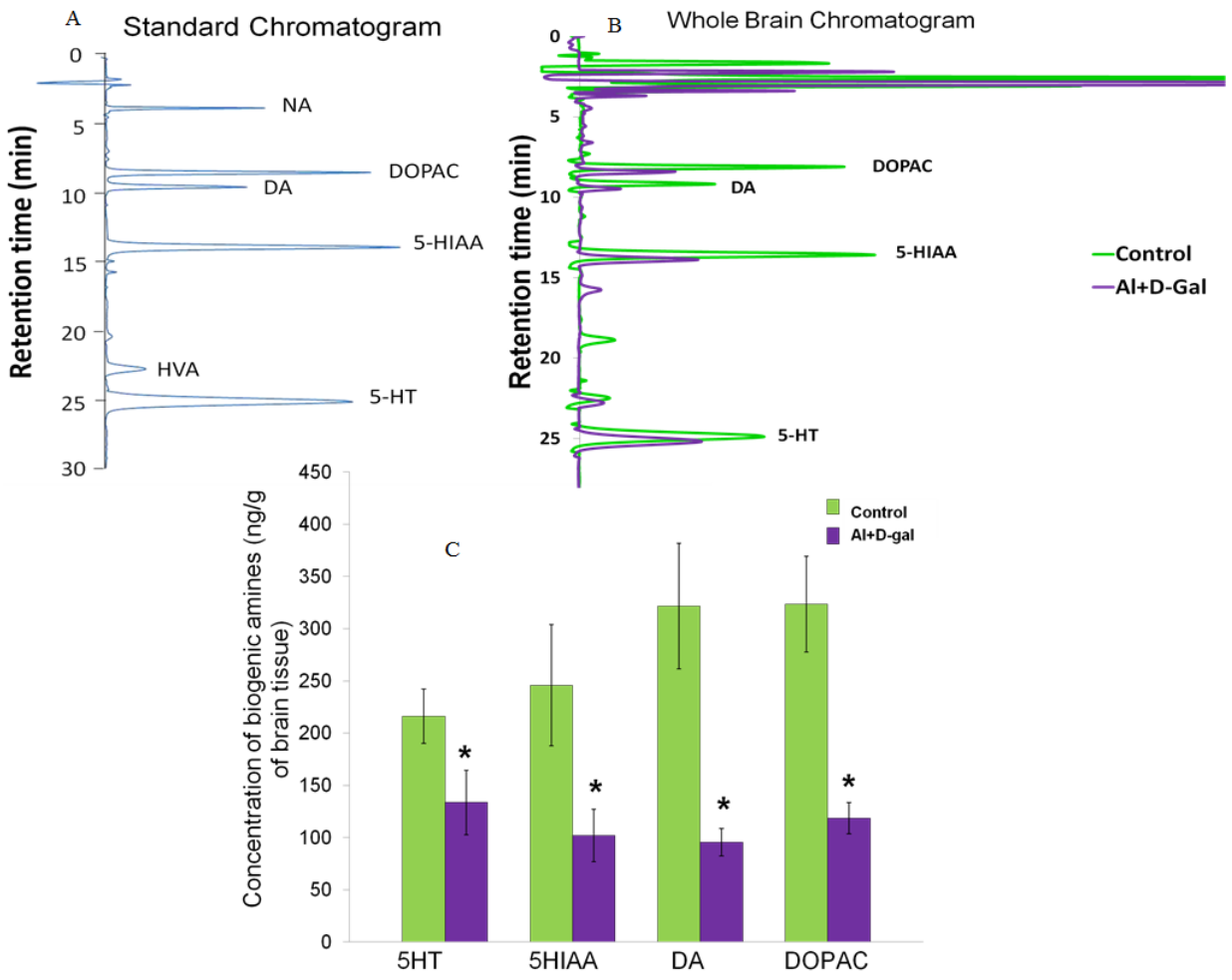


**Fig. 4**: **(**a) HPLC chromatogram showing the retention time of noradrenaline (NA), dihydroxyphenyl acetic acid (DOPAC), dopamine (DA), 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 5-hydroxytryptamine (5-HT) in standard solution and (b) whole brain of rats treated with saline and $AlCl_3$+D-gal (c) estimated levels of biogenic amines in whole brain of the control and test rats (***P*<0.01). Values are mean ± SEM (n=8). Mean differences for each group were evaluated by an independent sample t-test. Statistical difference is represented as **P*<0.01.

activity (Gulya *et al*., 1990). So their combined effect results in marked increased in AChE activity. These cholinergic deficiencies may also be linked to the neuropsychiatric aspects of the present study, as clinical observations suggest an important link between neuropsychological impairments observed in AD and cholinergic dysfunctions (Cummings and Kaufer, 1996). AChE activity was also correlated with escape latency, and a positive correlation was found and hence the resulted cognitive dysfunctions observed in present study may be strongly related to AChE activity.

