

THERAPEUTIC POTENTIAL OF HYDROGELS BASED ON PLANT EXTRACTS AND ZINC OXIDE NANOPARTICLES IN SKIN LESIONS

Catalin Ducu¹, Georgiana Cirstea^{2*}, Sorin Moga¹, Melania Motea¹, Albert Tudor³,
Diana Popescu⁴, Alin Din², Denisa Vilcoci²

¹S.C.GLOBAL RESEARCH S.R.L., 102 Aleea Salcamilor Street, 117715 Stefanesti, Arges, Romania

²Regional Research and Development Center for Innovative Materials, Products and Processes from Automotive Industry, University of Pitesti, 11 Doaga Street, 110440 Pitesti, Arges, Romania

³National Research & Development Institute for Non-Ferrous and Rare Metals — IMNR, 102 Biruinței Street, 077145 Pantelimon, Ilfov, Romania

⁴Cantacuzino National Military Medical Institute for Research and Development, Bucharest, Romania



Abstract

Colonization of skin lesions with various infectious agents is still a major health threat. The misuse and abuse of antibiotics and synthetic drugs has led to increased resistance of pathogens and therefore difficulties in treating these medical conditions, requiring the development of new materials with antimicrobial efficacy. Thus, the aim of the study was to determine the therapeutic potential of hydrogels based on plant extracts (*Chelidonium majus* L., *Arnica montana* L., *Calendula officinalis* L., *Aristolochia clematitis* L., *Hippophae rhamnoides* L.) and zinc oxide nanoparticles in skin lesions by assessing cytotoxicity and cell viability. The two types of zinc oxide nanoparticles were obtained at 100 atmospheres and oven dried as well as at 100 atmospheres and spray dried. The data from our study suggest that none of the hydrogels tested show a notable cytotoxic effect on fibroblast culture. Dilution of the stock product in all experimental samples resulted in a cell viability of over 80%, reflecting that these hydrogels may be a therapeutic alternative in the wound healing process.

Keywords: cell viability, plant extracts, ZnO nanoparticles, wound healing.

1. INTRODUCTION

Wounds are defined as disruptions to the anatomical or functional integrity of living tissue caused by various biological, chemical, physical or mechanical factors. The process of rebuilding injured tissue is called healing. The process of tissue repair involves the activity of inflammatory cells, thrombocytes and also fibroblasts and even collagen (Clark, 2001; Sharma et al., 2021).

Wounds can be of two types: closed wounds and open wounds. Open wounds are almost always associated with fungal or bacterial microbiota or viral infection. Most infections associated with skin lesions are bacterial in nature. Although these types of infections can be managed with synthetic drugs with antibacterial activity, the problem of multidrug resistance of bacteria is

increasingly acute. Alternative therapies are therefore needed to manage these types of skin conditions (Nolff et al., 2016; Marume et al., 2017).

Since ancient times, natural compounds with antimicrobial, anti-inflammatory or antipruritic properties extracted from medicinal plants have been excellent alternatives for the management of skin lesions (Barreto et al., 2014).

There are numerous studies in the literature attesting to the therapeutic activity of medicinal plants such as *Chelidonium majus* L. (Yang et al., 2011), *Arnica montana* L. (Marzotto et al., 2016), *Calendula officinalis* L. (Fronza et al., 2009; Nicolaus et al., 2017), *Aristolochia clematitidis* L. (Cristea et al., 2010) or *Hippophae rhamnoides* L. (Gupta et al., 2006).

Studies in the literature show that a very important aspect of drug delivery systems is the control of the targeted delivery of medicines. Thus, a technique has been developed to harness the therapeutic potential of medicinal plants in wound healing, involving the incorporation of bioactive compounds into different biocompatible forms of carriers of these secondary metabolites, such as nanoliposomes, nanoemulsions, nanoparticles and nanogels. These systems have been shown to have much diminished or even no side effects, improving the dissolution rate of drug agents and their efficacy, respectively (Andreu et al., 2015; Cui et al., 2017; El-Refaeie et al., 2015; Ghayempour et al., 2016; Singla et al., 2017;)

Zinc oxide nanoparticles are increasingly used in the field of nanomedicine because they are non-toxic and compatible with biological systems. Moreover the use of these types of vectors is promising because they show high antimicrobial activity, thus accelerating the tissue regeneration process (Kaushik et al., 2019).

