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# PLEUROTUS OSTREATUS (OYSTER MUSHROOM), A COMMONLY USED DIETARY SUPPLEMENT, MAY INCREASE THE ACUTE TOXIC EFFECTS OF HEXAVALENT CHROMIUM IN RATS

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Funghi are important resources of biologically active substances (1). Pleurotus ostreatus (oyster mushroom) is an edible mushroom. Pleurotus ostreatus and its main active substance, the polysaccharide pleuran (β-1,3/1,6 glucan, a major component of cell walls), are widely used as immunomodulatory nutritional supplements (2, 3). Pleurotus ostreatus and pleuran have been shown not only to enhance the defence against infections, but also to lower cholesterol, to have antitumor, anticoagulant, antiinflammatory, antinociceptive and hepatoprotective properties (4–9). Recently, the research has focused on their antioxidant properties (10–13). Administration of β-glucan or Pleurotus ostreatus was effective in decreasing oxidative stress in various organs (liver, kidneys, heart, brain) in rats intoxicated with carbon tetrachloride and acetaminophen and in the liver of mice intoxicated with thioacetamide (14–17).

Chromium (Cr) is a naturally occurring metal that exists in many oxidation states, but the trivalent form – Cr(III) and the hexavalent form – Cr(VI) occur most frequently in the environment (18). While Cr(III) is an essential nutrient involved in glucose and lipid metabolism and improving insulin sensitivity by enhancing intracellular signalling (19–22), Cr(VI) is a toxic and carcinogenic pollutant (23). Occupational exposure and Cr(VI) and contamination of the environment is a result of an extensive use of hexavalent chromium compounds in diverse industries (stainless steel production, chrome plating, leather tanning, manufacturing of colour pigments, wood treatment) (24, 25).

Reactive radical species play an important role in the pathogenesis of Cr(VI) toxicity. Cr(VI) readily enters cells and undergoes an intracellular reduction to Cr(III), during which reactive Cr intermediates Cr(V) and/or Cr(IV), as well as superoxide, hydrogen peroxide, and hydroxyl radical are generated (26–29).

The present study was designed to investigate the antioxidant and protective effects of Pleurotus ostreatus in acute intoxication with hexavalent chromium in the liver and kidneys of rats.

## Chemicals

Commercially available preparation of finely powdered Pleurotus ostreatus was used in the experiment (Terezia Company). Potassium dichromate ($K_2Cr_2O_7$) was purchased from Lachema Brno. Commercial kits for the determination of serum activities of ALT, AST and GLDH were purchased from DiaSys Diagnostic System, Germany. All other reagents were of analytical grade purity.

## Animals and treatment

Male Wistar rats, weighing (180–200 g Anlab Prague, CZ) were housed at standard laboratory conditions (temperature 22 ± 2 °C, relative humidity 50–60%, 12-h light–dark cycles). The animals had free access to standard pellet diet and drinking water. After 5 days of adaptation, the rats were randomly assigned to three groups of eight rats each: group I – the control, group II – Cr(VI) group, group III – Cr(VI) + Pleurotus.

Animals were treated as follows (tab. 1): Group III: Finely powdered Pleurotus ostreatus was administered via gavage once daily (500 mg/kg body weight/day as a suspension in distilled water) for 10 consecutive days. Groups I and II received distilled water. A single dose of $K_2Cr_2O_7$ was administered intraperitoneally (40 mg/kg body weight, dissolved in saline) to the animals in groups II and III on the 10th day of the experiment. Rats in group I were given saline alone.

**Tab. 1** The design of the experiment

| Group | | Treatment | Days 1–9 | Day 10 | Day 11 |
|---|---|---|---|---|---|
| **I** | **control** | distilled water p.o. | ↓ | ↓ | **end of the experiment** |
| | | saline i.p. | | ↓ | |
| **II** | **Cr(VI)** | distilled water p.o. | ↓ | ↓ | |
| | | $K_2Cr_2O_7$ i.p. | | ↓ | |
| **III** | **Cr(VI) + Pleurotus** | Pleurotus o. p.o. | ↓ | ↓ | |
| | | $K_2Cr_2O_7$ i.p. | | ↓ | |

24 hours after the last dose animals were killed in ether anaesthesia, blood and tissue samples were collected for analyses and used immediately or stored frozen at −70 °C until analysed.

