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Formulation and Cytotoxic Characterization of Trigonella Foenum Loaded Polymeric Nanoparticles

Sagar Trivedi[1*], B Priyanka[1], TD Nandkishor[2] and U Milind[2]

1Department of Quality Assurance, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee Nagpur, Maharashtra, India

2Department of Pharmaceutics, Smt. Kishoritai Bhoyar College of Pharmacy, Kamptee Nagpur, Maharashtra, India

***Corresponding author:** Sagar Trivedi, Department of Quality Assurance, Smt. KishoritaiBhoyar College of Pharmacy, Kamptee, Nagpur, Maharashtra, India, Phone No. +91 7276318609; E-mail: sagartrivedi2000@gmail.com

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Abstract

The aim of present study was to formulate herbal Trigonella Foenum (TF) loaded nanoparticles using hydrophilic polymer at different concentration and optimizing the nanoparticle formulation. Herbal drug having high molecular size or poor water solubility, suffers from less assimilation and poor bio accessibility and hence loading of TF in nanoparticles improves the systematic drug delivery approaches. The formulation containing 3% of polymer PVP showed optimum result with encapsulation efficiency of 77.73 ± 0.28 and loading efficiency of 16.98 ± 0.7. Simultaneously showing high stability with zeta potential of -23.7 ± 0.98 mV and PDI of 215. The mean average particle size of nanoparticles was found to be 432.1 ± 16.01. FT-IR studies suggested compatibility of polymer and drug when observed with major peaks and functional groups. The in-vitro SRB cytotoxicity assay showed a maximum inhibition of cell viability of 71 ± 2.3% and IC 50 value of 100 ± 10.2 ug/ml.

Keywords: Trigonella foenum; Poor bioavailability; Polymeric nanoparticle; Cytotoxicity assay

Introduction

Nanoparticles (NPs) and Polymeric Nanostructured Materials (PNPs) signify an vital role in terms of research field and techno-economic sector with its various application in diseases management . NPs and PNPs have gained prominence in technological advancements due to their enhanced physicochemical properties [1]. Nanoparticles are an emerging method for treating cancer. The major problems associated with treating cancer includes drug low specificity, rapid drug clearance and biodegradation, and limited targeting. The properties of nano carriers, including their nano scale sizes, high surface-to-volume ratios, favourable drug release profiles, and targeting modifications, can allow them to better reach target tumour tissue and release drugs in a stable, controlled manner [2].The high toxicity usually associated with synthetic chemotherapeutic agents increases the demand for search of herbal bioactive molecule, many plant extracts and/or isolated compounds such as herbal drugs has its vital role in maintaining the human health. Most of the active attributes of extracts are incompetent to pass the lipid. Phytosterols are specific phytochemicals that resemble cholesterol in structure but are found exclusively in plants.

The major problem associated with phytosterols is incapablity to pass the lipid membranes of the cells because either their molecular size is to a large extent or the water solubility is poor, and hence suffers from low assimilation and poor bioavailability [3]. Thereby causing decrease in very less or no *in-vivo* activity though having excellent *in-vitro* activity because of poor absorption .Due to these obstacles, some extracts are not used clinically. Herbal drugs with nanotechnology and nanostructured polymeric systems have proven to strengthen the action of herbal extracts decreasing the necessary dosage and side effects, and improving bioactivity [4]. Fenugreek is one of these medicinal plants used by cancer patients and the general population and is considered one of the oldest traditional medicinal herbs, cultivated in India, the Mediterranean region, and North Africa. Fenugreek extracts and compounds were found to target at least five hallmarks of cancer including, proliferation, inflammation, angiogenesis, invasion, and metastasis [5,6].The search for novel herbal drugs is still a precedence for cancer therapy, resistance to chemotherapeutic drugs is the key factor associated with assigning a delivery system. The high toxicity usually of cancer chemotherapy drugs and their detrimental side-effects which surge the demand for novel anti-tumour drugs active against untreatable tumours, with fewer side-effects and/or with greater therapeutic efficiency.

Materials and Methods

Materials

Petroleum ether , n-hexane . Chloroform , were purchased from Loba Chemie Pvt. Ltd. Mumbai, Disodium Hydrogen Phosphate was purchased from Fisher Scientific India, Pvt. Ltd, Polyethylene Glycol 400, Isopropyl Alcohol and Methanol from Merck Specialties Pvt. Ltd. Mumbai. The further chemicals were of the analytical grade of standard Merck (Merck Specialities Pvt. Ltd. Mumbai.).

