PROCEEDINGS

of THE XVI WORKSHOP WITH INTERNATIONAL PARCIPITATION

"BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS"

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PROCEEDINGS

OF THE XVIth WORKSHOP ON BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS

with international participation

24-26 November 2021

Institute of Experimental Morphology, Pathology and Anthropology with Museum

at the Bulgarian Academy of Sciences

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THE XVITH WORKSHOP

"BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS"

with international participation

IS ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)

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THE PROGRAM OF THE WORKSHOP

Monday, 24th November 2021

10.15 - 10.30 OPENING CEREMONY

Session A

Chairpersons:
Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Assist. Lora Dyakova, MSc, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

AO1 10.30 – 11.00	NATURAL DEEP EUTECTIC SOLVENTS – POTENTIAL NEW PLAYERS IN ANTI-AGEING THERAPY?	Bela Vasileva, Dessislava Staneva, George Miloshev, Boryana Trusheva, Kalina Alipievaa, Milena Popova, Vasya Bankova, and Milena Georgieva
AO2 11.00-11.15	Ин витро проучване на инхибиторните свойства спрямо пролил олигопептидаза на галотанини от листа на Cotinus coggygria Scop. (In vitro study on the inhibitory properties against prolyl oligopeptidase of gallotannins from leaves of Cotinus coggygria Scop.)	Машенка Димитрова, Иван Илиев, Инна Суликовска, Донка Ташева, Весела Михайлова, Ивайло Иванов
AO3 11.15-11.30	STUDY ON THE CLASTOGENIC EFFECT OF WASTEWATER OBTAINED AS A BY-PRODUCT OF THE DISTILLATION OF ROSA ALBA L. OIL	Tsvetelina Gerasimova, Margarita Topashka-Ancheva, Ana Dobreva, Almira Georgieva, Milka Mileva
AO4 11.30-11.45	TARGETED SUPPRESSION OF ALLERGEN-SPECIFIC B LYMPHOCYTES IN A MURINE ALLERGY MODEL	Nikola Ralchev, Nikolina Mihaylova, Nikola Kerekov, Andrey Tchorbanov
AO5. 11.45-12.00	ВЛИЯНИЕ НА МЕТАЛНИ КОМПЛЕКСИ С ШИФОВИ БАЗИ ВЪРХУ ПРЕЖИВЯЕМОСТТА И ПРОЛИФЕРАТИВНАТА АКТИВНОСТ НА ЧОВЕШКИ И ПЛЪШИ ТУМОРНИ КЛЕТКИ	Христо Христов, Вероника Батаклиева, Десислав Динев, Абедулкадир Абудалех, Лора Дякова, Daniela-Cristina Culita, Gabriela Marinescu, Радостина Александрова
AO6. 12.00-12.30	SARS-CoV-2 и COVID-19 – хипотези и факти	Радостина Александрова
12.30-12.45	**Discussion**	

Thursday, 25th November 2021

Session B

Chairpersons:

Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Assist. Desislav Dinev, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

BO1 10.30 - 11.00	THE ROLE OF PROBIOTICS FOR HONEYBEE HEALTH	D. Salkova
BO2 11.00-11.30	NMR METABOLOMICS APPLIED TO THE OLDEST ALCOHOLIC BEVERAGE (HONEY WINE) – SOURCE OF BIO-ANTIOXIDANTS	Десислава Гергинова, Светлана Симова
BO3 11.30– 11.45	BIOLOGICAL ACTIVITIES OF GLUCOSAMINE AND ITS RELATED SUBSTANCES	Lora Dyakova, Desislav Dinev Dinev, Abedulkadir Abudalleh, Tomori Toluwase Israel, Orlin Alexandrov, Alexandrova, Radostina
BO4 11.45-12.00	DEVELOPMENT OF EXPERIMENTAL IN VITRO-MODELS OF ANTI-VIRAL AND ANTI-MALIGNANT IMMUNOMODULATIONG ACTION OF PLANT EXTRACTS	Iskra Sainova, Iliana Ilieva
BO5 12.00-12.15	EVALUATION OF THE ANTI-INFLAMMATORY EFFECTS OF CROCUS SATIVUS EXTRACT IN MOUSE MODEL OF OSTEOARTHRITIS	Blagovesta Dimitrova Boneva, Andrey Ivanov Tchorbanov, Nikolina Mihaylova Mihaylova
BO6. 12.15-12.30	ANTI-TUMOR EFFECTS OF HEMOCYANIN - PEPTIDE EPITOPE CONJUGATES IN MURINE MODEL OF MELANOMA	Emiliya Stoyanova, Nikolina Mihaylova, Nikola Ralchev, Petya Ganova, Silviya Bradyanova, Iliyan Manoylov, Yuliana Raynova, Krassimira Idakieva, Andrey Tchorbanov
BO7. 12.30-12.45	ADHESION OF GUERIN TUMOR CELLS TO ECM AND CELL-SURFACE OLIGOSACCHARIDES	J. Stoyloff
12.45-13.00	**Discussion**	

Friday, 26th November 2021

Session C

Chairpersons:

Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Assist. Prof. Abedulkadir Abudalleh, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

CO1 10.30 – 10.45	ОРГАНИЧНО ПТИЦЕВЪДСТВО И ЕСТЕСТВЕНИ ИЗТОЧНИЦИ НА МИКРОМЕТАЛИ В ХРАНАТА	Пламен Киров, Радостина Александрова
CO2 10.45-11.00	CYTOTOXIC ACTIVITY OF POLYAMIDOAMINE DENDRIMER FROM ZERO GENERATION, MODIFIED WITH FOUR 1,8-NAPHTHALIMIDE UNITS CONTAINING N-GLUCOSAMINE, ON VIABILITY OF HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS	Tomori Toluwase Israel, Awad I. Said, Lora Dyakova, Desislav Dinev, Abedulkadir Abudalleh, Ivo Grabchev, Radostina Alexandrova.
CO3 11.00 – 11.30	THE FORMATION AND ANALYSIS OF MELT SPUN MULTIFILAMENT YARNS WITH NATURAL ADDITIVE	Evaldas Bolskis, Erika Adomavičiūtė, Egidijus Griškonis
CO4 11.30-11.45	ENDOMETRIAL CANCER	Glafy Paul
CO5 11.45 – 12.00	MY BACKGRPUND: INDIA	Glafy Paul
CO6. 12.00-12.15	METHODS FOR DRUG RESISTANCE DEVELOPMENT	Desislav Dinev, Abedulkadir Abudalleh, Tanya Zhivkova, Lora Dyakova, Radostina Alexandrova
CO7. 12.15-12.45	FACTORS ASSOCIATED WITH SEVERE COVID-19	Tsvetelina Velikova, Stanislav Kotsev, Dimitrina Miteva, Hristiana Batselova, Milena Gulinac, Snejina Lazova, Martina Shopova, Maria Pishmisheva-Peleva, Radislav Nakov, Spaska Angelova Stanilova
12.45– 13.00	**Discussion**	
13.00 – 13.15	**Final Discussion and Closing remarks**	

Session A

Chairpersons:
Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Assist. Lora Dyakova, MSc, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

AO6. NATURAL DEEP EUTECTIC SOLVENTS – POTENTIAL NEW PLAYERS IN ANTI-AGEING THERAPY?

Bela Vasileva[1*], Dessislava Staneva[1], George Miloshev[1], Boryana Trusheva[2], Kalina Alipieva[2], Milena Popova[2], Vasya Bankova[2], and Milena Georgieva[1]

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Abstract:

Recently, a new branch of chemistry, called green chemistry, has been gaining popularity, with the public shifting its eye towards a more natural, sustainable and environmentally friendly way of life, as well as health and personal care. In response to this, research is being focused on new approaches in the development of modern and more natural quality of life strategies. Following this trend, a new natural alternative to the already known and mass used toxic solvents has emerged, called Natural Deep Eutectic Solvents (NADES) – a combination of natural solid ingredients with a low melting point. So far, NADES have been proven to have a superior potential as they are non-toxic, biocompatible, and sustainable, as well as having the ability to increase the benefits of the extracted compounds.

Our work, using the yeast *Saccharomyces cerevisiae* as a well-known model organism in ageing research, focuses on the potential anti-ageing properties of extracted with specific NADES bioactive compounds from different Bulgarian plants, known for their biological activities. Results are promising and are a foundation for the deeper understanding of the different molecular mechanisms through which the extracted with NADES bioactive compounds can influence the ageing process.

This work is supported by the Bulgarian National Research Fund [grant number: DN 19/4].

AO2. *IN VITRO STUDY* ON THE INHIBITORY PROPERTIES AGAINST PROLYL OLIGOPEPTIDASE OF GALLOTANNINS FROM LEAVES OF *COTINUS COGGYGRIA* SCOP.

