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Preparation and Development of Black gram Seed Polysaccharide-Coated Nanostructured Lipid Carriers for Oral Delivery of Nifedipine

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Abstract

The present study is aimed at preparation and development of Nanostructured lipid carriers from natural polysaccharide extracted from Black gram seeds (Black gramus indica) for the sustained delivery of Nifedipine. The Nifedipine loaded NLCs were prepared by solvent injection technique with the isolated black gram seed polysaccharide. The formulations were optimized using two level factorial design using the polysaccharide, solid lipid and liquid lipid as independent variables and particle size (PS), drug entrapment efficiency as the dependent variables. The NLCs were characterized in terms of PS, entrapment efficiency, *in vitro*, *invivo* drug release and Scanning Electron Microscopy. Stable NLCs were obtained with average PS of 285.1 ± 4.6 nm. The entrapment efficiency of optimized batch was found to be 82.56 ± 2.5% (w/w). *In vitro* drug release showed controlled release pattern showing up to 90% release in 24 h. It may be concluded from the study that Black gram seed polysaccharides may be suitable for formulation of NLCs for better efficacy and sustained delivery of hypertensive drug Nifedipine.

Keywords: Nifedipine, NLCs, Characterization, *In Vitro* Drug Release, *In Vivo* Drug Release Black gram seed polysaccharide (BSP)

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.

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 **[3].** Black gram tree belongs to dicotyledonous family *Leguminosae*. Black gram seed polysaccharide (BSP) is extracted from the seed kernel of *Vigna mungo* which possesses high viscosity, broad pH tolerance, non-carcinogenicity, mucoadhesive property and biocompatibility**[4,5]** Black gram seed polysaccharide (BSP) shows sustained release behavior for both hydrophilic and lipophillic drugs **[6].** BSP has also shown high drug loading capacity **[7]** and high thermal stability **[8**]. BSP has been used as excipient in hydrophilic drug delivery system. Different mucoadhesive preparations have been formulated using BSP for drugs such as Gentamycin, Ofloxacin, **[9]** Paclitaxel,[**10]** Ketotifen Fumarate with enhanced efficacy.

2. Materials and Methods

2.1 Materials and Methods

The Black gram seeds were collected locally. All the chemicals used during the project are of analytical grade. Irinotecan hydrochloric acid (HCl) was purchased from Nice Laboratory Reagents, Kochi, India. Ethanol was purchased from Merck Ltd., India. Nifedipine, Glycerol monostearate (GMS), oleic acid (O.A), isopropyl alcohol were purchased from Balaji chemicals, Surat, India. Poloxamer 188 was purchased from Crystal Chemicals, Gangtok, India.

2.1 Isolation of BSP

250 g of Black gram seeds were taken and ground into a powder in a grinder. 20 g of this powder was taken in 1000 ml beaker having 800 ml of water and then the slurry was prepared. This slurry was boiled at a temperature below 60°C for 2 h under stirring condition. The viscous solution was kept overnight for the release of polysaccharides into water and squeezed in a muslin cloth to obtained the filtrate. To this filtrate equal amount of acetone was added to precipitate the polysaccharides. The polysaccharides were obtained by separating the filtration method, dried, powdered and stored at room temperature in air tight container **[11].**

2.2 Preparation of Nifedipine loaded NLCs with BSP

Nanostructured lipid carriers were prepared by solvent injection technique with slight modification **[12].** 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 **[13]** 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**. [14].** 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: Formulation Design of NLCs.

Formulation Code	GMS(mg)	O.A(mg)	BSP(mg)
B_1	100	10	40
B_2	200	10	40
B_3	100	20	40
B_4	200	20	40
B_5	100	10	50
B_6	200	10	50
B_7	100	20	50
B_8	200	10	50

2.3 Characterization of NLC

2.3.1 Particle size

The all preparations of NLCs were characterized for their size by utilizing digital microscope (BA-310, Motic, USA). NLCs were dispersed in 10ml of water. From the dispersion, a drop of sample was put down on glass slide and covered with cover slip. The slide then analysed under digital microscope under 40X magnification. The size of NLCs was also studied by Zetasizer at 25°C at an angle of 90°, taking the average of three measurements.

