



A Study on the Effect of Valeric Acid in Alzheimer's Induced Rats by the Estimation of A β 1-42 Biomarker

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Abstract

The effect of valeric acid on the behavior of Alzheimer's disease (AD)-induced rats by aluminum chloride (100 mg/kg body weight) was assessed using elevated plus maze (EPM) and the Hebb Williams maze (HWM). Amyloid β 1–42(A β 1–42) biomarker was estimated by ELISA. In this study, valeric acid-treated rats were compared with those treated with piracetam (200 mg/kg), rivastigmine (0.5 mg/kg), and the results showed the rats treated with valeric acid had a very less transfer latency of EPM and HWM when compared with other standard drugs. In addition, valeric acid-treated rats showed reduced levels of amyloid β 1–42 biomarker in the plasma. Hence, this study found that valeric acid may be suggested as a better drug for Alzheimer's disease.

Keywords

- ▶ Alzheimer's disease
- ▶ amyloid β 1–42
- ▶ elevated plus maze
- ▶ Hebb Williams maze
- ▶ valeric acid

Introduction

The ability of an individual to record sensory stimuli, information, and events and keep them over short or long periods and recollect the same later when needed is called memory. Lower retention, poor recollection, and slow recall are common problems with people going through stress and tension. Age, emotions, and stress are the conditions that possibly lead to amnesia, memory loss, anxiety, dementia, high blood pressure, or more ominous threats such as schizophrenia and Alzheimer's disease (AD). The central cholinergic system plays an important role in memory and learning. Cognitive deterioration occurring in patients with AD is correlated with the progressive loss of cholinergic neurons and a subsequent reduction in the levels of acetylcholine in the brain.^{1,2} The prevalence of AD has been increasing. The occurrence of moderate to severe dementia in different groups of the population is ~5% in the population older than 65 years of age, 20 to 40% in the general popula-

tion older than 85 years of age globally.³ The increasing incidence of AD is a social concern and financial burden for society. Memory-enhancing drugs are costly and have minimal efficacy. Hence, there is a need to develop an alternative and economical agent with high efficacy. Herbs are the source of most drugs.⁴

The perennial flowering plant *Valeriana officinalis* is used widely in different countries to treat insomnia and anxiety due to its sedative effects,⁵ its extracts contain free amino acids such as gamma aminobutyric acid (GABA) and other substances such as isovalerate.⁶ Many studies have reported that the effects of this plant when combined with other drugs to treat various central nervous system (CNS) diseases, most of them interfere with GABA neurotransmitters.^{5–8} Valeric acid is a key component of *V. officinalis* and prevents GABA from being broken down in the brain.⁹ It elevates the GABA levels through the inactivation of α -ketoglutarate dehydrogenase¹⁰ and reduces GABA degradation by mediating the GABA transaminase and thereby inhibiting GABA catabolism.

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It functions as a GABA-A agonist by increasing the response of GABA-A receptor II.^{11,12} The administration of AlCl₃ (100 mg/kg b.wt) for 42 days orally resulted in the loss of spatial as well as contextual memory in rats.¹³ Bhattacharjee et al. Exley reported that aluminum is a potential causative agent of AD.^{14,15} Aluminum accretion in the hippocampus causes anomalous (amyloid β) A β accumulation and neuroinflammation according to some epidemiological and animal studies. The stimulation of microglia is the main event in brain neuroinflammation. It could result in hippocampus-dependent memory, learning, and memory problems.¹⁶

This pre-clinical study is focused to identify a better drug for AD over the pre-existing drugs and aims to find the behavioral activity of valeric acid in the treatment of AD rats in comparison with that of the standard drugs such as piracetam and rivastigmine.

Piracetam and rivastigmine are established therapeutic agents used to treat AD. Studies have shown that piracetam increases mitochondrial function, plasticity, synaptic markers, oxidative stress, neuronal outgrowth, cognition, and also cerebral microcirculation.¹⁷

Rivastigmine increased the neurotrophic soluble amyloid precursor protein- α (sAPP- α) and decrease A β secretion. Rivastigmine treatment enhances neuronal β amyloid precursor protein (sAPP) in degenerating neuronal cultures. It preserves neuronal morphology and enhances cell viability in neurodegenerating cultures.¹⁸ By enhancing sAPP production, rivastigmine might protect the neurons from neurite retraction¹⁹ and apoptosis.²⁰

Materials and Methods

This study was conducted in the Department of Anatomy, Yenepoya Medical College, Mangalore, in 2018, after presenting it in the 20th IAEC meeting and getting approval from the Institutional Animal Ethics Committee (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.

