Modeling Stages Mimic Alzheimer’s Disease Induced by Different Doses of Aluminum in Rats: Focus on Progression of the Disease in Response to Time

Azza A Ali*, Hebatalla I Ahmed, Karema Abu-Elfotuh
Department of Pharmacology & Toxicology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

Introduction
Alzheimer’s disease (AD) is a degenerative disorder of the brain that leads to memory loss. There are two main forms of the disease; familial AD affects people younger than 65 years [1] while the remainder of AD cases occurs in adults aged 65 and older and is classified as sporadic AD [2]. It is a progressive disease, where dementia symptoms gradually worsen over a number of years. There are three stages of the disease; mild, moderate and severe. In early stage memory loss is mild, but with late-stage AD individuals lose the ability to carry on a conversation and respond to their environment [3,4]. The prevalence of AD varies among many different factors, including age, co-morbidities, genetics, and education level [5]. The changes in the brain caused by AD are not usually the primary cause of death. AD often causes complications such as immobility and trouble swallowing, thus can lead to malnutrition and increased risk of pneumonia, resulting in death [6]. AD affects people in different ways, thus each person experience symptoms or progress through the disease stages differently [7].

Aluminum (Al) is a constituent of antacids, deodorants and food additives which allowed easy access into the body. Its neurotoxicity in animals has been clearly established...
and shown to be involved in etiology of neurodegenerative diseases such as AD. It promotes the formation of amyloid-β (Aβ) protein plaques by aggregating tau proteins in the brain. Al has also been implicated in aging related changes and neurodegeneration. It is reported that Al toxicity is due to potentiating the activity of Fe $^{2+}$ and Fe $^{3+}$ ions to cause oxidative damage. It also interacts with calcium binding sites and disrupts calcium homeostasis and thereby induces neurodegeneration. Moreover, Al toxicity was found to be associated with reduced axonal length and dendritic branches in hippocampus [8,9]. Administration of AlCl$_3$ predominantly accumulates in the hippocampus and this region is known to be particularly susceptible in AD and has important role in learning and memory functions [8]. For these reasons, AlCl$_3$ model was selected for the present study. In general, Animal models of a disease are a cornerstone of the drug development process; their function is to closely mimic the disease or an aspect of the disease in human, thus AD animal model is a scientific tool for the testing of new drugs [10].

In the light of what was mentioned, the aim of this study was to establish model mimics of AD in rats by using different doses of AlCl$_3$. It also aimed to study stages and progression of AD in rat's brain in response to time by using these different doses of AlCl$_3$ for different periods. In addition, the present study aimed to determine mode of the disease progression in rat's brain despite stopping administration of AlCl$_3$. By modeling stages mimic AD and identifying its progression; prediction is possible, symptoms can be expected and the power to find real treatment will be enhanced.

**Materials and Methods**

**Animals**

Eighty eight male Sprague Dawley rats, weighing 250-280 g and obtained from The Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt were used. They were housed in stainless-steel cages, three to four per cage, at a temperature of 25 ± 1°C with alternatively 12 hour light and dark cycles. Water was given for rats ad-libitum and they were kept under the same controlled conditions and provided with their daily dietary requirements of standard diet pellets (El-Nasr; Abu Zaabal, Cairo, Egypt) contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture. Rats were taken to test situation one hour before each experiment for adaptation and after removing food and water from the cages. All experiments were usually carried out at a fixed time from 8 AM; 12 PM. The study was conducted in accordance with the ethical guidelines of Faculty of Pharmacy, Al-Azhar University, Egypt.

**Drugs and chemicals**

Aluminum chloride - hydrated (AlCl$_3$·6H$_2$O), was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was freshly dissolved in distilled water. All other chemicals and solvents were of highest grade-commercially available.

**Experimental design**

For modeling stages of neurotoxicity that mimick AD: 40 rats were randomly assigned to four groups (10/group) and treated daily for six weeks as follows; the first group served as control and was given saline. The other three groups were injected LP with different doses of AlCl$_3$·6H$_2$O; 100 mg/kg [11] and 70 mg/kg (chosen for this study), as well as 50 mg/kg [12]. All treatments were administered at dose volume not exceeding 0.5 ml/200 g body weight. Two behavioral tests (2 days time interval between them) were carried out at the end of the six weeks; Morris Water Maze (MWM) test and Conditioned Avoidance (CA) test. Rats were sacrificed 24 h after the last test and the brain tissues were dissected and washed with ice-cold saline. The brain tissues were either subjected for biochemical analysis immediately or kept frozen at -80°C till the time of analysis where they were homogenized in saline. The brain tissue homogenates were used to assess oxidative stress markers; lipid peroxides expressed as malondialdehyde (MDA), superoxide dismutase (SOD) and total antioxidant capacity (TAC). Acetylcholine esterase (ACHE) activity and Aβ content were also assessed for all groups. For histopathological examinations, the brain was removed, cleaned, washed with phosphate buffer saline (PH 7.4) and specimens from the different brain areas of treated groups were taken (24 h after administration of the last doses).

