Estee Lauder Companies
Research Park
125 Pinelawn Road
Melville N.Y. 11747

ESTEE
LAUDER
INC.

To: Tom Mammone

From: David Gan

Re: Effects of C-Med 100 on sunburn cell formation in Living Skin Equivalents

Date: 5/31/2002

cc: D. Collins, R. Foyouzi, E. Goyarts, M. Ingrassia, E. Pelle, C. Chen, M. Goldstein, L. Declercq, M. Matsui, K. Marenus, D. Maes, H. Gedeon

Summary: C-Med 100 containing "Cat's Claw" (*Uncaria Tomentosa*) from AF Neutraceuticals (BRD #3362) was found to reduce UVB induced sunburn cells in Organogenesis living skin equivalents. At 100mJ, we observed 95% less sunburn cells in C-Med 100 treated samples compared to the untreated control. As C-Med 100 is reported to effect DNA repair, we will further investigate if this reduction in sunburn cell is due to increased DNA repair by measuring the induction and removal of TT dimers in these living skin equivalents.

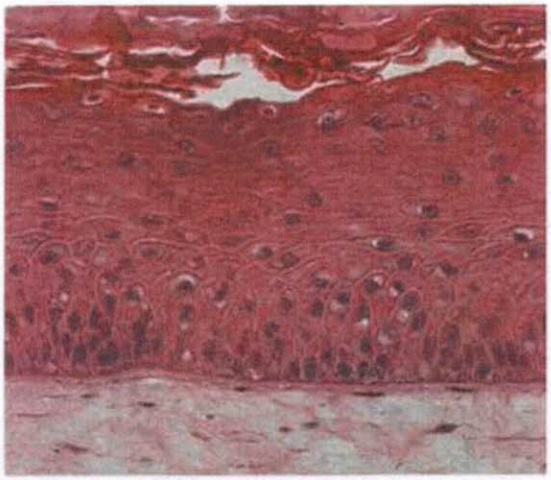
Introduction: Cat's claw (*Uncaria Tomentosa*), is traditionally used by South American natives in the Amazon jungle for its therapeutic properties, including its effects on inflammation and infections. Originally, it was reported in literature to that the bioactivity of Cat's claw is attributed mainly to several alkaloids found in the plant extract, but recently, there have been reports that the glycosides, and triterpenes such as quinovic acid and quinic acid are the main bioactive constituents of Cat's claw. Recently, oral consumption of C-Med 100 (the liquid phase extract of Cat's claw that contains the glycosides and triterpenes), was found to improve DNA repair caused by oxidative stress in humans. In order to examine the topical effects of C-Med 100 on skin, we decided to examine its effects on sunburn cell formation in living skin equivalents.

Methods: Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. These excised portions were pretreated topically with C-Med 100 at 5mg/ml (in sterile H_2O) for 6 hours. After the post-incubation, these excised portions were UVB irradiated at 0, 50, 75, and 100mJ/cm^2. These skins were then re-treated with C-Med 100. Following a 24hour post-incubation, these skin equivalents were fixed in formalin and stored at -4°C. These samples were then sent to Paragon Biotech for H&E staining. Sections were then evaluated using a microscope at 400X magnification. A section was selected from each sample and counts of sunburn cells were made.

Results: We observed sunburn cell formation with UVB but was unable to observed a dose dependent increase as doses of 50mJ and 75mJ did not induce the formation of any sunburn cells (refer to figures on the left side. Data for 50mJ not shown) At 100mJ, we observed 22 sunburn cells in a selected field of the untreated control. In the pre-treated sample, we observed 1 sunburn cell (95% less sunburn cells than the untreated control). Treatments with C-Med 100 reduced the formation of UVB induced sunburn cells in living skin equivalents.

Discussion: C-Med 100 was found to inhibit the formation of sunburn cells in UVB irradiated living skin equivalents. This effect was only observed at 100mJ because the treatments of 50mJ, and 75mJ of UVB alone in this set of skin equivalents did not induce any sunburn cells. As it has been reported in the literature that oral consumption of C-Med 100 increases DNA repair in humans, it is possible that topical use of C-Med 100 may improve DNA repair in the skin. In order to determine if this reduction in sunburn cell formation is due to increased DNA repair, we will be examining the induction and removal of TT dimers in these living skin equivalents. These results suggest that C-Med 100 (Cat's claw) may be a potent active in a sun protection and repair product.

