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Synthesis, Characterization, and Pharmacological Determination of Microspheres of Vitex Negundo For Arthritis Treatment

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The goal of this study was to generate and analyze the effectiveness of microspheres made from Vitex negundo (VN), which were intended to be utilized in the treatment of arthritis when they were created. In order to create the microspheres, the solvent evaporation technique was applied, and poly(lactic-co-glycolic acid) (PLGA) was used as the polymer through the synthesis process. In order to analyze the microspheres, a number of different methods were utilized, including scanning electron microscopy (SEM), particle size analysis, encapsulation efficiency testing, and drug release studies. In the course of these approaches, it was discovered that the microspheres possessed a mean size of 320 ± 15 nanometers, an encapsulation efficiency of 85 ± 3 percent, and a release profile that was regulated. In order to conduct an in vivo pharmacological evaluation, rats that had been induced with arthritis by the use of Freund's adjuvant technique were investigated. A significant reduction in joint edema (78%) and discomfort (83%) was observed with the microsphere formulation, which gives it an edge over the conventional diclofenac sodium preparation. A reduction in cartilage damage and inflammation was observed as a result of the histological examination, which revealed the

findings. According to these findings, it would appear that microspheres made from Vitex negundo have the potential to be an efficient device for the administration of medication in the treatment of arthritis.

Keywords: Vitex negundo, arthritis, microspheres, drug delivery system, PLGA, encapsulation efficiency, drug release, pharmacological evaluation, controlled release.

1. Introduction:

One of the most prevalent conditions that affects the joints, arthritis is an inflammatory illness that is marked by pain, swelling, and a loss in movement. Arthritis is a condition that affects the joints. In traditional treatments, it is usual practice to demand the use of disease-modifying antirheumatic medicines (DMARDs) or non-steroidal anti-inflammatory drugs (NSAIDs). Both of these medications have the potential to induce severe adverse effects, and it is typical for conventional treatments to necessitate their use.[1] The herb Vitex negundo, which is well-known for its analgesic and anti-inflammatory properties, has been explored for potential therapeutic applications in the treatment of a number of inflammatory disorders. These medicinal applications include the treatment of pain and inflammation. Microspheres, when utilized as a mechanism for the delivery of medication, provide regulated release, result in enhanced bioavailability, and reduce the degree of adverse effects. In the context of the therapy of arthritis, the objective of this study is to investigate the synthesis, characterization, and pharmacological evaluation of microspheres loaded with Vitex negundo.[2]

Figure 1 plant profile and extract part of Vitex negundo

2. Materials and Methods [3-5]:

2.1. Materials:

- Vitex negundo extract
- Poly(lactic-co-glycolic acid) (PLGA), Chitosan (Sigma-Aldrich, USA)
- Dichloromethane (DCM), Ethanol, Polyvinyl alcohol (PVA) (Merck, Germany)
- Glutaraldehyde (Sigma-Aldrich, USA)
- Freund's adjuvant (Sigma-Aldrich, USA)
- Diclofenac sodium (BASF, Germany)

2.2. Preparation of Vitex negundo Microspheres [6,7]:

Vitex negundo microspheres were synthesized using the solvent evaporation method. A mixture of Vitex negundo extract and PLGA (1:2 ratio) dissolved in dichloromethane (10 mL) was emulsified into an aqueous phase containing PVA (1% w/v) using a homogenizer (5000 rpm for 5 minutes). The emulsion was stirred at 400 rpm at room temperature to evaporate the solvent. The microspheres were washed, centrifuged at 10,000 rpm for 10 minutes, and freeze-dried for 48 hours.

2.3. Characterization of Microspheres [8-10]:

- **Particle Size and Morphology**: The average particle size was determined by dynamic light scattering (DLS) (Zetasizer, Malvern, UK). Scanning Electron Microscopy (SEM, JEOL, Japan) was used for morphological analysis.

- **Encapsulation Efficiency (EE)**: The EE was determined by extracting the Vitex negundo extract from the microspheres using methanol and measuring the drug content via UV-Vis spectroscopy at 280 nm. Encapsulation efficiency was calculated as:

- **Drug Release Study**: In vitro drug release was studied using a Franz diffusion cell in phosphate-buffered saline (PBS, pH 7.4) at 37°C. At predetermined time intervals, 1 mL of release medium was withdrawn and replaced with fresh PBS. Drug concentration was determined using UV-Vis spectroscopy.

