RESEARCH PAPER

Paclitaxel Loaded Nanoparticles a Potential Dosage Form for Brain Targeting-Design, Characterization and Evaluation

Hiranmoy Parya[1], Subhabrota Majumdar[2], Banhishikha Kar[3], Beduin Mahanti[4*]

[1]Department of Pharmaceutics, Gupta College of Technological Sciences, Ashram More, Grand Trunk Road, Asansol 713303, West Bengal, India.

[2]Department of Pharmaceutics, Calcutta Institute of Pharmaceutical Technology and AHS, Banitabla, Uluberia, Howrah-711316, West Bengal, India.

[3]Department of Pharmaceutics, Calcutta Institute of Pharmaceutical Technology and AHS, Banitabla, Uluberia, Howrah-711316, West Bengal, India.

[4*]Director (School of Pharmacy), Techno India University, Bidhannagar, Kolkata, West Bengal 700091. E-mail address: beduin.pharmacy@gmail.com (Correspondence)

ABSTRACT

Background: Paclitaxel (PTX) is an effective anticancer agent used against various tumors, including gliomas, but its clinical application in brain cancer is hindered by poor solubility, rapid clearance, multidrug resistance, and difficulty crossing the blood–brain barrier (BBB). Traditional formulations like emulsions, liposomes, and polymeric micelles struggle to achieve adequate drug delivery to the brain while minimizing toxicity. This study investigates protein-based nanoparticles loaded with paclitaxel as a novel targeted drug delivery method for brain tumors. These nanoparticles provide benefits such as biocompatibility, biodegradability, and potential for surface modification to enhance BBB transport. Proteins like albumin facilitate better drug loading and stability, enabling effective delivery of hydrophobic drugs and promoting prolonged circulation, passive targeting, and possible active targeting through ligand attachment.

Methods: In this study, paclitaxel-loaded protein nanoparticles are formulated using a desolvation technique and characterized for particle size, zeta potential, morphology, encapsulation efficiency, in-vitro drug release and in-vivo study.

Results: Resulted formulation showed narrow particle size distribution 178.1 to 285.6 nm, satisfactory PDI ranges from 0.160 to 0.316 with desire entrapment efficiency of drug loaded nanoparticles. In silico analysis indicated that paclitaxel potentially downregulates the expression of anti-cancer activity with TNF-α. In silico molecular docking studies targeting TNF-α revealed that certain metabolites of Paclitaxel exhibit strong binding affinities. In vivo study on wister rat shows that paclitaxel effectively crossed the blood brain barrier and shows the anti-cancer activity.

Conclusion: Further, cytotoxicity and cellular uptake studies are conducted to evaluate their therapeutic potential against brain cancer cells. The results are expected to demonstrate that protein-based nanoparticles significantly improve the brain delivery and anticancer efficacy of paclitaxel compared to conventional formulations.

Keywords: Paclitaxel, Blood brain barrier, Protein based nanoparticles, desolvation technique.

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Introduction

Cancer is regarded as one of the most severe health-related hazards on a global scale. Chemotherapeutic drugs, surgical excision, and radiation therapy have been the standard methods of treating cancer in recent decades. However, because of their severe side effects and toxicity, radiation and chemotherapy decrease quality of life. Additionally, they may cause cancer cells to develop resistance, making them immune to radiation and chemotherapy.

Of all brain tumors, 13-22% are malignant gliomas. The average duration of survival is less than one year, irrespective of the approach of treatment. These cancers frequently return within millimetres of their initial position even after surgery, external beam radiation therapy, and systemic chemotherapy. The blood–brain barrier (BBB) is the reason systemic chemotherapy is not as effective. The BBB can only be significantly penetrated by lipid-soluble, low molecular weight compounds, a small number of

peptides, and nutrients, either passively or by certain transport processes. Therefore, it is challenging for the majority of drugs to reach therapeutic levels at the intended location. The latest findings of the anticancer properties of several natural compounds have led to their use as substitutes for pharmaceuticals in the treatment and prevention of cancer. One of these drugs with less side effects is paclitaxel[1].

One of the most potent anticancer drugs now available on the market is paclitaxel, a chemotherapeutic agent. [2] A class IV drug according to the Biopharmaceutics Classification System, it has low water solubility (0.77 - 35 µM) and high lipophilicity (logP~4). It was initially isolated from the bark of the Pacific yew, or Taxus brevifolia [3]. It is widely used to treat brain tumors and a variety of malignancies, including breast, ovarian, and lung cancers. Paclitaxel works by interfering with the regular disintegration of microtubules, which halts cell division and ultimately leads to cell death [4]. Paclitaxel functions well against a variety of malignancies because of its capacity to stabilize microtubules. This drug's poor solubility in water is one of its main drawbacks. However, paclitaxel is produced in combination with Cremophor EL under the brand name "Taxol" to increase its restricted clinical application due to its weak water solubility of around 0.4 µg mL^{-1}. One of the most serious adverse effects linked to the administration of Taxol, acute hypersensitivity reactions, have been demonstrated to occur in 2-4% of individuals treated with Cremophor EL.[5] Since paclitaxel is a substrate of the efflux transporter P-glycoprotein (P-gp), it cannot significantly permeate the blood-brain barrier.[6] According to recent reports, novel nanoparticles may be employed as potential drug carriers that can pass through the blood-brain barrier. Nano drug delivery systems are less harmful and increase paclitaxel's solubility. When compared to free or conventional paclitaxel, the optimal nanoformulation exhibits superior action.

