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Preparation and *In-vivo* Evaluation of Tamarind Seed Polysaccharide Coated Nanostructured Lipid Carriers for Oral Delivery of Nifedipine

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Abstract

The present study is aimed at preparation and *in-vivo* evaluation of Nanostructured lipid carriers from natural polysaccharide extracted from Tamarind seeds (Tamarindus indica) for the sustained delivery of Nifedipine. The Nifedipine loaded NLCs were prepared by solvent injection technique with the isolated tamarind seed polysaccharide. The prepared eight preparations were characterized in terms of *in-vivo* study. Stable NLCs were obtained with average particle size of 322 nm. The entrapment efficiency of optimized batch was found to be 82.56 ± 2.5% (w/w). *In-vivo* drug release showed controlled release pattern in 24 h. It may be concluded from the study that tamarind seed polysaccharides is suitable for formulation of NLCs for better efficacy and sustained delivery of hypertensive drug Nifedipine.

1. Introduction

Lipid-based drug delivery systems are predicted as promising oral carriers due to their prospective to enhance the solubility and increased oral bioavailability of drugs having low water-solubility and/or lipophilicity [1]. Standard lipid-based formulations have a broad range of lipid solution, emulsions, liposomes, lipid microparticles and nanoparticles. In between the formulations above, the nanostructure lipid carriers (NLCs) are considered as the second-generation of lipid nanoparticles [2], and are attracting vital attention as substitute of colloidal drug carriers. Mucoadhesive NLCs coated with hydrophilic polysaccharides may also sustain the release of drug and hence also can improve bioavailability. Polysaccharides are the choice of materials among the hydrophilic polymers used, because they are nontoxic and acceptable by the regulating authorities [3]. The various polysaccharides used in drug delivery application are cellulose ethers [4], xanthan gum [5], locust bean gum [6] and guar gum [7]. Another natural polysaccharide, Tamarind seed polysaccharide (TSP) obtained from the seed kernel of *Tamarindus indica*, possesses properties like high viscosity, broad pH tolerance [8], noncarcinogenicity [9], mucoadhesive nature, and biocompatibility [10]. It is used as stabilizer, thickener, gelling agent, and binder in food and pharmaceutical industries. The tamarind seed polysaccharide constitutes about 65% of the tamarind seed components [11]. It is a branched polysaccharide with a main chain of β -d-(1,4)- linked glucopyranosyl units, and that a side chain consisting of single d-xylopyranosyl unit attached to every second, third, and fourth d-glucopyrnosyl unit through an α-d-(1,6) linkage. One d-galatopyranosyl unit is attached to one of the xylopyranosyl units through a β - d-(1,2) linkage[12].

Nifedipine, a poorly soluble drug with calcium channel blocker activity utilized in hypertension treatment. It is the most vascular selective dihydropyridine with antioxidant effect. Nifedipine exhibits a high first-pass hepatic metabolism with 45% bioavailability. The complete metabolism of Nifedipine occurs in the liver by cytochrome P450 3A4 to pharmacologically inactive metabolites. Nifedipine has small water solubility and could be classified as a BCS class II drug [13].

2. Materials and Methods

Materials and Methods The tamarind seeds were collected locally. All the chemicals used during the project are of analytical grade. Hydrochloric acid (HCl) was purchased from Nice Laboratory Reagents, Kochi, India. Ethanol.

2.1 Isolation of TSP

To isolate TSP 20 g of tamarind kernel powder was taken, 200 ml of cold distilled water was utilized to make slurry. The slurry was then poured into 800 ml boiling distilled water. The solution was boiled for 20 minutes with continuous stirring. The resulting clear solution was kept overnight so that most of the fibers settle down. The solution was then centrifuged at 5000 rpm for 20 min. The supernatant solution was separated out and poured into twice the volume of absolute Ethanol by continuous stirring to obtain the precipitate. The precipitate was washed twice with absolute ethanol and dried at room temperature for 2 days. The dried product was grounded and passed through BSS # 60 and stored in dessicator till further use [14].