Mashenka Dimitrova[1], Ivan Iliev[1], Inna Sulikovska[1], Donka Tasheva[2], Vesela Mihaylova[2], Ivaylo Ivanov[3]

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Prolyl oligopeptidase (POP, EC 3.4.21.26) is a widely distributed protease belonging to the small group of post-proline specific peptidases. A number of studies show that the enzyme is involved in pathogenesis of neurodegenerative and tumor diseases (reviewed in Dunaevsky et al., 2020). The specific inhibition of POP results in a suppression of the tumor growth *in vivo* (Jackson et al., 2015). Our experiments *in vitro* showed that the leaves extract of *C. coggygria*, obtained by ethyl acetate/water system inhibits tumour cells growth with the highest selectivity index (SI) for HeLa cells (cervical carcinoma; SI > 10) and HepG2 cells (hepatocellular carcinoma; SI = 2.7) (Iliev et al., 2021). Main components of the extract (analysed using high performance liquid chromatography - mass spectrometry (HPLC-MS)) proved to be oligogalloyl glucoses with different numbers of gallic acid residues (from 5 to 10) (gallotannins), as well as small quantities of quercetin and myricetin glycosides.

The aim of the present study is to evaluate *in vitro* the inhibitory properties against POP of the ethyl acetate extract from *C. coggygria* leaves in HeLa and HepG2 cell lines.

For the purpose, the cell lines were cultured and three concentrations of the extract, corresponding to IC_{20}, IC_{35} and IC_{50}, were applied for 48 hours. Then, the cells were homogenized and POP activity was evaluated using the fluorogenic substrate benzyloxycarbonyl-glycyl-prolyl-4-methylcoumarin-7-amide in phosphate buffer (pH = 7.4). The results for POP activity were compared to those in non-treated cells. According to the results, the effect on POP was concentration dependent in both cell lines. In HepG2 cells, the enzyme activity decreased with the increase of the extract concentration. However, in HeLa cells, the enzyme was activated and the degree of activation decreased with the increase of the extract concentration.

Gallotannins are known to have favourable effects on health by *per os* application, mainly due to gallic acid. The most studied of those compounds is pentagalloyl glucose (PGG) for which anticancer, anti-inflammatory, anti-diabetic and other properties have been described (reviewed in Torres-Leon et al., 2017). Pro-apoptotic activity of the compound is also documented (Kantapan et al., 2020). Additionally, PGG have been shown to inhibit protein phosphatase 1 (EC 3.1.3.16), which suppression is in parallel to the cytotoxic effect of PGG on HeLa cells (Kiss et al., 2013). Finally, our own studies have shown that gallotannins from *C. coggygria* inhibit fibroblast activation protein alfa (FAP, EC 3.4.21.B28) (Iliev et al., 2020; Iliev et al., 2021), as well. The inhibition of other enzymes involved in the development

of cervical carcinoma can be the cause of activation of POP in HeLa cell by small amounts of gallotannins. In any case, this interesting result will be a subject of our future studies.

Acknowledgement. This work is financially supported by the National Science Fund of the Bulgarian Ministry of Education and Science, Grant Nr KP-06-N31/1.

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AO3. STUDY ON THE CLASTOGENIC EFFECT OF WASTEWATER OBTAINED AS A BY-PRODUCT OF THE DISTILLATION OF *ROSA ALBA* L. OIL

Tsvetelina Gerasimova[1]*, Margarita Topashka-Ancheva[1], Ana Dobreva[2], Almira Georgieva[3,4], Milka Mileva[3]

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The most popular method for the production of rose oil is a classical method of water-steam distillation, which leaves a water fraction as a rest material of the technological process. The liquid residue - wastewater would represent an environmental problem as pollutant. Based on this data consideration, we formulated the objective of the present work - to investigate the clastogenic and cytotoxic effects of *Rosa alba* L. distillation wastewaters through classical cytogenetic methods on a laboratory animals test model.

Eight-week old male and female ICR strain albino mice were randomly assigned to four experimental groups (eight male/eight female animals each). Each mouse received a single dose (0.01 mL/b. w.) of 20% (v/v) or 11% (v/v) wastewater solution by intraperitoneal administration (Preston et al., 1987). The chromosomal aberrations frequency, micronuclei formation (MN) in peripheral blood, and mitotic index were scored.

The results about the percentage of cells with chromosomal aberrations showed a slight reduction in the percentage of mitoses with aberrations (0.75% ± 1.03 and 0.75% ± 1.49) in the bone marrow mice cells, treated with 11% wastewater at the 24th and 48th h compared to the data in 20% wastewater treated groups, but the difference is not statistically significant ($p>0.001$). The data of experimental groups are statistically undistinguishable from those calculated in the 0.9% NaCl control groups ($p>0.001$) ($tst=1.0755$ and $tst=0.579$, respectively)

Our experiments clearly showed that the tested wastewater concentrations from *Rosa alba*'s water-steam distillation does not cause a real clastogenic effect in the bone marrow cells of the experimental mouse line.

Mitotic index data indicate a slight cell proliferation inhibition in mice bone marrow of both wastewater concentrations, compared to the negative control group ($p<0.001$). MNPCE frequency also demonstrated a slight increase in treated with *Rosa alba* L. wastewater animals. Under the conditions employed in this study, our results suggested that the white oil-bearing rose *Rosa alba* L. distillation wastewater extracts in both concentrations applied showed a negligible genotoxic effect, but a slight antiproliferative effect. The flavonoid content is apparently the reason for the observed reduction in mitotic activity in bone marrow cells.

Acknowledgment: *The team expresses heartfelt thanks to the financial support of Project of Bulgarian National Sciences Fund KII-06 H36/17 (granted to Assoc. prof. M. Mileva, PhD)*

AO4. TARGETED SUPPRESSION OF ALLERGEN-SPECIFIC B LYMPHOCYTES IN A MURINE ALLERGY MODEL

Nikola Ralchev[1], Nikolina Mihaylova[1], Nikola Kerekov[1], Andrey Tchorbanov[1,2]

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Aim: Der p1 is a major allergen of *Dermatophagoides pteronyssinus* (Dpt) which causes house dust mite (HDM) allergy. The production of allergen-specific IgE antibodies by the pathological Der p1-specific B cells mediate most of the hypersensitivity allergic reactions.
It may be possible to influence Der p1-specific B cells by administration of chimeric molecule in a mouse model of HDM allergy, containing the 2.4G2 monoclonal antibody which targets the B cell inhibitory receptor FcγRIIb conjugated to a B cell epitope-carrying peptides from the Der p1 molecule. Co-crosslinking of the FcγRIIb receptors and the immunoglobulin receptors by this molecule is expected to deliver higher affinity and suppressive signal selectively silencing these B cells and the subsequent allergic response.

Material and methods: protein engineering, FACS, animal models, ELISpot

Results: The synthetic peptide Der p1 p52-71 and 2.4G2 monoclonal antibody were used for the construction of the protein engineered chimeric molecules, which bind to the inhibitory FcγRIIb receptor on murine splenocytes. A chronic mouse HDM allergy model was established. The chimeric molecules reduce the number of IgE anti-Dpt antibody producing plasma cells from splenocytes of allergic mice *in vivo*. Allergen driven proliferation of B and T cells was also reduced in the presence of chimera.

Discussion: The present study explores a different approach for suppression of the pathological Dpt-specific B cells. Targeted elimination of these B cells reduced the number of allergen-specific plasma cells, lead to reduction of allergen-induced lymphocyte proliferation and might result in silencing of the allergic immune response.

АО5. ВЛИЯНИЕ НА МЕТАЛНИ КОМПЛЕКСИ С ШИФОВИ БАЗИ ВЪРХУ ПРЕЖИВЯЕМОСТТА И ПРОЛИФЕРАТИВНАТА АКТИВНОСТ НА ЧОВЕШКИ И ПЛЪШИ ТУМОРНИ КЛЕТКИ

Христо Христов[1], Вероника Батаклиева[1,2], Десислав Динев[1], Абедулкадир Абудалех[1], Лора Дякова[3], Daniela-Cristina Culita[4], Gabriela Marinescu[4], Радостина Александрова[2]

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АО6. SARS-CoV-2 и COVID-19 – ХИПОТЕЗИ И ФАКТИ

Радостина Александрова

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Session B

Chairpersons:

Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Assist. Desislav Dinev, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

BO1. THE ROLE OF PROBIOTICS FOR HONEYBEE HEALTH

D. Salkova

Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., bl. 25, 1113 Sofia, Bulgaria

BO2. NMR METABOLOMICS APPLIED TO THE OLDEST ALCOHOLIC BEVERAGE (HONEY WINE) – SOURCE OF BIO-ANTIOXIDANTS

D. Gerginova, S. Simova

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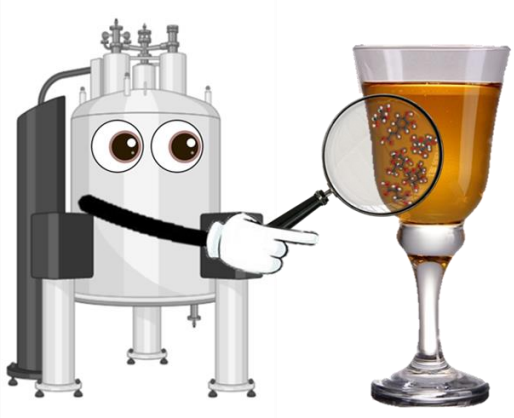
Honey wine (mead) is the oldest alcoholic beverage known for its antioxidant and therapeutic properties. Recently, its popularity has increased, especially in Slovenia, Poland, Russia, England, Germany, South Africa, Ethiopia, Australia, New Zealand and the USA. Traditional mead is made from honey and water via fermentation with yeast. Different types of honey wine can be obtained using fruits (melomel), herbs and spices (meteglin) during or after the fermentation. Literature information about mead analysis is limited with main components sugars, alcohols, organic acids, phenolic substances, volatile compounds and minerals.