The experimental treatment protocol was approved by the local Animal Care and Use Committee.

## Biochemical Assays

**Lipid peroxidation (LP)** was estimated in liver and kidney homogenates by measuring the products formed in the thiobarbituric acid (TBA) reaction (30). Tissue homogenates (0.25 g / 2.5 ml of 1.15% potassium chloride (KCl) were mixed with 1.5 ml of 1% phosphoric acid ($H_3PO_4$) and 0.5 ml of 0.6% TBA aqueous solution. The samples were heated at 95 °C for 1 hour. After cooling, 2 ml of n-butanol were added, mixed vigorously and the butanol phase was separated by centrifugation. The absorbance of butanol layer was measured at 520 and 535 nm; the difference between the determinations was used to calculate concentration of TBA reactive substances (TBARS). The results are expressed in nmole TBARS/gram of tissue.

**Reduced glutatione (GSH)** level was estimated in the deproteinized supernatant fraction of liver and kidney homogenates (0.2 g / 8 ml of 0.02 M EDTA) using 5,5′-dithiobis*itrobenzoic acid) (DTNB, Ellman's reagent) and reading absorption at 412 nm.(31). The results are expressed in µmol GSH/g of tissue.

**Glutathione peroxidase (GPx)** activity was assayed in liver and kidney homogenates by a coupled test system, in which GR is employed for the regeneration of reduced glutathione and butyl hydroperoxide used as the acceptor substrate (32). The decrease in NADPH concentration was registered photometrically at 340 nm. The GPx activity is expressed in µmol NADP+/min/g of tissue.

**Glutathione reductase (GR)** assay is based on the reduction of oxidized glutathione (GSSG) by NADPH in the presence of glutathione reductase. The formed GSH reacts with 5,5'-dithiobis(2-nitrobenzoic acid). The increase in absorbance at 412 nm was measured (33). The reaction system contained 0.1M phosphate buffer (pH 7.5), 1 mM EDTA, 2 mM GSSG and 3 mM DTNB solution. Reactions were started by the addition of 2 mM NADPH and the increase in absorbance was measured at 412 nm.

**Serum activities of ALT, AST and GLDH** were estimated photometrically using the commercial kits (DiaSys Diagnostic System, Germany), according to the manufacturer's protocol.

## Analysis of Cr and essential elements

For the estimation of Cr and essential elements, the tissues were dry-ashed in a muffle furnace under temperature-controlled program overnight; the ash was solubilized with 3 M HCl. Appropriately the diluted samples were analyzed for Cr, Zn, Cu and Fe by atomic absorption spectrometry (SpectrAA 220 FS Varian, Australia Ltd.).

## Statistical Analysis

The statistical significance of the differences between the groups was determined by unpaired Student's *t*-test after ascertaining the homogeneity of variance between treatment groups. The data are presented as mean ± SD values. Significant difference: * $P < 0.05$; ** $P < 0.01$ and *** $P < 0.001$.

## Effects of Pleurotus ostreatus and $K_2Cr_2O_7$ on markers of oxidative state (tab. 2, 3)

Administration of Cr(VI) caused a significant increase in LP in liver (by 494 % vs. controls, $p < 0.001$) and a decrease in renal LP (by 21% vs. controls, $P < 0.01$). Pretreatment with Pleurotus ostreatus further decreased the LP in kidneys – by 13% vs Cr(VI) group ($p < 0.05$) but had no effect on hepatic LP.

Hepatic content of GSH did not significantly change in Cr(VI) group compared to controls, but there was a significant increase in GSH level in Cr(VI) + Pleurotus group compared to Cr(VI) group (by 10%, $p < 0.05$). The content of GSH in kidneys was decreased by Cr(VI) administration (by 33% compared to control group, $P < 0.001$) and was not influenced by Pleurotus ostreatus (tab. 2).