0mJ UVB 24 hour post irradiation



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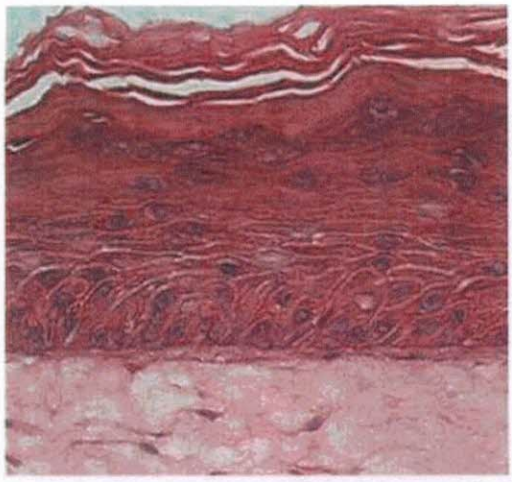
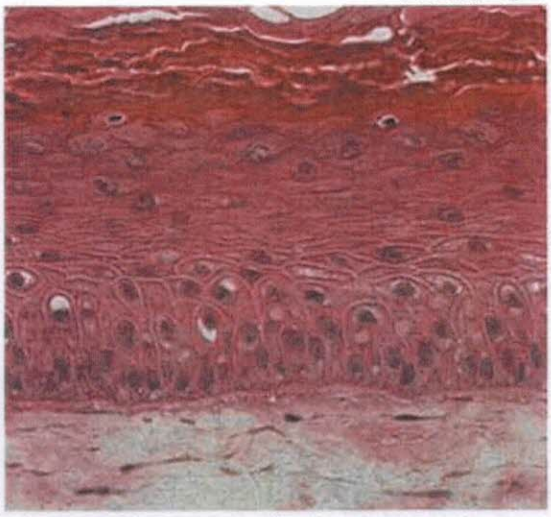


C-med 5mg/ml

75mJ UVB 24 hour post irradiation



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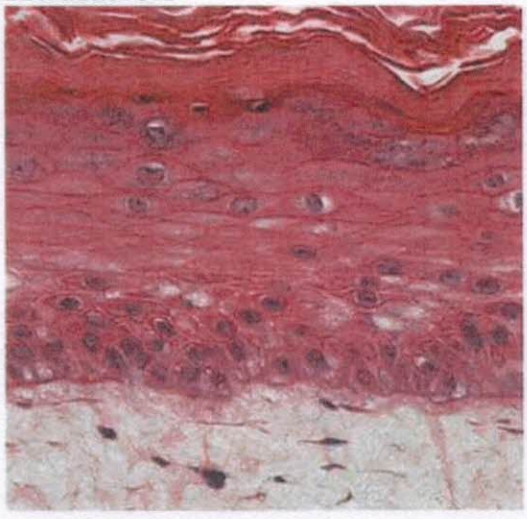
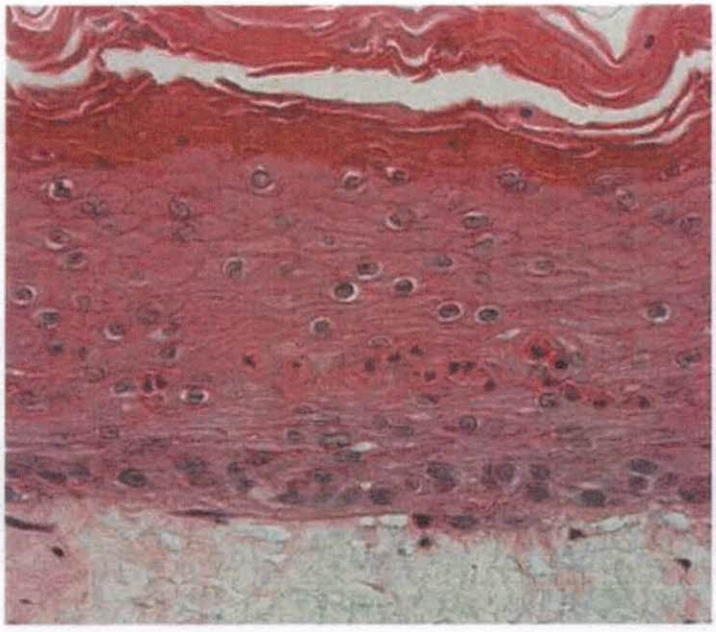
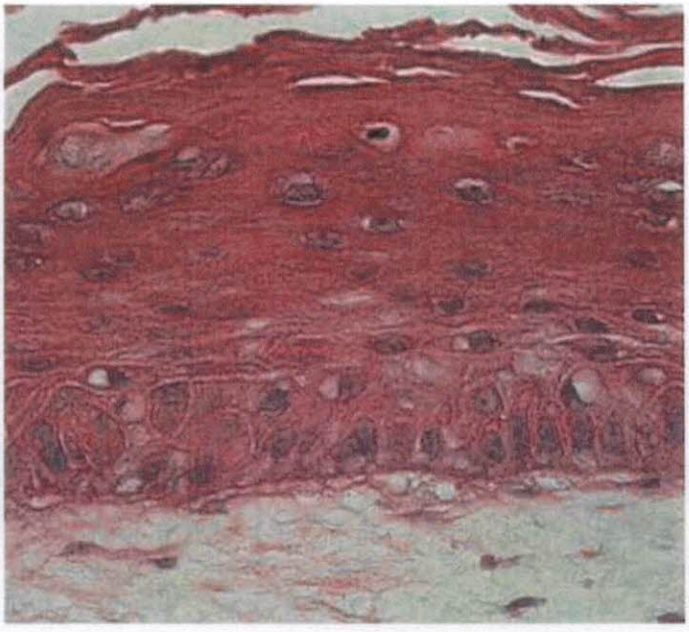


C-med 5mg/ml

100mJ UVB 24 hour post irradiation



Control



C-Med 5mg/ml

Estee Lauder Companies
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125 Pinelawn Road
Melville N.Y. 11747

ESTEE LAUDER INC.