2.2 Preparation of Nifedipine loaded NLCs with TSP

Nanostructured lipid carriers were prepared by solvent injection technique with slight modification [15]. Nifedipine and specified amount of Glycerol monostearate (GMS) and oleic acid were dissolved in 4 ml of isopropyl alcohol (boiling point 81–83°C) with heating at the melting temperature of GMS. The resulting solution was rapidly injected into the 100 mL aqueous phase containing polysaccharide at 0.4 mg/mL [16] and poloxamer at 0.4 mg/mL with continuously stirring at 400 rpm for 30 min on a magnetic stirrer and then 0.1 N HCl (8 ml) was added to the dispersion. Thereafter, the dispersion was centrifuged at 10,000 rpm for 30 min at 10°C in REMI cooling centrifuge (Model C- 24BL, VACO-779, Vasai, India), and aggregates were re-suspended in 10 ml double distilled water containing 4% poloxamer 188 (by weight) as stabilizer with stirring at 1000 rpm for 10 min. [17]. Purification of Nifedipine loaded NLCs was done by dialysis technique. Re-suspended suspension was taken in the dialysis bag and sealed at both ends. The dialysis bag then immersed into 100 ml of double distilled water containing 0.2% (w/v) sodium lauryl sulphate and stirred at 100 rpm for 20 min. The unentrapped drug has been removed in the 20 min. The HPLC was performed by using HPLC (Jasco) C18 column. A mixture of methanol: water (85:15 v/v) was used as mobile phase. The flow rate of mobile phase, injection volume and detection wavelength were 1.0 ml/min, 20µl and 350nm respectively. Nifedipine showed linear calibration curve with R^2 =0.997 in the range 50-250µg/ml.

Table 1: Composition of Nefidipine loaded NLCs with Tamarind polysaccharides

Formulation Code	GMS(mg)	O.A(mg)	TSP(mg)
T_1	100	10	40
T_2	200	10	40
T_3	100	20	40
T_4	200	20	40
T_5	100	10	50
T_6	200	10	50
T_7	100	20	50
T_8	200	10	50

3. Characterization of NLC

3.1 Estimation of drug by HPLC

The Nifedipine concentration in rat plasma samples were analyzed by HPLC. To 200 µl of each plasma sample, 600 µl of methanol was added and vortex-mixed in a microcentrifuge tube. The mixture was permitted to stand for about 10 min and centrifuged for 10 min at 3000 rpm. The clear supernatant was evaporated until it was completely dry, then it was redissolved with 100 µl methanol. After ultrasonic extraction for 3 min and centrifuging for 5min at 14,000 rpm, the clear supernatant was removed and evaporated repeatedly, and the dried residue was dissolved with 50 µl mobile phase and filtered through 0.45 µ syringe filter and injected directly into HPLC system. The mobile phase consisting of metanol: water (85:15) at a flow rate 1.0 ml/min. and the detection was carried out at 230 nm (18).

3.2 Drug Entrapment Efficiency (DEE %)

The drug entrapment was measured by RP-HPLC method using methanol: water (85:15 v/v) as a mobile phase. 1ml of Nifedipine loaded NLCs colloidal solution centrifuged for 10 min at 4000rpm. Then the solution was filtered through a 0.45µm membrane filter. After that, it analysed by HPLC [19]. Drug entrapment efficiency (DEE) of nanostructured lipid carriers calculated using the following equation

$$\text{DEE } (\%) = \frac{\text{Total amount of drug recovered}}{Total\ amount\ of\ drug\ added} \times 100$$

3.3 Pharmacokinetic studies

Rats (Albino), young, weighing 250-300 gm were divided into three groups, each group consisting of six animals. Rats were kept on fasting 12 h before drug administration. Water was given throughout the study. The dose of Nefidipine was 2.5mg/kg and given by oral administration **(20).** The study protocols were approved by Institutional Animal Ethics Committee under CPCSEA number 1355/PO/Re/L/10/ CPCSEA.

Number of groups:

Group 1 (n=6) - Vehicle treated
Group 2 (n=6) - Control
Group 3 (n=6) - Nefidipine loaded NLCs coated with TSP

Weighed amount of nanostructured lipid carriers with tamarind seed polysaccharide equivalent to dose 2.5mg/kg (animal dose) were suspended in 1.0 ml vehicle and administered orally using a rubber canula under non-anaesthetic condition **(20).** Blood samples were collected at 1, 2, 4, 6, 8, 12, 18, 24 h time intervals, from retro-orbital region in ependorff tubes and centrifuged at 4000rpm for 10 min. The blood sample volume withdrawn was immediately replaced with an equal volume of physiological saline. The serum was separated by placing the tubes in a centrifuge 20 min at 3000 rpm and then serum was stored at -20°C until drug analysis was carried out using HPLC.

