Design and In-Vitro Characterization of Paclitaxel Loaded Nanoparticles with Emphasis Piperine

Dr. Rama Prasad Padhy* (Corresponding Author)[1]
[1]Professor Cum Vice-Principal, Department of Pharmaceutical Chemistry, Danteswari College of Pharmacy, Borapadar, Raipur Road, Jagdalpur, Chhattisgarh, India--494221
Email id-drramaprasadpadhy@gmail.com
Dr.Vakkalagadda Ravi Kumar[2]
[2]Professor, Department of Pharmaceutical Biotechnology, School of Pharmacy,
Guru Nanak Institutions Technical Campus, Ibrahimpatnam, Hyderabad, Telangana, India-501506
Dr. Sandhya. S[3]
[3]Professor and Head, Department of Pharmacology,
PSM College of Dental Science and Research, Akkikavu, Thrissur, Kerala, India-680519
Dr. Anoop Kumar N[4]
[4]Associate Professor, School of Family Health Studies, Kerala University of Health Sciences, Thrissur, Kerala, Adjunct Faculty, Department of Oral Pathology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India-600077
Dr. B.Ravindra Babu[5]
[5]Professor, Department of Pharmaceutics
Pulla Reddy Institute of Pharmacy, Dommadigu Village, Gummadidala (M),
Sangareddy District, Hyderabad, Telangana, India-502313
Dr. Ghanshyam Panigrahi[6]
[6]Professor and Head, Department of Pharmacology,
Royal College of Pharmacy, Berhampur, Odisha, India-760002

Abstract: A cytotoxic medication with an excellent mechanism of action against cancer cells is paclitaxel. When given as an infusion, it revealed severe dose-limiting toxicity. A proper delivery method is widely desired to combat these negative effects. When used with pharmaceuticals, bioenhancers increase and promote their bioavailability while lowering the risk of adverse drug reactions and the incidence of drug resistance. Therefore, nanoparticles are chosen as a delivery mechanism in this investigation based on the literature review. The goal of this research was to include herbal bioenhancer and paclitaxel into a nanoparticle technology. In that study, the nanoparticles were created using the Eudragit RLPO1 polymer and the emulsion solvent evaporation process. The produced nanoparticles underwent testing for drug entrapment, in vitro drug release, zeta potential, and particle size. Additionally, the pharmacokinetic profile and mean transit time (MTT) assay on a lung cancer cell line were used to evaluate the nanoparticles for an in vitro cell cytotoxicity research. The bioenhancer-loaded nanoparticles exhibit better in-vitro performance with a particle size range of 124 to 200 nm. In a 24-hour period, every formulation that was released contained between 82.71 and 95.47% of the medication. The drug's release kinetics followed Fick's law of diffusion and were best described by Higuchi's model. Every batch of nanoparticles demonstrated a good drug entrapment capacity between 57.51 and 86.12%. Although the pure medication solution did not entirely stop cells from proliferating, nanoparticle formulations considerably slowed it down in the MTT assay. Unexpectedly, the FNP 6 formulation had a stronger

antiproliferative effect on A549 cells than formulations with lower bioenhancer loading. The plasma level of FNP-6 in the in vivo pharmacokinetic assay was higher than that of any other formulation, including control. FNP-6 had an absolute bioavailability of 7.89 and an AUC of 6.423 g/mL. The fact that FNP-5 and FNP-6 included more bioenhancer than the other formulations could be the cause of their higher absolute bioavailability. It follows that the proliferative effect and bioavailability of an anticancer medication can be improved by the inclusion of a bioenhancer.
Keywords- *Nanoparticle, Paclitaxel, Piperine, Bioenhancer, ERLPO, and Anti-Cancer.*

