

**COBALT ACCUMULATION AND IRON-REGULATORY PROTEIN PROFILE  
EXPRESSION IN IMMATURE MOUSE BRAIN AFTER PERINATAL  
EXPOSURE TO COBALT CHLORIDE**

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RASHEV, P., VLADOV, I., GLUHCHEVA, Y.

**Abstract**

Developing brain is very sensitive to the influence of environmental factors during gestation and the neonatal period. The aim of the study is to assess cobalt and iron accumulation in the brain as well as changes in the expression of iron-regulatory proteins transferrin receptor 1, hepcidin, and ferroportin in suckling mice. Perinatal exposure to cobalt chloride increased significantly cobalt content in brain tissue homogenates of 18-day-old (d18) and 25-day-old (d25) mice inducing alterations in brain iron homeostasis. Higher degree of transferrin receptor 1 expression was demonstrated in cobalt chloride-exposed mice with no substantial changes between d18 and d25 mice. A weak ferroportin expression was found in 18-day-old control and cobalt-treated mouse brain. Cobalt exposure of d25 mice resulted in increased ferroportin expression in brain compared to the untreated age-matched control group. Hepcidin level in cobalt-exposed groups was decreased in d18 mice and slightly increased in d25 mice. The obtained data contribute for the better understanding of metal toxicity impact on iron homeostasis in the developing brain with further possible implications in neurodegeneration.

**Keywords :** Cobalt, Iron, Immature brain, Transferrin receptor 1, Hepcidin, Ferroportin

## **EFFECT OF ACUTE SODIUM NITRITE INTOXICATION ON SOME ESSENTIAL BIOMETALS IN MOUSE SPLEEN**

PETROVA, E., GLUHCHEVA, Y., PAVLOVA, E., VLADOV, I., VOYSLAVOV, T., IVANOVA, J.

### **Abstract**

#### Background and aim

Sodium nitrite ( $\text{NaNO}_2$ ) is an inorganic salt with numerous applications in a variety of industries, as well as in medicine. Nevertheless, exposure to high levels of  $\text{NaNO}_2$  is toxic for animals and humans. Sodium nitrite intoxication is shown to decrease the activity of major antioxidant defence enzymes which is dependent on the maintenance of specific ion equilibrium. The aim of the present study was to investigate the effect of acute  $\text{NaNO}_2$  intoxication on the content of the essential metals iron (Fe), calcium (Ca) and zinc (Zn) in mouse spleen.

#### Methods

Mature male ICR mice were divided into four groups and subjected to acute  $\text{NaNO}_2$  exposure by a single intraperitoneal injection of 120 mg/kg body weight. Animals in each group were sacrificed at certain time interval after treatment (1 h, 5 h, 1 day and 2 days). Spleens were excised and processed for atomic absorption spectrometry analysis of Fe, Ca and Zn content.

#### Results

At the first hour after treatment, a decrease in Fe and Ca levels was observed. One day following  $\text{NaNO}_2$  administration, Zn concentration reached its lowest value and Ca levels remained lower, compared to the untreated controls. In contrast, Fe concentration increased on the first and second day after treatment.

#### Conclusion

The results of the present study demonstrate that acute  $\text{NaNO}_2$  intoxication provokes changes in the endogenous levels of Fe, Ca and Zn in mouse spleen. These findings suggest disruption of the ionic balance and impact on the activity of antioxidant defence enzymes.

**Keywords :** Sodium nitrite; Mouse spleen; Iron; Calcium; Zinc.

**COMPARATIVE STUDY ON THE EFFECTS OF SALINOMYCIN, MONENSIN AND MESO-2,3-DIMERCAPTOSUCCINIC ACID ON THE CONCENTRATIONS OF LEAD, CALCIUM, COPPER, IRON AND ZINC IN LUNGS AND HEART IN LEAD-EXPOSED MICE**

IVANOVA, J., KAMENOVA, K., PETROVA, E., VLADOV, I., GLUHCHEVA, Y., DORKOV, P.

**Abstract**

**Background and aim**

Environmental lead (Pb) exposure damages the lungs and is a risk factor for death from cardiovascular disease. Pb induces toxicity by a mechanism, which involves alteration of the essential elements homeostasis. In this study we compare the effects of salinomycin (Sal), monensin (Mon) and meso-2,3-dimercaptosuccinic acid (DMSA) on the concentrations of lead (Pb), calcium (Ca), copper (Cu), iron (Fe) and zinc (Zn) in the lungs and heart of lead-exposed mice.

**Methods**

Sixty days old male ICR mice were divided into five groups: control (Ctrl) – untreated mice obtained distilled water for 28 days; Pb-intoxicated group (Pb) – exposed to 80 mg/kg body weight (BW)  $\text{Pb}(\text{NO}_3)_2$  during the first 14 days of the experimental protocol; DMSA-treated (Pb + DMSA) – Pb-exposed mice, subjected to treatment with an average daily dose of 20 mg/kg BW DMSA for two weeks; Monensin-treated (Pb + Mon) – Pb-exposed mice, obtained an average daily dose of 20 mg/kg BW tetraethylammonium salt of monensic acid for 14 days; Pb + Sal - Pb-exposed mice, treated with an average daily dose of 20 mg/kg BW tetraethylammonium salt of salinomycinic acid for two weeks. On the 29<sup>th</sup> day of the experiment the samples (lungs and heart) were taken for atomic absorption analysis.

