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Development and Characterization of Guizotia abyssinica (L.f.) Cass. Ethosomes

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Abstract
In order to minimize issues with stability, toxicity, and poor bioavailability, various novel drug delivery systems are currently preferred. Formulation of herbal drugs is emerging as a promising tool for the treatment of many diseases with less toxic effects, better therapeutic effect, and patient compliance. Herbal extract is being used to develop NDDS such phytosomes, ethosomes, herbal transdermal patches, and nanoparticles. In the current study, ethanolic extract of *Guizotia abyssinica* (L.f.) Cass Fowers' ethosomes is being created. We assessed the developing ethosomes. The results show that the EEGAF formulation code GAF-5 has the highest EE, which is 92.89.

Key-words: Ethosomes, Leaves Extract, *Guizotia abyssinica (L.f.) Cass*

Introduction

The outermost layer of skin (stratum corneum) consists of corneocytes enclosed by the lipid bilayers. This is the main barrier to deliver the drug to the skin. Many techniques are being used to overcome the problem, including ethosomes. The composition of ethosomes is based on phospholipids and ethanol (20–40%) already developed by . Ethanol is used as a skin permeation enhancer, allowing it to transport through the skin easily. Studies have already shown the effectiveness of ethosomes across the skin in terms of depth and quantity [1-2]. Ethosomes are developed by mixture of phospholipids and ethanol in which the ethanol concentration is maintained at higher level. This carrier will penetrate into the skin deeply to improve the delivery of drugs in to deeper layers of the skin. Ethosomes is non invasive vesicles and are commonly formulated as a carrier for the delivery of the herbal drugs into deeper layers of the skin. *Guizotia abyssinica* (L.f.) Cass. belongs to family Asteraceae commonly known as Ramtil (H), Niger (E) Almost every parts of the plant is used medicinally in the treatment of pain, inflammation, microbial infection, as contraceptives etc. The plant parts are rich in various phytochemicals [3-4]. The present study was designed to formulate and evaluated ethosomes of ethanolic flowers extract of *Guizotia abyssinica (L.f.) Cass*.

Material and Methods

Extract

Ethanolic leaves extract of *Guizotia abyssinica (L.f.) Cass* (EEGAF) was taken after extraction. The extract was prepared using soxhlet apparatus. [5]

Development of Ethosomes containing extract

Ethosomal formulations were prepared by using the cold method. The ethanolic vascular system was composed of phospholipid (2.0% to 4%W/V), ethanol (20% to 40% V/V), propylene glycol (20 % V/V), extract (EEGAF, 0.5% W/V) and distilled water to 100% (V/V). Phospholipid was dissolved along with the drug in ethanol. This mixture was heated to 400 C ± 10 C and a fine stream of distilled water was added slowly, with constant mixing

at 700 rpm with a mechanical stirrer in a closed container. Mixing was continued for an additional 5 minutes, while maintaining the system at 400 C ± 10 C. The preparation was left to cool at room temperature for 30 min and then it was sonicated at 40 C for five cycles of 3 minutes each with a minute rest between cycles using a probe sonicator. Nine formulations were prepared using different concentration of phospholipid and ethanol among them optimized formulation was selected for characterization and evaluation studies. [6-7]

Table 1: Compositions of ethosomes of *Guizotia abyssinica* (L.f.) Cass.

Formulation Code	Conc. of Phospholipid (w/v)	Conc. of Ethanol (v/v)	Conc. of Propylene Glycol (v/v)	Conc. of Extract (w/v)	Conc. of distill water (v/v)
GAF-1	2%	20%	20%	0.5%	Up to 100%
GAF-2	3%	30%	20%	0.5%	Up to 100%
GAF-3	4%	40%	20%	0.5%	Up to 100%
GAF-4	2%	30%	20%	0.5%	Up to 100%
GAF-5	3%	40%	20%	0.5%	Up to 100%
GAF-6	4%	20%	20%	0.5%	Up to 100%
GAF-7	2%	40%	20%	0.5%	Up to 100%
GAF-8	3%	20%	20%	0.5%	Up to 100%
GAF-9	4%	30%	20%	0.5%	Up to 100%

Evaluation of Ethosomes [6-7]

Image analysis of ethosomes by optical microscope

Visualization done by image analysis optical microscope (Labomed Microscope, Leica ATC2000, India). The optical microscope is attached with the software Digipro V 4.0, through which image analysis was done, photographs were captured

Particle size analysis and Zeta potential

ZP, vesicle size and PDI were measured by zetasizer (Malvern Instruments, Malvern)