INTRODUCTION

A bioenhancer is a substance that, at the dosage employed, increases the bio-efficacy and bioavailability of the medicine with which it is coupled without exerting any other pharmacological effects. [1] Bioenhancers boost the availability of medications when combined with them without disclosing the impact of pharmacological interactions. [2] Adding a bioenhancer results in decreased medicine dosage, expenses, resistance and the possibility of any negative consequences or side effects. A brand-new cytostatic medication with an original mode of action is called paclitaxel (Taxol). When treating serious conditions including ovarian cancer, breast cancer, and non-small cell lung cancer, the medication is particularly effective [3]. When used as a myelosuppressant, the medication exposed dose-limiting toxicity when given by a sustained infusion. However, exposed neuropathy is a very prevalent condition [4]. A proper delivery method is widely desired to combat these negative effects. One of the most innovative and promising drug delivery techniques is nanoparticles (NPs), which are able to entrap the therapeutic agent in a smaller amount while yet assisting in their transport to the targeted site of action and increasing their therapeutic efficacy. Additionally, it facilitates drug release and combats harmful side effects [5]. Therefore, in this investigation, a delivery mechanism made of nanoparticles was chosen. This study's goal was to entrap paclitaxel and a herbal bioenhancer in a nanoparticle system and test it in vitro for a variety of characteristics, such as how it affected a lung cancer cell line and its pharmacokinetic profile. Breast cancer, ovarian cancer, non-small cell lung cancer, and other tumours respond very well to the medication. It has a bitter flavour and is an alkaloid. It is a phytochemical found in food that has a variety of physiological effects, including excellent lung cancer protection.[6] According to published evidence, piperine increases the bioavailability of various medications in healthy volunteers, including phenytoin, theophylline, propranolol, and rifampin. The primary mechanisms of action of piperine are inhibition of CYP3A4 enzyme activity and P-gp protein transporters. [7-10] All of these hopeful findings led us to hypothesise that adding piperine, a bioenhancer, to paclitaxel would enhance its anticancer effects and lessen its side effects. [11-13] Therefore, by encasing paclitaxel and piperine in nanoparticles, we investigated the In vitro effects on lung cancer cell lines and assessed the pharmacokinetic profile.

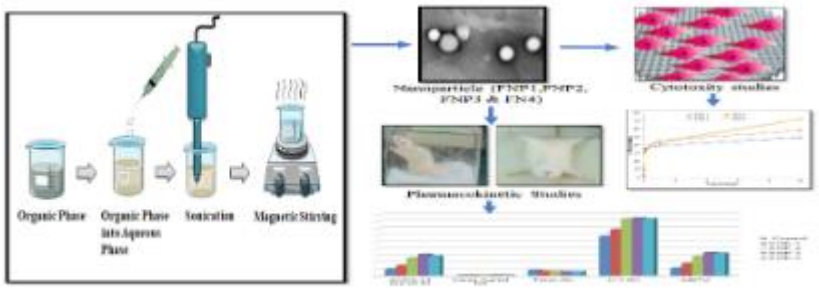

Fig1-Graphical Abstract

Formulation Code	*Drug (mg)*	*Bioenhancer (mg)*	*ERLPO (mg)*	*PVA (%)*
FNP-1	20	10	100	1.25
FNP-2	20	10	150	1.25
FNP-3	20	20	100	1.25
FNP-4	20	20	150	1.25
FNP-5	20	30	100	1.25
FNP-6	20	30	150	1.25

Table 1- Different formulation of PTX-PIP loaded ERLPO Nanoaprticles

Physical properties	*Observation*	*Standard*
Form	Crystalline powder	Crystalline powder
Colour	White	White to Off-white
Taste	-	Tasteless
Odour	Odourless	Odourless

Table 2- Physical appearance of Paclitaxel drug

METHODS AND MATERIALS

(i) Materials-The name of the medication used to treat cancer is paclitaxel (PTX). The source of the PTX was an Intas Pharmaceutical in Ahemdabad, India. It hardly dissolves in water. The chosen polymer for this project is called Eudragit RLPO (ERLPO), and other materials utilised in it include piperine (PPN), polyvinyl alcohol (PVA), acetone, and an emulsifying agent called polyvinyl alcohol. All of these materials were purchased from Merck in Mumbai, India.

(ii) Methods

(a)Pre-formulation Research- Prior to creating a new delivery system, it is crucial to conduct pre formulation studies on the drug molecule. To understand the qualities of the medicine, it is necessary to study its physicochemical properties. As per the recommended methodology, we examined the melting point, solubility, medication and excipient interaction research, and UV absorption in this investigation.

(iii) Creating Nanoparticles- In the current study, nanoparticles were created utilising the sonicatior and the emulsion solvent evaporation method. The PTX and ERLPO were initially acetone-dissolved. Then, to create an O/W type emulsion, this drug-polymer solution was combined with an aqueous phase that contained a surfactant (PVA) and a bioenhancer. The chosen oil to water phase ratio was 1 to 9. The entire procedure involved sonication in order to create nanodroplets from the O/W emulsion. To evaporate the organic phase, the aforementioned produced emulsion was sonicated and then held in magnetic stirring at 400 rpm under ambient conditions for two hours.[14,15] Six distinct formulations were created by adjusting various constituents. Table 1 displays the formulation variables.

