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Neuroprotective Effect of Valeric Acid in Combination with Piracetam and Rivastigmine by Evaluating the Expression of Caspase 3 in Alzheimer's Disease Induced Rats

Blessina Sugandhi.D[1], Bindhu.S[2], Nidhi.S[3], Shishir Kumar[4], Dhiren Kumar Panda[5]

[1] Assistant Professor, Department of Anatomy, Maheshwara Medical College, Isnapur, Sangareddy District, Telangana, India

[2] Additional Professor, Department of Anatomy, K S Hegde Medical College, Nitte (Deemed to be University), Mangalore, Karnataka, India

[3] Assistant Professor, Department of Anatomy, Yenepoya Medical College, Yenepoya (Deemed to be University), Mangalore, Karnataka, India

[4] Professor, Department of Anatomy, IMS & SUM Hospital, Bhubaneshwar, Odisha, India

[5] Assistant Professor, Department of Anatomy, IMS & SUM Hospital, Bhubaneshwar, Odisha, India

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Abstract

Background: Alzheimer's disease (AD) is associated with neuronal death, where caspases, especially Caspase-3, play a key role in apoptosis and neurodegeneration.

Objective: This study evaluates the neuroprotective effects of valeric acid in combination with piracetam and rivastigmine by analyzing Caspase-3 expression in AD-induced rats.

Methods: Forty-two male Wistar albino rats were divided into seven groups. Alzheimer's disease was induced using aluminum chloride (AlCl3), followed by treatment with valeric acid, piracetam, or rivastigmine. Caspase-3 expression in the hippocampus was evaluated using immunohistochemistry.

Results: Rats treated with a combination of valeric acid and either piracetam or rivastigmine showed reduced Caspase-3 expression in the hippocampus compared to those treated with individual drugs.

Conclusion: Valeric acid, when combined with piracetam or rivastigmine, exhibits neuroprotective effects by reducing Caspase-3 expression, making it a potential therapeutic option for Alzheimer's disease.

Introduction

Neurodegeneration in Alzheimer's disease (AD) is closely linked with the activation of various proteases, including caspases, calpains, cathepsins, and proteases involved in the lysosomal and ubiquitin-proteasome systems [1–3]. Among these, apoptosis-related caspases are believed to significantly contribute to the progressive neuronal death observed in AD [4–10]. Amyloid-beta (Aβ), a key protein in AD pathology, activates caspases in cultured hippocampal neurons, leading to apoptosis [11]. Several studies have demonstrated the abnormal expression of initiator caspases 8 and 9 and effector caspases 3 and 6 in postmortem AD brain tissues [12–17].

Caspases not only play a central role in apoptosis but also contribute to amyloid precursor protein (APP) processing, promoting the biogenesis of amyloidogenic Aβ peptide species [7]. Caspase-cleaved APP and activated caspase-9 have been identified in the brains of AD patients but are absent in controls [9]. Active caspases and their cleaved substrates,

such as fodrin, actin, tau, and APP, have been consistently detected in postmortem AD brain tissues and AD animal models, further supporting the hypothesis that caspases contribute significantly to neurodegeneration in AD [7,9,18-23]. Caspase-3, in particular, has been implicated in the processing of APP into amyloidogenic fragments, and the accumulation of caspase-cleaved APP may represent an early neurodegenerative event in the progression of AD [7,24]. Immunohistochemical analysis of AD brains has revealed increased levels of active caspase-3 in hippocampal neuron somata and neurites, showing a strong co-localization with neurofibrillary tangles and plaques [25].

Root extracts of *Valeriana officinalis* are commonly used as herbal supplements for treating sleep disorders, anxiety, and epilepsy [26]. Studies have demonstrated that valerian has neuroprotective effects in neurodegenerative diseases such as Parkinson's disease and AD, likely due to its interaction with multiple neurotransmitter systems [27–32]. Valeric acid, a straight-chain alkyl carboxylic acid found naturally in *Valeriana officinalis*, has been shown to exert therapeutic effects on various neurological disorders, including AD [31–32].

