Nanoparticulate Hydrogel Incorporated with *Mimosa pudica* Extract: Formulation and Characterization

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Abstract: Present study is designed at the preparation of *Mimosa pudica* extract and formulating into nanoparticulate hydrogel. The active ingredients were extracted by cold maceration of the *M. pudica* leaves in ethanol. These were then developed into polymeric nanoparticles by nanoprecipitation method using PLGA as polymer, and incorporated into gel matrix, using HPMC K4M as base. Reports showed that polymeric nanoparticles are closely spherical shape with z-average 95.7-185.2 nm, PDI in the range of 0.236- 0.298 and zeta potential is -2.07 mV to -3.5 mV, with sufficient drug entrapment of 69%. Nanoparticulate hydrogel formulations exhibited high viscosity, neutral pH with good spreadability which is appropriate for transdermal application. In vitro drug release showed initial burst release of 28.56 ± 0.93 % with prolonged drug release of 90.06± 0.93 % from optimized formulation up to 24 h. Thus, the prepared nanoparticulate hydrogel can be utilized as a carrier for transdermal delivery of extract of *M. pudica*.

Keywords: *Mimosa. pudica, Nanoparticle, hydrogel*

1. Introduction

Natural products are being used in the treatment of diseases since ancient times. The natural plant preparations have numerous constituents which work instantaneously all together against the diseases [1].

A scientific approach is needed for the phytochemists to carry the active constituents in a sustained fashion. This can be achieved by scheming novel drug delivery systems (NDDSs) for plant components. They have the benefit of mitigating the toxicity, enhancing the solubility, bioavailability and activity [2]. Therefore, in order to battle many diseases such as asthma, cancer, diabetes, and all the skin related diseases, loading of the nanocarriers as a NDDS is more important.

Polymeric nanoparticles are favourable formulation used for controlled drug delivery systems, and are made from biodegradable and biocompatible polymers, whereas hydrogels are polymeric networks with three-dimensional configuration capable of imbibing high amounts of water or biological fluids [3,4]. Due to their unique nature, hydrogel nanoparticles have attained substantial attention in the current period as one of the most potential nanoparticulate drug delivery systems.

*Mimosa pudica* (Family: Fabaceae / Mimosaceae) also called shy plant or sensitive plant, is a valued medicinal plant, which is simply existing throughout India. Its roots contain tannin, ash, calcium oxalate crystals and alkaloid mimosine, and therefore suggested for the treatment of diarrhoea, amoebic dysentery, high blood pressure and gynaecological disorders, skin diseases etc [5,6]. To make the extract into efficient candidate for topical application, study was conducted to develop nanoparticulate hydrogel formulation containing *M. pudica* extract which is expected to have sustained release as well as improved permeation characteristics due to nano size and hydrophilic nature.
2. Materials and Methods

2.1. Materials
Poly Lactic Glycolic Acid (PLGA 50:50) was procured from Sigma Aldrich, Bangalore and H.P.M.C K4M was obtained from Yarrow Chem products, Mumbai.

2.2. Preparation of Plant Extract and Phytochemical Analysis
Leaves of Mimosa pudica were collected from local area of Bantwal Taluk and authenticated by Prof (Dr) Nagalakhamma St. Aloysius College, Mangalore. Leaves were dried, powdered and then extracted by cold maceration method using 95% ethanol as solvent for 7 days. After the extraction solution was filtered and filtrate was evaporated to dryness and percentage yield was calculated. Prepared extract was subjected to different chemical tests according to standard procedure in order to determine the presence of various phytoconstituents [7].

2.3. Formulation of Polymeric Nanoparticles
Emulsion solvent evaporation method [9] was performed for preparing nanoparticles of plant extract using PLGA as polymer. PLGA (50 mg, 100 mg, 150 mg) and plant extract in DMSO as solvent, in different proportions was used to prepare the organic phase. The organic phase was then added dropwise at the rate of 1ml/min into an aqueous phase containing surfactant (PVA -0.5%) dissolved in water as aqueous solvent.

The nanoparticles suspension was stirred constantly at 300 rpm for 3 h at 30°C to allow the complete evaporation of DMSO, leaving behind the colloidal suspension of PLGA nanoparticles holding plant extract in aqueous phase. The colloidal nanosuspension was centrifuged at 12,000 rpm (Remi, Mumbai, India) for 30min at 4°C to get the final nanoparticulate containing pellet as encapsulated plant extract. The pellet was washed with deionized water twice to remove unentrapped drug from the surface of nanoparticles. Nanoparticulate pellets were redispersed in water [8].

