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Research Article

Design and Characterization of Paclitaxel Loaded Nanoparticles with Piperine

Gyanesh K. Sahu[1], Harish Sharma[2], Swarnali D. Paul[3], Ajazuddin[4], Chanchal D. Kaur[5]*

[1]*Rungta College of Pharmaceutical Sciences and Research, Kohka, Bhilai, Chhattisgarh, INDIA*
[2]*Rungta Institute of Pharmaceutical Sciences, Kohka, Bhilai, Chhattisgarh, INDIA*
[3]*Shri Shankaracharya College of Pharmaceutical Sciences, Bhilai, Chhattisgarh, INDIA*
[4]*Department of Pharmaceutics, School of Pharmacy and Technology Management, SVKM's NMIMS, Shirpur, Maharashtra, INDIA*
[5]*Rungta College of Pharmaceutical Sciences and Research, Raipur, Chhattisgarh, INDIA*

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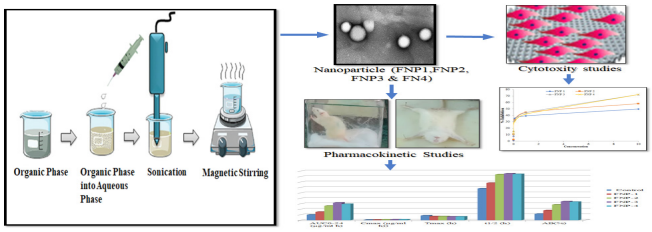
ABSTRACT

Paclitaxel is a cytotoxic drug having wonderful mechanism against cancer cells. However, it showed severe dose-limiting toxicity when administered as infusion. To overcome these side effects a suitable delivery system is highly desirable. Bioenhancers, when mixed with drugs promote and augment their bioavailability and reduced incidence of drug resistance and risk of adverse drug reaction. Therefore, based on the literature survey nanoparticles are selected as a delivery system in this study. The object of this work was to entrap paclitaxel and herbal bioenhancer in a nanoparticle system. In that work the nanoparticles prepared the by emulsion solvent evaporation method with Eudragit RLPO polymer. The prepared nanoparticles were evaluated for particle size, zeta potential, drug entrapment, in vitro drug release. Further the nanoparticles were evaluated for in vitro cell cytotoxicity study by mean transit time (MTT) assay on lung cancer cell line and pharmacokinetic profile. The bioenhancer loaded nanoparticles show betrer *in-vitro* properties with 124 to 200 nm particle size range. All the formulations released within the range of 82.71 to 95.47% of drug in 24 hours. The release kinetic of drug was best fitted to Higuchi's model and was following Fick's law of diffusion. All the nanoparticle batches had good drug entrapment capacity in the range of 57.51 to 86.12%. The pure drug solution could not inhibit the cell proliferation completely but the nanoparticle formulations significantly reduced the cell proliferation in MTT assay. Surprisingly, formulation with higher bioenhancer loading (FNP 6) showed a higher antiproliferative effect on A549 cells. In *in vivo* pharmacokinetic assay, the plasma level of FNP-6 was highest than other formulation including control. The AUC of FNP-6 was 6.423 µg/mL and the absolute bioavailability of FNP-6 was 7.89. FNP-5 and FNP-6 had a higher bioenhancer quantity as compare to the other formulation this may be possible reason of their higher absolute bioavailability. Therefore, it can be concluded that addition of bioenhancer with antitumor drug can enhance its proliferative effect and bioavailability.

Introduction

A bioenhancer is an agent that is able to increase bio-efficacy and bioavailability of a certain drug with which it is combined, without any other pharmacological action of its own at the dose used.[1] Bioenhancers, when mixed with drugs promote and increase their availability without indicating the effect of drug interactions.[2] The benefits of adding bioenhancer result reduced drug dosage, costs,

Graphical Abstract



***Corresponding Author:** Dr. Chanchal Deep Kaur

Address: Rungta Institute of Pharmaceutical Sciences and Research, Kohka, Bhilai, Chhattisgarh, INDIA

Email ✉**:** drcdkresearch@gmail.com

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Table 1: Different formulation of PTX-PIP loaded ERLPO nanoaprticles

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

resistance and risk of any adverse reactions or side effects.

