Determination of Antioxidative properties of LIPEX® PreAct™

ABSTRACT

This in vitro-study investigates the antioxidative properties of LIPEX® PreAct™, a tocopherol-rich canola-based emollient, using a validated human skin model. By evaluating tissue viability through calorimetry and assessing the protective effects against UV-induced oxidative stress, the research highlights LIPEX® PreAct™'s potential in safeguarding skin health.

LIPEX® PreAct™ demonstrated robust antioxidant properties by reducing multiple markers of oxidative stress after ultraviolet (UV) radiation exposure, with no cytotoxic or significant irritating effects observed.

INTRODUCTION

Oxidative Stress

Oxidative stress is a key factor in the deterioration of skin health, contributing to cellular damage, inflammation, and premature aging. The skin, as the body's primary barrier to environmental aggressors, is constantly exposed to sources of oxidative stress such as ultraviolet radiation. Antioxidants play a crucial role in neutralizing reactive oxygen species and protecting skin tissue from such damage.

This study focuses on evaluating the antioxidative properties and skin compatibility of LIPEX® PreAct™, a tocopherol-rich natural oil, using a validated human skin model to assess its protective effects against UV-induced oxidative stress.

LIPEX® PreAct™

LIPEX® PreAct™ (INCI: Canola oil) is a vegetable oil-based emollient with a high content of natural tocopherols and a fatty acid composition which is stable against oxidation. It is ideally suited for applications where a natural-based stable emollient is required, such as in body lotions and facial care products. The high concentration of Vitamin E (800-1300 ppm) makes it a candidate to be used in formulations where an added protection against UV induced skin damage is desired.

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ENDPOINTS USED IN THIS STUDY

Tissue viability
The UV-protecting properties of the test materials was determined using the calorimetric MTT assay, which evaluates mitochondrial activity as a marker of cell survival. This method provides insight into the overall health of the epidermal tissue by measuring the metabolic function of cells following exposure to test materials and UV-radiation

Inflammation response
The interleukin-1 (IL-1) family consists of key proteins that play significant roles in both inflammation and immune system responses. IL-1α cytokine release was quantified using an ELISA assay to assess the inflammatory response of the skin model after being subjected to the test materials and UV-radiation. Elevated IL-1α levels indicate increased inflammation, often associated with tissue irritation or damage induced by oxidative stress.

Lactate dehydrogenase (LDH) release
Lactate dehydrogenase release serves as a classical marker for assessing plasma membrane damage and cell death. In this assay, the cytosolic enzyme LDH is released into the culture medium as cells undergo membrane damage or cytotoxicity. The released LDH is then quantified using an ultra-sensitive assay based on a diaphorase-linked tetrazolium salt reaction. This method enables precise measurement of cell cytotoxicity, cell proliferation, and cell death.

Carbonyl protein formation
The formation of carbonyl groups on proteins was quantified using a colorimetric assay with dinitrophenylhydrazine (DNPH), as a marker of oxidative protein damage. Elevated carbonyl content suggests increased protein oxidation resulting from UV-induced oxidative stress, providing a measure of the protective effect of LIPEX® PreAct™ against protein damage.

STUDY OBJECTIVES

To evaluate the antioxidative efficacy and skin compatibility of LIPEX® PreAct™, a tocopherol-rich lipid, using a validated human skin model. The study aimed to determine whether LIPEX® PreAct™ could protect skin tissue from oxidative stress induced by UV radiation, as well as to assess its potential for skin irritation or cytotoxicity.

EXPERIMENTAL

In vitro, laboratory-based study using the EpiDerm™ (EPI-200) human skin model, which consists of multilayered, highly differentiated human keratinocytes that closely mimic the structure and function of real human epidermis.

Test Materials:

- LIPEX® PreAct™
- Negative control (viability): purified water
- Used solvent: high oleic sunflower oil (Akosun™)
- Negative control: medium chain triglyceride (Caprylic/Capric Triglyceride)

All materials were tested at 100% concentration

Application Protocol and UV Irradiation
Test materials were applied directly to the surface of the skin model tissues. Each material was tested in duplicate, with both 2-hour and 24-hour exposure times. Controls were treated in parallel. After pre-treatment, tissues were exposed to UV radiation to induce oxidative stress, simulating real-life environmental exposure.

