Aripiprazole ameliorates scopolamine-induced amnesia in mice

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Abstract

Aripiprazole, a known third generation anti-psychotic drug. The drug has shown to have lesser side effects on extrapyramidal system and enhance memory when compared with the first-generation anti-psychotic drugs. However, studies on the impact of aripiprazole on scopolamine-induced memory impairment in mice have been poorly reported. This study was designed to investigate the impact of aripiprazole on scopolamine-induced amnesia in mice. Thirty-six (36) mice weighing between 20-23g were randomly divided into six groups. Group 1 was given 10 ml/kg distilled water. Group 2 received 3 mg/kg scopolamine alone. Group 3 was given 1 mg/kg donepezil. Group 4 received 0.5 mg/kg aripiprazole. Group 5 was given 0.3 mg/kg aripiprazole. Group 6 received 0.1mg/kg aripiprazole. Thirty minutes after administration of either aripiprazole or donepezil, scopolamine (3 mg/kg) was administered, intraperitoneally. The administration was for 7days, during which their memory was assessed using Morris water maze and Y-maze models. The results showed that the anti-amnesic effect of aripiprazole appeared to be dose-dependent; the animals administered with 0.5 mg/kg aripiprazole showed the greatest improved memory performance against scopolamine-induced amnesia. The hippocampal and prefrontal cortex tissues displayed anti-amnesic potential of aripiprazole. Aripiprazole seems to improved memory performance against scopolamine-induced memory impairment in mice.

Keywords: Aripiprazole; Anti-amnesic; Scopolamine; Memory

INTRODUCTION

Memory is any indication that learning has persisted over time. It is our ability to store and retrieve information [1]. Memory plays a big role in our lives. It allows us to remember skills that we have learnt, or retrieve information that is stored in the brain, or recall precious moments. Memory also organizes information so that when we retrieve it, we can apply that information in the proper context and in the current event. Short term memory which can also be term working memory recall information we learnt recently and long-term memory is used to recall information that we learnt anytime in the recent past to childhood [2]. Amnesia is the general term for a condition

in which memory (either stored memories or the process of committing something to memory) is disturbed or lost, to a greater extent than simple everyday forgetting or absent-mindedness. Amnesia may result either from organic or neurological causes (damage to the brain through physical injury, neurological disease or the use of certain drugs), or from functional or psychogenic causes (psychological factors, such as mental disorder, post-traumatic stress or psychological defense mechanisms) [3].

Antipsychotic drugs are used mainly for the treatment of psychosis, a severe mental disorder characterized by disordered thought processes. The features are blunted or inappropriate emotional responses; bizarre behavior ranging from hypo-activity to hyperactivity with agitation, aggressiveness, hostility, and combativeness; social withdrawal in which a person pays less-than-normal attention to the environment and other people. Others may include deterioration from previous levels of occupational and social functioning hallucinations; and paranoid delusions [4].

Aripiprazole is an atypical antipsychotic agent with unique receptor and low side effect profiles that has been licensed by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) to treat schizophrenia in adults and adolescents [5], manic and mixed episodes associated with bipolar I disorder with or without psychotic features in children, adolescents and adults [6]. Also, it has been used in major depression when used along with antidepressants in adults and irritability associated with autistic disorder [7]. Anti-inflammation potential of aripiprazole inhibiting carrageenan-induced inflammation in laboratory animals have been reported [8]. The relatively safe profile in approved uses has led to off-label uses as well, including treatment of schizophrenia in children, treatment of dementia-related psychosis in geriatric patients and treatment of other behavior problems [9].

Aripiprazole, 7-{4-[4-(2,3-dichloro phenyl)-1-piperazinyl]butyloxy}-3,4-dihydro-2(1H) quinolinone, is the first atypical antipsychotic that may be classified as a Dopaminergic System Stabilizer (DSS). It is presumed to be active against both positive and negative symptoms of schizophrenia, to have a low propensity for extrapyramidal side effects. Also, there is minimal weight gain or sedation and to produce neither elevation in serum prolactin levels nor prolongation of QTc interval on electrocardiogram [10]. It might be considered a "third generation" antipsychotic agent and a relatively new antipsychotic that is differentiated from currently available atypical antipsychotics in its mechanism of action and adverse effect profile. The older generation antipsychotic drugs such as haloperidol have been reported with cardiac problems and extrapyramidal side effects (EPS) [11]. It is a partial agonist at dopamine D2 receptors and serotonin (5-HT) 1A receptors and an antagonist at 5-HT2A receptors [12]. However, studies on the anti-amnesic impact of aripiprazole on scopolamine-induced memory deficit in mice have been poorly reported. This study investigated the impact of aripiprazole on scopolamine-induced amnesia in mice.

EXPERIMENTAL

Chemicals and drugs. Department of Pharmacology, Obafemi Awolowo University, Ile-Ife supplied scopolamine used for the experiment. Aripiprazole was purchased from Consilient Health Ltd Clonskeagh Dublin, Ireland. Donepezil was purchased from a Registered Pharmacy, Alpha Pharmacy Ikeja Lagos, manufactured by Wilcare Pharma Limited, Watford, England WD 18 84H and expired September, 2019.

Experimental animals. Thirty mice weighing 20-23 g aged 11-12 weeks were obtained from the Laboratory Animal House of College of

Health Sciences, Bowen University, Iwo, Nigeria. The Animals were maintained under standard nutritional and environmental conditions of normal relative humidity, room temperature $25\pm2^{\circ}C$ and 12-hour light/dark cycle throughout the experiment. The mice were acclimatized for a period of two weeks to the laboratory environment and provided with standard feeds and clean water *ad libitum*. The mice were housed in the Experimental Animal House, College of Health Sciences, Bowen University Iwo, in a well-ventilated plastic cage with large holed wire mesh in the cover. Their cages were cleaned of waste daily. The mice were weighed on the first day of Scopolamine administration, during treatment of the animals with drugs, first day of trial of the Morris water maze test and last day of administration of using a weighing scale. The experimental procedures adopted in this study were in accordance with the United State National Institute of Health guidelines for care and use of laboratory Animal in Biomedical Research [NIH, 1985]. The work was approved by the College of Health Sciences ethical committee Bowen University, Iwo.

