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Research Article
Ibuprofen with Amino Acid Dipeptide Conjugation: A Novel Prodrug for the Management of Alzheimer's Disease Complications

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Abstract

Background and Objective: Alzheimer's disease (AD) is a severe, diverse and complex brain disorder that slowly degrades cognitive and memory abilities. Patients with a history of long-term use of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) have a lower risk of AD, according to epidemiological research. The objective of the research was to synthesize a novel dipeptide prodrug of ibuprofen and evaluate its anti-Alzheimer's potential. **Materials and Methods:** A Novel Ibuprofen compound (Ibu-GLVL) was synthesized by the catalyst microwave method. Characterization of the compound was done by IR, ^{1}[H] NMR and mass spectroscopy. The anti-Alzheimer's activity was investigated using the Rotarod test and Morris water test against aluminum chloride-induced neurodegeneration. Further, histopathology was done on the hippocampus isolated from the experimental animals. **Results:** The characterization analysis predicted the composition of the novel compound as 'C'-66.27%, 'H'-8.34%, 'N'-7.73% and 'O'-17.66%. The solubility studies indicated that the R^2 value was 0.9816, the compound was stable in both the pH of gastric and intestine and the partition coefficient (log P) value was 1.64. Further, the synthetic compound reduced significantly ($p<0.05$) the aluminum chloride defects using the rotarod and Morris-water test, which was confirmed with the histopathological examination. *In vitro* studies predicted that the newly synthesized compound inhibited the acetylcholinesterase activity. **Conclusion:** The data suggested that the newly synthesized prodrug form of ibuprofen is stable and could possess the potential to reduce the complications of AD. More research in this direction might aid in the discovery of a new therapeutic agent that has the potential to manage the symptoms of AD.

Key words: Alzheimer's disease, ibuprofen, prodrug, characterization, aluminum chloride, acetylcholinesterase activity

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Prodrugs are pharmacologically inactive chemical substances that undergo biotransformation into an active molecule before exhibiting pharmacological effects[1]. The purpose of designing the prodrug is to overcome the issues related to the release characteristics and therapeutic effects of the parent molecules[2]. Carrier-linked (Bipartite, Tripartite, Mutual), bioprecursor and chemically modified (alcohol group prodrugs, carboxylic group prodrugs, ester group prodrugs, amide prodrugs) are the three primary types of prodrugs. Amide prodrugs can improve drug stability, deliver targeted drugs and change the lipophilicity of parent drugs[3].

Alzheimer's disease (AD) is a complex degenerative brain disorder that gradually impairs cognitive functions and memory. Globally, life expectancy has increased because of medical advances and the aging population and rising number of AD patients need the identification of unmet needs to properly treat AD[4]. The etiology of AD is unclear; however, pathophysiology identifies the following active factors in AD: Amyloid beta aggregation[5], tau-phosphorylation, acetylcholine insufficiency[6], oxidative stress[7] and neuroinflammation[8]. Around 30 million people are suffering from AD internationally which is further expected to accelerate in the next decade making AD a global public health crisis. Because of the complexities of neurodegenerative disease causation, no effective therapeutic therapy has been established. Many ideas have been postulated based on AD pathogenesis variables, but only a few such as acetylcholinesterase inhibitors have been identified as AD therapy that can effectively delay the progression[9].

The currently approved medications work on a single target (ODOT; one drug, one target) and give brief relief from the symptoms of AD. As a result, efforts are being made to identify and develop innovative medicines for the treatment of Alzheimer's disease that can reach several targets. Long-term usage of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) has been found in trials to reduce the chance of acquiring and postponing the beginning of AD[10]. To be successful as a therapy for Alzheimer's and other neurodegenerative disorders, NSAIDs must reach sufficiently high concentrations in the CNS, particularly brain parenchyma. Due to the poor penetration of NSAIDs through the blood-brain barrier (BBB), substantial dosages of these drugs would be necessary, raising the risk of adverse effects. To reach their site of action on the CNS side, NSAIDs must have BBB permeability[11]. However, because NSAID distribution into the CNS is typically restricted, developing NSAID delivery strategies that allow them to be taken up by the brain more effectively is crucial.

The use of transporters at the BBB using a prodrug strategy might boost brain absorption of poorly penetrating medicines. The sodium-independent exchanger Large-Neutral Amino Acid Transporter 1 (LAT-1) is present in several organs including the brain, testis and placenta. It allows thyroid hormones and large-neutral amino acids, such as triiodothyronine, to flow across the BBB. Their brief chemical alteration via the prodrug approach is an attractive and satisfying chemistry-based strategy that has successfully increased the CNS transport of poorly penetrating medicinal drugs[12].