In the present study twenty-five meads from different countries (Bulgaria, Poland and Slovakia) were analysed. Fifty-five compounds were identified and quantified using ^{1}H NMR spectroscopy. Similarities in the chemical composition of traditional mead, honey (blossom and honeydew) and wine (white and red) were established using Venn diagram. Different statistical techniques (PLS-DA and OPLS-DA) have been applied for determination of the components important for differentiation of the geographical origin of meads and the variety of added ingredients – fruits or spices.



NMR metabolomics **Mead**

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ВОЗ. БИОЛОГИЧНА АКТИВНОСТ НА ГЛЮКОЗАМИН И НЕГОВИ ПРОИЗВОДНИ

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Резюме. Статията представя кратки обобщени данни за биологичната активност на глюкозамина, хитозана и хиалуроновата киселина и потенциалното им медицинско прлиложение.

Глюкозамин

Глюкозаминът ($C_6H_{13}NO_5$) е един от малкото естествено срещащи се в човешкото тяло аминозахариди съдържащи 6 въглеродни атома и е важен прекурсор в биохимичния синтез на гликолипиди и белтъци (1). В природата се среща в черупките на мидите, в костите, костния мозък и гъбите (3). D-глюкозамин е най-често под формата на глюкозамин-6-фосфат, който е предшественик на всички азот-съдържащи захари (4). У човека глюкозамин-6-фосфатът се синтезира от фруктозо- 6-фосфат и глутамин под действието на ензима глутамин-фруктозо-6-фосфат трансаминаза. Крайният продукт на този метаболитен път е уридин дифосфат N-ацетилглюкозамин (UDP-GlcNAc), който след това се използва като субстрат за получаване на гликозаминогликани, протеогликани и гликолипиди (5).

В промишлеността производството на глюкозамин се осъществява посредством хидролизата на черупки на ракообразни и гъби (*Aspergillus niger*) или чрез ферментация на зърнени култури (царевица, пшеница). За първи път е получен през 1876 г. от Georg Ledderhose чрез хидролиза на хитин с концентрирана солна киселина (6,7,8,9).

В човешкото тяло глюкозаминът се открива в редица органи и тъкани - в храносмилателната система, пикочния мехур, синовиалната течност, хрущялите, съединителната тъкан и кожата. Влиза в състава на хиалуроновата киселина и е важен субстрат за синтезирането на мукополизахариди – хондроитин сулфат, дерматан сулфат, кератан сулфат, хепарин и др. Съобщено е, добавянето на глюкозамин към клетъчни култури получени от хондроцити повишава образуването на агрекан. Редица експериментални данни и клинични проучвания подсказват възможен противовъзпалителен ефект, както и антиоксидантна активност в хондроцити. Доказано е, че глюкозаминът може да намали експресията на матриксни металопротеинази (MMP) в клетъчни култури от хондроцити и остеобласти и да повиши изявата на колаген тип 2A1 и сиртуин-1 (SIRT1) в хондроцити. При някои in vitro проучвания е установено, че той намалява нивата на провъзпалителните цитокини IL-1, IL-6 и TNF-α и предотвратява повишаването на серумните нива на NO при плъхове с остеоартрит; понижава експресията на p38 MAPK и c-Jun N-терминална киназа (JNK) и повишава изявата на извънклетъчната сигнално-регулирана киназа 1/2

(ERK-1/2) в хрущяла на плъхове с остеоартрит. Някои изследвания показват способността на глюкозамина да предизвиква автофагия в клетки от бъбрек на маймуна (клетъчна линия COS-7), трансформирани с SV-40 вирус, в пигментни епителни клетки на ретината у човек, както и в клетки от аденокарцином на шийката на матката (клетъчна линия HeLa).

През последните години се натрупват и редица данни за антимикробна и антивирусна активности на глюкозамин и негови производни. Установено е потискане на растежа на бактерии като *Bacillus subtilis, Micrococcus luteus, Staphylococcus saprophyticus* и *Pseudomonas fluorescens.* Производни на глюкозамина са потенциални инхибитори на гликозилирането на вирусната обвивка и намаляват скоростта на растеж на тумори у пъдпъдъци или пилета, индуцирани с *Rous sarcoma virus*; увеличават преживяемостта на мишки, инокулирани с човешки грипен вирус (2, 10).

Вниманието на учени и клиницисти все повече започва да се насочва към установяване на зависимост между глюкозамин и остеоартрит. Честотата на това заболяване се увеличава с възрастта (> 50 години) и е зависима от пола (при жените се среща 10 пъти по-често, в сравнение с мъжете) (2). При него се наблюдават не само увреждания на хрущяла, но и на цялата става, засяга се субхондралната кост и се образуват остеофити (шипове), както и възпалителни процес в синовията. Обикновено се засягат коленните, тазобедрените и раменните стави, ставите на ходилото, гръбначния стълб, дисталните и проксималните интерфалангеални стави на ръцете (11,12). Най-честите симптоми са болка, скованост, подуване и ограничение в движението на ставата, затопляне и евентуално зачервяване на кожата над ставата, отслабване на мускулатурата, осъществяваща движението на ставата, спазъм на околоставната мускулатура, деформация на ставата и ненормално положение на крайника и тялото. Лечението на остеоартрита е комплексно и включва използването на нестероидни противовъзпалителни средства, акупунктура, здравословен начин на живот, физиотерапия и рехабилитация, хранителни добавки, хиалуронова киселина и хирургическо лечение (13).

Установено е, че функционалният дефицит на глюкозоаминогликани може да бъде причина за развитието на редица патологични състояния- хиперпропускливост на червата и пикочния мехур, болест на Crohn, синдром на раздразнените черва, астма, болест на Алцхаймер, непоносимост към белтък, гастроезофагеален рефлукс, улцерозен колит, ревматоидни заболявания, артрити, включително и остеоартрит (2). Глюкозамин и негови производни се използват за профилактика и лечение на артритни заболявания. Редица клинични проучвания показват, че такива съединения намаляват загубата на протеогликани, забавя дегенерацията на хрущяла и стесняването на ставното пространство, намаляват болката, подобряват функцията и подвижността на ставите при животни и пациенти с остеоартрит.

Глюкозаминът се предлага на пазара като хранителна добавка като най-често е под формата на глюкозамин сулфат, глюкозамин хондроитин, глюкозамин хидрохлорид и N-ацетилглюкозамин. С най-добър ефект и най-добре усвоим от организма се отличава глюкозамин сулфатът. Тези му свойства и успешното му прилагане при артритни състояния са причина той да бъде признат като "вероятно ефективен" за лечение на остеоартрит (14,15,16). Смята се, че най-добър ефект се постига след прилагане на хранителната добавка между 2 и 4 месеца, като може да бъде перорално, трансдермално и интрамускулно. Препоръчителната дневна доза е 1.5 г/кг телесно тегло. Странични ефекти се наблюдават сравнително рядко, най-честите сред тях са стомашно разстройство, запек, диария, главоболие и обрив, алергични реакции, и др. (2).

Едни от производните съединения на глюкозамина, които намират широко приложение в редица сфери, са хитозанът и хиалуроновата киселина.

Хитозан

Хитозанът е линеен полизахарид, съставен от произволно разпределени β-(1 → 4)-свързани d-глюкозамин и N-ацетил-d –глюкозамин. Синтезира се чрез алкална обработка (например NaOH) и деацетилиране на хитин, извлечен от черупките на скариди и гъби. Намира широко приложение в селското стопанство като средство за естествен биоконтрол и увеличаване на добива. Използва се в производството на вино и бира, както и като средство за пречистване на водата- хитозанът е сред биологичните адсорбенти, използвани за отстраняване на тежки метали без отрицателно въздействие върху околната среда. Има данни, че е ефективен при понижаване на холестерола и телесното тегло. От 2003 г. е одобрен като подходящ материал за изработването на превръзки за лечение на рани и изгаряния. Хитозанът и негови производни са изследвани при разработването на наноматериали, биоадхезиви, подобрени системи за доставяне на лекарства и др. Редица данни разкриват способността му да постиска растежа на редица бактерии като *Staphylococcus aureus,Streptococcus spp., Enterobacter faecalis, Candida spp., Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa P. aeruginosa, Salmonella typhimurium, Vibrio cholerae* и *B. Subtilis* (10, 17, 18, 19).