Experimental design. Thirty-six (36) laboratory mice were randomly distributed into six groups with six mice in each group (n=6).

Group 1(Control)-The mice were administered 10 ml/kg distilled water alone through oral route of administration.

Group 2 (Scopolamine alone)-Memory impaired by administration of 3 mg/kg Scopolamine.

Group 3 (Reference)-Memory impaired by administration of 3 mg/kg Scopolamine 30min after pretreatment with 1 mg/kg Donepezil.

Group 4 – Memory impaired administration of 3 mg/kg Scopolamine 30min after pretreatment with 0.5 mg/kg Aripiprazole

Group 5 – Memory impaired administration of 3 mg/kg Scopolamine 30min after pretreatment with 0.3 mg/kg Aripiprazole

Group 6 – Memory impaired by administration of 3 mg/kg Scopolamine 30min after pretreatment with 0.1mg/kg Aripiprazole.

All drugs were administered intraperitoneally.

Memory studies. Memory impairment was induced by intraperitoneal administration of 3 mg/kg Scopolamine in the mice for 7 consecutive days after administration of Aripiprazole. Group 3 was pretreated with 1mg/kg Donepezil consecutively for the 7 days. Groups 4, 5, and 6 were also pretreated with 0.5 mg/kg Aripiprazole, 0.3 mg/kg Aripiprazole and 0.1 mg/kg Aripiprazole respectively consecutively for the seven days. On the third, fourth and fifth days of administration, the trials began. Three trials were carried out at an interval of 30 minutes each day using Morris water maze [13].

On the last day of administration, the probe trial test was carried out on the mice using Morris Water Maze post administration followed by another model of memory impairment assessment, Y maze.

MORRIS WATER MAZE. The Morris water-maze procedure was performed as described by Christopher and others [14]. Animals were placed in circular basin (124 cm in diameter) containing water. The water was made opaque using liquid peak milk. Water temperature was maintained at room temperature. The escape platform was $25cm^2$ in diameter and was placed in the centre of the North-East quadrant region. It was submerged $1cm^2$ beneath the water surface. The platform remained in the same position throughout the learning trials. On the first day of training, before the first trial, the mice were placed on the platform for 20 seconds to allow orientation. After orientation, the mice were then placed into the water facing the wall of the basin at the South-West quadrant and allowed to search for the platform. The trial ended when an animal climbed onto the platform or when a maximum of 60 seconds elapsed. If the mouse located the platform before 60 seconds had passed, it was immediately removed from the pool. But, if the platform was not located after 60 seconds of swimming, the mouse was gently guided to the platform and allowed to re-orient to the distal visual cues for an additional 20 seconds before

being removed from the pool. After removal from the pool, mice were manually dried with a towel and placed in a cage. Mice were visually inspected to ensure thorough dryness. Mice were tested in three trials per day with an inter-trial interval of approximately 30 minutes. All testing was conducted at roughly the same time each day in order to minimize variability in performance due to time of day. For each trial, the escape latency and time spent in target quadrant were recorded. To examine spatial reference memory, a probe test was administered on the last day of administration which was 24hour after the last training session. During the probe trial test, the platform was removed from the pool and the mouse was allowed to swim freely for 60 seconds.

Y-MAZE. Y-maze apparatus consisted of three arms made of wood joined in the middle to form a ''Y'' shape. The inside of the arms was identical, providing no intra maze cues. This ethologically relevant test is based on the rodents' innate curiosity to explore novel areas and presents no negative or positive reinforcers and very little stress for the mice. The Y-maze design and experimental procedure was based on published protocol with modifications to adapt the system to mice [15]. The Y-maze used was composed of three equally spaced arms (120°; 35 cm long $\times$ 5 cm wide $\times$ 8 cm high). Activity in a Y-maze was used to measure spontaneous alternation performance (spatial working memory). The mice were placed in one of the arm compartments and allowed to move freely for 5 minutes. The sequence of arm entries was manually recorded. The Y-maze was cleaned with 70 % ethyl alcohol and permitted to dry between tests. Alternation was defined as an entry into all three arms in consecutive choices. Spontaneous alternation percentage (SA%) was defined as the ratio of actual alternations (the arm entry choices that differed from the previous two choices) to the maximum alternation. The number of maximum

alternation was then calculated as the total number of arms entered minus 2.

$$\% \, Spontaneous \, alternation = \frac{Actual \, alternations}{Maximum \, alternations} X100$$

Histological studies. At the end of the experiment, two mice in each group were selected for light microscopy study. Under anesthesia, the brains were perfused through a transcranial perfusion of 200ml normal saline followed by 200 ml of 4% paraformaldehyde in 0.1M phosphate buffer. The brains were removed, weighed and preserved in 10% formalin saline. Hematoxylin and Eosin stain (H & E stain) procedures were carried out in the Department of Histopathology, University College Hospital (UCH) Ibadan to determine the morphological changes in the Hippocampus and Prefrontal Cortex. The results were interpreted by a neuropathologist in the Department of Histopathology, (UCH), Ibadan.