Therefore, given the above findings, it is thought worthwhile to synthesize the derivatives that can cross BBB and study their chemical stability, screen their anti-Alzheimer's activity and compare them with standard drugs. The current study attempts to create a dipeptide prodrug of ibuprofen conjugated with amino acids and to investigate the changes in activity induced by amino acid combination using the solution phase peptide synthesis approach. The *in vivo* anti-Alzheimer's activity was evaluated in experimentally induced symptoms of AD using aluminum chloride in animals.

MATERIALS AND METHODS

Study area: The study was conducted in the research laboratories of Krupanidhi College of Pharmacy, Bangalore, India as per the guidelines of good laboratory practice. The study was conducted between May, 2022 to September, 2023.

Materials: All chemical substances of laboratory grade were obtained from standard medical suppliers after placing the request in the chemical-reagent procurement department. These chemicals were supplied by the regular agents authorized by the institute.

Synthesis: The microwave synthesis was performed in a catalyst microwave. In brief, the scheme of synthesis of the novel ibuprofen prodrug was depicted in Fig. 1.

Synthesis of amino acid methyl ester hydrochlorides (microwave-assisted synthesis): A paste-like mass of methyl ester hydrochloride was obtained by adding thionyl chloride (20.0 mmol) to methanol (30 mL) at 0°C, followed by the addition of amino acid (20 mmol) and then irradiating the reaction mixture at 180 W for 15 min. Excess dimethyl sulfite was removed by triturating the mixture with ether at 0°C.

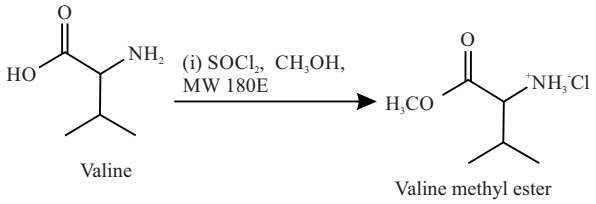

Fig. 1: Synthesis of amino acid methyl ester hydrochlorides

At 0°C, the solid (I) was recrystallized from methanol and diethyl ether[13].

Synthesis of a mono-peptide ibuprofen conjugates: In a conical flask, 7 mmol of ibuprofen was dissolved in 2.8 mL of triethylamine (TEA). At 0°C, add dicyclohexylcarbodiimide (DCC) (7 mmol), DIPEA or DMAP and stir continuously for 30 min. After 24 hrs of stirring at room temperature, 7 mmol of amino acid methyl ester hydrochloride dissolved in THF was added to the first mixture. The reaction mixture was filtered after 24 hrs, then $CHCl_3$ (30 mL) was added, the residue was separated and the extract was washed many times with 5% $NaHCO_3$ and 5% HCl. Vacuum drying, filtration and evaporation of the organic layer over anhydrous Na_2SO_4 were performed.

Coupling reaction to synthesize ibuprofen dipeptide conjugates: Ibuprofen (7 mmol) was dissolved in 2.8 mL of triethylamine (TEA) and DMAP (0.2 mmol) in a conical flask. To this solution add DCC (7 mmol), DIPEA or DMAP at 0°C with continuous stirring for 30 mins. Monopeptide Ibuprofen conjugate (7 mmol) was dissolved in Tetrahydrofuran (THF) in flask 2. After 30 min, mix the contents and stir the mixture at room temperature for 24 hrs. The reaction mixture was filtered after 24 hrs and the residue was washed with $CHCl_3$ (30 mL) and added to the filtrate. The mixture was then extracted and dried with 5% $NaHCO_3$ (20 mL).

Carboxyl group deprotection: At 0°C, LiOH (1.5 mmol) was added to a product solution (1 mmol) in THF: H_2O in 1:1 (36 mL). Adjust the pH 3.5 with 1N H_2SO_4 and then reflux for 15 min at 55-60°C. Ether was used to extract the aqueous layer (3×15 mL)[14].

IR (KBr, cm^{-1}) :3291 (N-H stretch of amide); 1539 (N-H bend of amide); 1229 (C-O stretch of -COOH); 1699 (C=O stretch of -COOH); 2926 (asymmetric C-H stretch of -CH_2-); 2852 (symmetric C-H stretch of -CH_2-), 1652, 1633 (aromatic C-C stretch). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 1.28 (s, 3H, -CH_3), 1.90-1.95 (d, 2H, CH_2), 5 and 5.15 (s and d, 2H, NH). Mass spectra (m/z, %): 363 (362).

Characterization: The FTIR spectrum was captured using VARIAN on a Shimadzu model IRAffinity-1 spectrophotometer between 4000 and 5000 cm^{-1}. The ^{1}H NMR spectrum data was collected using a Bruker 400 MHz Advance II NMR spectrometer using $CDCl_3$ as a deuterated solvent and chemical shifts are reported as δ (ppm) with tetramethylsilane (TMS) as an internal standard. The WATERS-XEVO C2-XS-QToF high-resolution mass spectrometer (HRMS) equipped with a custom-made electro-spray interface is used to generate the MASS spectra (ESI)[15].

