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Original Article

Assessment of 1, 3, 4- Oxadiazole Derivatives for Memory Enhancing Activity

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ABSTRACT

Background & Objective: Memory is a crucial process which assists human to record their experiences and use it to adapt them for living in the environment. Acetylcholine, the central neurotransmitter is responsible for the cognitive function. Memory impairment is main characteristics of neurodegenerative disorder like Alzheimer disease. Scopolamine – a muscarinic blocker blocks the acetylcholine receptor and causes defect in learning & memory process. The current study was conceived to investigate the effects of novel 1, 3, 4-oxadiazole derivatives on learning and memory in Swiss albino mice.

Materials and Methods: Two novel compounds (compound 1 and 2) were subjected for acute toxicity studies at 5,50,300,2000 mg/kg in wister rats weighing about 150-180g as per OECD guideline 423. In this study, Swiss albino mice weighing about 20-25g were used. Total 42 animals were divided into 7 groups of 6 animals in each group. The animals were administered with 2 novel compounds at 15 and 30 mg/kg for seven days and on the seventh day scopolamine (1mg/kg *i.p*) was given to induce memory loss. Morris water maze task was employed to assess the spatial learning memory. Estimation of brain acetyl cholinesterase activity was also done.

Results: The results were statistically analysed by using one way ANOVA method. Scopolamine-induced amnesia was reversed by novel 1, 3, 4-oxadiazole derivatives, the observed beneficial effects on learning and it may be due to of facilitation of cholinergic transmission in mouse brain.

Conclusion: Compound 1 dose 2(30mg/kg) showed promising action on both behavioural & biochemical parameters. Therefore, it would be worthwhile to investigate the therapeutic potential of novel 1, 3, 4-oxadiazole derivatives in the management of cognitive disorder. However, future studies are required to identify the exact mechanism of action. In the current investigation, 1, 3, 4-oxadiazole derivatives have shown promising protective effects as a memory enhancing agents.

Key words: 1,3,4-oxadiazole derivatives, Morris water maze, Time spent in the target quadrant, Escape latency time.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive, neurodegenerative disorder of brain characterized by dementia; by the loss of neurons and synapses in the cerebral

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cortex, sub-cortical regions results in the gross atrophy including temporal lobe, frontal lobe, parietal lobe, Cingulated gyrus .The central cholinergic pathways plays a vital role in learning and memory processes[1]. Centrally acting anti-muscarinic drugs (e.g. scopolamine) impairs learning and memory both in animals[2]and human beings[3].AD individuals' exhibit deterioration in mental functions rendering them debilitated to perform normal day-to-day activities. However, evidence indicates that AD can also affect individuals in their young age such as early as 40 years of age[4].Normal ageing in human beings known to cause memory deterioration. Oxygen free radicals[5], the harmful by-products of oxidative metabolism are known to cause organic damage to the living system[6]which may be responsible for the development of AD in elderly patients. The research-based pharmaceutical industry has increasingly employed modern medicinal chemistrymethods[7], including molecular modelling, as powerful tools for the study of structure-activity relationships Molecular docking approach allow us to designate the interaction between a small molecule and a protein at the atomic level, which further allow us to characterize the behaviour of small molecules in the binding site of target proteins as well as to elucidate fundamental biochemical processes[8]. 1, 3,4Oxadiazole is a thermally stable neutral aromatic molecule. Out of its four possible isomers such as 1,3,4oxadiazole, 1,3,4oxadiazolines, 1,3,4oxadiazolium cations, and the exocyclic conjugated mesoionic 1,3,4oxadiazole[9], 1,3,4 - oxadiazole is widely used for various applications. Literature survey reveals that 1,3,4 - oxadiazole exhibited a wide range of biological activities which includes antibacterial, anti-tubercular, anti-Alzheimer, anticonvulsant, hypoglycaemic, anti-allergic, enzyme inhibitor, vasodilator, antifungal, cytotoxic, anti-inflammatory, analgesic, hypolipidemic, anticancer, insecticidal activities etc. The current study was undertaken to scrutinize the memory enhancing effect of 1,3,4-oxadiazole derivatives in mice using Morris water maze model.

