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In vitro assessment of solubility and bioavailability of vinblastine using nanoparticulated formulations

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ABSTRACT

Catharanthus roseus (Madagascar periwinkle) is an ornamental plant belonging to the Apocynaceae family. Besides its importance as an ornamental plant, its interest today is centered on its capacity to biosynthesize a great variety of terpenoid indole alkaloids (TIAs) which are valued highly due to their wide spectrum of pharmaceutical application. Among these, vinblastine and vincristine are of particular importance because of their wide use in cancer chemotherapy. Vinblastine can have extensive use for the treatment of lung cancer, breast cancer, head and neck cancer, and testicular cancer. It is also used to treat Langerhens cell histocytosis.This study was done to assess the solubility of vinblastine. The extracted crude vinblastine was encapsulated with nanoparticles and solubility was determined at different pH range.Silver Nanoparticles was synthesized using reduction method, LDH was synthesized by Co-precipitation method while MMT was purchased from Sigma-Aldrich.Silver NP formulation showed maximum solubility at pH 10.4 and least at pH 6. In case of Layered Double Hydroxide (LDH) Nanoparticles and Montmorillonite nanoparticles (MMT) maximum solubility was obtained at pH 7 and pH 8.6, respectively. The increased solubility range of the drug with various nanoparticles formulation will enhance the bioavailability of the drug at physiological pH.This study shows that formulation of NP-TIA's has significant potential in the field of chemotherapy and drug delivery.

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INTRODUCTION

Plants have been an important source of valuable pigments, drugs and fine chemicals that are utilized by food, cosmetics or pharmaceutical industries. One such plant is *Catharanthus roseus* that belongs to Apocynaceae family and comprises off eight different families. The leaves and roots are specialized for monoterpenoids indole alkaloids(MIA) biosynthesis. It is known for the production of two potent anti-cancer agents, the bis-indole alkaloids Vincristine and

Vinblastine. These compounds are classified as terpene indole alkaloids that are used as anti- cancer, anti- malarial and anti-arrhythmic agents. Vinblastine, an anti microtubule drug is used to treat certain cancers like Non- small cell lung cancer, Head and Neck cancer, Hodgkin's lymphoma, Breast cancer and Testicular cancer (Aslam *et al*., 2010). Advances in drug discovery technologies and combinatorial chemistry techniques have led to identification of a number of compounds with good therapeutic potential. However, because of their complex chemistry majority of these compounds have poor aqueous solubility resulting in reduced and variable bioavailability (Lipinski, 2002). Nanoparticle formulation technologies have provided the pharmaceutical industry with solubility and bioavailability issues associated with poorly soluble compounds. In new chemical entities (NCE) development, the technology has been of great value when it is used as a screening tool during preclinical efficacy and / or safety assessment studies in the early development phase. For marketed products requiring lifecycle extension opportunities, nanoparticle formulation strategies provide a means to develop a new drug-delivery platform with improved therapeutic outcome incorporating the existing drug, thus creating new avenues for addressing unmet medical needs (Vijaykumar*et al*., 2012).

MATERIAL AND METHODS

Synthesis of nanoparticles :

Silver nanoparticle:

The silver nanoparticles were prepared by chemical reduction method. 1ml of 0.001M $AgNO_3$ aqueous solution was added in 100ml of distilled water along with 1ml of 0.1M PEG and 1ml of $NaBH_4$. The mixture was vigorously stirred and heated at 70-80°C till the colour changed to pale yellow. The mixture was then removed from heating and the solution was kept in amber glass to keep away from sunlight. Confirmation of silver nanoparticle was done using SEM and UV-Vis spectrophotometer to obtain a peak at 430nm. Calibration curve at 430nm was drawn for further calculations.

