Vasile Goldiș Western University of Arad

HABILITATION THESIS

HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL AND ULSTRASTRUCTURAL BIOMARKERS IN TOXICOLOGY, PHYTOTHERAPY AND TISSUE ENGENEERING STUDIES

ABSTRACT

AUTHOR:

Professor HERMENEAN ANCA, PhD

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The habilitation thesis entitled ***Histopathological, Immunohistochemical and Ultrastructural Biomarkers in Toxicology, Phytotherapy and Tissue Engineering Studies*** presents personal research conducted between 2004 and 2015, more specifically since obtaining my PhD in Biology and up until now.
This thesis mainly comprises results published in original and significant scientific papers in ISI journals.
The thesis consists of scientific contributions prior to obtaining my PhD, scientific and professional achievements corresponding to the 4 main thematic directions approached in postdoctoral research studies, a career progression and development plan and bibliographic references corresponding to the contents of the previous sections.
The first part of the thesis is structured into six chapters. Chapter 1 presents scientific contributions prior to obtaining my PhD in Biology, the objectives pursued and how the outcomes were leveraged.
Chapters 2, 3, 4 and 5 present in detail the scientific contributions published in the main stream, namely in ISI journals, separated into the 4 different thematic areas:

1. Morphological alterations of the liver and digestive tract induced under experimental conditions. Hepatoprotective effects of natural biocompounds.

Objectives:

- to investigate the hepatoprotective effects of natural compounds administered and establish the protective mechanism for the *Berberis vulgaris* extract, the flavonoid Naringenin, the native oil obtained from seeds of *Silybum marianum*, the formulated complex of native oil obtained from seeds of *Silybum marianum* in SMEDDS (Self-Micro Emulsifying Drug Delivery System);
- to investigate the effects of natural biocompounds (silymarin) in reducing the severity of mucositis symptoms following the administration of cytostatic drugs (epirubicin);

Publications:

1. Hermenean A., Popescu C., Ardelean A., Stan M., Hadaruga N., Mihali C.V., Costache M., Dinischiotu A. 2012. Hepatoprotective Effect of Berberis vulgaris L.extract/beta-cyclodextrin on carbon tetrachloride – induced acute toxicity in mice, *International Journal of Molecular Sciences*, 13, 9014-934, IF= 2,339.

2. **Hermenean A.**, Ardelean A., Stan M., Hadaruga N., Mihali C.V., Costache M., Dinischiotu A. 2014. Antioxidant and Hepatoprotective Effects of Naringenin and Its b-Cyclodextrin Formulation in Mice Intoxicated with Carbon Tetrachloride: A Comparative Study, *Journal of Medicinal Food*, 17(6): 670-7, IF= 1,699

3. **Hermenean A.**, Stan M., Ardelean A., Pilat L., Mihali C.V., Popescu C., Nagy L., Deák G., Zsuga M., Kéki S., Bácskay I., Fenyvesi F., Costache M., Dinischiotu A.,

Vecsernyés M., Antioxidant and hepatoprotective activity of milk thistle (Silybum marianum L. Gaertn.) seed oil, *Open Life Science*, 10: 147–158, IF= 0,633

4. Sasu A., Herman H., Mariasiu T., Rosu M., Balta C., Anghel N., Miutescu E., Cotoraci C., **Hermenean** A. 2014. Protective effects of silymarin on epirubicin - induced mucosal barrier injury of the gastrointestinal tract, *Drug an Chemical Toxicology*, on-line: 1-10, DOI:10.3109/01480545.2014.992072, IF= 1,098,

5. European patent *"Pharmaceutical composition useful for preventing and treating liver disease, comprises oil isolated from Silybum marianum seed"*, Patent Number: WO2013124700-A2; HU201300110-A1, Patent assignee: Univ. Debrecen, Hungary and Vasile Goldis Western University of Arad, Romania
Inventors: **Anca HERMENEAN**, Aurel ARDELEAN, Miklós ZSUGA, Sándor KÉKI, György DEÁK, Cristina POPESCU, Zoltán UJHELYI, Ildikó BÁCSKAY, Gavril ARDELEAN, Marieta COSTACHE, Anca DINISCHIOTU, Violeta TURCUS, Ciprian MIHALI, Miklós VECSERNYÉS, Ferenc FENYVESI, Tímea KISS, Judit VÁRADI, FEHÉR Pálma SIPOSNÉ

2. Renal morphological alterations induced by xenobiotics and mycotoxins in vitro and in vivo. Protective mechanisms of natural antioxidants.

Objectives:

- to establish renal cytotoxic and tissue mechanisms of xenobiotics and mycotoxins;
- to investigate the protective renal effects of natural antioxidants.

