Photoprotective potential of emulsions formulated with Buriti oil (*Mauritia flexuosa*) and Vitamin E against UV irradiation on human keratinocytes and fibroblasts cell lines

C. F. Zanatta[a,b], M. Mitjans[b] , V. Urgatondo[b], P. A. Rocha-Filho[a], M. P. Vinardell[b*]

[a] Dept. Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil.

[b]Dept de Fisiologia, Facultat de Farmácia, Universitat de Barcelona, Spain.

*Corresponding author. Dept de Fisiologia, Facultat de Farmácia, Av. Joan XXIII s/n, 08028 Barcelona, Spain. Tel.: +34934024505; fax: +34934035901

e-mail address: mpvinardellmh@ub.edu

ABSTRACT

Considering the belief that natural lipids and edible substances are safer for topical applications and that carotenoids are able to protect cells against photooxidative damage, wea have investigated whether topical creams and lotions, produced with Buriti oil and commercial surfactants, can exert photoprotective effect of against UVA and UVB irradiation. Emulsions and plain Buriti oil were diluted in DMEM medium supplemented with 10% FBS. Cell treatment was divided in two stages, prior and after being exposed to 30 minutes of UVA plus UVB radiation or 60 minutes to UVA radiation. Emulsions prepared with ethoxylated fatty alcohols as surfactants and containing α-tocopherol caused phototoxic damage to the cells, especially when applied prior to UV exposure. Damage reported was due to prooxidant activity and phototoxic effect of the surfactant. Emulsions prepared with *Sorbitan Monooleate* and *PEG-40 castor oil* and containing panthenol as active ingredient, were able to reduce the damages caused by radiation when compared to non-treated cells. When the different cells lines used in the study were compared, keratinocytes showed an increase in cell viability higher than fibroblasts. The Buriti oil emulsions can be considered potential vehicles to transport antioxidants precursors and also be used as adjuvant in sun protection.

Keywords: Buriti oil, emulsion, UV protection, skin irritation, cell culture.

1. Introduction

UV radiation can be divided into three categories, according to the wavelength range: UVC, 200–280nm; UVB, 280–315nm; UVA, 315–400 nm. Since the stratospheric ozone effectively absorbs wavelengths shorter than 290 nm, and also 70–90% of UVB, only UVA and part of UVB radiation reaches Earth's surface (Duthie et al, 1999). However a substantial decrease in the protective ozone layer occurred (Kerr, 1988) with a resultant increase in the incidence of UVB on Earth.

Exposure of human skin to ultraviolet-A (UVA) radiation can induce various biological responses, ranging from erythema to photoaging (Murphy, 1999; Wlaschek *et al.*, 2001), because this component of the solar UV spectrum penetrates through the dermis to the subcutaneous tissue and affects both the epidermal and dermal components of skin (Tarozzi et al., 2005).

At the cellular level, UVA radiation causes significant oxidative stress because it leads to the generation of reactive oxygen species (ROS), including singlet oxygen, hydroxyl radical, superoxide anion and hydrogen peroxide as well as the release of 'free' iron (Pourzand and Tyrell, 1999). ROS produced by UVA exert a variety of harmful effects including oxidation of nucleic acids, proteins and membrane lipids (Morita and Krutmann, 2000). Keratinocytes contain high levels of antioxidants such as glutathione and various enzymes involved in antioxidant defense, including superoxide dismutase and catalase (Liochev and Fridovich, 1994). Nevertheless, generation of high levels of ROS by UVA can overwhelm normal defenses to oxidative damage, leading to extensive cellular damage and eventual cell death either by apoptosis or necrosis (Pourzand and Tyrell, 1999; Morita and Krutmann, 2000, Tarozzi et al., 2005).

The UVB radiation is experimentally demonstrated to be the most effective light to induce skin cancers in animals, and can cause DNA damage, particularly cyclobutane pyridimine dimmers (CPDs) and photoproducts which induce mutations in the epidermal cells, leading to the development of cancer cells (Ichihashi et al., 2003).

Fundamentally, protection against solar UV-induced oxidative damage to human skin relies on avoiding excessive sunlight exposure and on the use of sunscreens. Topical and endogenous photoprotection by antioxidants could have the potential to complement these strategies (Chiu and Kimball, 2003; Tarozzi et al., 2005), since ROS are the main responsible for the biological effects caused by the photo-oxidative stress.

