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Visit Journal at <https://www.jacsdirectory.com/jpmr> Nano-Niosomal Formulation of Alkaloids from *Vinca rosea* for Improved Oral DeliveryV. Poorani^{1,*}, Vigneswaran², G. Venkat Kumar¹¹Department of Biochemistry, PRIST University, ECR Campus, Chennai – 603 102, Tamilnadu, India.²BioLim Centre for Science & Technology, Chennai – 600 023, Tamilnadu, India.

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ABSTRACT

Phytochemicals, the natural biochemical substances produced by plants possess a range of medicinal values. These phytochemicals get into our system through food and bring different physiological benefits. However, many of these phytochemicals lack essential physicochemical properties that can provide them effective drug-likeness properties. *Vinca rosea* alkaloids are known for their therapeutic values especially in the cancer treatment domain. However, the physicochemical properties of these molecules limit their bioavailability greatly. This current research aimed at improving the bioavailability of *Vinca rosea* alkaloids through the nanovector system, niosomes. *Vinca* alkaloids were extracted, purified, screened and quantified. Niosomal constructs of the alkaloids were made, characterized through scanning electron microscope and ex-vivo studies were carried out using goat intestine. The size of the niosomes was found to be in the range of 400 to 800 nm. Results shows that niosomal formulation can increase bioavailability of *Vinca rosea* alkaloids two folds compared to the native alkaloid extract.

1. Introduction

Pharmaceutical denotes any substance or compound that provides medicinal or health benefits. It is majorly used for curing a wide range of diseases. It is a multidisciplinary field, which has gained numerous dimensional roles in curing diseases. It deals with understanding the substance or compound in-depth, i.e. at the molecular level, for designing the drug. Traditional medicine serves as a lifesaver when compared with modern medicine [1]. Plants and their therapeutic compounds are effective in preventing diseases and one of the advantages over traditional medicine is that it does not accelerate the disease, for example, the deleterious effect of the drug post medication. Phytopharmacological compounds like alkaloids are highly soluble in the aqueous layer and have low absorption because they are unable to penetrate the lipid bilayer of the cells, which results in loss of bioavailability. As a result of this obstacle, the phytochemicals are formulated using a nanostructured system, which enables potentiating the action of the phytochemicals, reducing the side effects and improving the therapeutic activity [2]. Pharmaceutical industries henceforth focus on the plant compounds and their improvement through nanotechnology. Pharmaceutical nanotechnology represents revolutionary opportunities to fight against threatful diseases, such as cancer, diabetes mellitus, neurodegenerative diseases, etc., [3]. The activities of active compounds in plants are being researched to understand their complexity for further developing them into therapeutic formulations. Phytotherapeutics requires a delivery system to improve the bioavailability and sustained release in the system and reduces the effect of frequent administration [4]. Delivery of drug through the nanovector system has gained a potential future for the effective treatment and to overcome the drug's side effects, to reduce the toxicity and minimize the degradation of the compounds [5]. The advantages of using nanovectors are improved solubility of the drug, promotion of the sustained release of the active constituents and biological target reach [6]. Currently, nanotechnology is developing innovative delivery systems like nanovesicles using biodegradable and biocompatible substances for effective drug delivery in the system. Niosomes are one such nanovesicles that can be used for entrapping the active phytochemical compounds and delivering them effectively through different drug delivery routes, such as oral, topical, intravenous, etc. This research work have engaged in

developing niosomal nanoformulations of *Vinca* alkaloids. *Vinca* alkaloids are a series of secondary metabolites that are isolated from the medicinal plant *Vinca rosea*. These alkaloids are reported to possess a range of medicinal activities that include anticancer and antidiabetic activity, hypertension treatment, etc. Even though the therapeutic application profile of these alkaloids is high, there are many challenges in making these alkaloids reach the destination for treatment. This may be due to their physicochemical properties which lead to poor bioavailability and possible side effects. Therefore, *Vinca* alkaloids could be an effective target compound group among the range of phytochemicals to be experimented with for developing a nanoformulation.