**Results**

The results revealed that exposure of mice to Pb for 14 days significantly increased the concentration of the toxic metal in both organs and elevated the cardiac concentrations of Ca, Cu and Fe compared to untreated mice. Pb exposure diminished the lung concentrations of Ca and Zn compared to that of untreated controls. DMSA, monensin and salinomycin decreased the concentration of Pb in the lungs and heart. Among the tested chelating agents, only salinomycin restored the cardiac Fe concentration to normal control values.

**Conclusion**

The results demonstrated the potential application of polyether ionophorous antibiotic salinomycin as antidote for treatment of Pb-induced toxicity in the lungs and heart. The possible complexation of the polyether ionophorous antibiotics with  $\text{Ca}(\text{II})$  and  $\text{Zn}(\text{II})$ , which can diminish the endogenous concentrations of both ions in the lungs should be taken into account.

**Keywords :**Lead; Essential elements; Chelating agents; Lung; Heart.

**DYNAMICS OF TRACE METALS IN THE SYSTEM WATER – SOIL – PLANT – WILD RATS – TAPEWORMS (*HYMENOLEPIS DIMINUTA*) IN MAGLIZH AREA, BULGARIA**

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TEPAVITCHAROVA, ST., NANEV, V., DASSENAKIS, M., KARAVOLTSOS, S.

**Abstract**

**Background**

The impact of chemical elements on the biosphere is a function of their concentration and chemical form. Elucidation and prognosing of the latter in water basins and soil extracts is of particular significance for the assessment of their bioaccumulation in plants and animals.

**Objectives**

Trace metals dynamics in the system *water – soil–plant–wild rats – Hymenolepis diminuta* in two agro-industrial zones (East and West) around Maglizh city, Bulgaria were investigated through experimental studies and thermodynamic modelling of the chemical species.

**Methods**

Samples from surface waters of rivers, their nearby uncultivated soils, meadow uncultivated vegetation (*Ranunculus acris* and *Gramineae*) and field rats were collected. *In situ* measurements and laboratory analyses were performed for the determination of the physico-chemical characteristics and total concentrations of Al, Fe, Mn, Ni, Cu, Zn and Pb. The distribution of their dissolved chemical species in water samples and in the aqueous soil extracts was calculated using a thermodynamic approach. The relationship *chemical species - bioaccumulation* was discussed.

**Results**

Waters and soils in the East zone of Maglizh area were found to be more polluted compared to those in the West one, regarding Ni, Mn, Zn, Pb and Cu, while Mn and Cu displayed the highest mobility in West zone soils. Trace metals contents in *Ranunculus acris* exceed that in *Gramineae*, since the highest accumulation factors were calculated for Cu and Zn. The highest accumulation in rats was found for Zn followed by Cu, being higher in the West zone. Thermodynamic modelling shows that  $Mn^{2+}$  free ions are dominant in both waters and aqueous soil extracts.  $Ni^{2+}$  and  $Zn^{2+}$  ions followed by metal-organic complexes are dominant in waters of East zone while metal-organic complexes followed by free ions are dominant in waters of West zone and both soil extracts. Metal-organic complexes are dominant for Fe, Cu and Pb in all samples studied, while mainly hydroxy forms ( $Al(OH)_4^-$ ) followed by metal-organic complexes are typically for Al depending on pH.

**Conclusions**

Experimentally established bioaccumulation of trace metals in the studied vegetation and rats is a consequence of the total concentration of trace metals in waters and soils, their mobility and chemical species. The dominance of organic complexes of trace

metals is a prerequisite for their bioaccumulation in plants. Rats are in direct contact with the soil solution and therefore, of importance is the content of free ions of  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ , which are easily absorbed through the skin. The host-helminth system *wild rat/H. diminuta* could be used as a bioindicator for trace metals pollution.

**Keywords:** Trace metals; Chemical species; Accumulation in plants; Accumulation in rats; Water pollution; Soil pollution.

**AMELIORATIVE EFFECT OF THE ANTICANCER AGENT SALINOMYCIN ON CADMIUM-INDUCED HEPATOTOXICITY AND RENAL DYSFUNCTION IN MICE**

KAMENOVA, K., GLUHCHEVA, Y., VLADOV, I., STOYKOVA, S., IVANOVA, JU.