Paclitaxel (Taxol) is a new cytostatic drug with a unique mechanism of action. The drug is very effective in serious illnesses such as ovarian cancer, breast cancer, and non-small cell lung cancer.[3] However the drug exposed dose-limiting toxicity when administered on a prolonged infusion as myelosuppression. But in short exposed neuropathy is very common.[4] To overcome these side effects a suitable delivery system is highly desirable.

Nanoparticles (NPs) are one of the unique and promising drug delivery systems which are capable of entrapping the therapeutic agent in less amount although help them to reach at the specific site of action and enhances their therapeutic efficacy. Further it helps to sustain the drug release and overcome toxic side effects.[5] Therefore nanoparticles were selected as a delivery system in this study. The object of this work was to entrap paclitaxel and herbal bioenhancer in a nanoparticle system and evaluated it in vitro for different properties including its effect on lung cancer cell line and pharmacokinetic profile.

The drug is very effective in different cancers such as non-small cell lung cancer breast cancer, and ovarian cancer. It is an alkaloid with a bitter taste. It is, a dietary phytochemical with many physiological effects including wonderful lung cancer prevention action.[6] As per reported data piperine enhances the bioavailability of many drugs like phenytoin, theophylline, propranolol and rifampin in healthy volunteers. Piperine mainly acted by inhibiting the CYP3A4 enzyme activity and P-gp protein transporters.[7-10] All these encouraging data directed us to speculate that combining the bioenhancer piperine with paclitaxel may improve its antitumor effect and reduce different side effects.[11-13] Therefore, we explored the In vitro effects of paclitaxel and piperine by entrapping them into nanoparticles on lung cancer cells lines and evaluated the pharmacokinetic profile.

Material and Methods

Materials

The drug known to fight cancer its name is paclitaxel (PTX). PTX was procured from an Intas Pharmaceutical, Ahemdabad, India. It is almost insoluble in water. The selected polymer used in this work is Eudragit RLPO (ERLPO), Piperine (PPN) used as a bioenhancer, Polyvinyl alcohol (PVA) was used as an emulsifying agent and acetone was used as solvent which were obtained from Merck, Mumbai, India.

Methods

Pre-formulation Studies

Preformulation studies of the drug molecule are necessary to carry out at the very beginning before developing a new delivery system. To develop physicochemical properties are need to be studied for understanding the characteristics of the drug. In this study we checked melting point, solubility, drug and excipient interaction study and UV absorption as per reported method.

Formulation of Nanoparticles

In the present study, nanoparticles were prepared by emulsion solvent evaporation method by using sonicatior. At first, the PTX and ERLPO were dissolved in acetone. Then this solution of drug-polymer was mixed with an aqueous phase containing a surfactant (PVA) and bioenhancer to make an O /W type emulsion. The selected ratio of oil and water phase was 1: 9. The whole process was carried out under sonication to form nanodroplets from the O/W emulsion. After sonication, the above prepared emulsion was kept into magnetic stirring at 400 rpm under atmospheric condition for 2 hrs. to evaporate the organic phase.[14,15] Total six formulations were prepared by varying different ingredients. The formulation variables are presented in Table 1.

Particle Size, Zeta Potential and Polydispersity Index

To characterize particle size, polydispersity index and zeta potential dynamic light scattering technique was used by Zeta sizer (Malvern Co., Worcestershire, UK).