The use of natural ingredients in cosmetics is steadily increasing. As a result, formulators are being offered a host of newly derived lipids, most from renewable plants sources (Rieger, 1994). High levels of phytochemicals, especially antioxidants are commonly found in many of these plants. One of such plants is Buriti palm tree (*Mauritia flexuosa*), which oil is rich in carotenoids and has been frequently used in cosmetic production. The Buriti oil contains about 1706 $\pm$ 54 μg of total carotenoids/g and β-carotene is considered the major carotenoid performing 90% of total content (Garcia-Quiroz et al., 2003). The oil also presents high levels of oleic acid (60.3%) and considerable amounts of alfa-tocopherol (643.2 mg/g) (Costa, 2007).

Carotenoids are known to be powerful antioxidants and their ability to quench singlet oxygen has been studied (Conn et al. 1991) and reviewed (Edge R

and Truscott, 1999) extensively. Oxygen reactive species, such as superoxide radical, peroxide radical and hydroxyl radical, which can be generated by cytotoxic compounds and by exposure to UV radiation, are potentially damaging to cells through initiation of lipid peroxidation. Consequently, there has been great interest in the antioxidant properties of carotenoids with regard to human health. Several authors studied the antioxidant activity of carotenoids and vitamin E in the skin and the synergism of their association (Böhm et al.,1998; Biesalski and Obermüller-Jevic, 2001). According to Mortensen (2002), carotenoids showed a peroxide radical scavenging activity in human skin and also a capacity of inhibiting lipid peroxidation. Stahl and Sies (2002) also reported their ability in preventing formation of epidermal erythema during sun exposure.

Ethical, legal and financial motives have banned the *in vivo* method for testing cosmetic ingredients and formulations. Over the past few years, several *in vitro* test methods have been suggested as valid substitutes of the irritation tests and recently reviewed (Vinardell and Mitjans, 2008). To this end, a broad range of cell and tissue culture systems has been developed for assessing the irritation and phototoxic potential of chemicals, among them human keratinocytes and fibroblasts cultures (Dijoux et al., 2006). Although some authors (Maier et al., 1991) argue that keratinocytes [either primary cultures or cell permanent lines] are less sensitive than fibroblasts to irritation, for certain specific purposes, is preferable to use keratinocytes, since *in vivo* they are first cells to be exposed to topical formulations.

Considering the belief that natural lipids and edible substances are safer for topical applications and that carotenoids are able to protect cells against photooxidative damages, the aim of this study was to investigate whether topical creams and lotions, produced with Buriti oil and commercial surfactants, can exert photoprotective effect of against UVA and UVB irradiation, assessed on monolayers cultures of human keratinocytes HaCat and 3T3 embryonic mouse fibroblast.

2. Materials and methods

2.1. Materials

Culture media and reagents: Dulbecco's Modified Eagle's Medium [DMEM], Hepes buffer, penicillin [10,000 U/ml]–streptomycin [10,000 μg/ml] mixture and fetal bovine serum [FBS] were purchased from Bio-Whittaker [Verviers, Belgium]. The 75 cm^2 flasks and 96-well plates were obtained from TPP [Trasadingen, Switzerland].The dimetyl sulfoxide and the neutral red dye was acquired from Sigma-Aldrich (Deisenhofen, Germany)

For the emulsions preparation, Buriti oil was supplied by Croda [Brazil], *Ceteareth-5*, *Ceteareth-20*, *Sorbitan Monooleate*, *PEG-40 castor oil* by Oxiteno [Brazil]. The *Steareth-2* was obtained from Beraca Sabará Ingredients [Brazil] and sodium poliacrylate from ISP Corp [USA]. The vitamin E acetate and, D-panthenol were supplied by Sigma-Aldrich.

2.2. Products preparation

Five different emulsions were evaluated, two O/W macroemulsions containing liquid crystals [29R and 51LC], one simple O/W emulsion [51S], one W/O/W multiple emulsion [37M] and one O/W nanoemulsion [37N]. Each system was produced with and without an active ingredient at concentration of 1%. The DL-α-tocopherol or vitamin E acetate [VE] was used to produce the 29R.VE, 51S.VE, 51LC.VE and 37N.VE; and the D-panthenol [P] to produce the 37M.P and the 37N.P. The concentrations of the different components are described in Table 1.