To: Tom Mammone

From: David Gan

Re: Effects of C-Med 100on DNA repair in Living Skin Equivalents

Date: 6/3/2002

cc: D. Collins, R. Foyouzi, E. Goyarts, M. Ingrassia, E. Pelle, C. Chen, M. Goldstein, L. Declercq, M. Matsui, K. Marenus, D. Maes, H. Gedeon

Summary: C-Med 100, containing "Cat's Claw" (*Uncaria Tomentosa*) from AF Neutraceuticals (BRD #3362) was found to increase DNA repair in Organogenesis living skin equivalents. The increase in DNA repair was measured by increased removal of TT dimers in UVB irradiated skin models. At 100mJ, C-Med 100 treated living skin equivalents showed a 73% TT dimer removal compared to 11.1% in the untreated control. This increase in DNA repair explains previous observations of topical treatments of C-Med 100 reducing sunburn cell formation (refer to memorandum dated 5/31/2002). In conclusion, these results suggest that C-Med 100 (Cat's claw) may potentially be a potent active in a sun protection and repair product.

Introduction: Cat's claw (*Uncaria Tomentosa*), is traditionally used by South American natives in the Amazon jungle for its therapeutic properties. We previously tested C-Med 100 (the liquid phase extract of Cat's claw that contains glycosides and triterpenes), as it was reported that oral consumption of C-Med 100 (containing 40% Cat's claw extract, 60% dextran) was found to improve DNA repair caused by oxidative stress in humans. We found that topical treatments of C-Med 100 reduced UVB induced sunburn cell formation in living skin equivalents (please refer to memorandum dated 5/31/2002). In order to determine if this reduction was due to C-Med 100's effects on DNA repair, we decided to measure the induction and the removal of TT dimers in previously examined living skin equivalents.

Methods: Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. Excised portions (8mm) were taken from living skin equivalents (Organogenesis) and cultured over transwell membrane plates. These excised portions were pretreated topically with C-Med 100 at 5mg/ml (in sterile H_2O) for 6 hours. After the post-incubation, these excised portions were UVB irradiated at 0, 50, 75, and 100mJ/cm^2. One set of skins was fixed immediately after UVB irradiation to determine TT dimer induction. Another set of skins

were then re-treated with C-Med 100 at 5mg/ml. Following a 24hour post-incubation, these skin equivalents were fixed in formalin and stored at -4°C. These samples were then sent to Paragon Biotech for immunostaining for TT dimers. Sections were then evaluated using a microscope at 400X magnification. Representative sections were selected from each sample and counts of cells expressing TT dimer staining were made. TT dimer levels of each section is represented as number of cells expression TT dimer staining divided by the total number of cells in that section.

Results: We observed an increase in cells expressing TT dimers with UVB in both sets of living skin equivalents (samples irradiated and fixed at 0hr). At 75mJ, we observed 67% of keratinocytes in a selected field expressing TT dimers in the C-Med 100 treated set compared to 71% in the control set. At 100mJ, we observed 65% of keratinocytes in a selected field expressing TT dimers in the C-Med 100 treated set compared to 67% in the control set (refer to figures at 0hr, Table1. Data for 50mJ not shown). There was no significant difference in the induction of TT dimers between the C-Med 100 treated samples and the untreated samples. In order to determine the level of DNA repair, we compared the levels of cells expressing TT dimers in the 24hour post-irradiation group to the 0hr group (refer to figures at 0hr and 24hr, Table 2, & Graph 1.). At 75mJ, an 79% reduction of cells expressing TT dimers in the C-Med 100 treated skin was observed, while only a 54% reduction was observed in the control. At 100mJ, a 73% reduction of cells expressing TT dimers in C-Med 100 treated skin was observed while only a 11% reduction was observed in the control (refer to Table 3 & Graph 1).

Discussion: As there was no significant difference in the induction of TT dimers between the C-Med 100 treated and untreated skins, we concluded that C-Med 100 does not reduce UVB induced DNA damage. However, C-Med 100-treatment was found to significantly increase DNA repair in UVB irradiated living skin equivalents. This result explains the previous observation of C-Med100 reducing UVB induced sunburn cells. These findings are consistent with published work that demonstrated the oral consumption of C-Med 100 increasing DNA repair in humans. As we have shown the efficacy of topically applied C-Med 100 increasing DNA repair and reducing sunburn cell formation in living skin equivalents, we conclude that C-Med 100 (Cat's claw) may potentially be a potent active in a sun protection and repair product.

