Enhancement of UVB protection in fibroblasts using *Camellia sinensis* L. extract formulated in transfersomes for cosmetic applications

Immacolata Faraone[1,2], Nadia Benedetto[1], Maria Ponticelli[1], Daniela Russo[1], Claudia Mangieri[1], Ludovica Lela[1], Luigi Milella[1], Carla Caddeo[3], Antonio Vassallo[1,4]

[1] **University of Basilicata,** Department of Health Sciences, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy, immacolata.faraone@unibas.it, nadia.benedetto@unibas.it, maria.ponticelli@unibas.it, daniela.russo@unibas.it, claudia.mangieri@unibas.it, ludovica.lela@unibas.it, luigi.milella@unibas.it, antonio.vassallo@unibas.it;
[2] **Innovative Startup Farmis s.r.l.,** Via Nicola Vaccaro 40, 85100, Potenza, Italy;
[3] **Department of Life and Environmental Sciences,** University of Cagliari, S.P. 8 Km 0.700, 09042 Monserrato, Italy, caddeoc@unica.it;
[4] **Spinoff TNcKILLERS s.r.l.**, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.

INTRODUCTION

The use of nanoparticulate systems to enhance the protection of natural molecules from UVB rays (ultraviolet B rays) is an area of growing interest, particularly in the formulation of sunscreens and skin care treatments aimed at protecting the skin from sun damage that leads to skin aging.

Chronic exposure to UV radiation accelerates the breakdown of collagen and elastin fibres in the skin, leading to wrinkles, loss of elasticity, and the formation of age spots. Protection against UV-induced skin damage is essential to preventing premature aging and the development of skin cancers (Widel, M. *et al.* 2014).

This study focuses on natural sources formulated in transfersomes intended for UVB radiation protection. Plant extracts, such as *Camellia sinensis* L., are known for their antioxidant, anti-inflammatory and photoprotective properties. However, their active molecules often encounter difficulties in penetrating the skin barrier and transfersomes, due to their lipidic structure, can easily pass through the skin, allowing for deeper and more targeted delivery of the active compounds.

METHODS

Four different samples, white tea (WT), green tea (GT), black tea (BT), and decaffeinated black tea (DBT) are derived from different fermentation processes of *C. sinensis* leaves, each offering distinct chemical profiles and bioactive properties. Known for their potent antioxidant activities (Lee, L. S. *et al.* 2014), these teas have gained attention for their potential to combat skin aging.

This study aimed to evaluate the anti-aging effects of these tea extracts, with a particular focus on their ability to protect the skin from oxidative stress and environmental damage.

To assess the phytochemical composition, spectrophotometric assays were performed to determine the total content of flavonoids, phenols, and condensed tannins (Milella, L. *et al.* 2014). In addition, high-performance liquid chromatography (HPLC) coupled with photodiode array (PDA) detection and electrospray ionization-tandem mass spectrometry (ESI-MS/MS) was used to quantitatively analyze key polyphenolic compounds such as theaflavins and thearubigins (Akuli, A. *et al.* 2012), which are known for their antioxidant and anti-inflammatory properties.

The study involved the formulation of transfersomes containing the black tea extract and the evaluation of the Sun Protection Factor (SPF) of the tea extracts and transfersomes with black tea extract.

Transfersomes were prepared, empty or loaded with black tea extract, as reported in **Table 1**.

Formulation	Lecithin	Black tea extract	Tween 80	H_2O
Empty transfersomes	140 mg		0.05 mL	0.95 mL
Black tea transfersomes	140 mg	5 mg	0.05 mL	0.95 mL

Table 1. Composition of the transfersomes.

The characteristics of empty and black tea extract-loaded transfersomes, such as mean diameter, polydispersity index, zeta potential, and entrapment efficiency were measured.

Moreover, we evaluated the inhibitory effects of the extracts on enzymes implicated in the skin aging process, such as tyrosinase, which is involved in melanin synthesis, and elastase, which degrades elastin fibers and contributes to loss

of skin elasticity. By combining these analyses, the study aimed to identify the most promising tea extracts for their potential use in anti-aging skincare formulations.

RESULTS

Both black tea (BT) and decaffeinated black tea (DBT) demonstrated the highest Sun Protection Factor (SPF) values of 23.47 and 23.15, respectively, along with notable enzyme inhibition activity. This can be attributed to their rich content of theaflavins and thearubigins, potent antioxidants with anti-inflammatory properties. To further investigate their potential to protect against skin aging, the anti-photoaging effects of these tea samples were evaluated in UVB (100 mJ/cm²)-irradiated normal human dermal fibroblasts (NHDF) (Abidin, Z. 2017). Among the extracts tested, white tea (WT) and green tea (GT) exhibited significant protective effects at lower concentrations (50 µg/mL), effectively preventing UVB-induced cell viability loss. In contrast, black tea (BT) required a higher concentration (200 µg/mL) to achieve similar results, while decaffeinated black tea (DBT) showed limited efficacy at the concentrations tested.

