PROCEEDINGS

OF THE THIRTEENTH WORKSHOP WITH INTERNATIONAL PARTICIPATION ON BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS

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PROCEEDINGS

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

with international participation

19-21 November 2018

Institute of Experimental Morphology, Pathology and Anthropology with Museum

at the Bulgarian Academy of Sciences

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THE THIRTEENTH 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)

UNDER THE AUSPICES OF

THE BULGARIAN ACADEMY OF SCIENCES

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The responsibility for the content of published papers/abstracts belongs entirely to their authors

The Program of the Workshop

Monday, 19 November 2018

9.50 – 10.00 OPENING CEREMONY

Session A.

Chairpersons:

Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc
Medical University, Varna

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

Secretary: Tanya Zhivkova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

10.00 – 10.40
AO1. A NEW TARGET FOR AN OLD NATURAL MOLECULE
Gunay Yetik-Anacak, Ege University
Faculty of Pharmacy, Department of Pharmacology
Izmir, Turkey

10.40 – 11.20
AO2. THERAPEUTIC EPIGENOME EDITING: Use of Epi-CRISPR-induced targeted DNA methylation for cellular reprogramming in diabetes and cancer
Melita Vidakovic
Institute of Biological Research, Belgrade, Serbia

11.20 – 11.40 Coffee Break

11.40 – 11.55
AO3. HISTOPATHOLOGICAL EVALUATION OF THE EFFECT OF EUGENOL IN A MODEL OF TRINITROBENZENESULFONIC ACID-INDUCED COLITIS IN RATS
S. Valcheva-Kuzmanova[1], V. Marinov[1], M. Zhelyazkova-Savova[1], S. Gancheva[l], M. Tzaneva[2]
[1]Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University Prof. Dr. Paraskev Stoyanov, Varna, Bulgaria
[2]Department of Preclinical and Clinical Sciences, Faculty of Pharmacy, Medical University Prof. Dr. Paraskev Stoyanov, Varna, Bulgaria

11.55-12.25

AO4. Pd(II) AND Pt(II)-LINKED METALLO-SUPRAMOLECULAR CAPSULES WITH APPLICATION IN BIOINORGANIC CHEMISTRY

A. Ahmedova

Laboratory of Biocoordination and Bioanalytical Chemistry, Faculty of Chemistry and Pharmacy, Sofia University "St. Kliment Ohridski",

Sofia, Bulgaria

12.25 – 12.40

AO5. AMINOACIDURIA: PHENYLKETONURIA

Chukwuemeka Obinna Ekeh

Faculty of Biology, Sofia University "St. Kliment Ohridski",

Sofia, Bulgaria

Tuesday, 20 November 2018

Session B.

Chairpersons:

Assoc. Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences
Assoc. Prof. Petya Genova, MSc, PhD
National Centre of Infectious and Parasitic Diseases
Secretary: Boyka Andonova-Lilova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

14.00 – 14.30

BO1. **BlOOD ANTIOXIDANT/OXIDANT PARAMETERS IN RATS EXPERIMENTALLY INFECTED WITH FASCIOLA HEPATICA AND EXPOSED TO LEAD**

V. Nanev[1], I. Vladov[1], N. Tsocheva-Gaytandzieva[1], O Kandil[2], H Shalaby[2]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS
[2]Veterinary Research Division, National Research Center, Giza, Egypt

14.30 – 14.45

BO2. ТЕРЕННИ ПРОУЧВАНИЯ ВЪРХУ ПРОФИЛАКТИКАТА НА ХЕЛМИНТОЗИТЕ ПРИ МУФЛОНА

Василена И. Дакова, Мариана С. Панайотова-Пенчева
Институт по експриментална морфология, патология и антропология с музей – БАН, София, България

14.45-15.15

BO3. CHECKING THE FIELD EFFICACY OF MENTOTIM AGAINST *VARROA DESTRUCTOR*

D. Salkova[1], K. Gurgulova[2], I. Zhelyazkova[3], V. Popova[4], S. Takova[2]
[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]National Diagnostic & Research Veterinary Medical Institute „Prof. Dr. G. Pavlov“, Sofia, Bulgaria
[3]Department of Animal Science-Non-ruminants and other Animals, Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
[4]Primavet-Sofia Ltd., Sofia, Bulgaria

15.15 – 15.35 **Coffee break**

15.35 – 15.50

BO4. TESTING OF MEROCYANINES ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I IN CELL CULTURES

Ivanka Nikolova[1], Neli Vilhelmova-Ilieva[1], Tsonko Kolev[2], Petar Grozdanov[1]
[1] Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Institute of Molecular Biology "Roumen Tsanev", Bulgarian Academy of Sciences, Sofia, Bulgaria

15.50-16.05

BO5. TWO SALTS OF VIOLURIC ACID INHIBITORS OF THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I

Neli Vilhelmova-Ilieva[1], Petar Grozdanov[1] , Tsonko Kolev[2], Ivanka Nikolova[2]
[1]Department of Virology, The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Institute of Molecular Biology "Roumen Tsanev", BulgarianAcademy of Sciences, Sofia, Bulgaria

16.05 – 16.20

BO6. DRIED BLOOD SPOTS IN HEPATITIS B DIAGNOSIS

Ch. Ismailova[1], St. Krumova[2], T. Tenev[1], E. Golkocheva-Markova[1]
[1]NRL "Hepatitis viruses", National Center of Infectious and Parasitic Diseases, Sofia
[2]NRL "Measles, Mumps and Rubella", National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria

16.20 – 16.35

***BO7. IN VITRO* ANTITUMOR ACTIVITY OF *VACCINIUM VITIS-IDAEA L.*, PICKED IN BULGARIA**

Rosalina Uzunova[1], Ivayla Dincheva[2], Ivelina Trifonova[3], Silvia Voleva[3], Irina Georgieva[3], Stefka Ivanova[4], Asya Stoyanova[5], Svetla Angelova[3]
[1] Faculty of Biology, St. Kl. Ohridski Sofia University, Sofia, Bulgaria
[2] Agro Bio Institute, Sofia, Bulgaria
[3] National Reference Laboratory of Influenza and Acute Respiratory Diseases, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria
[4] National Reference Laboratory of Measles, Mumps, and Rubella, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria
[5] National Reference Laboratory of Enteroviruses, National Centre of Infectious and Parasitic Diseases, Sofia, Bulgaria

16.35 – 16.50

BO8. **ШАП ПО ЧИФТОКОПИТНИТЕ ЖИВОТНИ**

Симеон Димитров
НРЛ "Шап и ВБС" към НДНИВМИ

16.50 – 17.20

BO9. CYTOTOXIC ACTIVITY OF METAL [Zn(II), Co(II), Ni(II)] COMPLEXES WITH SCHIFF BASES

Milena Glavcheva[1], Gabriela Marinescu, Daniela Cristina Culita, Rossen Spasov, Zdravka Petrova[1], Radostina Alexandrova[1]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania

[3]Medical Faculty, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

16.50-17.30
Poster Session

BP1. CESTODOSES AND TUMORS

N. T. Tsocheva-Gaytandzhieva

Department of Experimental Parasitology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

BP2. CLINICAL CASES OF NEMATODOSES COMBINED WITH TUMORS IN HUMANS

N. T. Tsocheva-Gaytandzhieva

Department of Experimental Parasitology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

BP3. HISTOLOGICAL STUDY OF HOST AND HELMINTH TISSUES IN NATURALLY INFECTED WITH BRAIN COENUROSIS SHEEP

N. Tsocheva-Gaytandzhieva[1], P. Zhelyazkov[2], M. Gabrashanska[1], I. Vladov[1], V. Nanev[1]

[1]Department of Experimental Parasitology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]National Diagnostic and Research Veterinary Medical Institute "Prof. Dr. G. Pavlov", Sofia, Bulgaria

BP4. STRUCTURAL AND TRACE ELEMENTS CHANGES IN RAT LIVERS AND MATURE FASCIOLA HEPATICA AFTER EXPERIMENTAL FASCIOLOSIS AND DIETHYLNITROSAMINE TREATMENT

N. Tsocheva-Gaytandzhieva[1], M. Gabrashanska[1], V. Nanev[1], I. Vladov[1], K. Georgieva[2]

[1]Department of Experimental Parasitology, Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

[2]Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia, Bulgaria

Wednesday, 20 November 2018

Session C.

Chairpersons:

Assoc. Prof. Julia Radenkova-Saeva, **MD, PhD**
Toxicology Clinic, UMHATEM "N.I.Pirogov", Sofia, Bulgaria
Assoc. Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences
Secretary: Desislav Dinev, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

9.50 – 10.20

CO1. ACUTE INTOXICATION FOLLOWING AYAHUASCA INGESTION: CASE REPORT

Radenkova-Saeva J.
Toxicology Clinic, UMHATEM "N.I.Pirogov", Sofia, Bulgaria

10.20 – 10.50

CO2. COMPETITION BETWEEN ABIOGENIC Al^{3+} AND NATIVE Mg^{2+}, Fe^{2+} AND Zn^{2+} IONS IN PROTEIN BINDING SITES: IMPLICATIONS FOR ALUMINIUM TOXICITY

Todor Dudev, Diana Cheshmedzhieva and Lyudmila Doudeva
Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

10.50-11.05

CO3. CYCLODEXTRIN INCLUSION COMPLEXES OF Pt-BASED ANTICANCER DRUGS

Valya Nikolova[1], Silvia Angelova[2], Aleksandrina Krasteva[1], Jaroslav Burda[3], Todor Dudev[1]
[1] *Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria*
[2] *Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[3] *Faculty of Mathematics and Physics, Charles University, Prague, Czech Republic*

11.05 – 11.25 Coffee Break

11.25-11.40

CO4. EFFECTS OF SUBCHRONIC EXPOSURE TO LEAD ON RAT`S BLOOD MINERALS

Vladov[1], I., V. Nanev[1], M. Gabrashanska[1], A. Kovacheva[2]
[1]*Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria*
[2]*Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria*

11.40-12.10

CO5. INHIBITORY EFFECTS OF TANSY ON POSTPROLINE CLEAVING ENZYME ACTIVITY IN BREAST CANCER CELLS

A.Vasileva[1], I. Ivanov[1], M. Dimitrova[2], V. Lozanov[1], V. Mitev[1]
[1]Department of Medical Chemistry and Biochemistry, Medical University of Sofia, Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Sofia, Bulgaria

12.10-12.25

CO6. ANTIPROLIFERATIVE AND PROAPOPTOTIC ACTIVITY OF WATER EXTRACTS OBTAINED FROM GREEN MICROALGA *COELASTRELLA* Sp. BGV AGAINST HeLa TUMOR CELLS

T. Toshkova-Yotova[1], A. Georgieva[2], P. Pilarski[1], R.Toshkova[2]
[1]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Parasitology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

12.25 – 12.55

CO7. DIVALENT METAL IONS BINDING TO LACTOSE: A DFT COMPUTATIONAL STUDY

S. Angelova[1], V. Nikolova[2], T. Dudev[2]

[1] Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2] Faculty of Chemistry and Pharmacy, Sofia University "St. Kl. Ohridski", Sofia, Bulgaria

Session D.

Chairpersons:

Prof. Anna Tolekova, MD, PhD
Medical Faculty, Trakia University, Stara Zagora, Bulgaria
Assoc. Prof. Radostina Alexandrova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences
Secretary: Boyka Andonova-Lilova, MSc
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

14.00-14.30

DO1. VITAMIN D EFFECTS ON URIC ACID IN THE EXPERIMENTAL MODEL OF METABOLIC DISORDERS IN FRUCTOSE FED WISTER RATS

L. Pashova-Stoyanova[1], Zh. Tsokeva[1], Kr. Nancheva[2], P. Hadzhibozheva[1], Ts. Georgiev[1]
[1] *Deptartment of Physiology, Pathophysiology and Pharmacology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*
[2] *Department of Clinical Laboratory, Medical Faculty, Trakia University, Stara Zagora, Bulgaria*

14.30-14.45

DO2. HAEMOSTASIS: EVOLVED UNDERSTANDING

N. Begum, N. Allana, A. Ahmed, S. Mohammed, N. Sanhye
Department of Physiology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

14.45 – 15.15

DO3. APPLANATION TONOMETRY NON-INVASIVE METHOD FOR CARDIOVASCULAR RISK ASSESSMENT

Vesela Georgieva, Matthias Müller, Moritz Oestreich, Paul Adeyemo, Boryana Mineva
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

15.15 – 15.35 Coffee break

15.35-16.05

DO4. TWO PATIENTS AFTER ST-ELEVATION MYOCARDIAL INFARCTION WITH DIFFERENT CARDIOVASCULAR RISK

Moritz Oestreich, Vesela Georgieva, Matthias Müller, Godwin Adeniji, Lyubica Pop Trajkova
Medical Faculty, Trakia University, Stara Zagora, Bulgaria

16.05-16.20

DO5. ЕСЕНЦИАЛНИТЕ МЕТАЛИ – МОЛЕКУЛИТЕ НА ЖИВОТА

Вилиана Йончева[1], Тенчо Тенев[1], Елица Голкочева-Маркова[1], Чийдем Исмаилова[1]

[1]НРЛ „Хепатитни вируси“, Отдел „Вирусология“,
Национален Център по Заразни и Паразитни Болести (НЦЗПБ)

16.20 – 16 50

DO6. METAL COMPLEXES OF BILE ACIDS EFFECTIVE AGAINST SENSITIVE AND RESISTANT TO OXALIPLATIN HUMAN COLORECTAL CANCER CELLS

Lora Dyakova[1], Tanya Zhivkova[2], Milena Georgieva[3], George Miloshev[3], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[2]
[1]Institute of Neurobiology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[3]Institute of Molecular Biology, Bulgarian Academy of Sciences, Sofia, Bulgaria
[4]Institute of Physical Chemistry "Ilie Murgulescu", Bucharest, Romania

16.50-17.30
Poster Session

DP1. BRIEFLY ABOUT SOME OF THE MOST POPULAR CYTOTOXICITY ASSAYS

Radostina Alexandrova, Zdravka Petrova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

DP2. ANTITUMOR ACTIVITY OF DISULFIRAM

Radostina Alexandrova, Desislav Dinev
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

DP3. THE MANY FACES OF STATINS

Jula Danova[1,2], Milena Glavcheva[2], Radostina Alexandrova[2]
[1]Medical Faculty, Sofia University "St. Kliment Ohridski", Sofia, Bulgaria
[2]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria

DP4. 2D AND 3D BREAST CANCER CELL CULTURES IN EACCER RESEARCH AND EXPERIMENTAL ONCOPHARMACOLOGY

Desislav Dinev[1], Iva Gavrilova-Valcheva[2], Margarita Dosina[3], Ivan Gavrilov[2], Osama Azmy[4], Radostina Alexandrova[1]

[1]Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria
[2]University Specialized Hospital for Active Treatment in Oncology, Sofia, Bulgaria
[3]Institute of Physiology, National Academy of Sciences of Belarus, Minsk, Belarus
[4]Medical Research Division, Institute of National Research Centres, Gyza, Egypt

17.30 – 17.50
Final Discussion and Closing Remarks

Abstracts and Articles

Session A.

Chairpersons:

Prof. Stefka Valcheva-Kuzmanova, MD, PhD, DSc
Medical University, Varna

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

Secretary: Tanya Zhivkova, MSc, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

AO1. A NEW TARGET FOR AN OLD NATURAL MOLECULE

Gunay Yetik-Anacak,

Ege University, Faculty of Pharmacy, Department of Pharmacology
Izmir, Turkey

AO2. THERAPEUTIC EPIGENOME EDITING: Use of Epi-CRISPR-induced targeted DNA methylation for cellular reprogramming in diabetes and cancer

Melita Vidakovic

Institute of Biological Research, Belgrade, Serbia

AO3. HISTOPATHOLOGICAL EVALUATION OF THE EFFECT OF EUGENOL IN A MODEL OF TRINITROBENZENESULFONIC ACID-INDUCED COLITIS IN RATS

S. Valcheva-Kuzmanova[1], V. Marinov[1], M. Zhelyazkova-Savova[1], S. Gancheva[l], M. Tzaneva[2]
[1]*Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University Prof. Dr. Paraskev Stoyanov, Varna, Bulgaria*
[2]*Department of Preclinical and Clinical Sciences, Faculty of Pharmacy, Medical University Prof. Dr. Paraskev Stoyanov, Varna, Bulgaria*
E-mail: stefkavk@yahoo.com

Inflammatory bowel disease (IBD) is a chronic inflammatory condition within the gastrointestinal tract. Crohn's disease and ulcerative colitis are two major forms of IBD. Trinitrobenzensulfonic acid (TNBS)-induced experimental colitis in animals is commonly

used as a model of IBD. Eugenol is a natural phenolic compound possessing promising antioxidant and anti-inflammatory properties.

The present study investigated the effects of eugenol (Eug) in a TNBS-induced rat colitis model using criteria for histopathological evaluation of colonic damage.

