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CYTOTOXICITY OF THE FUNGICIDE MANCOZEB IN VITRO

Ani Georgieva, Anton Kril, Ivan Ivanov

Mancozeb, a polymeric complex of manganese ethylene – 1, 2-bis (dithio-carbamate) with a zinc salt, is a widely used fungicide. In the present study, the cytotoxic potential of the fungicide mancozeb was examined in primary cultures of Syrian golden hamster embryo cells (SHE cells) and in the continuous cell line of mouse embryo fibroblasts BALB/c 3T3. The cytotoxicity was measured by the Neutral Red Uptake assay. The cell sensitivity to the test chemical was determined by the IC_{50} values. A clear dose-dependent cytotoxic effect on both cell culture systems was observed after 24 h treatment with mancozeb. The SHE cell cultures were found to be more sensitive to the cytotoxic effect of mancozeb than BALB/c 3T3 cultures. Data obtained from our experiments suggest that SHE cells and BALB/c 3T3 cell line are useful models for studies on the cytotoxic potential of dithiocarbamate fungicides.

Key words: cytotoxicity, cell cultures, fungicide, mancozeb

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IN OVO STUDY ON THE EMBRYOTOXIC, MUTAGENIC AND CARCINOGENIC POTENTIAL OF THE ETHYLENE BISDITHIOCARBAMATE FUNGICIDE MANCOZEB

Ani Georgieva, Anton Kril, Ivan Ivanov

Mancozeb, an ethylene bisdithiocarbamate, has been one of the most commonly used fungicides in commercial use for several decades. The embryotoxic, mutagenic and carcinogenic potential of mancozeb was studied in 15I line, White Leghorn chicken embryos. The in ovo carcinogenicity assay (IOCA) was used to examine whether mancozeb induce toxicity and subsequently morphological alterations in the embryonic liver, compared to the carcinogen N-nitrosodimethylamine (NDMA). In this study, NDMA induced foci of altered hepatocytes (FAHs), tubular structure formation and hyperplasia of cholangiocytes. Histological examination of mancozeb-exposed embryos revealed a severe toxic damage of the liver tissue. Treatment of avian embryos with high mortality-inducing dose of mancozeb resulted in the appearance of basophilic zones, resembling the basophilic FAHs, observed in NDMA-treated embryos. The mutagenic potential of mancozeb was assessed by the hen's egg test for micronucleus induction (HET-MN). The application of this fungicide did not increase significantly the frequency of micronucleated erythrocytes, as opposed to well known mutagen NDMA.

Key words: mancozeb, in ovo assay, avian embryo, mutagenicity, carcinogenicity, embryotoxicity

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ANTHRACENE-DERIVED BIS-AMINOPHOSPHONATES: CRYSTAL STRUCTURE, IN VITRO ANTITUMOR ACTIVITY, AND GENOTOXICITY *IN VIVO*

Kraicheva , E. Vodenicharova, B. Shivachev, R. Nikolova, A. Kril, M. Topashka-Ancheva, I. Iliev, A. Georgieva, Ts. Gerasimova, T. Tosheva, E. Tashev, I. Tsacheva, K. Troev

The X-ray crystal structures of the anthracene-derived bis-aminophosphonates 4.4′-bis[N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]diaminodiphenylmethane (1) and 1,3-bis [N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]diaminobenzene (3) are reported. The X-ray analyses demonstrated that both compounds crystallize in a centrosymmetric manner containing a meso-form (1) and a pair of enantiomers (3).

The cytotoxic potential, genotoxicity, and antiproliferative activity of bis-aminophosphonates 1 and bis[N-methyl(diethoxyphosphonyl)-1-(9-anthryl)]benzidine (2), as well as their subcellular distribution in a tumor cell culture system, are also discussed. Compounds 1 and 2 showed optimal antiproliferative activity to human tumor cells from colon carcinoma line HT-29. *In vitro* and *in vivo* safety testing revealed that the compounds exert lower toxicity to normal cells as compared with well-known anticancer and cytotoxic agents.