For studying neurotoxicity that mimics AD progression:

Rats were divided into six groups (6/each) and received AlCl$_3$ (once daily, I.P) as follows; Group 1, 2: Received AlCl$_3$ (100mg/ kg) for four and five weeks respectively. Group 3, 4: Received AlCl$_3$ (70mg/kg) for four and five weeks respectively. Group 5, 6: Received AlCl$_3$ (50mg/kg) for four and five weeks respectively. All treatments were administered at dose volume not exceeding 0.5 ml/200 g body weight. 24 h after administration of the last doses, rats were weighed and sacrificed under light ether anesthesia. The brain of each rat was removed, cleaned, washed with phosphate buffer saline (PH 7.4), and used for histopathological examination for different brain regions.

For determination of neurotoxicity that mimics AD progression after the causative stopped:

Two groups (6/each) were treated according to the following schedule: Rats received AlCl$_3$ (LP once daily and at dose volume not exceeding 0.5 ml/200 g body weight) in a dose of 70 mg/kg (Group 1) or 50 mg/kg (Group 2) for six weeks then stop administration for one week. Then and after 24 h, rats were weighed and sacrificed under light ether anesthesia. The brain was removed, cleaned, washed with phosphate buffer saline (PH 7.4), and used for histopathological examination for different brain regions.

**Behavioral experiments**

Two behavioral experiments with different degree of stressfulness were used as an integrative testing battery to allow measuring the most behavioral changes in AD rat’s models.

Morris water maze (MWM) test: As previously described, it is a hippocampus dependent spatial learning task [13]. The MWM tank was 150 cm in diameter, 62.5 cm in height, painted black, and filled to a depth of 40 cm with water maintained at a temperature of 20 ± 1°C. Around the room, numerous visual cues were present which remained constant throughout the experiment. The maze was divided geographically into four quadrants northeast (NE), northwest (NW), southeast (SE), southwest (SW), and starting positions, north (N), south (S), east (E), west (W) that were equally spaced around the perimeter of the pool, a hidden circular platform (diameter: 13 cm) was located in the center of the NW quadrant, 1 cm below the surface of the water. All rats were required to learn to locate an escape platform in a pool of water, using visual cues surrounding the
maze. The position of the escape platform remained the same for all the animals across the training trials. During four consecutive daily sessions (each session consisted of four trials) after AlCl₃ treatments for six weeks, rats were trained to find a submerged escape platform located in a fixed position. Four different starting positions, equally spaced around the perimeter of the pool, were used in a fixed order. Each trial had a maximum duration of 60 sec began with releasing the rats in MWM then calculated escape latency (time in seconds taken to escape on to the submerged platform). For the training trials, escape latency was averaged per rat (four different positions) then calculated the averages of the groups. At the end of each trial the rats were allowed to remain on the platform for 20 sec in order to recognize the place well. Rats not finding the platform within 60 sec were placed on it. Two hours after the last training trial, rats were subjected to a memory probe trial during which they swam for 60 sec in the absence of the training platform. All rats started from the same position, opposite to the target quadrant (the quadrant where the escape platform had been positioned). Time spent in the target quadrant (probe trial time) was calculated in seconds. A video camera (Nikon, Melville, NY, USA) linked to a computer was mounted directly above the MWM pool to record the escape latency (indicating learning ability), as well as the time spent in the target quadrant (memory trial).

**Conditioned avoidance (CA) test:** The CA apparatus consists of five interconnected chambers; four of them can be electrified through their stainless steel grid floor, the fifth chamber represents the safety area (glass floor) as previously described and modified [14-16]. The stainless steel grid floor can be electrified using a laboratory DC power supply, model GPR-6060 D to deliver the foot shock (50 volts, 25 pulse/sec) which represented un-conditioned stimulus. The use of this test was extended and the parameters were manipulated for evaluating learning ability and memory consolidation in these high stressful conditions. Rats were alternatively introduced in the CA apparatus; training was conducted by using the auditory conditioned stimulus (electric bell for 5 sec.) followed by 5 sec. of foot shock. Number of trials at the 1st and 2nd day of training to avoid the electric shock and reach the safety area during 5 sec. of the conditioned stimulus were calculated for each rat indicating learning ability, as well as the time spent in the target quadrant (memory trial).