Layered double hydroxide (LDH) nanoparticles:

Synthesis of Mg-Al layered nanoparticles (LDH) was done through conventional co-precpitation route in which 20ml of 0.1 M solution of $Mg(NO_3)_2$ and 20 ml of 0.3 M solution of $Ag(NO)_3$ was mixed and titrated dropwise against 40 ml of 0.1 M solution of NaOH with vigorous stirring at 50° C in inert atmosphere. The pH value of the mixture was maintained at 9-10 during the entire reaction process. The precipitate was heated and stirred for 30-40 minutes. The precipitate was washed 3 times with distilled water and centrifuged at 5000 rpm for 10 min. and then resuspended in 20 ml of distilled water as stock. The concentration was calculated by drying 1 ml of stock. The synthesized hydroxides were characterized by SEM.

Montmorillonite nanoparticles:

2 g of MMT (Sigma) was dispersed in 10 ml of 0.1N NaCl solution and stirred for 12 hours and centrifuged. This process was repeated thrice. The slurry obtained was centrifuged at 5000 rpm for 10 min. and washed with distilled water. It was then suspended in 20 ml of distilled water and then Na-MMT was purified by sedimentation which was used for further studies. The concentration was calculated by drying 1 ml of prepared MMT stock and characterized by SEM.

Preparation of crude vinblastine :

Extraction and partial purification of Vinblastine from the roots of *Catharanthus roseus* was done using methanolic extraction (Tikhomiroff and Jolicoeur, 2002).

Various dilutions of 0.2, 0.4, 0.6, 0.8, 1 ml crude Vinblastine stock was used for the estimation of concentration at different pH ranges of 3, 4, 5, 6, 7, 8.6 and 9.

Nanoformulation of vinblastine :

Formulation of crude Vinblastine was done with different nanoparticles Ag, LDH and MMT by adding 0.5 ml of crude vinblastine to 9 ml of different pH solutions and 0.5 ml of NPs respectively to perform solubility test.

pH solubility :

Solubility test was done at different pH ranges of 4, 6, 7, 8.6, 9, and 10.4. The pH solution was prepared using Phosphate-Citrate buffer and Glycine-NaOH buffer in which di-sodium hydrogen phosphate of 0.2 M and Citric acid of 0.1 M and Glycine of 0.2 M and NaOH of 0.2 M were used, respectively to prepare buffer solution. 0.5 ml of crude vinblastine was added to 9.5 ml of pH solution and in case of pure solution of crude vinblastine and 0.5 ml of crude vinblastine was added to

9 ml of pH solution and 0.5 ml of NPs to prepare different pH solutions with different NPs, respectively. The solutions were vortexed for 5 min. and then filtered using Whatmann filter paper. The filtered solution was calibrated at 420 nm using UV-Vis spectrophotometer. The solubility was checked by calculating the concentration from the linear equation of the graph and compared at different pH ranges.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Nanoparticle synthesis and characterization :

Silver nanoparticle :

Silver nanoparticles were prepared by reduction of silver nitrate solution by Sodium borohydride in the presence of PEG. Silver nitrate and PEG is a colourless solution but after adding $NaBH_4$ to the solution the colour turned to pale yellow which indicated formation of silver nanoparticles. Characterization of silver nanoparticle was done using UV-Vis Spectroscopy and the peak showed at 430nm which indicated total conversion of silver ions to silver nanoparticles as shown in Fig. 1.

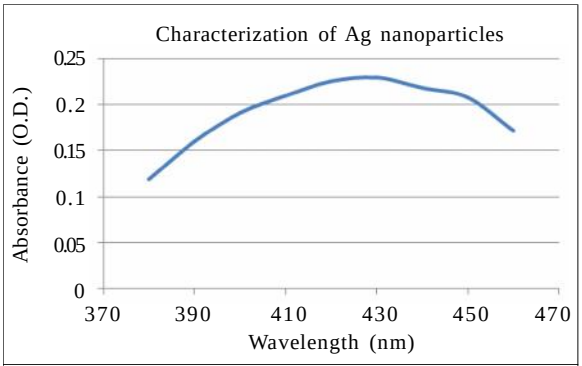

Fig. 1: Characterization of silver nanoparticles using UV-Vis spectrophotometer

Scanning Electron Microscope (SEM) image of Silver nanoparticle shown the morphology and the size approximately 50-150nm as shown in Fig. 2.

