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RESEARCH PAPER

# A comparative study of scopolamine, D-Galactose and AlCl3-induced Alzheimer's like disease by enhancing hippocampal neurodegeneration and memory impairment in male rats

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**A B S T R A C T:**

Alzheimer's disease (AD) is a debilitating neurodegenerative disorder identified by progressive loss of neurons, cognition decline, and memory deficits. The current study sought out to compare the effects of scopolamine (SCO), D-galactose (D-gala), and aluminum chloride ($AlCl_3$) on memory performance, histological alterations of the hippocampus, and to determine the best model for inducing Alzheimer's-like disease. Twenty-four male adult albino rats (250–300g) were used in this study, which were arbitrarily assigned into four groups: control group (normal saline), SCO group (2 mg/kg), D-gala group (125 mg/kg) and $AlCl_3$ group (50 mg/kg). The chemicals were given intraperitoneally for 30 days. All the rats were subjected to novel object recognition (NOR) and Barnes maze (BM) tests to assess memory performance. At the end of the period, all the rats were anesthetized, blood samples were taken for hematological and biochemical tests, and the brains were removed for morphometric analysis. The results have demonstrated that SCO, D-gala, and $AlCl_3$ caused memory impairment in BM and NOR tests, and malondialdehyde serum MDA levels significantly increased. Histological analysis showed degenerative pyknotic cells in the rat hippocampus. The impairment in memory performance and histopathological alterations were higher in the $AlCl_3$ than in the other models, but the hematological parameters were significantly changed. Therefore, it was concluded that SCO (2 mg/kg) is the best model for mimicking AD-like disease among D-gala and $AlCl_3$ by enhancing histopathological alternations and declining memory in rats without affecting other study parameters.

KEY WORDS: Alzheimer's disease, hippocampus, memory impairment, AlCl3
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## 1.INTRODUCTION :

Alzheimer's disease (AD), is the most common neurodegenerative disorder that about 46 million persons in the world suffer recently, and estimated to rise to 131.5 million by the year 2050 and progressively increase throughout the years (Mat Nuri et al., 2017). AD patients suffer from memory loss and other cognitive deficits due to cholinergic neurons degenerating or loss of neurons in the hippocampus and cortex (Kumar et al., 2018). The hippocampus is a section of the limbic system (Alsemeh et al., 2020). It has a very distinctive shape, composed of two areas: the dentate gyrus (DG) and the Cornu Ammonis (CA), which mainly contain the pyramidal cells and the granule cells, respectively.

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Based on the architecture of the pyramidal neurons, the CA area can be separated into the CA1, CA2, CA3, and CA4 subareas (Boccara et al., 2015, Brennan et al., 2019). The hippocampus is crucial for some processes involved in learning and memory (Um et al., 2017).

Many hypotheses about AD have been developed, including cholinergic neuron degeneration, oxidative stress, amyloid β (Aβ), tau, and inflammation (Ravi et al., 2018). Aggregation of Aβ allows for neural loss and synaptic degeneration. Tau protein hyperphosphorylation hinders axonal transport from the soma to nerve terminals and vice versa (Knopman et al., 2021). The development of AD is influenced by oxidative stress. AD patients have greater levels of malondialdehyde (MDA), a byproduct of lipid peroxidation brought on by free radicals, which causes neurodegeneration that is

facilitated by the excess creation of free radicals (Abulfadl et al., 2018).

Several kinds of AD rodent models are used to study different characteristics of the illness. Aluminum is the third most common element on the planet. It has also been suggested that an increased intake of aluminum ions may be one of the risk factors for AD and nervous system impairment (Klotz et al., 2017, Yang et al., 2019). Aluminum chloride ($AlCl_3$) was used in many manufactured such as medicines, foods, toothpaste, and in drinking water (Newairy et al., 2009, Colomina and Peris-Sampedro, 2017). $AlCl_3$ can reach the circulation system and quickly move to the brain by passing the blood brain barrier (BBB). When $AlCl_3$ reaches the brain, it accumulates in various areas involving the hippocampus as it causes neurodegenerative disease development (Alam and Bansal, 2020, Cao et al., 2017). The toxicity of $AlCl_3$ induces a wide range of physiological, behavioral, and biochemical dysfunctions in humans and animals (Aly et al., 2018).

D-galactose is a mono-saccharide sugar, used for senescence in age-associated neurodegenerative disorders (Shwe et al., 2018, Vlassara et al., 1994). Chronic exposure of rats to D-galactose leads to gradual memory impairment and neurodegeneration (Imbimbo et al., 2005). Malondialdehyde (MDA) levels may rise resulting from the increased oxidative stress caused by D-gala exposure (Yin et al., 2010).

Scopolamine (SCO) is an anticholinergic agent that can pass BBB and then interact with the cholinergic system to impair memory and learning and produce an AD disease (Budzynska et al., 2015, Fuji et al., 2018). Cognitive impairment in AD people is strongly associated with the loss of cholinergic neurons and abnormalities in cholinergic neurotransmission in the cortex and hippocampal regions (Abd-El-Fattah et al., 2014). The objective of this study is to determine the impacts of aluminum chloride, scopolamine, and D-galactose on rat hippocampal structure and to select the best model that mimics the AD-like disease.