This study aimed to evaluate the neuroprotective effects of valeric acid, in combination with piracetam and rivastigmine, in an AD-induced rat model by assessing Caspase-3 expression. We hypothesized that valeric acid, when combined with these standard AD therapies, could reduce neuronal apoptosis by modulating Caspase-3 activity.

Materials and Methods

Study Design and Ethical Approval

This experimental study was conducted at the Department of Anatomy, Yenepoya Medical College, Mangalore, India, between 2018 and 2020, following ethical approval from the Institutional Animal Ethics Committee (IAEC) in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval No: YU/IAEC/4/2018).

Animals

Forty-two male Wistar albino rats, aged 4-6 months and weighing 220–250 g, were used in the study. The animals were housed in polypropylene cages under controlled environmental conditions (22 ± 1°C, 12-hour light/dark cycle) and were provided with standard rat chow and water ad libitum. Rats were randomly assigned to one of seven experimental groups (six rats per group) for scientific and statistical analysis [33].

Drugs and Chemicals

Aluminum chloride ($AlCl_3$), valeric acid, piracetam, and rivastigmine were purchased from Sri Durga Laboratory, Mangalore, Karnataka, India, and Sanative Medical Stores, Mangalore, India.

Induction of Alzheimer's Disease

Alzheimer's disease was induced in rats by administering $AlCl_3$ at a dose of 100 mg/kg body weight orally for 42 days, as per established protocols [34]. The control group (Group 1) received distilled water for the same duration. After AD induction, the remaining groups received their respective treatments as follows:

- **Group 2 (Positive control)**: $AlCl_3$ only.
- **Group 3 (Valeric acid)**: Valeric acid 50 mg/kg [35].
- **Group 4 (Piracetam)**: Piracetam 200 mg/kg [36].
- **Group 5 (Rivastigmine)**: Rivastigmine 0.5 mg/kg [37].
- **Group 6 (Valeric acid + Piracetam)**: Valeric acid 50 mg/kg + Piracetam 200 mg/kg [38].
- **Group 7 (Valeric acid + Rivastigmine)**: Valeric acid 50 mg/kg + Rivastigmine 0.5 mg/kg [38].

Drugs were administered orally for 30 days following the induction of Alzheimer's disease.

Tissue Collection and Processing

At the end of the treatment period, rats were euthanized under anesthesia, and hippocampal tissue was harvested for further analysis. Tissue samples were fixed in 10% formalin for 24 hours, dehydrated using graded alcohol, cleared with xylene, and embedded in paraffin blocks. Serial sections (3–5 µm thick) were cut using a microtome and mounted on glass slides for immunohistochemical staining [39].

Caspase-3 Immunohistochemistry

Caspase-3 expression was evaluated using immunohistochemistry (IHC). The formalin-fixed, paraffin-embedded sections were deparaffinized in xylene and rehydrated through a graded alcohol series. Antigen retrieval was performed by boiling the sections in sodium citrate buffer (pH 6.0) for 30 minutes at 95–100°C [40,41]. After cooling, the sections were treated with peroxidase blocking solution to inhibit endogenous peroxidase activity.

The sections were incubated with a primary anti-Caspase-3 antibody (1:200 dilution) at room temperature for 1 hour, followed by incubation with a horseradish peroxidase-conjugated secondary antibody for 20 minutes. The antigen-antibody complexes were visualized using diaminobenzidine (DAB), and counterstaining was done with hematoxylin. The slides were then dehydrated and mounted for microscopic analysis [42,43].

Statistical Analysis

Data were analyzed using one-way ANOVA followed by Tukey's post-hoc test for group comparisons. Results were expressed as mean ± standard error (SE), and a p-value of <0.05 was considered statistically significant.

