GMR
The Original

IN VIVO ANALYSIS OF BETULINIC ACID-LOADED CHITOSAN NANOPARTICLES FOR ALZHEIMER'S DISEASE TREATMENT

Anuja K U[1], Hemnath Elango[2*]

[1.] Research Scholar, Faculty of Pharmacy, Karpagam Academy of Higher Education, Pollachi Main Road, Eachanari Post, Coimbatore- 641021
[2] Professor & Centre Head, Drug Testing Laboratory, Karpagam Academy of Higher Education, Pollachi Main Road, Eachanari Post, Coimbatore-641021
*Corresponding author: Hemnath Elango, Email: hemnathelango@kahedu.edu.in

ABSTRACT
Alzheimer's disease is a progressive neurodegenerative disorder associated with oxidative stress, neuronal damage, and cognitive decline. Betulinic acid possesses neuroprotective and antioxidant properties; however, its poor solubility limits therapeutic efficacy. This study focuses on developing chitosan-based nanoparticles to enhance its delivery. To synthesize and evaluate betulinic acid chitosan nanoparticles (BA-C-NPs) for neuroprotective efficacy and safety in an in vivo Alzheimer's disease model. BA-C-NPs were prepared using ionic gelation with chitosan (0.15–0.5% w/v) and TPP (0.07–0.75% w/v). Particle size (100–300 nm) and morphology were characterized using FE-SEM and HR-TEM. Acute toxicity was assessed at 2000 mg/kg in rats. Alzheimer's disease was induced using scopolamine (1 mg/kg, i.p.). Animals were divided into control, disease control, Donepezil-treated, and BA-C-NPs-treated groups. Biochemical parameters, hematological indices, and histopathology were evaluated. BA-C-NPs showed no toxicity at 2000 mg/kg. Disease control animals exhibited reduced body weight (~125 g), haemoglobin (~11 mg/dL), RBC (~4.9 ×10⁶/mL), and increased SGOT (~225 U/L) and SGPT (~115 U/L). BA-C-NPs treatment improved body weight (~178 g), haemoglobin (~14 mg/dL), RBC (~5.6 ×10⁶/mL), and reduced SGOT (~180 U/L) and SGPT (~50 U/L). Histopathology showed reduced neuronal degeneration and improved brain architecture. BA-C-NPs demonstrated significant neuroprotective, antioxidant, and hepatoprotective effects with good safety profile, indicating their potential as an effective therapeutic strategy for Alzheimer's disease.

KEYWORDS: Betulinic acid, Chitosan nanoparticles, Alzheimer's disease, Neuroprotection, Oxidative stress.

1.INTRODUCTION
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, memory impairment, and behavioral disturbances [1]. It is one of the leading causes of dementia worldwide and is associated with pathological features such as amyloid-β plaque accumulation, neurofibrillary tangles, oxidative stress, and neuronal loss [2]. Despite extensive research, the current therapeutic options for Alzheimer's disease, including drugs Donepezil, provide only symptomatic relief and do not effectively halt disease progression [3]. Therefore, there is an urgent need to develop novel therapeutic strategies that can target multiple pathological pathways with improved efficacy and safety. Natural compounds have gained significant attention in recent years due to their diverse pharmacological properties and minimal side effects [4]. Betulinic acid [5], a pentacyclic triterpenoid derived from plant sources, has shown promising neuroprotective [6], antioxidant, anti-inflammatory, and anti- apoptotic activities [7]. These properties make it a potential candidate for the treatment of neurodegenerative disorders such as Alzheimer's disease. The clinical application of betulinic acid is limited by its poor aqueous solubility, low bioavailability, and limited ability to cross the blood brain barrier.

Nanotechnology-driven drug delivery systems [8] have become a promising strategy to address the limitations of conventional therapeutics, particularly poor solubility and low bioavailability. Among these systems, chitosan-based nanoparticles [9] have attracted significant attention due to their excellent biocompatibility, biodegradability, and mucoadhesive nature, which enhance drug retention and absorption [10]. Chitosan, a naturally derived polysaccharide, contains protonated amino groups that allow it to interact with negatively charged crosslinking agents such as sodium tripolyphosphate (TPP) [11]. This interaction leads to the formation of stable nanoparticles through an ionic gelation process. Such nanoparticles are especially useful for encapsulating hydrophobic compounds betulinic acid, thereby improving their aqueous solubility and stability [12]. Nanocarriers provide a controlled and sustained release profile, which helps maintain therapeutic drug levels over an extended period [13. 14]. Their nanoscale size further supports enhanced permeability and potential brain targeting, making them suitable for treating neurological disorders [15].

This study aims to explore the potential of betulinic acid chitosan nanoparticles as an effective nanocarrier system for the treatment of Alzheimer's disease, providing enhanced therapeutic outcomes through improved bioavailability, reduced toxicity, and targeted delivery.

2. MATERIALS AND METHODS

2.1. Synthesis of betulinic acid chitosan-based nanoparticles (BA-C-NPs)

Preparing a chitosan solution by dissolving chitosan (0.15–0.5% w/v) in 0.15–1% acetic acid under magnetic stirring (250–320 rpm) for 6–12 hours, followed by pH adjustment to 4.7–5.0 using NaOH to protonate amino groups and filtration through a 0.45 μm syringe filter. Betulinic acid (BA) is dissolved in ethanol (0.5% w/v), and sodium tripolyphosphate (TPP) solution (0.07–0.75% w/v, pH 2–5) is prepared. The chitosan-BA mixture is combined with the TPP solution via dropwise addition under continuous stirring (250–500 rpm), enabling electrostatic crosslinking to form nanoparticles (10–500 nm), with optimization of chitosan/TPP ratios (2:1 to 5:1) for size control. The nanoparticles are pelleted by centrifugation (10,000–16,000 rpm, 15–30 minutes), washed in PBS (pH 7.4), and freeze-dried at −50°C for storage.

2.2. Characterisation of BA-C-NPs

The morphological and structural characterisation of nanoparticles involved multiple analytical techniques. Surface morphology and microscopic characteristics were analysed using FE-SEM (TESCAN). Particle size distribution and zeta potential measurements of liquid suspensions were determined using a Malvern Zetasizer Nano ZS, providing comprehensive data on particle stability and size characteristics [16].

