

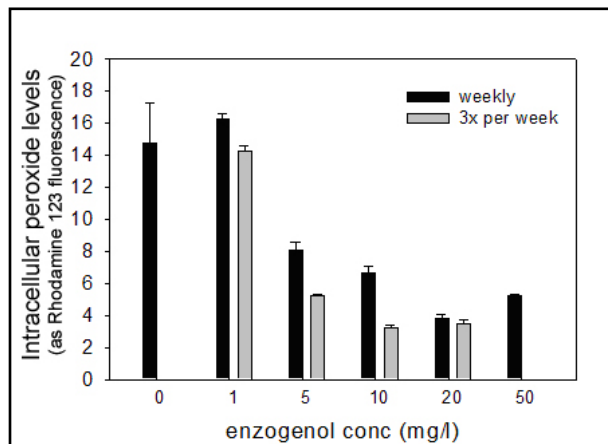


Enzogenol[®] Skin Cell Research

1. Enhancing the Skin's Internal Free Radical Defense

Human fibroblasts (P43) were treated for 3 weeks with weekly or 3-weekly doses of Enzogenol[®] at indicated concentrations and accumulation of peroxides in cells was assessed by staining with Dihydro-rhodamine123 (DHR) and analysis by flow cytometry. In the living cells the DHR is oxidised by peroxides that otherwise need to be neutralized by the cells antioxidant defence mechanisms.

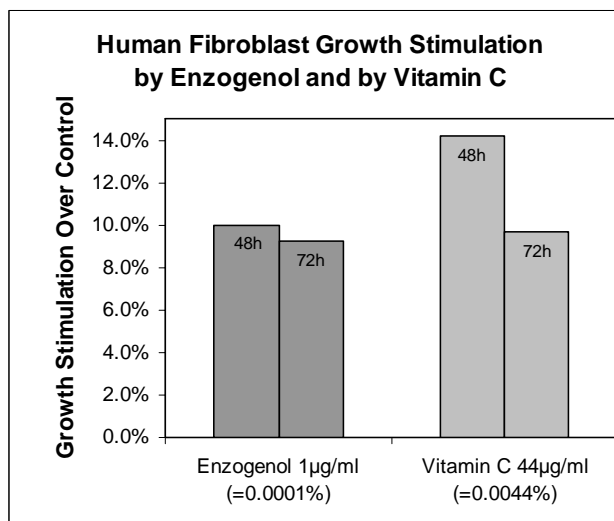
Enzogenol[®], at ≥ 5 mg/l, reduced the internal peroxide load in the human skin cells by more than 50% demonstrating its potent antioxidant effects in the skin cells.



2. Stimulating Skin Cell Growth

Human fibroblasts (CCL110) were treated for 48 and 72 hours with Enzogenol[®] (1 μ g/ml) or, as a positive control, with Vitamin C (44 μ g/ml). Cell growth was determined by MTT assay. Shown here is the % increase in growth over the untreated control cells.

Results show that Enzogenol[®] and Vitamin C stimulate fibroblast growth. Enzogenol[®] at 1 μ g/ml, stimulated fibroblast growth by 9.2 - 10.0%. In comparison, Vitamin C used as a positive control at 44-times the concentration of Enzogenol[®], stimulated growth by 9.7 - 14.2%. Expressed in concentration equivalents Enzogenol was up to 30 times more effective at stimulating cell growth than vitamin C.



This experiment demonstrates the growth activation of cultured human skin fibroblasts by Enzogenol[®], *pinus radiata* bark extract.

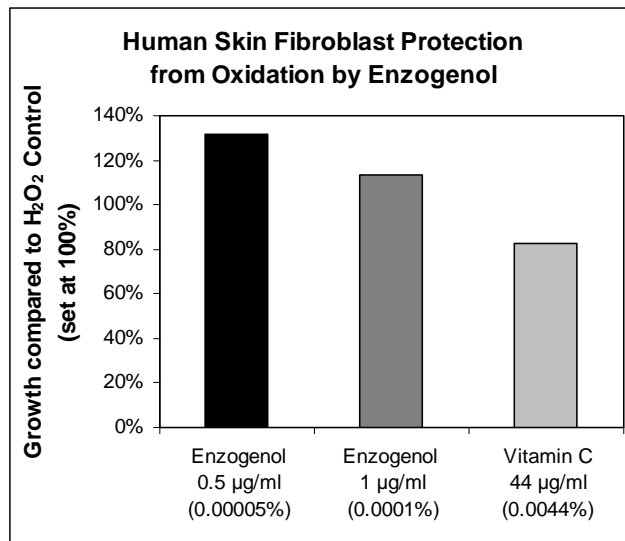


3. Protecting Skin Cells from Oxidative Stress

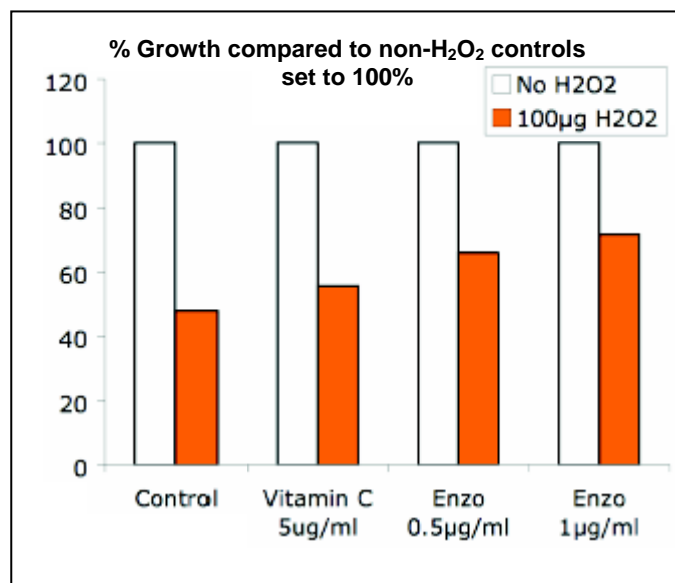
Human fibroblasts (CCL110) were treated for 16 hours with Enzogenol® or with Vitamin C. The fibroblasts were then challenged with 100 µM hydrogen peroxide (H₂O₂) for 6 hours. The cells were then given fresh growth medium and incubated for an additional 48 hours.