Mg-Al (LDH) Nanoparticles :

Synthesis of LDH nanoparticles was done through conventional co-precpitation route. Scanning electron microscopy (SEM) image of LDH nanoparticles shows that the morphology and size distribution of LDH nanoparticle was approximately -300nm as shown in the Fig. 3.

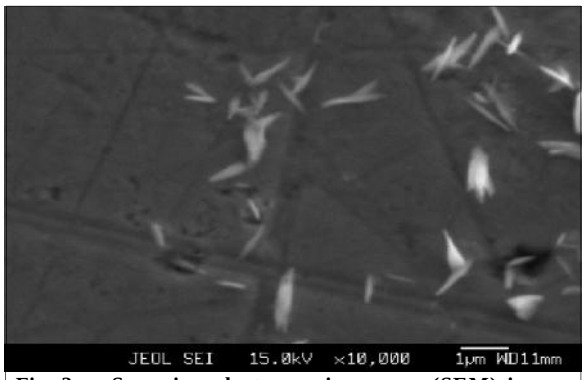

Fig. 2: Scanning electron microscopy (SEM) image of silver nanoparticle

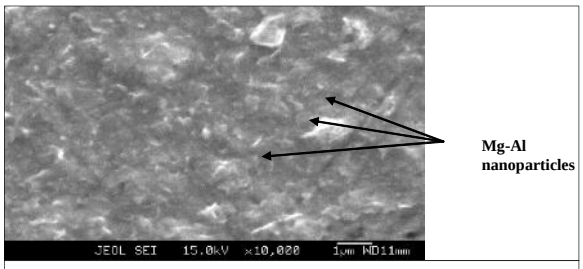

Fig. 3: Scanning electron microscopy (SEM) image of Mg-Al nanoparticles

MMT Nanoparticles :

Synthesis and purification of MMT nanoparticles was done through sedimentation of MMT. Scanning electron microscopy (SEM) image of MMT nanoparticles shows that the morphology and size distribution of MMT nanoparticle was as shown in Fig. 4.

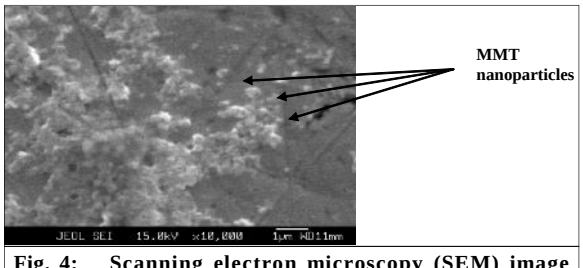

Fig. 4: Scanning electron microscopy (SEM) image of MMT nanoparticles

Characterization and calibration of crude vinblastine :

Characterization of crude vinblastine was done using UV-Vis Spectroscopy which showed a peak at 420 nm as shown in Fig. 5.

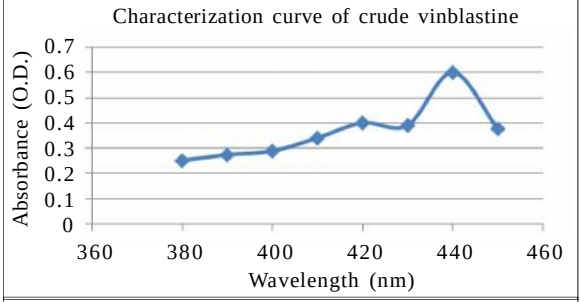

Fig. 5: Characterization of crude vinblastine using UV-Vis spectra

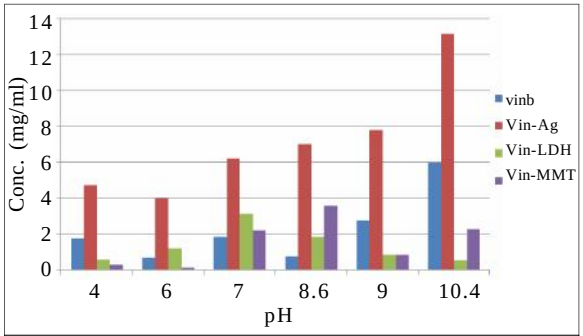

Fig. 7 : Analysis of bioavailiblity of pure and NP formulated Vinblastine

Crude Vinblastine was calibrated by UV-Vis spectra at 440 nm at different concentrations which is calculated using a relation between OD (y) and concentration (x) equation

y = 0.003x+ 0.014 ..... (Equation 1)

Solubility test by pH analysis :

Solubility test was done by mixing pure crude vinblastine, pure crude vinblastine with Ag, LDH and MMT nanoparticles with different pH solutions of 4, 6, 7, 8.6, 9 and 10.4, respectively and concentration was calculated by substituting the value of absorbance using equation 1.