Results and Discussion

Caspase-3 is one of the most crucial proteases in the caspase cascade, playing a pivotal role in apoptosis. In the present study, Caspase-3 immunopositive cells were absent in the control group, indicating no significant apoptosis (**Figure 1**). However, in the group treated with aluminum chloride ($AlCl_3$), a substantial increase in Caspase-3-positive cells was observed (**Figure 2**), signifying elevated levels of apoptosis induced by $AlCl_3$ administration.

Control group

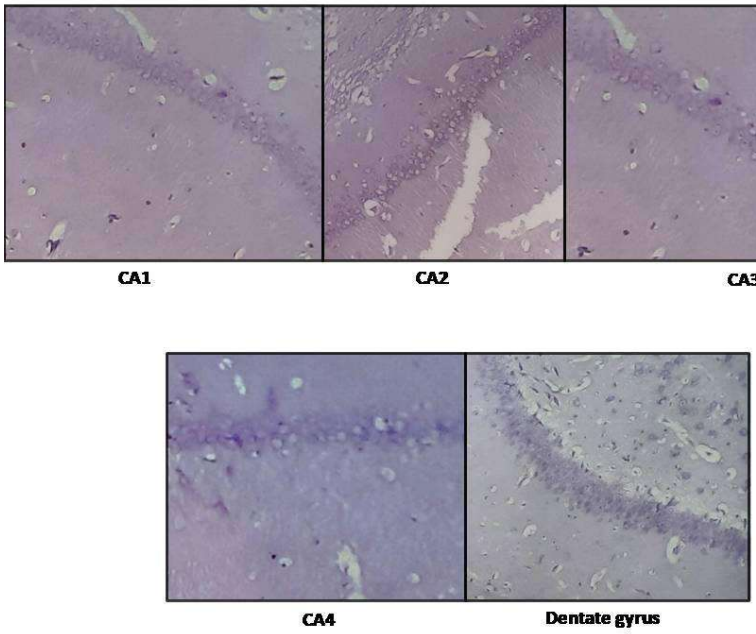

Figure 1: A section of the rat hippocampus from the control group, showing all subregions with Caspase-3 immunohistochemistry at 40x magnification.

No Caspase-3-positive neurons were detected, and normal hippocampal architecture was preserved.

