CYTOTOXIC AND GENOTOXIC EFFECTS OF LIPOSOME PARTICLES WITH ENCAPSULATED NATURAL EXTRACTS

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Abstract

Easy scale up of production led to an increase in cosmetic industry interest in lipid nanostructures. There are currently some liposome formulations on the market and the amount of these products will indoubtedly continue to grow. For this, detailed studies about their potential genotoxicity and cytotoxicity are neccesary.

Presented work deals with preparation and characterization of liposome particles with aqueous and oil extracts of plant origin. The aim of this work was to observe cytotoxic and genotoxic effects of prepared liposomes.

Total phenolics, flavonoids and antioxidant activity of plant extracts were determined using spectrophotometry as well as encapsulation efficiency. Stability of liposome particles was evaluated using zeta potential and size of liposomes was determined by dynamic light scattering. Potential genotoxic effects of liposomes were analysed using SOS Chromotest. Cytotoxicity of liposome particles was observed in human epidermal keratinocytes HEK (102-05a).

Micro- and nanoparticles with encapsulated antioxidant and antimicrobial active compounds can enhance the effectiveness of cosmetics and pharmaceuticals. In this work, the potential use of liposome particles with natural plant extracts in appropriate concentration was proposed.

Keywords: Liposome, cosmetics, cytotoxicity, genotoxicity, antioxidant

1. INTRODUCTION

1.1. Encapsulation of natural antioxidants in cosmetics

Many modern cosmetics contain antioxidants as their active agents and as protectors of other ingredients against oxidation. Antioxidants can prevent oxidative damage by the chain-breaking of radical scavengers and by inhibiting the oxidation reaction [1].

Phyto-antioxidants consist mainly of phenolic compounds. The structure of polyphenols contains -OH groups attached to the benzene ring and is responsible for their ability to inhibit lipid peroxidation (as chain-breaking peroxyl radical scavengers). Moreover, phenolic compounds possess anti-inflammatory, antibacterial and anitviral functions. However, the effectiveness of polyphenols depends on preserving their bioavailability and bioactivity. The utilization of encapsulated polyphenols, instead of free compounds, can effectively prolong the stability of the formulation in the production process and storage. Final application of the product, concentration, required partice size, release mechanism and manufacturing costs are the main factors that influence the selection of appropriate encapsulation technique and carrier material. Lipid-based delivery systems could protect and maintain the stability of phenolics. This technology can also contribute to improvement of the skin penetration and has protective effects against skin dehydration. Various kinds of lipid carriers have been developed and used, such as liposomes, emulsions, solid lipid nanoparticles, etc [1,2].

1.2. Safety in cosmetics

In recent years, as engineered nanomaterials have been widely produced and applied, people are increasingly exposed to different kinds of manufactured nanoparticles. According to their scale, nanoparticles are provided with many unique physicochemical properties, and thereby may pose a hazard for human health [3].

Nowadays cosmetic formulation contains nano-sized components, such as nanoparticles. nanoemulsions, nanosomes or noisome. These microscopic vesicles consist of traditional cosmetic materials and their scales range from 50 to 5000 nm [4].

In Europe, the standard reference for the testing of cosmetic ingredients is the SCCP´s Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation that follows the OECD guidelines for the testing of chemicals. Compounds that are classified as mutagenic, carcinogenic or teratogenic are forbidden for the use in cosmetic products, according to the cosmetics directive [76/768/EEC]. The most challenging task in the risk assessment of nanoparticles in cosmetic products is that nanomaterials cannot be classified as a homogeneous group of chemicals but is necessary to address the risks in individual cases. Size distribution, surface chemistry and reactivity of nanomaterials with regard to biological tissue must be explored. Uptake of nanomaterials by viable skin cells and their genotoxic, proinflammatory or sensitising effects are the main concerns about nanomaterials in cosmetic products [5].