**Abstract**

This study presents experimental data on the effects of the tetraethylammonium salt of salinomycinic acid (Sal) on Cd-induced hepatotoxicity and renal dysfunction in Cd-treated mice compared to those of *meso*-2,3-dimercaptosuccinic acid (DMSA). Forty 60-day-old male ICR mice were randomized into five groups: control group (untreated mice), Cd group (Cd(II) acetate 20 mg/kg body weight provided orally once per day for 14 days), Cd + DMSA group (exposed to Cd(II) acetate as the Cd-exposed group followed by DMSA 20 mg/kg body weight provided orally once per day for 14 days), and Cd + Sal group (exposed to Cd(II) acetate as the Cd-exposed group followed by Sal 20 mg/kg body weight once per day for 14 days). Cd intoxication of mice induced significant liver and kidney injury and a significant elevation of the concentration of Cd in both organs. Treatment of Cd-exposed mice with DMSA or Sal restored the levels of the renal and hepatic functional markers and significantly decreased the concentration of the toxic metal ion in both organs. Administration of Sal improved Cd-induced alterations of the endogenous levels of the essential metal ions. Histological studies revealed that the antibiotic more effectively ameliorated the Cd effect on the liver morphology compared to DMSA. Taken together, the results confirm that the anticancer agent salinomycin is a promising antidote to Cd poisoning.

**Keywords:** Cadmium . Chelation therapy .DMSA . Salinomycin . Hepatotoxicity . Renal dysfunction

## **ANTIBIOFILM POLY(CARBOXYBETAIN METHACRYLATE) HYDROGELS FOR CHRONIC WOUNDS DRESSINGS**

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### **Abstract**

The current study demonstrates the benefits of poly(carboxybetaine methacrylate) hydrogels in chronic wound healing. These hydrogels demonstrate high absorbing capacity upon swelling in salt solutions thus revealing great potential as dressings for highly exuding chronic wounds. Moreover, upon swelling they expand, increasing their volume by 25%, which makes them patient friendly ensuring also the proper wound healing. Poly(carboxybetaine methacrylate) hydrogels were also shown to absorb collagenase and myeloperoxidase, two enzymes that are specific for chronic wounds, reducing in this way their amount by 30–45% in the wound bed without entirely inhibiting their activity, as the latter is necessary for the wound healing process. The hydrogels were also shown to be non-cytotoxic as well as to prevent the biofilm formation of *S. aureus*. The *in vivo* implantation in rats showed no immune response to moderate immune reaction for both studied PCB hydrogels. Thus, the properties of the PCB networks revealed in the study demonstrate their potential as chronic wounds dressing materials.

**Keywords:** Polyzwitterions, Chronic wound dressings, Antipolyelectrolyte behavior, pH and salt responsiveness, Antibiofilm activity, Biocompatibility.

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

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**Abstract:** 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  $\alpha$ -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



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

KRAICHEVA I., VODENICHAROVA E., SHENKOV S., TASHEV E., TOSHEVA T., TSACHEVA I., KRIL A., TOPASHKA-ANCHEVA M., GEORGIEVA A., ILIEV I., VLADOV I., GERASIMOVA TS., TROEV K.

**Abstract**

Novel polyphosphoesters containing anthracene-derived aminophosphonate units, poly(oxyethylene aminophosphonate)s (**4** and **5**) and poly[oxyethylene(aminophosphonate-co-H-phosphonate)]s (**6** and **7**), were synthesized via an addition of poly(oxyethylene H-phosphonate)s to 9-anthrylidene-*p*-toluidine. The IR, NMR (<sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P) 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

**THE IMPACT OF PERINATAL COBALT CHLORIDE EXPOSURE ON EXTRAMEDULLARY ERYTHROPOIESIS, TISSUE IRON LEVELS, AND TRANSFERRIN RECEPTOR EXPRESSION IN MICE**

GLUHCHEVA, Y., PAVLOVA, E., PETROVA, E., TINKOV, A. A., AJSUVAKOVA, O. P., SKALNAYA, M. G., VLADOV, I., SKALNY, A. V.

**Abstract**

The objective of the present study was to elucidate the effect of perinatal cobalt chloride (CoCl<sub>2</sub>) exposure on extramedullary erythropoiesis in suckling mice in relation to iron (Fe) content and transferrin receptor (TfR) expression. Pregnant ICR mice were subjected to a daily dose of 75 mg CoCl<sub>2</sub>/kg body weight 2–3 days prior and 18 days after delivery. Co exposure significantly increased erythrocyte count (RBC), and reduced the erythrocytic parameters mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the offspring. Total iron-binding capacity (TIBC) was decreased while bilirubin values were ~ 1.2-fold higher in the metal-exposed mice. Perinatal CoCl<sub>2</sub> treatment also induced pathohistological changes in target organs (spleen, liver, and kidneys) as altered organ weight indices, leukocyte infiltration, abundant Kupffer cells in the liver, increased mesangial cellularity, and reduced capsular space in the kidney. CoCl<sub>2</sub> administration induced significant 68-, 3.8-, 41.3-, and 162-fold increase of Co content in the kidney, spleen, liver, and RBC, respectively. Fe content in the target organs of CoCl<sub>2</sub>-treated mice was also significantly elevated. Immunohistochemical analysis demonstrated that TfR1 was well expressed in the renal tubules, hepatocytes, the red pulp, and marginal zone of white pulp in the spleen. TfR2 showed similar expression pattern, but its expression was stronger in the spleen and liver samples of Co-treated mice compared with that of the untreated controls. The results demonstrate that exposure to CoCl<sub>2</sub> during late pregnancy and early postnatal period affects body and organ weights and alters hematological and biochemical parameters, iron content, and TfR expression in target organs.