$AlCl_3$ group

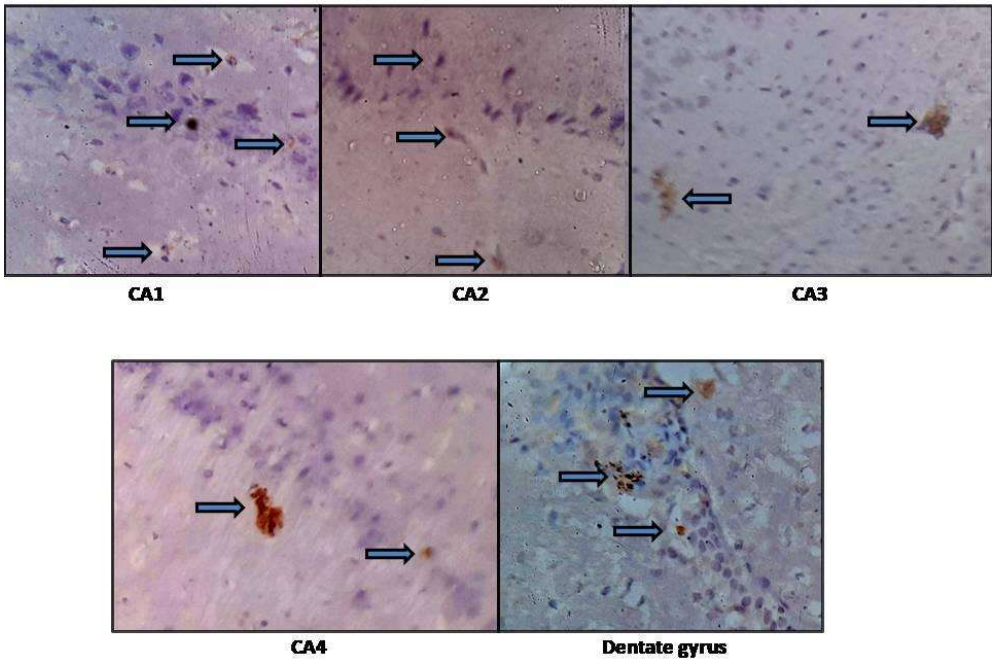

Figure 2: **A section in a rat hippocampus showing all the sub-regions in the $AlCl_3$ group with Caspase 3 IHC in 40X magnification.**

illustrating the effect of 100 mg/kg $AlCl_3$ on Caspase-3 protein expression across all hippocampal subregions, with increased immunostaining indicating heightened apoptosis.

In rats treated with valeric acid (50 mg/kg), there was a significant reduction in Caspase-3-positive cells compared to the $AlCl_3$ group (**Figure 3**), demonstrating the neuroprotective effects of valeric acid. Similarly, piracetam (200 mg/kg) and rivastigmine (0.5 mg/kg) also reduced Caspase-3 expression, although not as extensively as valeric acid (**Figures 4** and **5**, respectively).

Valeric acid group

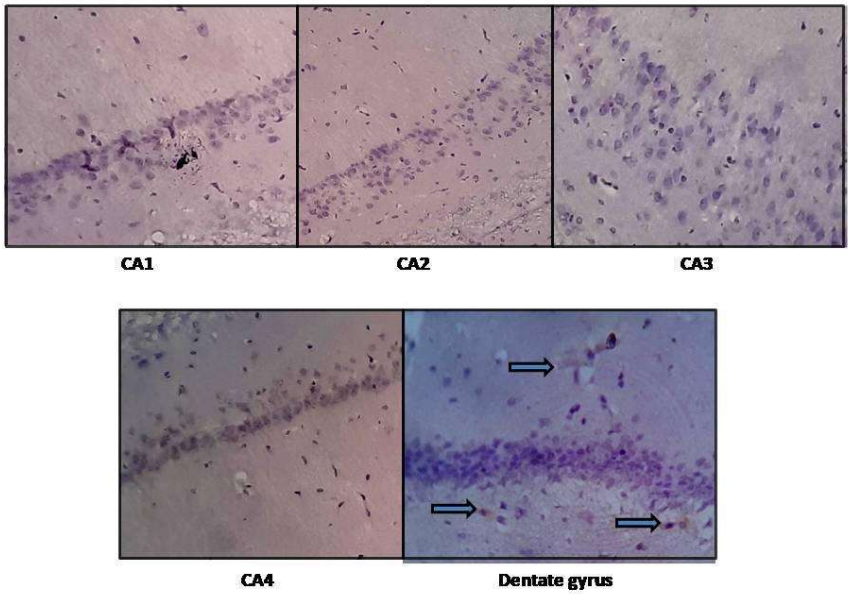

Figure 3: A section of the rat hippocampus from the valeric acid group, showing reduced Caspase-3 expression in all hippocampal subregions following treatment (40x magnification).
Valeric acid (50mg/kg b.wt) reduced the expression of Caspase 3 protein in all the sub-regions of rat hippocampus.