The O/W macroemulsions [51S, 51S.VE, 51LC, 51LC.VE, 29R and 29R.VE] and the multiple emulsions [37M and 37M.P] were prepared using the emulsification by phase inversion [EPI] method (Santos et al., 2005), while the nanoemulsions [37N and 37N.P and 37N.VE] were obtained by the phase inversion temperature method (Tadros et al., 2004). All emulsions were produced under constant stirring [Mechanic Mixer Fisatom Mod. 713 D] at 600 rpm until they reached room temperature [25±2°C].

2.3. Culture of HaCaT and 3T3 cell line

The spontaneously immortalized human keratinocyte cell line HaCaT and the mouse embryonic fibroblast cell line 3T3 were grown in DMEM medium [4.5 g/l glucose] supplemented with 10% fetal bovine serum [FBS], 2mM l-glutamine, 10 mM Hepes buffer and 1% penicillin [10.000 U/ml]–streptomycin [10.000 μg/ml] mixture at 37 °C. 5% CO_2. Both cell lines were routinely cultured into 75 cm^2 culture flasks.

When the cells were approximately 80% confluent, they were harvested with trypsin/EDTA and seeded into a 96-well plates, at a cell density of 8.5×10^4 cells/mL

for fibroblasts and of 10×10^4 cells/mL for keratinocytes and then incubated for 48 h at 37°C, 5% CO_2. For these experiments, half-plates were seeded with HaCat and the other half with 3T3.

2.4. Cell treatment and UV irradiation

Emulsions were diluted in DMEM medium supplemented with 10% FBS at a final concentration of 125 mg/mL according to previous studies of cytotoxicity (Zanatta et al., 2008) The plain Buriti oil was also tested, diluted in DMEM 10% FBS, after previous solubilisation in DMSO (final concentration not higher than 5%), at a final concentration of 100 µL/mL.

Cell treatment was divided in two stages, prior and after the irradiation to determine which treatment would be more effective.

When culture cells in the 96-well reached confluence, the medium was removed from the wells corresponding to pre-treatment condition. These cells were treated with 100 µL of the emulsions with and without active ingredient (1% of α-tocopherol or panthenol) for 15 minutes at 37°C, 5% CO_2. Before UV exposure, the treated cells were washed with PBS and the culture medium of the whole plate was replaced by PBS.

Two types of UV sources were used, an UV TLK 40 W lamp with UVA and UVB emission and a TL-D 15W/10 UVA lamp (Royal Philips Eletronics–Netherlands). Irradiance was measured using a photoradiometer Delta OHM (HD2302 - Italy) to determine the time of exposure, using the following equation:

$$E\ (J/cm^2) = t(s) \times P\ (W/cm^2)$$

Where E represents the UV energy, t stands for the time of exposure (in seconds), and P is the light intensity.

We have established a dose of 2.5 J/cm2 to assays performed in fibroblasts and keratinocytes as reported in literature (Offord, *et al.,* 2002; Tarozzi *et al.*, 2005; Flamand *et al.,* 2006). To reach this irradiation, cells remained exposed 30 minutes in the case of the UVA and UVB lamps and 60 minutes for the UVA lamp.

After UV exposure, the PBS was removed from non treated wells and 100 µL of the emulsions with and without active ingredient (1% of α-tocopherol or panthenol) were added for 15 minutes at 37°C, 5% CO_2 to the wells corresponding to post-treatment condition. The remaining wells had their PBS replaced by culture medium with 10% FBS. Removed the product, the cells were washed with PBS and medium was added. Plates were then incubated at 37°C, 5% CO_2 for 3 hours to run the effects of the UV.

2.6. NRU assay

The NRU assay was performed as described Riddell et al., (1986). Following treatment, the medium was removed and Neutral Red solution (50 µg/mL in culture medium) was added. After 3 h of incubation at 37°C, 5% CO_2 medium was removed, cells were washed twice with PBS and a solution containing 50% ethanol absolute, 1% acetic acid in distilled water was added to extract the dye. After 10 min on a microtitre-plate shaker, the absorbance of neutral red was measured at a wavelength of 550 nm in a Bio-Rad 550 microplate reader. Results are expressed as percentage of control.

