CHAPTER IV

RESULTS

1. Preparation of green tea extracts

Extraction was carried out with 10 g of ground, dried green tea leaves using a 100 mL portion of different extracting solvents (distilled water, citrate buffer pH 3, 3.5, 4, 4.5).No brown precipitate occurred when the eleventh portion of solvent reacted with ferric chloride solution. The total amount of catechins in the leaves was transferred to the solvent. Caffeine, aromatic compounds and pigment were removed by chloroform extraction. The fifth chloroform extract was colorless and odorless. Catechins were then removed from the green tea aqueous solution by extracting with ethyl acetate. No precipitate formed in the reaction between the tenth ethyl acetate extraction and ferric chloride solution. Catechins-rich ethyl acetate fractions were mixed and freeze dried, light brown solid matters were obtained. The total yield of all extracts was above 20 % (w/w) of dried leaves (Table 4).

Table 4. The total yield of freeze dried extracts using citrate buffer p H 3.0, 3.5, 4.0, 4.5 and distilled water

Extracting solvent	% FD extract / dried leaves (w/w)
Citrate buffer p H 3.0	29.18 ± 2.61
Citrate buffer p H 3.5	29.42 ± 3.10
Citrate buffer p H 4.0	27.85 ± 2.01
Citrate buffer p H 4.5	26.89 ±1.92
Distilled water	23.17± 1.65

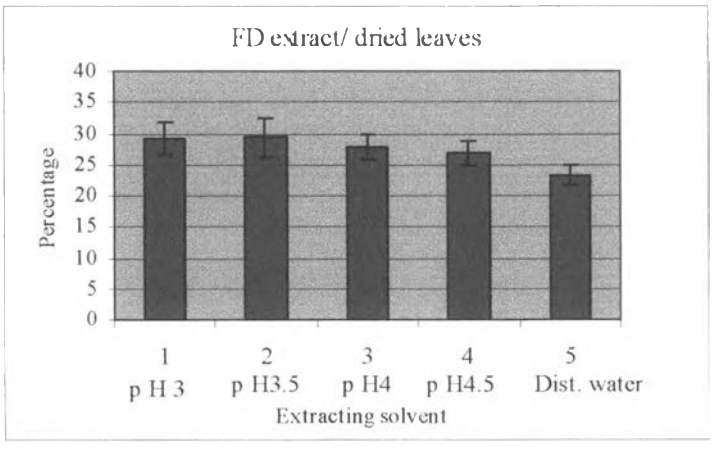

Figure 13. The total yield of freeze dried extracts using citrate buffer p H 3.0, 3.5, 4.0, 4.5 and distilled water

2. Determination of green tea polyphenols (GTP_S) in green tea extracts

2.1 HPLC condition development

Each catechin standard, caffeine standard and sample solution was injected into the reverse phase HPLC column. The chromatogram are shown in Figure 51, 52, 53, 54 and 55. Retention times were in the range of 3.78 min for EGC, 6.1 min for caffeine, 8.24 min for EGCG, 9.86 min for EC and 25.98 min for ECG. No peak of caffeine was found in the green tea extract chromatogram. Resolution and asymmetry were calculated by integrating program as shown in Figure 60. Resolution between peak of EGC and EGCG was 1.21, EGCG and EC was 2.64, EC and ECG was 13.30. Asymmetry of peak EGC, EGCG, EC and ECG were 1.21, 1.22,1.29 and 1.28, respectively. The standard curves of EGC, EGCG, EC and ECG were performed by plotting peak areas versus concentration as shown in Figure 56, e 57, 58 and 59 ; R^2 (correlation) of EGC, EGCG, EC and ECG standard curves were 0.9991, 0.9994, 0.9986 and 0.9989, respectively.

The developed HPLC conditions provide resolution between peaks of the interested substances (EGC, caffeine, EGCG, EC and ECG) more than 1.5 and with a tailing factor (as shown by asymmetry) less than 2. Thus according to USP 26 this condition can be concluded to be able to determine the amount of catechins in green tea extracts.

2.2 HPLC Assay

The amount of epicatechin derivatives present in green tea extracts were calculated from the peak area of each substance obtained from the HPLC chromatogram with the substitution in the equation of each standard curve. Calculated data of each epicatechin in dried leaves are summarized in Table 5.

Table 5. Contents of epicatechin derivatives in dried green tea leaves; extracting with citrate buffer p H 3.0, 3.5, 4.0, 4.5 and distilled water

Extracting solvent	% GTPs in the dried leaves (w/w)			
	EGC	EGCG	EC	ECG
Citrate buffer p H 3.0	4.54 ± 0.29	7.69 ± 0.36	0.79 ± 0.1	2.4 ± 0.14
Citrate buffer p H 3.5	4.7 ± 0.25	7.33 ± 0.29	0.82 ± 0.12	2.12 ± 0.13
Citrate buffer p H 4.0	4.1± 0.23	7.43 ± 0.32	0.95 ± 0.09	2.48 ± 0.15
Citrate buffer p H 4.5	4.92 ± 0.26	7.75 ± 0.41	0.76 ± 0.08	2.21 ± 0.11
Distilled water	7.86 ± 0.41	2.08 ± 0.16	1.49 ± 0.12	0.28 ± 0.05

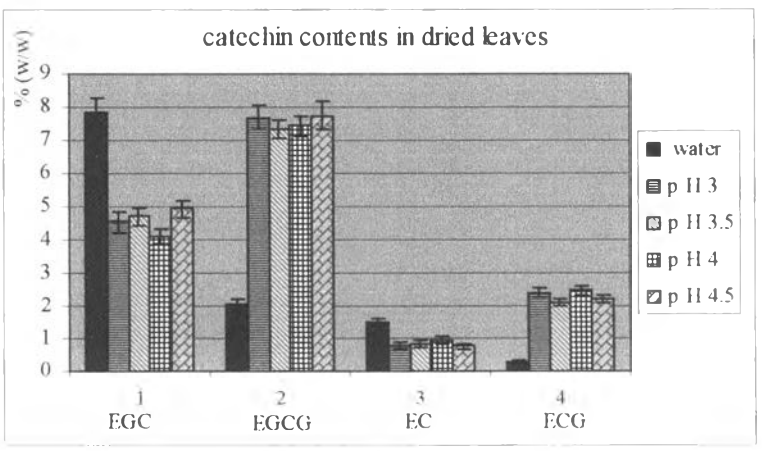

Figure 14. Catechin contents (EGC, EGCG, EC and ECG) in the dried leaves; extracting with citrate buffer p H 3.0, 3.5, 4.0, 4.5 and distilled water

There were no significant differences in the percentage yield of EGC, EGCG, EC and ECG obtained from extraction with citrate buffer p H 3.0, 3.5, 4.0 and 4.5 (p= 0.05). However, a significant difference (p= 0.05) was observed when the extraction with distilled water is compared to the other methods. For further studies, Green tea extract from citrate buffer pH 4.5 extraction was chosen because it gave high yields of EGCG and EGC (Table 5) and was close to skin pH.

3. Analytical Method Validation

Each reference standard and sample solution was injected through the HPLC system. The retention time of EGC, EGCG, EC and ECG from standard solutions (Figure 51, 53, 54 and 55) were 3.78, 8.24, 9.86 and 25.98 min, respectively, and from sample solutions (Figure 60) were 3.78, 8.25, 9.88 and 25.01 min, respectively. From these results, it can be seen that the peaks of the catechins did not overlap, hence the developed method is specific for analyzing catechins in this study.

Accuracy of the method was studied at five different concentrations of sample solution. It was found that % recovery of each catechin (EGC, EGCG, EC and ECG) at five concentrations (Table 16, 17, 18 and 19) was between 98.73-101.52 % and % relative standard deviation (RSD) was between 0.20-0.87.

Precision of the method was studied in six replicates of the same concentration of sample solution. The results in Table 20, 21, 22 and 23 show that % recovery of EGC, EGCG, EC and ECG was between 98.73-101.44 % and % RSD was between 0.69-1.05 %.

The linearity of the method was studied at 5 different concentrations of sample solutions. The results are shown in Table 24, Table 25, Table 26 and Table 27. It can be seen that the graph of each catechin (Figure 61, 62, 63 and 64) obtained by plotting the observed concentration versus the actual concentration is a straight line. The R^2 for EGC, EGCG, EC and ECG was 0.999,0.998,0.999 and 0.996, respectively.