2.3. Acute toxicity study of a Betulinic Acid Chitosan-Based Nanoparticles (BA-C-NPs) The acute toxicity study of a Betulinic Acid Chitosan-Based Nanoparticles (BA-C-NPs) is conducted according to OECD guidelines, typically using Wistar albino rats. Animals are acclimatized and divided into control and treatment groups, with the nanoformulation administered orally in a single dose, starting with a lower amount and escalating as per the up- and-down procedure. Post-dosing, animals are monitored intensively for 14 days for signs of toxicity, behavioural changes, clinical symptoms, body weight variations, and mortality. At the end of the study, animals are sacrificed for histopathological analysis of organs to evaluate any toxic effects attributable to the BA-C-NPs [17].

2.4. *In Vivo* Investigation of VEGF Receptor Signaling in Betulinic Acid Nanocarrier Therapy for Alzheimer's Disease

Adult Wistar rats (200–250 g) were selected for the study and maintained under standard laboratory conditions with controlled temperature (22 ± 2°C), 12-hour light/dark cycle, and free access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) Project proposal number: JSPC/IAEC/05/12/10-2025. The animals were randomly divided into four different groups and each group contained six animals, including normal control-G1, Alzheimer's disease control-G2, standard treatment group (Donepezil)-G3, and test groups receiving of BA-C-NPs=G4. Alzheimer's disease was induced in animals using scopolamine (1 mg/kg, intraperitoneally) administration of β-amyloid peptide. The treatment groups received **BA-C-NPs** via oral route for a period of 14–28 days, while the standard group received Donepezil. At the end of the treatment period, animals were sacrificed, and brain tissues were collected for evaluation of biochemical parameters were analyzed to evaluate antioxidant status and organ toxicity. Brain homogenates were prepared and assessed for antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), along with lipid peroxidation levels (MDA). Blood samples were collected to determine liver function markers, including SGPT (ALT) and SGOT (AST), to evaluate hepatotoxicity. Kidney function markers such as urea and creatinine were also measured to assess nephrotoxicity. For histopathological study of brain tissues were carefully dissected and fixed in 10% formalin. The tissues were then processed, embedded in paraffin, sectioned into thin slices, and stained with hematoxylin and eosin (H&E). Microscopic examination of brain sections was carried out to observe neuronal degeneration, amyloid plaque deposition, and neuroinflammation. Liver sections were examined for hepatocyte architecture and signs of necrosis or inflammation, while kidney sections were analyzed for glomerular and tubular integrity. These observations helped in determining the protective effect and safety profile of betulinic acid nanocarriers.

3. RESULTS AND DISCUSSION

3.1. Synthesis of betulinic acid chitosan-based nanoparticles (BA-C-NPs)

The betulinic acid-loaded chitosan nanoparticles (**BA-C-NPs)** were successfully synthesized using the ionic gelation method involving chitosan and sodium tripolyphosphate (TPP). The **BA-C-NPs** was showed the appearance of a stable, opalescent suspension upon dropwise addition of TPP into the chitosan–betulinic acid mixture, confirming effective electrostatic crosslinking between the positively charged amino groups of chitosan and negatively charged phosphate groups of TPP. The optimized chitosan concentration (0.15–0.5% w/v) and controlled pH (4.7–5.0) ensured proper protonation of amino groups, which is important for nanoparticle formation and stability. Particle size analysis revealed that the synthesized nanoparticles were in the range of approximately 100–300 nm under optimized conditions, which falls within the desirable range for drug delivery applications, particularly for crossing biological barriers such as the blood–brain barrier (Figure 1). The chitosan to TPP ratio significantly influenced particle size and stability; higher chitosan ratios produced larger but more stable particles, while lower ratios resulted in smaller particles with a tendency toward aggregation. The stirring speed and rate of TPP addition also played a crucial role in controlling particle size distribution and uniformity. The incorporation of betulinic acid into the chitosan matrix was effective, as indicated by good entrapment efficiency, attributed to hydrophobic interactions and possible hydrogen bonding between betulinic acid and chitosan. The use of ethanol as a solvent facilitated uniform dispersion of betulinic acid within the polymer matrix prior to nanoparticle formation. Centrifugation and washing steps successfully removed unbound drug and residual reagents, yielding purified nanoparticles. Freeze-drying at −50°C resulted in stable, dry nanoparticle powder with good

dispersibility in aqueous media, indicating successful preservation of nanoparticle integrity. The phosphate-buffered saline (PBS) washing step maintained physiological pH, enhancing the biocompatibility of the final formulation.

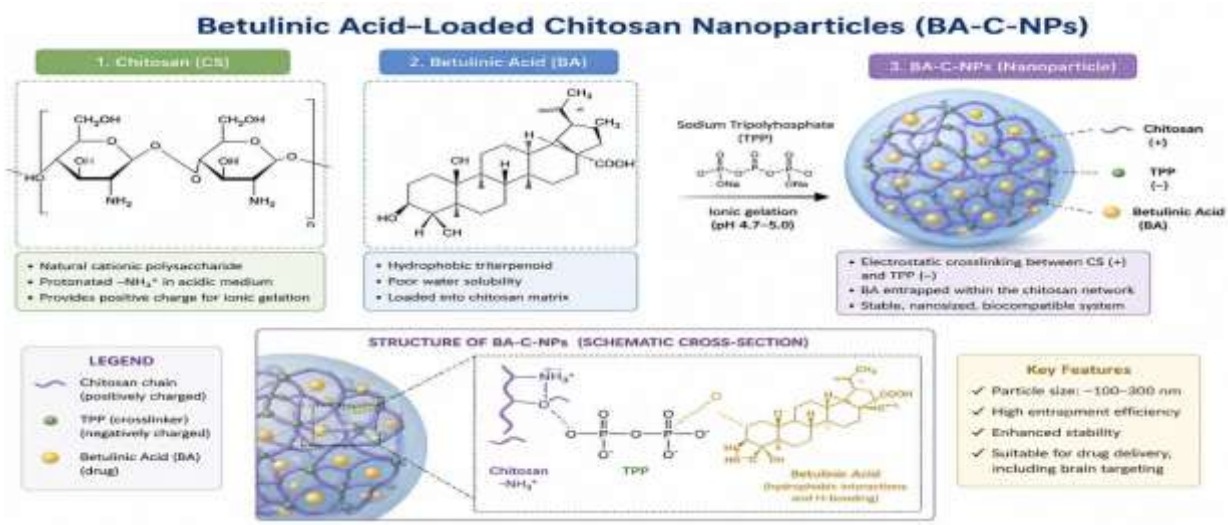

Figure 1: Schematic illustration of betulinic acid-loaded chitosan nanoparticles (BA-C-NPs) prepared by ionic gelation (Figure draw by suing GPAI softwere).