Nanoparticles (NPs) are solid colloidal particles and can be produced in various shapes and sizes. Their sizes range from about 10 to 1000 nm.[7] Its benefits include slow controlled release characteristics, protective function, and unique drug targeting, particularly when it comes to the transfer of hydrophobic drugs.[8] To improve therapeutic efficacy and minimize side effects of the loaded drugs, nanoparticles have been widely used in recent decades to transport chemotherapeutic drugs to the tumor microenvironment.[9] One such type of nanoparticle is protein-based nanoparticle (PNPs). Proteins have distinct features and roles in biological products and can be employed as a base for nanoparticle formation. PNPs are nano-sized particles composed of proteins, either naturally occurring or recombinantly engineered, that are designed to encapsulate, deliver, or target drugs to specific sites in the body. PNPs offer an advantage over other forms of nanoparticles because they are capable of controlling the size of particles, surface alterations, biodegradability, and stability. Additionally, they are less immunogenic and hazardous.[10,11]

The role of the present research was to prepare paclitaxel loaded protein-based nanoparticles for effective brain delivery and evaluate its physicochemical characteristics. Furthermore, the in vitro drug release behaviour and in vivo studies of the prepared nanoparticles were investigated.

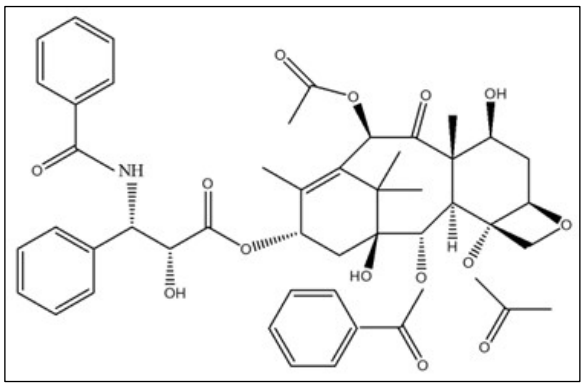

Figure 1: Structure of Paclitaxel

2. Materials and Methods

2.1. Materials used in the study

We purchased Paclitaxel from Neon Lab. Ltd. in Palghar. HiMedia provided the human serum albumin (HSA), whereas Loba Chemie Pvt. Ltd. provided the glutaraldehyde (GTA). The supplier of ethanol was Sisco Research Laboratories Pvt. Ltd. The study's other substances and reagents were analytical in nature and adhered to pharmaceutical regulations.

2.2. Preparation methods

2.2.1. Formulation of paclitaxel loaded protein-based nanoparticles

Protein-based nanoparticles loaded with paclitaxel were produced using the desolvation technique listed in Table 1. [12] HSA was dissolved in 10 millilitres of distilled water to create the protein's aqueous solution in a beaker. A suitable quantity of drug was dissolved in 20 millilitres of ethanol in a different beaker. After that, the HSA solution was put in a magnetic stirrer and rotated at room temperature at 700 rpm. Until the solution turned turbid, the drug solution was added dropwise to the HSA solution using a syringe at a rate of 1 ml min-1. The nanoparticles were cross-linked

with GTA after 30 minutes, and they were then constantly agitated for 8 hours at room temperature at 700 rpm. [13]

Table 1. Study RSM results for several formulations of protein-based nanoparticles loaded with paclitaxel using a 3^2 factorial design

Formulation Code	Factors		Response		
	X1: Concentration of HSA (%w/v)	X2: Concentration of GTA (%v/v)	Y1: Entrapment efficiency (%)	Y2: Drug release at 8 hours (%)	Y3: Drug release at 12 hours (%)
F1	1	4	74.69	34.91	57.35
F2	1.5	6	66.33	37.19	52.79
F3	1.25	2	75.24	35.51	58.67
F4	1.5	2	72.59	41.15	59.27
F5	1.5	4	68.97	40.43	61.91
F6	1.25	6	61.27	38.51	52.31
F7	1	2	67.76	33.11	56.99
F8	1	6	78.93	39.47	58.19
F9	1.25	4	57.21	36.71	51.95

2.2.2. Characterization of paclitaxel loaded protein-based nanoparticles

2.2.2.1. FTIR Study

FTIR is essential for evaluating possible chemical interactions between drug molecule and other additives. With a resolution of 4 cm^{-1}, the IR absorption peak of Paclitaxel was investigated using this method within the frequency range of 4400 cm^{-1} to 400 cm^{-1}. To interpret FTIR, the potassium bromide (KBr) pellet technique was used.[14]

2.2.2.2. XRD Study

X-ray diffraction (XRD) is an analytical technique to discover about the structure of crystalline materials. The properties of materials, including their chemical composition, lattice strain, and preferred orientation, can be analysed, along with their crystal structure and orientation. In addition, XRD can be used to assess thermal expansion in crystal structures and ascertain the dimensions and structure of crystals.[15]

2.2.2.3. Particle Size; PDI; and ZP Study

PDI serves as a gauge of particle size distribution heterogeneity within a sample for the nanoparticles. Zeta potential is a significant physicochemical parameter which has significant effects on the stability of nanoparticles.[16,17] The paclitaxel loaded protein-based nanoparticles underwent particle size, PDI and zeta potential analysis which were determined using Photo Correlation Spectroscopy. A Malvern Zeta-sizer (Malvern Instruments, UK) was used to measure the particle size, PDI, and zeta potential for each formulation in polystyrene curettes at a temperature of 25° C and at a predetermined position of 90°.

