RESEARCH PAPER

Development And Evaluation of Brivaracetam Loaded Nanoparticular Bionanosuspension for Nose to Brain Delivery in Management of Epilepsy

Anurag Jaiswal[1], Dr. Sushant Kumar[2*], Dr Amita Singh[3], Kirti Rajput[4], Akash Prajapati[5], Pooja Rajpoot[6], Dr Praveen Kumar[7]

[1,2,4,5,6,7]*Faculty of Pharmacy, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, UP*
[3]*Veterinary officer & In-charge Animal House, Uttar Pradesh University of Medical Sciences, Saifai, Etawah, UP*
[*2]*Corresponding Author: Dr. Sushant Kumar, email id: k.sushant25@gmail.com*

Received: 16th September, 2026; Revised: 8th October 2026; Accepted: 12th October, 2026; Available Online: 18th October, 2026

ABSTRACT
Epilepsy is a chronic neurological disorder characterized by recurrent seizures, and effective treatment may be limited by inadequate drug delivery to the brain and unwanted systemic exposure. The present study was undertaken to develop and evaluate a brivaracetam-loaded bionanosuspension using a natural biopolymer isolated from Buchanania lanzan (chironji) seeds for intranasal nose-to-brain delivery. The isolated biopolymer was evaluated for its physicochemical characteristics, chemical tests, spectral analysis profile like SEM, DSC, FTIR, NMR, MASS, and in-vitro cytotoxicity. The isolated biopolymer showed low cytotoxicity toward H9c2 rat cardiomyoblast cells, with an IC_{50} value greater than 500 µg/mL. Brivaracetam-loaded bionanosuspensions were prepared by probe sonication using the isolated biopolymer along with polyvinyl alcohol, dextrose, sodium benzoate, and purified water. Sodium carboxymethyl cellulose-based nanosuspension was also prepared as a standard-polymer comparator. The formulations were evaluated for pH, entrapment efficiency, particle size, polydispersity index, zeta potential and in-vitro drug release. Among the Buchanania lanzan formulations, the optimized formulation BF6 showed maximum entrapment efficiency of 89.42 ± 1.50%, whereas the standard-polymer formulation CF5 showed 90.34 ± 0.25%. The developed formulations exhibited pH values ranging from 6.8 to 7.4 ±0.5 and demonstrated sustained drug release. In the in-vivo study using Wistar rats, the optimized intranasal bionanosuspension produced greater plasma exposure and delayed the onset of pentylenetetrazole-induced seizures compared with the relevant comparator. Overall, the findings indicate that Buchanania lanzan biopolymer can serve as a natural excipient for the development of brivaracetam-loaded bionanosuspensions for intranasal administration and potential nose-to-brain drug delivery.
Keywords: Brivaracetam; Buchanania lanzan; Natural biopolymer; Epilepsy; Bionanosuspension; Nose-to-brain delivery; Pentylenetetrazol
How to cite this article: Jaiswal, A., Kumar, S., Singh, A., Rajput, K., Prajapati, A., Rajpoot, P., & Kumar, P. (n.d.). Development and evaluation of brivaracetam loaded nanoparticular bionanosuspension for nose to brain delivery in management of epilepsy. Int J Drug Deliv Technol. 2026;16(87s): 553-567; DOI: 10.25258/ijddt.16.87s.56
Source of support: Nil.
Conflict of interest: None

1.INTRODUCTION

Epilepsy affects more than 50 million people globally and is a chronic neurological disorder defined by recurrent, unprovoked seizures caused by an overactivity and synchronization of the brain's cortical networks. Today, epilepsy is well-known as a multifaceted neurological disorder with a huge range of causes- genetic, metabolic, immune, infectious, idiopathic factors, and lifestyle factors of individuals. **(1)** It impacts nearly 1% of the world's population and remains a major concern for global public health. In response to this challenging neurological disorder, extensive research has been conducted over the years to gain a deeper understanding of epilepsy and develop effective therapeutic interventions for the affected people, and improve public health & safety. **(2)** According to the ILAE, seizures are classified into focal onset, generalized onset, and unknown onset. Focal seizures originate in one hemisphere and may occur with preserved or impaired awareness, while

**Author for Correspondence: gowricivil@gmail.com*

generalized seizures involve both hemispheres from onset and include tonic-clonic, absence, myoclonic, atonic, tonic, and clonic seizures. Unknown-onset seizures are classified when the seizure origin cannot initially be determined and may be reclassified as more information becomes available [3]**.** Brivaracetam is a 2-pyrrolidone derivative structurally related to levetiracetam, differing by an n-propyl substituent at the 4-position of the pyrrolidone ring and a butanamide rather than acetamide side chain. This modification was identified through a target-based structure–activity relationship program in which a large series of pyrrolidone butanamide analogues was systematically screened for SV2A binding affinity following the discovery that SV2A was the site of action of levetiracetam. Brivaracetam emerged from this program as a compound with 15- to 30-fold higher affinity for SV2A than levetiracetam across various experimental conditions, together with substantially higher lipophilicity, properties that together confer more rapid and more complete brain penetration. Brivaracetam binds SV2A with 15- to 30-fold higher affinity than levetiracetam and penetrates the brain more rapidly, translating into a broad-spectrum preclinical antiepileptic profile [4]. Nose to brain administration is a non-invasive approach that allows the rapid transport of the drug directly to the brain and minimizes its systemic exposure. This route has attracted considerable attention over the past two decades as a promising alternative for the treatment of central nervous system disorders, including epilepsy, Alzheimer's disease, Parkinson's disease, depression, and brain tumors. Compared with oral, intravenous, and other parenteral routes, intranasal delivery offers several distinctive pharmacokinetic advantages that are particularly relevant to acute and chronic epilepsy management, including rapid onset of action, avoidance of hepatic first-pass metabolism, ease of self-administration, and improved patient compliance, especially in emergency settings such as status epilepticus or acute repetitive seizures where intravenous access may be difficult to obtain [5]. Biopolymers isolated from natural sources can be utilized as novel excipients having a polymeric nature. These have the brilliant film-framing capacity and biocompatibility properties. The isolated bio-polymers have great drug release rate controlling capacities. Since these are biodegradable, act as an option in contrast to standard manufactured synthetic and semisynthetic polymers [6].

2. Material and methods

2.1 Materials

Brivaracetam was a gift sample from SMILEX Drugs Pvt. Ltd., Jharmarzi, Baddi India. The *Buchanania lanzan* (Chironji) seed were purchased from the Ram Gopal market of Saifai, Uttar Pradesh. The remaining solvents and chemicals were of analytical reagent grade and were procured from chemicals storeroom of Faculty of Pharmacy, UPUMS, Saifai, Etawah, U.P.

2.2 Methods

2.2.1 Isolation of biomaterial from *Buchanania lanzan* seeds:

250 gm of *Buchanania lanzan* seeds were purchased from Ram Gopal market of Saifai, U.P. soaked overnight in purified water. The upper covering of seed was then removed. About 100 ml of purified water was added to this and then this blend was properly mixed in mixer. This slurry was filtered with the help of muslin cloth and thus the biomaterial was separated by filtration. The resultant was obtained as filtrate. After that the mixture was subjected to centrifugation at about 4000 rpm for 15 minutes and then the resultant supernatant layer was properly separated and taken. Then, half of the supernatant was treated with acetone in 1:1, 1:2, and 1:3 ratios. Another half of supernatant was treated with the methanol in the same ratios as acetone. This mixture was kept in refrigerator for 24 hours and then the solution was centrifuged at 4000 rpm for 30 minutes. Residue was collected having biomaterial and dried in desiccators for 24 hours. This residue of biomaterial was washed with n-hexane and the biomaterial was dried naturally for 10 hours for getting free flowing powder. The collected biomaterial was stored for further use in airtight containers after passing through sieve no. 120. The schematic flow chart of isolation procedure has been summarized in Flow chart-1. This procedure was repeated for six times and optimized after that percentage yield was calculated and reported [7].



Fig 1: Flow Chart for Isolation of Biomaterial from *Buchanania Lanzan*

2.2.2 Physicochemical characterization and evaluation of isolated biomaterial

The physicochemical properties of isolated biopolymer were characterized for color, odor, taste, and solubility. The chemical tests for presence of carbohydrate, starch, and proteins were also performed [8]. The isolated biopolymer was also characterized for Scanning electron microscopy (SEM) analysis, Differential scanning calorimetry (DSC) testing, Fourier transformed infrared spectroscopy (FTIR), Mass spectroscopy, and Nuclear magnetic resonance spectroscopy (NMR).

