Development of Anti-Melanogenic Agent for Skin Whitening

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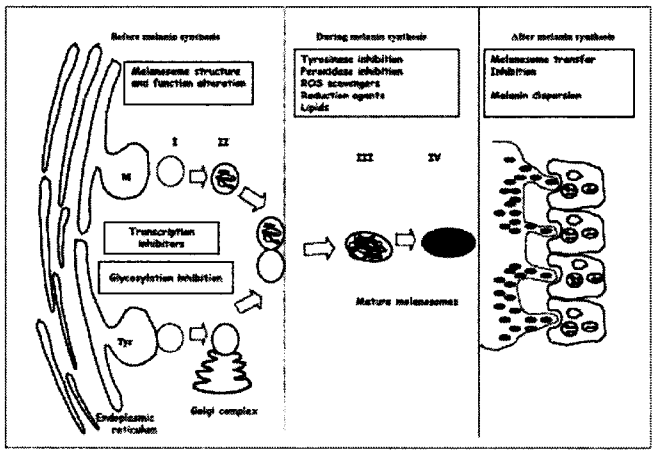
Many modalities of treatment for acquired skin hyperpigmentation are available including chemical agents or physical therapies, but none are completely satisfactory. The ideal depigmenting compound should have a potent, rapid and selective bleaching effect on hyperactivated melanocytes, carry no short- or long-term side-effects and lead to a permanent removal of undesired pigment, acting at one or more steps of the pigmentation process. Depigmentation can be achieved by regulating (i) the transcription and activity of tyrosinase, tyrosinase related protein-1 (TRP-1), tyrosinase related protein-2 (TRP-2), and/or peroxidase; (ii) the uptake and distribution of melanosomes in recipient keratinocytes and (iii) melanin and melanosome degradation and turnover of pigmented keratinocytes. One of the interesting point for development of skin whitening agent is Mitf(Microphthalmia-associated transcription factor). Mitf belongs to the basic helix-loop-helix-zip family of trabscription factors and it is crucial as it regulates both melanocyte proliferation as well as melanogenesis and is the major regulator of tyrosinase and the related enzymes (TRPs), as well as many melanosome structural proteins such as pMel17. Recently, we developed MITF-down-regulating agents from natural and synthetic sources, which have anti-melanogenic effect on *in vitro* and *in vivo*. We suggested that potent MITF-down regulating agents might be used for skin whitening cosmeceuticals.

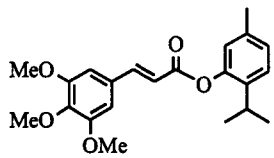
Key words : Depigmentation, melanocyte, melanin, MITF, Whitening

Schematic illustration of the possible approaches to interfere with melanogenesis pathway.



PIGMENT CELL RES 16: 101–110. 2003

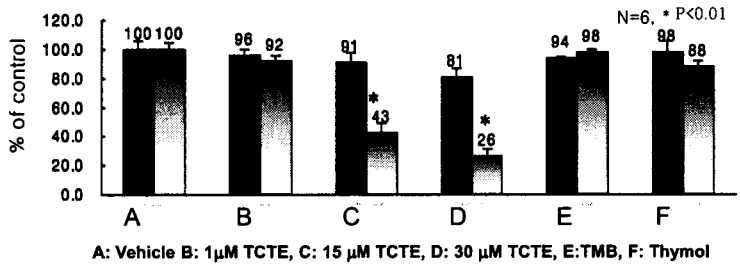
Inhibitory Mechanism of 3, 4, 5-Trimethoxy Cinnamic Acid Thymol Ester on Melanogenesis



M.W=374.44

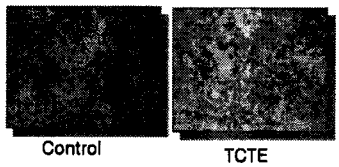
TCTE
(3,4,5-trimethoxy cinnamic acid thymol ester)

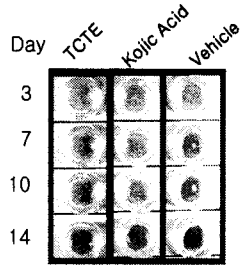
TCTE decrease Melanin synthesis in Melan-a cell