Male Wistar rats (250-350 g) were divided into 6 experimental groups, each of 10 rats: Control, TNBS, TNBS+Eug1, TNBS+Eug5, TNBS+Eug25 and TNBS+Eug125. Eugenol or the solvent (sunflower oil) were applied orally using an orogastric cannula. Groups Control and TNBS were treated with sunflower oil (10 ml/kg). Groups TNBS+Eug1, TNBS+Eug5, TNBS+Eug25 and TNBS+Eug125 were treated with Eug at doses of 1 mg/kg, 5 mg/kg, 25 mg/kg and 125 mg/kg dissolved in sunflower oil to a total volume of 10 ml/kg. There was a pretreatment for 6 days. Then the colitis was induced by TNBS (10 mg dissolved in 0.25 ml of 50% ethanol) applied in the colon by a soft cannula at a depth of 8 cm from the anus. Control rats received 0.25 ml of 50% ethanol. The oral treatment of the animals was resumed 24 hours after the induction of colitis and lasted 8 days. On the 10^{th} day after colitis induction, the severity of colitis was evaluated histopathologically and scored with numbers from 0 to 3 regarding epithelium injury, inflammatory cell infiltration and formation of granulation tissue, respectively.

The histopathological results showed that TNBS caused a variable degree of alteration on the colon wall ranging from focal and zonal destructions of the epithelial surface to diffuse ulcerations involving the submucosa. Inflammatory cell infiltration varied from subepithelial or in lamina propria to reaching the submucosa and muscularis propria. The scores of epithelium injury and inflammatory cell infiltration were significantly elevated in TNBS group in comparison with the Control group. In all TNBS+Eug groups, no significant effects on the scores of epithelium injury and inflammatory cell infiltration were observed. The lowest epithelium injury score was observed in TNBS+Eug5 rats but it was not significantly different from that of TNBS group. Inflammatory cell infiltration scores of TNBS+Eug1 and TNBS+Eug5 groups were lower (but not significantly) in comparison with the score of TNBS group. Regarding the formation of granulation tissue, the score of TNBS group was not significantly different from the control score while it was significantly higher for groups TNBS+Eug1 ($p<0.05$ vs. Control), TNBS+Eug25 ($p<0.001$ vs. Control; $p<0.05$ vs. TNBS) and TNBS+Eug125 ($p<0.05$ vs. Control).

In conclusion, eugenol did not improve the signs of TNBS-induced epithelial injury and inflammatory cell infiltration but it stimulated the formation of granulation tissue which might be considered as a sign of healing.

Key words: TNBS, colitis, eugenol, rats

AO4. Pd(II) AND Pt(II)-LINKED METALLO-SUPRAMOLECULAR CAPSULES WITH APPLICATION IN BIOINORGANIC CHEMISTRY

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The design and development of metallosupramolecular systems has resulted in construction of fascinating structures with highly diverse properties and potential applications. Assessment of biomedical applications of metallosupramolecular assemblies is an emerging field of research that stems from the recently demonstrated promising results on such systems.

The structure and properties of the complexes can be freely modified to enhance their biological activity. The unique structural characteristics of the metallosupramolecular assemblies, however, is their discrete cavity that renders a whole range of additional applications resulting from specific host-guest interactions [1].

Herein, we present our recent results on the anticancer activity of anthracene based coordination capsules that have earlier been designed and synthesised by Yoshizawa and co-workers [1]. The capsules have M_2L_4 composition (with M= Pd(II) or Pt(II)) and provide large hydrophobic cavity that is capable of encapsulating various guest molecules. Recently, we have discovered that they have appropriate stability in presence of small biomolecules and exert very high cytotoxicity against a panel of human cancer cells (HL-60, SKW-3, HT-29, and T-24). The anticancer activity of the capsules and their host-guest complexes correlate very well with their stability in presence of glutathione, which was estimated by NMR-based kinetic experiments. The overall results suggested the glutathione-triggered disassembly of the capsular structures as a potential activation pathway for the observed cytotoxicity [3].

Our findings illustrate the high potency of the Pt(II)- and Pd(II)-linked capsules for improved therapeutic applications in terms of better selectivity to cancer cells and ability to overcome multi-drug resistance.

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AO5. AMINOACIDURIA: PHENYLKETONURIA

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Session B.

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BO1. BlOOD ANTIOXIDANT/OXIDANT PARAMETERS IN RATS EXPERIMENTALLY INFECTED WITH *FASCIOLA HEPATICA* AND EXPOSED TO LEAD

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ABSTRACT

The aim of this study was to study antioxidant/oxidant status of blood of rats infected experimentally with *Fasciola hepatica* combined with chronic lead administration. Parameters of antioxidant/oxidant status were malondialdehyde (MDA), CuZn- superoxide dismutase,total antioxidant capacity in the blood of rats experimentally infected with *Fasciola hepatica*. The level of lead was determined as well. The data showed that oxidative/ antioxidative imbalance was developed due to an increased MDA level, reduced TAC concentration and SOD- activity in double treated animals compared to control animals and these with only one treatement.. The Pb level was significantly increased in all rats received Pb (non-infected and infected with *F.hepatica*). Our study leads us to conclude that co-exposure to Pb and helminths causes a more pronounced increase in the blood oxidative stress in the hosts.

Key worlds: lead, *Fasciola hepatica*, oxidative/antioxidative imbalance

Heavy metals are non-degradable environmental pollutants that can negatively affect the human and animal health. Lead (Pb) is a common environmental pollutant with widespread distribution, and oxidative stress has been implicated in the pathogenesis of its toxicity. Lower total antioxidant capacity, high lipid peroxidation and deviations in the activity of antioxidant enzymes have been reported in tissues in experimental rat models of lead toxicity (Ujowundu et al. 2017). Parasites are widely distributed in animals and can interfere with bioindicative processes including oxidative parameters in their host. Under environmental conditions organisms are exposed not only to parasites but are also confronted with a variety of other endogenous and exogenous factors. The information on combined effect of helminths and lead on oxidative status of animals is very short.

Fasciolosis is a neglected water- and food-borne disease. It is an economically important helminth disease, caused by two trematode species: *Fasciola hepatica* (Linnaeus, 1758) and *Fasciola gigantica* (Cobbold, 1855). It is neglected water- and food born disease. Fasciolosis has an important worldwide distribution due to parasite proliferation in a wide range of freshwater snail species and domestic as well as wild mammals, including human.

It is known that parasites strongly interact with pollutant- induced biomarker responses of their hosts by influencing their physiology in a multitude of different ways. The host responds to *F. hepatica* infection stimulating immune cells and activating reactions associated with the generation of reactive oxygen species, including superoxide radicals, causing oxidative stress.

Commonly markers used in oxidative stress assessment include the evaluation of total antioxidant capacity including oxidant and antioxidant parameters.

The aim of this study was to study antioxidant/oxidant status of blood of rats infected experimentally with *Fasciola hepatica* combined with chronic lead administration.

Material and methods

The experiment was carried out on 32 male Wistar albino rats aged 30 days, divided into 4 groups: The control group with healthy animals is a group 1; a group 2 - *F. hepatica* infected rats ; groups 3 - rats with lead administration and the group - 4 rats experimentally infected with *F. hepatica* and Pb administration. Every one control or experimental group consists of 8 animals.

The laboratory animals were orally infected with 15 viable *F. hepatica* L. encysted metacercariae per animal, suspended in dechlorinated water and passed through a stomach tube on the 1st day of the experiment. Metacercariae were obtained from experimentally cultivated snails *Galba truncatula* after experimental infection with 5 miracidiae per snail, hatched from eggs of mature *F. hepatica* L. The freshwater snails from family Lymnaeidae G. truncatula are intermediate hosts of *F. hepatica* L.

Rats were exposed to Pb salt ($Pb(CH_3COO0_2)$ dissolved in drinking water in a dose of 100 mg/L drinking water after 2 week post infestation. Pb- administration lasted 2 weeks.
The experiments were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations.

The rats were euthanized by anesthesia on the 35th day post infestation.. The blood was collected for the further biochemical investigations. Measurements of MDA, SOD and TAC in blood. Malondyaldehide (MDA), as a lipid peroxidation product was measured by thiobarbituric acid reactive substances according Londero and Lo Greco (1996).

Blood Pb level was determined using ICP-OES. Cu,Zn-SOD activity was established according the method of Misra and Fridovich (1972). TAC was determined according Rubio et al (2016).

Results

Parameters of oxidant/antioxidant status are presented in Table 1:

	Serum MDA mmol/L	SOD (blood) U/ml	TAC mmol/L serum	Pb blood μg/L
control	4,85±0,65	115,12±10,20	816,50±44,20	8,66±2,00
infected	6,17±0,30	81,20±5,16	720,20±30,15	9,53±3,70
Control+Pb	6,70±1,10	93,45±11,22	781,66±18,70	47,50±6,20
Infected+Pb	7,10±1,05	89,00±9,60	722,40±26,10	45,10±9,80

The serum MDA content is significantly increased in rats infected with *F. hepatica* compared with the control level. Similar MDA increased is observed in rats treated with Pb. MDA content in serum from rats with a double treatment (lead and parasite) is slightly increased compared to this in rats with only Pb – administration or infected with *F. hepatica*.

Blood SOD activity is significantly reduced in rats received Pb as well as in infected rats compared to control. The differences between both groups was non-significant. Reduced SOD activity is seen in the group with combined treatment compared to control but it is higher than that in group 2nd and higher than that in gr. 3rd.

Serum TAC is reduced in both treated groups – in higher degree in infected rats. TAC is significantly reduced in the group of rats with double treatment compared to controls.

Blood lead concentration is not changed in infected rats but is significantly increased in groups with only Pb administration and with dual impacts (Pb and *F. hepatica*).

Discussion

Blood is the best indicator of internal exposure of an individual to lead. A lot of studies demonstrated that in rat experimentally infected with *Fasciola hepatica* a high production of reactive oxygen species was developed causing oxidative stress (Kolodziejczyk et al, 2006; Anisimova et and Gabrashanska, 2007). Increased lipid peroxidation and disturbed antioxidant status were observed in the infected rats. The increased MDA level is an indication of increased LPO Oxidative cell injury may occur in the course of fasciolosis. The increased MDA concentration in Pb-treated groups could be due to decrease activity of the defense system protecting tissues from ROS – damage. Rat experimental studies focused on the combined effect of heavy metals and parasites on the antioxidant defence system of the host are very important for pathogenesis of parasitosis. In our experiment rats infected with *F. hepatica* and administered with Pb salt showed deviations in antioxidant status compared with those in non-intoxicated rats. The decrease activity of SOD proved that Pb indeed induced generation of ROS leading to increase ptoduction of MDA which was much higher than the level which could be compensated by the cellular defense systems, thus these compounds may not be converted to less harmful or ineffective metabolites at the sufficient levels (Valko et al., 2005).

The rats only infected with *F. hepatica* (gr. 2) developed antioxidant disturbances. They were manifested by reduced activity of the main antioxidant enzymes such as Cu,Zn-SOD. It was shown that the infection with *F. hepatica* was accompanied by rising level of the superoxide radical (Bottari et al.2015). Enhancement of lipid peroxidation was observed, shown by MDA and was seen 5 weeks pi. Our finding showed that reduced antioxidant capacity and enhanced generation of ROS are most pronounced during acute stage of fasciolosis.

Oxidant and antioxidant parameters were influenced after Pb exposure. It is an adaptive process protected the cell from toxic effects of free Pb. Free radical damage after Pb administration was demonstrated by increased MDA level. Activity of Cu, Zn-SOD was reduced in the supplemented animals compared to the controls. SOD activity may be reduced as a result of the response against an oxidative challenge. It could a part of preparation for the intense metabolic activity. Increased blood Pb concentration clearly suggests that toxic manifestation are due to increased Pb absorption. It is consistent earlier finding of Valko et al, (2005). Helminths may be induced gastrointestinal permeability modulations and they may contribute to increased Pb absorption and finally high blood Pb burden. Synergistic reactions which may be attributed to 1. high body Pb burden; 2. essential metal deficiency (Ca, Mg) caused due to helminthic exposure; 3. Pb and helminths facilitate ROS production her independently or though common mechanisms causing oxidative stress and GSH depletions either via direct thiol binding or via ROS (Jomova and Valko, 2011).

Our study leads us to conclude that co-exposure to Pb and helminths causes a more pronounced increase in oxidative stress. It shows that increased of free radicals regeneration due to dual impact of lead and *F. hepatica*, leads to disturbances in the body metabolism as well as oxidative-antioxidative balance in the host in a high degree.

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ВО2. ТЕРЕННИ ПРОУЧВАНИЯ ВЪРХУ ПРОФИЛАКТИКАТА НА ХЕЛМИНТОЗИТЕ ПРИ МУФЛОНА

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BO3. CHECKING THE FIELD EFFICACY OF MENTOTIM AGAINST *VARROA DESTRUCTOR*

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BP1. CESTODOSES AND TUMORS

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Literature data were summarized in this review article about combinations of natural and experimental cestodoses with tumors in animals and humans. Relations between some parasitic infections and development of the tumors were detected. The data about investigations on experimental combined models of cestodoses and tumors were collected. Clinical cases of cestodoses combined with tumors in humans and animals were described. Although the pathogenic mechanisms of interactions between parasite infections and carcinogenesis remained unclear some hypotheses existed for explanation of this phenomenon. One of that was that the initial carcinogenic effect was probably a function of duration and severity of the infection, changed from the parasites host immune response or such variables as ingestion of dietary carcinogens. It was supposed that parasitic associated tissue and cell injury and non-specific compensatory tissue regeneration or proliferation may play an important role in parasite-promoted carcinogenesis. Many chronic inflammatory conditions increase the risk of cancer in the affected tissues. Also, it was supposed that complex biochemical and immune mechanisms probably may take part in parasite-promoted or parasite-inhibited carcinogenesis. Different bioactive substances, inhibitors of cell proliferation and immune modulators, aroused in the infected hosts or secreted from parasites were investigated about their responsibility for the tumor growth stimulation or inhibition at the background of some parasitic diseases.

BP2. CLINICAL CASES OF NEMATODOSES COMBINED WITH TUMORS IN HUMANS

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In the present work literature data were summarized about described clinical cases of nematode infections combined with malignant tumors in humans. Some nematode infections like *Strongyloides stercoralis* infection had been recognized and proved as causative factors for human cancer. Possible pathogenic mechanisms of the carcinogenic effect of these parasites on the infected hosts were discussed. It was supposed that the development of chronic inflammation in the host tissues elicited by the parasites, the changed host immune response or possible carcinogenic effects of certain parasitic excretory-secretory products may take part in these pathogenic processes.

BP3. HISTOLOGICAL STUDY OF HOST AND HELMINTH TISSUES IN NATURALLY INFECTED WITH BRAIN COENUROSIS SHEEP

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Coenurosis is a parasite disease of the central nervous system in sheep, caused by the helminth *Coenurus cerebralis*, the larval stage of tapeworm *Taenia multiceps*, a platyhelminth in the class Cestoda, which infests the small intestine of carnivores. There the eggs secreted from parasites develop into tapeworm larvae that group within cysts known as coenuri, which can be seen in the central nervous system, muscles, and subcutaneous tissues of infected hosts. The disease is more complicated and severe when the oncospheres settle in the central nervous system tissue.

Macroscopic and histological investigations of host brain and parasite cysts developing in the brain tissue in naturally infected with *Coenurus cerebralis* sheep with brain coenurosis were carried out.

During the first stage of coenurosis clinical symptoms like nervousness of the animals, grinding, etc., were appeared. Macroscopically little parasite cysts about 3-5 mm in size, with whitish-grey color, smooth white surface, good turgor, filled up with lightly opalescent liquid were observed in the brains of the autopsied animals. Brain tissue surrounding coenurus cysts was found morphologically injured from the localized there parasites. Distrophic changes, wide necrotic zones, hemorrhages, leucocytes infiltration, connective tissue and blood vessels proliferation, dilatation of brain blood vessels were observed in the brain tissue by histological investigation in the damaged regions of the brain. Near the reactive zones the brain changes were the strongest but they were observed also far away from the parasite localization.

Second clinical stage of coenurosis is a period of remission of clinical symptoms. Zones of necrosis in the brain tissue formed by the growing of the parasites, surrounded by

distrophically changed cells, infiltration with leucocytes, blood vessels and fibroblasts proliferation were observed by the histological investigation.

Third clinical stage of coenurosis is presented with heavy symptoms like ring rotation of the animals, blindness, etc. Morphological changes detected in the brain tissue of the infected animals were similar to that observed during the earlier stages of the parasite disease.

One-chamber cysts of the parasites *C. cerebralis* with two-layer walls and groups of scoleces on the internal germinative layer were observed in the brains of the investigated naturally infected with *C. cerebralis* sheep similarly during all clinical stages of the parasite disease.

BP3. STRUCTURAL AND TRACE ELEMENTS CHANGES IN RAT LIVERS AND MATURE *FASCIOLA HEPATICA* AFTER EXPERIMENTAL FASCIOLOSIS AND DIETHYLNITROSAMINE TREATMENT

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The combination of *Fasciola hepatica* (L., 1758) infection with exogenously introduced or endogenously formed nitrosamines is possible in nature and being not well studied it stimulated our interest for investigation the character of their interaction. Morphological changes and contents of trace elements zinc (Zn), iron (Fe), molybdenum (Mo), chrome (Cr), cobalt (Co), copper (Cu) selenium (Se), bromine (Br) and rubidium (Rb) were investigated in infected host livers and tissues of mature helminths *F. hepatica* under the combined effect of experimentally influenced chronic fasciolosis and toxic compound diethylnitrosamine (DENA) application in rats. Changes in trace elements contents correlated with the alterations observed in the tissues of host livers or mature parasites. Slight changes in structure and trace elements contents were established in *F. hepatica* infected rat livers where Zn, Fe, Co and Se contents were decreased and zones with dystrophic and necrotic changes were observed. DENA treatment caused severe structural changes in rat livers and decreasing of levels of all investigated liver trace elements. The combination of helminth infection and DENA treatment leaded to dystrophic and necrotic changes in the livers and decreasing of rat liver trace elements Zn, Fe, Co and Cr. The combined application of the both pathogenic factors did not cause more severe damaging of liver tissue than the single treatment with DENA. Specific structural changes in the tegument, decreasing of Zn, Fe and Rb contents and increasing of Cr content were established in the tissues of mature helminths *F. hepatica*, isolated from DENA-treated rats, in comparison with these in intact parasites (controls). Typical for the investigated combined model of host-parasite system was decreasing of Zn and Fe contents in all investigated kinds of tissues, which suggested for presence of alterations of oxy-reduction processes in the cells. Probably this was as a result of toxic effects of the both pathogenic factors, which caused increasing of permeability of the cell membranes.

