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PhD THESIS

**ORAL CAVITY DISEASES AND INNOVATIVE PREVENTIVE
TREATMENTS BASED ON NANOPARTICLES LOADED WITH
BIOMOLECULES**

A B S T R A C T

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TABLE OF CONTENTS

List of published articles	VI
List of abbreviations	VII
Index of Figures	VIII
Index of Tables	XII
Acknowledgment.....	XIII
INTRODUCTION	XV
 GENERAL PART	 1
CHAPTER1. ORAL CAVITY AND FREQUENT AFFECTIONS	1
1.1 Introduction	1
1.2 Bacteria and oral diseases	5
1.2.1 Bacteria and biofilm formation	5
1.2.2 Factors that influence the oral microbiota	10
1.2.3 The link between the oral microbiome and oral diseases.....	16
CHAPTER 2. MEDICINAL PLANTS USED IN ORAL DISEASES	19
2.1 Introduction	19
2.2 Medicinal plants used in oral hygiene products	21
CHAPTER 3. TYPES OF MODERN FORMULATIONS FOR INCREASING THE BIOAVAILABILITY OF ACTIVE COMPOUNDS.....	25
3.1 General presentation.....	25
 SPECIAL PART	 32
CHAPTER 4. EVALUATION OF THE CYTOTOXICITY OF LIPOSOMES LOADED WITH BIOLOGICALLY ACTIVE SUBSTANCES ON ORAL TUMOR CELLS.....	34
4.1 Introduction	34
4.2 Materials and methods	37
4.2.1 Reagents.....	37
4.2.2 Preparation and characterization of botulin liposomes.....	37
4.2.3 Cell culture	38
4.2.4 Cellular viability assessment.....	38
4.2.5 Cellular morphology.....	39
4.2.6 Nuclear staining.....	39
4.2.7 Statistical analysis	40
4.3 Results.....	40
4.3.1 Cellular viability assessment.....	40
4.3.2 Cellular morphology assessment.....	43

4.3.3 Nuclear morphology assessment.....	47
4.4. Discussions.....	51
4.5. Conclusions	53
CHAPTER 5. ASSESSMENT OF THE CYTOTOXIC POTENTIAL OF RUTIN FORMULATIONS ON HUMAN ORAL CELLS.....	54
5.1 Introduction	54
5.2 Materials and methods.....	57
5.2.1 Reagents.....	57
5.2.2 Preparation and characterization of liposomes	58
5.2.3 Cell culture	58
5.2.4 Cellular viability assessment.....	59
5.2.5 Statistical analysis	59
5.3 Results.....	60
5.4 Discussions.....	66
5.5 Conclusions	70
CHAPTER 6. ASSESSMENT BY IN VITRO METHODS THE CYTOTOXIC PROPERTIES OF PHYTOCOMPOUNDS.....	71
6.1 Introduction	71
6.2 Materials and methods.....	73
6.2.1 Reagents.....	73
6.2.2 Cell culture	73
6.2.3 Cellular viability assessment.....	74
6.2.4 Cellular morphology and confluence evaluation.....	74
6.2.5 Nuclear morphology assessment.....	74
6.2.6 Senescence detection	75
6.2.7 Statistical analysis	75
6.3 Results.....	76
6.3.1 Cellular viability assessment.....	76
6.3.2 Cellular morphology assessment.....	78
6.3.3 Nuclear morphology assessment.....	80
6.3.4 Senescence assessment.....	83
6.4 Discussions.....	84
6.5 Conclusions	89
GENERAL CONCLUSIONS AND PERSONAL CONTRIBUTIONS.....	90
REFERENCES	93
ANNEX	I

ABSTRACT

Diseases, both chronic and acute, among the population are increasingly common and, as expected, include all age groups. The research carried out in different areas related to the dental field highlighted the major role that the oral cavity plays in the propagation of systemic diseases. An optimal state of health of the oral cavity makes a major contribution to the general well-being of the entire human body and has an obvious impact on the quality of life. Oral diseases, which are often accompanied by severe pain, damage normal physical functions (for example speaking, eating), affecting social life at the same time. In general, most oral pathologies can be prevented, but considering that they are very widespread throughout life and have important adverse reactions, it is necessary to study them in detail and pay them due attention.

