

## Development and In-Vitro Anticancer Evaluation of Dual Loaded Nanoparticles on Human Glioblastoma Multiforme Cell line T98G

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### Abstract

Cancer treatment suffers with the failure of chemotherapeutic agents because of multi drug resistance. Investigation of new molecules involves huge expenditure and time. Present investigation aims at the dual loading of anticancer agent Imatinib Mesylate along with Piperine on to bovine serum albumin nanoparticles in order to overcome multi drug resistance and to achieve the maximum therapeutic effect. Desolvation method with the addition of acetone is used to prepare the nanoparticles. Drugs and polymer are subjected to differential scanning calorimetry. Nanoparticles are evaluated for encapsulation efficiency, particle size, zeta potential and drug release studies. In-vitro anticancer activity of the nanoparticles against Human Glioblastoma Multiforme (GBM) cell line T98G is determined. Results indicated compatibility in DSC, an encapsulation efficiency of 52.456%-88.254%, particle size of 208.3nm -497.3nm, zetapotential of -16.5mV to -63.2mV and drug release of 86.256% to 94.56% in 24H. In in-vitro anticancer activity % of cell death is 68.257% to 79.658%. Results suggest increased anticancer activity with the nanoparticles dual loaded with Imatinib mesylate and Piperine.

**Keywords:** Piperine, Imatinib mesylate, anticancer activity, bovine serum albumin and desolvation method.

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### INTRODUCTION

Even with the advents in cancer research, multi drug resistance is still a big challenge for cancer chemo therapy. This resistance may be because of multiple mechanisms like, decreased uptake of drug, more drug efflux, DNA repair mechanisms and many more [1]. This ultimately results in the failure of chemotherapeutic agents. The cancer drug resistance is a complex phenomenon and more difficult to overcome. A combination therapy which can show synergy or an additive effect with reduced drug resistance, along with anticancer drug may provide a new strategy in drug resistant cancers [2]. Imatinib mesylate (IMB) is a selective tyrosine kinase inhibitor approved for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors. Several clinical studies reported the use of IMB in the treatment of patients with malignant glioma [3]. IMB is a substrate for multidrug resistance proteins. An increased expression of P glycol protein (PGP) plays an important role in drug resistance to IMB in treating cancer [4]. Piperine (PIP) being natural antioxidant can act as anticancer agent by detoxification of enzymes, suppression of cell self renewal and also by inhibition of cancer cell

proliferation [5]. Literature also supports PIP, a substrate for PGP can prevent the efflux and can be an interesting novel modulator of multidrug resistance [6]. Present investigation aimed at dual loading of the PIP and IMB on to Bovine serum albumin (BSA) nanoparticles (NPs) in order to evaluate their anticancer activity on Human glioblastoma multiforme (GBM) cell line T98G.

### MATERIALS AND METHODS

#### Materials

Imatinib Mesylate is a kind gift sample from MSN laboratories, Hyderabad. Piperine and Bovine serum albumin are from Sigma Aldrich, Mumbai. All the other chemicals and reagents used are of analytical grade.

#### Preparation of dual loaded NPs

BSA dual loaded NPs were prepared by desolvation method with the addition of acetone as the desolvating agent. 1% BSA solution was prepared in double distilled water. pH of the solution was adjusted by using 0.1M NaOH. 0.5g of (IMB) and (PIP) in 1:1 ratio was added to acetone. Acetone was added

dropwise at a rate of 1ml/min in to BSA solution under a constant stirring rate of 500rpm until the solution becomes turbid. Finally 0.01ml of glutaraldehyde (25%) was added and stirred for 3 hours to harden the NPs by intra particle crosslinking. The BSA nanoparticles formed were purified by two cycles of centrifugation and redispersion to remove unreacted chemicals and free BSA molecules. For each centrifugation step, the BSA nanoparticle solutions were centrifuged at 20,000g (Remi RM-12C, Remi Electrotechnik ltd, India) for 30 min. The supernatant was subjected for encapsulation efficacy and the pellet was collected for further characterization [7-9]. Nanoparticles were prepared by dual loading of the IMB and PIP at different pH of 9 (NP1), 7 (NP2) and 4 (NP3).