2. MATERIALS AND METHODS

Chemicals and drugs:

1, 3, 4 - oxadiazole derivatives were synthesized and obtained from Department of Pharmaceutical Chemistry , JSS College of Pharmacy, Mysuru. Scopolamine Hydro bromide was obtained from Sigma Aldrich, Lt. Louis, MO, and USA. It was dissolved separately in normal saline and injected *i.p*, Piracetam (Nootrpil®, UCB India Pvt. Ltd., Marg, Mumbai) was obtained from the local market.

Animals

The experiments were carried out on male Swiss albino mice weighing 20-25g. Animals used in the study were procured from Animal house, JSS Medical College, Mysore. The animal care and handling was carried out in accordance with guidelines issued by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Animals were acclimatized to the experimental room for one week prior to the experiment. Animals were maintained under controlled conditions of temperature (27 ± 2ºC) and were caged in polypropylene cages containing sterile paddy husk as bedding material with maximum of six animals in each cage. The animals were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) approval number: 175/2015, JSS College of Pharmacy, Mysore, and Karnataka.

Animal Grouping:

In this study, Swiss albino mice weighing about 20-25g were used. Total 42 mice were used. Animals were divided into 7 groups as shown table 1.

Acute toxicity Studies:

1,3,4-oxadiazole derivative 1 and 2 were administered at various doses(5,50,300,2000 mg/kg)as per OECD guidelines 423 (Acute toxic class method) for 14 days[10]. The starting dose selected was 300mg/kg. During the first four hours after the drug administration, the animals were observed for gross behavioural changes, if any for another 3 days. No mortality or moribund status was observed.

Morris water maze

Morris water maze was performed according to Mohan *et al* 2014 [11] and Achliya *et al* 2004 [12] with slight modification according to laboratory conditions. The results were statistically analyzed using one way ANOVA method.

Estimation of Brain Acetyl Cholinesterase Activity:

The whole brain AChE activity was measured using the Ellman method [13, 14]with slight modification according to laboratory conditions. The results were statistically analyzed using one way ANOVA method.

3. RESULTS

Acute toxicity study

The result of the acute oral administration of 1,3,4-oxadiazole derivatives (compound 1 and 2) in various doses of 5, 50, 300, 2000 mg/kg indicated no mortality up to 14 days after treatment.

Morris water maze

The improvement effects of the 1,3,4-oxadiazole derivatives(compound 1 and 2)on special learning and memory process were assessed by Morris water maze test. The escape latency for finding the hidden platform is shown in Table 2 & Graph 1-3. In addition, the result exhibited a significance difference of escape latencies when compared the 0^{th} day and 4^{th} day. The escape latencies of compound 1 and 2(15mg/kg and 30mg/kg) and Piracetam (200 mg/kg) - treated mice were rapidly reduced after day 1. These groups also exhibited a significant reduction in escape latencies when compared the day 0 and day 4. The Time Spent in the Target Quadrant of Morris water maze at 0^{th}, 4^{th}& 8^{th} day are shown in Table 3& Graph 4-6. The time spent in the target quadrant (TSTQ) were increased when compared day 0 with day 4.on the assessment day(day 8)after the scopolamine

administration, the ELT was found to be reduced in the standard and the compound 1 and 2 treated groups and there was a significant increase in the ELT in the control group. The TSTQ was decreased for the control group compared to the standard and test groups.