2.2.2.1 Scanning electron microscopy (SEM) analysis

The isolated biomaterial's surface morphologies were characterized by Scanning Electron Microscope (JSM-IT200 Jeol, Japan). In SEM analysis the external surface and internal structure was characterized. The small quantity of biomaterial was taken and fixed on aluminum studs and the coated with gold with using the sputter under vacuum atmosphere. The scanning electron micrograph was taken for the biopolymer under observation. The SEM images were interpreted and reported.

2.2.2.2 Differential scanning calorimetry (DSC) testing

DSC testing is the thermal analytical method in which the heat flow in or out of the sample for testing is determined as the function of temperature. Here the biopolymer sample was taken and exposed to controlled temperature program. The glass transition temperature was determined. The heat flow range was 50-300°C. The DSC thermogram was recorded by (DSC-60 Shimadzu, Japan), finally interpreted and reported [9].

2.2.2.3 Fourier transformed infrared spectroscopy (FTIR)

Fourier-transform infrared (FTIR) spectroscopy was performed using a PerkinElmer Spectrum Two FT-IR spectrometer (Model L160000A, PerkinElmer Inc., USA) equipped with KBr optics and operated using Spectrum 10 software. The isolated biopolymer was dried to remove residual moisture prior to analysis. Approximately 1 mg of the isolated biopolymer was accurately weighed and thoroughly triturated with 100 mg of dried, IR-grade potassium bromide (KBr) using a clean and dry mortar and pestle. The resulting homogeneous mixture was subjected to drying under an IR lamp to minimize the effect of residual moisture. The dried mixture was then compressed using a hydraulic press to obtain a transparent KBr disc/pellet. The prepared KBr disc was placed in the sample holder in the path of the infrared beam, and the FTIR spectrum was recorded over the 4000–400 cm^{-1} range. The obtained spectrum was examined for characteristic absorption bands corresponding to the functional groups present in the isolated biopolymer [10].

2.2.2.4 Mass spectroscopy

Mass spectroscopy is an accurate method for determining the molecular mass of compound. This is the laboratory technique in which the sample of biopolymers was introduced through the inlet system. The gas phase ions of the compound were produced. Then molecular ion fragmentation, the ions separated in mass spectrometer according to their mass to charge ratio. A mass spectrum of ion abundance versus Mass to Charge ratio was recorded. Mass spectra were interpreted and reported.

2.2.2.5 Nuclear magnetic resonance spectroscopy (NMR)

In Nuclear magnetic resonance spectroscopy (NMR) sample was dissolved in specific solvent like CDCl3. The mixture was pumped in the instrument at high-rate flow. The switch for valve was used to stop the flow. Then the measurement was performed. After the finishing of measurement, the spectrum was processed and analyzed in automation computer. NMR spectra were interpreted and reported.

2.2.3 *In-vitro* cell line toxicity of isolated biopolymer

The *in-vitro* cytotoxicity of the isolated biopolymers was evaluated using the MTT assay in H9c2 cells. The cells were exposed to different concentrations of the isolated biopolymers (25–500 µg/mL) for 48 h. Following treatment, cell viability was assessed using the MTT assay, based on the reduction of MTT to purple formazan by metabolically active cells. The absorbance of the resulting solution was measured at 540 nm using a microplate reader. Untreated cells served as the control, and cell viability was expressed as a percentage of the control. The results were used to assess the cytotoxic potential of the isolated biopolymers at different concentrations.

2.3 Drug -excipient compatibility study

2.3.1 FTIR spectroscopy

Brivaracetam and Isolated *Buchanania lanzan* biopolymer

Fourier transform infrared (FTIR) spectroscopy was employed to investigate the possible interaction between Brivaracetam and the isolated biopolymer of *Buchanania lanzan*. Before analysis, the samples were dried appropriately to minimize interference from moisture. A physical mixture of Brivaracetam and the isolated biopolymer was prepared by mixing the two components in a 1:1 ratio by weight, followed by gentle trituration to obtain a homogeneous mixture. The FTIR spectra were recorded using an FTIR spectrophotometer within the range of 4000–400 cm^{-1}. The compatibility was evaluated by determining whether the characteristic functional-group peaks of brivaracetam remained identifiable in the physical mixture without substantial spectral alterations.

2.4 Formulation of Bio-nanosuspension

The different formulations of bio-nanosuspension were prepared by using different drug-biopolymer ratio as given in tables. The Bio-nanosuspension was prepared by ultrasonication method of the mixture of drug-biopolymer along with other excipient like polyvinyl alcohol as suspending agent, sodium benzoate as the preservative, dextrose as nanosizent and double distilled water to make up the volume. The Brivaracetam, biopolymer and other excipient were accurately weighed and triturated with addition of the double distilled water. The mixture was kept in beaker and placed in ultrasonic probe sonicator. The beaker was placed in such a manner that tip of probe dipped in about 1-2cm in mixture inside of beaker. After placing the sample, the various parameter was set on the Ultrasonic Probe Sonicator, like Pulse on for 40 seconds, Pulse of for 5 seconds, Power rate at 70% and time for 30 minutes. The mixture was sonicated for 5 cycles, then 0.5ml of 0.5 % polyvinyl alcohol was added during sonication. The volume of the formulation was made up to 10ml with double distilled water along with 0.5% sodium benzoate and 5% dextrose. The mixture was allowed for sonication for 30 minutes. After sonication the prepared bio-nanosuspension was refrigerated for two days. The prepared formulations were observed after two days and if no settlement is there then it means the formulation is optimized. If settlement is there, then 0.5 ml of 0.5 % polyvinyl alcohol was again added and allowed for sonication for 30 minutes and refrigerated for 48 hours. In the same way the nanosuspension was also prepared by using Brivaracetam with standard polymer Carboxy Methyl Cellulose -Sodium salt. The different formulations were prepared and optimized according to stability. After formulation their stability was tested and then evaluated for different parameters like pH, Entrapment efficiency, particle size, polydispersity index, zeta potential, *In-vitro* drug release and best formulation was selected for *In-vivo* studies [11].

S.no.	Formula	BF1	BF2	BF3	BF4	BF5	BF6	BF7	BF8	BF9	BF10
1.	Drug:Polymer ratio	1:0.8	1:1	1:2	1:3	1:4	1:5	1:8	1:10	1:12	1:15
2.	Drug (mg)	10	10	10	10	10	10	10	10	10	10
3.	*Buchanania lanzan* biopolymer (mg)	8	10	20	30	40	50	80	100	120	150

4.	**PVA (ml)**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
5.	**Dextrose (%)**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
6.	**Sodium Benzoate (%)**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
7.	**Double Distilled Water (ml)**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 1: Brivaracetam loaded Bio-nanosuspension using *Buchanania lanzan* biopolymer

S.no.	**Formula**	**CF1**	**CF2**	**CF3**	**CF4**	**CF5**	**CF6**	**CF7**	**CF8**	**CF9**	**CF10**
1.	**Drug:Polymer ratio**	**1:0.8**	**1:1**	**1:2**	**1:3**	**1:4**	**1:5**	**1:8**	**1:10**	**1:12**	**1:15**
2.	**Drug (mg)**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**
3.	**Sodium CMC Standard polymer (mg)**	**8**	**10**	**20**	**30**	**40**	**50**	**80**	**100**	**120**	**150**
4.	**PVA (ml)**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
5.	**Dextrose (%)**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**	**5**
6.	**Sodium Benzoate (%)**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**	**0.5**
7.	**Double Distilled Water (ml)**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**	**10**

Table 2: Brivaracetam loaded Nanosuspension using Sodium CMC polymer

3. Characterization and Evaluation of Formulations

3.1 pH study

The pH studies were measured at 25 degrees Celsius using a calibrated pH digital meter The Bio-nanosuspension was brought in contact with the electrode of pH meter and equilibrated for 1min. This method was done in triplicate and mean was calculated along with standard deviation.

3.2 % Entrapment efficacy

The prepared bio-nanosuspension was centrifuged at 20,000 RPM for 20 minutes at 5 C using ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance at 217 nm of the appropriately diluted supernatant solution using UV spectrophotometer. The experiment was performed in triplicate for each wedge and the average was calculated [12].

