International Journal of Medical Science in Clinical Research and Review
Online ISSN: 2581-8945
Available Online at http://www.ijmscrr.in Volume 05|Issue 05 (September-October)|2022|Page: 636-644
NLM ID: 101768774

Original Research Paper

EFFECT OF VALERIC ACID ON RAT MODEL OF ALZHEIMER'S DISEASE: A BEHAVIORAL AND HISTOPATHOLOGICAL STUDY

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Article Received: 10-08-2022 **Revised:** 31-08-2022 **Accepted:** 21-09-2022

ABSTRACT:

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the presence of neuritic plaques and neurofibrillary tangles. Aluminum has been reported to play an important role in the etiology and pathogenesis of this disease. Hence, the present study aimed to evaluate the neuroprotective role of Valeric acid on aluminum chloride ($AlCl_3$) induced neurobehavioral and histopathological changes in AD induced rats. $AlCl_3$ (100 mg/kg body weight, orally) for 42 days significantly elevated the transfer latency in Morris water maze (MWM) and also resulted in histological disruption in the hippocampus as compared to control group. In the present study, the rats treated with Valeric acid (50mg/kg) and its combination with the other standard drugs like Piracetam (200 mg/kg), Rivastigmine (0.5 mg/kg) showed less transfer latency on Morris Water Maze(MWM) and reversing of histological alteration. Hence, Valeric acid can be suggested as a better drug for Alzheimer's disease.

Key words: Valeric acid, Alzheimer's disease, Aluminum chloride, Morris water maze.

1. INTRODUCTION:

Alzheimer's disease (AD) is a progressive neurological disease that causes memory loss, language problems, and other cognitive impairments in the elderly people. The accumulation of extracellular amyloid peptide (Aβ) containing plaques through the amyloidogenic pathway and intraneuronal fibrillary tangles in the brain are two key aspects in the neuropathogenesis of Alzheimer's disease, which slowly destroys neurons. The origin of Alzheimer's disease appears to be multifaceted, involving a variety of factors such as head trauma, genetics, oxidative stress, inflammation, proteosomal dysfunction, and environmental factors such as aluminium (Al) toxicity. Al is a common neurotoxin that is highly exposed to humans and has been proven to accumulate in AD prone brain regions such as the cortex and hippocampus.[1-3]There is yet no effective treatment for AD that stops or even slows the destruction to neurons. The currently available treatments only work to alleviate the disease's symptoms. As a result, there is a high demand for safe, effective, and multi-mechanistic medicines to effectively manage and control AD.[4] Valeric acid, a major component of *Valeriana officinalis*, which is utilized in home grown medication of many societies as tranquilizers and sedatives.[5] It increases GABA levels by inactivating -ketoglutarate dehydrogenase[6] and decreases GABA breakdown via regulating GABA transaminase and therefore inhibiting GABA catabolism. It acts as a GABA-A agonist by enhancing GABA-A receptor II response.[7] This pre-clinical study is focused to compare the behavioral activity of Valeric acid in Alzheimer's disease-induced rats to that of conventional medicines such as piracetam

and rivastigmine in order to produce a better treatment for AD than currently available drugs.

2.MATERIAL AND METHODS:

This Experimental study was conducted in the Dept of Anatomy, Yenepoya Medical College, Mangalore in 2018, after getting the permission of the Institutional Animal Ethics Committee in 20th IAEC meeting (YU/IAEC/4/2018). The study was conducted in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India guidelines.

2.1 Animals

Forty two male Wistar albino rats weighing 220-250g (4-6 months age group) were used. They were housed in polypropylene cages, at a temperature of 22±1ºC and kept in under adequate environmental conditions. All the rats were divided into 7 groups and 6rats were assigned to each group for scientific and statistical basis.[8]

Material

Aluminum chloride salt (500ml bottle), Valeric acid solution were purchased from Sri Durga Laboratory, Mangalore, Karnataka. Piracetam and Rivastigmine tablets were purchased from Ganesh medical stores, Mangalore.

