Research article

Effect of valeric acid on the neurotransmitter levels of hippocampus on Alzheimer's induced rats

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ABSTRACT

Introduction and Aim: Though there are many drugs approved for treatment of Alzheimer's disease (AD), there is scope for exploring newer molecules for AD management, especially considering the side effects and drug interaction profile of the existing drugs. The present study was conducted to assess the neuroprotective properties of valeric acid by estimating the five neurotransmitters in the hippocampus of Wistar albino rats in an aluminium induced AD model.

Materials and Methods: In this experimental study design effects of valeric acid, piracetam and rivastigmine were evaluated for changes in neurotransmitter levels. Seven groups were made out of 42 Wistar albino rats (male). Aluminum chloride ($AlCl_3$) (100 mg/kg body weight) was given orally for 42 days to cause AD. As a treatment, valeric acid (50 mg/kg body weight) was administered to rats in group 3, piracetam was given to group 4, rivastigmine to group 5, and a combination of piracetam and valeric acid to group 6 and valeric acid and rivastigmine to group 7 rats. After this, rat hippocampus is used to estimate acetylcholine (ACH), GABA, glutamate, dopamine, and serotonin levels.

Results: ACH levels of the hippocampus in all treated groups (groups 3 to 7) showed more increase in comparison to positive control (group 2) group. And remaining all groups demonstrated a considerable rise in GABA, glutamate, dopamine, and serotonin levels, demonstrating the reversed $AlCl_3$-induced impairment.

Conclusion: The neurotransmitter levels in aluminum chloride-induced neurological impairment appear to be significantly improved by valeric acid and its use in combination with piracetam and rivastigmine.

Keywords: Alzheimer's disease; acetylcholine; GABA; glutamate; dopamine; serotonin; valeric acid.

INTRODUCTION

Cholinergic dysfunction as a component of Alzheimer's disease (AD) is well established. Among many animal models to evaluate the effects of new molecules and treatment modalities, aluminum chloride induced AD in rats has carved its own importance in terms of ease of performance of behavioral studies, brain tissue accessibility and histopathological documentation of amyloid bodies specifying the neurodegeneration (1). It is proven on several studies that aluminum chloride at doses of 100 to 200 mg/kg body weight produces cognitive impairments mimicking AD in rats (2,3). Quantification of neurotransmitters at after exposure have shown that there is increase in aluminum in hippocampus of the rat brains proving its neurodegenerative effects (4). These accumulations are significant given the fact that hippocampus and cerebral cortex are the areas of learning and memory. Therefore, it is prudent to utilize this established AD rat model for evaluation of study drugs among many researchers across the globe.

There is growing evidence that a plethora of drugs that were hitherto indicated for other medical conditions improve cognitive dysfunction in aluminum induced AD models. Simvastatin, a lipid lowering agent belonging to the statin group, has been shown "per se" protecting its role against neuronal injury caused by aluminium salts in hippocampus and cortex (3). An active component of green tea, epigallocatechin-gallate, loaded in nanoparticles, is shown to have a protective role against aluminum induced neurobehavioral changes in AD induced rats (2). Further, it is shown that co-administration of rivastigmine and aluminium chloride normalizes the expression of BACE1 (5), AChE and IL1B genes indicating neuroprotection (6). Medicinal plants and herbal treatments are currently attracting more attention and are a great source for developing medicine for Alzheimer's (7). The anti-inflammatory and antioxidant properties are suggested as possible mechanisms for beneficial effects (8). Systematic reviews of herbal medicines for dementia conclude positively indicating a promising role in AD management (7,9). However, the quest for newer and better molecules for AD is ongoing. Though many drugs are approved for treatment of AD, there is scope for exploring newer molecules for AD management, especially considering the side effects and drug interaction profile of the existing drugs.

