EFFECT OF ZINC-COPPER HYDROCHLORIDE MIXED CRYSTALS APPLICATION IN RABBITS WITH ACUTE EXPERIMENTALLY INDUCED FASCIOLOSIS

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ABSTRACT
Effects of zinc (Zn) – copper (Cu) hydroxochloride mixed crystals were tested on body weights, body weight gain, mortality, liver vitamins A, C, E, trace elements Zn and Cu and enzyme Cu-Zn superoxide dismutase. Investigated parameters were not changed significantly compared to controls in Zn-Cu hydroxochloride mixed crystals supplemented rabbits. Body weights and body weight gain were reduced, mortality was increased and liver biochemical parameters were decreased in F. hepatica infected animals. All affected parameters were increased near to controls after Zn-Cu hydroxochloride mixed crystals application in infected rabbits, so they may be used for restoration of disturbances from fasciolosis.

Key words: zinc-copper hydrochloride mixed crystals, fasciolosis.

Introduction
Fasciolosis is widespread in animals and men plant-borne zoonotic helminth disease with considerable economic and health importance. Compared with the other helminthoses, this food borne trematodosis is often neglected as a serious problem for animal health.

Trace element imbalance and oxidative stress develop during many parasitoses including fasciolosis (Gabrashanska et al., 1993; 2005; 2008 a, b; Dede et al., 2000; 2002). Different mineral compounds (neutral and basic) are used for treatment of biochemical and metabolic disturbances appeared under the parasitic infections (Gabrashanska et al., 1993; 2005; 2008 a; b; Galvez Morros et al., 1995; Tsocheva-Gaitandjieva et al., 2002). Neutral salts are more often used but their longer application can cause some negative adverse effects in the body. Recently hydroxi (basic) salts of 3d transition elements are tested for restoration of the disturbed elemental deficiencies in infected with helminths animals and they are better tolerated by the body than neutral salts (Gabrashanska et al. 1993, 2005; 2008 a, b; Galvez-Morros et al., 1995, Tsocheva-Gaitandjieva et al., 2002).

The present study aims to examine the effect of mixed zinc (Zn) and copper (Cu) hydroxochloride crystals: (Cu_{0.78}Zn_{0.22})_2(OH)_3Cl on live performance and antioxidant abilities of experimentally F. hepatica infected rabbits in acute stage of parasitic disease, by estimating animal body weights, body weight gain, mortality, levels of liver vitamins A, C, E; liver trace elements Zn and Cu and liver enzyme Cu-Zn superoxide dismutase (CuZn-SOD) activity.

Materials and methods
The experiments were performed on thirty 32-days old male Chinchilla rabbits, divided into 4 groups: Group I – healthy animals (controls), Group II – rabbits treated with Zn-Cu hydroxochloride mixed crystals, Group III – rabbits experimentally infected with F. hepatica and Group IV – F. hepatica infected and Zn-Cu hydroxochloride mixed crystals supplemented animals.
Mixed crystals ($\text{Cu}_{0.78}\text{Zn}_{0.22})(\text{OH})_3\text{Cl}$ were synthesized by the method of continuous co-precipitation under standard conditions with a pH = 7 (Markov, 1987). The mixed crystals were highly soluble in mineral acids but not in water and their composition was determined by chemical X-ray and thermal analyses. The supplemented amounts of Zn and Cu were applied according the pharmacological doses used for young rabbits.

The rabbits were orally infected with 60 metacercariae of *F. hepatica*. The treatment with Zn-Cu hydroxochloride mixed crystals was initiated on day 14 post-infection (p.i.) in acute phase of fasciolosis. Salt was applied per os (with food) at a dose of 0.020 g per rabbit for 7 days. The animals were fed on a basal semi synthetic diet for young rabbits and were euthanized by anesthesia on day 60 p.i. The rabbit livers were collected for the further biochemical investigations. All procedures with the animals 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.

Rabbit body weights, body weight gain, mortality, liver vitamins A, C, E contents, liver transitional elements Zn and Cu levels and liver enzyme CuZn-superoxide dismutase (CuZn-SOD, E.C. 1.15.1.1) activity were investigated. Rabbit body weights, body weight gain and mortality were established at the end of experiments. The levels of rabbit liver vitamins A, C and E were determined by HPLC methods using fluorescent detector or spectrophotometer (Bieri et al., 1979; Omaye et al., 1979). Liver trace elements Zn and Cu contents were established by atomic absorption spectrophotometer (Varian Techtran, Model AA 220) and were expressed as µg per g of dry liver tissue (Anonymous, 1982). Liver CuZn-SOD activity was determined by the method of Beauchamp and Fridovich (1971) (U/mg protein). Analysis of variance and Student's t-test of the results were done. Values of P<0.05 were considered statistically significant.

