

## CHEMICAL ELEMENTS IN FASCIOLA HEPATICA (TREMATODA) AFTER DIETHYLNITROSAMINE TREATMENT

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### ABSTRACT

The aim of the work was to be investigated the trace elements content in *Fasciola hepatica* under the toxic effect of diethylnitrosamine (DENA). The study of the trace elements in the injured from DENA helminthes showed specifically changed picture. The levels of Zn and Fe were heavily decreased but Rb – slightly reduced. The contents of Cu, Co, Br, Mo and Se were similar to those of the intact helminthes. The content of Cr was increased. We supposed that the morphological changes of *F. hepatica* on the background of DENA intoxication lead to a mineral imbalance related to the pathophysiology of the helminthes.

**Key words:** Helminthes tissues, chemical intoxication, trace elements.

### Introduction

The parasitic way of life of the helminthes leads to specific biochemical adaptations to the host tissues in which they develop and inhabit (Tsocheva et al., 1992; Breshahen & Tanumihardjos, 2014). As *Fasciola hepatica* (Trematoda) inhabits the liver and the bile ducts of the host, the trace elements content of *F. hepatica* infected liver and the trace elements content of the tissues of *F. hepatica* have been investigated and compared (Gabrashanska & Damyanova, 1987). The results for the trace elements content in the host liver and the helminth's tissues are similar. Significant differences are established only in the zinc content which is less in the tissues of *F. hepatica* than in the liver tissue.

The aim of the present study is to exam the trace elements status of the tissues of mature helminthes *F. hepatica* under the toxic action of diethylnitrosamine (DENA) on *F. hepatica* infected host.

### Materials and Methods

The experiments were carried out on 12 male albino Wistar rats aged 30 days, divided as follows: Group I – *F. hepatica* infected animals – 6; Group II – animals infected with *F. hepatica* and treated with DENA – 6.

The rats were infected per os on the 1st day of the experiment with 15 methacercariae of *F. hepatica*. DENA was injected intraperitoneally 4 times at 7-day intervals at a dose of 100 mg/ kg body weight from the 6th week p. i. The animals were sacrificed on the 10th week of the experiment. The mature helminths *F. hepatica* were collected and investigated.

The experiment was 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 trace element content in the helminth tissues was determined by a non-destructive method of neutron activation analysis (Gabrashanska & Damyanova, 1987). The contents of zinc (Zn), iron (Fe), copper (Cu), cobalt (Co), molybdenum (Mo), chrome (Cr), selenium (Se), rubidium (Rb) and

bromine (Br) were determined in mature *F. hepatica*. The results were statistically processed after variation analysis and Student's t-test.

## Results and Discussion

The results are presented in Figures 1, 2 and 3.

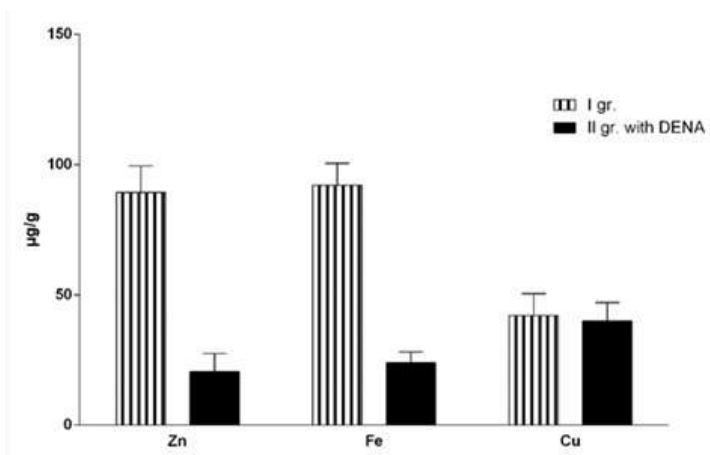


Figure 1: Content of Zn, Fe and Cu in tissue of *F. hepatica* after *F. hepatica* infection and DENA treatment.

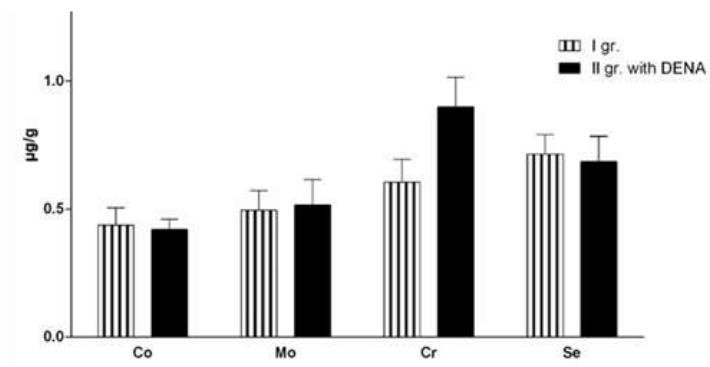


Figure 2: Content of Co, Mo, Cr and Se in tissue of *F. hepatica* after *F. hepatica* infection and DENA treatment