Epilepsy, anxiety, and sleep disorders are routinely treated with herbal supplements that are widely used and generated from root extracts of *Valeriana*

officinalis (10). It has preventive effects against neurodegenerative diseases like Parkinson's disease (11) and interacts with various neurotransmitter systems. It can also control anxiety (12) and insomnia (13) by interacting with various neurotransmitter systems (14). Neurological diseases have been treated with valeric acid, a straight chain alkyl carboxylic acid that is naturally present in the *Valeriana officinalis* (10,14). By measuring the five neurotransmitters in the hippocampus of Wistar albino rats using an aluminum-induced AD model, valeric acid neuroprotective effects were assessed in the current work.

MATERIALS AND METHODS

The effects of valeric acid, piracetam, and rivastigmine on alterations in neurotransmitter levels were assessed in this experimental study design which was carried out in the Dept. of Anatomy, Yenepoya Medical College, Mangalore in 2020. The study was approved by the Institutional Animal Ethics Committee (approval letter number: YU/IAEC25/01/2020) and it was confined to the CPCSEA's (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules and standards.

Experimental animals

We used 42 male Wistar albino rats, aged 4-6 months, weighing 220-250g. They were kept in appropriate climatic conditions and housed in polypropylene cages at a temperature of 22±1°C. Rats used in the study were bred at the Liveonbiolabs in Bangalore (Registration no. 1610/ROBiBt/S/2012/CPCSEA). They were maintained during the tests in dry polypropylene cages with husk. Four rats were housed in a single cage. Rats were marked on the head, body, and tail with their cage number to help with identification (15,16).

Rat model of Alzheimer's disease

Rats were categorized into seven groups. Group 1(negative control) rats were given distilled water orally, whereas remaining group rats were given aluminum chloride ($AlCl_3$) at a dosage of 100 mg/kg body weight orally for 42 days to induce Alzheimer's disease (17). Group 2 was considered as positive control and was not administered any medication to address the aluminum induced defects.

Study drug administration

Study drugs were administered on the 47th day. Group 3 rats were given valeric acid (50 mg/kg body weight). Group 4 was administered with piracetam (200mg/kg body weight) (13). Group 5 was given rivastigmine (0.5 mg/kg body weight) (14). Group 6 rats were given a combination of valeric acid (50 mg/kg body weight) and piracetam (200 mg/kg body weight). Group 7 rats were given a combination of valeric acid (50 mg/kg body weight) and rivastigmine (0.5 mg/kg body weight) (12). These oral medications were administered for 30 days (15) to treat the aluminum chloride-induced cognitive alterations in rats. In group 6 and 7 with combination oral medication, a second drug was administered after 30 minutes of first drug administration.

All rats were sacrificed by cervical dislocation 24 hours after the treatment and their hippocampuses separated from their brains on ice. The hippocampus was homogenized in phosphate buffer with a pH of 7.4, the homogenates centrifuged at 2000 rpm for 20 min, and the supernatant of the homogenates was utilized for the quantitative determination of the neurotransmitters.

Quantification of neurotransmitters in homogenate of hippocampus was performed using specific ELISA kits (Rat Acetylcholine, Rat Gamma-aminobutyric Acid, Rat Dopamine D2 Receptor, Rat Glutamate and Rat Serotonin, Bioassay Technology Laboratory, China), according to the manufacturer's instructions.

Quantification of neurotransmitters

ACH, GABA, glutamate, dopamine, and serotonin levels

In these tests, high sensitivity conjugates were used to find the protein in samples of hippocampal homogenate. For a 40µl sample, 10µl anti-GABA antibody is added for GABA estimation. For a 40µl sample, 10µl anti-ACHR antibody is added for glutamate estimation. For a 40µl sample, 10µl anti-GLM antibody is added for glutamate estimation. For 40µl sample, 10µl anti-D2R antibody is added for the dopamine estimation. For a 40µl sample, 10µl anti-ST antibody is added for the serotonin estimation. The sample wells were then incubated for 1 hour at 37°C with 50µl of streptavidin-HRP. After 5 times washing with a wash buffer, soaked wells with 0.35 ml wash buffer for 1 minute for each wash. Positive samples obtained blue color when 50µl of substrate solution A and 50µl of substrate solution B added to each well and incubated (10 min) at 37°C in the dark. After adding 50µl of stop solution to the reaction, it was stopped, and within 10 minutes the absorbance at 450 nm was measured. By comparing the samples' OD value to the standard curve, it was possible to estimate the amounts of neurotransmitters present in the samples.