(iv) Zeta Potential, Polydispersity Index, and Particle Size- Zeta sizer employed the polydispersity index and zeta potential dynamic light scattering approach to quantify particle size (Malvern Co., Worcestershire, UK). The stability of colloidal dispersions and the potential difference between the stationary and dispersion medium are also crucial indicators of zeta potential. The water used to dilute the 1-mL sample of paclitaxel-piperine NPs was filtered through a 10-mL volumetric bottle ten times. One millilitre of this solution is put into a disposable cuvette.[13]

(v) Percentage of Drug Loading and Encapsulation Efficiency- 10 mg of NPs were combined with 10 mL of distilled water until the equilibrium solubility was reached in order to assess the effectiveness of the encapsulation. Then, using high-speed cooling centrifugation, this was separated.

Filters were used to collect and purify the clear supernatant. 4 mL of methanolic HCl were added to 1 mL of the filtrate. The final sample was examined at 230 nm using a UV-visible spectrophotometer. Using Equation, the percentage of encapsulation efficacy was calculated (1).

$$\% \text{ Encapsulation efficacy} = [\frac{\text{Drug in supernatant liquid}}{\text{Total drug added}}] \times 100 \quad (1)$$

The drug loading capacity was determined by dissolving 5 mg of NPs in 5 mL of methanolic HCl and filtering the resulting solution through an Axiva syringe filter with a 0.2 m pore size. With the aid of a UV visible spectrophotometer, the concentration of PTX in the sample was detected at 230 nm. Equation was used to determine the drug loading capacity (2).

$$\% \text{ Drug loading} = (\frac{\text{Mass of PTX} - \text{NPs}}{\text{Mass of NP recovered}}) \times 100 \quad (2)$$

(vi) Using the dialysis bag diffusion technique- an in-vitro drug release research was conducted. The dialysis bag was filled with nanoparticles that had been pre-weighed and were equivalent to 20 mg of PTX. This bag was then placed inside the USP type-II dissolution device with phosphate buffer, pH 7.4. Under 100 rpm, the temperature was kept at 37°C and 1°C. To keep the sink condition throughout the experiment, five millilitres of aliquots were periodically taken and replenished with brand-new buffer. By calculating absorbance at a maximum of 227 nm in a UV-vis spectrophotometer (UV 1800 Shimadzu), the filtered aliquots were identified.[13]

(vii) MTT Assay- In A549 cells, the cytotoxicity of free PTX NPs and enhanced PTX-PPN NPs was assessed using the MTT assay. In a 96-well plate, A549 cells were planted at a density of 3 to 4 103 cells per well. Different nanoparticle formulations (ranging in drug concentration from 0.001 to 10 g/mL) were added after 12 hours, and the plates were then incubated for 24 hours. The PTX was dissolved in ethanol at concentrations ranging from 0.25 to 2.5 mg/mL to create the PTX standard solution, which was then appropriately diluted with distilled water. Microplate readers were employed to take measurements.

(viii) Study of Pharmacokinetics- For this investigation, male Sprague-Dawley rats weighing between 240 and 300 g were chosen. In each experiment, four or five animals were confined in a cage with the temperature and humidity kept constant. Prior to testing, SD rats were allowed unrestricted access to water after going without food for at least 24 hours. Each rat received ether exposure to put it to sleep. Right femoral artery was cannulated using polyethylene tubing to collect blood samples. Throughout the course of the trial, paclitaxel was administered orally at a dose of 40 mg/kg body weight to 21 animals, who were then separated into 7 groups at random: Control group (group A), FNP-1 group (group B), FNP-2 group (group C), FNP-3 group (group D), and FNP-4 group (group E). FNP-5 Group F and FNP-6 Group G Blood samples were taken from each group (A to G) after 0, 25, 0, 50, 1, 2, 4, 6, 8, 12, and 24 hours. After receiving medication, the animals were exsanguinated by cardiac stick under isoflurane anaesthesia, and blood samples were then taken. In heparinized tubes, blood samples were put, and they were promptly centrifuged to separate them. The collected plasma was centrifuged and then kept at -20°C for analysis.