Не са малко и данните за неговата антивирусна активност, както срещу растителни (*potato virus X (PVX)* and *tobacco mosaic virus (TMV)*), така и срещу човешки вируси (*Human cytomegalovirus, H1N1 influenca virus A, Herpes simplex virus-1 (HSV-1), Coxsackie viruses, Human immunodeficiency virus (HIV), Human papillomavirus (HPV), SARS-CoV-2* и *Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV)).*

Физикохимичните и биологичните свойства на хитозана, неговите биоразградимост, биосъвместимост, антимикробни свойства, антивирусно действие, ниска токсичност, рентабилност, мукоадхезивността и екологичен характер го превръщат в подходяща суровина за проектиране и разработване на нови производни на него съединения, системи за доставка на лекарства и подходи за насочена антивирусна терапия.

Редица клинични проучвания са посветени на изясняване на антивирусната активност на хитозана и неговите производни. Има доказателства, че тези съединения подобряват профила на безопасност и ефективността на едни от най-често прилаганите антивирусни агенти, например: хитозанови наночастици подобряват профила на безопасност на Sofosbuvir (насочен срещу хепатит С вируса) и Bay 41-4109 (инхибитор на хепатит В вируса); разработена е нано-емулсия на Acyclovir (противохерпесен препарат) чрез капсулиране в хитозанови наносфери за локално приложение върху кожата за лечение на лезии, предизвикани от HSV; стабилизирани с цинк хитозан-хондроитинсулфатни нано-комплексни носители повишават антиретровирусната активност на Tenofovir disoproxil срещу инфекция с HIV-1.

Биологичните свойства на хитозана го правят подходящ материал и при разработване на ваксини. Създадена е хитозанова интраназална ваксина, която при проучвания *in vivo* показва обещаваща ефективност срещу грипен вирус тип А (H1N1) и птичен грипен вирус (H9N2). Хитозанови наночастици са използвани за интраназално доставяне на плазмидна ДНК, кодираща гени на SARS-CoV-2 при разработване на ваксина срещу този вирус (10).

Хиалуронова киселина

Хиалуроновата киселина ($C_{14}H_{23}NO_{11}$) е дълъг, линеен, високомолекулен, неразтворим биополимер, изграден от повтарящи се дизахаридни единици от D-глюкоронова киселина и N–ацетил-глюкозамин, свързани с редуващи се β1–3- и β1–4-гликозидни връзки. За първи път е изолирана от Джон Палмър и Карл Майер през 1934 година от стъкловидното тяло на телешко око (наименованието ѝ произхожда от гръцката дума "хиалос", която означава "стъклен"). Синтезира се във всички живи организми (с изключение на водораслите) и изгражда междуклетъчните пространства при бозайниците. В най-големи количества се среща в кожата, стъкловидното тяло на окото, в синовиалната течност, съединителната тъкан, аортата, белите дробове и др. В тялото на човек се съдържа приблизително 15 g хиалуронова киселина, като над 50 % от това съединение са в кожата. Едно от основните свойства на хиалуроновата киселина е да хидратира - може да свързва големо количество водни молекули (1000 до 4000-кратна стойност на собственото ѝ тегло) (27).

Биологичните и свойства са причина за широкото ѝ приложение в козметиката под формата на кремове и гелове (за хидратация и свежест на кожата) (20), в клиничната практика (под формата на капки за очи за предпазване на роговицата от изсушаване (23); в лечението на рани, поради нейните противовъзпалителни свойства (21); за облекчаване на болка при интестициален цистит (26); предотвратяване загуба на костно вещество (25); диагностичен маркер при ревматоиден артрит (23), пътологични състояния на черния дроб, включително злокачествени новообразувания (27). Смята се, че продължителният прием на хиалуронова киселина във високи дози може да намали значително болката в коленете при хора, страдащи от остеоартрит (23, 24).

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BO4. DEVELOPMENT OF EXPERIMENTAL *IN VITRO*-MODELS OF ANTI-VIRAL AND ANTI-MALIGNANT IMMUNOMODULATION ACTION OF PLANT EXTRACTS

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ABSTRACT

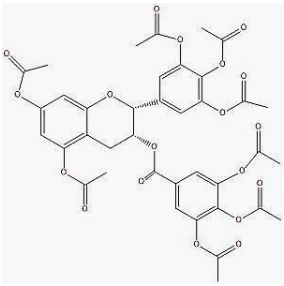
Anti-viral and anti-malignant immunomodulatory properties of total extract of the medical plant *Aronia melanocarpa* (*Black Chockeberry*) were investigated on experimental *in vitro*-cellular models. For this goal, cultures of mouse embryonic fibroblasts 3T3, of containing endogenous *retrovirus* mouse malignant myeloma cells, as well as mixed of both cellular types, were incubated. After formation of semi-confluent monolayer of 3T3 mouse fibroblasts, they were pre-incubated in cultural fluid from previously incubated in it bearing endigenous *retrovirus* mouse malignant myeloma cells, after previous centrifugation and filtration. Subsequently, total *Aronia melanocarpa* extract was added to separate sub-populations of the control cultures of non-malignant 3T3 cells, as well as of the pre-incubated in cultural fluid of malignant myeloma cells, together with respective control samples. The so incubated cellular cultures were observed at each 24 hours by light microscope. Signs of differentiation of to myeloid and lymphoid directions were noted, probably in result of the presence of virus antigens, of malignant antigens, as well as of plant extract. In cultures of pre-incubated cells were established signs of initial of differentiation phagocytes and plasmatic cells, respectively, probably on the influence of viral and malignant antigens. When the plant extract was added, the initial differentiation to initial myeloid and lymphoid directions was observed. The established changes could be explained with eventual existence of able to differentiate to various directions sub-populations of stem-like cells in the general embryonic cell line. Furthermore, a possibility for production of membrance receptor glycoproteins by non-lymphoid and non-myeloid cellular types in appropriate conditions was proposed. In this way, possibilities about activation of both anti-malignant and anti-viral immune response by *Aronia melanocarpa* plant extract were suggested.

Key words: experimental *in vitro*-models, containing endogenous retrovirus malignant cells, *Aronia melanocarpa*, phases of myeloid/phagocyte and lymphoid/plasmatic cells differentiation

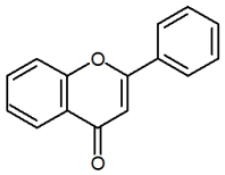
Introduction

The plants have been used for many years is almost all cultures all over the world as traditional drugs about therapy of many chronic infections, including viruses-caused diseases and malignancies [21]. The functionally-active contents in the plants have been proposed to perform these functions by their immunomodulation and anti-oxidant activities, against the Reactive Oxygene Species (ROS) and Oxidative Stress (OS), caused by many pathogenic microorganisms, heavy metal ions and other xenobiotics. It is well known that these therapeutic properties are due to the chemicals in different parts of the plant. In the last has been marked years increased interest to the application of different classes of plant substances, including flavons, terpens, saponins, alkaloids and polysaccharides (Fig. 1), which have been proved to possess immunomodulation effects, but less toxicity in comparison to the chemical drugs [26]. The possible immunomodulatory function of plants is a recent concept in the field of phytomedicines. For instance, plant terpens are widely applied because of the

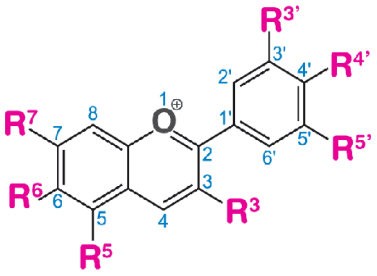
content of aromatic substances, which have been found to perform antimicrobial, antineoplastic and pharmacological properties, similarly to saponins [23]. Plant immunomodulators could not only enhance cell-mediated and humoral immunity [19], but could also activate nonspecific immune responses such as activation of natural killer (NK) cells, macrophages, granulocytes and complement systems, that increase the resistance to infections by non specophic immune mechanisms [2]. Activation of these important immune cells leads to production of various molecules such as interferons (IFNs), chemokines and other cytokines, which are involved in the enhancement of immune responses [1].



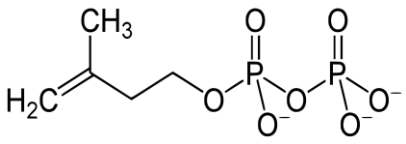
Polyphenols (general formula)



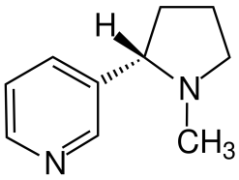
Flavons (general formula)



Antocyanins (general formula)



Isopentenyl pyrophosphate (terpen)



Nicotine (alkaloid)

Fig. 1. Plant bioactive substances.