4. Preparation of monolayer cell cultures.

4.1 Preparation of melanocyte / keratinocyte co-cultures

Epidermal cells were isolated from normal human foreskins. The cells were cultured in melanocyte / keratinocyte medium, 5 days later the cells were attached to the dish surface and started to grow (Figure 15). After 12 days keratinocyte colonies with melanocyte attachment were obtained (Figure 16). Around 20 days the cells were confluent (Figure 17) and ready to use for the preparation of melanocyte cell cultures or melanin inhibition assays.

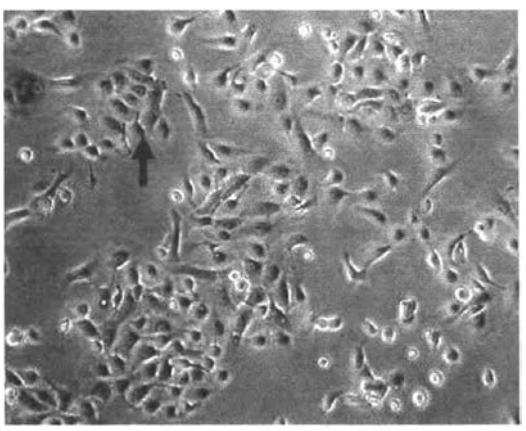

Figure 15. Keratinocytes and melanocytes at 5 days of culture

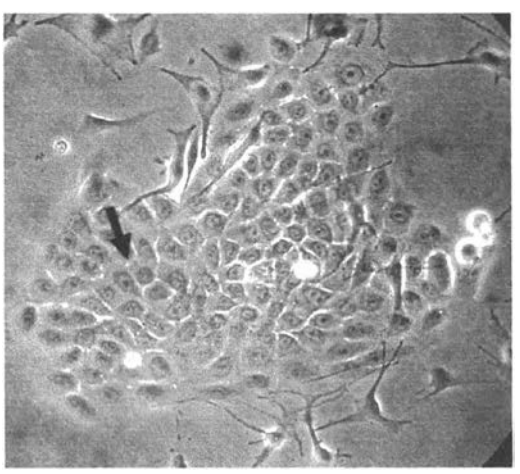

Figure 16. Melanocyte / keratinocyte coculture at 12 days of culture

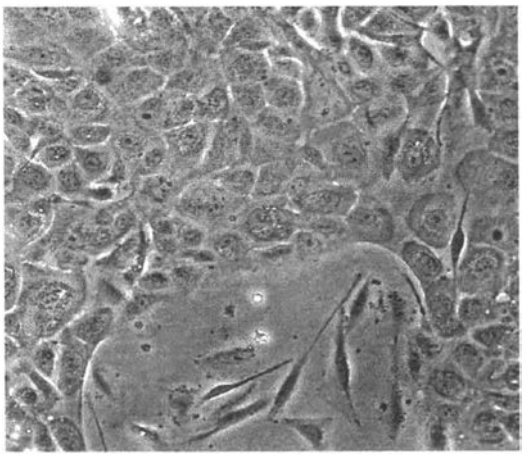

Figure 17. The confluent melanocyte / keratinocyte co-culture at 20 days of culture

4.2 Preparation of Normal Human Foreskin Keratinocyte (NHFK) cell cultures

Normal Human Epidermal cells were cultured in keratinocyte growth medium. After 4 days keratinocytes with some melanocytes attached to the dish surface and started to grow (Figure 18). After 14 days the cultures were ready to subculture into selective medium to eliminate the melanocytes from keratinocytes (Figure 19). About 10 days after subculture keratinocytes were confluent and ready to use for cytotoxicity assays or UV protection activity studies (Figure 20)

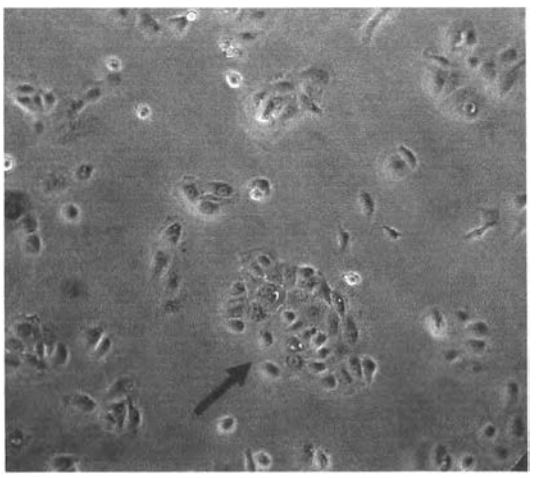

Figure 18. Keratinocytes with some melanocytes at 4 days of culture

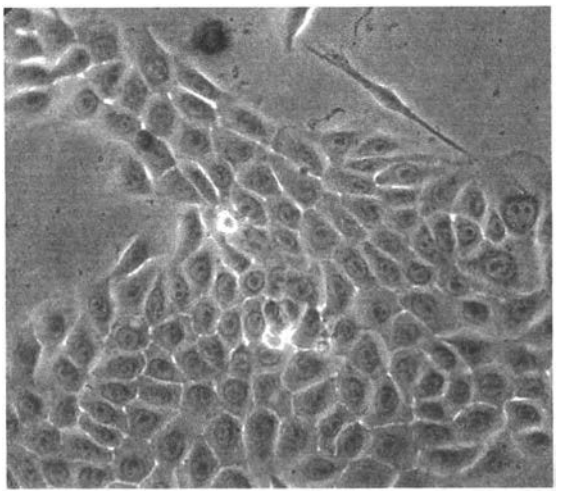

Figure 19 The keratinocyte/melanocyte co-culture at 14 days of culture ready to subculture.

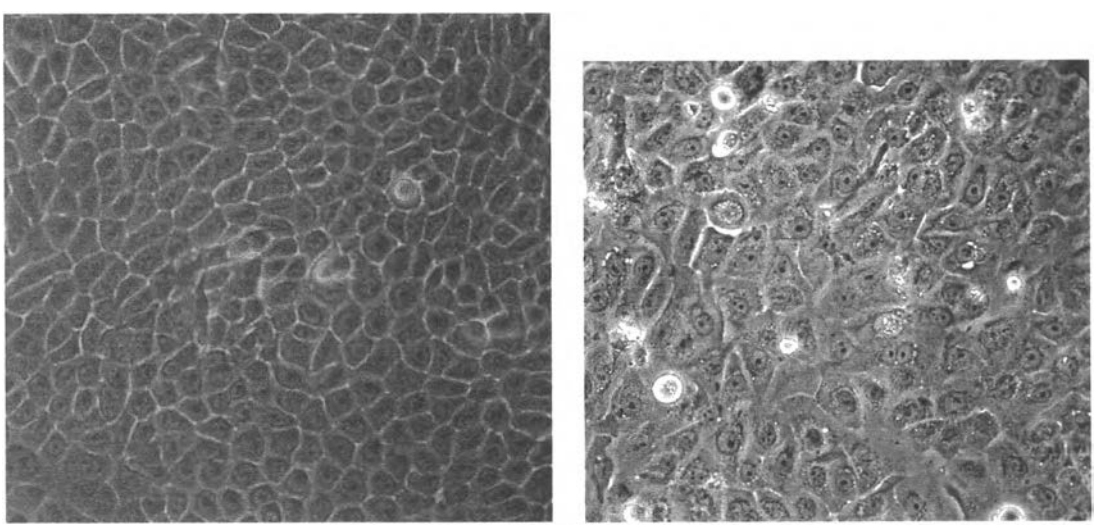

Figure 20. The confluent keratinocytes

4.3 Preparation of Normal Human Foreskin Melanocyte (NHFM) cell cultures

Melanocytes were separated from the co-culture and cultured in melanocyte growth medium. Five days later they proliferated with dendrites (Figure 21). After 10 days they were about 70% confluent with less dendrites (Figure 22) and ready to use for cytotoxicity.