**Tab. 2** The effect of $K_2Cr_2O_7$ administered alone and in a combination with Pleurotus ostreatus on the levels of LP and GSH in the liver and kidneys of rats

| | Group | | Liver | | | Kidneys | | |
|---|---|---|---|---|---|---|---|---|
| **LP** (nmol TBARS/g) | I | control | 37.6 | ± | 10.9 | 63.4 | ± | 8.2 |
| | II | Cr(VI) | 200.6 | ± | 89.3 *** | 49.9 | ± | 4.7 ** |
| | III | Cr(VI) + Pleurotus | 243.7 | ± | 85.6 *** | 43.8 | ± | 5.0 ***,# |
| **GSH** (μmol/g) | I | control | 5.18 | ± | 0.37 | 3.23 | ± | 0.16 |
| | II | Cr(VI) | 4.64 | ± | 0.80 | 2.22 | ± | 0.15 *** |
| | III | Cr(VI) + Pleurotus | 5.70 | ± | 0.65 # | 2.17 | ± | 0.18 *** |

Values represent means ±SD, n = 8. Significant difference: ** $p < 0.01$ and *** $p < 0.001$ vs. controls; # $p < 0.05$ vs. Cr(VI) group.

**Tab. 3** The effect of $K_2Cr_2O_7$ administered alone and in a combination with Pleurotus ostreatus on the activities GPx and GR in the liver and kidneys of rats

| | Group | | Liver | | | Kidneys | | |
|---|---|---|---|---|---|---|---|---|
| **GPx** (μmol/g/min) | I | control | 22.8 | ± | 2.4 | 13.0 | ± | 0.8 |
| | II | Cr(VI) | 22.8 | ± | 2.4 | 9.5 | ± | 0.6 *** |
| | III | Cr(VI) + Pleurotus | 23.0 | ± | 0.9 | 9.0 | ± | 0.8 *** |
| **GR** (U/g) | I | control | 4.01 | ± | 0.43 | 7.01 | ± | 0.76 |
| | II | Cr(VI) | 4.59 | ± | 0.30 ** | 4.29 | ± | 0.50 *** |
| | III | Cr(VI) + Pleurotus | 4.03 | ± | 0.31 ## | 3.67 | ± | 0.40 ***,# |

Values represent means ±SD, n = 8. Significant difference: ** $p < 0.01$ and *** $p < 0.001$ vs. controls; # $p < 0.05$ and ## $p < 0.01$ vs. Cr(VI) group.

No change was seen in the activity of GPx in the liver of Cr(VI) or Cr(VI)+ Pleurotus groups. On the contrary a significant decrease in GPx activity was seen in kidneys of both Cr(VI) and Cr(VI) + Pleurotus groups (by 27% and 31% resp., $p < 0.001$).

The activity of hepatic GR was significantly increased in Cr(VI) group (by 14%, $p < 0.01$). Pretreatment with Pleurotus ostreatus prevented this effect. In kidneys, the activity of GR was significantly decreased by Cr administration (by 39% vs. control, $p < 0.001$) and the decline was further enhanced by preatreatment with Pleurotus ostreatus – by 14% vs. Cr(VI) group ($p < 0.05$).

## Effects of Pleurotus ostreatus and $K_2Cr_2O_7$ on markers of hepatotoxicity in serum (Fig. 1)

Chromium significantly ($p < 0.001$) increased the activities of ALT, AST and GLDH (by 59%, 71% and 72% resp. compared to controls). Pretreatment with Pleurotus ostreatus did not influence the activity of ALT but significantly augmented the increase in the activities of AST (by 34%, $p < 0.05$) and GLDH (by 103%, $p < 0.01$) induced by Cr(VI).