Keywords: aminophosphonic acids, single crystal, antitumor activity, *in vitro* cytotoxicity, genotoxicity

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LOW CYTOTOXICITY AND CLASTOGENICITY OF SOME POLYMERIC AMINOPHOSPHONATE DERIVATIVES

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Poly(oxyethylene aminophosphonate)s synthesized on the basis of biodegradable poly(phosphorester)s and Schiff bases were tested *in vitro* for antitumor activity against a panel of six human epithelial cancer cell lines, for cytotoxicity to mouse fibroblast cells and *in vivo* for clastogenicity and antiproliferative effects. The polymers showed lower cytotoxicity, both *in vivo* and *in vitro* and lower clastogenicity *in vivo* than the corresponding low-molecular aminophosphonates. The biological activities of the tested polymers correlate with their low *in vitro* antitumor activity.

Keywords: poly(aminophosphonate)s, human epithelial cancer, cell lines, safety testing

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SYNTHESIS, CHARACTERIZATION, ANTITUMOR ACTIVITY AND SAFETY TESTING OF NOVEL POLYPHOSPHOESTERS BEARING ANTHRACENE-DERIVED AMINOPHOSPHONATE UNITS

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Novel polyphosphoesters containing anthracene-derived aminophosphonate units, poly(oxyethylene aminophosphonate)s (4 and 5) and poly[oxyethylene(aminophosphonate-co-H-phosphonate)]s (6 and7), were synthesized via an addition of poly(oxyethylene H-phosphonate)s to 9-anthrylidene-p-toluidine. The IR, NMR (1H, 13C and 31P) and fluorescence emission spectral data of the polymers are presented. The copolymers 6 and 7 were tested for *in vitro* antitumor activity on a panel of seven human epithelial cancer cell lines. Safety testing was performed both *in vitro* (3T3 NRU test) and *in vivo* on ICR mice for genotoxicity and antiproliferative activity. The copolymer 7 showed excellent antiproliferative activity to HBL- 100, MDA-MB-231, MCF-7 and HepG2 cell lines. However, the *in vitro* safety testing revealed significant toxicity to Balb/c 3T3 mouse embryo cells. In contrast, the copolymer 6 showed complete absence of cytotoxicity to Balb/c 3T3 cells, but inhibited the growth of breast cancer cells, cervical carcinoma cells (HeLa) and hepatocellular carcinoma cell cultures after prolonged (72 h) exposure. The polymers (4–6) exhibited low (4 and 6) to moderate (5) clastogenicity *in vivo* and slightly inhibited bone marrow cell division, compared to Mitomycin C. The subcellular distribution of the copolymers 6 and 7 were studied in model cell culture systems. The tested polyphosphoesters are expected to act *in vivo* as prodrugs of aminophosphonates and could be valuable as a new class of biodegradable polymer drug carriers.

Keywords: polyphosphoesters, aminophosphonic acids, NMR, antitumor activity, cytotoxicity, genotoxicity

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FUMONISIN B1 CYTOTOXICITY AND SUBCELLULAR LOCALIZATION IN DUCK EMBRYO CELL LINE DEC 99

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A comparative study on the cytotoxic effect of fumonisin B1 (FB1) was carried out on BALB/c 3T3 and DEC 99 cell lines. The newly tested cell line DEC 99 appeared as more sensitive than BALB/c 3T3 line according to the performed Neutral red uptake assay. Light microscopic investigations of DEC 99 cultures treated with FB1 showed altered monolayer with free of cells spaces and abundance of dead cells. The immunocytochemical techniques (immunofluorescent and immunogold labelling) proved the influx of the toxin through the cell membranes. The toxin was visualized in the cytoplasm and in the nucleus of the treated cells.