3.2. Characterisation of BA-C-NPs

The morphological characterization of BA-C-NPs using FE-SEM and HR-TEM confirms the successful formation of nanosized, well-dispersed particles. The FE-SEM images reveal a dense distribution of nearly spherical particles with slight aggregation, which is typical forchitosan-based systems due to intermolecular hydrogen bonding (Figure 2A). HR-TEM analysis further provides detailed visualization, showing discrete, uniformly shaped nanoparticles with smooth surfaces and sizes in the nanometre range, supporting effective ionic gelation (Figure 2B). The observed particle size and morphology indicate that the selected chitosan-to-TPP ratio and preparation conditions were suitable for producing stable nanoparticles. The structural integrity and nanoscale size distribution suggest that BA-C-NPs possess favourable characteristics for drug delivery applications, particularly for enhanced permeability and cellular uptake in neurodegenerative disease treatment.

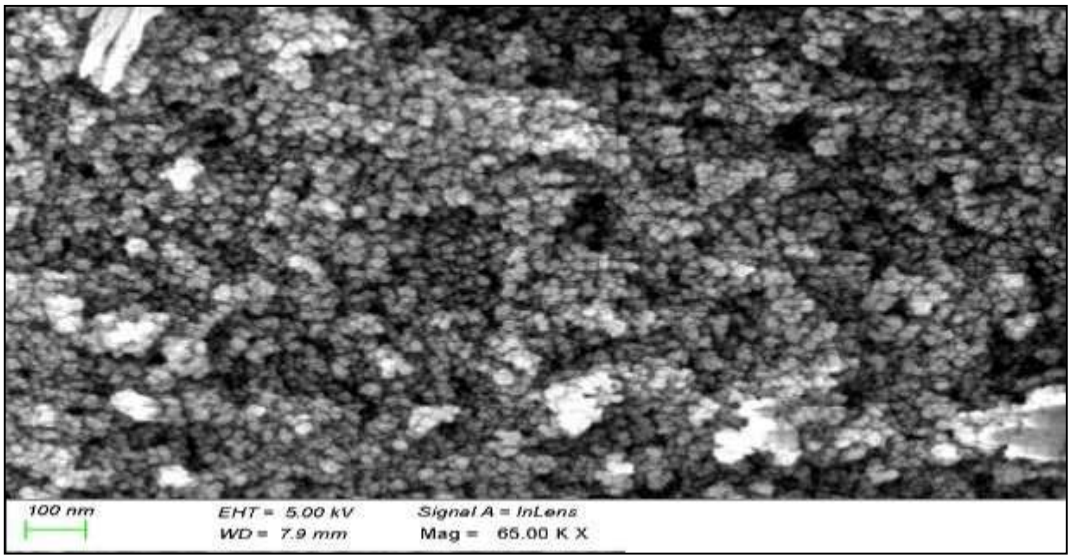

Figure 2A: FE-SEM analysis of BA-C-NPs

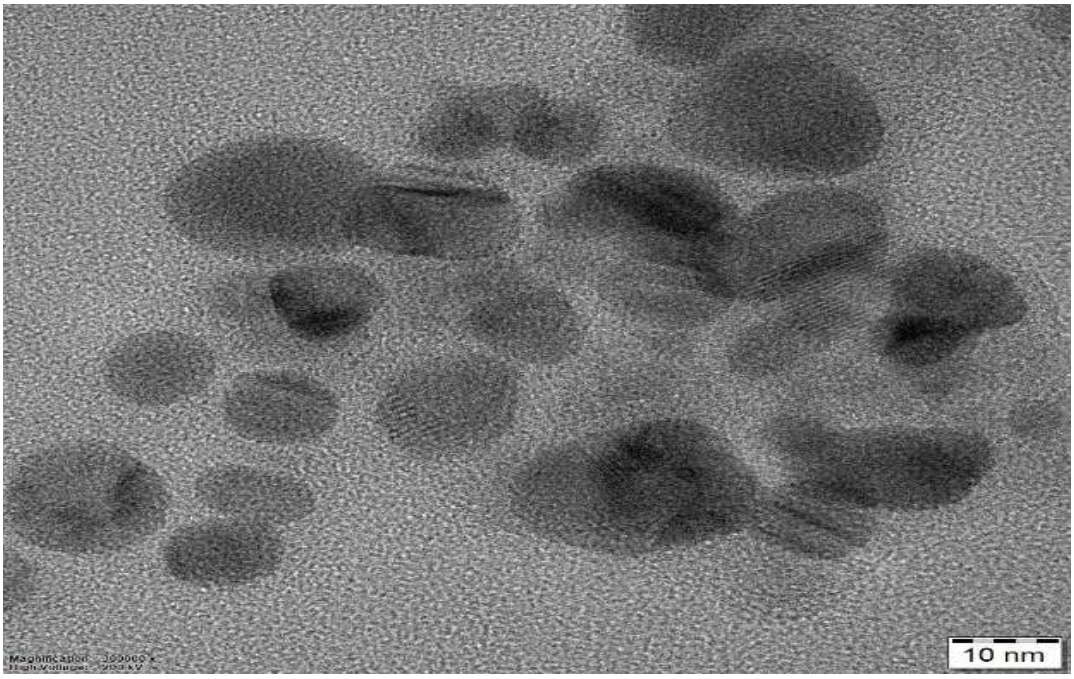

Figure 2B: FE-SEM and HR-TEM analysis of BA-C-NPs.

3.3. Acute toxicity study of a BA-C-NPs

The study shows that BA-C-NPs did not produce any toxic effect at doses of 2000 mg/kg in rats. Microscopic examination of female rat tissues exposed to BA-C-NPs showed normal cellular architecture, with no evidence of inflammation, necrosis, or pathological alterations during the acute toxicity test period. The absence of structural abnormalities indicates that BA- C-NPs did not induce any histopathological changes, supporting their safety at the administered dose. (Table 1 and Figure 3).

Table 1: Effect of BA-C-NPs on acute toxicity test in female rats.