**Keywords:** Cobalt . Iron . Sucklingmice . Hematological indices . TfR.

**TRACE ELEMENT CONTENTS IN RAT TISSUES AFTER EXPERIMENTALLY INDUCED *FASCIOLA HEPATICA* INFECTION**

NANEV, V., GABRASHANSKA, M., GEORGIEVA, K., VLADOV, I., KANDIL, O., TSOICHEVA-GAYTANDZHIEVA, N.

**Abstract**

The structural, functional and metabolic changes in host tissues caused during penetration, migration and localization of helminths in the host body, are associated with specific changes in trace element levels in body tissues, including these of liver and spleen. The aim of the present study is determination of the quantities of trace elements zinc (Zn), copper (Cu), cobalt (Co) and iron (Fe) in liver and spleen tissues of rats at acute or chronic phases of fasciolosis. Statistically significant reducing of Zn, Cu, Co and Fe quantities is established in the livers of rats with acute and chronic fasciolosis compared to the controls. The decreasing of trace elements levels was slighter in chronic phase of fasciolosis. Significant reducing of spleen Fe quantity and slighter decreasing of Zn and Cu levels were established in the spleens of rats with acute fasciolosis. Statistically significant increased quantity of Zn, markedly increased level of Fe, slightly increased Cu and unchanged level of Co were detected in the spleens of rats with chronic fasciolosis compared to controls. The results we obtained point that the infection with *F. hepatica* leads to specific mineral imbalance in the host body as a result of parasite-associated host liver and spleen structural changes and alterations of biochemical and immunological mechanisms and show the important role of the investigated biogenic trace elements in pathogenesis of fasciolosis.

**Key words:** acute and chronic fasciolosis, trace elements contents, livers, spleens, rats.

**A COMPARATIVE STUDY OF SIX *TRICHINELLA* SPECIES BY REAL - TIME PCR ASSAY**

DILCHEVA, V., VLADOV, I., PETKOVA, S.

**Abstract**

Six *Trichinella* isolates (ISS03, ISS13, ISS10, ISS02, ISS029, ISS035) were detected by real-time PCR. C<sub>T</sub> values connected with melting curve analysis allowed a distinct species identification from the beginning of the reaction and HRM curve analysis showed individual melting curve for each of the studied species which can be used for genomic control on subsequent diagnostic tests. Obtained results determined the method as sensitive, accurate and appropriate for prevention of trichinellosis. The data of this study provide an additional and re-evaluate the existing information through which a new preventative and disease-fighting approach can be accomplished.

**Key words:** trichinellosis, real-time PCR.

**RAT SPLEEN TRACE ELEMENT CONTENTS UNDER COMBINED EFFECT  
OF CHRONIC FASCIOLOSIS AND BASIC ZINC-COPPER COMPOUND**

NANEV V., VLADOV I., GABRASHANSKA M., KANDIL OMNIA M., HASSAN NOHA  
M. F., SEDKY DOOA, TSOICHEVA-GAYTANDZHIEVA N. T.

**Abstract**

The contents of spleen trace elements copper (Cu), zinc (Zn), iron (Fe), and cobalt (Co) were determined after basic Cu-Zn salt application in rats with experimental chronic fasciolosis. The Cu-Zn salt treatment of healthy animals caused statistically significant increased quantity of spleen Zn and nearly unchanged levels of Co, Cu and Fe compared to controls. Statistically significant increased quantity of Zn, markedly increased level of Fe, slightly increased Cu and unchanged level of Co were detected in the spleens of rats with chronic fasciolosis compared to the controls. The application of basic Cu-Zn salt at the background of chronic fasciolosis caused statistically significant increasing of the quantities of spleen Zn and Cu, higher than in the other experimental groups, increasing of Fe level, which was lower than in the rats with chronic fasciolosis, and not changed Co content in the spleens of experimental animals compared to the control levels. May be the application of basic Cu-Zn salt caused an improvement of some altered from chronic *Fasciola hepatica* infection biochemical and immunological parameters in the spleens of infected hosts.

**Key words:** spleen, trace elements, basic zinc-copper salt, chronic fasciolosis, rats.

**EFFECT OF MIXED CU-ZN BASIC SALT ON SPLEEN TRACE ELEMENT  
CONTENTS IN RATS WITH ACUTE FASCIOLOSIS**

NANEV V., VLADOV I., GABRASHANSKA M., TSOICHEVA-GAYTANDZHIEVA N.