## 2. Materials and methods

### 2.1 Drug and Chemicals

These chemicals were used in this study: Scopolamine (Gulf Pharmaceutical Industries, Ras Al Khaimah, U.A.E), aluminum chloride (AlCl3), and D-galactose (BDH, England).

### 2.2 Experimental Animals

Adult male rats (250–300 gram) were kept in plastic cages shielded with steel grids, six rats per cage. The environment in the room was regulated at a temperature of (23 ± 3 $C^o$), with a 12-hour cycle for light and dark. During the experiment, drinking, water, and a standard food were provided to each cage *ad libitum*. Before the start of the experiment, all the rats were acclimatized for 2 weeks. Only the smallest number of rats was required to create reliable logical data. Any stress and pain during the experiment were reduced.

### 2. 3 Experimental Design

The idea was to use intraperitoneal injection (I.P) with of scopolamine (SCO), aluminum chloride ($AlCl_3$), or D-galactose (D-gala) so as to induce memory impairment in rats and to evaluate behavioral, biochemical, and histological effects on rats. Twenty four adult male Wistar rats were arranged arbitrarily into 4 groups containing six animals each: the control group received normal saline intraperitoneally, scopolamine group received scopolamine at a dose of 2 mg/kg (Hafez et al., 2017). The $AlCl_3$ group received aluminum chloride (50 mg/kg body weight), which was melted in normal saline (Chavali et al., 2020). The D-galactose group was injected with D-gala at a dose of 125 mg/kg body weight, which was melted in normal saline (Li et al., 2020). The treatments were carried out for 30 days.

### 2.4 Body weight evaluation

Rat body weight was recorded on the first day and the last day of the experiment.

### 2.5 Behavioral experiments

The behavioral tests were performed after the end period of treatment to evaluate the effects of D-gala, SCO, and $AlCl_3$ on memory performance by novel object recognition and the Barnez maze tests. A camera (C270 HD Webcam Logitech) was used to record the activity of animals.

#### 2.5.1 Novel object recognition test (NOR)

Hippocampal function and memory recognition, especially spatial and working memory in rats, were evaluated by using the NOR test (Singh et al., 2018). This test was carried out in an open box (45 × 40× 46 cm). The NOR test

was done (9:30 AM to 5:30 PM) under red light exposure (40 LX) (Retinasamy et al., 2019). In order to habituate the animals, they were allowed to explore the box for ten minutes. After 24 hours, the rat was placed inside the open box between two identical objects (familiar) and was permitted to explore the familiar objects for 4 minutes. Then, the rat was removed from the box and transported into their cage, and the box was cleaned with 60% ethanol to eliminate the effect of odor on rat behavior. After 20 minutes, the rat was exposed to a new object (novel) in addition to the familiar object for three minutes (Justin-Thenmozhi et al., 2018). The discrimination index (DI) was defined as the percentage of time spent on a new object to the total time spent on familiar and novel objects together (Batool et al., 2016).

**2.5.2 Barnes Maze Test (BM)**

The Barnes maze is a broadly accepted test of hippocampal-dependent learning and memory in rodents (Zhang et al., 2018). In brief, the current test was performed on a white circular stage (126 cm in diameter) with 18 holes (10 cm in diameter), 4 cm away from the edge, and no surrounding walls. The maze was raised 90 cm from the base and light intensity (1250 LX) was determined by using a digital lux meter. A light-emitting diode (LED) light source was installed on the ceiling nearly 120 cm from the top of the maze to increase rats' motivation to escape from the circular platform and to search for the target hole. A small removable black box (escape box) was put at the button of one hole (target hole) in which the animal could hide. The BM test involves the following stages: habituation (1 day), acquisition stage (five days), and probe phase (1 day). The maze and escape box were cleaned with 60% alcohol after each rat was tested. Based on the techniques previously used by other researchers, the experimental design was developed (Sahraei et al., 2019). Latency time and time spent to reach the target zone were documented during the acquisition phase and the probe phase, respectively.

**2.6 Organs and Blood Samples Collection**

After BM and NOR tests, rats were fasted for 13 hours and then anesthetized with an intraperitoneal (I.P) injection of ketamine (90 mg/kg) and xylazine (12 mg/kg) (Farzampour et al., 2016). Blood was collected via the left ventricle. Then it was separated into two parts. The first part was collected into centrifuge tubes without anticoagulant. The blood was centrifugated at 2400 rpm for 10 minutes, and then the serum samples were obtained, which were used for the determination of the MDA and serum electrolyte. The second portion was collected into sterilized tubes containing dipotassium salts of ethylenediaminetetraacetic acid (EDTA) for hematological study. Each brain was swiftly removed after it was sacrificed by decapitation.

**2.7 Blood glucose determination**

Control and experimental rats were fasted for 13 hours but provided with tap water *ad libitum*. Blood samples from the rats were collected by the heart puncture method. Blood glucose was measured by using a blood glucose monitor (VivaChek Laboratories, Wilmington, USA).

**2.8 Hematological analysis**

An automated blood cell analyzer (Boule Medical AB, Stockholm, Sweden) was used to determine platelet distribution width (PDW), red blood cells (RBCs), white blood cells (WBCs), and hemoglobin (HGB).