2.3.2 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 **[15].** Drug entrapment efficiency (DEE) of nanostructured lipid carriers calculated using the following equation;

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

2.3.3 *In vitro* release studies

The dialysis technique was utilized for *in vitro* drug release from the NLCs **[16].** Dialysis bag of cellulose dialysis membrane (MW cut- off 10,000 Da) was soaked in the distilled water overnight and then 1ml of drug loaded NLCs formulation was taken in dialysis bag with both the ends sealed with threads. Initial studies were carried out in 100 ml of 0.1N HCl (pH 1.2) for 2 hours and then in phosphate-buffered saline (PBS) pH 6.8 at 37°C on magnetic stirrer moving at a speed of 50 rpm for 24hrs[17]. The pH of formulation was adjusted with 2N HCl or 2N NaOH. Samples were taken out at predetermined time intervals and replaced with fresh media. Samples were filtered and analysed by using HPLC at λmax of

350 nm **[18].** All the values obtained were expressed as mean ± standard error mean (S.E.M.). Each data represents mean ± SD (n=3).

2.3.4 Scanning electron microscopy

Nifedipine loaded NLCs with Black gram polysaccharide was visualized by scanning electron microscopy for the surface morphology [**19].** Before observation, the NLCs were fixed on a double-sided sticky tape which had previously been secured on aluminum stubs and then coated with gold with thickness about 450 Å using Sputter gold coater and were visualized under scanning electron microscope.

2.3.5 *In vivo* pharmacokinetics

In vivo pharmacokinetic study was carried out by using male albino rats. The study protocols were approved by Institutional Animal Ethics Committee under CPCSEA number 1355/PO/Re/L/10/ CPCSEA. The rats were fasted overnight with free access to water before drug administration. Nifedipine suspension and Nifedipine-NLCs suspension, these two types of formulations were administered orally to the rats. The administration dose of Nifidipine was 2 mg/kg. At defined time points (1, 2, 4, 6, 8, 12 and 24 h), the collected blood sample were centrifuged for 10 min at 4000 rpm [20]. The withdrawn blood sample volume was replaced immediately with an equal volume of physiological saline. The tubes were placed in a centrifuge for 20 min at 3000 rpm to separate out the serum and then serum was stored at -20°C until drug analysis was carried out by utilizing HPLC. The standard pharmacokinetic parameters were collected from each of the individual rat plasma and plasma concentration Vs. time profiles of Nifedipine were determined by non compartmental method utilizing the Win Nonlin computer program. The Cmax and tmax were calculated from the plasma concentration vs. time graph of NIfedipine. The trapezoidal method was used to obtain the AUC. The t1/2 was calculated by linear regression of log linear portion of the plasma concentration time profile. The apparent plasma clearance (CL) was determined by dividing the dose with AUC. Win Nonlin software was utilized to calculate the mean residence time (MRT). The relative bioavailability of NLCs formulations was determined by using the following formula:

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

3.0 Results and discussion

Nifedipine loaded NLCs with coating of Black gram 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 Black gram polysaccharide can't be refused. The *in vitro* and *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.

3.1 **Optimization of various parameters by Full Factorial Design**

The test factors for optimization of process parameters are summarized in **Table 2** and the results obtained after implementing 2^3 Full Factorial Design are summarized in **Table 3**.

Table 2: Test factors for optimization of process parameters for Nifedipine loaded NLCs with Black gram Seed Polysaccharide

Factor	Name	Low level (-)	High level (+)
A (X_1)	Amount of Oleic acid	10mg	20mg
B (X_2)	Amount of Glyceryl monostearate	100mg	200mg
C (X_3)	Amount of Black gram Seed Polysaccharide	40mg	50mg

Characterization of optimized nanostructured lipid carrier formulations

Table 3: Effect of Various Parameters on Characteristics of Black gram seed polysaccharide coated NLCs