Publications:

1. Dinu D., Bodea G.O., Ceapa C.D., Munteanu M.C., Israel Roming F., **Hermenean A.**, Costache M., Zărnescu O., Dinischiotu A. 2011. Adapted response of the antioxidant defense system to oxidative stress induced by deoxynivalenol in Hek-293 cells, *Toxicon*, 57 (7): 1023-1024, IF=2,581
2. **Hermenean A.**, Ardelean A., Stan M., Herman H., Mihali C.V., Costache M., Dinischiotu A. 2013. Protective effects of naringenin on carbon tetrachloride-induced acute nephrotoxicity in mouse kidney, *Chemico-Biological Interactions*, 205 (2): 138-147, IF=2,982

3. Biomarkers correlated to oxidative stress induced by nanoparticles at cellular and subcellular level

Objectives:
- to investigate the specific antioxidant response of MRC-5 cells and human pulmonary cells to Si/SiO_2 QDs nanoparticles by analyzing the redox status and the intracellular distribution of glutathione;
- to establish the *in vitro* protective effect of silver-doped hydroxyapatite;
- to investigate the effects of α-Fe2O3 (hematite) nanoparticles on MRC-5 pulmonary fibroblasts by analyzing the function of the antioxidant system and lipid peroxidation;

Publications:
1. Munteanu M.C., Radu M., **Hermenean A.**, Sima C., Dinu D., Costache M., Grigoriu G., Dinischiotu A. 2010. Antioxidative response induced by SiO_2 nanoparticles in MRC5 cell line, *Romanian Biotechnological Letters*, 15 (1): 5000-5007, IF=0.343
2. Radu M., Munteanu M.C., Petrache S., Serban A.I., Dinu D., **Hermenean A.**, Costache M., Dinischiotu A. 2010. Depletion of intracellular glutathione and increase of lipid peroxidation mediate cytotoxicity of hematite nanoparticles in MRC-5 cells, *Acta Biochimica Polonica*, 57 (3): 355-260, IF=1,389
3. Ciobanu C.S., Iconaru S.L., Pasuk I., Vasile B.S., Lupu A.R., **Hermenean A.**, Dinischiotu A., Predoi D. 2013. Structural properties of silver doped hydroxyapatite and their biocompatibility, *Materials Science and Engineering: C*, 33 (3):1395-1402, IF=2,736
4. Stan M.S., Memet I., Sima C., Popescu T., Teodorescu V.S., **Hermenean A.**, Dinischiotu A. 2014. Si/SiO2 quantum dots cause cytotoxicity in lung cells through redox homeostasis imbalance. *Chemico-Biological Interactions,* 220: 102–115, IF=2,982

4. ***Biomarkers in tissue engineering studies***

Objectives:
- to develop a new composite 3D scaffold based on the natural polymer chitosan and proportion of GO allowing the efficient interaction of cells with the scaffold; - to evaluate its cytocompatibility with cells of the pre-osteoblast type in terms of cytotoxicity and the ability to sustain metabolic activity and cell proliferation.
- to evaluate the cytocompatibility of composite PSF/GO membranes in contact with murine mesenchymal stem cells (MSC), in order to characterize the degree of applicability in tissue engineering;
- to establish the appropriate composition of collagen/sericin supports to sustain cellular processes and evaluate the biocompatibility of this new scaffold based on collagen and sericin, enriched with prochondrogenic factors type HA and CS, in

relation to stem cells isolated from adipose tissue (ASC), utilized in cartilage regenerative medicine;
-to evaluate the biocompatibility of gelatin/alginate/polyacrylamide scaffolds and their ability to sustain chondrogenesis processes;
- to evaluate the ability of stem cells isolated from adipose tissue (ASC) to generate mature and functional chondrocytes in these 3D structures

Publications:
1. Dinescu S., Galateanu B., Radu E., **Hermenean A**., Lungu A., Stancu I. C., Jianu D., Tumbar T., Costache M. 2015. A 3D porous gelatin-alginate-based-IPNs acts as an efficient promoter of chondrogenesis from human adipose-derived stem cells, *Stem Cells International ,* article ID 252909, IF=2.806
2. Ionita M., Vasile E., Crica L.E., Voicu S.I., Pandele A.M., Dinescu S., Predoiu L., Galateanu B., **Hermenean A**., Costache M. 2015. Synthesis, characterization and in vitro studies of polysulfone /graphene oxide composite membranes, *Composites: Part B*, vol. 72, 108-115, IF: 2,602
3. Dinescu S., Ionita M., Pandele A.M., Galateanu B., Iovu H., Ardelean A., Costache M., **Hermenean A.** 2014. In vitro cytocompatibility evaluation of chitosan/graphene oxide 3D scaffold composites designed for bone tissue engineering, *Bio-Medical Materials and Engineering*, 24: 2249–2256, IF=0.847
4. Dinescu S., Galateanu B., Albu M., Lungu A., Radu E., **Hermenean A.**, Costache M. 2013. Biocompatibility Assessment of Novel Collagen-Sericin Scaffolds Improved with Hyaluronic Acid and Chondroitin Sulfate for Cartilage Regeneration, *Journal of Biomedicine and Biotechnology* (BioMed Research International), ID 598056, 11 pages, IF=2,706

Each of these 4 chapters begins with a presentation of the level of knowledge in that specific field and a statement of research objectives. Chapter 6 lists the publications included in this habilitation thesis.