Biochemical assay. Mice in each group, at the end of the experiment, were decapitated under ethyl ether anaesthesia and the brains were immediately removed, weighed and kept in the refrigerator with ice block for 30mins. Thereafter, the whole brain was homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4). Each brain tissue homogenate was separated into various portions for the different biochemical assays. Acetylcholinesterase and antioxidant activities were carried out at the Department of Biochemistry, University of Ilorin to assess memory function.

Statistical analysis. The data were analyzed using GraphPad Prism software version 7.0 and were expressed as Mean $\pm$ Standard error of mean (S.E.M). Statistical analysis was done using ANOVA, followed by Dunnett's post hoc tests. Comparison between the means of control group and the treated groups were made and values of $P < 0.05$ were considered significant. Comparison between the means of Scopolamine group and the treated groups

were also made and values of $P < 0.05$ were considered significant.

RESULTS

Behavioural studies. The results of the experiment were tabulated as mean ± SEM as shown in Table 1. When the mean values for the weight of mice in all groups were compared with the control and Scopolamine group, there was no statistically significant difference. The results obtained as shown (Figure 1) after 7 days of treatment revealed that: The percentage alternation for the Scopolamine group was significantly decreased [F(5,30)=17.6; p=0.0001] (****P<0.0001) when compared with the control group. The percentage alternation for the treatment groups – Donepezil + Scopolamine, 0.5mg/kg ARI+Scopolamine, 0.3mg/kg ARI+Scopolamine, 0.1mg/kg ARI+Scopolamine groups showed no significant difference (p>0.05) when compared to the control group. The percentage alternation for the treatment groups (Donepezil+Scopolamine, 0.5mg/kg ARI, 0.3mg/kg ARI, 0.1mg/kg ARI) showed significant [F(5,30)=17.6; p=0.0001] increase (####P<0.0001) when compared with Scopolamine group (26.50 ± 3.753). After 7 days of treatment, statistical analysis of the results showed (Figure 2) that the time spent in quadrant for the 0.5mg/kg ARI+ Scopolamine and 0.3 mg/kg ARI+ Scopolamine groups showed significant increase [F(5,28)=7.191; p=0.0002] (p<0.01) when compared with the control group. The time spent in quadrant for Scopolamine (9.50±0.29) group decreased (p>0.05) in comparison with control group. However, the time spent in quadrant for the Donepezil+Scopolamine and 0.1mg/kg ARI+ Scopolamine groups increased though not significantly when compared to the control group. The time spent in quadrant for the 0.5mg/kg ARI+Scopolamine, 0.3 mg/kg ARI+Scopolamine and 0.1 mg/kg ARI+ Scopolamine groups showed significant

[F(5,28)=7.191; p=0.0002] increase when compared with Scopolamine group. The time spent in quadrant for the Donepezil + Scopolamine group increased though not significantly (p>0.05) when compared to the Scopolamine group. The results obtained in Figure 3 after 7 days of treatment showed that the first day of trial, the percentage target quadrant time for all the treatment groups showed no significant difference (p>0.05) when compared to the control group and Scopolamine group.

On the second day of trial, the percentage target quadrant time for the Scopolamine groups showed significant decrease (p<0.05) when compared with control group. The time spent in target quadrant for the 0.5 mg/kg ARI+Scopolamine and 0.3mg/kg ARI+Scopolamine, 0.1 mg/kg ARI+Scopolamine and 1 mg/kg Donepezil+Scopolamine increased though not significantly (p>0.05) when compared to control group. There was increase though not significantly (p>0.05) when the treatment groups were compared with Scopolamine group.

On the third day of trial, the percentage target quadrant time for the Scopolamine group, 0.3 mg/kg ARI+Scopolamine and 0.1 mg/kg ARI+Scopolamine groups showed significant difference (p<0.05) when compared with control group. However, the time spent in quadrant for the 0.5 mg/kg ARI+Scopolamine and 0.3 mg/kg ARI + Scopolamine and 1mg/kg Donepezil + Scopolamine showed significant increase [F(4,75)=7.13;p=0.0001] (p<0.05) when compared with scopolamine group.

The results obtained (Table 2) after 7 days of treatment showed that the escape latencies of the treatment groups were compared with the control and Scopolamine group, there was no significant difference. However, there was a steady increase in the escape latency of the Scopolamine group when compared with the control group. The mean values of the weights of the brain at the onset

of administration, the trials, and the last day of administration are shown in Table 3, the mean values of the weight of the brain in the treatment groups were compared with the control and Scopolamine group, and there was no significant difference.

Biochemical assay result of impact of aripiprazole in mice. The mean values of Glutathione Concentration (GSH), Lipid peroxidation (Malondialdehyde) (MDA), Superoxide Dismutase enzyme activity (SOD) and Nitric Oxide (NO) are shown in Table 4, when the mean values of GSH, MDA, SOD and NO concentration in the brain in all groups were compared with the control and Scopolamine group, they were not statistically significant.

DISCUSSION

The findings of this study revealed that aripiprazole (ARI) seem to improved behavioural memory performance against scopolamine-induced memory deficit in mice. There is regeneration of neurons in the hippocampus and prefrontal regions of the mice brain as shown in the gross histology of the mice brains. This is an evidence that aripiprazole improves memory performance in the brain region of the mice. There is improvement in antioxidant activity but were not significant in the results obtained.

Scopolamine-induced memory impairment is useful in detecting active anti-amnesic agents and to predict the value of anti-amnesic agents by impairing cholinergic neurotransmission and blocking muscarinic receptors [16]. Blokland [17] reported that cholinergic system plays an important role in learning and memory functions. Impairment of the cholinergic system is a major neuropathological feature that is linked with memory loss and closely associated with the severity of cognitive dysfunction in Alzheimer's disease (AD) [18].