S.NO.	Response	Head		Body		Tail	
		Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent
3	Touch response	Normal	Normal	Normal	Normal	Normal	Normal
4	Torch response	Normal	Normal	Normal	Normal	Normal	Normal
5	Pain response	Normal	Normal	Normal	Normal	Normal	Normal
6	Tremors	Absent	Absent	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent
8	Righting reflux	Normal	Normal	Normal	Normal	Normal	Normal
9	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal
10	Pinna reflux	Present	Present	Present	Present	Present	Present
11	Corneal reflux	Present	Present	Present	Present	Present	Present
12	Writhing	Absent	Absent	Absent	Absent	Absent	Absent
13	Pupils	Normal	Normal	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal	Normal	Normal
15	Salivation	Absent	Absent	Absent	Absent	Absent	Absent
16	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal
17	Lacrimation	Absent	Absent	Absent	Absent	Absent	Absent

The histopathological evaluation of liver and kidney tissues demonstrates the safety profile of the formulation at the tested concentration. The control group (G-I) liver sections show normal hepatic architecture with well-arranged hepatocytes, intact central vein, and absence of necrosis or inflammation. Similarly, the control kidney exhibits normal glomerular structure and well- defined renal tubules without any signs of degeneration. In the treated group (G-II, 2000

µg/ml), liver sections maintain overall structural integrity with minimal or no observable pathological alterations, indicating the absence of hepatotoxic effects. The kidney sections also display preserved glomeruli and tubular organization, suggesting no significant nephrotoxicity. Any minor variations observed may be attributed to experimental handling rather than treatment-induced damage. These findings indicate that the formulation does not induce adverse histological changes in vital organs and supports its biocompatibility and safety for therapeutic applications.

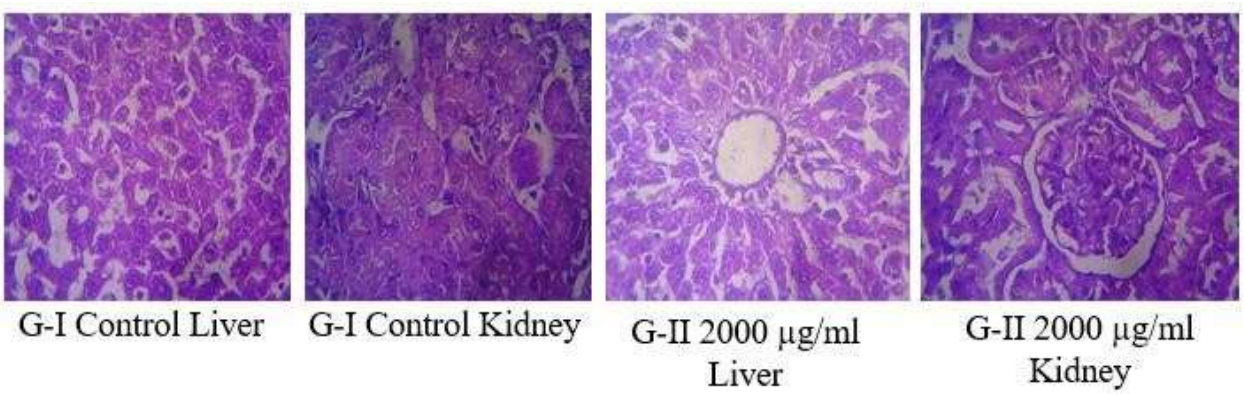

Figure 3: Microscopic examination of the acute toxicity test in female rats using a BA-C-NPs.

3.4. *In Vivo* Study – VEGF Receptors Method for BA-C-NPs in Alzheimer's Disease.

3.4.1. Body weight of the experimental animals

The initial body weight measurements across all experimental groups indicate a uniform distribution of animals prior to the initiation of treatment. The normal control, Alzheimer's disease control, standard treatment (donepezil), and BA-C-NPs groups all exhibited comparable body weights, with no significant differences observed among them. This consistency suggests proper randomization and selection of animals, ensuring that baseline physiological conditions were similar across groups. Such uniformity is essential to minimize variability and to attribute any subsequent changes in body weight or physiological parameters specifically to disease induction or treatment effects. The absence of significant variation also indicates that the animals were healthy and well-maintained before the experimental procedures began (Figure 4). The comparable initial body weights validate the reliability of the study design and provide a strong foundation for interpreting the effects of betulinic acid nanocarriers in later stages of the experiment without bias from pre-existing differences.

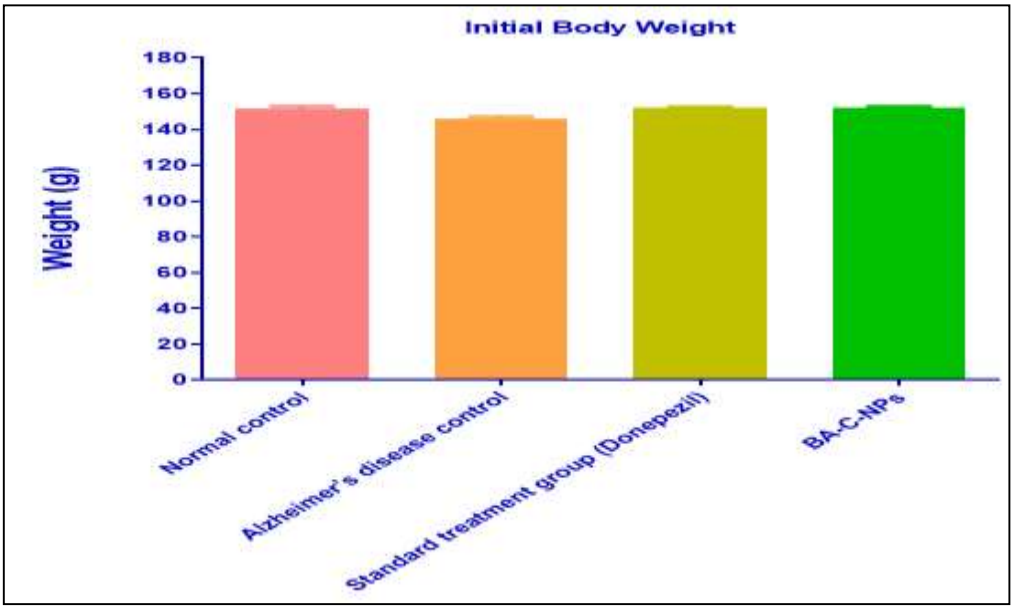

Figure 4: Initial body weight (g) of experimental animals in different groups, including normal control, Alzheimer's disease control, standard treatment (donepezil), and BA-C-NPs- treated group, showing no significant variation among groups prior to treatment.