**2.9 Determination of serum electrolytes**

Serum electrolytes ($Ca^{2+}$, $Na^{+}$, $K^{+}$ and $Cl^{-}$) were determined from different groups by using the Centromeric GmbH Wartenberg, Germany reagent kit.

**2.10 Serum malondialdehyde (MDA) determination**

Malondialdehyde (MDA) was measured according to this method (Ohkawa et al., 1979). The principle is the reaction between the MDA in the serum and thiobarbeturic acid (TBA). The optical density was scanned spectrophotometrically (SQU10111012002, USA) at 535 nm. The optical density was changed to concentration (µmol/L).

**2.11 Histological section preparation**

At the end of the period, animals were killed, and the whole brains of all the animals were swiftly removed. The brains were directly fixed in formalin (10%) for 48 hours, washed and dehydrated with graded ethanol, cleared in xylol, then set in wax. Paraffin blocks were cut at 4-µm thickness via a rotary microtome (SRM 200 CW, Netherland) and dyed with haematoxylin and eosin (H&E) (Tamizhazhagan and Pugazhendy, 2017) for studying the histological structure of the

rat hippocampus by using a light microscope (Olympus, 810812, Japan).

**2.12 Statistical analysis**

To perform the statistical analysis, GraphPad Prism (Version 8.0.1) was used. Kolmogorov-Smirnov and Shapiro-Wilk tests were used for all data to check normality tests. All data are represented as mean ± SEM. One-way and or two–way ANOVA was performed for comparison, followed by a Tukey post hoc test. A difference is considered statistically significant if $p \leq 0.05$.

**3. Results**

**3.1 Effect of SCO, D-gal and $AlCl_3$ on body weight, blood glucose, and hematological parameters**

The results of rat body weight, blood glucose levels, and hematological parameters in the SCO, D-gal, and $AlCl_3$ groups are shown in Table 1. The results revealed that, administration of $AlCl_3$ to the experimental animals significantly reduced (***$p < 0.001$) in the RBC, HGB, and body weight in comparison with other groups. The WBC count in the SCO treated groups increased but not significantly, while in the $AlCl_3$ treated group, WBC count and PDW significantly increased when compared to WBC count and PDW of other treatment groups. The automated blood cell analyzer could not measure the platelet count except for one sample, which was 1159 $\times 10^3/\mu L$, due to high count level. Blood glucose did not change significantly among groups.

**Table 1** Effect of SCO, D-gal and $AlCl_3$ on body weight, blood glucose, and hematological parameters.

| Parameter | N | Control | SCO | D-gala | $AlCl_3$ |
|---|---|---|---|---|---|
| Initial weight (g) | 6 | 267.5±3.09[a] | 265±5.24[a] | 271±6.09[a] | 281.7±4.59[a] |
| Final weight (g) | 6 | 332.2±7.23[a] | 335.8±2.93[a] | 337.2±17.28[a] | 253.2±7.41[b] |
| Body weight gain (g) | 6 | 76.67±9.43[a] | 71.67±3.50[a] | 58.33±6.80[a] | -45.17±15.34[b] |
| Blood glucose (mg/dl) | 6 | 110.5±10.18[a] | 113.2±5.64[a] | 120.2±4.57[a] | 121.3±7.23[a] |
| RBC ($10^6/\mu L$) | 6 | 7.745±0.18[a] | 6.922 ± 0.36[a] | 7.646±0.06[a] | 5.979±0.22[b] |
| HGB (g/dL) | 6 | 14.957±0.45[a] | 13.18±1.10[a] | 13.92±0.70[a] | 11.77±0.26[b] |
| WBC ($10^3/\mu L$) | 6 | 6.040±1.13[a] | 7.240±0.47[ab] | 6.860±0.33[ab] | 9.218±0.78[b] |
| PDW (fl ) | 6 | 8.733±0.15[a] | 11.50±4.62[a] | 8.833±0.25[a] | 19.65±5.16[b] |

Values are represented as mean ± SEM (n=6). Different alphabetical letters (a and b) represent significant difference among the groups. One-way ANOVA with Tukey's post hoc test was applied, $p < 0.05$. RBC: red blood cells, HGB: hemoglobin, WBC: white blood cells, PDW: platelet distribution width, SCO: scopolamine, D-gala: D-galactose and $AlCl_3$ aluminum chloride.

**3.2 Effect of SCO, D-gala and $AlCl_3$ on serum electrolytes and malondialdehyde (MDA)**

The results of the mean values of serum electrolytes ($Ca^{2+}$, $Na^+$, $K^+$ and $Cl^-$) and MDA are shown in Table 2. Statistical analysis in this present study showed that serum $Ca^{2+}$ in the $AlCl_3$ treated group significantly decreased (*$p < 0.05$) in the concentration compared to control group. In D-gala group, there is a significant increase ($^*p < 0.05$) in the serum $Cl^-$ than SCO, $AlCl_3$ and control groups. In addition, a significant reduction ($^{***}p < 0.001$) in the concentration of serum $Na^+$ was detected in the D-gala when compared to the SCO, $AlCl_3$, and control groups However, serum $K^+$ did not show a significant change among groups. MDA levels in serum were significantly increased in SCO ($^{***}p < 0.001$), D-gala ($^*p < 0.05$) and $AlCl_3$ ($^{***}p < 0.001$) groups than control group.