At higher concentrations (200 and 100 µg/mL), black tea (BT) significantly reduced cell viability compared to the control. Given the results obtained, black tea was nanoformulated in transfersomes and in **Table 2** the characteristics of empty and black tea extract-loaded transfersomes are shown. The transfersomes were small in size, around 60 nm, homogeneously dispersed, and negatively charged (-40 mV).

Formulation	Mean diameter nm ± SD	Polydispersity Index	Zeta Potential mV ± SD
Empty transfersomes	60 ± 3.1	0.23 ± 0.01	-40 ± 2.6
Black tea transfersomes	*63 ± 4.8	**0.25 ± 0.02	-40 ± 3.4

Table 2. Characteristics of empty and black tea extract-loaded transfersomes. Each value represents the means±standard deviations (SD; *n*>10); **p*<0.05 and ***p*<0.001 *vs*. empty transfersomes.

The entrapment efficiency of the transfersomes, determined as a function of caffeine and epigallocatechin gallate, the main components of *C. sinensis*, was high (86.29 ± 2.31% and 92.86 ± 4.79%, respectively). Then, the protective effects of black tea (BT) extract and transferosome-encapsulated black tea (TBT) against UVB-induced loss of viability was investigated (**Figure 1**). UVB exposure significantly decreased cell viability compared to the CTRL group ($p < 0.0001$). Notably, TBT showed significantly greater protection at 100 µg/mL and 200 µg/mL compared to BT alone, which was able to restore the loss of viability only at lower concentrations.

Moreover, treatment with black tea extract and black tea extract-loaded transfersomes partially restored collagen levels. These findings suggest that while green tea has well-documented photoprotective properties, black tea in transfersomes also holds promising potential.

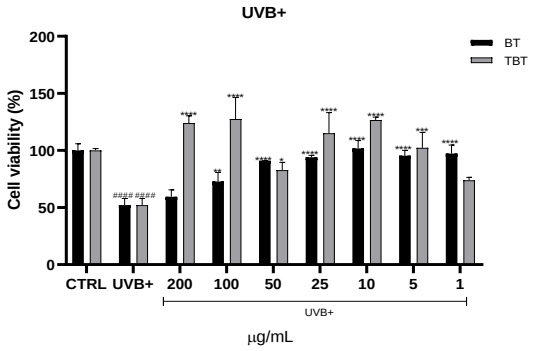

Figure 1. Cell viability of Normal Human Dermal Fibroblasts (NHDF) of Black Tea extract (BT) or black tea extract-loaded transfersomes (TBT) irradiated with UVB 100 mJ/cm². Data are expressed as the mean ± SD of three independent experiments and were analyzed by one-way ANOVA followed by Tukey's post-hoc test. CTRL: untreated cells (100% viability). *****p*<0.0001, ****p*<0.001, ***p*<0.01, **p*<0.05 *vs* UVB+, ####*p*<0.0001 *vs* CTRL.

CONCLUSIONS

The ability of black tea to mitigate UVB-induced damage could open new avenues for its use in cosmeceutical formulations, especially as an ingredient in products aimed at preventing photoaging and improving skin health. Treatment with black tea extract and its nanoformulation effectively mitigated the decline in fibroblast viability, with the nanoformulation offering significantly superior protection compared to the extract alone. This enhanced efficacy is likely due to the nanoformulation's ability to facilitate the delivery of bioactive molecules across cell membranes.

REFERENCES

1. Widel, M., Krzywon, A., Gajda, K., Skonieczna, M. and Rzeszowska-Wolny, J. Induction of bystander effects by UVA, UVB, and UVC radiation in human fibroblasts and the implication of reactive oxygen species. Free Radic. Biol. Med., 68, 278-287 (2014).
2. Lee, L. S., Kim, S. H., Kim, Y. B. and Kim, Y. C. (2014). Molecules, 19(7), 9173-9186.
3. Milella, L., Bader, A., De Tommasi, N., Russo, D. and Braca, A. Food Chem., 160, 298-304 (2014).
4. Akuli, A., Joshi, R., Dey, T., Pal, A., Gulati, A. and Bhattacharyya, N. In 2012 Sixth International Conference on Sensing Technology (ICST), 23-28 (2012).
5. Abidin, Z. In International Symposium on Natural Medicines, 1-17 (2017