Analysis of Skin Damage
Several key assays were used to evaluate the effects of the test materials on the human skin model. Tissue viability was determined using the MTT assay, which measures mitochondrial activity as an indicator of cell survival. The release of the cytokine IL-1α, a marker of inflammatory response, was quantified by ELISA. To assess cell membrane damage and cytotoxicity, LDH release was measured. Additionally, carbonyl protein formation, serving as a marker of oxidative protein damage, was quantified using a colorimetric assay.

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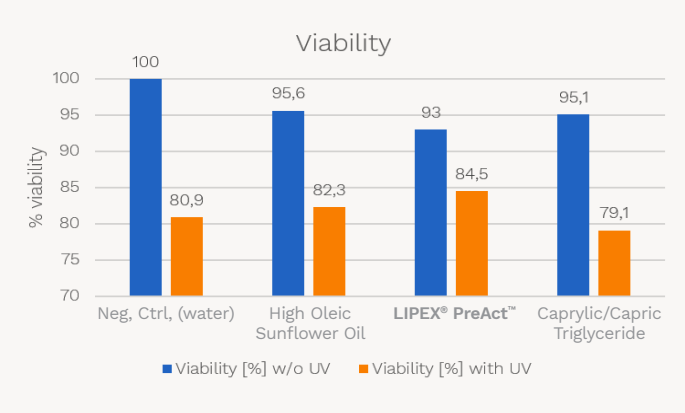
RESULTS

General Morphology & Tissue Viability

All test groups, including those treated with LIPEX® PreAct™, maintained high tissue viability after both 2-hour and 24-hour exposures. UV irradiation caused a minor reduction in cell viability across all samples, with LIPEX® PreAct™ providing a higher UV-protective effect than the other test materials.

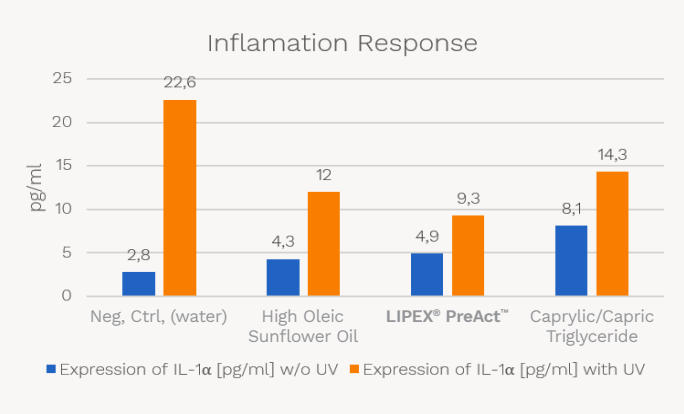
Test Material	Viability [%] w/o UV	Viability [%] with UV
Neg. Ctrl. (water)	100.0	80.9
High Oleic Sunflower Oil	95.6	82.3
LIPEX® PreAct™	**93.0**	**84.5**
Caprylic/Capric Triglyceride	95.1	79.1



Inflammation response by Cytokine Analysis (IL-alpha)

UV exposure led to a marked increase in IL-1α levels in negative control tissues, indicating an inflammatory response. Tissues treated with LIPEX® PreAct™ showed the lowest IL-1α output after UV irradiation, suggesting that LIPEX® PreAct™ effectively reduced inflammation and protected against UV-induced cytokine release.