Drug interventional studies

Table 1 shows how 42 rats were split into seven groups. There were six rats in each group. Group 1(Negative control) rats were given distilled water orally, whereas Group 2 (Positive control) rats were given aluminum chloride (AlCl3) at a dosage of 100 mg/kg b.wt. orally for 42 days to develop Alzheimer's disease.[9] The remaining ten group rats (Groups 3–7) were likewise given the same dosage of AlCl3 to develop AD. The Morris water maze (MWM) was used to compare the memory and learning behaviour of aluminum chloride treated rats with the control group rats after 42 days. After inducing Alzheimer's disease, Group 3 rats were given Valeric acid (50 mg/kg b.wt)[10], Piracetam (200mg/kg b.wt) was given to Group 4 rats[11]. Rivastigmine (0.5 mg/kg b.wt) was given to Group 5 rats[12], Group 6 rats were given Valeric acid + Piracetam and Group 7 rats were given Valeric acid +Rivastigmine14 for 30 days[13] to treat the aluminum chloride-induced cognitive impairment in rats. The memory enhancing effect of Valeric acid in comparison with the other standard drugs was assessed after the treatment which was confirmed by Morris water maze (MWM) test.

Table. 1 Animal groups

S.No	Groups	Dose
1	Normal/Positive control	Distilled water
2	Disease/Negative control	100mg/kg ($AlCl_3$)
3	Valeric acid	50mg/kg
4	Piracetam	200mg/kg
5	Rivastigmine	0.5mg
6	Valeric acid + Piracetam*	50mg/kg + 200mg/kg
7	Valeric acid + Rivastigmine*	50mg/kg + 0.5mg/kg

Piracetam* - administered after 30min , Rivastigmine* - administered after 30min.

2.2 Background of Experimental rats

Wistar albino rats weighing 220–250g were used in the experiment. As indicated in Table 1, the rats were randomly assigned to one of seven treatment groups. Rats used in the study were bred in the Liveonbiolab, Bangalore (registration number 1610/ROBiBt/S/2012/CPCSEA). Throughout the tests, they were kept dry in polypropylene cages containing husk. Four rats were housed in a single cage. Rats were identified by marking them on the head, body, and tail, as well as their cage number. The animals were kept under standard laboratory settings, which included a room temperature of 22±1ºC and a 12-hour light/dark cycle. Rat chow was provided to them. All behavioural tests were conducted in a room next to the one where the rats were monitored under the same temperature, humidity, and light cycle conditions.[14]

2.3 Behavioral test on Morris water maze (MWM) (Fig.1)

This behavioural test was conducted in the Behavioral Testing Facility at the Department of Pharmacology, in a closed, silent, and light-controlled environment. Each day, rats were acclimatized to the facility's environment for at least 30 minutes before being tested.