2. METHODS

Total phenolics and antioxidant activity of aqueous and oil extracts of plant origin were determined spectrophotometrically. Prepared extracts were then packed into liposome particles. Liposomes were prepared from mixture of egg lecithin and cholesterol using ultrasonic homogenization.

The encapsulation efficiency was evaluated spectrophotometrically as well as concentration of phospholipids in prepared liposome suspension. For sterile filtration 0.22 mm filter membrane was used. Stability of liposome particles was evaluated using zeta potential and size of liposomes was determined by dynamic light scattering.

Potential genotoxic effects of liposomes were analysed using commercial kit EBPI SOS Chromotest™. After overnight incubation of bacteria strain E. Coli PQ 37, cells were diluted with fresh medium to absorbance of 0.08 at 630 nm. 100 µl of suspension were transferred into well containing 10 µl of tested compound. Enzyme activity was evaluated spectrophotometrically after 2 hours incubation and adding colored reaction solution.

Cytotoxicity of liposome particles was observed in human epidermal keratinocytes HEK (102-05a). Cells were cultured in a serum-free medium containing 1% antibiotics and antimycotics. Cell viability was measured by colorimetric MTT assay according to the protocol [6]. After exposure to liposome suspensions and controls for 24 hours, MTT reagent in PBS solution was applied for 3 hours at 37 °C. Crystals of converted dye were diluted in 10% SDS reagent and colour was measured at 562 nm by a plate reader.

3. RESULTS AND DISCUSSION

In plant extracts prepared from dried oak bark, clove, oregano and powdered ginger the total amount of phenolics, flavonoids and antioxidant capacity was evaluated (**Table 1**). Antioxidant activity was expressed as milligrams of Trolox equivalents per 1 g sample.

In **Figure 1** the comparison of encapsulation efficiency of aqueous and oil extracts into liposomes were compared. Both water and oil extracts were encapsulated with very high efficiency except aqueous ginger extract. According to characterization of extracts (**Table 1**), most ginger bioactives are lipophilic.

Table 1 Characterization of water and oil plant extracts

	Oak bark		Clove		Oregano		Ginger	
	water extract	oil extract	water extract	oil extract	water extract	oil extract	water extract	oil extract
Total phenolics [mg/g]	33.15 ±0.09	14.04 ±0.05	10.11 ±0.40	65.78 ±2.15	36.47 ±2.16	32.39 ±0.39	7.55 ±0.90	33.52 ±5.45
Flavonoids [mg/g]	18.33 ±0.07	20.43 ±1.35	4.67 ±0.03	5.49 ±0.11	22.04 ±0.03	9.97 ±0.19	2.32 ±0.01	31.60 ±2.16
Antioxidant activity [mg/g]	30.7 6±1.05	17.68 ±0.56	13.75 ±0.42	7.50 ±0.69	37.76 ±1.26	15.21 ±0.46	9.20 ±0.31	14.61 ±0.43

