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Short communication

Deficiency of macro- and micronutrients induced by *Lentinula edodes*

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ABSTRACT

Mushroom *Lentinula edodes* has been widely studied therapeutically. However, there is no data regarding its daily intake level safety. Since *L. edodes* has many active compounds known to bind to metals, we evaluated macro and micronutrients in liver and kidney of healthy rats after subchronic exposure to *L. edodes*. Rats were divided into four groups, receiving water and *L. edodes* at 100, 400 and 800 mg/kg/day. The treatment lasted 30 days. Essential elements (Zn, Cu, Mg, Fe, Mn, Se, Co, Mo, and Li) were analyzed in an inductively coupled plasma mass spectrometer. Our results demonstrated a significant decrease in Cu, Fe, Mn and Co levels in liver of rats receiving *L. edodes* at the highest doses. In kidney, Mn, Mo and Li concentrations significantly dropped in the groups exposed to the highest doses. In this way, an important point is revealed concerning the food safety from *L. edodes*, once its chronic and high consumption could contribute to macro and micronutrients deficiency. Additionally, we speculate that the daily use of *L. edodes* could be unsuccessful for patients in mineral therapy besides being able to be unsafe for individuals with some propensity to mineral deficiency.

1. Introduction

Mushrooms are widely used around the world as food due their delicious flavor and particularly by their medicinal properties [7].

Among the edible mushrooms, *Lentinula edodes* stands out for its nutritional content and active compounds of pharmacological value. We can mention β-glucans (lentinan) [29], high proteins and essential amino acid levels [4,14], vitamins (C, D, B_1, B_2, B_{12}, niacin) [4,16] among others [4]. These compounds provide for *L. edodes* several therapeutic applications as antitumor activity [9,29], antihyperlipidemic effect [28], antioxidant [9], and antiviral activity [26].

Indeed the positive effects of *L. edodes* are well established. However, the positive outcomes occur in disease states, such as cancer (both *in vitro* as *in vivo*). Thus, there are no studies regarding the safe daily intake level for *L. edodes* in healthy individuals. One way to verify the food safety is quantifying the levels of essential elements in

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different body tissues, once foods can bind to chemical elements, decreasing essential minerals and altering the physiological framework. Hence, we evaluated macro and micronutrients in healthy rats (liver and kidney) exposed to *L. edodes* in different doses.

2. Materials and methods

2.1. Preparation of rat diets

Fresh *L. edodes* mushrooms were sliced and dried in a ventilated stove at the temperature of 38 ± 2 °C until constant mass. Dried mushroom was ground in a mill. A homogeneous powdered mushroom was obtained, similar to flour. The mushroom powder was given to the animals every day, as described below.

2.2. Experimental design

Male Wistar rats weighing from 140 to 160 g and about 45 days old were obtained from Anilab – Laboratory Animals Conception and Market, São Paulo State. Animals were kept in the Toxicological Research Laboratory facility in accordance with the Guide for the Care and Use of Laboratory Animals [20] and the Organization for Economic Co-operation & Development guidance document [22]. The experiment was approved by the University of Sorocaba Committee on the Care and Use of Experimental Animals, under protocol number Process Approbation 008/2012 (issued on the 24th of April of 2013). Animals were kept in 12 h light/dark cycles at a controlled temperature of 22–24 °C with food and water *ad libitum*. Animals were randomly assigned to one of the four groups ($n = 6$/each group). Group I: control group, received water. Group II: received *L. edodes* at 100 mg/kg. Group III: received *L. edodes* at 400 mg/kg. Group IV: received *L. edodes* at 800 mg/kg.

All administrations were conducted by gavage, and *L. edodes* was solubilized in water. Doses were chosen based on the consumption per capita in China (about 10 kg/person/year) [24], which is about 400 mg/kg/day. A higher and a lower dose were also given. After 30 days of treatment, animals were euthanized by anesthesia overdose with ketamine and xylazine and the liver and kidney were collected.

2.3. Tissue treatment

Essential elements zinc (Zn), cupper (Cu), magnesium (Mg), iron (Fe), manganese (Mn), selenium (Se), cobalt (Co), molybdenum (Mo) and lithium (Li) were determined in an inductively coupled plasma mass spectrometer (ICP-MS) (ELAN DRCII, PerkinElmer, SCIEX, Norwalk, CT, USA) operating with high-purity argon (99.999%). The sample introduction system was composed of a quartz cyclonic spray chamber and a Meinhard® nebulizer connected by Tygon® tubes to the peristaltic pump of the ICP-MS.