Animals

Forty-two male Wistar albino rats weighing 220–250 g (4–6 months age group) were used. They were housed in polypropylene cages at 22 \pm 1°C and kept under adequate environmental conditions. All rats were divided into seven groups, and six rats were assigned to each group for scientific and statistical analyses.²¹

Materials

Aluminum chloride salt (500 mL bottle) and valeric acid solution were purchased from Sri Durga Laboratory, Mangalore, Karnataka. Piracetam and rivastigmine tablets were purchased from Ganesh Medical Store, Mangalore.

Drug Interventional Studies

Forty-two rats were divided into seven groups as shown in **Fig. 1**. Each group contained six rats. The control group (Group 1) was administered distilled water orally and

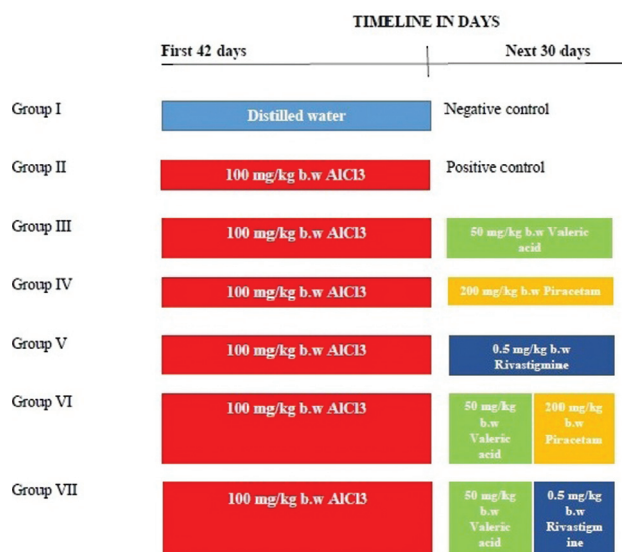


Fig. 1 Experimental animal groups piracetam*-administered after 30 min, rivastigmine*-administered after 30 min.²⁶

Group 2 rats were administered aluminum chloride (AlCl₃) at a dose of 100 mg/kg b.wt. for 42 days orally to induce AD.¹³ AD was also induced in the remaining rats (Group 3 to Group 7) with the same dose of AlCl₃. After 42 days, elevated plus maze (EPM) and Hebb Williams maze (HWM) models were used to evaluate the memory and learning behavior of aluminum chloride-treated rats in comparison with the control group rats. After inducing AD, Group 3 rats were treated with valeric acid (50 mg/kg b.w.t),²² Group 4 rats were treated with piracetam (200 mg/kg b.w.),²³ Group 5 rats were treated with rivastigmine (0.5 mg/kg b.w.),²⁴ Group 6 rats were treated with valeric acid + piracetam,²⁵ and Group 7 rats were treated with valeric acid + rivastigmine²⁵ for 30 days to treat the cognitive impairment in rats induced by aluminum chloride. The memory-enhancing effect of valeric acid in comparison with the other standard drugs was assessed after the treatment and confirmed by EPM and HWM tests.

Background of Experimental Rats

The study was performed on Wistar albino rats weighing ~220–250 g. The rats were randomized into seven different treatment groups as shown in **Fig. 1**. Rats used in the study were bred in the Liveon Biolabs, Bangalore (registration number - 1610/ROBiBt/S/2012/CPCSEA). They were housed in polypropylene cages containing husk to keep them dry throughout the experiments. Four rats were kept in one cage. The identification of rats was done by marking them on the head, body, and tail and with cage numbers. Animals were housed under standard laboratory conditions such as room temperature (22 \pm 1°C) with 12 hours light and dark cycle. They were fed with rat chow. All behavioral experiments were performed in a room adjacent to that where the rats were monitored under the same conditions of temperature and humidity and light cycle.

Behavioral Experiments

Elevated Plus Maze

Elevated plus maze served as the exteroceptive behavior model to evaluate learning and memory in rats. This model has two open arms (30 × 5 cm) and two covered arms (30 × 5 × 12 cm) expanded from a central platform (5 × 5 cm) and the maze was lifted to a height of 50 cm from the floor. At the time of the experiment, each rat was placed at the end of an open arm, opposite from the central platform. Transfer latency (TL) is the time (in seconds) taken by the animal to move from the open arm into one of the closed arms with its four legs inside. TL was recorded on the initial day (training session) for each animal. The rats were allowed to walk around the maze for another 2 min and then taken back to their home cage. The retention of the learned-task (memory) was observed after a complete day.²⁶