Collection of plant material

The seeds of Trigonella foenum were collected from an authentic medical store.

Preparation of plants extract

A powdered form of Trigonella foenum was made with the help of its seeds. The powdered seeds (50 g) were extracted with n-hexane (300 ml) in Soxhlet apparatus for 6 h to remove the fatty substances. Further the marc was mined with ethanol (95%) for 6 h with the assistance of Soxhlet apparatus. After exostive extraction were distilled (60-80ºC) off to get semisolid extract [7].

Formulation of TF loaded Nanoparticles

Polymeric Nanoparticles (PNP) were prepared by O/W Solvent evaporation method, accurately weighed quantity of TF (25mg) was dissolved in 10 ml of Ethanol and added into Aqueous phase (25 ml Distilled water) containing 1%,2%,3% w/v PVP (**Table 1**) with respect to drug, using a high share homogenizer at 8000 rpm for 20 min at 40ºC, by adding organic phase into aqueous phase. Then emulsion was passed through the high-pressure homogenizer at a pressure of 500 bar for one cycle. The emulsion stirred at 1000rpm for 2hrs at room temperature for the evaporation of organic solvent, after that the nanoparticles were collected by centrifugation for 40 minutes at 8000 rpm at the controlled temperature 4ºC (Remi Cooling centrifuge, Remi Elektrotechnik limited, India). The supernatant was discarded and precipitate was washed 3 times with distilled water. The nanoparticles thus obtained were dried overnight at 600C and stored in a desiccator [8,9].

Characterization of TF loaded Nanoparticles

Loadinge efficiency

The amount of drug in the formulation was done by leaching the drug out from the polymeric nanoparticles using 0.1M HCL as extracting solution. Accurately weighed 50 mg of nanoparticles were sonicated for 60 minutes using 50 ml of 0.1M HCL as a solvent and further subjected to filtration through Millipore filter and determining the drug content at suitable dilution at 271nm using UV/visible spectrophotometer (Model: SPECTRO 2060 PLUS, Analytical Technologies Ltd., Gujarat, India). The loading efficiency (%LE) of polymeric nanoparticles using the equation 1

$$\%LE = (DN/WN) \times 100 \quad (1)$$

Where DN is amount of drug in nanoparticles and WN is weight of nanoparticles [10].

Encapsulation efficiency

For determining Encapsulation efficiency (%EE) Nanoparticles (50 mg) were drenched in 10 ml of phosphate buffer for 30 mins. The amount of drug in clear supernatant was determined after centrifuging at 10,000 rpm for 1 hr. at the controlled temperature of 4°C (Remi cooling centrifuge, Remi Elektrotechnik limited, India) using UV/visible spectrophotometer (Model: SPECTRO 2060 PLUS, Analytical Technologies Ltd., Gujarat, India) at 271nm. The complete extraction procedure of the drug was done by repeating the abstraction process on the earlier extracted polymeric debris [11].

Particle size

Nanoparticle size for the prepared formulation was determined by dynamic light scattering method based on laser diffraction in a multimodal mode using the Malvern particle sizing system (Malvern, Nano Series ZS90, Malvern Instruments, Ltd., UK) with He-Ne laser at 632.8 nm at a scattering angle of 90.0°, which is capable of measuring vesicles in the 1nm to 5 nm size range. The preparation was diluted with distilled water. The sample was then transferred to a quartz cuvette and examined at room temperature.

Zeta potential

The zeta potential is a measurement of stability of Nanoparticles. It was reported that if zeta potential values are lower than (-30 Mv) or higher than (+30 Mv) the formulation is considered to be stable respectively.

Scanning electron microscopy (SEM)

PNP were visualized using a SEM electron microscope with an accelerating voltage of 100kv and magnification up to 20.00 kx. Samples were stained with 1% aqueous solution of phosphotungstic acid (PTA) as negative stain. Nanoparticle solution (10ml) was dried on a microscopic carbon-coated grid for staining. The excess solution was removed by blotting. After drying, the stained samples were examined in SEM electron microscope accelerated.

Drug-excipient compatibility studies

Drug-excipient compatibility studies were done using Fourier Transform Infrared Spectrophotometer (FTIR) (Shimadzu FTIR 8400s), the FTIR spectra of drug polymer were obtained and were compared for the incompatibilities.