2.6. Statistical Analysis

Results were expressed as a percentage of viability compared with control wells [the mean optical density of untreated cells was set to 100% viability], calculated from the dose–response curves by linear regression analysis.

Each experiment was performed at least three times using triplicates for each emulsion and condition analyzed. Photoprotective potential of emulsions was evaluated by one-way analysis of variance conducted by ANOVA test [Sigmastat 3.5]. Differences were considered statistically significant at a $p < 0.05$

3. Results and discussion

The photoprotective potential of the Buriti oil emulsions was based on the high levels of carotenoids present in the oil, especially the beta-carotene, which is known as a potent antioxidant. The distinctive pattern of alternating single and double bonds in the polyene chain of carotenoids is what allows them to absorb the excess of energy from other molecules, explaining the antioxidant properties, while the nature of the specific end groups may influence their polarity; and consequently the way that individual carotenoids interact with biological membranes (Britton, 1995).

The capacity of absorbing the excess of energy would allow the carotenoids to absorb part of the UV radiation and also to reduce the oxidative stress caused by the radiation. Analysis showed that when the red dye was applied immediately after the post-treatment, cellular viability did not varied when compared to control values. However, when the red dye was applied after 3 hours of post-treatment,

the results obtained were distinctive. Same pattern was observed by other authors (Liebler and Burr, 2000, Merwald et al., 2005).

Control plates that remained in the dark, passed through all stages except the radiation, allowed us to compare cells' response to the products with and without UV exposure and also the activity of plain Buriti oil. The results of the NRU of the control plate showed that the formulations and the Buriti oil caused a small decrease in cell viability (Table 2) when compared to the controls cells without product.

The assays performed with UVA/UVB irradiation showed a reduction in cell viability and the pre and post treatment with F29, F29.VE, F51LC, F51LC.VE, F51S and F51S.VE increased phototoxicity (Table 3). These results could be partially explained by the use of a surfactant (*Ceteareth-20*) that was considered cytotoxic in a moderate grade in a previous study (Zanatta et al., 2008), where all surfactants and emulsion had their cytotoxicity evaluated. By contrast, the treatments with F37N, F37N.P, F37M, F37M.P and Buriti oil not only did not increase cytotoxicity, but also Pre and post-treatment of HaCaT cells with F37N, F37N.P, F37M, F37M.P and Buriti oil increased cell viability of irradiated cells.

Results also demonstrated that the keratinocytes viability values were significantly higher than those presented by the 3T3 fibroblasts when the cells were pre-treated with the formulations F29, F51LC, F51S, F29.VE. F51LC.VE and F51S.VE. For the post-treatment condition, the HaCat presented significantly higher values when exposed to emulsions F51LC and F51LC.VE and to the plain oil.

Similar results were observed by Dijoux *et al.* (2006). The authors reported that the HaCat keratinocytes showed to be less sensitive to photo-irritant compounds when compared to 3T3 fibroblasts. However, for some specific purposes, like cosmetic's safety assays, keratinocytes are preferred since they are the first cells exposed to the products and to the sun light, although the phototoxicity test had been validated by the ECVAM (European Centre for the Validation of Alternative Methods) in fibroblasts 3T3 (Spielmann *et al.,* 1998a, b) due to the high correlation with *in vivo* tests.

Regarding the cells' response to treatment condition, we have observed that in general, all emulsions showed to be more phototoxic to the 3T3 fibroblasts, when applied before UV exposure in the pre-treatment condition. However, significant differences in viability values were only found for the plain oil (Table 3). The same pattern was observed for the HaCat keratinocytes, except for the formulations F51S and F37M.P, which presented higher viability values in pre-treatment when compared to the post-treatment (Table 3). Significant differences in viability values between the treatments were only found for F29 and F29.VE in the case of HaCaT cell line.

UVA radiation for 60 minutes induced less reduction on cell viability than UVA/UVB radiation. But the assays, performed with UVA radiation only, presented same tendency observed for UVA/UVB assays' results in the sense that once more, keratinocytes showed higher viability values in comparison to fibroblasts. Except for the plain oil, all other samples analyzed resulted in lower values in fibroblasts and significant differences were observed for emulsions F29, F29.VE,

F51LC, F51LC.VE, F51S, F51S.VE and F37M, which viability values are presented in Table 4.