**Biochemical measurements**

**Protein estimation:** The protein content was measured in the brain homogenates using bovine serum albumin as a standard and according to Bradford method [17].

**Assessment of oxidative stress markers:** In the brain homogenate of each rat, SOD, TAC and MDA were measured. Relying on the ability of the enzyme to inhibit the phenazine methosulphate mediated reduction of nitroblue tetrazolium dye, the SOD activity was assessed [18], while TAC activity determination is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide H2O2. The residual H2O2 is determined colorimetrically by an enzymatic reaction which involves the conversion of 3, 5-dichloro-2-hydroxybenzene sulphonate to a colored product [19]. On the other hand, by estimating the level of thiobarbituric acid reactive substances measured as MDA, Lipid peroxidation was determined [20].

**Determination of ACHE activity:** In the brain tissue homogenate, ACHE activity was assessed using ELISA Kits (Ray Biotech, Inc., USA) according to their manufacturer’s instructions.

**Determination of Aβ content:** In brain tissue homogenate, determination of Aβ content was assessed using ELISA Kits (USCN Life Science, Inc., Product Number MBS702915) according to their manufacturer’s instructions.

**Histopathological examinations in different brain regions**

For histopathological examinations, rat’s brain specimens were prepared and stained [21]. They were fixed in 10% formalin for 24 h and then washed with tap water. For dehydration, serial dilutions of alcohol were used. Specimens were cleared in xylene embedded in paraffin at 56°C in hot air oven for 24 h. Paraffin wax tissue blocks were prepared for sectioning at 4 microns thickness by microtome. On glass slides, the obtained tissue sections were collected, deparaffinized and were stained with hematoxylin & eosin stain for light microscopy and histological examination.

**Statistical analysis**

Data were presented as mean ± SEM. Multiple comparisons were performed using one-way ANOVA followed by Tukey Kramer as a post hoc test. As the criterion for significance, the 0.05 level of probability was used. Statistical analyses were performed using Instat (version 3) software package and Graphs were sketched using GraphPad Prism (IST®, USA) software (version 5).

**Results**

**Behavioral changes induced by different doses of AlCl₃ in MWM test**

As shown in Figure 1a, there was a decrease in learning ability of rats treated with different doses of AlCl₃ (100, 70, 50 mg/kg I.P, for 6 weeks) as indicated by the significant increase in escape latency (sec) from the first to the fourth day of training in MWM test. The escape latency increased to approximate 400.00%, 428.08%, 573.33%, 714.08% respectively with AlCl₃ 50 mg/kg and to 345.79%, 336.41%, 270.00%, 254.00% respectively with AlCl₃ 70 mg/kg as well as to 297.02%, 301.04%, 241.66%, 218.00% respectively for AlCl₃ 50 mg/kg as compared to control group.

![Figure 1a: Effect of different doses of AlCl₃ on escape latency (sec) in Morris water maze test.](attachment://image_url)

Data expressed as Mean ± SEM (n = 10).

a, b, c: Significantly different from control, AlCl₃ 100 mg/kg, between AlCl₃ 70 and 50 mg/kg respectively at P<0.05 using one way ANOVA followed by Tukey-Kramer as a post hoc test.
The changes in the time spent in target quadrant (sec), indicating memory retention, is shown in Figure 1b. It significantly decreased in rats treated with different doses of AlCl$_3$ (100, 70, 50 mg/kg I.P for 6 weeks) reached to approximately 17.112%, 33.422% and 45.721% respectively from the control values.

**Behavioral changes induced by different doses of AlCl$_3$ in CA test**

Rats treated with different doses of AlCl$_3$ (100, 70, 50 mg/kg I.P) showed marked increase in the number of trials to avoid the electric shock at the 1st day of the experiment, indicating decrease in learning ability, amounted to 725%, 481.25% and 300% respectively compared to control group (Figure 2a). Administration of different doses of AlCl$_3$ (100, 70, 50 mg/kg I.P) caused marked increase in the number of trials to avoid the electric shock at the 2nd day of the experiment amounted to 925.2%, 560% and 425.1% respectively with respect to control group (Figure 2b).

