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Oxidant/antioxidant potentials and heavy metal levels of *Pisolithus arhizus* and its effects on cardiovascular diseases

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Pisolithus arhizus (Scop) Rauschert is a cosmopolitan mushroom in warm temperate regions of the world and forms ectomycorrhizas associations with a wide range of tree species (both angiosperms and gymnosperms). *P. arhizus* samples were collected from a pine forest in Antalya province (Turkey). Powdered mushroom samples were extracted with ethanol (EtOH) using a Soxhlet apparatus at 50 °C, then concentrated under pressure at 40 °C in a rotary evaporator, and stored at 4 °C in airtight containers. Rel Assay Kits were used to determine the total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) of mushroom extracts. The elemental contents of the mushrooms were then determined using the atomic absorption spectrophotometer. It was determined that the heavy metal content in *P. arhizus* were found to be generally at optimal levels and the TOS value of mushroom was exceptional. Heavy metals are toxic, they may have chronic degenerative changes on organs. Vascular effects of heavy metals may contribute to a variety of pathologic conditions. Heavy metals resulting in pathophysiological changes causes atherogenic events like increased oxidative stress, inflammatory response, and coagulation activity. It has been determined that *P. arhizus* may be an important source of antioxidants.

Keywords: Antioxidant, Heavy metal, Oxidant, Oxidative stress, *Pisolithus arhizus*.

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Introduction

A number of mushroom species are consumed by humans as medicines and nutrients. Edible mushrooms are not only preferred for their taste and texture but also for having good nutrients inside, such as protein & amino acids[1]. Having a good antioxidant potential, mushrooms can also help in reducing oxidative stress. Some serious health problems such as cardiac disease, cirrhosis, necrosis, leukaemia, diabetes may occur due to the oxidative stress stimulated by free radicals[2,3]. Mushrooms are reported to have medicinal properties such as anti-microbial, anticancer, antioxidant, and immunomodulatory properties[4-6]. *Pisolithus arhizus (Scop.) Rauschert (Dyeballs)* is counted among the best mushrooms for dyeing and imparts wool with a deep brown to reddish-brown to blackish colour. The pigments come from the tar-like gel between the peridioles, so younger Dyeballs work better[7,8]. One of the most significant aspects of *P. arhizus* is its exceptional ability to form ectomycorrhizas. The Dyeball is one of the most generous mycorrhizal partners, so foresters and others often inoculate tree seedlings with *P. arhizus* spores to give the plants an early growth boost. There are other mushrooms that can be used for the same purpose, but *P. arhizus* is the easiest because it forms mycorrhizas with a wide variety of tree species[9]. Mushrooms have a higher content of heavy metals than vegetables and fruits and agricultural plants[10]. During organic matter, catabolism mushrooms are considered to be used as natural pollution indicators as element level builds up in their body because of substrate content[11]. Living organisms require trace amounts of elements such as Fe, Co, Cu, Mn, Mo, Sr, V, and Zn which can accumulate in living organisms ultimately leading toward detrimental consequences[12].

Heavy metals are commonly found in the earth's crust. They are found in very low concentrations in the organisms. Increased amounts in the atmosphere, soil, and water can cause serious problems for all

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organisms. It is possible to accumulate with human occupational exposure, environmental pollution, and vegetables grown in dirty pellets. Heavy metals are toxic and may cause chronic degenerative changes in organs. They have effects on the nervous system, liver, kidneys, and cardiovascular system[13]. Heavy metals are known to have oxidant effects[14]. The toxicity of heavy metals at high levels are well known. Constant exposure to low levels of heavy metals causes chronic health problems. Although heavy metals do not have a metabolic function in the body, they disrupt normal cellular processes and lead to toxicity. Their absorption is weak, their excretion is slow after absorption, and they accumulate in the body causing organ damage. The severity of adverse health effects is related to the chemical form of heavy metals and is also time and dose-dependent. Human intoxication has both acute and chronic effects on health and the environment. Cardiovascular disease (CVD), including coronary heart disease (CHD), cardiomyopathy, heart failure, hypertension, and valvular heart disease, is a leading cause of death worldwide[15]. Smoking, hypertension, hypercholesterolemia, and other factors (age, sex, and air pollution) are among the major risk factors for CHD. In addition, non-occupational exposure to several heavy metals has been associated with CHD[16]. Nevertheless, their contribution to CVD is still incompletely understood. Recent studies have shown that vascular effects of heavy metals may contribute to a variety of pathologic conditions including diabetes mellitus and hypertension[17]. Thus, it is important to determine the elemental level of mushrooms to avoid serious illnesses that may arise due to the utilizations through the food chain.