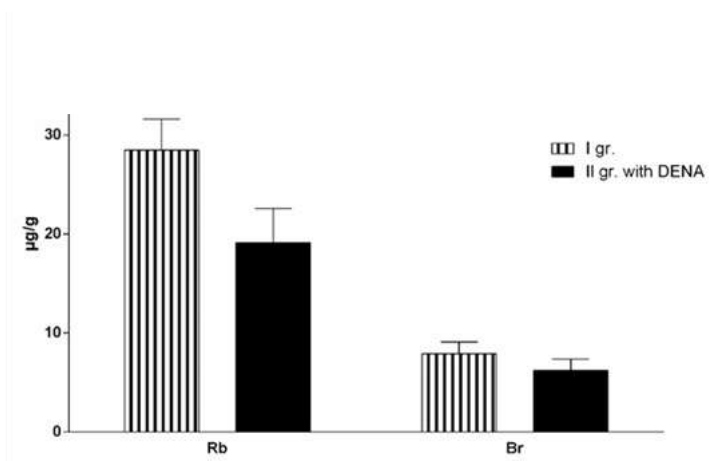


Figure 3: Content of Rb and Br in tissue of *F. hepatica* after *F. hepatica* infection and DENA treatment.

The values of the quantitatively established trace elements in the tissues of intact helminths *F. hepatica* (Group I) are used as controls.

The study of the trace elements status in the tissues of intact *F. hepatica* (Group I) shows the following quantitative sequence:

$\text{Fe} > \text{Zn} > \text{Cu} > \text{Rb} > \text{Br} > \text{Se} > \text{Cr} > \text{Mo} > \text{Co}$ .

The study of the trace elements spectrum in the tissues of injured from DENA helminths *F. hepatica* (Group II) shows specifically changed picture. The levels of Zn and Fe are heavily decreased ( $P < 0.001$ ) (Fig. 1). The Rb content is slightly reduced ( $P < 0.05$ ) (Fig. 3). The contents of Cu, Co, Br, Mo and Se are similar to that of the controls (Group I) ( $P > 0.05$ ) (Fig. 2 and Fig. 3). The content of Cr is increased ( $P < 0.05$ ) (Fig. 2). The quantitative sequence of the trace elements content in Group II is the following:

$\text{Cu} > \text{Fe} > \text{Zn} > \text{Rb} > \text{Br} > \text{Cr} > \text{Se} > \text{Mo} > \text{Co}$ .

The results of the trace elements content in *F. hepatica* isolated from DENA treated rats (Group II) show significant changes in the quantity of Fe and Zn (Fig.1). The strong decrease of Fe and Zn contents exerts influence of on the cell antioxidants-oxidant processes and on the permeability of the cell membranes which are significantly injured in the parasites (Gajewska et al., 2005; Gabrashanska et al., 2008). Similar decrease of the quantity of these two biogenic elements is established in DENA-treated host liver investigation too (Tsocheva-Gaytandzhieva et al., 2016). The quantitative changes for the trace elements Cu, Co, Cr, Mo, Se, Br and Rb are different in the DENA-treated host and the injured helminth tissues which are investigated (Tsocheva-Gaytandzhieva et al., 2016). The alterations in the trace elements content caused from DENA correlate with the structural changes caused from DENA both in the tissues of the helminth *F. hepatica* and the host tissues. The data propose an interesting discussion on the results from the electron microscopic investigation of the tegument structure of the DENA-injured *F. hepatica* (Gorchilova et al., 1986). A decrease of the ATP-ase activity in the tegument of injured from DENA *F. hepatica* is demonstrated enzymocytochemically (Gorchilova et al., 1986). As the Co takes part in the cell synthesis of phospholipids, it can be assumed that these enzymocytochemical data correlate with the data from the present investigation for the decreased content of Co in the DENA-treated *F. hepatica*. The Co takes part in the

regulation of the cellular oxidant-antioxidant processes and its decrease is in the directly proportional dependence from the decreased quantity of Fe in the investigated helminth tissues (Miller & Britigan, 1997).

The increased quantity of Cr in the tissues of DENA-treated *F. hepatica* is an interesting result. It is known that Cr takes part in the carbohydrate metabolism of the cell (Evans & Halliwell, 2001). Stoitsova et al. (1992) investigate the changes in the glycocalix structure of injured with DENA *F. hepatica* by ruthenium red staining which gives indirect data for the injured synthesis of carbohydrates in the glycocalix of these helminthes. These structural-functional changes might be in connection with the changed quantity of Cr in the tissues of DENA-injured helminthes which has been established.

## Conclusions

The results we obtained give grounds for the assumption that the concrete ultrastructural changes in *F. hepatica* on the background of DENA intoxication lead to a mineral imbalance related to the pathophysiology of the helminthes.

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