Oral tumor diseases are mostly associated with carcinomas of the oral cavity, which in turn involve a vast category of neoplasms. The incidence of oral malignant tumors is increasing in our country, it is among the most frequently developed malignant diseases and is directly influenced by factors such as tobacco and alcohol consumption. Squamous cell carcinomas of the head and neck generally include epithelial cancers, including those of the lips, pharynx, larynx, and salivary glands.

The therapeutic protocols involve several interventions (surgical, chemotherapeutic, radiotherapeutic) which are often followed by significant adverse effects. The need to find complementary and alternative methods is of major importance. Natural resources are rich, they provide a series of compounds with important and varied pharmacological actions. The disadvantage of using compounds of natural origin is mainly related to their poor bioavailability, thus reducing the mode of action and disease fighting. Lipid-based systems are evaluated as effective carrier systems in delivering natural compounds, due to the fact that lipids are biodegradable and can enhance transcellular transport by disrupting lipid bilayers. In dentistry, liposomes have been used in a series of actions, including the prevention of caries, combating gingivitis and even in the treatment of oral lesions and periodontitis. The potential of liposome-based transport systems in the treatment of ulcerated oral mucosa was investigated and the results obtained were promising.

The present thesis is structured in two parts and includes the general part and the special part. In the general part, current aspects related to the oral cavity, pathologies of the oral cavity, natural resources, and their role in addressing oral pathologies and, last but not least, current techniques for obtaining transport systems for pharmacologically active biomolecules are treated. In the special part, the original research directions are presented.

The topic addressed is of international interest, since oral pathologies, whether they are acute or chronic, leave their mark on the state of health. Moreover, malignant pathologies are on the rise and therapeutic protocols are not without consequences, both in the short term and in the long term, and the results are very different and depend on several factors. At the national level, oral cancers occupy a prominent place, prevention and control measures being imperatively necessary. The search for complementary and alternative methods to combat the increased incidence of oral malignant pathologies is a topic of real interest, starting from the level of the regional research group and up to the highest international levels.

The research methods that were the basis of the experimental studies are recognized and used internationally. By applying them, reproducible results are obtained, and specialized protocols are developed that are the basis of research starting from the preclinical stage until the introduction into clinical trials if the results obtained in the preclinical are of interest.

Dental diseases are mostly chronic and progressive diseases. The general state of health is most often affected by some of the most common oral pathologies, dental caries, periodontal diseases, and oral malignancies. The latest statistics rank our country among the most affected countries in terms of the general rate of occurrence and progression of oral malignant diseases. Oral melanoma is an extremely rare malignant disease, which is known for its rapid evolution and aggressiveness. The disease represents a challenge in terms of treatment because most of the time, it is discovered at an advanced stage and dentists play a key role, in terms of prompt diagnosis and the major contribution related to the diagnosis.

The therapeutic approach to these conditions generally consists of a combination of surgical procedures with chemotherapy, immunotherapy, hormonal therapy. However, these treatments are not without effects, and the vast majority of side effects are often severe. Alternative methods are intensively sought and studied at present, being necessary therapeutic approaches that present effective therapeutic effects, and the side effects are as less severe as possible.

Over time, resources of natural origin (plants and natural compounds) have shown their pharmacological capacity in numerous types of pathologies (acute and chronic), as well as in the case of malignant diseases. Despite these demonstrated beneficial actions, the effectiveness of products of natural origin, of compounds derived from natural sources, as well as of complexes and preparations obtained from natural resources, is strongly influenced by stability and bioavailability. Currently, these drawbacks are addressed either by synthesizing complexes to obtain hydrophilic formulations, or by producing a derivatization to obtain more water-soluble molecules. Some of the transport systems frequently used in the medical field are represented by liposomes. These are phospholipid,

biocompatible vesicles that have the property of incorporating an extremely varied range of bioactive molecules (hydrophobic, hydrophilic and amphiphilic).

