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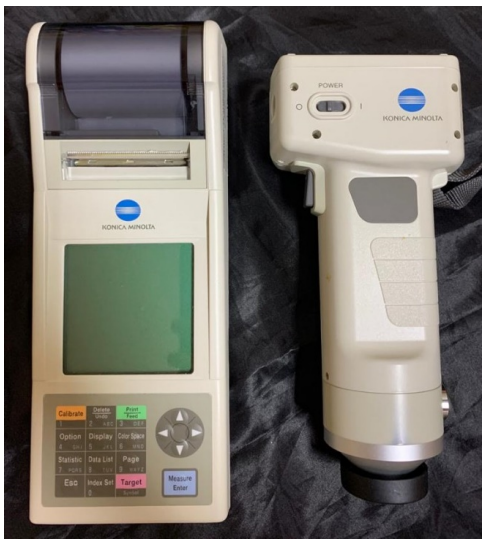
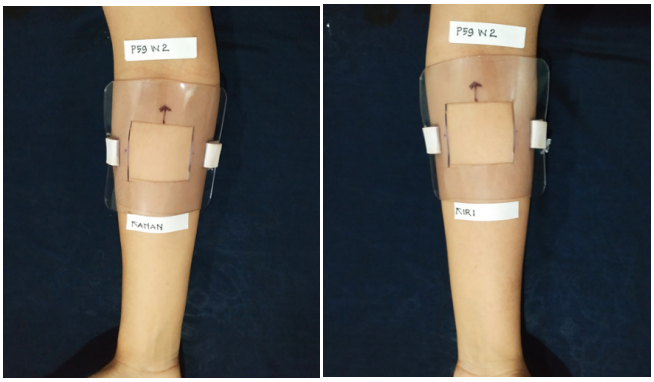
LAMPIRAN

Lampiran 1.Konica Minolta Chroma Meter Model CR-400 ®

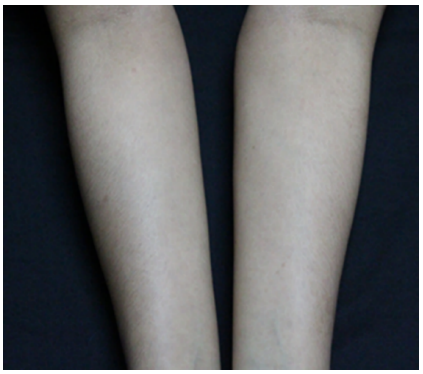


Lampiran 2.Serum yang diujikan dengan kode CT01R dan CT01L

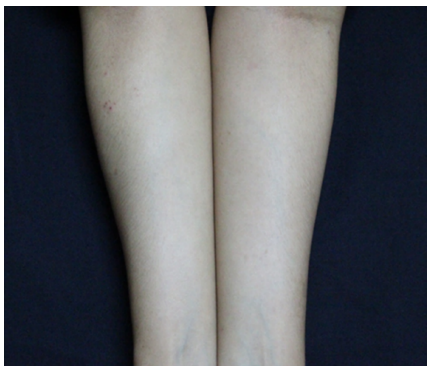
Lampiran 3.Patron khusus ukuran 4x4cm



Lampiran 4.Foto klinis subjek sebelum perlakuan



Lampiran 5.Foto Klinis pasien setelah 12 minggu perlakuan



Lampiran 6. Diskripsi statistik nilai L pada kelompok deoxyarbutin 2%*

Descriptives

			Statistic	Std. Error
Baseline_R	Mean		52,4071	,44411
	95% Confidence Interval for Mean	Lower Bound	51,5181	
		Upper Bound	53,2961	
	5% Trimmed Mean		52,3661	
	Median		52,1300	
	Variance		11,637	
	Std. Deviation		3,41127	
	Minimum		45,39	
	Maximum		59,90	
	Range		14,51	
	Interquartile Range		5,54	
	Skewness		,170	,311
	Kurtosis		-,731	,613
Week2_R	Mean		52,7966	,42515
	95% Confidence Interval for Mean	Lower Bound	51,9456	
		Upper Bound	53,6476	
	5% Trimmed Mean		52,8266	
	Median		53,3600	
	Variance		10,664	
	Std. Deviation		3,26560	
	Minimum		46,30	
	Maximum		59,13	
	Range		12,83	
	Interquartile Range		5,31	
	Skewness		,006	,311
	Kurtosis		-,973	,613
Week4_R	Mean		53,0020	,42870
	95% Confidence Interval for Mean	Lower Bound	52,1439	
		Upper Bound	53,8602	
	5% Trimmed Mean		53,0233	
	Median		53,1600	
	Variance		10,843	
	Std. Deviation		3,29292	
	Minimum		46,36	
	Maximum		59,83	

	Range		13,47	
	Interquartile Range		4,96	
	Skewness		-,078	,311
	Kurtosis		-,584	,613
Week6_R	Mean		53,2454	,43017
	95% Confidence Interval for Mean	Lower Bound	52,3843	
		Upper Bound	54,1065	
	5% Trimmed Mean		53,2606	
	Median		53,4500	
	Variance		10,918	
	Std. Deviation		3,30421	
	Minimum		46,91	
	Maximum		60,04	
	Range		13,13	
	Interquartile Range		5,64	
	Skewness		-,076	,311
	Kurtosis		-,855	,613
Week8_R	Mean		53,4998	,40470
	95% Confidence Interval for Mean	Lower Bound	52,6897	
		Upper Bound	54,3099	
	5% Trimmed Mean		53,5557	
	Median		53,3500	
	Variance		9,663	
	Std. Deviation		3,10852	
	Minimum		47,22	
	Maximum		59,21	
	Range		11,99	
	Interquartile Range		5,02	
	Skewness		-,181	,311
	Kurtosis		-,856	,613
Week10_R	Mean		53,6712	,40406
	95% Confidence Interval for Mean	Lower Bound	52,8624	
		Upper Bound	54,4800	
	5% Trimmed Mean		53,7051	
	Median		53,8000	

	Variance		9,633	
	Std. Deviation		3,10366	
	Minimum		47,27	
	Maximum		59,38	
	Range		12,11	
	Interquartile Range		4,22	
	Skewness		-,161	,311
	Kurtosis		-,681	,613
Week12_R	Mean		54,0832	,40495
	95% Confidence Interval for Mean	Lower Bound	53,2726	
		Upper Bound	54,8938	
	5% Trimmed Mean		54,0957	
	Median		54,3300	
	Variance		9,675	
	Std. Deviation		3,11045	
	Minimum		47,60	
	Maximum		60,67	
	Range		13,07	
	Interquartile Range		4,78	
	Skewness		-,133	,311
	Kurtosis		-,691	,613

Lampiran 7. Uji normalitas nilai L kelompok deoxyarbutin 2%*

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Baseline_R	,075	59	,200*	,982	59	,540
Week2_R	,083	59	,200*	,971	59	,162
Week4_R	,057	59	,200*	,983	59	,563
Week6_R	,088	59	,200*	,975	59	,264
Week8_R	,075	59	,200*	,970	59	,160
Week10_R	,061	59	,200*	,979	59	,398
Week12_R	,063	59	,200*	,984	59	,631

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Lampiran 8. Paired samples test kelompok deoxyarbutin 2%

NO	*PAIRED SAMPLE TEST*	NILAI P
1	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 1 Baseline_R - Week2_R: -,18122 | -3,743 | 58 | ,000	Nilai L* antara baseline dan minggu ke 2→ perubahan signifikan
2	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 2 Baseline_R - Week4_R: -,27392 | -3,710 | 58 | ,000	Nilai L* antara baseline dan minggu ke 4→ perubahan signifikan
3	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 3 Baseline_R - Week6_R: -,53366 | -5,508 | 58 | ,000	Nilai L* antara baseline dan minggu ke 6→ perubahan signifikan
4	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 4 Baseline_R - Week8_R: -,74010 | -6,203 | 58 | ,000	Nilai L* antara baseline dan minggu ke 8→ perubahan signifikan
5	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 5 Baseline_R - Week10_R: -,90084 | -6,966 | 58 | ,000	Nilai L* antara baseline dan minggu ke 10→ perubahan signifikan
6	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 6 Baseline_R - Week12_R: -1,32022 | -9,428 | 58 | ,000	Nilai L* antara baseline dan minggu ke 12→ perubahan signifikan