This research work deals with the extraction of alkaloids from the *Vinca* leaves, developing niosomal formulation of *Vinca* alkaloids, characterizing the nanovesicle formulation and studying the ex vivo applications of the nanoformulation to determine the improved deliverability of *Vinca* alkaloids through nanovectors.

2. Experimental Methods

2.1 Extraction of *Vinca rosea* Alkaloids

About 4 g of fresh leaves of *Vinca rosea* was taken and rinsed with sterile distilled water, and air-dried. Leaves were then crushed with 0.1 M HCl solution using a mortar and pestle. It was then kept for stirring in a magnetic stirrer for 2 h and centrifuged. The supernatant was then added with petroleum ether and shaken well in a separating funnel. The acid fraction was carefully removed and transferred to a separating funnel containing an equal volume of 0.1 N NaOH solution and shaken well. The funnel was kept undisturbed for 30 min. To that, diethyl ether (1:1) was added and mixed by inverting gently and the internal pressure was released slowly. The mixing action was repeated thrice and observed for two layers of separation. The upper organic layer containing alkaloids was separated, evaporated and stored for further experiments [7].

2.2 Purification of *Vinca rosea* Alkaloids using Column Chromatography

Column chromatography was performed on a glass column (20 × 2 cm) packed with 90 g of alumina. The fresh *Vinca* alkaloid extract (5 mL) was applied to the column by using a pipette and the column was eluted with methanol. Five fractions were collected and each fraction was tested for the presence of alkaloids using Dragendorff's reagent. The fractions were then evaporated and dissolved in 5 mL of methanol and refrigerated for further analysis [8].

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2.3 Identification of *Vinca rosea* Alkaloids by Thin Layer Chromatography (TLC)

Silica gel-coated aluminium plates (obtained from Merck, India) were taken in the size of 2 x 9 cm. The *Vinca* alkaloids sample in methanolic solution was loaded at the base of the TLC plate using an applicator stick. The solvent system containing 80% ethanol and 1 N HCl (in the ratio 25:1) was taken in the TLC chamber and a thin layer chromatogram was developed. The plate was then allowed for drying and Dragendorff's reagent was sprinkled over the plate and observed for yellow spots [9].

2.4 Separation of *Vinca rosea* Alkaloids by High-Performance Thin Layer Chromatography (HPTLC)

HPTLC was performed on 10 x 20 cm aluminum-backed silica gel F254 HPTLC plates from Merck, India. In order to avoid possible interference of manufacturing-based impurities on silica plates, the plates were prewashed with methanol, dried and activated for 30 min at 110 °C. *Vinca* alkaloids dissolved in methanolic solution was applied on plates as 6 mm bands, by 6 mm apart and 1 cm from the edge of the plate, by means of automatic sample applicator, fitted with a syringe. Vinblastine sulphate was used as standard and was applied to the parallel track. The mobile phase used in the analysis was hexane: ethylacetate: glacial acetic acid (3:1:0.1). The plate was then moved with the mobile phase and developed to a distance of 90 mm and then removed from the chamber, dried and scanned at 210 nm using scanner densitometer [10].

2.5 Estimation of *Vinca rosea* Alkaloids using Bromocresol Green (BCG) Method

Vinca alkaloids dried in rotary vacuum evaporator was taken and dissolved in 2 N HCl and mixed well. The solution was then filtered. 1 mL of this solution was transferred to a separating funnel and washed with 10 mL of chloroform (3 times) followed by pH adjustments to neutral using 0.1 N NaOH. Then, 5 mL of BCG solution and 5 mL of phosphate buffer were added to this solution and mixed well. The complex thus formed was then extracted with chloroform (1, 2, 3 and 4 mL) by continuous and vigorous shaking. The absorbance of the complex in the chloroform was measured at 470 nm. Vinblastin sulphate obtained from Sigma chemicals was used as the standard [11].