**Abstract**

Contents of spleen trace elements copper (Cu), zinc (Zn), iron (Fe), and cobalt (Co) were investigated after mixed Cu–Zn basic salt treatment in rats with acute fasciolosis. The application of Cu–Zn salt on healthy rats caused increasing of spleen Zn level. Significant decreasing of spleen Fe quantity and slight decreasing of Zn and Cu levels were established in the spleens of rats with acute fasciolosis. The application of Cu–Zn salt at the background of acute fasciolosis caused normalization of Zn and Cu quantities and slight increasing of the decreased from the parasite Fe level in the rat spleens.

**Key words:** spleen, trace elements, mixed basic salt, acute fasciolosis

## **INTERACTION BETWEEN EXPERIMENTAL FASCIOSIS AND CARCINOGENESIS**

TSOCHEVA-GAYTANDZHIEVA N., TOSHKOVA R., ROEVA I., TOPASHKA-ANCHEVA M., FILCHEV A., VLADOV I.

### **Abstract**

*Introduction:* A new property of the mature trematode *Fasciola hepatica* was established in previous investigations of ours - its cell growth inhibiting effect on the development of experimental liver carcinogenesis with diethylnitrosamine (DENA) (3). This formed the basis for a hypothesis on the possible roles of some biologically active substances, inhibitors of cell proliferation, of parasite or host origin, in the pathogenesis of the interaction fascioliasis - carcinogenesis.

Later, biologically active substances (BASes) were isolated from the tissues of *Fasciola hepatica* and from *F. hepatica* infected rat liver and their inhibiting effect on the proliferation of primary hepatocyte and lymphocyte cell cultures was established (4, 5). The aim of the present study is to investigate the antitumor effect of mature *F. hepatica* and the newly isolated BASes from rat liver and tissues of *F. hepatica* on DNA synthesis *in vivo* on tumor bearing mice and *in vitro* on tumor cell cultures.

**Key words:** *Fasciola hepatica* , carcinogenesis, diethylnitrosamine

## **ITS2 AMPLIFICATION OF *HAEMONCUS CONTRORUS***

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### **Abstract**

*Introduction and aim:* Gastrointestinal nematodes are among the most destructive pathologies affecting ovine production, given high prevalence in flocks and serious consequences of infection. The gastrointestinal nematodes from the *Haemonchus* species are major blood feeding parasite of ruminants worldwide. Infection can lead to anaemia, emaciation, oedema and gastric disturbances resulting principally from blood loss. Heavy infections may be fatal especially in young animals.

The aim of the study was evaluation the genetic diversity and patterns of gene flow in isolates of *Haemonchus* sp. from cattle and sheep from different localities of Bulgaria based on the DNA marker (Internal transcribed spacer - ITS2)

**Key words:** *Haemoncus controrus* , Internal transcribed spacer - ITS2, PCR.



## **COMBINED EFFECTS OF *FASCIOLA HEPATICA* INFECTION AND COPPER INTOXICATION ON OXIDATIVE/ANTIOXIDATIVE STATUS IN RATS**

NANEV, V., VLADOV, I., GABRASHANSKA, M.

### **Abstract**

The aim of our study was to investigate parameters of oxidative/antioxidative status in rats experimentally infected with *Fasciola hepatica* and treated with a copper salt. The experiment was carried out on 24 male Wistar albino rats, divided into 4 groups with 6 animals in each: group 1 – healthy untreated animals, group 2 – rats orally infected with *F. hepatica*; group 3 – rats treated with CuSO<sub>4</sub>·5H<sub>2</sub>O and group 4 – rats experimentally infected with *F. hepatica* and treated with CuSO<sub>4</sub>·5H<sub>2</sub>O. Rats from group 2 and 4 were orally infected with 15 viable *F. hepatica* metacercariae per animal. Rats from group 3 and 4 received CuSO<sub>4</sub>·5H<sub>2</sub>O dissolved in drinking water at a dose of 150 mg/kg body weight after post infestation week 2. Copper administration lasted 2 weeks. The rats were euthanised on the 35th day post infestation. The levels of malondialdehyde, glutathione, and the activity of Cu, Zn-superoxide dismutase and glutathione peroxidase in the liver of all rats were established. Increased liver MDA level was observed in groups infected and untreated with copper compared to control level. Reduced Cu,Zn-SOD activity was found in all infected rats as well as insignificant increase of the enzyme in group 3 compared to control group value. GPx activity was reduced in similar manner in the treated and infected groups compared to the control group. GSH level was lower in all treated rats than in controls ( $P < 0.01$ ). Copper liver content was increased in groups receiving CuSO<sub>4</sub>·5H<sub>2</sub>O compared both to control and infected only group. Substantial imbalance in oxidative/antioxidative status in groups 2, 3 and 4 was demonstrated compared to the control group. Combined effect of chronic copper administration and experimental *F. hepatica* infection increased significantly MDA level, reduced the activity of Cu,Zn-SOD and the GSH content in host livers. Elevated copper level influenced defense system in *F. hepatica* infected rats at a high extent. Parasites and copper acted together to increase the oxidative stress. Parasitism in the presence of copper pollution compromises the health of the host, even at low intensities.

