

# *In Vitro* Cytotoxicity and Pharmacokinetic Study for Bosutinib Solid Lipid Nanoparticles

Baddela Nagaiah<sup>1</sup>, S. Nirmala<sup>2\*</sup>

<sup>1</sup>Department of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Selaiyur, Chennai, Tamilnadu-600073, India

<sup>2</sup>Department of Pharmacy, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chrompeat, Chennai, Tamilnadu-600064, India

**\*Correspondence Author:**

Dr. S. Nirmala,  
Professor, Department of Pharmacognosy, Faculty of Pharmacy,  
Sree Balaji Medical College and Hospital Campus, Bharath  
Institute of Higher Education and Research, Chrompeat, Chennai,  
Tamilnadu-600064, India  
E-mail: nirmala.cognosy@gmail.com  
Contact no.: +91-9505421144

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**Abstract** **Objective:** Bosutinib (BST) is a Biopharmaceutics Classification System Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of BST lead to poor bioavailability, Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of BST. Animal data suggest that the absolute bioavailability of BST is about 14–34% due to an extensive first-pass effect. To overcome hepatic first-pass metabolism and to enhance oral bioavailability, lipid-based drug delivery systems such as solid lipid nanoparticles (SLNs) can be used.

**Methods:** SLNs are submicron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. BST can be conveniently loaded into SLNs to improve the oral bioavailability by exploiting the intestinal lymphatic transport. An optimal system was evaluated for bioavailability study in rats compared with that of BST suspension (SUS).

**Results:** An *in vitro* cytotoxicity study was done by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay method through ATCC cell lines; the percent inhibition was more in SLN when compared with SUS. The pharmacokinetics of BST-SLNs after oral administration in male Wistar rats was studied. The bioavailability of BST was increased by 2.28 fold when compared with that of a BST SUS.

**Conclusion:** The results are indicative of SLNs as suitable lipid-based carrier system for improving the oral bioavailability of BST.

**Keywords** Bosutinib, Solid lipid nanoparticles, In vitro cytotoxicity, Pharmacokinetic study

## Introduction

The drugs with poor oral bioavailability are unable to reach the minimum effective concentration to exhibit

therapeutic action. Some of the reasons for poor bioavailability include poor solubility, inappropriate partition coefficient as it influences the permeation of drug through lipid membrane, first-pass metabolism,

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P-glycoprotein mediated efflux, and degradation of drug in the gastrointestinal (GI) tract due to pH of the stomach or enzymatic degradation or by chemical [1].

Solid lipid nanoparticles (SLNs) are sub-micron colloidal carriers having a size range of 50–1000 nm. These are prepared with physiological lipid and dispersed in water or aqueous surfactant solution. SLNs were developed in the past decade as an alternative system to the existing traditional carriers, i.e., emulsions, liposomes, and polymeric nanoparticles [2,3]. These are related to emulsions, where the liquid lipid oil is substituted by a solid lipid. SLNs offer unique properties such as small size, large surface area, and high drug loading and are attractive for their potential to improve the performance of active pharmaceutical ingredients. The advantages of SLNs include drug targeting, biocompatibility, nontoxicity, drug release modulation, and small-scale production [4].

SLNs are also useful for the improvement of bioavailability of poorly water-soluble drugs, such as cyclosporine A [5], and to prolong the release of lipophilic drugs, such as camptothecin [6]. The mechanism proposed for enhancement of bioavailability of poorly water-soluble drugs by use of oral lipids includes promotion of lymphatic transport, which delivers drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing GI membrane permeability [7,8]. It is known that drug delivery through SLNs improved the pharmacokinetic (PK) behavior. SLNs of Bosutinib (BST) were not yet reported till now; hence, there is a need to improve the solubility and bioavailability through this delivery system.

## Materials

BST was obtained as gift sample from Aurobindo labs, Hyderabad. ATCC-Leukemia Cell Line Panel was obtained from Sigma-Aldrich, USA. Culture media contains Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin from Sigma-Aldrich, USA. Male Wistar rats were obtained from M/s. Sri Venketeswara Enterprises, Bangalore.