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BO4. TESTING OF MEROCYANINES ON THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I IN CELL CULTURES

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Abstract

The cytotoxicity of four merocyanines (L-6, L-8, L-9 and L-10) on monolayer cell culture MDBK was determined. The lowest toxicity of all compounds showed L-9 (CC_{50} = 843.5μM), followed by L-10 (CC_{50} = 603.5μM). Highest toxicity showed L-8 (CC_{50} = 11μM).

The antiviral activity of the substances on the replication of herpes simplex virus type 1 (HSV-1) was determined. The significant effect on the replication of intracellular HSV-1 showed substances L-10 with a selective index (SI) = 20.8.

The effect of the test substances on the activity against the virulence of the extracellular HSV–1 virions was also investigated. Activity was determined in five time intervals: 15, 30, 60, 90and 120 minutes. The strongest activity showed L-8 which decreases the virus titer with Δlg = 2, followed by L-6, L-9 and L-10 with Δlg = 1.75.

Keywords: Herpes simplex virus type I, viral replication, antiviral drugs, therapy, synthesis, acyclovir (ACV)

Introduction

Herpes simplex virus (HSV) exists as two types: HSV-1 and HSV-2, of which HSV-1 primarily causes infections of the mouth, throat, face, eyes and central nervous system but can also cause genital infection. HSV can cause various diseases but is most often characterized by the formation of lesions on the skin and mucous membranes of the infected area. After the primary infection, the virus always forms latent lifetime infection. Especially severe herpes infections are those in the eye [17] and herpes encephalitis [23]. HSV-1 may lead to partial damage to the nervous system and increase the risk of developing Alzheimer's disease [8, 9, 6]. One of the diseases ending often fatal is Neonatal herpes simplex caused by vertical transmission of HSV (type 1 or 2) from the mother to the newborn [1, 20].

Acyclovir (ACV) as well as other nucleoside analogues are effective in the treatment of HSV infections, but in a number of cases therapy fails due to the occurrence of ACV-resistant mutants [4, 14, 22]. Therefore, it is necessary to find new therapies whose mechanism of action is different from that of acyclovir.

For the past decades, many studies were conducted in the field of photodynamic inactivation of viruses. Numerous efforts have been made to seek new methods of virus inactivation because of their ability to develop resistance to antiviral agents. Particular attention is paid to photosensitizers and their antiviral activity in the treatment of contaminated blood as no side effects in cells or plasma proteins are seen in this process [3, 7, 11,13].

During the photo dynamic process, various oxygen-containing derivatives are formed, such as a hydroxyl radical, a superoxide radical anion, a peroxide radical and a singlet oxygen [15, 18]. Singlet oxygen is the most important molecule for the virucidal effect of these compounds since inactivation of viruses is inhibited by oxygen removal or addition of oxygen tails such as beta-carotene or sodium azide or improved in the presence of heavy water (D_2O) which prolongs the life of singlet oxygen [5, 16].

One such photosensitizer compound is Merocyanin 540 which has been used to treat leukemia cells and enveloped viruses. Although there are many literature on the biological action of Merocyanin 540, very little has been done to study the antiviral activity of newly synthesized derivatives of this compound.

This study demonstrates the antiviral activity of four newly synthesized merocyanines on the replication of HSV type 1.

Materials and Methods

Cells. Monolayer cultures of Madin-Darbey bovine kidney (MDBK) cells (National Bank for Industrial Microorganisms and Cell Cultures, Sofia) were grown in DMEM medium containing 10% fetal bovine serum (Gibco BRL, USA), supplemented with 10 mM HEPES buffer (Merck, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml) in CO_2 incubator (HERA cell 150, Heraeus, Germany) at 37 °C/5% CO_2.

Virus. Herpes simplex virus type1, Victoria strain (HSV-1) was received from Prof. S. Dundarov, National Center of Infectious and Parasitic Diseases, Sofia. The virus was replicated in monolayer MDBK cells in a maintenance solution DMEM Gibco BRL, Paisley, Scotland, UK, plus 0.5% fetal bovine serum Gibco BRL, Scotland, UK. The virus titers were estimated from cytopathogenicity by the limit-dilution method and expressed as 50% cell culture infectious dose per ml ($CCID_{50}$/ml).

Compounds tested. Four merocyanines (L-6, L-8, L-9, L-10) were first dissolved in DMSO to a concentration of 0.01 M and then diluted in DMEM to the required concentration. The structural formulas of the substances are presented in Fig. 1.

Synthesis of the compounds. L-6 Synthesis. The starting compound, 3,5,5-trimethyl(cyclohex-2-enylidene)-malonodinitrile, $C_{12}H_{14}N_2$, was prepared by means of Knoevenagel condensation of malonodinitrile (660 mg, 10 mmol, Fluka) and isophorone (1.382 g, 10 mmol, Fluka). Both compounds were dissolved in N,N-dimethylformamide (50 ml). The solution was stirred for eight hours at room temperature. Piperidinium acetate (600 mg) was used as a catalyst. A yellow precipitate was obtained from the resulting dark-yellow solution after evaporation of half of the solvent. The product was filtered and recrystallized from 95% ethanol. The title compound was prepared according to a published procedure [19] from 3,5,5-trimethyl(cyclohex-2-enylidene)malonodinitrile (1.86 g, 10 mmol) and 4-hydroxybenzaldehyde (1.22 g, 10 mmol) in a 150 ml trichloromethane solution with continuous stirring for two days at room temperature. Piperidinium acetate was used as a catalyst. The solution was purified by column chromatography on silica gel. The orange precipitate was recrystallized from glacial acetic acid [12]. Crystals were grown by slow (severaldays) evaporation from ethyl acetate.

The **compound L-8** was given us from Dr. Uli Bohne, University of Dortmund, Germany.

L-9 Synthesis. The compound was synthesized by prof. Ts. Kolev [10] and the synthesis will be published in separate paper. In was characterized by IR, UV-vis, elemental analysis and HPLC MS MS and single crystal X-rayanalysis.

L-10 Synthesis. The starting compounds for the synthesis of (1), 1,4-dimethylpyridinium iodid and 3-methoxy-4-hydroxybenzaldehyde were Merck (Germany) products. The reaction scheme for obtaining of 1-methyl-4-[2-(3-methoxy-4-hydroxyphenyl)ethenyl)]pyridinium iodide is: 2.3500 g (10.0 mmol) 1,4-dimethylpyridinium iodide is mixed with 1.2200 g (10.0 mmol) 3-methoxy-4-hydroxybenzaldehyde in 50.0 ml toluene. 5.00 ml acetic acid and 0.77 g (10.00 mmol) ammonium acetate are also added to the reaction mixture. The resulting suspension is stirring for 24 h at room temperature. Then 0.50 ml concentrated HI and 10.00 ml ethanol are added and the obtained reaction mixture left to stand for 16 h at room temperature. The resulting orange precipitate is filtered off, washed with C_2H_5OH and dried on P_2O_5 at 298 *R.* 1-methyl-4-[2-(3-methoxy-4-oxocyclohexadienilydene) ethylidene]-1,4-dihydropyridine is then obtained in the following way: 10.0 mg of iodide salt are dissolved in 10.00 mmol ethanol and then 5 ml 1.0 M KOH are added. The obtained lila solution is heated for 2 h at a temperature of 70°C, and then is kept at 4°C for 16 h. The resulting violet precipitate is filtered off and, dried on P_2O_5 at 298 K. Finally, is obtained by mixing equimolar amounts of the quinolid derivative and squaric acid in 20 ml ethanol. The obtained mixture is stirred for 2 h at 40°C. The obtained solution is cooled to 4°C and held at this temperature for 10 h. Finally, the resulting orange crystals are filtered off, washed with methanol and dried on P_2O_5 at 298 K. Yield 42%.

Cytotoxicity assay. The *in vitro* cytotoxicity of the merocyanines were examined using MDBK cells. Confluent monolayer in a 96-well plate was treated with culture medium containing no or increased concentrations of the compounds. The cells were incubated at 37ºC for 2 days. The viability of the cells after drug treatment was measured using a neutral red uptake assay and ELISA reader at OD_{540nm}.

Antiviral activity assay. Cytopathic effect (CPE) inhibition test used confluent cell monolayer in 96-well plates infected with 100 $CCID_{50}$ in 0.1ml. After 1 h of virus adsorption compounds were added in various concentrations and cells were incubated for 48h at 37ºC. Inhibition of cytopathic effect was determine using a neutral red uptake assay and an ELISA reader at OD_{540nm}. The IC_{50} concentration of the amines and bromide derivative was identified as that concentration that inhibited development of CPE by 50%.

The selectivity index of substances was defined as the ratio between CC_{50}/IC_{50}.

Virucidal assay. Virus suspension containing 10^5 $CCID_{50}$ HSV-1 was mixed in a 1:1 ratio with or without the compounds in maximal non-toxic concentration (MNC). Samples were incubated for 15, 30, 60, 90 and 120 min., at room temperature. Then the infectious virus titer was determined of all contact samples by the method of the final dilution. The results are compared to those from control virus - equal volume of virus suspension and maintenance medium, incubated for the same time intervals and determine Δlg.

Statistical analysis. Data on compounds cytotoxicity and antiviral effects were analysed statistically. The values of CC_{50} and IC_{50} were calculated using non-linear regression analysis (GraphPad Prism5 Software) and presented as means ± SD. The differences' significance between the effects of each tested substance compared to the corresponding acyclovir value [21] was done through the One-Way ANOVA analysis of variance followed by Bonferroni's post hoc test, where p-values of <0.05 were regarded significant.

Results and Discussion

To demonstrate that the resulting anti-herpesvirus activity of the tested dyes was not a result of cytotoxicity, we evaluated the effect of the compounds on monolayer cell culture, MDBK in order to conduct more stringent studies with non-toxic concentrations (Table I). The lowest toxicity of all compounds showed L-9 with CC_{50} = 843.5μM (MNC = 90μM), followed by L-10 with CC_{50} = 603.5μM (MNC = 95μM). L-6 showed significantly higher

toxicity with a value of CC_{50}= 37.5μM (MNC = 10μM). Highest toxicity showed L-11 with CC_{50} = 11μM (MNC = 2μM).

The antiviral activity of the substances on the replication of HSV-1 was determined. The highest effect of all dyes tested showed L-10 with a selective index (SI) = 20.8 followed by L-6 with SI = 12.5 and L-8 with SI = 11. The dye L-9 does not demonstrate an effect on the replication of HSV-1

The virucidal activity of the tested substances against the extracellular HSV-1 virions was investigated. From the results presented in Table II, it can be seen that the strongest effect in 15 minutes has L-8 with Δlg = 2.0 followed by L-6, L-9 and L-10 with Δlg = 1.75. The effect of the four substances is maintained at the same values without change to 120 minutes.

In the literature, for years, there has been evidence that the merocyanines has a higher affinity for small liposomes, presumably because the dyes bind preferentially to liposomes with the higher radius of curvature and thus the more widely spaced polar head groups [2, 19, 24]. Perhaps enveloped viruses should be more sensitive to merocyanins than naked viruses.

These data require a more detailed study of the anti-viral activity of merocyanine dyes and theirs other derivatives or complexes.

Conflict of interest

The authors have no conflicts of interest to declare.

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Table I. Cytotoxicity and antiviral activity of the merocyanines

Compounds	CC_{50}±SD(μM/ml)[1]	IC_{50}±SD(μM/ml)[1]	SI = CC_{50}/IC_{50}
L-6	37.5 ± 3.2 ***	3.0 ± 0.7	12.5
L-8	11.0 ± 2.7 ***	1.0 ± 0.08	11.0
L-9	843.5 ± 14.7 ***	-	-
L-10	603.5 ± 17.3 ***	29.0 ± 3.6 ***	20.8
ACV	1296 ± 49.4	1.47 ± 0.06	881.6

**p<0.01, ***p<0.001, compared to ACV

[1] Values are means ± standard deviation from three consecutive experiments.

Table II. Effect of merocyanines on the extracellular HSV-1 virions

Compounds	Δlg				
	15 min	30 min	60 min	90 min	120 min
L-6	1.75	1.75	1.75	1.75	1.75
L-8	2.0	2.0	2.0	2.0	2.0
L-9	1.75	1.75	1.75	1.75	1.75
L-10	1.75	1.75	1.75	1.75	1.75

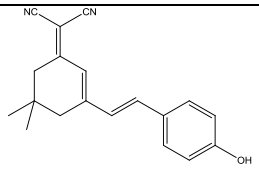
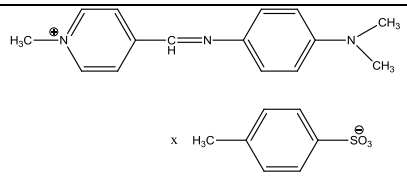
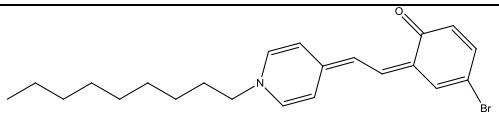
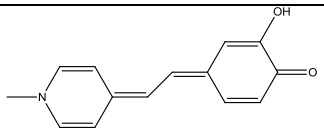
L-6		L9	
L8		L10	

Fig. 1. Structural formulas of the merocyanines

BO5. TWO SALTS OF VIOLURIC ACID INHIBITORS OF THE REPLICATION OF HERPES SIMPLEX VIRUS TYPE I

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Abstract

The cytotoxicity of two salts of violuric acid (K-12 and K-98) on monolayer cell culture MDBK was determined. Both substances showed close toxicity values K-12 (CC_{50} = 640μM) and K-98 (CC_{50} = 630 μM).

The antiviral activity of the substances on the replication of herpes simplex virus type 1 (HSV-1) was determined. Stronger effect on the replication of intracellular HSV-1 showed substances K-98 with a selective index (SI) = 74. Significant activity also demonstrates and the other substance K-12 (SI = 64).

The effect of the test substances on the activity against the virulence of the extracellular HSV–1 virions was also investigated. Activity was determined in five time intervals: 15, 30, 60, 90 and 120 minutes. A low virucidal effect is marked at 30 minutes with Δlg = 1.5 for both compounds. K-12 showed a higher inhibition value of Δlg = 1.75 when measured at 90 min which has time-dependent properties.

Keywords: Herpes simplex virus type I, viral replication, salts of violuric acid, therapy, synthesis, acyclovir (ACV)

Introduction

According to the World Health Organization data, more than 3.7 billion people aged up to 50 years (67%) of the world's population have HSV-1 infection. Primary infection with the virus is most common in infancy through oral-to-oral contact. More than 417 million people aged between 15-49 years (11%) worldwide have an HSV-2 infection that is defined as sexually transmitted. The results are even more disturbing, considering that more than 140 million people aged between 15-49 years have an already established genital HSV-1 infection, also taking into account the fact that both HSV-1 and HSV-2 are lifetime infections (8).

Therapy against the replication of herpes simplex viruses based on the use of nucleoside analogues, the largest application of which received acyclovir (ACV), has been developed. The disadvantage of this therapy is the relatively rapid formation of resistant mutants, leading to failure of the treatment (3).

Therefore, numerous studies have been carried out in different directions, both with natural and synthetic products for the detection of new therapies against herpes infection (3, 7).

Materials and Methods

Cells. Monolayer cultures of Madin-Darbey bovine kidney (MDBK) cells (National Bank for Industrial Microorganisms and Cell Cultures, Sofia) were grown in DMEM medium containing 10% fetal bovine serum (Gibco BRL, USA), supplemented with 10 mM HEPES buffer (Merck, Germany) and antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml) in CO_2 incubator (HERA cell 150, Heraeus, Germany) at 37 °C/5% CO_2.

Virus. Herpes simplex virus type1, Victoria strain (HSV-1) was received from Prof. S. Dundarov, National Center of Infectious and Parasitic Diseases, Sofia. The virus was replicated in monolayer MDBK cells in a maintenance solution DMEM Gibco BRL, Paisley, Scotland, UK, plus 0.5% fetal bovine serum Gibco BRL, Scotland, UK. The virus titers were estimated from cytopathogenicity by the limit-dilution method and expressed as 50% cell culture infectious dose per ml ($CCID_{50}$/ml).

Compounds tested. Two salts of violuric acid K-12 and K-98 were first dissolved in DMSO to a concentration of 0.01 M and then diluted in DMEM to the required concentration. The structural formulas of the substances are presented in Fig. 1.