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total Drug} - \text{Drug in supernatant}}{\text{Total drug}} \times 100$$

#### Measurement of Particle Size and zeta potential

Mean particle size and zeta potential of the NPs were determined by Photon Correlation Spectroscopy (PCS) attached with a Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The prepared nanoparticles were dispersed in deionized water and sonicated for 30 min. The resultant dispersions were analyzed for mean particle size, particle size distribution and zeta potential values. Both size and zeta-potential measurements were performed in triplicate.

#### Scanning electron microscopy

The size-controlled BSA NPs were examined in a low vacuum scanning electron microscope (S-3700N, Hitachi Science Systems, Ltd., Japan). Shade dried particles were deposited on aluminum stubs using double-faced adhesive and coated with gold-palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator before observation.

#### Drug release from the NPs

For determining the drug release from NPs, 10 mg of the NPs was dispersed in 10 ml of phosphate buffered saline (PBS; pH 7.4). This solution was then enclosed in a dialysis bag and submerged in 100 ml of PBS at 37°C under a steady stirring rate of 100 rpm min. At regular time intervals, 2 ml of medium was withdrawn and the same volume of fresh medium was added. Each sample was characterized for drug contents using UV-vis spectroscopy [9].

#### DSC analysis

The thermal behavior of drugs and polymer BSA individually and as physical mixtures was studied using DSC (Mettler Toledo DSC823, Switzerland). The heating rate and nitrogen purges were 10 K/min and 20 ml/min, respectively.

#### Encapsulation Efficiency

To check the encapsulation efficiency, the free IMB present in the supernatant after centrifugation was measured by UV Spectrophotometer (Elico SL 159) at 281nm. The encapsulation efficiency was calculated using the formula:

#### Screening of NPs against Morphology of T98G

GBM cell line T98G was used for the study. Cells were grown Dulbecco's Modified Eagles medium (Gibco Invitrogen, Paisley, UK). Cell confluence (80%) was confirmed by microscopic observance. For the Screening of Nano-formulations against Morphology of T98G, cells were treated 6 hours post-seeding with 2.5 µl/ml and 5 µl/ml concentration of each formulation namely Control (PBS), NP IMB with PIP, NP IMB and IMB for morphological study. Cells were observed for 24, 48, 72 H, after treatment. Images were taken by Axiovert 200M phase contrast microscope at the magnification of 10X. Axiovision Rel.4.2 software was used to acquire the images. To check the Cell Viability Trypan blue Assay was done. T98G cells (10<sup>5</sup>/ml) were treated 6 hours post-seeding with 5 µl/ml concentration of each formulation namely NP IMB with PIP, NP IMB and IMB. Cells were removed after 48H of incubation by trypsinizing with 0.05% trypsin-EDTA solution and mixed with fresh DMEM growth medium [10].