In the last years of XX century, medical plant *Aronia melanocarpa* has become popular in many countries all over the world not only with its valuable food qualities, but also as a therapeutic and prophylactic supplement [6, 8, 11]. This plant has been proved to contain big amounts of fibres, phytoncydes, antioxidant substances and vitamins. The *Aronia*-extract is rich particularly in beta-carotene and folic acid, of A and E, and the content of съдържанието vitamin C is almost equivalent of this in the lemons. Additionally, the fruits are valuable because of the content of many microelements as K, Ca, Mg, Fe, Zn, Cu, Mn, P, B, I. Because of the described content, the whole plant has been determined among the strongest natural antioxidants. It helps the immunity and organism detoxication mechanisms, including protection against the influence of heavy metals [16]. For example, a high amount of rutin (vitamin P) in it has been proved to play a key role in the blood vessels permeability by the support of their walls, prevention against plaques, decreasing the levels of LDL in the blood and thus – availability of anti-hypertensive influence. As this plant is rich of polyphenols and anthocyanins, besides the established anti-hypertensive, anti-atherosclerotic, anti-proliferative, anti-diabetic, hepato-protective and chemoprotective properties [10, 12, 22, 24, 27, 30, 31], its total extract, but also separate ingradients, have also been proved to possess a lot of properties as immunostimulators and immunomodulators [9, 10, 13, 32]. Intra-cellular anti-oxidant mechanisms have been suggested as underlying in the immuno-modulatory influence of the plant ingredients, both in *in vitro* and *in vivo* conditions [5, 7, 15, 29].

In this relation, the main **goal** of the current study was directed to investigation on the anti-viral and anti-malignant immunomodulatory properties of plant extract in *in vitro*-conditions.

Materials and Methods

Mouse embryonic fibroblasts from line 3T3 were incubated Dulbecco's Modified Minimal Essential Medium (DMEM) (Sigma-Aldrich), supplemented with 10% Fetal Calf Serum (FCS) (Sigma-Aldrich), 100 U/ml penicillin (Sigma-Aldrich) and 100 μg/ml streptomycin (Sigma-Aldrich). Also, suspension cultures of mouse malignant myeloma cells, containing endogenous *retrovirus* (possessing RNA-genome), were incubated in analogically supplemented medium RPMI 1640. All cellular types were seeded at initial dilution 1 x 10^6 cells/ml cultural fluid. After formation of semi-confluent minolayers of 3T3 fibroblasts, they were tripsynized, resuspended and pre-seeded in 24-well micro-plates. In separate sub-populations of *in vitro*-incubated 3T3 fibroblasts, was added *Aronia melanocarpa* total water extract; cultural fluid from previously *in vitro*-incubated mouse malignant myeloma cells (after centrifugation and filtration), as well as from the plant extract plus cultural fluid from malignant cells. *Aronia*-extract and cultural fluid from malignant myeloma cells were diluted in incubation medium at 1:1 (500 μl/well cultural fluid plus 500 μl/well *Aronia*-extract or cultural fluid from malignant myeloma cells, respectively). All cellular cultures were cultivated at 37^0C in incubator with 5% CO_2 and 95% air humidification. Fixed light microscopy slides were prepared by ethanol fixation, followed by washing with PBS (Sigma) and subsequent staining by Giemsa dye (Sigma). The cells were observed by inverted light microscope (Leica). The so prepared cell cultures were observed by inverted light microscope, supplied with mega-pixel CCD-camera.

Results

The prerformed morphological studies showed some differences between the cells from the control culture of non-malignant 3T3 mouse embryonic fibroblasts (Fig. 2 a) and cultures of 3T3 cells, pre-incubated with total water *Aronia*-extract (Fig. 2 b) and with cultural fluid from previously incubated in it containing endogenous retrovirus mouse malignant myeloma cells, in the absence and presence of the plant extract (Fig. 2 c, d). In pre-incubation of embryonic fibroblasts in the presence of *Aronia*-extract, signs of initial differentiation to myeloid-like and lymphoid-like progenitors were assessed (Fig. 2 b), compared to the control non-treated cells (Fig 2 a). In many of the non-malignant cells, pre-incubated in cultural fluid from mouse malignant myeloma cells, containing endogenous *retrovirus*, were noted signs of initial phagocyte and plasmatic cellular differentiation, which were expressed with changed nuclei/cytoplasm ratio in both cases, but also in the appearance of granular cytoplasm structure and formation of cytoplasmic pseudopodia (in phagocyte-like cellular progenitors), as well as the appearance of large rounded cells with dark centrally-located nuclei (in plasmatic cells-like progenitors) (Fig. 2 c), compared to the control culture of normal cells (Fig. 2 a). However, when the same cells were pre-incubated in the presence of cultural fluid from malignant cella plus plant extract, partial regeneration of the features, observed in their pre-incubation in the presence of the plant extract only (to initial myeloid-like and lymphoid-like progenitors), was noted (Fig. 2 d). These differences in comparison with the control non-treated 3T3 cells suggested adequate immune reaction in *in vitro*-conditions in response to the influence of immunomodulators, malignant and virus antigens, respectively.

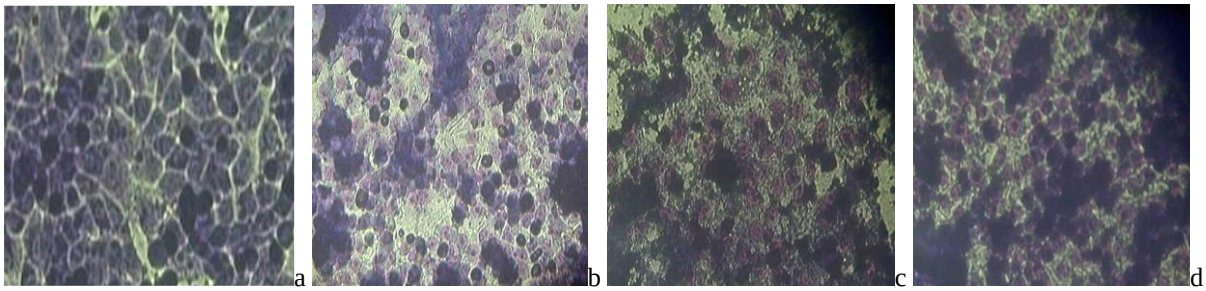


Fig. 2. *In vitro*-incubated cultures of mouse embryonic fibroblasts 3T3: a – control culture; b - pre-incubated in total extract from *Aronia melanocarpa*; c - pre-incubated in cultural fluid from previously incubated in it containing endogenous *retrovirus* mouse malignant myeloma cells; d - pre-incubated in cultural fluid from previously incubated in it containing endogenous retrovirus mouse malignant myeloma cells plus total extract from *Aronia melanocarpa* (fixed light-microscopy preparations, stained by Giemsa-dye, X 100).

Discussion

The results obtained were in agreement with the literature data, concerning similar cultivation conditions of immature normal cells [15]. In this way, the observed morphological changes in the normal cells could be accepted as primary signs of initial myeloid differentiation, probably in particular sub-populations of embryonic stem-like cells in the entire 3T3 line. The noted changes could be explained by the probable existence of the ability to differentiate to various directions sub-populations of stem-like cells in the general embryonic cell line. According to literature messages about cellular malignant transformation by endogenous *retroviruses*, the mechanism could be by *in vivo*-infection of newborn mice [20] or xenograft inbred animals [25], but also by inoculation of *in vitro*-incubated cells [28]. The current data also confirmed literature findings of activation of the neutrophil differentiation on the influence of *Aronia* ingredients (about 5461 mg/l polyphenols, 3122.5 mg/l proanthocyanidins and 221.4 mg/l anthocyanidins), probably by intracellular antioxidant mechanisms [7, 15, 33]. Furthermore, the noted signs of initial myeloid-like and lymphoid-like cellular phenotype in pre-incubation in cultural fluid from previously incubated in it containing endogenous *retrovirus* malignant myeloma cells, including in the presence of *Aronia melanocarpa* extract, are in agreement with literature suggestions about the possibility for the appearance of initial lymphoid and myeloid differentiation signs in subpopulations of immature stem-like cells [4]. These explanations were based on “intrinsic immunity” changes, as internal protection of the cell in response to its infection [29]. Another hypothesis was associated with the eventual appearance of initial signs of immune cellular differentiation of immature embryonic cells in the presence of appropriate immunomodulators [3, 14, 17, 18]. In this way, activation of both anti-malignant and anti-viral immune mechanisms by *Aronia melanocarpa* extract could be proposed.

Taking in consideration these properties, *Aronia melanocarpa* extract, but also its separate ingredients, could be applied as antioxidants, neutralizing the effects of Reactive Oxigene Species (ROS) and thus, the generated by the last oxidative stress (OS) [5, 10, 27]. Future studies are necessary in this direction.

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BO5. EVALUATION OF THE ANTI-INFLAMMATORY EFFECTS OF *CROCUS SATIVUS* EXTRACT IN MOUSE MODEL OF OSTEOARTHRITIS

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Aim: Osteoarthritis is age-related disease, which affects a high percentage of population over the age of 60, and it leads to a decline in quality of life and disability. The disease is detected at a late stage of progression and there is no adequate treatment. The aim of our research is to study and observe the effect of *Crocus sativus* extract and the possibility of its long-term administration on mice model of collagenase- induced osteoarthritis (CIOA).