Briefly, liver and kidney samples (75–100 mg) were weighed and transferred to a conical tube (15 mL). A volume of 1 mL of tetramethyl ammonium hydroxide (TMAH 50%, v/v) was added to the tube, which was homogenized rotationally for 24 h. After that the volume was made up to 10 mL with a diluent containing 0.5% (v/v) HNO_3 and 0.01% (v/v) Triton X-100 [3]. Analytical calibration standards for Zn, Cu, Mg, Fe, Mn, Se, Co, Mo and Li were prepared daily over the range from 0 to 5000 ng g^{-1} in the same diluent. The correlation coefficient for calibration curves was better than 0.9999 to all essential elements. In all experiments, 10 μg L^{-1} of the internal standard Rh was used.

2.4. Statistical analysis

Data were reported as mean ± standard deviation (SD). Differences between the treatments were evaluated by one-way non-parametric ANOVA, followed by Duncan's multiple range tests. P values <0.05 were considered significant. Data were analyzed using Statistica® 8.0 (Statsoft software, Tulsa, OK, USA).

3. Results

Data regarding Zn, Cu, Mg, Fe, Mn, Se, Co, Mo and Li levels in liver samples are shown in Table 1. A significant decrease in Cu and Fe levels was observed in rats receiving *L. edodes* at 800 mg/kg/day compared to the control group. Moreover, *L. edodes* at 400 mg/kg/day decreased the Mn level compared to the control group. And finally a reduction in Co levels was observed in both groups exposed to 400 and 800 mg/kg/day compared to 100 mg/kg/day *L. edodes*.

Levels of Zn, Cu, Mg, Fe, Mn, Se, Co, Mo and Li in kidney samples are presented in Table 2. The manganese (Mn) and

Table 1
Essential elements (mean ± standard deviation) in liver samples from rats exposed to *Lentinula edodes* in different concentrations. Chemical elements were determined by Inductive Coupled Plasma Mass Spectrometry. Data are reported as μg g^{-1} or ng g^{-1} (wet weight).

Essential elements	Control	*L. edodes* 100 mg/kg	*L. edodes* 400 mg/kg	*L. edodes* 800 mg/kg
Zn (μg g^{-1})	44.5 ± 3.2	46.9 ± 2.4	43.1 ± 6.1	47.1 ± 3.9
Cu (μg g^{-1})	10.1 ± 1.2	9.0 ± 0.8	8.5 ± 1.0	8.0 ± 0.4[a]
Mg (μg g^{-1})	261.4 ± 33.5	245.2 ± 54.0	241.9 ± 53.7	274.9 ± 38.8
Fe (μg g^{-1})	92.9 ± 15.4	85.3 ± 12.9	84.4 ± 15.9	70.1 ± 16.7[a]
Mn (ng g^{-1})	2.879 ± 320	2.730 ± 467	2.275 ± 394[a]	2.560 ± 357
Se (ng g^{-1})	781 ± 75	835 ± 91	743 ± 79	788 ± 65
Co (ng g^{-1})	146 ± 21	165 ± 44	130 ± 12[b]	122 ± 16[b]
Mo (ng g^{-1})	625 ± 62	678 ± 102	630 ± 93	661 ± 102
Li (ng g^{-1})	11.6 ± 2.8	12.0 ± 1.4	9.6 ± 2.5	10.6 ± 2.7

[a] Statistically significant difference from control group.
[b] Statistically significant difference from *L. edodes* 100 mg/kg group.

Table 2
Essential elements (mean ± standard deviation) in kidney samples from rats exposed to *Lentinula edodes* in different concentrations. Chemical elements were determined by Inductive Coupled Plasma Mass Spectrometry. Data are reported as $\mu g\,g^{-1}$ or $ng\,g^{-1}$ (wet weight).