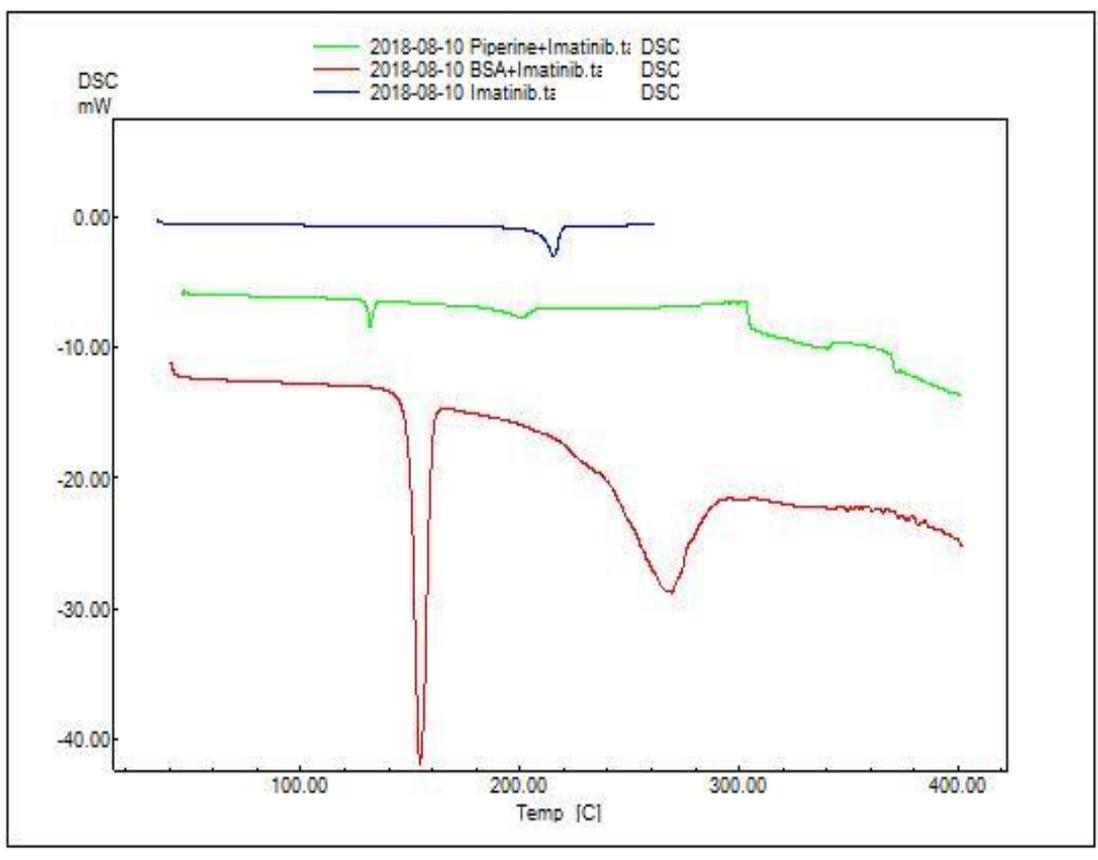
## RESULTS AND DISCUSSION

#### Preparation of dual loaded NPs

NPs were prepared by dual loading of the IMB and PIP at different pH, NP1 at pH 9, NP2 at pH 7 and NP3 at pH 4 to check the effect on drug loading. All the conditions during the process are maintained constant except for the pH.

#### DSC analysis

DSC analysis (Fig-1) of the physical mixtures confirms the absence of interactions between IMB, PIP and BSA. DSC curves clearly show the endothermic peaks at 215.49°C, 131.91°C and 154.51°C respectively for IMB, PIP and BSA.

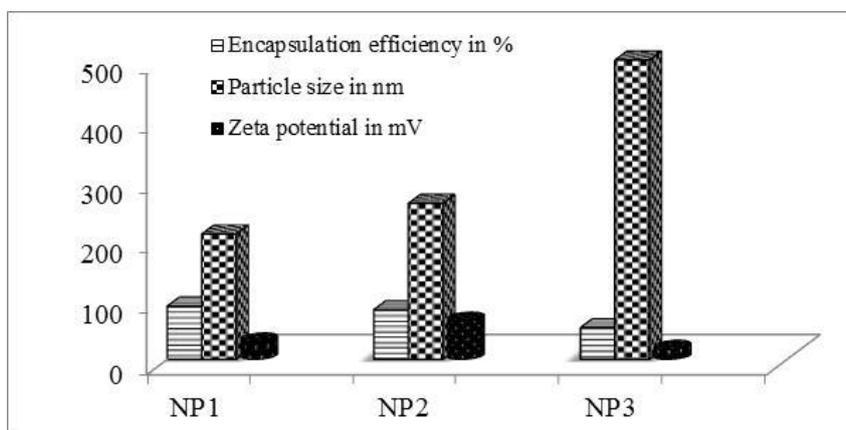


**Fig-1: DSC curves of Piperine, Imatinib mesylate and BSA in combination**

**Encapsulation Efficiency**

Encapsulation of drug (Fig-2) on to BSA NPs occurs due to the electrostatic and hydrophobic interactions between the drug and polymer [14]. In order to understand the effect of electrostatic charge on the encapsulation efficiency, drug loading was done at different pH. According to literature, IMB was reported to be predominantly neutral, monocationic, and tricationic at intestinal, blood, and gastric pH,