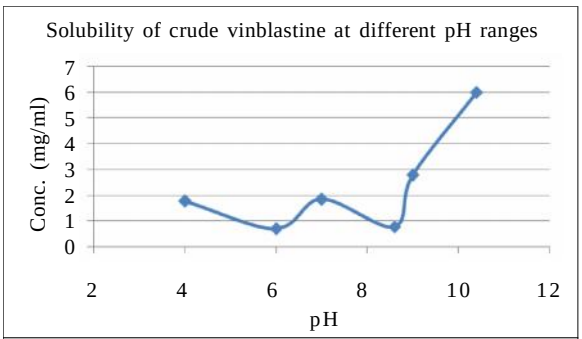

Fig. 6 : Solubility curve of crude vinblastine at different pH ranges

Comparative analysis of vinblastine bioavailibility:

The comparative study of solubility of pure vinblastine and vinblastine attached with different nanoparticles showed increased solubility with pH range. In case of LDH and MMT since this is physiological range thus LDH and MMT can be considered as good stabilizer for physiological conditions. It can be concluded that nanoparticle stabilized vinblastine generally showed higher solubility as shown Fig. 7.

Researches on solubility of the drug in pure form and formulation with nanoparticles have not been performed extensively. For any drug to be effective it must be soluble and have increased bioavailability at physiological pH.

Crude Vinblastine was extracted from *Catharanthus roseus* and incorporated into the nanoparticles Ag, LDH and MMT. Silver NPs were coated with PEG which acts as a capping agent and a linker between vinblastine and nanoparticles. The current study demonstrates that the attachment of crude vinblastine into the nanoparticles foe the enhancement of its solubility and stability compared to the pure drug. Vinblastine formulation with silver NP showed the best results in comparison to LDH and MMT. The solubility tests of vinblastine attached with different nanoparticles showed increased solubility and stability with pH range. In case of LDH and MMT since this is physiological range thus LDH and MMT can be considered as good stabilizer for physiological conditions. Vinblastine attached with nanoparticles the increased stability of drug increases to pH 9- 10, which will show better efficacy and drug delivery system than the normal one. The silver attached nanoparticles showed maximum solubility at pH 10.4 and least at pH 6. In case of LDH and MMT maximum solubility was obtained at pH 7 and pH 8.6.

For commercial production, a semi-synthetic route has been used to couple vindoline and catharanthine; both compounds are present in higher concentrations within the leaves (Heijden *et al.*, 2004).

Surface modification of NPs and artificial control of NPs size and shape, are effective ways to reduce the toxicity of NPs. But some scholars believe that artificially coated and modified NPs have lost their original features, which from a fundamental sense not the original NPs

can be compared with. Surface modification methods can be divided into the surface coating and chemical modification. Through the surface modification of NPs, the inherent toxicity of NPs can be reduced, which also can greatly improve the biocompatibility of NPs (Li *et al*., 2012).

Conclusion :

The current study demonstrates that Vinblastine formulated with nanoparticles enhanced its solubility compared to the pure drug. The silver formulated drug showed the highest bioavailability in comparison to LDH and MMT showing maximum solubility at pH 10.4 and least at pH 6.The comparative study of solubility of pure vinblastine and vinblastine attached with different nanoparticles showed increased solubility with pH range. In case of LDH and MMT, the maximum bioavailability was obtained at pH 7 and pH 8.6 and therefore can be considered as good stabilizer for physiological conditions. They can also be utilized for imaging studies, increasing the bioavailability of drugs and targeted delivery of drugs at cellular and nuclear level.

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