In Vivo Study of a pH-Responsive Mesoporous Silica Nanoparticle (MSN) System Containing Bawang Dayak Bulb Fraction as an Anti-inflammatory Agent

Abbigail Wynna[1], Benny Permana[2], Neng Fisheri Kurniati[3], Satrialdi[1], Diky Mudhakir1

[1] Department of Pharmaceutics, School of Pharmacy ITB, [2]Department of Pharmacochemistry, School of Pharmacy ITB
[2]Department of Pharmacology-Clinical Pharmacy, School of Pharmacy ITB

Abstract

Prolonged inflammation contributes to chronic disease, while long-term use of non-steroidal anti-inflammatory drugs (NSAIDs) is limited by gastrointestinal side effects. The n-hexane fraction of Bawang Dayak (*Eleutherine palmifolia*) bulb (FBD) is a promising natural anti-inflammatory candidate, but its low solubility, stability and bioavailability restrict its use.

This study developed a pH-responsive mesoporous silica nanoparticle (MSN) system loaded with FBD and capped with an imidazole-polyethyleneimine gatekeeper. MSN was synthesised with a CTAB template, calcined, aminated with APTES, loaded with FBD and coated with PEI and imidazole-PEI; FTIR, ^{1}H NMR and UV-Vis confirmed every step, with a loading capacity of 3.38% and a loading efficiency of 36.26%.

Activity was tested in carrageenan-induced rat paw edema (n = 5 per group) with serum interleukin-6 (IL-6) measured by ELISA. MSN-FBD at 200 and 400 mg/kg reduced the total edema response comparably to diclofenac sodium 20 mg/kg, and 400 mg/kg gave the lowest serum IL-6 level.

Keywords: anti-inflammatory, *Eleutherine palmifolia*, interleukin-6, mesoporous silica nanoparticles, pH-responsive delivery.

Introduction

Inflammation is a normal physiological response to injury and infection, but when it persists it contributes to chronic disease, which makes its control an important therapeutic strategy. NSAIDs are effective acutely, yet long-term use causes gastrointestinal adverse effects and drives the search for safer alternatives.

Bawang Dayak (*Eleutherine palmifolia*) is an Indonesian medicinal plant whose bulb shows anti-inflammatory potential, but the active compounds in its n-hexane fraction suffer from low solubility, limited stability and poor bioavailability, so a delivery system is required.

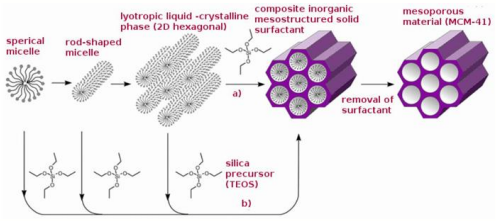
Mesoporous silica nanoparticles offer a large surface area and tunable pores. Capping those pores with a pH-responsive polyethyleneimine-imidazole gatekeeper lets the cargo be retained and then released in a controlled way in the acidic microenvironment of inflamed tissue.

Objectives. (1) To develop a pH-responsive MSN system containing the n-hexane fraction of Bawang Dayak bulb and confirm it by physicochemical characterisation. (2) To evaluate its *in vivo* anti-inflammatory activity from paw edema volume in carrageenan-induced rats and from serum IL-6.

Research Method

Synthesis and modification. CTAB served as the surfactant template forming the micelles that shape the mesoporous structure, and TEOS was added as the silica source to build the framework around them. The product was calcined in ethanolic HCl at 70 °C for 24 h to remove CTAB.

Silanisation with APTES (24 h) formed siloxane bonds and introduced amine (-NH_2) groups on the surface. FBD was loaded by adsorption (24 h) through hydrogen bonding, van der Waals forces and hydrophobic interactions. PEI was then adsorbed electrostatically, and imidazole-PEI – made by activating the carboxyl group of 4-imidazoleacetic acid to react with PEI amines – was conjugated as the pH-responsive gatekeeper.