**Table 2** Effect of SCO, D-gal and $AlCl_3$ on serum electrolytes ($Ca^{2+}$, $Na^+$, $K^+$ and $Cl^-$) and MDA

| Parameter | N | Control | SCO | D-gala | $AlCl_3$ |
|---|---|---|---|---|---|
| $Ca^{2+}$ (mg/dl) | 6 | $10.07\pm0.217^{a}$ | $9.543\pm0.91^{ab}$ | $9.497\pm143^{ab}$ | $9.258\pm0.255^{b}$ |
| $Na^+$ (mmol/L) | 6 | $162.5\pm2.74^{a}$ | $155.9\pm3.57^{a}$ | $136.2\pm2.58^{b}$ | $157\pm1.65^{a}$ |
| $K^+$ (mmol/L) | 6 | $3.022\pm0.223^{a}$ | $3.230\pm0.193^{a}$ | $3.428\pm0.093^{a}$ | $3.460\pm0.187^{a}$ |
| $Cl^-$ (mmol/L) | 6 | $96.32\pm0.812^{a}$ | $96.72\pm0.745^{ab}$ | $104.2\pm2.750^{cd}$ | $97.94\pm1.414^{ad}$ |
| MDA (µmol/L) | 6 | $4.0\pm0.447^{a}$ | $8.66\pm0.557^{b}$ | $6.83\pm0.477^{b}$ | $10\pm0.816^{b}$ |

Values are represented as mean ± SEM (n=6). Different alphabetical letters (a, b, c, and d) represent significant difference among the groups. One-way ANOVA with Tukey's post hoc test was applied, $p < 0.05$. SCO: scopolamine, D-gala: D-galactose, $AlCl_3$: aluminum chloride, MDA: malondialdehyde.

### 3.3 Behavioural experiments

#### 3.3.1 Effects of SCO, D-gala and $AlCl_3$ on recognition memory impairment using NOR Test

Recognition memory was detected by NOR test in rats. In our results, statistical analysis showed a significantly decreased time spent with the new object in SCO (**$p < 0.01$), D-gala (**$p < 0.01$) and $AlCl_3$ (***$p < 0.001$) groups in comparison to control group. This is indicated by the alteration of memory recognition. Also, there was a significant difference ($^{\#}p < 0.05$) in $AlCl_3$ compared to SCO, and D-gala groups (Figure 1A). The discrimination index (DI) was significantly lower in SCO (*$p < 0.05$), D-gala (*$p < 0.05$) and $AlCl_3$ (*$p < 0.05$) groups in rats than control group (Figure 1B). All results proved that SCO, D-gala, and $AlCl_3$ induced loss of recognition memory.

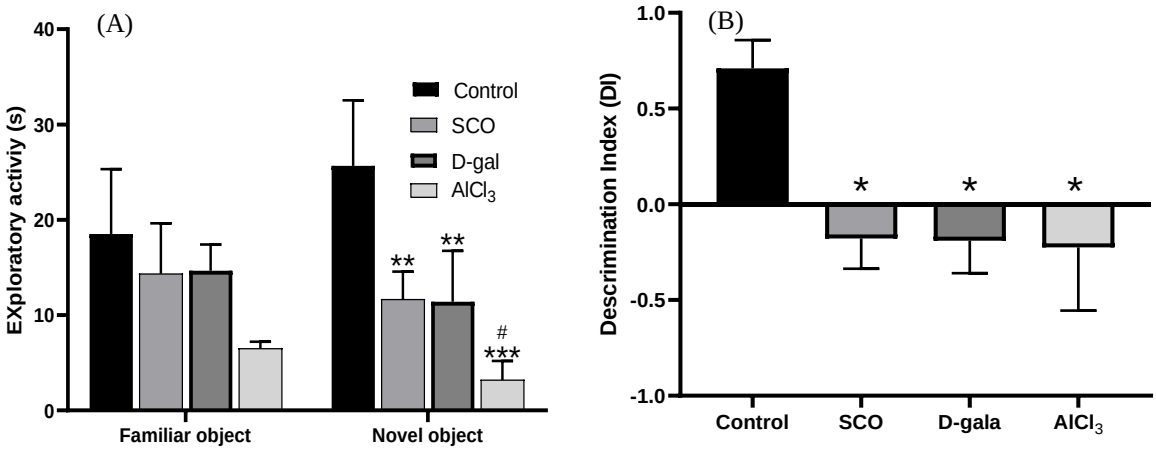


**Figure 1**: Effect of SCO (2 mg/kg), D-gal (125 mg/kg) and $AlCl_3$ (50 mg/kg) groups on working memory. (A) Exploratory activity. Animals in the SCO, D-gala, and $AlCl_3$ groups spent less time with novel object than familiar object when compared with control group. (B) discrimination index decreased in the SCO, D-gala, and $AlCl_3$ when compared with control group. Data are represented as means ± SEM (n=6). Statistical analysis was done by one-way ANOVA for discrimination index and two-way ANOVA for exploratory activity, followed by Tukey's Multiple Comparisons test. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$: statistically different than control group. $^{\#}p < 0.05$ statistically different than SCO and D-gala groups. SCO: scopolamine, D-gala: D-galactose and $AlCl_3$: aluminum chloride.