Material and Methods: Balb/C mouse line, collagenase type AI and *Crocus sativus* extract have been used to develop sufficiently effective model to allow the monitoring of osteoarthritis` stages to define the optimal period for treatment of the experimental animals and a subsequent set of studies to track the changes in bone marrow cells – osteoclasts and osteoblasts. Sera were collected from the tested animals and changes in the knee joint were monitored during the disease stages.

Results: The studied model allowed to observe different aspects of CIOA because of strong reaction of the organism and different manifestations of the disease not only on local level but also in the lymphoid organs. It was proven by changes in the cell populations within splenocytes from the treated animals, and the main differences were found in the populations of the T-, NK- cells and macrophages. The *in vitro* cultivation of bone marrow cells with different concentrations of C*rocus sativus* showed an effect on the differentiation of the main players in the osteoarthritis pathology - osteoclasts and osteoblasts.

Conclusion: At the current stage of the research, the therapy with the C*rocus sativus* extract showed positive effects on the control and restriction of the disease progression, and improved disease symptoms in the treated groups.

This study is funded by National Science Fund – Bulgaria with grant KП-06-H33/8 - 16.12.2019.

BO6. ANTI-TUMOR EFFECTS OF HEMOCYANIN - PEPTIDE EPITOPE CONJUGATES IN MURINE MODEL OF MELANOMA

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Cancer is one of the major reasons for human death in the last decade. Different cancer vaccines have been developed for the treatment of malignant diseases. A group of potential anti-cancer agents are the hemocyanins - oligomeric copper-containing glycoproteins used so far for therapy of superficial bladder cancer and melanoma. Their unique structures are often linked to special mechanisms of action, through which they can trigger unexpected biological activities such a directly affecting tumor processes at the cellular and tissue level by blocking various growth factors. More attractive for modern medicine are molecules with limited adverse reactions affecting tumor progression through enhancing the effective antitumor response by various mechanisms provided by acquired and innate immunity. The aim of the present work was the selective suppression of tumor progression in a mouse melanoma model by a chimeric protein vaccine that contains a hemocyanin molecule conjugated to a mimotope peptide structurally resembling the tumor-associated carbohydrate epitope GD3.

The hemocyanins isolated from marine snail Rapana thomasiana (RtH) and the terrestrial snail Helix aspersa (HaH) were used as carriers in the composition of antitumor vaccines after conjugation with a ganglioside mimotope GD3P4. Vaccine chemical conjugation was performed. Murine melanoma cell line B16F10 was used for solid tumor establishment. Antitumor effects of hemocyanin-peptide conjugates were monitored on the survival of animals, tumor incidence, and tumor growth. Flow cytometry was performed for phenotyping of tumor suspensions. Cytotoxicity assay was used to demonstrate the generation of tumor-specific cytotoxic T cells.

Both protein-engineered vaccines exhibited strong anti-cancer effects in the developed murine model of melanoma. The administration of the conjugates RtH-GD3P4 or HaH-GD3P4 suppressed tumor growth, decreased tumor incidence, and prolonged the survival of treated animals. The immunization of experimental mice induced infiltration of immunocompetent cells into the tumors and generated cytotoxic tumor-specific T cells in the spleen. Generated anti-tumor antibodies to intact B16F10 cells were visualized by fluorescent microscope analysis.

Our study demonstrated that the protein-engineered vaccines RtH-GD3P4 and HaH-GD3P4 exhibited a strong anti-tumor immune response in the B16F10 murine melanoma model. This study demonstrated a promising approach for cancer therapy with potential applications for cancer vaccine research.

BO7. ADHESION OF GUERIN TUMOR CELLS TO ECM AND CELL-SURFACE OLIGOSACCHARIDES

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Abstract:
Metabolic radioactive labeling of tumor cell surface oligosaccharides showed that 51 kDa gCBP binds to cell surface with subsequent formation of dimers and trimers. Double radioactive labeling of cell surface oligosaccharides and immobilized glycoproteins of the extracellular matrix (ECM) showed formation of double radioactive labeled tetramers, pentamers and sextamers. Our results show that adhesion of Guein tumor cells (GTC) to cell-surface and ECM glycoproteins start with ligand-induced oligomerization of 51 kDa gCBP.
Key words: galectin, cancer, adhesion

Introduction:
Galectins are secreted by non-classical pathway. There is abundant evidence for the extracellular localisation of some galectins at cell surfaces and in the extracellular matrix, pointing out for the extracellular roles of galectins as modulators of cell adhesion [2]. Cells are attached to the extracellular matrix, consisting mainly of proteoglycans and glycoproreins. Metastasizing cancer cells from a primary tumor infiltrate into the ECM, which may facilitate cancer cell migration or act as barrier [3]. Secreted galectins could participate in cell-to-ECM adhesion through bridging carbohydrate moieties of cell-surface and ECM glycoproteins [11]. In this way secreted galectins could facilitate cancer cell migration through the extracellular matrix [7].
The aim of our study was to evaluate adhesion of Guerin tumor cell to both ECM and cell-surface glycoproteins, mediated by secreted 51 kDa gCBP.

Materials and Methods:
Radioactive labeling of ECM oligosaccharides with [3H]borohydride:

ECM glycoproteins (20 mg) were dissolved in 0.1 M sodium acetate buffer, pH 4.5, containing sodium metaperiodate (0.005 M) and oxidized for 1 h in dark at 0°C. The reaction was stopped by the addition of 1 ml of ethylene glycol, followed by incubation for 30 min at 0°C. After extensive dialysis against 0.1 M sodium phosphate buffer, pH 8.0, the solution was concentrated to 1 ml by ultrafiltration and added to an ampule containing 2.6 mg of sodium [^{3}H]borohydride (177.8 mCi/mmol). Reduction proceeded overnight at 0°C in the dark and excess borohydride was decomposed by lowering the pH to 5.0 with acetic acid.
Metabolic labeling of cell-surface glycoproteins with [^{14}C] glucosamine:

Guerin tumor cells (1 x 10^{6}) were inoculated into peritoneal cavity of Wistar rats. Ascitic fluid was withdrawn aseptically from mice 7 days following routine tumor inoculation. The fluid was centrifuged at 2000 rpm for 3 minutes. The sedimented cells were resuspended in approximately 4 volumes of RPMI culture medium and centrifuged again at 800 rpm, for 3 minutes. The packed cells were resuspended for growing in RPMI medium supplemented with calf serum.

Monolayer cells were detached by trypsinization, resuspend in complete RPMI medium containing 1 μCi/ml D-[14C] glucosamine (45 to 60 mCi/mmol), and grown for two days.

Cells were pelleted for 5 min at ~500 × g, 4°C. Glycans were removed from cell-surface with Peptide-N-Glycosidase F (PNGase F). Samples were emulsified with toluene-based scintillation fluid containing 6 g 2,5-diphenyloxazole (PPO), 0.5 g 2,2 '- phenylene - bis (5 – phenyloxazole) (POPOP), 333 ml Triton X-100 and 667 ml toluene and counted in a Beckman liquid scintillation spectrometer.

Gel Filtration is described in [12].

Secretion of 51 kDa CBP:

Guerin tumor cells were centrifuged and resuspended in complete RPMI medium. After addition of PNGase F at specific intervals (given in 'Results' section), samples were subjected to gel filtration to assess binding of 51 kDa CBP to cell-surface and ECM glycopeptides.

Results:

Harvesting 51 kDa CBP - oligosaccharide complexes showed dimeric and trimeric forms of 51 kDa gCBP (Fig. 1). Above results suggest that 51 kDa gCBP binds to cell-surface oligosaccharides and ECM glycoproteins, followed by its oligomerization.

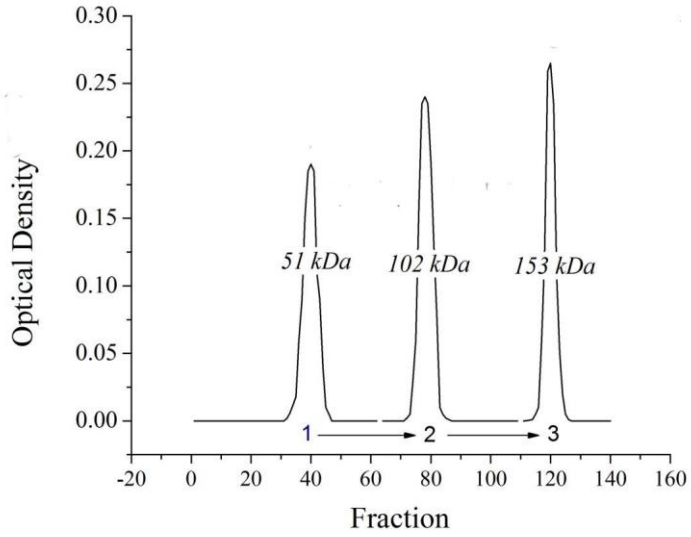

Fig. 1: Binding and oligomerization of 51 kDa gCBP on cell-surface glycoproteins of Guerin tumor cells.

Guerin tumor cells were added to solid-phase immobilized ECM glycoproteins. Labeled cell-surface and ECM glycoproteins were harvested with PNGase F and subjected gel filtration, see Fig. 2.