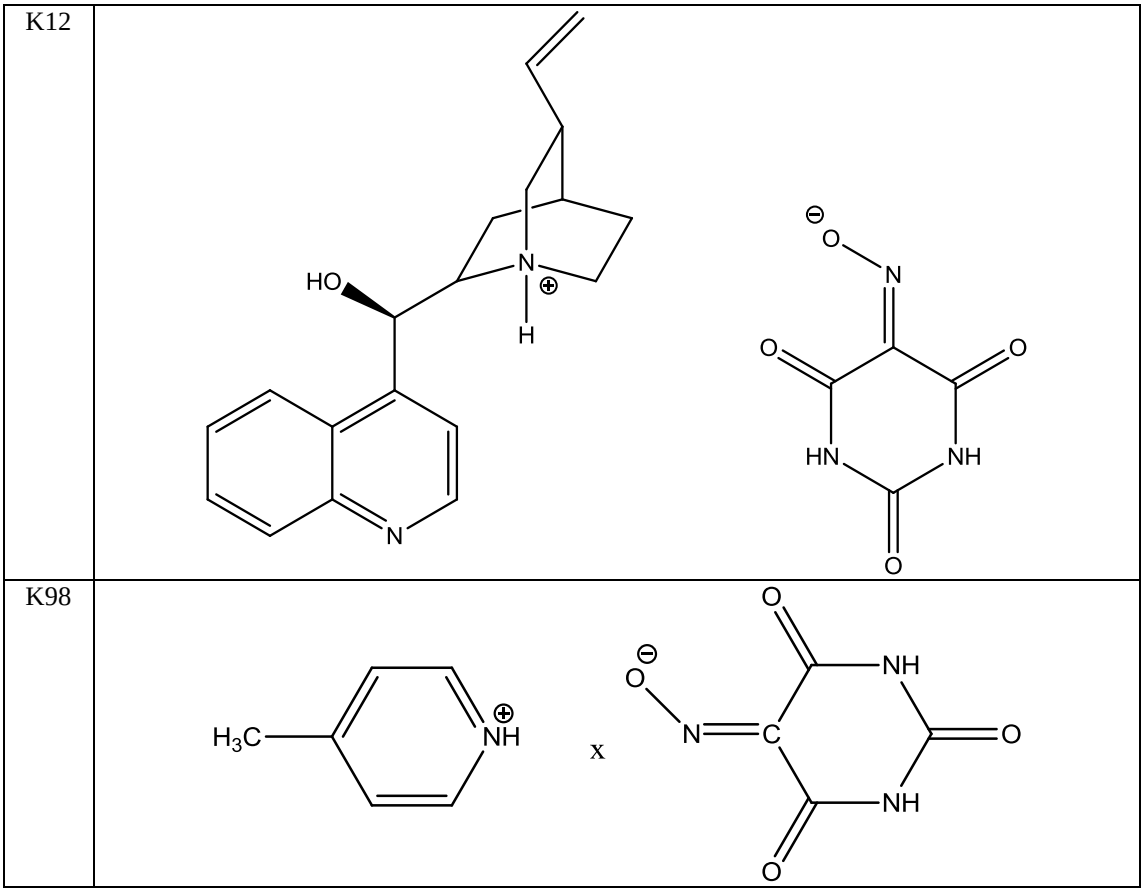

Fig. 1. Structural formulas of the salts of violuric acid.

Synthesis of the compounds.

L-12 Synthesis. Cinchoninium violurate monohydrate (1) was synthesized by mixing of equimolar amounts of cinchonine (0.2944 g) and violuric acid (0.1570 g) preliminarily dissolved in 25 ml water with continuous stirring and heating at 100 °C for 10 h. After leaving to stand red crystals were obtained from the resulting red solution and were filtered off and dried under air. The most intensive signal in the positive ESI mass spectrum is that of the

peak at m/z 259.71, corresponding to the singly charged cation $[C_{19}H_{23}N_2O]^+$ with a molecular weight of 259.40. The TGV and DSC data in the temperature range of 300–500 K exhibit a molecular weight loss of 3.88% and an enthalpy effect of 3.55 kcal/mol at 124°C corresponding to loss of the water molecule included in the crystal structure.

L-98 Synthesis. This salt of violuric acid was synthesized by a common scheme, by mixing equimolar amounts of the corresponding amine 4-methyl pyridine (930 mg – 10 mmols) and violuric acid (1.75 10 mmols) in 200 mL of water, under continuous stirring at a temperature within 40-50°C for 24 h. Precipitate and crystalline sample with violet color were obtained after leaving the resulting solutions to stand at 25°C for about a week.

Cytotoxicity assay

The *in vitro* cytotoxicity of the compounds was examined using MDBK cells. Confluent monolayer in a 96-well plate was treated with culture medium containing no or increased concentrations of the compounds. The cells were incubated at 37ºC for 48h. The viability of the cells after drug treatment was measured using a neutral red uptake assay and ELISA reader at OD_{540nm}.

Antiviral activity assay

Cytopathic effect (CPE) inhibition test used confluent cell monolayer in 96-well plates infected with 100 $CCID_{50}$ in 0.1ml. After 1 h of virus adsorption compounds were added in various concentrations and cells were incubated for 48 h at 37ºC. Inhibition of cytopathic effect was determine using a neutral red uptake assay and an ELISA reader at OD_{540nm}. The IC_{50} concentration of the two salts was identified as that concentration that inhibited development of CPE by 50%.

The selectivity index of substances was defined as the ratio between CC_{50}/IC_{50}.

Virucidal assay

Virus suspension containing 10^5 $CCID_{50}$ HSV-1 was mixed in a 1:1 ratio with or without the salt of violuric acid in maximal non-toxic concentration (MNC) of each compound. Samples were incubated for 15, 30, 60, 90 and 120 min., at room temperature. Then the infectious virus titer was determined of all contact samplesby the method of the final dilution. The results are compared to those from control virus - equal volume of virus suspension and maintenance medium, incubated for the same time intervals and determine Δlg.

Statistical analysis

Data on compounds cytotoxicity and antiviral effects were analysed statistically. The values of CC_{50} and IC_{50} are presented as means ± SD. The differences' significance between the effects of each salt is compared to the corresponding acyclovir value (6) was done through the One-Way ANOVA where p-values of <0.05 were regarded significant.

Results and discussion

To eliminate the possibility that the effect on the replication of HSV-1 due to the toxicity of the substances was predetermined their cytotoxicity on monolayer cell culture, MDBK in order to conduct more stringent studies with non-toxic concentrations (Table 1). Both salts show almost the same cytotoxicityas the K-12 was lower CC_{50} = 640 μM (MNC = 100 μM), and that of the K-98 was CC_{50} = 630 μM (MNC = 110 μM).

The antiviral activity of the two salts of violuric acid on the replication of HSV-1 was determined. Both substances show close activity as a stronger effect indicates K-98 with a selective index (SI) = 74 followed by K-12 with SI = 64.

The activity of violuric salts was also determined against the virulence of extracellular virions. From the results presented in Table 2, it can be seen that both salts exhibit weak virucidal activity Δlg = 1.5 per 30 minutes. The K-98 substance retained this activity at all the

time intervals tested, while the K-12 slightly increased its activity at 90 minutes Δlg = 1.75 as the influence is time-dependent.

Table 1. Cytotoxicity and antiviral activity of the salts of violuric acid

Compounds	CC_{50}±SD(μM/ml)[1]	IC_{50}±SD(μM/ml)[1]	SI = CC_{50}/IC_{50}
K-12	640.0 ± 9.6 ***	10.0 ± 2.4 ***	64.0
K-98	630.0 ± 10.4 ***	8.5 ± 2.2**	74.0
ACV	1296 ± 49.4	1.47 ± 0.06	881.6

**p<0.01, ***p<0.001, compared to ACV
[1] Values are means ± standard deviation from three consecutive experiments.

Table 2. Effect of salts of violuric acid on the extracellular HSV-1 virions

Compounds	Δlg				
	15 min	30 min	60 min	90 min	120 min
K-12	1.25	1.5	1.5	1.75	1.75
K-98	0.75	1.5	1.5	1.5	1.5

Considering the structure of the two substances – K-12 and K-98 (Table 1), it is noticed that they are complexes containing in their structure anion of violuric acid which can be attributed to their high activity. There is evidence that violuric acid can act as fungicide or bactericides (1, 2). Violuric acid is a derivative of barbituric acid, the use of which is most often related to its sedative properties. Some derivatives or complexes of barbituric acid with other substances show antifungal or anti-tumor activity (4, 5).

These data require a more detailed study of the anti-viral activity of violuric acid and its other derivatives or complexes.

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BO6. DRIED BLOOD SPOTS IN HEPATITIS B DIAGNOSIS

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It is estimated that 257 million people worldwide were infected with hepatitis B (HBV), they were defined as HBsAg positive [1]. In order to facilitate more widespread uptake of testing for HBV, there needs to be greater access to diagnostic assays. The use of dried blood spots (DBS) for transportation after fingerstick sampling in adults and older children, or heel pricking sampling in neonates and infants, of capillary blood and subsequent analysis with serologically based EIA represents another affordable alternative to testing [2,3]. The use of filter papers is an attractive alternative to the use of larger volume tubes for blood collection and storage for several reasons. Only a few drops of blood are applied to the paper, and this amount can be obtained by a heel stick. Venepuncture of small infants is not always successful; the amount of blood obtained is sometimes insufficient and mothers of small infants are often more comfortable with a heel stick than venepuncture. Dried blood samples on filter can be stored at room temperature, eliminating the need to store and transport whole or separated blood samples in cold chain. The use of filter papers also provides fewer chances for mislabeling because there are no transfer steps once the blood is applied to the paper [4]. Sero-diagnosis of HBV is traditionally carried out by collecting venous blood and initial screening for HBsAg with subsequent detection of other HBV markers. In the case of HBV infection, DBS samples have been used for detection of viral antigens and antibodies, such as HBV DNA, HBV core gene, anti-HBs, anti-HBc, HBsAg, hepatitis B e antigen (HBeAg) and for genotyping [5]. However, the main disadvantage of DBS is that the existing commercial assays have not been validated or received regulatory approval with this method of sample collection and transport. Also most of the studies did not assess all HBV markers on the same card and they generally did not use commercial assays for elusion [6]. In fact, some studies published detailed protocols on how to collect and analyze DBS [7], but such instructions are not provided by manufacturers on how to use their assays with DBS or how to manage with the appearance of different cut-offs. Further data is needed to validate serological assays and provide instructions for the use of DBS.

Acknowledgements
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BO7. IN VITRO ANTITUMOR ACTIVITY OF *VACCINIUM VITIS-IDAEA L.*, PICKED IN BULGARIA

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A major goal in antitumor therapy is to find active ingredients that selectively suppress the proliferation of tumor cells. In this connection, the antitumor activity of natural products with proven phytochemical properties and pharmacological significance are actively investigated. A promising candidate are Bulgarian cranberries from high mountain plant populations, which are rich in various bioactive compounds including phenolics and anthocyanins.

Currently they belong to a group of functional foods and many studies have demonstrated their beneficial effects on different functions in the human body
Aim: The present study aims to evaluated *in vitro*, antitumor potential of total methanol extracts and purified fractions (B- nonanthocyanin / C- anthocyanins) of *Vaccinium Vitis-Idaea L.*, picked in Bulgaria on human cervical cancer (HeLa) cell line.
Materials and methods: A total of four methanol extracts and respective number purified fractions (B- nonanthocyanin / C- anthocyanins) of cranberry picked in Bulgaria were used. Antitumor effect was established by Trypan Blue method and MTT cell viability assay.
Results: The results from MTT analyses showed that B- nonanthocyanin fractions of Bulgarian cranberry have a dose-dependent inhibitory effect on survival of cells of the cervical tumors.
Conclusion: Evaluation of antitumor activities of Bulgarian cranberries using modern molecular methods, could contribute to establish the natural substances useful for human health.

Key words: *Cranberries, antitumor activity, HeLa*
Acknowledgment: This work was supported by grant № DM 13/3 from 15.12.2017.

BO8. ШАП ПО ЧИФТОКОПИТНИТЕ ЖИВОТНИ

Симеон Димитров
НРЛ "Шап и ВБС" към НДНИВМИ

Шапът е остро контагиозно вирусно заболяване по двукопитните домашни и диви животни, проявяващо се с треска, везикулозни поражения по устната кухина, обезкосмените части на тялото, копитния венец и междукопитната цепка, съпроводено с обилна саливация и трудности при движение. Причинява се от вирус (Aphtovirus), който се среща в 7 серотипа с над 60 подтипа. Протичането на заболяването при различните видове животни се манифестира с различна клиника. При домашните животни, най-различими са симптомите, които проявяват говедата, докато при овцете и козите заболяването обикновено протича със слаба клинична симптоматика, а понякога без явни или с трудно забележими клинични признаци. При епизоотологичното проучване на нововъзникнало огнище на шап определянето на най-вероятната дата за проникване на вируса е от решаващо значение за налагане на мерките, определени от Директива 85/2003/ЕС/. Фактът, че симптомите на шапа при говедото са най-типични, дава възможност с най-голяма достоверност да се определи вероятното време за проникване на вируса в стадото или фермата. За по-точно определяне на времето за това проникване е необходимо да се прегледат възможно повече заболели животни. Най-подходящият материал за доказване на вируса на шапа за лабораторията представлява афтозната течност и епитела на млада афта, взети принудително или само епител от току-що разкъсана афта. Доказването на вируса на шапа в епител от лезии на възраст по-голяма от 4-5 дни е вече проблематично. Най-подходящи за пробовземане в периода 4-10-15-и ден от началото на шапна инфекция е орофарингеалният секрет (ОР). В този период доказването на вируса в лезиите може да даде фалшиво негативни резултати. ОР проба задължително се взема и при животни в много по-късните фази на шапната инфекция, дори когато клиничните симптоми на болестта напълно отсъстват. Вирусът на шапа засяга лимфоретикуларната тъкан. На този факт се основава феномена на безсимптомното 48 носителство ("carrier status") на вируса в назофарингеалния лимфен пръстен и лигавици при преживните животни с различен период след

преболедуването от шап. Диагностицирането е свързано с проследяване на клиничните признаци, патолого –анатомичните изменения и от специфичното откриване на вируса антиген/антитяло/геном във проби от изследвани животни с серологични тестове (ELISA) и молекулярно-биологични анализи.
ключови думи: Aphtovirus, шап, епизоотия, ELISA

BO9. **CYTOTOXIC ACTIVITY OF METAL [Zn(II), Co**(II), Ni(II)] COMPLEXES WITH SCHIFF BASES

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Session C.

Chairpersons:

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Assoc. Prof. Radostina Alexandrova, MSc, PhD
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CO1. ACUTE INTOXICATION FOLLOWING AYAHUASCA INGESTION: CASE REPORT

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Ayahuasca is a hallucinogenic tea that is comprised of the vine Banisteriopsis caapi alone or in combination with other plants such as Psychotria viridis. This tea originates from the Amazon Basin where it is used in religious ceremonies. Banisteriopsis caapi is rich in β-carbolines, especially harmine, tetrahydroharmine and harmaline, which have monoamine oxidase inhibiting (MAOI) activity. Psychotria viridis contains the 5HT2A/2C/1A receptor agonist hallucinogen N,N-dimethyltryptamine (DMT). This concoction results in an orally active form of dimethyltryptamine (DMT), a hallucinogenic amine. Consuming this combination leads to psychoactive effects which indigenous peoples in the Amazon region utilized for centuries. Ayahuasca is a sacrament, a movement that has spread to some countries around the world. Because interest in these religious groups spreading as well as awareness of use of ayahuasca for therapeutic and recreational purposes, its use is increasing.

For the first time in Bulgaria is presented the case of a patient who ingested ayahuasca tea for recreational purposes. A review of ayahuasca toxicity and evaluation of serious adverse effects is also presented.

Keywords: Ayahuasca, dimethyltryptamine (DMT), psychoactive eff ects

CO2. COMPETITION BETWEEN ABIOGENIC AL^{3+} AND NATIVE MG^{2+}, FE^{2+} AND ZN^{2+} IONS IN PROTEIN BINDING SITES: IMPLICATIONS FOR ALUMINIUM TOXICITY

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The abiogenic aluminium has been implicated in some health disorders in humans. Protein binding sites containing essential metals (mostly magnesium) have been detected as targets for the "alien" Al^{3+}. However, the acute toxicity of aluminium is very low. Although substantial body of information has been accumulated on the biochemistry of aluminium, still the underlying mechanisms of its toxicity are not fully understood. Several outstanding questions remain unanswered: (1) Why is the aluminium toxicity, unlike that of other "alien" metal cations, relatively low? (2) Apart from Mg^{2+} active centers in proteins, how vulnerable are other essential metal binding sites to Al^{3+} attack? (3) Generally, what factors do govern the competition between 'alien" Al^{3+} and cognate divalent metal cations in metalloproteins at physiologically relevant conditions? Here, we endeavor to answer these questions by studying the thermodynamic outcome of the competition between Al^{3+} and a series of biogenic metal cations, such as Mg^{2+}, Fe^{2+} and Zn^{2+}, in model protein binding sites of various structures, compositions, solvent exposure and charge states. Density functional theory (DFT) calculations in combination with polarizable continuum model (PCM) computations are employed. For the first time the presence of different Al^{3+} soluble species at physiological pH is properly modeled in accordance with the experimental observations. The results imply that the combination between concentration and physicochemical factors renders the $Al^{3+} \rightarrow M^{2+}$ (M = Mg, Fe, Zn) substitution and subsequent metalloenzyme inhibition a low-occurring event at ambient pH: the more active aluminium species, $[Al(H_2O)_6]^{3+}$, presents in very minute quantities at physiological conditions, while the more abundant soluble aluminium hydrate, $\{[Al(OH^-)_4](H_2O)_2\}^-$, appears to be thermodynamically incapable of substituting for the native cation.