respectively [15]. The isoelectric point of BSA is at pH 4.5-5.0. The protein is negatively charged at neutral pH and positively charged in acidic conditions [16]. The encapsulation efficiency was found to be less in acidic pH and neutral than the basic pH. This may be because of the same charge (positive) of IMB with the BSA charge (positive) in acidic pH. There is almost no difference in the encapsulation efficiency at neutral and alkaline pH.



**Fig-2: Evaluation parameters of the prepared NPs**

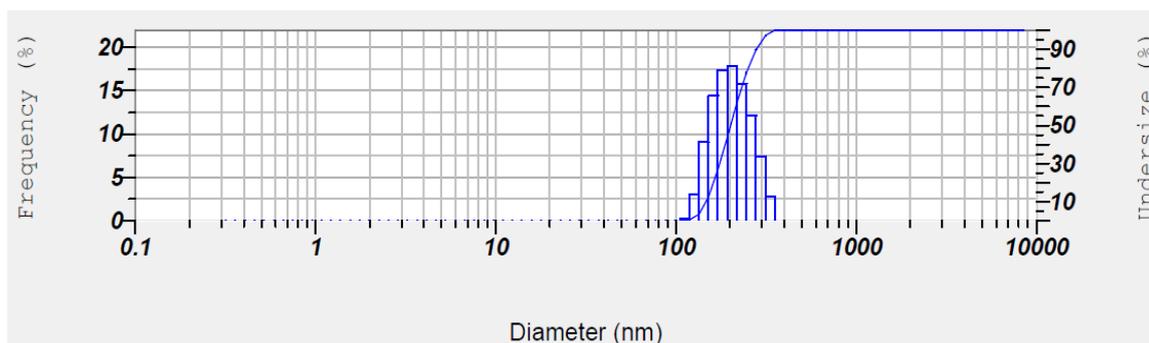
**Measurement of Particle Size and zeta potential**

The size of the NPs was found to range between 208.3±50.3nm (alkaline) to 497.3±172.8nm (acidic). There is not much difference in particle size

between neutral (259.2±65.0nm) and alkaline, but the particle size was found to be more at acidic pH. This may be because of the accumulation of positive charge of the drugs and BSA at acidic pH. It was reported that,

NPs with the size range above 300 nm were inclined to be blocked or captured by RES as well as liver sinusoids [11]. The prepared NPs from neutral and

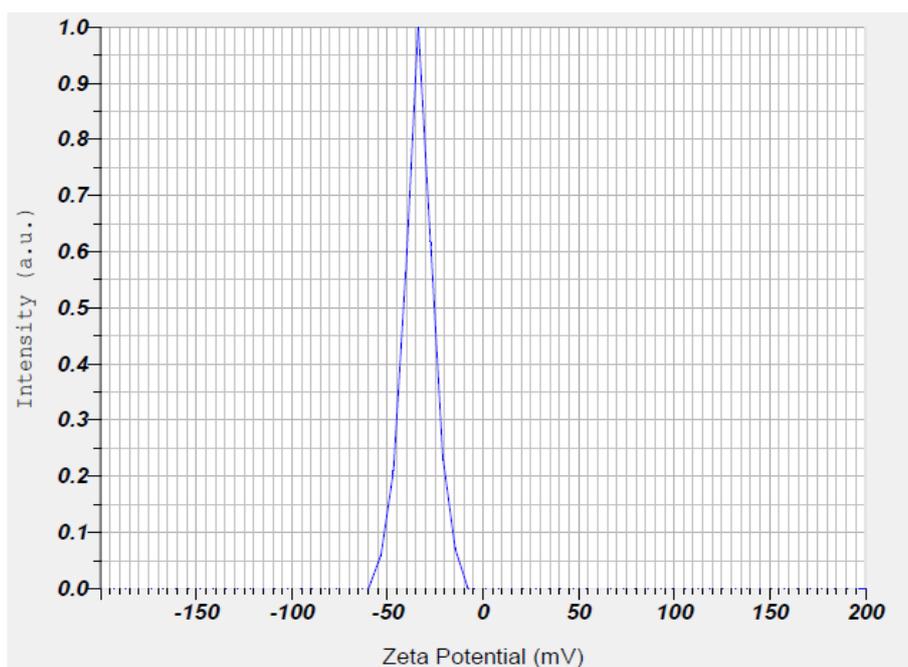
alkaline  $p^H$  have the size range suitable for long circulation without capture by RES.