Lampiran 9. Diskripsi statistik nilai L kelompok hydroquinone 4%*

Descriptives

			Statistic	Std. Error
Baseline_L	Mean		52,5790	,45968
	95% Confidence Interval for Mean	Lower Bound	51,6588	
		Upper Bound	53,4991	
	5% Trimmed Mean		52,5639	
	Median		52,6900	
	Variance		12,467	
	Std. Deviation		3,53086	
	Minimum		46,19	
	Maximum		59,94	
	Range		13,75	
	Interquartile Range		6,17	
	Skewness		,058	,311
	Kurtosis		-,843	,613
Week2_L	Mean		52,8537	,44538
	95% Confidence Interval for Mean	Lower Bound	51,9622	
		Upper Bound	53,7452	
	5% Trimmed Mean		52,8231	
	Median		53,1300	
	Variance		11,703	
	Std. Deviation		3,42100	
	Minimum		46,64	
	Maximum		59,78	
	Range		13,14	
	Interquartile Range		6,11	
	Skewness		,123	,311
	Kurtosis		-,921	,613
Week4_L	Mean		53,0600	,43415
	95% Confidence Interval for Mean	Lower Bound	52,1909	
		Upper Bound	53,9291	
	5% Trimmed Mean		53,0616	
	Median		53,3100	
	Variance		11,121	
	Std. Deviation		3,33479	
	Minimum		46,29	
	Maximum		59,93	

	Range		13,64	
	Interquartile Range		5,03	
	Skewness		,030	,311
	Kurtosis		-,926	,613
Week6_L	Mean		53,2502	,44362
	95% Confidence Interval for Mean	Lower Bound	52,3622	
		Upper Bound	54,1382	
	5% Trimmed Mean		53,2737	
	Median		53,6900	
	Variance		11,611	
	Std. Deviation		3,40749	
	Minimum		46,52	
	Maximum		59,52	
	Range		13,00	
	Interquartile Range		5,42	
	Skewness		-,077	,311
	Kurtosis		-,930	,613
Week8_L	Mean		53,4095	,43668
	95% Confidence Interval for Mean	Lower Bound	52,5354	
		Upper Bound	54,2836	
	5% Trimmed Mean		53,4157	
	Median		53,6500	
	Variance		11,251	
	Std. Deviation		3,35419	
	Minimum		46,42	
	Maximum		59,91	
	Range		13,49	
	Interquartile Range		5,77	
	Skewness		,004	,311
	Kurtosis		-,895	,613
Week10_L	Mean		53,4492	,44009
	95% Confidence Interval for Mean	Lower Bound	52,5682	
		Upper Bound	54,3301	
	5% Trimmed Mean		53,4946	
	Median		53,7800	

	Variance		11,427	
	Std. Deviation		3,38036	
	Minimum		46,64	
	Maximum		60,03	
	Range		13,39	
	Interquartile Range		5,54	
	Skewness		-,115	,311
	Kurtosis		-,948	,613
Week12_L	Mean		54,0807	,44219
	95% Confidence Interval for Mean	Lower Bound	53,1955	
		Upper Bound	54,9658	
	5% Trimmed Mean		54,0847	
	Median		54,3400	
	Variance		11,537	
	Std. Deviation		3,39655	
	Minimum		47,28	
	Maximum		60,34	
	Range		13,06	
	Interquartile Range		5,75	
	Skewness		-,057	,311
	Kurtosis		-,897	,613

Lampiran 10. Uji normalitas nilai L kelompok hydroquinone 4%*

Tests of Normality

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Baseline_L	,068	59	,200*	,977	59	,320
Week2_L	,087	59	,200*	,971	59	,166
Week4_L	,101	59	,200*	,974	59	,232
Week6_L	,096	59	,200*	,973	59	,220
Week8_L	,092	59	,200*	,976	59	,304
Week10_L	,080	59	,200*	,969	59	,144
Week12_L	,090	59	,200*	,971	59	,170

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Lampiran 11. Paired samples test kelompok hydroquinone 4%

NO	NILAI P	KELOMPOK
1	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 1 Baseline_L - Week2_L: -,04977 | -2,445 | 58 | ,018	Nilai L* antara baseline dan minggu ke 2→ perubahan signifikan
2	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 2 Baseline_L - Week4_L: -,17215 | -3,117 | 58 | ,003	Nilai L* antara baseline dan minggu ke 4→ perubahan signifikan
3	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 3 Baseline_L - Week6_L: -,32720 | -3,906 | 58 | ,000	Nilai L* antara baseline dan minggu ke 6→ perubahan signifikan
4	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 4 Baseline_L - Week8_L: -,49840 | -5,006 | 58 | ,000	Nilai L* antara baseline dan minggu ke 8→ perubahan signifikan
5	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 5 Baseline_L - Week10_L: -,24444 | -2,341 | 58 | ,000	Nilai L* antara baseline dan minggu ke 10→ perubahan signifikan
6	**Paired Samples Test** Paired ... 95% Confidence Interval of the ... Upper | t | df | Sig. (2-tailed) Pair 6 Baseline_L - Week12_L: -1,16383 | -8,897 | 58 | ,000	Nilai L* antara baseline dan minggu ke 12→ perubahan signifikan

Lampiran 12. Diskripsi statistik nilai delta L kelompok deoxyarbutin 2% dan hydroquinone 4%*

Descriptives

	Type			Statistic	Std. Error
Week2_B	R	Mean		.3895	.10405
		95% Confidence Interval for Mean	Lower Bound	.1812	
			Upper Bound	.5978	
		5% Trimmed Mean		.3880	
		Median		.2900	
		Variance		.639	
		Std. Deviation		.79920	
		Minimum		-1.58	
		Maximum		2.63	
		Range		4.21	
		Interquartile Range		.89	
		Skewness		.047	.311
		Kurtosis		.892	.613
	L	Mean		.2747	.11239
		95% Confidence Interval for Mean	Lower Bound	.0498	
			Upper Bound	.4997	
		5% Trimmed Mean		.2883	
		Median		.3100	
		Variance		.745	
		Std. Deviation		.86328	
		Minimum		-2.07	
		Maximum		2.28	
		Range		4.35	
		Interquartile Range		.62	
		Skewness		-.411	.311

		Kurtosis		1.079	.613
Week4_B	R	Mean		.5949	.16036
		95% Confidence Interval for Mean	Lower Bound	.2739	
			Upper Bound	.9159	
		5% Trimmed Mean		.6395	
		Median		.6400	
		Variance		1.517	
		Std. Deviation		1.23174	
		Minimum		-3.77	
		Maximum		3.45	
		Range		7.22	
		Interquartile Range		1.07	
		Skewness		-.685	.311
		Kurtosis		2.394	.613
	L	Mean		.4810	.15430
		95% Confidence Interval for Mean	Lower Bound	.1721	
			Upper Bound	.7899	
		5% Trimmed Mean		.4026	
		Median		.4200	
		Variance		1.405	
		Std. Deviation		1.18523	
		Minimum		-1.32	
		Maximum		4.51	
		Range		5.83	
		Interquartile Range		1.55	
		Skewness		1.101	.311
		Kurtosis		2.084	.613
Week6_B	R	Mean		.8383	.15219
		95% Confidence Interval for Mean	Lower Bound	.5337	
			Upper Bound	1.1430	
		5% Trimmed Mean		.8530	
		Median		.7900	
		Variance		1.367	
		Std. Deviation		1.16902	
		Minimum		-1.97	