3.4 Data analysis

The standard pharmacokinetic parameters obtained from each of the individual rat plasma and plasma concentration Vs. time profiles of Nifedipine were calculated by non-compartmental method using the Win Nonlin computer program. The C_{max} and t_{max} were determined directly from the plasma concentration Vs. time graph of Nifedipine loaded NLCs coated with tamarind seed polysaccharide. The AUC was determined by trapezoidal method. The $t_{1/2}$ was determined linear regression of log linear portion of the plasma concentration time profile**.** The apparent plasma clearance (CL) was calculated by dividing the dose by the AUC. The mean residence time (MRT) was determined using WinNolin software.

3.5Relative bioavailability

The relative oral bioavailability of different formulations was evaluated by the equation:

$$\textbf{Relative bioavailability} = \frac{\text{AUC Sample}}{\text{AUC Standard}}$$

4. Results And Discussion

Nifedipine loaded NLCs with coating of tamarind seeds polysaccharide were successfully formulated by solvent injection technique which depends upon high speed diffusion of the solvent over the solvent lipid interfaced with the aqueous phase and this physical phenomenon is used to evaluate for the precipitation of nano sized lipid particle. The small size NLCs found may couple with low density of lipids. To control this limitation, the pH was decreased to 1.5–2 to maintain the zeta potential to a level that raise the aggregation of NLCs. The purity of the NLCs obtained is another significant characteristic in formulation of NLCs. A feasibility of free Nifedipine particles in the sediment of Nifedipine loaded NLCs with tamarind polysaccharide can't be refused. The *in vivo* release behaviour of drug can affect the free drug particles. Therefore, dialysis technique was utilized to remove out the free drug particles from the sediment of NLCs formulation. Nifedipine have low molecular weight of 346.335 g/mol so that this method was considered appropriate to remove the free drug particles.

Table 2: Effect of Various Parameters on Characteristics of Tamarind seed polysaccharide coated NLCs

Batch Code	Amount of GMS acid(lipid) (mg)	Amount of oleic acid (lipid) (mg)	Amount of Tamarind Seed Polysaccharide (mg)	Particle size ±S.D	Entrapment Efficiency (%w/w)
T_1	100	10	40	459.5±6.2	69.16±1.2
T_2	200	10	40	357.1±7.3	79.12±2.5
T_3	100	20	40	362.3±3.5	65.47±7.2
T_4	200	20	40	453.8±7.3	76.04±3.5
T_5	100	10	50	359.2±1.2	63.22±2.9
T_6	**200**	**10**	**50**	**322.3±1.2**	**82.56±2.5**
T_7	100	20	50	485.1±4.6	77.38±5.6
T_8	200	20	50	389.1±6.2	71.10±4.2

4.1 Pharmacokinetic Analysis

The plasma concentration-time data of NIfedipine for each animal was analyzed by interactive, pharmacokinetic computer software, Winnolin® 6.3.0.395 Core version (2007)

The program yielded kinetic parameters of absorption, distribution and elimination for individual data set. Comparative plasma concentrations of active drug Nifedipine, and optimized NLCs formulation (T_6) respectively are shown in **Table 3** and **Figure 1.** The pharmacokinetic parameters based on the plasma level time curve have been recorded in **Table 4.** The peak plasma drug concentration (C_{max}) and time of its occurrence (T_{max}) were read directly from each concentration-time data. Area under the plasma level time curve (AUC and moment plasma level time curves (AUMC) were calculated by trapezoidal method. Area under the plasma level time curves for optimized formulation T6 is shown in **Figure 2**. The ratio of the above areas was used to estimate the mean residence time (MRT) of the drug.