In a comparative experimental study of two types of burn treatments, zinc oxide was shown to be significantly more effective than silver sulfadiazine, which is a sulfa antibiotic commonly used in the treatment of bacterial wound infections and burns. Topically applied zinc oxide accelerated the processes of epithelialization, dermal maturation and scar formation, unlike the antibiotic tested (Arslan et al., 2012).

Taking all these aspects into account, the aim of the study was to determine the therapeutic potential of hydrogels based on plant extracts and zinc oxide nanoparticles in skin lesions by evaluating cytotoxicity and cell viability.

2. MATERIALS AND METHODS

Obtaining plant extracts of *Chelidonium majus* L., *Arnica montana* L., *Calendula officinalis* L., *Aristolochia clematitidis* L., *Hippophae rhamnoides* L.

Different parts of the target plants were used as plant material: *Chelidonii Herba*, *Arnicae Flos*, *Calendulae Flos*, *Aristolochiae Herba*, *Hippophae Fructus*.

Briefly the plant material, previously dried, was subjected to pulse milling for 3 minutes at 4000 rpm at a laboratory mill. For the two advanced extraction methods used in this study (ultrasound-assisted extraction-UAE, microwave-assisted extraction-MAE) a plant:solvent ratio of 1:10 and a water:pharmaceutical ethyl alcohol ratio of 30:70 (v/v) were used.

UAE was carried out for 10 minutes in the extraction vessel with cooling of the Hielscher UP200ST at 70% amplitude and MAE was carried out for 10 minutes in the extraction vessel of the Neos Milestone GR under continuous stirring. The extracts obtained were subjected to centrifugation, vacuum filtration, and evaporation of pharmaceutical ethyl alcohol (Dent, 2015).

Obtaining hydrogels based on plant extracts and zinc oxide nanoparticles

Two types of zinc oxide nanoparticles were used:

- obtained at 100 atmospheres and dried in an oven (ZnO 100.E1)
- obtained at 100 atmospheres and spray-dried (ZnO 100.SD1).

Ten formulations of hydrogels were produced, consisting of: plant extract, zinc oxide nanoparticle powder, non-ionic surfactant, carbomer, triethylamine, pure water:

R100.E1 = 10% extract *C. majus* L. + 63,95% pure water+ 0,05% ZnO 100.E1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

R100.SD1 = 10% extract *C. majus* L. + 63,95% pure water+ 0,05% ZnO 100.SD1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

A100.E1 = 10% extract *A. montana* L. + 63,95% pure water+ 0,05% ZnO 100.E1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

A100.SD1 = 10% extract *A. montana* L. + 63,95% pure water+ 0,05% ZnO 100.SD1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

G100.E1 = 10% extract *C. officinalis* L. + 63,95% pure water+ 0,05% ZnO 100.E1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

G100.SD1 = 10% extract *C. officinalis* L. + 63,95% pure water+ 0,05% ZnO 100.SD1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

ML100.E1 = 10% extract *A. clematitis* L. + 63,95% pure water+ 0,05% ZnO 100.E1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

ML100.SD1 = 10% extract *A. clematitis* L. + 63,95% pure water+ 0,05% ZnO 100.SD1 + 25% non-ionic surfactant + 0,5% carbomer + 0,5% triethylamine

C100.E1 = 10% extract *H. rhamnoides* L. + 63,95% pure water+ 0,05% ZnO 100.E1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

C100.SD1 = 10% extract *H. rhamnoides* L. + 63,95% pure water+ 0,05% ZnO 100.SD1 + 25% non-ionic surfactant+ 0,5% carbomer + 0,5% triethylamine

Half the amount of non-ionic surfactant and nanoparticle powder was added to half the mixture of pure water and plant extract, homogenised and heated on a laboratory hotplate. This system was then sonicated for better dispersion of the nanoparticles. At the same time, in the other half of the mixture: distilled water: extract, also subjected to homogenization, carbomer was added until solidification and the remaining non-ionic surfactant. A final step in obtaining the hydrogel consisted of homogenising the two mixtures and adding the pH modifier, in this case triethylamine. The hydrogels thus obtained were stored in plastic vials at 4°C.

***In vitro* biocompatibility testing of hydrogels. MTT test.**

The MTT assay is based on the cleavage of MTT tetrazolium yellow salt by viable cells to form a purple formazan. A decrease in the number of viable cells causes a decrease in metabolic activity in the culture tested. This reduction is directly related to the amount of purple formazan formed. Cell proliferation with the MTT assay and cell viability after treatment with the test substance are quantified spectrophotometrically at 500-600 nm (Ogbole et al., 2017).