Estimation of Brain Acetyl Cholinesterase Activity:

The Effect of 1, 3, 4 – Oxadiazole derivatives on Acetyl Cholinesterase activity in mice are shown in Table 4 & Graph 7. In control group mice, brain acetyl cholinesterase activity was more significantly ($p<0.001$) increased, when compared with normal group of young mice. Piracetam (200 mg/kg. i.p.) significantly ($p<0.05$) diminished brain acetyl cholinesterase activity, when compared with control group of mice. The groups treated with compound 1 dose 2(30mg/kg) significantly reduced the brain acetyl cholinesterase activity, when compared with control group of mice. The current study was aimed to investigate the memory enhancing activity of 1,3,4-oxadiazole derivatives(compound 1 and 2). For assessment of efficacy, scopolamine was used to induce memory impairment in mice, and impairment was gauged using Morris water maze.

Compound 1 at doses of 15mg/kg and 30mg/kg dose shortened the escape latencies which were prolonged by scopolamine, where compound 1 dose 2 shows less escape latency time compared to dose 1 of compound 1 as well as compound 2 (dose 1 and 2) and it was almost similar to the standard drug Piracetam. On the assessment day (day 8) trail of Morris water maze, scopolamine treated group spent considerably less time in escape quadrant indicating a failure to learn the location of the hidden platform. Whereas animals treated Compound 1 and 2 at 15mg/kg and 30mg/kg dose remarkably spent more time in the escape quadrant compared to scopolamine treated group which in turn supports the explanation of Compound 2 to show better memory enhancing activity.

4. DISCUSSION

Alzheimer's disease (AD) is a progressive neurodegenerative disease and the most common form of adult-onset dementia. AD is characterized by loss of neuronal integrity and consequent memory impairment and cognitive decline as a result of loss of neurons andsynapses[15]. Oxidative stress has been implicated to play a crucial role in the pathogenesis of a number of diseases, including neurodegenerative disorders. Thus synthetic compounds possessing antioxidant activities appear to be the potential targets for developing new remedies in neuroprotection as the intake of antioxidants appears to benefit in conditions of oxidative stress by maintaining the balance between the generation and scavenging of free radicals. 1, 3, 4 Oxadiazole derivatives are heterocyclic compounds which have potent antioxidant property and anti-Alzheimer's activity**.**

Acute Toxicity Studies:

Acute toxicity studies are done according to OECD guideline 423 (Acute toxic class method)

300 mg/kg was administered and was found as safe doses where no mortalities of the animals were observed for 14 days[10].

Spatial learning by Morris water maze task

Morris developed this behavioral procedure designed initially to study spatial localization in the mice where an animal learns to escape from opaque water onto a hidden platform[16, 17, 18]. MWM task has often been used in the validation of rodent models for neurocognitive disorders and the evaluation of possible neurocognitive treatments. The simplicity and versatility of the tank makes it the most widely acceptable experimental model for the assessment of cognitive skills in the animals[16]. The functional integrity of forebrain cholinergic systems, which are essential for efficient performance of the MWM, appears to be consistently disrupted in patients who suffer from AD. Extensive evidence of its validity as a measure of hippocampally dependent spatial navigation and reference memory, its specificity as a measure of place learning, and its relative immunity to motivational differences across a range of experimental treatment effects .Escape latency time has been linked to long-term potentiation (LTP),reference memory and NMDA receptor function[19], making it a key technique in the investigation of hippocampal circuitry, whereas Time spent in the target quadrant has been linked to the spatial memory.

Compound 1 at doses of 15mg/kg and 30mg/kg dose showed efficacious result of reducing

ELT by almost equal to the effect of Piracetam. Whereas Compound 2 shortened the escape

Latencies prolonged by scopolamine. In the assessment day of Morris water maze, scopolamine treated group spent considerably less time in escape quadrant indicating a failure to learn the location of the hidden platform. Whereas animals treated Compound 1 and 2 at 15mg/kg and 30mg/kg dose remarkably spent more time in the escape quadrant compared to scopolamine treated group. And the compound 1 showed increased time spent in the target quadrant shows it has better activity than compound 2.