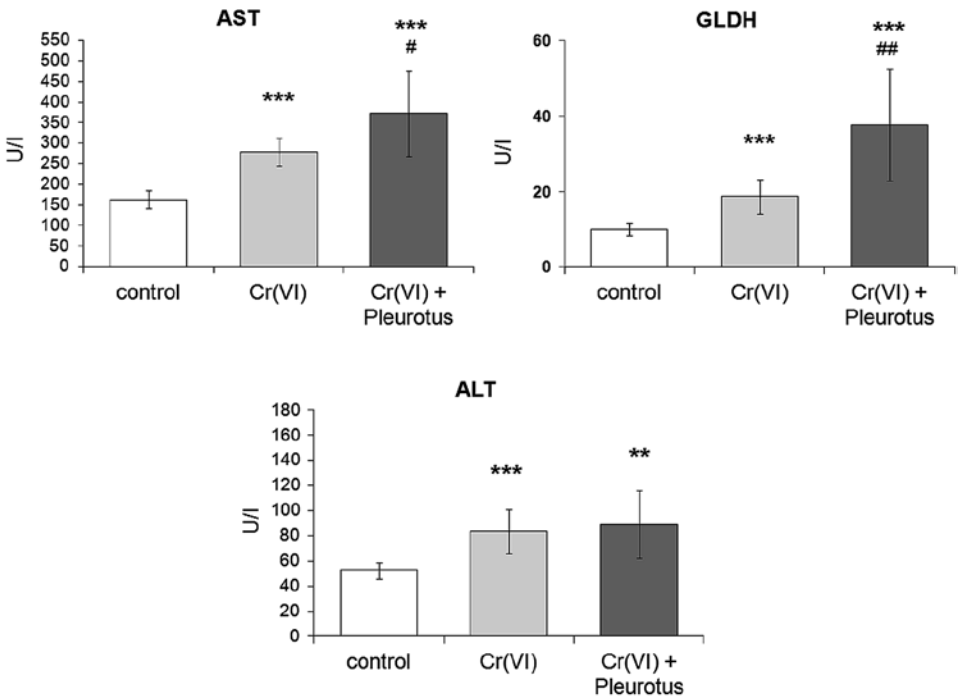


**Fig. 1** The effect of $K_2Cr_2O_7$ administered alone and in a combination with Pleurotus ostreatus on the activities of ALT, AST and GLDH in the serum of rats. Values represent means ±SD, n = 8. Significant difference: ** $P < 0.01$, *** $p < 0.001$ vs. controls; $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$ vs. Cr(VI) group

## The level of essential elements in the liver and kidneys (tab. 4)

A significant increase in hepatic Zn and Fe levels and a decrease in renal Cu and Fe levels were observed in Cr(VI)-exposed rats. Pretreatment with Pleurotus ostreatus influenced just the effect of Cr(VI) on Fe level in the liver: In Cr(VI) + Pleurotus group the increase of hepatic Fe was not significant vs. controls.

**Tab. 4** The effect of $K_2Cr_2O_7$ administered alone and in a combination with Pleurotus ostreatus on zinc, copper and iron concentrations in the liver and kidneys of rats. Element concentrations are expressed as microgram per gram of wet tissue weight

| | Group | | Zn | | | Cu | | | Fe | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Liver** | I | control | 26.8 | ± | 1.7 | 3.14 | ± | 0.20 | 84.5 | ± | 12.7 |
| | II | Cr(VI) | 34.7 | ± | 2.2 *** | 3.21 | ± | 0.19 | 102.2 | ± | 12.6 * |
| | III | Cr(VI) + Pleurotus | 32.9 | ± | 4.1 ** | 3.12 | ± | 0.24 | 91.4 | ± | 18.0 |
| **Kidneys** | I | control | 21.7 | ± | 0.7 | 6.34 | ± | 1.09 | 29.8 | ± | 3.0 |
| | II | Cr(VI) | 21.1 | ± | 0.8 | 3.66 | ± | 0.49 *** | 23.4 | ± | 1.6 *** |
| | III | Cr(VI) + Pleurotus | 20.3 | ± | 1.3 | 3.66 | ± | 0.58 *** | 24.2 | ± | 4.0 ** |

Values represent means ±SD, n = 8. Significant difference: ** $p < 0.01$ and *** $p < 0.001$ vs. controls.

## Chromium content in the liver and kidneys

A significant increase in the content of Cr was found in the liver (by 35%, $p < 0.001$) and kidneys (by 12%, $p < 0,01$) of rats in Cr(VI) + Pleurotus ostreatus group compared to animals in Cr(VI) group (Fig. 2).