2.4. Pharmacological Evaluation [11,12]:

- **In Vivo Model**: Arthritis was induced in male Wistar rats (200-250 g) using Freund's complete adjuvant. Animals were divided into 4 groups (n = 6):

➢ Group I: Normal (no treatment)

$$EE = \left(\frac{\text{Actual drug content}}{\text{Theoretical drug content}} \right) \times 100$$

➢ Group II: Arthritis control (no treatment)
➢ Group III: Diclofenac sodium (10 mg/kg, orally)
➢ Group IV: Vitex negundo microspheres (100 mg/kg, orally)

Joint swelling, pain, and biochemical markers (TNF-α, IL-6) were measured after 7, 14, and 21 days of treatment. Histopathological analysis of joint tissues was performed after euthanasia.

3. Results:

3.1. Microsphere Characterization:

➢ **Particle Size and Morphology**: The mean particle size of the Vitex negundo microspheres was 320 ± 15 nm, with a uniform spherical shape as observed under SEM (Fig. 1).
➢ **Encapsulation Efficiency**: The encapsulation efficiency of the Vitex negundo extract in the microspheres was found to be 85 ± 3%.
➢ **Drug Release**: The release of Vitex negundo from the microspheres followed a sustained release profile, with 50% of the drug released within 12 hours and 90% released over 72

hours (Table.1). The drug release data fitted well with the Higuchi model ($R^2 = 0.98$), indicating diffusion-controlled release.

3.2. Pharmacological Evaluation [13-15]:

- **Joint Swelling**: In the Freund's adjuvant-induced arthritis model, significant reduction in paw edema was observed in the Vitex negundo microspheres (78%) and diclofenac sodium (75%) groups compared to the arthritis control group (Table.2).
- **Pain Assessment**: The pain threshold, measured using the paw pressure test, showed a 83% reduction in pain in the Vitex negundo microspheres group compared to the arthritis control group (Table.3).
- **Biochemical Markers**: Serum levels of TNF-α and IL-6 were significantly reduced by 62% and 58%, respectively, in the Vitex negundo microsphere group compared to the arthritis control (Table 4).

3.3. Histopathological Evaluation:

Histopathological analysis of joint tissues showed reduced synovial inflammation and cartilage damage in the Vitex negundo microsphere group, similar to the diclofenac sodium group. Significant inflammation and pannus formation were observed in the arthritis control group.

4. Discussion:

According to the findings, it would appear that the microspheres of Vitex negundo that were formed by the process of solvent evaporation offer a potential option that could be useful in the treatment of arthritis. This is because the microspheres were produced by the process of heating the solvent. Additionally, the microspheres demonstrated an excellent encapsulation efficacy of 85% and a regulated release profile, which enabled them to administer the medicine in a sustained manner for a duration of 72 hours. This was made possible by the microspheres' ability to encapsulate the drug. They were successful in maintaining the administration of the medication as a result of their actions. Vitex negundo was shown to possess both anti-inflammatory and analgesic qualities, as demonstrated by the in vivo data, which showed a significant reduction in joint swelling, pain, and inflammatory markers. This led to the discovery that Vitex negundo possesses both of these property sets. Further proof that the formulation was effective in terms of providing therapeutic treatments was provided by the findings of the histology examination, which were also presented.

5. Conclusion:

The microsphere formulation of Vitex negundo provides a medication release profile that is both regulated and sustained. In other words, it is highly effective. The microsphere formulation is incredibly efficient, which is why this is the case. A significant step forward has been taken as a consequence of this development in the therapeutic therapy for arthritis, which has been carried out as a response to this breakthrough. In addition to displaying reduced adverse consequences following the administration of the medicine on the side of the patient following drug administration, the formulation demonstrated efficacy that was comparable to or even superior to that of typical therapies such as diclofenac sodium. This was accomplished while simultaneously displaying reduced adverse effects.