Cell viability SRB assay

Determination of cytotoxic effect of TF loaded polymeric nanoparticles was mainly done on MCF-7 breast cancer cell line. Initially a medium was prepared containing 10% fatal bovine serum and 2mM L-glutamine called as RPMI medium and the cell line under study were grown in this particular medium. Supplementary a microliter plate was taken having 96 well in 100 µL and the MCF-7 cells were inoculated in that wells, depending on the doubling time of individual cell lines. For effective estimation of potency of TF nanoparticles and plain TF, both were initially solubilized in dimethyl sulfoxide at 100mg/ml and diluted to 1mg/ml using water and stored frozen prior to use. During the process of drug addition an aliquot of frozen concentrate (1mg/ml) was softened and diluted to 100 µg/ml, 200 µg/ml, 400 µg/ml and 800 µg/ml with complete medium comprising of the samples under examination. Aliquots of 10 µl of these different drug dilutions were auxiliary to the appropriate microliter wells already comprehending 90 µl of medium, ensuing in the required final drug concentrations i.e.10

µg/ml, 20 µg/ml, 40 µg/ml, 80 µg/ml. Once the test article addition is completed , the microliter plates are kept for incubation for duration of 48 h under standard condition. Assay is being determined by addition of cold trichloroacetic acid (TCA), by moderate addition 50 µl of cold 30 % (w/v) TCA (final concentration, 10 % TCA) and incubated for 60 minutes at 4°C leading to *in situ* fixation of cells. Abandon the supernatant and the microliter plated were several times with tap water and were air dried. Sulforhodamine B (SRB) solution (50 µl) at 0.4 % (w/v) in 1 % acetic acid was added to each of the wells and further followed by incubation of those plates at room temperature for 20 minutes. To remove the unbound and residual dye the washing process was opted for approximately five times with 1 % acetic acid. The bound stain was illuminated with 10 mM trizma base and the reader plate gave absorbance at a wavelength of 540 nm with 690 nm reference wavelength. The technique chosen calculating percent growth was plate-by-plate basis for test wells relative to control wells. Ratio of average absorbance of the test well to the average absorbance of the control wells * 100 gave percent growth. By means of the six absorbance dimensions [time zero (Tz), control growth (C), and test growth in the existence of drug at the four deliberation levels (Ti)], the % growth was calculated at each of the drug concentration levels [12].

Growth inhibition percentage was determined as :

[Ti/C] x 100 % (2)

Stability of TF Loaded PNP

The polymeric Nanoparticles were stored for three months at 4°C. The size, zeta potential and PDI of drug-loaded polymeric Nanoparticles were taken as parameters for stability testing.

Results and Discussion

TF loaded polymeric nanoparticles (PNP) were prepared by O/W Solvent evaporation method, evaluation was done and determinates were recorded in triplicates. The selection of polymer was based on the solubility and miscibility of extract of TF with these emulsifying agent. It is generally accepted that the entrapment efficiency of the active constituent would be higher if it is miscible/soluble with the solvents and emulsifying agents.

Characterization of TF loaded Polymeric nanoparticles

Drug loading denotes the expanse of pharmacology active ingredient encapsulated to the amount equivalent to nanoparticles, and entrapment efficiency helps in extracting the ratio of experimental drug content with relating to the theoretical mass of drug used for formulation. Loading of drug into the nanoparticles depends on the extent of polymer used and the method which is mainly obtained for formulation of nanoparticles. Encapsulation efficiency & drug loading of TF nanoparticles were in range of 74.935 ± 1.06% to 77.73 ± 0.28 % to and 11.43 ± 0.9 to16.98 ± 0.7%. TF nanoparticles shows maximum encapsulation efficiency, after that there was no change in encapsulation efficiency due to saturation of the polymer dispersion. It was observed that formulation F3 containing 3% of polymer PVP (75mg) showed maximum encapsulation of drug and simultaneously low for the formulation having 1% of polymer PVP (25 mg). This phenomenon may be observed because of a larger portion of drug forming cross linkage with the hydrophilic polymer and the TF also being hydrophilic. The loading efficiency may be further increased by optimizing the amount of polymer and maximum entrapment of drug may not be achieved because of hydrophilic nature of TF extract [13].