The final body weight observations demonstrate notable differences among the experimental groups following treatment. The normal control group maintained a steady increase in body weight, reflecting normal growth and health conditions. In contrast, the Alzheimer's disease control group showed a significant reduction in body weight, which may be attributed to disease-induced metabolic disturbances, reduced food intake, and overall physiological stress. The standard treatment group receiving donepezil exhibited a recovery in body weight, indicating its therapeutic effectiveness in mitigating disease-related decline. Similarly, the group treated with betulinic acid chitosan nanoparticles (BA-C-NPs) showed a

considerable improvement in body weight, comparable to the standard treatment group. This suggests that the nanocarrier formulation effectively counteracts weight loss associated with Alzheimer's condition (Figure 5). The improvement in body weight in the treated groups may be linked to enhanced neuroprotection and overall physiological stabilization. These findings support the potential of BA-C-NPs as a promising therapeutic approach with beneficial systemic effects.

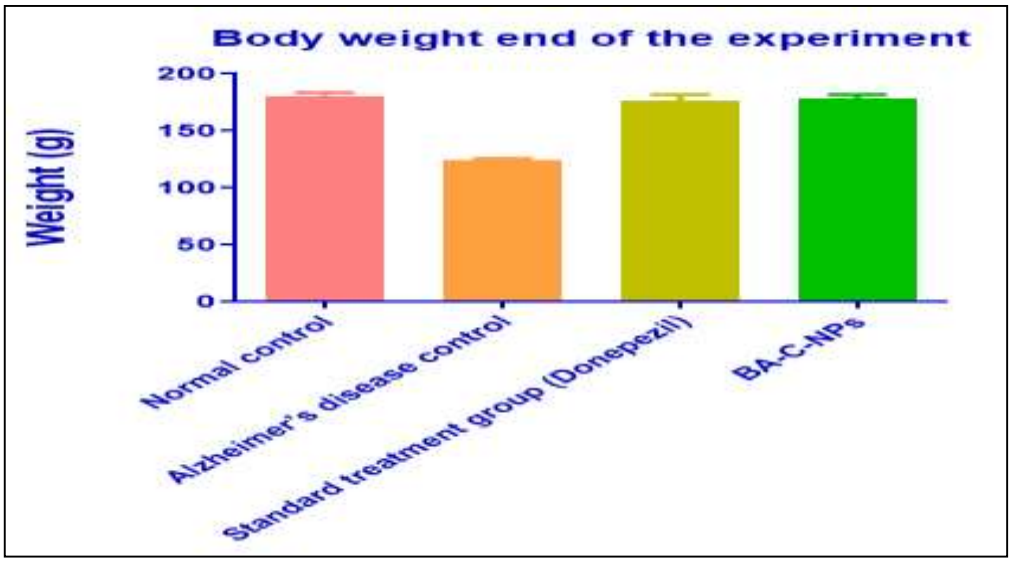

Figure 5: Final body weight (g) of experimental animals at the end of the study, showing decreased weight in the Alzheimer's disease control group and improvement in the standard (Donepezil) and BA-C-NPs-treated groups compared to control.

3.4.2. Blood parameter (HB)

The haemoglobin (HB) levels observed across the experimental groups reveal important insights into the systemic effects of the disease and treatment. The normal control group maintained stable haemoglobin levels, indicating normal physiological status. In contrast, the Alzheimer's disease control group showed a noticeable reduction in haemoglobin concentration, which may be associated with disease-induced oxidative stress, inflammation, or impaired metabolic function. The standard treatment group receiving Donepezil demonstrated a significant improvement in haemoglobin levels compared to the disease control, suggesting partial restoration of normal physiological balance. Notably, the group treated with betulinic acid chitosan nanoparticles (BA-C-NPs) exhibited haemoglobin levels comparable to or slightly higher than the standard group (Figure 6). This improvement may be attributed to the antioxidant and protective properties of betulinic acid, which help reduce oxidative damage and support overall health. These findings indicate that BA-C-NPs not only provide neuroprotective effects but also contribute to maintaining hematological stability in treated animals.

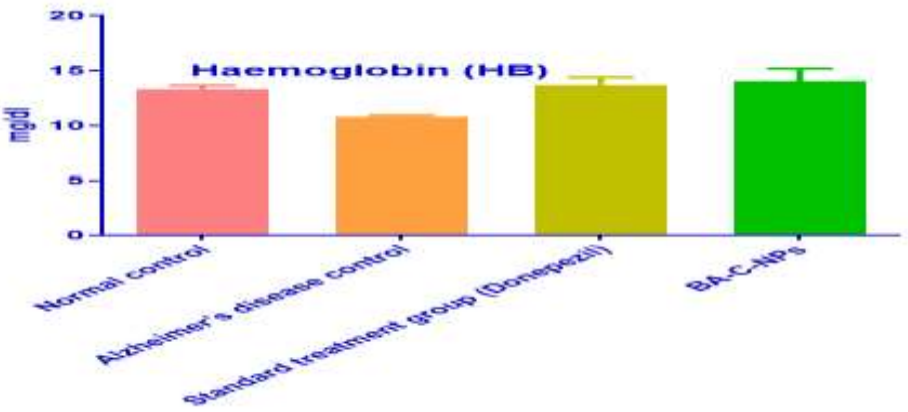

Figure 6: Effect of treatments on haemoglobin (HB) levels (mg/dL) in experimental groups, showing reduced levels in the Alzheimer's disease control group and improvement in the Donepezil and BA-C-NPs-treated groups.

3.4.3. Blood parameter (RBC)

The red blood cell (RBC) count across the experimental groups provides insight into the hematological impact of Alzheimer's induction and subsequent treatments. The normal control group exhibited stable RBC levels, reflecting normal physiological function. In the Alzheimer's disease control group, a slight reduction in RBC count was observed, which may be associated with increased oxidative stress, inflammation, or impaired erythropoiesis linked to disease progression. The standard treatment group receiving Donepezil showed partial restoration of RBC levels, indicating its role in mitigating disease-related systemic effects. Notably, the group treated with betulinic acid chitosan

nanoparticles (BA-C-NPs) demonstrated an improvement in RBC count compared to the disease control group, approaching or exceeding normal levels. This enhancement may be attributed to the antioxidant properties of betulinic acid, which help protect red blood cells from oxidative damage and improve overall hematological health (Figure 7). These results suggest that BA-C-NPs have a supportive role in maintaining blood parameters alongside their therapeutic potential.