The results are shown in Figure 3a, b, c. Administration of different doses of AlCl$_3$ (100, 70, 50 mg/kg I.P) to rats resulted in marked increase in MDA level with respect to control group. 100 mg/kg of AlCl$_3$ caused marked increase in the MDA level than 70 mg/kg and 50 mg/kg, while 70 mg/kg of AlCl$_3$ showed marked increase in MDA level as compared 50 mg/kg treated rats. Different doses of AlCl$_3$ treated group showed significant increase in MDA level by approximately 581.8%, 503.7% and 396.07% respectively as compared to control group.

**Figure 1b:** Effect of different doses of AlCl$_3$ on the time spent in target quadrant (sec) in Morris water maze test. Data expressed as Mean ± SEM (n = 10).

a, b, c: Significantly different from control, AlCl$_3$ 100 mg/kg, between AlCl$_3$ 70 and 50 mg/kg respectively at P<0.05 using one way ANOVA followed by Tukey-Kramer as a post hoc test.

**Figure 2a:** Effect of different doses of AlCl$_3$ on the number of trials to avoid the electric shock at the 1st day in the conditioned avoidance test. Data expressed as Mean ± SEM (n = 10).

a, b, c: Significantly different from control, AlCl$_3$ 100 mg/kg, between AlCl$_3$ 70 and 50 mg/kg respectively at P<0.05 using one way ANOVA followed by Tukey-Kramer as a post hoc test.

**Figure 2b:** Effect of different doses of AlCl$_3$ on the number of trials to avoid the electric shock at the 2nd day in the conditioned avoidance test. Data expressed as Mean ± SEM (n = 10).

a, b, c: Significantly different from control, AlCl$_3$ 100 mg/kg, between AlCl$_3$ 70 and 50 mg/kg respectively at P<0.05 using one way ANOVA followed by Tukey-Kramer as a post hoc test.

**Figure 3a, b, c:** Effect of different doses of AlCl$_3$ on brain oxidative stress biomarkers [MDA (3a), SOD (3b), TAC (3c)] during induction and progression of AD. Data expressed as Mean ± SEM (n = 10).

a, b, c: Significantly different from control, AlCl$_3$ 100 mg/kg, between AlCl$_3$ 70 and 50 mg/kg respectively at P<0.05 using one way ANOVA followed by Tukey-Kramer as a post hoc test.
On the other hand, rats treated by AlCl$_3$ 100 mg/kg showed marked decreases in the activities of SOD and TAC levels than AlCl$_3$ 70 and 50 mg/kg. While 70 mg/kg showed marked decreases in their activities as compared to AlCl$_3$ 50 mg/kg treated rats. AlCl$_3$ treated groups (100, 70 and 50 mg/kg) showed significantly decreases in SOD activity by approximately 8.98%, 11.03% and 20.44% respectively compared to control group, as well as in TAC activities by approximately 11.46%, 17.49% and 26.35% respectively compared to control.

4- Effect of AlCl$_3$ on brain ACHE activity

The results are shown in Figure 4. Rats received AlCl$_3$ 100 mg/kg showed marked increase in the ACHE activity than 70, 50 mg/kg. But 70 mg/kg treated rats showed marked increase in its activity as compared to 50 mg/kg treated rats. Different doses of AlCl$_3$ (100, 70 and 50 mg/kg IP) showed significant increase in the ACHE activities by approximately 1268.237%, 826.139% and 587.841% respectively as compared to control values.

Administration of AlCl$_3$ by 100 mg/kg caused marked increase in Aβ content in rat brain than AlCl$_3$70 and 50 mg/kg. But 70 mg/kg induced marked increase in the content of Aβ as compared to dose 50 mg/kg. However, different doses of AlCl$_3$ (100, 70 and 50 mg/kg IP) caused increase in Aβ content by approximately 560.244%, 483.395% and 373.46% respectively as compared to control group.

Mortality rate: Mortality rate reaches 70% at dose 100 mg/kg of AlCl$_3$ for 6 weeks. But at dose 70 and 50 mg/kg of AlCl$_3$, the mortality rate reached 10%. Mortality rates were taken in consideration for choosing the model of AD in rats.