2.4. Characterization of Polymeric Nanoparticles

2.4.1. Measurement of Particle size, PDI and Zeta Potential of the nanoparticles
Average particle size (z-average) and Polydispersity index (PDI) of the developed nanoparticles were determined by laser dynamic light scattering using Malvern Zetasizer (Nano ZS, Malvern Instruments, UK). The PDI value directs the particle size distribution of nanoparticles in a given sample [9].

2.4.2. Entrapment Efficiency of the Polymeric Herbal Nanoparticle
The nanoparticle suspension formulated with the extract and polymer was ultra-centrifuged at 18,000 rpm for 30 minutes in a cooling centrifuge apparatus and then the supernatant solution was diluted suitably to measure the absorbance, from which the concentration of drug in supernatant was calculated using the standard calibration data [10]. The entrapment efficiency of the extract in the polymeric nanoparticles was calculated using the formula,

\[
\text{Entrapment Efficiency (\%)} = \left( \frac{\text{Total amount of Drug added} - \text{amount of Drug is supernatant}}{\text{Total amount of Drug added}} \right) \times 100
\]

Based on the outcomes of particle size analysis, PDI and entrapment efficiency, optimised formulation of nanoparticles was chosen and scanning electron microscopy and transmission electron microscopy was carried out.
2.4.3. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)
Shape and surface morphology of the nanoparticles were studied using SEM (JEOL, JSM 50A, Tokyo, Japan). An appropriate amount of colloidal dispersion of polymeric nanoparticle was mounted onto metal (aluminium) using double-sided adhesive tape and fractured with a razor blade. The samples were sputter-coated with gold/palladium for 120 sec at 14 mA under argon atmosphere for secondary electron emissive SEM and observed for morphology, at acceleration voltage of 15 KV. The morphology of formulation was observed under TEM.

2.4.4. Formulation of Nanoparticulate Hydrogel
All the formulations (MN1-MN3) were found in nanosize range and therefore incorporated in gel matrix. Hydroxypropyl methylcellulose K4m (HPMC K4M), was selected as gel matrix base. Required quantity of gelling agent was weighed and dispersed in a small quantity of distilled water to form a homogeneous dispersion. Prepared nanoparticle formulation was added to the above solution to obtain hydrogel formulation NG1, NG2, NG3. Other excipients (methyl paraben and propyl paraben) were also added with stirring. The pH values were subsequently regulated to 6–9 by using triethanolamine [11].

2.4.5. Characterization of Nanoparticulate Hydrogel
The pH of the formulation was determined [12] with digital pH meter (digital pH meter, 335, systronics). The spreadability of prepared hydrogel formulation was determined by measuring the spreading diameter of formulation between the two glass plates after 1 min. The viscosity of the prepared formulations [13] was determined by using spindle number 4 (Brookfield DV-II+ Pro viscometer).

2.4.6. In Vitro Drug Release Study of Hydrogel
Prepared nanoparticulate hydrogel formulations (NG1, NG2, NG3) was evaluated for the percentage of release of the extract constituents, for 24 h. In vitro release of drug across the dialysis bag (12 Kda, Hi Media) soaked in deionized water for 12 h before use was performed by using diffusion cell (containing 1 ml of sample) and 80 ml of phosphate buffer pH 6.8 as the dissolution medium (n=6). The dissolution medium was maintained at 37 ± 0.5°C and the medium was stirred at 100 rpm with the help of small teflon coated magnetic bead. Aliquots of the medium were withdrawn at suitable time interval and were replaced with the same volume of fresh medium to maintain the sink condition. These samples were filtered through 0.45 µm membrane filter and the collected samples were analyzed using UV-visible spectrophotometer at the λmax of 203 nm [14].

3. Results and Discussion
Ethanolic extract of Mimosa pudica were greenish in colour and percentage yield was found to be 14% respectively. The preliminary phytochemical analysis showed the presence of flavonoids, tannins, steroids, carbohydrates, proteins in the extract.

3.1 Preparation and Characterisation of Polymeric Nanoparticles
The characterization of the nanoparticles are shown in Table 1, where MN1, MN2, MN3 are nanoparticles of Mimosa pudica ethanolic extract with 25 mg, 50 mg and 100 mg PLGA respectively.

The average size of the synthesised nanoparticles was found to lie within the range of 95.7-185.2 nm. Polydispersibility index (PDI) of formulations was found to be in a range of 0.236- 0.298. The zeta potential of the synthesized herbal nanoparticles was found to be -2.07 mV to -3.5 mV. Entrapment efficiency determination showed that the extract of different nanoparticle formulation had higher entrapment efficiency in the range of 56– 69%.