2.2.2.4. Entrapment efficiency

Amount of drug entrapped within nanoparticles is measured by the entrapment efficiency. 2 ml of paclitaxel loaded protein-based nanoparticle was taken and centrifuged at 12000 rpm at room temperature for 30 minutes using a centrifuge tube. The supernatant was separated without disturbing the sediment layer using a micropipette. After appropriate dilution, absorbance was measured using a UV-Visible spectrophotometer (Shimadzu 1900i, Japan) at a wavelength of 230 nm.[18] The percentage of drug entrapped was calculated by the following formula:

Entrapment Efficiency (%) = [(W-w) × 100

Where '**W**' is the total amount of drug in the preparation and '**w**' is the free drug present in supernatant.

2.2.2.5. In-vitro Drug Release Profile

In vitro drug release of various formulations of paclitaxel loaded protein-based nanoparticles was conducted through a Franz diffusion cell by utilizing a 47 mm diameter dialysis membrane with 0.45 µm pores. The receptor chamber was filled with 70 ml phosphate buffer pH 7.4 at 37± 0.5°C to provide a perfect sink condition. To keep the receptor compartment's temperature stable, water was poured into the jacket that encircled it. A magnetic stirrer running at 100 rpm was used to continuously agitate entire arrangement. At different predefined intervals, 1 ml sample were withdrawn and they were quickly replaced with equivalent volume of fresh buffer medium to maintain the equilibrium. After diluting the samples, they were analyzed at 230 nm using a UV-Visible spectrophotometer (Shimadzu-1900i, Japan).[19] The cumulative percentage of drug released was estimated using the pre-generated calibration curve.

2.2.2.6. Study of Release Kinetics

To understand in vitro drug release kinetics, the drug release data was analysed by different release kinetic models such as zero-order, first-order, Higuchi model, and Korsmeyer-Peppas model for highest correlation coefficient value (R^2). The release coefficient (n) was calculated using the Korsmeyer-Peppas model in order to investigate a drug release mechanism from nanoparticles.

2.2.2.7. Experimental Design

A 3^2-factorial design was employed to achieve the optimal result from the Paclitaxel loaded protein-based nanoparticles by using concentration of HSA and concentration of GTA as two independent variables. Three levels of variation were applied to the two independent variables: low (-1), medium (0), and high (+1). Various levels of responses for optimization of nanoparticle have been embraced based on experimental groups. Three different dependent responses were selected as an optimization parameter which were entrapment efficiency (%) (Y1), drug release at 8 hrs (%) (Y2), and drug release at 12 hrs (%) (Y3). Stat Ease Design Expert 13 trial version was

employed for the data collection and experimental design, as shown in Table 1. Using a polynomial equation, Paclitaxel loaded protein-based nanoparticles were evaluated in terms of optimizing the different responses. The following equation is:

$$Y = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$$

Y is the response parameter in this investigation. Equation AB denotes an interaction between two variables, with A and B serving as response variables. Intercept is denoted by b_0, while the coefficients of regression are b_1, b_2, b_3, b_4, and b_5. The validity of the model and the individual parameters of the response can be determined with an ANOVA one-way model.[20]

Cryo FEG SEM

According to the cryo FEG SEM imaging investigation, nanoparticles containing Paclitaxel were spherically formed, smooth-surfaced, and between 100 and 200 nm in size [21]. The findings were identical to those of the DLS particle size measurements and SEM analysis.

2.2.3. In-vivo Study

To perform the in vivo study, male Wistar rats of body weight 200 ± 20 g were used. The entire investigation was carried out in accordance with CPCSEA criteria. The Institutional Animal Ethical Committee, Calcutta Institute of Pharmaceutical Technology & AHS, West Bengal, India (2075/PO/Re/S/19/CPCSEA) approved the study protocol CIPT/IAEC/2025/20 The animals were given free access to food and water for seven days before the actual experiment, during which time they were acclimated to the laboratory environment at 22 ±1° C and 60 ± 10% humidity. This study was carried out to compare the targeting efficiency of paclitaxel loaded protein-based nanoparticle with that of paclitaxel aqueous suspension in the brain. 15 rats were divided into 3 groups, each containing 5 animals. One group of animals was treated as normal control (saline solution). The second group was treated with paclitaxel aqueous suspension, and the third group was treated with the optimized formulation of paclitaxel loaded protein-based nanoparticle at an equivalent dose of 10 mg/kg body weight of rats of paclitaxel intravenously through tail vein. At a predetermined time interval of 0, 1, 2, 4, 6, 8, 10 and 12 hr of dosing, the brain was isolated from the animals. The isolated brain was homogenized, centrifuged and stored at -80 ° C until further analysis. HPLC was used to estimate the drug content.[22,23,24]

Network pharmacology

The rapidly developing discipline of network pharmacology seeks to understand the complexities of pharmacological actions and illness within intricate biological networks. This method has transformed research by offering a methodical framework for exploring scientific problems on several levels. Scholarly emphasis in network pharmacology research aimed at applying Traditional Chinese Medicine (TCM) to cancer treatment has increased in recent years[25].

The key steps in network pharmacology for cancer –

- Target identification
- Drug-target interaction mapping by using computational tool
- Network construction by build network on Drug-target, Protein-Protein interaction.
- Pathway enrichment analysis
- Validation by computational docking and molecular dynamics.