The *in-vivo* doom and the stability of the prepared formulation is mainly affected by particle size distribution and zeta potential. The primed nanoparticles were assessed for its particle size and morphological features which were observed with scanning electron microscopy SEM (Figures 1). The PNPs assessed with SEM were in the accepted range of nanoparticles (10-1000 nm). Their SEM images bare the particle size of TF PNPs and were found to be 355.2-312.6 nm (**Table 1**), 456.63-339.2 nm and 432.1-472.8 nm, respectively. Statistical analysis using unpaired t-test reveals that there was a significant difference ($p<0.05$) between the particle sizes obtained for nanoparticles containing different ratios of polymer. The polymer are comparatively having higher molecular weight, hence the amount of increased polymer may proportionally increase the mean particle size of prepared nanoparticles [14]. Dissolution velocity, wetting, particle surface area and saturation solubility are the vital parameter which can be increased to an extent by homogenization process of prepared herbal PNPs.

This particularly bring about augmented bioavailability and enhanced *in-vivo* discharge because only solubilized particles can be captivated through hydrophilic polymeric cellular crusts [15]. The herbal extract lacks the value-added potent activity and due added side effects and combined with large dose size, hence these nanostructured systems will possible help in overcoming these shortcomings. PNPs are proficient with dispersal of the bioactive molecule at optimum level during the whole process of treatment and amassed the drug moieties at the preferred site of action. Low value of PDI indicated that using ideal conditions, we could fabricate unwavering herbal nanoparticles with are fairly narrow size distribution. The zeta potential of the prepared herbal polymeric nanoparticle was measured (Malvern, Nano Series ZS90, Malvern Instruments, Ltd., UK). It was reported that if zeta potential values are lower than (-30 mV) or higher than (+30 mV) the formulation is considered to be stable respectively. The negative values of the zeta potential of TF loaded nanoparticles might be attributed to the adsorption of counter ions or the preferential adsorption of hydroxyl ions at the nanoparticle surface. Negatively charged nanoparticle exhibited lower negative ZP values, ranging from 19.23mV to -23.7mV. Characterization values of polymeric TF nanoparticles are represented in **Table 1**.

Sr.No	Formulation Code	Drug Polymer Ratio (mg)	Loading efficiency ± SD %	Entrapment efficiency ± SD %	Particle Size ±SD nm	PDI	Zeta Potential ±SD mV

1	PNP 1	01:25	11.43 ± 0.9	74.935 ± 1.06	355.1 ± 21.66	200	-19.23 ± 1.22
2.	PNP 2	01:50	13.28 ± 0.2	75.73 ± 0.76	456.63 ± 150.34	231	-21.65 ± 0.45
3.	PNP 3	0.09375	16.98 ± 0.7	77.73 ± 0.28	432.1 ± 16.01	215	-23.7 ±0.98

Table 1: Characterization of TF Loaded Polymeric Nanoparticles.

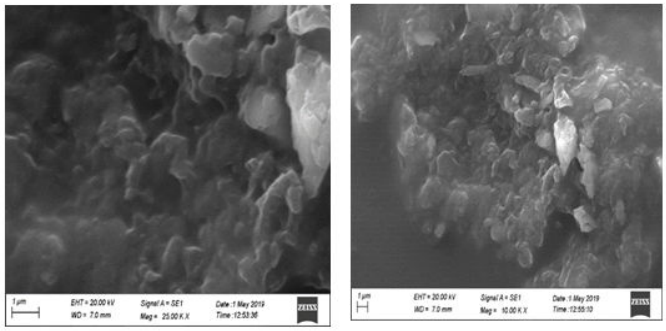

Figure 1 : SEM Images of TF loaded PNP.

Drug excipient compatibility studies:

The upshots of Drug excipient compatibility studies are illustrated in **Table 2**, from the IR data it was clear that functional group of the formulations was found to be unchanged and similarly corresponding intensities of peak. This clearly deceits that during formulation process the drug and polymer haven't undergone any changes and ultimately not resulting into a new reactant product. Therefore there was no interaction and formulation indigenously were compatible and results were in favour and formulation can be proceeded.