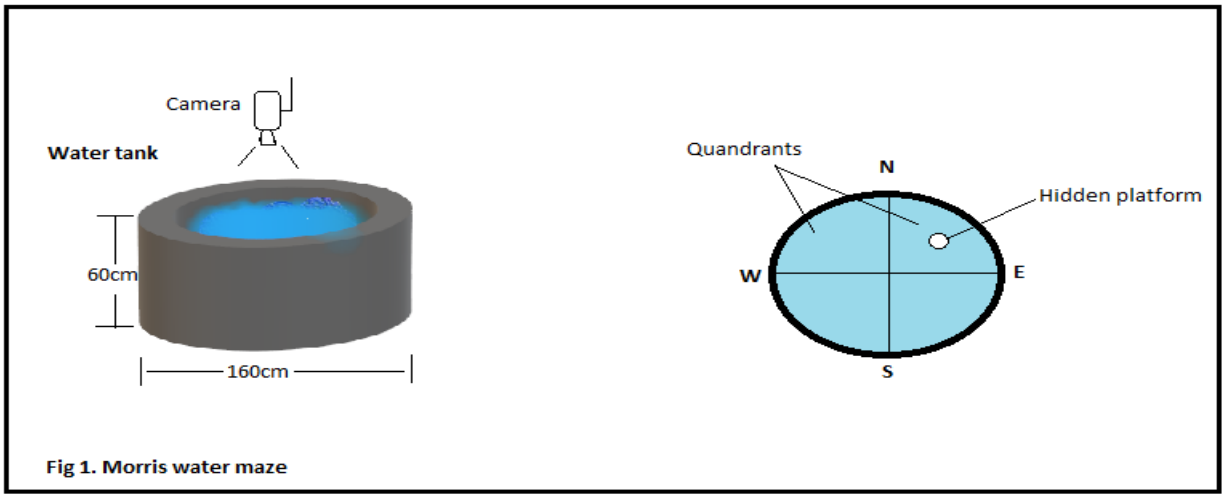

Figure.1 Morris water maze

Morris water maze consists of a circular water tank with 160 cm in diameter and 60 cm height, filled with water(23±1ºC) to a depth of 25 cm. The pool was divided into 4 quadrants (North, South, East and West). These were used as start points. A submerged platform (10 cm diameter) was placed in the middle of one of the randomly selected quadrants of the pool, 1 cm above the water level. Each rat was placed gently at one of the four starting points and allowed 90 seconds to locate the platform. The position of the platform was kept constant for each individual rat throughout the whole experiment. If unsuccessful in the 90-s trial, the rat was gently guided to the platform. Once the rats reach the platform, they were allowed to remain there for 30 seconds and then they were removed from the pool, towel dried, and returned to their home cage. This training was given to the rats for 4 days before the formal experiment. On the day of experiment (i.e. on 5th day), the water level was increased 1 cm above the platform. And the time taken by the rat to reach the platform was regarded as the transfer latency (TL).[14]

2.4 Histological procedure

After the behavioral test, rats were sacrificed using ketamine anaesthesia and brains were removed to carry out the histological procedure in the hippocampus. The brains were fixed in 10% paraformaldehyde for 24 h. Following routine processing in paraffin, serial coronal sections of the brain were cut at 5µm thickness in a rotary microtome (Leica, RM2125 RTS, Germany). The part of each brain section were stained with cresyl violate stain. Cresyl violet is a reliable stain to study the morphological changes in the neurons as it highlights the structural features of it. This stain helps to count the number of neurons.[15] Slides were immersed in xylene (Sigma) twice, for 5 minutes each time, followed by two immersions in 100% ethanol (Sigma) for 5 minutes each. Then slides were immersed in 95% ethanol once and70% ethanol once for 2 minutes each time before being placed in a container with distilled water for 2 minutes. The slides were then immersed in cresyl violet staining solution for 15-17 minutes and washed again in a container with distilled water for another 2 minutes. The previous steps were repeated in reverse, first by dipping in 70% ethanol for 2 minutes, then 90% ethanol for 2 minutes, followed by two immersions in 100% ethanol for 5 minutes each. The final two immersions were in xylene solution for 5 minutes each. Slides were then cover slipped with permanent mounting medium and left air dried overnight.[16]

3. STATISTICAL ANALYSIS:

The data, expressed as mean ± standard deviation, was subjected to one-way analysis of variance (ANOVA) followed by Tukey Kramer's test. $P < 0.05$ was considered to be statistically significant.

4. RESULTS AND DISCUSSION:

4.1 Transfer latency on Morris water maze (MWM)

In the present study, Transfer latency (Fig.2) on Morris water maze was marked in all the groups. Group 1 (Negative control +Distilled water) rats showed a TL of only 4sec showing good memory retention. Group 2 rats

(Positive control+$AlCl_3$) showed an increase in TL period showing impairment in memory retention. All the treatment groups showed significant reduction in the transfer latency after the treatment showing reversal of impairment caused by $AlCl_3$ and improved memory. Among all the treatment groups, Group 7 (Valeric acid+ Rivastigmine) showed very less TL period.