TT dimer levels in LSE with 0mJ UVB

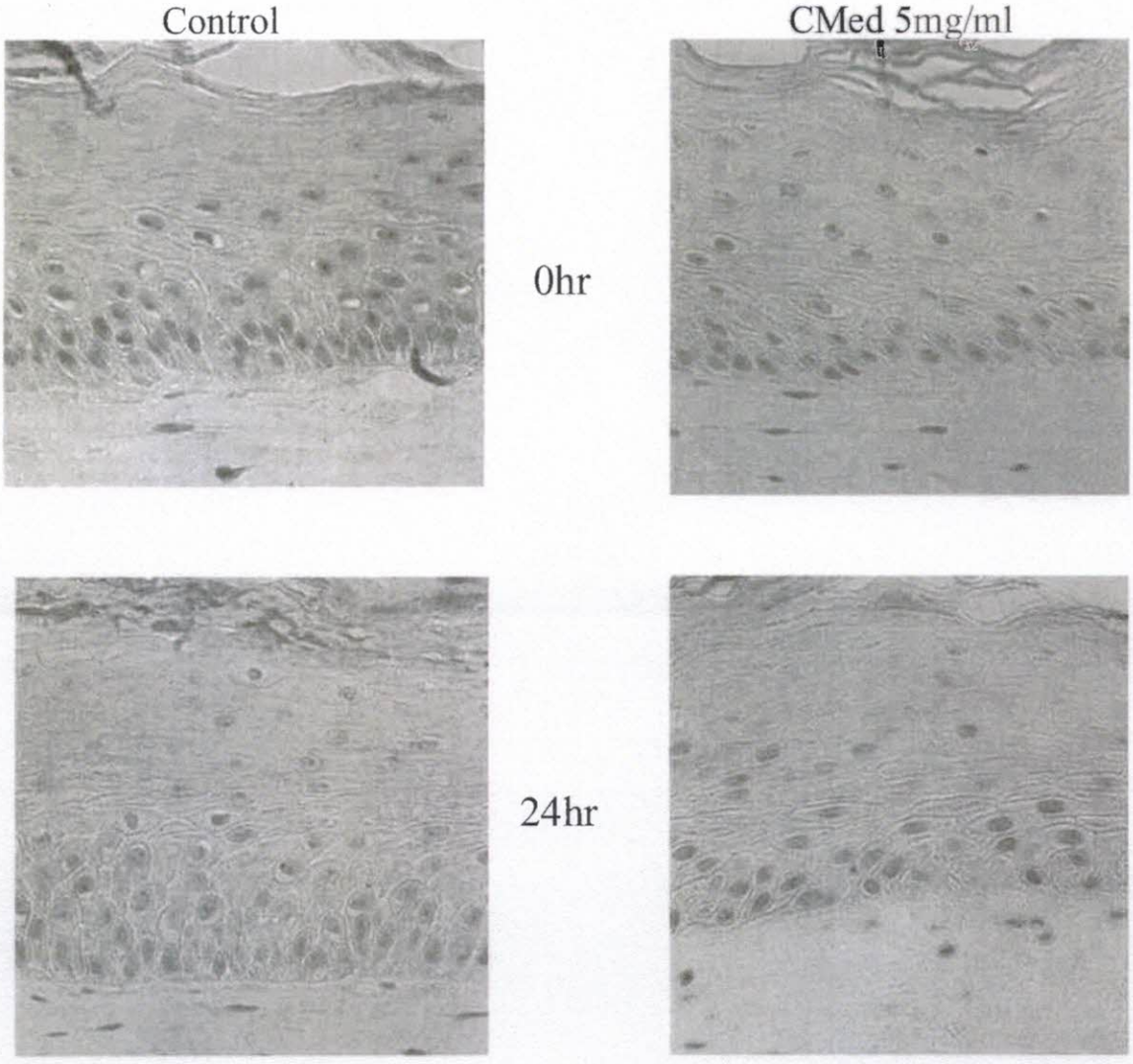


Figure 1.

