“VICTOR BABEȘ” UNIVERSITY OF MEDICINE AND PHARMACY FROM TIMIȘOARA
FACULTY OF MEDICINE
DEPARTMENT V

MOATĂR AURICA ELISABETA

PHD THESIS

THE STUDY OF THE ANTITUMOR POTENTIAL OF BIOLOGICALLY ACTIVE AND SYNTHETIC COMPOUNDS ON COLONIC NEOPLASTIC CELLS

– R E S U M E –

Scientific Coordinator
PROF. DR. DUMITRAŞCU VICTOR

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INTRODUCTION

Colorectal cancer (CRC) represents one of the most frequent causes of malignancy, the mortality in second place among malignancies. Despite the medical progress, the actual medication is sometimes inefficient, being meet the resistance and recidivous phenomena.

The present dissertation aims to evaluate the antitumoral potential of some biological and synthesis compounds, *in vitro*, on colon neoplastic cells. The study's general objective was to analyse which form the extracts in diverse solvents or the synthesis substances present the capacity to influence tumorigenesis, primarily to act as agents with inhibiting roles on cell multiplication, and neoplastic cell adhesion or to determine cell death. With this purpose, we evaluated the antitumor capacity of the extracts of *Myrmecodia pendans*, and also the antineoplastic potential of the ionic liquids based on tetrabutylammonium, tetrahexylammonium, metilimidazolium, and metilpirimidin. The specific objectives of this study followed more hypotheses: the quantification of the antiproliferative potential and the analysis of the antioxidative capacity of the plant extracts, the effect of the plant extracts on the interleukin 8 synthesis, the evaluation of the antiproliferative potential and adhesion inhibiting of the neoplastic cells under the influence of the ionic liquids considered in the study.

CHAPTER 1
COLORECTAL CANCER

1.1. EPIDEMIOLOGIE AND ETIOPATHOGENIE DATA

Although many public health systems have colorectal cancer (CRC) screening programs, it remains one of the most important malignancy causes worldwide. The CRC identified well-defined molecular pathways which seem to play an important role in sporadic colorectal cancer, familial adenomatous polyposis, and the associated polyposis syndrome, hereditary colorectal cancer, the development of cancer in the intestinal inflammatory disease, and familial colorectal cancer. There were identified various biologic pathways which can

lead to the same phenotypic expression. As risk factors were identified the forwarded age, sex (more often diagnosed by men), adiposity, alcohol consumption, red meat, and processed meat products consumption, smoking, reduced physical activity, intestinal inflammatory disease (colitis ulcerosa and Crohn's disease), and the genetic factor.

1.2. THE BIOLOGY OF THE COLORECTAL CARCINOGENESIS

The CCR represents a challenge for medical research. Near the sporadic form which is the most often, this disease can be familial or hereditary. The hereditary factor but also lifestyle contribute to disease development. The accumulation of the mutations at the oncogene genes involved in the DNA repair suggests the existence of different molecular signalizing pathways which can lead to malignant lesions. The cellular and molecular research targets new specific therapy alternatives for CRC.

1.2.1. CARCINOGENESIS

It begins at the mucosa and suffers genetic modifications, provoking intraepithelial neoplasia and the growth of adenomatous lesions which can or can not progress to invasive cancer. It proposed a model, the so-called sequence adenoma-carcinoma which binds the genetic modifications and their appearance order up to the stages of development of the tumor. Unlike the early models, carcinogenesis is now known to be subjected to heterogeneity.

1.2.2. 3D TUMOR CELL SYSTEMS *in vitro*

The extracellular matrix (ECM) is a complex network of proteins and glycans that offer architectural support for the cells. Additionally, the matrix proteins connect with cell growth factors and expose those to the cell. ECM offers the cells the biophysical and biochemical parameters necessary for the proper function and represents a major compound of the regulation of cell comportment. Because of the gaps of the traditional 2D cell cultures, they can not reproduce integrally the *in vivo* architecture and the tumor micromedium, this leads to the fact that cell cultures in 2D systems are different from the cells *in vivo* concerning the phenotype and cell development, the cell-cell and cell-ECM connections and cell signaling. To improve the simulation of the cell micromedium *in vivo*, tridimensional cell cultures are the new frontier of tumor cell research.