Pharmacokinetic studies

Solubility studies: The equilibrium solubility of the synthesized prodrug was evaluated using the "shake-flask" method. In brief, an excess of synthesized prodrug was dissolved in 0.5 mL of different solvents i.e., the solutions were prepared in methanol at pH 1.2, pH 6.8 and pH 7.4 and stirred for 24 hrs at room temperature. The solid-liquid phases were separated by centrifuging each solution at 2000 rpm for 20 min. The supernatant was examined using UV-vis spectroscopy at 264 nm[16].

Stability studies: Twenty milliliters of 0.1 N HCl (pH 1.2) and phosphate buffer (pH 7.4) were used to completely dissolve five milligrams of the produced prodrug. These were divided into Eppendorf tubes as 1 mL solutions. The tubes were examined sequentially at different time intervals (0, 24, 48, 72, 96 and 120 hrs) using an established UV spectroscopic technique[17].

Partition coefficient: The neutral solubility of a substance in an immiscible biphasic system of lipids and water is described by its partition coefficient. The partition coefficient of the synthesized prodrugs is determined by the shake flask method. In a separating funnel, 100 mg of the drug was transferred and 30 mL of octanol. At room temperature, the contents of the separating funnel were shaken for 1 hr. The quantity of prodrug present in the solutions was then determined using the absorbance at 264 nm and the log P value was calculated[18]. The following formulas were used to calculate the partition coefficient and log P:

$$P = \frac{O}{A}$$

Where:

P = Partition coefficient

O = Concentration of organic partition

A = Concentration of aqueous partition

$$\text{Log (P)} = \log_{10} \text{(partition coefficient)}$$

***In vivo* anti-Alzheimer's activity:** Animal experiments were carried out by industry standards. The experiment methodology was approved by the Krupanidhi College of Pharmacy's Institutional Animal Ethic Committee (IAEC) with the number KCP/IAEC/PCOL/PCHEM/97/2022 and the animals were obtained from the central animal house.

The animals (Wistar rats weighing 230-250 g of either sex) were given limited access to food and water and housed under the same laboratory conditions, which included a 12 hrs light/dark cycle, humidity of 5 to 15% and a temperature of 22°C, under CPCSEA guidelines. After 5 days of acclimation, the Wistar rats were randomly divided into four groups (n = 6 in each group). The animals were treated with a test drug dissolved in 5% DimethylSulfoxide (DMSO), the standard drug (rivastigmine) (2.5 mg kg^{-1} p.o.) (once daily) along with Aluminum Chloride ($AlCl_3$) which serves as an inducing agent, orally for 30 days. On "the 31st day" the animals were euthanized and the brain was assessed for various parameters of neurotoxicity:

Group 1: Vehicle control group (5% DMSO).

Group 2: Positive control group (aluminum chloride induced Alzheimer's, 32.5 mg kg^{-1}, p.o.)

Group 3: Treatment group (prodrug Ibu-glvl, 50 mg kg^{-1} p.o.)[19]

Group 4: Standard drug (rivastigmine 2.5 mg kg^{-1} p.o.)[20]

Rotarod apparatus: Motor learning, balance and coordination were measured using a rotarod apparatus (Model # 2213, Dolphin Labs, Mumbai, India). The rats were suspended on a horizontally rotating rod (8 cm diameter; 20 rpm rotation speed) for 5 min, or until they fell off. A switch was triggered by falling off the pole, which turned off a timer. Five rats were evaluated simultaneously, separated by large discs. Each rat was subjected to three trials separated by a10 min interval[21].

Morris water maze test: The morris water maze (MWM) test (Model # A442, SNS Labs Pvt. Ltd., Mumbai, India,) is used to assess spatial memory. A big circular swimming pool with four equal quadrants of water (25°C) (Northwest, Northeast, Southeast and Southwest) was used. Red and blue colored tapes were utilized to offer visual indicators around the water tank for assistance. During the acquisition phase, the submerged platform (10×10 cm) was kept one centimeter above the water's surface. Throughout each experiment, animals were gently placed in different quadrants of the swimming pool for 120 sec and allowed to identify the platform. The water surface was rendered opaque during the retention phase by concealing the platform with milk powder and keeping it one centimeter below the tank's water level. On the tenth, twentieth and thirtieth days, the animal was placed in one of the tank's quadrants, facing the tank's wall and its memory recall was tested. Escape latency was calculated using the time it took the animal to find the hidden platform within the water maze[22].