(ix) Short-term stability- experiments of six months were conducted on the produced nanoparticles to ascertain any physical and chemical alterations. For this aim, the microcrystals were maintained in a stability chamber at a variety of temperatures and relative humidity (RH) levels: 5 °C, 25 °C, 30 °C, and 40 °C. [16] They were FTIR analysed after six months to determine how the functional groups had changed as a result of the chemical instability. The measurement of crystal size, drug release, and permeability were also investigated.

(x) Statistical Analysis- The experimental data were analysed using a one-way analysis of variance (ANOVA). Data analysis was done using the Graph Pad Prism software-5, San Diego, CA, USA. The mean and standard deviation (SD) were used to determine all of the data, and mean variations were deemed significant at p 0.05.

CONCLUSION AND DISCUSSION

(i) Physical appearance- is useful in defining the physical qualities of drugs and other pharmaceutical products, according to pre-formulation research. Understanding things like look, colour, flavour, and odour is helpful. The physicochemical characteristics of the paclitaxel medication as discovered for this investigation are shown in Table 2. A solid's melting point is the temperature at which it will melt. Pressure affects a substance's melting point. The melting point apparatus, type MPA350, was used to determine the melting point. It was examined three times, and the average temperature is recorded as 214.6°C. UV spectroscopy was carried out by UV 1800 Shimadzu to determine the maximum value and build the standard curve. In Fig. 1, the standard curve is shown. Dimethyl sulfoxide (DMSO) and DMFO had a high solubility for the medication, while water and chloroform had low solubility. Table 3 displays the solubilization test data. From the ester groups presented in Fig. 2, the FTIR investigation identified the drug's primary peaks at 3479-3300 cm-1 (N-H stretching vibrations), at 2976-2885 cm-1 (CH2 asymmetric and symmetric stretching vibrations), and at 1734 assigned to C=O stretching vibration. These results demonstrated the drug's purity because these peaks are remarkably comparable to the typical paclitaxel peak.

(ii) Particle Size and Zeta Potential- According to the results of the zetasizer investigation, the particle sizes of all the batches ranged from 124 to 204 nm. However, FNP-2, FNP-4, and FNP-6 were larger (179, 195, and 204 nm, respectively), which may have been caused by their increased polymer content. For each formulation, an excellent polydispersity index of less than 0.5 was discovered, indicating evenly dispersed particles. The FNP3 batch's zeta potential shows that there has been no particle agglomeration. The zeta potentials of the batches FNP-1, FNP-2, FNP-3, and FNP-4 showed that the higher piperine concentrations had no effect on it. The zeta potential graph for batch FNP3, which was the best example of an average value, was shown in Figure 3.

(iii) Study on in vitro drug release- In vitro drug release was examined using the dialysis bag diffusion method in phosphate buffer, pH 7.4. Over the course of 24 hours, it was discovered that these NPs maintained their cumulative percentage drug release (Fig. 4). All of the formulation batches of NPs that were tested for drug release in vitro displayed an initial burst of drug release, which might be explained by the presence of weakly bound drug on the NPs' surface. The range of medicines released across all batches in a 24-hour period was between 82.71 and 95.47%. First order analysis, zero order, and Higuchi kinetic models all included data. In Higuci's kinetic, the regression coefficient value, or R^2, was highest. Thus, it may be said that the kinetic output was in accordance with Fick's distribution law.

(iv) Evaluation of entrapment effectiveness- The drug entrapment capacities of all batches of nanoparticles were good, ranging from 57.51 to 86.12%. The concentration of the polymer improved the entrapment efficiency. As a result, compared to other FNPs, FNP-2, FNP-4, and FNP-6 showed a higher entrapment efficiency. The outcomes were displayed in Table 4. The range of the loading capacity was 0.55 to 0.92 mg/mL. Additionally, factors like entrapment and particle size increased with polymer concentration.

(v) MTT Assay- Using the MTT Assay, the effects of several preparations on A549 cells were discovered (Fig. 5). As can be shown, the examined cell lines were cytotoxic to drug- and bioenhancer-loaded nanoparticles in a dose- and time-dependent manner. Despite being a potent anticancer agent, the pure drug solution did not entirely block cell multiplication. On the other hand, a medication and bioenhancer combination drastically decreased cell proliferation. Surprisingly, formulation FNP 6 had a greater antiproliferative effect on A549 cells compared to formulation FNP 1 because it had a larger bioenhancer loading. This could be as a result of a potential synergistic impact of a medicine and a bioenhancer. It can be inferred from this that adding a bioenhancer to an anticancer medication will increase its proliferative effect. Bioavailability information can be used to support this conclusion further. After receiving paclitaxel treatment for 24 hours, the IC50 value for samples of A549 cancer cell was calculated to be 242.58 M. In comparison to the control, the results of cell inhibition of A549 cells have dramatically lowered to almost 49.93 4.11%, 58.17 4.55%, 72.30 4.76%, 75.85 4.25%, 79.38 4.36%, and 88.06 3.33%.