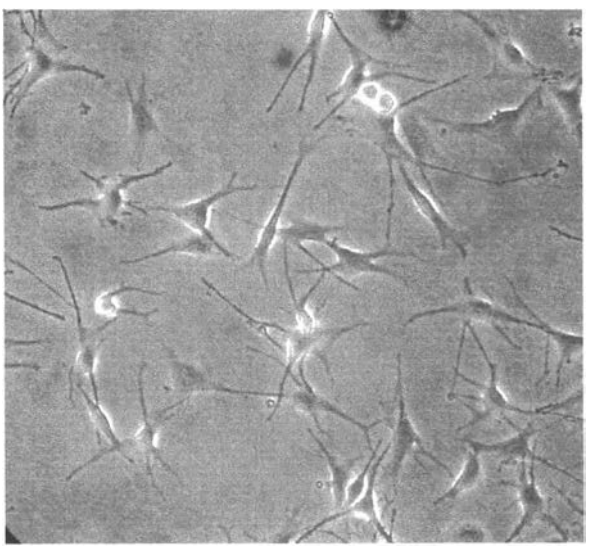

Figure 21. Melanocytes 5 days of culture

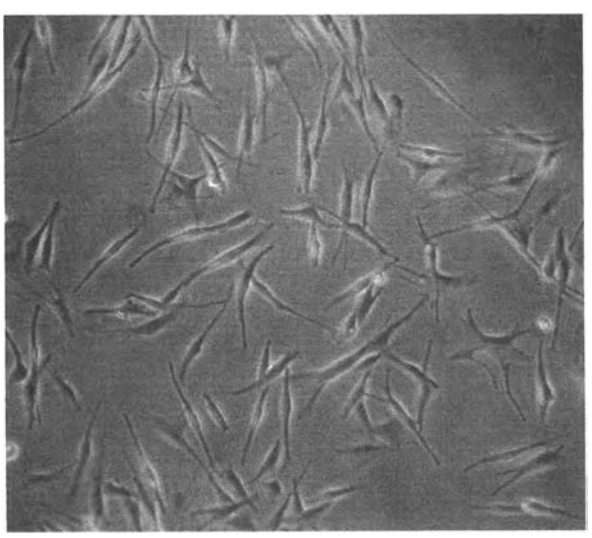

Figure 22. Melanocytes 10 days of culture

4.4 Preparation of Normal Human Foreskin Fibroblast (NHFF) cell cultures

Fibroblasts were prepared from dermis of foreskins. After 4 days of cultivation, fibroblasts started growing from the edges of the attached tissue (Figure 23). They proliferated to be about 50% confluent at 10 days and 100 % confluent at 17 days (Figure 24 and 25)

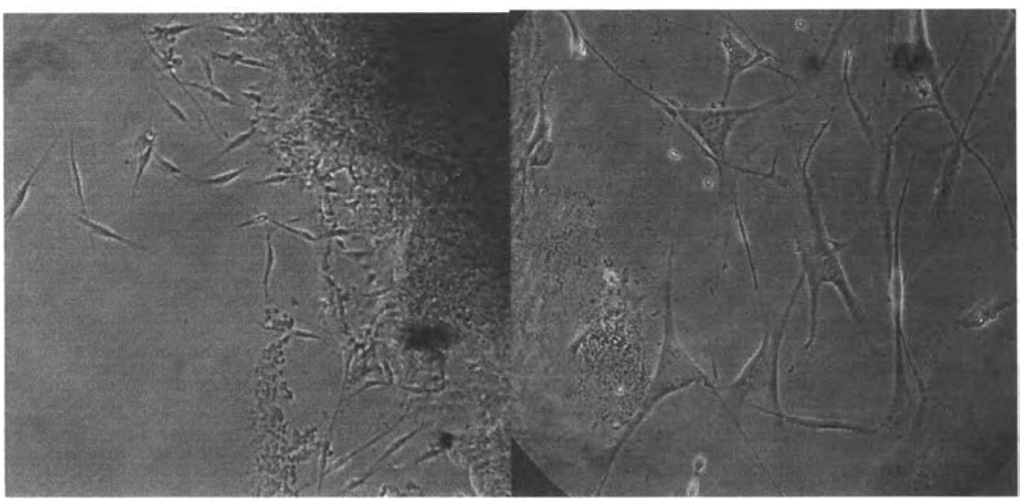

Figure 23. Fibroblasts started growing from the edges of the attached tissue after 4 days of culture.

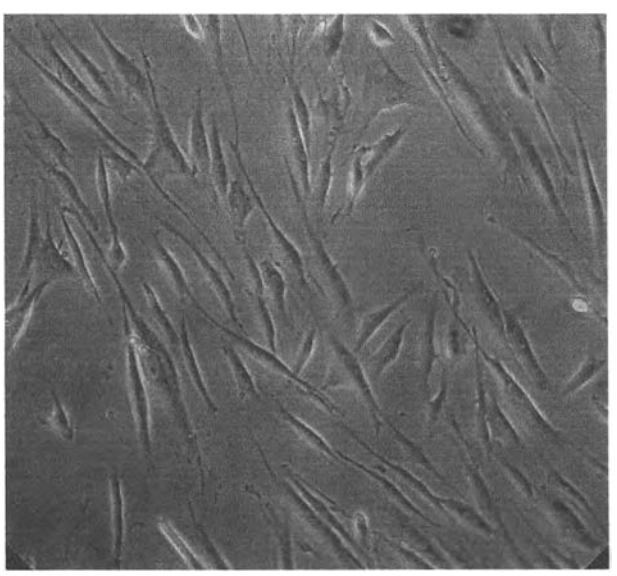

Figure 24. Fibroblasts 10 days of culture.

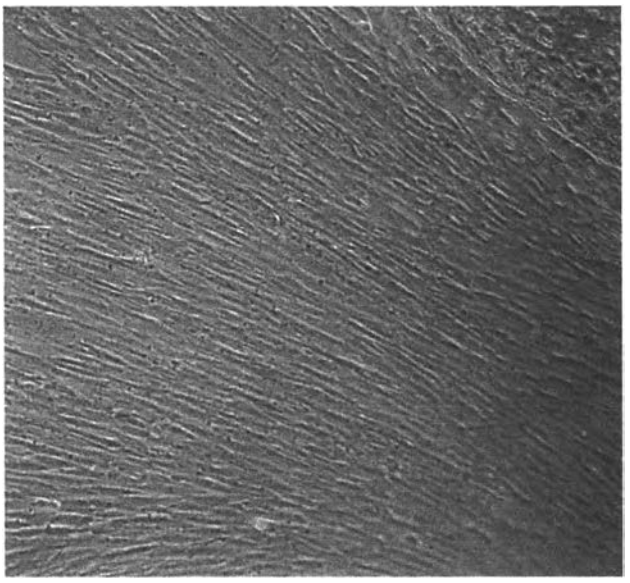

Figure 25. The confluent fibroblasts 17 days of culture.

5. Preparation of Reconstituted 3-dimentional human skin (3D skin)

Rat tail tendon collagen was prepared to the final concentration of 2 mg/ml.

Collagen rafts consisting of NHFF in collagen and Minimum Essential Medium (MEM) were prepared. After 7 days, there was no more contraction of raft (Figure 26) and fibroblasts could be seeded on to the raft (Figure 27). Epidermal cells in FBS were added on the top of the raft and incubated for 1 day. After that the raft with seeded epidermal cells was cultured in keratinocyte growth medium, changing medium every 2 days for 10 days. About 3 days after seeding, the cells proliferated and could be seen on the surface (Figure 28). After 10 days, a monolayer of cells (keratinocytes, melanocytes and fibroblasts) could be seen (Figure 29) then the medium was changed to 1:1 keratinocyte/fibroblast medium. Around 30 days, the 3D skins were formed and ready to use, see histology Figure 30.

