Microencapsulated extract of green tea as a prospective cosmeceutical with anti-inflammatory properties

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INTRODUCTION

The skin is a complex organ considered a mechanical and also an active barrier.. Cosmeceuticals are the borderline products between cosmetics and pharmaceuticals. The increasing number of people with skin inflammations has imposed the need for search of new anti-inflammatory cosmeceuticals. Regarding growing consumer demand for natural and organic products, anti-inflammatory botanical extracts as cosmeceutical actives may play a significant role based on their long-standing traditional usage and present bioactives. Although positive skin effects of green tea leaves have been reported, wide topical usage of its extracts has been hindered by chemical sensitivity to the environmental factors (1). On the other hand, myriad of bioactives can present a safety issue. We used spray drying to develop a chemically stable green tea powder with good technological and skin safety characteristics, and biological activities that would serve as a prospective anti-inflammatory cosmeceutical active with pectin as an encapsulating agent. The higher dosage of bioactive compounds *per* g of dry powder compared to liquid extracts may enable smaller amounts of the preparation to exert greater *in vivo* biological activity, at the same time providing more comfort/better compliance of consumers/patients (2). The aim of this paper was to investigate and compare skin irritation potential and anti-inflammatory effects of pure and encapsulated green tea extract.

MATERIALS AND METHODS

Ethanolic extract of green tea leaves (*Theae folium*, *Camellia sinensis* (L.) Kuntze, Theaceae) was obtained from the Institute for Medicinal Plants Research “Dr. Josif Pančić”, Belgrade, Serbia. It was spray-dried without (sample A). and with the addition of pectin (sample C, 1:1 ratio). Dry weight to pectin ratio was set to provide adequate flowability of the encapsulant solution into the spray dryer. (data not shown). Pure pectin was marked as P. The morphology of the materials was examined by scanning electron microscope ((JSM-5300, JEOL). Samples were gold-coated (Vacuum Evaporator JEOL, Tokyo, Japan) and then observed (magnification 3,500). SEM images also served for the assessment of particle size distribution using ImageJ, a Java-based software for processing and analysing digital images and microscopy data, as previously described (2).

In vitro anti-inflammatory activity of the samples was assessed using protein denaturation assay with 5% w/v aqueous solution of BSA (bovine serum albumin) (2). 100 µg/mL extract served as test solution. The test control solution was pure distilled water. The aqueous solution of diclofenac sodium served as the standard. The samples were first incubated. After cooling, phosphate buffer saline (pH=6.3) was added, and the absorbance was measured at 340 nm. The inhibition (%) of protein denaturation was calculated using the equation.

In vivo skin irritation potential was investigated by measuring skin biophysical parameters: transepidermal water loss (TEWL), often used in support of claims of product mildness (3); electrical capacitance (EC) and pH on the skin of healthy volunteers (2) using MPA®9 (Courage & Khazaka Electronic GmbH, Germany).

14 healthy female volunteers (27.4±1.1 years) were recruited. Skin sites on the flexor side of the forearms were treated with samples A, C and P, 16 cm^2 each. Additional skin site was left as a non-treated control under occlusion (UCO). Samples were applied in quantities of 0.016 g/cm^2. Skin was covered with Parafilm® and then with cotton adhesive tapes. Parameters were measured prior to (baseline values) and 60 min upon cessation of 24h occlusive treatment. To compare the parameters as a function of time, Student t-test was used (SPSS 20.0). A one-way ANOVA followed by a Tukey post-hoc test was carried out to compare the *in vivo* effects, (statistical significance $p < 0.05$).