Histopathological evaluation: After 31 days, the rats were killed by cervical dislocation and the brains were removed, rinsed in cold isotonic saline and stored at 80°C in neutral buffered formalin (NBF) (10%). The brain sample was then sent for histopathology to Koushik Laboratory and Clinic, Bengaluru. Thin transverse slices of the hippocampus, cortical region and pyramidal region of the brain were produced using a microtome. The sections were stained with Hematoxylin and Eosin (H&E) and Congo red dye before being examined under a digital microscope (Model No. E4625, ESAW Labware Pvt. Ltd., Ambala, India) at 100X magnification. Six slides from each group were evaluated for hippocampal histology. The degree of neuronal degeneration and deposition of amyloid-beta in the brain were assessed using morphometric analysis; the resulting severity index was reported as 0+, 1+, 2+ and 3+, correspondingly, for 1% (minimal), 1-25% (mild), 26-50% (moderate), 51-75% (marked or moderately severe) and 76-100% (severe). By examining the images, the optical density (OD) of amyloid plaque accumulation in hippocampus areas stained with Congo red was studied[23].

***In vitro* acetylcholinesterase activity:** Randomization was used to divide the Wistar rats into four groups (n = 6 each group) Group 1: Vehicle control (5% DMSO), Group 2: Control group (aluminum chloride 32.5 mg kg^{-1} p.o.), Group 3: Ibu-GLVL and Group 4: Standard medicine (rivastigmine 2.5 mg kg^{-1} p.o.). The animals were administered Ibu-GLVL dissolved in 5% DMSO for 30 days, as well as the conventional medicine (rivastigmine) (2.5 mg kg^{-1} p.o.) once daily in combination with aluminium chloride inducing agent. On the 31st day, the animals were euthanized and the brain was homogenized in heparin and PBS solution and centrifuged at 2000 rpm for 10 minutes. The anti-acetylcholinesterase activity in brain tissue was measured using a modified approach reported by Havas *et al.*[24]. The test used supernatant (0.1 mL), sodium phosphate buffer (0.1M, pH 8.0, 2 mL) with 0.1% BSA, dithiobis-nitrobenzoic acid (DTNB) (0.1 mL) and acetylthiocholine iodide (AChI) (0.05 mL). A spectrophotometer (Model # UV 3200, LabIndia Analytical Ltd., Thane, Maharashtra, India) was used to record the absorbance change at 412 nm at 1 min intervals for 2 min. The activity of

acetylcholinesterase was measured in micromoles of acetylthiocholine hydrolyzed per minute per mg protein.

Statistical analysis: Mean and Standard Deviation (SD) are used to summarize the study's findings. GraphPad Prism version 5.0 software (California, United States) was used for statistical analysis, which included One-way ANOVA followed by Dunnett's test. The results of various studies were compared and a value of $p<0.05$ was used to signify the level of significance.

RESULTS

Characterization of the newly synthesized compound: The spectroscopical analysis using FTIR, ^{1}[H]-NMR and MASS revealed that $+CH_3<RCH_2+<R_2CH+<R_3C+$. Different composition in the newly synthesized compound is 'C' (66.27%), 'H' (8.34%), 'N' (7.73%) and 'O' (17.66%). The SMILE analysis for the compound was found to be CC(C)CC1=CC=C(C=C1)C(C)C(=O)NCC(=O)NC(C(C)C)C(O)=O. Further, proposed mechanisms of MS/MS collisional induced dissociation (CID) for Ibu-GLVL (m/z 362), (m/z 363) and (m/z 364). Collectively, the data was represented in Fig. 2(a, b).

Pharmacokinetic studies

Solubility study: The UV-Vis spectroscopy was used to measure solubility at 264 nm. The UV-Vis spectra of a series of standard solutions of the synthesized prodrug (Ibu-GLVL) of various concentrations (100, 200, 300, 400, 500, 600, 700, 800 and 900 mg mL^{-1}) were recorded to generate an absorbance vs. concentration calibration curve (Fig. 3a). The standard deviation and mean absorbance were averaged across three replicates to get the calibration curve. Figure 3b shows a linear calibration curve. The slope value found was 0.0006 and the R^2 value was 0.9816.

Stability studies: The stability characteristics of the novel agent were depicted in Table 1, the percentage drug release of all synthesized prodrugs remained approximately constant for 120 hrs. The percentage amount of drug released at different time intervals suggested a consistency when the drug was tested in the solution of pH 1.2 and pH 7.4.

Partition coefficient: The data for pH-dependent hydrolytic stability study conducted for the Ibuprofen prodrug (Ibu-GLVL) is represented in Table 2. The stability study of the compound was conducted in two phases viz., organic layer and aqueous layer. The concentration of the drug isolated in organic layer was found to be 4.38 µg mL^{-1}, whereas in aqueous layer, it was 0.3 µg mL^{-1}. Furthermore, the partition coefficient and log P values for the Ibu-GLVL was found to be 14.6 and 1.64, respectively.

***In vivo* anti-Alzheimer's activity:** In the rotarod test for motor learning, the aluminum chloride-treated group spent much less time on the spinning rod than the normal control group. Rivastigmine (standard) treatment had a significant impact on spatial navigation task retention performance. When compared to the diseased control group, the synthesized prodrug Ibu-GLVL-treated rats spent more time on day 10 ($p<0.001$) and significantly much more time on days 20 and 30 ($p<0.001$), which was nearly identical to the standard group (Fig. 4 a, b).