1.2.3. INTERLEUKINS IN THE COLORECTAL CANCER

Interleukins (IL) are classified in many families, each of these having approximately 40 members divided into subfamilies. They can interact with diverse cell types which act over immune system in different cancer types. In the last years, the IL throw attention because of its distinct role in CRC, offering a new and promising strategy for the management of the CRC. Generally, IL facilitates CRC by promoting tumorgenesis, tumor developing, angiogenesis, invasion, and metastasis of the cancer cells, absconding from the immune system. On the other side, certain types of IL inhibit colorectal tumorigenesis acting on diverse complex cell signal pathways. In colorectal cancer at the tumor, micromedium takes place nut only the immune stimulation but also the immune suppression, with a pronounced immune suppression in advanced stages of the disease. The cytokines profile can provide data about the immune state of the tumor micromedium, contributing to the assessment of the prognosis.

CHAPTER 2

BIOLOGICALLY ACTIVE COMPOUNDS FROM VEGETABLE AND SYNTHETIC SOURCES WITH THERAPEUTIC POTENTIAL

2.1. PHYTOTHERAPY

2.1.1. PHYTOTHERAPY IN HUMAN HEALTH

Biologically active compounds derived from natural sources have been used since ancient times to treat various human or animal ailments, while therapeutic products obtained from plant sources have been a commonly used medicinal alternative, dominating human pharmacopeia over the years.

2.1.2. APPLICATIONS OF PHYTOTHERAPY IN CANCER

The use of herbal products or other natural sources in cancer is the basis of adjuvant treatments in neoplastic pathology. The first therapeutic agents introduced in antineoplastic medical practice were the vinca alkaloids: vinblastine and vincristine, obtained from *Catharanthus roseus G. Don*. Between the 1960s and 1970s, therapeutic strategies were developed isolated from species of the *Podophyllaceae* family - etoposide and teniposide, used to treat

lymphomas, bronchial and testicular cancer. Paclitaxel (taxol) was isolated from the bark of *Taxus brevifolia Nutt (Taxaceae)*. Another class of chemotherapeutic drugs is represented by active camptothecin derivatives isolated from *Camptotheca acuminate Decne (Nyssaceae)*. This class includes topotecan, which is used as an antineoplastic agent in ovarian and lung cancers, as well as irinotecan administered in colorectal cancers.

2.2. MYRMECODIA PENDANS

2.2.1. NOTIONS OF BOTANY

Myrmecodia pendans (M. Pendans, Rubiaceae) (MP), locally known as Sarang Semut or ant plant, is a member of the *Rubiaceae* family. Myrmecodia belongs to the genus of epiphytic myrmecophytes or ant plants. It belongs together with *Anthorrhiza, Hydnophytum, Myrmephytum*, and *Squamellaria* from the *Rubiaceae* family. It is a plant that has its origins in Southeast Asia, in the Papua Islands in the eastern part of Indonesia, and has multiple biological properties thanks to its active compounds. Myrmecodia has 45 species.

2.2.2. THERAPEUTIC APPLICATIONS

M. Pendans is known for its antitumor, and anticancer effect. Among the forms of cancer on which it has an anti-cancer effect are colon, liver, prostate, hematological diseases, breast, brain, cervix, lung, and skin. It is also used as a treatment for systemic diseases such as infectious diseases, tuberculosis, leukemia, diseases of the heart, prostate, kidneys, hemorrhoids, rheumatism, migraines, but also in the treatment of various allergies. The therapeutic effect of this plant is given by the active substances, it contains flavonoids, tannins, polyphenols with antioxidant function, and glycosides.

2.3. IONIC LIQUIDS

Ionic liquids (IL) are ionic compounds with numerous applications due to their physicochemical properties. By definition, they are a class of salts formed by asymmetric anions and cations, which can keep their liquid state at low temperatures, below 100°C. They have attracted the attention of research groups since the 1980s, in recent years numerous studies have been reported in the field, starting from the idea that millions of cation-anion combinations will generate the ideal ionic liquid for any type of application.

2.3.3. BIOMEDICAL APPLICATIONS OF IONIC LIQUIDS

2.3.3.1. Biological activities of ionic liquids

The development of intelligent therapeutic formulations, such as the improvement of drug delivery systems, in parallel with the effective management of human pathologies, represents a difficult task for the medico-pharmaceutical field. Most pharmaceutical products under research or development do not reach the formulation stage due to problems related to their low solubility or insolubility in currently accepted pharmaceutically acceptable solvents. Various pharmaceutical methods have been developed to increase bioavailability: nanoemulsions and nanoparticles, liposomes, micellization, inclusion complexes with cyclodextrin, solid dispersions. Although some of these approaches have proven therapeutic efficacy, some organic solvents used in their preparation exhibit important side effects for biological systems. Consequently, ecological techniques, the so-called green technologies, are desirable to make the delivery of insoluble drugs more efficient, with minimal adverse effects.