2.6 Synthesis of *Vinca rosea* Alkaloids Niosomal Formulation

Vinca alkaloids niosomal formulation was prepared by thin-film hydration technique. Niosomes were prepared using Span-60 and cholesterol in the ratio 1:1 and 1:2 respectively. For each ratio, non-ionic surfactant (Span-60) and cholesterol was weighed accurately and dissolved in 15 mL of diethyl ether. The contents of the above ratio were taken in a round-bottomed flask and left in a rotary shaker evaporator for 24 h. The surfactant/lipid thin film was formed by the evaporation of chloroform. Purified *Vinca* alkaloids dissolved in the methanolic solution was then taken in a syringe and injected slowly through a 16 gauge needle into the beaker containing vesicles maintained at 60–65 °C and agitated slowly. The solution thus obtained was transferred to the centrifuge tube and centrifuged at 4500 rpm for 30 min. The supernatant which consists of extra untrapped *Vinca* alkaloids was carefully removed [12].

2.7 Entrapment Efficiency of *Vinca rosea* Alkaloids Niosomal Formulation

Vinca alkaloids niosomal formulation was centrifuged at 4500 rpm for 30 min and the supernatant obtained was separated from the sediment which forms the nanoformulations. The separated niosomal suspension (1 mL) was disrupted using 3 mL of 50% propanol for 5 min, which was then analyzed spectrophotometrically for alkaloid concentration at λ_{max} 210 nm to calculate the amount of entrapped *Vinca* alkaloids against 50% propanol as blank.

The percentage of entrapped *Vinca* alkaloids was calculated by the following equation:

$$\% \text{ Entrapment} = A_e \times 100 / A_i$$

where, A_e is the amount of entrapped *Vinca* alkaloids and A_i is the initial amount of *Vinca* alkaloids in the lipid phase [13].

2.8 Scanning Electron Microscope (SEM) Analysis

Samples of *Vinca* alkaloids niosomal formulations were mounted on the cover glass fixed on the specimen stub using adhesive and coated with gold to a thickness of about 100 Å. Coated samples were viewed in SEM operated at 15 kV with different magnifications and photographed [14].

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2.9 Ex vivo Drug Release Analysis of *Vinca rosea* Alkaloids Niosomal Formulation

Phosphate-buffered saline was prepared and its pH was adjusted to 7.4. The buffer has been used as a donor solution to dissolve *Vinca* alkaloids niosomal formulation. The surface area of the receiver cell opening was 2 sq. cm and the cell volume was 50 mL. Isotonic phosphate buffer with a pH of 7.4 was prepared and used as the receptor solution. Domestic goat intestine was obtained from the local market, cleaned well using deionized water and with phosphate-buffered saline. The intestine was then dissected longitudinally and trimmed (2.3 x 2.3 cm²) and used in the drug release study. 5 mg of *Vinca* alkaloids niosomal formulation was weighed and dissolved in 10 mL of fresh phosphate-buffered saline (pH 7.4) and vortex mixed. The receptor cell was filled with a receptor solution (fresh phosphate-buffered saline - pH 7.4). The intestine (2.3 x 2.3 cm²) was mounted on the receptor opening and the donor cell was placed above and clamped carefully. The donor cell was filled with 5 mL of *Vinca* alkaloids niosomal formulation. The receptor compartment was agitated uniformly using a teflon-coated magnetic stir bar. 3 mL of samples from the reservoir compartment were collected through the sample collection port at every 60 min for a period of 12 h. All the samples were subjected to UV-spectrophotometrical absorbance analysis at the wavelength of 210 nm for quantification of released *Vinca* alkaloids. Purified total *Vinca* alkaloids were taken as control, and a release study was performed. Readings were tabulated and compared [15].