In the Morris water maze test for spatial learning, aluminum chloride-treated group displayed a significant increase in escape latency when compared to the normal control group. Standard had a significant impact on spatial navigation task retention performance. Therapy with the synthetic prodrug Ibu-GLVL, however, significantly reduced the increase in escape latency produced by aluminum chloride

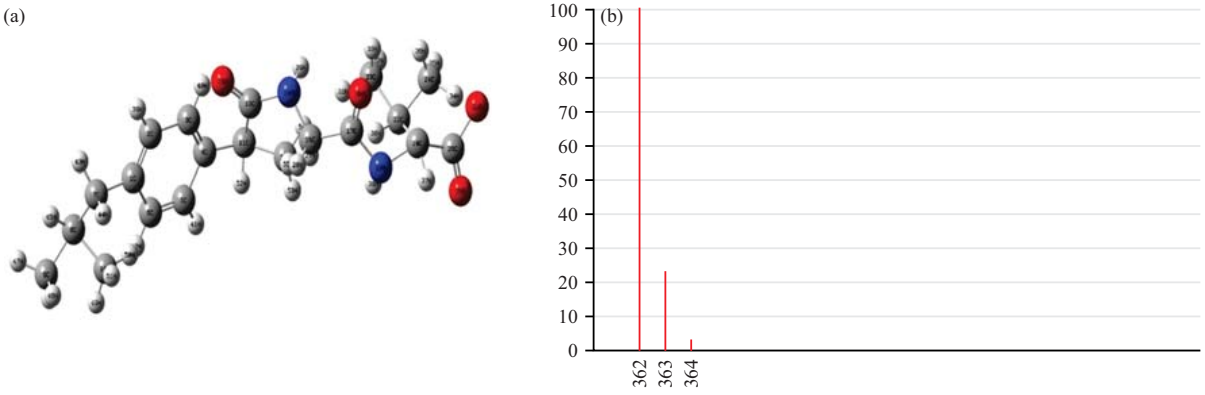

Fig. 2(a-b): Optimized structure and mass spectrum of Ibuprofen-GLVL, (a) 3-dimensional structure and (b) Mass spectrum

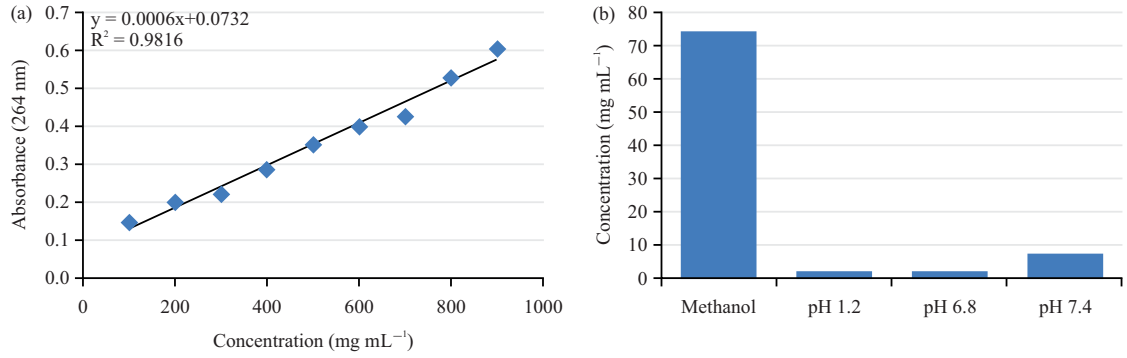

Fig. 3(a-b): Solubility characteristics of novel ibuprofen prodrug, (a) Standard solubility graph for ibuprofen and (b) Solubility of synthesized graphs in different solvents

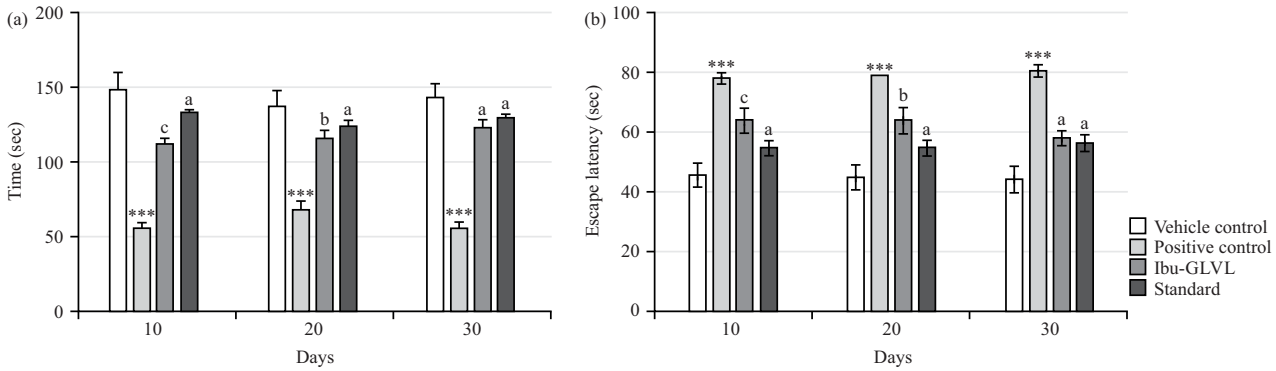

Fig. 4(a-b): Effect of Ibu-GLVL on (a) Transfer latency and (b) Escape latency in aluminum chloride-induced neurotoxicity in rats at different day intervals

