



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

V. NANEV, I. VLADOV & M. GABRASHANSKA

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

Summary

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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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and group 4 – rats experimentally infected with *F. hepatica* and treated with $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Rats from group 2 and 4 were orally infected with 15 viable *F. hepatica* metacercariae per animal. Rats from group 3 and 4 received $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ 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

INTRODUCTION

Heavy metals are non-degradable environmental pollutants that can negatively affect the human and animal health. Copper (Cu) is an essential trace element and an integral component of many enzymes and proteins. Excess copper, however, is highly toxic leading to abnormal activity of antioxidant enzymes in liver tissue as reported in an experimental rat model of copper toxicity (Pal *et al.*, 2013). Fasciolosis is an economically important helminthic disease, caused by two trematode species: *Fasciola hepatica* L. 1758 and *Fasciola gigantica* Cobbold, 1855. Fasciolosis is a widespread parasitosis affecting small mammals as well.

Under environmental conditions organisms are exposed not only to parasites but also to a variety of other endogenous and exogenous factors. It is known that parasites strongly interact with pollutant-induced biomarker responses of their hosts by influencing their physiology in a multitude of different ways (Marcogliese & Retrock, 2011). Little attention has been paid to how parasites and pollutants affect the defense system of their animal hosts. *Fasciola hepatica* infection is frequently encountered in small terrestrial mammals in the heavy metal polluted environment. That is why we used a host-parasite system Wistar rat/*Fasciola hepatica* in our study.

The aim of the study was to investigate liver antioxidative/oxidative status in rats infected experimentally with *F. hepatica* and/or chronic copper intoxication.

MATERIALS AND METHODS

Experimental design

The experiment was carried out on 24 male Wistar albino rats aged 30 days,

weighing about 150 g. The rats were divided into 4 groups (6 animals in each). Group 1 comprised healthy untreated animals. Every rat from group 2 was orally infected with 15 viable *F. hepatica* metacercariae suspended in dechlorinated water and passed through a stomach tube on the 1st day of the experiment. Metacercariae were obtained from experimentally cultivated snails *Galba truncatula* after experimental infection with 5 miracidiae per snail, hatched from eggs of mature *F. hepatica* L. The freshwater snails from family Lymnaeidae *G. truncatula* are intermediate hosts of *F. hepatica* L. Animals from group 3 were treated orally with 150 mg/kg copper (II) sulfate pentahydrate (CuSO₄·5H₂O) dissolved in drinking water after the 2nd week of infestation. Copper administration lasted 2 weeks. The rats from group 4 were experimentally infected with *F. hepatica* and treated with copper as described above.

The experiments were conducted in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Specific Purposes and the current Bulgarian laws and regulations. The rats were euthanised by anaesthesia on the 35th day post infection. The livers were collected for biochemical investigations.

Biochemical studies

Part of liver samples were weighed and homogenised in 0.15 M NaCl for assay of oxidative stress parameters. Another part was used for copper content analysis. Homogenates of 29% were obtained and were centrifuged at 500 rpm for 10 min and at 12,500 rpm for 15 min, respectively. The supernatants were used for analysis. The assayed parameters were

expressed per milligram of protein. Protein was determined by the method of Lowry *et al.* (1951).

Glutathione (GSH) level was measured according to Beutler *et al.* (1963), using metaphosphoric acid for protein precipitation and 5',5-dithiobis (2-nitrobenzoic acid) for colour development. Cu, Zn-superoxide dismutase (Cu,Zn-SOD) activity was established according the method of Misra & Fridovich (1972). The assay involves inhibition of nitro blue tetrazolium reduction by the superoxide anion. Glutathione peroxidase activity (GSH-Px) was measured spectrophotometrically according to Paglia & Valentine (1967), in which GPH-Px catalyses the oxidation of GSH by hydrogen peroxide. Malondyaldehyde (MDA) content, a product of lipid peroxidation product, was measured by thiobarbituric acid reactive substances assay according to Londero & Lo Greco (1996).