The Morris Water Maze test is an approved model and has been extensively used in the study of spatial learning and memory [19]. The Y-maze spontaneous alternation test is also widely used in the international classic learning and behaviour evaluation techniques and methods [20]. In Morris water maze model, the animals administered with 0.3mg/kg ARI showed significant increase in time spent in training quadrant on the last day of trial (Day 5 of administration) and during the probe trial after Scopolamine-induced amnesia. Also, 0.1mg/kg groups' time spent in target quadrant during the probe trial phase was significant. However, the increase in target quadrant time observed during the training period in the animals treated with 0.1mg/kg ARI was not statistically significant.

Table 1: Mean value of the weights (g) of the mice at the onset of administration (Day 1), onset of Trials (Day 3) and last day of administration (Day 7).

Groups	Day 1	Day 3	Day 7
Control (10ml/kg)	21.33 ± 1.58	20.67 ± 1.41	22.33 ± 1.31
Sc (3mg/kg)	21.83 ± 0.87	21.33 ± 0.61	21.25 ± 0.85
Dp (1mg/kg) + Sc (3mg/kg)	21.50 ± 1.43	21.83 ± 1.33	21.83 ± 1.45
0.5mg/kg ARI + Sc (3mg/kg)	23.00 ± 0.93	21.83 ± 1.08	22.5 ± 0.99
0.3mg/kg ARI + Sc (3mg/kg)	20.83 ± 1.66	19.83 ± 1.47	21.17 ± 1.62
0.1mg/kg ARI + Sc (3mg/kg)	21.33 ± 0.99	20.17 ± 1.08	20.33 ± 1.31

Values are expressed as Mean ± Standard error of mean for 6 animals per group.
Sc represents Scopolamine, Dp represents Donepezil and ARI represents Aripiprazole
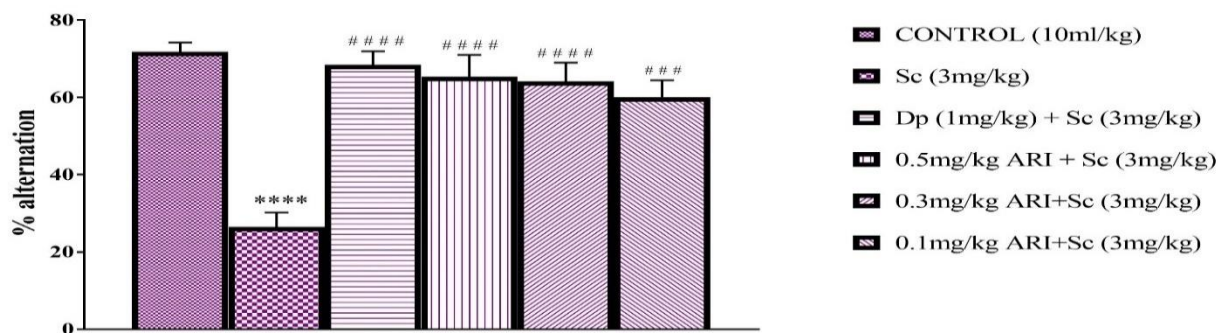
Figure 1: Chart showing the mean value of percentage alternation of the mice in Y-maze test in mice
Values are expressed as Mean ± Standard error of mean for 6 animals per group. ****P < 0.0001 compared to control group (ANOVA followed by Dunnett's test post-hoc); ###P<0.001, ####P < 0.0001 compared to Scopolamine group (ANOVA followed by Dunnett's test post-hoc)   Sc = Scopolamine, Dp = Donepezil and ARI = Aripiprazole.
* Indicates the significant difference when compared with the control group using the one-way ANOVA followed by Dunnett's test post-hoc. # Indicates the significant difference when compared with Scopolamine group using the one-way ANOVA followed by Dunnett's test post-hoc
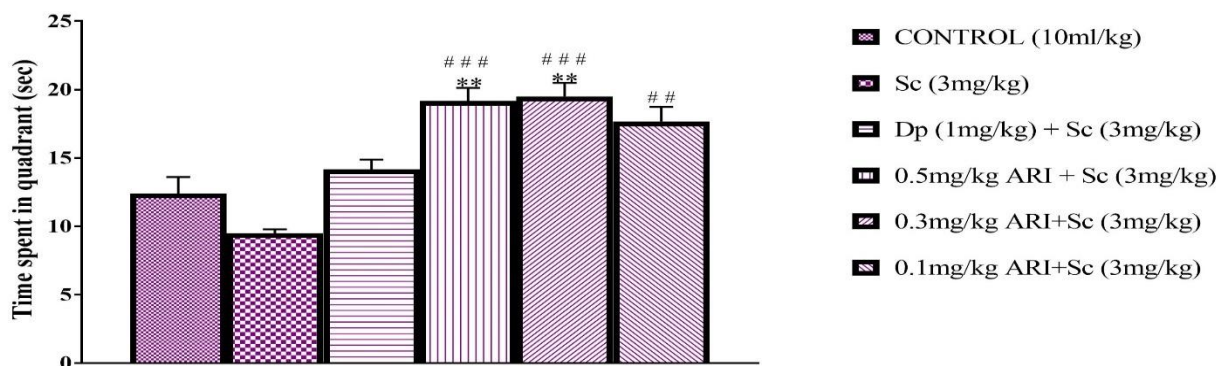