**Results and discussion**

Effects of Zn-Cu hydroxochloride mixed crystals are tested on body weights, body weight gain, mortality, levels of liver vitamins A, C, E; liver trace elements Zn and Cu and liver enzyme Cu-Zn superoxide dismutase (CuZn-SOD) activity in experimentally *F. hepatica* infected rabbits. The results are presented on Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Contents of liver vitamins C and E after acute <em>Fasciola hepatica</em> infection and Zn-Cu hydroxochloride mixed crystals application in experimental rabbits (nmol/g)</th>
<th>Rabbit liver trace element contents (µg/g dry tissue)</th>
<th>Body weights at the end of experiment (g)</th>
<th>Body weight gain (g)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.56±0.99 15.8±2.15 5.3±1.07 229.1±0.29 34.2±1.15 3080 2137 20</td>
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<tr>
<td>2</td>
<td>4.30±1.01 16.1±2.77 6.8±1.58 307.1±3.36 41.5±1.92 3119 2174 0</td>
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<tr>
<td>3</td>
<td>2.36±0.85 12.6±3.97 2.1±0.97 174.6±2.38 22.6±0.59 2300 1355 60</td>
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<tr>
<td>4</td>
<td>2.44±0.96 13.1±4.11 3.5±1.46 196.9±8.51 32.3±1.59 2614 1669 20</td>
<td></td>
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</tbody>
</table>
Body weights, body weight gain, levels of liver vitamins A, C, E; trace elements Zn and Cu and activity of liver enzyme CuZn-SOD in the treated with Zn-Cu hydroxochloride mixed crystals rabbits are slightly increased but not significantly changed compared to the controls. Mortality of the animals is not affected (Table 1, Figure 1).

Body weights and body weight gain in *F. hepatica* infected rabbits are reduced and mortality is increased. The levels of liver vitamins A, C, E; contents of liver trace elements Zn and Cu and activity of liver enzyme CuZn-SOD are decreased (Table 1, Figure 1). Rabbit liver hypovitaminoses A, C and E, trace element imbalance and alterations of enzyme CuZn-SOD activity are developed as a result of the oxidative stress and antioxidant imbalance due to the acute fasciolosis (Gabrashanska et al., 1993; 2005; 2008 a; b). Decreased activities of some liver enzymes (SOD, GSH-Px, GSSG-R and CAT) are established in acute phase of fasciolosis in rats (Kolodziejczyk et al., 2004; 2006). The reduced CuZn-SOD activity in the livers of *F. hepatica* infected rabbits may be due to the inactivation of the enzyme by interaction with oxygen radicals. Some authors are reported for fall of SOD activity by the action of hydroxyl radicals and H$_2$O$_2$ (Retsky & Frei, 1995). It may be a result of injury of the enzyme by the superoxide radical. It is established that vitamin C enhances antioxidant ability of vitamin E by reducing the tocopheroxyl radicals back to their active form of vitamin E (Halliwell & Gutteridge, 1995) or sparing available vitamin E (Jacobs, 1995). Regarding to their antioxidant properties vitamins A, C and E have a synergistic effect on the immune response (Barja et al, 1996). Vitamin A deficiency in the livers of *F. hepatica* infected rabbits is developed probably because the storage of vitamin A in affected liver is disturbed. Similar antioxidant imbalances with reduced levels of vitamins-antioxidants C, E, A, carotene, retinal and glutathione are determined in rats, goats or sheep infected with *Fasciola* spp. (Gameel, 1982; Barja et al., 1996; Dede et al., 2000; 2002; Gabrashanska et al., 2005; 2008 a; b). The trace elements-antioxidants are in the interrelationships with the vitamins-antioxidants because they affect and
control redox metabolism (Evans & Halliwell, 2001). Based on the above results we could estimate that trace elements Cu and Zn, as well as vitamins C and E plays an important role in the antioxidant status under fasciolosis.

A beneficiation and normalization of liver antioxidant status is established in *F. hepatica* infected and treated with Zn-Cu hydroxochloride mixed crystals rabbits compared to that of only *F. hepatica* infected animals. Supplementation with Zn-Cu hydroxochloride mixed crystals influences a restoration of affected after acute fasciolosis liver antioxidant defence system in experimental rabbits. The application of Zn-Cu hydroxochloride mixed crystals on the infected with *F. hepatica* rabbits is not affecting but beneficing the live performance of the animals. Body weights and body weight gain of the rabbits supplemented with Zn-Cu hydroxochloride mixed crystals after acute *F. hepatica* infection are slightly decreased compared to the controls but increased in comparison with that in only *F. hepatica* infected animals (Table 1; Figure 1). Rabbit mortality is kept near to the control. Decreased under acute fasciolosis levels of liver vitamins A, C and E; trace elements Zn and Cu and fold activity of liver enzyme CuZn-SOD were increased near to the control values (Table 1, Figure 1).

There are not a lot of data about application of basic salts of biogenic metals in parasitology. Data exist about the beneficial effects of basic salts on body weights, mortality and parasitic burden of *Ascaridia galli* infected chickens (Gabrashanska et al., 1993). Effects of restoration of the decreased contents of liver trace elements Zn and Cu was established after application of Zn-Cu basic salt in rats with experimental fasciolosis (Tsocheva-Gaytandjieva et al., 2002).

The presented results clearly show imbalances and decreasing of levels/activities of investigated rabbit liver nonenzymatic and enzymatic antioxidants in acute stage of fasciolosis. Antioxidant-oxidant imbalance is developed in the infected hosts. The investigated Zn-Cu basic salt can be used to correct the antioxidant imbalance due to fasciolosis. Our results are in a good agreement with the literature data. The supplementations with other chemical compounds containing Zn, Co and Mn show positive effects and almost normalize the the antioxidant imbalance due to *F. hepatica* infection in experimental rabbits.

**Conclusion**

Our present study was oriented toward searching of new sources for prophylactic and therapeutic application causing increasing of rabbits productivity and improving their live performances. Supplementation with pharmacological doses of the here investigated and previously synthesized Zn-Cu hydroxochloride mixed crystals could be usefully applied for correction and restoration of the parameters of affected after fasciolosis live performance and antioxidant defence system in *Fasciola hepatica* infected animals.

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