Figure 26. Collagen raft

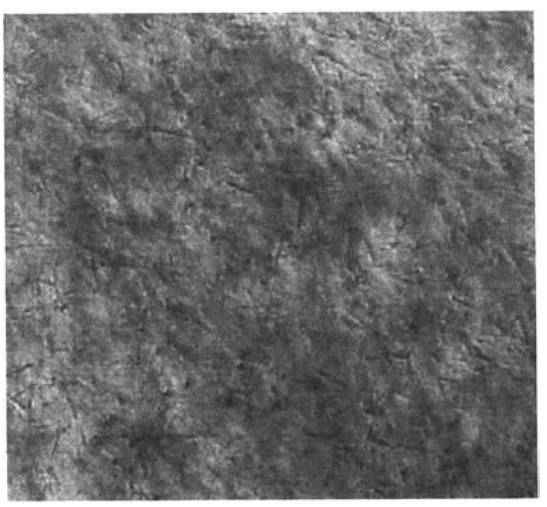

Figure 27. Fibroblasts in collagen raft

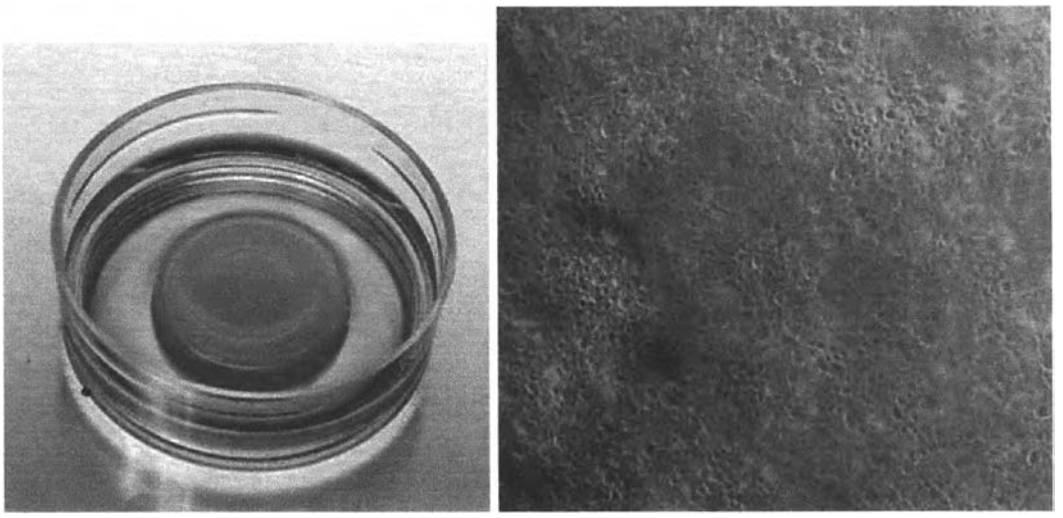

Figure 28. Epidermal cells on collagen raft

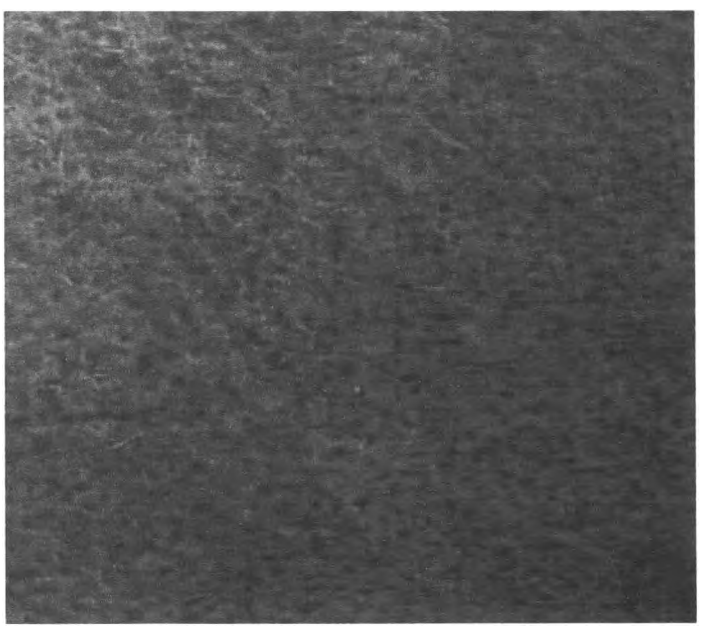

Figure 29. Monolayer of keratinocytes and fibroblasts on collagen raft

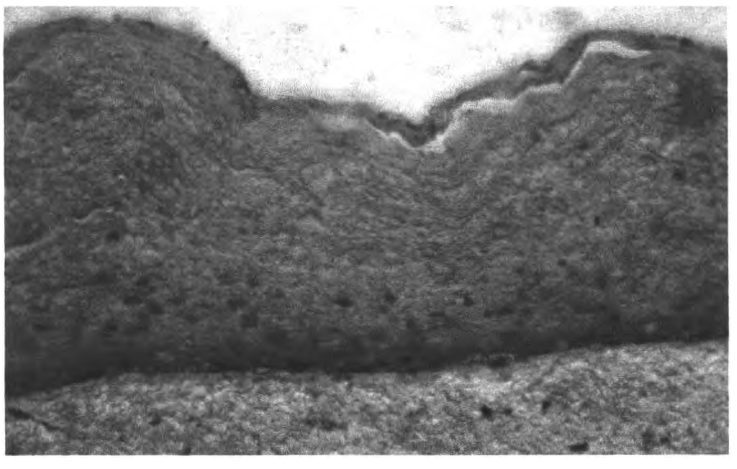

Figure 30. Paraffin cross sections of 3D skin model stained with Haematoxylin and Eosin

6. Cytotoxicity testing for green tea extract and EGCG

To assess the cytotoxicity of green tea extract (GTE pH 4.5) and EGCG *in vitro*, the cells (L929, NHFM, NHFK,and NHFF) were incubated with different concentrations of GTE or EGCG and survival was measured by MTT assay as described in methods.

The experimental results are summarized in Tables 6 and Tables 7. The dose response curve of GTE and EGCG with the calculated % survival of each cell type against concentrations were plotted and shown in Figure 31 and Figure 32.

Table 6. Percent survival data of EGCG on L 929, NHFM, NHFK and NHFF by MTT assay (the values are the means ± SD of 12 measurements).

Concentration	% Survival			
of EGCG (µg/ml)	L 929	NHFM	NHFK	NHFF
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
10	90.42 ± 3.96	106.98 ± 4.87	_	93.20 ± 1.16
20	91.61 ± 0.57	104.59 ± 4.63	97.06 ± 23.76	94.23 ± 1.98
30	98.16 ± 4.95	107.11 ± 7.68	143.63 ± 11.26	86.95 ± 0.89
40	91.69 ± 4.44	111.36 ±5.48	169.30 ± 4.49	88.56 ± 2.85
50	84.63 ± 0.77	115.97± 10.21	192.89 ± 6.17	90.01 ± 0.18
60	_	104.89 ± 7.31	221.33 ± 17.73	_
70	87.66 ± 2.49	98.60 ± 7.82	241.16 ± 4.69	81.43 ± 1.46
80	_	65.42 ± 6.19	241.13 ± 3.55	_
90	80.39 ± 11.64	_	_	66.52 ± 0.21

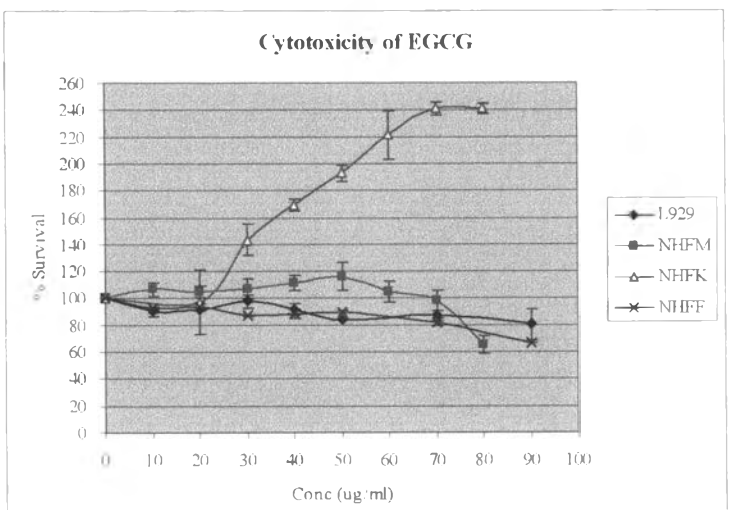

Figure 31 The dose-response curve from the cytotoxicity studies of EGCG on L929, NHFM, NHFK and NHFF by MTT assay

Below 60 µg/ml of EGCG the percent survival of L929, NHFM and NHFF was above 80%.. In contrast the percent survival of NHFK exposed to EGCG was found to increase dramatically with increased in concentrations from 20 µg/mL to 70 µg/mL.

