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PHOTOPROTECTIVE EFFECT OF AN EXTRACT OF WINE FROM *JACQUEZ* GRAPES.

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Inflammatory and degenerative processes following acute and chronic UV light skin irradiation are known to be mediated by overproduction of reactive oxygen species (ROS) and free radicals and by impairment of antioxidant systems. Thus it is today largely recognized that, under conditions of environmentally challenged, skin topical application and systemic administration of antioxidants could support physiological mechanisms to maintain or restore a healthy skin barrier. Clear experimental evidence supports the development of new effective pharmaceutical and cosmetic strategies involving antioxidant ingredients to prevent UV radiation-induced skin pathological conditions and photoaging. Particularly, a number of recent studies are focussed on the effect of several natural antioxidant compounds (lipoic acid, flavonoids, vitamin E, silymarin, pycnogenol, etc.) in protecting human skin against solar UV-simulated light-induced damage.

Jacquez (*Vitis aestivalis-cinerea* x

¡Vitis vinifera) grapes are a famous red grape cultivar esteemed in Sicily for its potential to produce wines with a high proanthocyanidin and anthocyanin content. We have previously demonstrated that a lyophilized extract of wine from *Jacquez* grapes (JW-E; Bionap, Rome, Italy) possesses strong antioxidant/free radical scavenging effectiveness in different *in vitro* tests (bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical; peroxidation, induced by the water-soluble radical initiator 2,2'-azobis(2-amidinopropane) hydrochloride, on mixed dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles; UV radiation-induced peroxidation in phosphatidylcholine multilamellar vesicles); furthermore, when topically applied, a gel formulation containing the JW-E afforded significant *in vivo* protection against UV-B light-induced skin erythema in healthy human volunteers (Spagna G. et al., J. Sci. Food Agric., 2002, in press).

In the present study, by means of *in vitro* experiments, the capability of the JW-E in protecting human skin against UV-B induced photooxidative damage was investigated. We have, therefore, used a commercially available and well-characterized tissue culture model of human skin (Rosdy M. et al., Br. J. Dermatol., 129, 227, 1993).

Samples of human reconstituted epidermis were purchased from SkinEthic Laboratories (Nice, France). Individual tissue cultures (0.63 cm^2) were placed into disks containing 1 ml of maintenance medium (MCDB 153). At the beginning of the experiment, 1-2 mg/cm^2 of the JW-E (dissolved in 100 µl) were applied on a single epidermis skin. After incubation for 18 h in a humidified atmosphere at 37 °C and 5% CO_2, the epidermis samples were

rinsed with phosphate buffer saline (PBS), covered with 100 µl of the vehicle and irradiated (150-200 mJ/cm^2) using a Polymer 400 apparatus equipped with a quartz UV mercury vapour lamp type Zh (Helios Italquartz srl, Milan, Italy; max emission: 320-360 nm). Then the JW-E (at the same dose reported above) was added and the coltures incubated for 24 h. After the exposure period in a humidified atmosphere at 37°C and 5% CO_2, the cultured tissues were rinsed with PBS. The cell survival in exposed tissue cultures was measured by the quantification of mitochondrial dehydrogenase activities (MTT assay) and expressed as a percentage of the not-irradiated control. The concentrations of PGE_2, IL-1α and malondialdehyde/hydroxynonenal (MDA/HNE) in the incubation medium and the tissue amounts of GSH was measured by commercially available kits.

Our findings show that UV-B irradiation significantly reduced cell survival (72.22 % and 40.03 % after 150 and 200 mJ/cm^2 respectively); furthermore the amounts of $PGE2057_2$, IL-1α and MDA/HNE released in the incubation medium and the GSH tissue concentrations increased significantly following UV-B irradiation. The treatment with the JW-E markedly reduced, in a dose-dependent way, epidermis oxidative responses following UV-B exposure. In fact, in tissue cultures treated with the JW-E and exposed to UV-B irradiation cell survival was similar to that calculated in not-irradiated control samples; furthermore the levels of PGE_2, IL-1α and MDA/HNE released in the incubation medium and the tissutal content of GSH show an evident tendency to return to values measured in controls.

The present *in vitro* findings demonstrate that the JW-E tested in this study is an efficient vegetable mixture able to prevent, after topical application, skin oxidative damage induced by UV-B exposure. This interesting effect of the JW-E is very likely due to phenolic compounds contained in it. In fact, the JW-E employed in our study is rich in phenolic compounds (proanthocyanidins 270.95±14.44 mg/g, anthocyanins 50.83±4.21 mg/g, hydroxycinnamic acids 34.32±0.72 mg/g). Apart from greatly contributing to their sensory and organoleptic characteristics, phenols contained in musts and wines also possess several biological properties, that have been ascribed to their free radical scavenging/antioxidant properties (Burns J. et al., J. Agric. Food Chem. 48**,** 220, 2000; Rigo A. et al., J. Agric. Food Chem., 48**,** 1996, 2000). In conclusion, the JW-E could be efficaciously employed in certain skin diseases caused, initiated or exacerbated by ROS or free radical overproduction.