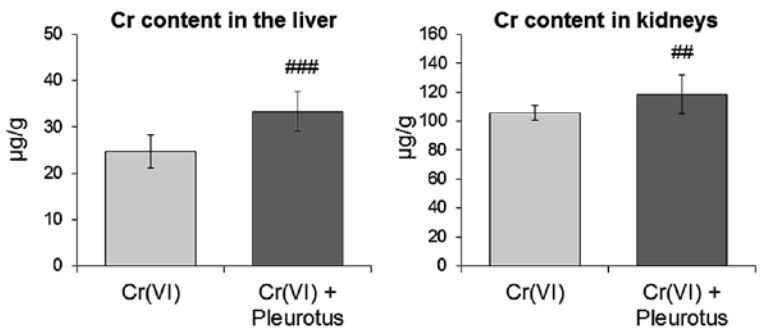


**Fig. 2** Chromium content in the liver and kidneys of rats. Concentrations are expressed as microgram per gram of wet tissue weight. Cr content in controls was < 0.3 microgram/g tissue.
Values represent means ±SD, n = 8. Significant difference: ## $p < 0.01$ and ### $p < 0.001$ vs. Cr(VI) group

## DISCUSSION

Administration of $K_2Cr_2O_7$ at a dose of 40 mg/kg body weight caused a significant increase in LP in liver 24 h after the administration (by 49.4% vs. controls, $p < 0.001$) and a decrease in renal LP (by 21% vs. controls, $p < 0.01$). Our previously published results showed that a single i. p. dose of 20 mg/kg $K_2Cr_2O_7$ induced LP in both organs; however the levels of LP were markedly higher in the liver compared to kidneys (34). The dose of Cr(VI) and the duration of exposure is considered to be of great importance for the effect of Cr(VI) on LP and a dual role of Cr(VI) in inducing LP has been already described (29, 35, 36). A decrease in LP in both the liver and kidneys, and an increase in testicular and cerebral LP was described by Dey (37) in rats exposed to subchronic Cr(VI) intoxication.

Pretreatment with Pleurotus ostreatus in our experiment did not significantly influenced the increase in hepatic LP, but further decreased the LP lowered by Cr(VI) in kidneys.

We observed a decrease in GSH in kidneys (by 33% compared to control group, $p < 0.001$), but no significant change of GSH was seen in the liver in Cr(VI) group. In our previously published results, we described slightly different effect of potassium dichromate: a single dose of $K_2Cr_2O_7$ (20 mg/kg) increased hepatic GSH and did not change renal GSH levels 24 hours after the injection (34). The difference could be due to the higher dose of Cr(VI) we used in the present experiment. Standeven and Wetterhahn (38) reported an initial decrease in hepatic and renal GSH levels followed by marked increase between 5th and 26th hour after i.p. injection of 20 mg $Na_2Cr_2O_7$/kg to rats. The possible dose-dependent response of GSH level in the liver can be also seen in contradictory results of published studies of subacute Cr(VI) intoxication in rats: Dey (37) described increased hepatic and decreased renal GSH content after subacute administration of 8 mg/kg chromium trioxide daily for 28 days; Soudani (39) observed a significant decrease in glutathione (GSH) levels in the liver of rats which were given 700 ppm $K_2Cr_2O_7$ in drinking water for 21 days.

Pretreatment with Pleurotus ostreatus had not influenced the effect of Cr(VI) on renal GSH. In the liver, the combination of Pleurotus ostreatus + Cr(VI) in lead to an increase in GSH content compared to Cr(VI) group. This effect of Pleurotus ostreatus could be considered to be protective, but contradictory data exist about the role of GSH in Cr(VI) toxicity. The protective effect of GSH as well as potentiation of the Cr(VI) toxicity has been described (40–43).

In the presence of reducing agents reactive intermediates Cr(V) and Cr(IV) are generated. GSH can act as a reductant for Cr(VI) and reactive intermediates and the generated hydroxyl radical or another reactive oxidative species may be responsible for the toxic effects of Cr(VI) (44). It was reported, that GSH depletion provided partial protection from the effects of Cr (VI) on cell viability in freshly isolated hepatocytes (45). Gunaratnam (46) also showed that during the metabolism of Cr (VI), the activity of GR was markedly reduced and GSH levels decreased in isolated hepatocytes, whereas the expression of the protein was increased 2-fold concomitantly, presumably as a compensatory mechanism. In pre-treated cells where GR was inhibited, the effects of Cr (VI) were ameliorated.