Keywords: fumonisin B1, DEC 99 cell line, immunocytochemistry

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THE INFLUENCE OF ANTI-CANCER AGENT ERUFOSINE ON GRAFFI MYELOID TUMOUR CELLS BEHAVIOUR. CYTOTOXICITY AND CYTOSKELETON REORGANISATION STUDY

Veselina Uzunova, Sonia Apostolova, Ani Georgieva , Martin Berger, Reneta Toshkova, Rumiana Tzoneva

In the present study we aimed to evaluate the role of cytotoxicity of erufosine for eliciting changes in cytoskeleton organization and induction of apoptosis in Graffi myeloid tumour cells. The cytotoxicity of erufosine was revealed by MTT assay. The effect of erufosine on cytoskeleton and cell nuclei was evaluated by immunostaining for α-tubulin and F-actin, as well as by DAPI staining. We show that IC_{50} dose for EPC_3 treatment of Graffi tumour cells was obtained at 20 μM. Fluorescent images showed existence of apoptosis at the same EPC_3 concentration. The induction of apoptosis by EPC_3 was accompanied by actin and tubulin reorganization. The obtained results revealed reorganization of actin cytoskeleton and induction of adhesive cell phenotype by erufosine treatment.

Keywords: erufosine, reorganization of cytoskeleton, apoptosis, Graffi tumour cells

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IN VITRO ANTITUMOUR ACTIVITY, SAFETY TESTING AND SUBCELLULAR DISTRIBUTION OF TWO POLY [OXYETHYLENE(AMINOPHOSPHONATE-CO-H-PHOSPHONATE)]S IN EHRLICH ASCITES CARCINOMA AND BALB/C 3T3 CELL CULTURE SYSTEMS

Ani Georgieva, Ivan Iliev, Margarita Topashka-Ancheva, Ivanka Kraicheva, Ivelina Tsacheva, Emil Tashev, Tania Tosheva and Anton Kril

Two polyphosphoesters containing anthracene-derived aminophosphonate and hydrophilic H-phosphonate repeating units, poly[oxyethylene(aminophosphonate-co-H-phosphonate)]s (1 and 2), were tested for the *in vitro* antitumour activity on cell cultures derived from ascitic form of Ehrlich mammary adenocarcinoma by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-dye reduction assay. The *in vitro* safety testing of the copolymers was performed by BALB/c 3T3 neutral red uptake assay. A study on their uptake and subcellular distribution in nontumourigenic and tumour cells was performed by means of fluorescence microscopy. Both copolymers showed significant antitumour activity towards Ehrlich ascites carcinoma (EAC) cells. However, the *in vitro* safety testing revealed significant toxicity of polymer 2 to BALB/c 3T3 mouse embryo cells. In contrast, polymer 1 showed complete absence of cytotoxicity to BALB/c 3T3 cells. The fluorescent studies showed that the substances were diffusely distributed in the cytoplasm in both cell culture systems. As opposed to BALB/c 3T3 cells, in EAC cells, intense fluorescent signal was observed in the nuclei and in the perinuclear region. The tested polyphosphoesters are expected to act under physiological conditions as prodrugs of aminophosphonates.

Keywords: aminophosphonates, polyphosphoesters, antitumour activity, subcellular distribution

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POLY(3-HYDROXYBUTYRATE)/CAFFEIC ACID ELECTROSPUN FIBROUS MATERIALS COATED WITH POLYELECTROLYTE COMPLEX AND THEIR ANTIBACTERIAL ACTIVITY AND *IN VITRO* ANTITUMOR EFFECT AGAINST HELA CELLS

Milena G. Ignatova, Nevena E. Manolova, Iliya B. Rashkov, Nadya D. Markova, Reneta A. Toshkova, Ani K. Georgieva, Elena B. Nikolova

The purpose of this work was to investigate the possibility for the preparation of new poly(3-hydroxybutyrate) (PHB)/poly(ethylene glycol) (PEG)-based fibrous materials containing natural phenolic compound caffeic acid (CA) of diverse architectures, as well as to study the impact of the fiber composition on the *in vitro* CA release profile and on the biological properties of the fibrous materials. The application of the one-pot electrospinning enabled the fabrication of nanofibrous materials from PHB and PEG loaded with the CA.Materials with targeted design were obtained by coating with polyelectrolyte complex of alginate (Alg) and N,N,N-trimethylchitosan (TMCh). Three different processing paths were used to obtain coated mats: (i) with CA incorporated in the PHB/PEG core; (ii) with CA embedded in the Alg layer; and (iii) with CA included in the TMCh layer. The *in vitro* release of CA was modulated by controlling the composition and the architecture of the nanofibrous mats. The performed microbiological screening and MTT cell viability studies revealed that in contrast to the bare mats, the CA-containing nanofibrous materials were effective in suppressing the growth of the Grampositive bacteria Staphylococcus aureus and the Gram-negative bacteria Escherichia coli and displayed good cytotoxicity against human cervical HeLa tumor cells. In addition, the proliferation of murine spleen lymphocytes and peritoneal macrophages was increased by the prepared CA-containing nanofibrous materials. The obtained materials are promising for antibacterialwound dressing applications aswell as for application in local treatment of cervical tumors.

