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Research Article

Comparative Study of Herbal Bioenhancer Containing Nano Formulation for Oral Delivery of Paclitaxel: Pharmacokinetic and Cytotoxicity Analysis

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ABSTRACT

The main objective of this study was to develop novel formulation containing herbal bioenhancer to increase the bioavailability of poorly water-soluble anticancer drug. Paclitaxel (PTX) loaded bioenhancer (Piperine and Quercetin) nanoparticle prepared by emulsification solvent evaporation method by using Eudragit RLPO as a polymer and to evaluate its *in-vivo* pharmacokinetics (PK) and cytotoxicity studies. The result is reduced oral bioavailability, which limits the use of paclitaxel, a crucial anticancer medication, when taken orally. Though multiple formulation approaches have been taken to fight this low absorption profile but consequence of different herbal bioenhancer, happening CYP3A and P-gp inhibitor on augmentation of bo plasma. Paclitaxel loaded nanoparticles with both bioenhancer used for this study and pharmacokinetic, cytotoxic study (MTT Assay) was used for comparative study. The prepared nanoparticles were evaluated for in vitro cell cytotoxicity study by MTT assay on lung cancer cell line and pharmacokinetic profile. The result revealed good in vitro properties with bioenhancer. The goal of the present work is to asses any modification in oral pharmacokinetics of paclitaxel. Therefore, it can be concluded that addition of bioenhancer with antitumor drug can enhance its prolifertive effect. However, further *in vivo* studies are recommended to establish the fact.

Introduction

Recent advancements in herbal-based technology that improve drug bioavailability have resulted in a paradigm shift in how medicines are delivered. Although phytochemical and phytopharmacological studies have long established the overall health-promoting abilities of various plant products, there is a growing interest and medical need to improve the bioavailability of a large number of herbal drugs and plant extracts that are poorly lipid soluble and thus less bioavailable.[1]

A bioenhancer is a substance that increases the bioavailability and efficacy of a medicine when given together with it, without having any pharmacological activity of its own at the therapeutic dose. They tend to reduce the dose of active drug required for the optimal endpoint of the treatment strategy, bypassing the need to use injectable routes of drug administration to a greater extent, which could aid in overcoming antimicrobial resistance and saving precious raw materials used in medicine manufacturing. Fixed drug combinations (FDCs) are also cost-effective.[2]

Lung cancer is the second most frequent cancer in both men and women, and it is the leading cause of cancer-related death. It is connected with a significant death rate in the majority of patients because it is diagnosed at an advanced stage. In the last few years, tremendous progress

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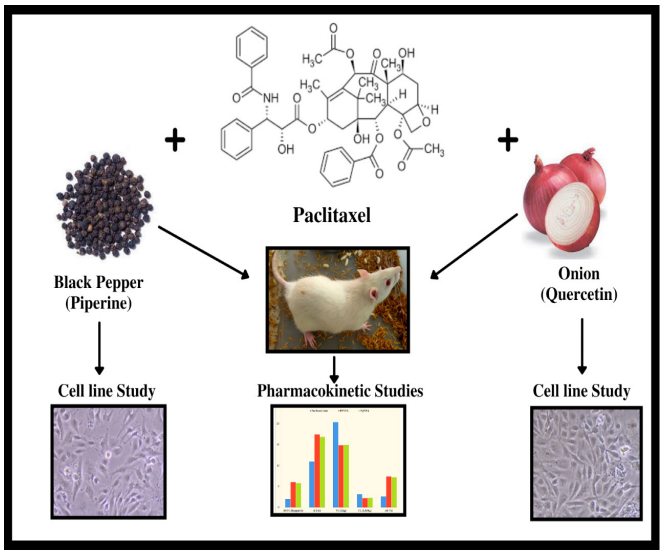
Graphical Abstract



has been made in the development and application of nanotechnology in cancer detection, diagnosis, and therapy. As a result of this advancement, the field of "cancer nanomedicine" has emerged. Bioavailability, intestinal absorption, in vivo stability, solubility, sustained and targeted distribution, and therapeutic efficiency of various anticancer drugs have all boosted the popularity of nanoparticle-based therapeutic systems.[3]

The exact mechanism of action for increasing bioavailability by the bioenhancers is unknown. The objective of the study was to estimate bidirectional of paclitaxel with piparin, paclitaxel with quaracitin and paclitaxel with both bioenhancer. For this the pharmacokinetic, hemolysis and cytotoxicity study was performed.

Material and Methods

Materials

The drug known to fight cancer its name is 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) and Quercetin (QUR) 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.