Molecular Docking Analysis

The protein data bank provided the X-ray crystallographic structures of the protein targets catalytic domains in combination with their co-crystallized ligands. The "Molecular Operating Environment 2019.0102 software" (MOE) was implemented for all molecular modelling mathematical calculations and docking investigations. Utilising the quick preparation tool in MOE, the protein was prepared by eliminating both the uninvolved ligands and molecules of water. The target compounds were docked following the assembly of the enzyme. The following approach was adopted: The "site finder" tool was used to identify the enzyme's active site. The program guidelines have been updated to use the alpha triangle as the placement methodology alongside ligand atoms to be the docking site. After loading the ligand's MDB file, the docking algorithms were carried out consequently. Both 2D and 3D models of the complexes' receptor-ligand interactions were investigated. For energy calculations, the positions exhibiting optimal ligand–enzyme associations were determined and secured. Based on their RMSD data and binding scores, position were chosen[26].

2.7. In silico study

AUTODOCK Vina software was implemented to conduct a molecular docking analysis. Two proteins' co-crystal 3D atomic coordinates were obtained from PDB. PyMol software (Oberhauser) was used to configure the receptor. All non-standard atoms at first. Using MGL Tools 1.5.6, the receptor coordinates were obtained, polar hydrogens were inserted, and water molecules were eliminated before being stored (The

Scripps Research Institute). Chemical structure PDB open bable[27] was used to save energy and draw ligand structures. In Table 2, every docking parameter was mentioned. UCSF Chimera 1.18[28] is used to create all structures with docking posture visualization and graphics.

Table 2: The molecular docking parameter as well as PDB IDs of various wound healing proteins used for molecular docking.

Sl. No	Proteins name	*PDB ID*	X*Y*Z dimension (Å3)	The docking box centre (Å)
1	TNF α	2AZ5	60×66×66	X=-9.181, Y= 67.363, Z=20.045

3. Results

3.1. Characterization of paclitaxel loaded protein-based nanoparticles

3.1.1. FTIR Study

The purpose of the IR analysis is to demonstrate that there was no chemical interactions between the drug (paclitaxel) and the protein component utilized to prepare the formulation. The FTIR spectra of paclitaxel, and the physical mixture of paclitaxel and the excipient are shown in Figure 2.

3.1.2. XRD study. XRD is an effective technique for characterizing the physical properties of drug substances, particularly in terms of their crystallinity and polymorphism. It helps to determine the crystalline structure, phase composition, and other properties of the drug and excipients, aiding in the development of stable and effective dosage forms.[23] Figure 2 shows the XRD graphs of Paclitaxel (pure drug) and physical mixture of drug and excipient respectively.

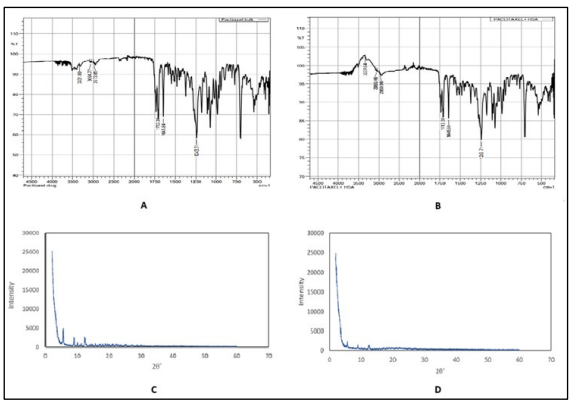

Figure 2. (A) FTIR Spectra of Paclitaxel, **(B)** FTIR Spectra of Paclitaxel along with excipient, (**C**) XRD graph of Paclitaxel, and (**D**) XRD graph of Paclitaxel along with excipient

3.1.3. Estimation of Particle Size, Polydispersity Index (PDI) and Zeta Potential

The particle size of paclitaxel loaded protein-based nanoparticle ranges from178.1±1.87 nm and 285.6±2.10 nm. Particle size analysis revealed that as the amount of protein increased, the particle size decreased. PDI values of the nanoparticles ranges from 0.160±0.06 to 0.316±0.03. The zeta potential for various formulations of paclitaxel loaded protein-based nanoparticle were found to be between -14.66±0.49 mV to -34.15±0.56 mV. Table 2 displays the results for particle size, PDI, and zeta potential.

3.1.4. Entrapment efficiency

EE is a physicochemical parameter that measures the amount of drug entrapped within the nanoparticles. The EE (%) of the prepared nanoparticles varies between 57.21±0.92 % and 78.9±0.75 % which is represented in Table 2.

Table 2. Particle size, PDI, zeta potential, and entrapment efficiency of various formulations of paclitaxel loaded protein-based nanoparticles (mean±SD; n=3)

Formulation Code	Particle Size (nm)	PDI	Zeta Potential (mV)	Entrapment Efficiency (%)
F1	197.2±1.52	0.238±0.07	-31.12±0.68	74.69±1.04
F2	245.6±2.34	0.294±0.06	-24.59±0.35	66.33±1.32
F3	218.4±3.17	0.250±0.02	-27.34±1.08	75.24±0.95
F4	285.6±2.10	0.316±0.03	-14.66±0.49	72.59±0.76
F5	259.5±1.96	0.273±0.08	-18.26±0.71	68.97±1.24
F6	231.4±1.39	0.299±0.02	-20.37±0.32	61.27±1.67
F7	178.1±1.87	0.189±0.05	-16.25±0.44	67.76±0.55

F8	194.4± 2.15	0.160± 0.06	- 34.15± 0.56	78.93±0. 75
F9	241.7± 1.48	0.209± 0.04	- 15.78± 0.59	57.21±0. 92

Cryo Field Emission Gun Scanning Electron Microscopy (Cryo FEG SEM) According to the cryo FEG SEM imaging investigation, the nanoparticles carrying Paclitaxel were spherically formed, smooth-surfaced, and between 100 and 200 nm in size (Figure:). The findings were identical to those of the DLS particle size measurements and SEM analysis.