Batch Code	A: Amount of GMS acid (mg)	B: Amount of oleic acid (lipid) (mg)	C: Amount of BSP (mg)	Y1: Particle size ±S.D	Y2: Entrapment Efficiency (%w/w)
T_1	100 (-)	10 (-)	40(-)	262.12±6.2	66.38±8.4
T_2	200(+)	10 (-)	40(-)	282.12±6.5	62.43±2.6
T_3	100 (-)	20 (+)	40(-)	300.21±5.5	65.21±1.6
T_4	200 (+)	20(+)	40(-)	295.41±2.3	69.08±2.4
T_5	100 (-)	10(-)	50(+)	255.44±2.2	77.42±3.7
T_6	**200(+)**	**10(-)**	**50(+)**	**212.15±6.5**	**80.92±5.4**
T_7	100(-)	20 (+)	50(+)	265.11±2.1	71.26±3.5
T_8	200 (+)	20 (+)	50(+)	280.72±1.1	62.08±2.6

3.2 Calculation of Main and Interaction Effects

a) For Y_1: Particle sizes

The main and interaction effects of Black gram seeds polysaccharide coated NLCs are summarized in **Table 4** and **Table 5**.

Table 4: Effect of Independent variables on Dependent variables

Batch Code	Main Effects			Interaction Effects				Response
	A	B	C	AB	AC	BC	ABC	Y_1
B_1	-	-	-	+	+	+	-	262.12±6.2
B_2	+	-	-	-	-	+	+	282.12±6.5
B_3	-	+	-	-	+	-	+	300.21±5.5
B_4	+	+	-	+	-	-	-	295.41±2.3
B_5	-	-	+	+	-	-	+	255.44±2.2
B_6	+	-	+	-	+	-	-	251.15±6.5
B_7	-	+	+	-	-	+	-	265.11±2.1
B_8	+	+	+	+	+	+	+	280.72±1.1
Effect	**+1.940**	**+4.380**	**-6.645**	**-0.715**	**+1.900**	**+7.120**	**-4.625**	**71.815**

Table 5: Summarized results of main and interaction effects (Y_1)

Coefficient	Effect
A	+1.940
B	+4.380
C	-6.645
AB	-0.715
AC	+1.900
BC	+7.120
ABC	-4.625

The regression equation obtained after calculation of main and interaction effect is represented in **Eq. 1** and the corresponding Pareto chart is shown in **Figure 1**.

$$Y_1=71.81500+1.94000A+4.38000B-6.64500C-0.71500AB+1.90000AC+7.12000BC-4.62500ABC \quad \textbf{(Eq. 1)}$$

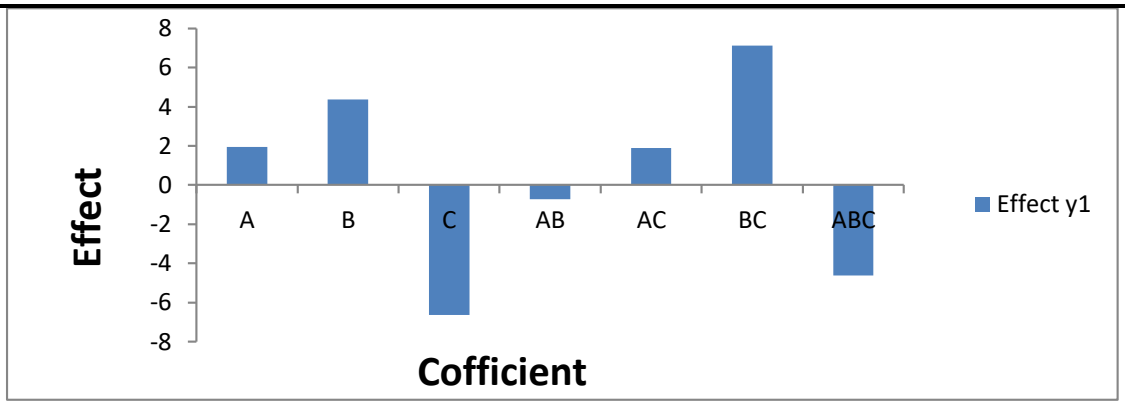

Figure 1

b) For Y_2: Entrapment Efficiency (%w/w)

The main and interaction effects are summarized in **Table 6** and **Table 7**.