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Based on results of particle size analysis, PDI and entrapment efficiency, nanoparticle formulation MN1 containing Mimosa pudica ethanolic extract with 25 mg of PLGA polymer was selected as optimised formulation as it has nanosize range particles with low PDI and sufficient entrapment efficiency.

3.2 Scanning Electron Microscopy (SEM) and TEM
The SEM study reveals that polymeric nanoparticles were spherical in shape with an average particle size around 97.3 nm as shown in Fig 1. The transmission electron microscope revealed a positive image in which nanoparticles appeared dark with bright surroundings as shown in Fig 2. The average droplet size of sample was less than 1000 nm. These results confirmed that the droplets were in nanosize range. Moreover, z-average gives the hydrodynamic size when the particles are suspended in aqueous media. TEM images would give a better understanding of the real geometric size of the particles.

Fig. 1: SEM Image of Optimized Nanoformulation Loaded with Extract

Fig. 2: TEM Image of Optimized Nanoformulation Loaded with Extract
3.3 Formulation and Characterisation of Nanoparticulate Hydrogel Formulation

Hydrogel formulations NG1, NG2, NG3 were prepared by incorporating the nanoparticle formulation MN1, MN2, MN3 into HPMC K4M gel matrix respectively. Prepared hydrogel were subjected for determination of pH, spreadability, viscosity and drug content and results of which is shown in Table 2.

The drug content of nanoparticulate hydrogel formulation was in the range of 90.6 ± 0.38% to 95.4±0.21%. The results showed that the drug was uniformly distributed throughout the formulation and drug loss was minimum while formulating nanoparticulate hydrogel. The pH values of different nanoparticulate hydrogel formulations were found to be in a range of 6.4–6.8 (nearly neutral), permitting the use of the formulation on the skin. The spreadability of the all formulations exhibited slip and drag phenomenon with higher diameters.

<p>| TABLE I: Physicochemical characterization of nanoparticles loaded with extract of Mimosa pudica |</p>
<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Particle Size (nm)</th>
<th>PDI</th>
<th>Zeta Potential (mV)</th>
<th>Entrapment Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN1</td>
<td>95.7</td>
<td>0.236</td>
<td>-2.07</td>
<td>56.5</td>
</tr>
<tr>
<td>MN2</td>
<td>140.3</td>
<td>0.256</td>
<td>-3.14</td>
<td>59.3</td>
</tr>
<tr>
<td>MN3</td>
<td>185.2</td>
<td>0.298</td>
<td>-3.50</td>
<td>65.9</td>
</tr>
</tbody>
</table>

<p>| TABLE II: Characterization of Hydrogel |</p>
<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug content</th>
<th>pH</th>
<th>Spreadability g.cm/sec</th>
<th>Viscosity (m.PaS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG1</td>
<td>94.8±0.23</td>
<td>6.4±0.2</td>
<td>6.0±0.32</td>
<td>3231±0.21</td>
</tr>
<tr>
<td>NG2</td>
<td>95.4±0.21</td>
<td>6.5±0.3</td>
<td>5.5±0.22</td>
<td>3295±0.18</td>
</tr>
<tr>
<td>NG3</td>
<td>90.6±0.38</td>
<td>6.8±0.1</td>
<td>5.8±0.42</td>
<td>3311±0.16</td>
</tr>
</tbody>
</table>

All the values are expressed as mean ± SD (n= 3).

3.4 Release Studies from Nanoparticulate Hydrogel Formulation

Release of the extract from the nanoparticulate hydrogel formulation prepared with nanoparticles containing different concentrations of polymer were compared [15] (Fig.3). the highest release of 90% was shown at the end of 24 hours, by hydrogel formulation NG1 containing nanoparticles loaded with extract of Mimosa pudica and polymer (25mg). It was found that when the polymer concentration was increased (50 mg and 100 mg), the drug release was found to decrease to 80% and 66%, it may be due to the higher polymer coating or encapsulation.

Fig 3: In-vitro drug release profile of nanoparticulate hydrogel loaded with extract of Mimosa pudica

4. Conclusion

The innovative nanoparticulate hydrogel formulation loaded with herbal extract of appropriate viscosity was effectively formulated for transdermal application. PLGA nanoparticles with Mimosa pudica incorporated into
the hydrogels showed controlled release of active constituents which might be a likely carrier for transdermal delivery of active constituents in the extract.

5. Acknowledgment

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6. References