## Methods

### In vitro cytotoxicity studies

Leukemia Cell Line Panel (ATCC<sup>®</sup> TCP-1010™) cells are used for the cytotoxicity of Bosutinib (DS)-SLNs. ATCC is a perpetual cell line consisting of human leukemia

cells, derived from the genomic mutations in one or more of the following genes: CDKN2A, KDM6A, TP53, NRAS, NOTCH1, PTEN, FBXW7, FLT3, KRAS, MLH1, and PIK3R1. ATCC was cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in a 5% CO<sub>2</sub> incubator. This cell line has been well characterized for its relevance to the toxicity models in human.

### Cell culture and treatment

Cells were seeded at 2, 50, 000 cells/flask in a total volume of 10 mL. When confluent, cells were trypsinized and seeded in 96 well plates at the rate of  $1.0 \times 10^4$  cells/0.1 mL. All the cell cultures were maintained in a 5% CO<sub>2</sub> incubator at 37°C. The control and SLNs were prepared as dispersions in different concentrations (10–100 µg/mL) in phosphate-buffered saline as a solvent with <1% polyethylene glycol as a stabilizer and used for different assays.

### Cell viability assay

The effect of BST-SLNs on the cellular proliferation and viability was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay method. The procedure for this assay is as follows: Briefly, the cells were seeded in 96 well plates and to it, SLN and control suspension (SUS) (100 µL, in the triplicate) in media were added. The microtiter plates were then incubated at 37°C for 48 h in 5% CO<sub>2</sub> incubator, and 20 µL of MTT (5 mg/mL) was added to each well. The plate was again incubated for 2 h, and dimethyl sulfoxide (80 µL) was added to each well and was wrapped in aluminum foil to prevent the oxidation of the dye. The plate was placed on a rotary shaker for 2 h for thorough mixing. The absorbance was recorded on the enzyme-linked immunosorbent assay reader at 321 nm. The absorbance of the test was compared with that of solvent control to get the cell viability [9,10].

### In vivo bioavailability study

Male Wistar rats were obtained from M/s. Sri Venketeswara Enterprises, Bangalore. The animals were quarantined in the animal house. Rats had free access to food and water ad libitum. Twelve male Wistar rats weighing 210–230 g were kept for overnight fasting with free access to water. The animals were divided into two groups (six animals per group), optimized BST-SLN formulation for one group and another one with BST-SUS was administered by oral route [11].

### Oral administration

Two groups of rats were administered with their respective formulations using oral feeding needle based on body weight 10 mg/kg. For comparison, Bosutinib suspension was administered to the second group. Rats were anesthetized and blood samples (0.5 ml) were collected from the retro-orbital venous puncture at 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 h after drug administration. The blood samples were centrifuged (5000 rpm, 15 min) and serum was collected and stored at  $-20^{\circ}\text{C}$  until high-performance liquid chromatography (HPLC) analysis [12].

### Serum drug analysis by HPLC

For the determination of Bosutinib in 100  $\mu\text{l}$  of serum, add 25  $\mu\text{l}$  of methanol and 25  $\mu\text{l}$  of internal standard (Imatinib, 1  $\mu\text{g}/\text{ml}$ ) was added and vortexed for 2 min. The samples were made alkaline by the addition of 100  $\mu\text{l}$  of 1N NaOH and vortexed for 3 min. To this sample, 750  $\mu\text{l}$  of dichloromethane was added and vortexed for 5 min and then centrifuged at 4000 rpm for 15 min. The organic layer was separated and evaporated. The residue was reconstituted with mobile phase and a volume of 20  $\mu\text{l}$  of sample was injected into HPLC (LC 10AT, Shimadzu, Japan) with ultraviolet detector at 321 nm (SPD 10A, Shimadzu, Japan) using Lichrospher  $\text{C}_{18}$  (250 mm  $\times$  4.6 mm, i. e., 5  $\mu\text{m}$  particle size) analytical column for the analysis. Acetonitrile:water at a ratio 66:34 v/v was used as mobile phase with a flow rate of 1 ml/min. Retention time was 10.9 min for DS and 7.2 min for internal standard. The standard graph was plotted in the linearity range of 0.1–10  $\mu\text{g}/$

ml concentration with  $r^2 > 0.995$  [13].

