INVESTIGATIONS ON THE LIPOSOMES CONTAINING HORSE CHESTNUT EXTRACTS TO BE USED AS COSMECEUTICS

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INTRODUCTION

Horse chestnut (*Aesculus hippocastanum* L.), a medically used herb of turkish flora and its active substance escin, are widely used for their antiedemic, astringent and antiinflammatory activities in pharmaceutics and cosmetics. Horse chestnut extracts and escin increase circulation of the blood and nutrient intake of scalp, prevent degeneration of the elastic fiber and hair falling, and give softness and luster to hair (1). In this study, ethanolic extract from the seeds of horse chestnut has been encapsulated in liposomes to provide prolonged release of the active substance and these encapsulated liposomes have been added to hair care preparations for investigation.

EXPERIMENTAL METHODS

Preparation of the horse chestnut extract

After collection of horse chestnut seeds, they were dried, powdered and extracted with ethanol. The extract obtained was lyophilised and stored for further studies at 4 °C ±0.5. The yield and the amount of escin in the seeds and extract were determined spectrophotometrically at 540 nm (2).

Preparation of liposomes

Film and dehydration – rehydration methods have been applied for the preparation of liposomes (3,4). Egg and soya phosphatidylcholine (PC) and equimolar amounts of cholesterol were used for MLV and DRV types of liposome production (Table 1). The amount of the extract was 5 mg/mL per liposome preparation.

Determination of particle size and encapsulation capacity of liposomes

Fresh and one month aged samples of liposomes were subjected to particle size analysis using Malvern Autosizer. These data were used to determine any possible change occuring in the formulations. In order to determine the encapsulation capacity, liposome preparations were ultracentrifuged at 4 °C±0.5 (40000 g, 30 min, 3 times) and both phases (supernatant and pellet) were analysed spectrophotometrically at 540 nm. Using these data, the extract amount encapsulated in liposomes was calculated.

Preparation of hair care formulations

Hair care products are an important group of cosmetic preparations. They are widely used for several purposes such as; hair repearing, hair strengthening and healing effects (5). Hair care formulations in this study were chosen as two hair curing cream vehicles (FA and FB) which are applied on clean hair and are not rinsed out to provide more and longer effectiveness. Encapsulated liposomes were added to hair care formulations given in Table 2.

Diffusion Studies

Diffusion from encapsulated liposomes and formulas was investigated by an *in vitro* method using a diffusion cell at 37 °C±0.5 [receptor phase: 30 ml distilled water, cellophane membran (Visking Tubing, UK)]. The samples were collected at certain time intervals and were analysed

spectrophotometrically at 540 nm. Diffusion of escin from formulas was compared with that of the formulas containing the plain extract.

Table 1.Liposome formulations

Formula Code	Molar Composition (128μmol:128μmol)	Type of Liposome
L1	Egg PC: Cholesterol	MLV
L2	Soya PC: Cholesterol	DRV
L3	Egg PC: Cholesterol	MLV
L4	Soya PC: Cholesterol	DRV

Table 2. Hair care formulations

FORMULA CODE	
FA + extract	Formulation A with plain extract.
FB + extract	Formulation B with plain extract
FA+L1 FB+L1	Formulations including MLVs (egg PC)
FA+L2 FB+L2	Formulations including MLVs (soya PC)
FA+L3 FB+L3	Formulations including DRVs (egg PC)
FA+L4 FB+L4	Formulations including DRVs (soya PC)

RESULTS AND DISCUSSION

The extract yield obtained from dried horse chestnut seeds was 33.88±1.289%. Active substance, escin, was determined as 6.059 ±0.30% in the seeds and as 21.813 ±0.38% in the extract. The particle size of liposomes prepared in this study was in aggreement with literature and liposomes remained stable after storage at 4 C°±0.5 for one month.

Encapsulation capacity of L1 and L2 (MLVs) were 7.543 ±1.25% and 8.467 ±1.25%, of L3 and L4 (DRVs) were 20.721 ±1.21% and 21.036 ±1.15% successively.

Diffusion of escin from formulas containing the plain extract was high in the first hour and did not show much difference in time. On the other hand with formulas containing encapsulated liposomes, maximum diffusion level was reached after 8 hours and continuous liberation was observed during this time interval.

CONCLUSIONS

Based on data obtained from the diffusion studies, liposome encapsulated extracts of horse chestnut can be used to prepare prolonged release cosmeceutical hair care formulations.

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