THE SPECIAL PART

CHAPTER 3

THE STUDY OF THE ANTITUMOR POTENTIAL OF *MYRMECODIA PENDANS* EXTRACTS

3.1. EVALUATION OF ANTIPROLIFERATIVE POTENTIAL OF *MYRMECODIA PENDANS* EXTRACTS ON NEOPLASTIC CELLS IN THE COLON

3.1.1. PURPOSE OF THE STUDY

This study aimed to evaluate the antioxidant capacity of *Myrmecodia Pendans* extracts in different solvents, as well as the extent to which these extracts influence the growth and development of colon neoplastic cells.

3.1.2. OBJECTIVES

The specific objectives of this study were: combining extracts of *Myrmecodia Pendans* in different solvents, assessing the effective ability to inhibit DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals, assessing the antiproliferative potential of the extracts, assessing the cellular phenotype of incubated neoplastic cells with these extracts.

3.1.3. HYPOTHESES

The working hypotheses from which this study started are: the antioxidant and antiproliferative activity of *Myrmecodia Pendans* extracts is different depending on the solvents used for extraction and the concentrations; there is a direct correlation between dose/incubation time and tumor cell proliferation in culture.

3.1.4. MATERIAL AND METHODS

Vegetal material

The plant material was represented by the plant *Myrmecodia Pendans* which was purchased from the Papua region.

Human biological material

Human biological material was represented by two colon cancer cell lines: CaCo2 and HCT116.

Extraction of the vegetal material

The plant material was identified by an experienced biologist. Next, the plant material was ground until a powder was obtained. After a preliminary weighing, the extraction was performed (10g powder/100ml solvent). The sealed containers were kept for 24h in the dark with continuous shaking. The extraction was carried out in the following solvents: ultrapure water, ethanol, methanol, and N-hexane. Subsequently, the solvents were evaporated and stock solutions of 1000 µg/ml in DMSO were made.

DPPH activity analysis

The capacity to capture free radicals from the obtained extracts was tested by the DPPH test. The activity percentage of captured DPPH was calculated by using the following equation:

$$\%\ DPPH\ radical\ capturing\ activity = [(A0 - A1)/A0] \times 100$$

Cell culture technique

The two cell lines were thawed, and cells were seeded for multiplication. Passage 3 was used for the CaCo2 line and passage 10 for the HCT116 line, respectively. The cells were cultured in a specific medium (DMEM supplemented with FBS 10% and antibiotic, antimycotic 1%), in an incubator in an atmosphere of 5%CO2 and 370C. When the cells reached 80-85% confluence, they were detached from the substrate, and a suspension of isolated cells was obtained by trypsinization and centrifugation, steps performed after a prior washing with PBS. Cell viability was assessed by the 0.4% trypan blue test using the automated cell counter (Countess Automated Cell Counter, Invitrogen).

The analysis of the cytotoxic potential of the extracts

The cells were cultured in a specific medium, in 96-well culture plates, at a concentration of 2x104/ml in 100µl medium (2x103) for the HCT116 cell line and 1.5x104/ml in 100µl medium (1.5x103) for the CaCo2 cell line. For each solvent, we made several experimental groups, testing 5 different concentrations (50-150-300-450-600 µg/ml).

The assessment of cellular phenotype

The evaluation of the cellular morphology, respectively the cellular phenotype, was carried out by microscopic analysis with the inverted phase contrast microscope (MCF) Nikon Eclipse TE 2000-U, the microscopic images being taken by the Nikon D200 camera.

Statistical analysis

Quantifiable data obtained from spectrophotometric readings were entered into Excel databases and analysed with the ANOVA test. Means and standard deviations were calculated. Statistical significance was assessed according to the p-value: *p≤ 0.001 statistically significant, **p≥ 0.001 statistically insignificant.

3.1.5. RESULTS

ANTIOXIDANT ACTIVITY

The DPPH inhibition capacity decreases in the following order: E > M > A > H. This result suggests that most of the antioxidant compounds were extracted in the alcoholic (ethanolic and methanolic) extracts.