Keywords: electrospinning, caffeic acid, poly(3-hydroxybutyrate), quaternized chitosan, antibacterial activity, antitumor activity

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IN OVO HEPATOCARCINOGENICITY OF N-NITROSODIMETHYLAMINE AND N-NITROSODIMETHYLAMINE IN WHITE LEGHORN CHICKENS

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Avian embryos have been gaining an increasing scientific interest as a valuable model system for the experimental cancer research that could contribute to a significant reduction of the number of laboratory animals. In the present study, the liver lesions induced by N-nitrosodimethylamine and N-nitrosodiethylamine in 15I line, White Leghorn embryos were identified and studied by routine histopathological methods. Foci of altered hepatocytes with basophilic and eosinophilic phenotype, well known as preneoplastic alterations were identified in the avian embryonal livers after *in ovo* exposure to both N-nitroso compounds. These studies were further extended by histopathological, haematological and biochemical examinations on the effects of N-nitrosodimethylamine in chickens hatched from carcinogen-inoculated eggs. In addition to the preneoplastic lesions observed in the avian livers, proliferations of oval and hepatocellular carcinoma cells, with clearly expressed signs of malignancy were found. The *in ovo* application of the chemical carcinogen was found to affect both hematological and blood biochemistry parameters measured in experimental birds. The established conditions such as thrombocytopenia and increased levels of liver enzymes, as an essential part of the paraneoplastic syndrome, were associated with the process of hepatocarcinogenesis. The results of this study confirm the preneoplastic nature of the focal lesions in embryonal avian liver and their progression to liver neoplastic alterations after a single *in ovo* application of known hepatocarcinogens. Moreover, the results indicate that 15I line, White Leghorn embryos are a new, valuable *in ovo* model for studies on hepatocarcinogenicity of chemical compounds and underline the importance of research on the development of different avian models of carcinogenicity.

Keywords: in ovo models, avian embryos, nitrosamines, hepatocarcinogenesis

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CHITOSAN/FERULIC ACID-COATED POLY(ε-CAPROLACTONE) ELECTROSPUN MATERIALS WITH ANTIOXIDANT, ANTIBACTERIAL AND ANTITUMOR PROPERTIES

Gyuldzhan Yakub, Milena Ignatova, Nevena Manolova, Iliya Rashkov, Reneta Toshkova, Ani Georgieva, Nadya Markova

Novel fibrous materials from poly(ε -caprolactone) (PCL), chitosan (Ch) and natural phenolic acid ferulic acid (FA) of diverse design were successfully prepared by electrospinning or electrospinning combined with dip-coating. FA incorporated in the PCL fibrous mats or in the Ch coating was in the amorphous state as evidenced by the performed differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analysis. The release of FA was affected by the composition and design of the polymer matrix. The incorporation of a combination of FA and Ch in the fibrous mats imparted to these materials higher killing rates against pathogenic bacteria S. aureus than that of FA-containing mats or Ch-coated mats alone. TheFA-containing fibrous materials as well as those coated with Ch or Ch-FA inhibited the adhesion of S.aureus bacteria. Moreover, FA preserved its antioxidant activity when incorporated in the fibers or in theCh coating. It was found that the cytotoxicity of all types of FA-containing mats against HeLa tumor cellswas higher than that of the free FA. Thus, the obtained fibrous materials can be suitable candidates for wound dressing applications and for application in local treatment of cervical tumors.