Discussion

In the present study, neurotoxicity rat model that mimics AD was induced by injection of AlCl$_3$ (100, 70, 50 mg/kg, IP) daily for consecutive six weeks. Al has been suggested as a causal factor in AD, in part because of reports showing the toxicity of Al, the elevation of Al concentrations in the brains of patients with AD, and an association between Al concentrations in water and the prevalence of AD [22,23]. Also Al is a well-known neurotoxicant reported to accelerate oxidative damage to biomolecules. Furthermore, Al salts have been reported to cause cell depletion in the hippocampus [24] and degeneration of cholinergic terminals in the cortical areas. It accumulates in the cingulated bundle and thereby induces learning deficits [25].

Results of the present study showed that injection of different doses of AlCl$_3$ for six weeks induced a significant increase in escape latency to reach the hidden platform accompanied by a significant decrease the time spent in target quadrant in MWM which indicate impairment of learning ability and spatial memory. Administration of 100 mg/kg of AlCl$_3$ showed marked impairment of learning ability and spatial memory than both 70 and 50 mg/kg, but the impairment was more pronounced at dose 70 than dose 50 mg/kg of AlCl$_3$. These results clearly indicate progressive deterioration of learning ability and spatial memory (as determined by Morris water maze task) by increasing the dose of AlCl$_3$, which accumulates in all the brain regions and maximum being in the hippocampus, the key site of memory and learning.

Experimentally, it was previously demonstrated that intracerebral administration of AlCl$_3$ caused learning deficits in the MWM task in rabbits [26], while intraperitoneal administration caused learning deficits in rats [11]. The present findings were in agreement with the previous results and may be attributed to the ability of Al to interfere with downstream effector molecules, such as cyclic GMP involved in memory potentiating [27]; this disruption could then explain the memory impairment and neurobehavioral deficits observed. Moreover, results of the present study showed that different doses of AlCl$_3$ induced marked elevation in the number of trials to avoid the electric shock in the 1st and 2nd day of the CA experiment. These results could be attributed to the deficits in the learning, memory and retrieval abilities (Cognitive functions), where number of trials demonstrated by the animal in the CA technique is known as a valuable parameter to assess the memory and learning behaviors of the animal [28]. Administration of AlCl$_3$ at dose 100 mg/kg showed marked elevation in the number of trials to avoid the electric shock in the 1st and 2nd days of the experiment than 70 mg/kg which showed in turn more pronounced effect than dose 50 mg/kg of AlCl$_3$. These impairments of learning and memory abilities indicating progressive deterioration of the memory and learning behaviors of the animal.

Results of the present study also showed that injection of different doses of AlCl$_3$ significantly increased ACHE activity; a marker of loss of cholinergic neurons in the brain. Administration of AlCl$_3$ at dose 100 mg/kg showed significant increase in the ACHE activity than 70 mg/kg and 50 mg/kg, while AlCl$_3$ at dose 70 mg/kg has more pronounced effect than at dose 50 mg/kg. Functionally, Al can alter the blood brain barrier and produce changes in the cholinergic and noradrenergic neurotransmission [29]. A possible explanation of the present results could be also depending on the hypothesis that Al is a potent cholinotoxin [30]; it also has a slow accumulation rate in the brain [31]. The present results were also in parallel with those previously recorded that there was a significant increase in ACHE activity in rats treated with AlCl$_3$ (50 mg/kg) daily for three months [12]. It is also reported that ACHE activity in different brain regions increased after administration of AlCl$_3$. This elevation in ACHE activity may be attributed to the direct neurotoxic effect of Al or perhaps a disarrangement of the cell membrane caused by increased lipid peroxidation as previously reported [32].
Results of the present study also showed that injection of different doses of AlCl$_3$ significantly increased the MDA. Administration of AlCl$_3$ at dose 100 mg/kg showed a significant increase in MDA as compared to 70 mg/kg which in turn has more pronounced effect than dose 50 mg/kg. Al is a potent pro-oxidant known to enhance lipid peroxides in the cortex and hippocampus [33]. On the other hand, the present results showed that injection of different doses of AlCl$_3$ significantly decreased SOD and TAC activities. Administration of AlCl$_3$ at dose 100 mg/kg showed significantly decreased in the SOD and TAC activities than 50 mg/kg, but not significantly decreased than 70 mg/kg, while administration of AlCl$_3$ at dose 70 mg/kg showed more pronounced decrease than at dose 50 mg/kg of AlCl$_3$. It has been reported that Al induce lipid peroxidation and alter physiological and biochemical characteristics of biological systems [34]. In addition, it was previously found to be a potent pro-oxidant known to enhance lipid peroxides in the cortex and hippocampus [29]. Moreover, as oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes which are the first line of defense against free radical damage under oxidative stress conditions. In this study, administration of AlCl$_3$ resulted in marked elevation in oxidative stress as indicated by increases in lipid peroxidation (measured as MDA level) and decreases in SOD and TAC. These changes could be attributed to the reduced axonal mitochondria turnover, disruption of the golgi or reduction of synaptic vesicles which induced by Al treatment [35,36].