Considering the above, the main objectives of the current thesis were:

- determining the efficiency of incorporating a pentacyclic triterpene with proven pharmacological properties (betulin) into liposomes (obtaining a betulin release system) and evaluating the cytotoxicity of the formulation obtained, on pharyngeal tumor cells (Detroit-562 cells).
- determining the efficiency of incorporating a glycosylated flavonoid with proven pharmacological properties (rutin) into liposomes (obtaining a rutin release system) and evaluating the cytotoxicity of the formulation obtained, on pharyngeal tumor cells (Detroit-562 cells) and squamous carcinoma cells (SCC-4 cells).
- offering an *in vitro* perspective on the cytotoxic potential of a glycosylated flavonoid (rutin) on melanoma cells, both of epithelial and polygonal morphology, by evaluating the impact on cell viability and morphology but also on the nuclear aspect and the properties related to the induction of senescence.

The aim of the first study was to determine the efficiency of betulin incorporation into liposomes and to evaluate their cytotoxicity on pharyngeal cancer cells (Detroit-562 cells). More precisely, it was desired to obtain a betulin release system, with a strong cytotoxic effect in the case of oral tumor cells.

The therapeutic actions of phytochemicals are well known in various pathologies, but most of the time natural compounds cannot be administered due to their low solubility, and it is necessary to find suitable formulations to increase their biomedical properties. The present study revealed the efficiency of betulin incorporation into liposomes and the cytotoxic effect of liposome-based formulation on pharyngeal cancer cells (Detroit-562) by morphology, viability, and nuclear staining analyses. Depending on the concentrations tested, betulin liposomes showed a pronounced cytotoxic effect, with a decrease in cell viability. Additionally, changes in cell morphology were observed with signs of cell death, with cells becoming round and then detached from the plaque. The liposomal system also significantly decreased the number of nuclei, causing nuclear fragmentation and the formation of apoptotic bodies.

The aim of the second study was to obtain a rutin-loaded liposomal formulation and test it on oral cancer cells (SCC-4 and Detroit-562 cell lines) compared to the healthy gingival keratinocyte (PGK) cell line, in terms of identifying possible beneficial effects on oropharyngeal carcinomas.

Local factors on the periodontal support tissues lead to the appearance of chronic inflammatory phenomena that result in the appearance and development of periodontitis.

One of the current therapeutic targets focuses on the regeneration of the affected periodontal tissue and more recently the engineering techniques associated with the periodontal tissue have gained considerable ground becoming successful therapeutic protocols in terms of regeneration, recovery of functionality. The periodontal ligaments are made up of numerous stem cells of the periodontal ligament (undifferentiated mesenchymal stem cells), which have multidirectional regeneration and differentiation capacity, contributing to the restoration of the periodontal tissue, maintaining the dynamic balance, and regulating the regeneration of the periodontal tissue. It was highlighted that these constituent cells of the periodontal ligament are some of the most useful types of cells that contribute to the regeneration processes of the affected periodontal tissue, continuously differentiating and proliferating, having the ability to secrete growth factors that contribute to refreshing the environment locally and finally regeneration of the affected tissue occurs. In general, chronic inflammatory processes are associated with the possibility of the development of tumor cells.

Treatments for malignant diseases are recognized for their aggressiveness and the multitude of adverse effects exerted. For years, efforts were made in search for different treatments, either preventive or curative, that do not produce adverse effects or that the adverse effects produced are significantly reduced.

Oral and oropharyngeal cancers are some of the most common types of cancer worldwide and present an alarming health problem. Available treatment options are radiotherapy, surgery and chemotherapy with cisplatin or cetuximab. However, these treatment options induce severe acute toxicities, swallowing dysfunction, and long-term morbidities and call for improvements and/or replacement.

The therapeutic efficacy associated with medicinal plants in the case of malignant tumor cells has attracted the attention of researchers from all over the world. Flavonoids are among the most studied phytocomponents, especially due to their significant presence in medicinal plants. The consumption of medicinal plants and derived products has proven to be beneficial for human health, especially due to the significant impact shown in the reports that studied their effectiveness against various viral, allergenic, inflammatory, oxidative stress, etc. A series of studies have highlighted the ability of flavonoids and proanthocyanidins in terms of preventing oral and head cancers and reducing the incidence of malignant pathologies associated with the colon, breast, kidneys and ovaries, thus becoming potential candidates for antitumor therapeutic protocols.