Keywords: electrospinning, ferulic acid, chitosan, antioxidant activity, antibacterial activity, antitumor activity

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ELECTROSPUN CELLULOSE ACETATE MEMBRANES DECORATED WITH CURCUMIN-PVP PARTICLES: PREPARATION, ANTIBACTERIAL AND ANTITUMOR ACTIVITIES

Petya Tsekova, Mariya Spasova, Nevena Manolova, Iliya Rashkov, Nadya Markova, Ani Georgieva, Reneta Toshkova

Curcumin (Curc) exhibits anti-inflammatory, antibacterial and antitumor activity. However, its clinical application is limited by its poor bioavailability related to its extremely low water solubility. Novel materials allowing enhanced release of Curc in aqueous medium were obtained. The new materials consisted of electrospun fibers from cellulose acetate (CA) (mean fiber diameter ca. 780 nm ± 110 nm) with electrosprayed Curc/polyvinylpyrrolidone (Curc/PVP) particles. Scanning electron microscopy (SEM) showed that separated and evenly distributed particles of Curc/PVP were deposited on the surface of the mats and on the inner layers of the mat. X-ray diffraction studies showed that Curc was in amorphous state. *In vitro* studies demonstrated that Curc release was facilitated from Curc/PVP-on-CA mats (ca. 78% for 24 h) compared with the materials in which Curc was incorporated in CA fibers (17% for 24 h). Moreover, the curcumincontaining materials exhibited antibacterial activity against Gram-positive bacteria Staphylococcus aureus (S. aureus) and Gram-negative bacteria Escherichia coli (E. coli). Curc/PVP-on-CA fibrous mats exhibited high *in vitro* cytotoxicity towards HeLa tumor cells. Therefore, the obtained materials are promising for antibacterial wound dressing applications as well as for application in local treatment of cervical tumors.

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METAMIZOLE (DIPYRONE) – CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS ON HELA, HT-29 AND MCF-7 CANCER CELL LINES

Irina Nikolova, Lyubomir Marinov, Ani Georgieva, Reneta Toshkova, Martin Malchev, Yulian Voynikov and Ivanka Kostadinova

Cancer pain treatment is a big challenge for healthcare providers and patients as well. The wide range of non-steroidal anti-inflammatory drugs (NSAIDs) used as painkillers in cancer patients, requires in-depth characterization of their effect on the disease process. The effects of NSAIDs have been widely studied over the last decades as preventive drugs in some oncological diseases. Metamizole is an NSAID belonging to the non-narcotic analgesics group and is highly recommended in oncology either alone or in combinations with opioid analgesics. There is a dearth of information regarding the cytotoxicity profile of metamizole and hence the present study evaluated the potential anticancer activity of metamizole in some permanent human tumour cell lines: HeLa, human cervical cancer cells; HT-29, a human colorectal adenocarcinoma cell line; and MCF F-7, human breast adenocarcinoma cells. The studied tumour cells were sensitive to metamizole at doses higher than 25 µg/mL. Metamizole induced a statistically significant decrease in the viability of HeLa, HT-29 and MCF-7 cells in *in vitro* tests as measured by the MTT assay; the highest effect was observed at the 48th hour of the treatment. Metamizole could induce cell death by apoptosis. Metamizole also suppressed the migration of the three tumour cell lines. This was most clearly pronounced in HeLa cells. The results obtained indicate that metamizole is a suitable choice for the treatment of cancer pain and has prospects for further in-depth studies.

Keywords: metamizole, apoptosis, cytotoxicity, wound-healing assay, proliferation, migration

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MOLECULAR DETECTION AND PHYLOGENETIC ASSESSMENT OF SIX HONEYBEE VIRUSES IN *APIS MELLIFERA* L. COLONIES IN BULGARIA

Rositsa Shumkova, Boyko Neov, Daniela Sirakova, Ani Georgieva, Dimitar Gadjev, Denitsa Teofanova, Georgi Radoslavov, Maria Bouga and Peter Hristov

Honey bee colonies suffer from various pathogens, including honey bee viruses. About 24 viruses have been reported so far. However, six of them are considered to cause severe infection which inflicts heavy losses on beekeeping. The aim of this study was to investigate incidence of six honey bee viruses: deformed wing virus (DWV), acute bee paralysis virus (ABPV), chronic bee paralysis virus (CBPV), sacbrood virus (SBV), kashmir bee virus (KBV), and black queen cell virus (BQCV) by a reverse transcription polymerase chain reaction (RT-PCR). A total of 250 adult honey bee samples were obtained from 50 colonies from eight apiaries situated in three different parts of the country (South, North and West Bulgaria). The results showed the highest prevalence of DWV followed by SBV and ABPV, and one case of BQCV. A comparison with homology sequences available in GenBank was performed by phylogenetic analysis, and phylogenetic relationships were discussed in the context of newly described genotypes in the uninvestigated South Eastern region of Europe. In conclusion, the present study has been the first to provide sequencing data and phylogenetics analyses of some honey bee viruses in Bulgaria.