Table 3: Plasma drug concentrations (ng/ml) of optimized batch

Time	Plasma drug concentration (ng/ml)* of T_6
0	0
1	100.12798
2	256.21557
4	375.59548
6	460.654
8	425.659
12	456.35.215
18	390.546
24	264.216

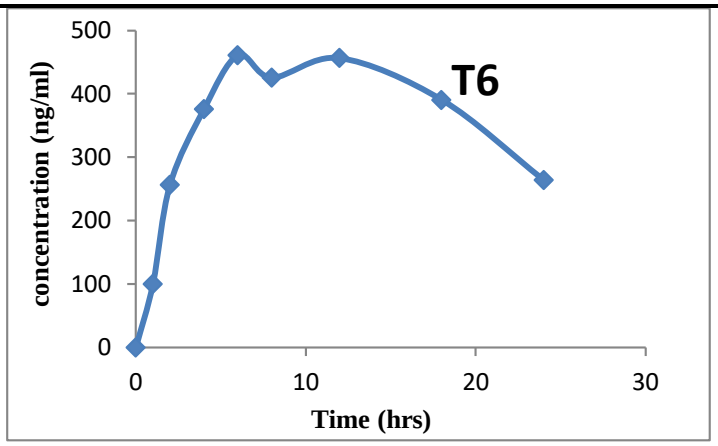

Fig.1 Plasma- concentration time profile for optimized formulation

For the computation of the Elimination rate constant (K_e), the program used a minimum of three data points. Where the computation of K_e was not possible for all the animals, best fit implemented in software was used. The biological half life ($t_{1/2}$), total clearance (Clt) and volume of distribution (Vd) were estimated with the help of following equations;

$t_{1/2} = 0.693 / K_e$; Cl_{total} = Dose / $AUC_{0\text{-}inf}$ and $Vd = Clt / K_e$ respectively.

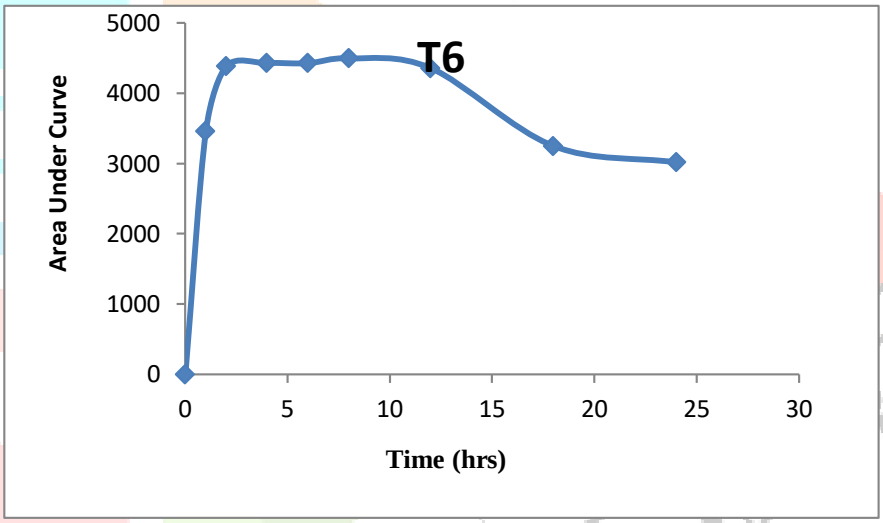

Fig.2: Area under the plasma level time curve for optimized formulation (T6)

Table 4: Mean pharmacokinetic parameters of optimized formulations

Formulation	AUC (h*ng/ml)	C_{max} (ng/ml)	Plasma clearance (ml/hr)	MRT (h)	$t_{1/2}$ (h)	Relative bioavailability
T_6	5592.275	460.65	0.3461	23.69	19.47	82.59%

Optimized formulation Nifedipine loaded NLCs coated with TSP **(T6)** shows peak plasma concentration (C_{max}) of 460.65ng/ml with half life ($t_{1/2}$) of 19.47 hrs and Mean Residence Time (MRT) of 23.69 hrs. The relative bioavailability of **T_6** formulation was calculated as 89.25%.

Conclusion

Using solvent injection technique, Nifedipine loaded NLCs coated with tamarind seed polysaccharide formulations which can be potentially useful for delivery of this drug. From *in vivo* drug release study, it was concluded that the NLCs with tamarind seed polysaccharide formulation delayed the drug release for two hours and controlled drug release upto 24 hrs. The result obtained from the study showed that the NLCs developed for oral delivery of Nifedipine possessed site specific targeting ability, better stability and higher entrapment efficiency, easy to scale up. The results of the present investigation showed that the problems associated with the oral bioavailability of Nifedipine could be overcome by incorporating it into a new gastrointestinal drug delivery system, nanostructured lipid carriers.