Statistical analysis

All the groups were subjected to one-way analysis of variance (ANOVA) followed by Tukey Kramer's test. Statistical significance was set at $P < 0.05$.

RESULTS

ACH levels of the hippocampus in all treated groups (groups 3 to 7) showed significant increase in comparison to positive control group (group 2) (table 1). This indicated significant reversal of impairment

caused by $AlCl_3$. Among all the treatment groups, Group 3 (valeric acid) showed more ACH levels. There was a statistical difference between ACH levels between negative control and piracetam group (group 4).

Table 1: Comparison of acetylcholine (ACH) levels of hippocampus between animal groups

Group number	Animal Groups	ACH levelsin µ/ml (mean ± SD)
1	Distilled water (DW) (Negative control)	177.57 ± 20.77
2	$AlCl_3$(Positive control)	112.53±21.58[a]
3	Valeric acid	221.04±21.58[b]
4	Piracetam	189.02±12.78[b]
5	Rivastigmine	215.79±26.31[b]
6	Valeric acid+ Piracetam	199.72±31.16[b]
7	Valeric acid+ Rivastigmine	198.52±26.12[b]

Animal groups (n=6 rats in each group); [a]p<0.05 (values in comparison with negative control group) and [b]p<0.05 (values in comparison with positive control group)

All the treatment groups (groups 3 to 7) showed marked increase in the level of GABA after the treatment showed reversal of impairment in the neurotransmitter levels caused by $AlCl_3$ (table 2). Group 6 (valeric acid+ piracetam) reported the highest values in the treatment groups. There was statistical difference between GABA levels between negative control and valeric acid group (group 3) and rivastigmine group (group 5).

Table2: Comparison of GABA levels of hippocampus between animal groups

Group number	Animal Groups	GABA levels in nmol/L (mean ± SD)
1	DW (Negative control)	440.52±72.61
2	$AlCl_3$(Positive control)	181.46±63.8[a]
3	Valeric acid	449.07±74.15[b]
4	Piracetam	403.05±104.03[b]
5	Rivastigmine	436.34±97.29[b]
6	Valeric acid+ Piracetam	480.05±123.3[b]
7	Valeric acid+ Rivastigmine	409.48±48.16[b]

Animal groups (n=6 rats in each group); [a]p<0.05 (values in comparison with negative control group) and [b]p<0.05 (values in comparison with positive control group)

All the treatment groups (groups 3 to 7) showed significant increase in the dopamine after the treatment showing reversal of impairment caused by $AlCl_3$ (table 3). Among all the treatment groups, valeric acid group (group 3) showed no statistical difference in comparison to negative control. There was a statistical difference among groups 4 to 7.

Table3: Comparison of Dopamine levels of hippocampus between animal groups

Group number	Animal Groups	Dopamine in ng/L (mean ± SD)
1	DW (Negative control)	1158.4±62.29
2	$AlCl_3$(Positive control)	594.6±94.11[@]
3	Valeric acid	1185.29±123.56*
4	Piracetam	1024.9±158.08*
5	Rivastigmine	936.12±175.3*
6	Valeric acid+ Piracetam	932.43±126.96*
7	Valeric acid+ Rivastigmine	981.15±374.5*

Animal groups (n=6 rats in each group); [@]p<0.05 (values in comparison with negative control group) and *p<0.05 (values in comparison with positive control group)

All the treatment groups (groups 3 to 7) showed significant increase in the glutamate after the treatment showing reversal of impairment caused by $AlCl_3$ (table 4).