TT dimer levels in LSE with 75mJ UVB

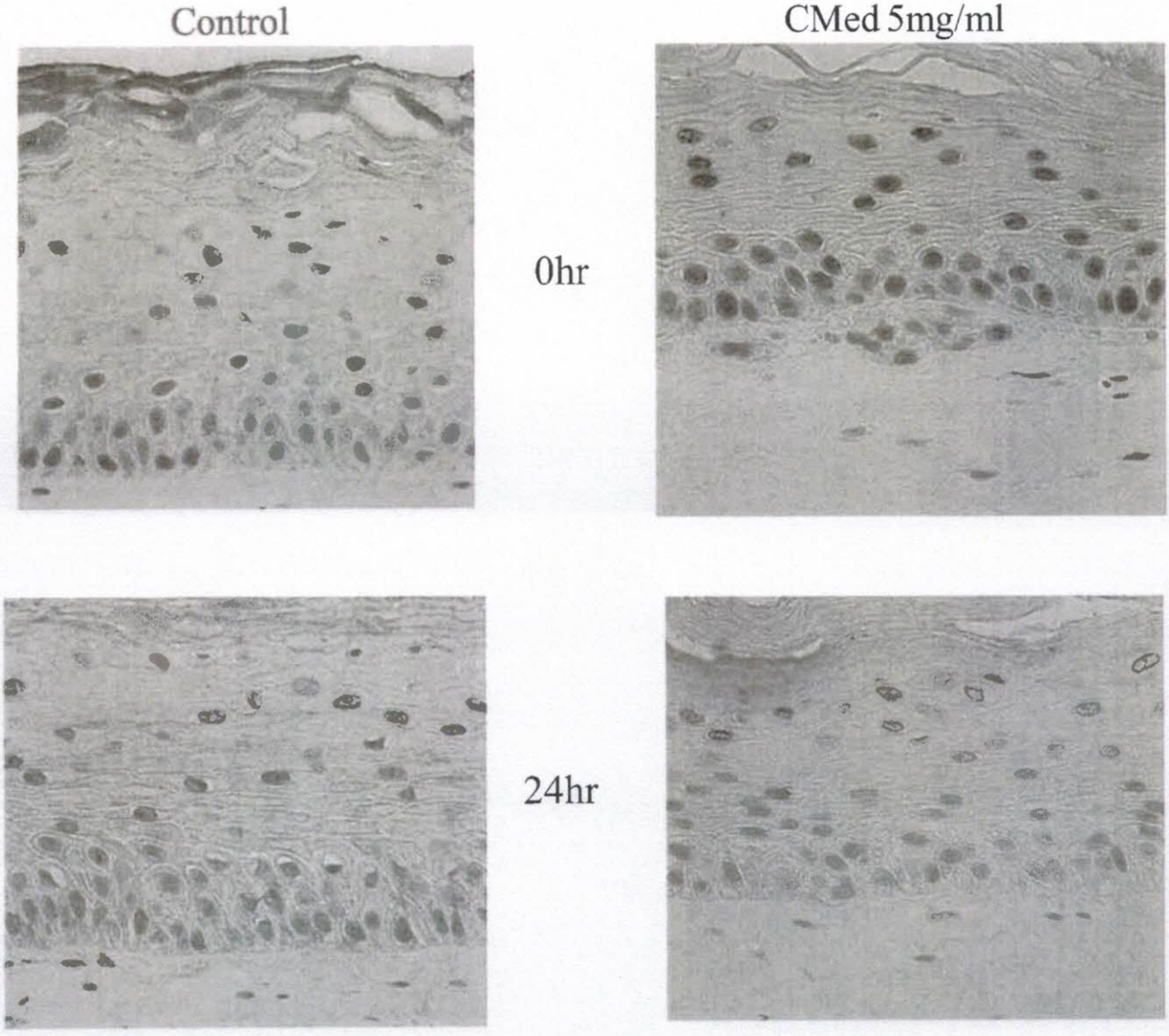


Figure 2.