Copper liver content was determined by flame atomic absorption spectrophotometry (PU-900 spectrometer, Cambridge, UK). The samples were burned in a muffle furnace and ashes were treated with a mixture of H₂SO₄ and HNO₃. Wet residues were dissolved in 1 mL HCL.

Copper content was expressed as µg per g of dry liver tissue.

Statistical analysis

It was carried out with the Prism 4.0 software. The distribution of data (a Gaussian one) was determined using the test of Kolmogorov-Smirnov and D'Agostino-Pearson. In the Grubb's test, no extreme values were found. The mean values, the standard deviations (SD) and the significance criterions (P) of four groups were determined and compared using the Student's *t*-criterion, Bonferroni's multiple comparison test. Analysis of variance and Student's *t*-test were used for statistical processing of the results.

RESULTS

The liver copper content (Fig. 1) was significantly higher in Cu-exposed animals from groups 3 and 4. It was reduced in infected only rats.

Enhancement of lipid peroxidation (LPO) in infected animals was noted – 2.5 fold increase in liver MDA compared to the control group (Fig. 2). MDA level was twice higher in the group supple-

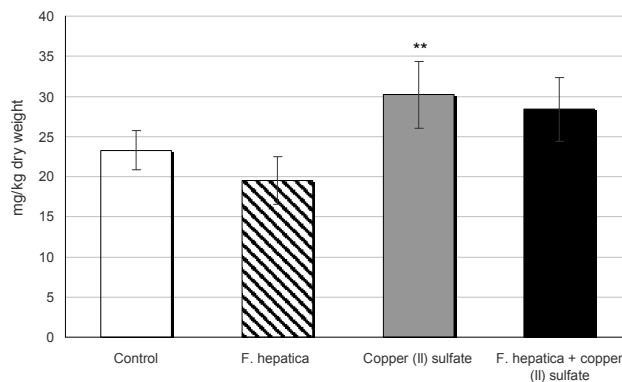


Fig. 1. Liver copper content (mg/kg dry weight) in control rats, rats infected with *F. hepatica* only, rats treated with copper (II) sulfate only and rats infected with *F. hepatica* and treated with copper (II) sulfate. Values are presented as mean ± SD (n=6); ** P<0.05 vs control group.

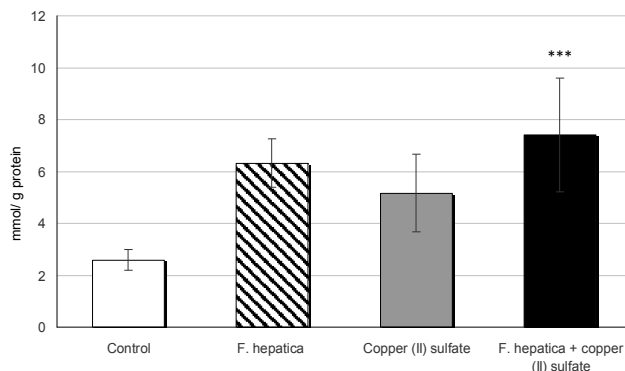


Fig. 2. Liver malondialdehyde content (mmol/mg protein) in control rats, rats infected with *F. hepatica* only, rats treated with copper (II) sulfate only and rats infected with *F. hepatica* and treated with copper (II) sulfate. Values are presented as mean \pm SD (n=6); *** P<0.001 vs control group.