Table 7. Percent survival data of GTE on L929, NHFM, NHFK and NHFF by MTT assay (the values are the means ± SD of 12 measurements)

Concentration	% Survival			
of GTE (µg/ml)	L 929	NHFM	NHFK	NHFF
0	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
10	103.69 ± 0.17	_	_	_
20	104.61± 1.56	_	_	82.06 ± 5.35
30	101.58 ± 1.46	102.84 ± 2.81	140.03 ± 14.02	77.29 ± 11.46
40	97.75 ± 5.32	_	_	_
50	95.23 ± 4.18	_	_	_
60	_	103.47 ± 6.25	145.22 ± 17.77	90.53 ± 3.22
70	79.77 ± 3.66	_	_	86.70 ± 3.76
90	74.41 ± 1.93	110.92 ± 2.86	199.35 ± 14.85	87.31 ± 7.39
120	_	90.57 ± 6.05	191.82 ± 16.15	84.82 ± 0.46
150	_	43.09 ± 1.19	215.95 ± 16.55	73.54 ± 2.62
200	13.48 ± 0.64	27.12 ± 2.36	250.87 ± 10.05	_

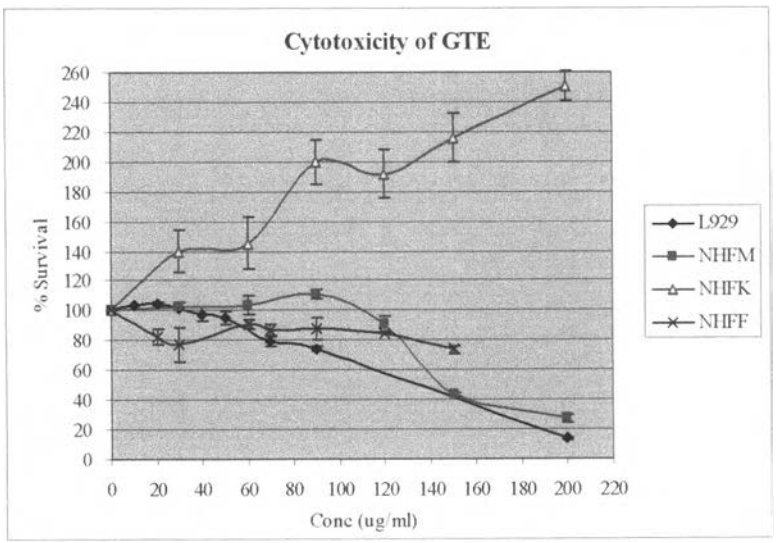

Figure 32. The dose-response curve from the cytotoxicity studies of GTE on L 929, NHFM, NHFK and NHFF by MTT assay

The GTE affects the cells in similar to EGCG. Below 90 µg/ml concentration of GTE the percent survival on L929, NHFM and NHFF cells tend to be constant with no remarkable change. While the effect of GTE on percent survival of NHFK was found to increase with increased in concentrations up to 250 mg/ml.

For these studies 80% survival of cells was accepted to indicate no effect. Thus, concentrations of GTE below 90 μg/ml and of EGCG below 60 μg/ml should be safe. In further studies, 60 μg/ml of GTE and 30 μg/ml of EGCG concentrations were selected to be used as test samples.

7. Inhibition of melanin synthesis studies in melanocyte / keratinocyte co-cultures

The test samples were applied to melanocyte/keratinocyte co-culture, after 2 days the medium was replaced with fresh test sample and cultured for another 2 days. The cells were evaluated for melanin content by Dopa reaction and OD measurement.

7.1 Dopa reaction

The inhibition of melanin synthesis as depicted by the L-dopa assay is shown in the Figure 33, 34 and 35. By visual comparison of the photos, it was found that the color of cells treated with kojic acid were lighter than untreated cells. While the color of cells treated with green tea extract were lighter than untreated cells but darker than the cells treated with kojic acid.

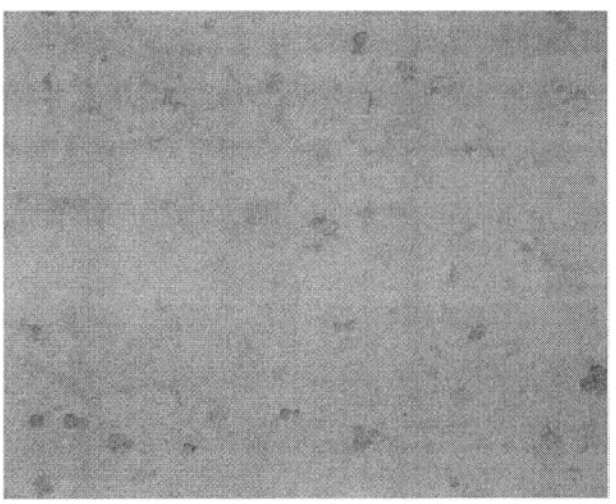

Figure 33. The photograph of untreated (control) melanocyte/keratinocyte co-culture, taken after L-dopa reaction.

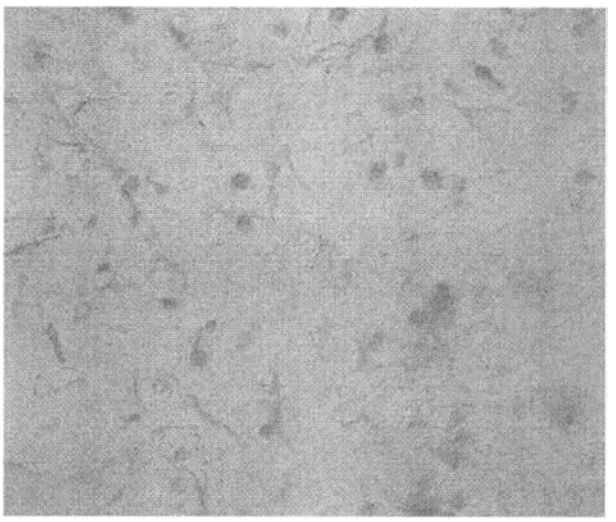

Figure 34. The photograph of melanocyte/keratinocyte co-culture treated with kojic acid, taken after L-dopa reaction.

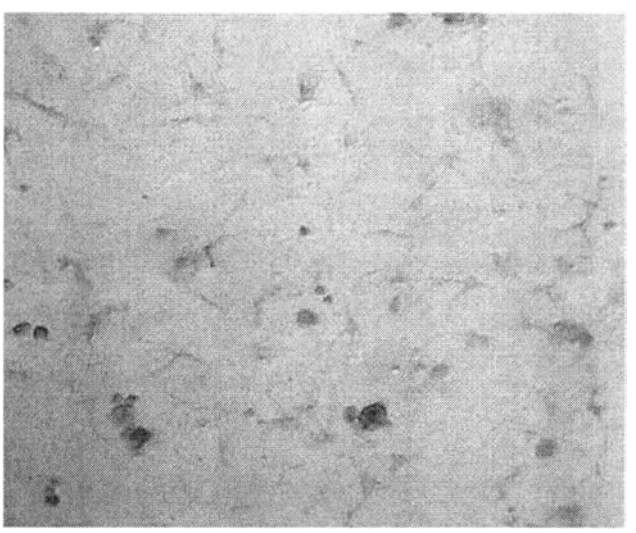

Figure 35. The photograph of melanocyte/keratinocyte co-culture treated with GTP 30 mg/ml, taken after L-dopa reaction.

7.2 Melanin content measurements (Hedle, *et al.*, 1998)

The optimum wave length for measurement of melanin content was determined. Melanin standard solutions were prepared at concentrations from 10- 500

μg/ml and the optical density (OD) read at 405, 450 and 475 nm. The OD data are summarized in Table 29. It was found that at 405 nm, the optical density of every concentration was highest (Figure 65). Therefore the calibration curve at 405 nm (Figure 66) was used to analyze melanin content in this study..

The melanin content of melanocyte/keratinocyte co-cultures after exposure to kojic acid or GTE were determined by weight and as a percentage of the untreated control cultures. The results by weight are shown in Table 8 and Figure 36, and as a percentage in Table 9 and Figure 37. The data show the melanin content in the culture treated with kojic acid was 33% less than untreated cells. While melanin content in the culture treated with GTE was 14. 47% less than untreated cells. These results quantify the preliminary results observed visually.

Table 8. Analysis data of melanin content in cell cultures.