Lipid peroxidation is one of the main manifestations of oxidative damage and it has been found to play an important role in toxicity [37]. Concerning the data obtained in the present work, the elevation of lipid peroxidation in brain of Al-treated rats was evidenced by increased production of MDA, similar result were stated that Al induced significant increase in MDA concentration in hippocampus and frontal cortex of rats administered daily AlCl$_3$ via drinking water for six weeks [38]. Nearly similar findings were obtained; it demonstrated that administration of AlCl$_3$ in dose level of (50 mg/kg/day) in drinking water for a month induced oxidative damage with accumulation of lipid damage peroxidation [39]. It is also reported that intraperitoneal injection of AlCl$_3$ for 60 days at different doses can accelerate lipid peroxidation in rat's brain which may represent one of the most important intoxication mechanisms [40]. The elevation of MDA could be also attributed to the ability of Al itself to accelerate oxidative damage to biomolecules like lipids, protein and nucleic acids [41]. Also, Al is able to cross the blood brain barrier, deposits in rat brain and increase lipid damage peroxidation. Therefore, the estimation of free radical generation and antioxidant defense has become an important aspect of investigation in mammals. The obtained data revealed also significant inhibition in the activities of SOD and TAC in brain tissue of AlCl$_3$ treated rats. The present findings were consistent with the results of several investigators who revealed marked decrease in endogenous antioxidant after administration of different salts of Al [42-44]. It is also reported that administration of AlCl$_3$ significantly increased free radicals (TBARS), decreased the activity of glutamate-s-transferase [45] and induced decline in the activity of GST and level of sulphhydryl group (SH) in the brain and other organs [46,47]. These enzymes activities may be also included in the declining effect of Al on the expression of mRNA of endogenous antioxidants [48,49].

As a direct effect intrahippocampal injections of AlCl$_3$ in rats induced significant increase in MDA concentration [50] (Figure 5).

Results of the present study also showed that injection of different doses of AlCl$_3$ significantly increased the $\beta$-content. Dose 100 mg/kg of AlCl$_3$ induced significant increase in $\beta$-content more than 70 and 50 mg/kg; however AlCl$_3$ at dose 70 mg/kg produced more pronounced effect than at dose 50 mg/kg. Al is known as a chelotninox agent; its neurotoxic effect could be also exerted by additional mechanisms such as induction of oxidative stress. The increased production of the ACHE may be due to a direct action of $\beta$-Ap which binds to nicotinic receptors or due to over expression of $\beta$-Ap precursor (APP). Consequently, $\beta$-Ap induced by Al resulted in increased activity of ACHE within and around $\beta$-Ap plaques [51]. Al is also bound by the $\beta$-Ap and was found co-localized with it in the AD brain [52]. Amyloid fibrils formed in the presence of Al were slightly thicker significantly longer and spirally wound around each other. Subsequent studies have been conducted to confirm that amyloid beta sheets $\beta$-Ap (1-40) will bind up to 4 Al atoms and that binding increased the $\beta$- sheet content of the peptide [53]. The neurotoxicity of $\beta$-Ap in whatever form may involve the formation of reactive oxygen species. Al is a pro-oxidant and is known to promote the oxidation activity of $\beta$-Ap in the presence of iron. It has also been linked to $\beta$-Ap production through the immune response. It is also linked to activate compliment which in turn has been linked to the enhanced aggregation of $\beta$-Ap [52]. Moreover, self-aggregation of $\beta$-Ap due to Al administration leads to generation of hydrogen peroxide and hydroxyl radical via certain chemical reactions. The production of these reactive oxygen species induces membrane lipid peroxidation [54]. The present results are confirmed by the histological examination of different brain regions which showed that administration of AlCl$_3$ 100 mg/kg for 6 weeks caused neuronal degeneration in hippocampus, focal neuronal degeneration and gliosis with focal hemorrhage in cerebrum, focal haemorrhage in cerebral striatum and focal plaque formation in cerebrum, while 70 mg/kg caused neuronal degeneration and atrophy in the hippocampus associated with focal gliosis in striatum, the medulla oblongata showed encephalomalacia. However, administration of AlCl$_3$ by 50 mg/kg for 6 weeks caused encephalomalacia in the medulla oblongata but the hippocampus was histologically intact (Figure 8).
On the other hand, administration of AlCl$_3$ at dose 100 mg/kg for 6 weeks caused severe mortality in rats reached to 70% as compared to control group (Figure 7). Since this very high mortality rate of AlCl$_3$ exceeded that of LD50, so 100 mg/kg of AlCl$_3$ for 6 weeks was inappropriate to be considered as an ideal dose for modeling AD in rats. The present finding showed that AlCl$_3$ at dose 70 mg/kg for 6 weeks induced more pronounced effects as AD model regarding all measured parameters than AlCl$_3$ 50 mg/kg where the hippocampus neuronal degeneration and pyknosis were more pronounced. This model was sharply confirmed by biochemical and behavioral examinations. Consequently, 70 mg/kg of AlCl$_3$ for 6 weeks (dose chosen for this study), was the ideal dose of AlCl$_3$ for modeling neurotoxicity that mimics AD in rats (Table 1).