The 3T3 fibroblast cell line was used to perform the cytotoxicity test (MTT) of the 10 experimental models (hydrogels).

Line 3T3 was cultured using Dulbecco's Modified Eagle's Medium (DMEM). Fetal bovine serum (FBS) and 0.05 µg/ml antibiotic was added to the medium used.

After trypsinization, centrifugation and resuspension in specific growth medium, cells were stained with Trypan blue solution for quantification.

The 10 test samples were subjected to extraction (as recommended by ISO10993-12).

The fibroblast cell line together with the test samples (in binary serial dilutions) were incubated for 24 hours at 37°C, 5% CO₂. After addition of MTT reagent all the samples were subjected to spectrophotometric analysis at 570 nm wavelength.

Cell line viability (CV) was determined using the formula (Karakas et al., 2017):

$$\% \text{ CV} = (\text{positive control-blank})/(\text{negative control-blank}) \times 100$$

Where:

positive control = cells + compound (hydrogel) + MTT + solvent MTT

negative control = cells + MTT + solvent MTT

blank = medium (complete growth) + MTT + solvent MTT

3. RESULTS AND DISCUSSIONS

In the initial stages of the wound healing process, fibroblasts play a vital role by actively proliferating, migrating into the wound area and inducing the formation of new extracellular matrix (Li et al., 2004). Studies in the literature, including the experimental study by Kumar et al., 2007, attest to the fact that a variety of herbs can be traditionally used in folk medicine showing potential in wound healing.

The results of our study highlighted in Figure 1 show that:

- the 10 experimental variants showed low cytotoxicity with no significant difference between the plant extracts used;
- the cytotoxicity of the samples was directly proportional to the concentration of the test product;
- the highest cell viability was recorded for the highest dilutions;
- it is noted that cell viability on fibroblasts had values above 70% starting from the stock sample for all 10 experimental models.

Of all 10 hydrogel formulations tested, the highest percentage of fibroblast cell viability was calculated for sample R100SD1, representing the variant based on *A. clematitis* L. extract and spray-dried zinc oxide nanoparticles.