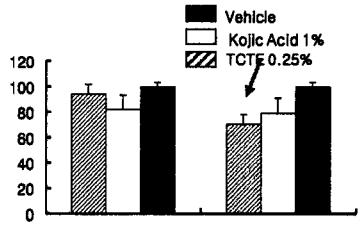
A: Vehicle B: 1μM TCTE, C: 15 μM TCTE, D: 30 μM TCTE, E:TMB, F: Thymol

Depigmentation Effects of TCTE in Reconstituted Human Epidermis



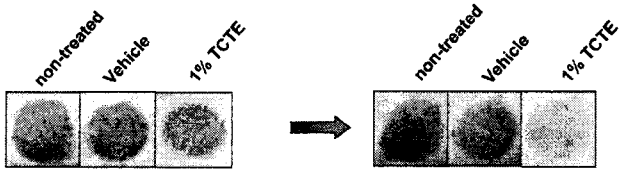


Melanoderm Skin



Melanin Content

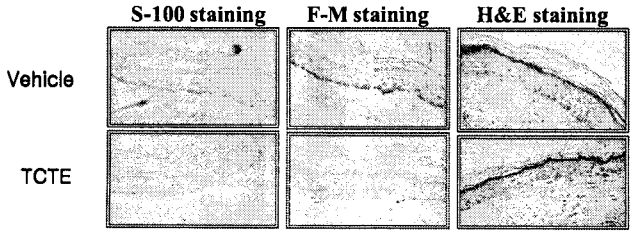
Depigmentation Effects of TCTE in Guinea pig skin



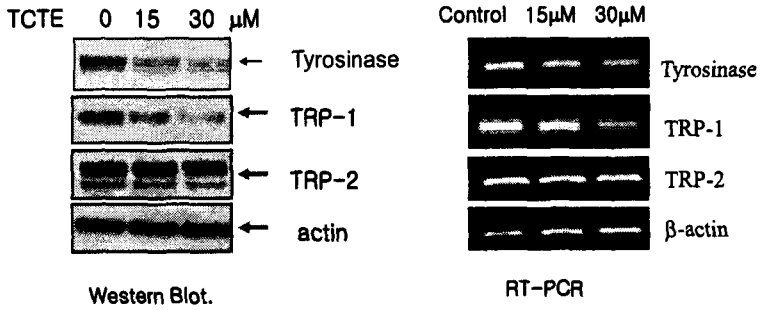
1st week

7th week

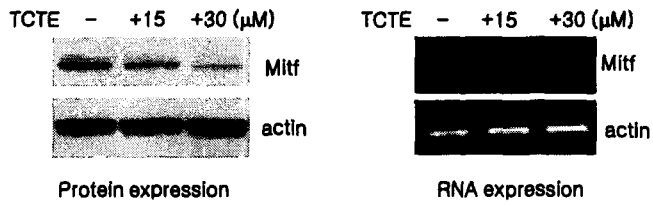
* Vehicle (PG:EtOH:D.W. = 5 : 3 : 2)



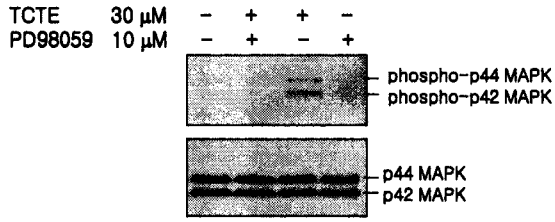
Tyrosinase and TRP-1 expression is decreased by TCTE treatment