Piracetam group

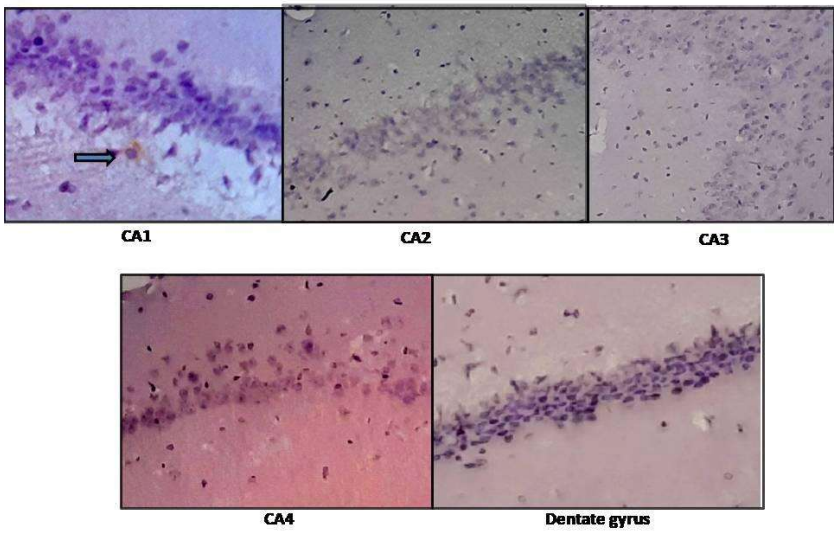

Figure 4: A section of the rat hippocampus from the piracetam group, showing a moderate reduction in Caspase-3-positive cells in all subregions (40x magnification).
Piracetam (200mg/kg b.wt) reduced the expression of Caspase 3 protein in all the sub-regions of rat hippocampus.

Rivastigmine group

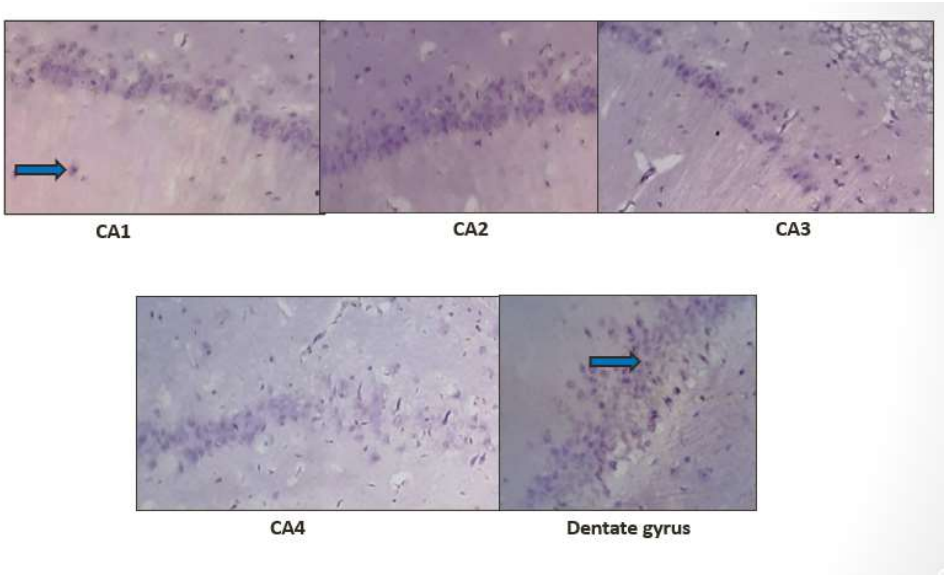

Figure 5: A section of the rat hippocampus from the rivastigmine group, displaying reduced Caspase-3 expression, although to a lesser extent than the valeric acid group (40x magnification).

Rivastigmine (0.5mg/kg b.wt) reduced the expression of Caspase 3 protein in all the sub-regions of rat hippocampus.

The combination therapies, particularly valeric acid with piracetam or rivastigmine, resulted in a more pronounced decrease in Caspase-3 expression. The valeric acid + piracetam group exhibited a significant reduction in Caspase-3-positive cells in all hippocampal subregions compared to the groups treated with valeric acid, piracetam, or rivastigmine alone (**Figure 6**). A similar result was seen in the valeric acid + rivastigmine group, where a substantial decline in Caspase-3-positive cells was evident (**Figure 7**), suggesting an enhanced neuroprotective effect from the combination therapies.