UVA irradiation also induced a reduction in cell viability and the pre and post treatment with F29, F29.VE, F51LC, F51LC.VE, F51S and F51S.VE increased phototoxicity. The cells that were treated after UVA irradiation exposure, also presented higher viability values when compared to those that were previously treated with the emulsions. Viability values were significantly higher when 3T3 fibroblasts were post-treated with formulations F29, F51LC.VE, F37N.P and F37M.P when compared to the values of the cells pre-treated with the same emulsions. As for the keratinocytes, significant differences were found in those wells post-treated with the following emulsions F29, F29.VE, F51LC, F51LC.VE, F51S, F51S.VE, F37N.P and F37M.P.

The phototoxic damages caused to the cells by the emulsions, could be attributed to the surfactants used in their production, since their cytotoxic effect could be enough to make more sensitive-labile the cells prior to UV exposure. According to literature, the exposure of the cell membrane to non ionic surfactants, like *Polysorbate 80* and Cremophor EL, leads to a reduction in intracellular glutathione levels, resulting in an increase in hydrogen peroxide concentration, which is responsible for part of the oxidative stress that causes damages to the cells (Hirama et al., 2004; Tatsuishi et al., 2004).

Considering that the surfactants caused an initial oxidative damage, cells' defenses were reduced even before they were exposed to UV, justifying the lower viability values found for the pre-treatment condition.

Another possibility is that the antioxidants added to the emulsions combined with carotenoids present in Buriti oil might have exerted a prooxidant effect in the cells submitted to UV radiation. That would explain the fact that the emulsions with α-tocopherol, in general, led to lower viability values, when compared to the emulsion without these components, especially in the pre-treatment condition. These results were more evident in the assays where UVA and UVB radiation were employed.

Obermüller-Jevic *et al.* (1999) observed that beta-carotene strongly enhances the stress response in UVA-irradiated skin fibroblasts, determined by induction of heme oxygenase-1 (HO-1). The heme oxygenase-1 (HO-1) is a sensitive marker for oxidative stress, which can be induced by the substrate heme itself, as well as a wide variety of other cellular stressors, including reactive oxygen species, such as hydrogen peroxide, hydroxyl radical, nitric oxide, and $^1O_2$ (Tyrrell,1999; Applegate et al., 1991). Consequently, authors hypothesized that the $^1O_2$ quencher activity of beta-carotene in UVA-irradiated fibroblasts was not effective and that the observed prooxidant potential of beta-carotene could also have enhanced inflammatory responses to UVA-induced oxidative stress such as production of pro-inflammatory cytokines. Nevertheless, these effects showed to be dose-dependent, prooxidant activity was observed at higher levels and was reported as adverse effects (Lowe et al., 1999; Eichler et al., 2002) and also could

be related to the interactions of the carotenoids and the α-tocopherol (Black and Guerguis, 2003).

Among the effects of UVB, there are the induction of DNA photodamage that leads to mutation in critical genes; tumor promotion by activation of intracellular signaling and also the generation of free radicals and related oxidants that contributes to carcinogenesis (Liebler and Burr, 2000; Merwald *et al*., 2005). When the UVB photons reach the cells, tocopheroxyl radicals are formed. These radicals can be consumed by divergent pathways (Liebler and Burr, 2000). They can be rapidly reduced by other cellular reductants, however, the accumulation of this oxidation products associated to excess of antioxidants (carotenoids and the α-tocopherol) leads to chain reaction due to a prooxidant activity.

Initially our purpose was to verify if the antioxidant properties of the Buriti oil emulsions were able to diminish or even retard the damages caused by the UV radiation. On the contrary, the results showed us that the emulsions containing high levels of a combination of antioxidants (carotenoids associated to α-tocopherol) did not protect the cells but contributed to undesirable phototoxic effects.

On the other hand, although no significant differences were found, viability values were higher when the cells were post-treated with the emulsions containing panthenol in comparison to those that were treated with formulations without this compound (Tables 3 and 4). This might be explained because panthenol is not chemically considered an antioxidant compound but a precursor of an intracellular

antioxidant, the gluatione (Slyshenkov et al., 2004). Based on this, we postulated that not only the active ingredient did not act as a prooxidant, but also had time to induce glutathione's synthesis during incubation period after irradiation.