Keywords: Honey bee viruses, RT-PCR, Apis mellifera, Bulgaria

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BIOTIC AND ABIOTIC FACTORS ASSOCIATED WITH COLONIES MORTALITIES OF MANAGED HONEY BEE (*APIS MELLIFERA*)

Boyko Neov, Ani Georgieva, Rositsa Shumkova, Georgi Radoslavov and Peter Hristov

Despite the presence of a large number of pollinators of flowering plants worldwide, the European honey bee, *Apis melifera*, plays the most important role in the pollination of a number of crops, including all vegetables, non-food crops and oilseed crops, decorative and medical plants, and others. The experience of isolated cases of complete extinction of honey bees in individual regions has shown that this phenomenon leads to a dramatic pollination crisis and reduced ability or even total inability to grow insect-pollinated crops if relying solely on native, naturally occurring pollinators. Current scientific data indicate that the global bee extinction between the Cretaceous and the Paleogene (Cretaceous-Tertiary) occurred, which led to the disappearance of flowers because they could not produce viable fruit and germinate due to lack of pollination by bees or other animals. From the Middle Ages to the present day, there has been evidence that honey bees have always overcome the adverse factors affecting them throughout the ages, after which their population has fully recovered. This fact must be treated with great care given the emergence of a new, widespread stress factor in the second half of the 20th century—intoxication of beehives with antibiotics and acaricides, and treatment of crops with pesticides. Along with acute and chronic intoxication of bees and bee products, there are other new major stressors of global importance reducing the number of bee colonies: widespread prevalence of pathogenic organisms and pest beetles, climate change and adverse climatic conditions, landscape changes and limitation of natural habitats, intensification of agricultural production, inadequate nutrition, and introduction of invasive species. This report summarizes the impact of individual negative factors on the health and behavior of bees to limit the combined effects of the above stressors.

Keywords: *Apis mellifera*, honey bee colony losses, biotic factors, abiotic factors

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ANTIPROLIFERATIVE AND APOPTOGENIC EFFECTS OF MYOSMINE ON ERYTHROLEUKEMIA AND HEPATOCELLULAR CARCINOMA CELLS

Rada Mateva, Ani Georgieva, Ivan Iliev, Reneta Toshkova and Tamara Pajpanova

Myosmine, 3-(1-pyrroline-2-yl) pyridine is a minor tobacco alkaloid that has also been found in various widely used foods. Recently, this phytochemical has been gaining an increasing interest as a potential risk factor for the development of oesophageal adenocarcinoma. This study aimed to examine the effects of myosmine on the cell viability and proliferative activity of erythroleukemia and hepatocellular carcinoma cells and to obtain additional information about the mechanisms underlying its cytotoxic activity. The *in vitro* cytotoxic effect of myosmine on the HepG2 and MEL tumour cell lines was assessed by MTT dye reduction and trypan blue dye exclusion assays. The alterations in the tumour cell morphology induced by myosmine were analysed by fluorescent microscopy after staining with acridine orange (AO)/ethidium bromide (EtBr) and 40,6-diamidine-20-phenylindole dihydrochloride (DAPI). Annexin V-FITC/propidium iodide (PI) staining was used to assess the apoptosis-inducing ability of myosmine. The modulating action of antioxidant treatment on myosmine-induced cytotoxicity against the HepG2 tumour cell line was also examined. The cell viability tests indicated that myosmine induced a significant dose-dependent reduction of the viability and proliferative activity of both tumour cell lines. Fluorescent microscopy studies revealed marked alterations in the morphology of myosmine-treated tumour cells with signs of cell cycle arrest and apoptosis. The results of the simultaneous treatment with myosmine and vitamin C showed modulating activity of vitamin C on the cytotoxic effect of myosmine with concentration- and time-dependent variations. The presented results could contribute to the assessment of the potential health risks associated with the dietary myosmine exposure.