Entrapment efficiency

The total volume of the ethosomal suspension was measured. 5ml of this formulation was diluted with distilled water up to 8 ml and centrifuged at 15,000 rpm for 45 min at 40Cusing a cooling centrifuge. After centrifugation, the supernatant and sediment were recovered, their volume was measured.Then sediment was lysed using n-propanol and filtered through a 0.45 µm nylon disk filter. The concentration of extract in the supernatant and sediment was analyzed by UV- spectroscopic methodat 260 nm. The percent drug entrapment was calculated using the following equation:

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of entrapped drug recovered}}{\text{Total amount of drug}} \times 100$$

Stability testing

The ability of vesicles to retain the drug (i.e., drug-retentive behavior) was assessed by keeping the ethosomal suspensions at different temperatures, i.e., 4 ± 2, 25 ± 2 (room temperature, RT), 37 ± 2 and 45 ± 2 ºC for different periods of time (1, 20, 40, 60, 80 days). The vesicular suspensions were kept in sealed vials (10 ml capacity) and their image analysis done by optical microscopy.

Results and Discussion

Nine different batches of ethosomes were prepared by using the cold method. In the investigation extract (EEPFL, 0.5% W/V) is used and along with phospholipid (2.0% to 4%W/V), ethanol (20% to 40% V/V), propylene glycol (20 % V/V), and distilled water to 100% (V/V). Nine formulation of i.e., GAF-1 to GAF-9 were prepared and further evaluated for various parameters.All the images depict smooth surface. It has been reported that if the vesicular size is <300 nm, they can deliver their contents into deeper layers of skin to some extent. For all developed formulation of *Guizotia abyssinica (L.f.) Cass* the vesicle size was found be in range. Size analysis of the formulation depending upon the concentration of SPC and ethanol was found to range from 207.08 to 248.29 nm. ZP is an important parameter that affects stability. All the ethosomal formulation was found to have negative ZP due to the net charge of the lipid composition in the formulation. The negative ZP is responsible for enhanced percutaneous permeation of drug. The ranges of EE of ethosomes were between 39.02% and 92.89%. The ethanol concentration in the ethosome system should not be too high, and generally, should be kept below 40%. As increasing concentration of ethanol results in leaking of the drug from the lipid bilayer due to which EE decreases therefore ethanol concentration only up to 40% was considered. The ability of vesicles to retain the drug (i.e., drug-retentive behavior) was assessed by keeping the ethosomal suspensions at different temperatures, i.e., 4 ± 2 ºC, 25 ± 2 ºC (room temperature, RT), 37 ± 2 and 45 ± 2 ºC for different periods of time (1, 20, 40, 60, 80 days). The results of stability study were presented in Table 3.

Table 2: Evaluation of Ethosomes of *Guizotia abyssinica (L.f.) Cass*

Formulation Code	Vesicle Size (nm)	Zeta Potential (mV)	Entrapment Efficiency (%)
SAF-1	207.08 ±0.05	−23.3	39.02±0.03
SAF-2	210.20±0.18	−29.5	42.89±0.21
SAF-3	230.03±0.23	−28.6	71.28±0.11
SAF-4	220.22±0.02	−25.8	89.06±0.05
SAF-5	248.29±0.09	−30.7	92.89±0.06
SAF-6	230.302±0.06	−26.9	87.31±0.19
SAF-7	202.23±0.01	−25.5	78.18±0.04
SAF-8	211.11±1.02	−28.7	62.17±0.19
SAF-9	216.32±0.16	−29.9	56.20±0.16

Note: All values are Mean±SEM; n=3

Table 3: Stability studies of ethosomes containing *Guizotia abyssinica (L.f.) Cass* flower extract

S/No.	Ethosomes	Stability condition	20 days	40 days	60 days	80days
1.	GAF-5	4±2 ºC	Stable	Stable	Stable	Stable
2.		25±2 ºC	Stable	Stable	Stable	Stable
3.		37±2 ºC	Fusion of vesicles	Fusion of vesicles	Complete deformation	Complete deformation of

					of vesicles	vesicles
4.		45±2 ºC	Fusion of vesicles	Complete deformation of vesicle	Complete deformation of vesicles	Complete deformation of vesicles

Conclusion

From the results obtained it was concluded that the formulation code GAF-5 have maximum EE i.e., 92.89±0.06 having vesicle size 248.29±0.09 nm with zeta potential of −30.7 mv. Also results of stability studies indicate that temperature of 4±2 ºC and 25±2 ºC have stable ethosomes while in other two temperature there is deformalities. The ethanol concentration in the ethosome system should not be too high, and generally, should be kept 40%. As increasing concentration of ethanol results in leaking of the drug from the lipid bilayer due to which EE decreases therefore ethanol concentration only up to 40% was considered as best for formulation of ethosomes.

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