Effect of TCTE on MITF expression



Western analysis of ERK1/2 after TCTE treatment



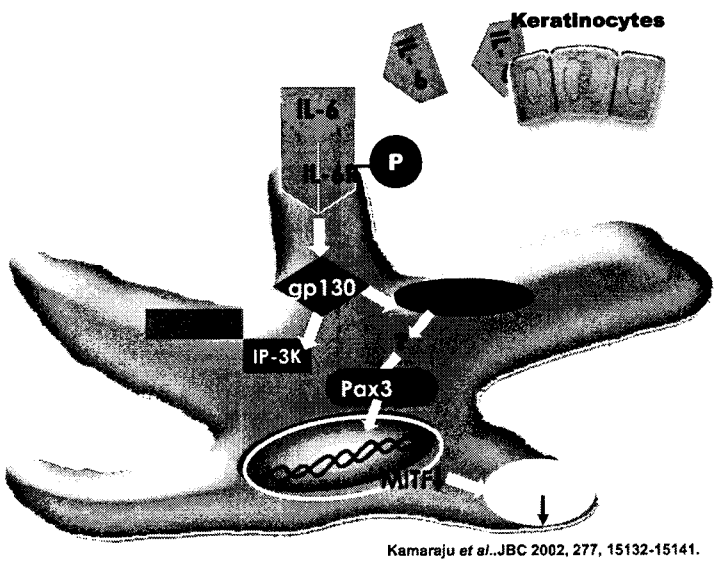
Melan-a melanocytes were treated with DMSO,
10 μM PD98059 with/without 30 μM TCTE for 1 hr

Summary

- TCTE, a new synthetic ester of cinnamic acid and thymol, inhibit melanin synthesis by reducing the expression of tyrosinase and TRP-1 at the transcriptional level
- The down regulated expression of tyrosinase and TRP-2 was caused by the reduced Mitf level in melan-a melanocytes
- It is possible that reduced level of Mitf is associated with ERK1/2 activation by TCTE

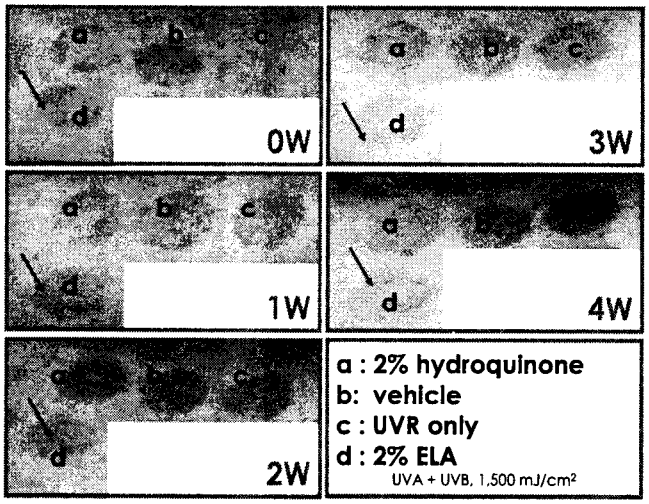
Inhibition of skin pigmentation by an extract of *Lepidium apetalum* (ELA) and its possible implication in IL-6 mediated signaling

Inhibition of melanogenesis by IL-6



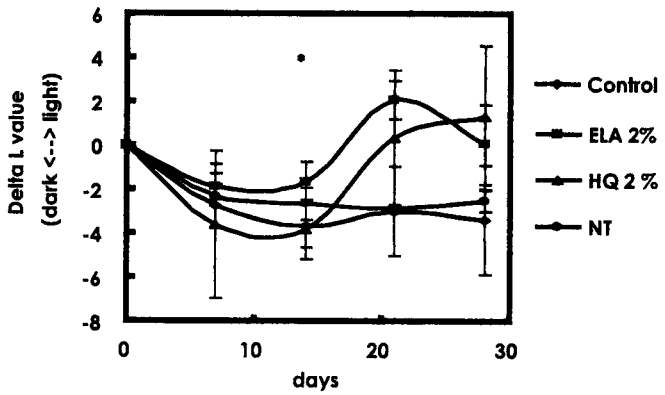
Kamaraju *et al.*,JBC 2002, 277, 15132-15141.