Figure 2: Chart showing the mean value of time spent in quadrant (s) of the mice in Morris water maze test.
*Values are expressed as Mean ± Standard error of mean for 6 animals per group. **P< 0.01 compared to control group (ANOVA followed by Dunnett's test post-hoc).##P<0.001, ###P< 0.001 compared to Scopolamine group (ANOVA followed by Dunnett's test post-hoc).* Sc = Scopolamine, Dp = Donepezil and ARI Aripiprazole * Indicates the significant difference when compared with the control group using the one-way ANOVA followed by Dunnett's test post-hoc. # Indicates the significant difference when compared with Scopolamine group using the one-way ANOVA followed by Dunnett's test post-hoc.
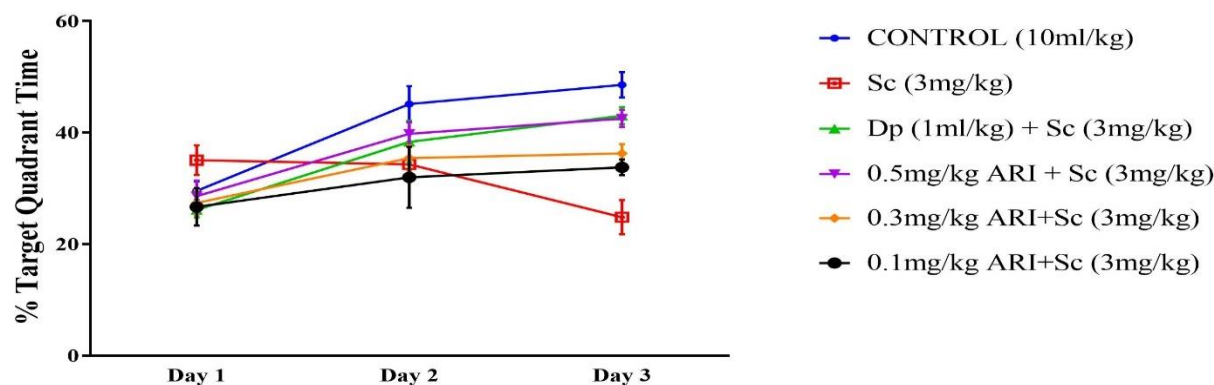

FIGURE 3: Chart showing the mean value of percentage time spent in target quadrant in Morris water maze test.
Values are expressed as Mean ± Standard error of mean for 6 animals per group. *P< 0.05,   **P< 0.01, ***P< 0.001 compared to control group (ANOVA followed by Dunnett's test post-hoc). #P< 0.05, ##P< 0.01, ###P< 0.001 compared to Scopolamine group (ANOVA followed by Dunnett's test post-hoc).   Sc = Scopolamine, Dp = Donepezil and ARI = Aripiprazole.
* Indicates the significant difference when compared with the control group using the one-way ANOVA followed by Dunnett's test post-hoc. # Indicates the significant difference when compared with Scopolamine group using the one-way ANOVA followed by Dunnett's test post-hoc.

Table 2: Mean value of Escape Latency (sec) of the mice in Morris water maze test

Groups	Day 1 (Secs)	Day 2 (Secs)	Day 3 (Secs)
Control (10ml/kg)	40.22 ± 4.76	15.28 ± 2.09	12.11 ± 2.05
Sc (3mg/kg)	34.934 ± 4.32	20.07 ± 2.03	13.27 ± 2.50
Dp (1mg/kg) + Sc (3mg/kg)	34.11 ± 4.87	15.61 ± 4.35	10.17 ± 4.98
0.5mg/kg ARI + Sc (3mg/kg)	36.89 ± 3.09	16.33 ± 2.12	11.45 ± 1.91
0.3mg/kg ARI + Sc (3mg/kg)	37.61 ± 4.98	16.89 ± 3.72	12.50 ± 3.52
0.1mg/kg ARI + Sc (3mg/kg)	41.28 ± 4.68	20.45 ± 4.43	12.17 ± 4.46

Values expressed as Mean ± Standard error of mean for 6 animals per group. Sc = Scopolamine, Dp Donepezil ARI = Aripiprazole.

Table 3: Mean value of the weights (g) of the brain at the onset of administration (Day 1), onset of Trials (Day 3) and last day of administration (Day 7).

Groups	Grams (g)
Control (10ml/kg)	0.3625 ± 0.017
Sc (3mg/kg)	0.395 ± 0.012
Dp (1mg/kg) + Sc (3mg/kg)	0.405 ± 0.020
0.5mg/kg ARI + Sc (3mg/kg)	0.400 ± 0.009
0.3mg/kg ARI + Sc (3mg/kg)	0.3825 ± 0.021
0.1mg/kg ARI + Sc (3mg/kg)	0.3625 ± 0.003

Values are expressed as Mean ± Standard error of mean
Sc represents Scopolamine, Dp represents Donepezil and ARI represents Aripiprazole

Table 4: Impact of Aripiprazole on Glutathione Concentration (GSH), Lipid peroxidation (Malondialdehyde) (MDA), Superoxide Dismutase enzyme activity (SOD) and Nitric Oxide (NO)

Treatment and Dose		
Control (10ml/kg)		0.1513 ± 0.0515
Sc (3mg/kg)		0.1010 ± 0.0175
Dp + Sc (3mg/kg)	GSH Conc. (mM) →	0.1557 ± 0.0240
0.5mg/kg ARI + Sc		0.1387 ± 0.0049
0.3mg/kg ARI + Sc		0.1477 ± 0.0177
0.1mg/kg ARI + Sc		0.1143 0.0092
Control (10ml/kg)		2.5345 ± 0.2413
Sc (3 mg/kg)		2.9978 ± 0.0930
Dp + Sc (3 mg/kg)	MDA Conc. (uM) →	2.4389 ± 0.3535
0.5 mg/mg ARI + Sc		2.7153 ± 0.2338
0.3 mg/mg ARI + Sc		2.8664 ± 0.7462
0.1 mg/mg ARI + Sc		2.9133 ± 0.1865
Control (10 ml/kg)		0.8431 ± 0.0934
Sc (3 mg/kg)		0.7122 ± 0.1036
Dp + Sc (3 mg/kg)	SOD Conc. (U/ml) →	0.8185 ± 0.0340
0.5 mg/kg ARI + Sc		0.9084 ± 0.1648
0.3 mg/kg ARI + Sc		0.7462 ± 0.0885
0.1 mg/kg ARI + Sc		0.7284 ± 0.067
Control (10ml/kg)		53.503 ± 1.1016
Sc (3mg/kg)		26.0323 ± 1.7354
Dp + Sc (3mg/kg)	NO Conc. (uM) →	56.1827 ± 2.6383
0.5 mg/mg ARI + Sc		59.7503 ± 1.0685
0.3 mg/mg ARI + Sc		48.6247 ± 2.0450
0.1 mg/mg ARI + Sc		34.955 ± 6.6480