CELLULAR PROLIFERATION

After 24 hours of incubation with the five concentrations of extracts, for each solvent, we observed a significant reduction in cell proliferation, especially in cells exposed to the ethanolic extracts. Statistically significant differences were observed between the extracts obtained in the four solvents, for the same applied concentration (p≤ 0.001). After the 48h exposure, we noticed the same effect on cell multiplication. Incubation of the CaCo2 cell line over a period of 72h revealed a dose-dependent decrease in the multiplication rate for the ethanolic extract and a moderate inhibition for the extract obtained in N-hexane.

CELL MORPHOLOGY

I highlighted changes in the phenotype of CaCo2 cells, especially in cells exposed to aqueous and alcoholic extracts. Cellular damage ranged from

minimal changes to severe cellular alterations and cell death. Cell morphological changes were proportional to extract dose and exposure time. After extraction in hexane, the tested solutions did not cause significant cell damage.

3.2. ACTIVITY OF *MYRMECODIA PENDANS* ON IL-8 SECRETION IN COLON NEOPLASTIC CELLS

3.2.1. PURPOSE OF THE STUDY

The previously obtained results demonstrated that the alcoholic and aqueous extracts of MP play an important role in the cell growth process. The study aimed to analyse the effects of Myrmecodia ethanolic and aqueous extracts on IL8 production.

3.2.2. OBJECTIVES

The objective was to quantify IL8 secretion in neoplastic colon carcinoma cells incubated 24h, 48h, and 72h with *M. pendans* extracts.

3.2.3. HYPOTHESES

The working hypotheses were:

(I) there are correlations between the type of solvent and the antiangiogenic effect expressed by IL8 synthesis.

(II) MP extracts affect colon carcinoma cells in a dose- and time-dependent manner of incubation with extracts.

MATERIAL AND METHOD

The analysis of IL8 synthesis was performed by the Elisa technique (IL8 Human Elisa Kit, Boster Biological Technology Pleasanton CA, USA). For the quantification of IL8 levels, we made the standard calibration curve (optical density vs. concentration), using the following calibrator solutions: 0 - 15.6 - 31.2 - 62.5 - 125 -250 - 500 - 1000 pg/mL. The test range for this method was 15.6 pg/mL to 1000.0 pg/mL. Optical density was measured at 450 nm using the Tecan microplate reader.

RESULTS

Although we demonstrated the antioxidant and antiproliferative effects of ethanolic and aqueous extracts of *M. pendans*, the activity of these extracts on CaCo2 cells did not produce statistically significant changes in IL8 synthesis. The level of IL8 in the supernatant obtained after centrifugation of the cell

suspensions was not significantly different from the control, regardless of the concentrations or periods of incubation with the extracts. In contrast, upon exposure of HCT116 cells to the different concentrations of extracts, we obtained statistically significant values between the experimental groups compared to the control group (*p≤0.05, ** p≤0.01, and *** p≤0.001).

CHAPTER 4

EVALUATION OF ANTITUMOR POTENTIAL OF IONIC LIQUIDS

4.1. PURPOSE OF THE STUDY

Carrying out investigations on the applications of ionic liquids (IL) based on ammonium, hexylammonium, methylimidazolium, methylpyrimidine (tetrabutyl ammonium chloride, 1-butyl-3-methylimidazolium bromide, 1-butyl-4-methylpyrimidine chloride, tetrahexylammonium bromide), in medicine in what concerns their action on the process of cell development and tumorigenesis.

4.2. OBJECTIVES

The general objective of this study was to highlight the potential of IL in biomedical applications, namely to evaluate their activity in colon cancer, considering that IL can be adapted to meet a wide range of medico-pharmaceutical needs. The specific objectives were: the analysis of the cytotoxic potential of IL on CaCO2 colon neoplastic cells in standard 2D (two-dimensional) cell culture systems, the establishment of the minimum inhibitory concentration, the generation of 3D constructs, the analysis of the effects of IL on the cell adhesion process in 3D (three-dimensional) cell systems.

4.3. HYPOTHESES

The working hypotheses were: IL structure, respectively the length of the alkyl chain and the attached ion, and the influence of the cytotoxic effect if the concentration and the incubation time affect tumorigenesis.