Solvent	*Observation*	*Standard*
DMSO	Very soluble	Very soluble
Dimethyl formamide	Very soluble	Very soluble
Aqueous buffer	Sparingly soluble	Sparingly soluble
Water	Practically insoluble	Practically insoluble
Chloroform	Practically insoluble	Practically insoluble
Ethanol	Freely soluble	Freely soluble

Table 3- Solubility chart of PTX in different solvents

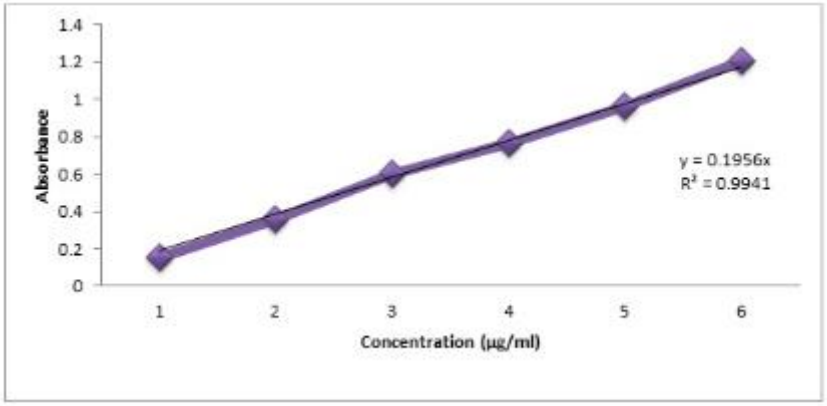

Fig2- The standard curve of PTX

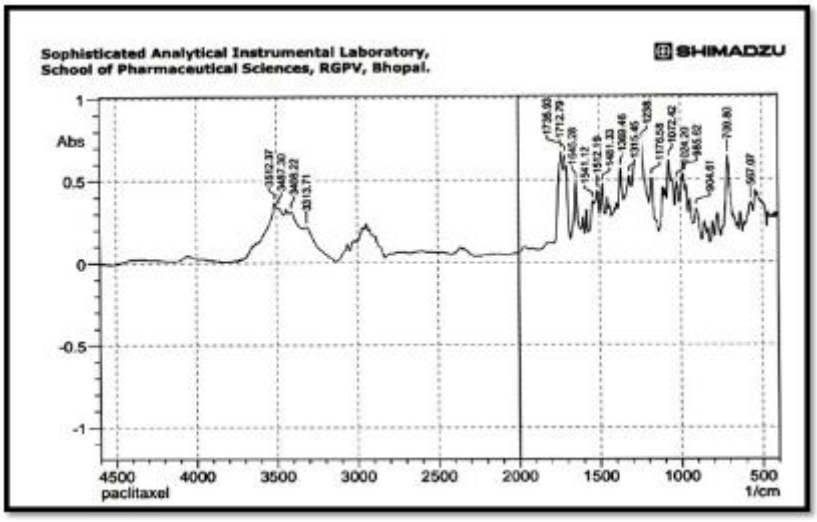

Fig3- FTIR spectra of PTX

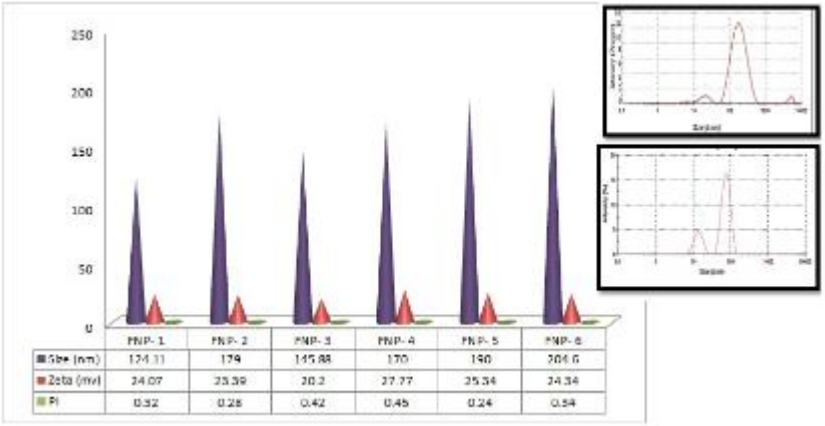

Fig4- Particle size, zeta potential and poly dispersity index of different formulations