Keywords: myosmine, apoptosis, antioxidants, vitamin C, oxidative stress

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RESISTANCE OF NATIVE HONEY BEES FROM RHODOPE MOUNTAINS AND LOWLAND REGIONS OF BULGARIA TO NOSEMA CERANAE AND VIRAL PATHOGENS

R. Shumkova, B. Neov, A. Georgieva, D. Teofanova,G. Radoslavov, P. Hristov

The Western honey bee (*Apis mellifera* L., Hymenoptera: Apidae) is a species of fundamental economic, agricultural and environmental importance. The aim of this study was to compare the prevalence of some parasitic and viral pathogens in local honey bees from the Rodope Mountains and plain regions. To achieve this goal, molecular screening for two of the most distributed *Nosema* spp. And molecular identification of six honey bee viruses – Deformed wing virus (DWV), Acute bee paralysis virus (ABPV), Chronic bee paralysis virus (CBPV), Sacbrood virus (SBV), Kashmir bee virus (KBV), and Black queen cell virus (BQCV) was performed. Molecular analysis was carried out on 168 honey bee samples from apiaries situated in three different parts of the country where a mix of different honey bee subspecies were reared. In South Bulgaria (the Rhodope Mountains), a local honey bee called *Apis mellifera rodopica* (a local ecotype of *A. m. macedonica*) was bred, while in the other two regions (plains) different introduced subspecies existed. The results showed that the samples from the lowland regions in the country were outlined with the highest prevalence (70.5%) of *N. ceranae,* while those from the mountainous parts had the lowest rate (5.2%). Four of the honey bee viruses were identified – DWV (10/5.9%), followed by SBV (6/3.6%) and ABPV (2/1.2%), and one case of BQCV. In conclusion, the local honey bee *A. m. rodopica* (despite the higher number of samples) has shown lower prevalence of both nosemosis and viral infections. Therefore, this honey bee has to be preserved as a part of the national biodiversity.

Keywords: *Apis mellifera*, Bulgaria, honey bee diseases, molecular detection

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HEMOCYANINS FROM HELIX AND RAPANA SNAILS EXHIBIT *IN VITRO* ANTITUMOR EFFECTS IN HUMAN COLORECTAL ADENOCARCINOMA

Georgieva A., Todorova K., Iliev I., Dilcheva V., Vladov I., Petkov, S. Toshkova, R., Velkova L., Dolashki A., Dolashka P.

Hemocyanins are oxygen-transporting glycoproteins in the hemolymph of arthropods and mollusks that attract scientific interest with their diverse biological activities and potential applications in pharmacy and medicine. The aim of the present study was to assess the *in vitro* antitumor activity of hemocyanins isolated from marine snail *Rapana venosa* (RvH) and garden snails *Helix lucorum* (HlH) and *Helix aspersa* (HaH), as well the mucus of H. aspersa snails, in the HT-29 human colorectal carcinoma cell line. The effects of the hemocyanins on the cell viability and proliferation were analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and the alterations in the tumor cell morphology were examined by fluorescent and transmission electron microscopy. The results of the MTT assay showed that the mucus and α-subunit of hemocyanin from the snail H. aspersa had the most significant antiproliferative activity of the tested samples. Cytomorphological analysis revealed that the observed antitumor effects were associated with induction of apoptosis in the tumor cells. The presented data indicate that hemocyanins and mucus from *H. aspersa* have an antineoplastic activity and potential for development of novel therapeutics for treatment of colorectal carcinoma.