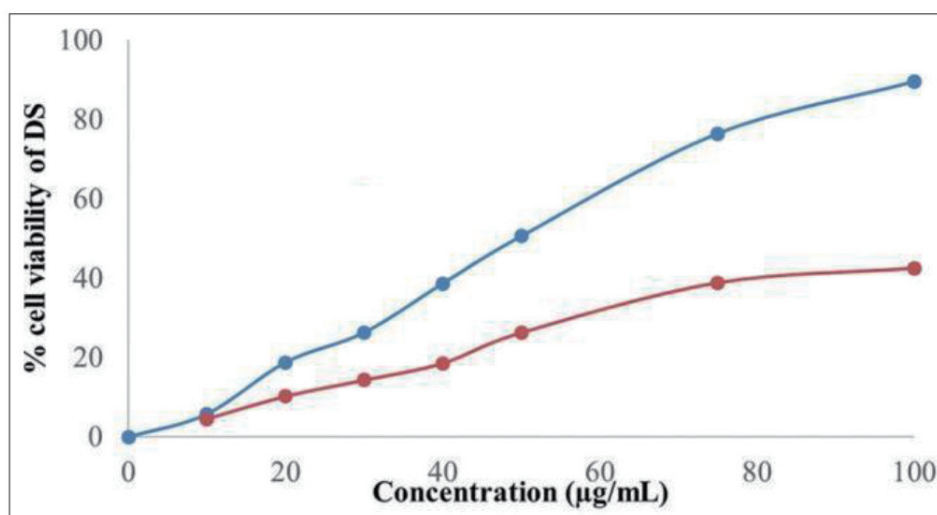
### Calculation of PK parameters and statistical significance

PK parameters such as  $C_{\text{max}}$ ,  $T_{\text{max}}$ , area under the curve (AUC),  $t_{1/2}$ , and mean residence time (MRT) were calculated using Kinetic software (version 5.0). The statistical significance of observed differences in  $C_{\text{max}}$ , AUC,  $t_{1/2}$ , and MRT of different groups was assessed by ANOVA test using GraphPad Prism software. All the data were expressed as mean  $\pm$  SD.  $p < 0.05$  was considered to be statistically significant [14,15].

## Results and Discussion

### *In vitro* cytotoxicity studies

MTT assay method was used for the determination of cytotoxicity of BST-SLNs in ATCC cell lines. In this method, the yellow tetrazolium salt was reduced by dehydrogenase enzymes present in mitochondria of metabolically active cells, to generate reducing equivalents, NADH, and NADPH. The formazan product has low aqueous solubility and was observable as purple crystals. The resulting formazan was dissolved using suitable detergent, permitted the convenient quantification of product formation. The intensity of the color of the product was measured at 321 nm. From the results, the *in vitro* cytotoxicity of BST-SLN and BST-SUS was dose dependent up to the concentration of 10–100  $\mu\text{g}/\text{mL}$ . The percentage inhibition was more (89%) with SLN formulation when compared to SUS (42%) (Fig.1).



**Fig. 1:** *In vitro* cytotoxicity profiles of Bosutinib (BST)-Solid lipid nanoparticle and BST-Suspension in ATCC cell lines (mean  $\pm$  standard deviation,  $n=3$ )