Values are expressed as Mean ± Standard error of mean   Sc = Scopolamine, Dp = Donepezil and ARI = Aripiprazole
From the above table, when the mean values of GSH, MDA, SOD and NO concentration in the brain in all groups were compared with the control and Scopolamine group, they were not statistically significant.
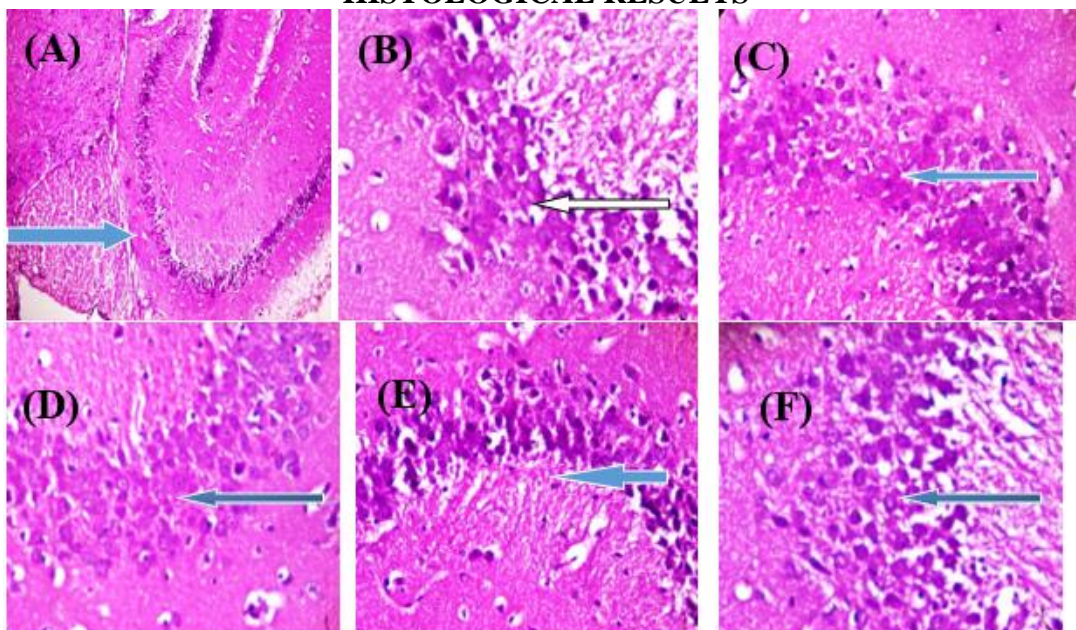
HISTOLOGICAL RESULTS

Plate 1: PHOTOMICROGRAPH OF THE HIPPOCAMPAL REGION (H&E STAIN)
Haematoxylin and Eosin staining of hippocampal tissue of mice pretreated with: **(A)** Distilled water (10ml/kg), **(B)** Scopolamine (3 mg/kg), **(C)** Donepezil (1 mg/kg) + Scopolamine (3 mg/kg), **(D)** 0.5 mg/kg ARI + Scopolamine (3 mg/kg), **(E)** 0.3 mg/kg ARI + Scopolamine (3mg/kg), **(F)** 0.1 mg/kg ARI + Scopolamine (3 mg/kg). Each photomicrograph is representative of six specimens for each group. All figures were magnified by 400X. White arrow indicates depleted neuronal cells. Blue arrow indicates normal structural organization of the cornu Ammonis including CA1, CA2 AND CA3 subfields.
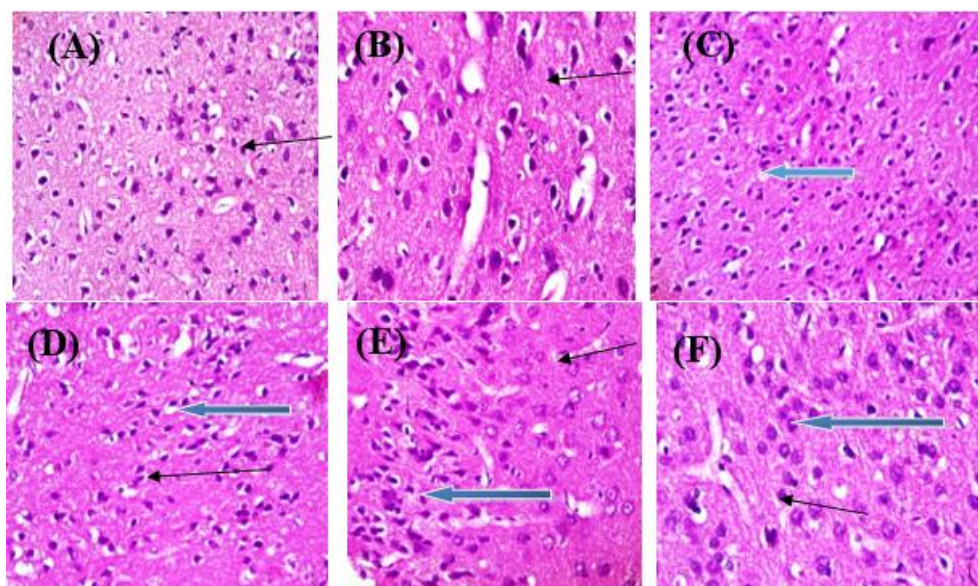