Valeric acid + Piracetam

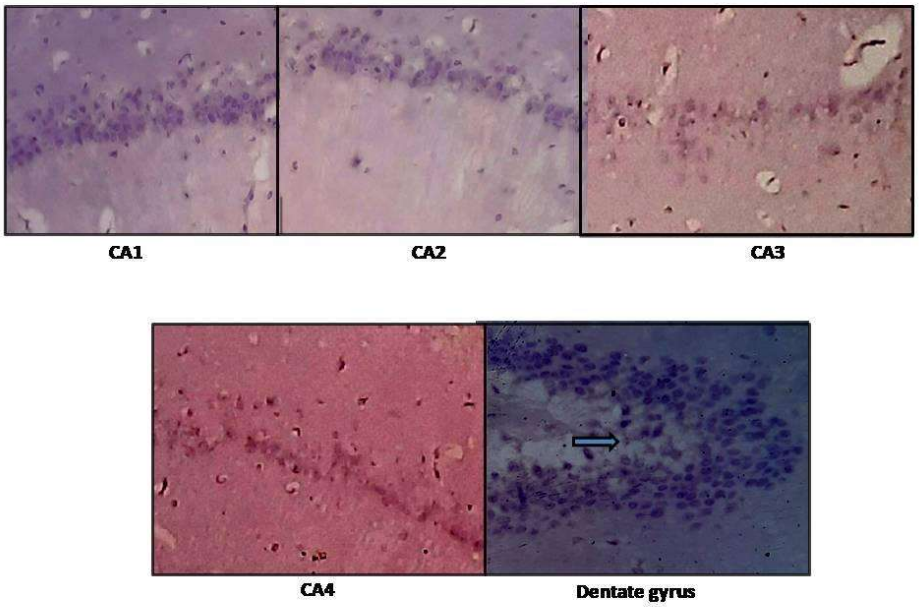

Figure 6: A section of the rat hippocampus from the valeric acid + piracetam group, demonstrating a notable reduction in Caspase-3-positive cells across all subregions (40x magnification).
A combination of Valeric acid and piracetam reduces the expression of Caspase 3 protein in all the sub-regions of rat hippocampus.