4.4. MATERIAL AND METHODS

The biological material was represented by the colon adenocarcinoma cell line, CaCo2 (human colon adenocarcionoma) (ATCC cell line). Cell culture materials and supplies were obtained from the manufacturer Invitrogen and ThermoScientific. Ionic liquids were purchased from the supplier Sigma-Aldrich. Cells of the CaCo2 line were cultured *in vitro* for multiplication. After reaching

confluence, they were seeded in a specific medium and incubated with different concentrations of ionic liquids.

IL cytotoxicity analysis on 2D cell systems: cells were incubated with IL 24h, 48h, and 72h. We subsequently assessed cell proliferation and cell phenotype.

Analysis of the antitumor potential of IL, *in vitro* on 3D cell aggregates

4x104 cells/ml in 100µl medium (4x103 cells/well) were seeded in 96-well plates (NunclonSphera, Thermo Fisher Scientific, Inc.). After 4 days and after adding fresh specific medium with different concentrations of IL, the plates were kept in an incubator at 37°C with 5% CO2 for 72 hours. All analyses were carried out in triplicate. To determine the effects of IL on Caco-2 cell proliferation, we performed the Alamar Blue bioassay. Cell morphology was analysed using the Zeiss Axio Observer A1 phase-contrast inverted microscope (Zeiss), and images were captured with the Axio Cam 1CC1 video camera. Statistical analysis was performed with the Medcalc v20:015 program.

4.5. RESULTS

Assessment of cell proliferation

We evaluated the *in vitro* antitumor capacity of IL, on CaCo2 colon neoplastic cells. Cells were treated with different concentrations, ranging from 1mM to 10mM, solutions obtained by dilution in the specific culture medium of TBAC_LI, [BMIM][BR]_LI, 4MBPC_LI, and THABr_LI. The cells were initially cultured in a 2D monolayer system. At 24h, 48h, and 72h post-treatment, respectively, we analysed the rate of cell proliferation/inhibition of cell proliferation by MTT assay.

After a 24h incubation, we observed a dose-dependent decrease in cell proliferation for IL1 and IL4. For the other liquids, we noted a decrease in cell multiplication independent of the dose applied. The inhibition rate was marked at 10mM dose of IL1 (about 40%). For the other liquids, at all applied concentrations cell proliferation was over 50%.

At 48h, the inhibition of cell proliferation was more marked, but for IL2 and IL3, at the small doses applied (1mM-0.1mM) no significant decrease in the proliferation of neoplastic cells was revealed. A marked decrease in the proliferation rate (≤50%) was observed at concentrations between 10mM – 0.5mM IL1, respectively 10mM for IL2, IL3, and IL4. At the 72h incubation, a similar pattern of the inhibition rate was noted.

Assessment of cell morphology and 3D spheroids

The assessment of cell morphology was carried out with a phase contrast microscope. At 24h of incubation, we observed severe cell damage in the groups exposed to tetrabutyl ammonium chloride, especially at the 10mM and 5mM doses. If during the incubation with lower doses of TBAC_IL we revealed minimal lesions of cellular degeneration, at the previously mentioned concentrations we observed frequent lesions of agony and cell death, evidenced by apoptosis and necrotic lesions. For the same incubation period, in groups exposed to 1-butyl-3-methylimidazolium bromide and 1-butyl-4-methylpyrimidine chloride, we observed a similar cellular behaviour, respectively, at increased doses of ionic liquid microscopically observable lesions were occasional at the minimum incubation dose and moderate at the other doses used in the experimental study. In the experimental groups treated with tetrahexylammonium bromide, at 24h of exposure to IL, the cells expressed moderate morphological alterations, mostly reduced in intensity. In the case of treating colon adenocarcinoma cells CaCo2, for 24h with tetrabutylammonium chloride, for doses between 10mM and 0.5mM, we observed severe and frequent changes in cell morphology: numerous floating cells, cell elongation, apoptotic bodies, such as and cell lysis and fragmentation. The same pattern was observed at 48h exposure, at a dose of 10mM with 1-butyl-3-methylimidazolium bromide, 1-butyl-4-methylpyrimidine chloride, and tetrahexylammonium bromide. The 72h incubation with the ionic liquids resulted in frequent severe alterations of the cellular phenotype for tetrabutylammonium chloride (10mM-0.5mM concentrations) and tetrahexylammonium bromide (10mM). At the other doses, the lesions were minimal or similar to the control group.