Essential elements	Control	*L. edodes* 100 mg/kg	*L. edodes* 400 mg/kg	*L. edodes* 800 mg/kg
Cu ($\mu g\,g^{-1}$)	15.9 ± 2.6	15.2 ± 1.9	16.3 ± 3.5	15.6 ± 1.1
Mg ($\mu g\,g^{-1}$)	253.3 ± 18.8	275.1 ± 23.2	242.1 ± 21.1	233.2 ± 28.6
Fe ($\mu g\,g^{-1}$)	85.2 ± 19.3	72.3 ± 10.1	72.6 ± 16.1	68.5 ± 15.1
Mn ($ng\,g^{-1}$)	1.032 ± 95	1.039 ± 97	916 ± 172	835 ± 109[a]
Se ($ng\,g^{-1}$)	1.383 ± 95	1.589 ± 140[a]	1.425 ± 117	1.490 ± 165
Co ($ng\,g^{-1}$)	235 ± 34	246 ± 34	213 ± 32	215 ± 40
Mo ($ng\,g^{-1}$)	285 ± 40	290 ± 23	250 ± 16	248 ± 32[a]
Li ($ng\,g^{-1}$)	13.3 ± 1.7	12.7 ± 1.2	8.9 ± 1.2[a]	8.7 ± 1.3[a]

[a] Statistically significant difference from control group.

molybdenum (Mo) concentrations decreased significantly in the group given *L. edodes* at 800 mg/kg/day, and the levels of lithium (Li) declined in both groups given *L. edodes* at 400 and 800 mg/kg/day compared to the group control. On the other hand, the level of selenium (Se) increased in the group receiving *L. edodes* at 100 mg/kg/day.

4. Discussion

It was observed that *L. edodes* mushroom in high doses and even at the dosage usually consumed by the population (population of China) can chelate metals, inducing deficiency in macro and micronutrients.

Copper (Cu) is a cofactor for various enzymes such as superoxide dismutase, responsible for removal of oxygen radicals, cytochrome c-oxidase, and ceruloplasmin [1,12]. Regarding Fe, it is required in several enzymes and proteins, but it is of particular importance in heme synthesis. Iron deficiency induces anemia, a worldwide problem [1].

Manganese (Mn) is also a cofactor for enzymes as superoxide dismutase, sulfite oxidase, aldehyde oxidase and others enzymes [2,12]. Manganese deficiency impairs growth, induces skeletal abnormalities and disturbs reproductive function [12]. In the same way, Co is a core constituent of vitamin B_{12} complex, as the central coordinated ion in cyclic tetrapyrroles. Cobalt deficit impairs growth and causes pernicious anemia [15].

In turn, magnesium (Mg) is essential for the glycolytic and citric acid cycles and beta-oxidation of fatty acids. Magnesium deficiency is associated with fatigue, convulsions, and inflammatory syndrome [17]. Molybdenum is a transition element required, as a dynamic metal, during enzyme catalysis. Sulfite oxidase, xanthine oxidoreductase, aldehyde oxidase, and mitochondrial amidoxime reductase belong to a group of molybdenum-dependent enzymes [18]

Finally, lithium (Li) is also an essential chemical element, but is required in moderate amounts. Lithium deficiency is related to behavioral deficits, such as anxiety and depression [19]. We speculate that the consumption of *L. edodes* by patients undergoing lithium therapy can leave the plasma lithium concentration below the therapeutic dosage.

In addition to these implications, multiple macro and micronutrient deficiencies can be related to the etiopathogenesis of other diseases. Vural et al. [25] showed that patients with Alzheimer's disease had lower levels of Mg, Fe, Cu, Zn and Se compared to healthy subjects. Moreover, deficiency of Zn, Mg and Ca appears to be related to the occurrence of autism in children [27].

A feasible interpretation of these findings is that the chemical constituents of *L. edodes*, for instance, chitin, a natural polysaccharide, and chitosan, the deacetylated form of chitin, are excellent adsorbents for metal ions [5,10,11]. These compounds have been studied for wastewater treatment for environmental purposes [23]. However, there are no reports in the literature concerning the safety of intake of chitin and chitosan from food. The cell wall of mushrooms produces chitosan, and the remaining fiber from mushroom glucans isolation of comprise chitin [21].

Other components from *L. edodes* are phenolic compounds [6]. Quercetin, one of the most typical dietary phenolic compounds, was demonstrated to play a significant role in iron [13] and copper complexation [8]. Thus, because these compounds have the ability to bind to metals, a reduction in the levels of Cu, Fe, Mn, Co, Mn, Mo and Li was found after subchronic intake of *L. edodes* in all studied doses.

In conclusion, for the first time the *L. edodes* mushroom, known for its pharmacological properties (particularly antitumor activity), is reported as an unsafe food, by sequestering essential chemical nutrients *in vivo*. This result is particularly prominent for those individuals who have some genetic predisposition to mineral deficiency, those that depend of mineral therapy, children and pregnant women.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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