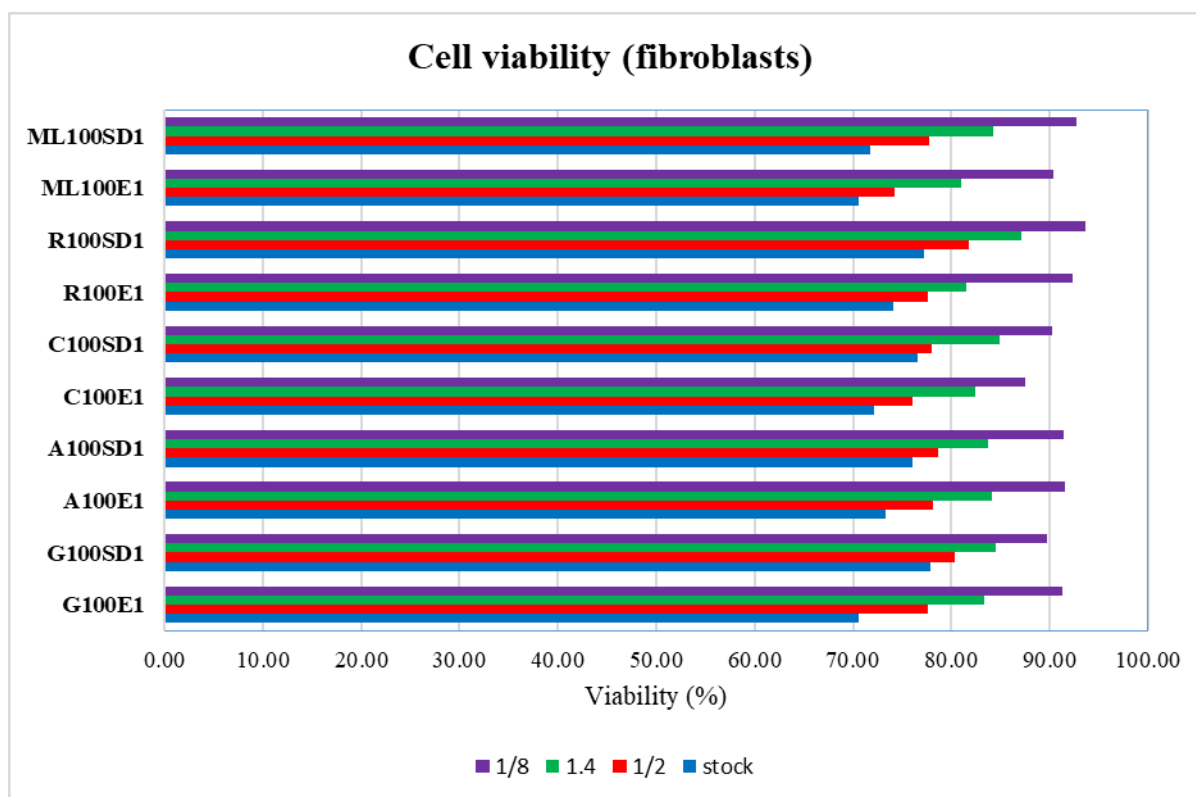


Figure 1. Fibroblast cell viability for the 10 experimental models

4. CONCLUSIONS

The data from our study suggest that none of the hydrogels tested exhibit a notable cytotoxic effect on fibroblast culture. Dilution of the stock product in all experimental samples resulted in a cell viability of over 80%, reflecting that these hydrogels may be a therapeutic alternative in the wound healing process.

5. ACKNOWLEDGEMENTS

This work was supported by a grant of the Romanian Ministry of Research, Innovation and Digitization, CNCS– UEFISCDI, **GELINT FINANCING CONTRACT NO. 18PTE/2020, project number PN-III-P2-2.1-PTE-2019-0314.**

6. REFERENCES

- Abbassi, K., Mergaoui, L., Atay-Kadiri, Zn., Stambouli, A., Ghaout, S. (2003). Effets des extraits de *Peganum harmala* (*Zygophyllaceae*) sur le criquet pèlerin (*Schistocerca gregaria*, Forskål, 1775) [Effects of *Peganum harmala* (*Zygophyllaceae*) extracts on desert locust (*Schistocerca gregaria*, Forskål, 1775)]. *Zoologica baetica*, 14, 203-217.
- Andreu, V., Mendoza, G., Arruebo, M., Irusta, S. (2015). Smart dressings based on nanostructured fibers containing natural origin antimicrobial, anti-inflammatory, and regenerative compounds. *Materials*, 8, (8), 5154–5193.
- Arslan, K., Karahan, O., Okuş, A., Unlü, Y., Eryılmaz, M.A., Ay, S., Sevinç, B. (2012). Comparison of topical zinc oxide and silver sulfadiazine in burn wounds: an experimental study. *Ulus Travma Acil Cerrahi Derg*, 18, (5), 376-383.
- Barreto, R.S., Albuquerque-júnior, R.L., Adriano, A.A., Almeida, J.R., Santos, M.R., Barreto, A.S., DeSantana, J.M., Siqueira-Lima, P.S., Quintans, J.S., Quintans-Júnior, L.J. (2014). A systematic review of the wound-healing effects of monoterpenes and iridoid derivatives. *Molecules*, 19, 846–862.