CONCLUSIONS

- The antioxidant activity was dependent on the concentration of the extract, being at the same time influenced by the extraction solvent. Most of the antioxidant compounds were obtained by extraction with alcoholic solvents, followed by aqueous extracts, and finally those in N-hexane.
- The extracts of MP determined an inhibition of CaCo2 cell proliferation, in a dose-dependent manner for all solvents studied at 24h exposure, for aqueous and alcoholic extracts at 48h incubation, as well as for ethanolic extracts at 72h. The extract made in hexane solvent did not cause significant inhibition of cell growth. The IC50 index was reached by the aqueous and ethanolic extracts, for the increased concentrations, at 48h

and 72h hours post-treatment. In the case of experimental groups with HCT116 cells, treatment with alcoholic extracts resulted in significant inhibition of cell proliferation, although the IC50 was only reached in the case of high-concentration methanolic extracts at a 24h exposure and high-concentration aqueous extracts, upon incubation of 72h. At the same time, hexane solutions caused a slight decrease in the multiplication of HCT116 cells.

- The results obtained from the MTT test correlate with those revealed by the analysis of the antioxidant potential of the extracts, the antioxidant compounds extracted in alcoholic solvents and water causing a decrease in the rate of cell multiplication and/or causing cell death.
- We highlighted changes in the phenotype of colon adenocarcinoma neoplastic cells, especially in the groups of cells treated with aqueous and alcoholic extracts. Cell phenotypic changes varied from minimal damage (intracytoplasmic vacuolations, cell ballooning), reaching more severe damage characteristic of the phenomenon of cell death, through necrosis and apoptosis. Cell morphological changes were proportional to the type and dose of extract, as well as exposure time. Extracts obtained in hexane caused minimal damage, mostly the microscopic images were similar to those of the control group. We highlighted the same pattern in the groups of HCT116 cells, the morphological changes being present, but of lower intensity in the groups incubated with the solutions extracted in hexane.
- IL8 secretion was not influenced in a statistically significant manner in CaCo2 cells. The quantification of IL8 synthesis *in vitro*, in colon cancer cells incubated with *M. pendans* extracts, was at the time of publication the first report in the specialized literature.

The severity of cell damage correlated with the antioxidant and antiproliferative profile of the extracts.

TBAC_IL caused inhibition of the multiplication of CaCo2 cells in 2D systems, in a manner dependent on the incubation time. Dose-dependence was observed at 48h, 72h at all doses, while at 24h only at concentrations of 10-5mM. At 24h of exposure to doses of 0.5-0.1mM, the inhibition was insignificant. IC50 was observed at 24h exposure for 10mM, for all other concentrations at 48h and 72h exposure (except 0.1mM). The inhibition did not depend on concentration in 3D systems.

In 2D systems, the neoplastic cell exposure to [BMIM][BR]_IL resulted in moderate concentration-dependent inhibition for LI exposure over 48h and 72h. IC50 was observed upon incubation with 10mM, 48h. At low doses of [BMIM][BR]_IL, the stimulation of cell multiplication was observed at 48h (0.1mM), as well as at 72h (0.5mM and 0.1mM).

4MBPC determined a statistically significant reduction at doses of 5-10mM, applied at all time intervals, as well as for 1mM at 24h and 72h, respectively. IC50 was reached at the 10mM dose, exposure time 48h. Concentrations of 0.1mM (48h) and 0.5mM-0.1mM (72h) stimulated the development of the neoplastic cell culture.

THABr_IL determined a dose- and time-dependent response in the cell multiplication rate, but the inhibition was moderate and the minimum inhibitory concentration was not reached.

The analysis of 3D experimental groups highlighted a moderate reduction in the cell proliferation rate for groups incubated with TBAC_IL, [BMIM][BR]_IL and THABr_IL and 4MBPC, independent of concentration, except for the group treated with 4MBPC.

The degree of damage to the cell phenotype was dependent on the applied dose.

In cells cultured in the 3D system, ILs did not cause increased inhibition of cell growth (IC50 was not reached at any applied concentration), however, TBAC_IL and THABr_IL inhibited cell aggregation in the 3D structures.

The IL dose which determined a minimum inhibitory concentration varied depending on the type of ionic liquid. However, the characteristic value of IC50 was 10m for all liquids.

The ionic liquids with cation ammonium and anion chloride produced superior cytotoxic effects on tumor cells compared to other liquids.

Ammonium-based ionic liquids induced a decrease in cell adhesion, most likely through structural alterations of the plasmalemma.

The degree of novelty of our study is represented by the comparative analysis of the cytotoxic effects of ionic liquids in 2D and 3D cell cultures.