In our experiment, the activity of renal GR was significantly decreased by Cr (VI) administration (and the decline was further enhanced by preatreatment with Pleurotus ostreatus.

The depletion of GSH in kidneys after Cr(VI) administration could explain the difference in the levels of LP we found between the liver and kidneys. The depletion of GSH in kidneys could decrease the production of reactive Cr intermediates. The inhibition of GR activity and GSH depletion could also activate compensatory antioxidant responses that lead to the observed decrease of renal LP.

A different situation was seen in the liver: the activity of hepatic GR was increased, possibly as a compensatory response and the content of GSH in the liver was not significantly changed in Cr (VI) intoxicated rats compared to controls. There was significant increase in GSH level in the liver in Cr(VI) + Pleurotus group. We also observed the highest level of hepatic LP in this group.

No change in the activity of GPx was seen in the liver of Cr(VI) intoxicated rats, whereas a significant decrease in GPx activity was seen in kidneys. The experiment confirmed a difference in the susceptibility of renal and hepatic GPx to Cr(VI), that we have already described (34).

Cr(VI) significantly elevated the activities of ALT, AST and GLDH. Pretreatment with Pleurotus ostreatus further significantly augmented the increase in the activities of AST and GLDH. The increase in GLDH, an important marker of toxic liver damage, shows, that Pleurotus ostreatus augments the toxic effect of Cr(VI) in the liver. The mechanism of this effect is not clear yet.

Significantly higher content of Cr(VI) was found in liver tissue of animals in Cr(VI) + Pleurotus group in comparison with animals in Cr(VI) group. This influence of Pleurotus ostreatus on Cr distribution may be partly responsible for the increased toxicity of Cr(VI) in the presence of Pleurotus ostreatus. The content of Cr(VI) was also increased in kidneys in animals pretreated with Pleurotus ostreatus.

Our pilot experiments in rats as well as a search in the literature confirmed that administration of Pleurotus ostreatus alone does not induce any significant changes in the oxidative state in liver or kidney tissues or in markers of liver damage in serum (47–49). The only effect we observed in pilot experiments in rats was a decrease in copper content in liver tissue (by 11% vs. controls; $p < 0.01$). Higher doses (5% in the diet), compared to our experiment, increased the levels of reduced glutathione in the liver and stimulated the activities of catalase and glutathione peroxidase in rats after 52 weeks of administration (50).

The doses of Pleurotus ostreatus we used in the experiment are higher, than is the usual recommended human dosage for Pleurotus ostreatus in nutritional supplements. The doses in our experiment were based on published studies in rats where the hepatoprotective and antioxidant effects of Pleurotus ostreatus were described (10, 14, 15, 17). However, some nutritionists and nutrition websites recommend taking up to 15,000 mg of Pleurotus ostreatus per day. Similarly high doses were used in clinical studies where the antihyperlipidemic effects of Pleurotus ostreatus (15 g /day) was studied (47).

Pleurotus ostreatus is widely used immunomodulatory nutritional supplement, which is sold over the counter. The dosage that is used and length of the treatment are usually not consulted with the doctor. The ability of Pleurotus ostreatus to increase some of the hepatotoxic effects of hexavalent chromium (a known environmental pollutant) has not been previously described. Because the occupational exposure or a contact with Cr(VI) in the environment are not rare, the fact that Pleurotus ostreatus can influence the distribution of Cr in tissues is of high importance. The described effect and the exact mechanism of it should be further studied.