Preparation of Nanoparticles: Paclitaxel-piperin Nanoparticle (PPNP) and Paclitaxel-quercetin Nanoparticle (PQNP)

Paclitaxel-loaded polymeric nanoparticles were prepared by emulsification solvent evaporation Eudragit RLPO (ERLPO) was selected as a polymer for this technique. DMSO and acetone were used as a solvent.

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

Formulation Code	*Drug and bioenhancer Ratio*	*Eudragit RLPO (mg)*	*PVA (%)*
PPNP-1	1:0.5	100	0.5
PPNP -2	1:0.5	150	0.5
PPNP -3	1:1	100	0.5
PPNP -4	1:1	150	0.5
PPNP -5	1:1.5	100	0.5
PPNP -6	1:1.5	150	0.5

Table 2: Different formulation of PTX-QUR loaded ERLPO nanoaprticles

Formulation Code	*Drug and bioenhancer Ratio*	*Eudragit RLPO (mg)*	*PVA (%)*
PQNP-1	1:0.5	100	0.5
PQNP -2	1:0.5	150	0.5
PQNP -3	1:1	100	0.5
PQNP -4	1:1	150	0.5
PQNP -5	1:1.5	100	0.5
PQNP -6	1:1.5	150	0.5

Emulsification Solvent Evaporation Method

The six-six different formulations of PTX loaded piperine and quercetin (PPNP-1, PPNP -2, PPNP -3, PPNP-5, PPNP-6 and PQNP-1, PQNP -2, PQNP -3, PQNP-5, PQNP-6) were prepared by emulsification solvent evaporation method using sonication. An organic phase consisting of polymer (ERLPO), PTX-PIP and PTX-QUR dissolved in DMSO. The organic phase was then added to the aqueous phase containing 0.5% PVA surfactant with continuous stirring at 300 rpm for 3 hours (Tables 1 and 2). The organic solvent was evaporated under a vacuum evaporator for 24 hours. The collected sample was under refrigerated condition for further evaluation.[4,5]

Sample Collection

Blood samples were collected into the tubes containing EDTA-Vitamin K3 from each animal at a predetermined time interval of 0.25, 0.5, 1, 2, 4, 8 and 24 hours. Plasma was separated by centrifuging the blood samples at 7500 rpm for 10 mins. Plasma sample was processed and analyzed by HPLC (at 227 nm).

Pharmacokinetic Parameters Evaluation

The mean SEM of plasma concentrations recorded at several time points was used to depict the data. The non-compartmental method was used to determine pharmacokinetic parameters such as area under the plasma-concentration time curve from zero to the last measurable plasma sample time and infinity (AUC_{0-t} and $AUC_{0-\infty}$), maximum plasma concentration (C_{max}), time to reach maximum plasma concentration (T_{max}), volume of distribution (Vd), clearance (CL), and elimination half-life ($t_{1/2}$) for the period of 0 to 24 hrs.

Characterization

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 (Table 1). The area under curve (AUC) for the time period of 0 to 24 hours (AUC_{0-24}) was calculated by means of linear trapezoidal rule.[6,7] The pharmacokinetic parameters for each formulation were shown in Table 3 and 4. The plasma level of PPNP-6 was highest than other formulation including control. The AUC of PPNP-6 was 6.029 µg/mL and the absolute bioavailability of PPNP-6 was 7.41%. PPNP-6 and PQNP-6 had a higher bioenhancer quantity as compare to the other formulation this may be possible reason of their higher absolute bioavailability. PPNP-6 and PQNP-6 (showed in Fig. 1 & Fig. 2) contained same amount of different bioenhancer and higher quantity as compare to other formulations.

MTT Assay

The MTT assay was used to assess the cytotoxicity of free PTX, optimised PPNP, and PQNP in A549 cells. A549 cells were planted at a density of 3 to 4 $x10^3$ cells per well in a 96-well plate. After 12 hours, several nanoparticle formulations (ranging from 0.001 to 10 g/mL medication concentrations) were introduced to the plates, which were then incubated for another 24 hours. The PTX standard solution was made by dissolving the PTX in ethanol at a concentration of 0.25 to 2.5 mg/mL and then diluting with distilled water to the desired concentration. A microplate reader was used to take measurements.[8-10]

Result and Discussion

Various pharmacokinetic parameters obtained in mice after oral administration of paclitaxel alone as well as in combination with PIP and QUR are summarized in Fig. 3. Plasma concentrations obtained at different time points for the two treatment groups were plotted against time to obtain the plasma concentration profile curves, are shown in Fig. 3.