### 3.3.2 Effects of SCO, D-gala and $AlCl_3$ on long term memory impairment using BM Test

The Barnes maze (BM) is a well-known test of learning and memory in rats that depends on the hippocampus region. Learning and memory

were evaluated by the measure of escape latency to find the escape box in the acquisition phase (days 1-5) and time spent in the target zone, which before had the escape box in the probe phase (day 7). Statistical analysis revealed no significant variation in the escape latency between treatment groups on the day one of the acquisition phase. The post-hoc analysis demonstrated a significant increase (*p < 0.05) in the latency time (the time to reach the escape box was greater) in the rats treated with $AlCl_3$ was noted on the second day of the acquisition phase than control group. However, there was no significant variation among SCO and D-gala than control group. On the third and fourth days of the acquisition phase, rats in SCO (*p < 0.05) and $AlCl_3$ (**p< 0.01) treated groups significantly need more time to find the escape box than control group. In contrast, there was no significant variation in the escape latency of the D-gala treated group (Figure 2A).

The last day of the BM test is considered as a probe phase. In this phase, escape box was removed. The post-hoc analysis indicated on day seven of BM test, time spent in the target zone in SCO (**p < 0.01), D-gala (*p < 0.05) and $AlCl_3$ (**p < 0.01) groups significantly decreased when compared to the control group (Figure 2B).

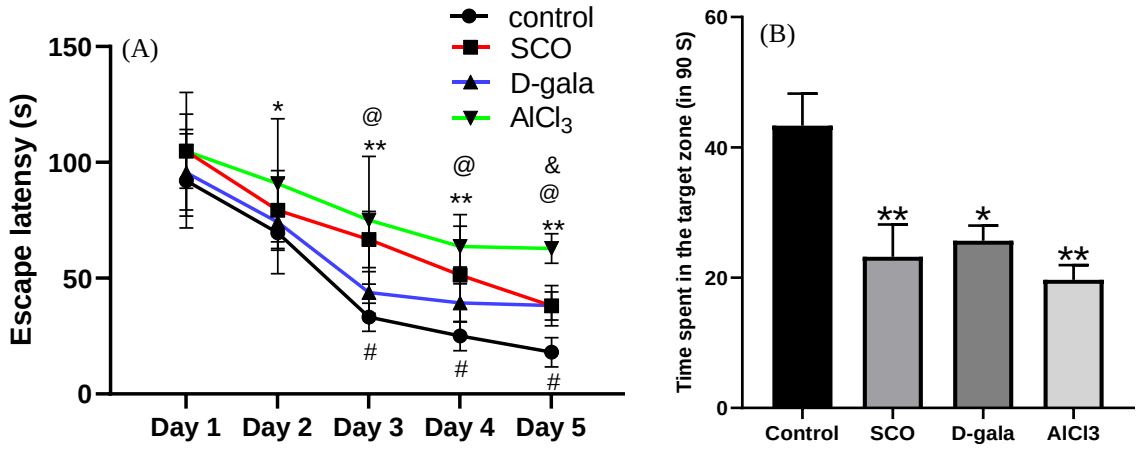


**Figure 2**: Impairment of learning and memory performance by SCO (2 mg/kg), D-gala (125 mg/kg), and $AlCl_3$ (50 mg/kg) in branes maze test. (A) Escape latency to find the target hole which contained an escape box on the first day of acquisition phase to find the escape box no significant difference between groups. On the second day of acquisition phase, no significant difference was found between groups. However, significant variation was only found between control and $AlCl_3$ groups (*p < 0.05). On the third and fourth day of acquisition phase rats in SCO and $AlCl_3$ treated groups significantly ($^{@}$p < 0.05 and **p < 0.01) respectively need more time to find the escape box. Also, there was a significant difference ($^{\#}$p < 0.05) between D-gala, and $AlCl_3$ groups. On the fifth day of the acquisition phase, rats in SCO, D-gala, and $AlCl_3$ treated groups significantly need more time to find the escape box. Also, $AlCl_3$ differ ($^{\#}$p < 0.05) from SCO and D-gala groups. (B) Time spent in the target zone. During the probe phase on the seventh day of the Barnes maze test, rats in SCO, D-gala, and $AlCl_3$ groups ($^{@}$p < 0.05, $^{\&}$p < 0.05 and **p < 0.01) respectively spent significantly less time in the target zone, which contained escape box. In each group data are represented as means ± SEM (n=6). Statistical analysis was done by two-way ANOVA for escape latency and one-way ANOVA for time spent in the target zone, followed by Tukey's Multiple Comparisons test. *p < 0.05; **p < 0.01 significantly difference compared to control group, @ p < 0.05 differ significantly in comparison with SCO and D-gala groups.