Test sample	Melanin content (pg/cell)
Control (untreated)	205.93 ± 34.83
Kojic acid (500 μg/mg)	139.35 ± 18.37
GTE (60 μg/mL)	176.12 ± 19.19

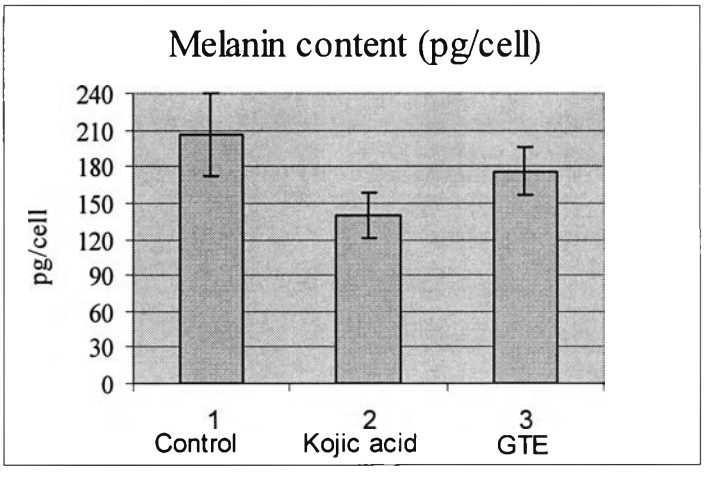

Figure 36. Comparison of melanin content (pg/cell) in cell cultures treated with kojic acid and green tea extract

Table 9. Percentage of melanin in the cultures

Test sample	Melanin content (%)
Control (untreated)	100 ± 16.91
Kojic acid (500 μg/mg)	67 ± 8.92
GTE (60 μg/mL)	85.53 ± 9.32

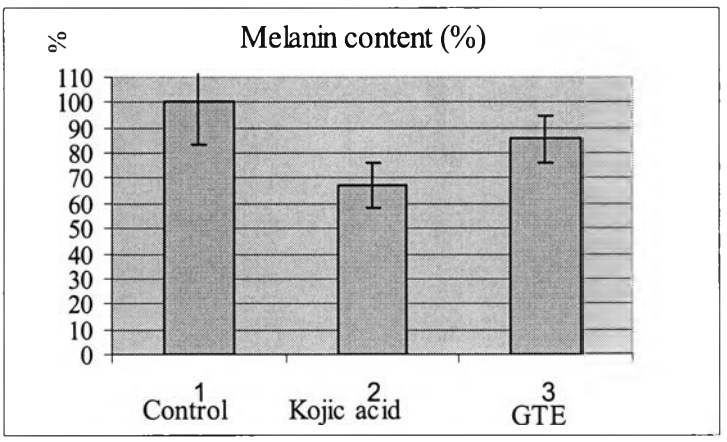

Figure 37. Comparison of melanin content (%) in cell cultures treated with kojic acid and green tea extract

From the graph shown in Figure 33 and Figure 34, melanin content in the culture treated with kojic acid was less than untreated cells. While melanin content in the culture treated with GTE was only slightly less than untreated cells (Figure 35).

The treatment of cells with either GTE or kojic acid reduced the amount of melanin. The GTE while less effective than kojic acid still consistently reduced the amount of melanin in the keratinocyte/melanocyte co-cultures.

8. UV protection studies in keratinocyte cell cultures

Keratinocytes were analysed for cell viability by the MTT assay method following exposure to 400 or 600 mj UVB in the presence of Ectoin, EGCG or GTE. The survival data are summarized in Table 10 and graphically in Figure 37.

Table 10. Percent survival data of keratinocytes exposed to Ectoin, EGCG and GTE prior to UVB exposure

Test sample	% survival		
	NI	400 mj	600 mj
Control	100 ± 3.26	73.34 ± 1.68	48.86 ± 3.07
Ectoin (4mM)	104.77 ± 3.81	101.05 ± 3.32	54.37 ± 4.9
EGCG (30µg/ml)	104.02 ± 2.37	96.32 ± 1.72	50.02 ± 5.37
GTE (60µg/ml)	102.19 ± 1.04	98.15 ± 2.42	46.27 ± 4.46

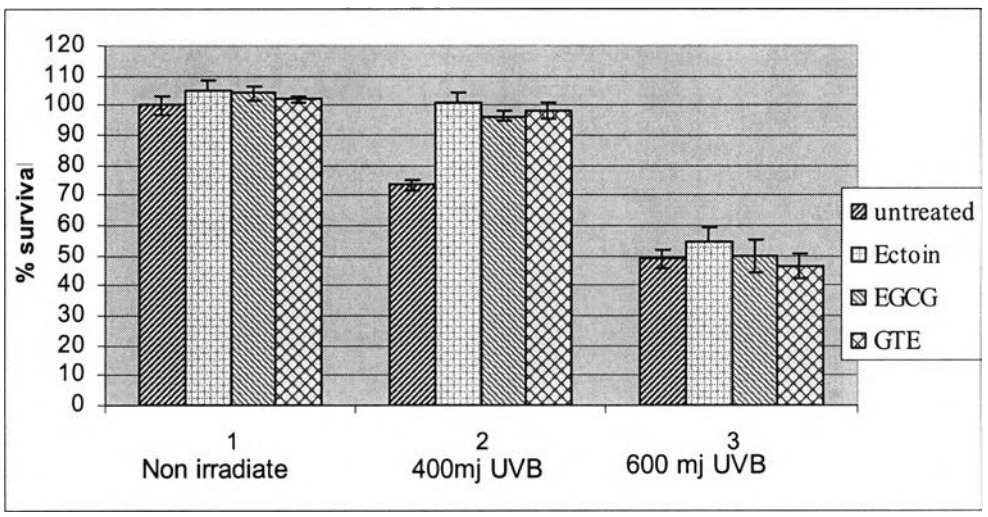

Figure 38. Survival of keratinocytes after UVB irradiation

It was found that in nonirradiated cultures, the percent survival of cells cultured in medium alone, medium with Ectoin, medium with EGCG or medium with GTE were not different. In 400 mj UVB irradiated cultures; the survival of untreated cells was 26.66% less than nonirradiated untreated (control) cells while the survival of treated cells with GTE or EGCG or Ectoin were similar and not different from control cells. In 600 mj UVB irradiation cultures; the survival of untreated cells or treated

cells with GTE or EGCG or Ectoin were similar at around 50% less than control cells. These results indicate that with 400 mj UVB irradiation; GTE, EGCG and Ectoin could protect keratinocytes from harmful effect of UVB. But 600 mj UVB irradiation, was too harmful and none of the test samples could protect the cells. Hence, up to a dose of 400mj, GTE has been shown to protect the keratinocyte/melanocyte co-cultured cells as efficiently as EGCG or Ectoin.

9. UV protection studies on 3D skin model derived from excised breast skin

Due to the lack of newborn foreskin, epidermal cells were prepared from excised human breast skin. These cells were seeded on collagen raft containing fibroblasts just as those from the newborn foreskin. When the skin models derived from excised breast cells were compared with the skin models prepared from newborn foreskin (Figure 30), it was found that there were fewer epidermal cells and those present had an irregular distribution in basal layer. After 4 weeks of culture, the skin model was treated with test samples and irradiated with 400 mj of UVB. The skin models were evaluated for sunburn cells and cell viability. Supernatant samples were assayed for IL 1α.

9.1 Evaluation of sunburn cells

Vertical sections of paraffin embedded skin models were prepared and stained with Haematoxylin /Eosin. The sections were examined under microscope and photos were taken. Living cells were seen as dark nuclei. Sunburn cells could not be identified and counted. UV irradiation damaged the epidermis and raft (Figure 40). Ectoin could protect both epidermis and the raft from UV radiation (Figure 41), while GTE could protect the raft but not epidermis (Figure 42).

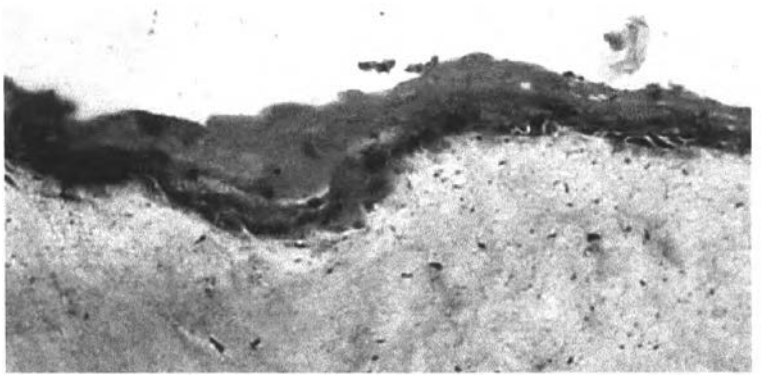

Figure 39. H/E staining of cultured 3D skin model in medium

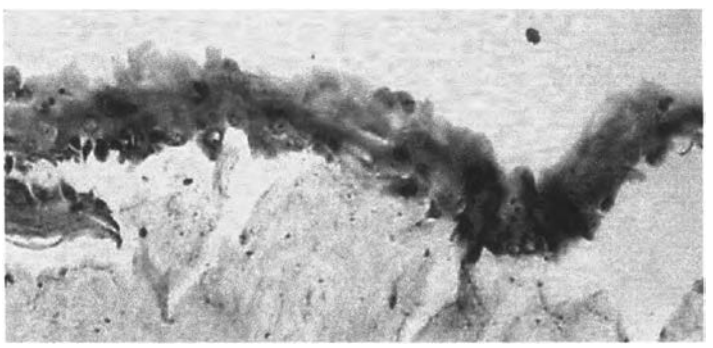

Figure 40. H/E staining of cultured 3D skin model in medium and irradiated with 400 mj UVB