Table 6: Effect of Independent variables on Dependent variables (Y_2)

Batch Code	Main Effects			Interaction Effects				Response
	A	B	C	AB	AC	BC	ABC	Y_2
B_1	-	-	-	+	+	+	-	66.38±8.4
B_2	+	-	-	-	-	+	+	62.43±2.6
B_3	-	+	-	-	+	-	+	65.21±1.6
B_4	+	+	-	+	-	-	-	69.08±2.4
B_5	-	-	+	+	-	-	+	77.42±3.7
B_6	+	-	+	-	+	-	-	78.92±5.4
B_7	-	+	+	-	-	+	-	71.26±3.5
B_8	+	+	+	+	+	+	+	62.08±2.6
Effect	-7.632	-21.65	+20.85	-2.222	-0.027	+4.032	+10.17	286.94

Table 7: Summarized results of main and interaction effects (Y_2)

Coefficient	Effect
A	-7.632
B	-21.65
C	+20.85
AB	-2.222
AC	-0.027
BC	+4.032
ABC	+10.17

The regression equation obtained after calculation of main and interaction effect is represented in **Eq. 2** and the corresponding Pareto chart is shown in **Figure 2**.

Y_2=286.94750-7.63250A-21.65250B+20.85750C-2.22250AB
0.02750AC+4.03250BC+10.17250ABC (Eq. 5.6)

Figure 2

3.3 **Particle Size Distribution of Optimized Batch**

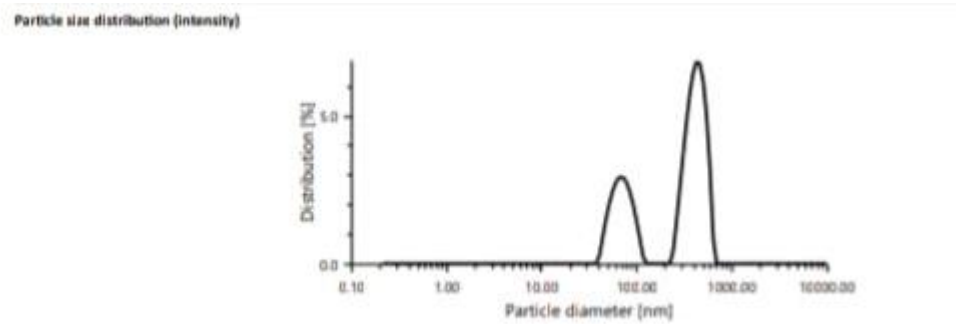

Figure 3

3.4 *In vitro* drug release

The optimized batch was subjected to *in vitro* drug release studies for 2 hrs in 0.1N HCl and further upto 24 hrs in phosphate buffer (pH 6.8) by dialysis bag technique using dialysis membrane. In vitro release rate of Nifedipine loaded NLCs is graphically presented in Figure 4. As both particle size and entrapment efficiency are determinants of drug release from a given carrier system, the *in vitro* release profile were expected to vary accordingly. In 2hrs, 12hrs and 24hrs of the release study, the %CDR observed was 16.06±0.004%, 69.73±0.13% and 92.99±0.19 %. The formulation B_6 with particle size of 212.15±6.5 nm and highest entrapment efficiency displayed maximum %CDR in a sustained manner.