### 3.5 Histological Observations

Microscopical examination of coronal sections of rats' hippocampal tissues from control, SCO, D-gala, and $AlCl_3$ groups showed the same histological features. H&E staining of the hippocampus revealed it was composed of two regions, hippocampus proprius and the dentate gyrus (DG). The hippocampus proprius can be divided into 4 Cornu Ammonis regions. These regions (CA1, CA2, CA3 and CA4) consist of molecular layer (ML), pyramidal layer (PCL), polymorphic cell layer (POL) and the dentate gyrus appeared as a coiled structure with upper and lower limbs were directed to the hippocampus proprius. The DG composed of 3 layers, polymorphic cell layer (POL), granular cell layer (GCL), and molecular cell layer (ML) (Figure 3). The pyramidal layer of the CA1 area in the control group contained small pyramidal cells with round vesicular nuclei, visible nucleoli (Figure 4: A). In the current study, histological analysis of the pyramidal cell layer of CA1 region in the SCO, D-gala, and $AlCl_3$ groups showed pyramidal cells with shrunken degenerated nuclei and darkly pyknotic nuclei and vaculation around cells (Figure 4: B, C, D). However. more neural cells damage in $AlCl_3$ than in other models.

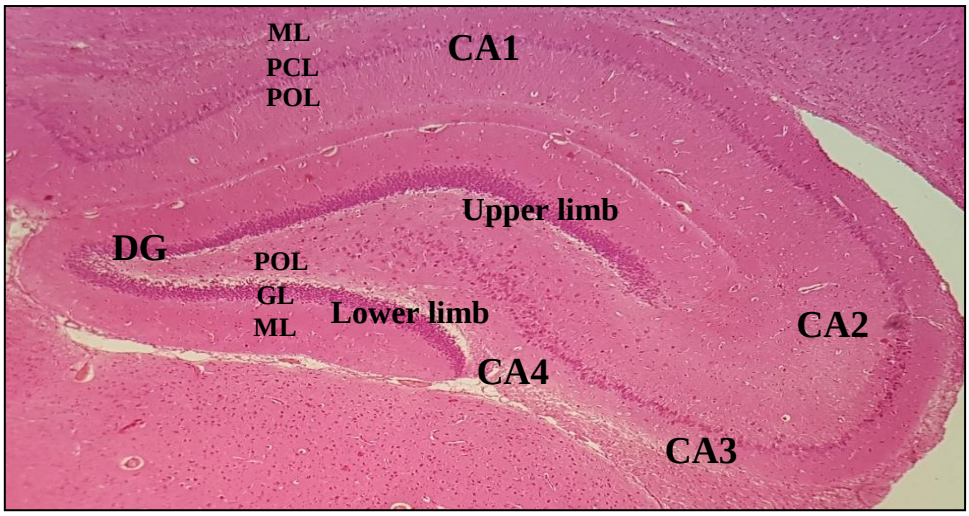


**Figure 3: A** coronal section of adult rat hippocampus of the control group shows dentate gyrus (DG) and hippocampus proper stained with H&E (10X). The hippocampus proper, which consists of the Cornu Ammonis (CA4, CA3, CA2, and CA1). ML: molecular layer, PCL; pyramidal cell, POL; polymorphic cell layer, GL; granular cell layer.