Valeric acid + Rivastigmine group

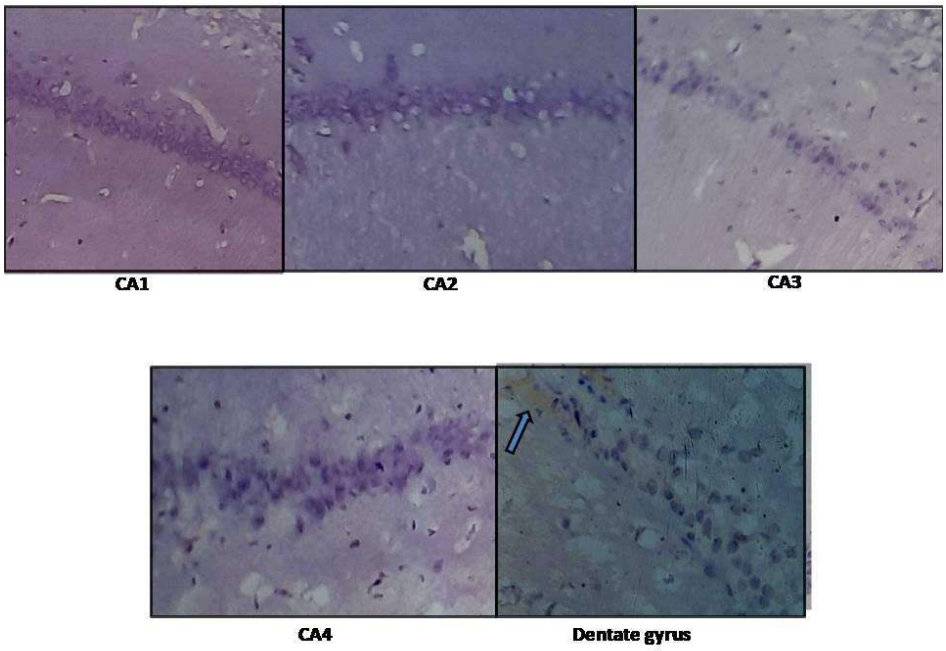

Figure 7: A section of the rat hippocampus from the valeric acid + rivastigmine group, showing a significant decrease in Caspase-3 expression in all hippocampal subregions (40x magnification).

Combination of Valeric acid and rivastigmine reduced the expression of Caspase 3 protein in all the sub regions of rat hippocampus.

Caspase-3, a member of the cysteine-aspartic protease family, plays a crucial role in the cleavage of specific target proteins, leading to apoptosis. In its inactive form, procaspase-3 exists as a zymogen composed of two subunits: a larger p20 and a smaller p10, along with an N-terminal prodomain. Upon activation by internal or external stimuli, the mature enzyme cleaves target proteins, contributing to the characteristic low-molecular-weight DNA oligomers seen during apoptosis [40,41].

Two critical features of apoptosis include the release of cytochrome C from mitochondria and the activation of caspases, especially caspase-3, within the cytosol [42,43]. Caspase-3 serves as a key mediator in apoptosis-related intracellular signaling pathways [44]. Targeting caspase-3 in Alzheimer's disease treatments has the potential to improve therapeutic outcomes [45]. Recent studies, such as that by Wang Y et al. (2023), have demonstrated increased caspase-3 expression in an AD rat model, consistent with the findings of the current study [41].

Elmorsy E et al. (2021) further explored the impact of $AlCl_3$ on gene expression related to apoptosis and oxidative stress. They found that $AlCl_3$ treatment caused significant downregulation of anti-apoptotic genes, including Bcl-2, as well as antioxidant enzymes like SOD1, SOD2, and CAT, which aligned with our results showing elevated Caspase-3 levels in the $AlCl_3$-treated group [42]. In our study, the overexpression of activated caspase-3 in the hippocampus of $AlCl_3$-administered rats was significantly mitigated by valeric acid, particularly when combined with piracetam or rivastigmine. Supporting these findings, Qin et al. (2009) reported that *Valeriana officinalis* extract promoted neural stem cell growth while reducing the formation of Caspase-3-positive neurons [44]. Jayaraj RL et al. (2020) similarly demonstrated that valeric acid treatment decreased Bax expression and increased Bcl-2 levels through activation of the mTOR pathway, thereby preventing apoptosis [45]. These observations are consistent with our findings, wherein valeric acid, particularly in combination with piracetam or rivastigmine, demonstrated potent neuroprotective effects by inhibiting caspase-3-mediated apoptosis in AD-induced rats.

Conclusion

This study highlights the neuroprotective effects of valeric acid in an Alzheimer's disease (AD) rat model induced by aluminum chloride ($AlCl_3$). The administration of valeric acid, especially in combination with piracetam or rivastigmine, significantly reduced Caspase-3 expression, a key marker of apoptosis in neuronal cells. The findings suggest that valeric acid offers enhanced protection against neurodegeneration when used alongside established AD treatments.

Given that Caspase-3 plays a crucial role in the apoptosis cascade leading to neuronal death, targeting this pathway may provide a promising therapeutic strategy in the management of Alzheimer's disease. The combination of valeric acid with either piracetam or rivastigmine demonstrated synergistic effects, indicating its potential for clinical applications in preventing or delaying AD progression.

Further research is needed to validate these findings in clinical settings and explore the underlying mechanisms in greater detail. However, this study supports the potential of valeric acid as a valuable adjunct in AD therapy, offering hope for improved therapeutic outcomes in neurodegenerative diseases.

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