Fenugreek-based exosome like-nanoparticles (FEN-ELN): A point of care vehicle for alleviating the pain intensity produced at primary dysmenorrheal condition in female of child bearing age.

Amoolya Sree & Prof (Dr.) Tamilvanan Shunmugaperumal

Departments of Pharmaceutics, National Institute of Pharmaceutical Education and Research (NIPER), Sila Katamur, Changsari, Kamrup, Assam 781101, India

Abstract

Fenugreek seeds is a traditional medicine that has been used for ages. Fenugreek (*Trigonella foenum-graecum*) has multiple pharmacological properties. This study aimed to evaluate the potential of fenugreek-derived exosome-like nanoparticles (FEN-ELN) in alleviating pain intensity during dysmenorrhea.

Formulation by design approach was used to find systematically a formula for making FEN-ELN. The influence of critical material attributes like PEG concentration, pH of the extracting solvent, homogenization time and speed on the critical quality attributes such as size, zeta potential, trigonelline content, protein concentration, etc. was studied using response surface methodology.

FEN-ELN had an average diameter of 122.2 nm and a negative surface charge of −21.9 mV. Substantial amounts of nanoparticles of FEN-ELN were counted using NTA, with a concentration of 8.3 × 10^{10} particles/mL. The total protein and RNA concentration was found satisfactory. The results suggest the method adopted can be used for scale up of FEN-ELN.

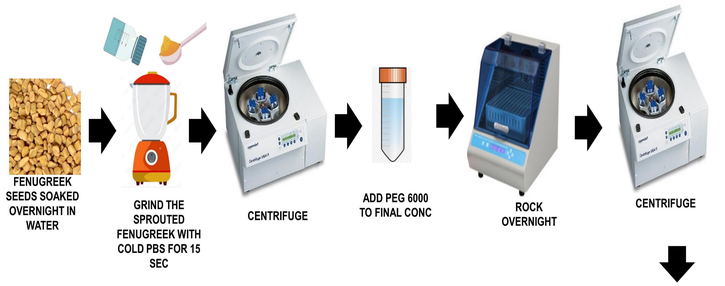
Introduction

Dysmenorrhea, a condition characterized by painful menstrual cramps of uterine origin. It is prevalent among females of reproductive age worldwide, affecting around 81% of the population. Despite its high incidence, dysmenorrhea is often overlooked as a significant cause of absenteeism in this population.

Current treatment options include nonsteroidal anti-inflammatory drugs (NSAIDs) and steroids, which can have long-term side effects. Therefore, there is a growing interest in **natural products** that offer greater efficacy with fewer side effects.

One promising approach is the use of exosome-like nanoparticles, which have several advantages like biocompatibility, biodegradability, low immunogenicity, and the ability to be easily taken up by cells. **Plant-derived exosome-like nanoparticles** are vesicles derived from plants that contain bioactive elements such as microRNAs, lipids, and plant secondary metabolites. These particles have a size range between 100 to 500 nm and are structurally similar to mammalian exosomes [1].

Methods

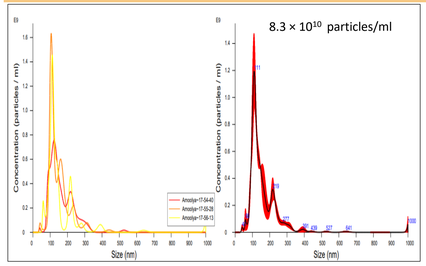


Fenugreek-based exosome like-nanoparticles generation using the QbD concept

FENUGREEK DERIVED EXOSOME LIKE-NANOPARTICLES	
SIZE	Dynamic light scattering
ZETA POTENTIAL	Dynamic light scattering
DISTRIBUTION	Nanoparticle Tracking analyzer
TOTAL PROTEIN ESTIMATION	Bradford assay
RNA CONCENTRATION	Using TRIzol reagent
TOTAL POLYPHENOLIC CONTENT	Using Folin-Ciocalteu reagent
TRIGONELLINE CONTENT	Using RP-HPLC method

Results

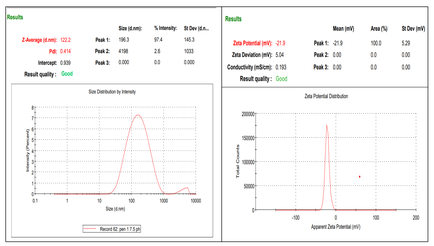
NANOPARTICLE TRACKING ANALYSIS



Stats: Mean +/- Standard Error

Mean:	159.8 +/- 4.7 nm
Mode:	113.6 +/- 4.9 nm
SD:	88.3 +/- 21.4 nm
D10:	94.3 +/- 1.9 nm
D50:	131.7 +/- 6.0 nm
D90:	245.5 +/- 5.4 nm
Concentration (Upgrade):	8.36e+10 +/- 3.74e+09 particles/ml
	44.7 +/- 0.7 particles/frame
	50.9 +/- 0.9 centres/frame