Estimation of Acetyl cholinesterase Activity

The brain acetylcholine level is responsible for memory and level of acetylcholine depends on the activity of metabolizing enzyme acetylcholine esterase (AChE) [13, 14]. In this study we have determined the level of AChE in whole brain homogenate of all animal groups (Table 4) AChE activity was significantly increased in animals treated with Scopolamine. Compound

1at dose 30 mg/kg attenuated Scopolamine induced AChE activity in a dose dependent manner. Standard (Piracetam) significantly reversed the increase in AChE activity induced by Scopolamine (Graph 7).

5. CONCLUSION

Since scopolamine induced amnesia was reversed by 1,3,4-Oxadiazole, the observed beneficial effect on learning and memory was may be because of facilitation of cholinergic transmission in mouse brain. The current findings conclude that dose 2 of compound 1 (30mg/kg) has potent nootropic activity which shows beneficial effect on learning and memory ability in mice brain. However, Future studies are required to identify the exact mechanism of action by which 1, 3, 4 – oxadiazole acts on learning and memory.

Table 1: Animal grouping and treatment

Group	No of animal	Treatment	Treatment and evaluation
Normal	6	Vehicle treated	All treatments were given for 7 days and on seventh day Scopolamine was given all groups except normal group animals. Morris Water Maze task was employed to assess the memory and learning. Brain cholinesterase activity was also determined after evaluation of behavioural parameters.
Control	6	Scopolamine	
Std	6	Piracetam 200 mg/kg	
C1D1	6	Compound 1 dose 1(15mg/kg)	
C1D2	6	Compound 1 dose 2(30mg/kg)	
C2D1	6	Compound 2 dose 1(15mg/kg)	
C2D2	6	Compound 2 dose 2(30mg/kg)	

Table 2: Effect of 1, 3, 4 Oxadiazole derivatives on Escape Latencies in Morris Water Maze Task

	Escape Latency Time (secs)		
	0^{th} day	4^{th} day	8^{th} day (Assessment)
Normal	35.7±0.33	28.0±.96	35.8±0.47
Control	37.5±0.9	28.5±1.50	40.8±1.16
Std	34.3±0.80	$18.1±0.79^{a,b}$	$25.6±1.08^{a,b}$
C1D1	35.1±0.47	$25.5±1.25^{a,b,c}$	$32.6±0.55^{a,b,c}$
C1D2	34.7±0.66	$22.3±0.88^{a,b}$	$28.6±0.84^{a,b}$
C2D1	35.3±0.33	$27.0±0.89^{a,b,c}$	$35.1±0.54^{b,c}$
C2D2	35.8±0.32	$27.1±0.79^{a,b,c}$	$33.3±0.76^{b,c}$

All values are expressed as Mean ± SEM, n=6. Latency data (day 8) were analyzed by one-way ANOVA followed by Tukeys-test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug, piracetam.

Table 3: Effect of 1, 3, 4 - Oxadiazole derivatives on time spent in the target quadrant in Morris Water Maze Task

Time spent in the target quadrant (secs)			
	0^{th} day	4^{th} day	8^{th} day
Normal	25.1±0.60	30.50±0.81	29.61±0.55
Control	23.8±0.30	29.45±0.79	$5.3±0.55^{a}$
Std	25.3±0.55	$37.6±0.71^{a,b}$	$17.±0.57^{a,b}$
C1D1	23.6±0.66	$33.1±0.60^{a,b,c}$	$11.5±0.61^{a,b,c}$
C1D2	25±0.57735	$35.8±0.47^{a,b}$	$15.±0.57^{a,b}$
C2D1	24.8±0.70	$34.3±1.14^{a,b}$	$13.1±0.60^{a,b,c}$
C2D2	23.5±0.84	$34±1^{a,b}$	$12.±0.57^{a,b,c}$

All values are expressed as Mean ± SEM, n=6. Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard piracetam.