3. Results and Discussion

The alkaloids of *Vinca rosea* are one of the most important and widely used antineoplastic agents. The *Vinca* alkaloids arrest cell growth during metaphase and exhibit strong cytotoxic activity [16–18]. These *Vinca* alkaloids are more specific to the stages of the cell cycle in order to exhibit their potential therapeutical activity. Hence, it is highly required for the compounds to get exposed more to the site of tumor cells in order to maximize their efficacy. Various delivery systems have been under research to increase the bioavailability of these *Vinca* alkaloids. This current research is also focused on developing such a novel drug delivery system to increase the bioavailability of *Vinca* alkaloids using nanosized particulate bodies called niosomes [19–23].

3.1 Extraction of *Vinca rosea* Alkaloids by Acid-Base Extraction Methodology

Vinca rosea contains different compounds, namely vindoline, catharanthine, vinblastine, vincristine, etc. under the class of alkaloids and hence the study is focused on extracting these alkaloids in total from the fresh leaves of *Vinca rosea* and furthering nano research with them. Fresh leaves of the herb *Vinca rosea* were collected (Fig. 1) and washed with distilled water, and further subjected to alkaloid extraction methodology. The hydrochloric acid reduces the pH of the extraction solution and alkaloids which are basically amines are converted into salts at this pH. The salt remains soluble in the acidic solution. The solution is then treated with the organic solvent (petroleum ether) in order to remove the non-polar constituents present in the solution. The acidic portion was again treated with alkaline solution which helped in increasing the pH at which the acidic salts become free bases which was then collected in the diethyl ether layer and applied in further experiments [7, 24].

Fig. 1 *Vinca rosea* leaves

3.2 Purification of *Vinca rosea* Alkaloids using Column Chromatography

The total alkaloid extract obtained by the end of extraction methodology was further continued with the purification procedure which involved the alumina-based column chromatography. The

chromatographic procedure was preceded following the Jóźwiak and Hajnos work [8]. Since alumina (aluminium oxide) is a better versatile sorbent to produce the best results in wide varying pH ranges in practice, it was used here for purification of *Vinca* alkaloids which was obtained by acid-base extraction procedure. Alumina possesses both Lewis acid and basic sites and is found to be more excellent at adsorbing plant alkaloids, possibly through strained Al-O bonds. The sample was eluted with methanol, and 5 different fractions were collected. All the fractions were qualitatively tested for the presence of alkaloids using Dragendorff's reagent test method. Of the five fractions collected, fraction five has shown maximum turbidity which helped us to sense the presence of alkaloids in the fraction. To strengthen the screening, further the fraction sample was subjected to TLC and HPTLC [25, 26].

3.3 Screening of *Vinca rosea* Alkaloids by TLC

TLC was performed following Lin Wu & Sharp method [9]. The silica plate was then developed with Dragendorff's reagent which formed yellow spots (Fig. 2) and confirmed the presence of the alkaloids [27].

Fig. 2 Thin layer chromatographic screening for *Vinca* alkaloids

3.4 Screening of *Vinca rosea* Alkaloids by HPTLC

TLC was followed with HPTLC, which produced a more standard screening result that confirms the presence of alkaloids in the fifth fraction of alumina column chromatography. The HPTLC procedure was performed following Hamrapurkar et al. [9]. The results are shown in Fig. 3. HPTLC chromatogram of both the vinblastine sulfate (standard) and all the *Vinca* alkaloids samples was compared, and closely similar peaks were found on the following R_f values, such as 0.78, 0.91, 0.98, and 1.04. This confirms the presence of alkaloid-like compounds in the chromatographic fraction of the extract [28].

Fig. 3 HPTLC – Densitometric scanning analysis of samples vinblastine sulphate (standard) and *Vinca* alkaloids
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3.5 Estimation of *Vinca rosea* Alkaloids using BCG Method

Usually a variety of methods including high-performance liquid chromatography (HPLC), fluorimetry, ion chromatography, colorimeter, gas chromatography, and electrochromatography were involved for the determination of alkaloids along with the simple spectrophotometrical methods. Here, the spectroscopical method which is simple, sensitive, and rapid was employed for the determination of total alkaloids in the extract. The estimation procedure was performed based on the principle of BCG reaction with alkaloids, which produced a yellow colored complex. The total alkaloids present in 100 g of *Vinca* leaves material were found to be 58 mg [11].