Zeta potential is an important indicator of the stability of colloidal dispersions and potential difference between the stationary and dispersion medium. The 1-mL sample of paclitaxel-piperine NPs was diluted 10 times with water filtered through a 10 mL volumetric bottle. In this solution, 1-mL is placed in a disposable cuvette.[13]

Percentage of Encapsulation Efficacy and Drug Loading

To determine the encapsulation efficacy, 10mg of NPs were mixed with 10 mL distilled water till the equilibrium solubility was achieved. Then this was centrifuged by high-speed cooling centrifugation. The clear supernatant was collected and filtered. 1-mL of the filtrate was mixed with 4 mL of methanolic HCl. Resulting sample was analyzed on UV visible spectrophotometer at 230 nm. The percentage of encapsulation efficacy was determined by using the Equation (1).

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

For the calculation of drug loading capacity, NPs (5 mg) were dissolved in 5 mL of methanolic HCl and the solution was filtered through 0.2 µm filter (Axiva syringe filter). PTX concentration in the sample was determined using UV visible spectrophotometer at 230 nm. The drug loading capacity was determined using Equation (2).

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

In-vitro Drug Release Study

In vitro drug release study was carried out by dialysis bag diffusion technique. Pre-weighed nanoparticles (equivalent to 20 mg PTX) were placed inside the dialysis bag. This bag was then immersed in phosphate buffer, pH 7.4 in the USP type- II dissolution apparatus. The temperature was maintained at 37 ± 1°C under 100 rpm speed. At regular time intervals, five millilitres of aliquots was collected and replaced with fresh buffer to maintain the sink condition during the experiment. The filtered aliquots were determined in UV–vis spectrophotometer (UV 1800 Shimadzu) by calculating absorbance at λ max of 227 nm.[13]

MTT Assay

The cytotoxicity of free PTX NPs and optimized PTX-PPN NPs was evaluated in A549 cells by MTT assay. Briefly, A549 cells were seeded in a 96-well plate at a density of 3 to 4×10^3 cells per well. After 12 hrs, different nanoparticle formulations (0.001 to 10 µg/mL drug concentrations) were added and plates were incubated for 24 hrs. PTX standard solution was prepared by dissolving the PTX in ethanol ranging from 0.25 to 2.5 mg/mL and then suitably diluted with distilled water. Measurements were taken using a microplate reader.

Pharmacokinetic Study

Male Sprague- Dawley rats (240–300 gm) were selected for this study. In all experiments, the animals were housed, four or five in each cage, in a maintained temperature and moisture. SD rats were fasted for at least 24 hours before testing and were given water freely. Each rat was exposed to ether for anesthetized. For blood sampling polyethylene tubing was cannulated to right femoral artery. The dose of paclitaxel used for oral administration was 40mg / kg body weight throughout the study, 21 animals were randomly divided into 7 groups: Group A for control, Group B for FNP-1, Group C for FNP-2, Group D for FNP-3, and Group E for FNP-4. Group F for FNP-5 and Group G for FNP-6. Blood samples was performed to all group (A to G) 0.25, 0.5,1,2,4,6,8,12 and 24 hrs. Following treatment, the animals were killed by cardiac stick exsanguination under isoflurane anaesthesia followed by collection of blood samples. Blood samples were placed into heparinised tubes and separated immediately by centrifugation. After centrifugation, the obtained plasma was stored at -20°C until analyzed.

Stability Studies

The prepared nanoparticles were subjected to six months short term stability studies to determine their physical and chemical changes. For this purpose the microcrystals were kept in stability chamber at different temperatures and humidity**:** 5 °C, 25 °C/60% RH; 30 °C/65% RH; 40 °C/ 75% RH.[16] After six months, they were studied with FTIR to find the changes in functional groups as a result of chemical instability. They also studied for crystal size measurement, drug release and permeability.

Statistical Analysis

A one-way analysis of variance (ANOVA) was performed for analysis of the experimental data. Graph Pad Prism software-5, San Diego, CA, USA was used for statistical analysis of the data. All the data were determined by the mean ± standard deviation (SD) and mean variations were considered to be significant at $p < 0.05$.

Result and Discussion

Pre-formulation studies

Physical appearance is helpful to define the physical properties of drug and other pharmaceutical products. It helps to know about appearance, color, taste, odour etc. Table 2 represented physical appearance of paclitaxel drug as found for this study.