Hebb-Williams Maze

This is an incentive-based exteroceptive behavioral model employed for determining the spatial working memory of rats. It has three components: the animal chamber (or start box) attached to the middle chamber (or exploratory area) and a reward chamber at the other end of the maze in which the food (reward) is kept. All three components are supplied with guillotine removable doors. On the initial day, the rats were placed in the start box and the door was opened to smooth the progress of the entry of the animals into the next chamber. The door of the start box was immediately closed after the rats moved into the next chamber to prevent back entry. For each animal, the time taken by it to reach the reward chamber from the start box was recorded (i.e., TL in seconds) on the first two days (training session). Each animal was permitted to explore the maze for 3 min with all the doors opened before returning to its home cage. The retention of this learned task (memory) was examined 24 h after the trial days.²⁷

Estimation of Amyloid β 1-42 ($A\beta_{1-42}$) Levels in Plasma

The presence of extracellular plaques composed mainly of 42 amino acid amyloid β peptide ($A\beta_{1-42}$) is one of the pathological traits of AD.²⁸ Amyloid β 1-42 biomarker was estimated in blood plasma using an ELISA kit (GeneTex, Sri Durga Laboratory, Mangalore). For plasma collection, the blood was collected in tubes containing EDTA as an anticoagulant. These plasma samples were kept at -80°C until biochemical analysis after centrifugation (2000 rpm, +4°C, 10 min).

Statistical Analysis

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

Results and Discussion

Transfer Latency on Elevated Plus Maze

In the present study, the TL (\rightarrow Fig. 2) on EPM was marked in all the groups. Group 1 (negative control + distilled water)

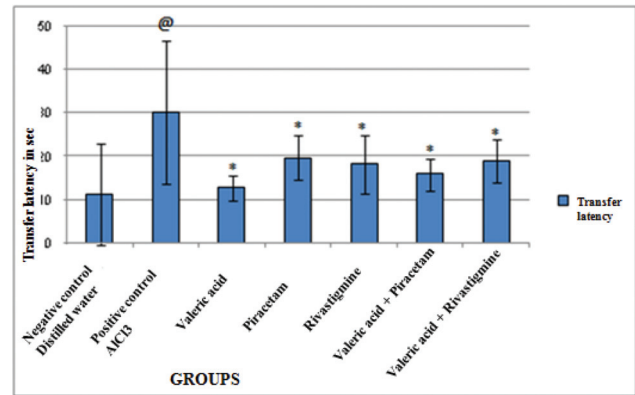


Fig. 2 Effect of valeric acid on the memory of Alzheimer's disease-induced rats using the elevated plus maze. The values are means \pm SEM ($n = 6$); @ $p < 0.05$ (versus negative control group), * $p < 0.05$ (versus positive control group).

rats showed a TL of only 10 s showing good memory retention in the EPM. Group 2 rats (positive control + AlCl₃) showed an increase in the TL period showing impairment in memory retention. Group 3 (valeric acid) showed a decrease in the TL period showing reversal of impairment caused by AlCl₃ and improved memory retention in the experimental group. Group 4 (piracetam) and Group 5 (rivastigmine) rats treated with standard drugs showed reduced TL period. Group 6 (valeric acid + piracetam) showed improvement in memory retention in AlCl₃-induced rats. Group 7 (valeric acid + rivastigmine) also showed a reduced TL period.

Lin et al and Thippeswamy et al reported that the TL in EPM was considerably enhanced in the group of rats treated with aluminum chloride when compared with the normal control group rats.^{29,30} Similarly, the present study showed increased TL in EPM test in aluminum chloride-administered rats. When these rats were treated with respective drugs, Group 3 rats treated with valeric acid showed marked improvement in memory with decreased TL period, indicating the reversal of AlCl₃-induced neurotoxicity in comparison to Group 4 (piracetam), Group 5 (rivastigmine), Group 6 (valeric acid + piracetam), and Group 7 (valeric acid + rivastigmine).

Transfer Latency on Hebb-Williams Maze

Transfer latency (\rightarrow Fig. 3) on HWM was marked in all groups. Group 1 (negative control + distilled water) rats showed a TL of only 12 s showing good memory retention in HWM. Group 2 rats (positive control + AlCl₃) showed an increase in TL period indicating impairment in memory retention. Group 3 (valeric acid) and Group 6 (valeric acid + piracetam) showed a decrease in TL period showing reversal of impairment caused by AlCl₃ and improved memory retention in the experimental groups. Reduction in the TL period was less in Group 5 (rivastigmine) followed by Group 7 (valeric acid + rivastigmine) and Group 4 (piracetam).