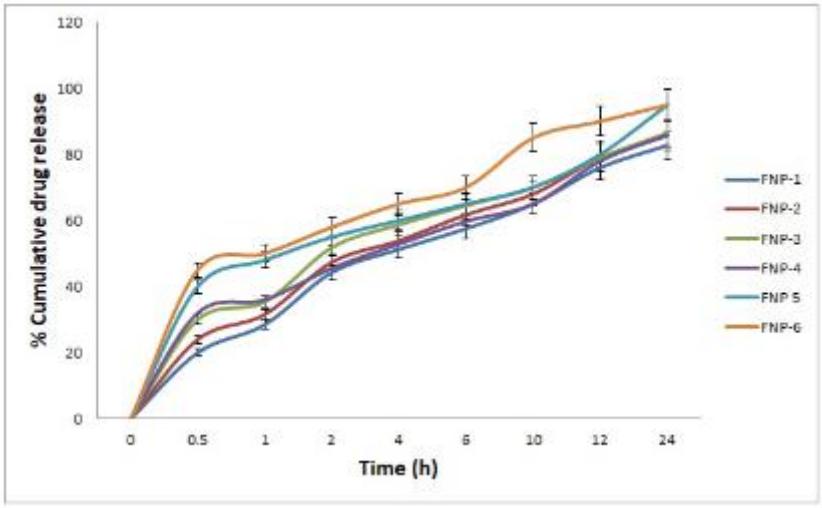

Fig5- Comparison of in vitro drug release data of different formulations

(vi) The Institutional Committee for Animal Ethics accepted- the experimental procedure for the pharmacokinetic study with approval number 1189/PO/Re/S/08/CPCSEA. The drug's maximum plasma level (Cmax) and time to attain Cmax (Tmax) were directly derived from the observed data (Fig. 6). Using the linear trapezoidal technique, the area under curve (AUC) for the time range of 0 to 24 h (AUC0-24) was calculated. Table 5 displayed the pharmacokinetic parameters for each formulation. FNP-6 had the highest plasma level of every formulation, including control. FNP-6 had an absolute bioavailability of 7.89 and an AUC of 6.423 g/ml. The fact that FNP-5 and FNP-6 included more bioenhancer than the other formulations could be the cause of their higher absolute bioavailability. The bioenhancer content of FNP-5 and FNP-6 was comparable and higher than that of other formulations. As a result, the outcomes of the bioavailability investigation for these two formulations were quite identical.

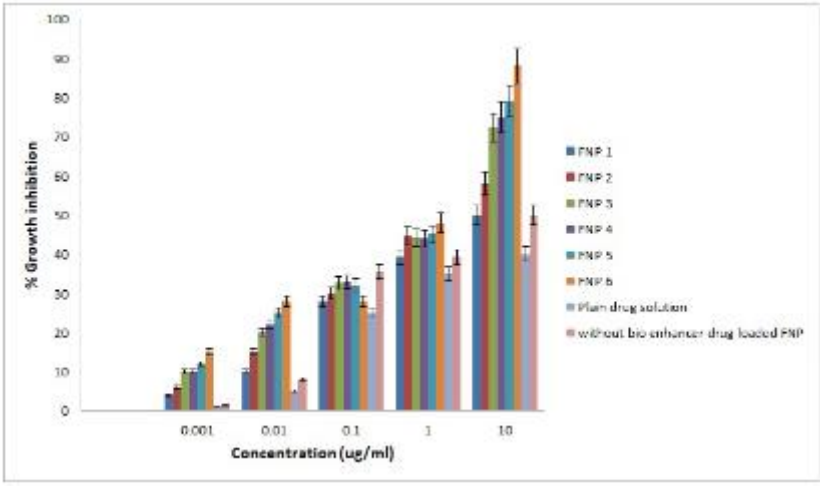

Fig6- Percentage cellular inhibition of different formulations at different concentration by MTT assay