Melting point is defining the temperature at which a given solid will melt. The melting point of a substance depends on pressure. Melting point was found by the Melting point apparatus model MPA350. It was studied in triplicate and average data is reported as 214.6°C

For determination of ƛ max and construction of standard curve UV spectroscopy was performed by UV 1800 Shimadzu. The standard curve is displayed in Fig. 1.

The drug was highly soluble in Dimethyl sulfoxide (DMSO) and DMFO and poorly soluble in water and chloroform. The data for solubilisation test is presented in Table 3.

FTIR study revealed the major peaks of the drug 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 from the ester groups shown in Fig. 2. From this data it was confirmed that the drug was pure as these peaks are very similar to standard paclitaxel peak.

Table 2: Physical appearance of Paclitaxel drug

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

Particle Size and Zeta Potential

Particle size of all the batches were ranged between 124 to 204 nm as found in zetasizer study. However FNP-2, FNP-4 and FNP-6 had a greater size (179, 195 and 204 nm respectively) may be due to higher amount of polymer content. A good polydispersity index of below 0.5 was found for every formulation which indicated uniformly dispersed particles. Zeta potential of FNP3 batch was indicates no agglomeration of the particles. The increased concentrations of piperine have not affected the zeta potential as revealed by the zeta potentials of the batches FNP-1, FNP-2, FNP-3 and FNP-4. Fig. 3 represented the zeta potential graph of batch FNP3 which was best of an average value.

In-vitro drug release study

In-vitro drug release from these prepared PTX loaded NPs were tested by using dialysis bag diffusion process in phosphate buffer, pH 7.4.The cumulative percentage drug release from these NPs was found sustained over a period of 24 hrs. (Fig. 4). The *in-vitro* drug release from all the formulation batches of NPs showed an initial burst release of drugs, which might be attributed to the presence of weakly bound drug on the surface of the NPs. The percentage of drugs released in all batches in 24 hrs. ranged from 82.71 to 95.47%. Data were included in the kinetic model of first order analysis, zero order and Higuchi kinetic. The regression coefficient value or R^2 was highest in Higuci's kinetic. It can therefore be concluded that the kinetic output was following Fick's distribution law.

Determination of entrapment efficiency

All the nanoparticle batches had good drug entrapment capacity in the range of 57.51 to 86.12%. The entrapment efficiency was increased with polymer concentration. Therefore FNP-2, FNP-4 and FNP-6 had a greater entrapment efficiency as compare to others. The results were presented in Table 4. Loading capacity was found in the range of 0.55 to 0.92 mg/mL. it was also increased with polymer concentration like entrapment and particle size.

MTT Assay

The effects of different preparations on A549 cells were detected by MTT assay (Fig. 5). It can be seen that drug

Table 3: Solubility chart of PTX in different solvents

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

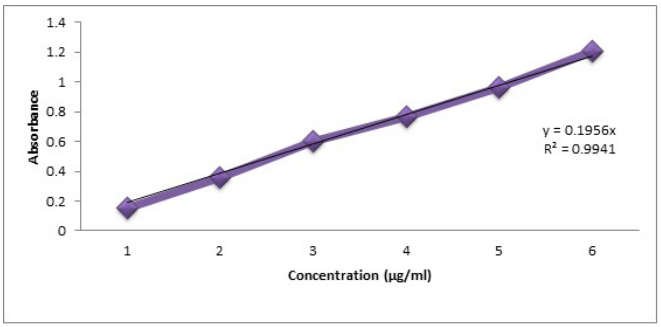

Fig. 1: The standard curve of PTX.