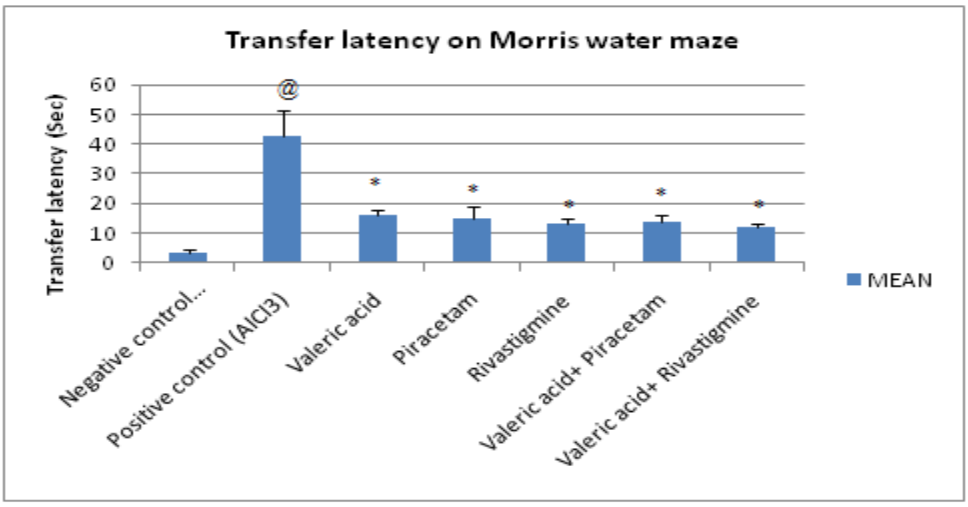

Fig 2. Effect of Valeric acid on memory of Alzheimer's disease induced rats on Morris water maze. Values are means±S.E.M. (n=6); *p<0.05 (vs. Positive control group), $^{@}$p<0.05 (vs. Negative control group)

Lakshmi B V et al reported that transfer latency in morris water maze was considerably enhanced in the group of rats treated with the aluminum chloride when compared to the normal control group rats.[17] Similarly present study showed increased transfer latency in morris water maze test in aluminum chloride administered rats. When these rats were treated with respective drugs, Group 7 rats treated with Valeric acid in combination with Rivastigmine showed marked improvement in memory with decreased TL period indicating reversal of $AlCl_3$ induced neurotoxicity in comparison to the group 3 (Valeric acid), group 4 (Piracetam), group 5 (Rivastigmine) and group 6 (Valeric acid+ Piracetam).

4.2 Histopathological studies

Microscopical examination of hippocampus was observed by staining with cresyl violet. The disease control group showed various histopathological changes like multifocal moderate neuronal degeneration with pyknotic nuclei, multifocal moderate reduced layer of neuronal cell in hippocampus when compared with normal control group.