In addition, the viability values of the non-treated cells that were irradiated with UVA and UVA plus UVB, revealed that radiation by itself caused more damage to 3T3 fibroblasts and HaCat keratinocytes in comparison to the cells that were treated with the emulsions prepared with *Sorbitan Monooleate* and *PEG-40 castor oil*, as surfactant system (Tables 3 and 4). The fibroblasts showed higher viability values when were post-treated with the formulations F37N, F37NP, F37M and with the plain oil in comparison to control wells irradiated with UVA plus UVB. As for the keratinocytes, their viability values were higher when they were pre and post-treated with F37N, F37NP, F37M, F37MP and with the pure Buriti oil. The significant differences detected are described in Tables 3 and 4.

Based on these results, not only the formulations F37N, F37NP, F37M, F37MP and the plain oil, did not caused phototoxic damages to the cells (especially keratinocytes), but also were able to reduce part of the damages caused by the UV radiation. That effect was possible, firstly because the surfactants present in those formulations was less harmful to the cells and secondly, because the panthenol is precursor of intracellular antioxidants, which were able to defend the cells against the oxidative stress generated by UVA and UVB radiation, without having prooxidant activity. Furthermore, it proofed that existed a synergism between the α-tocopherol and the carotenoids which led to a prooxidant activity.

Conclusion

The Buriti oil emulsions, produced with *Steareth-2* associated to *Ceteareth-5* or *Ceteareth-20*, containing or not α-tocopherol, were not capable to avoid or reduce the damages caused by UVA and UVB radiation. The damages caused by these emulsions can be attributed to a prooxidant activity, caused by an excess of antioxidant, summed to phototoxic effect of the surfactant system.

The emulsions prepared *Sorbitan Monooelate* and *PEG-40 castor oil*, as well as the plain oil were able to reduce damages caused by UV radiation, especially those containing panthenol, due to its indirect antioxidant activity.

In conclusion, the Buriti oil emulsions can be considered potential vehicles to transport antioxidants precursors and also be used as adjuvant in sun protection formulations and their effectiveness against UVA/UVB radiation should be reevaluated in association with chemical UV filters to investigate if the antioxidants can provide increased photoprotection when compared just with the chemical filters.

Table 1. Concentration of emulsion's components.

Formulation	Oil (%)	Water(%)	Surfactant System (%)		
			Total	Surfactant A	Surfactant B
F29R [a]	10	80	10	4.53	5.47
F51S [b]	5	80	15	12.10	2.90
F51LC [b]	5	80	15	12.10	2.90
F37M [c]	5	90	5	2.30	2.70
F37N [c]	5	90	5	2.30	2.70

[a] Surfactant system composed by surfactant A: *Steareth-2* and surfactant B: *Ceteareth-5* and 0.1% of sodium poliacrylate.
b Surfactant system composed by surfactant A: *Steareth-2* and surfactant B: *Ceteareth-20.*
[c] Surfactant system composed by surfactant A: *Sorbitan Monooleate* and surfactant B: *PEG-40 castor oil.*

Table 2. Cellular viability values of non irradiated control plates of 3T3 and HaCat pre and post-treated with Buriti oil emulsions.

Formulation tested	Non irradiated 3T3 cells (%)		Formulation tested		
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment
F29	46.3 ± 9.4	57.3 ± 3.9	**F29.VE**	31.2 ±.9.6	64.6 ± 8.4
F51LC	62.4 ± 5.7	60.2 ± 2.3	**F51LC.VE**	66.5 ±.3.2	67.0 ± 5.7
F51S	69.8 ± 5.8	61.5 ± 3.9	**F51S.VE**	71.4 ±.3.4	68.8 ± 3.3
F37N	99.6 ± 5.7	100.5 ± 6.8	**F37N.P**	96.8 ± 3.1	100.0 ± 6.9
F37M	106,3 ± 2.1	107.5 ± 7.3	**F37M.P**	106.6 ± 7.8	101.1 ± 2.7
Buriti oil	88,5 ± 3.6	94.9 ± 4.0			
	Non irradiated HaCat cells (%)				
F29	98.3 ± 2.2	100.5 ± 5.2	**F29.VE**	93.2 ± 4.9	101.6 ± 10.3
F51LC	81.2 ± 3.2	89.5 ± 2.2	**F51LC.VE**	84.2 ± 3.0	93.8 ± 5.4
F51S	87.6 ± 6.0	93.5 ± 6.0	**F51S.VE**	81.5 ± 0.7	92.7 ± 1.5
F37N	86.9 ± 4.9	90.4 ± 8.6	**F37N.P**	82.2 ± 2.0	94.5 ± 5.8
F37M	84.6 ± 3.7	101.9 ± 1.4	**F37M.P**	89.6 ± 1.2	91.8 ± 6.6
Buriti oil	94.9 ± 2.6	91.5 ± 2.5			