%Entrapment efficiency =Total drug – free drug *100/Total drug

3.3 *In-vitro* drug release studies and release kinetics

The in-vitro drug release was carried out in MS diffusion apparatus this is static method and require complete replacement of sample. The biological membrane separates the two compartment that is donor and acceptor compartment. 2 ml of Bio-nanosuspension was kept in the donor compartment and the receiver compartment was filled with phosphate buffer pH 6.8 The complete sample was withdrawn at different time interval (0,0.5,1,2,4,6,8,12,24,36 hrs.) and refilled with fresh medium. The amount of drug release is assessed by measuring the absorbance at 217 nm using UV spectrophotometer. The %cumulative drug release(%CDR) was calculated at different time interval. Apart of this, *In-vitro* release study was also performed for Sodium CMC nanosuspensions by using the same procedure. The procedure was repeated three times for each formulation and the results were expressed as mean ± SD (n=3) [13].

3.4 Particle size Analysis of best optimized formulation

The hydrodynamic diameter and polydispersity index (PDI) of the nanoparticles were measured by dynamic light scattering and zeta potential was determined by electrophoretic light scattering (ELS) on a Malvern Zetasizer (Malvern Panalytical, UK) using Zetasizer software version 6.34 at 25 °C. Nanoparticle dispersions were appropriately diluted in filtered distilled water to avoid multiple scattering, and measurements were performed in triplicate using disposable cuvettes (size) and folded capillary cells (zeta potential). Water was selected as the dispersant. Data are reported as Z-average diameter (nm) ± (SD), PDI and zeta potential (mV) ± SD [14].

3.5 *In-vivo* studies

3.5.1 Pharmacodynamics Study - Pentylenetetrazol Induced Model

The anticonvulsant activity of brivaracetam formulations was assessed using the PTZ-induced seizure model in Wistar rats. Animals were randomly divided into four groups: normal control, standard, Test 1, and Test 2. The normal-

control group received normal saline 10 mg/kg orally. The standard group received brivaracetam pure solution intravenously at 10 mg/kg. Test Group 1 received the BRV-*Buchanania lanzan* bionanosuspension formulation intranasally, while Test Group 2 received the BRV-NA CMC Nanosuspension formulation intranasally. The intranasal formulations were designed for nose-to-brain delivery and were administered at 4 µg/g body weight per nostril. After a predefined pretreatment interval 15, 30 or 45 min, seizures were induced by intraperitoneal administration of PTZ at the dose specified in the approved experimental protocol. Animals were observed individually, and the time interval between PTZ administration and onset of the first convulsive episode was recorded as seizure latency. Prolongation of seizure latency, reduction in seizure severity, shorter seizure duration, and increased protection from generalized convulsions were considered evidence of anticonvulsant activity[15].

3.6 Stability Studies

Stability studies of the optimized bio-nanosuspension were performed under long-term and accelerated storage conditions in accordance with the principles of ICH Q1A(R2). The formulation was filled into tightly closed container and stored in a stability chamber at $25 \pm 2°C/60 \pm 5\%RH$ and $40 \pm 2°C/75 \pm 5\%RH$. Samples were withdrawn at the initial time point and after 30, 60, and 90 days of storage. At each sampling interval, the formulation was evaluated for changes in physical appearance, color, pH, entrapment efficiency, drug content, and in-vitro drug-release. The stability test for nanosuspension loaded with Brivaracetam by using Sodium-CMC as standard polymer was also performed and reported. All measurements were performed in triplicate and results were expressed as mean ± standard deviation.

4. Result and discussion

4.1 Isolation of Biomaterial

The process of isolation was optimized by repeating the determinations six times and means standard deviation (±) SD was calculated. Percentage yield of *Buchanania lanzan* was 7.9±0.8%.

4.2 Characterization of Isolated Biomaterial

The isolated biopolymer was creamy-white in appearance. The biopolymer was found to be characteristic odor with characteristic taste. It was found to be partial soluble in water and Soluble in methanol. It showed positive test for carbohydrates, proteins and starch. The characterization of isolated biopolymer of *Buchanania lanzan* with observation is shown below.

Extracted Biopolymer	Color	Odor	Taste	Solubility	
Buchanania lanzan	Creamy-White	Characteristic	Characteristic	Partial soluble in water,	Soluble in methanol

Tabel 3: characterization of isolated biomaterial

4.3 Scanning electron microscopy (SEM) analysis of biomaterial

Scanning electron microscopy (SEM) analysis of the isolated biopolymer revealed an irregular, rough, and heterogeneous surface morphology with aggregated, compact particles of varying shapes and sizes. The particles exhibited uneven and wrinkled surfaces with several folds and protrusions, indicating a non-uniform structural arrangement. The observed agglomeration may be attributed to intermolecular interactions and drying during sample preparation. Overall, the SEM micrograph demonstrates the heterogeneous and irregular surface morphology of the isolated biopolymer.

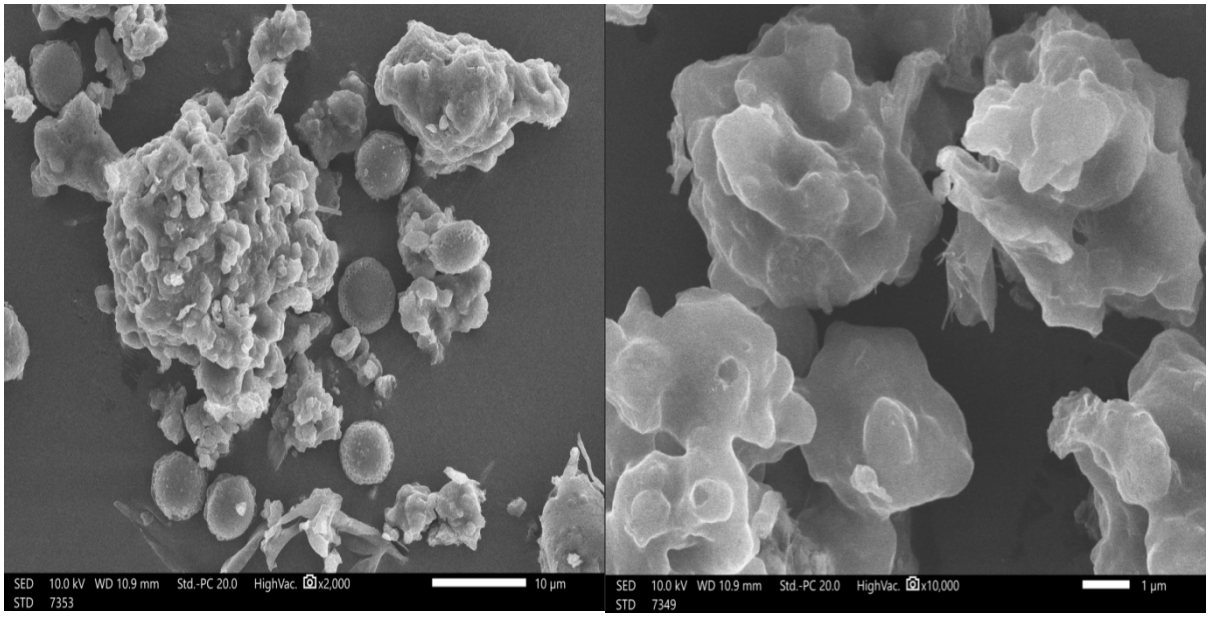


Fig 2: SEM images of isolated *Buchanania lanzan* biomaterial

4.4 Differential scanning calorimetry of biomaterial

The DSC thermogram of the biopolymer showed endothermic peak at 71.6°C. The area was found to be 103.4 mJ/mg. The broad peak indicates amorphous nature of the biopolymer. The absence of a sharp, well-defined melting endotherm suggests a predominantly amorphous nature of the isolated biopolymer.