CO3. CYCLODEXTRIN INCLUSION COMPLEXES OF PT-BASED ANTICANCER DRUGS

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Because of their biocompatibility and low toxicity, CDs are excellent excipients for drug formulations and can significantly improve the solubility, stability, bioavailability and dosing of variety of active pharmaceutical ingredients [1]. Although the use of CDs in drug

delivery has been documented for decades, some questions regarding the CD/drug interactions still wait to be answered in the quest for more operative and precise CD-based drug delivery systems. CD/drug interactions are quite intriguing when CD serves as an excipient for drug formulation of Pt-based anticancer drugs. Aim of our study is to elucidate the factors determining the interactions of the CDs (α-, β-,γ-) with cisplatin (the oldest platinum drug, in routine clinical use for treatment of ovarian, testicular, bladder, cervical, and other solid tumors [2]) and other Pt-based anticancer drugs - transplatin, picoplatin and carboplatin.

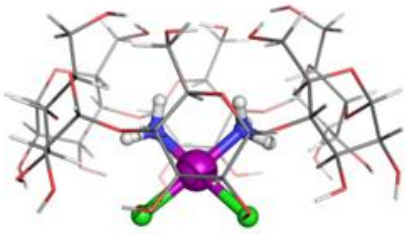

Figure 1. α-CD/cisplatin complex.

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CO4. EFFECTS OF SUBCHRONIC EXPOSURE TO LEAD ON RAT`S BLOOD MINERALS

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ABSTRACT

The aim of our study was to evaluate the effects of subchronic exposure to dietary lead on serum mineral content in rats. Levels of calcium (Ca), phosphorous (P), iron (Fe), copper (Cu) and zinc (Zn) were determined in the serum of control and lead treated rats. 100 mg lead acetate were added to the 1 L drinking water for 2 weeks. Results showed a significant imbalance among minerals from the treated rats. Serum concentration of Ca, P, Fe and Cu were significantly increased in the group received Pb compared to the control group. Serum zinc was significantly decreased in the group with Pb as compared to the control group.

Lead exposure resulted in mineral imbalance which affects the various biochemical processes taking part in oxidative stress. The oxidative stress could play a significant role in the protection of organism of Pb exposed animals.

Key worlds: lead, minerals, serum, rats.

INTRODUCTION

Lead is a toxic metal that induces a wide range of behavioral, biochemical, and physiological effects on humans and animals. As lead exposure tends to be sub- acute, produces only subtle chemical symptoms. Some mineral disturbances were established by Bafundo et al., 1984 and Taha et al., 2012. Lead is known to modify the metabolism of trace elements and nutrients (Taha et al, 2012). Lead administration decreased liver copper level whereas additional dietary copper increased the level of lead. It was postulated that Pb interferes whit Cu and Fe metabolism.

The aim of our study was to evaluate the effects of subchronic exposure to dietary lead on blood mineral content in rats.

MATERIAL AND METHODS

Twenty albino rats, weighting about 200 g were divided into 2 groups 1st gr. – control and 2nd – gr. – received 100mg Pb in 1 liter drinking water for 2 weeks. At the end of the experiment rats were sacrificed and blood samples were collected and were subjected to the calcium, phosphorous, zinc, copper and iron. Their levels were determined using ICP-OES Prodigy 7, Teledyne Leeman Labs USA.

RESULTS

Fig. 1-5 illustrated that serum Ca, P, Fe and Cu are significantly increased in the group received Pb compared to the control group. Serum zinc is significantly decreased in the group with Pb as compared to the control group.

Fig. 1- Concentration of Ca in serum of rats with Pb administration

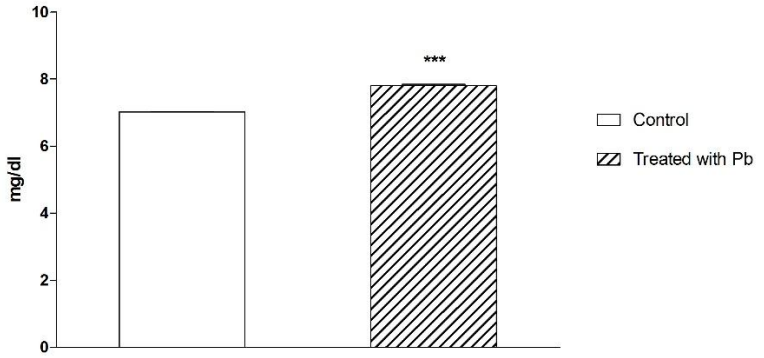


Fig. 2- Concentration of P in serum of rats with Pb administration

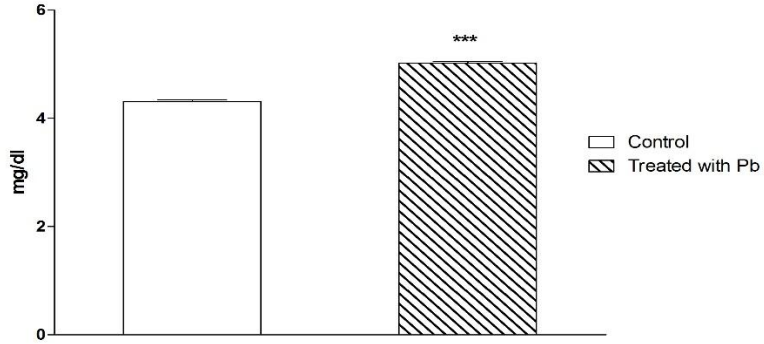


Fig. 3- Concentration of Fe in serum of rats with Pb administration

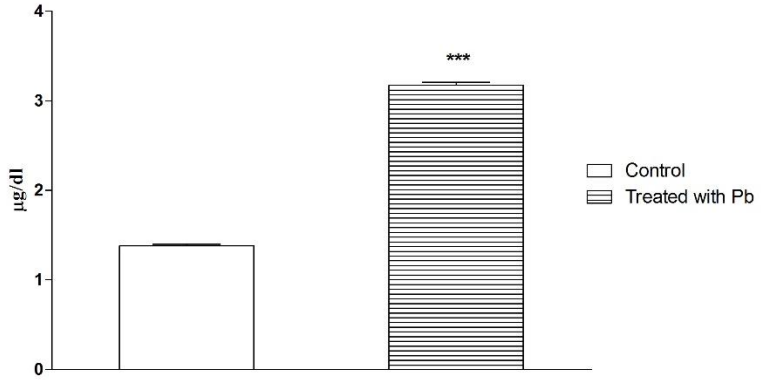


Fig. 4- Concentration of Cu in serum of rats with Pb administration

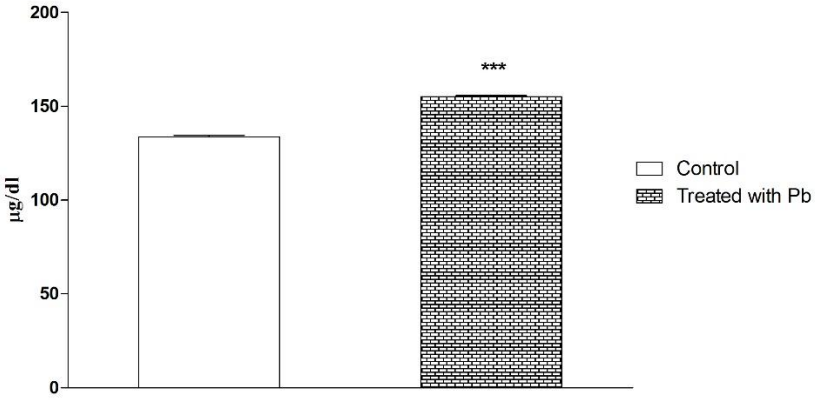
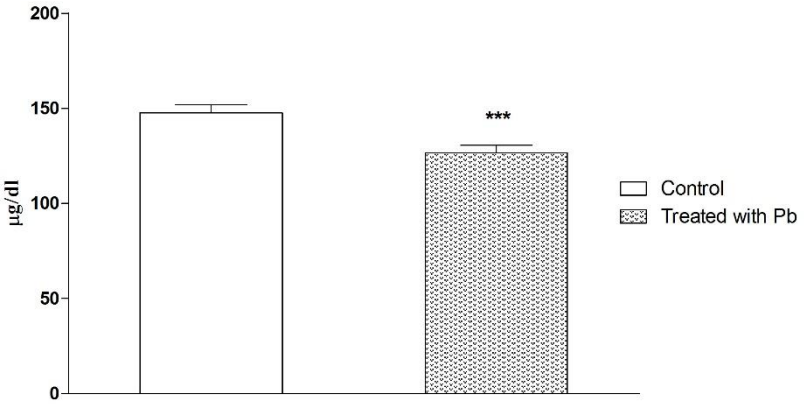


Fig. 5- Concentration of Zn in serum of rats with Pb administration



The decreased order of minerals in control rats is Ca>P>Zn>Cu>Fe
and in rats with lead administration is Ca>P>Cu>Zn>Fe

DISCUSSION

Lead is one of the major environmental pollutants in the world. In our study Ca and P levels in the rats received lead show significant increase compared with controls. Our result is similar to that of Missoun et al.(2010) recorded that the levels Ca an P were increased in serum of rats received lead acetat in drinking water for 8 weeks. According the above authors this results are due to impairment of renal function or inhibitory effects of Pb on cation transport in the tissues of treated rats. In these cases Pb has direct effect on osteoblast function including inhibition of active vitamin D3 stimulated synthesis of osteocalcin, acting in mineralization (Ronis, 2001). This is comfirmed that Pb administration had direct effects via

interference with calcium homeostasis. Hypercalcaemia observed by us in serum of rats with Pb administration is in agreement with the results of Taha et al. (2012).The increase in phosphate in our study may be due to cell membrane damage as a result of exposure to Pb. Similar phosphorus elevation was observed in bucks exposed to 8 mg Pb/kg b.w. (Desouky et al., 2001). In contrast to our results unchangeable inorganic phosphorous content was noticed in rabbits exposed to oral dose of Pb for a long period (Walid, 1997).

In this study the copper increase was observed. It is in a good accordance with the data presented by Kasperczyk et al. (2012). They found that plasma Cu level was higher than the control level. According the above authors these data of Cu elevation may be due to an elevated activity of Cu, Zn- SOD in both serum and erythrocytes. The increase plasma Cu level may also be caused by competitive displacement of the metal from tissues by Pb ion. Cu and Pb ions compete for binding sites on proteins, also the increased bioavailability of displaced Cu may induce reactive oxygen species (ROS) generation and contribute to oxidative stress enhancement (Qian et al., 2005).

Significant zinc serum decrease was observed in the group received Pb-salt, compared with controls. It may be due to the imbalance of metabolism produced by impairing Zn status in Zn-dependent enzyme acting in many metabolic processes (Nabil et al., 2012). Exposure to Pb may decrease the absorption rate and biologic availability of Zn ions in the body due to their competition for binding to the sulfhydryl group site in enzymes, proteins and tissues (Nabil et al., 2012).

There was significant increase in serum iron level in our study, whenever anemia caused on account of Pb poisoning can be hemolytic anemia Vij (2009). Pb- acetate affected the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting many key enzymes involved in in the heme synthesis pathway (Guidotti et al., 2008).

Lead exposure resulted in mineral imbalance which affects the various biochemical processes taking part in oxidative stress. It was logical to hypothesized that oxidative stress plays a significant role in the protection of organism of Pb exposed animals.

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CO5. **INHIBITORY EFFECTS OF TANSY ON POSTPROLINE CLEAVING ENZYME ACTIVITY IN BREAST CANCER CELLS**

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Abstract

The effect of the crude extract from tansy on the postproline-specific enzyme activity in three human cell lines MCF-10A, MCF-7 and MDA-MB-231 was investigated. In all the three cell lines, a concentration-dependent inhibition was observed, with the degree of inhibition in MDA-MB-231 being highest and the lowest in MCF-7. The type of inhibition of the extract on proline-specific enzyme activity in MDA-MB-231 cell lines proved to be uncompetitive. Our results show that different tansy extracts, obtained from the crude extract, have a pronounced inhibitory dose-dependent effect on the enzyme activity in the cancer cell line MDA-MB-231. The highest effect was observed using the ethyl acetate/aqueous extract of the herb. Since the proline specific enzymes are known to participate in different tumors growth, it could be concluded that the natural inhibitors from tansy have a potential to be used as therapeutic anti-cancer agents.

Key words: Tansy (*Tanacetum vulgare L.*), Postproline-specific enzymes, Inhibition, Breast cancer cell line, Dicaffeoylquinic acids, Flavonoides

Introduction

Studies on the identification of proteolytic enzymes, markers of pathological processes, could lead to the development of new therapeutic methods with high efficiency and good biological tolerance. Proteolytic enzymes could be considered as a potentially attractive therapeutic target. Proline is an important amino acid in many biologically relevant polypeptide sequences. The presence of proline in a peptide affects its interaction with other proteins, which prevents their degradation by the most common proteases. Postproline proteases constitute a subset of serine proteases involved in the regulation of many signaling events and are emerging as promising therapeutic targets for prevalent diseases such as diabetes and cancer [11]. This protease subset belongs to the S9 family of serine peptidases and includes such diverse and important enzymes as prolyl oligopeptidase (POP; EC 3.4.21.26) [7], dipeptidyl peptidase IV (DPP-IV, EC 3.4.14.5) [9] and fibroblast activation

protein alpha (FAP, EC 3.4.21.B28) [6]. Increased activity levels of these enzymes are observed in various pathological conditions, including malignancies [2, 12]. Their inhibitors are potential therapeutic agents [10].

Plants produce a broad range of bioactive compounds via their secondary metabolism. These compounds may elicit a long range of different effects on humans and animals. Recently, there is increasing interest towards substances of natural origin that are selective inhibitors of proline-specific enzymes. For example, it has been found that certain flavonoids and caffeoylquinic acids, as well as derivatives thereof, are POP inhibitors with good selectivity to DPP-IV [1, 5]. The main nonvolatile components in tansy (*Tanacetum vulgare L.*) blossom extract are caffeoylquinic acids and flavonoid derivatives [3], which are potential inhibitors of these peptidases.

In the present study, the effect of the extracts and fractions from flowers of tansy on the postproline-specific enzyme activity in the above three human mammary gland cell lines (normal and cancerous) was investigated. The IC_{50} values of inhibition from fractions and type of inhibition were determined.

Materials and Methods

Chemicals and reagents

Acetonitrile mass spectrometry (MS) grade was obtained from Merck (Germany). Ethyl Acetate, diethyl ether, diisopropyl ether and hexane were from Fisher Chemical (UK), formic acid, 98% was from Fluka (Germany). High-purity water was generated using a Purelab UHQ II system from ELGA (Netherland). All reagents were of the highest purity available.

Plant material and extracts preparation

The flowers of tansy and the crude extract were provided by Vemo 99 Ltd (Sofia, Bulgaria).

Twenty mL of water were added to 5 g of the crude tansy extract while stirring. After that, 6N HCl was added dropwise until pH 3.00. Ethyl acetate (15 mL) was applied to the aqueous phase while stirring. The organic phase was separated and the aqueous phase was extracted with 10 mL ethyl acetate. The combined organic phases were filtered, washed with brine and dried over Na_2SO_4. The ethyl acetate was removed under vacuum and small volume of diisopropyl ether was added. The formed dark yellow solid was filtered off and dried.

Dicyclohexylammonium salts fraction was obtained from the ethyl acetate extract as follows: The volume of the ethyl acetate extract was reduced to 1/4 and dicyclohexylamine was added dropwise. The obtained precipitate was filtered, washed with diisopropyl ether and dried.

Sixteen mL of water and 48 mL of ethyl acetate were added to 4 g tansy blossoms while stirring. Then, 6N HCl was added dropwise until the pH of aqueous phase equals 3.00 and the mixture was stirred for an additional hour. After a filtration, the organic phase was separated and processed as above. Finally, diisopropyl ether was added and the obtained precipitate was filtered and dried.

Cell culturing

Three permanent cell lines were used – MCF-10A (normal immortalized human epithelial cells from mammary gland), MCF-7 (human tumor cells obtained from mammary gland carcinoma of low invasiveness – liminal type A) and MDA-MB-231 (human tumor cells from mammary gland carcinoma of high invasiveness – triple negative). The cancer cells were cultured in 75 cm^2 tissue culture flasks in Dulbecco's Modified Eagle's Medium – high glucose 4.5‰ (DMEM), supplemented with 10% fetal calf serum and antibiotics in usual concentrations. Normal cells were cultivated in the same conditions but with the addition of 20 mg/L human epidermal growth factor (EGF), 0.5 mg/L hydrocortisone, 0.1 mg/L cholera

toxin and 10 mg/L insulin. Cell cultures were maintained at 37.5°C in a humidified atmosphere and 5% CO_2 until 95% confluence was achieved.

The cells were harvested by a rubber policeman and homogenized using homogenizer MSE (England) in 5 mL 0.1 M phosphate buffer (pH 7.4) with 0.1 M NaCl and 1 mM EDTA.

Enzyme activity measurement

Enzyme activity in homogenate of cell lines was measured in the presence of 0.1 or 0.2 mg/mL of tansy extract in 0.1 M phosphate buffer (pH 7.4), containing 0.1 M NaCl and 1 mM EDTA at 37°C using 80 μM fluorogenic substrate Z-Gly-Pro-AMC. Enzyme assays were carried out in 96-well plates, in a multifunctional spectrofluorimeter Varioscan Fluorescence at 360 nm excitation and 460 nm emission every 3 minutes. The software program EnzFitter V2 was used for data processing. The enzyme activity was determined from the initial rate of the reaction.