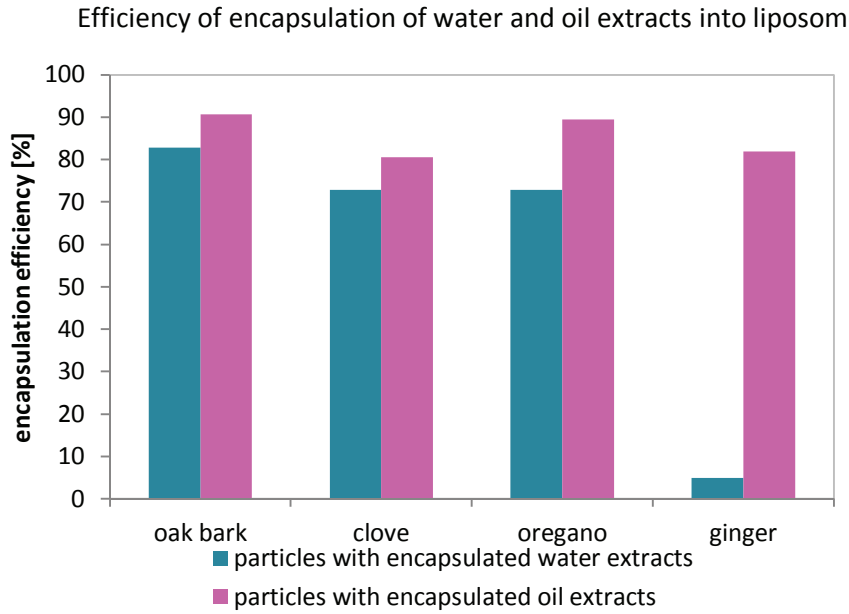

Figure 1 Encapsulation efficiency in individual liposomes measured as % of released phenolics

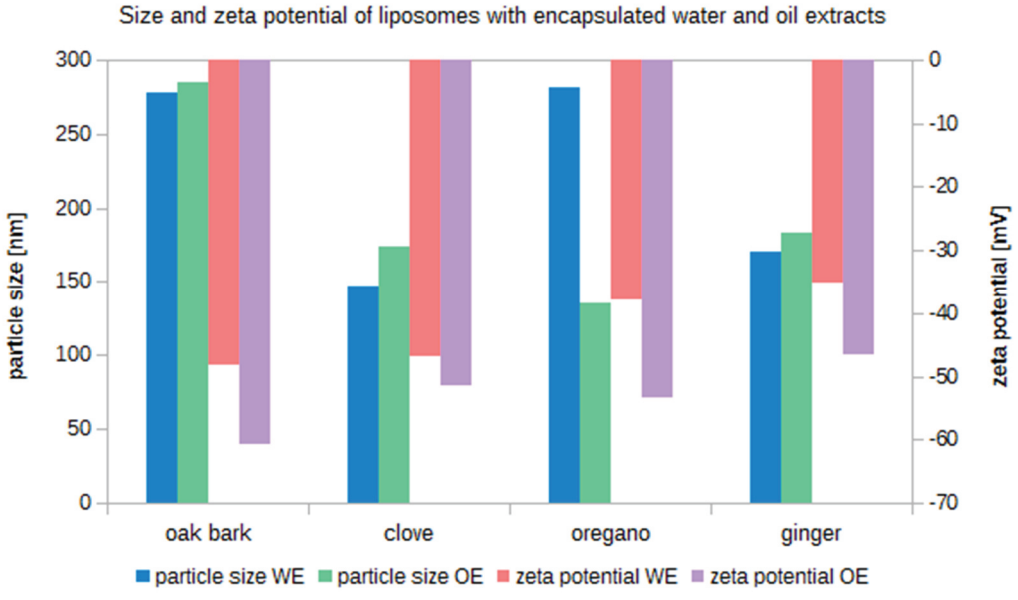

Figure 2 Size and zeta potential of particles

Size of liposomes was determined by dynamic light scattering and the stability of liposome particles was evaluated using zeta potential. Average size of particles was found in the range of 130 nm to 280 nm. Due to their zeta potential ranging from -35 mV to -60 mV, all types of particles were found as stable (**Figure 2**).

Cytotoxicity of liposome particles was observed in human epidermal keratinocytes. Liposome concentration was expressed as the concentration of lecithin, evaluated spectrophotometrically using Stewart method (**Table 2**). Liposome suspensions were diluted with medium and exposed to cells.

Table 2 The highest concentration of liposome suspensions exposed to cells

	Concentration of lecithin [mg/ml]		**Concentration of phenolics [µg/ml]**	
	water extract	oil extract	water extract	oil extract
Empty liposomes	148	148	-	-
Oak bark liposomes	191.5	132	274.5	309.0
Clove liposomes	120	65.5	73,5	2056.5
Oregano liposomes	157	144	265.5	579.0
Ginger liposomes	221.5	175	3.5	1927.0

It was found that neither aqueous nor oil plant extract liposomes are toxic for human epidermal keratinocytes, except of liposomes with encapsulated ginger extracts (**Figure 3** a **4**). Despite unexceptionable antimicrobial effects of ginger, its use in cosmetics product has to be further clarified due to its potential cytotoxic effects.