TT dimer levels in LSE with 100mJ UVB

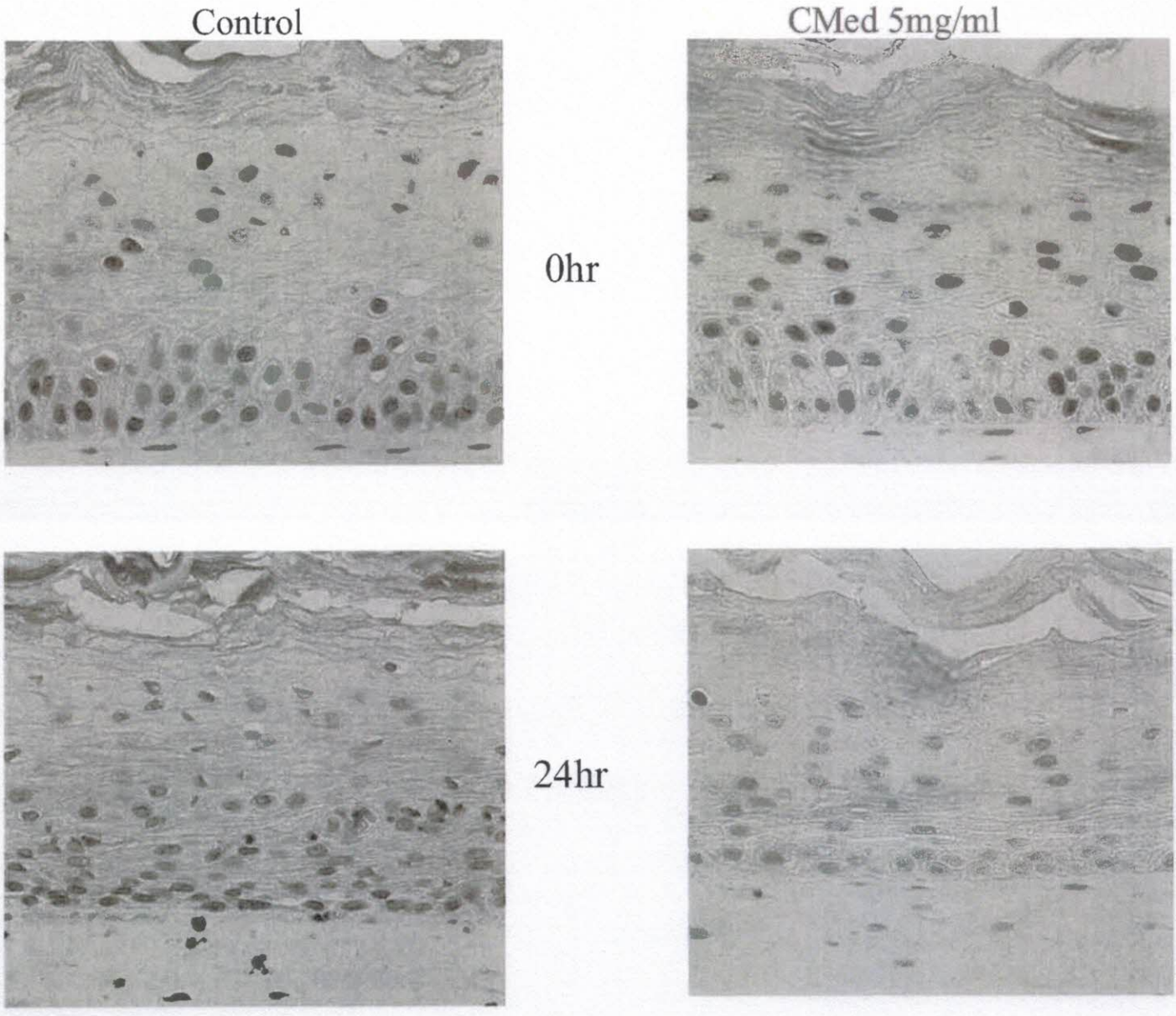


Figure 3.

	Control 0mJ	Control 75mJ	Control 100mJ	C-Med 0mJ	C-Med 75mJ	C-Med 100mJ
0hr (TT dimer expressing cells	0	79	94	0	65	89
0hr (total number of cells)	110	112	140	117	97	138
24hr (TT dimer expressing cells)	0	43	122	0	20	24
24hr (total number of cells)	124	133	205	126	144	138

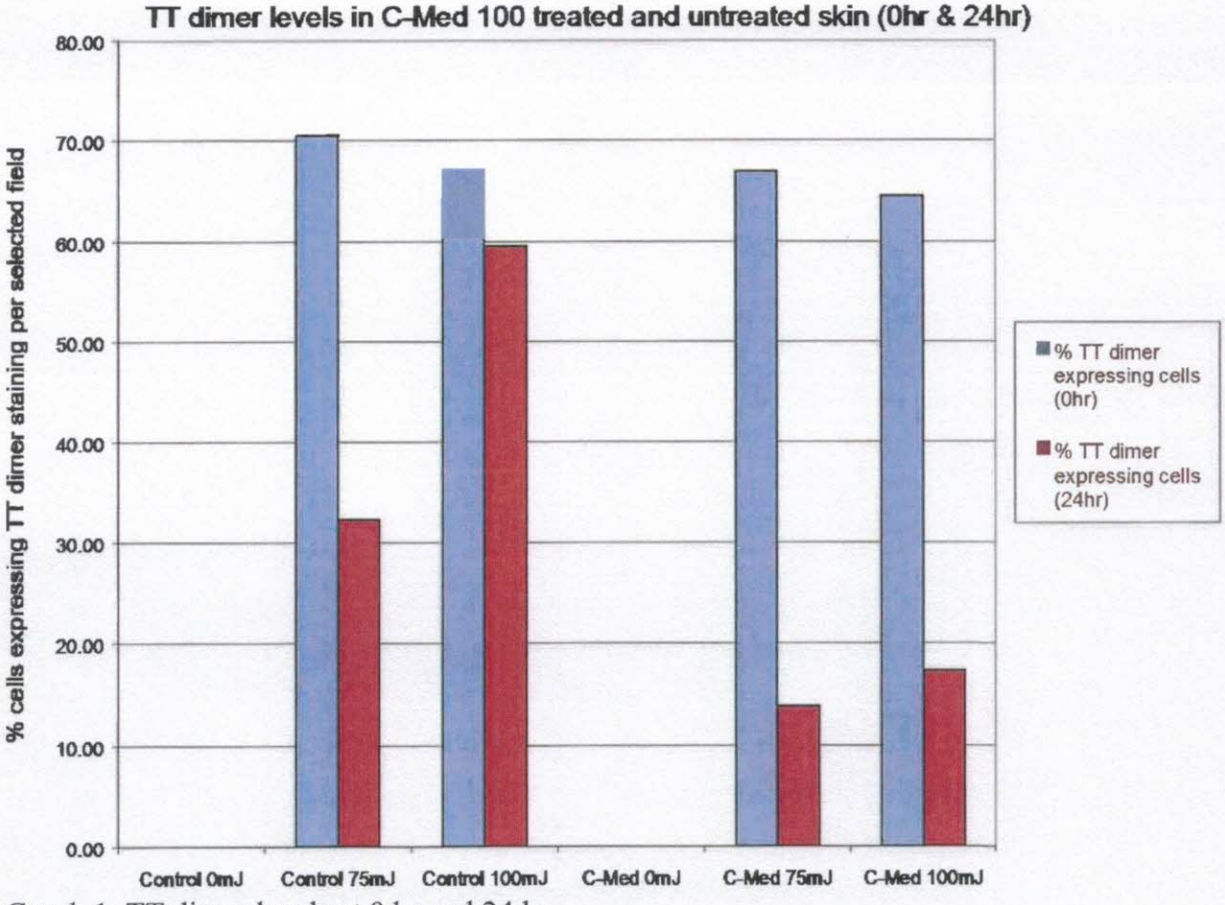
Table 1. Data displaying number of cells expressing TT dimer staining and total number of cells in the sections evaluated.