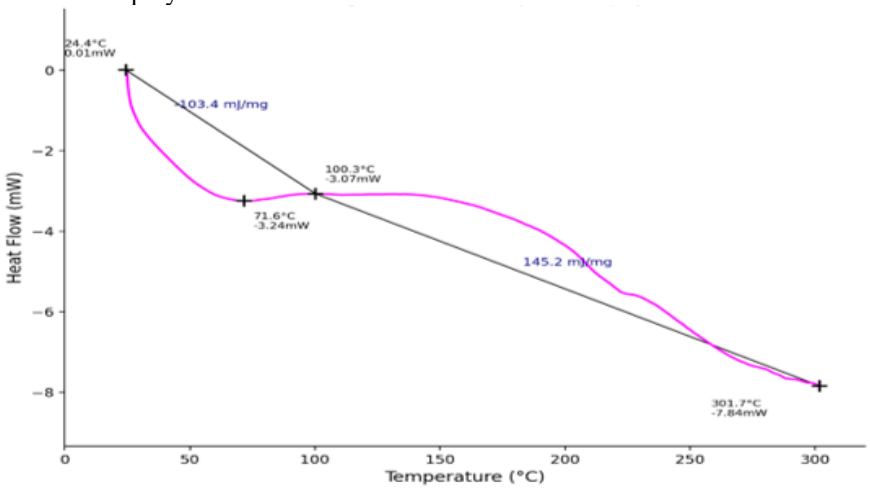


Fig 3: DSC thermogram of isolated *Buchanania lanzan* biomaterial

4.5 Fourier transformed infrared spectroscopy (FTIR)of biomaterial

The FTIR spectrum of the isolated biopolymer (Fig. 4) exhibited characteristic absorption bands corresponding to various functional groups. A broad absorption band around 3400 cm^{-1} was attributed to O–H stretching vibrations, indicating the presence of hydroxyl groups. The bands observed in the 2920–2850 cm^{-1} region were associated with C–H stretching vibrations. The absorption band in the 1650–1600 cm^{-1} region may be attributed to C=O/amide-related vibrations, while bands around 1400–1380 cm^{-1} were associated with C–H bending and carboxylate-related vibrations. The prominent absorption band in the 1100–1000 cm^{-1} region was attributed to C–O stretching vibrations. Additional bands in the fingerprint region indicated the presence of other C–C and C–O skeletal vibrations. Overall, the observed functional groups confirmed the polymeric nature of the isolated biopolymer and supported its potential use as a natural excipient in formulation development.

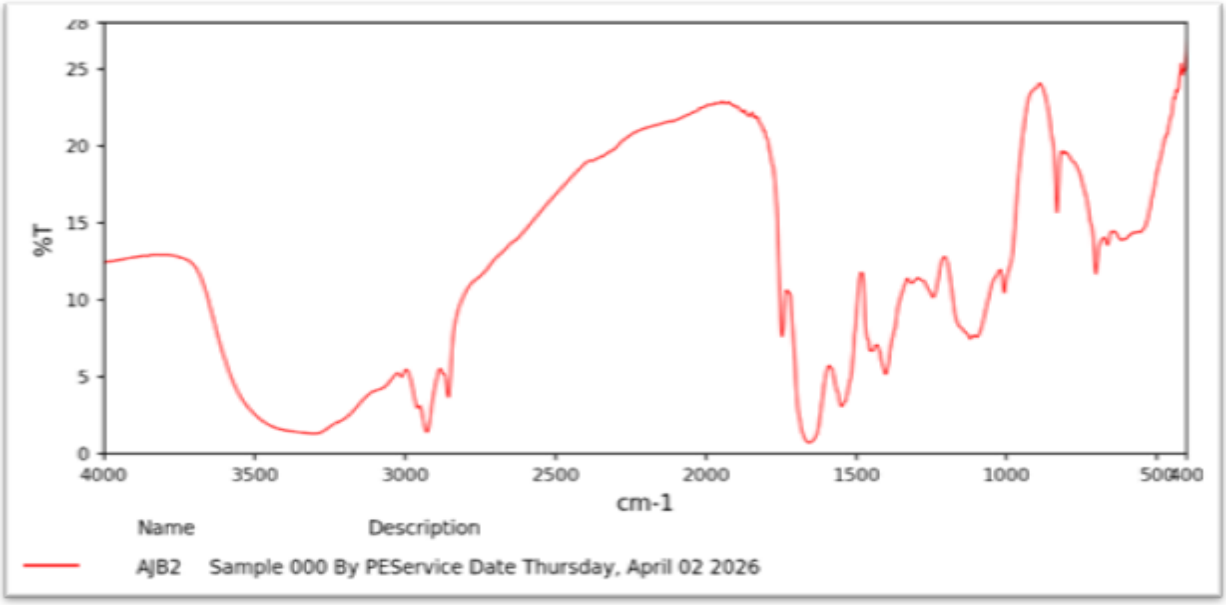


Fig 4: FTIR of isolated *Buchanania lanzan* biomaterial

4.6 Mass spectroscopy of biomaterial

The mass spectrum (Fig. X) of the isolated biopolymer exhibited several characteristic ions at **m/z 127.0739, 168.1469, 196.1312, 235.1756, 303.1217, 347.1811, 371.1090, 415.1480, 447.3297, 515.2776, 551.2617, 583.2668, 651.2619, 719.2493, 787.2325 and 855.2229**. Among these, prominent signals were observed at **m/z 235.1756 and 447.3297**. The observed ion pattern indicates the complex molecular composition of the isolated biomaterial and provides evidence of its heterogeneous polymeric nature.

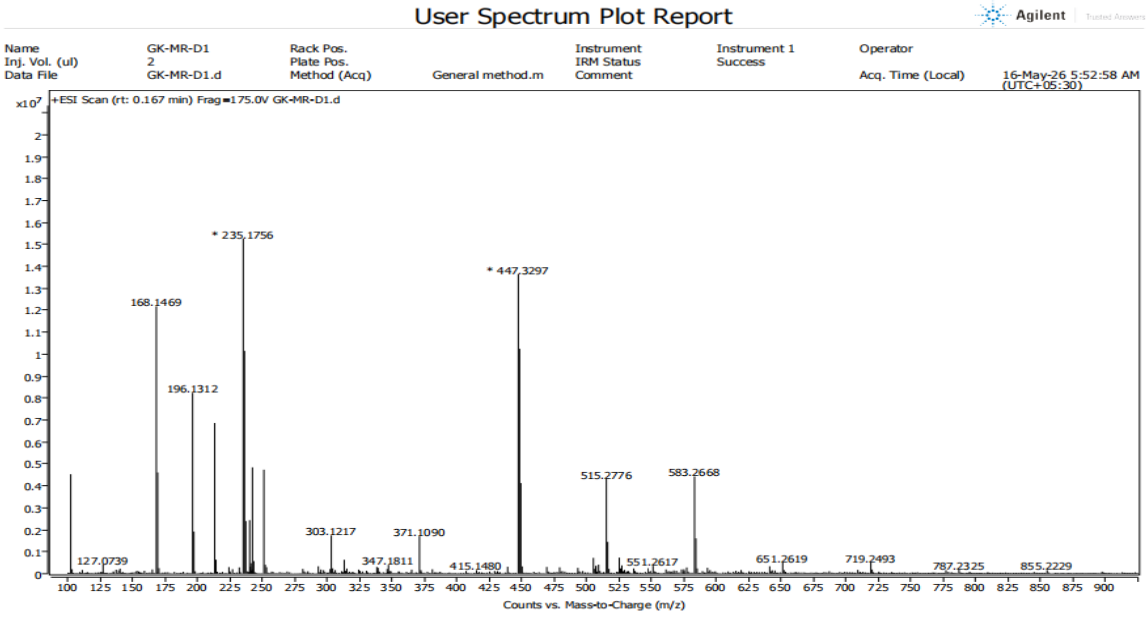


Fig 5: Mass spectroscopy of isolated *Buchanania lanzan* biomaterial

4.7 Nuclear magnetic resonance spectroscopy of biomaterial

The 1HNMR spectrum displayed major resonances at approximately δ3.41, 1.25–1.35, 1.15, and 0.87 ppm (Fig. X). The resonance at δ3.41 ppm suggests the presence of protons in an oxygenated chemical environment, while the upfield signals between δ0.87 and 1.35 ppm correspond to aliphatic proton environments, including methyl and methylene groups. The peak at δ2.52 ppm was attributed to residual DMSO-d_6 solvent. The spectral profile indicates the presence of aliphatic and oxygenated moieties, supporting the polymeric character of the sample.