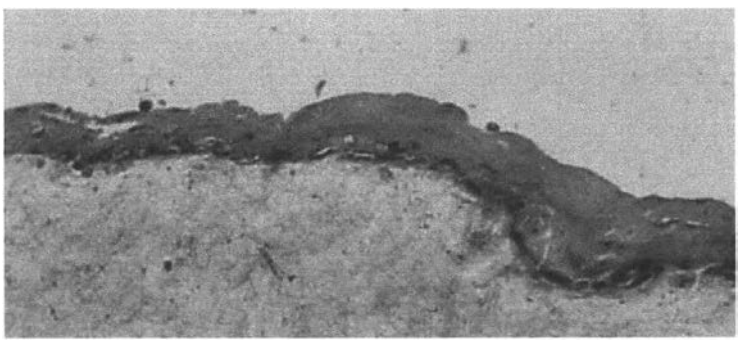

Figure 41. H/E staining of cultured 3D skin model in medium containing Ectoin and irradiated with 400 mj UVB

Figure 42. H/E staining of cultured 3D skin model in medium containing GTE and irradiated with 400 mj UVB

9.2 Analysis of tissue viability

Tissue viability was measured by MTT assay method. The percent survival of cells is reported in Table 11.

Table 11. The percent survival of cells in 3D skin

3 D skin model	% survival	% reduction
Untreated, non irradiated (control)	100.00	0
Untreated, irradiated with 400 mj UVB (UT/UVB)	59.29	40.71
GTE (60 µg/ml), irradiated with 400 mj UVB	94.08	5.92
Ectoin (4mM) , irradiated with 400 mj UVB	97.29	2.71

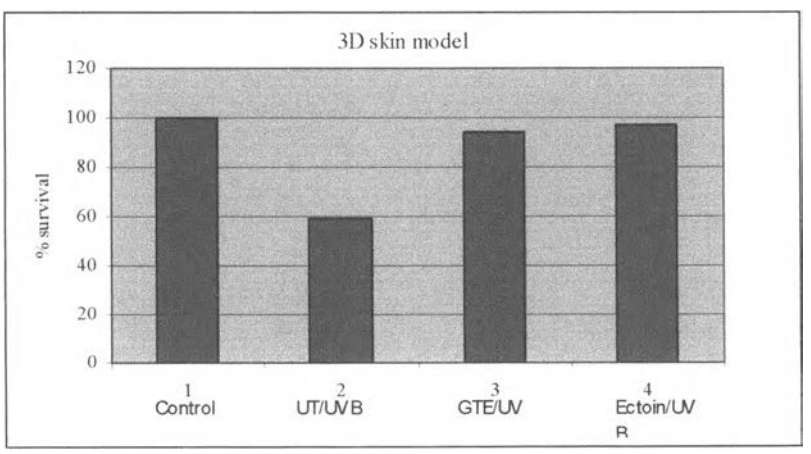

Figure 43. Tissue viability of 3D skin

It was found that 400 mj UVB irradiation reduced the cell viability of untreated skin, treated skin with GTE or Ectoin by 40.71, 5.92 and 2.71% respectively (Figure 43). GTE protected the cells against UVB irradiation as effectively as Ectoin.

9.3 Analysis of the released cytokines after UV irradiation

The amount of cytokine (IL-1α) in the culture medium samples was measured using an ELISA commercial kit. Amount of cytokine (Table 12)was calculated using the prepared standard calibration curve (Figure 67) . There was 13.56 pg/ml of IL 1 α in control skin. After irradiation, the amount of IL 1 α in untreated skin, treated skin with GTE or Ectoin were 15.13, 14.52 and 14.05pg/ml, respectively. These small amounts of IL 1 α might be due to the few cells in the epidermis. The amounts of IL 1 α in all samples were quite similar, no effect could be measured.

Table 12. IL 1α content in 3D skin model

3 D skin model	IL 1a (pg/ml)
Untreated, non irradiated (control)	13.56
Untreated, irradiated with 400 mj UVB (UT/UVB)	15.13
GTE (60 μg/ml), irradiated with 400 mj UVB	14.52
Ectoin (4mM) , irradiated with 400 mj UVB	14.05

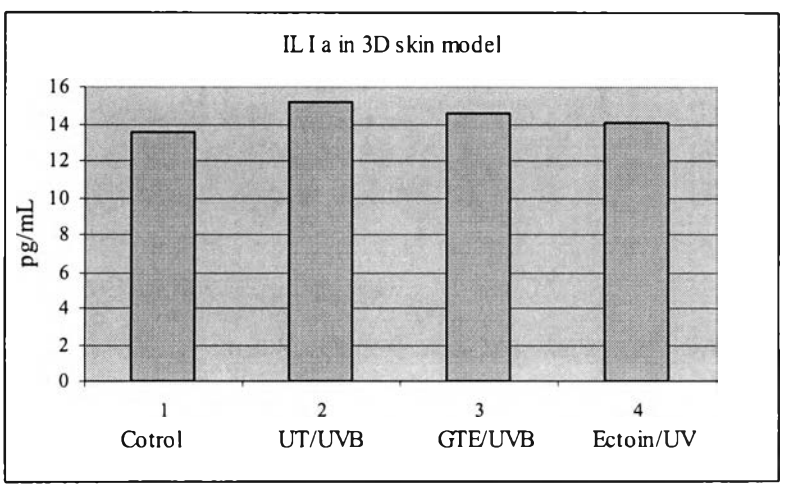

Figure 44. IL 1α content in 3D skin

10. UV protection studies on excised human breast skin

Excised breast human skins (less than 24 h after surgery) were treated with test samples and irradiated with 400 mj of UVB. The skins were evaluated for sunburn cells and cell viability. Supernatant samples were assayed for IL 1α.

10.1 Evaluation of sunburn cells

Vertical sections of paraffin embedded skins were prepared and stained with Haematoxylin /Eosin. The sections were examined microscopically and photos were taken (Figure 45, 46, 47 and 48). Sunburn cells were seen as dark condensed nuclei with vacuole. It was found that GTE and Ectoin applied before UV irradiation reduced the number of sunburn cells by 59 and 65% respectively (Table 13).

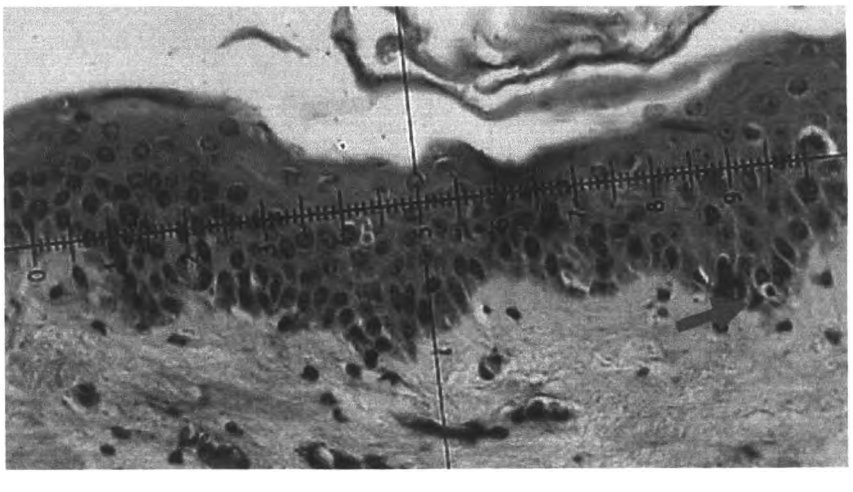

Figure 45. H/E staining of cultured excised human breast skin in medium, Sunburn cells were seen as dark condensed nuclei with vacuole (arrow)