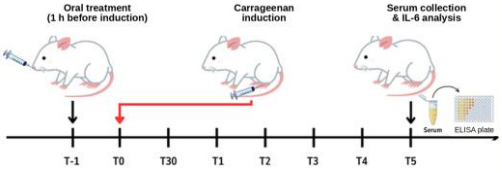


Picture 1. CTAB-templated synthesis of mesoporous silica nanoparticles

Characterisation. FTIR for bond interactions, ^{1}H NMR for the imidazole-PEI polymer, UV-Vis at 333 nm for loaded FBD, TEM for morphology, BET for surface area and pores, PSA and zeta potential for size, distribution and charge, and TGA for thermal stability.

Rat paw edema test. Rats were fasted for 18 h and divided into five groups (n = 5): negative control (CMC-Na 0.5%), positive control (diclofenac sodium 20 mg/kg) and MSN-FBD at 100, 200 and 400 mg/kg, given orally. One hour later, 0.1 mL of 1% carrageenan was injected subplantarly into the left hind paw. Paw volume was measured with a plethysmometer and analysed as percentage inflammation, inhibition and area under the curve (AUC).

Serum IL-6. Blood was taken at T5; standards and serum were incubated with anti-IL-6 (37 °C, 60 min), washed, incubated with streptavidin-HRP and TMB (37 °C, 10 min), stopped and read at 450 nm. Data were analysed by one-way ANOVA with Tukey post hoc test.



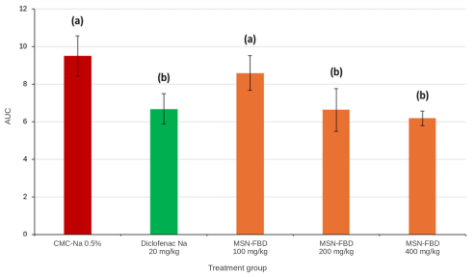
Picture 2. Timeline of the in vivo anti-inflammatory study

Discussion & Result

Characterisation. FTIR confirmed the silica framework (1098, 802, 3436 cm-1) and complete CTAB removal, then amination (1562, 1492 cm-1), FBD loading (1630-1680; 3300-3500 cm-1) and PEI conjugation (3363, 2935, 1473 cm-1); ^{1}H NMR confirmed the imidazole signals. Loading capacity was 3.38%, loading efficiency 36.26%.

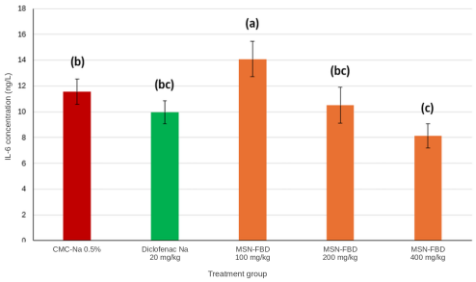
Model validity. Paw volume in the CMC-Na 0.5% group rose significantly from T0 to T3, and the diclofenac group differed from it at T3 ($p < 0.05$), validating both model and method.

Edema inhibition. At T4, MSN-FBD inhibited edema more strongly than diclofenac sodium 20 mg/kg, consistent with its gradual, controlled release. Over the whole period, 200 and 400 mg/kg reduced the AUC comparably to diclofenac.



Picture 3. AUC as the total edema response over the observation period

Serum IL-6. MSN-FBD 400 mg/kg gave the lowest IL-6 level, significantly below the 100 mg/kg and CMC-Na groups ($p < 0.05$).



Picture 4. Serum IL-6 concentration across treatment groups

Conclusion

1. A pH-responsive MSN system carrying the n-hexane fraction of Bawang Dayak bulb was successfully developed and characterised.

2. At 200 and 400 mg/kg it showed anti-inflammatory activity in carrageenan-induced rat paw edema and lowered serum IL-6 levels.