Each value is expressed as Mean±SEM, where n = 6, analyzed using ANOVA followed by Dunnett's test, ***p<0.001, vehicle control group vs positive control group and [a]p<0.001, [b]p<0.01 and [c]p<0.05, respectively when all treated group vs positive control on corresponding days

Table 1: pH-dependent hydrolytic stability study of Ibu-GLVL prodrug

	Drug release (%)	
Time	pH 1.2	pH 7.4
0	1.512	0.854667
24	1.58	0.881333
48	1.576	0.914667
72	1.647	0.924667
96	1.7	0.848
120	1.71	0.951333

Table 2: pH-dependent hydrolytic stability study of Ibu-GLVL prodrug

Prodrug	Phase	Absorbance (264 nm)	Concentration (μg mL^{-1})	Partition coefficient	Log P
Ibu-GLVL	Organic layer	0.215	4.38	14.6	1.64
	Aqueous layer	0.104	0.3		

treatment on day 30 (p<0.001), which was essentially equal to the standard group (Fig. 4).

Histopathological evaluation: Hematoxylin and eosin staining were used to examine the hippocampus under the microscope. Figure 5(a-d) depicted the observed features following various treatments. The disease control group (Fig. 5b) had multifocal moderate pyknotic nuclei, gliosis and apoptosis in the cortical area and gliosis and apoptosis in the hippocampal region, compared to the normal control group

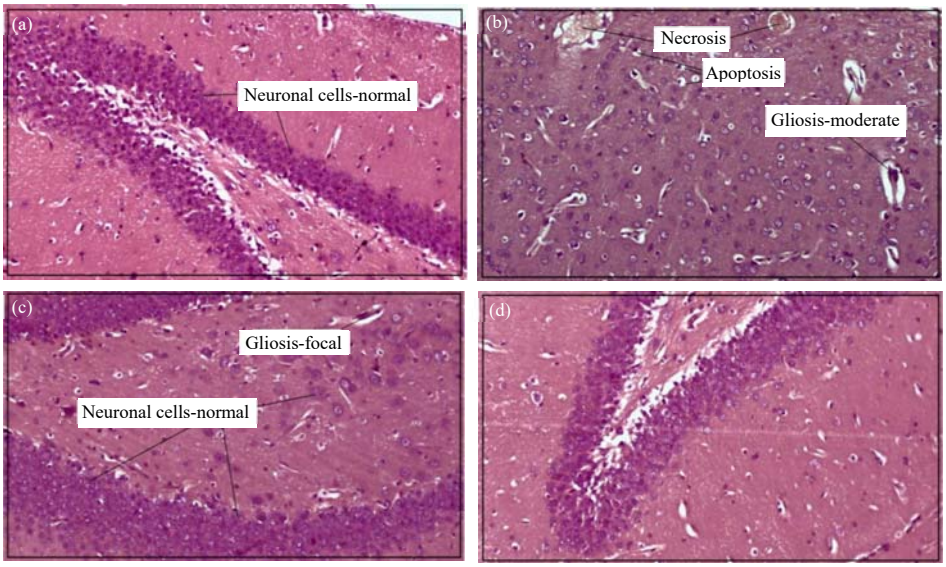

Fig. 5(a-d): Histopathological findings of hippocampus of rat brain, (a) DMSO-control, rat brain: Hippocampus region showing normal morphology (×100), (b) AlCl3, rat brain: Hippocampus showing gliosis and apoptosis was moderate 3+ (×100), (c) Ibu-GLVL, rat brain: Hippocampus region showing normal morphology as well as gliosis-focal: 1+ (×100) and (d) Standard (rivastigmine)
Rat brain: Hippocampus region showing normal morphology (×100)