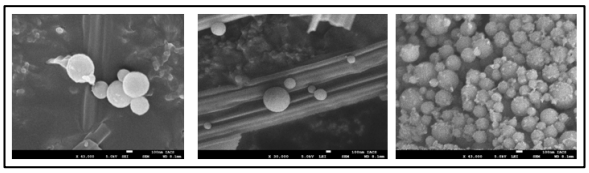

Figure 1: SEM image of Paclitaxel nanopaticles

3.1.5. In- vitro Drug Release Profile

Dissolution profile of numerous paclitaxel loaded protein-based nanoparticles are showed in Fig. 2. The highest release was observed in F5 formulation (61.91 % at 12 h), which contained 1.5 % HSA and 4 % GTA.

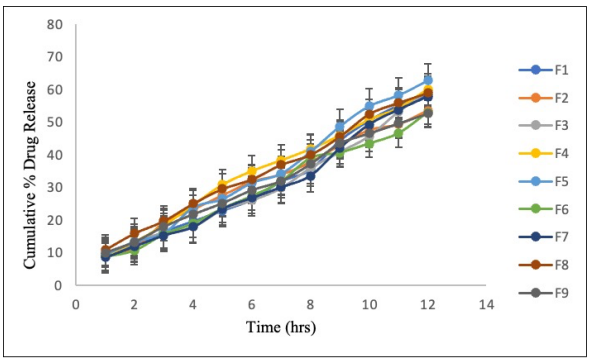

Figure 4. *In-vitro* drug release profile of different formulations of paclitaxel loaded protein-based nanoparticles

3.1.6. Release Kinetics

The correlation coefficient (R^2) result, which was obtained from a variety of kinetic models and the in vitro dissolution data of the paclitaxel loaded protein-based nanoparticle formulation was used to calculate the release mechanism. In this research, the formulation F8 demonstrates better result which obeyed the zero-order release model (R^2 value 0.9962) and follows non-fickian diffusion mechanism (n value 0.698).

3. 1.7. Statistical optimization

Paclitaxel loaded protein-based nanoparticle was analysed by 3^2 experimental designs as shown in Table 1. In this factorial design, the concentrations of HSA and GTA, which are independent variables, differ between experimental groups and are confirmed at three different levels: low (-1), medium (0), and high (+1). Additionally, there are three distinct dependent variables i.e., entrapment efficiency (%), drug release at 8 hours (%), and drug release at 12 hours (%). In order to indicate the analyzed response, the quadratic and 2FI equations are applied. The ANOVA summary for the various models are listed in Table 3.

Entrapment efficiency = 79.45 + 2.45 A - 4.53 B + 1.98 AB - 2.82 A^2 - 11.43 B^2

F = 11.77, R^2 = 0.8706, p = 0.0347

Drug release at 8 hrs = 37.67 + 1.22 A + 0.8578 B - 2.82 AB

F = 5.45, R^2 = 0.6251, p = 0.0494

Drug release at 12 hrs = 52.97 - 0.6453 A - 2.30 B - 2.85AB + 2.71 A^2 + 2.31 B^2

F = 9.14, R^2 = 0.8357, p = 0.0491

Table 3. Summary of ANOVA

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	
Entrapment Efficiency (ANOVA for Quadratic Model)						
Model	370.88	5	74.18	11.77	0.0347	significant
A-Conc. of HSA	26.39	1	26.39	4.19	0.1333	
B-Conc. of GTA	137.81	1	137.81	21.86	0.0185	
AB	15.64	1	15.64	2.48	0.2133	
A^2	16.79	1	16.79	2.66	0.2012	
B^2	183.87	1	183.87	29.17	0.0124	
Drug Release at 8 hrs (ANOVA for 2F1 Model)						
Model	43.98	3	14.66	5.45	0.0494	significant
A-Conc. of HSA	7.33	1	7.33	2.72	0.1597	
B-Conc. of GTA	5.07	1	5.07	1.88	0.2284	
AB	31.81	1	31.81	11.82	0.0185	
Drug Release at 12 hrs (ANOVA for Quadratic Model)						
Model	91.39	5	18.28	9.14	0.0491	significant
A-Conc. of HSA	1.83	1	1.83	0.9124	0.4099	
B-Conc. of GTA	35.51	1	35.51	17.75	0.0244	
AB	32.49	1	32.49	16.24	0.0275	
A^2	15.53	1	15.53	7.76	0.0686	
B^2	7.52	1	7.52	3.76	0.1479	