Table4: Comparison of glutamate levels of hippocampus between animal groups

Group number	Animal Groups	Glutamate in µg/ml (mean ± SD)
1	DW (Negative control)	26.32±4.45
2	$AlCl_3$(Positive control)	11.42±4.81[@]
3	Valeric acid	35.67±6.48*
4	Piracetam	31.66±6.96*
5	Rivastigmine	35.58±5.415*
6	Valeric acid+ Piracetam	35.26±6.11*
7	Valeric acid+ Rivastigmine	35.53±5.47*

Animal groups (n=6 rats in each group); [@]p<0.05 (values in comparison with negative control group) and *p<0.05 (values in comparison with positive control group)

All rats in the treatment groups (groups 3 to 7) showed significant increase in the serotonin after the treatment (table 5). Among all the treatment groups, rivastigmine group (group 5) showed lowest level of serotonin. statistical difference in comparison to negative control. There was no statistical difference between negative control and groups 3, 4, 6 and 7.

Table5: Comparison of serotonin levels of hippocampus between animal groups

Group number	Animal Groups	Serotonin in ng/ml (mean ± SD)
1	DW (Negative control)	52.41±5.27
2	$AlCl_3$(Positive control)	16.59±12.8$^{@}$
3	Valeric acid	55.96±9*
4	Piracetam	56.56±18.35*
5	Rivastigmine	45.88±6.13 *
6	Valeric acid+ Piracetam	53.7±11.59*
7	Valeric acid+ Rivastigmine	55.08±5.55*

Animal groups (n=6 rats in each group); $^{@}p<0.05$ (values in comparison with negative control group) and *$p<0.05$ (values in comparison with positive control group)

DISCUSSION

Systematic review of 13 studies evaluating herbal medicines either as monotherapy or adjunct to routine medications resulted significantly better outcomes (15). However, there are innumerable methodological drawbacks and differences among these studies that hinder unequivocal recommendation of herbal medicines for regular human use. Many of these herbal extracts employ either direct or indirect effects of ACH and acetylcholinesterase inhibition (8). Considering multiple targets including anti-oxidative neuroprotective mechanism of actions by use of combination of approved drugs and herbal extracts provide extended opportunity to address the cognitive impairment at early stages.

First neurotransmitter imbalance implicated in cognitive changes in dementia is ACH (16). A marker for cholinergic neuronal loss in the brain, acetyl cholinesterase, is utilized to quantify the neuroprotective role of green tea component epigallocatechin-gallate and it was proven that the administration of nanoparticles of this component resulted in significant decrease on cholinergic neuronal loss among aluminum induced AD rats (2). Memory problems linked to brain monocyte infiltration were lessened by simvastatin treatment (17). In another perspective the inhibition of degradation of synaptic acetylcholine by rivastigmine is found effective in AD rats (6). Rivastigmine is different from other cholinesterase inhibitors. It inhibits both acetylcholinesterase and butyrylcholinesterase in a partially irreversible way (18). Therefore, in these lines, along with rivastigmine, donepezil and galantamine are in vogue for AD management. In the present study, cholinesterase is not quantified. However, as increase ACH levels found in this study may be partly attributable to increase in synaptic ACH. Acetylcholinesterase quantification would have provided details of cholinergic neuronal loss. Similarly, quantification of amyloid beta 1-42 would have increased the overall effect of cognitive impairment.

Imbalance between GABAergic and glutaminergic neurotransmission contributes to the neuro degenerative process in animal model AD (19). Among many functions of astrocytes, maintenance of glutamate-glutamate shuttle is necessary for normal cognitive functions and for AD progression (20). Lavender extract effects on Amyloid beta 1–42 induced AD rat model showed significant lower levels of GABA and glutamate in comparison to positive controls (21). Outcomes of our study agree with this study in aluminum model of rat AD.

There are no established direct links between dopamine-serotonin alterations and cognitive dysfunctions of AD in humans (22). However, links of serotonin and early depression characteristics of AD is well established (23). Functional interdependence between dopamine and serotonin is the key factor in designing and exploring new opportunities to address early cognitive deterioration in AD (24). In this regard, as pointed out from dopamine and serotonin normalization after use of valeric acid in our study aids in understanding the interdependence of these two varied neuro-signaling pathways.

CONCLUSION

When aluminum chloride causes neurological impairment, valeric acid and its combination with piracetam and rivastigmine seem to be effective at raising neurotransmitter levels. Valeric acid may therefore be a helpful choice in the treatment of neurodegenerative diseases like AD, which justifies more investigation into this area.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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