	Control 0mJ	Control 75mJ	Control 100mJ	C-Med 0mJ	C-Med 75mJ	C-Med 100mJ
% TT dimer expressing cells (0hr)	0.00	70.54	67.14	0.00	67.01	64.49
% TT dimer expressing cells (24hr)	0.00	32.33	59.51	0.00	13.89	17.39

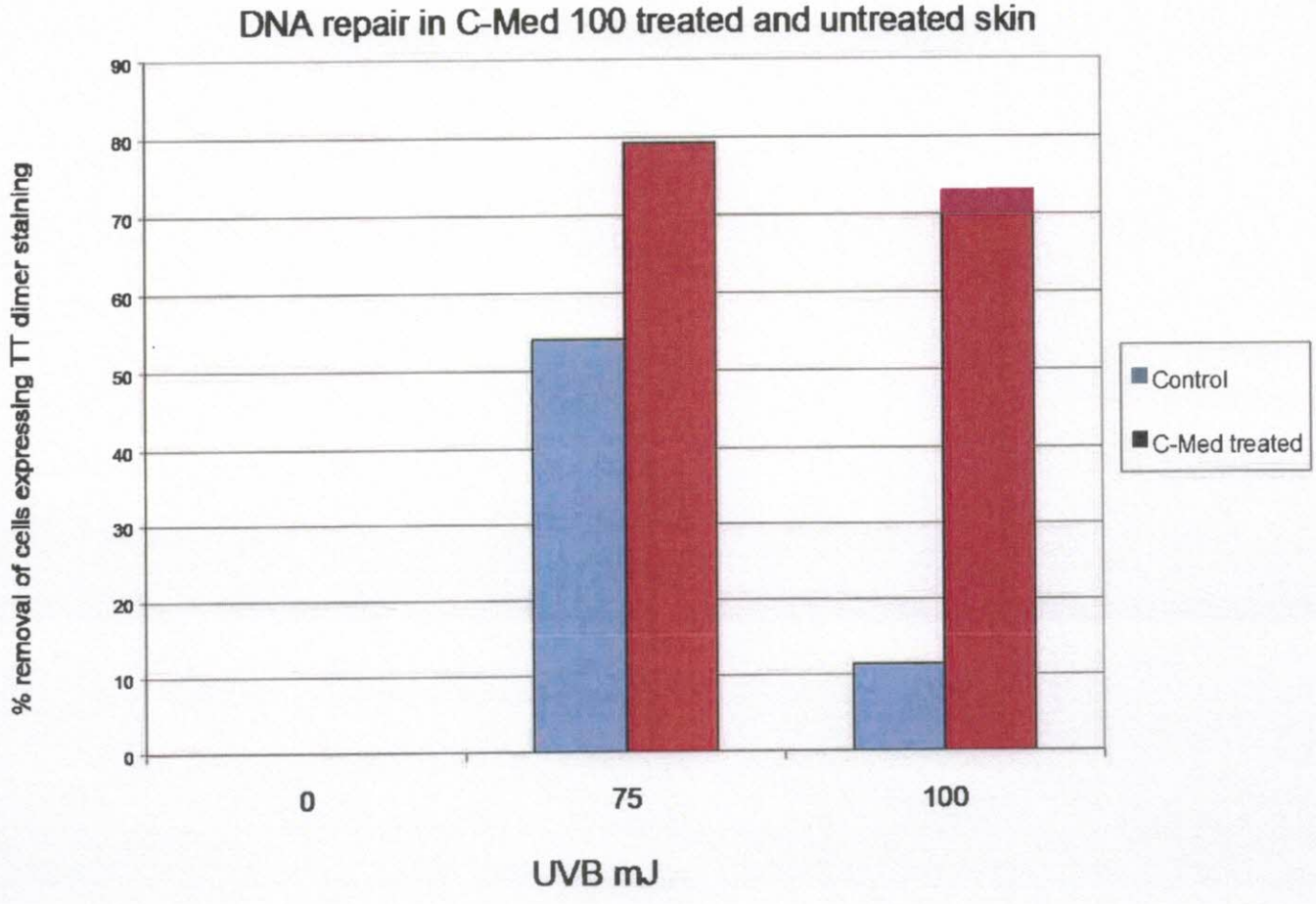
Table 2. % TT dimer expressing cells in C-Med 100 treated and untreated samples.