References

1. O'Driscoll, C.M., Griffin, B.T: Biopharmaceutical challenges associated with drugs with low aqueous solubility—the potential impact of lipid-based formulations. Adv. Drug Deliv. Rev. 2008; 60, 617–624.
2. Müller, R.H: Solid nanoparticle (SLN) and nanostructured lipid carrier (NLC) in cosmetic and dermatological preparations. Adv. Drug Deliv. Rev. 2002; 54, 131–155.
3. M.C. Bonferoni, S. Rossi, M. Tamayo, J.L. Pedraz, G.A. Dominguez, C. Caramella, J Control Release, 1993, 26, 119-27.
4. J.L. Ford, M.H. Ribinstein, F. McCaul, J.E. Hogan, P.J. Edgar. Int J Pharm, 1987, 40, 22334.
5. M.M. Talukdar, J. Plaizier-Vercammen. Drug Dev Ind Pharm, 1993, 19(9), 1037-46.
6. J. Sujja-areevath, D.L. Munday, P.J. Cox, K.A. Khan, Int J Pharm,1996, 139, 53-62.
7. P. Khullar, R.K. Khar, S.P. Agarwal, Drug Dev Ind Pharm, 1998, 24(11): 1095-99.
8. P.S. Rao, T.P. Ghosh, S. Krishna, J Sci Ind Research, 1946, 4, 705.
9. M. Sano, E. Miyata, S. Tamano, A. Hagiwara, N. Ito, T. Shirai.. Food and Chem Toxicol, 1996, 34, 463-467.
10. S. Burgalassi, L. Panichi, M.F. Saettone, J. Jacobsen, M.R. Rassing, Int J Pharm, 1996, 133:1-7. [11]. P.S. Rao, H.C. Srivastava, Tamarind, in Whistler RL (ed), Industrial Gums. 2nd ed., Academic Press, New York, 1973, 369-411.
11. M.J. Gidley, P.J. Lillford, D.W. Rowlands, Carbohydrate Res., 1991, 214: 299-314.
12. Ekambaram, P., Sathali, A.S., Priyanka, K: Solid lipid nanoparticles: A review. Sci Revs Chem Commun.2012; 2(1), 80- 102.
13. Rao PS, Ghosh TP and Krishna S., 1946. Extraction and purification of Tamarind seed Polysaccharide. J Sci Ind Research (India), 4,705.
14. Pathak kamla, Tiwari Radheshyam: Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: Comparative analysis of characteristics, pharmacokinetics and tissue uptake, International Journal of Pharmaceutics 2011; 415, 232– 243.
15. Taoran Wang, Qiaobin Hu, Mingyong Zhou, Jingyi Xue, Yangchao Luo: Preparation of ultra-fine powders from polysaccharidecoated solid lipid nanoparticles and nanostructured lipid carriers by innovative nano spray drying technology International Journal of Pharmaceutics 2016; 511, 219–222.
16. Pathakkamla, TiwariRadheshyam: Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: Comparative analysis of characteristics, pharmacokinetics and tissue uptake, International Journal of Pharmaceutics 2011; 415, 232– 243.
17. M. Pellegatti, P. Grossi, J. Ayrton, G. L. Evans and A. J. Harker. Absorption, distribution and excretion of lacidipine, a dihydropyridine calcium antagonist, in rat and dog. 1990; 20(8): 765-777.
18. Chun-Yang Zhuang, Ning Li, Mi Wang, Xiao-Ning Zhang, Wei-San Pana, Jun-Jie Penga, YuSheng Pana, Xin Tang. Preparation and characterization of vinpocetine loaded nanostructured lipid carriers (NLC) for improved oral bioavailability. International Journal of Pharmaceutics.2010; 394: 179–185.
19. Revathi R, Ethiraj T, Saravanan SV, et al. New spectrophotometric method of nifedipine using hydrotropic solubilization. Int J Pharm Pharm Sci. 2010; 2(4): 74-76.