Auti et al reported cognitive impairments in aluminum chloride-induced rats and the TL period in HWM was

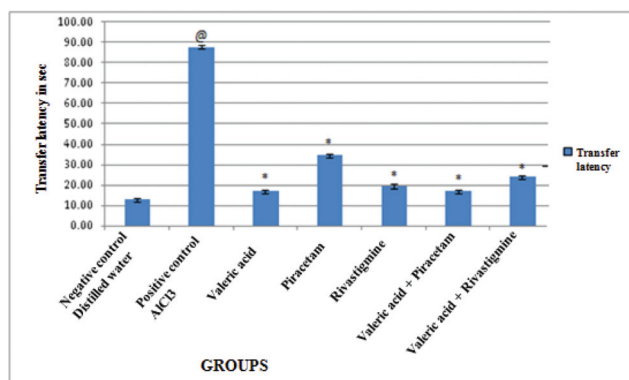


Fig. 3 Effect of valeric acid on the memory of Alzheimer's disease-induced rats using the Hebb Williams maze. The values are means \pm SEM ($n=6$); @ $p < 0.05$ (versus negative control group), * $p < 0.05$ (versus positive control group).

considerably increased in the group of rats treated with aluminum chloride when compared with the normal control group rats.³¹ Similarly, the present study showed increased TL in HWM test in aluminum chloride-administered rats. When these rats were treated with respective drugs, Group 3 (valeric acid) and Group 6 (valeric acid + piracetam) rats showed marked improvement in memory with decreased TL period indicating a reversal of AlCl₃-induced neurotoxicity in comparison to the Groups 4 (piracetam), 5 (rivastigmine), and 7 (valeric acid + rivastigmine).

Effect of Valeric Acid on the Level of Amyloid β 1–42 in Plasma

When amyloid β 1–42 was estimated in plasma using an ELISA kit, Group 1 (negative control + distilled water) rats showed a concentration of 1183.91 ± 110.13 pg/mL showing a slight increase in the plasma (\rightarrow Table 1). Matsubara et al and Fukumoto et al reported that the increase in age alone correlated with the increase in plasma amyloid β levels in their studies.^{32,33} Group 2 rats (positive control + AlCl₃) showed more levels of amyloid β showing impairment in

Table 1 Effect of valeric acid on A β _{1–42} levels in plasma

S. No.	Groups	Concentration in pg/mL (Mean \pm SD)
1	Distilled water (negative control)	1183.91 \pm 110.13
2	AlCl ₃ (positive control)	1693.58 \pm 212.56*
3	Valeric acid	97.53 \pm 9.80**
4	Piracetam	72.36 \pm 14.19**
5	Rivastigmine	66.70 \pm 29.14**
6	Valeric acid+ piracetam	61.20 \pm 20.12**
7	Valeric acid+ rivastigmine	45.95 \pm 42.65**

Note: Values are means \pm SD. ($n=6$); * $p < 0.05$ (versus positive control group), ** $p < 0.05$ (versus negative control group).

memory retention. Group 3 (valeric acid), Group 4 (piracetam), and Group 5 (rivastigmine) showed a marked reduction. Group 6 (valeric acid + piracetam) and Group 7 (valeric acid + rivastigmine) showed less levels compared with other treatment groups.

Liu et al found that the plasma levels of amyloid β 1–42 increased in the AD patients.³⁴ Similarly, AlCl₃-treated rats showed a significant increase in the concentration of A β _{1–42} as compared with that in the negative control group (distilled water) in the present study. When these rats were treated with respective drugs, Group 7 (valeric acid + rivastigmine) and Group 6 (valeric acid + piracetam) rats showed a marked reduction in the level of A β _{1–42} showing improvement in the reversal of increased levels of A β _{1–42} in AlCl₃-induced AD rats.

The findings of this study showed aluminum chloride-induced rats showed significant impairment in learning and memory in the positive control and experimental groups. A marked improvement in memory, reversing the impairment caused by aluminum chloride, was seen in valeric acid-treated rats in the EPM experiment. In the HWM experiment, valeric acid-treated rats and valeric acid in combination with piracetam showed promising results. The amyloid β 1–42 estimation in plasma however showed a marked reduction in amyloid β deposits in valeric acid-treated rats in combination with rivastigmine when compared with other treated groups.

Conclusion

Valeric acid and its use in combination with standard drugs such as piracetam and rivastigmine appear to play a significant role in improving learning and memory in aluminum chloride-induced neural impairment. Thus, it would be worth exploring the potential of valeric acid in the treatment of neurodegenerative disorders such as AD.

Limitations

The EPM and HWM experiments were not recorded on the 15th day of treatment with valeric acid (Group 3), piracetam (Group 4), rivastigmine (Group 5), valeric acid + piracetam (Group 6), and valeric acid + rivastigmine (Group 7).

Conflict of Interest

None declared.

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