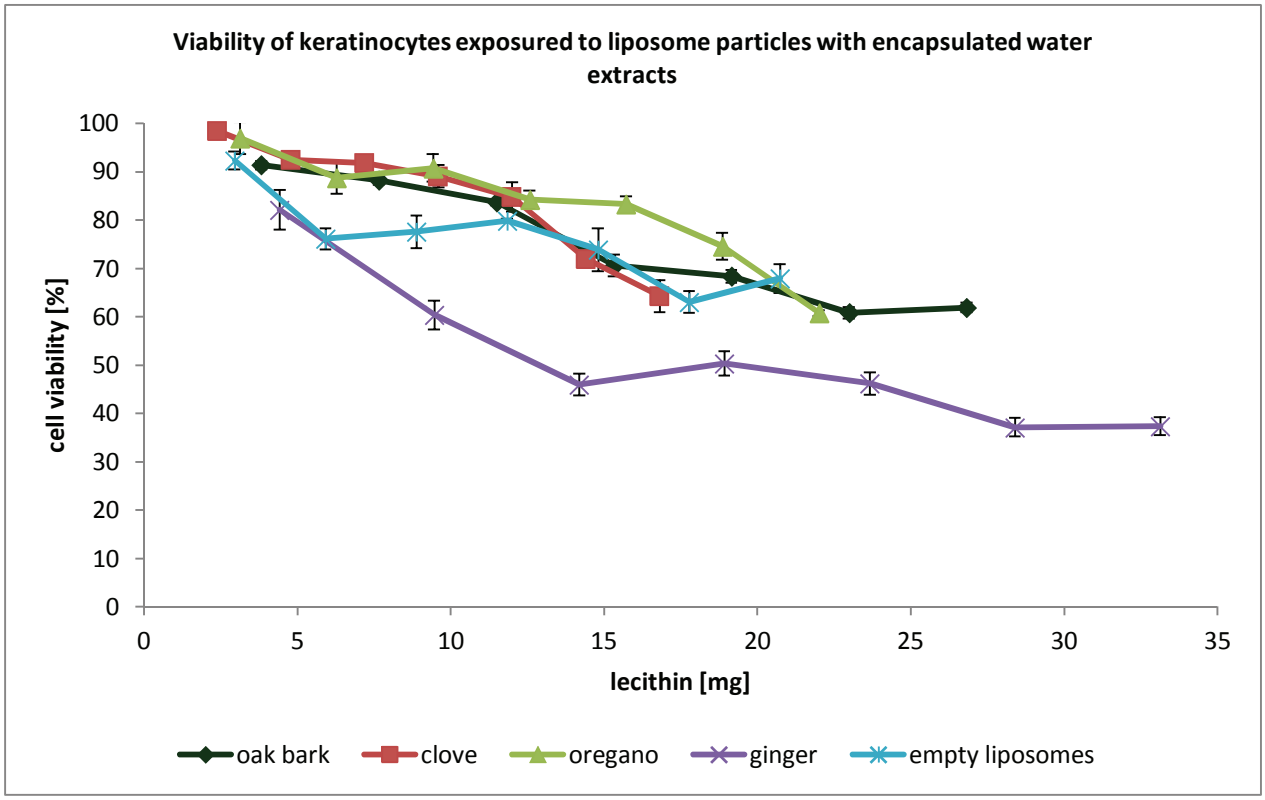

Figure 3 Cell viability after treatment with water extract liposome particles

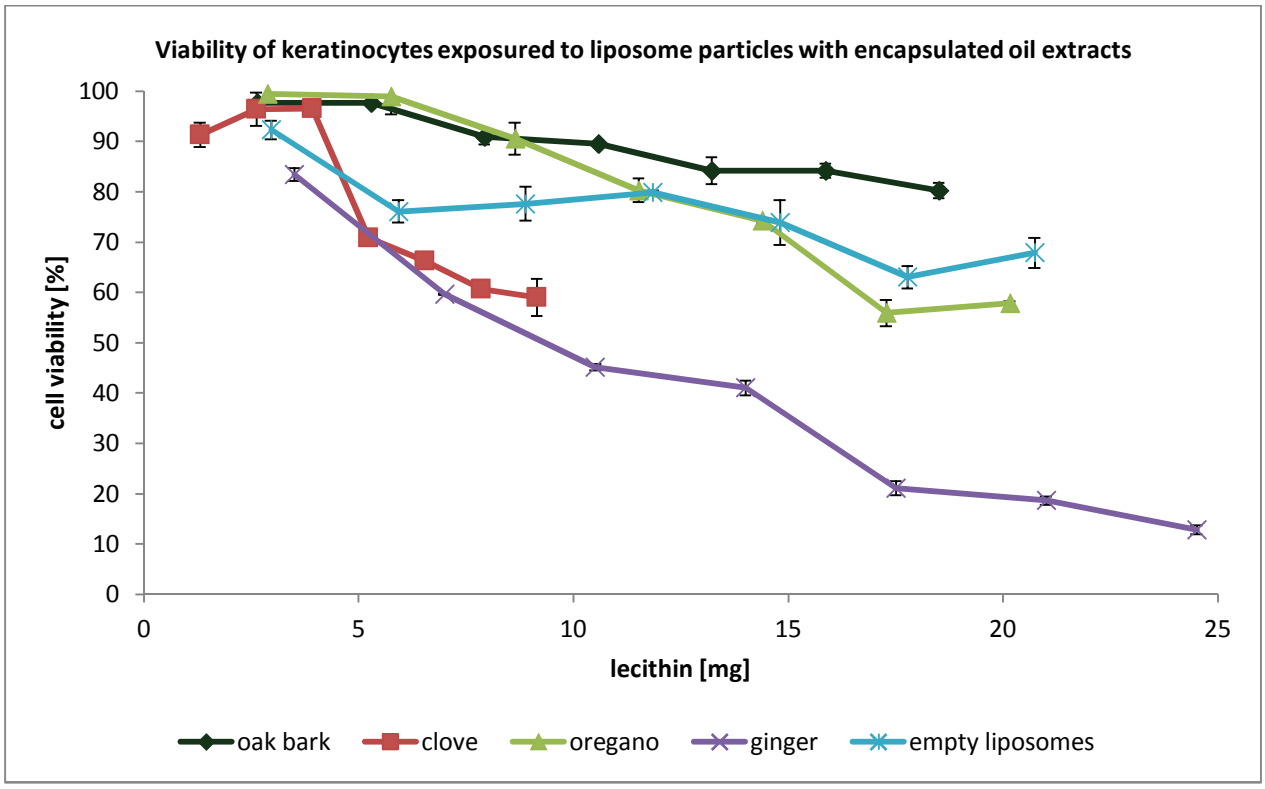

Figure 4 Cell viability after treatment with oil extract liposome particles

Potential genotoxic effects of aqueous and oil plant extracts as well as liposome suspensions were evaluated using commercial SOS Chromotest kit. Specific activity of β-galactosidase was determined spectrophotometrically after 2 hours incubation with samples. Genotoxic potency of samples was expressed as the induction factor IF, that was calculated as a proportion of β-galactosidase specific activity of a sample and negative control. IF value of 1.5 is considered as a critical threshold for labelling substance as genotoxic [7]. It was found, that neither aqueous and oil extracts nor particles show any genotoxic effect.

4. CONCLUSION

Liposome formulation with antioxidant compounds can contribute to the protection of a skin against the free radical stress. Lipid-based delivery systems help to protect the stability of phenolics and participate in improvement of the skin penetration. However, lipid nanostructures are provided with unique physicochemical properties and may pose a hazard for human health. Therefore assessment of potential cytotoxic and genotoxic effects of these formulations is necessary.

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