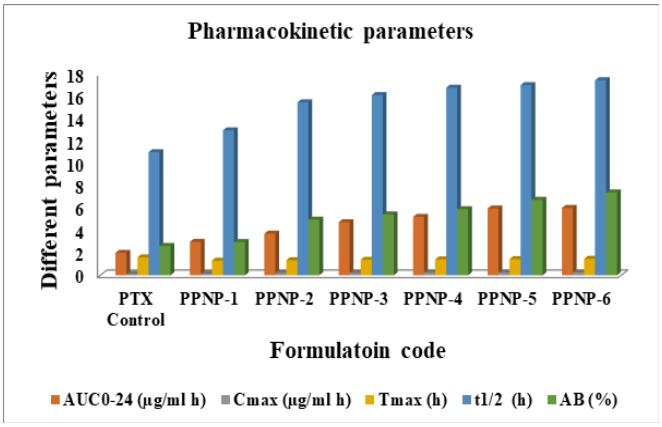


Fig. 1: Pharmacokinetic parameters of different PTX-PIP loaded ERLPO nanoparticles

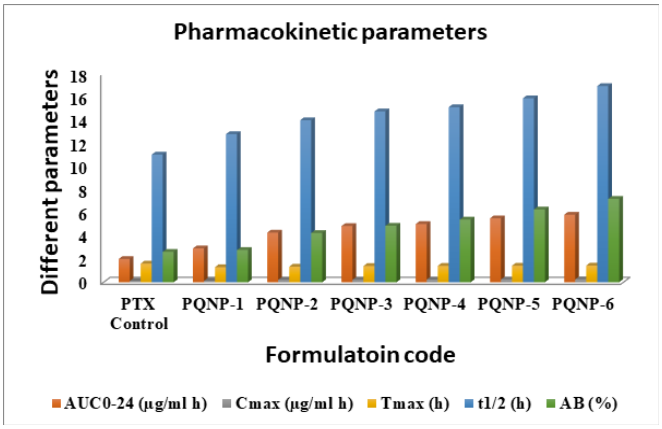


Fig. 2: Pharmacokinetic parameters of different PTX-QUR loaded ERLPO nanoparticles.

Table 3: Various pharmacokinetic parameter of PTX-PIP loaded ERLPO nanoparticles.

Parameters	*PTX Control*	*PPNP-1*	*PPNP-2*	*PPNP-3*	*PPNP-4*	*PPNP-5*	*PPNP-6*
AUC_{0-24} (µg/ml h)	2.005 ± 0.490	2.992 ± 0.410	3.720 ± 0.230	4.753 ± 0.870	5.223 ± 0.510	5.972 ± 0.523	6.029 ± 0.429
C_{max} (µg/ml h)	0.112 ± 0.023	0.128 ± 0.037	0.202 ± 0.042	0.212 ± 0.047	0.237 ± 0.052	0.242 ± 0.027	0.251 ± 0.031
T_{max} (h)	1.61 ± 0.62	1.31 ± 0.31	1.35 ± 0.27	1.39 ± 0.23	1.42 ± 0.32	1.45 ± 0.21	1.47 ± 0.30
$t_{1/2}$ (h)	11.01 ± 2.32	12.97 ± 3.03	15.47 ± 3.15	16.12 ± 3.12	16.78 ± 3.27	17.02 ± 3.56	17.45 ± 3.25
AB (%)	2.62	2.98	4.98	5.45	5.91	6.75	7.41

Table 4: Various pharmacokinetic parameter of PTX-QUR loaded ERLPO nanoparticles.

Parameters	*PTX Control*	*PQNP-1*	*PQNP-2*	*PQNP-3*	*PQNP-4*	*PQNP-5*	*PQNP-6*
AUC_{0-24} (µg/ml h)	2.005 ± 0.490	2.920 ± 0.320	4.278 ± 0.441	4.845 ± 0.514	5.021 ± 0.320	5.512 ± 0.411	5.823 ± 0.421
C_{max} (µg/ml h)	0.112 ± 0.023	0.120 ± 0.047	0.205 ± 0.048	0.210 ± 0.049	0.230 ± 0.042	0.239 ± 0.035	0.247 ± 0.031
T_{max} (h)	1.61 ± 0.62	1.30 ± 0.37	1.34 ± 0.41	1.40 ± 0.14	1.41 ± 0.37	1.42 ± 0.42	1.44 ± 0.39
$t_{1/2}$ (h)	11.01 ± 2.32	12.78 ± 3.14	13.98 ± 3.45	14.74 ± 4.45	15.10 ± 4.45	15.87 ± 3.41	16.92 ± 3.98
AB (%)	2.62	2.78	4.25	4.87	5.41	6.28	7.19

Table 5: Percentage inhibitions with different concentrations used to calculate the IC50 values.