**Fig-3: Mean particle size (208.3±50.3) and particle size distribution curve of the prepared NPs**

Zeta potential is the electrokinetic value associating a realistic magnitude of surface charge [12]. The prepared NPs are with a zeta potential values of, -33.5mV, -63.2mV and -16.5mV for NP1, NP2 and NP3 respectively. This indicates NPs prepared at acidic pH has less negative charge. Zetapotential not only depends on nature of particles but also on ionic strength and pH of the solution. This may be the reason for negative

zetapotential, in spite of the positive charges of BSA, IMB and PIP at acidic pH. To prevent the agglomeration of the NPs, a minimum of 20mV zeta potential is required for the electrostatic and steric stabilization of NPs [13]. NP3 were observed to have less negative zeta potential may be because of the domination of the positive charges of drugs and polymer.

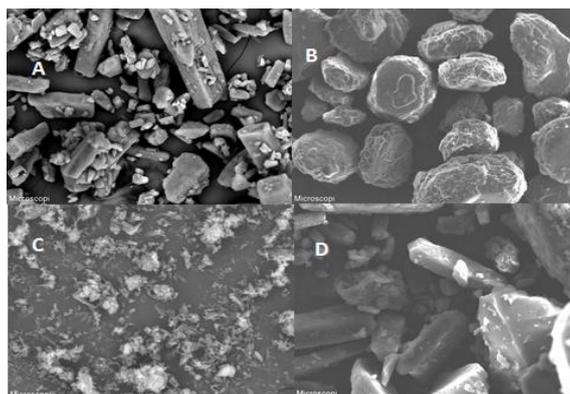


**Fig-4: Zetapotential (-33.5mV) of the prepared NPs**

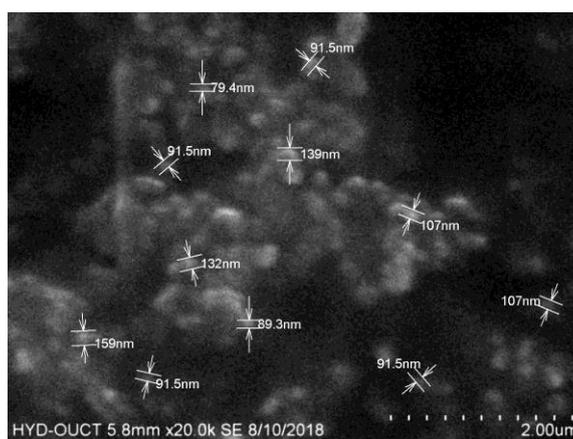
#### Scanning electron microscopy

SEM pictures (Fig-5) of the drug and polymer powders clearly indicated the crystalline morphology.

SEM picture of NPs (Fig-6) indicated loose aggregates of spherical nanosized particles.