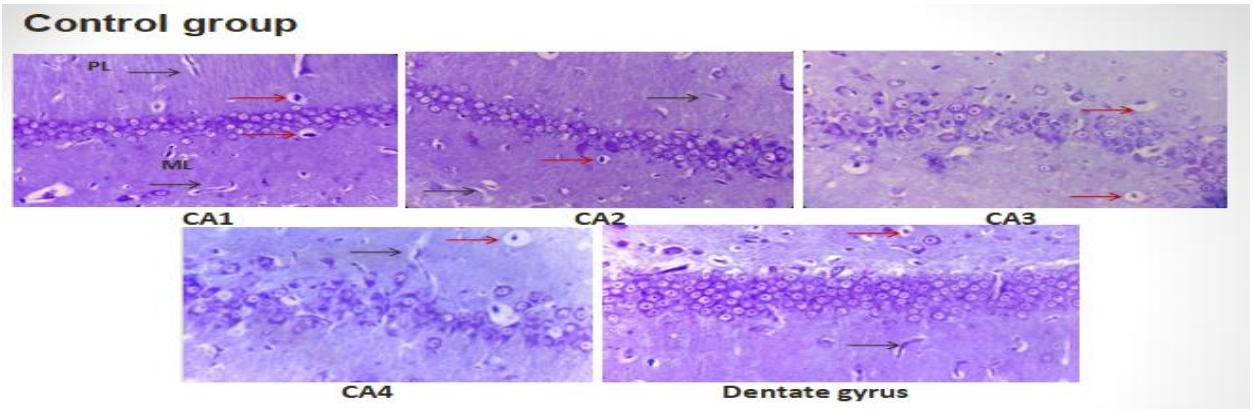

Fig 3. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

magnification. Cornu ammonis (CA) is merged between the molecular layer (ML) and polymorphic layer (PL). CA1 and CA2 subfields show small pyramidal cells with large vesicular nuclei. CA3 and CA4 subfields show more layers of large pyramidal cells with large vesicular nuclei compared to CA1 and CA2 subfields. Glial cells (GC) and the blood capillaries (BC) are noticed in the molecular and polymorphic layers. Cells are intact with proper cell membrane, nuclei and less intercellular space. Dentate gyrus shows the normal features of granular cells.

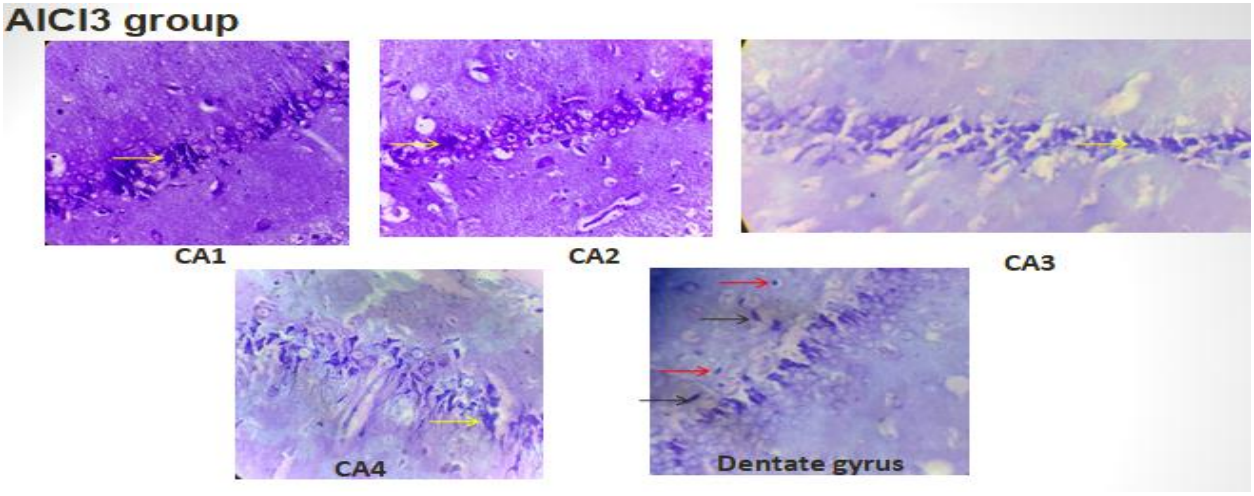

Fig 4. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

magnification (AlCl3 group). CA 1and 2 subfields showed neuronal damage with swollen neuronal cell bodies, cell debris, dense and dead neuronal cells and cells with pyknotic nuclei. CA 3 and 4 subfields showed less cell density, swollen irregular hyper dense cells with no well defined boundary between cytoplasm and nucleus, decreased cell size and more intercellular space. Dentate gyrus subfield shows conspicuous degenerative aberrations and many pyknotic granule cells, as well as hilar cells (black arrow) with moderate records of edema and vacuolation of neuropil (red arrow).