		Maximum		3.20	
		Range		5.17	
		Interquartile Range		1.41	
		Skewness		-.130	.311
		Kurtosis		-.032	.613
	L	Mean		.6712	.17184
		95% Confidence Interval for Mean	Lower Bound	.3272	
			Upper Bound	1.0152	
		5% Trimmed Mean		.6427	
		Median		.6900	
		Variance		1.742	
		Std. Deviation		1.31997	
		Minimum		-2.25	
		Maximum		4.33	
		Range		6.58	
		Interquartile Range		1.74	
		Skewness		.289	.311
		Kurtosis		.388	.613
Week8_B	R	Mean		1.0927	.17616
		95% Confidence Interval for Mean	Lower Bound	.7401	
			Upper Bound	1.4453	
		5% Trimmed Mean		1.0980	
		Median		.9900	
		Variance		1.831	
		Std. Deviation		1.35307	
		Minimum		-2.60	
		Maximum		4.53	
		Range		7.13	
		Interquartile Range		1.58	
		Skewness		-.060	.311
		Kurtosis		.777	.613
	L	Mean		.8305	.16591
		95% Confidence Interval for Mean	Lower Bound	.4984	
			Upper Bound	1.1626	
		5% Trimmed Mean		.7904	

		Median		.6900	
		Variance		1.624	
		Std. Deviation		1.27438	
		Minimum		-1.53	
		Maximum		4.41	
		Range		5.94	
		Interquartile Range		1.62	
		Skewness		.411	.311
		Kurtosis		.360	.613
Week10_B	R	Mean		1.2641	.18146
		95% Confidence Interval for Mean	Lower Bound	.9008	
			Upper Bound	1.6273	
		5% Trimmed Mean		1.3003	
		Median		1.1700	
		Variance		1.943	
		Std. Deviation		1.39382	
		Minimum		-3.81	
		Maximum		4.35	
		Range		8.16	
		Interquartile Range		1.55	
		Skewness		-.516	.311
		Kurtosis		1.890	.613
	L	Mean		.8702	.16274
		95% Confidence Interval for Mean	Lower Bound	.5444	
			Upper Bound	1.1959	
		5% Trimmed Mean		.8482	
		Median		.8600	
		Variance		1.563	
		Std. Deviation		1.25003	
		Minimum		-2.87	
		Maximum		4.54	
		Range		7.41	
		Interquartile Range		1.43	
		Skewness		.202	.311
		Kurtosis		1.672	.613

Week12_B	R	Mean		1.6761	.17779
		95% Confidence Interval for Mean	Lower Bound	1.3202	
			Upper Bound	2.0320	
		5% Trimmed Mean		1.7110	
		Median		1.6400	
		Variance		1.865	
		Std. Deviation		1.36560	
		Minimum		-3.49	
		Maximum		4.64	
		Range		8.13	
		Interquartile Range		1.88	
		Skewness		-.668	.311
		Kurtosis		2.354	.613
	L	Mean		1.5017	.16879
		95% Confidence Interval for Mean	Lower Bound	1.1638	
			Upper Bound	1.8396	
		5% Trimmed Mean		1.4812	
		Median		1.3200	
		Variance		1.681	
		Std. Deviation		1.29649	
		Minimum		-1.71	
		Maximum		4.80	
		Range		6.51	
		Interquartile Range		1.71	
		Skewness		.268	.311
		Kurtosis		.537	.613

Lampiran 13. Uji normalitas nilai delta L kelompokdeoxyarbutin dan hydroquinone 4%*

Lampiran 14. . Independent t test kelompok deoxyarbutin 2% dan hydroquinone 4%

Tests of Normality

	Type	Kolmogorov-Smirnov[a] Statistic	df	Sig.	Shapiro-Wilk Statistic	df	Sig.
Week2_B	R	.096	59	.200*	.978	59	.372
	L	.151	59	.002	.950	59	.017
Week4_B	R	.114	59	.054	.944	59	.009
	L	.080	59	.200*	.928	59	.002
Week6_B	R	.069	59	.200*	.985	59	.699
	L	.074	59	.200*	.986	59	.722
Week8_B	R	.080	59	.200*	.982	59	.545
	L	.066	59	.200*	.979	59	.408
Week10_B	R	.057	59	.200*	.965	59	.086
	L	.086	59	.200*	.972	59	.182
Week12_B	R	.103	59	.185	.954	59	.025
	L	.091	59	.200*	.980	59	.423

*. This is a lower bound of the true significance.

a. Lilliefors Significance Correction

Lampiran 15. Independent t test kelompok deoxyarbutin dan hydroquinone 4%

NO	NILAI P	KELOMPOK
1	t-test for Equality of Means Week4_B, Equal variances assumed: Sig. (2-tailed) .610; Mean Difference .11390; Std. Error Difference .22254; 95% Confidence ... Lower -.32687 Equal variances not assumed: Sig. (2-tailed) .610; Mean Difference .11390; Std. Error Difference .22254; Lower -.32688	Delta L* kelompok deoxyarbutin 2% dan hydroquinone 4% minggu ke 4 → tidak ada perbedaan yang signifikan
2	t-test for Equality of Means Week6_B, Equal variances assumed: Sig. (2-tailed) .468; Mean Difference .16712; Std. Error Difference .22955; 95% Confidence ... Lower -.28754 Equal variances not assumed: Sig. (2-tailed) .468; Mean Difference .16712; Std. Error Difference .22955; Lower -.28761	Delta L* kelompok deoxyarbutin 2% dan hydroquinone 4% minggu ke 6 → tidak ada perbedaan yang signifikan
	t-test for Equality of Means Sig. (2-tailed); Mean Difference; Std. Error Difference; 95% Confidence ... Lower	Delta L* kelompok deoxyarbutin 2% dan

3	Week8_B	Equal variances assumed	.281	.26220	.24198	-.21708	hydroquinone 4% minggu ke 8 → tidak ada perbedaan yang signifikan
		Equal variances not assumed	.281	.26220	.24198	-.21710	
4			t-test for Equality of Means				Delta L* kelompok deoxyarbutin 2% dan hydroquinone 4% minggu ke 10→ tidak ada perbedaan yang signifikan
			Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower	
	Week10_B	Equal variances assumed	.109	.39390	.24375	-.08887	
		Equal variances not assumed	.109	.39390	.24375	-.08893	
5			t-test for Equality of Means				Delta L* kelompok deoxyarbutin 2% dan hydroquinone 4% minggu ke 12 → tidak ada perbedaan yang signifikan
			Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence ... Lower	
	Week12_B	Equal variances assumed	.478	.17441	.24515	-.31114	
		Equal variances not assumed	.478	.17441	.24515	-.31115	

Lampiran 16. Uji Mann-Whitney delta L kelompok deoxyarbutin 2% dan hydroquinone 4%*

NO	NILAI P	KELOMPOK
1	**Test Statistics[a]** Week2_B Mann-Whitney U 1653.500 Wilcoxon W 3423.500 Z -.468 Asymp. Sig. (2-tailed) .640 a. Grouping Variable: Type	Delta L* kelompok deoxyarbutin 2% dan hydroquinone 4% minggu ke 2 → tidak ada perbedaan yang signifikan

Lampiran 17.Lembar inform consent

KEMENTERIANRISET, TEKNOLOGI DAN PENDIDIKAN TINGGI
UNIVERSITAS HASANUDDIN
FAKULTAS KEDOKTERAN
KOMITE ETIK PENELITIAN KESEHATAN
Sekretariat: Lantai 3 Gedung Laboratorium Terpadu
JL. PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM.10, Makassar. Telp. (0411) 5780103, Fax (0411) 581431.
Contact person **dr. Agussalim Bukhari, M.Med, PhD, Sp.GK** (HP.081241850858), email: agussalimbukhari@yahoo.com

Lampiran 2

FORMULIR PERSETUJUAN MENGIKUTI PENELITIAN
SETELAH MENDAPAT PENJELASAN

Setelah membaca informasi penelitian serta mendengar penjelasan dan menyadari pentingnya penelitian:

"Perbandingan Efektivitas Topikal Serum Deoxyarbutin 2% dan Serum Hydroquinone 4% Sebagai Agen Pencerah Kulit "

Maka saya yang bertandatangan di bawah ini:

Nama	: .....
Umur	: .....
Jenis kelamin	: .....
Pekerjaan	: .....
Alamat	: .....

Saya bersedia dilakukan pemeriksaan dan pengaplikasian serum untuk meningkatkan kecerahan kulit. Pengolesan serum akan dilakukan pada malam hari pada lengan bawah kanan dan kiri bagian dalam selama 12 minggu. Serum A dioleskan pada lengan kanan dan serum B dioleskan pada lengan kiri, masing-masing sebanyak 2 tetes menggunakan spet yang ada pada botol kemasan serum. Untuk memastikan tempat pengolesan serum sama setiap kali pengolesan, dibuat patron khusus. Patron terbuat dari pewarna kulit semi permanen berupa garis berbentuk segi empat berukuran 4x4 cm. Setelah pengolesan saya harus selalu memakai manset (disiapkan) bila beraktivitas di luar ruangan.