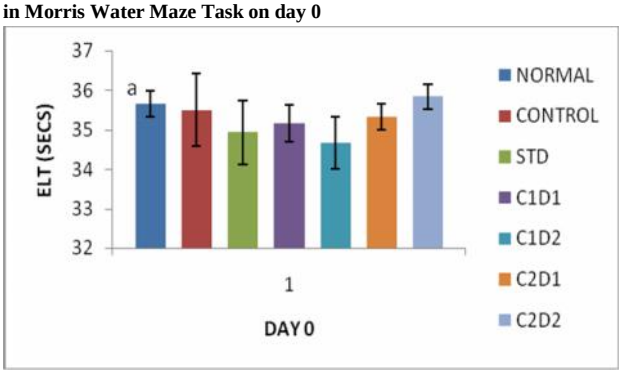
Table 4: Effect of 1, 3, 4 - Oxadiazole derivatives on Acetyl cholinesterase activity in mice

Treatment	AChE Activity(µM/min/mg)
Normal	21.05±1.25
Control	$27.66±1.28^{a}$
Std	$11.50±0.84^{a,b}$
C1D1	$19.5±1.28^{a,b,c}$
C1D2	$15.33±0.42^{a,b}$
C2D1	$20.16±0.54^{a,b,c}$
C2D2	$19.83±1.07^{a,b,c}$

All values are expressed as Mean ± SEM, n=6 .Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard Piracetam.

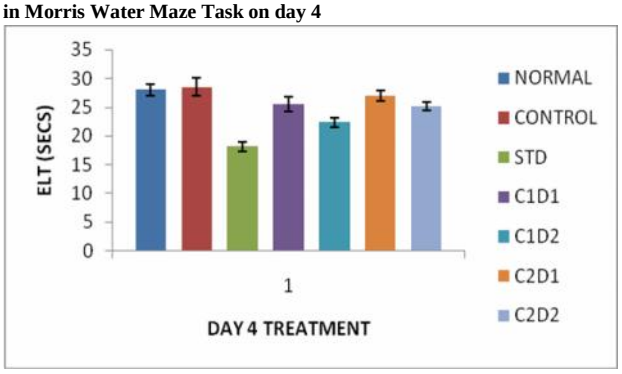
GRAPHS

Graph 1: Effect of 1, 3, 4 - Oxadiazole derivatives on Escape Latencies in Morris Water Maze Task on day 0



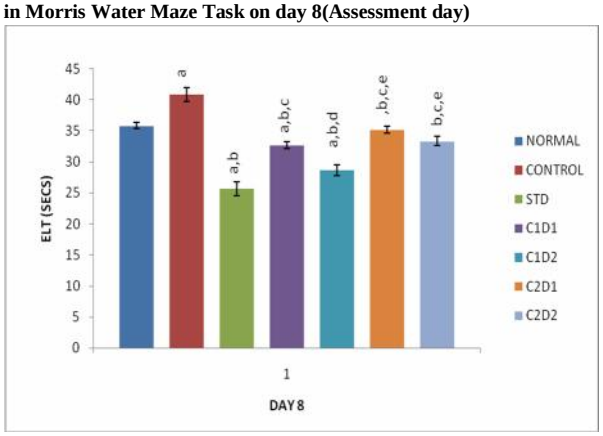
All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug Piracetam.

Graph 2: Effect of 1, 3, 4- Oxadiazole derivatives on Escape Latencies in Morris Water Maze Task on day 4



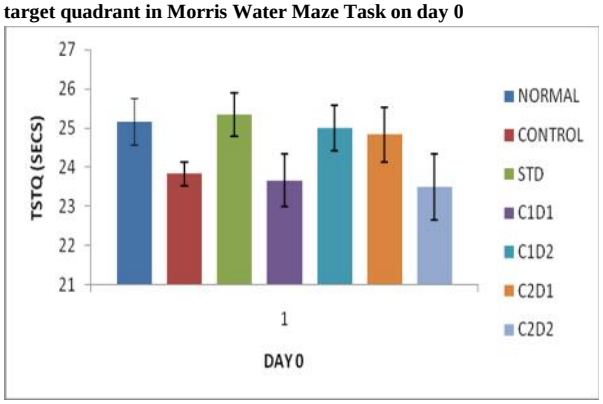
All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug piracetam