**Key words:** copper, *Fasciola hepatica*, oxidative/antioxidative status, rat.

## **OXIDATIVE STRESS STATUS IN MOUFLONS INFECTED WITH LUNG WORM *Dictyocaulus viviparus***

NANEV V., GABRASHANSKA M., VLADOV I., GUNCHEV Y.

### **Abstract**

The purpose of our study is to examine the lung oxidative stress status in mouflons naturally infected with the nematode *Dictyocaulus viviparus* by determination of the malondyaldehyde (MDA) concentration as a biomarker of lipid peroxidation and its relation to the antioxidant status (glutathione, vitamin A, vitamin E, vitamin C, enzymes SOD, CAT, GPx and the trace elements selenium and zinc ). 16 mouflons, non-infected and infected with *D. viviparus* were studied. Animals were shot in a hunt during the hunting season in ecological areas (naturally and anthropogenically non-polluted) in the Rhodope Mountains in Bulgaria. Antioxidant/ oxidant imbalance occurred in the animals with dictyocaulosis. Levels of vitamin A, C and E, GSH and selenium were reduced, SOD, GPx and CAT activity was suppressed, whereas MDA level was increased in the lungs infected with *D. viviparus* compared to the non-infected. The zinc level was not changed in the infected animals. The results of the present study indicate that the antioxidant/oxidant imbalance was expressed mainly by significant reduction of the antioxidant enzyme activities. The present findings confirm that parasitic infection correlates well with OS status which can be assessed using enzymatic and nonenzymatic biomarkers. The pathogenesis of *D. viviparus* infection needs to be re-examined in order to understand more deeply the consequences of such an infection in the animal body.

**Key words:** dictyocaulosis, mouflons, oxidative stress.

**ALTERATIONS IN BLOOD METABOLIC PARAMETERS OF IMMATURE MICE AFTER SUBCHRONIC EXPOSURE TO COBALT CHLORIDE**

VLADOV, I., PETROVA, E., PAVLOVA, E., TINKOV, A.A., AJSUVAKOVA, O.P., SKALNY, A., GLUHCHEVA, Y.

**Abstract**

The wide use of cobalt (Co) in food, industry, and medical devices requires full elucidation of its biological effects on tissues and organs. The aim was to assess serum metabolic alterations in immature mice after subchronic exposure to  $\text{CoCl}_2$ . Pregnant ICR mice were subjected to a daily dose of 75 mg cobalt chloride/kg body weight ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ) 2–3 days before they gave birth, and treatment continued until days 25 and 30 after delivery. The compound was dissolved in and obtained with regular tap water. ICP-DRC-MS analysis showed significantly elevated serum  $\text{Co}^{2+}$  and diverse alterations in metabolic parameters of 25- and 30-day-old pups after exposure to  $\text{CoCl}_2$ . Cholesterol and urea levels were significantly elevated in day 25 mice while HDL-C and LDL-C were reduced. In day 30, Co-exposed mice LDL-C and triglycerides were significantly increased while the total cholesterol level remained unchanged. Alkaline phosphatase was significantly reduced in day 25 Co-exposed mice. Blood glucose level of Co-exposed mice remained close to the untreated controls. Total protein content was slightly increased in day 30 mice. Co-exposure reduced albumin content and albumin/globulin ratio but increased significantly globulin content. Co administration showed strong correlation with cholesterol, urea, and HDL-C in both day 25 and 30 mice. Inverse correlation was found with alkaline phosphatase and albumin for day 25 and with triglycerides, globulin, and total protein content in day 30 Co-exposed mice. Subchronic  $\text{CoCl}_2$  exposure of immature mice induced significant changes in key metabolic parameters suggesting possible further disturbances in energy metabolism, osteogenesis, and reproduction.

**Keywords:** Cobalt . Cholesterol . Urea . Alkaline phosphatase . Serumproteins . Immature mice

**TRACE METALS ACCUMULATION IN THE ECO-SYSTEM WATER – SOIL – VEGETATION (*AGROPYRON CRISTATUM*) – COMMON VOLES (*MICROTUS ARVALIS*) – PARASITES (*HYMENOLEPIS DIMINUTA*) IN RADNEVO REGION, BULGARIA.**

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**Abstract**

**Background**

Coal and coal processing industries provoke trace metal pollution, which has a negative effect on the water – soil – vegetation – small mammals eco-system, constituting part of the food chain and exerting a serious impact on human health.

**Objectives**

Assessment of the environmental impact of Maritza iztok coal complex, situated east of Radnevo town, Bulgaria, by tracking the dynamics and accumulation of trace metals in the eco-system water – soil – vegetation – common voles – parasites.