Saya mengerti sepenuhnya bahwa pemeriksaan dan pengaplikasian serum yang dilakukan tidak akan mempengaruhi kondisi kesehatan saya dan hal ini semata-mata dilakukan untuk kepentingan penelitian. Saya mengetahui bahwa saya berhak untuk menolak ikut serta dalam

penelitian ini tanpa kehilangan hak saya untuk mendapatkan pelayanan kesehatan yang seharusnya saya peroleh.

Semua biaya pemeriksaan dan biaya pengobatan bila terjadi keluhan apa pun sehubungan dengan penilitian ini, ditanggung oleh peneliti.

Bila masih ada hal yang masih belum saya mengerti atau saya ingin mendapatkan penjelasan lebih lanjut, saya bisa mendapatkannya dari dokter peneliti. Demikian persetujuan ini saya buat dengan penuh kesadaran dan tanpa paksaan.

Makassar, ……

Yang menyatakan,

……
(Nama subjek penelitian dan tanda tangan)

Saksi 1 (suami) …….. ……..

Saksi 2 …….. ……..

Penanggung Jawab Penelitian
Nama : dr. Yulia asmarani
Alamat : Jl. Cemara III Blok GL 09, Perumahan NTI, Makassar
Telepon : 081279083271

Penanggung Jawab Medis
Nama : Prof. DR. Dr. Anis Irawan Anwar, Sp.KK (K), FINSDV, FAADV
Alamat : Jl. Sungai Saddang A 11/7A, Makassar
Telpon : 0811412678

DISETUJUI OLEH KOMISI
ETIK PENELITIAN
KESEHATAN FAK.
KEDOKTERAN UNHAS.
Tgl:……..2019

Lampiran 18.Investigational brochure

PLOS | ONE

RESEARCH ARTICLE

Deoxyarbutin Possesses a Potent Skin-Lightening Capacity with No Discernible Cytotoxicity against Melanosomes

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Abstract

Safe and effective ingredients capable of removing undesired hyperpigmentation from facial skin are urgently needed for both pharmaceutical and cosmetic purposes. Deoxyarbutin (4-[(tetrahydro-2H-pyran-2-yl) oxy] phenol, D-Arb) is a glucoside derivative of hydroquinone. Here, we investigated the toxicity and efficacy of D-Arb at the sub-cellular level (directly on melanosomes) and skin pigmentation using *in vivo* and *in vitro* models to compare with its parent compound hydroquinone (1,4-benzenediol, HQ). At first, we examined the ultrastructural changes of melanosomes in hyperpigmented guinea pig skin induced by 308-nm monochromatic excimer lightand/or treated with HQ and D-Arb using transmission electron microscopy. The results showed that prominent changes in the melanosomal membrane, such as bulb-like structure and even complete rupture of the outer membranes, were found in the skin after topical application of 5% HQ for 10 days. These changes were barely observed in the skin treated with D-Arb. To further clarify whether membrane toxicity of HQ was a direct result of the compound treatment, we also examinedultrastructural changes of individual melanosomes purified from MNT1 human melanoma cells. Similar observations were obtained from the naked melanosome model *in vitro*. Finally, we determined the effects of melanosomal fractions exposed to HQ or D-Arb on hydroxyl radical generation in the Fenton reaction utilizing an electron spin resonance assay. D-Arb-treated melanosomesexhibit a moderate hydroxyl radical-scavenging activity, whereas HQ-treated melanosomessignificantly generate more hydroxyl free radicals. This study suggests that D-Arb possesses a potent ability in skin lightening and antioxidation with less melanosome cytotoxicity.

Introduction

Melasma (chloasma) is a common skin pigmentary disorder characterized by irregular light to darkbrown patches on the face, which usually cause significant psychiatric and psychological burdens for affected individuals. However, the inciting events for the pathogenesis of melasma

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DeoxyArbutin: a novel reversible tyrosinase inhibitor with effective *in vivo* skin lightening potency

Boissy RE, Visscher M, deLong MA. DeoxyArbutin: a novel reversible tyrosinase inhibitor with effective *in vivo* skin lightening potency. Exp Dermatol 2005: 14: 601–608. © Blackwell Munksgaard, 2005

Abstract: Modulation of melanogenesis in the melanocytes can be achieved using chemicals that share structural homologies with the substrate tyrosine and as thus competitively inhibit the catalytic function of tyrosinase. We have developed a new tyrosinase inhibitor, deoxyArbutin (dA), based on this premise. DeoxyArbutin demonstrates effective inhibition of mushroom tyrosinase *in vitro* with a Ki that is 10-fold lower that hydroquinone (HQ) and 350-fold lower than arbutin. In a hairless, pigmented guinea pig model, dA demonstrated rapid and sustained skin lightening that was completely reversible within 8 weeks after halt in topical application. In contrast, HQ induced a short but unsustained skin lightening effect whereas kojic acid and arbutin exhibit no skin lightening effect. Results from a panel of safety tests supported the overall establishment of dA as an actionable molecule. In a human clinical trial, topical treatment of dA for 12 weeks resulted in a significant or slight reduction in overall skin lightness and improvement of solar lentigines in a population of light skin or dark skin individuals, respectively. These data demonstrate that dA has potential tyrosinase inhibitory activity that can result in skin lightening and may be used to ameliorate hyperpigmentary lesions.

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Key words: melanocyte – pigments – hydroquinone – complexion coloration

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Introduction

Tyrosinase [EC 1.14.18.1] is the primary, rate-limiting enzyme responsible for conversion of the substrate tyrosine into melanins by melanocytes (1,2). Initially, tyrosinase hydroxylases tyrosine to dihydroxyphenlyalanine (DOPA) and subsequently oxidizes DOPA to DOPAquinone. Inhibition of the catalytic activity of tyrosinase, either by genetic mutations as in albinisms (3) or pharmacologically with competitive inhibitors (4), results in reduced or absent melanin synthesis by melanocytes and hypopigmentation of skin.

Various compounds that bind to the active site of tyrosinase and inhibit the synthesis of melanin have been developed as agents to lighten skin and ameliorate hyperpigmented lesions. These agents include hydroquinone (HQ) (5,6), kojic acid (7), arbutin (8,9), etc. (10). However, the efficacy and safety of these compounds has been a concern. Specifically, HQ can be very irritating, induce Ochronosis (11), and cause permanent leucoderma or scarring with long-term use (12). In addition, animal studies (13,14) and *in vitro* cell studies (15) have demonstrated that HQ is possibly carcinogenic probably related to the metabolites of benzene. Kojic acid and arbutin, although quite safe, are ineffective at levels allowed by the quasi-drug regulations in force. In addition, kojic acid has been demonstrated to be mutagenic (16) and has recently been banned for quasi-drug usage in Japan.

In an attempt to develop a more effective and safer tyrosinase inhibitor, we have synthesized and screened various candidate compounds in which the polar tail of tyrosine had been structurally altered in a systematic way. One compound, (per) dA, appears to be an excellent tyrosinase inhibitor. We demonstrate herein that this compound effectively inhibits tyrosinase activity and reduces pigmentation of guinea pig and human skin.

Materials and methods

Compounds

Figure1 presents the chemical structure of dA and arbutin compared to tyrosine. DeoxyArbutin (dA) was initially synthesized by the authors and scaled up by Girindus America Inc. (Cincinnati, OH, USA) and Starks' Associates, Inc. (Buffalo, NY, USA). Hydroquinone, kojic acid, and arbutin were either synthesized by the authors or purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA).