Sample preparation for MS/AIF analyses

Extracts of tansy (3 mg) were dissolved in 1 mL 0.1 % formic acid buffer by ultrasound assisted extraction for 15 min and were subjected to LC–MS analyses.

LC-MS analysis

Analysis were carried out using Q Exactive hybrid quadrupole-Orbitrap mass spectrometer (ThetmoScientific Co, USA) equipped with TurboFlow® LC system, heated electrospray model HESI II on IonMax® (ThetmoScientific Co, USA).

The chromatographic separation of analytes was carried out by Hypersil Gold column (100 mm × 2.1 mm i.d., 1.9 μm) using the following mobile phases: A: 0.1% formic acid in water and B: 0.1% formic acid in acetonitrile at flow rate of 300 μL/min and gradient: 0 % B for 1 min, 30 – 90 % B for 30 min, 90 % B for 5 min, 90 – 0 % B for 2 min. and 0% B for 2 min. Injection volume was 10.0 μL.

Full-scan spectra over the m/z range 80-1200 were acquired in negative ion mode at resolution settings of 70 000. All MS parameters were optimized for sensitivity to the target analytes using the instrument control software program. Q Exactive parameters were: spray voltage 4.0 kV, Sheath gas flow rate 32, Auxiliary gas flow rate 10, Spare gas flow rate 3, Capillary temperature 320 ºC, Probe heater temperature 300 ºC and S-lens RF level 50. All Ion Fragmentation (AIF) was used for qualification of the caffeoylquinic acids. Optimized values of the collision energy were HCD 25 %. Data acquisition and processing were carried out with Xcalibur 2.4® software package (ThetmoScientific Co, USA). Calculations for theoretical m/z values were made by Mass Frontier 5.1 Software program (ThetmoScientific Co, USA)

Results and Discussion

The crude extract from *Tanacetum vulgare L.* was provided by Vemo 99 Ltd (Sofia, Bulgaria). The effect of the extract on postproline-specific enzyme activity in three human cell lines (MCF-10A, MCF-7 and MDA-MB-231) was studied using the nonspecific fluorogenic substrate Z-Gly-Pro-AMC. The tested extracts were with 0.1 mg/mL and 0.2 mg/mL. In all three cell lines, concentration-dependent inhibition was observed (Fig. 1), with the degree of inhibition in MDA-MB-231 being the highest: 0.40 at concentration 0.1 mg/mL and 0.61 at 0.2 mg/mL. The lowest degree of inhibition was observed in MCF-7: 0.30 at concentration 0.1 mg/mL and 0.48 at 0.2 mg/mL respectively. In view of the obtained results it can be concluded that crude extract of *Tanacetum vulgare L.* has a marked dose-dependent inhibitory effect on the enzyme activity of homogenates from breast cancer cells. The here reported results are initial. More detailed investigations are necessary for screening the active compounds.

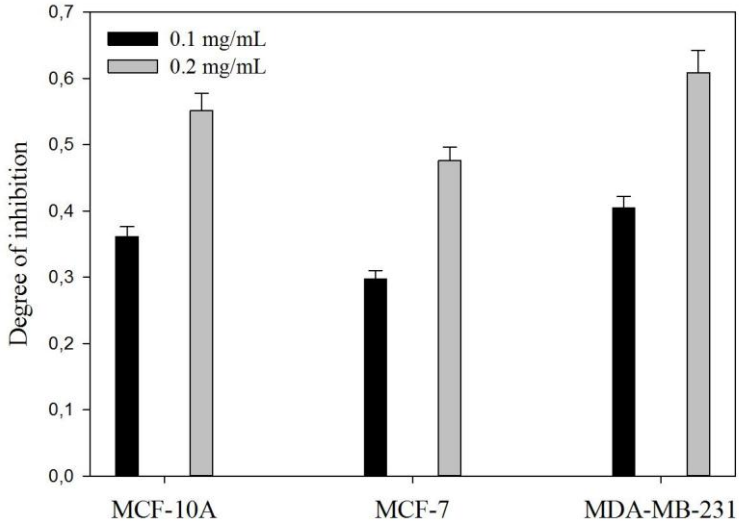

Figure 1. The degree of inhibition of the postproline-specific enzymatic activity from the total tansy extract in homogenates from three human cell lines.

Cell line MDA-MB-231 (triple negative human mammary gland carcinoma) is usually used as a negative control for FAP, since it is known to lack this enzyme activity [4, 8]. The tansy extract inhibits mildly recombinant DPP-IV, specifying a high selectivity of the natural tansy inhibitors to the postproline endopeptidase activity. On the other hand, the lack of FAP-activity in MDA-MB-231 cells indicates that an inhibition of POP-activity only was observed in our study.

Due to the variation in the chemical and quantitative composition of the herb blossom depending on the habitat and extraction method, it is important to establish the composition of the extract used by us. Using the LC-HRMS method, we determined the major nonvolatile compounds in the extract of tansy. In the crude extract from tansy we detected the presence of 3-caffeoylquinic acid, 5-caffeoylquinic (chlorogenic) acid, 3,4-, 3,5- and 4,5-dicaffeoylquinic acids, O-glucuronides of apigenin, luteolin and quercetin and O-hexosides of luteolin and quercetin.

Further on, the crude extract was partitioned between water and ethyl acetate and the effect of the aqueous and organic fractions on postproline-specific enzyme activity in the homogenates of MDA-MB-231 cell line were investigated. Ethyl acetate fraction exhibited a higher activity (0.68 degree of inhibition at concentration 0.1 mg/mL and 0.83 at 0.2 mg/mL respectively) (Fig. 2) as compared to the crude extract (Fig. 1). On the other hand, aqueous fraction showed a very low activity. Investigations by LC-HRMS indicated that ethyl acetate fraction contains a higher concentration of the major nonvolatile compounds, compared to theirs concentration in the crude extract.

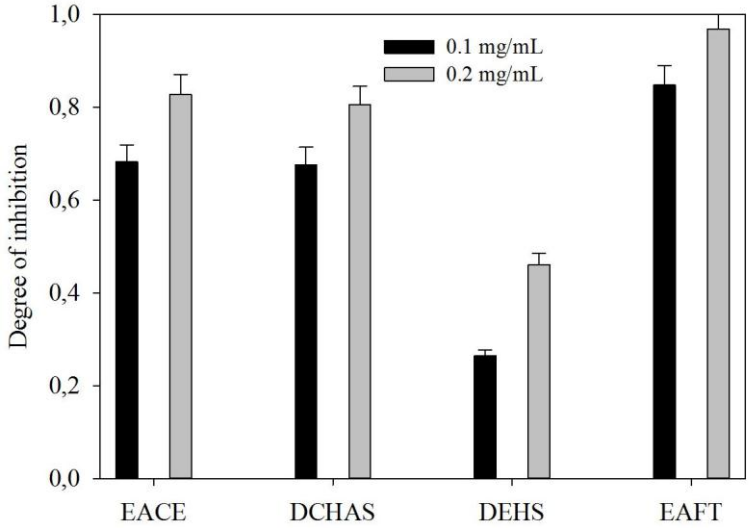

Figure 2. The inhibition effect of the fractions on postproline-specific enzyme activity in homogenate from MDA-MB-231 cell line. EACE – ethyl acetate fraction of the crude extract of tansy; DCHAS – dicyclohexylammonium salts fraction; DEHS – solid substance from diethyl ether/hexane separation; EAFT – ethyl acetate extract from *Flores Tanaceti*.

From these data, we can assume that some of these compounds are effective inhibitors of postproline endopeptidase activity, respectively POPs. Dicyclohexylamine was added to the ethyl acetate fraction, whereby a precipitate of the dicyclohexylammonium salts of 3-caffeoylquinic acid, chlorogenic acid, isomeric dicaffeoylquinic acids and O-glucuronides of apigenin, luteolin and quercetin formed. The inhibitory activity of these dicyclohexyl-ammonium salts proved to be essentially the same as the activity of ethyl acetate fraction (Fig. 2). After separating the precipitate from dicyclohexylammonium salts, the filtrate was concentrated and treated with diethyl ether and hexane, whereby a precipitate formed. The degree of inhibition by these substances is twice lower relative to of the ethyl acetate fraction (Fig. 2). These results confirm our hypothesis that some of the major components in the extract are potential selective inhibitors of the POP.

The powder from the flower of the plant was extracted with two phase system water/ethyl acetate. The crude ethyl acetate extract showed the highest effect with respect to the degree of inhibition of postproline endopeptidase activity (Fig. 2). Using the LC-HRMS we established that the crude ethylacetate fraction has differences in the quantitative and qualitative composition with respect to the content of the components in the organic fraction of the raw extract of *Tanacetum vulgare L.*, provided by Vemo 99 Ltd.

The crude extract from the herb was fractionated by flash chromatography. Some of the fractions showed inhibitory effect of 75-95% on the enzyme activity in the homogenate of MDA-MB-231 cell line. The IC_{50} (10 μg/mL) of the fraction showed the highest degree of inhibition. The UHPLC analysis of the most effective fractions showed the presence of one or two basic components, which structures will be elucidated by HRMS and NMR.

We conducted experiments to determine the type of inhibition of postproline-specific enzyme activity in MDA-MB-231 cell lines from the fractions with highest activity using the non-specific fluorogenic substrate Z-Gly-Pro-AMC. The obtained data are presented in Dixon coordinates (Fig. 3). According to the type of graph, the type of inhibition is uncompetitive. The apparent K_i is 3.2 μg/mL. Our results show that tansy extracts have a pronounced inhibitory dose-dependent effect on the enzyme activity in the cancer cell line MDA-MB-231.

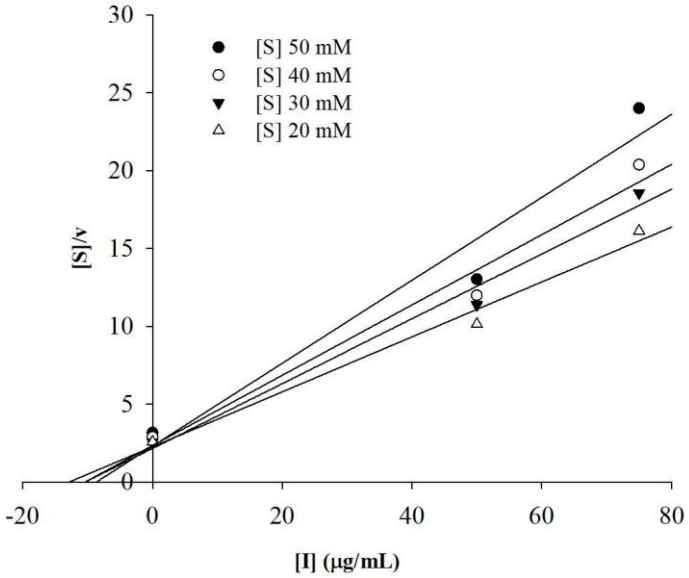

Figure 3. Determination of the type of inhibition of proline-specific enzyme activity in homogenate from MDA-MB-231 by Dixon plot

Conclusions

The components in the extracts from flower of *Tanacetum vulgare L.* show high selectivity with respect to the inhibition of proline-specific peptidases. These inhibitors have a potential to be used as therapeutic agents at least for mammary gland carcinoma.

Acknowledgement

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CO6. ANTIPROLIFERATIVE AND PROAPOPTOTIC ACTIVITY OF WATER EXTRACTS OBTAINED FROM GREEN MICROALGA *COELASTRELLA* Sp. BGV AGAINST HeLa TUMOR CELLS

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ABSTRACT

Microalgae are ubiquitous - they occur in freshwater, sea, acid or alkaline waters, wastewater, hot springs, mountain, polar regions, etc. The ability to survive in extreme habitats is due to their high adaptability, which involves the synthesis of various metabolites with high biological activity.

The present study show results on the antiproliferative activity and the pro-apoptotic effects of total aqueous extracts (low temperature-LT and high temperature-HT) and culture medium (CM), obtained from newly isolated Bulgarian strain green microalgae *Coelastrella* sp. BGV against Hela cells. The antiproliferative activity of the water extracts and CM was examined by MTT assay. The evaluation of cell death was done through morphological changes of HeLa cells after AO/EtBr and DAPI staining by fluorescence microscopy. The results indicated that LT and HT suppressed more pronounced the proliferation of HeLa cells at 24 h, whereas at 48 h, a significantly inhibition of proliferation was observed at concentrations of 500 μg/ml and 1000 μg ml. After treatment with CM, the tumor cell vitality at 48 hours is about 3 times lower than that seen at 24 hours. Morphological observation of DAPI and Acridine Orange/Ethidium bromide staining revealed typical characteristics of early and late apoptotic cell death of treated HeLa cells.

The observed antiproliferative and proapoptotic effects of total water extracts and CM from the Bulgarian strain *Coelastrella* sp. BGV can be explained with the water-soluble metabolites contained in them. The results obtained are promising and are the basis for further research to clarify better the biological activities of products and secondary metabolites from the Bulgarian strain *Coelastrella* sp. BGV.

Key words: microalgae; *Coelastrella* sp. BGV; aqueous extracts, HeLa cells; antitumor activity; apoptosis

Introduction

Over the last decades the interest of scientists in biological activity of microalgal compounds has increased significantly. Microalgae are eukaryotic plants that contribute up to 40% of world productivity. Their significance in marine drug discovery is their metabolic plasticity, which can trigger the production of a large group of structurally unique natural substances with a wide range of possible applications in various biotechnology areas - food, energy, health, environment, biomaterials etc. [3, 4, 11, 20, 30, 33]. Approximately 28,500 marine natural products had been identified by the end of 2016 [2]. Most of these compounds show pronounced cytotoxic and anticancer properties and are promising candidates for new drugs primarily for cancer treatment [33, 42]. Many studies showed that algae substances exert antiproliferative and cytotoxic effects against several human cell lines from the breast

[23, 28], colon [1], cervix uteri [21], prostate [11, 18], liver [1, 23, 36], lung, kidney and melanoma [5], etc. Other reports also demonstrated that the algae bioactive compounds inhibited angiogenesis [1] and induced apoptosis in leukemia and lymphoma cells [27], HT-29 human colon carcinoma cells [19], oral cancer [41], etc.

Many studies have focused on the antitumor properties of water soluble active substances from various marine algae, however, the data about the effects of aqueous extracts from green microalga *Coelastrella* species has been limited. In an effort to explore the anticancer activity of local algal strains, this study was conducted to investigate the antiproliferative and the pro-apoptotic activity of the aqueous extracts (LT and HT) and culture medium (CM) from the new Bulgarian strain green microalgae *Coelastrella* sp. BGV on human cervical tumor cell line HeLa. The induction of apoptosis of the tested extracts on HeLa cells is directed to outline the mechanism of action.

Materials and methods

Chemicals. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, antibiotic solution (penicillin–streptomycin), phosphate buffered solution (PBS) and trypsin–EDTA solution(2.5 g/L trypsin and 0.2 g/L EDTA), 3-[4,5-dimethylthiazol-2-yl]-2,3-diphenyl tetrazolium bromide (MTT), Acridine Orange (AO), Ethidium Bromide (EtBr), were purchased from Merck (Darmstadt, Germany). All the solvents and reagents used are in analytical grade.

Microalgal cultivation. The microalga strain used in this study was *Coelastrella* sp. BGV from the Culture Collection of Algae of the department of Experimental algology, of the Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences. The microalga was collected from a stagnant water in metal trough near Varvara village, Bulgaria (N 42° 10′; E 24° 0-7′), identified as *Coelastrella* sp and marked as strain BGV [8]. The axenic culture of this strain was grown in glass bottles containing 200 mL of Šetlik medium [35] modified by Georgiev et al. [14] on a block for the intense cultivation of microalgae at 28°C under continuous lateral illumination with cool-white fluorescent lamps at a photon flux density of 132 μmol m^{-2} s^{-1} for 20 days. During the incubation the algal culture was given air, enriched with CO_2 at 2-3% (v/v).

Preparation of algal extracts. Culture medium (CM) with extracellular secretions. At the stationary phase of growth of *Coelastrella* sp. BGV, the culture samples containing microalgae were centrifuged at 5000 rpm^{-1} for 10 min at room temperature and the respective supernatant was dried by lyophilization. The microalgal biomass was harvested, then was frozen and freeze-dried for further use. **Low temperature (LT) aqueous extract.** Lyophilized dry algal biomass of *Coelastrella* sp. BGV was flooded with boiling distilled water at a ratio of 1:8. After cooling to 40°C, the mixture was placed in a refrigerator (4°C) for 48 hours with periodic stirring. After removal of the cell mass by centrifugation, the aqueous extract was dried by lyophilization. **High temperature (HT) aqueous extract.** The HT aqueous extract from dried biomass of green microalga *Coelastrella* sp. BGV was prepared in distilled water at a ratio of 1: 8 and subsequent boiling the algal biomass at a temperature of 80-90°C for 90 minutes on magnetic stirrer under constant stirring. The extract was centrifuged at 4000 rpm for 10 min, and was lyophilized.

Assessment of Antitumor Activity against HeLa cells *in vitro*. Cell culture. Human cervical cancer cells (HeLa) were grown in DMEM medium, supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), streptomycin (100 μg/mL), at 37^{o}C in an humidified atmosphere with 5%CO_2. Tested microalgal samples (LT and HT extracts, CM) were dissolved in DMEM culture medium to obtain a stock solution with concentration of 10

mg/mL, which was further diluted to desired concentrations according to subsequent assay protocol.