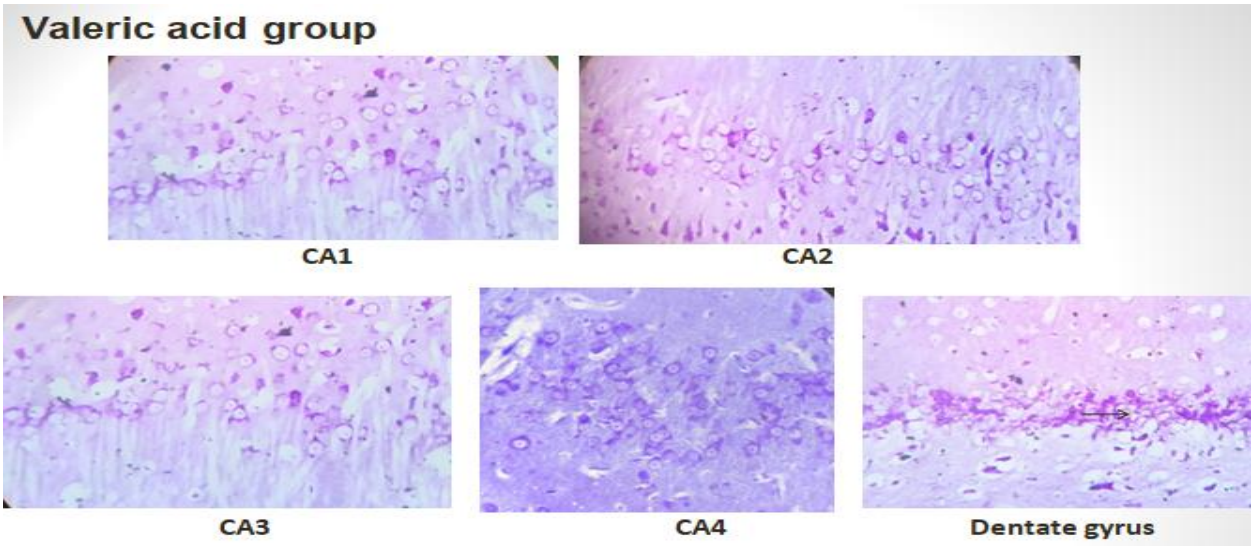

Fig 5. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

magnification (Valeric acid group). All the subfields of hippocampus showed some improvement in cell morphology, number of cells and size of cell body. CA 3 and 4 subfields showed less cell density with increased cell. Dentate gyrus subfield shows thin degenerative aberrations and many pyknotic granule cells (black arrow).

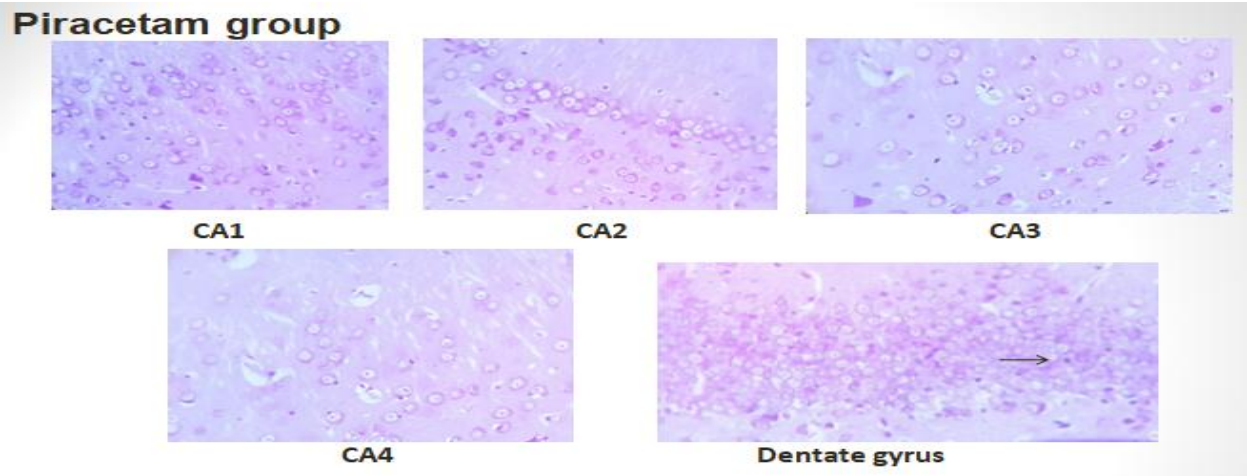

Fig 6. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

Magnification (Piracetam group). All the subfields of hippocampus showed some improvement in cell morphology, number of cells and size of cell body. CA 3 and 4 subfields showed less cell density with increased cell size. Dentate gyrus subfield less thin degenerated and pyknotic granule cells (black arrow).