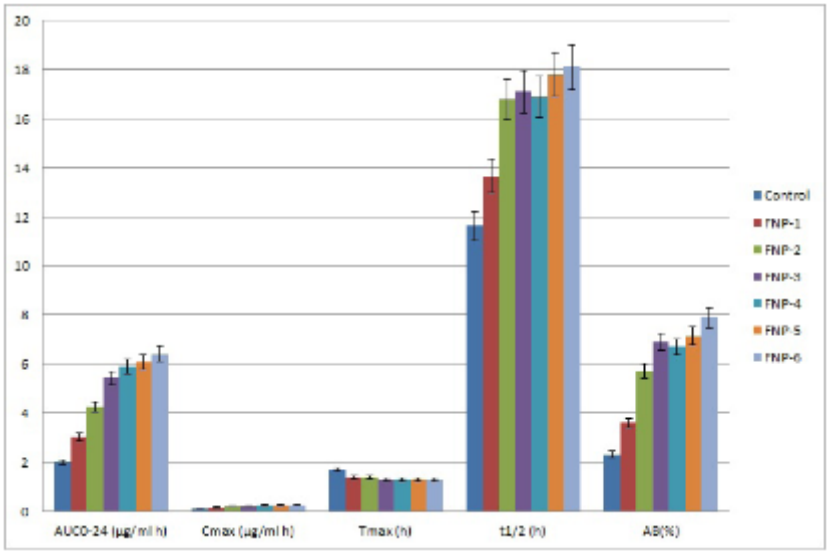

Fig7- In vivo pharmacokinetic study result of different formulations

Formulations	*%EE*	*LC (mg/ml)*
FNP 1	57.51 ± 1.2	0.55 ± 0.8
FNP 2	64.57 ± 0.8	0.64 ± 1.1
FNP 3	68.78 ± 1.0	0.68 ± 0.5
FNP 4	70.36 ± 1.0	0.70 ± 1.2
FNP 5	79.98 ± 0.5	0.89 ± 1.3
FNP 6	86.12 ± 1.1	0.92 ± 1.1

Table 4- Entrapment efficiency and loading capacity of formulations

Parameters	*Paclitaxel Control*	*FNP-1*	*FNP-2*	*FNP-3*	*FNP-4*	*FNP-5*	*FNP-6*
AUC_{0-24} (µg/ml h)	2.011 ± 0.520	3.042 ± 0.490	4.270 ± 1.070	5.423 ± 1.036	5.920 ± 1.430	6.070 ± 1.087	6.423 ± 1.021
C_{max}(µg/ml h)	0.120 ± 0.028	0.158 ± 0.040	0.218 ± 0.054	0.221 ± 0.055	0.247 ± 0.057	0.258 ± 0.032	0.261 ± 0.034
T_{max}(h)	1.7 ± 0.65	1.4 ± 0.35	1.4 ± 0.45	1.3 ± 0.39	1.3 ± 0.38	1.3 ± 0.45	1.3 ± 0.39
$t_{1/2}$ (h)	11.63 ± 2.47	13.67 ± 3.12	16.78 ± 3.89	17.10 ± 4.35	16.89 ± 4.30	17.78 ± 3.54	18.10 ± 4.35
AB(%)	2.32	3.61	5.72	6.89	6.70	7.14	7.89

Table 5- Various pharmacokinetic parameter of paclitaxel formulation

(vii) Studies on Stability - In this investigation, all the formulations were individually heated to 4°C, 25°C, and 40°C for three months, after which they were reexamined for factors such physical appearance, drug entrapment, particle size, and FTIR. Particle size results revealed no appreciable changes, while the poly dispersity index rose. The agglomeration of the nanoparticles during storage may be to blame for the effect that was observed. However, no colour change or microbiological growth was discovered after a physical examination. Drug entrapment in nanoparticles was somewhat lower than before, however the difference was not statistically significant. For the improved batch FNP-6, only 0.8 to 1.2% variations in drug entrapment were discovered. The synthesised formulation's FTIR spectra showed no alterations in the main peak or any other chemical modifications.

CONCLUSIONS

This research sought to combine paclitaxel with a natural bioenhancer in a nanoparticle technology. The nanoparticles were created for this purpose using emulsion solvent evaporation. The produced nanoparticles demonstrated favourable in vitro characteristics and a notable decrease in cell proliferation in the MTT assay of the six formulations, FNP-6 was deemed to be the best. In comparison to other cells, it had a greater anti-proliferative effect on A549 cells. The plasma level of FNP-6 was likewise higher than that of the control and other formulations. It follows that the proliferative effect and bioavailability of an anticancer medication can be improved by the inclusion of a bioenhancer. (Expressed as a graphical abstract).