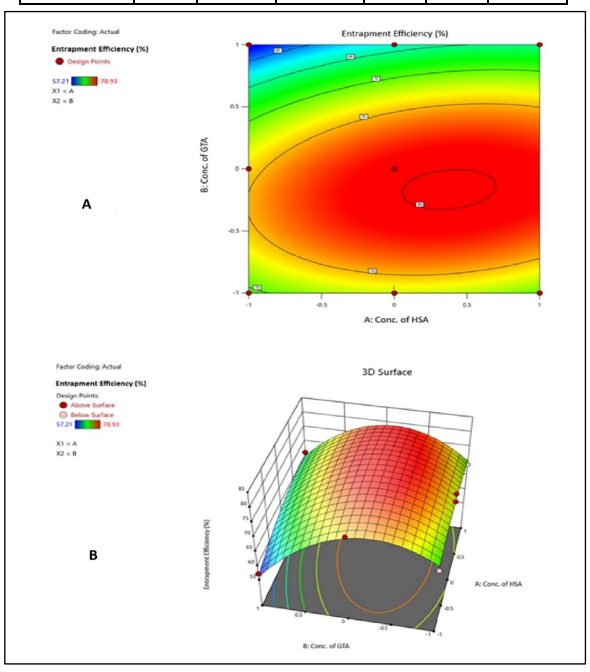

Figure 5. Impact of concentration of HSA and GTA on % of Entrapment Efficiency **(A)** Contour Plot, **(B)** Response Surface 3D Plot.

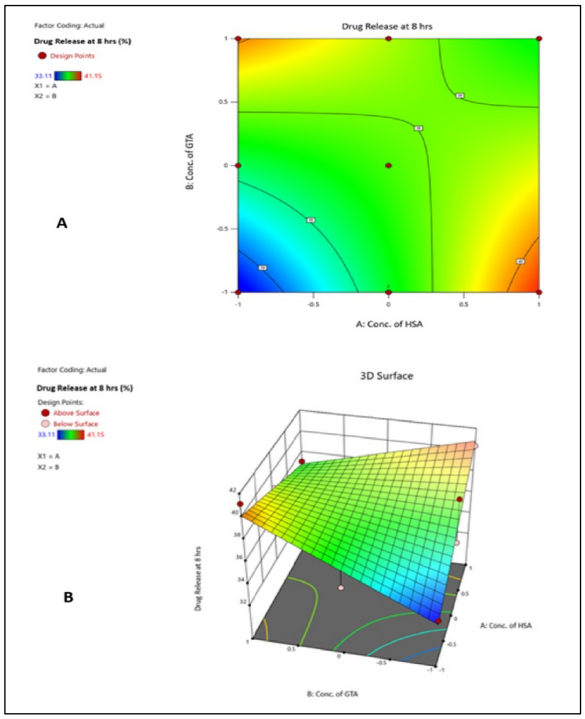

Figure 5. Impact of concentration of HSA and GTA on % of drug release at 8 hrs **(A)** Contour Plot, **(B)** Response Surface 3D Plot.

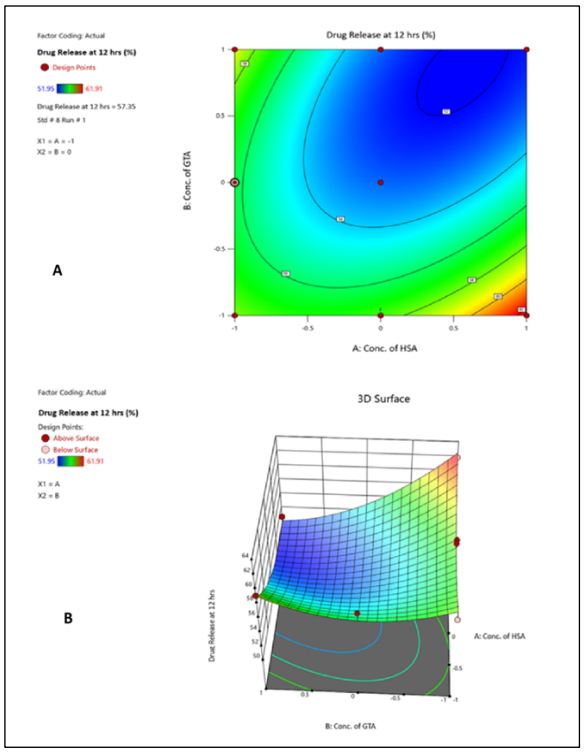

Figure 7. Impact of concentration of HSA and GTA on % of drug release at 12 hrs **(A)** Contour Plot, **(B)** Response Surface 3D Plot.

3.2. In vivo study

Saline solution, paclitaxel aqueous suspension and optimized paclitaxel loaded protein-based nanoparticle were given to male Wistar rats. The mean drug concentration of paclitaxel in brain was graphically explained in connection of the time figure (Figure 61). Significant pharmacokinetic characteristics of paclitaxel aqueous suspension and optimized paclitaxel loaded protein-based nanoparticle were demonstrated after being given intravenously to animals. Optimized paclitaxel loaded protein-based nanoparticle showed improvements in $AUC_{0\text{-}12}$ when compared to paclitaxel aqueous solution. The C_{max} of the optimized paclitaxel loaded protein-based nanoparticle was observed to be greater than that of the paclitaxel aqueous solution.