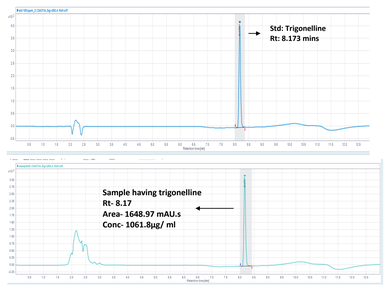
SIZE & ZETA POTENTIAL USING DLS



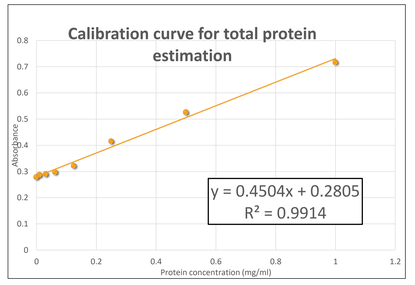
The average size was found to be 122.2 nm
PDI- 0.414
Zeta potential -21.9 mV

Total RNA concentration measurement	
260	0.064
280	0.033
260/280	1.917
ng/ml	51.276

QUANTIFICATION OF TRIGONELLINE



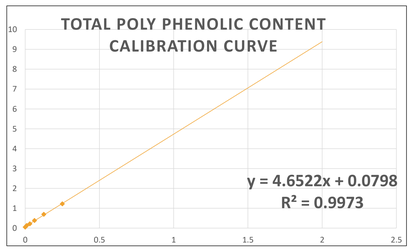
TOTAL PROTEIN ESTIMATION



total protein estimation in FE-ELN

1019.596 µg/ml

TOTAL POLYPHENOLIC CONTENT

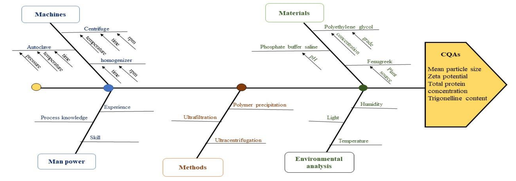


total polyphenolic estimation in FE-ELN

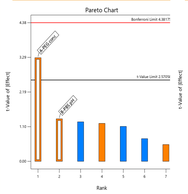
1019.596 µg/ml

QbD concept to screen the factors influencing generation of FEN-ELN

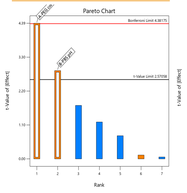
ISHIKAWA BONE DIAGRAM



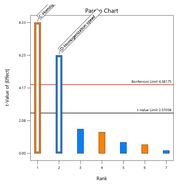
Mean particle size



Zeta potential



Protein concentration



PEG concentration, **pH of the extracting solvent**, **homogenization speed** and **time** are the major factors that affect the yield and quality of FEN-ELN.

Discussion and Conclusion

- Exosome like-nanoparticles were **successfully extracted** from fenugreek seeds.
- The FEN-ELN contained **1061.8 µg/ ml Trigonelline.**
- The size, zeta potential, yield, RNA concentration, and protein concentration were found satisfactory.

In this study, FEN-ELN was extracted with a less laborious, simple and reproducible way using a polymer precipitation technique. The physical, chemical attributes of FEN-ELN were verified. The phytoconstituent trigonelline was also quantified in the FEN-ELN. The results were found satisfactory. Besides, we suggest that FEN-ELN are potential nanovesicles with exosome-like properties and conclude that FEN-ELN could mediate intercellular communication as a drug to target. We anticipate that FEN-ELN with high reproducibility can be applied in pain intensity alleviation during primary dysmenorrhea.

Contact Information

Prof (Dr.) Tamilvanan Shunmugaperumal
HOD, Department of Pharmaceutics
National Institute of Pharmaceutical Education and Research- Guwahati
Email: tamilvanan1@yahoo.co.in
Phone: 8054002847

References

1. Ly, N. P., Han, H. S., Kim, M., Park, J. H., & Choi, K. Y. (2023). Plant-derived nanovesicles: Current understanding and applications for cancer therapy. Bioactive Materials, 22, 365-383.
2. Kalarikkal, S. P., Prasad, D., Kasiappan, R., Chaudhari, S. R., & Sundaram, G. M. (2020). A cost-effective polyethylene glycol-based method for the isolation of functional edible nanoparticles from ginger rhizomes. *Scientific reports*, *10*(1), 1-12.
3. Kim, J., Lee, Y. H., Wang, J., Kim, Y. K., & Kwon, I. K. (2022). Isolation and characterization of ginseng-derived exosome-like nanoparticles with sucrose cushioning followed by ultracentrifugation. *SN Applied Sciences*, *4*(2), 63.

Future direction

In vitro and *in vivo* studies to ascertain the effectiveness of fenugreek derived exosome like-nanoparticles in alleviating pain intensity during primary dysmenorrhea.