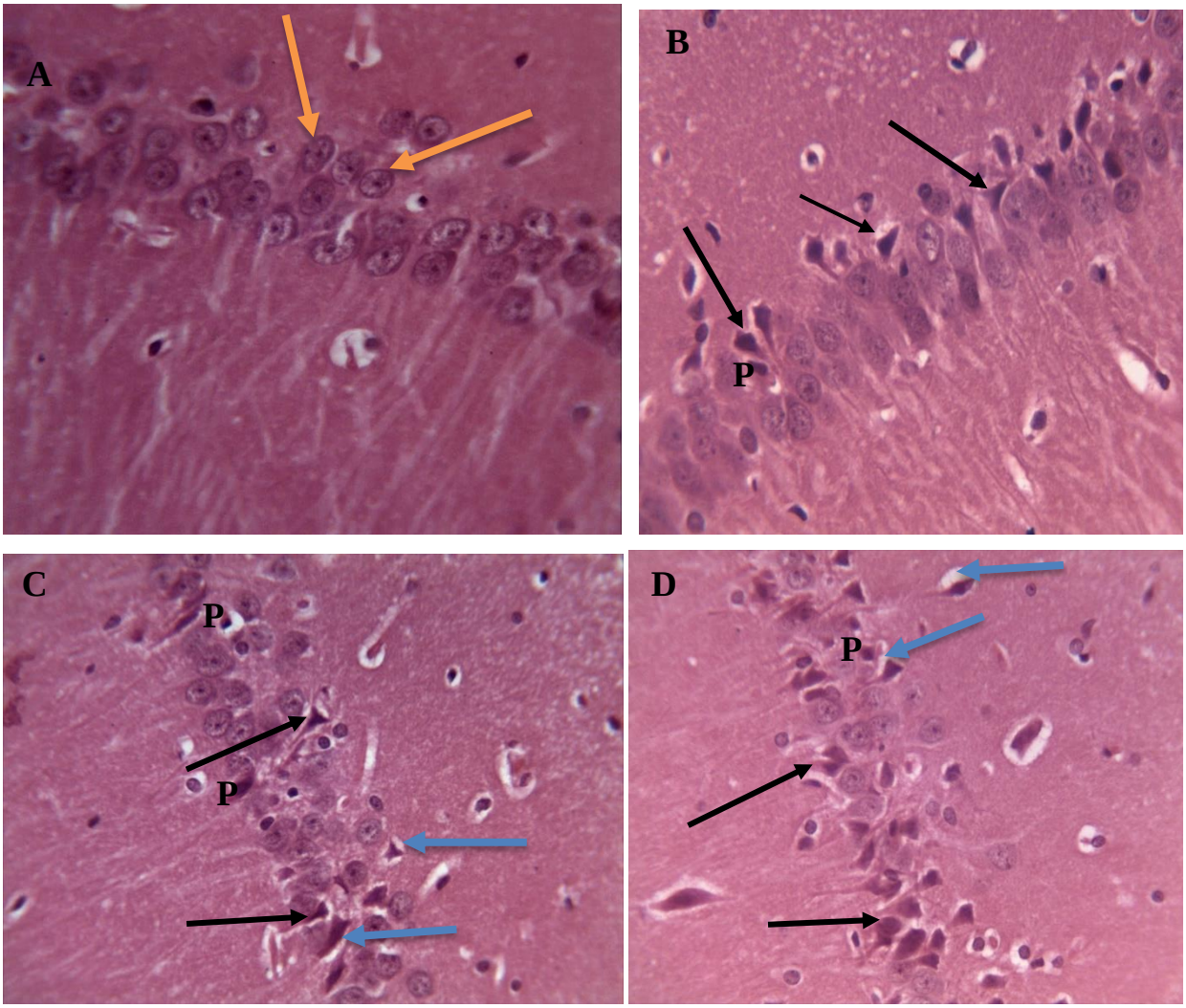


**Figure 4**: Section of rat hippocampus brain showing CA1 reagion treated with (A) normal saline (B) scopolamine (2 mg/kg), (C) D-galactose (125 mg/kg) and (D) aluminum chloride ($AlCl_3$) (50 mg/kg) treated groups intraperitoneally injected for 30 days. In the control group, showed healthy neurons (normal pyramidal cells) with round vesicular nuclei and visible nucleoli (green arrows). In scopolamine, D-galactose and aluminum chloride treated groups, pyramidal cells with shrunken elongated nuclei (black arrows), vacuolation around them (blue arrows) or pyknotic nuclei (P). Stained with Hematoxylin-Eosin dye (H&E, 40 x).

## 4. Discussion

Alzheimer's disease (AD), is the most common neurodegenerative disease (Karran and De Strooper, 2016). It is mainly categorized by the loss of cognitive and memory functions (Livingston et al., 2020) as a result of the loss of pyramidal neurons and cholinergic neurons in the brain cortex and hippocampus being degenerated (Hansson et al., 2006). The hippocampus is a portion of the brain in humans and vertebrates. It plays a vital role in memory formation (Knierim, 2015, Saeed et al., 2021). In this study, to induce memory disorders in rodents, and to select the best model that mimics the AD-like symptoms, the SCO, D-gala, and $AlCl_3$ models were used. This work, revealed noticeable impairments in learning and memory along with damaged neurons in the hippocampus regions in rats. In the present study, the tests for memory assessment and learning related to hippocampus were used, including Barnes maze (BM) as well as novel object recognition (NOR). These tests can assess animals' cognitive impairment (Kim et al., 2014).

The NOR test in our study showed injections of SCO, D-gala, and $AlCl_3$ in rats caused a significant reduction in the time need for exploring the novel (new) object by measuring

time spent between novel and old objects when we compared it to the familiar object. However, the $AlCl_3$ group is statistically more significant in comparison to the SCO and D-gala groups. Also, the NOR test revealed a significant decrease in discrimination index in the SCO, D-gala, and $AlCl_3$ treated groups. This current study suggests that prolonging SCO, D-gala, and $AlCl_3$ administration led to recognition memory impairment, which is also confirmed by other studies (Wong-Guerra et al., 2017, Alghamdi, 2018, Saenno et al., 2022).

Barnes maze (BM) test is used for measuring spatial learning and memory in animals. The first phase of the test refers to the acquisition phase, which allows evaluation of spatial learning, while the second phase of the test refers to the probe phase to evaluate spatial memory. These phases are thought to be related to hippocampus function (Barnes, 1979, Gawel et al., 2019). In the current work, the BM test revealed that injection of SCO, D-gala, and $AlCl_3$ in rat models had effects on memory and learning capability by measuring time spent in the target zone and escape latency. In this test, we noticed that SCO, D-gala, and $AlCl_3$-treated groups spent more time to find the escape box location in the acquisition phase. Additionally, decrease spent time in the target zone than control rats in the probe phase (Fuji et al., 2018, F Ewida and A Mansour, 2015). However, $AlCl_3$ was more effective than other models.