Sr.No	Bands	Drug(TF)	PNP 1	PNP 2	PNP3
	O-H	2917.38 cm -1	2976.67 cm -1	2898.33 cm -1	2997.23 cm -1
	C=O	2848.91 cm -1	2791.87 cm -1	2898.24 cm -1	2798.35 cm -1
	C=C multiple bends	1705.10 cm -1	1754.23 cm -1	1809.22 cm -1	1754.87 cm -1
	C-O	1472.68 cm -1	1401.3 cm -1	1509.32 cm -1	1442.92 cm -1
	=C-H	1056.04 cm -1	992.34 cm -1	1200.98 cm -1	1134.45 cm -1
	=C-H Strong bending	978.89 cm -1	1011.23 cm -1	901.23 cm -1	934.47 cm -1

Table 2: Main Peaks Observed in FT-IR Spectrum.

In-vitro anticancer potential

The cytotoxicity of TF polymeric nanoparticles, against MCF-7 breast Cell line by SRB Assay was determined. The inhibiting activity was increased in TF polymeric nanoparticles against MCF-7 breast cells when compared to plain TF and represented in **Table 3** and **Figures 2 and 3**.

Sr.No	Test Sample	Test Conc. Ug/ml	% Cytotoxicity	IC 50 ug/ml
1	TF PNP	10	23±1.2	100±10.2 ug/ml
		20	38±2.7	
		40	54±1.9	
		80	71±2.3	
2	Plain TF	10	11.23±1.5	>150±20.1 ug/ml
		20	23±0.9	
		40	38±2.4	
		80	54±3.1	

Table 3: % Inhibition of Test Sample And IC 50.

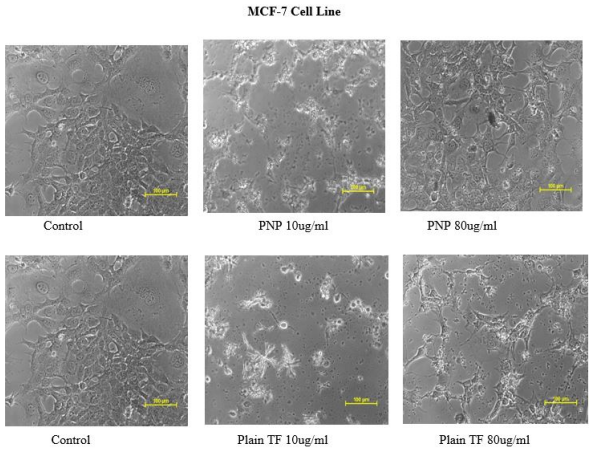

Figure 2: Breast Cell lines treated TF Plain and TF PNP Formulation.

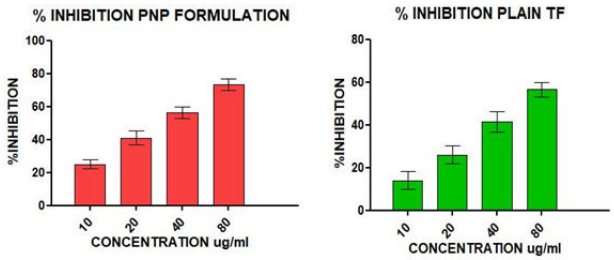

Figure 3: % Inhibition in breast Cell lines treated TF Plain and TF PNP Formulation.

Stability of TF Loaded PNP

Stability after three months of storage at 4°C, non-aggregation and no creaming were observed. The size and zeta potential and polydispersibility index of TF loaded polymeric Nanoparticles had slight change from 344.7to 478.9 nm, -21.3 ± to -17.7mv and 0.200 to 0.278 respectively, showing non-significant difference and indicating that the formulation was stable.

Conclusion

Among three different TF loaded polymeric nanoparticles formulated, PNP 3 i.e. 1:75 drug is to polymer ratio showed satisfactory results. The PNP 3 showed mean particle size of 432.1 ± 16.01nm and all remaining particles were in range of (200-500 nm), with having zeta potential of 23.7 ± 0.98 with high stability and PDI 215.TF an herbal drug showed higher encapsulation of 77.73 ± 0.28 and loading efficiency of 16.98 ± 0.7. FTIR study concluded that there was no any interaction between drug and polymers used. SRB assay of TF PNP and Plain TF was performed to exact the percent inhibition cell viability of TF and results showed that increased in inhibition of cell viability was observed in NPN formulations with IC50 100 ± 10.2 ug/ml. Currently, there is universal interest in the use of eco-friendly and cost-effective drug delivery systems to improve drug performance. This opens up opportunities to explore polymeric nanoparticles as carriers for effective delivery of phytopharmaceuticals.

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Compliance with ethical standards Ethical statement

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

Authors shows no conflict of interest.

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