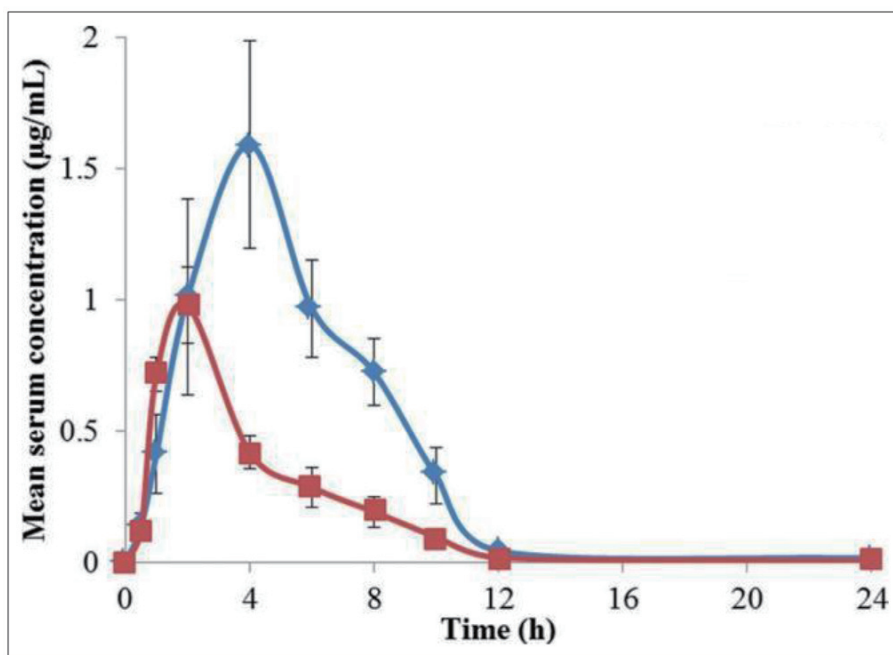
**In vivo-bioavailability studies**

Oral bioavailability studies of BST-SLN and BST-SUS formulation were conducted in male albino Wistar rats. The serum concentration levels of BST-SLN and BST-SUS are represented in Tables 1-3. The PK parameters were calculated using Kinetica software and the profiles are represented in Tables 4-6 and Fig. 2. From the results, the peak plasma concentration and peak time concentration were doubled compared with BST-SUS formulation. This is the indication of prolonged BST levels in the serum. Further, the MRT of BST-SLN was almost more compared with BST-SUS, confirmed the increased residence time and it is responsible for

sustained release behavior from the in vitro release studies also.

**Conclusion**

BST is a Biopharmaceutics Classification System Class II drug having very low solubility and high permeability. Low aqueous solubility and poor dissolution of BST lead to poor bioavailability, Thus, limited aqueous solubility is the bottleneck for the therapeutic outcome of BST. Animal data suggest that the absolute bioavailability of BST is about 14–34% due to an extensive first-pass effect. In the in vitro



**Fig. 2:** Mean serum concentration-time profiles of Bosutinib (BST) from BST-Solid lipid nanoparticles and BST-Suspension in rats (Mean±standard deviation, n=6)

**Table 1:** Serum concentrations of BST-SLN in rats

Time (h)	Serum levels of BST (µg/mL)					
	R1	R2	R3	R4	R5	R6
0.5	0.09	0.11	0.24	0.13	0.13	0.1
1	0.26	0.38	0.604	0.53	0.47	0.23
2	0.82	1.1	1.62	1.12	0.88	0.51
4	2.06	1.16	1.66	1.33	1.28	2.05
6	0.91	1.02	0.86	0.79	0.91	1.31
8	0.85	0.71	0.68	0.52	0.71	0.87
10	0.48	0.33	0.29	0.23	0.43	0.21
12	0.03	0.04	0.06	0.04	0.05	0.04
24	0.01	0.02	0.01	0.01	0.03	0.01

BST: Bosutinib, SLN: Solid lipid nanoparticles

**Table 2:** Serum concentrations of Bosutinib -suspension in rats

Time (h)	Serum levels of DST ( $\mu\text{g/mL}$ )					
	R1	R2	R3	R4	R5	R6
0.5	0.09	0.08	0.17	0.12	0.14	0.11
1	0.76	0.65	0.81	0.69	0.73	0.65
2	1.01	1.21	0.85	0.91	0.83	1.06
4	0.38	0.42	0.35	0.47	0.38	0.51
6	0.28	0.33	0.21	0.29	0.2	0.4
8	0.18	0.26	0.18	0.14	0.13	0.26
10	0.1	0.08	0.12	0.09	0.05	0.14
12	0.02	0.02	0.01	0.02	0.015	0.02
24	0.007	0.005	0.007	0.006	0.001	0.005