ELA reduced UV-induced skin pigmentation in brown guinea pig



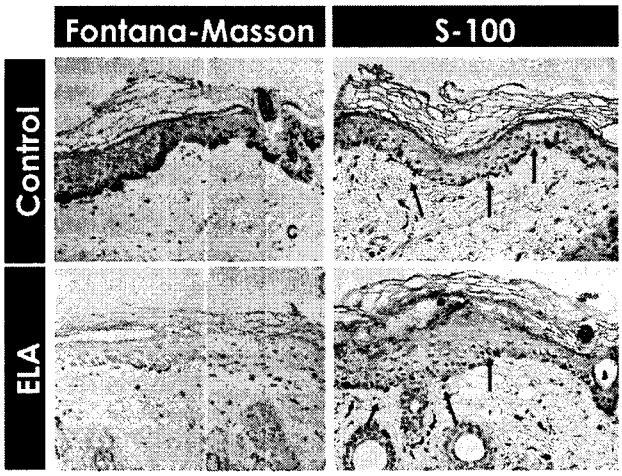
ELA : Extract of Lepidium apetalum

ELA reduced UV-induced skin pigmentation in brown guinea pig

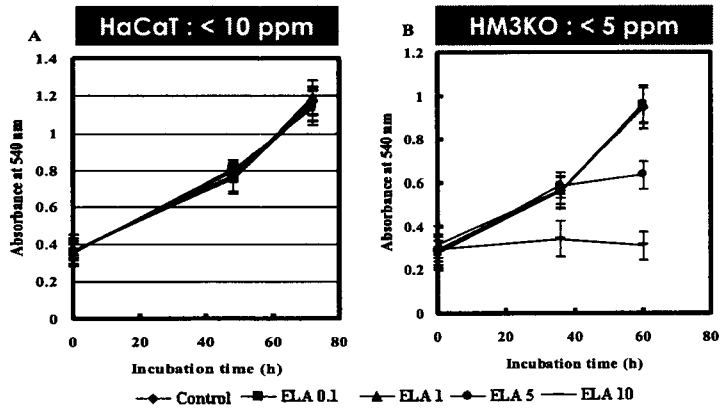


Data are expressed as mean ΔL values ± SEM (n = 4). Student's t-test was used for the statistical analysis of the data. (* P < 0.05, vs control)

Histological analysis of skin from brown guinea pig

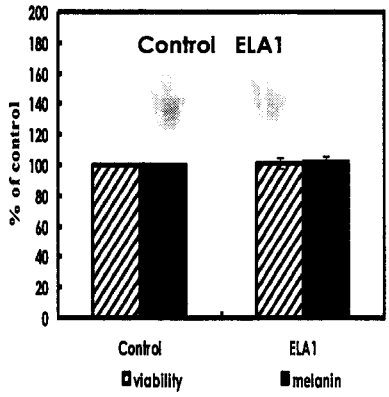


Effects of ELA on the growth of on the growth of HaCaT and HM3KO cells



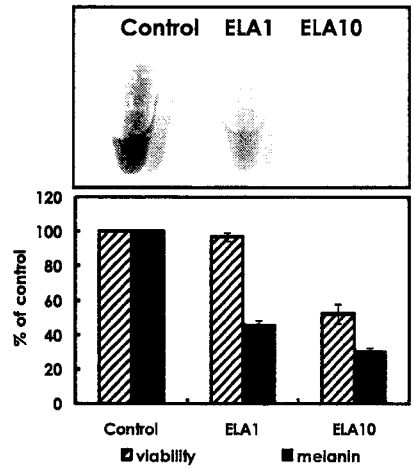
The degree of cell growth was evaluated using a modified thiazolyl blue assay. The value of absorbance at 540 nm increased with cell proliferation. The control sample was taken as the vehicle. ELA (0.1, 1, 5 or 10 ppm) was added to the culture medium at different time-points throughout the experiment. Data are presented as the means ± SD of six determinations.