3.6 Synthesis of *Vinca rosea* Alkaloids Niosomal Formulation

Niosomes are nano-shaped vesicular bodies formed by the process of self-assembly of non-ionic amphiphilic molecules in aqueous media, which results in closed bilayer structures that entrap both hydrophilic and lipophilic therapeutic agents irrespective of their chemical origin, namely synthetic or natural [29]. Niosomes show more chemical stability, lower toxicity, less requirement of handling care, biodegradability and biocompatibility. Niosomes possess more ability to improve the performance of therapeutic agents by increasing their bioavailability and controlled delivery [30, 31]. Hence, niosomes were chosen to enhance the retention properties of these *Vinca* alkaloids. Niosomal formulation of extracted *Vinca* alkaloids was prepared using thin-film hydration technique [32]. The technique was found to be more simple and effective in terms of synthesizing niosomes for therapeutic research purposes.

3.7 Entrapment Efficiency of *Vinca rosea* Alkaloids Niosomal Formulation

Various factors are involved in determining the entrapment efficiency of the niosomal formulations, which include the nature of the surfactant used in synthesizing niosomal formulation, cholesterol ratio, etc., [33]. The total amount of *Vinca* alkaloids entrapped in the niosomal vesicles are determined by the propanolysis method [34] and was found to be 74.02%.

3.8 SEM Analysis of *Vinca rosea* Alkaloids Niosomal Formulation

SEM is an important tool, capable of producing high-resolution images of the sample surface. The scanning electron microscopical photograph of the *Vinca* alkaloids niosomal formulation was observed to have spherical and uniform morphology. The size of the niosomes was found to be in the range of 400 to 800 nm (Fig. 4).

Fig. 4 SEM image of *Vinca* alkaloids niosomal formulation

3.9 Ex Vivo Drug Release Analysis of *Vinca rosea* Alkaloids Niosomal Formulation

Niosomal formulation of *Vinca* alkaloids was synthesized and tested for its improving efficacy in terms of bioavailability. Broadly, bioavailability refers to the absorption of the administered dosage of a therapeutic compound that reaches the systemic circulation. Usually, bioavailability decreases due to incomplete absorption and first-pass metabolism. This current study has focused to improve the bioavailability of *Vinca* alkaloids using niosomal suspensions. Intestinal skin of domestic goat was used for the ex vivo bioavailability study and the bioavailability experiment was performed in the modified Franz diffusion cell chamber.

The purified total *Vinca* alkaloid extract and the niosomal formulations of *Vinca* alkaloids were tested through ex vivo release studies and the results have shown that the bioavailability has been increased to two folds in terms of niosomal formulations than the total extract (Fig. 5). Apart from increasing the bioavailability, the niosomal formulations aid in the consistent release of the *Vinca* alkaloids over the tested period (Fig. 6). The niosomal formulations and their oral delivery could be a wise route for the steady release of the active alkaloid molecules of the wonder plant *Vinca rosea*.

Fig. 5 UV spectrum of ex vivo release of total alkaloids and niosomal formulations

Fig. 6 Steady release of *Vinca* alkaloids through niosomes in ex vivo experiments

4. Conclusion

The present research was carried out to develop a novel drug delivery system to increase the bioavailability of the active compound (*Vinca* alkaloids) present in the plant *Vinca rosea* using the nano-sized vesicles called niosomes. From the SEM analysis, it was observed that it has spherical and uniform morphology. The size of the niosomes was found to be in the range of 400 to 800 nm. Through ex vivo release studies the results shows that the bioavailability has been increased to two folds in terms of niosomal formulations than the total extract. The current study with its positive results, demonstrated the possibility to improve the bioavailability of the compound using a nanotechnological approach.

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