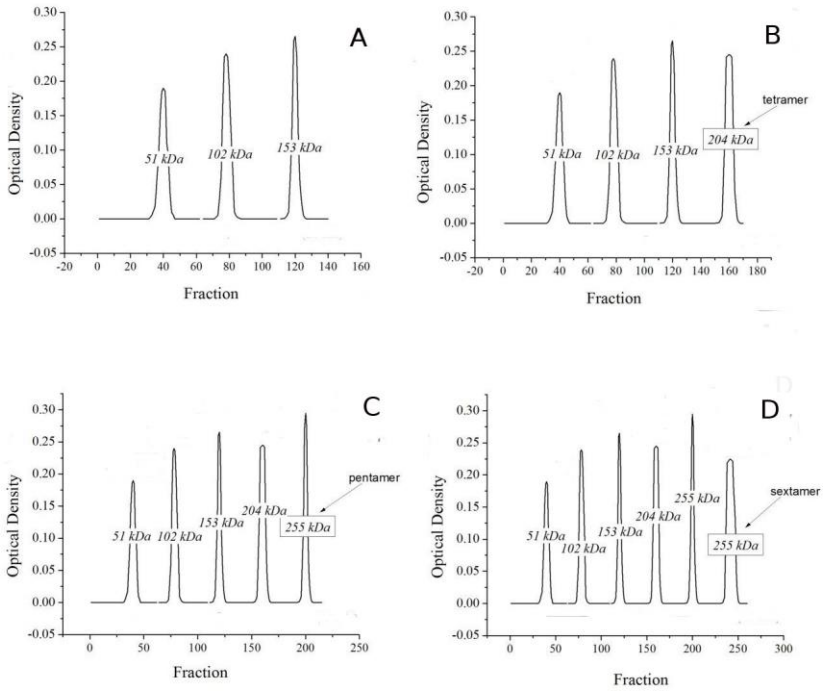

Fig. 2: Binding and oligomerization of 51 kDa gCBP on cell-surface and ECM glycoproteins.

We detected monomeric, dimeric and trimeric forms of 51 kDa gCBP attached to both [^{14}C]cell-surface (Fig. 1) and [^{3}H]ECM glycopeptides (Fig.2A). Double radioactive labeled ([^{14}C] & [^{3}H]) tetramers appeared later on (Fig.2B), followed by double radioactive labeled pentamers (Fig.2C), and double radioactive labeled sextamer (Fig.2D). This experiment shows that adhesion of tumor cells to ECM start by formation of rafts of the 51 kDa protein on cell surface and solid-phase immobilizes ECM. The two types of rafts are then bridged to effect adhesion of the tumor cells [^{12}].

Discussion:

Oligomerization is a unique feature of secreted galectin-3 leading to formation of ordered galectin-glycan lattices on cell surface. Balan et al. [1] show that galectin-3 is monomer in solution but in the presence of a ligand, galectin-3 polymerizes up to pentamers. In this way galectin-3 form ordered galectin-glycan structures on the cell surface [1]. Oligomerization of secreted Gal-3 is also induced by its interaction with lipopolysaccharides (LPS) [5]. Ligand-induced oligomerization is also observed in binding of human alpha defensin 5 (HD5) with its ligands. Extensive binding of HD5 to (neo) glycoproteins results from multivalent interactions of individual HD5 molecules with carbohydrate moieties of the target molecule. Primary binding events are magnified and enhanced by subsequent *in situ* assembly and oligomerization of HD5 [9]. CEL-III, a hemolytic lectin from Cucumaria echinata, also oligomerize after binding to cell-surface ligands [6]. Oligomerization of galectin-3, after ligand binding, occurs on cell surfaces within the physiological concentrations of the lectin. Galectin-3 plays a role in cell adhesion. Those activities may be associated with ligand cross-linking by galectin-3. However, unlike other members of the galectin family, galectin-3 exists as a monomer. It has thus been proposed that oligomerization of the N-terminal domains of galectin-3 molecules, after ligand binding by

the C-terminal domain, is responsible for this cross-linking. [10]. High-affinity interactions between collectins and microorganisms depend on the degree of oligomerization of the collectin [13]. Same is true for CD23 molecules which oligomerization is required for high affinity IgE binding to CD23 [8]. Oligomerization is also required for high affinity binding to HIV glycans by langerin [4].
According to the 'ligand-induced' model, receptors oligomerize only in presence of their ligands. Low MW ligand galactose was able to induce oligomerization of 51 kDa gCBP. We found also that multivalent ligands were able to induce di- and trimers of 51 kDa gCBP. It can be concluded that 51 kDa gCBP form ligand-induced and ligand-valency dependable oligomers in oxidative conditions [[12]].

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Session C

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CO1. ОРГАНИЧНО ПТИЦЕВЪДСТВО И ЕСТЕСТВЕНИ ИЗТОЧНИЦИ НА МИКРОМЕТАЛИ В ХРАНАТА

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РЕЗЮМЕ

Постоянно нарастващото търсене от страна на потребителите на продукти с органичен произход води до непрекъснатото увеличаване на на дела на екологичното животновъдство. Причините, обуславящи този повишен интерес, включват: животните се отглеждат в условия максимално близки до естествената им среда, хуманното отношение към тях, подобрените вкусови и хранителни качества на месните продукти. Редица наблюдения показват, че последно посоченият фактор е в тясна връзка с начина на живот на животните както и с достъпа до естествени хранителни вещества. Представеният обзор разглежда свободното отглеждане на птиците и това как храненето им директно в условията на обкръжаващата ги среда влияе върху качеството на произвежданите от тях продукти – месо и яйца. Вниманието ни е насочено основно към микроелементите, и в частност металите, като съществен компонент, необходим за растежа и метаболитните процеси в птичия организъм.

УВОД

Микроелементите в диетата са от важно значение за нормалния растеж на птиците, развитието на имунната система, метаболитните процеси, размножителните функции. Наличието на микроелементи във фуражите от растителен произход както и от минералните добавки към тях е сравнително ниско. При системите на интензивно отглеждане това е довело до значително повишаване на количествата на съставките съдържащи микроелементи над нивата, които са необходими на птиците (Aksu et al., 2012). Това води до натрупване на различни микроелементи в птичия организъм и

замърсяване на почвите с екскретираните излишъци от микрометали (Mohanna et al., 1997). Увеличените количества на микроелементи в диетата на птиците оказва директно влияние върху качеството и вкусовите характеристики на получаваните месо и яйца (Naber 1979).

Яйцата намират широко приложение в хранително-вкусовата промишленост. Това обуславя провеждането на редица изследвания върху връзката между състава им (в качествено и количествено отношение) и начина на отглеждане на носачките (Anderson 2011, 2013), типа на хранене и здравословното им състояние (Dvorák et al., 2010), както и в зависимост от породата и възрастта им (Scheideler, et al., 1998; Petek et al., 2009). Повишеното търсене на яйца, получени от носачки в системи за органично отглеждане нараства ежегодно, като основните причини за това са здравословните условия на живот и хуманното отглеждане на птиците, а цената е факторът с най-ниска важност (Bejaei et al., 2011). Като резултат от това повишено търсене се наблюдава постоянен растеж в броя и размера на фермите за органичноите производства на територията на Европейския съюз (ЕС, 2019).

Противно на нагласите сред потребителите, проучвания проведени през първото десетилетие на 21^{ия} век показват завишени количества на микроелементи в произвежданите яйца от птици отглеждани в интензивни системи в сравнение със свободно отглежданите такива (Minelli et al., 2006; Matt et al., 2009). Въпреки че при свободно отглежданите носачки нивата на омега-3 мастните киселини може да надвишават значително тези при отглежданите в клетки (Anderson 2011), това не означава непременно, че произведените в органичните ферми яйца надвишават тези от интензивните системи по хранителните си характеристики (Hidalgo et al., 2008).

Анализът на данните за съдържанието на цинк и фосфор в яйцата води до противоречиви резултати: в едни случаи, съдържанието на двата елемента е завишено при свободно отглежданите носачки (вероятно в резултат от поглъщането на земни частици и трева) (Giannenas, et al., 2009), то според друго изследване количествата са по-ниски в сравнение с тези при интензивно отглежданите носачки (въпреки че приемът на хранителни вещества отговаря на приетите норми) (Küçükyilmaz et al., 2012).

Като допълнение към вече посочените причини, поради които потребителите предпочитат едни яйца пред други, е също така и мнението, че породата на носачките оказва влияние върху качеството на продукцията. Израз на това виждане е и предпочитанието към яйцата с кафява черупка пред тези с бяла (Bejaei et al., 2011). Противно на тези разбирания, проучванията показват липса на връзка между хранителните качества на яйцата и оцветяването на черупката. Нещо повече, съобщено е за около 3% по-високо съдържание на жълтък при яйцата с бяла черупка и съответно понижено ниво на албумин (Anderson, 2013). Тъй като основното съдържание на микроелементи е съсредоточено в жълтъка (Giannenas et al., 2009), може да се заключи, че яйцата с бяла на цвят на черупката не отстъпват по хранителна стойност на тези с кафява черупка, а е възможно дори да ги надвишават.

