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Utilisation of *Moringa oleifera* Seed Protein in Pharmaceutical Formulation.

Jain Ashish, Rege Madhura, and Pananchery Jeeja*

Shri. D. D. Vispute College of Pharmacy and Research Centre, New Panvel, Maharashtra, India.

Research Article

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***For Correspondence**

Shri. D. D. Vispute College of Pharmacy and Research Centre, New Panvel, India

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ABSTRACT

The aim of our study was to isolate a novel biomaterial from the seeds of *Moringaoleifera*, and to evaluate its bio-emulsifying ability by formulating liquid paraffin emulsion. The bio-material was isolated by simple economic process and subjected to various physicochemical parameters like colour, chemical tests. Liquid paraffin emulsion was formulated using bio-material. The formulated emulsions were subjected for evaluation parameters like globule size, pH, phase separation, viscosity. The emulsions showed a globule size in the range 17.86-21.31µm and viscosity of 85.76.4±0.5cp to 92.79±1.2 cp. The formulation F3 was found to be best formulation on the basis of evaluation parameters. The optimized formulation's stability studies were carried out for a period of 12 weeks, at 8°C, 25°C as well as 40°C with respect to colour, phase separation, pH, globule size and viscosity. The stability studies revealed that formulation were found to be stable after a period of 12 weeks at different storage conditions. Thus, the bio-material from *Moringaoleifera*can serve as bio-emulsifier for formulating various drug loaded emulsion.

INTRODUCTION

*Moringaoleifera*is the most widely cultivated species of a monogeneric family, the Moringaceae, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly-growing tree (also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree), was utilized by the ancient Romans, Greeks and Egyptians; it is now widely cultivated and has become naturalized in many locations in the tropics. It is a perennial softwood tree with timber of low quality, but which for centuries has been advocated for traditional medicinal and industrial uses [1].

*Moringaoleifera*is esteemed as a versatile plant due to its multiple uses. The leaves, fruits, flowers and immature pods of this tree are edible and they form a part of traditional diets in many countries of the tropics and sub-tropics[2]. The leaves of *M. oleifera*are a good source of protein, vitamin A, B and C and minerals such as calcium and iron[3]. In addition to its substantial uses and nutritional benefits, *M. oleifera*also has a great potential as a medicinal plant. The flowers, leaves and roots are used for the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulants in folkremedies. The roots of the young tree and also root bark are rubefacient and vesicant[4]. The seeds yield 38-40% edible oil.

The seeds from this plant contain active coagulating agents characterized as dimeric cationic proteins, having molecular weight of 13 kDa and an isoelectric point between 10 and 11. The seeds also have antimicrobial activity and are utilized for waste water treatment. In some developing countries, the powdered seeds of *M. oleifera*aretraditionally utilized as a natural coagulant for water purification because of their strong coagulating properties for sedimentation of suspended undesired particles[5]. The coagulating protein form the seeds has been reported to have surfactant property[6]. In the current

research a protein from the seed of *Moringaoleifera*is studiedas a bioemulsifier for the formulation of emulsion.

Emulsion formulation requires extensive study in order to check the most important parameters to obtain stable emulsions. Optimizing a process implies determination of the experimental conditions giving optimal performance. Emulsion stability is estimated by the average size of the droplets and the variation of emulsion viscosity. Smaller the emulsion droplet size and smaller the variations in viscosity better stability of the emulsion hencebioemulsifier can be used to replace the existing synthetic polymers used for the preparation of pharmaceutical dosage forms.

Hence, an attempt was made to study the emulsifying property of the seed protein and effect of its concentration on globule size and viscosity of formulation.

Thus, our study aimed to utilize the seed protein of *Moringaoleifera* as bioemulsifier for the formulation of emulsion.

MATERIALS AND METHODS

Materials

The dried seeds of *Moringaoleifera* were procured from the local market of Princess street,Mumbai and authenticated at Agharkar Research Institute, Pune. The sample is cotyledon of seeds of *Moringaoleifera* Lam. (Family-*Moringaceae*) and the voucher specimen no. is S-132.All other reagents used were of highest purity and analytical grade. Double distilled water was used throughout the experimental work.

Bio-emulsifier extraction

The seeds were washed, sun-dried and finely powdered. The powder of *M. oleifera*was extracted with 0.15M NaCl for 30 min. at room temperature. Proteins were precipitated using 0-60% ammonium fractionation and wasdialysed with distilled water overnight.

Standardization of bio-emulsifier

The dialysed protein was estimated by Lowry et al's method with Boveine serum albumin as reference.