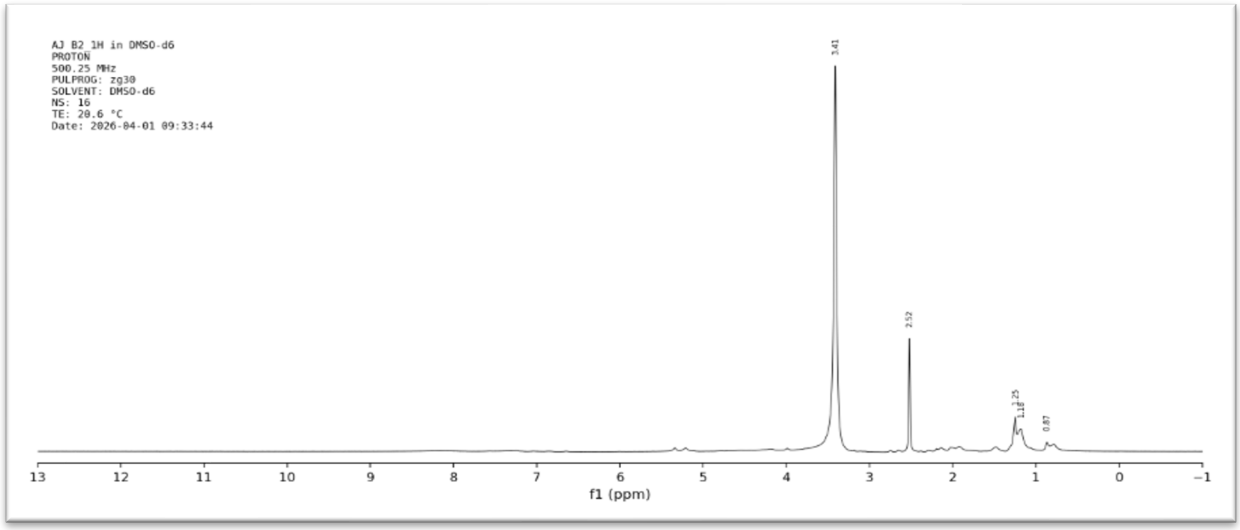


Fig 6: NMR spectroscopy of isolated *Buchanania lanzan* biomaterial

4.8 *In-vitro* cell line toxicity of isolated biopolymer:

The isolated *Buchanania lanzan* seed biopolymer was evaluated for cytotoxicity using the MTT assay in H9c2 rat cardiomyoblast cells at concentrations of 25–500 µg/mL for 48 h. Cell viability ranged from 138.00 ± 3.82% at 25 µg/mL to 67.00 ± 2.49% at 500 µg/mL, demonstrating a concentration-dependent reduction in viability. The IC_{50} was found to be >500 µg/mL, as 50% cell viability was not reached at the highest tested concentration. Thus, the isolated *B. lanzan* biopolymer exhibited low cytotoxicity under the tested conditions. So, it can be safely used for the preparation of drug loaded bionanosuspension.

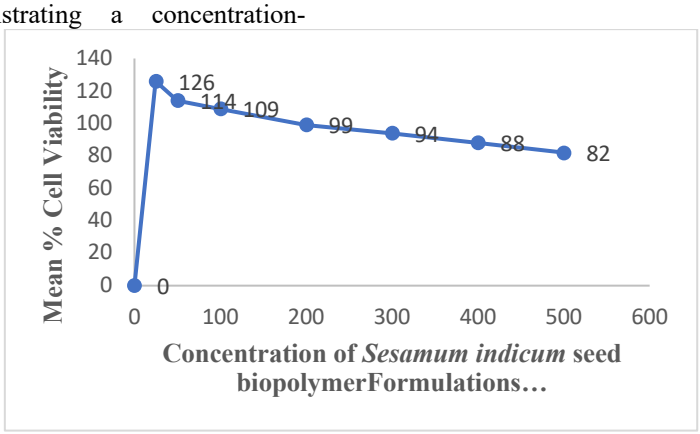


Fig 7: *In-vitro* cell line toxicity of isolated biopolymer *Buchanania lanzan*

4.9 Drug -excipient compatibility study:

The FTIR spectrum of brivaracetam with *Buchanania lanzan* biopolymer showed characteristic peaks at 3300, 2927, 2856, 1733, 1659, 1549, 1402, 1244, 1121, 1009, 819 and 740 cm^{-1}. The characteristic functional groups of Brivaracetam were retained without significant peak shifting or disappearance, and no new prominent peaks were observed. This indicates the absence of significant chemical interaction and confirms the compatibility of brivaracetam with *Buchanania lanzan* biopolymer.

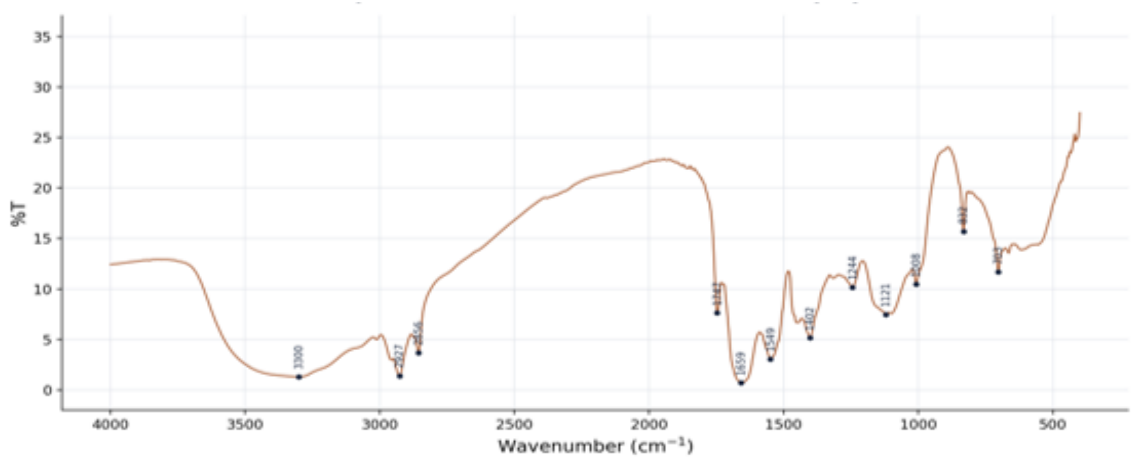


Fig 8: FTIR of Brivaracetam and isolated *Buchanania lanzan* biomaterial

5. Characterization and Evaluation of Formulations

5.1 pH

Brivaracetam-loaded bio-nanosuspensions were prepared successfully and evaluated for pH, entrapment efficiency, particle size, polydispersity index, and in-vitro drug release. The obtained pH values range between 6.8-7.4±0.05.

5.2 Entrapment Efficiency:

Among the formulations of the *Buchanania lanzan*-biopolymer loaded bionanosuspension batch BF6 and batch CF5 of the sodium carboxymethyl cellulose loaded nanosuspension showed maximum entrapment efficiency of 89.42 ±1.50% and 90.34 ±0.25% respectively.

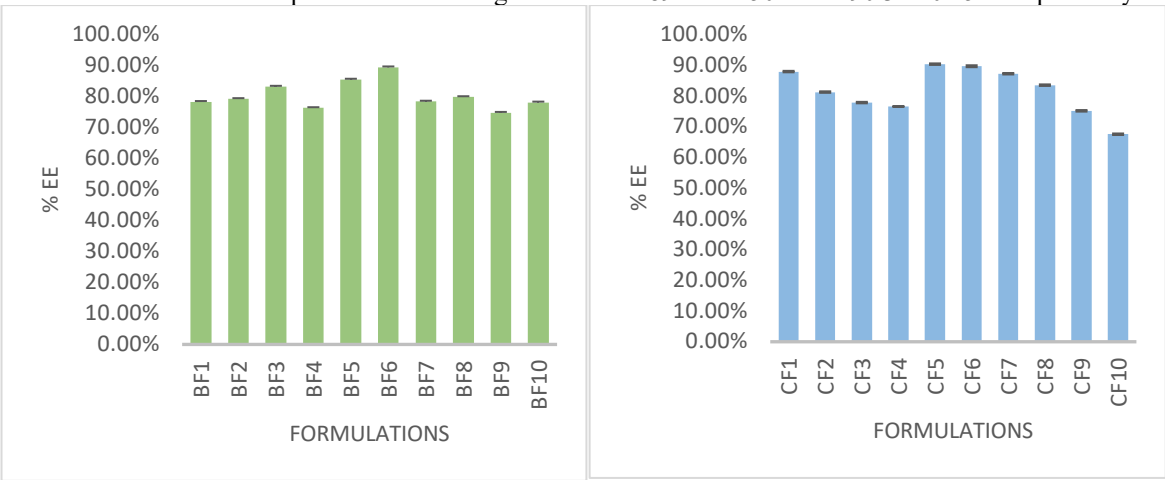


Fig 9: % Entrapment efficacy of Bio-nanosuspensions using *Buchanania lanzan* (BF1-BF10) and Nanosuspension using Na-CMC polymer (CF1-CF10), mean of three observations ± SD (n=3).