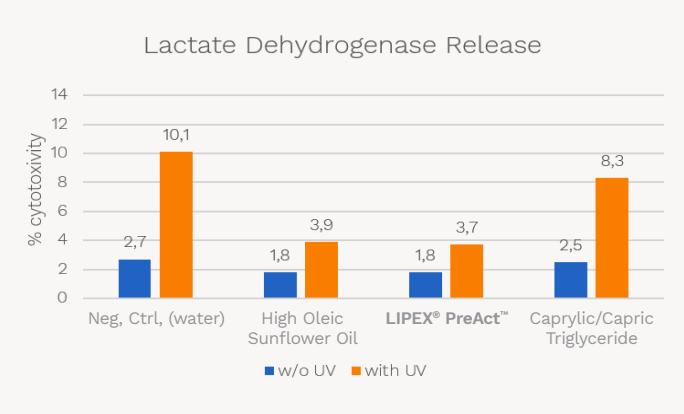
Test Material	Expression of IL-1α [pg/ml] w/o UV	Expression of IL-1α [pg/ml] with UV
Neg. Ctrl. (water)	2.8	22.6
High Oleic Sunflower Oil	4.3	12.0
LIPEX® PreAct™	**4.9**	**9.3**
Caprylic/Capric Triglyceride	8.1	14.3



Lactate Dehydrogenase Release

Baseline LDH levels were low in all non-irradiated tissues, indicating minimal spontaneous cell damage. After UV exposure, LDH release increased in the reference materials, but was significantly reduced in LIPEX® PreAct™ and sunflower oil-treated tissues, demonstrating a protective effect against cell membrane damage.

Test Material	w/o UV	with UV
Neg. Ctrl. (water)	2.7	10.1
High Oleic Sunflower Oil	1.8	3.9
LIPEX® PreAct™	**1.8**	**3.7**
Caprylic/Capric Triglyceride	2.5	8.3



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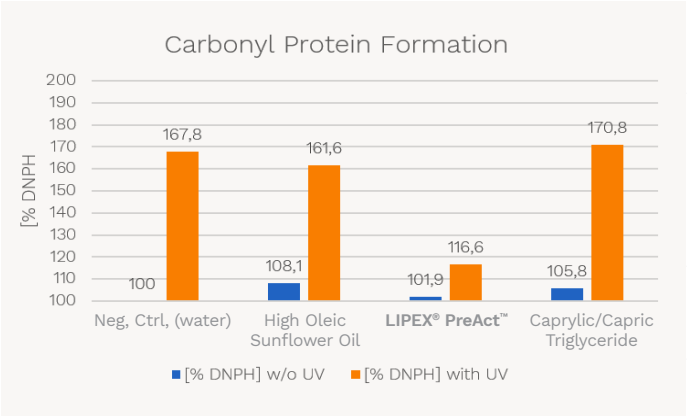
Carbonyl Protein Formation
Baseline levels of carbonyl proteins (oxidative protein damage measured with DNPH) were similar across all groups. UV irradiation increased carbonyl protein formation in water, sunflower oil and Caprylic/Capric Triglyceride, but this increase was suppressed in LIPEX® PreAct™-treated tissues, indicating antioxidant protection.

Test Material	[% DNPH] w/o UV	[% DNPH] with UV	Δ
Neg. Ctrl. (water)	100.0	167.8	
High Oleic Sunflower Oil	108.1	161.6	=
LIPEX® PreAct™	**101.9**	**116.6**	-
Caprylic/Capric Triglyceride	105.8	170.8	=



DISCUSSION

Antioxidant Efficacy
LIPEX® PreAct™ demonstrated robust antioxidant properties by reducing multiple markers of oxidative stress (IL-1α, LDH, carbonyl proteins) after UV exposure. The use of three independent endpoints provided a comprehensive assessment of antioxidant activity.

Cytotoxicity & Irritation
No cytotoxic or significant irritating effects were observed for LIPEX® PreAct™. The product was well tolerated by the skin model, supporting its suitability for topical applications.

CONCLUSION

LIPEX® PreAct™ provides effective antioxidant protection in a human skin model, suppressing oxidative stress markers and maintaining cell viability after UV exposure. It is non-cytotoxic and non-irritating under the tested conditions. The study supports the use of LIPEX® PreAct™ as an active ingredient in topical formulations aimed at protecting skin from oxidative damage. However, as with all antioxidant-based products, it should be considered a complement to, not a replacement for traditional UV filters and sunscreens.