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ΓΕΝΙΚΗ ΓΡΑΜΜΑΤΕΙΑ ΕΡΕΥΝΑΣ & ΤΕΧΝΟΛΟΓΙΑΣ

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Laboratory SOL - GEL

Toxicity Evaluation of NOD-1E

MTT Assay

MTT assay was used aiming at investigating the cell viability after 24 h incubation, of material **NOD -1E (**S. Patitungkho, S. Adsule, P. Dandawate, S. Padhye, A. Ahmad, F. H. Sarkar, Bioorg.Med.Chem.Lett. 21 (2011) 1802-1806**.,** V. Uivarosi, Molecules. 18 (2013) 11153-11197.**3.** A.-E. Radi, The opened chemical and biomedical methods journal 3 (2010) 27-36.), in different dilution from initial concentration. It is known that MTT is absorbed by cell mitochondria, where it is transformed into formazan by the enzyme hydrogenase. Initially $1*10^4$ cells seeded in a 96 well plate and treated for 24 h with the material in different dilution of each compound and then incubated at 37 ◦C, 5% CO_2 after the 24 h treatment for logarithmic phase. Subsequently, MTT was added at a final concentration of 0.5 mg/ml, and the cells were incubated for additional 4 h at the same conditions, aiming at measuring the MTT (yellow) transformation into formazan crystals (purple) by the viable cells. The formazan crystals were solubilized for 4 h upon addition of DMSO and incubated at 37◦C. The absorbance of the lysate solution of each well was measured with a UV spectrometer at 520 nm (Reference wavelength 640 nm). The results from the MTT assay are presented based on the absorptions at 520 ± SD, using data of two different experiments (triplicate experiments) **.** MTT assay was used in order to investigate the cell viability after 24 h incubation of the material **1** in different dilutions (x, x/2, x/4).(E. K. Efthimiadou, H. Thomadaki, Y. Sanakis, C. P. Raptopoulou, N. Katsaros, A. Scorilas, A. Psomas, J. Inorg. Biochem. 101 (2007) 64-73. **,** E. K. Efthimiadou, M.E. Katsarou, A. Karaliota, G. Psomas, J. Inorg. Biochem. 102 (2008) 910-920., M. E. Katsarou, E. K. Efthimiadou, G. Psomas, A. Karaliota, D. Vourloumis, J. Med. Chem. 51 (2008) 470)

Cell Viability Assay

NOD-1E were tested for their in vitro antiproliferative activity against NCTC cell line (Ceratinocytes cell line). The results of the cytotoxic study of **NOD-1E** presented in Fig. 1.

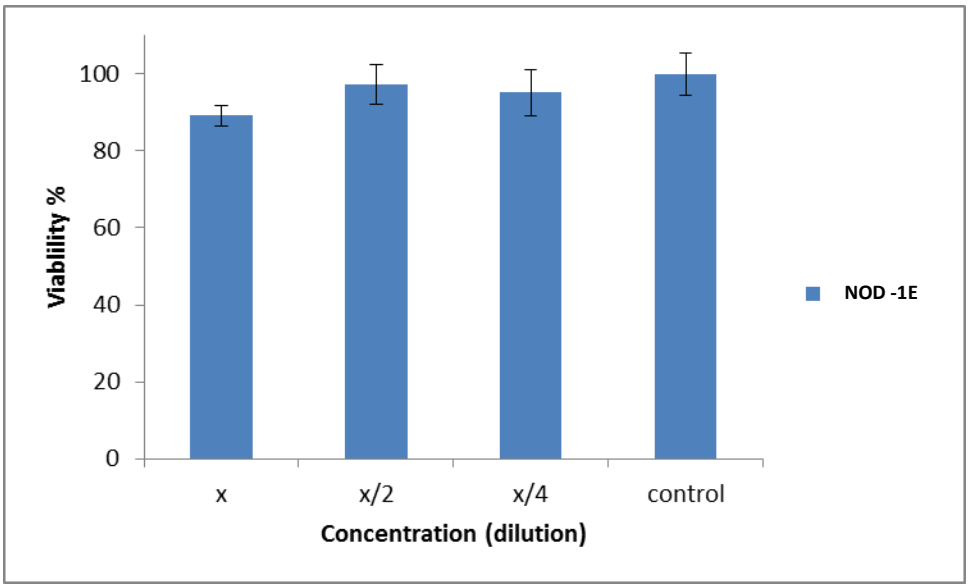

Figure 1.
Toxicity study of NOD-1E in different dilution

Based on the MTT assay results, the % cell **viability not affected by the tested** material as a function of their concentration. In addition, IC_{50} value **cannot be calculated** due to the fact that **the material lucks of toxicity**.

Wound Healing assay

The scratch wound-healing assay has been widely adapted and modified to study cell migration in vitro. Wound gap in a cell monolayer is created by scratching on monolayer cells and the gap could heal by cell migration while occurring subsequently capturing at regular time intervals images of the cells filling the gap, by a transmitted-light microscope equipped for live-cell imaging. More precisely, in order to create the monolayer, a plastic-bottomed 6-well dish was filled with the desired cell line. A suspension of the cells with specific density in culture media will yield a confluent monolayer within one day of plating (5*106cells). After that, the plate was incubated at 37oC under 5% CO2 in humidity. When the cells were confluent, the growth media removed from the plate and the creation of a scratch took place in the same direction. The scratched monolayer was washed one time with media to remove detached cells, and then the media replaced with fresh one. **NOD-1E** in high concentration introduced and monitored by optical microscopy in order to test cell proliferation. By using the wound assay we confirm that when we treat the cells with the material for 24 hours the wound close is a great number (Figure 2).

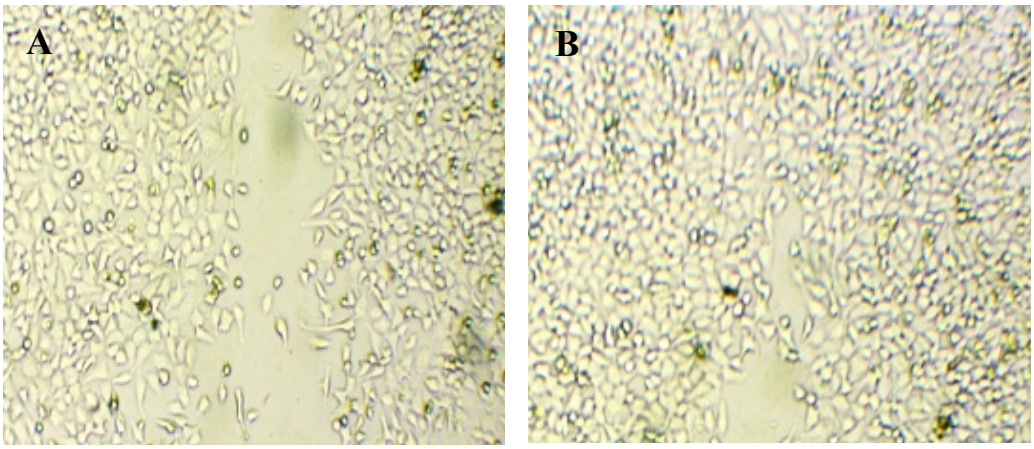

Figure 2
Wound healing assay, tratment of the keratincytes with NOD-1E
A: t=0
B: t=24h

Conclusion

According the MTT assay and would healing results it is strongly supported that the NOD-1E is not toxic in comparison to control sample. As it is observed from figure 1 and 2 the growth of healthy cells not affected from material's treatment.

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