5.3 *In-vitro* release and release kinetics

The *in-vitro* drug release study was performed for all brivaracetam-loaded *Buchanania lanzan* bio-nanosuspensions (BF1–BF10). The release data were fitted to zero-order, first-order, Higuchi matrix, Korsmeyer–Peppas, and Hixson–Crowell kinetic models, and the corresponding R^2values were determined. The % CDR profile showed gradual drug release, reaching approximately 80–85% at 36 h. Among the tested formulations, BF6 exhibited the sustained release at the final time point. For optimized formulation F6, the R^2values for zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models were 0.6358, 0.7385, 0.9128, 0.9280, and 0.7050, respectively. The Korsmeyer–Peppas model showed the highest R^2value (0.9280). These findings suggest that the release profile was best described by the Korsmeyer–Peppas model.

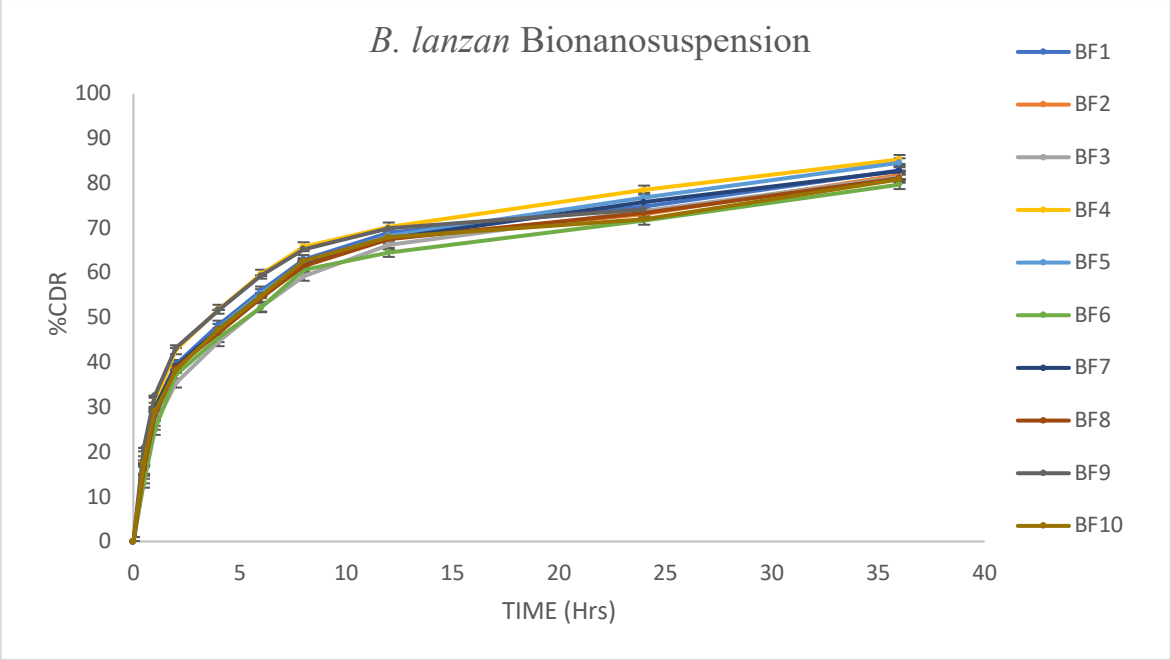


Fig 10: % Cummulative drug release of Bio-nanosuspension using *Buchanania lanzan*

Table 4: Release Kinetics of Best formulation of *Buchanania lanzan* Bio-nanosuspension

Best Formula-tion	Zero-order R^2	First-order R^2	Higuchi matrix R^2	Korsmeyer-Peppas Model R^2	Hixon Crowell model R^2	n	Best fit model	Mechanism of release
BF6	0.6358	0.7385	0.9128	0.9280	0.7050	0.4092	Peppas Korsmeyer	Fickian Diffusion (Higuchi Matix)

The Na-CMC nanosuspension formulations exhibited a progressive increase in cumulative drug release over the 36-h study period. The formulation CF5, showed sustained release approximately 76.68% cumulative drug release at 36 h, lower than that of the other formulations evaluated. The release data for CF5 were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell kinetic models. The corresponding R^2 values were 0.8383, 0.9504, 0.9258, 0.9735, and 0.9182, respectively. The Korsmeyer–Peppas model provided the best fit, exhibiting the highest coefficient of determination (R^2 = 0.9735). The release exponent (n = 0.6514) was consistent with the reported anomalous transport mechanism, suggesting contributions from both drug diffusion and polymer relaxation.

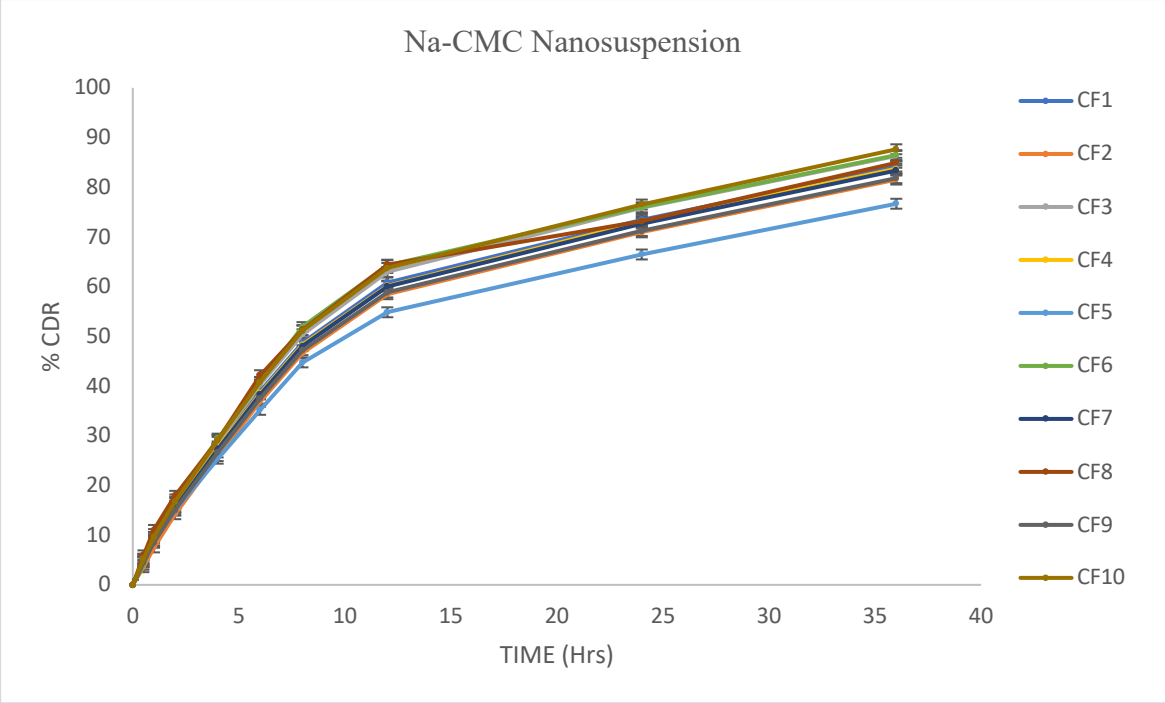


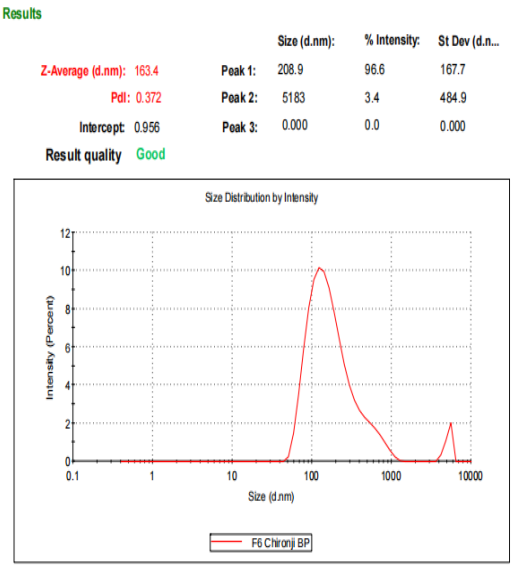
Fig 11: % Cummulative drug release of nanosuspension using of Na-CMC