The histopathology characteristics of AD include the continuous degeneration of specific brain neurons (Ravi et al., 2018, Chiroma et al., 2019). The first area affected by this degenerative process in AD patients is the hippocampus. It plays a vital function in memory processes (Padurariu et al., 2012, Heo et al., 2014). The hippocampus is highly susceptible to neuronal damage. In the recent study, histopathological analysis of the brain tissues from the SCO, D-gala, and $AlCl_3$ treated groups revealed major morphological changes and degenerate neurons in the hippocampal CA1 regions, pyramidal cells with shrunken degenerated nuclei, darkly pyknotic nuclei, and vacuolation around cells in the CA1 hippocampal region compared to the control group. These results are in accordance with those of (Fawzi et al., 2020, Aboelwafa et al., 2020, Firdaus et al., 2022). However, $AlCl_3$ causes more neural cell damage compared to the SCO, and D-gala models. SCO is related to cholinergic dysfunction and oxidative stress in the hippocampus. (Pattanashetti et al., 2017). In the body, d-galactokinase and galactose-one-phosphate uridyl transferase metabolize the reducing sugar D-gala. Yet high levels of D-gala result in abnormal metabolism. The conversion of D-gala to galactitol, which is not processed but aggregated in the cells, made osmotic stress and the creation of oxidative stress in the cell, which causes neurodegeneration and causes behavioral disorders and cognitive disorders (Kumar et al., 2011, Wang et al., 2020). $AlCl_3$ can induce neurological conditions for instance AD (Maya et al., 2016, Inan-Eroglu and Ayaz, 2018). The mechanism of $AlCl_3$-induced neurodegeneration is not obviously known. However, $AlCl_3$ increases the activity of ferrous ($Fe^{2+}$) and ferric ($Fe^{3+}$) ions as a reason to oxidative damage, leading to neurodegeneration, which is also confirmed by another study (Aljarari and Bawazir, 2019).

In the current study, body weight was elevated in the control, SCO, and D-gala groups with age increasing, but in the groups treated with $AlCl_3$ it was decreased significantly. These results we obtained indicate the impact of $AlCl_3$ on body weight but that the effects of SCO and D-gal on this parameter were not significant. The underlying cause of the effect $AlCl_3$ on body weight might be due the damage in pancreatic tissue and reduced glucose transporter 4 (GLUT4) expression level, which in turn results in glucose metabolism change (Wei et al., 2018). The glucose level in the blood of the groups did not show a significant difference (Harandi et al., 2015, Nam et al., 2016).

One of the factors has an effect on pathophysiology of AD is oxidative stress (Ferreira et al., 2015). AD patients have higher levels of MDA, which is a free radical byproduct of lipid peroxidation reaction. MDA causes cross-linking and polymerization of large molecules like nucleic acids and proteins, and has certain cytotoxicity (Xu et al., 2019). In the recent study, serum MDA levels were significantly increased in the SCO, D-gala, and $AlCl_3$ groups in comparison to the control group (Ghanbari et al., 2019, Kou et al., 2017, KANDİŞ et al., 2022).However, in the $AlCl_3$ group it was statistically highly significant.

According to hematological results, $AlCl_3$ led to a significantly decreased RBC and HGB compare to control group. Also, administration of

SCO, and D-gala led to decreased RBC and HGB but no significantly lower than control group. By decreasing $Fe^{2+}$ binding to ferritin and disrupting heme production, $AlCl_3$ can alter membrane structures and enzyme activity for RBC production. The reduction in HGB content might be due to an increased rate of destruction or decrease in the rate of development of RBCs (Aziz and Zabut, 2011, Adedosu et al., 2018). Also, reductions in RBC and HGB might be attributed to hyper-activity of the bone marrow, leading to synthesis of RBCs with decreased integrity that are easily destroyed in the blood circulation. The reduction of RBC, HGB by $AlCl_3$ in this study suggests the induction of anemia. Also, hematological results indicated that $AlCl_3$ led to a significantly increased WBC when compared to SCO, D-gala, and control groups (Yakubu et al., 2017). Increase in WBC is suggestive of leukocytosis and anemia as seen from the corresponding decreases in RBC which may indicate the activation of the immune system to form defensive cells (Adedosu et al., 2018).

Electrolytes have a significant role in the maintenance of the function of muscle and nerve and also in the hydration process of the body. In this work, $AlCl_3$ produced a significant reduction in the serum concentration of $Ca^{2+}$ but the serum levels of $Na^+$, $K^+$, and $Cl^-$, did not change significantly. D-gala produced a significant decrease in the serum concentration of $Na^+$ and a significant rise in the serum concentration of $Cl^-$. On the other hand, SCO did not affect serum electrolytes (Ugbaja et al., 2017, Ogunlade et al., 2022).

## 5. Conclusion

The present study indicated that administration of SCO, D-gala, and $AlCl_3$ induced learning and memory impairment in the Barnes Maze and novel object recognition tests by increasing the escape latency to find the escape box, decreasing the time spent in the target zone in the BM test, and lowering the recognition index in the NOR test. Additionally, histopathology results showed an increase number of shrinking or degenerating pyramidal cells in the rat hippocampus. We concluded that SCO (2 mg/kg) is the best model for mimicking AD-like disease among D-gala, and $AlCl_3$ by enhancing histopathological alternation and impairing memory in rats without changing hematological and electrolyte parameters.

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## Conflicts of Interest

No conflict of interest

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