Трябва да се отбележи, че естественият начин на хранене на птиците, въпреки всичките си положителни страни, предразполага носачките към по-висок риск от поглъщане на замърсители от околната среда. Тези замърсители може да са ДДТ, различни пестициди, бавно разграждащи се органични замърсители, тежки метали и др. (Overmeire et al., 2006).

ПРЕГЛЕД НА РЕЗУЛТАТИ ОТ ЕКСПЕРИМЕНТАЛНИ ИЗСЛЕДВАНИЯ

През последните години са проведени редица проучвания относно зависимостта между характеристиките на яйцата, произведени в интензивни и органични системи. Тези изследвания имат за теоретична основа обобщителното изследване на Naber, в което използвайки известните до момента експериментални резултати, той демонстрира взаимовръзката между съдържанието и количеството на приеманите храни от една страна и състава на яйцата от друга (Naber, 1979).

Данните от експериментални наблюдения в Турция, публикувани през 2009 година показват тясната връзка между типа на системата за отглеждане на носачките и тяхната възраст с продуктивността и качеството на яйцата (Petek et al., 2009). Според получените резултати, продуктивността при свободно отглежданите носачки е понижена: количеството получени яйца при интензивните системи е по-високо. Консумацията на фуражи при органичните системи на отглеждане е била по-ниска, вероятно поради хранене с треви и насекоми при прекараното време на открито. Броят на спуканите яйца при свободно отгледаните носачки надвишава този при отглежданите в клетки. Това се обяснява с понижената консумация на калций и фосфор, комбинирана с повишената физическа активност, оказващи влияние върху минералния метаболизъм в птичия организъм. Оцветяването на яйчения жълтък на яйцата получени във ферми за органично отглеждане е по-наситен, дължащо се на поглъщането на каротеноиди от различни растителни източници, липсващи или с намалено количество при интензивно отглежданите птици.

Различия между двете системи на отглеждане на носачки и производство на яйца се наблюдава и при последвали изследвания. Проучвания в Естония показват по-добри показатели при яйцата от интензивните системи на отглеждане в сравнение с органичните ферми (Matt et al., 2009). Наблюдава се сравнително по-голям обем на яйчения жълтък при клетъчното отглеждане птици (30.3%) спрямо свободно отглежданите (27.4%). Вероятно това обяснява и повишеното съдържание на витамин A, витамин D3, α-токоферол и γ-токоферол в яйцата от конвенционално отглеждани носачки, единствено съдържанието на β-токоферола е по-високо в яйцата от при органичните ферми. При свободно отглежданите птици, съдържанието на калций е значително по-ниско от това при клетъчно отглежданите – 13.6 спрямо 38.2 мг/100г. При холестерола тенденцията е в обратна насока: 341 мг/100г при интензивното и 489 мг/100г при органичното отглеждане. Тези резултати потвърждават съобщените от Minelli и колектив наблюдения (Minelli et al., 2006). Авторите отбелязват, че съдържанието на витамини в яйцата лесни може да бъде повлияно чрез повишаване на съдържанието на някои добавки във фуражите и, понеже няма информация относно съставките на храните, използвани при двете системи, коректността на изнесените от проучването данни е под въпрос и са необходими допълнителни изследвания в тази област.

През 2012 година Küçükyulmaz и колектив публикуват техните резултати от проучване, което са провели на територията на Турция (Küçükyilmaz et al., 2012). Екипът е разполагал с достъп до информация за съдържанието на хранителни съставки в използваните и при двете системи фуражни смески, както и с възможност за избор и разпределение на птиците. Това е направено с цел максимално уеднаквяване на условията при двете групи носачки – интензивно отглеждани и свободни такива. Измерванията на дебелината на яйчната черупка и здравината на спукване при двете системи на отглеждане потвърждават заключенията от вече известни проучвания (Rizzi et al., 2006), които показват, че при свободното отглеждане на носачки, черупката на яйцата е по-дебела в сравнение с яйцата в конвенционалните системи. Това се обяснява

с поглъщането на песъчинки и директното излагане на слънчева светлина в откритите площи, което подобрява минералния метаболизъм, и води до повишаване на концентрацията на калций в черупката и нейната дебелина. При носачките в интензивните системи е наблюдавано понижение в качеството на яйчения белтък (единица на Хю), вероятно дължащо се на промени в pH на албумина – резултат от повишената концентрация на амоняк в клетъчните системи на отглеждане. Качеството на албумина при свободно отглежданите носачки се обяснява с допълнителното количество поемана храна под формата на треви, червеи и насекоми. Приемът на различни видове треви и растителност от околното пространство обяснява също така и по-високите качествени показатели на яйчения жълтък. При конвенционалните системи на отглеждане по-интензивното оцветяване на жълтъка е резултат от повишеното количество на добавки, съдържащи каротеноиди.

Учени от САЩ осъществиха наблюдения върху пет различни метода на отглеждане на носачки: клетъчно в батерии, обогатено клетъчно, обогатени колонии, подово отглеждане и свободно отглеждани (Heflin et al., 2018). Получените резултати показват, че при обогатените системи съдържанието на магнезий и манган е по-ниско в сравнение със свободно отглежданите. И тук причината за тези различия е достъпът на носачките до различни видове треви и минерали (под формата на пясък или земни маси). Като допълнителен фактор е отбелязано и пониженият стрес при свободно отглежданите птици. Въпреки повишените нива на някои метали (калций, магнезий, цинк) при яйцата от органичните системи, изследователите отбелязват, че разликите са сравнително ниски в сравнение с тези от другите типове системи, което от гледна точка на консумация – 50 гр. яйца на ден от един възрастен, не оказва съществено влияние върху диетата (съдържанието на посочените метали в яйцата е около 1% от препоръчителната дневна доза при хората).

Колективът на Szymanek провежда сравнително изследване върху яйцата, получени от органично отглеждани носачки и тези от отглеждани в батерии птици (Szymanek et al., 2019). Изследователите установяват значителни различия в минералното съдържание при двете групи. Измерванията са извършени по отношение на количествата на калций, магнезий и цинк в яйчения белтък, в жълтъка както и в смес от белтък и жълтък. Съдържанието на анализираните елементи в трите вида проби е значително повишено при яйцата от свободно отглежданите носачки (органично/клетъчно отглеждане): калций 55.99/47.77, магнезий 15.49/9.11 и цинк 2.15/1.17 мг/100г. Разглеждайки данните за съдържанието на цинк, авторите установяват, че освен от вида на системата на отглеждане то е в пряка зависимост и от възрастта на носачките – при по-младите съдържанието на цинк е по-високо. Посочено е също така, че яйцата биха могли да са алтернативен източник на минерали в диетата на хората затруднение в това отношение би било липсата на ясна индикация в търговската мрежа относно съдържанието на микроелементи в яйцата.

ЗАКЛЮЧЕНИЕ

Направеният преглед върху наличните данни относно хранителните навици при свободно и интензивно отглежданите носачки показва наличието на зависимост между съдържанието на микроелементи в яйцата и възможността за свободен достъп до открити площи на птиците. Въпреки че при някои от изследванията, показателите на яйцата от конвенционалните системи са с по-добри характеристики от тези при органичните системи, последните остават предпочитани от потребителите. Отчетено е

че тези подобрени показатели може да са повлияни от изкуствено повишаване на минералното съдържимо в използваните фуражи (например чрез добавяне на каротеноиди с цел по-наситено оцветяване на жълтъка). Сравняването на съдържанието на калций в черупката както и нейната чупливост, демонстрират че допълнителният прием на микроминерали под формата на песъчинки и насекоми, последвано от подобреното усвояване на калция в резултат от директното излагане на слънчеви лъчи, осигуряват по-високо и получено по естествен път качество на яйцата. Наличието на естествени каротеноиди в растителността, до която имат достъп свободно отглежданите носачки, осигурява естествено оцветяване на яйчения жълтък и подобрени вкусови качества. Достъпът до песъчинки и земни маси при органичните системи допринасят за здравословните за човешка консумация нива на микроелементите в яйцата. Трябва да се отбележи обаче, че нито едно от изследванията не намира съществени разлики между качествените показатели на яйцата, получени при отделните системи на отглеждане на носачките, на база оцветяването на яйчената черупка.

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CO2. CYTOTOXIC ACTIVITY OF POLYAMIDOAMINE DENDRIMER FROM ZERO GENERATION, MODIFIED WITH FOUR 1,8-NAPHTHALIMIDE UNITS CONTAINING N-GLUCOSAMINE, ON VIABILITY OF HUMAN TRIPLE NEGATIVE BREAST CANCER CELLS

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CO3. THE FORMATION AND ANALYSIS OF MELT SPUN MULTIFILAMENT YARNS WITH NATURAL ADDITIVE

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CO4 ENDOMETRIAL CANCER

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CO5. MY BACKGROUND: INDIA

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CO6. METHODS FOR DRUG RESISTANCE DEVELOPMENT IN CANCER CELL CULTURES

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