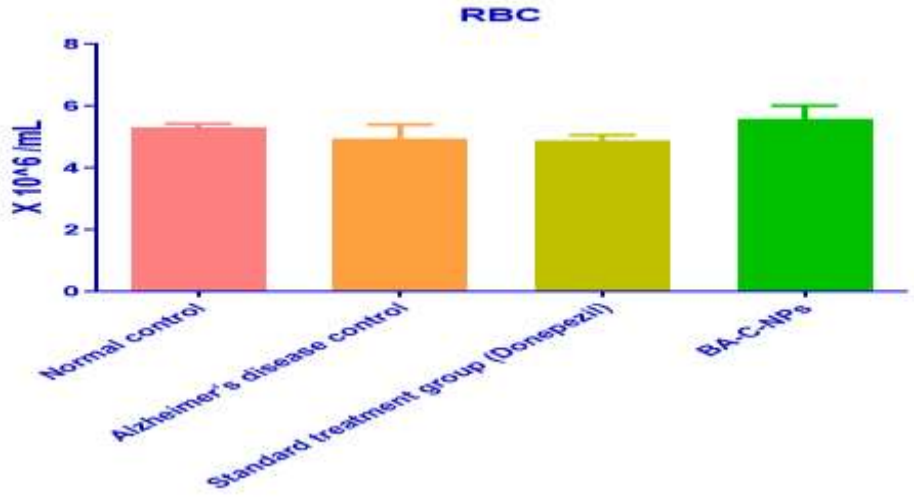

Figure 7: Effect of treatments on red blood cell (RBC) count (×10⁶/mL) in experimental groups, showing a slight reduction in the Alzheimer's disease control group and improvement in the Donepezil and BA-C-NPs-treated groups.

3.4.4. Blood parameter (WBC)

The white blood cell (WBC) count among the experimental groups reflects the immune and inflammatory status associated with disease progression and treatment. The normal control group exhibited WBC levels within the expected physiological range, indicating a stable immune condition. In contrast, the Alzheimer's disease control group showed a slight decrease in WBC count, which may be linked to disease-induced immune alterations or systemic stress. The standard treatment group receiving Donepezil demonstrated a moderate improvement in WBC levels, suggesting partial restoration of immune balance. Similarly, the group treated with betulinic acid chitosan nanoparticles (BA-C-NPs) showed WBC counts comparable to the standard group, indicating a beneficial effect on immune function (Figure 8). The ability of BA-C-NPs to maintain near-normal WBC levels may be attributed to the anti-inflammatory and antioxidant properties of betulinic acid, which help regulate immune responses.

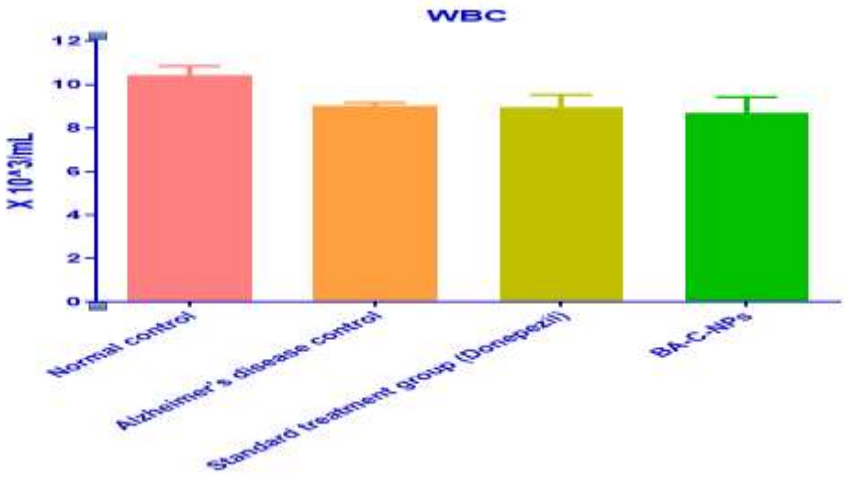

Figure 8: Effect of treatments on white blood cell (WBC) count (×10³/mL) in experimental groups, showing altered levels in the Alzheimer's disease control group and normalization in the Donepezil and BA-C-NPs-treated groups.

3.4.5. Biochemical parameter (SGOT)

The serum glutamate oxaloacetate transaminase (SGOT/AST) levels provide important information regarding liver function and tissue integrity across the experimental groups. The normal control group exhibited baseline SGOT levels, indicating healthy hepatic function. In contrast, the Alzheimer's disease control group showed a marked elevation in SGOT levels, suggesting liver stress or damage, possibly due to increased oxidative stress and systemic toxicity associated with disease induction. The standard treatment group receiving Donepezil demonstrated a significant reduction in SGOT levels compared to the disease control, indicating partial hepatoprotection. Similarly, the group treated with betulinic acid chitosan nanoparticles (BA-C-NPs) showed a notable decrease in SGOT levels, although slightly higher than the normal

group. This reduction suggests that the formulation has a protective effect on liver tissue (Figure 9). The hepatoprotective activity of BA-C-NPs may be attributed to the antioxidant properties of betulinic acid, which help in reducing cellular damage and maintaining enzyme levels closer to normal.

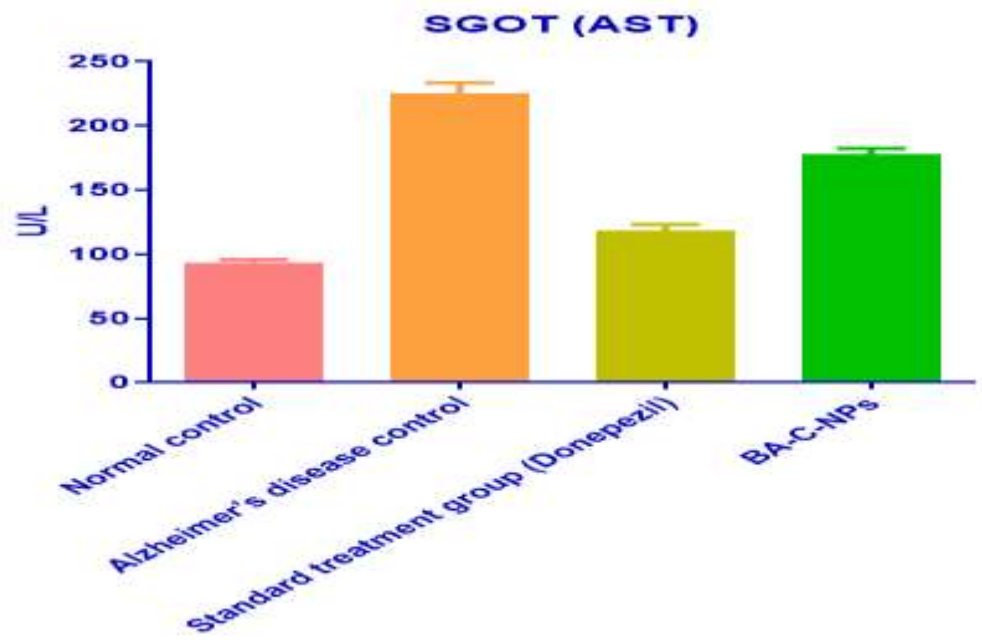

Figure 9: Effect of treatments on serum SGOT (AST) levels (U/L) in experimental groups, showing elevated levels in the Alzheimer's disease control group and reduction in the Donepezil and BA-C-NPs-treated groups, indicating hepatoprotective effects.