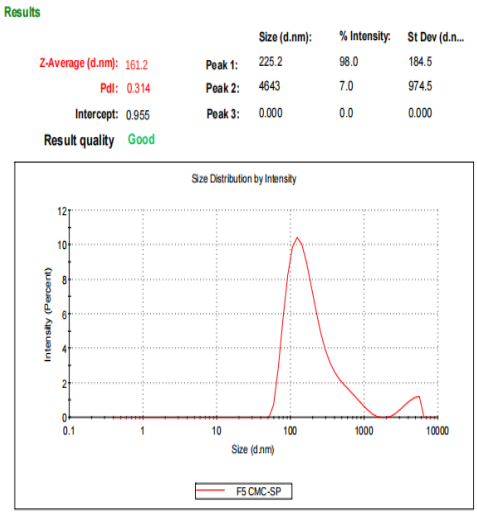
Table 5: Release Kinetics of Best formulation of Na-CMC nanosuspension

Best Formula-tion	Zero-order R^2	First-order R^2	Higuchi matrix R^2	Korsmeyer -Peppas Model R^2	Hixon Crowell model R^2	n	Best fit model	Mechanism of release
CF5	0.8383	0.9504	0.9258	0.9735	0.9182	0.6514	Peppas Korsmeyer	Anomalous Transport

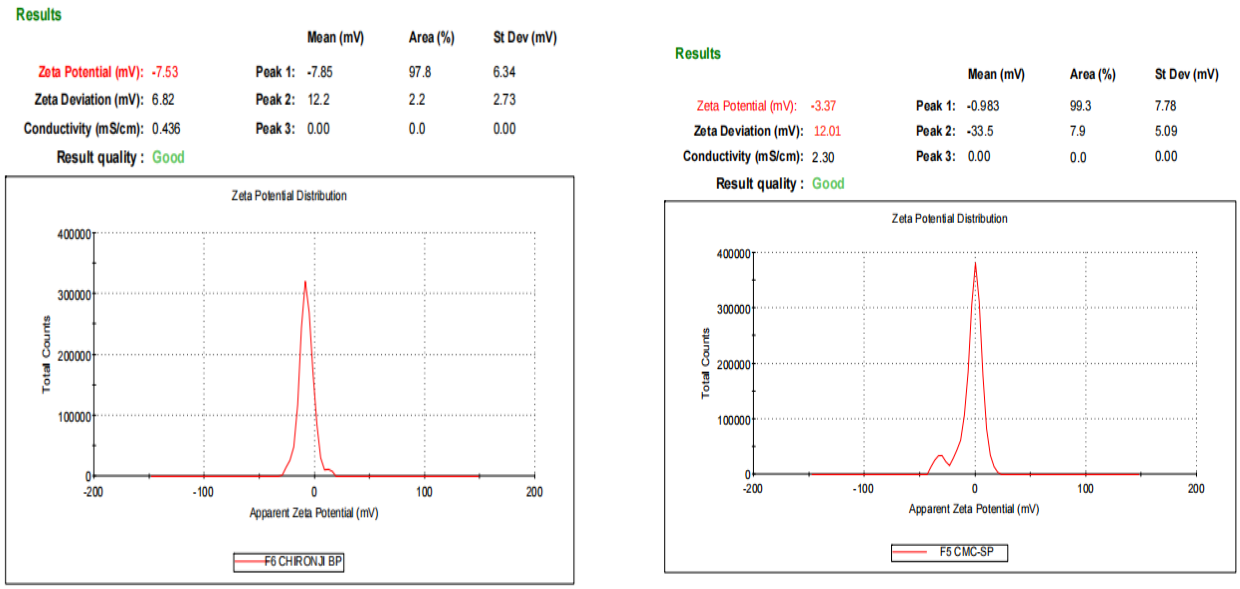
5.4 Particle size Analysis of best optimized formulation

The optimized *Buchanania lanzan* bionanosuspension (BF6) showed a Z-average of 163.4 nm (PDI 0.372) and a zeta potential of −7.53 mV, comparable to the optimized Na-CMC nanosuspension (CF5) (161.2 nm, PDI 0.314, −3.37 mV) (Fig. 1a–d). Both systems were in the nanometer range with moderately broad distributions and near-neutral surface charge, which indicates that stability relies mainly on steric stabilization by the polysaccharide.





(a) (b)



(c) (d)

Fig 12: (a,c)-Particle size and PDI of optimized bionanosuspension of *Buchanania lanzan* and optimized nanosuspension of Na-CMC respectively, (b,d) zeta potential of optimized bionanosuspension of *Buchanania lanzan* and optimized nanosuspension of Na-CMC respectively.

5.6 *In-vivo* studies

5.6.1 Pharmacodynamic studies

The PTZ-induced seizure study demonstrated differences in seizure-onset time among the treatment groups. The *Buchanania lanzan*-based bionanosuspension showed seizure-onset times of 380.33 ± 15.89 s, 351.83 ± 7.15 s, and 275.17 ± 7.43 s at 15, 30, and 45 min pretreatment, respectively. The corresponding values for the standard-polymer bionanosuspension were 361.67 ± 7.18 s, 285.17 ± 5.58 s, and 188.33 ± 4.91 s, while the brivaracetam pure-solution group showed 190.17 ± 7.47 s, 171.33 ± 7.48 s, and 156.67 ± 7.36 s, respectively. The normal control group showed substantially shorter seizure-onset times, ranging from 84.17 to 87.33 s. Within the observed values, the *B.lanzan* bionanosuspension produced the longest seizure-onset time at each pretreatment interval. These findings indicate a measurable antiepileptic response, as reflected by prolongation of the time to seizure onset following brivaracetam treatment.

5.7 Stability Study

The stability of the optimized *B. lanzan* bio-nanosuspension (BF6) and the Na-CMC nanosuspension (CF5) over 90 days is presented in Table (6,7). Both formulations remained homogeneous suspensions at 25 ± 2 °C/60 ± 5% RH and 40 ± 2 °C/75 ± 5% RH, with no visible change in appearance. Under long-term conditions, BF6 showed only marginal changes: pH ranged from 6.87 ± 0.02 to 6.84 ± 0.02, entrapment efficiency (% EE) decreased from 89.42 ± 0.26% to 88.90 ± 0.64%, and in-vitro drug release changed from 79.71 ± 1.35% to 78.10 ± 1.31%. CF5 showed a slightly larger decline, with pH falling from 6.87 ± 0.01 to 6.77 ± 0.05, % EE from 90.34 ± 0.25% to 86.11 ± 0.28%, and drug release from 76.68 ± 3.83% to 74.89 ± 3.71%. Under accelerated conditions, both formulations showed a gradual, time-dependent decrease in % EE and drug release. For BF6, % EE fell from 89.42 ± 0.26% to 85.63 ± 0.72% and release from 79.71 ± 1.35% to 73.80 ± 1.67%, while pH stayed between 6.86 and 6.93. For CF5, % EE fell from 90.34 ± 0.25% to 86.13 ± 0.15%, release from 76.68 ± 3.83% to 70.42 ± 4.12%, and pH from 6.92 ± 0.02 to 6.72 ± 0.03. Overall, both systems were physically stable and retained more than 90% of their initial EE and release over three months, with BF6 showing smaller pH changes than CF5.

Table 6: Stability Study of BF6 best optimized Bionanosuspension.