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## SUMMARY

Pleurotus ostreatus (P. o.), oyster mushroom, is widely used immunomodulatory nutritional supplement. P. o. and pleuran (β-1,3/1,6 glucan from P. o.) have been shown not only to enhance the defence against infections, but also to lower cholesterol, to have antitumor, anticoagulant, anti-inflammatory, antinociceptive and hepatoprotective properties. Recently, the research has focused on their antioxidant properties. Hexavalent chromium [Cr(VI)] is a toxic and carcinogenic pollutant. Production of reactive oxygen species plays an important role in the pathogenesis of Cr(VI) toxicity. The study was designed to investigate possible protective and antioxidant effects of Pleurotus ostreatus in acute intoxication with hexavalent chromium. Wistar albino male rats were divided into three groups: control, Cr(VI), and Cr(VI) + P. o. Finely powdered P. o. (500 mg/kg b.w./day) was administered in a form of suspension via gavage for ten consecutive days. $K_2Cr_2O_7$ (40 mg/kg b.w./day) was administered i.p. as a single dose on the tenth day of the experiment. 24 hours after the administration of Cr the experiment was terminated and liver and tissue and blood samples were collected. Lipid peroxidation, reduced glutathione, the activities of glutathione peroxidase and glutathione reductase and Cr content were estimated in liver and kidney homogenates. ALT, AST and GLDH were determined in serum. The supposed antioxidant effect has not been proved. Administration of Cr(VI) significantly elevated the activities of ALT, AST and GLDH. Pretreatment with P. o. further significantly augmented the increase in the activities of AST and GLDH. Significantly higher content of Cr(VI) was found in the liver and kidneys of animals in Cr(VI) + P. o. group in comparison with Cr(VI) group. The influence of P. o. on Cr distribution may be partly responsible for the increased toxicity of Cr(VI). The ability of P. o. to increase some of the hepatotoxic effects and the tissue distribution of hexavalent chromium has not been previously described. Because P.o. is commonly used, the observed effect and the exact mechanism of it should be further studied.

## *Pleurotus ostreatus (hlíva ústřičná), běžně používaný doplněk stravy, má schopnost zvyšovat akutní toxické účinky šestimocného chromu u potkanů*


SOUHRN

Pleurotus ostreatus (P. o.), hlíva ústřičná, se často používá jako imunomodulační doplněk stravy. Bylo prokázáno, že P. o. a pleuran (β-1,3/1,6 glukan obsažený v P. o.) kromě zvýšování obranyschopnosti proti infekcím také snižují cholesterol, mají protinádorové, antikoagulační, protizánětlivé, antinociceptivní a hepatoprotektivní účinky. V poslední době se výzkum zaměřuje i na jejich antioxidační účinky. Šestimocný chrom je toxický polutant s karcinogenními účinky. V mechanismu jeho toxicity hraje významnou roli produkce reaktivních forem kyslíku. Cílem studie bylo ověřit možné protektivní a antioxidační účinky při akutní intoxikaci chromem. Potkani – samci kmene Wistar byli rozděleni do tří skupin: kontrola, Cr(VI) a Cr(VI) + P. o. Sušený P. o. ve formě prášku byl podáván perorálně sondou jako suspense v destilované vodě (500 mg/kg těl. hmotnosti/den) po dobu deseti dní. $K_2Cr_2O_7$ (40 mg/kg těl. hmotnosti/den) byl aplikován i.p. jednorázově desátý den od začátku pokusu. Zvířata byla usmrcena 24 hodin po podání chromu a byly odebrány vzorky tkání a krve. V homogenátech jater a ledvin byla stanovena hladina peroxidace lipidů, redukovaného glutathionu, aktivity glutathionperoxidázy a glutathionreduktázy a obsah chromu. V séru byly stanoveny hladiny ALT, AST a GLDH.

Předpokládaný antioxidační účinek nebyl prokázán. Podání chromu významně zvyšovalo hladiny ALT, AST a GLDH. Ve skupině Cr(VI) + P. o. byly hodnoty AST a GLDH proti skupině Cr(VI) ještě významně vyšší. Také obsah chromu v játrech a ledvinách byl ve skupině Cr(VI) + P. o. významně vyšší než ve skupině Cr(VI). Schopnost zvyšovat toxické účinky chromu v játrech a jeho distribuci do tkání nebyla u P. o. až dosud popsána. Vzhledem k běžnému používání doplňků stravy s P. o. by měl být pozorovaný efekt a jeho mechanismus dále studován.


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