Formulation of emulsion

The o /w emulsions were prepared by taking the specified amount of Liquid paraffin, glycerin, chloroform water, sodium benzoate and double distilled water were emulsified using bio-emulsifier at 4000 rpm for 30 min with the addition of peppermint oil at the latter stage. The volume was made up by adding excess of water. Similar procedure was followed for other formulations. The emulsions were prepared in three ratios(F1-F3) (Table No. 1) by varying the amount of biomaterial (30mg-50mg). The formulated emulsions were evaluated for the various evaluation parameters.

EVALUATION OF EMULSION

Globule size determination

The diameter of the droplets was measured with an optical microscope equipped with a calibrated eyepiece micrometer. The mean diameter was calculated on the basis of at least 100 droplets. Mean of three readings was reported. All measurements were taken after 24 h[7].(Table No. 2)

Rheological study

Viscosity measurements were made by using Brookfield viscometer, at constant temperature (25°C) at 100 rev/ min. Readings performed in triplicate. (Table N0. 2)

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Stability test

Stability tests were performed for optimized formulation at different storageconditions to see theeffect of these conditions on the storage emulsions. These tests were performed on samples kept at8˚C ± 0.1˚C (in refrigerator), 25˚C ± 0.1˚C (in oven), 40˚C ± 0.1˚C (in oven) with 75% RH. Organoleptic characteristic of emulsions, i.e. color, liquefaction and phase separation were notedat various intervals for 12 weeks [9](Table No.3). The samples were also analysed for globule size and viscosity on 12th week (Table No.4)

RESULTS AND DISCUSSION

The seed fraction obtained from saline extraction of *Moringaoleifera*showed presence of proteins. The total protein content of the dialysed seed fraction was found to be 10mg/ml[10]. The seed protein was studied for its emulsifying property since it is reported to have surfactant property [6]. On this basis Liquid paraffin emulsion was formulated using dialysed protein. The emulsion formulated was milky white in colour with no creaming. The emulsion showed globule size of 18.675±3.12 µm and viscosity of 88.52±1.12.It is observed that with increase in the concentration of bio emulsifier there is decrease in the globule size and increase in viscosity of the formulation. Formulation F-2 was selected for stability study of liquid paraffin emulsion containing bioemulsifier and toxicity study of the bioemulsifier in the preparation.

The emulsion stability were evaluated for a period of 12 weeks , at 8ºC, 25ºC as well as 40ºC with respect to colour, liquefaction, phase separation, globule size and viscosity (Table 3.1, and 3.2). There were no significant changes in organoleptic properties for emulsion after 12 weeks at different conditions of storage. The data does not show any significant changes with respect to globule size and viscosity after 12 weeks 8ºC, 25ºC as well as 40ºC. Thus, formulation was found to be stable after a period of 12 weeks at different storage conditions.

Table No 1: Formula of Liquid paraffin emulsion

Sr. No.	Ingredients	Quantity		
		F-1	F-2	F-3
1	Liquid paraffin IP	50ml	50ml	50ml
2	Glycerin IP	12.5ml	12.5ml	12.5ml
3	Chloroform water IP	1ml	1ml	1ml
4	Sodium benzoate IP	0.5gm	0.5gm	0.5gm
5	Peppermint oil	2.5ml	2.5ml	2.5ml
6	Seed protein	30mg	40mg	50mg
7	Distilled water	100ml	100ml	100ml

Table No 2: Globule size and viscosity of Liquid paraffin emulsion Undergone: For optimizing formula

Formulation	Globule size in µm	Viscosity in centipoise
F-1	21.312±4.1	85.76±43
F-2	18.675±3.12	88.52±1.12
F-3	17.864±6.5	92.79±49

Table No 3: Sensory Evaluation of Liquid paraffin emulsion: undergone stability studies

Sensory evaluation	Observation For Day 0	Observation after 12 weeks for 8ºC	Observation after 12 weeks for 25ºC	Observation after 12 weeks for 40ºC
Colour	Milky white	Milky white	Milky white	Milky white
Liquefaction	Nil	Nil	Nil	Nil
Phase separation	Nil	Nil	Nil	Nil
Flavour	Peppermint	Peppermint	Peppermint	Peppermint

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Table No 4: Globule size and viscosity of Liquid paraffin emulsion Undergone: Stability studies.

Formulation	Day 0	After 12 weeks		
		8°C	25°C	40°C
Liquid paraffin emulsion with bioemulsifier (globule size in µm)	18.675±3.12	22.35±2.52	21.50±3.19	23.25±2.34
Liquid paraffin emulsion with bioemulsifier (viscosity in centipoise)	88.52±1.12	89.5±1.34	88.79±1.22	90.04±1.47

CONCLUSION

The research work gives an insight to the use of potential bio-materials for the formulation of various dosage forms. It shows the potential bio-emulsifying ability of bio-material from *Moringaoleifera*. Finally, conclusion was drawn that the bio-material can serve as bio-emulsifier for formulating various drug loaded emulsion.

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