Nanotechnology is the science that aims to obtain particles of nanometric size, located in the range of 1-100 nm, with the aim of obtaining a significant increase in surface area and improving physical and chemical properties in terms of bioavailability and stability. Therefore, nanotechnology has found its applications in various extremely varied fields,

including the medical fields. In the case of medical fields, the synthesized nanoparticles must meet a series of characteristics to achieve the necessary biocompatibility and their shape, size, and stability are defining parameters. The final formulations intended for biomedical use must also present an adequate solubility, which in turn directly influences cellular absorption. Another important characteristic of nanoparticle-based formulations is targeting, a process that is accompanied by several advantages, including reducing the dose of active substance required to achieve the desired effect.

The second objective evaluating the cytotoxic potential of rutin formulations on human oral cells involved obtaining a liposomal formulation loaded with rutin (a phytochemical from the flavonoid class) and testing it on oral tumor cells to identify possible beneficial effects. The main conclusions that can be drawn are the following: a biocompatible formulation was obtained for the transport of rutin to the site of action from the biological environment; the phytochemical rutin is a flavonoid with good integration capabilities in liposomes; liposomes prepared to play the role of a transport system, containing cholesterol and phosphatidylcholine, did not show cytotoxic effects; the nanoformulations loaded with rutin showed a significant action on the viability of both types of tumor cells, an action that proved to be superior to that exerted by the pure compound, not incorporated in the liposomal system.

The last study aims to provide an *in vitro* insight into the cytotoxic potential of the phytochemical against two different human melanoma cell lines: RPMI-7951 (epithelial morphology) and SK-MEL-28 (polygonal morphology) by evaluating its impact on cell viability, cell morphology and evaluation of nuclear appearance but also senescence-inducing properties.

The studies on cell cultures are of real help in terms of the analysis of biological processes through the lens of the mechanisms that occur in different pathologies, especially in the detailed study of certain mechanisms involved in the processes of tumor cell development and their response in the presence of different factors.

Ultraviolet radiation is one of the most well-known risk factors for the appearance and development of malignant skin diseases. Various biological mechanisms associated with carcinogenesis instrumented by ultraviolet radiation are recognized and among these are DNA damage, induction of immunosuppression, action of different viruses (for example HPV), mutagenesis, etc. The interaction between certain viral proteins and DNA repair processes is detrimental to the repair processes of damaged DNA following exposure to ultraviolet radiation. Some research has linked exposure to ultraviolet radiation to the development of oral malignancies. Exposure to UV radiation was taken into account depending on the latitude at which cases were identified, highlighting the fact that there were

important correlations between exposure to UV radiation and the incidence of oral tumor diseases.

The third objective, the evaluation by in vitro methods of the cytotoxic properties of the phytochemicals, aimed to obtain an in vitro perspective on the cytotoxic potential of rutin on epithelial and polygonal morphology melanoma cells. The main conclusions are reproduced in the following: cell viability and morphology, appearance and senescence-inducing properties were analysed; the obtained results showed that the phytochemical rutin shows dose-dependent cytotoxic activity against human melanoma cells; the cytotoxic effect was associated with a low rate of cell viability, with the signalling of changes in terms of cell morphology, with the highlighting of nuclear changes similar to apoptotic changes and with a reduced confluence; furthermore, the phytochemical rutin increased senescence in cells with polygonal morphology at the highest concentrations. Further studies are needed to confirm and elucidate the mechanisms underlying the anti-proliferative and pro-senescent properties of rutin in tumor cells.

The current techniques are of real help regarding the deepening of the mechanisms exercised and the detailed study of the compounds presented in the present work is necessary.

Future research directions should focus on deepening the development of up-to-date transport systems, loaded with substances of natural origin, to help prevent non-tumor and tumor oral diseases. The ultimate goal of nanoparticles loaded with biomolecules is to improve the prognosis of oral diseases, to reduce the side effects of conventional medication and to promote a good quality of life for our patients.