Tyrosinase inhibition assay

An assay was developed to measure the conversion of tyrosine to DOPA by mushroom tyrosinase with quantitative detection by high-performance liquid chromatography (HPLC). This assay was used to determine the effective concentration range and strength of inhibition (K_i). Briefly, 20 µl of mushroom tyrosinase (3.2 µg) in 3-(N-Morpholino)-propanesulfonic acid buffer (100 mM; pH 7.0) was added to 10 ml of 0.5 µM DOPA and incubated for 10 min at 70 °C. About 100 µl of test inhibitor (ranging from 0.1 to 10.0 µM) plus 200 µl of tyrosine (10 µM) were added and incubated for an additional 22 min. The reaction solution was cooled to room temperature and used for HPLC quantification of tyrosine consumption (µM tyrosine/min/mg tyrosinase). About 6 min, run times of a 5 µl sample through a HPLC Supelcosil LC-CN column (25 cm × 4.6 mm) using a 470 scanning fluorescence detector were employed to allow separation of tyrosine from DOPA and inhibitor. The mobile phase used was 0.05 M acetic acid/20 mM triethylamine. Units of inhibition represent percent activity of sample treated with inhibitor compared to sample treated with vehicle only.

Animal study

A hairless pigmented (black) guinea pig model was developed. Viral/pathogen-free hairless, albino guinea pigs (Charles River Laboratories, Inc., Kingston, NY, USA) and haired, pigmented guinea pigs (Kuiper's Rabbit Ranch, Gary, IN, USA) were crossbred for several generations with selection for hairless animals with interfollicular pigmentation. For experiments comparing skin lightening effects of test compounds, skin was treated daily for 9 weeks with dA, HQ, kojic acid, or arbutin (3%) prepared in I base cream consisting of water (25%), 0.2 M triethanolamine hydrochloride (44.5%), glycerin (3.75%), Parson MCX (7.5%), cutina GMS (2.5%), and tegin 90 (2.5%). Ten animals were in each treatment group, and three independent experiments were performed. Skin lightness (L-value) was measured at baseline and then weekly with a colorimeter (Minolta Chroma Meter, CR-300, Konica Minolta Photo Imaging, Mahwah, NJ, USA). For experiments comparing dose–response and reversibility of dA, skin was treated daily for 6 weeks with 0, 0.1, 0.3, 1, and 3% dA, and evaluated for eight additional weeks after halt of treatment. Ten animals were in each treatment group.

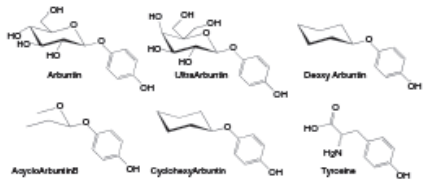

Figure 1. Chemical structures of arbutin, ultraarbutin, deoxyarbutin, acycloarbutin b, cyclohexylarbutin, and tyrosine.

Metabolism studies

Depilated, pigmented guinea pigs (n = 10/group) were topically treated once with 30 µCi of ^{14}C-dA or ^{14}C-HQ (3.0%) in I base cream. Urine was collected daily for 7 days. Volume of urine and amount of radioactivity per sample was determined. Excretion profiles for the time course of the experiment were calculated. In a second experiment, pigmented guinea pigs (n = 10/group) were topically treated with 30 µCi of ^{14}C-dA or ^{14}C- HQ in buffered SC-23 cream at 3.0% applied to 16 cm^2 daily for 4 days. Urine was collected twice daily until day 8. Excretion profile of radiolabeled metabolites was determined by LC-MS for presence of glucuronide and sulfate metabolites.

Safety assays

Volume eye irritation. Preliminary ocular examinations were performed in three female New Zealand rabbits (Myrtle's Rabbitry, Thompson Station, TN, USA). About 10 µl of 3% dA in a standard cream formulation was dosed into one eye, the other eye was left as untreated control. (10) Animals were scored for irritation at 24, 48, and 72 h post dosing using the Ocular Grading System (17).

Murine local lymph node assay. This assay evaluated for potential to induce contact hypersensitivity by evaluating cellular proliferation of auricular lymph node cells. Female CBA/J mice (n = 25) were treated with 12.5 µl of 3, 10, and 20% dA w/v in acetone, acetone only, or untreated control to ventral and dorsal surfaces of both pinnae. Animals were treated daily (4 days) and injected with 20 µCi of ^{3}H-thymidine (specific activity of 6.7 µCi/mmole) on day 5. Under anesthesia, auricular nodes were excised, dissociated, washed, resuspended in 5% trichloroacetic acid, and radioactivity assessed.

Ames test and Escherichia Coli assays. The Ames test assessed mutagenic activity of dA. Tester strains were Salmonella typhimurium histidine auxographs TA98, TA100, TA1535, TA1537, and TA1538 (18) and *E. coli* strains WPs uvrA (pKM101) and WP2 (pKM101). Cultures were prepared, and following dose determination, six dose levels (in triplicate) of test article and controls were plated with tester strains in presence and absence of rat liver S9 activation. Using plate incorporation, 500 µl of S9 or sham mix, 100 µl of tester strain, and 50 µl of vehicle or test substance were added to 1.0 ml of molten selective top agar at 45 ± 2°C. For positive control, test article was replaced by 50 µl of the control. Vortexed mixture was overlaid onto 25 ml of minimal bottom agar. Plates were inverted, incubated for 48–72 h at 37 ± 2°C, and stored at 4 ± 2°C. Plates were evaluated for evidence of test article toxicity using a dissecting microscope and scored relative to vehicle control.

Up/down procedure LD_{50}. Single-dose oral toxicity of dA was determined using Sprague-Dawley Crl:CD® BR VAF/Plus® rats (Charles River Laboratories, Inc., Portage, MI, USA). Six females were given single oral doses (mg/kg): 923 (n = 2), 1200 (n = 3), and 1560 (n = 1). Results for first dose level were observed prior to establishing second dose level. Seven males were dosed at 2275 (n = 1), 2958 (n = 3), 3846 (n = 2), and 5000 (n = 1) mg/kg, respectively. Three males and three females served as controls at

a dose of >10 ml/kg of vehicle (propylene glycol). Animals were observed daily, weighed on day 7 and examined after euthanasia.

Up/down intraperitoneal LD_{50}. Single-dose up/down intraperitoneal toxicity study of dA in seven male and nine female Sprague-Dawley Crl:CD®BR VAF/Plus® rats (Charles River Laboratories) was performed. Vehicle was propylene glycol/absolute ethanol/saline at 60:20:20 V:V:V. Vehicle was evaluated at a volume of 5 ml/kg in three males and three females to assess toxicity. Animals received a single intraperitoneal injection at 240, 310, 400, 520, 680, and 1150 mg/kg body weight. Animals were observed daily, weighed on day 7 and examined after euthanasia.

Human repeat insult patch test (HRIPT). Ninety-four male and female subjects completed the HRIPT (19). Exclusion criteria were use of immunosuppressive drugs, current or frequent use of topical or systemic anti-inflammatory medications, significant skin disease (excluding facial acne), skin cancer at or near test sites, insulin-dependent diabetes, chronic respiratory disease, ongoing allergy injections, slight erythema in test site, recent malignancy treatment, lactation, or pregnancy. Occlusive patches containing 0.2 g of topical moisturizer with 3% dA was applied for 24 h to the upper arm. Subjects removed patches and returned for application of another patch on M, W, and F for 3 weeks during induction phase, followed by a 2-week rest period. In the challenge phase, patches were applied to the previous sites and to contralateral naïve sites for 24 h. Patch sites were scored for irritation, prior to application of the next patch during the induction phase and then for sensitization 24 and 96 h after challenge. Irritation was scored on a 4-point scale (0 none, 1 slight, 2 moderate, and 3 severe).

Dermal toxicity. Dermal toxicity of dA was evaluated in male ($n=46$) and female ($n=46$) New Zealand rabbits (Hazelton Research Products, Kalamazoo, MI, USA). In the 14-day pilot phase, two animals (one male and one female) were treated with 5, 10, 20 or 40% dA in a solvent vehicle, vehicle alone, or 3% dA in a cream. The 28-day trial evaluated 3% dA in a cream vehicle vs. vehicle alone in two groups (five males and five females per treatment), dosed daily for 6 h at 2.0 ml/kg. The test sites were then washed and dried. The 91-day study evaluated 1, 5, and 40% dA in solvent vehicle (propylene glycol/ethanol, 75:25, v/v) and vehicle alone in four groups (five males and five females), dosed daily for 6 h at 2.0 ml/kg. Test animals were assessed for weight changes and dermal irritation after 2 and 4 weeks of treatment in the 28-day study and after 2, 4, and 13 weeks in the 91-day study. Animals were euthanized and microscopic evaluations performed.