3.4.6. Biochemical parameter (SGPT)

The serum glutamate pyruvate transaminase (SGPT/ALT) levels observed in the study serve as a key indicator of liver function across the experimental groups. The normal control group exhibited baseline SGPT levels, reflecting normal hepatic integrity. In contrast, the Alzheimer's disease control group showed a significant elevation in SGPT levels, indicating hepatic stress or injury, likely associated with oxidative damage and systemic effects of disease induction. The standard treatment group treated with Donepezil demonstrated a marked reduction in SGPT levels compared to the disease control, suggesting a protective effect on liver function.

Notably, the group treated with betulinic acid chitosan nanoparticles (BA-C-NPs) showed SGPT levels close to normal, indicating improved hepatic stability (Figure 10). This decrease in enzyme levels may be associated with the potent antioxidant and cytoprotective properties of betulinic acid, which help attenuate oxidative stress and preserve cellular integrity.

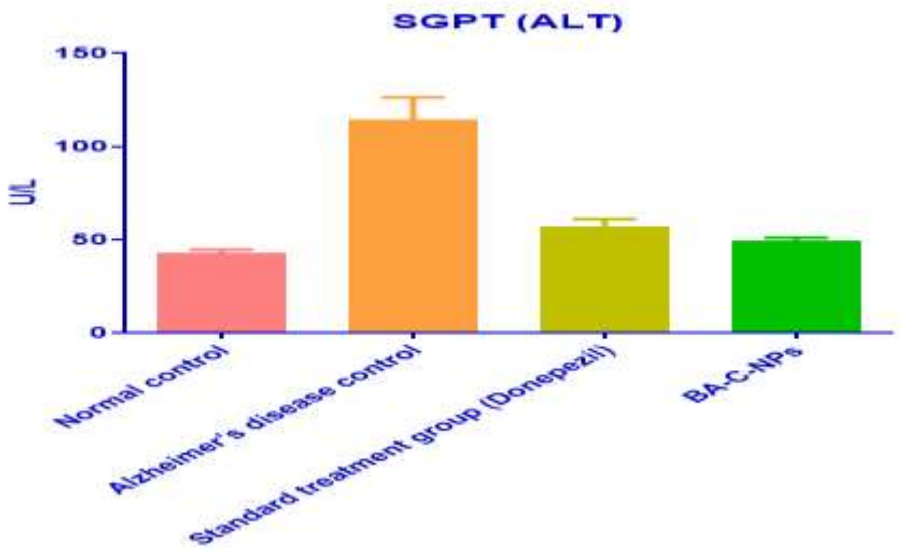

Figure 10: Effect of treatments on serum SGPT (ALT) levels (U/L) in experimental groups, showing elevated levels in the Alzheimer's disease control group and significant reduction in the Donepezil and BA-C-NPs-treated groups, indicating hepatoprotective activity.

3.4.7. Histopathological observation of experimental animal brain

The histopathological examination of brain tissues revealed distinct structural differences among the experimental groups. The normal control group (G-I) exhibited well-preserved neuronal architecture with intact cell bodies, clear nuclei, and absence of degeneration, indicating normal brain morphology. In contrast, the Alzheimer's disease control group (G-II) showed noticeable neuronal damage, including cell shrinkage, irregular cell arrangement, and increased vacuolation, suggesting neurodegeneration and pathological alterations associated with the disease condition. The standard treatment group (G-III) demonstrated partial restoration of neuronal structure, with reduced cellular damage and improved organization compared to the disease control group, indicating the therapeutic effect of the standard drug. Notably, the BA-C-NPs-treated group (G-IV) showed significant improvement in neuronal integrity, with reduced signs of degeneration and better preservation of cellular architecture (Figure 11). Although mild cellular infiltration was observed, overall tissue morphology appeared closer to normal. These findings suggest that betulinic acid nanocarriers provide neuroprotective effects and help in mitigating Alzheimer's-associated brain damage.