**Fig-5: SEM pictures of Piperine (A), Bovine serum albumin (B), Imatinib (C) and physical Mixture (D)**

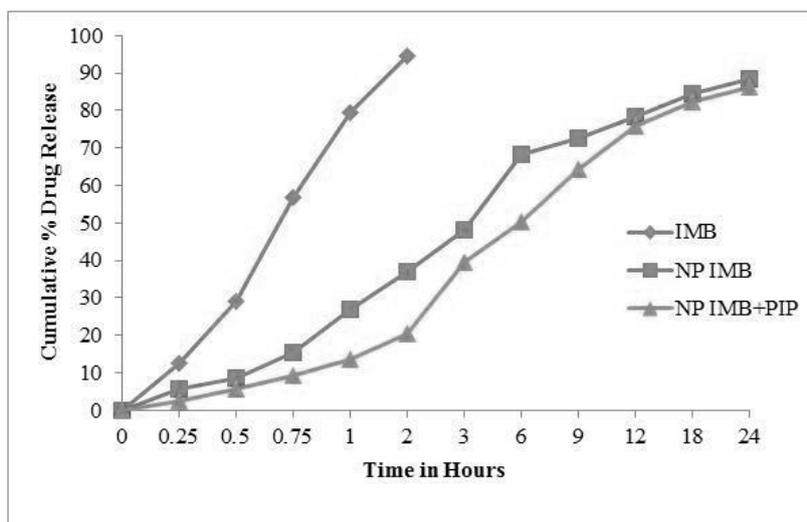


**Fig-6: SEM pictures showing the formation of NPs**

**Drug release profile of the NPs**

The prepared NPs loaded with IMB alone (NP IMB), dual loading with IMB & PIP (NP IMB+PIP) and IMB are evaluated for the cumulative % drug release. It was found from the Fig-7 that, the cumulative

drug release of 94.56 % (f2= 75.468) was within 2 H for the powder form of IMB. The same was found to be sustained upto 24H with 88.45% (f2= 69.452) and 86.254% (f2= 78.869) for NPs of IMB and NPs of IMB+PIP respectively.



**Fig-7: Cumulative % drug release of prepared nanoparticles**

**Screening of Nano-formulations against Morphology of T98G:**

From the cell viability study using trypan blue assay shown in Fig-8, there is a clear vision of cell

necrosis after 72H with the higher concentration of 5µl/ml. Fig-9 shows no necrosis of cells even after 72H with the PBS used as control.