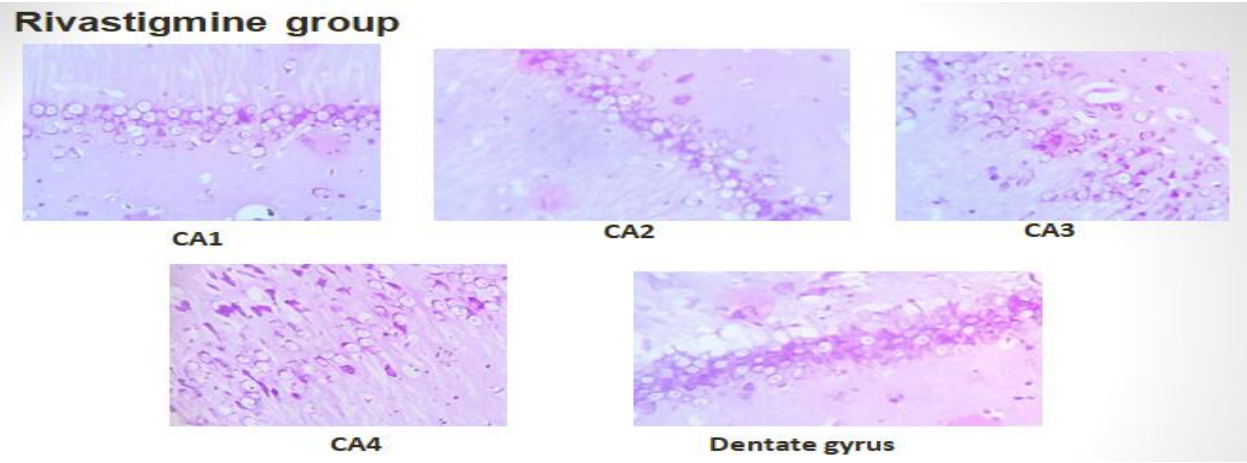

Fig 7. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

Magnification (Rivastigmine group). All the subfields of hippocampus showed improvement in cell morphology, number of cells, size of cell body and the arrangement. CA 3 and 4 subfields showed improvement in cell density with increased cell size. Dentate gyrus subfield shows some improvement in number and arrangement of granular cells.

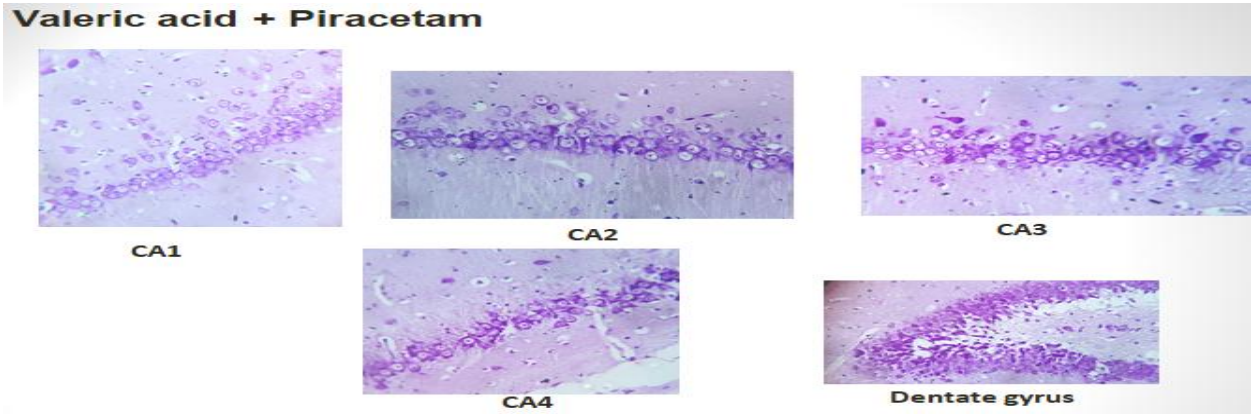

Fig 8. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

magnification (Valeric acid + piracetam group). All the subfields of hippocampus showed improvement in cell morphology, number of cells and size of cell body. CA 3 and 4 subfields showed improvement in less density with increased cell size and less intercellular space. Dentate gyrus subfield shows improvement in number of granular cells with less degenerated cells.