DNA repair (% removal)		0	75	100mJ
	Control	0	54.17	11.3643134
	C-Med treated	0	79.27	73.034579

Table 3. DNA repair expressed as % removal of cells expressing TT dimer staining.



Graph 1. TT dimer levels at 0 hr and 24 hr



Graph 2. DNA repair in C-Med100 treated and untreated skin

PRODUCT	C-med 100		
COMPANY	AFN for Optigenex Inc.		
BRD #	3362		
DATE	02/24/2003		
INVESTIGATOR	Denise Dicanio (MB Research)		
TEST	In-vitro Ocular (IVM 2852)	TEST 2	
DATE REQUESTED 1	02/24/2003	DATE REQUESTED 2	
DATE COMPLETED 1	02/24/2003	DATE COMPLETED 2	
Memo 1	\\RD_BRD\	Memo 2	
WHY			
RESULTS	passed, score is low, 2/24/03		

PRODUCT	C-MED 100		
COMPANY	AFN for Optigenex Inc.		
BRD #	3362		
DATE	10/31/2002		
INVESTIGATOR	Marie Randazzo (CPT)		
TEST	RIPT	TEST 2	
DATE REQUESTED 1	10/31/2002	DATE REQUESTED 2	
DATE COMPLETED 1	01/06/2003	DATE COMPLETED 2	
Memo 1		Memo 2	
WHY	2% in 25% BG		
RESULTS	no Indication of irritation and sensitization at 2% (verbal ok received from Marie, MT# 2085609), 1/6/03		

Conclusion

- C-MED was moderately effective in whitening skin tan, having whitening factors of 1.85.

Summary

Test material	Whitening Factor
KM4512/1 0.5% C-MED	1.85
CS-2003-04G (CS-2000-08B) Internal standard KM3211/2,	1.70

Introduction

Clinical study CS-02-03 was designed to study lightening effect of various materials using the fading of UV-B induced tan as a marker. The study was conducted at Consumer Product Testing Inc., Fairfield, N.J. The following materials were tested:

CS-2003-04C: KM4512/1 0.5% C-MED consisted of Carboxyl Alkyl esters extracted from a Peruvian Plant for DNA repair and Sunburn cell reduction
CS-2003-04G (CS-2000-08B) Internal standard KM3211/2,

Procedure

Ten female volunteers age 18-45 skin type I-II (Fitzpatrick, 1986) who were interested in taking part in this study were recruited from a local population. In order to qualify, the panelists had to be in normal health with no evidence of acute or chronic disease including dermatologic problems. The subjects expressed willingness to cooperate with the investigator and demonstrated the ability to understand the purpose of the study and the risks associated with participation. They signed an informed consent form prior to initiation.

Subjects exhibiting current sunburn, rashes, scratches, burn marks etc., which might interfere with evaluation of test results were excluded from the study. Pregnant or lactating females were also excluded. On examination the test site was devoid of excessive warts nevi, moles, sunburn. suntan, scars and active dermal lesions. The subjects were not using systemic or topical retinoids, antihistamines or similar agents during the course of the study and two weeks prior to commencement.

The source of radiation was a Xenon Arc Solar Simulator (150 Watt) with filters (mm UG-5) to allow UV-B and UV-A. Distinct areas corresponding to the test materials and an additional untreated irradiated, (approximately 4 cm^2) were marked on the backs of the panelists. The panelists received **twice** MED of UV-B on each site. Tanning was observed every 3-4 days at which point baseline color measurements were obtained with the Minolta Chromameter.

The test materials were applied on their respective sites at the rate of 2 mg/cm^2 after the chromameter measurements and allowed to dry for 10 minutes. Product treatment was continued once a day for 7-10 days. Chromameter readings were obtained at alternate days for 15 after irradiation.

Reflectance values for all the time points were observed and area under the curve for each test site was calculated. Whitening Factor was calculated as the area under the curve of treated site subtracted from the untreated site.

Results

Kojic acid; the internal standard exhibited an area under the curve differential (whitening factor) of 1.7 which is within the range observed in previous studies. C-MED was moderately effective in whitening skin tan, having ehitening factors of 1.85.