Photoirritation. Dorsal surfaces of albino guinea pigs [Crl:(HA)BR strain, Charles River Laboratories, $n=9$ males, $n=9$ females] were depilated with Neet®. Duplicate 0.3 ml applications of test or control material were applied to four sites per animal under 2-h occlusion (Hill Top Chamber, 25-mm diameter). Test formulas were 0, 1, 5, 20, 50% w/v dA in propylene glycol. The positive control was 8-methoxypsoralen (8-MOP, Sigma Chemical Company), 0.01% w/v in acetone. Patches were removed, left side treated sites wiped, and covered with aluminum foil. Animals were exposed to 10 J/cm^2 of Ultraviolet A (UVA) light (320–400 nm) and patches removed. At 1, 4, 24, and 48 h following UV exposure, skin was evaluated for dermal irritation (0, no erythema; 1, slight but confluent or moderate patchy; 2, moderate; 3, severe with or without edema).

Photoallergy. Nnuchal regions of albino guinea pigs ($n=25$) were depilated with Neet® and tape stripped repeatedly to glistening layer. In induction phase, sites were treated for 2 h with 0.3 ml of 50% w/v dA in propylene glycol ($n=10$ animals) or 10% w/v musk ambret in acetone (positive control, $n=5$), both applied via an adhesive patch (Hill Top Chamber®, 25-mm diameter). For the primary challenge ($n=5$) and rechallenge ($n=5$) controls, dry patches were applied. Patches were removed and sites wiped. Untreated lumbar regions were shielded, and animals were exposed to 10 J/cm^2 of UVA light (320–400 nm). Induction was performed on days 2, 4, 7, 9, and 11 with depilitation performed on days 4, 7, and 11. On day 22, a naïve lumbar site was depilitated prior to application of 0.3 ml/patch of test material and positive control. Sites were occluded for 2 h, patches removed and sites wiped. The left side challenge site and the induction areas were shielded with aluminum foil under tape (right challenge site uncovered). Animals were exposed to 10 J/cm^2 of UVA light, and foil was removed. Dermal reactions were evaluated at 24 and 48 h following irradiation.

Human clinical trial

A paired comparison, vehicle-controlled, double-blind study was implemented with 3% dA /formula or vehicle/formula applied to a 100 cm^2 area on dorsal surface of each forearm. The formula utilized was composed primarily of water (79.4%), Butylene Glycol (1.5%), Parson MCX (7.5%), Cetyl Alcohol (1.25%), and Stearyl Alcohol (1.25%). The population consisted of 50 post-menopausal female subjects stratified into two subgroups: 34 Caucasian light skin individuals (mean baseline L-value = 62.7) with solar lentigines and 16 non-Caucasian darker skin individuals (mean baseline L-value = 60.6) with solar lentigines. Daily treatment was for 12 weeks from mid-November to mid-February at a clinical site in the United Kingdom. For overall skin lightness evaluation, L-values were obtained using the Minolta Chroma Meter at onset (baseline) and termination (12 weeks). For assessment of solar lentigines, digital images were obtained, coded, and evaluated by manual grading by three independent expert judges at onset of the trial (baseline) and at termination (12 weeks). Images were evaluated for improvement in solar lentigines by a side-by-side comparison of treated and control images, using a 0–4 comparative scale (where 0 = there is no difference; 1 = I think there is a difference; 2 = I know there is a difference; 3 = there is a moderate difference; and 4 = there is a very large difference).

Statistics

For most studies, statistical differences between control and treatment groups were assessed using Student t-test.

For local lymph node assay, data were long-transformed and analyzed by Bartlett's test for homogeneity and analysis of variance (ANOVA). Wilcoxon's rank sum test was used to compare control to treatment groups. A P-value of ≤ 0.01 was considered significant. For 28 and 91-day dermal toxicity trials, analysis of variance techniques were used to compare treatments to controls. For non-normally distributed data, ANOVA procedure of Conover and Iman was used (20). The Mann–Whitney U-test with Bonferroni correction was used for comparisons of treatments with control. Statistical significance of $P=0.05$, two-sided risk level, was used. For human clinical trial, all data for Week 12 change from baseline L-value or composite scores from evaluators were analyzed by 2-sided analysis of variance.

Results

Tyrosinase inhibition

The catalytic activity of mushroom tyrosinase was assessed in the presence of dA in comparison to HQ, arbutin, and kojic acid (Fig. 2). DeoxyArbutin was equally effective in inhibiting tyrosinase activity as HQ, whereas kojic acid and arbutin exhibited no tyrosinase inhibitory effect. The Ki (μM) of dA, HQ, kojic acid, and arbutin for purified mushroom tyrosinase were 0.05, 0.54, 7.70, and 17.60μM, respectively, indicating that dA was the most effective tyrosinase inhibitor of the compounds tested.

Animal studies

DeoxyArbutin, HQ, kojic acid, and arbutin were evaluated for topical effect on skin pigmentation of a guinea pig model (Fig. 3a,b) through daily application of 3% solutions for 9 weeks. Both dA and HQ resulted in skin lightening during the initial 3 weeks of treatment. From week 4 until week 9 of treatment, the extent of dA-induced skin lightening plateaued while the HQ-treated skin darkened and returned to the pretreatment value (Fig. 3a,b). The dA treated sites showed no signs of skin irritation. In contrast, HQ treated skin showed signs of irritation that resulted in irregular darkening, beginning at 3 weeks of treatment (Fig. 3b). In contrast, kojic acid, and arbutin were not significantly different from the vehicle control, vehicle at any time during the treatment period (Fig. 3a,b).

DeoxyArbutin was evaluated for dose–response and reversibility of effect upon discontinued use (Fig. 3c). By 6 weeks of treatment, 3, 1, and 0.3% dA were significantly better ($P < 0.05$) than the vehicle control. The 3 and 1% concentrations reached significance as early as 3 weeks of treatment. The 0.3% concentration produced significantly greater skin lightening than the vehicle at 5 weeks of treatment. The 0.1% level was not significantly different than the vehicle control. Following discontinuation of treatment at week 6, the skin repigmented for each concentration of dA tested (Fig. 3c). The rate of repigmentation correlated with dose of dA.

Pigmented guinea pigs treated with radiolabeled 3.0% dA were assessed for the presence of metabolites in urine in two independent studies. The total amount of radioactivity excreted in urine after one topical treatment indicated that by 48 h approximately, 90% of the radioactivity was detected in urine. Subsequently, urinary metabolism of topically applied radiolabeled dA and HQ was evaluated. DeoxyArbutin was excreted more rapidly than HQ with a $t_{1/2}$ of 9 h for dA and >167 h for HQ. At 38 h, 91–97% of dA was excreted vs. 52% for HQ. In the dA-treated guinea pigs, 52–57% and 29–30% of the inhibitor was retrieved in urine as dA-glucuronide and dA-sulfate, respectively (Table 1). In contrast, in HQ-treated guinea pigs, only 5–14% of the inhibitor was retrieved in urine collectively as HQ-glucuronide and HQ-sulfate.

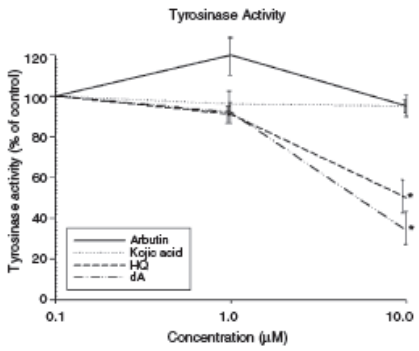

Figure 2. DeoxyArbutin (dA) exhibits tyrosinase inhibition. The consumption of tyrosine (i.e. tyrosinase activity) was quantitated in the presence of various concentrations of dA, hydroquinone (HQ), kojic acid, and arbutin compared to control as described in *Materials and Methods*. Both dA and HQ exhibit statistically significant inhibition of tyrosinase at 10 μM, whereas kojic acid and arbutin exhibited no inhibition of tyrosinase. *$P < 0.05$ for each line compared to the vehicle-treated control.