**Progression of AD after four weeks**

Administration of AlCl$_3$ 100 mg/kg for 4 weeks caused neuronal degeneration in the cerebral cortex, as well as in the striatum associated with few focal eosinophilic, plagues formation with diffuse gliosis in the striatum but there was no histopathological alteration in the hippocampus. While administration of AlCl$_3$ at dose 70 mg/kg for 4 weeks induced...
congestion in the cerebral blood vessels associated with neuronal degeneration in the cerebral cortex but there was no histopathological alteration in the hippocampus. On the other hand, at dose 50 mg/kg of AlCl$_3$ for 5 weeks no alteration in the hippocampus had been shown. On the other hand, at dose 50 mg/kg of AlCl$_3$ for 5 weeks the hippocampus was histologically intact, while the cerebral cortex showed neuronal degeneration.

Thus, Administration of AlCl$_3$ at doses 100, 70 mg/kg for 5 weeks caused atrophy and neuronal degeneration in the hippocampus, which means destruction or neurodegeneration in the specific area of memory in rat brain, in addition to neuronal degeneration in the cerebral cortex. Dose 50 mg/kg of AlCl$_3$ for 5 weeks showed no alteration in the hippocampus but showed neuronal degeneration in the cerebral cortex. Mortality rates were taken in consideration, where it was sever at dose 100 mg/kg. These results showed that by increasing the dose and time of exposure to AlCl$_3$, brain neuronal degeneration, brain destruction and severity of AD increased.

The progression of AD at all used dose levels was time dependent, however the mortality rate was severe at dose 100 mg/kg at all times, while no or slow degeneration was achieved at dose 50 mg/kg of AlCl$_3$ indicating incomplete induction or progression of AD. These results were illustrated in Figure 8 and Table 3 and can be explained, as administration of AlCl$_3$ induced cognitive dysfunction in time dependent manner and its toxicity is thought to be one of the causative agents of AD [55]. Moreover, administration of AlCl$_3$ predominantly accumulates in the hippocampus; this region is known to be particularly susceptible in AD and has important role in learning and memory functions [8]. In AD brain: the cortex shrivels up damaging areas involved in thinking, planning and remembering. Shrinkage is especially severe in the hippocampus, an area of the cortex that plays a key role in formation of new memories [56].

Alzheimer’s worsens over time

It is well known that AD is a progressive disease where dementia symptoms gradually worsen over a number of years [57]. In its early stage, memory loss is mild, but with late-stage AD individuals lose the ability to carry on a conversation and

<table>
<thead>
<tr>
<th>Histopathological alterations</th>
<th>Control</th>
<th>AlCl$_3$ 100 mg/kg</th>
<th>AlCl$_3$ 70 mg/kg</th>
<th>AlCl$_3$ 50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degeneration &amp; pyknosis in hippocampus neurons</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>Eosinophillic plaque formation in stratum</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Gliosis</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Encephalomalacia in medulla oblongata</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Congestion</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

+++ Very severe +++ Sever ++ Moderate + Mild - Nil

Table 3: Effect of different doses of AlCl$_3$ for 5 weeks on the severity of histopathological alterations in the brain of rats.