Cell growth was determined by MTT assay. Shown here is the % increase or decrease in growth compared to the H₂O₂-treated control cells set to 100%.

Hydrogen peroxide treatment leads to growth depression and cell death and is used in this experiment to simulate external oxidative stress for the skin. The results show that Enzogenol® protects the cells during hydrogen peroxide treatment and allows the cells to recover after removal of the oxidant. This resulted in up to 131% of growth compared to the control cells that did not have the protection of Enzogenol. Vitamin C was used at much higher concentration, due to the lower antioxidant activity when compared to Enzogenol. Yet, vitamin C failed to protect the cells resulting in only 83% growth compared to controls.



The same experiment as above was carried out here with a lower Vitamin C concentration. Results of the % growth of the skin cells are displayed relative to the controls that were not challenged with hydrogen peroxide. These results show that the hydrogen peroxide treatment causes severe growth depression to approximately 48% of normal levels. Vitamin C at 5 µg/ml was able to protect the cells to a some degree reducing the growth depression to 55.5%. Enzogenol at 0.5 and 1 µg/ml was more effective and reduced growth depression to 65.7% and 71.4%. This data shows that Enzogenol protects the skin cells 2.3-3 times better than vitamin C at a concentration 5-10 times lower than that of vitamin C. Expressed in concentration equivalents Enzogenol was 15-23 times more effective than vitamin C in protecting skin fibroblasts from oxidation.



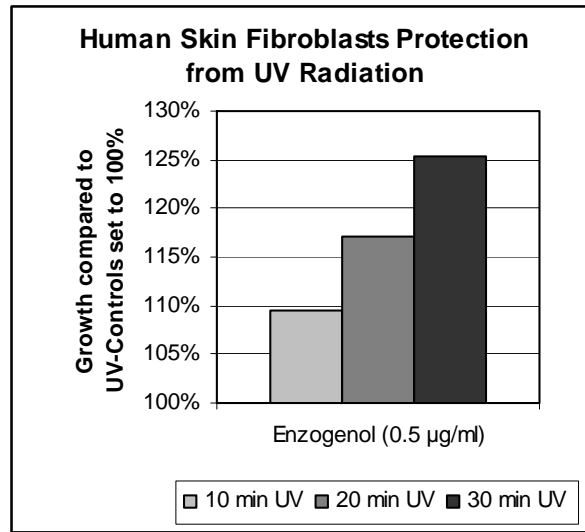
These experiments demonstrate the superior level of protection from external oxidation that Enzogenol®, *pinus radiata* bark extract, can afford the human skin fibroblasts.



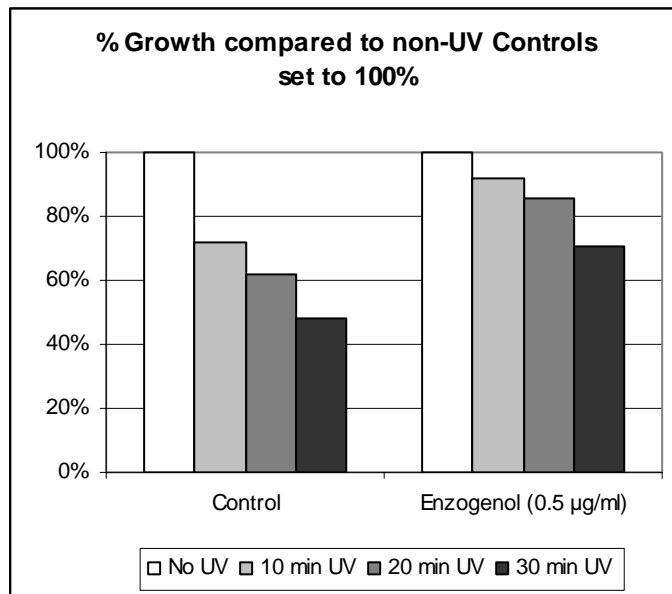
4. Protecting Skin Cells from UV Radiation

Human fibroblasts (CCL110) were treated for 16 hours with Enzogenol®. The fibroblasts were then challenged with 10, 20 or 30 min of UV radiation. The cells were then given time to recover from the UV challenge by further incubation for 48 hours. Cell growth was determined by MTT assay. Shown here is the % increase or decrease in growth compared to the respective UV-treated control cells that received equal radiation set to 100%.

UV treatment leads to growth depression and cell death and is used in this experiment to simulate sun exposure to the skin. The results show that Enzogenol® protects the cells from the consequences of UV radiation, and allows the cells to recover after exposure. At a concentration of 0.5 µg/ml Enzogenol this resulted in 109-125% of growth compared to the control cells that received equal UV radiation.



Results of the % growth of the skin cells are displayed here relative to the controls that were not challenged with UV radiation. These results show that the UV treatment causes severe growth depression of up to 47.9% of normal levels after 30 min exposure. Enzogenol at 0.5 µg/ml was able to reduce the growth depression to 70.5%. This data shows that Enzogenol was able to protect the skin cells from the growth inhibiting effects of UV radiation.



These experiments demonstrate the UV-protective effect of Enzogenol®, *pinus radiata* bark extract, for human skin fibroblasts.