Formulations	Temperature	Parameters Evaluated	Study periods (Days)			
			0	30	60	90
BF6	$25 \pm 2°C/60 \pm 5\%RH$	Appearance	homogeneous suspension	homogeneous suspension	homogeneous suspension	homogeneous suspension
		pH	6.87±0.021	6.89±0.020	6.85±0.031	6.84±0.023
		%Entrapment efficiency	89.42 ±0.26%	88.92±0.35%	88.30±0.87%	88.90±0.64%
		In-vitro drug-release	79.71 ±1.35	79.20±1.28	78.65 ±1.42	78.10±1.31
	$40 \pm 2°C/75 \pm 5\%RH$	Appearance	homogeneous suspension	homogeneous suspension	homogeneous suspension	homogeneous suspension
		pH	6.92±0.019	6.93±0.025	6.86±0.066	6.88±0.025
		%Entrapment efficiency	89.42 ±0.26%	87.96 ± 0.41%	86.74 ± 0.58%	85.63 ± 0.72%
		In-vitro drug-release	79.71 ± 1.35	77.85 ± 1.46	75.92 ± 1.52	73.80 ± 1.67

Conclusion

The present study successfully demonstrated the potential of an isolated *Buchanania lanzan* seed biopolymer as a natural carrier for the development of brivaracetam-loaded bio-nanosuspension intended for intranasal brain delivery. The isolated biomaterial was obtained with a percentage yield of $7.9 \pm 0.8\%$and exhibited suitable physicochemical characteristics for pharmaceutical application. It was creamy white, partially soluble in water and soluble in methanol, and showed the presence of carbohydrate, starch, and protein components. SEM analysis revealed an irregular, rough, and heterogeneous surface morphology, whereas DSC analysis showed a broad endothermic transition at 71.6 °C, supporting the predominantly amorphous nature of the isolated material. FTIR, mass spectrometry, and NMR findings further supported the polymeric and chemically heterogeneous nature of the biomaterial.

The MTT cytotoxicity study in H9c2 cardiomyoblast cells indicated that the isolated *B. lanzan* biopolymer possessed low cytotoxicity within the tested concentration range. Cell viability remained 67.00 ± 2.49% even at the highest tested concentration of 500 µg/mL, and the IC_{50} value was greater than 500 µg/mL. These findings support the preliminary biocompatibility of the isolated biomaterial for its application as a natural pharmaceutical excipient. In addition, FTIR compatibility analysis of brivaracetam with the isolated biopolymer retained the characteristic peaks of the drug without major shifting, disappearance, or formation of new peaks, indicating the absence of significant drug–polymer interaction.

Among the prepared formulations, BF6 containing brivaracetam and *B. lanzan* biopolymer at a drug:polymer ratio of 1:5 was selected as the optimized bio-nanosuspension based on its high entrapment efficiency, nanosized particle distribution, and sustained drug-release profile. BF6 exhibited an entrapment efficiency of 89.42 ± 1.50%, mean particle size of 163.4 nm, PDI of 0.372, and zeta potential of −7.53 mV. The optimized conventional polymer formulation, CF5 containing sodium CMC, showed comparable particle size and entrapment efficiency; however, BF6 produced a more sustained release profile. The release of brivaracetam from BF6 followed the Korsmeyer–Peppas model, with an R^2 value of 0.9280 and release exponent of 0.4092, indicating that Fickian diffusion was the predominant drug-release mechanism.

The PTZ-induced seizure model demonstrated that intranasal administration of the *B. lanzan*-based bio-nanosuspension prolonged seizure-onset time more effectively than both the sodium-CMC nanosuspension and brivaracetam pure solution. The maximum seizure latency was observed with the BF6 formulation at the 15-min pretreatment interval, suggesting rapid anticonvulsant activity following intranasal administration. This response may be associated with efficient nasal absorption and potential direct transport of brivaracetam through nose-to-brain pathways.

Stability testing showed that BF6 remained physically stable as a homogeneous suspension for 90 days under both long-term and accelerated conditions. Under long-term storage, only minor changes in pH, entrapment efficiency, and in-vitro drug release were observed. A comparatively larger decrease in entrapment efficiency and drug release occurred under accelerated storage, indicating that the formulation is preferably stored at 25 ± 2 °C/60 ± 5% RH. Overall, the findings establish *Buchanania lanzan* seed biopolymer as a promising natural, biocompatible, and sustainable excipient for the development of intranasal brivaracetam bio-nanosuspension. Further studies should evaluate

nasal mucosal safety, brain pharmacokinetics, biodistribution, long-term stability, and therapeutic performance in additional epilepsy models before clinical translation.

Acknowledgement: The authors gratefully acknowledge the support and facilities provided by Faculty of Pharmacy and Animal House, Uttar Pradesh University of Medical Sciences for conducting this research work.

Author Contribution: AJ-Conceptualization, methodology, experimentation, data analysis and manuscript preparation, SK-Supervision, review and critical revision of the manuscript, KR- Data interpretation, AS-Animal experiments, AP- *In-Vitro* experiment, PR- Data formatting.

Funding: No External funding received

Ethical Approval: The study was conducted in accordance with the applicable institutional ethical guidelines of Uttar Pradesh University of Medical Sciences, Saifai, Etawah, U.P, India. (IAEC/07/AH/2025-26)

Conflict of Interest: The authors declare that they have no conflict of interest.

Data Availability: The data are available from the corresponding author upon reasonable request.

References:

1. Abdelaziz, Heba Ali, Ghada M. Suddek, Manar A. Nader, and Hamdy A. Ghoneim. 2024. "Advances and Challenges in Understanding and Treating Epilepsy: A Concise Review." *Delta University Scientific Journal* 7 (2): 70–79
2. Brodie, Martin J. 2010. "Antiepileptic Drug Therapy the Story so Far." *Seizure* 19 (10): 650–55.
3. Fisher, Robert S., J. Helen Cross, Carol D'Souza, Jacqueline A. French, Sheryl R. Haut, Norimichi Higurashi, Edouard Hirsch, et al. 2017. "Instruction Manual for the ILAE 2017 Operational Classification of Seizure Types." *Epilepsia* 58 (4): 531–42
4. Nicolas JM, Hannestad J, Holden D, et al. Brivaracetam, a selective high-affinity synaptic vesicle protein 2A (SV2A) ligand with preclinical evidence of high brain permeability and fast onset of action. Epilepsia. 2016;57(2):201-209.
5. Crowe TP, Greenlee MHW, Kanthasamy AG, Hsu WH. Mechanism of intranasal drug delivery directly to the brain. Life Sci. 2018;195:44–52.
6. Kumar S, Pandey S, Madhav NVS. Biopolymer: a novel bioexcipient. In: Biocomposites. London: IntechOpen; 2021. doi:10.5772/intechopen.97191.
7. Madhav, N.V.S. and Raina, D., 2017. Formulation and evaluation of Duloxetine loaded bio-nano suspension for brain specificity via acoustic meatus. SOJ Pharm Pharm Sci., 4(1), pp. 1-5
8. Raut VR, Ghotankar AM. Review of Khuskhus (khaskhas)(seeds of Papaver somniferum linn.) with special reference of ayurveda medicine. Journal of Ayurveda and Integrated Medical Sciences. 2019 Dec 31;4(06):228-31.
9. Sampathkumar, L., Christopher Selvin, P., Selvasekarapandian, S. *et al.* Synthesis and characterization of biopolymer electrolyte based on tamarind seed polysaccharide, lithium perchlorate and ethylene carbonate for electrochemical applications. *Ionics* **25**, 1067–1082 (2019).
10. Raut VR, Ghotankar AM. Review of Khuskhus (khaskhas)(seeds of Papaver somniferum linn.) with special reference of ayurveda medicine. Journal of Ayurveda and Integrated Medical Sciences. 2019 Dec 31;4(06):228-31.
11. Yogita Tyagi & NV Satheesh Madhav (2020) Inbuilt novel bioretardant feature of biopolymer isolated from cucumis sativa for designingdrug loaded bionanosuspension, Journal of Drug Assessment, 9:1, 72-81
12. Raina D, Singh S, Negi RS, Sharma B. An Approach for Drug Delivery to the Brain Via External Acoustic Meatus. Electrochemical Society Transactions. 2022 Apr 24;107(1):3609-18
13. Kumar S, Madhav NVS, Verma A, Pathak K. Development and evaluation of novel bionanoparticles loaded with nanosized lamotrigine by using the novel isolated biopolymer from *Phaseolus vulgaris* seeds. Int J Pharm Sci Drug Res. 2020;12(3):247-254. doi:10.25004/IJPSDR.2020.120306.
14. Goswami, L., Madhav, N.V.S. and Upadhyaya, K., 2016. Development and evaluation of bionanoparticles as novel drug carriers for the delivery of Selegiline. International Current Pharmaceutical Journal, 5(4), pp.33-37.
15. Nour SA, Abdelmalak NS, Naguib MJ, Rashed HM, Ibrahim AB. Intranasal brain-targeted clonazepam polymeric micelles for immediate control of status epilepticus: in vitro optimization, ex vivo determination of cytotoxicity, in vivo biodistribution and pharmacodynamics studies. Drug Deliv. 2016;23(9):3681-3695. doi:10.1080/10717544.2016.1223216.