**Methods**

Samples from surface waters, their nearby uncultivated soils, meadow uncultivated vegetation (*Agropyron cristatum*) and field common voles (*Microtus arvalis*) were collected. *In situ* measurements and laboratory extraction procedures and analyses were performed. Accumulation and mobility indices were calculated. The distribution of dissolved Mn, Ni, Cu, Zn and Pb chemical species in water-soil extracts was calculated using a thermodynamic approach. Two thermodynamic models were applied – the classical ion-association model for calculating the inorganic trace metal species and the Stockholm Humic Model (SHM) accounting for the complexation reactions of trace metals with organic matter. Visual Minteq computer program, Version 3.1 was used. The relationship chemical species - bioaccumulation was discussed.

**Results**

Pb and Mn, together with  $\text{SO}_4^{2-}$  and  $\text{PO}_4^{3-}$  were found to be the main pollutants of waters in the region. The soils studied exhibited low concentrations of trace metals, not exceeding the specified MACs. The content of Mn was the highest, followed by Zn, Pb, Cu and Ni. The highest phytoaccumulation coefficients in the studied uncultivated grass vegetation were calculated for Cu and Zn, being 1–2 orders of magnitude higher than those of Mn and Ni. The accumulation of trace metals was explained on the basis of ions mobility and chemical species distribution. In the case of the host-parasite system *Microtus alvaris* - *Hymenolepis diminuta*, Zn displays the highest accumulation coefficient, followed by those of Cu and Pb. The parasite showed a higher bioaccumulation compared to infected common voles, with the highest bioaccumulation found for Ni.

**Conclusions**

The bioaccumulation of trace metals depends on their mobility, concentration and chemical forms in water-soil solutions. Metal-organic species stimulate the

phytoaccumulation of trace metals while inorganic ones suppress it. The sequence of trace metals bioaccumulation in common voles is analogous to that of soil contamination. The parasite exhibited higher bioaccumulation levels compared to infected common voles.

**Keywords :**Trace metals, Water pollution, Soil pollution, Chemical species, Accumulation in vegetation, Accumulation in common voles.

**PERINATAL AND EARLY-LIFE COBALT EXPOSURE IMPAIRS ESSENTIAL METAL METABOLISM IN IMMATURE ICR MICE**

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**Abstract**

The objective of the present study was to assess the impact of cobalt (Co) exposure on tissue distribution of iron (Fe), copper (Cu), manganese (Mn), and zinc (Zn), as well as serum hepcidin levels in immature mice (18, 25, 30 days). Pregnant mice were exposed to 75 mg/kg b.w. cobalt chloride ( $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ ) with drinking water starting from 3 days before delivery and during lactation. At weaning (day 25) the offspring were separated and housed in individual cages with subsequent exposure to 75 mg/kg b.w.  $\text{CoCl}_2$  until 30 days postnatally. Evaluation of tissue metal levels was performed by an inductively coupled plasma-mass spectrometry (ICP-MS). Serum hepcidin level was assayed by enzyme linked immunosorbent assay (ELISA). Cobalt exposure resulted in a time- and tissue-dependent increase in Co levels in kidney, spleen, liver, muscle, erythrocytes, and serum on days 18, 25, and 30. In parallel with increasing Co levels,  $\text{CoCl}_2$  exposure resulted in a significant accumulation of Cu, Fe, Mn, and Zn in the studied tissues, with the effect being most pronounced in 25-day-old mice. Cobalt exposure significantly increased serum hepcidin levels only in day18 mice. The obtained data demonstrate that Co exposure may alter essential metal metabolism *in vivo*.

**Keywords :** Cobalt, Iron, Copper, Hepcidin, Toxicity.

## **POTENTIAL THERAPEUTIC PROPERTIES OF PLASMONICALLY HEATED GOLD NANOPARTICLES IN HELA CELL LINE**

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### **Abstract**

Local application of heat is a well-known concept in therapeutic medicine that has been explored extensively for the treatment of cancer and other conditions. The plasmon resonance for gold nanoparticles (GNPs) is at  $\sim 520$  nm. The aim of the present investigation was to elucidate the effect of different size GNPs combined with laser irradiation on permanent human cancer cell line HeLa. The samples were irradiated at  $\lambda = 532$  nm, pulse duration  $\tau_p = 15$  ns and repetition rate 1 Hz. Laser pulses with energy densities of  $F = 80 \text{ mJ/cm}^2$  and  $F = 25 \text{ mJ/cm}^2$  were used. The irradiation of the samples was performed using 2, 3 and 10 laser pulses. The potential anti-tumor effect in vitro of GNPs and laser treatment on HeLa cells were studied by MTT assay. Cells treated with GNPs or laser irradiation alone showed no significant difference from the control. The combination of GNPs and laser treatment showed cytotoxic effect when cells were incubated with GNPs (100 nm), irradiated with 3 pulses and  $F = 80 \text{ mJ/cm}^2$ . Lower results were obtained with GNPs with the same size and irradiation with 2 and 10 pulses and  $F = 25 \text{ mJ/cm}^2$ ; while in cells treated with GNPs (40 nm), the best effect was observed after irradiation with 2 pulses and  $F = 25 \text{ mJ/cm}^2$ . Data obtained for irradiation with 3 pulses and  $F = 80 \text{ mJ/cm}^2$  and 10 pulses and  $F = 25 \text{ mJ/cm}^2$  were almost the same. Induction of apoptosis in treated with GNPs and irradiated cells were established using acridine orange and propidium iodide double staining method. Well defined morphological features of apoptosis were observed in order to confirm the results from MTT cytotoxicity assay. Based on the results obtained the combination of GNPs (both sizes) and laser treatment with different characteristics of the laser ray resulted in localized heating and causing irreversible thermal cellular destruction. This approach could have a potential application in clinical practice for local treatment of tumors.