The second part of the habilitation thesis presents the main strategies for professional career progression and development. This part comprises 3 chapters. The first chapter present research conducted as part of ongoing scientific projects. The second chapter describes research directions and how they are implemented for future research projects, whereas the last chapter presents plans for teaching and academic career development in histology.

My work, materialized in the scientific papers published throughout this period, was financed through the various national and international research projects I have supervised as a manager or responsible:

1. Hungary-Romania Cross-Border Cooperation Programme 2007-2013 CBC/1101/173 "**Integrated Cross-Border Research Platform to Identify Cellular Processes as Potential Targets for Personalized Cancer Therapies"**; implementation period: 2013-2015; Coordinator: Vasile Goldis Western University of Arad; Partners: University of Sezged and Biological Research Centre of Hungarian

Academy of Science, Szeged, Hungary

2. Hungary-Romania Cross-Border Cooperation Programme 2007-2013, CBC 1001/311 **"Establishing the homeostasis status of crayfish population in Cris and Mures rivers crossing the Romanian-Hungarian border"**; implementation period: 2012-2013; Coordinator: Research Institute for Fisheries, Aquaculture and Irrigation (HAKI); Partener: Vasile Goldis Western University of Arad

3. Hungary-Romania Cross-Border Cooperation Programme 2007-2013, CBC HURO/0901/058/2.2.2. "**Research on the development of *Sylibum marianum* nanoparticles with high bioavailability**"; implementation period: 2009-2011; Coordinator: University of Debrecen, Hungary; Partners: Vasile Goldis Western University of Arad

4. PN-II-PCCA-140 **"Developing new graphene-polymercomposites biomaterials for scaffold fabrication with aplicability in bone repair by coupling multiscale molecular modelling and experiments"**; perioada de implementare: 2012-2016; Coordinator: Politehnica University of Bucharest; Parteners: University of Bucharest, Vasile Goldis Western University of Arad, National Institute for Laser, Plasma & Radiation Physics (INFLPR), University Dunarea de Jos of Galati

5. Programme 4 Partnerships in priority areas, Area 6 Biotechnologies, National Research Plan II 2007-2013, contract no. 62072/2008, contract no. 62072/2008 " **Hepatoprotective nanoparticles with increased bioavailability**", implementation perios: 2008-2011; Coordinator: Vasile Goldis Western University of Arad; Parteners: Babes-Bolyai University, University of Bucharest, Politehnica University of Timisoara, King Michael Banat University of Agricultural Sciences and Veterinary Medicine Timisoara

6. Romanian Academy Grant – GAR nr. 89/2006: **"Research on the involvement of xenobiotics in hepatic pathology and hepatoprotection achieved by natural bioprotectors of vegetal origin" ,** perioada de implementare: 2006-2008 ; pozitia in proiect: director proiect

7. Grant Academia Romana - GAR nr. 64/2005 **"Structural-functional correlations regarding the effects of bioactive extracts from Chelidonium majus and Berberis vulgaris at hepatic and renal level"** ; perioada de implementare: 2005-2006 ; pozitia in proiect: director proiect

The research studies whose results are comprised in the habilitation thesis were conducted at the Institute for Life Sciences within Vasile Goldiș Western University of Arad, using its research infrastructure, especially in the area of morphological investigations: histopathology laboratory (microtomes, thermostats, SLEE- MAINZ-MNT cryostat), optical and fluorescence microscopy (Olympus BX43 microscope), confocal microscopy (Leica TCS SP8 microscope), electron microscopy (Tecnai 12 Biotwin TEM; Quanta 250 Scanning Electron Microscope), flow cytometry (Beckman Coulter, Cytomics FC 500), cell cultures (cell culture hood, phase contrast

microscope, CO_2 incubator), molecular biology (real-time PCR Qiagen, BIRAD electrophoresis and ChemiDoc gel imaging station (Bio-Rad), centrifuges, icemaker, refrigerators, -20°C and -80°C freezers, plus Animal Facility with IVC systems for animal hosting, with automatic control of light-dark cycles and electronic control of air ventilation, operating room, In vivo Xtreme - Carestreem imaging system (capturing images in fluorescence, luminescence, radioisotopes, Rx).