- Clark, R.A. (2001). Fibrin and wound healing. *Annals of the New York Academy of Sciences*, 936, 355-367.
- Cristea, M.I., Gruia, A.T., Anghel, S., Gai, E., Tatu, C., Paunescu, V. (2010). *Aristolochia clematitis* in medicine: the good and the bad. *Fiziologia – Physiology*, 20(1), 26-28.
- Cui, M.D., Pan, Z.H., Pan, L.Q. (2017). Danggui buxue extract-loaded liposomes in thermosensitive gel enhance *in vivo* dermal wound healing via activation of the VEGF/PI3K/Akt and TGF- β /Smads signaling pathway. *Evidence-based complementary and alternative medicine*. eCAM, 8407249.
- Dent, M. (2015). Comparison of conventional and ultrasound-assisted extraction techniques on mass fraction of phenolic compounds from sage (*Salvia officinalis* L.). *Chemical and Biochemical Engineering Quarterly*, 29, 475-484.
- El-Refaie, W.M., Elnaggar, Y.S., El-Massik, M.A., Abdallah, O.Y. (2015). Novel curcumin-loaded gel-core hyalurosomes with promising burn-wound healing potential: Development, *in-vitro* appraisal and *in vivo* studies. *International journal of pharmaceutics*, 486, (1-2), 88–98.
- Fronza, M., Heinzmann, B., Hamburger, M., Laufer, S., Merfort, I. (2009). Determination of the wound healing effect of *Calendula* extracts using the scratch assay with 3T3 fibroblasts. *Journal of Ethnopharmacology*, 126(3), 463-467.
- Ghayempour, S., Montazer, M., Mahmoudi, Rad, M. (2016). Encapsulation of *Aloe vera* extract into natural tragacanth gum as a novel green wound healing product. *International journal of biological macromolecules*, 93, 344–349.
- Gupta, A., Kumar, R., Pal, K., Singh, V., Banerjee, P.K., Sawhney, R.C. (2006). Influence of sea buckthorn (*Hippophae rhamnoides* L.) flavone on dermal wound healing in rats. *Molecular and Cellular Biochemistry*, 290(1-2), 193-198.
- Karakaş, D., Ari, F., & Ulukaya, E. (2017). The MTT viability assay yields strikingly false-positive viabilities although the cells are killed by some plant extracts. *Turkish journal of biology = Turk biyoloji dergisi*, 41(6), 919–925.
- Kaushik, M., Niranjan, R., Thangam, R., Madhan, B., Pandiyarasan, V., Ramachandran, C., Oh, D.H., Devanand Venkatasubbu, G. (2019). Investigations on the antimicrobial activity and wound healing potential of ZnO nanoparticles. *Applied Surface Science*, 479, 1169-1177.
- Kumar, B., Vijayakumar, M., Govindarajan, R., Pushpangadan, P. (2007). Ethnopharmacological approaches to wound healing—exploring medicinal plants of India. *Journal of Ethnopharmacology*, 114, (2), 103–113.
- Li, W., Fan, J.H., Chen, M., Guan, S.X., Sawcer, D., Bokoch, G.M., Woodley, D.T. (2004). Mechanism of human dermal fibroblast migration driven by type I collagen and platelet-derived growth factor-BB. *Mol. Biol. Cell.*, 15, 294–309.
- Marume, A., Matope, G., Katsande, S., Khoza, S., Mutingwende, I., Mduluzi, T., Munodawafa-Taderera, T., Ndhkala, A.R. (2017). Wound healing properties of selected plants used in ethnoveterinary medicine. *Frontiers in Pharmacology*, 8, 544.
- Marzotto, M., Bonafini, C., Olioso, D., Baruzzi, A., Bettinetti, L., Di Leva, F., Galbiati, E., Bellavite, P. (2016). *Arnica montana* stimulates extracellular matrix gene expression in a macrophage cell line differentiated to wound-healing phenotype. *PLOS ONE*, 11(11), e0166340.
- Nicolaus, C., Junghanns, S., Hartmann, A., Murillo, R., Ganzera, M., Merfort, I. (2017). *In vitro* studies to evaluate the wound healing properties of *Calendula officinalis* extracts. *Journal of Ethnopharmacology*, 196, 94-103.
- Nolff, M.C., Reese, S., Fehr, M., Dening, R., Meyer-Lindenberg, A. (2016). Assessment of wound bio-burden and prevalence of multi-drug resistant bacteria during open wound management. *Journal of Small Animal Practice*, 57, 255–259.
- Ogbole, O.O., Segun, P.A., Adeniji, A.J. (2017). *In vitro* cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC Complement Altern Med*, 17, 494.
- Sharma, A., Khanna, S., Kaur, G., Singh, I. (2021). Medicinal plants and their components for wound healing applications. *Future Journal of Pharmaceutical Sciences*, 7, 53.
- Singla, R., Soni, S., Kulurkar, P.M., Kumari, A., Mahesh, S., Patial, V., Padwad, Y.S., Yadav, S.K. (2017). In situ functionalized nanobiocomposites dressings of bamboo cellulose nanocrystals and silver nanoparticles for accelerated wound healing. *Carbohydrate polymers*, 155, 152–162.
- Yang, G., Lee, K., Lee, M.H., Kim, S.H., Ham, I.H., Choi, H.Y. (2011). Inhibitory effects of *Chelidonium majus* extract on atopic dermatitis-like skin lesions in NC/Nga mice. *Journal of Ethnopharmacology*, 138(2), 398–403.