Collaborative applied research projects

Project no. 147/2012

PHASE III: Dermatological products based on new biomaterials derived from metal nanoparticles functionalized with fruit extracts from *Cornaceae* family

SUMMARY

In the Phase III were prepared extracts from fruit from *Cornus sanguinea L*, an ornamental plant that grows in the Balkan Peninsula. The extract was obtained in distilled water at room temperature. The hydrophilic active principles have been physico-chemically characterized. The content in Cy-3-glu / L equivalent (total anthocyanin content) was found to be 42.36 mg. We determined the values of the kinetic parameters of the reaction of anthocyanins degradation at different temperatures. The resulting aqueous extracts were purified by partition with ethyl acetate and then subjected to solid phase extraction using a Dowex -50W-X8 cationic resin. Aromatic and polar compounds free sugars were removed by washing with distilled water. Adsorbed anthocyanins were eluted with acidified methanol (0.01% HCl) and this fraction was characterized by thin layer chromatography and UV-VIS spectroscopy. (**Partner P1**)
The extracts were further used in the preparation of gold and silver nanoparticles. Colloids were characterized by UV-Vis spectroscopy, Transmission Electron Microscopy (TEM), Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (RX) and Differential Scanning Calorimetry (DSC). Analysis showed the formation of gold/ silver nanoparticles. The solutions colors changed from yelow to dark red, respectively from incolor to yellow-brown. The average diameter of AuNPs has been found 19 nm and for AgNPs 9 nm. From FTIR spectra one can see the functional groups linked on the surface of metal nanoparticles (**Project Coordinator CO**).
We aimed assessing the effect of extract and of nanomaterials on the viability of keratinocytes and the secretion of inflammatory cytokines in basal conditions and after stimulation (exposure UVB ultraviolet B radiation). Our team studied the toxicity of nanoparticles functionalized with Au/ Ag and with extract on normal HaCaT keratinocyte cell line and A431 epidermoid carcinoma line.
In summary:
Gold nanoparticles do not show toxicity tests than those two lines at the highest concentrations, when A431 tumor cells are more affected compared to normal cells.
Gold nanoparticles have effects on inflammatory cytokine release follows: • not significantly alter the release of IL-1α; UVB exposure does not lead to a further increase in the concentration of IL-1α. • secretion of TNF-α does not change but when exposed to UVB addition, increased secretion augments radiation time; • alone did not lead to increased secretion of IL6, 24h and 48h but after exposure to NP and irradiation have the same effect as with TNF-α. Through these effects, gold nanoparticles prove that the keratinocytes are not toxic and have immunomodulatory properties;
Silver nanoparticles have high toxicity than Au NP on both lines, but especially on tumor cells. • Ag nanoparticles in cells leads to the release of IL-1α, exacerbated by exposure to UVB; • secretion of TNF-α increases to 24 hours following administration of silver nanoparticles, but does not change the exposure to UVB; decreases secretion of IL6 after radiation do not result in an increase in the secretion of IL6 in any period in which the determination is made. These effects suggest toxicity of Ag NP at concentrations tested.The extract is not toxic to the keratinocytes in culture and lowers IL6 and

TNF-α secretion when administered 30 min before exposure to UVB, proving anti-inflammatory properties. (**Partner P2**)
Nanomaterials based on metallic nanoparticles were evaluated in terms of toxicity and the anti-inflammatory effect *in vivo*. The evaluation was done by determining the effects of dynamics at intervals of 30 minutes, 24 hours, 7 days and 14 days, the biochemical and hematological parameters and histopathological changes in the assessment of normal coloration of liver, spleen and kidneys. Acute toxicity studies showed that the doses used and tested at intervals not present significant changes in hematological, biochemical and histopathological. Experimental inflammation induced by carrageenan in Wistar rats was evaluated by assessing plantar edema inflammatory, oxidative stress parameters, markers of inflammation by western blot and histological sections, hematoxylin eosin stained. Creams based on extract, metallic nanoparticles (gold and silver) and cream without active ingredients (used as control) were tested for anti-inflammatory effect by topical application to the skin humans. The study concludes that the creams do not show anti-inflammatory effect on psoriatic lesions as expected. (**Partner P3**). The results were disseminated as follows: 5 papers in national and international journals, one patent (OSIM), 9 international conferences, one mobility.

All activities have been completed.