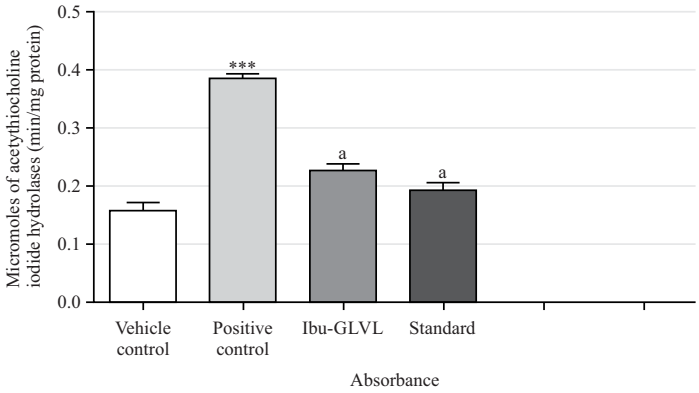

Fig. 6: Effect of Ibuprofen amino acid dipeptide conjugates on acetylcholinesterase assay in brain homogenate in $AlCl_3$-induced neurotoxicity in rats
Ibu-GLVL is Ibuprofen prodrug, each value is expressed as Mean±SEM, where n = 6, analyzed using ANOVA followed by Dunnett's test, ***p<0.001, vehicle control group vs positive control group and [a]p<0.001 vs positive control

(Fig. 5a). These alterations were observed to be minimized with the therapy of both Ibu-GLVL (Fig. 5c) and the conventional medication (rivastigmine) (Fig. 5d).

***In vitro* acetylcholinesterase activity:** Figure 6 summarized the effect of several therapies on *in vitro* acetylcholinesterase activity. When compared to the vehicle control, aluminum chloride significantly ($p<0.001$) increased acetylcholinesterase activity. The Ibu-GLVL injection was observed to reduce enzyme activity and the level of reduction was significant ($p<0.001$) when compared to the aluminum chloride group. Acetylcholinesterase levels in the standard drug treatment group were similarly significantly ($p<0.001$) lower than in the positive control group.

DISCUSSION

In the present study, a novel dipeptide prodrug of ibuprofen (Ibu-GLVL) was synthesized using the catalyst microwave technique. Characterization of the compound suggested the composition such as 'C' (66.27%), 'H' (8.34%),

'N' (7.73%) and 'O' (17.66%). The SMILE analysis for the compound was found to be C(C)CC1=CC=C(C=C1)C(C)C (=O)NCC(=O) NC(C(C)C)C(O)=O.

Mass spectrometry is used to explore and produce new molecular structures in industry and related sectors. Mass spectrometry measures molecule weight and structure destructively. Ionizing the substance without electromagnetic radiation distinguishes it from other approaches[25]. Fragmentation happens when ionized chemicals are energized. The arrangement of molecules can be deduced from the analysis of such fragments[15]. Figure 2b shows an optimized structure of Ibu-GLVL, it was computed using density functional theory with B3LYP/6-311++G(d, p).

Each fragment is identified by its mass-to-charge ratio, m/z and such ions can be distinguished and detected based on this property. The mass discrepancies between molecular ions and fragments must match a genuine chemical composition. Bond fragmentation is influenced by bond strength, the likelihood of a low-energy transition and the stability of emerging pieces[25]. The relative peak height of straight-chain ions is greater than that of branched-chain ions. It has been shown that when molecular mass increases, the relative height of the molecular ion peak falls[15,26].

The mass/charge ratio (m/z) values of Ibu-GLVL, as well as the fragments formed by electrospray ionization, were shown in Fig. 2b. Figure 2 depicted the proposed mechanisms of MS/MS collisional-induced dissociation (CID) for Ibu-GLVL (m/z 362), (m/z 363) and (m/z 364). The m/z values found in this analysis agree with earlier reports in samples containing anthocyanidins[26,27]. With an ion gate tuned to the appropriate masses and increased laser power, the final identification was achieved for every compound. The cleavage of a glycosidic bond, which has been found to be shared by all anthocyanin derivatives, is responsible for the peak. Such fragment monitoring significantly facilitates the identification of this class of drugs since it allows one to determine the core of the anthocyanin molecule[26,27].

The AD is characterized by a deficiency in cognition, memory and other kinds of memory-related defects in the population. Several studies have indicated that over the past few years, the prevalence of the disease has increased globally. The disease is reported to be caused by a combination of pathophysiological disturbances and genetic and environmental factors[8]. Long-term exposures to environmental metals have also been linked to neurodegenerative disorders. Aluminum chloride has been widely used to stimulate dementia in a variety of animal models. Aluminum is a cholinotoxin that promotes apoptotic neuronal death and neurodegeneration in Alzheimer's patients[7]. Aluminum can operate as an amyloid-protein cross-linker, resulting in oligomerization and neurotoxicity and affects the integrity and permeability of the blood-brain barrier (BBB) by modifying its lipophilic characteristics[26]. Exposure to aluminum chloride could occur through various sources such as toothpaste, foods, medicines and packaged drinking water. Even aluminum is a common commodity in several cookware used in most of the kitchen[11].