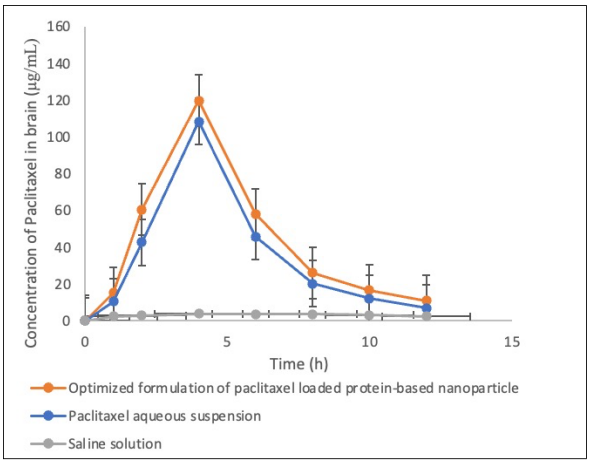

Figure 8: Drug Concentration Versus Time Profile of Paclitaxel

Table 4: Pharmacokinetic parameters of optimized Paclitaxel loaded protein-based nanoparticle with Paclitaxel aqueous suspension (n=5, Mean ± S.D.)

Parameters	Paclitaxel Aqueous Suspension	Optimized Paclitaxel loaded protein-based Nanoparticle
C_{max} (µg.ml^{-1})	108.43±12.06	119.81±17.13
T_{max} (h)	4.21±1.05	4.09±0.95
$AUC_{0\text{-}12}$ (µg.h.ml^{-1})	574±10.6	652.09±84.23
V_d (mL)	88.14±21.49	116.39±18.26
MRT (h)	19.4±8.3	22.4±6.45
Elimination rate (h^{-1})	0.07±0.01	0.11±0.02
Elimination half-life (h)	8.75±3.42	10.27±5.06

* C_{max}: maximum concentration, t_{max}: time, AUC: area under the curve, V_d: volume of distribution, MRT: mean residence time.

Network Pharmacology

Identifying gene targets for paclitaxel in cancer

We discovered 290 genes that paclitaxel targets based on the TCMSP database. We used the UniProt database to standardize the data in order assure consistency in the gene information, resulting in a final list of 289 genes. Concurrently, 1149 genes linked to this disease were retrieved from the GeneCards database. As seen in Figure 9, 92 overlapped genes, or 0.2% of the total genes examined, were found when the two data sets were combined for co-analysis.

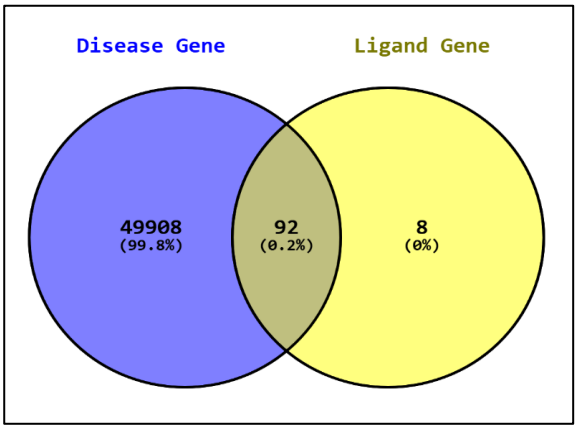

Figure 9: The Venn diagram of overlapping target genes between disease gene and ligand gene.

Construction of Compound-Target-Disease (CTD) network

The Cytoscape software was used to create a network of paclitaxel-common targets-cancer in order to visualise the pharmacological potential of the drug for treating cancer (Figure.10). Two additional nodes (shown in yellow) represent paclitaxel and cancer, while the network's 92 nodes (shown in green) represent shared target genes. The connections between the nodes were represented by the 155 edges in the network diagram. The results show that by targeting several different sites, paclitaxel inhibits the growth of malignant cells.

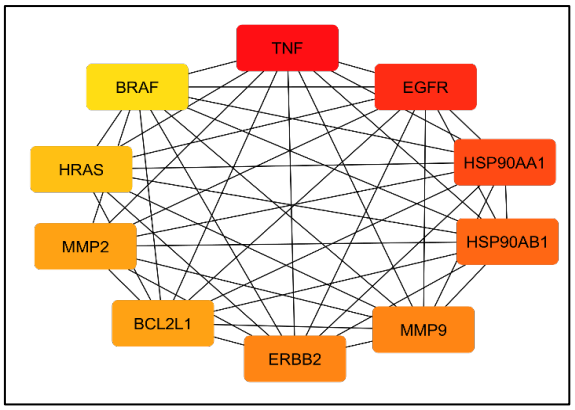

Figure 10: Cytoscape visualizes of the common targets in the PPI network

Construction and analysis of PPI network

PPI analysis was performed for the 92 genes commonly targeted by paclitaxel based on data in the STRING database (Figure. 11).

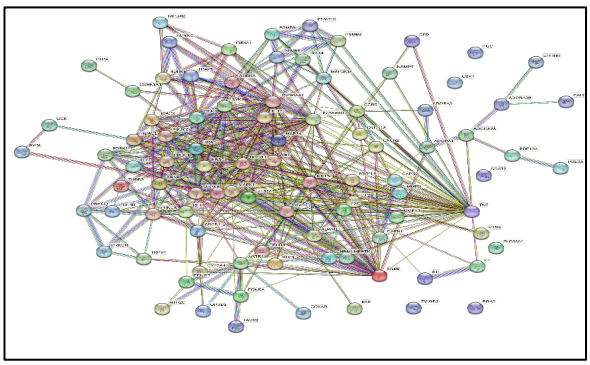

Figure 11: PPI network of common targets generated by STRING

In-silico study

A molecular docking was performed with two wound healing proteins like TNF-α to understand the possible mechanism action of Paclitaxel. TNF-alpha play crucial roles in cancer treatment. The docking of the Paclitaxel with the TNF-α protein was done using the crystal structure of PDB 2AZ5. The blind manner of docking allowed the ligand to attach to any cavity. As shown in Figure 6 & 7. and Table 8, the molecular docking of Paclitaxel with TNF-α reveals a docking score of -5.57 kcal/mol, forming two hydrogen bonds with amino acid residues Leu120 and Tyr151.