Safety studies

Safety analyses for dA were performed. Specifically, these tests included [i] low-volume eye irritation, [ii] murine local lymph node assay, [iii] Ames test, [iv] *E. coli* assay, [v] up/down oral and intraperitoneal LD50, [vi] HRIPT, [vii] dermal toxicity, [viii] photoirritation, and [ix] photoallergy using standard protocols as described in *Material and Methods.* The low-volume eye irritation assay demonstrated no irritation (maximum average score of 0.0) with a 1-day median time for eye clearance indication, no complications. The murine local lymph node assay indicated no significant clinical signs and demonstrated no statistical differences between any of the treatment groups and the carrier (vehicle) and untreated control groups. The Ames test and the *E. Coli* assay demonstrated no positive responses

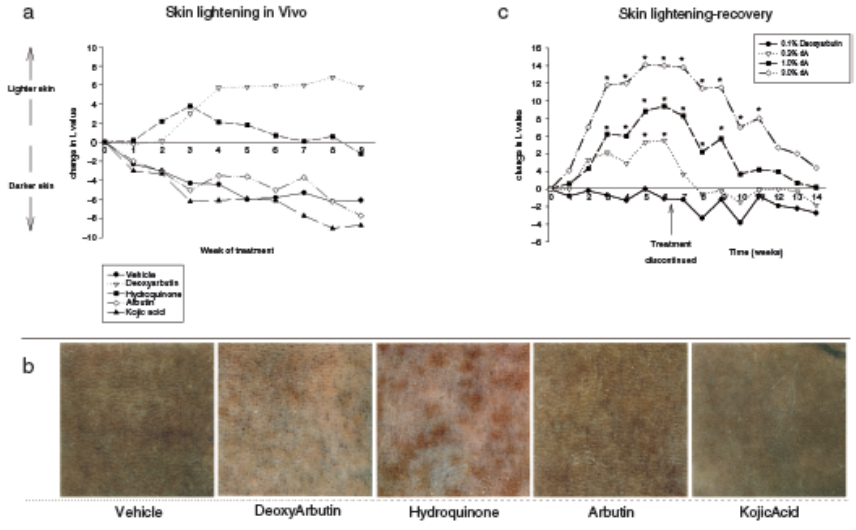

Figure 3. DeoxyArbutin (dA) effectively lightens skin pigmentation in a hairless, pigmented Guinea Pig model. Animals ($n = 10$/group) were topically treated daily for 9 weeks with vehicle, or dA, hydroquinone, arbutin, and kojic acid at 3% in I base. (a) Skin lightness was assessed weekly with a chromameter and change in L-value plotted. DeoxyArbutin exhibited a sustained skin lightening affect by the third week of application. (b) Photographs at the 9-week time point of skin areas treated with vehicle, dA, Hydroquinone, Arbutin, and Kojic Acid, respectively. (c) The skin lightening effect of dA is dose dependant reversible. Hairless, pigmented guinea pigs ($n = 10$/group) were topically treated daily with dA at 0.1, 0.3, 1.0, and 3.0% for 6 weeks and then maintained without treatment for an additional 8 weeks. Skin lightness was assessed weekly throughout the 14-week period with a chromameter and change in L-value plotted. DeoxyArbutin exhibited a dose-dependant skin lightening effect during treatment and recovery to base line within 8 weeks of halting of treatment. $^*P < 0.05$ for each line compared to the vehicle treated control.

indicating that dA did not cause a mutagenic response. In the dose–range finding study, the maximum dose tested was 5000 mg per plate, and this dose was used. No positive responses were observed. No precipitate was seen with any tester strains, but toxicity was found with tester strain WP2 (pKM101). Therefore, under the test conditions, dA did not cause a positive response in the Ames test and the *E. coli* WP2 *uvr*A mutagenesis assay. In the up/down oral toxicity study for LD_{50}, animal deaths occurred on day 1. The acute oral LD_{50} was 1148 mg/kg for females [95% confidence interval (CI) of 843–1564 mg/kg] and 3068 mg/kg for males (95% CI of 2270–4146 mg/kg). The up/down intraperitoneal LD50 assay for dA indicated an acute intraperitoneal LD_{50} of 367 mg/kg in males (95% CI of 264–511 mg/kg) and 314 mg/kg in females (95% CI of 235–419 mg/kg). All deaths occurred by day 5. No significant toxicity was found for vehicle. HRIPT demonstrated that the formulation caused a low level of irritation, presumably due to the occlusive nature of the patch. One subject showed indications of skin sensitization, but a rechallenge test confirmed the sensitization response to both the original product and the vehicle, but not for a 1.5% solution of dA in ethanol, indicating that the sensitization response was not due to dA. Dermal application of 3% dA in a cream vehicle for 28 days resulted in transient depression in weight gain in males and moderate to marked dermal irritation in females. The incidence of skin lesions (subacute dermal inflammation and epidermal hyperkeratosis and acanthosis) was slightly higher for the cream vehicle. No abnormalities were observed on organ weights or in hematological, serum, or

Table 1. Metabolites in guinea pig urine trough 8 days after topical application of deoxyArbutin (dA) or hydroquinone (HQ) for 4 days

Experiment number	dA-glucuronide	dA-sulfate	HQ-glucuronide + HQ-sulfate
1	57%	29%	5%
2	52%	30%	14%

urinalysis. Dermal application of dA in solvent vehicle produced no adverse effects clinically or on weight gain. Slight erythema was observed for all groups, with moderate levels at the higher dose only. Upon microscopic examination, chronic dermal inflammation and epidermal hyperkeratosis, and acanthosis, the photoirritation assay demonstrated no dermal reactions to any test mixtures at either the UVA irradiated or non-irradiated sites except for a very slight patchy erythema (grade of 1.0) at two sites with 1% dA and one with 50% dA. The responses were not indicative of photoirritation. The 8-MOP positive control resulted in moderate to severe erythema at the UVA irradiated sites in all animals. Slight erythema was observed at three times on two animals for the corresponding non-irradiated sites. In the Photoallergy assay, three out of 10 animals treated with 50% dA exhibited slight erythema at the irradiated challenge site at 24 and 48 h ($n=1$). One of 10-challenge sites shielded form UVA radiation had slight erythema at 24 and 48 h. Two of five animals in dA primary challenge control group (naïve site) had slight erythema at the UVA-treated site. No significant differences were observed in incidence or severity of the challenge reactions for the dA group sites treated with UVA and those shielded from UVA. There were no significant differences in incidence or severity of challenge reactions in the UVA sites in test and primary challenge control groups, indicating that dA was not a photoallergen. The musk ambrette positive control was a photoallergen, as evidenced by the increased incidence and severity of reactions (grades of 1.0–2.0) at the UVA sites in all five animals compared with non-UVA exposed challenge site.

Human clinical trial

A human clinical trial was performed to assess the skin lightening effect of dA. A 12-week paired forearm test comparing 3% dA in a moisturizing cream with the placebo control on 50 panelists was performed. Panelists consisted of Group 1, 34 Caucasian and Group 2, 16 mixed ethnic individual with darker basal skin tone; who were assessed for change in overall skin lightness and reduction of solar lentigines. The 3% dA containing formulation significantly reduced skin color compared to the placebo in the total population and in the Caucasian subset (Table 2a). Basal skin tone of the pigmented subset was reduced by dA but not significantly compared to the placebo at the 12-week point (Table 2a). However, it should be noted that although skin lightened by 12 weeks in both groups, there was a concomitant lightening response to placebo (*Discussion*). Expert blind visual assessment of the improvement in solar lentigines from baseline to week 12 in both Group 1 and Group 2 indicated an improvement for the dA formula that was statistically significant for Group 1 but not statistically significant for Group 2 vs. placebo control (Table 2b).

Discussion

The central premise that underlies medicinal chemistry research asserts the existence of a causal relationship between structure of a molecule and its physiochemical properties. However, the relationship is seldom obvious and often difficult to discern. One approach to building a clear understanding of structural characteristics responsible for observed properties of a chemical substance is termed quantitative structure-activity relationships (QSAR). While there are many different techniques used in QSAR studies, all have the same general foundation. The structure of the molecule, or parts of the structure, is converted to numerical values. These values are then examined for correlations with observed properties of interest for a set of known compounds.