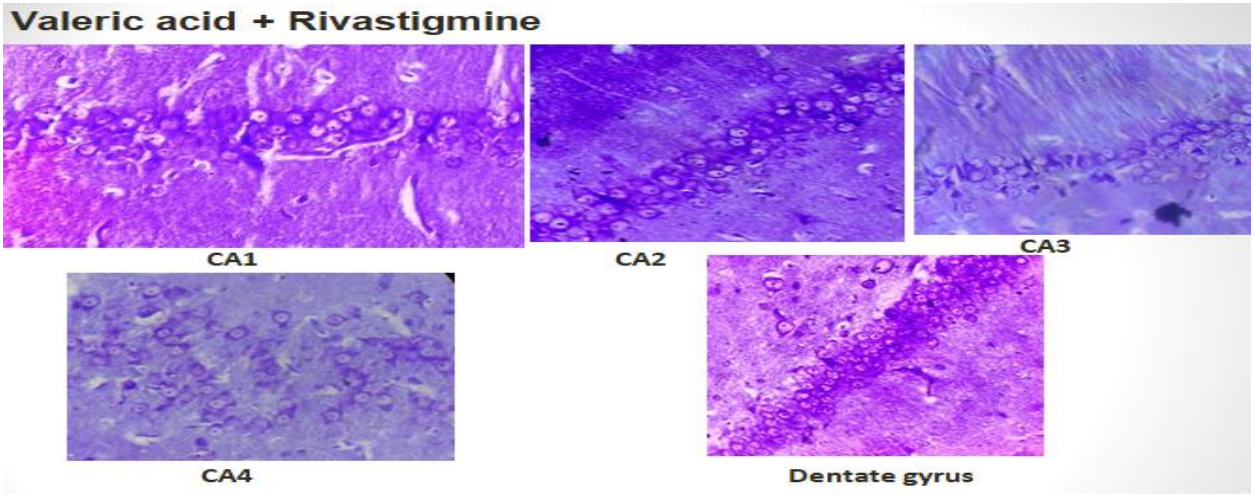

Fig 9. Represents the photomicrographs of subfields in hippocampal sections with cresyl violet staining under 40X

magnification (Valeric acid + Rivastigmine group). All the subfields of hippocampus showed improvement in cell morphology, number of cells and size of cell body. CA 3 and 4 subfields showed improvement in less density with increased cell size and less intercellular space. Dentate gyrus subfield shows improvement in number of granular cells with less degenerated cells.

Vishwakarma S et al., reported that Valeric acid showed neuroprotective effect through amelioration of intracerebroventricular streptozotocin induced neurodegeneration in Wistar rats.[10] In this study, treatment with Valeric acid did not prevent the neuronal degeneration completely. However, Valeric acid in combination with Piracetam and Rivastigmine showed a decrease in neuronal degeneration and showed normal histology, normal layer of neuronal cell in when compared with disease control group.

5. CONCLUSION:

The present findings suggest that Valeric acid exhibits neuroprotective effects for aluminum chloride – induced dementia. The behavioral impairments caused by aluminum chloride were significantly attenuated by Valeric acid. Histopathological studies of the hippocampus also indicate that Valeric acid in combination with Piracetam and Rivastigmine markedly reduced the toxicity of $AlCl_3$ and preserved the normal histoarchitecture of the hippocampus. Our results

suggest that Valeric acid might be beneficial in the prevention of dementia development and progression.

CONFLICT OF INTEREST:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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