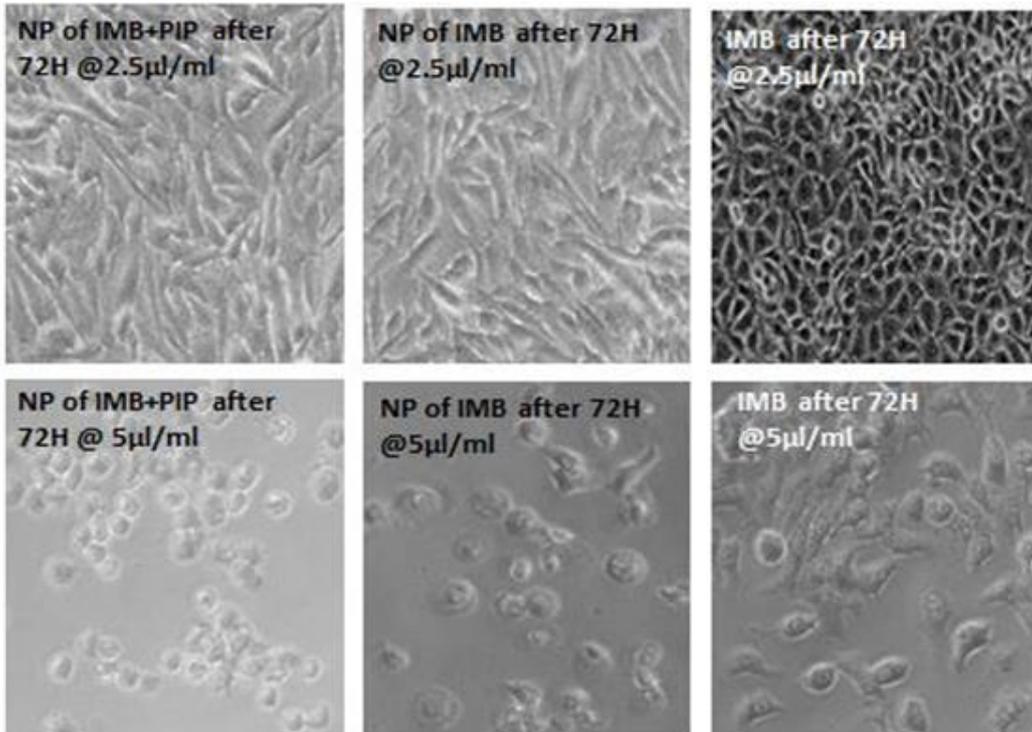


Fig-8: Contrast microscopic pictures showing cell necrosis

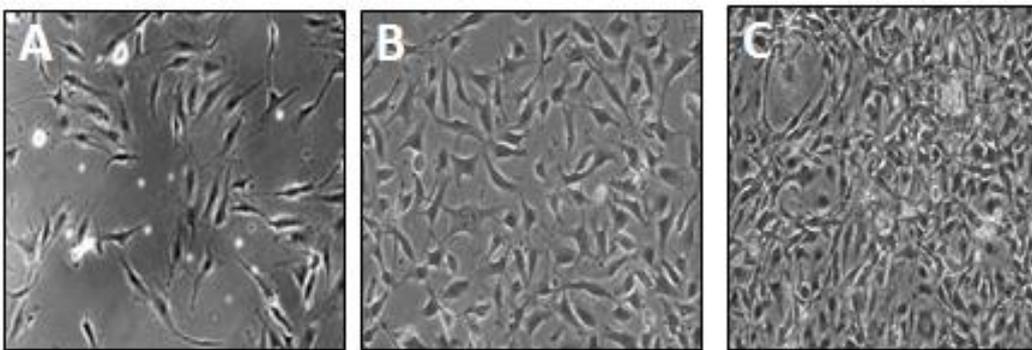


Fig-9: Contrast microscopic pictures showing cell viability in control A at 24H, B at 48H and C at 72H

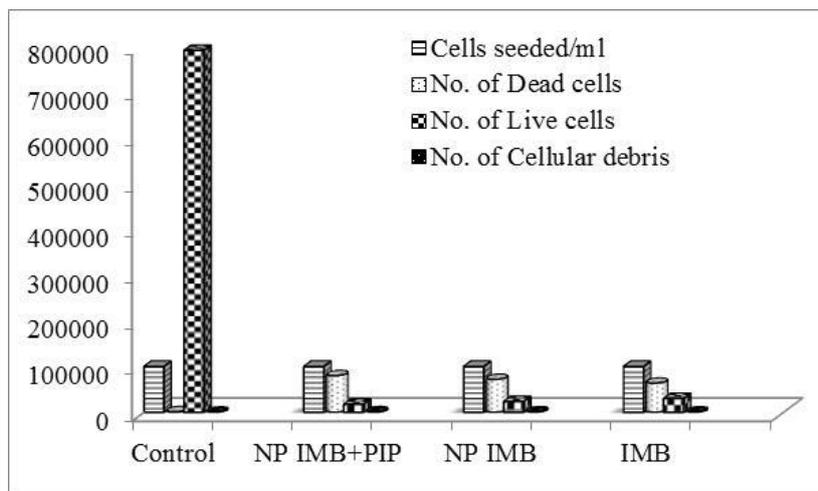


Fig-10: Screening of Nano-formulations against Morphology of T98G

Effect of dual loaded nanoparticles (NP1) on cell proliferation and survival of T98G cells was determined at different concentrations of 2.5µl/ml and

5µl/ml after incubating for 24, 48 and 72H. Fig-10 shows the cell death of 0.842%, 79.658%, 71.584% and 68.257% for control, NP of IMB along with PIP, NPs

of IMB and IMB respectively. NPs of IMB showed enhanced activity of 11.279% than the pure drug. NPs of IMB along with PIP showed 13.163% more than NPs of IMB alone. Dual loading of PIP with IMB on NPs was found to have comparatively more anti-cancer activity may be because of the additive effect.

## CONCLUSION

Cancer has a major impact on society throughout the world. It needs effective treatment option. It is necessary to overcome multi drug resistance, the major drawback in cancer treatment. Combination therapy, especially with the components like Piperine can give the way forward by preventing the drug resistance along with the provision of additive anticancer effect.

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