Rotarod and Morris-water tests were regularly utilized in literature to evaluate the influence of the treatment on motor function as well the memory, respectively[21,22]. The observations from this study indicated that exposure to aluminum chloride reduced significantly ($p<0.001$) the transfer latency in animals at three tested durations (10, 20 and 30 days). Further, when Ibu-GLVL was administered to the animals, a significant ($p<0.001$) improvement in the latency was observed. Similar improvements ($p<0.001$) in transfer latency were seen when the standard medication rivastigmine was evaluated in aluminum chloride-induced rats. Furthermore, the escape latency in the Morris water maze used to test spatial memory increased significantly ($p<0.001$) after treatment with aluminum chloride. The Ibu-GLVL treatment was reported to minimize ($p<0.05$) escape delay in aluminum chloride-induced rats. Rivastigmine was found to have a significant ($p<0.01$) reduction in escape delay (Fig. 4).

Earlier studies have indicated that exposure to aluminum changes the integrity of blood, blood-brain barrier resulting in the accumulation of the metal in neurons[28]. Besides causing toxic effects, the accumulated aluminum in the brain alters the antioxidant status and neurochemistry and produces damaging effects on the DNA of brain cells. These changes in the earlier studies have been reported to show similarity with clinical AD[29]. Furthermore, the excess presence of aluminum in the brain cells causes modification in neurofilaments and contributes to histopathological changes in areas such as the hippocampus[30]. Current study has indicated that exposure to aluminum chloride induced alteration in morphological setup in the hippocampus. The administration of both Ibu-GLVL as well rivastigmine was observed to prevent the histopathological changes induced by aluminum chloride (Fig. 4).

Another important finding of the study is that the observations indicated that Ibu-GLVL showed improved solubility compared to the parent drug in pH 1.2, 6.8 and 7.4, respectively (Fig. 3). Also, from the Table 1, it can be inferred that the synthesized dipeptide prodrug did not dissociate in the physiological pH of the gastric and intestine, therefore it was anticipated that the dipeptide prodrug is stable in both biological fluids[16]. The log P of 1.64 is optimal for drugs to

cross the BBB and enter brain tissue (Table 2). These values suggest that the amino acid dipeptide conjugate of ibuprofen has the requisite properties to be stable and can attain the therapeutic levels in the brain essential for the management of symptoms of AD[17].

As it is reported, degeneration of cholinergic neurons in the hippocampus of the brain causes AD by reducing acetylcholine (Ach) production and release and deposition of Amyloid beta (Aβ). Aluminum chloride induced AD in rats by suppressing oxidative stress and neuro-inflammation, resulting in a modest gradation of pyknotic nuclei, gliosis and apoptosis[31]. Treatment with Ibu-GLVL reduced these aluminum chloride-induced defects probably by suppressing oxidative stress and neuroinflammation and could have reinstated the brain histological architecture[31].

One of the known classes of drugs used in the treatment of AD is acetylcholinesterase inhibitors. It is reported in the literature that the neurodegenerative effect of AD causes a marked fall in the level of acetylcholine[23]. The administration of acetylcholinesterase inhibitors such as rivastigmine was found to cause a significant reduction in the level of enzymes which otherwise was elevated by aluminum chloride (Fig. 5). Interestingly, the administration of Ibu-GLVL also induced a significant diminishing effect on the acetylcholinesterase enzyme induced by aluminum chloride (Fig. 5), suggesting the possibility that a novel compound could have produced its action by mitigating the aluminum chloride-induced acetylcholinesterase activity in the brain.

The observations recorded from the present study indicated that novel ibuprofen prodrug synthesized by amino acid dipeptide conjugation is stable in different solvents. Besides, the compound exhibits the characteristics that aids in penetration of ibuprofen in to brain tissues where action of the drug is needed in AD. The *in vivo* studies supported the promising action of ibuprofen-prodrug in treating the symptoms of AD as well in reducing the complications in brain tissues. However, additional studies invoking other experimental parameters are needed to establish the safety and efficacy of novel ibuprofen prodrug in the management of AD.

CONCLUSION

The present study evaluated the newly synthesized ibuprofen amino acid dipeptide conjugate for the anti-Alzheimer's activity. Being a prodrug of ibuprofen with required stability characteristics to cross the blood-brain barrier as well as the effectiveness, could be useful in the management of AD. Such types of therapeutic agents might have the potential to maintain the functional integrity of neuronal cells because they attain optimal therapeutic levels at the site of action. However, more research in this direction could determine the actual influence of ibuprofen prodrug on the treatment of neurodegenerative disease that still lacks an effective therapeutic option.

SIGNIFICANCE STATEMENT

This study was done to explore the anti-Alzheimer's potential of ibuprofen prodrug using established animal experimental models. The dipeptide conjugate of the ibuprofen was synthesized and characterized using FTIR, NMR and mass spectroscopy. The synthesized compounds were tested against aluminum chloride defects using the rotarod and Morris-water test. The outcome of the study indicates that a dipeptide prodrug of Ibuprofen conjugated with amino acids (glycine and valine) is a promising compound for Alzheimer's disease and can be suggested for further studies.

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