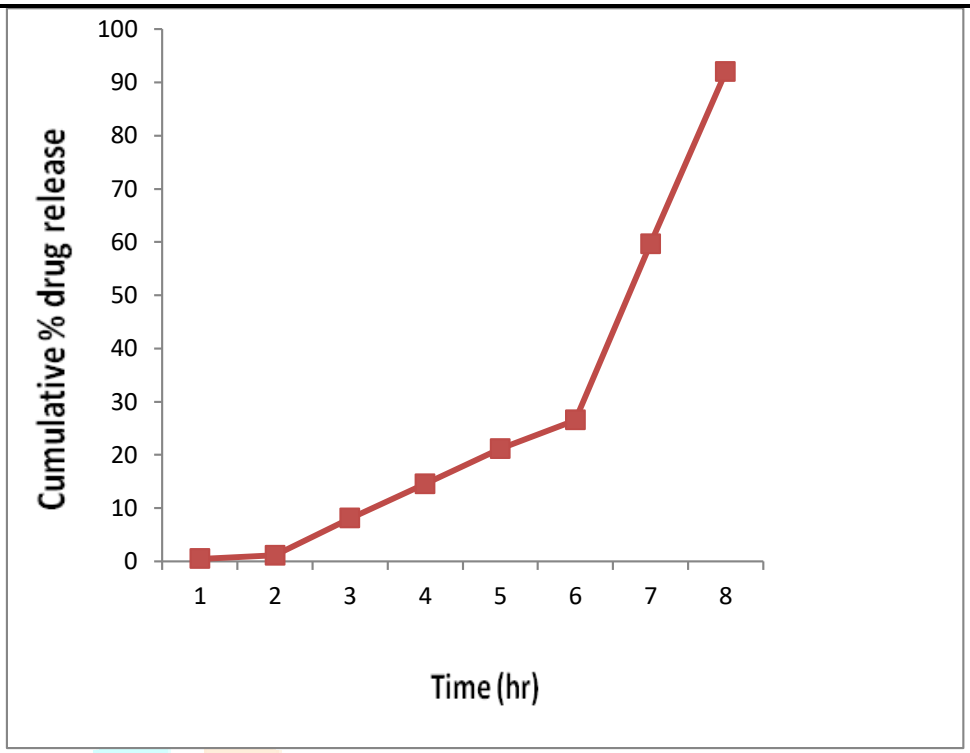

Figure 4

3.5 **SEM image of optimized NLCs**

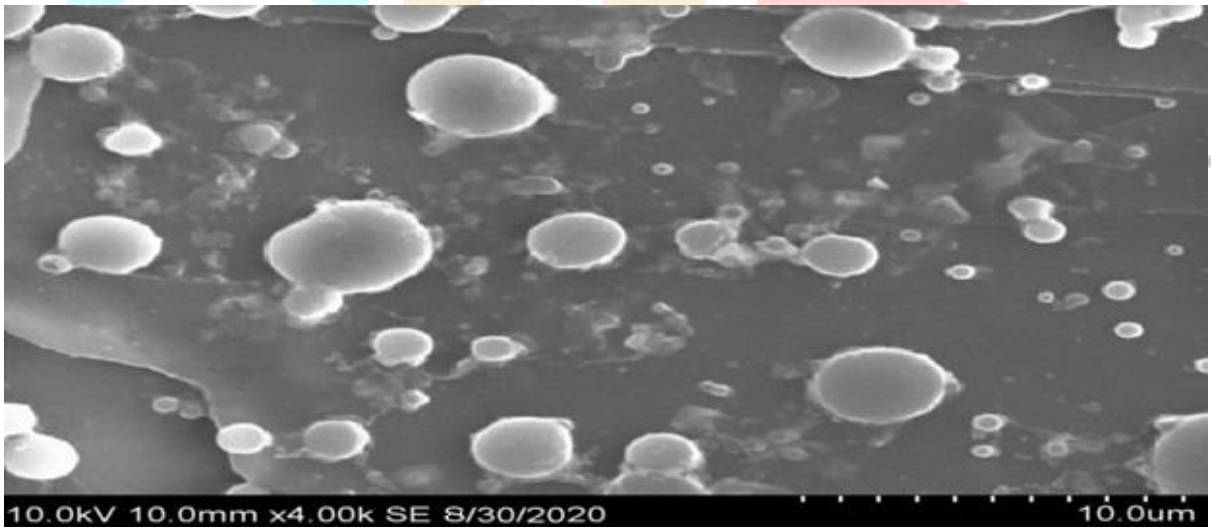

Figure5

3.6 In vivo studies

Nifedipine loaded NLCs coated with FSP and Nifedipine suspension were orally administrated to Male Albino rats. The Plasma concentration– time plots in rats after oral administration are shown in **Figure 5**.The was $t_{1/2}$ 2h and the Cmax value was 571.770ng/ml after oral administration of Nifedipine suspension. However, the $t_{1/2}$ value (21.24h) of Nifedipine NLCs was two hours later than that of Nifedipine suspension. The visible difference between $\mathbf{t_{1/2}}$ **(h)** value of Nifedipine NLCs and Nifedipine suspension manifested that the rates of absorption of two formulations were not the same. Nifedipine in suspension dissolved in the intestinal tract and absorbed directly into systemic circulation. However, Nifedipine in NLC could hardly be released into the gastrointestinal tract, as was supported in the in vitro release studies. Therefore, the intact Nifedipine NLCs were directly absorbed into the blood circulation and released the drug gradually. The C_{max} value of Nifedipine NLCs was 428.54ng/ml, which was significantly higher than that obtained with the Nifedipine suspension. The corresponding pharmacokinetic parameters are listed in Table 6. The AUC after oral administration of Nifedipine NLCs was 7668.415 ng/ml/h, which was approximately 3.9fold higher