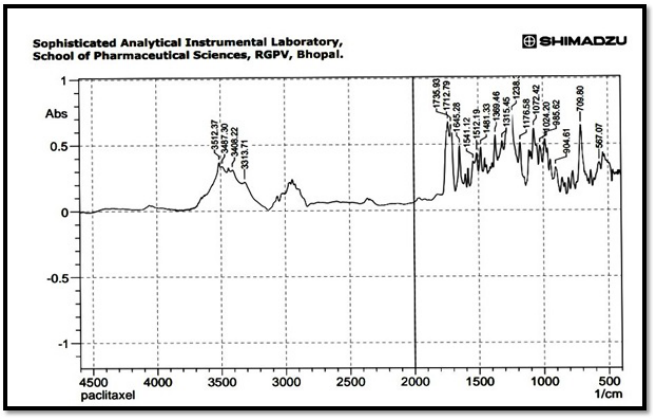

Fig. 2: FTIR spectra of PTX.

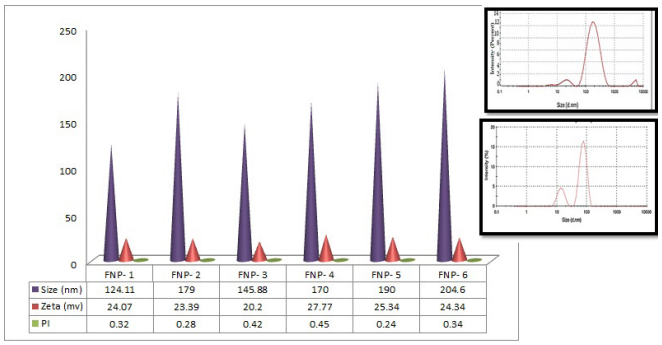

Fig. 3: Particle size, zeta potential and poly dispersity index of different formulations.

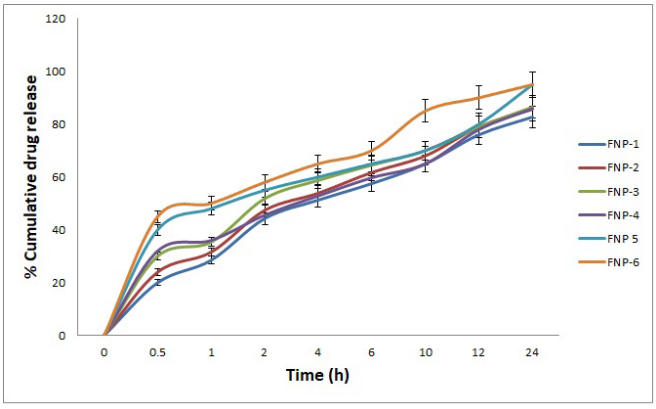

Fig. 4: Comparison of *in vitro* drug release data of different formulations.

and bioenhancer loaded nanoparticles exhibited a time-dependent and dose-dependent cytotoxicity in the cell lines tested. The pure drug solution could not inhibit the cell proliferation completely although a powerful antitumor drug. On the other hand, drug coupled with bioenhancer significantly reduced the cell proliferation. Surprisingly, formulation with higher bioenhancer loading (FNP 6) showed a higher antiproliferative effect on A549 cells in compassion to formulation FNP 1. This may be due to a possible mechanism of synergistic effect of drug and bioenhancer. Therefore it can be concluded that addition of bioenhancer with antitumor drug can enhance its proliferative effect. This result can be further established with bioavailability data. The IC50 value for A549 cancer cell samples was estimated to be 242.58 µM after 24 hours of paclitaxel treatment. The outcomes of % cell inhibition of A549 cells have decreased significantly to nearly 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% respectively for FNP 1 to FNP 6, as compared to that of the control.

Pharmacokinetic Study

The experimental protocol was approved by the Institutional Committee for Animal Ethics approval No. 1189/PO/Re/S/08/CPCSEA. The maximum plasma level (C_{max}) and the time to reach C_{max} T_{max} of the drug were obtained directly from the actual observed data (Fig. 6). The area under curve (AUC) for the time period of 0 to 24 h (AUC_{0-24}) was calculated by means of linear trapezoidal rule. The pharmacokinetic parameters for each formulation were shown in Table 5. The plasma level of FNP-6 was highest than other formulation including control. The AUC of FNP-6 was 6.423µg/ml and the absolute bioavailability of FNP-6 was 7.89. FNP-5 andFNP-6 had a higher bioenhancer quantity as compare to the other formulation this may be possible reason of their higher absolute bioavailability. FNP-5 and FNP-6 contained same amount of bioenhancer and higher quantity as compare to other formulations. Therefore, these two formulations had almost similar results in bioavailability study.