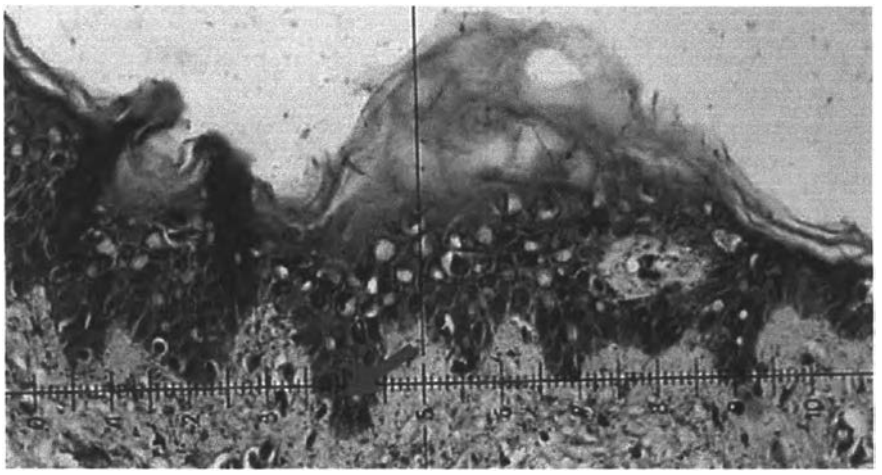

Figure 46. H/E staining of cultured excised human breast skin in medium and irradiated with 400 mj UVB

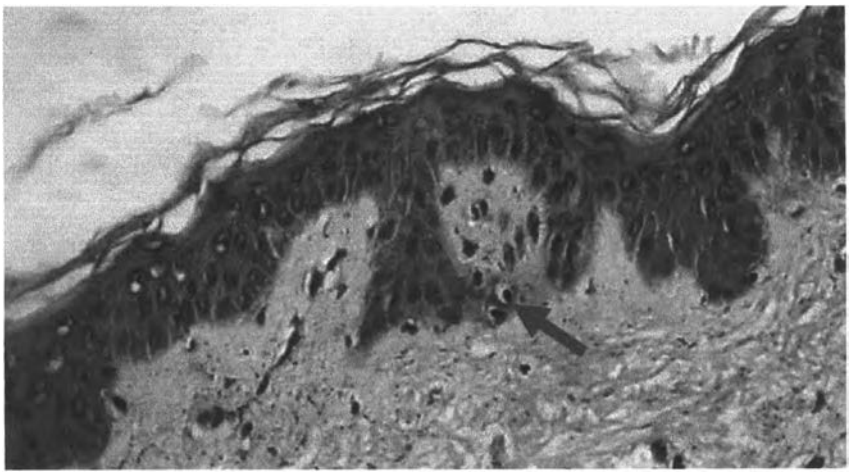

Figure 47. H/E staining of cultured excised human breast skin in medium containing Ectoin and irradiated with 400 mj UVB

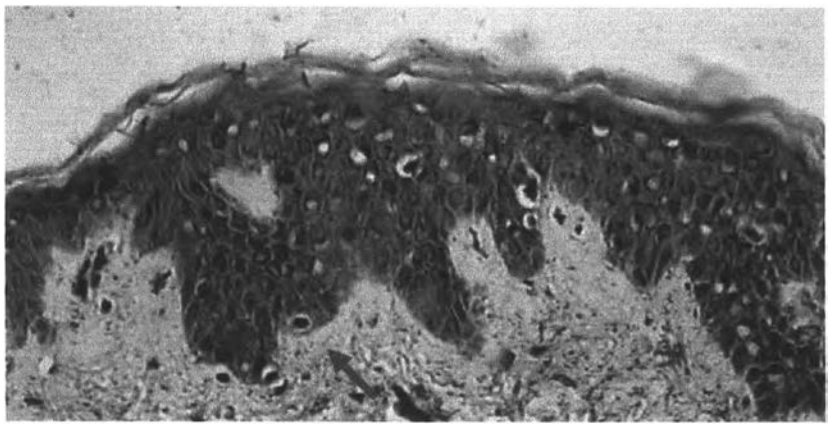

Figure 48. H/E staining of cultured excised human breast skin in medium containing GTE and irradiated with 400 mj UVB

Table 13. Number of sunburn cells in excised human breast skin

Excised human breast skin	Number of sunburn cells (SBC)		% SBC
	per 0.01mm	per mm	
Untreated, non irradiated (control)	3 ± 1	300	0
Untreated, irradiated with 400 mj UVB (UT/UVB)	20 ± 3	2000	100
GTE (60 µg/ml), irradiated with 400 mj UVB	10 ± 2	1000	41
Ectoin (4mM) , irradiated with 400 mj UVB	9 ± 2	900	35

10.2 Analysis of tissue viability

Tissue viability was measured by MTT assay method. The percent survival of cells is reported in Table 14.

Table 14. The percent survival of cells in excised human breast skin

Excised human breast skin	% survival	% reduction
Untreated, non irradiated (control)	100 ± 5.02	0
Untreated, irradiated with 400 mj UVB (UT/UVB)	68.81 ± 11.73	31.19
GTE (60 µg/ml), irradiated with 400 mj UVB	85.24 ± 11.38	14.76
Ectoin (4mM) , irradiated with 400 mj UVB	92.02± 6.49	7.98

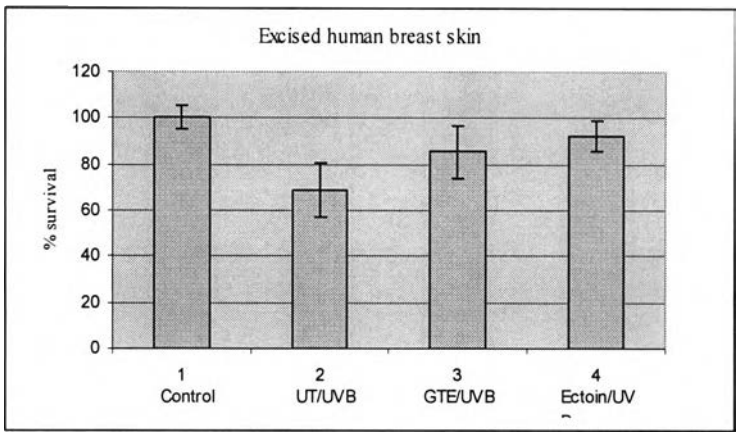

Figure 49. Tissue viability of excised human breast skin

It was found that 400 mj UVB irradiation reduced the cell viability of untreated skin, treated skin with GTE or Ectoin by 31.19, 14.76 and 7.98 %, respectively (Table 14). For these studies, 80% survival of cells was accepted, this means that GTE and Ectoin could prevent toxic effect of UVB at 400mj.

10.3 Analysis of the released cytokines after UV irradiation

The amount of cytokine (IL-1α) in the culture medium samples were measured using ELISA commercial kit. Amount of cytokine was calculated using prepared standard calibration curve and reported in Table 15. It was found that there was 62.13 pg/ml of IL-1 α in control skin which is the original value of this skin. After irradiation, the amounts of IL-1 α in untreated skin, treated skin with GTE or Ectoin were 105.36, 79.28, and 77.04 pg/ml, respectively. GTE reduced the increased production of IL-1α as effectively as Ectoin. The levels of IL-1α with GTE or Ectoin treatment approached those of untreated, unexposed skin.

Table 15. IL 1α content in excised human breast skin

Excised human breast skin	IL 1a (pg/ml)
Untreated, non irradiated (control)	62.13 ± 8.36
Untreated, irradiated with 400 mj UVB (UT/UVB)	105.36 ± 10.14
GTE (60 µg/ml), irradiated with 400 mj UVB	77.04 ± 11.78
Ectoin (4mM) , irradiated with 400 mj UVB	79.28 ± 9.75

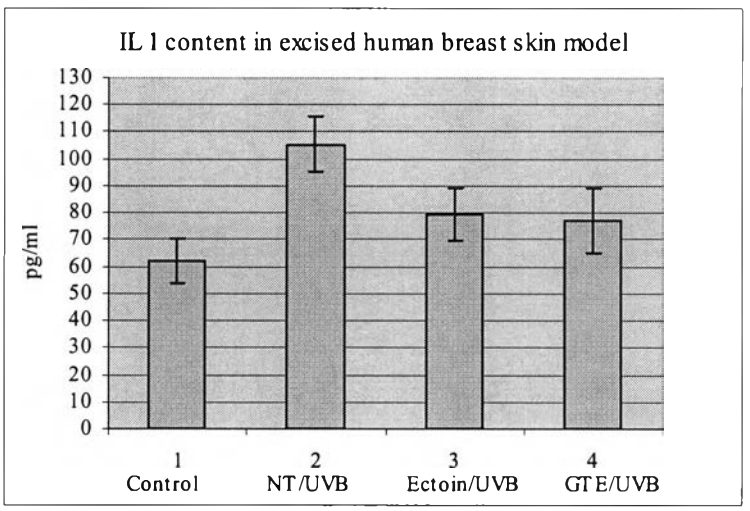

Figure50. IL-1α content in excised human breast skin