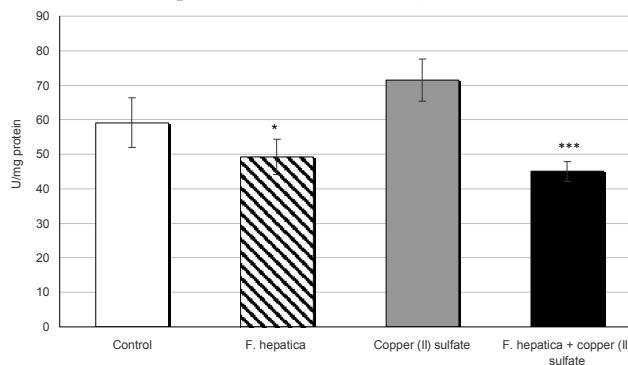


Fig. 3. Liver Cu,Zn-superoxide dismutase activity (U/mg protein) in control rats, rats infected with *F. hepatica* only, rats treated with copper (II) sulfate only and rats infected with *F. hepatica* and treated with copper (II) sulfate. Values are presented as mean \pm SD (n=6); * p<0.05; *** P<0.001 vs control group.

mented with Cu in relation to the controls. MDA content in group 4 exceeded that of all other groups and was 3 times higher vs controls (P<0.05).

The activity of antioxidant enzymes in groups 2, 3 and 4 was changed as compared to the controls. The activity of Cu,Zn-SOD in group 2 (rats infected with *F. hepatica*) was significantly (P<0.05) reduced compared to the control one (Fig. 3). The reduction in Cu,Zn-SOD activity in group 4 was similar to that in group 2. Increased Cu, Zn-SOD activity was ob-

served in group 3 compared to all other groups. The observed reduction in GPx activity was similar in all treated groups.

The GSH content (Fig. 5) was reduced in all treated groups, but the reduction in group 4 was at the highest extent.

DISCUSSION

A lot of studies demonstrated that in rats experimentally infected with *F. hepatica* a high production of reactive oxygen species was developed causing oxidative

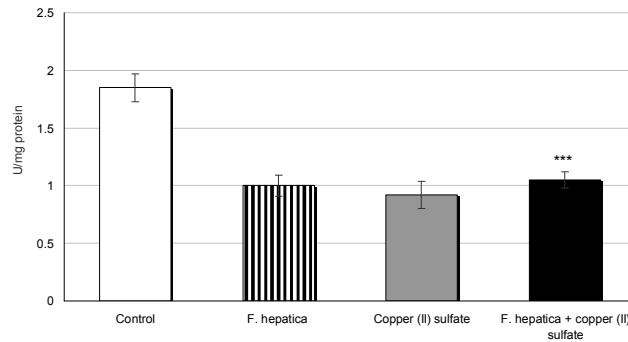


Fig. 4. Liver glutathione peroxidase activity (U/mg protein) in control rats, rats infected with *F. hepatica* only, rats treated with copper (II) sulfate only and rats infected with *F. hepatica* and treated with copper (II) sulfate. Values are presented as mean \pm SD (n=6); * p<0.05; *** P<0.001 vs control.

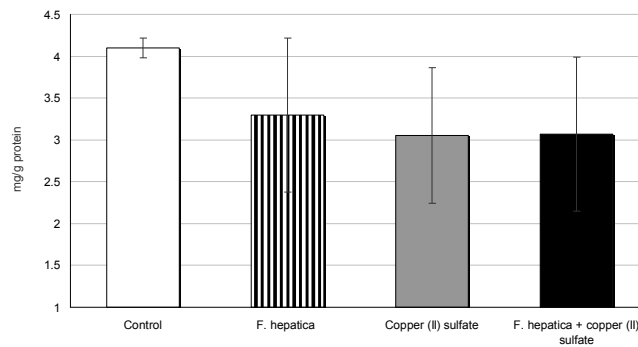


Fig. 5. Liver glutathione content (mg/g protein) in control rats, rats infected with *F. hepatica* only, rats treated with copper (II) sulfate only and rats infected with *F. hepatica* and treated with copper (II) sulfate. Values are presented as mean \pm SD (n=6).

stress (Kolodziejczyk *et al.*, 1995; Anisimova *et al.*, 2007; Gabrashanska *et al.*, 2008). Enhanced lipid peroxidation and disturbed antioxidative status were observed in infected rats. Oxidative cell injury may occur in the course of fasciolosis. Rat experimental studies focused on the combined effect of heavy metals and parasites on the host antioxidative defense system are very important for understanding the pathogenesis of parasitic infections.