Table 4: Entrapment efficiency and loading capacity of 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

Stability Studies

In this study all the formulations were subjected to a temperature of 4°C, 25°C and 40°C separately for three months and again studied for parameters like physical appearance, drug entrapment, particle size and FTIR. The results of particle size showed no significant changes however poly dispersity index was increased. This effect was found may be due to aggregation of the nanoparticles during storage. However in physical examination no colour change and microbial growth was found. Drug entrapment

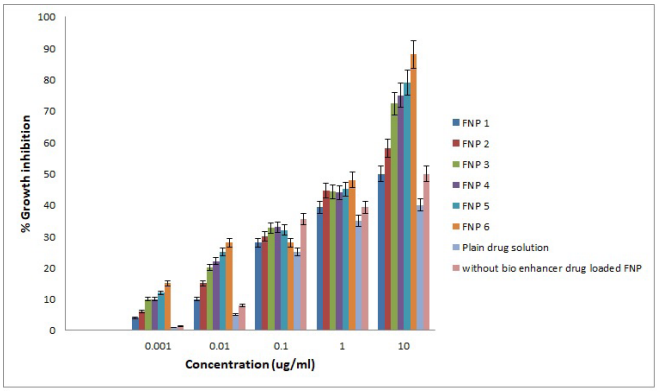

Fig. 5: Percentage cellular inhibition of different formulations at different concentration by MTT assay.

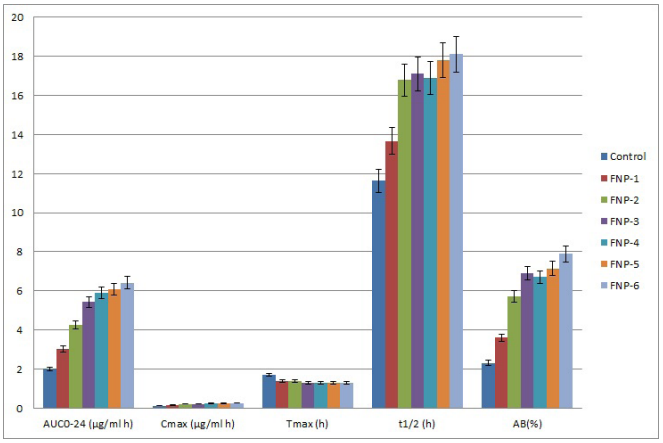

Fig. 6: *In vivo* pharmacokinetic study result of different formulations.

Table 5: Various pharmacokinetic parameter of paclitaxel formulation

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

of nanoparticles was slightly decreased as earlier but the value was not significant. Only 0.8 to 1.2% changes in % drug entrapment was found for the optimized batch FNP-6. FTIR spectra displayed no changes in major peak revealed no chemical changes in prepared formulation.

Conclusion

The object of this work was to entrap paclitaxel and a herbal bioenhancer in a nanoparticle system. For this purpose an emulsion solvent evaporation method was used to prepare the nanoparticles. The prepared nanoparticles revealed good in vitro properties and significant reduction in cell proliferation in MTT assay. Formulation FNP-6 was found best amongst the six formulations. It showed the higher anti-proliferative effect on A549 cells as compare to others. The plasma level of FNP-6 was also highest than other formulation including control. Therefore, it can be concluded that addition of bioenhancer with antitumor drug can enhance its proliferative effect and bioavailability. (Shown in 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, Desmukh R. Design of Eudragit RL 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 proinflammatory cytokine gene expression 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. Pandita D, Ahuja A, Velpandian T, Lather V, Dutta T, Khar 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, Nguyen LH, Chien DM. Preparation 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. https://www.tandfonline.com/action/journalInformation?journalCode=tjen20
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.

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