In the present study combined treatment of $AlCl_3$ and D-gal leads to decline levels of 5-HT, 5-HIAA, DA and DOPAC in whole brain of test rats as compared to control rats. Aging has been associated with declined levels of DA and 5-HT and their metabolites in hippocampus, cortex and whole brain (Haider *et al*., 2014). Psychological alterations and decline in cholinergic and serotonergic functions are excessively reported in aged and AD patients (Cummings and McPherson, 2001).This is parallel with our neurochemical results and also with our behavioural observations. A significant decline in 5HT, DA and their metabolites was observed in whole brain of rats treated with combination of $AlCl_3$ and D-gal compared to control rats. The deposition of Aβ plaques in brain is the characteristic pathological feature of AD. It has been shown that combined treatment of $AlCl_3$ and D-gal caused remarkable alterations in the expression of Aβ metabolism-associated protein and has a stronger effect on Aβ deposition (Luo *et al*., 2009). Deposition of plaques and tangles induce neuronal dysfunction and destruction of synapse and neurons that eventually results in deprived neurotransmission (Mesulam, 1996). Taken together this could explain neurochemical dysfunctions and behavioural alterations observed in the present study.

Although histopathological studies were not done in the present study but observed changes may be correlated with plaques and tangles associated neurodegeneration.

We further investigated the mechanism involved in the deleterious effects of co-administration of $AlCl_3$ and D-gal on antioxidant defense system. Increased LPO results in neuronal damage and may lead to neurodegenerative disorders like Alzheimer's and Parkinson's disease (Kaizer *et al*., 2005). Under the oxidative stress conditions, SOD attacks first by converting overloaded superoxide ($O_2^-$) radicals to hydrogen peroxide radicals ($H_2O_2$). These $H_2O_2$ radicals further scavenged by GPx and CAT result in production of water and molecular oxygen (Naidu *et al*., 2013; Haider *et al*., 2015). The free radical scavenging properties of SOD is only advantageous when it is proceeds by the scavenging activity of GPx and CAT (Bhattacharya *et al*., 2001). Excess of SOD with decrease CAT and GPx activity is known to have destructive tissue effects (Blake *et al*., 1987). MDA is widely used as oxidative stress biomarker, which is formed as an end product of LPO induced by free radicals (Cui *et al*., 2006). In the present study one week co-administration of $AlCl_3$ and D-gal to rats caused significant increased in oxidative stress. Following $AlCl_3$ and D-gal administration an increase in LPO in terms of MDA levels, increase in SOD activity with reduced activities of CAT and GPx was observed in the brain of test rats as compared to control rats. Enhanced LPO may interfere with cholinergic pathways possibly causing cholinergic dysfunction and leads to learning and memory impairment (Kaizer *et al*., 2005). The results of the present study showed that combination of $AlCl_3$ and D-gal has more toxic effects on antioxidant defense system and promote ROS induced oxidative damage. Activities of SOD, CAT and GPx were also correlated with MDA levels. A positive correlation was found between SOD and MDA levels whereas negative correlation was found between GPx and MDA levels and CAT and MDA levels in $AlCl_3$ and D-Gal treated rats.

## CONCLUSION

Although $AlCl_3$ and D-gal work through their own pathways but eventually result in similar pathological conditions and symptoms observed in AD. Present findings suggest that combined administration of $AlCl_3$ and D-gal for one week could induce AD like symptoms and may produce neurochemical and neurobehavioral alterations similar to AD. Hence co-administration of $AlCl_3$ and D-gal would produce an ideal AD animal model for understanding the underlying mechanism involved with neurodegenerative alteration in AD brain. This AD animal model may be more appropriate choice to understand pathogenesis, diagnosis, prevention and even treatment of AD in future studies as it is less time consuming and inexpensive.

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