Table 2. Effect of topical deoxyArbutin (dA) treatment after 12 weeks on (a) change in overall skin lightness and (b) improvement in solar lentigines in a human clinical trial.

Population	Average baseline L^*	dA	Placebo	P-value
a. Change in overall skin lightness (L^* value) vs. baseline				
Total group ($n=50$)	62.07	3.33[1]	3.13	0.04
Caucasian subset ($n=34$)	62.75	3.23	3.00	0.03
Dark skin subset ($n=16$)	60.60	3.57	3.46	0.29
b. Improvement in solar lentigines vs. baseline				
Caucasian subset ($n=34$)		1.0[2]	0.67	0.05
Dark skin subset ($n=16$)		2.0	1.35	0.08

[1]Amount change from baseline (i.e. delta L).
[2]Average score (*Materials and Methods*).

High correlations found between the numerical form of the structural features, and the observed properties constitute structure-activity relationship. Quantitative aspect of the relationship found is based on the ability to then estimate property of interest for new compounds not involved in original set. Thus, if one has observed property data for a set of compounds of sufficient size and diversity, it is possible to develop a QSAR and then propose new structures and to obtain estimates on the property of interest for these proposed molecules, strictly on the basis of their structure. In our case, the competitive (active site binding) inhibition of mushroom tyrosinase, the resistance of the inhibitor to oxidation by tyrosinase (thus serving as an alternate substrate), and the ability of the molecule to penetrate skin were properties optimized.

To create effective and non-irritating tyrosinase inhibitors, a classic pharmacophore determination and subsequent optimization approach was used (21,22). Compounds were selected based on the above parameters, synthesized or purchased and then tested for their ability to competitively inhibit tyrosinase *in vitro*. Our preliminary *in vitro* screening of published actives revealed that arbutin and HQ were the most attractive candidates for further exploration. However, *in vivo* tests of molecules such as arbutin failed to show any skin lightening ability, despite its tyrosinase inhibition. The effect could not be attributed to metabolism, as the galactose-derived ultraArbutin (*Scheme*), with a large and non-hydrolyzable sugar attached, had a similar effect. An analysis of these molecules revealed that they both had very poor skin penetration ability, which to a first approximation may be correlated to their logP. The synthesis of many alternative sugar-coupled inhibitors demonstrated that removal of pendant hydroxyls from the sugar backbone of both hexoses and furanoses results in molecules that were better at skin penetration than arbutin and ultraArbutin. Thus a series of increasingly deoxygenated sugars was synthesized. Removal of every hydroxyl group resulted in a series of per-deoxy sugars, the lead molecule of which has been named dA. Interestingly, all of these 'deoxysugars' retain arbutin's resistance to oxidation by tyrosinase, while steadily increasing both their skin-penetration ability and their binding affinity for the enzyme.

In addition, following the principles of pharmacophore minimization, a series of acyclic, even more simple, highly penetrating analogs were made. One example of this series of inhibitors is AcycloArbutin B. However, these smaller molecules, while excellent at skin penetration, were quickly oxidized. Ultimately, by selecting for the three variables in the screening, after the synthesis and screening of about 80 compounds, dA surfaced as the optimal candidate molecule, with a 50 nM EC_{50} in the mushroom tyrosinase assay.

Deoxyarbutin demonstrated effective inhibition with a Ki that was 10-fold lower that HQ, and 350-fold lower than arbutin itself. The rationale for the remarkable inhibition of dA relies on a combination of factors. The lipophilicity of the substrates is one factor. Deoxyarbutin has a clogP of 1.96 as compared to a −0.5 for arbutin; while the clogP for the oxidized substrates such as CyclohexylArbutin range as high as 3.6. Part of the effectiveness is found in its relatively rigid shape, because AcyclicArbutins had similar clogP's but were less resistant to both hydrolysis as well as oxidation. However, it should be noted that when tyrosinase is uncoupled by inhibitors, it produces H_2O_2 that can be a bleaching agent for melanin (23), thus providing an additional mechanism resulting in depigmentation common for dA and HQ.

When applied topically to a guinea pig model, dA demonstrated a more sustained lightening effect than HQ. In fact, HQ treatment of guinea pigs produced irritation; which results in moderate post-irritation hyperpigmentation that nullifies its lightening benefits. It has been reported that in human long-term HQ use can induce cutaneous damage manifested as scarring (12). Wounding of skin that results in scarring can cause a dysregulation of cutaneous cytokines (21) that can influence melanization by melanocytes and thus cause hyperpigmentation (22). The skin lightening effect of dA in our guinea pig model was also rapidly reversible. This is another benefit of dA over HQ because the latter has been associated with permanent damage of the melanocyte resulting in leukoderma similar to vitiligo (12). Hydroquinone has been reported to be readily oxidized either spontaneously or metabolically *via* tyrosinase into cytotoxic species causing oxidative damage to cells (24). It should also be noted that the guinea pig has more sensitive skin than humans. In the present study, daily vehicle treatment caused some mild irritation followed by darkening of the skin color (i.e. post-inflammatory hyperpigmentation). On the other hand, because dA inhibited tyrosinase competitively, melanogenesis was downregulated, overcoming the pigmentary consequence of vehicle treatment irritation.

The results of the clinical study demonstrated that dA provided a significant effect on overall

skin lightening and moderate resolution of solar lentigines in Caucasian subjects. We attribute these significant but modest effects to design/time of the study. The test period for our study was from November to February, a period when complexion coloration generally lightens in the Northern hemisphere. Therefore, dA probably was less effective in augmenting this natural lightening and may be more effective in summer months or in inhibiting to a better degree sun-induced tanning. Dark skinned subjects in our clinical study appeared minimally (i.e. not significantly) responsive to the effects of dA. It is possible that prolonged treatment or higher concentrations of dA will be needed for effective tyrosinase inhibition in more pigmented skin. Additional clinical tests are required to confirm these hypotheses.

In conclusion, we have demonstrated that our new tyrosinase inhibitor dA is effective *in vitro* and in a guinea pig model. Further development of dA may improve its effectiveness in human for use as a general skin lightener and/or to ameliorated lesions in hyperpigmentary disorders.

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Official report

Opinion of the Scientific Committee on Consumer safety (SCCS) – Opinion on the safety of the use of deoxyarbutin in cosmetic products

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ABSTRACT

Conclusion of the opinion: Although on the basis of the provided scientific data the use of deoxyarbutin as such can be considered safe for consumers in cosmetic products in a concentration up to 3% in face creams, hydroquinone will be formed at levels which raise concerns with regard to the safety of such products during life-cycle of the product (e.g. storage conditions and stability under in-use conditions). Therefore, the overall conclusion of the SCCS is that the use of deoxyarbutin up to 3% in face creams is not safe.

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http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_183.pdf.

Deoxyarbutin CAS n. 53936-56-4 (4-[(tetrahydro-2H-pyran-2-yl)oxy]phenol) with INCI name Tetrahydropyranyloxy Phenol is a skin lightening agent synthesised through removal of hydroxyl groups from the glucose side-chain of β-arbutin.

In the first opinion (SCCP/1158/08) on β-arbutin adopted on 15th April 2008 the SCCP raised concerns with other substances resulting in the release and/or formation of hydroquinone.

However, Hydroquinone (CAS 123-31-9) is listed in Annex II/1339 of the Cosmetic Regulation No 1223/2009; therefore it is banned as cosmetic ingredient with the exception of entry 14 in Annex III. It is only permitted for professional use in artificial nail systems in a concentration in the final product up to 0.02%. Since Hydroquinone could not be used as a skin whitener after introduction of a ban, other substances have been used for that purpose, including Arbutin.

A dossier on the related substance, deoxyarbutin, was submitted to the European Commission by Girindius AG in 2008.

Although on the basis of the provided scientific data the use of deoxyarbutin as such can be considered safe for consumers in cosmetic products in a concentration up to 3% in face creams, hydroquinone will be formed at levels which raise concerns with regard to the safety of such products during life-cycle of the product (e.g. storage conditions and stability under in-use conditions).

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