**Keywords :** gold nanoparticles; Hela cell line; MTT assay.

## **IMPACT OF STREPTOZOTOCIN-INDUCED DIABETES MELLITUS ON TESTIS CELL POPULATIONS IN DEVELOPING RAT**

PAVLOVA, E., PETROVA, E., GLUHCHEVA, Y., VLADOV, I., ZAPRYANOVA, S., RASHEV, P., LAKOVA, E., ATANASSOVA, N.

### **Abstract**

Impaired glucose metabolism can lead to a variety of complications affecting reproductive function. The study aimed to evaluate the impact of hyperglycemia in early life on the number of germ cells (GCs), expression of marker proteins of Sertoli (SCs) and Leydig cells (LCs) in tandem with serum testosterone (T) levels in developing rat. To induce diabetes mellitus pups were injected i.p. with 100 mg/kg b.w. streptozotocin on day 1 (neonatally, NDM) or day 10 (prepubertally, PDM). Testes were sampled on day 25. 5  $\mu$ m paraffin sections were used for ABC-HRP immunohistochemistry for androgen receptor (AR) and 3 $\beta$ -Hydroxysteroid dehydrogenase (3 $\beta$ -HSD). Serum T was assayed by ELISA. Stereological techniques were used to determine the nuclear volume of GCs and SCs per testis. Diabetic status of animals was confirmed by increased blood glucose levels, more pronounced in PDM compared to NDM. AR was localised in peritubular, LCs and SCs of the testis with stage-specific expression in SCs even on day 25. Immunohistochemical investigation of AR shows weaker intensity and uniform model of receptor expression in SCs of PDM but not in NDM. These data corresponded to reduced serum T levels in PDM (64% lower) and NDM (40% lower) and weaker 3 $\beta$ -HSD expression in steroid producing LCs. Spermatogenesis in PDM was delayed and manifested by reduced total GC population and in particular mitotic spermatogonia (40% lower) and meiotic spermatocytes (12% lower). The marker for supporting function of SCs (ratio total GCs/SCs) was significantly suppressed. In conclusion PDM affected androgen production and action in the pubertal testis and data suggest different sensitivity of GCs to hyperglycemia.

**Keywords :** streptozotocin; induced diabetes mellitus; testis cell populations.



## **ALTERATIONS IN SPERMATOGENESIS FOLLOWING CHRONIC EXPOSURE TO COBALT CHLORIDE**

PAVLOVA, E., MIHAYLOVA, V., TINKOV, A., PETROVA, E., VLADOV, I., SKALNY, A., GLUHCHEVA, Y.

### **Abstract**

Cobalt (Co) is an endocrine disruptor and a hypoxiamimicking agent. Its effect on spermatogenesis after prolonged administration remains unclear. The aim of the study was to assess chronic cobalt treatment on spermatogenesis. Pregnant mice were exposed to 75 mg (low dose) and/or 125 mg (high dose)/kg b.w. cobalt chloride ( $\text{CoCl}_2 \times 6\text{H}_2\text{O}$ ) with drinking water for 3 days before delivery and treatment continued until postnatal day 90. Age-matched control animals obtained regular tap water. At the end of the treatment, testes of control and Co-treated mice were excised and processed for histological and ICPMS analysis for metal content. Spermatozoa were isolated from both ductus deference and counted. Results showed a significant dose-dependent reduction in testicular index (testis weight to body weight ratio), up to 60% after treatment with 125 mg/kg and up to 34% after treatment with 75 mg/kg. Morphology of the testis was considerably affected in both doses of  $\text{CoCl}_2$  although in some tubules spermatogenesis was normal and completed. Diameter of many seminiferous tubules was significantly reduced and there was lack of lumen formation. We observed disorganization of seminiferous epithelium, islet of undifferentiated germ cells in luminal region of the tubules and presence of multinuclear germ cells. These findings corresponded to the significant reduction of sperm count by 2.15-fold for the low dose and 3.35-fold for the high dose. Chronic  $\text{CoCl}_2$  administration resulted in accumulation of the metal ions in the testes of the treated mice. The considerable impact of Co on male reproduction after chronic exposure shows negative correlation between metal concentration and male fertility.

**Keywords :** cobalt chloride, testicular index; spermatogenesis.