Graph 3: Effect of 1, 3, 4 - Oxadiazole derivatives on Escape Latencies in Morris Water Maze Task on day 8(Assessment day)



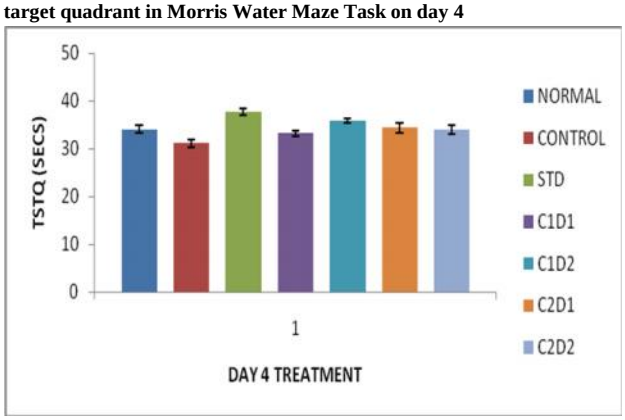
All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug Piracetam

Graph 4: Effect of 1, 3, 4 - Oxadiazole derivatives on time spent in the target quadrant in Morris Water Maze Task on day 0



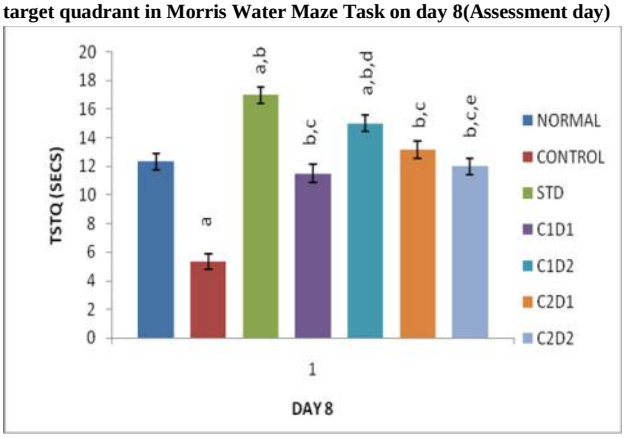
All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug piracetam

Graph 5: Effect of 1, 3, 4- Oxadiazole derivatives on time spent in the target quadrant in Morris Water Maze Task on day 4



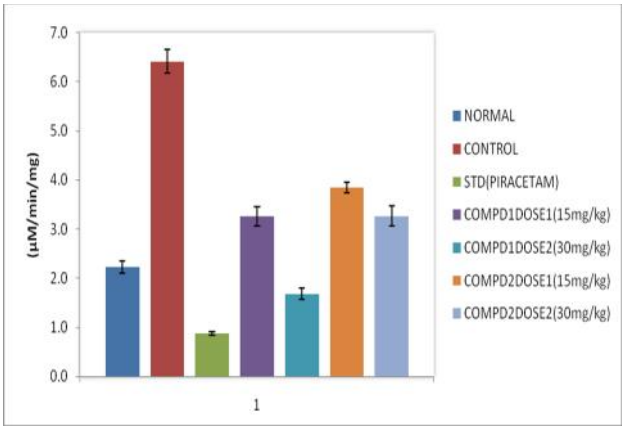
All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug piracetam

Graph 6: Effect of 1, 3, 4 Oxadiazole derivatives on time spent in the target quadrant in Morris Water Maze Task on day 8(Assessment day)



All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug Piracetam.

Graph 7: Effect of 1,3,4 - Oxadiazole derivatives on Acetyl cholinesterase Activity in Mice



All values are expressed as Mean ± SEM, n=6 Data were analyzed by one-way ANOVA followed by Tukey's test. a-(p <0.05) when compared to normal, b –(p <0.05) when compared to control, c –(p <0.05) compared to standard drug Piracetam.

6. ACKNOWLEDGEMENT

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