Plate 2: PHOTOMICROGRAPH OF THE PREFRONTAL CORTEX (H&E STAIN)
Haematoxylin and Eosin staining of prefrontal cortex tissue of mice pretreated with: **(A)** Distilled water (10 ml/kg), **(B)** Scopolamine (3 mg/kg), **(C)** Donepezil (1 mg/kg) + Scopolamine (3 mg/kg), **(D)** 0.5 mg/kg ARI + Scopolamine (3 mg/kg), **(E)** 0.3 mg/kg ARI + Scopolamine (3 mg/kg), **(F)** 0.1 mg/kg ARI + Scopolamine (3 mg/kg). Each photomicrograph is representative of six specimens for each group. All figures were magnified by 400X. Slender arrow indicates normal stroma. Blue arrow indicates piknotic nuclei.
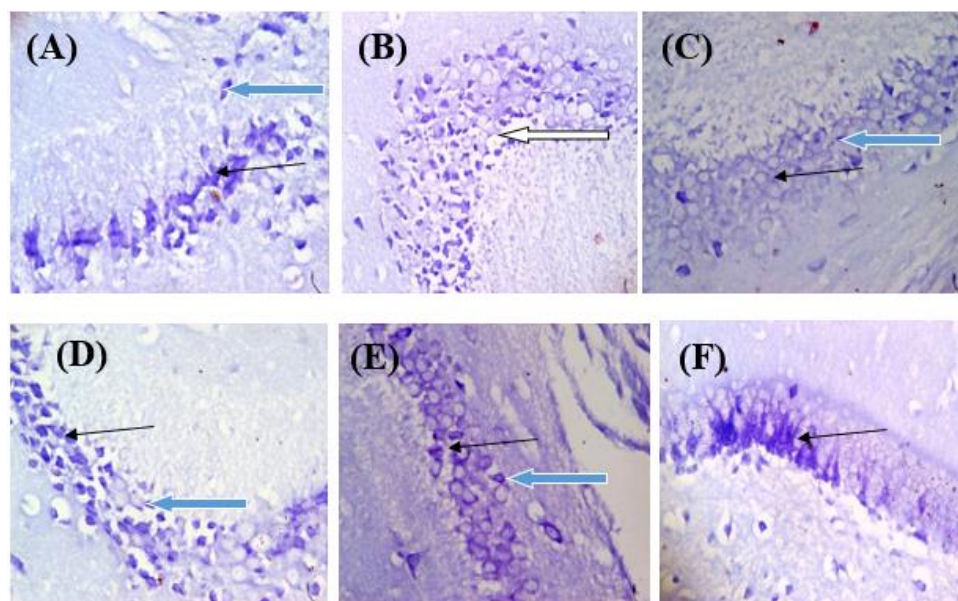
Plate 3: PHOTOMICROGRAPH OF THE HIPPOCAMPAL REGION (CRESYL FAST VIOLET STAIN)
Cresyl fast violet staining of hippocampal tissue of mice pretreated with: **(A)** Distilled water (10 ml/kg), **(B)** Scopolamine (3 mg/kg), **(C)** Donepezil (1 mg/kg) + Scopolamine (3 mg/kg), **(D)** 0.5 mg/kg ARI + Scopolamine (3 mg/kg), **(E)** 0.3 mg/kg ARI + Scopolamine (3 mg/kg), **(F)** 0.1 mg/kg ARI + Scopolamine (3 mg/kg). Each photomicrograph is representative of six specimens for each group. All figures were magnified by 400X. Blue arrow indicates the cells of the CA1 to CA3 are well organized and not dispersed. Slender arrow indicates normal distribution of nissl bodies within the neurons including the pyramidial cells. White arrow indicates moderately depleted neuronal cells at CA2
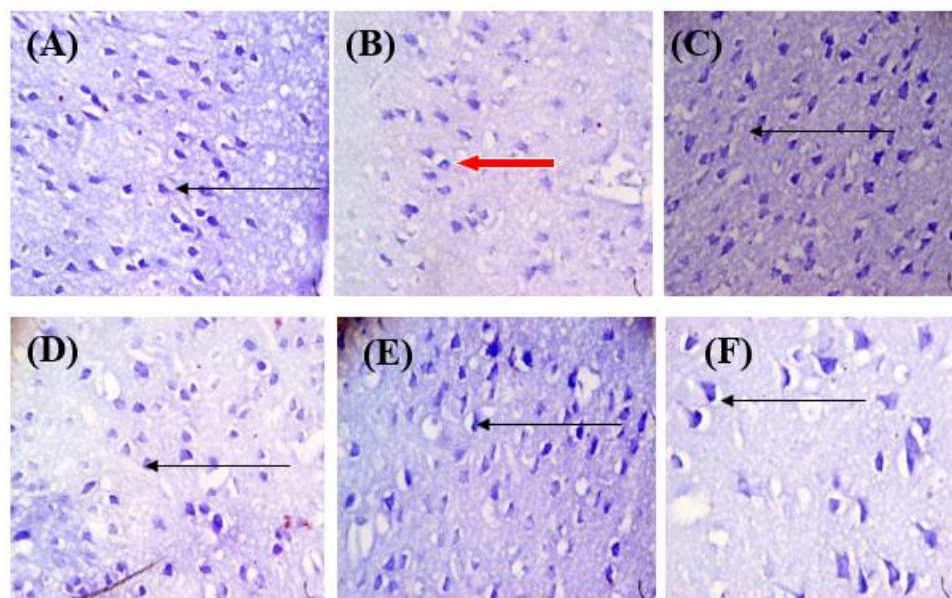