Keywords: hemocyanin, snail *Rapana venosa*, snail *Helix lucorum*, snail *Helix aspersa*, antitumor activity, colorectal adenocarcinoma, apoptosis

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ANTIPROLIFERATIVE AND ANTITUMOUR ACTIVITY OF SAPONINS FROM *ASTRAGALUS GLYCYPHYLLOS* ON MYELOID GRAFFI TUMOUR

Ani Georgieva, Georgi Popov, Aleksandar Shkondrov, Reneta Toshkova, Ilina Krasteva, Magdalena Kondeva-Burdina, Vasil Manov

Ethnopharmacological relevance: Astragalus glycyphyllos L. has been extensively used in Bulgarian folk medicine as an antihypertensive, diuretic, anti-inflammatory, anti-tumour, in cases of cardiac insufficiency, renal inflammation, calculosis, etc.

Aim of the study: To evaluate the possible *in vitro/in vivo* anti-proliferative/anti-tumour activity of a purified saponins' mixture (PSM) obtained from the plant.

Materials and methods: Viability and proliferative activity of the Graffi myeloid tumour cells was assessed by MTT test. The morphological alterations were visualized and analysed by fluorescent microscopy after intravital double staining. An *in vivo* model of Graffi tumour bearing hamsters was used to examine the influence of PSM on transplantability, tumour growth, survival and mortality as well as to observe pathomorphological changes.

Results: Graffi tumour cells were sensitive to purified saponins' mixture after 24 and 48 h treatment. The treatment induced a statistically significant decrease of the viability/proliferation of the Graffi tumour cells. These effects were concentration- and time-dependent. Fluorescent microscopy studies showed that these antiproliferative effects were connected to the induction of apoptosis. The *in vivo* study showed the presence of a stromal component, single mononuclear cells in the stroma. Multiple incorrect mitotic figures were observed in the tumour tissue from the control group. Well-formed stroma with accumulation of mononuclear cells and mitotic cells were found in the group, treated with PSM. The tumour weight was decreased in the group, treated with PMS.

Conclusion: The results indicate that PSM exhibited *in vitro/in vivo* antiproliferative/anti-tumour effects.

Keywords: *Astragalus glycyphyllos*, anti-tumour, cytotoxicity, saponins

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ANTINEOPLASTIC EFFECTS OF ERUFOSINE ON GRAFFI MYELOID TUMOUR IN HAMSTERS

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Cancer has become one of the most significant health challenges for both human and veterinary medicine. The present study examined the antineoplastic and antimetastatic activity of the novel membrane-targeting anticancer agent erufosine. The antitumour effects of erufosine on Graffi virusinduced experimental myeloid tumour in hamsters was assessed by histopathological methods and evaluation of some biometric parameters of tumour growth. Two schemes of experimental antitumour therapy were applied one that started simultaneously with the tumour transplantation and a second one that started after the appearance of palpable tumours. The results demonstrated protective antitumour effect of erufosine, expressed by decrease of transplantability, tumour growth inhibition, suppression of metastatic activity and extension of mean survival time. The effectivity of the experimental therapy was more pronounced when it was started simultaneously with the transplantation of the tumour cells. Presented results suggest that erufosine is a promising drug candidate for treatment of haematological malignances.

Key words: alkylphosphocholines, antitumour activity, erufosine, myeloid tumour

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CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF THE NONSTEROIDAL ANTI-INFLAMMATORY DRUG DICLOFENAC IN HUMAN TUMOUR CELL LINES

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Diclofenac is one of the most prescribed non-steroidal anti-inflammatory drugs (NSAIDs) for acute and chronic inflammatory conditions. Taking into consideration the extensive use of diclofenac, the crucial role of inflammation in tumorigenesis and data on the effects of NSAIDs on cancer cell lines, we investigated the cytotoxic potential of diclofenac on a panel of human cell lines originating from breast (MCF-7), cervical (HeLa) and colorectal cancer (HT-29). The cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay and the half maximal inhibitory concentrations (IC_{50}) were determined. Diclofenac had higher potency against MCF-7 and HT-29 than against HeLa. The cellular and nuclear changes were examined by fluorescent microscopy using 4′,6′-diamino-2-phenylindole (DAPI) stain and acridine orange/ethidium bromide (AO/EB). The wound-healing scratch assay showed significant reduction in the migration capacity of all tested cancer cell lines. The observed antineoplastic activity implies that the anticancer potential of NSAIDs and diclofenac, in particular, necessitates further investigation.

Keywords: diclofenac, apoptosis, cytotoxicity, wound-healing assay, proliferation, migration