Cell proliferation/viability assay. Cell proliferation was assessed according to the method of Mossmann [24]. Briefly, 100 μL(2.0×10^4) of freshly prepared HeLa cell suspension was seeded in each well of 96-well microtiter plates and were allowed to adhere for 24 h.The tumor cells were treated with different concentrations of algal extracts ranging from 62.5 to 2000 µg/mL (for CM) and 31.3 to 1000 µg/mL (for LT and HT extracts) and incubated for 24 and 48 hrs at 37oC, 5% CO2 with 95% relative humidity. Each concentration was examined in 6 replications. Untreated tumor cells incubated only in culture medium were used as negative controls and tumor cells cultivated in the presence of Doxorubicin (DOX) (1.25–40 µg/mL) were used as positive controls. After the incubation the medium was replaced with 100 µL of MTT solution containing 0.5 mg/mL 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) for 3 hrs. The supernatant was discarded and formazan crystals were dissolved in 100 µL of lysis solution (DMSO:Ethanol 1:1v/v). The absorbance at 570 nm with reference at 650 nm was measured using a micro plate ELISA reader (TECAN, Sunrise TM, Groedig/Salzburg, Austria). All the experiments were done in triplicates. The effect of tested microalgal samples was expressed as the percent of cell viability, using the following formula: (Optical density of treated cells/Optical density of control cells x 100). The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of treated cells, was estimated from the graph using Graphpad Prism software

Assessment of morphological changes in HeLa cells by fluorescent tests.

Dual acridine orange (AO)/ethidium bromide (EB) fluorescent staining was performed according to Wahab et al., [40]. HeLa cells were seeded in a final concentration of 5×10^5/mL on glass slides placed on the bottom of 24 well plates in complete DMEM medium containing 10% fetal bovine serum and the plates were incubated 24 hrs in CO_2 incubator. The test substances were added at a concentration equal to the half of the maximal concentration used in the MTT assay. After additional incubation of 24 hours, the glass slides were washed in PBS, stained with 5 µL of fluorescent solution containing 10 µg/mL AO and 10 µg/mL EB and then were mounted on microscope slides. The morphology of the stained cells was observed immediately using a fluorescent microscope (Leica DM 500B, Wetzlar, Germany).

DAPI staining was used to determine the cell nuclear morphology. Analysis was done by the method of Radhika et al., [29]. Hela cells were seeded and cultured with and without the algal test compounds as described in the previous paragraph. After 24 h of treatment, the glass lamellas with adherent tumor cells were washed, fixed with methanol and stained for 15 minutes with 1 µg/mL 4′,6-Diamidine-2′-phenylindole dihydrochloride (DAPI) in methanol in the dark. Stained cells were mounted with glycerol on microscope slides and analyzed by a fluorescent microscope (Leica DM 500B, Wetzlar, Germany).

Statistical Analysis. The experimental results are expressed as the mean ± S.D. (standard deviations). One-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test was performed using GraphPad PRISM software, Version 5 (GraphPad Prism Software Inc., San Diego, USA). Values of $*p < 0.05$ were considered statistically significant.

Results and discussion

Antitumor activities against HeLa tumor cells *in vitro.* Antitumor activity of Culture medium (CM) and total high temperature (HT) and low temperature (LT) aqueous extracts from *Coelastrella* sp. BGV were examined for antitumor activities against the HeLa tumor cells in *in vitro* experiments by MTT assay. The tumor cells were incubated with different

concentrations of tested samples (as pointed in section "Materials and methods") for 24 and 48 hrs at 37°C, 5% CO_2 with 98% humidity.

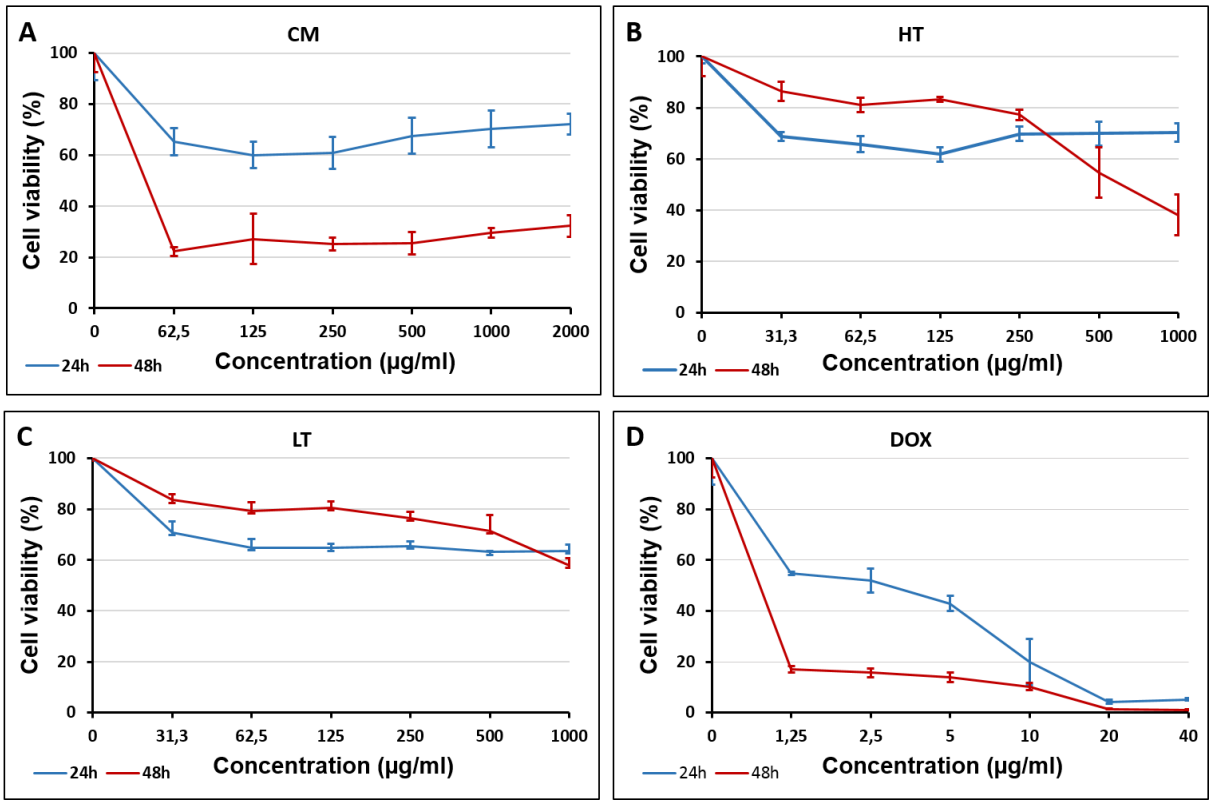

Figure 1. Antiproliferative effect of culture medium (A), high-temperature (B) and low-temperature (C) water extracts obtained from green microalga *Coelastrella* sp. BGV on the HeLa tumor cells estimated at 24h and 48 h after treatment by MTT test. DOX (D) was used as positive control in the experiments.

Culture medium from *Coelastrella* sp. BGV induced a statistically significant inhibition of the proliferation of HeLa tumor cells in the range of 60-72% at the 24th hour and about 3 times lower - 22-32% relative to the control at 48th hour (Fig.1, A). HT and LT total aqueous extracts showed statistically significant inhibition of cell proliferation at both time intervals (Fig.1, B,C). The HT significantly decreased the viability of tumor cells between 65.80 and 70.74% after 24 hours of treatment at all concentrations. It is evident at 48h that the vitality of tumor cells treated with HT at concentrations of 500 µg/ml and 1000 µg/ml is the lowest - 54.71% ± 9.612% and 38.13 ± 7.958% respectively (Fig.1, B). The half maximal inhibitory concentration IC_{50} was calculated - IC_{50}=486.9µg/mL for HT. The lowest vitality of HeLa cells was observed at the highest concentration of LT of 1000 µg/mL at 48 h - 57.98 ± 2.428% (Fig.1, C). The anti-tumor antibiotic DOX, applied in clinical practice for the treatment of a number of malignancies in humans, has been used as a positive control, to compare the effect of algal products.The dose and time-depended cytotoxic effect of DOX have been observed on HeLa cells, with IC_{50}=2.254 µg/mL at 24h and IC_{50} = 0.076 µg/mL at 48h of examination, using MTT assay (Fig. 1, D). Among the two tested aqueous extracts, the HT extract has higher cytotoxicity, however it was lower compared to the standard antitumor drug DOX as evidenced by its IC_{50}. At the 48th h of treatment the order of antiproliferative effect of the tested algal compounds was LT < HT < CM (Fig. 1, C, B, A).

Gacheva [12] and Gigova et al., [15] investigated the cytotoxicity of green microalgal extracts (ethanol cell, aqueous and lipophilic extracts, culture fluid) against HeLa tumor cells. The culture fluid from *Coelastrella* sp., *Scenedesmus incrassatulus* and 3B1, and water

extracts from *Chlorella* sp., 3B1 and *Coelastrella* sp. suppressed the proliferation of HeLa cells, with the effect on the culture fluid being concentration dependent. It was observed that the aqueous extracts show a stronger cytotoxic action than the culture fluids. Carotenoid extract from *Coelastrella oocystiformis* from Mumbai, India was capable of inhibiting 5% of human prostate cancer cell line DU-145 [18]. Some reports have shown that the *Spirulina platensis* cold water extracts inhibit the proliferation of human colon carcinoma (HCT116) and human liver (HepG2) cancer cell lines with the half-maximal inhibitory concentrations (IC_{50}) –18.8 μg/mL and 22.3 μg/mL, respectively [1]. Algal water extracts from nine microalgal species including eight cyanobacteria and one green microalgal species (*Spirulina platensis*) were investigated against Ehrlich ascites carcinoma cells (EACC) and human hepatocellular cancer cell line (HepG2). Higher antioxidant activity and moderate anticancer activity (60,67% and 54,8% respectively) of *S. Platensis* were established, which may be due to the content of total phycobiliprotein pigments, and highly produced secondary metabolites in this alga [36]. Water and ethanol extracts from ten green microalgae of the genera *Chlorella*, *Desmococcus* and *Scenedesmus* exhibited significant antibacterial and antitumor (against MCF7-human breast adenocarcinoma; CEM-human lymphoblastoid leukaemia and G361-human malignant melanoma and NIH3T3-mouse fibroblasts modified normal cell line) activities [28]. A glycoprotein prepared from *Chlorella vulgaris* culture supernatant exhibited protective activity against tumor metastasis and 5-fluorouracil -induced immunosuppression in mice [16, 26]. Marennine, a hydrosoluble bluegreen pigment synthesized and excreted by *Haslea ostrearia* showed an antiproliferative effect on three solid tumor cell lines [5], and also exhibited antibacterial (*Vibrio aesturianus*), antiviral (HSV1) and anticancer activities [13]. Mohd-Syahril *et al*. [23] have found that *Chlorella vulgaris* and *Spirulina platensis* crude extracts display anticancer properties towards the breast (MCF-7) and liver (HepG2) cancer cell lines. They suggested that new anticancer natural products from unicellular green algae and filamentous microalgae are possible.

Estimation of the morphological changes in HeLa cells by fluorescent tests. The morphological alterations induced by the algal samples in the HeLa tumor cells were examined. For this purpose, cells were incubated with CM, LT and HT at a concentration equal to half of the higher concentration used in the MTT assay. The changes at the cell and nucleus level were observed under fluorescent microscope after staining with AO/EB mixture and DAPI respectively.

As shown in Fig. 2a, the control (untreated HeLa cells) were uniformly green stained with monolayer growth and normal morphology. They were slightly elongated in shape, greenish-colored with pale green nuclei containing 3-4 yellow-green nucleolus and perinuclear located clusters of yellow-orange granules.

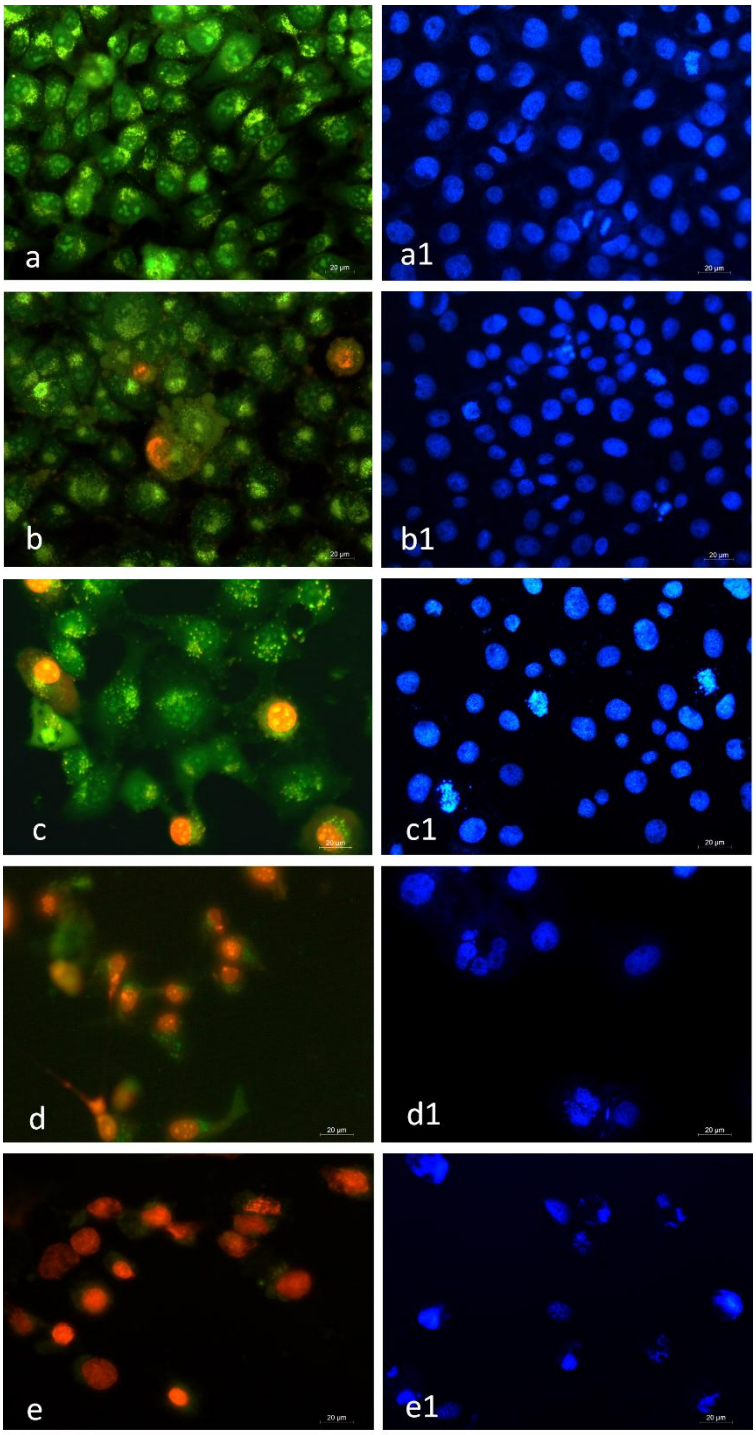

Figure 2. Fluorescent microscopy images of the morphological changes at cell and nucleus levels induced by *Coelastrella* sp. BGV algal samples in HeLa tumor cells. a,b,c,d,e - Acridine orange/ethidium bromide staining**;** a1,b1,c1,d1,e1 - DAPI staining; Objective 40X; bar = 20 µm.

a, a1- control HeLa cells; b, b1- HeLa cells treated with CM; c, c1- HeLa cells treated with LT extract; d, d1- HeLa cells treated with HT extract; e, e1- HeLa cells treated with DOX (10 µg/ml). HeLa cells were treated with test substances at a concentration equal to the half of the maximal concentration used in the MTT assay.

Fluorescence microscopic images of stained HeLa cells cultured in the presence of algal samples (Fig.2-b,c,d) showed morphological changes such as shrinkage, rounding up,

detachment of the cells and impaired monolayer growth. The cells in early apoptosis stage - pale green in color with a bright green nucleus and irregularly distributed chromatin into the nucleus in the form of dense green areas, cytoplasmic blebbings are dominant after treatment with CM and LT extract (Fig.2-b,c). Typical signs of apoptosis in HeLa cells after treatment with HT extract were observed (Fig.2-d). Tumor cells at late apoptosis were predominant. Late apoptotic cells have a bright orange colored nucleus with condensation and margination of chromatin, nuclear fragmentation and the apoptotic bodies formation (Fig.2-d). Cells with a reduction in cell and nuclear volume (picnosis) and karyorrhexis similar to that in the positive control with DOX were observed also. The DOX induced marked alterations in the growth and morphology of the HeLa cells. The presence of dead and destructed cells with picnotic nuclei was indicative of late apoptosis and necrosis. The nuclei were polymorphic and varying in size, with chromatin condensation and nucleus fragmentation (Fig.2-e).

Further the apoptosis in Hela cells, treated with algal samples was morphologically confirmed by DAPI staining. Fluorescence microscopic images of the control (untreated HeLa cells) (Fig. 2,a1) revealed cells with intact nuclei, round to slightly oval in shape with smooth edges and homogeneously distributed chromatin. Cell nuclei in different phases of mitosis were observed. HeLa tumor cells cultured in the presence of CM, LT and HT showed nuclear polymorphism (different shape and size), unequal outlines of the nuclei, irregularly distributed (condensation) chromatin, nuclear fragmentation and formation of apoptotic bodies (Fig.2 - b1,c1,d1). The highest changes were observed in cells treated with HT extract (Fig.2-d1). A strongly reduced number of nuclei, condensed chromatin and nuclear cleavage with multiple apoptotic bodies in DOX-treated cells were observed. A similar morphological picture of HeLa cells treated with HT and DOX respectively was impressed (Fig. 2,e and Fig.2,e1).