Plate 4: PHOTOMICROGRAPH OF THE PREFRONTAL CORTEX (CRESYL FAST VIOLET STAIN)
Cresyl fast violet staining of prefrontal cortex tissue of mice pretreated with: **(A)** Distilled water (10 ml/kg), **(B)** Scopolamine (3 mg/kg), **(C)** Donepezil (1 mg/kg) + Scopolamine (3 mg/kg), **(D)** 0.5 mg/kg ARI + Scopolamine (3 mg/kg), **(E)** 0.3 mg/kg ARI + Scopolamine (3 mg/kg), **(F)** 0.1 mg/kg ARI + Scopolamine (3 mg/kg). Each photomicrograph is representative of six specimens for each group. All figures were magnified by 400X. Slender arrow Showing normal layer and several normal neuronal cells with normal cytoplasmic distribution of nissl bodies. Red arrow indicates few neuronal cells with poor cytoplasmic distribution of nissl bodies

In the Y maze model, all animals treated with the different doses of aripiprazole (0.5 mg/kg, 0.3 mg/kg, 0.1 mg/kg) showed statistically significant increase in percentage alternation against scopolamine-induced amnesia which represents an index in improved memory. There was no significant effect of ARI on the weight of the animals and on the weight of the brain.

Casey and co-workers reported that aripiprazole is a partial agonist at dopamine D2 receptors and at serotonin 5-HT1A receptors but an antagonist at 5-HT2A receptors [21]. Also, the drug is known for its neutral effect on body weight, triglyceride and prolactin levels and sedation [22]. Furthermore, Dopaminergic (DA) neurons densely innervate the dorsal and ventral striatum, and project to the hippocampus (HPC), prefrontal cortex cortical and other certain subregions. DA is a major catecholamine neurotransmitter in the brain of mammal and prominently involved in motor control, motivation, emotional, learning and memory [23]. Studies have shown that 5-hT system consists of 14 receptor subtypes. They are involved in the regulation of cognitive process and that 5-HT1A receptor is well known to play a crucial role in learning and memory [24]. Also, several reports have shown that 5-HT1A receptor is a potent therapeutic target for memory deficits, such as cognitive dysfunction in schizophrenia, Alzheimer's disease and epilepsy [25-27]. The hippocampus region of the brain structure has been implicated for learning, memory and cognition [28].

The aripiprazole enhancement of memory functions observed in this study might seems to improve memory impairments via the 5-HT1A and D2 receptors as evidence in the Nissls stains and Haematoxylin. Also, Eosin staining of the hippocampus regions and the prefrontal cortex of the experimental animals are consistent with literature.

Furthermore, aripiprazole (partial agonist of D2 receptor) improved working memory of the experimental animals as seen in Morris water maze task and the Y-maze task in this study which is consistent with the studies reported by Castro-Caldas et al. [29] and Costa et al. [30] that Dopamine receptor agonist such as pramipexole, piribedil and pergolide, significantly improved executive functions and working memory performance in Parkinsonism (PD) patients with mild cognitive impairment. Also, the results of this study are consistent with other Scopolamine-induced memory impairment animal model showing reduced spatial memory compared with control group in the Y-Maze test and Morris Water Maze test [31-32].

Pharmacological evaluation of memory functions usually involves both behavioural and biochemical approaches. The biochemical approach involves evaluation of the underlying pathological process that may influence the behaviour component of memory functions. The biochemical assays carried out in this study revealed that 0.5 mg/kg aripiprazole demonstrated potential antioxidant activity. It is generally accepted that oxidative stress is significant in the formation of the pathology of AD [33]. Several reports highlight the potential role of anti-oxidant therapeutic agents in the treatment of AD [34].

Aripiprazole also decreased the concentration of oxidative stress markers in the brain like Malondialdehyde (MDA) in a dose-dependent manner. This further buttressed its antioxidant potential. Similar effects were also observed in the group treated with Donepezil (1mg/kg). In contrast, Scopolamine group increased the concentration MDA in mice brain.

The histological findings in the study showed that aripiprazole is able to protect against structural damage of neurons of hippocampal and prefrontal cortex (PFC) caused by scopolamine. The scopolamine extensively destroys the hippocampal region and induces neurotoxicity in the brain, as

revealed by the observed cellular clusters with pyknotic characteristics, which were observed in the PFC layers. The cornus ammonis regions of the hippocampus were poorly organized in the group treated with scopolamine. The findings of this study are consistent with research study indicating that administration of scopolamine causes cell loss in hippocampal neurons [35].

Further histological analysis revealed that treatment of mice with aripiprazole prevented scopolamine-induced memory impairment and neuronal degeneration especially at higher dose of 0.5 and 0.3 mg/kg. We also observed that the effect was similar to Donepezil, a well-known rapidly-reversible cholinesterase inhibitor widely used in the treatment of Alzheimer's disease [36].

Inge and Arjan [36] reported that agents that ameliorate Scopolamine-induced memory deficit significantly may have anti-amnesic potential. Therefore, the findings from this study are indications that atypical anti-psychotic drug like Aripiprazole may be of potential benefit against memory impairment associated with psychotic disorders through dopaminergic and serotonergic pathways.

Conclusion. The results of the study showed that aripiprazole seems to improve memory loss from the behavioural models and the histology of the hippocampus as expressed in the neuronal changes of the cornus ammonis and the prefrontal cortices of the mice.

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