REFERENCES

[1]. DB M, S S, KR M. Role of Piperine as an Effective Bioenhancer in Drug Absorption. Pharm Anal Acta [Internet]. 2018 [cited 2021 Jun 24];09(07):7–10.

[2]. Gottesman MM, Pastan IH. The Role of Multidrug Resistance Efflux Pumps in Cancer: Revisiting a JNCI Publication Exploring Expression of the MDR1 (P-glycoprotein) Gene [Internet]. Vol. 107, Journal of the National Cancer Institute. Oxford University Press; 2015 [cited 2021 Jun 24].

[3]. Key A, Sch T, Pharm AJ, Verma S, Rai S. Scholars Academic Journal of Pharmacy (SAJP) Bioenhancers from Mother Nature : A Paradigm for Modern Medicines. 2018;

[4]. Ramalingam S, Belani CP. Paclitaxel for non-small cell lung cancer. Expert Opin Pharmacother. 2004;5(8):1771–80.

[5]. Das S, Suresh PK , Desmuk h R . Desig n of Eudrag it R L 100 nanoparticles by nanoprecipitation method for ocular drug delivery. Nanomedicine Nanotechnology, Biol Med [Internet]. 2010;6(2):318–23.

[6]. Yang W, Xia Y, Fang Y, Meng F, Zhang J, Cheng R, et al. Selective Cell Penetrating Peptide-Functionalized Polymersomes Mediate Efficient and Targeted Delivery of Methotrexate Disodium to Human Lung Cancer In Vivo. Adv Healthc Mater. 2018;7(7):1–8.

[7]. Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, Fromm MF. Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4. J Pharmacol Exp Ther. 2002;302(2):645–50.

[8]. Pradeep CR, Kuttan G. Piperine is a potent inhibitor of nuclear factor-κB (NF-κB), c-Fos, CREB, ATF-2 and proinf lammatory c y tok ine gene ex pression in B16F-10 melanoma cells. Int Immunopharmacol. 2004;4(14 SPEC.ISS.):1795–803.

[9]. Greenshields AL, Doucette CD, Sutton KM, Madera L, Annan H, Yaffe PB, et al. Piperine inhibits the growth and motility of triple-negative breast cancer cells. Cancer Lett [Internet]. 2015;357(1):129–40.

[10]. Selvendiran K, Prince Vijeya Singh J, Sakthisekaran D. In vivo effect of piperine on serum and tissue glycoprotein levels in benzo(a)pyrene induced lung carcinogenesis in Swiss albino mice. Pulm Pharmacol Ther [Internet]. 2006 Apr 21 [cited 2021 Jun 29];19(2):107–11.

[11]. Volak LP, Ghirmai S, Cashman JR, Court MH. Curcuminoids inhibit multiple human cytochromes P450, UDP- glucuronosyltransferase, and sulfotransferase enzymes, whereas piperine is a relatively selective CYP3A4 inhibitor. Drug Metab Dispos. 2008;36(8):1594–605.
[12]. Motiwala MN, Rangari VD. ScienceDirect Combined effect of paclitaxel and piperine on a MCF-7 breast cancer cell line in vitro : Evidence of a synergistic interaction. Synergy [Internet]. 2015;2(1):1–6.
[13]. Pandit a D, A huja A, Velpandian T, Lather V, Dut t a T, K har RK. Characterization and in vitro assessment of paclitaxel loaded lipid nanoparticles formulated using modified solvent injection technique. Pharmazie [Internet]. 2009 [cited 2021 Jun 29];64(5):301–10.
[14]. Gandhi A, Jana S, Sen KK. In-vitro release of acyclovir loaded Eudragit RLPO® nanoparticles for sustained drug delivery. Int J Biol Macromol [Internet]. 2014 Jun 1 [cited 2021 Jun 22];67:478–82.
[15]. Hoa LTM, Chi NT, Ng uyen LH, Chien DM. Preparat ion and characterisation of nanoparticles containing ketoprofen and acrylic polymers prepared by emulsion solvent evaporation method. J Exp Nanosci [Internet]. 2012 Mar [cited 2021 Jun 23];7(2):189–97.
[16]. Deshmukh R, Harwansh RK, Paul S Das, Shukla R. Controlled release of sulfasalazine loaded amidated pectin microparticles through Eudragit S 100 coated capsule for management of inflammatory bowel disease. J Drug Deliv Sci Technol. 2020;55:101495.