Table 8: Results of Molecular docking of Paclitaxel with various cancerous proteins

Sl. no	Inflammatory proteins name	The binding energy of Paclitaxel with different cancerous protein ΔG (Kcal/mol)	The binding energy of existing ligand with different cancerous protein ΔG (Kcal/mol)	H-bond interactions
1	TNF α	-5.57	-5.09	Tyr151 Leu120

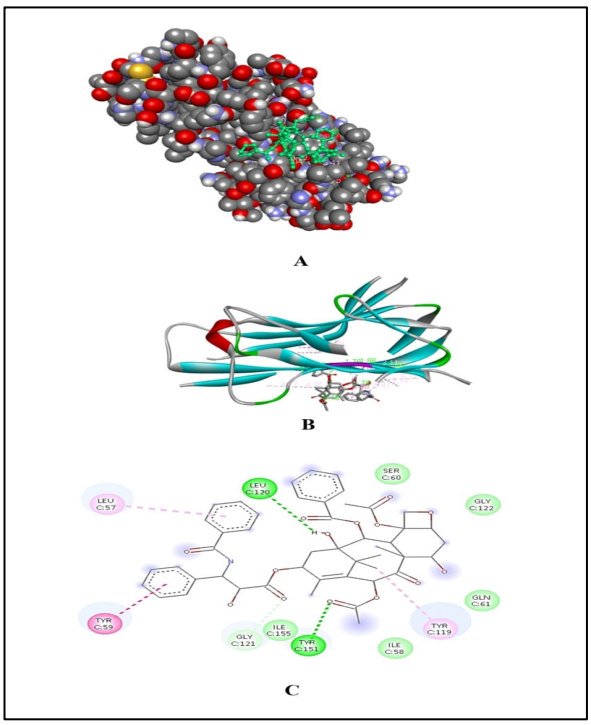

Figure 12: Molecular docking interactions of Paclitaxel with protein (TNF- α) (A) surface view of Paclitaxel and protein (TNF- α) binding complex. (B) binding pose of Paclitaxel (C) visualization of the docked complex with ligplot+ shows the binding interaction of Paclitaxel with protein (TNF- α)

4. Discussion

Paclitaxel loaded protein-based nanoparticles were developed utilizing a 3^2-factorial design, with concentrations of HSA and GTA acting as independent variables that underwent three distinct degrees of variations: low (-1), medium (0), and high (+1) which provides relevant result. In the FTIR analysis distinct peaks of ivermectin were identified. The C=O stretching, C-C stretching, C-N stretching, N-H stretching, C-H stretching and O-H stretching were detected at 1734.88 cm^{-1}, 1645.84 cm^{-1}, 1629.64 cm^{-1}, 11243.71 cm^{-1}, 3337.64 cm^{-1}, 2969.98 cm^{-1}, and 3060.46 cm^{-1}. It was discovered from the spectra that the drug and physical mixture showed no significant shifting or loss of functional peaks. The results indicated that the selected excipient was found to be compatible with the selected drug. The phase constituents of paclitaxel and paclitaxel with excipient were characterized by XRD. The crystalline nature of both compositions can be evidenced from the presence strong, symmetric fundamental reflections. In addition to this, no new phase formation can be evidenced from the XRD spectrum. The particle size of paclitaxel loaded protein-based nanoparticles ranges from 178.1 nm to 285.6 nm. The research demonstrated that the formulation with low concentration of HSA resulted in smaller particle size and the formulation with high concentration of HSA resulted in bigger particle size. The unimodal particle size distribution shown by the PDI values for all formulations denoted homogeneity within the samples. The formulation F8 showed the highest entrapment efficiency with 78.93%. The paclitaxel loaded protein-based nanoparticle had a zeta potential that ranges between -14.66 mV to -34.15 mV, signifying reduced amalgamation and stabilizing the nanoparticle as a result of electrostatic repulsion between particles. Furthermore, *in-vivo* investigation was conducted for comparison of the targeting efficiency of Paclitaxel loaded protein-based nanoparticle with that of Paclitaxel aqueous suspension in the brain. It can be concluded that the optimized formulation of Paclitaxel loaded protein-based nanoparticle was the better formulation and capable of entering the brain through the blood-brain barrier (BBB).

5. Conclusion

Paclitaxel loaded protein-based nanoparticles were efficiently formulated by desolvation method. It was statistically optimized using 3^2 factorial designs. The independent variables (concentrations of HSA and GTA) and the dependent variables (entrapment efficiency, drug release at 8 hrs and 12 hrs) served as the base for the study. Following a comprehensive evaluation of the formulation, it was discovered that formulation F8, which has low concentration of HSA, shows better results. From *in-vivo* study of the optimized formulation it was found out that the optimized formulation of Paclitaxel loaded protein-based nanoparticle effectively crossed the blood-brain barrier (BBB) and reached the brain.

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