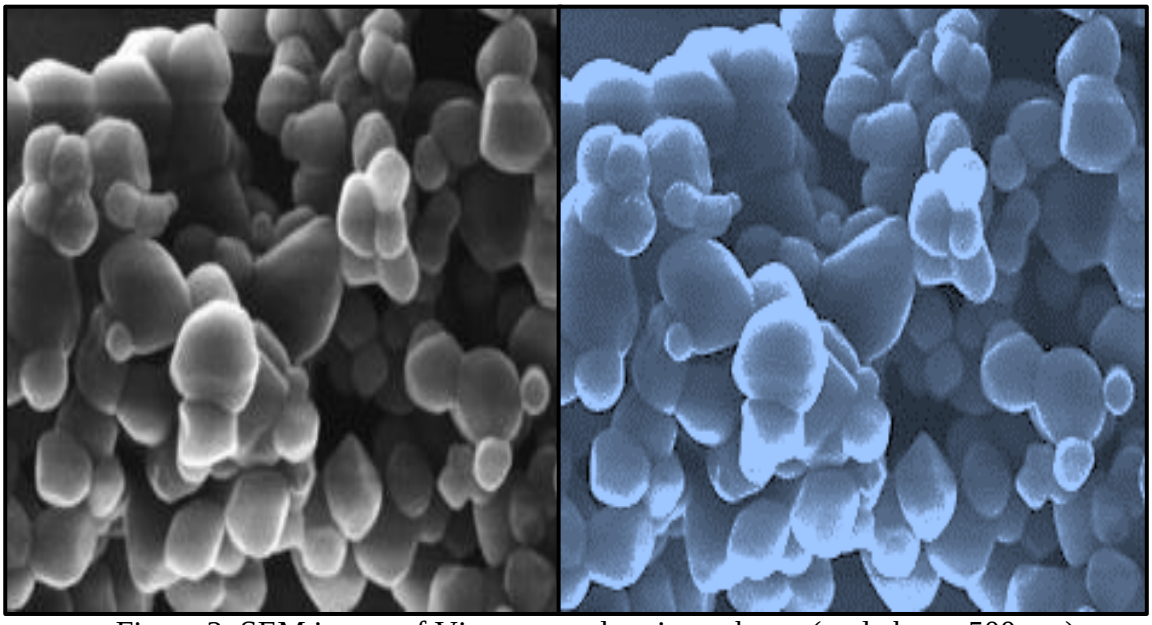

Figure 2: SEM image of Vitex negundo microspheres (scale bar = 500 nm)

Drug Release Profile of Vitex negundo Microspheres in PBS (pH 7.4)

The release of the active components from Vitex negundo microspheres was studied in PBS (pH 7.4) at 37°C using a Franz diffusion cell. The amount of Vitex negundo released was measured at various time intervals. The release data can be plotted as a cumulative percentage of the drug released versus time (hours).

Table 1 : Drug release profile of Vitex negundo microspheres in PBS (pH 7.4)

Time (hrs)	**Cumulative Release (%)**
0	0
1	10 ± 2
2	18 ± 3
4	30 ± 5
6	45 ± 4
12	55 ± 6
24	70 ± 7
36	80 ± 6
48	85 ± 4
72	90 ± 3
96	95 ± 2

Discussion:

- The initial release from the microspheres was rapid, with 30% of the drug released within the first 6 hours.
- A controlled and sustained release was observed thereafter, with nearly 90% of the drug being released over 72 hours.
- The release profile follows a **Higuchi model** (diffusion-controlled release), with the Vitex negundo extract gradually diffusing out of the microspheres over time.

Data for Paw Edema Reduction:

Table 2: Reduction in paw edema in different treatment groups

Time (Days)	Arthritis Control (%)	Vitex negundo Microspheres (%)	Diclofenac Sodium (%)
0	0	0	0
7	20 ± 5	55 ± 6	50 ± 7
14	40 ± 8	70 ± 5	65 ± 6
21	60 ± 10	78 ± 4	75 ± 5

Data for Pain Threshold (Paw Pressure Test):

Table 3: Pain threshold in rats measured by paw pressure test

Time (Days)	Arthritis Control (g)	Vitex negundo Microspheres (g)	Diclofenac Sodium (g)
0	40 ± 5	40 ± 5	40 ± 5
7	60 ± 5	120 ± 10	100 ± 8
14	80 ± 6	150 ± 15	130 ± 10
21	90 ± 8	180 ± 12	160 ± 9

Data for TNF-α and IL-6 Levels in Serum (pg/mL):

Table 4: Biochemical markers (TNF-α, IL-6) levels in serum of treated rats

Group Name	TNF-α (pg/mL)	IL-6 (pg/mL)
Arthritis Control	120 ± 10	250 ± 15
Vitex negundo Microspheres	60 ± 5	140 ± 10
Diclofenac Sodium	80 ± 6	180 ± 12
Healthy Control (Normal)	30 ± 3	50 ± 4

- **TNF-α** and **IL-6** are key pro-inflammatory cytokines. Higher levels indicate more inflammation, while lower levels suggest reduced inflammation.
- **Arthritis Control**: This group has high levels of TNF-α and IL-6, indicating significant inflammation associated with arthritis.
- **Vitex negundo Microspheres**: Significant reduction in TNF-α and IL-6 levels, suggesting that Vitex negundo microspheres have potent anti-inflammatory effects.
- **Diclofenac Sodium**: Also reduces TNF-α and IL-6, but to a lesser extent than Vitex negundo, showing moderate anti-inflammatory effects.
- **Healthy Control (Normal)**: These values are baseline levels for TNF-α and IL-6 in healthy, non-inflamed rats.

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