Melanin content was not affected by direct treatment with ELA



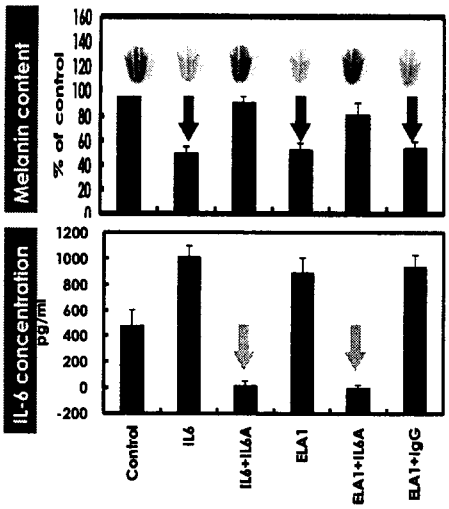
Photograph and melanin content of HM3KO melanoma cells Suspensions of HM3KO melanoma cells were photographed (A) and analysed to determine melanin content (B), as described in Materials and Methods. Each tube has the same number of cells, i.e. 6 X 10^5 cells. ELA was directly added to HM3KO melanoma cells at the concentration of 1 ppm (ELA1). The melanin content of HM3KO melanoma cells treated with vehicle (Control) was regarded as reference of 100 %. Values are expressed as mean ± SD of two independent experiments, each using duplicate culture flasks.

ELA-treated HaCaT -conditioned medium decrease melanin synthesis in HM3KO



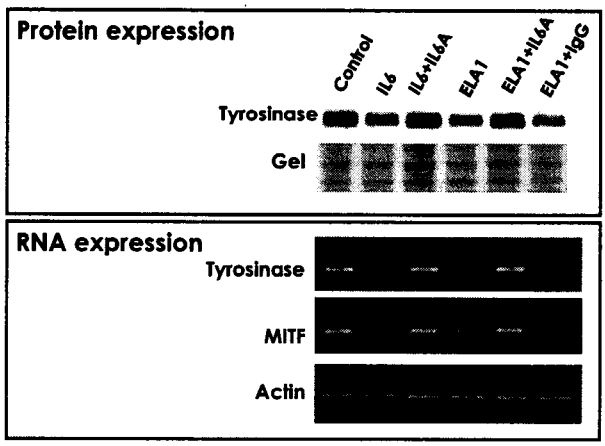
Photograph and melanin content of HM3KO melanoma cells Suspensions of HM3KO melanoma cells cultured with HaCaT keratinocyte-conditioned media were photographed (A) and analysed to determine melanin content (B), as described in Materials and Methods. Each tube has the same number of cells, i.e. 6 X 10^5 cells. ELA was added to culture medium for keratinocyte to the concentration of 1 (ELA1) or 10 ppm (ELA10). The melanin content of HM3KO melanoma cells treated with vehicle indirectly (Control) was regarded as reference of 100 %. Values are expressed as mean ± SD of two independent experiments, each using duplicate culture flasks.

Anti-IL6 antibodies partially abolished the depigmenting effect of ELA



melanin content of HM3KO melanoma cells and the IL6 level in HaCaT keratinocyte-conditioned medium. Each tube contained the same number of cells, i.e. 6 X 10^5 cells. IL6 was added to culture medium for keratinocytes to a concentration of 1 μg /ml (IL6) without or with 1 μg /ml anti-human IL6 antibody (IL6+IL6A). ELA was added to culture medium for keratinocytes to a concentration of 1 ppm (ELA1) without or with 1 μg /ml anti-human IL6 antibodies (ELA1+IL6A) or IgG (ELA1+IgG). The melanin content of HM3KO melanoma cells treated with vehicle (control) was taken as the reference value of 100%. Values are expressed as means ± SD of two independent experiments, each using duplicate culture flasks.

ELA-treated conditioned medium suppresses tyrosinase and MITF expression



Summary

- Extract of *Lepidium apetalum* (ELA) has inhibitory effect on skin pigmentation induced by UV radiation.
- Melanogenesis of HM3KO melanoma cells was not affected by direct treatment with ELA.
- HaCaT keratinocyte-conditioned medium treated with ELA decrease melanin synthesis in HM3KO melanoma cell by inhibiting MITF and tyrosinase expression in the cell.
- ELA increases IL6 production by HaCaT keratinocyte and IL6 antibody removed inhibitory effect of ELA on melanogenesis in HM3KO melanoma cells.

Conclusion

these results indicate that extract of *Lepidium apetalum* (ELA) inhibits melanogenesis of HM3KO melanoma cells by increasing IL6 production of HaCaT keratinocyte cells.