Sr. No.	*Concentration*	*PPNP-6*	*PQNP-6*
1.	10	78.3	59.72
2.	1	45.58	44.99
3.	0.1	36.60	34.33
4.	0.01	18.74	11.08
5.	0.001	10.9	4.86
6.	IC50 value µg/mL	2	2

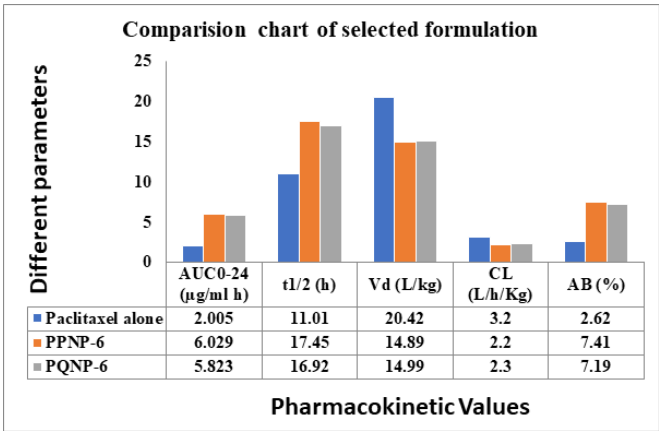


Fig 3: Pharmacokinetic parameters of paclitaxel alone as well as paclitaxel in combination with bioenhancer.

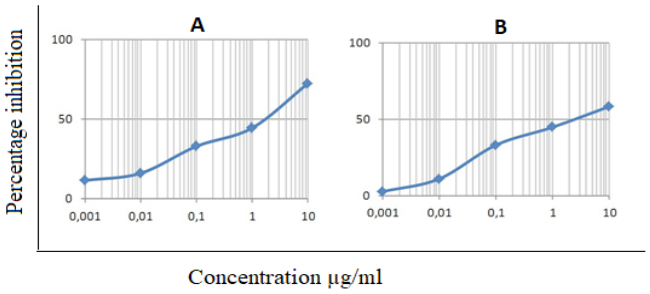


Fig. 4: Percentage inhibitions of selected nanoparticle sample at different concentration, A: PPNP-6 and B: PQNP-6.

It has been observed that bioenhancer enhance the bioavailability of PTX, which in turn increase their therapeutic potential. In the present study, pharmacokinetics profile of paclitaxel was explored specially emphasizing with PIP and QUR which causes inhibition of CYP3A4 and P-gp activities. Paclitaxel, on the other hand, is eliminated systemically by hepatic metabolism involving CYP3A4. Paclitaxel is also a substrate for P-gp, a transporter that is encoded by the ABCB1 (mdr-1) gene and is thought to play a key contributor in drug resistance to absorption and biliary elimination, including paclitaxel. Therefore, improvement in pharmacokinetics of P-gp and CYP3A4 substrates could be achieved by co-administration with P-gp or CYP3A4 inhibitors. Results suggest that PIP can potentiate the therapeutic potential of paclitaxel by enhancing its oral bioavailability.

MTT assay was used to detect the impact of various preparations on A549 cells (Fig. 4). The cytotoxicity of drug and bioenhancer loaded nanoparticles (PPNP and PQNP) was observed to be time and dose dependent in the cell lines evaluated. The pure drug solution could not inhibit the cell proliferation completely although a powerful antitumor drug. Drugs combined with bioenhancer, on the other hand, drastically inhibited cell proliferation. Surprisingly, formulation with higher bioenhancer loading (PPNP-6) showed a higher anti-proliferative effect on A549 cells in compassion to other formulation.

Conclusion

The incorporation of pharmacokinetic data in preclinical experimental models can help to establish safe and effective dose regimens, which is a critical problem in cancer medication development. Orally administered paclitaxel loaded-Piperine and Quercetin nanoparticles increased C_{max} and AUC by 1.4 to 1.5-fold compared to oral paclitaxel alone therapy, resulting in improved oral bioavailability. Piperine and quercetin may exert its bioavailability enhancing properties on the drug molecule because of the following mechanisms: (a) by enhancing the absorption of orally administered drugs by inhibiting CYP metabolism (b) by modulating the active transporters located in the intestine viz P-gp is an efflux pump which pumps out drugs and prevent it from reaching the target site. As a result, piperine appears to be an excellent candidate for regular administration in order to increase paclitaxel bioavailability and reduce dose-related adverse effects. Furthermore, piperine's anticancer properties, both alone and in combination with paclitaxel, could be used to treat cancer in a clinical setting.

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