than that of Nifedipine suspension (975.8±109.4 ng/ml/h). The results indicated that systemic absorption of Nifedipine as significantly enhanced by incorporating into NLC compared with Nifedipine suspension. The NLCs showed a promising potential for enhancing oral bioavailability of poorly water-soluble drug

Table 6: Mean pharmacokinetic parameters of nanostructured lipid carriers formulations

Formulation	**AUC (h*ng/ml)**	**C_{max} (ng/ml)**	**Plasma clearance (ml/hr)**	**MRT (h)**	**$t_{1/2}$ (h)**	**Relative bioavailability**
Drug suspension	**3064.29**	**351.770**	**539.32**	**4.34**	**1.32**	**49.67%**
Nifedipine loaded NLCs coated with BSP	7668.415	428.54	0.2585	22.56	21.24	83.48%

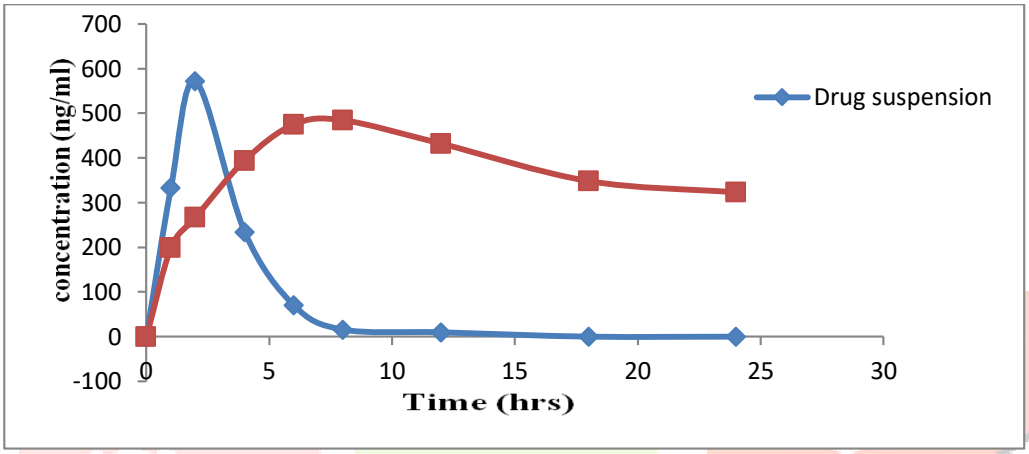

Fig. : Plasma- concentration time profile for optimized formulations

4. Conclusion

Using solvent injection technique, Nifedipine loaded NLCs coated with Black gram seed polysaccharide formulations which can be potentially useful for delivery of this drug. From *in vitro* drug release study, it was concluded that the NLCs with Black gram seed polysaccharide formulation delayed the drug release for two hours and controlled drug release upto 24 hrs. *In vivo* release study confirmed that SLNs system is very suitable to improve oral delivery of poor water soluble drug like Nifedipine with increased solubility which in turn enhanced bioavailability with sustained release of drug. In future perspectives, the produced Nifedipine loaded NLCs could potentially be transformed into solid and liquid dosage form followed by in vitro and in vivo assessments.

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