Table 3. Cellular viability values obtained by the NRU assay in 3T3 and HaCat cell lines pre and post-treated with Buriti oil emulsions and exposed for 30 minutes of UVA and UVB irradiation.

Formulation tested	UVA/UVB irradiated 3T3 cells (%)		Irradiated 3T3 controls (%)	UVA/UVB irradiated HaCat cells (%)		Irradiated HaCat controls (%)
	Pre-treatment	Post- treatment		Pre-treatment	Post- treatment	
F29	39.4 ± 24.8	53.1 ± 38.8	66.0 ±4.0	45.0 ± 23.6	77.6 ± 21.5 [a]	83.1 ±4.6
F29.VE	40.7 ± 29.2	44.4 ± 27.7	66.8 ±1.5	40.8 ± 15.0	72.6 ± 13.5 [a b]	85.6 ±7.2
F51LC	30.3 ± 17.3	42.9 ± 23.1	52.8 ±4.9	71.3 ± 7.4 [b]	75.1 ± 8.5 [b]	84.8 ±2.7
F51LC.VE	23.1 ± 7.5	37.9 ± 20.6	53.2 ±2.4	68.0 ± 9.2 [b]	72.4 ± 15.0 [b]	81.7 ±3.4
F51S	38.2 ± 13.9	40.8 ± 19.9	59.9 ±3.1	57.3 ± 8.7 [b]	44.9 ± 20.2	74.5 ±2.4
F51S.VE	24.8 ± 6.0	40.8 ± 19.4	62.4 ±3.2	48.7 ± 8.4 [b]	57.8 ± 10.9	73.6 ±3.4
F37N	69.5 ± 18.5	72.9 ± 19.9	73.0 ±2.9	79.0 ± 17.1	87.9 ± 17.3 [c]	70.7 ±4.6
F37N.P	62.6 ± 23.8	72.8 ± 19.0	67.5 ±3.6	78.1 ± 26.4	92.8 ± 16.6 [c]	69.7 ±2.2
F37M	62.4 ± 25.4	77.7 ± 10.8	75.8 ±2.0	82.6 ± 14.8	84.7 ± 11.8	74.7 ±5.9
F37M.P	55.1 ± 20.5	68.4 ± 11.8	76.2 ±3.3	81.8 ± 5.6 [b c]	80.2 ± 11.2 [c]	69.9 ±9.3
Buriti oil	59.9 ± 4.0	65.9 ± 5.5 [a]	65.9 ±9.2	87.1 ± 5.5 [b c]	92.6 ± 7.8 [b c]	77.7 ±4.6

Results are expressed as mean ± standard error of almost three independent experiments. Percentage of viability related to control cells non-irradiated.
[a] Indicates significant difference in comparison to the pre-treatment condition, at significance level of 95%.
[b] Indicates significant difference in comparison to the 3T3 fibroblasts in the same treatment condition, at significance level of 95%.
[c] Indicates that it is significantly higher than viability of the irradiated control wells, at significance level of 95%.

Table 4. Cellular viability values obtained by the NRU in 3T3 and HaCat pre and post-treated with Buriti oil emulsions exposed to UVA radiation for 60 minutes.