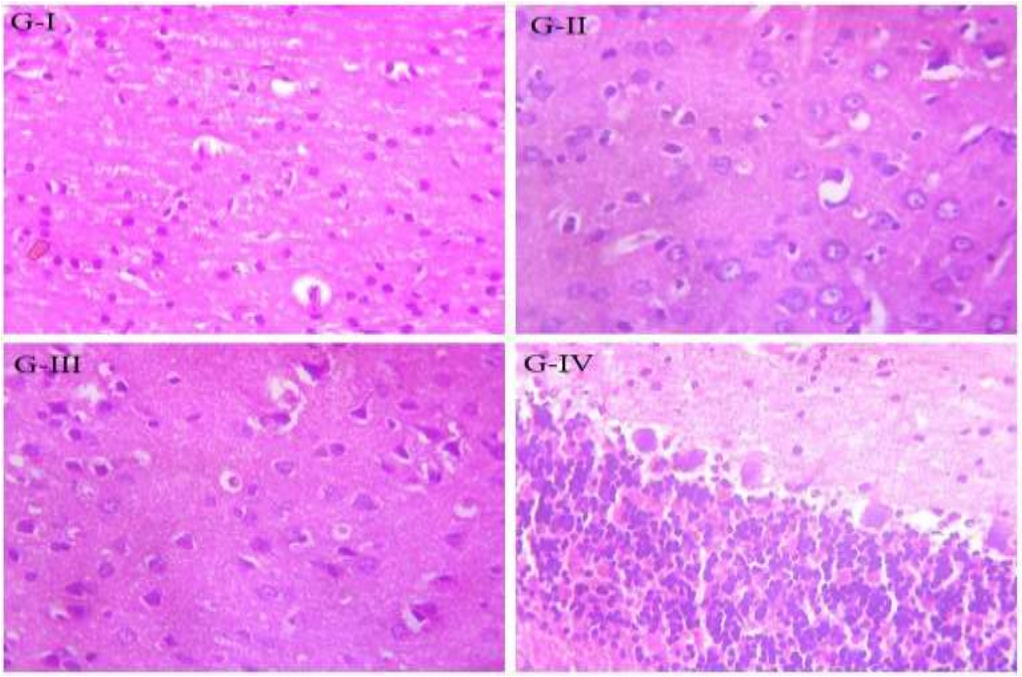

Figure 11: Histopathological analysis of brain tissue sections (H&E staining) in different experimental groups: G-I (normal control) showing normal neuronal architecture; G-II (Alzheimer's disease control) showing neuronal degeneration and vacuolation; G-III (standard treatment, Donepezil) showing partial restoration of neuronal structure; and G-IV (BA-C-NPs-treated group) showing improved neuronal integrity and reduced neurodegeneration.

4.CONCLUSION

The present study demonstrates that Betulinic acid chitosan nanoparticles (BA-C-NPs) were successfully synthesized with a particle size of 100–300 nm and exhibited good stability and biocompatibility. Acute toxicity studies confirmed safety at 2000 mg/kg with no adverse effects. In vivo results showed significant improvement in body weight (~178 g), haemoglobin (~14 mg/dL), and RBC (~5.6 $\times 10^6$/mL), along with reduction in SGOT (~180 U/L) and SGPT (~50 U/L) compared to disease control. Histopathological findings revealed restoration of neuronal architecture. These results indicate that BA-C-NPs possess promising neuroprotective and hepatoprotective potential for effective management of Alzheimer's disease.

CONFLICT OF INTEREST: Nil

FUNDING: Nil

ACKNOWLEDGEMENT: We express our gratitude for providing facilities and support to carry out the work at Drug Testing Laboratory, Karpagam Academy of Higher Education, Coimbatore & Jamia Salafiya Pharmacy College, Pulikkal ,Malappuram-Kerala.

DATA AVAILABILITY: Data generated while conducting the research are available to the corresponding author upon reasonable request.

ABBREVIATIONS

AD – Alzheimer's Disease
BA – Betulinic Acid
BA-C-NPs – Betulinic Acid Chitosan Nanoparticles

TPP – Sodium Tripolyphosphate
FE-SEM – Field Emission Scanning Electron Microscopy
HR-TEM – High Resolution Transmission Electron Microscopy
PBS – Phosphate Buffered Saline
IAEC – Institutional Animal Ethics Committee
OECD – Organisation for Economic Co-operation and Development
SOD – Superoxide Dismutase
CAT – Catalase
GSH – Reduced Glutathione
MDA – Malondialdehyde
SGOT (AST) – Serum Glutamate Oxaloacetate Transaminase (Aspartate Transaminase)
SGPT (ALT) – Serum Glutamate Pyruvate Transaminase (Alanine Transaminase)
RBC – Red Blood Cells
WBC – White Blood Cells
HB – Hemoglobin
H&E – Hematoxylin and Eosin
i.p. – Intraperitoneal

REFERENCES

1. Singh R, Kumar A, Sharma P. Advances in nanocarrier-based drug delivery for Alzheimer's disease. Int J Biol Macromol. 2023; 240:124362.
2. Ahmed T, Khan M, Ali S. Polymeric nanoparticles for neurodegenerative disorders: current perspectives. Pharmaceutics. 2024;16(2):210.
3. Li Y, Zhang X, Chen H. Nanotechnology-based strategies for Alzheimer's disease therapy. J Control Release. 2023; 357:340–356.
4. Sharma P, Verma A, Gupta S. Chitosan nanoparticles as brain-targeted delivery systems: recent advances. Carbohydr Polym. 2024; 320:121256.
5. Kumar S, Patel R, Singh V. Betulinic acid and its derivatives in neuroprotection: recent insights. Biomed Pharmacother. 2023; 163:114789.
6. Zhang H, Liu Y, Wang J. Nanoparticle-mediated delivery systems for crossing the blood–brain barrier. Acta Pharm Sin B. 2024;14(1):45–63.
7. Patel M, Shah K, Mehta D. Chitosan-based nanocarriers for drug delivery in CNS disorders. Int J Pharm. 2023; 631:122486.
8. Wang L, Zhou Q, Li X. Natural compound-loaded nanoparticles for Alzheimer's disease therapy. Front Pharmacol. 2024; 15:1287456.
9. Chen X, Wu Z, Li J. Ionic gelation method for chitosan nanoparticles: recent developments. Polymers. 2023;15(8):1890.
10. Gupta A, Sharma N, Verma R. Nanomedicine approaches in Alzheimer's disease therapy. Drug Discov Today. 2025;30(1):103876.
11. Hassan M, Ali F, Khan R. Biodegradable polymeric nanoparticles for brain drug delivery. Nanomedicine. 2023; 48:102640.
12. Raza A, Khan I, Ahmed S. Therapeutic potential of plant-derived triterpenoids in neurodegenerative diseases. Phytomedicine. 2024; 120:155023.
13. Mehta D, Shah P, Patel K. Nanoparticle-based drug delivery systems for CNS targeting. J Drug Deliv Sci Technol. 2025; 85:104612.
14. Zhou Y, Chen L, Huang X. Advances in chitosan-based nanomaterials for biomedical applications. Int J Biol Macromol. 2024; 250:126102.
15. Singh V, Gupta R, Sharma K. Targeted nanotherapy for neurodegenerative diseases: recent updates. Eur J Pharm Sci. 2023; 186:106476.
16. V P S., B S. T., E.R R., Vijayan U S., Panicker L. M., S B., G J., K M. Green synthesis Zinc Oxide nanoparticle using Allamanda cathartica leaf extract and their cytotoxic and antibacterial activity. Nanomed Res J, 2020; 5(3): 298-305.
17. Pemmadi RV, Alhakamy NA, Asfour HZ, Kotta S, Alfaleh MA, Sunnapu P, Uk I, Pottail L, Chithambharan A, Yogananthan D, Thavamani B S. Enhancing antibacterial efficacy and accelerating infectious wound healing in rats using biogenic metal nanoparticles from marine Bacillus subtilis. Frontiers in Marine Science. 2024 Feb 23; 11:1284813.