In our experiment, rats infected with *F. hepatica* and treated with Cu showed deviations in antioxidative/oxidative status compared with those in non-intoxi-

cated rats. The rats infected only with *F. hepatica* (group 2) developed antioxidative disturbances manifested by reduced activity of the main antioxidant enzymes such as Cu,Zn-SOD, GPx and reduced level of GSH. It was shown that the infection with *F. hepatica* was accompanied by increased level of the superoxide radical (Zhang *et al.*, 2000). GPx together with GSH reduce peroxides so that the content of GSH is significantly decreased during fasciolosis. It could be attributed to enhanced oxidation of GSH into glutathione disulphide catalysed by free radicals. The loss of GSH may compromise cellular antioxidant defence and lead to the higher

production of ROS. GPx and GSH represent a major pathway for metabolising hydrogen peroxide and lipid peroxides. Enhancement of lipid peroxidation was observed, as shown by MDA at post infection day 35. Our findings showed that reduced antioxidative status in the rat liver and enhanced generation of ROS were most pronounced during acute stage of fasciolosis.

Oxidative and antioxidative parameters were influenced after Cu exposure. Free radical damage after Cu administration was demonstrated by increased MDA level. Activity of Cu, Zn-SOD was increased in the Cu-treated animals compared to the controls. Cu,Zn-SOD activity may increase as a result of the response against an oxidative challenge. It could be a part of preparation for the intense metabolic activity (Halliwell & Gutteridge, 1999).

GSH is an essential intracellular reducing agent for the maintenance of thiol groups on intracellular proteins and for antioxidant molecules. GSH depletion occurs as a result of excessive GSH consumption during oxidative stress (Facino *et al.*, 1993). Hepatocyte GSH protect the cells against Cu because GSH-depleted cells are vulnerable to Cu toxicity. A small excess of copper is toxic for the organism. It increases lipid peroxidation (LPO) and depletes GSH reserves, making the organism more vulnerable to other oxidative challenges.

The combined effect of chronic Cu administration and experimental *F. hepatica* infection changed the liver MDA level, the activity of antioxidant enzymes and the GSH content in the present rat model. Oxidative stress occurred due to *F. hepatica* infection and/or Cu administration. Protective antioxidative response against combined treatment with the heavy metal and *F. hepatica* infection was

not significantly increased compared to that in only infected/ or only Cu-intoxicated rats. In our case the dose of administered copper did not increase Cu liver content very significantly because it was a moderate one and not high enough to accumulate in very high amounts in the liver. Furthermore, adaptive changes favouring antioxidative defence were involved (Ozcelik *et al.*, 2003; Jomova & Valko, 2011).

Different impact of contaminants on parasites and their hosts affects the dynamics of the host-parasite relationship. Endoparasites live within their hosts and environmental effects usually manifest first at the host level subsequently can be detected at the parasite level.

The greater induction of lipid peroxidation in group 4 was due to Cu exposure and *F. hepatica* infection. The effects of combined stress on rats shows the important role of MDA. Parasites and toxicants act together to exacerbate the oxidative stress. Parasitism in the presence of pollution may further compromise the health of the host, even at low intensities and lead to disease and parasitism (Kaya, 2007).

Our results show that lipid peroxidation can be used to measure the degree of pathogenicity exerted by parasites and pollutants. The combination of heavy metal pollution and parasitism may act individually or synergistically on systemic health. Measurements of oxidative stress status can be effective means of detecting pathological effects of various endoparasites and chronic intoxications where typical pathology is not manifested.

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Correspondence:

Veselin Nanev
Institute of Experimental Morphology,
Pathology and Anthropology with Museum,
Bulgarian Academy of Sciences,
Acad. G. Bonchev Str Bl. 25,
1113 Sofia, Bulgaria
e-mail: veselinnanev@gmail.com