Formulation tested	UVA irradiated 3T3 cells (%)		Irradiated 3T3 controls (%)	UVA irradiated HaCat cells (%)		Irradiated HaCat controls (%)
	Pre-treatment	Post- treatment		Pre-treatment	Post- treatment	
F29	16.7 ± 1.2	25.1 ± 3.3[a]	105.2 ±8.7	44.9 ± 3.0 [b]	61.9 ± 4.6 [a b]	96.3 ± 1.1
F29.VE	23.4 ± 1.5 [c]	23.8 ± 1.7	98.7 ±7.2	25.6 ± 2.5 [c]	54.3 ± 11.5 [a b]	100.0 ± 5.8
F51LC	18.1 ± 0.6	20.1 ± 1.5	113.8 ±7.6	37.1 ± 0.6 [b]	51.7 ± 3.8 [a b]	98.5 ± 5.5
F51LC.VE	18.2 ± 1.1	20.7 ± 0.3 [a]	102.9 ±4.0	29.2 ± 5.5 [b]	51.2 ± 3.8 [a b]	93.6 ± 7.2
F51S	16.2 ± 0.5	22.4 ± 0.6	99.6 ±7.8	33.5 ± 2.3 [b]	74.6 ± 9.2 [a b]	95.2 ± 4.2
F51S.VE	18.7 ± 1.2 [c]	22 ± 2.3	96.2 ±8.0	32.6 ± 2.1 [b]	77.4 ± 8.8 [a b]	103.1 ± 4.5
F37N	57.3 ± 12.9	78.2 ± 4.3	91.0 ±1.8	68.9 ± 4.7	78.2 ± 11.4	86.9 ± 4.6
F37N.P	44.9 ± 16.3	87.1 ± 5.6 [a]	95.4 ±4.9	69.1 ± 1.3	89.4 ± 5.9 [a d]	81.5 ± 2.9
F37M	60.2 ± 19.2	84.8 ± 8.6	96. 6 ±4.5	79.8 ± 4.1 [b]	89.6 ± 4.7	89.1 ± 2.63
F37M.P	45.2 ± 13	94.7 ± 3.2 [a]	97.3 ±7.5	80.8 ± 3.6	102.1 ± 6.3 [a]	92.1 ± 4.0
Buriti oil	66.7 ± 4.7	71.9 ± 2.5	99.0 ±3.9	64.4 ± 3.0	67.9 ± 4.3	88.6 ± 6.3

Results are expressed as mean ± standard error of almost three independent experiments. Percentage of viability related to control cells non-irradiated.
[a] Indicates significant difference in comparison to the pre-treatment condition, at significance level of 95%.
[b] Indicates significant difference in comparison to the 3T3 fibroblasts in the same treatment condition, at significance level of 95%.
[c] Indicates significant difference in comparison to the formulation without active ingredient, at significance level of 95%.
[d] Indicates that it is significantly higher than viability of the irradiated control wells, at significance level of 95%.

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Photoprotective potential of emulsions formulated with Buriti oil (*Mauritia flexuosa*) and Vitamin E against UV irradiation on human keratinocytes and fibroblasts cell lines

C. F. Zanatta[a,b], M. Mitjans[b] , V. Urgatondo[b], P. A. Rocha-Filho[a], M. P. Vinardell[b*]

[a] Dept. Ciências Farmacêuticas, Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Brazil.

[b]Dept de Fisiologia, Facultat de Farmácia, Universitat de Barcelona, Spain.

*Corresponding author. Dept de Fisiologia, Facultat de Farmácia, Av. Joan XXIII s/n, 08028 Barcelona, Spain. Tel.: +34934024505; fax: +34934035901

e-mail address: mpvinardellmh@ub.edu

UNIVERSITAT DE BARCELONA

Dr M.Pilar Vinardell

Departament de Fisiologia.

Facultat de Farmàcia
Edifici B, 3a planta
08028 Barcelona
Tel. 93 402 45 05
Fax 93 403 59 01
e-mail: mpvinardellmh@ub.edu

Dear Editor of Food Chem Toxicology

I am pleased to enclose herewith our study entitled: "Photoprotective potential of emulsions formulated with Buriti oil (Mauritia flexuosa) and Vitamin E against UV irradiation on human keratinocytes and fibroblasts cell lines" for consideration by the Editorial board of Food Chem Toxicology .

The study is an original study and has not been previously published in any language anywhere. This work represent the continuation of our previous study published in Food Chem Toxicol. 2008 Aug;46(8):2776-81.

Looking forward to your news, sincerely yours

Dr Mª Pilar Vinardell
e-mail: mpvinardellmh@ub.edu