RESULTS AND DISCUSSION

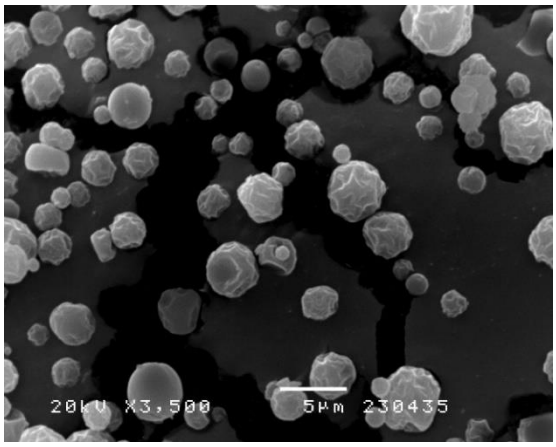
In vivo parameters were shown as relative changes to baseline values (Table 1). After 24 hours, **Δ** EI, a parameter which could indicate an unfavorable skin irritation profile showed a statisticaly significant increase compared to baseline values for A sample.

There were no significant variations in TEWL, EI and pH second vs. first day for samples C and P relative change. Significant changes were not observed between tested samples, also between tested sample and UCO. These results indicate enhanced skin safety profile of extract microencapsulated with pectin.

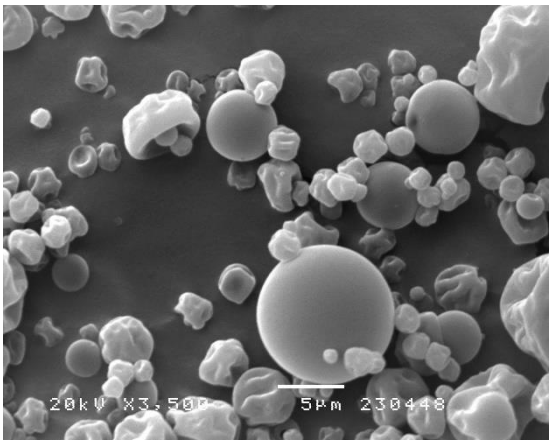
Sample	Δ TEWL	Δ EI	Δ pH
A	1.62 ± 31.77	11.19 ± 14.11*	-3.88 ± 9.65
C	12.07 ± 40.64	4.28 ± 9.75	-5.83 ± 11.30
P	22.93 ± 41.30	2.09 ± 7.47	-14.92 ± 9.32
UCO	14.32 ± 27.13	-5.16 ± 14.63	-11.89 ± 10.4

Table 1. Skin irritation test: The influence of the samples A, C and P on SCH, EI, TEWL and UCO; the results are shown as relative changes of mean values on the second vs. first day and standard error of means.

In protein denaturation assay, samples A and C showed significant anti-inflammatory activity with a percentage inhibition of BSA denaturation of 81.42 ± 0.013% and 81.28 ± 0.005% respectively, (Table 2). Pectin *per se* also showed anti-inflammatory potential. There were statistical difference for A, C and P activity compared to Diclofenac (marked with *), but without statistical significances between samples A and C in percentage inhibition of BSA denaturation.



a)



b)

Figure 1. SEM micrographs of the samples: a) A and b) C

SEM image of sample A revealed the presence of particles of uniform size, irregular spherical shape with a wrinkled surface (Figure 1a). With the addition of pectin in sample C, the appearance of a fraction of spherical particles may be seen, which can be related to the presence of the encapsulating agent. Particle size distribution was in line with the findings of the visual inspection of SEM images. Sample A revealed a narrow distribution pattern with a peak around 2.5 µm. The addition of pectin in sample C led to almost identical frequency distributions of particle size with the slight shift of the peak towards lower size around 1.4 µm.

Tested extracts (100 µg/mL)	Inhibition of protein denaturation in %
A	81.42 ± 0.013*
C	81.28 ± 0.005*
P	55.96 ± 0.038*
Diclofenac sodium	95.60 ± 0.001

Table 2. Inhibition of protein denaturation induced by different tested extracts in BSA assay

CONCLUSION

Bearing in mind many health benefits of green tea on the one hand, and the sensitivity of its main bioactive compounds to environmental factors and its problematic skin safety profile, the main goal of our work was to investigate if extract microencapsulated using spray dry method with pectin as carrier will have additional benefits on skin in terms of anti-inflammatory activity and safety. This paper showed that incapsulated green tea ethanolic extract could serve as prospective cosmeceutical active with anti-inflammatory properties and enhanced skin safety profile compared to spray dried extract *per se*.

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