DST: Bosutinib, DST-SUS: Bosutinib-suspension

**Table 3:** Mean serum concentration-time profiles of BST-SLN and BST-SUS in rats

Time (h)	DS-SLN	DST-SUS
	Mean $\pm$ SD	Mean $\pm$ SD
0	0 $\pm$ 0	0 $\pm$ 0
0.5	0.13 $\pm$ 0.05	0.12 $\pm$ 0.03
1	0.41 $\pm$ 0.15	0.72 $\pm$ 0.06
2	1.01 $\pm$ 0.37	0.98 $\pm$ 0.14
4	1.59 $\pm$ 0.40	0.42 $\pm$ 0.06
6	0.97 $\pm$ 0.18	0.29 $\pm$ 0.08
8	0.72 $\pm$ 0.13	0.19 $\pm$ 0.06
10	0.33 $\pm$ 0.11	0.10 $\pm$ 0.03
12	0.04 $\pm$ 0.01	0.02 $\pm$ 0.00
24	0.015 $\pm$ 0.01	0.005166667 $\pm$ 0.00

BST: Bosutinib, SLN: Solid lipid nanoparticles, DS-Dasatinib, SUS: Suspension, SD: Standard deviation

**Table 4:** PK parameters of DST-SLN in rats

Parameter	R1	R2	R3	R4	R5	R6
C-max ( $\mu\text{g/ml}$ )	2.06	1.16	1.66	1.336	1.288	2.05
t-max (h)	4	4	4	4	4	4
AUC total ( $\mu\text{g/ml}$ ) h	10	8.75	10.21	8.06	8.91	9.94
t <sub>1/2</sub> (h)	3.39	3.83	3.08	3.19	4.29	3.52
MRT (h)	5.72	5.91	5.14	5.25	6.42	5.78

BST: Bosutinib, SLN: Solid lipid nanoparticles, MRT: Mean residence time, AUC: Area under the curve, PK: Pharmacokinetic



**Table 5:** PK parameters of DST-SUS in rats

Parameter	R1	R2	R3	R4	R5	R6
C-max ( $\mu\text{g/ml}$ )	1.01	1.21	0.854	0.91	0.83	1.06
t-max (h)	2	2	2	2	2	2
AUC total ( $\mu\text{g/ml}$ ) h	4.06	4.5	3.69	4	3.36	4.79
t1/2 (h)	3.36	3.43	3.42	3.47	2.53	3.38
MRT (h)	4.57	4.48	4.37	4.47	3.83	4.7

DST: Bosutinib, SUS: Suspension, MRT: Mean residence time AUC: Area under the curve, PK: Pharmacokinetic

**Table 6:** PK parameters of BST from BST-SLN and BST-SUS in rats (Mean $\pm$ standard deviation, n=6)

Parameter	BST-SLN	BST-SUS
	Mean $\pm$ SD	Mean $\pm$ SD
C-max ( $\mu\text{g/mL}$ )	1.59 $\pm$ 0.39	0.98 $\pm$ 0.14
t-max (h)	4.00 $\pm$ 0.00	2.00 $\pm$ 0.00
AUC total ( $\mu\text{g/ml}$ ) h	9.31 $\pm$ 0.86	4.07 $\pm$ 0.52
t1/2 (h)	3.55 $\pm$ 0.45	3.27 $\pm$ 0.36
MRT (h)	5.70 $\pm$ 0.47	4.40 $\pm$ 0.30

BST: Bosutinib, SLN: Solid lipid nanoparticles, SUS: Suspension, MRT: Mean residence time, AUC: Area under the curve

cytotoxicity study, the percentage inhibition was more with BST- SLN formulation when compared to BST-SUS. The indication of the enhanced oral bioavailability is evidenced from the AUC of the test and control formulation. BST-SLN and BST-SUS shows the AUC<sub>total</sub> levels at 9.31 $\pm$ 0.86 ( $\mu\text{g/ml}$ ) h and 4.07 $\pm$ 0.52 ( $\mu\text{g/ml}$ ) h, respectively. From the AUC parameter, about 2.28-fold enhancement in the BA was observed in BST-SLN compared with BST-SUS, which is statistically significant at a level of p<0.05.

#### Conflict of interest

Authors declare that we have no Conflict of Interest.

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