Progression of AD after five weeks

Administration of AlCl$_3$ 100 mg/kg for 5 weeks caused neuronal degeneration in the cerebral cortex with pyknosis in the nuclei as well as focal gliosis and congestion in the blood vessels. Neuronal degeneration with nuclear pyknosis in the hippocampus had been also shown; there was also focal haemorrhage in the medulla oblongata. While at dose 70 mg/kg of AlCl$_3$, for 5 weeks atrophy and neuronal degeneration in the hippocampus associated with encephalomalacia in the striatum had been shown. On the other hand, at dose 50 mg/kg of AlCl$_3$ for 5 weeks the hippocampus was histologically intact, while the cerebral cortex showed neuronal degeneration.
Figure 8: Representative photomicrographs of brain sections stained by Hematoxylin–Eosin stain (magnification 40x).

(A–C) Sections taken from brain of rats treated daily by AlCl₃ (100 mg/kg for four weeks). It showing neuronal degeneration in the cerebral cortex and in the striatum associated with few focal eosinophilic plagues formation with diffuse gliosis in striatum (8A, 8B) and no histopathological alteration in the hippocampus (8C) respectively.

(D–I) Sections taken from brain of AlCl₃ (100 mg/kg for five weeks) treated rats showing neuronal degeneration in the hippocampus (8D), congestion in the blood vessels (8E), focal haemorrhage in the medulla oblongata (8F), nuclear pyknosis in the hippocampus (8G), neuronal degeneration with pyknosis in the nuclei in the cerebral cortex (8H) and focal gliosis in the cerebral cortex (8I).

(J and K): Sections taken from brain of AlCl₃ (70 mg/kg for four weeks) treated rats showing neuronal degeneration in the cerebral cortex with congestion in the cerebral blood vessels (8J) and no histopathological alteration in the hippocampus (8K).

(L-N): Sections taken from brain of AlCl₃ (70 mg/kg for five weeks) treated rats showing atrophy and neuronal degeneration in the hippocampus (8L, 8M) associated with encephalomalacia in the striatum (8N).

(O-P): Sections taken from brain of AlCl₃ (50 mg/kg for four weeks) treated rats showing no histopathological alteration in the hippocampus (8O) and cerebral cortex (8P).

(Q-R): Sections taken from brain of AlCl₃ (50 mg/kg for five weeks) treated rats showing neuronal degeneration in the cerebral cortex (8Q). The hippocampus was histologically intact (8R).

(Rs): Section taken from brain of AlCl₃ (50 mg/kg for 6 weeks) treated rats after stopping AlCl₃ for 7 days, showing mild congestion in the blood vessels and capillaries in the striatum of the cerebrum.

(T-U): Sections taken from brain of AlCl₃ (70 mg/kg for 6 weeks) treated rats after stopping AlCl₃ for 7 days, showing sever neuronal degeneration and atrophy in the hippocampus (8T) as well as hemorrhages in the brain fissures (8U).
respond to their environment [4]. It progresses slowly in three general stages mild (early-stage), moderate (middle-stage) and severe (late-stage).

Progression of AD after the causative stopped

Administration of AlCl3 at 50 mg/kg for 6 week daily then stopping administration for one week showed mild congestion in the blood vessels and capillaries of the stratum of the cerebrum. While 70 mg/kg of AlCl3 injected daily for 6 week then stopping its administration for one week caused severe neuronal degeneration in the hippocampus and atrophy as well as hemorrhages in the brain tissues. Severe mortality at 100 mg/kg reaching 70% after 6 weeks and the liability to be increased by time preventing its use for completing this part of the experiment. This very high mortality rate of AlCl3, exceeded that of LD50. These results showed that AD spread spontaneously even after the causative stopped. Mortality rate which increased at dose 100 mg/kg can be discussed as death occurred by time in AD. The changes in the brain caused by AD are not usually the primary cause of death. AD often caused malnutrition and increased risk of pneumonia, resulting in death in the patients [6].

Consequently, the present results cleared that rats injected with AlCl3 at dose 70 mg/kg LP for 6 weeks represents the most exact model mimics AD, where the hippocampus neuronal degeneration and pyknosis were more pronounced. This model was sharply confirmed by biochemical and behavioral examinations. Mortality rates were taken in consideration.

Conclusion

Animal models of AD are a cornerstone of the drug development process and represent a scientific tool for testing of new drugs or disease-modifying agents. In the present study, 70 mg/kg of aluminum injected daily (AlCl3.6H2O, IP) for 6 weeks has been established as an ideal model for induction and progression of AD in rats. The progression of the disease is time dependent and just starts spread spontaneously without more aluminum exposure. By modeling AD stages and identifying its progression; prediction is possible, symptoms can be expected and the power to find real treatment or new protective agents will be enhanced.

References