Programmed cell death, apoptosis, is an important biological mechanism that contributes to the maintenance and integrity of multicellular organisms. Apoptosis of cells is accompanied by various morphological and biochemical changes typically referred to as hallmarks: shrinkage of the cytoplasm, nuclear condensation, membrane blebbing, externalization of phoshatidylserine, formation of apoptotic bodies etc. [6, 10, 17, 32, 38]. Apoptosis is an important factor in preventing cancer, but cancer cells have lost the ability to enter the apoptosis phase for several reasons [10]. Efforts are directed to the induction of apoptosis in cancer cells. In this aspect, there are reports in the literature that some microalgal agents cause cell death through the induction of apoptosis [19, 22, 41]. Many types of blue-green algae (cyanobacteria) are considered as a promising source of anticancer agents [7, 9, 27, 39]. Extracts derived from *Cyanothece* sp. strain had high anticancer impact on T-lymphoma cells but not against myelogenic leukemia cells. Their action is associated with the presence of long-lasting effective apoptotic compounds in them [27]. Semary and Fouda, [34] reported the anticancer effect of eight cyanobacterial hydrophilic extracts derived from the unicellular *Cyanothece* sp. from Egypt on Ehrlich ascites carcinoma cell line and MCF-7 cells and their potential as a plausible candidate for future mass biotechnological applications. Extracts from *Synechocystis* sp., induced apoptosis in HL-60 tumor cells. After treatment with the extracts, tumor cells showed morphological lesions, with typical signs of apoptosis - shrinkage and rounding of cells, fragmentation of the nucleus, and presence of apoptotic bodies [22]. Extracts of *Anabaena* sp. induced apoptosis in a leukemic myeloid cell line [27]. Some *Nostoc* species produce cryptophycin, which is several hundred to a thousand times more active than vinblastine or taxol against human colorectal adenocarcinoma cancer cells [37]. The extract from Oscillatoria boryana showed activity against human breast cancer [25].

The microalga strain *Coelastrella* sp. BGV has high growth rate under a wide range of conditions and is able to produce a balanced amount of secondary metabolites [8] which motivated our choice for this study. The current experiment was conducted to investigate the

anticancer activity of the total water extracts from the local strain *Coelastrella* sp. in order to explore the biological activities of local algal strains.

Data in the literature on the antitumor activity of algal metabolites from genus *Coellastrella* are scarce and mainly refer to *Coelastrella oocystiformis*, from India [18] and reports of Cacheva [12] and Gigova et al.[15]. The tested samples from *Coelastrella* sp. BGV showed varying degrees of antitumor activity and severe morphological changes characteristic of early and late apoptosis. By the MTT colorimetric method, a statistically significant inhibition of the viability of HeLa tumor cells was detected. The results of the fluorescence methods are consistent with the data obtained from the MTT test and show that the antitumor activity of the algal samples isolated from the Bulgarian strain *Coelastrell*a sp. BGV versus HeLa tumor cells is mediated by induction of apoptosis. The current study represents the first report on the anticancer/antiproliferative effect and the mechanism of action of aqueous extracts and culture fluid derived from the *Coelastrella sp*. BGV in HeLa tumor cells.

Despite considerable progress in research, cancer remains one of the leading causes of death in the world. Overall, we aim to emphasize the potential of using local unexploited microalgae that are easily cultivated and extracted as a promising source of anticancer agents. Based on the results obtained, further studies should be carried out searching for new compounds from green microalgae *Coelastrella* sp. BGV in order to develop alternative therapeutics against neoplastic diseases.

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CO7. DIVALENT METAL IONS BINDING TO LACTOSE: A DFT COMPUTATIONAL STUDY

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In recent years, there has been a growing interest in searching ways to enrich various food products with minerals essential for good health. One of the food products which is important and it is a main part of the people's diet is milk. The main minerals with which the milk is enriched are Ca, Mg, Zn, etc. However, very little information is available about the competition between metals for binding to natural or artificial nutrients in milk. The goal of this study is to elucidate the factors determining the interactions of lactose, one of the natural ingredients of milk with Ca^{2+}, Mg^{2+} and Zn^{2+} cations. The crystal structure of the hydrated calcium bromide complex of lactose (4-O-β-D-galactopyranosyl-D-glucopyranose) (Figure 1; [1]) was taken as a basis for our study. DFT calculations of complexes of lactose (represented in a simplified manner by using a model) and Ca^{2+}, Mg^{2+} and Zn^{2+} cations are performed at M062X/6-31G(d,p) level of theory. The influence of physicochemical properties, such as ionic radius, preferred coordination and hydration numbers of the metal cation, and influence of the medium on the process of metal binding are estimated.

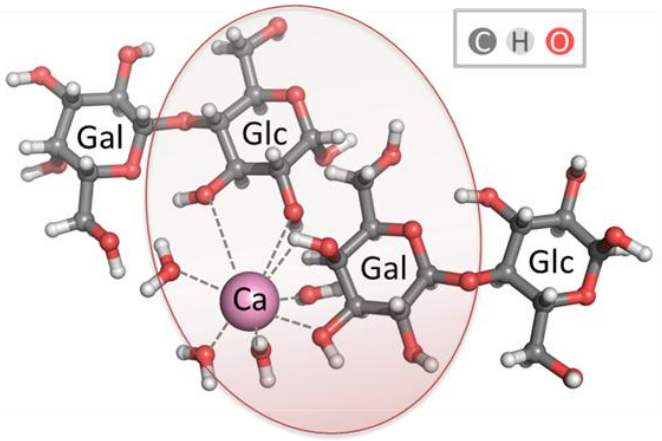

Figure 1. Environment of the calcium ion in the Ca-lactose complex [1].

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Session D.

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DO1. VITAMIN D EFFECTS ON URIC ACID IN THE EXPERIMENTAL MODEL OF METABOLIC DISORDERS IN FRUCTOSE FED WISTER RATS

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Background
Vitamin D is known to be beneficial for regulation of calcium homeostasis, bone health and can also affect blood sugar levels. Uric acid is the product of oxidation of xanthine and hypoxanthine oxidoreductase and takes part in various homeostatic, metabolic and hemodynamic processes. High uric acid is associated with the development and progression of metabolic syndrome, hypertension, insulin resistance, diabetes mellitus, dyslipidemia, obesity, cardiovascular disease. There has been evidence that vitamin D and uric acid levels are connected and there is a strong relationship between their levels in patients with type 2 diabetes mellitus and metabolic disorders. Hyperuricemia has been shown to suppress 1-alfa hydroxylase possibly by nuclear factor k B and hence lower 1,25(OH)2D and may cause hypovitaminosis D and vice versa.

Aim
The present study aims to investigate the role of vitamin D effects on uric acid levels in the experimental model of metabolic disorders in fructose fed Wister rats.

Materials and methods
High fructose diet has been shown to be involved in the progression and pathogenesis of metabolic disturbances like obesity, glucose intolerance, dyslipidemia.
Male Wister rats (n = 30) were randomly divided into four groups: control group (n = 9), fructose fed group (n = 9), fructose fed group supplemented with vitamin D (n = 6) and group with normal diet (without fructose) supplemented with vitamin D (n = 6). The whole experiment lasted 12 weeks. One week from the start of the experiment to the groups supplemented with vitamin D was given vitamin D 500 UI/kg (Vigantoil dissolved in olive

oil), orally three times per week till the end of the experiment. The fructose fed groups were injected with streptozotocin 20 mg/kg b.w. i.p. two weeks after the start of the experiment. During this period changes in the morphometric characteristics (body weight, waist circumference, distance from muzzle to tail) of the animals and glucose levels were monitored. At the end of the experiment we studied the lipid profile, creatinine levels, uric acid and glucose levels. Fasting serum glucose, triglycerides levels, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and uric acid were measured by standard laboratory methods.

Results

The levels of uric acid, glucose, LDL and LDL/HDL were lower in the supplemented with vitamin D groups. Uric acid levels in the control group were 74 mmol/l, 86 mmol/l for the fructose fed group, 24 mmol/l in the group with normal diet and supplemented with vitamin D and 53 mmol/l in the fructose fed group supplemented with vitamin D. There is not a significant difference between the levels of HDL, triglycerides and cholesterol concentrations.

Conclusions

The effects of vitamin D are significant in uric acid levels and blood sugar levels. Vitamin D decreases uric acid concentrations and glucose in the fructose fed group and improves the cardiogenic lipid indexes (such as LDL/HDL and cholesterol/HDL).

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DO2. HAEMOSTASIS: EVOLVED UNDERSTANDING

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Introduction

Haemostasis is the physiological process involved with stopping the bleeding that occurs at the site of injury while ensuring the maintenance of normal blood flow throughout the remaining circulation. Our initial proposed explanation of coagulation was in theory correct

to an extent in terms of one factor activating the next and for this reason it was referred to as the coagulation cascade. Although this theory could be supported by in vitro findings, it did not account for the roles of cofactors and we now breakdown the cascade into intrinsic and extrinsic pathways. Despite there being two pathways, we know that both pathways work together with the common pathway to trigger coagulation. With time we have learned more about coagulation, while there is the cascade which works to achieve coagulation, there is also a system which works in the background constantly as an antithrombogenic, with elements such as heparin and thrombomodulin which prevent the formation of clots in the normal state. The end goal of the body's haemostatic mechanisms is to form a clot therefore preventing blood loss. This all begins with tissue factor being exposed to blood cells at the site of injury, from this point several other factors are activated, and the result is activation of factor X of the common pathway which eventually leads to a fibrin clot.

Following vascular injury, any escaping blood must rapidly be converted into a gel ("clot") to plug the hole and minimize further blood loss. The plasma portion of blood contains a collection of soluble proteins that act together in a cascade of enzyme activation events, culminating in the formation of a fibrin clot. While these steps are not enough to cause a clot to form, they initiate the process and other factors in the cascade are activated and von Willebrand factor, amongst other chemicals (ADP) causes platelet aggregation. Once platelets are activated and aggregate, large amounts of thrombin can be activated and stabilise the clot. Activated factors 10 and 5 play a large role in this. It is important to understand that these are not isolated reactions that occur independently rather a continuum of events.

Plasmin is the key to undoing the haemostatic clot. It cuts through the fine meshwork laid down by the platelets and dissolves the clot by cleaving fibrin at specific lysine and arginine residues. However, for plasmin to be available, tissue plasminogen activators must act on plasminogen the precursor to plasmin. Fibrinolysis is also key for wound healing to begin. When analysing aPTT and PT we need to understand what definitive conclusions we can draw from them and what they are able to tell us. aPTT measures intrinsic pathway while PT measures efficiency of the extrinsic pathway. More importantly, we also need to be aware of what the tests cannot tell us, so we can be aware of other factors that many influence bleeding or clotting in clinical scenarios. Coagulopathy can be caused by several things, but one of the major signs of a coagulopathy within a patient is microvascular bleeding, this can lead to major blood loss. Some causes are, hypothermia, acidosis, depletion of coagulation components. When the blood clot has been broken down healing can finally begin. There are many leucocytes at the site of injury and M2 macrophages can begin repair with the help of fibroblasts. Cytokines such as platelet derived growth factor (PDGF) and TGFβ play significant roles in the repair process.

New findings:

Since its inception, the cascade or waterfall description of haemostasis has remained unchallenged. Currently, a significant number of scientists and researchers have questioned the role of cellular components in the cascade process. Cellular components are believed to predominantly provide a surface for coagulation to take place. This belief, although greatly supported by laboratory findings, leaves a great deal of important questions unanswered. The recent work has expanded the role played by cell components and has introduced a new model to describe haemostasis known as cell-based haemostasis. Under the cell-based model, coagulation occurs as a result of three steps working simultaneously with each other. In the initiation phase, cells which express TF initiate coagulation when a break in vascular endothelium exposes TF to plasma. This allows the necessary factors and proteases to step in and interact with TF in a controlled manner. Amplification stage focuses on the processes which occur on the surface of platelets as they are activated and bind several cofactors.

Coagulation is brought to a close during the final phase of propagation as thrombin synthesis takes place. Up to this point, the cascade and cell-based model remain similar. The key difference is the concept of localization of procoagulants which is preformed by specialized cellular features. These include TF-bearing cells, platelets and endothelial cells.

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DO3. APPLANATION TONOMETRY NON-INVASIVE METHOD FOR CARDIOVASCULAR RISK ASSESSMENT

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The assessment of cardiovascular risk in the particular patient is important for the prevention, treatment and prognosis of cardiovascular disease. There are various methodologies for cardiovascular risk assessment, including clinical, laboratory and other criteria based on extensive studies. Their main disadvantage is the underestimation of cardiovascular risk in young individuals and its overestimation in closely monitored and treated patients with very high risk. Applanation tonometry (AT) is a non-invasive method for examining the augmentation pressure and pulse wave velocity to assess the elasticity and biological age of the blood vessels. Its use as a method for personalized assessment cardiovascular risk is recommended by the latest guidelines. Cardiologists and GPs know the different methods for assessing cardiovascular risk. However, personal doctors prefer to use existing questionnaires. And cardiologists are oriented to modern non-invasive methods for assessing cardiovascular risk including AT.

Key words: cardiovascular risk, augmentation pressure, applanation tonometry, pulse wave

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DO4. TWO PATIENTS AFTER ST-ELEVATION MYOCARDIAL INFARCTION WITH DIFFERENT CARDIOVASCULAR RISK

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Vascular accidents determine the highest percentage of adult mortality in developed countries. According to previous publications, 1 out of 4 patients with acute coronary syndrome have a new vascular accident within 5 years. Therefore, current recommendations accent that all patients who have undergo acute coronary events must be evaluated as patients with very high coronary risk. We present two clinical cases. One is a 44-year-old male, smoker, with no concomitant illnesses. Percutaneous coronary angiography evidence a vascular spasm and a small atherosclerotic plaque without thrombosis. The applanation tonometry one month later demonstrate normal systolic, diastolic, mean, pulse and augmentation pressure and normal pulse wave velocity. The patient refuses the cigarettes and strictly adheres to the prescribed therapy. The second case is a 68-year-old non-smoker, obese, diabetic, and long-lasting history of arterial hypertension. Due to clinical evidence of transmural myocardial infarction, percutaneous coronary angiography evidence double-stranded coronary artery disease, two plaques causing more than 90% stenosis. Despite successful revascularization and strict medical treatment, the patient fails to achieve the LDL-targets targets. The applanation tonometry one month later demonstrate high systolic, diastolic and mean pressure and very high augmentation pressure and pulse wave velocity. These are just two clinical cases of real practice not a representative sample, but thew show how performing AT provides an individual cardiovascular risk assessment for patients belonging to the same risk group.

Key words: individual cardiovascular risk assessment, applanation tonometry, myocardial infarction with ST-elevation

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DO5. ЕСЕНЦИАЛНИТЕ МЕТАЛИ – МОЛЕКУЛИТЕ НА ЖИВОТА

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Деветдесет елемента се срещат на Земята, като 61 са метали, от които 9 са радиоактивни, а останалите 81 са поддържащи живота. Есенциални метали са: Na, Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo и Se. Те са жизнено необходими за нормалната дейност на организма. Влизат в състава на редица ензими, витамини и хормони. Подпомагат и някои ключови процеси в организма като растежа на клетките, кръвообразуването, размножаването, доставката на хранителни вещества, различни хормонални процеси, укрепват имунната система, съдействат за нормалната функция на различни органи и системи и др. Недостигът или излишъкът им води до нарушения в обмяната на веществата. Според някои изследвания приемането на достатъчно количество есенциални метали като цинк и селен например може да бъде добра превенция на рака. При внасянето на повече натрий в организма пък, намалява калият. Дефицитът на цинк отслабва имунната система, а недостигът на мед може да предизвика болки в ставите и загуба на вкусова сетивност и др.

Поради това, специалистите в областта на храненето и здравето препоръчват консумацията на храни, богати на изброените микроелементи. Балансираното хранене, което залага на голямо количество прясна храна е всичко, от което имаме нужда, за да си набавим необходимите вещества за правилно функциониране на организма и доброто му здравословно състояние. Затова е желателно да научим още малките си, невръстни деца от най- ранна детска възраст да консумират „сурови“ храни, за да укрепят своето здраве и да подсилят от имунна си система, така че да са максимално защитени от ежедневните „сблъсъци“ с патогенните за организма вируси, бактерии и паразити.

DO6. METAL COMPLEXES OF BILE ACIDS EFFECTIVE AGAINST SENSITIVE AND RESISTANT TO OXALIPLATIN HUMAN COLORECTAL CANCER CELLS

Lora Dyakova[1], Tanya Zhivkova[2], Milena Georgieva[3], George Miloshev[3], Gabriela Marinescu[4], Daniela-Cristina Culita[4], Luminita Patron[4], Radostina Alexandrova[2]

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DP1. BRIEFLY ABOUT SOME OF THE MOST POPULAR CYTOTOXICITY ASSAYS

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DP2. ANTITUMOR ACTIVITY OF DISULFIRAM

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DP3. THE MANY FACES OF STATINS

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DP4. 2D AND 3D BREAST CANCER CELL CULTURES IN EACCER RESEARCH AND EXPERIMENTAL ONCOPHARMACOLOGY

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