The present study aimed to investigate the effect of *P. arhizus* (Scop) as total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OST), and heavy metal contents (Fe, Cu, Zn, Pb, Cd, Cr, Mn, and Ni).

Materials and Methods

Sample collection, identification, and extract preparation

P. arhizus samples (Fungarium number: ANK Akata 7312) used in the study were collected from near the pine forest in Muğla province (Turkey) in 2017. Collected mushroom samples were dried at 40 °C. Then, the mechanical grinder was used to pulverize the mushroom samples. The pulverized mushroom sample was then extracted with ethanol (EtOH) in a soxhlet apparatus at 50 °C (Gerhardt EV 14). In a rotary evaporator (HeidolphLaborater 4000 Rotary Evaporator), the mushroom extract was then concentrated under pressure at 40 °C and stored at 4°C in airtight containers.

Determination of TAS, TOS, and OSI

TAS, TOS, and OSI of mushroom extracts were determined using Rel Assay Kits (Rel Assay Kit Diagnostics, Turkey). TAS value was expressed as mmol Trolox equiv./L and Trolox was used as the calibrator[18]. The TOS value was expressed as μmol H_2O_2 equiv./L and hydrogen peroxide was used as the calibrator[19]. The OSI (Arbitrary Unit) was calculated with the formula below[20].

$$\text{OSI (AU)} = \frac{\text{TOS, } \mu\text{mol } H_2O_2^{\text{Equiv.}} / \text{L}}{\text{TAS, mmol Trolox }^{\text{Equiv.}} / \text{L} \times 10}$$

Determination of heavy metal content

Mushroom samples were dried at 80 ºC to constant weight in order to determine Cr, Cu, Mn, Fe, Ni, Cd, Pb, and Zn contents. About 0.5 g of these samples were taken and mineralized in a mixture of HNO_3 (9 mL) + H_2O_2 (1 mL) in a microwave solubilizer (Milestone Ethos Easy). The elemental contents of the mushrooms were then determined using the atomic absorption spectrophotometer (Agilent 240FS AA)[21].

Results and Discussion

TAS, TOS, and OSI Values

TAS (mmol/L), TOS (μmol/L), and OSI values of *P. arhizus* collected from (pine forest in Muğla province, Turkey) were determined with Rel Assay commercial kits (Table 1).

The TAS value of *P. arhizus* was found to be 2.881 mmol/L, the TOS to be 5.043 μmol/L, and the OSI value to be 0.175. A previous study observed the TAS value of *Lentinus tigrinus* (Bull.) Fr. as 1.748 mmol/L, TOS value as 19.294 μmol/L, and OSI value as 1.106[22]. TAS value of *Cyclocybe cylindracea* (DC.) Vizzini & Angelini was reported as 4.325 mmol/L, TOS value as 21.109 μmol/L, and OSI value as 0.488[23]. TAS value of *Fomitopsis pinicola* (Sw.) P. Karst was reported as 1.57 mmol/L, TOS value as 2.03 μmol/L, and OSI value as 0.13[24]. TAS value of

Table 1 — TAS, TOS and OSI values

	TAS mmol/L	TOS μmol/L	OSI
P. arhizus	2.881±0.037	5.043±0.042	0.175±0.001

Values are presented as mean±S.D.; n=6 (Experiments were made as 5 parallel)

Laetiporus sulphureus (Bull.) Murrill. was reported as 2.195 mmol/L, TOS value as 1.303 μmol/L, and OSI value as 0.059[25]. TAS value of *Cerioporus varius* (Pers.) Zmitr. & Kovalenko was reported as 2.312 mmol/L, TOS value as 14.358 μmol/L, and OSI value as 0.627[26]. TAS value of *Infundibulicybe geotropa* (Bull.) Harmaja was reported as 1.854 mmol/L, TOS value as 30.385 μmol/L, and OSI value as 1.639[27]. TAS value of *Helvella leucopus* Pers. was reported as 2.181 mmol/L, TOS value as 14.389 μmol/L, and OSI value as 0.661[28]. TAS value of *Lepista nuda* (Bull.) Cooke was reported as 3.102 mmol/L, TOS value as 36.920 μmol/L, and OSI value as 1.190[29]. TAS value of *Leucoagaricus leucothites* (Vittad.) Wasser was reported as 8.291 mmol/L, TOS value as 10.797 μmol/L, and OSI value as 0.130[30].

In the present study, a significantly higher TAS value of *P. arhizus* was observed as compared to *L. tigrinus, F. pinicola, L. sulphureus*, *H. leucopus*, *I. geotropa* and *C. varius*. However, it was found to be lower than *L. nuda* and *L. leucothites*. The high TAS value of *P. arhizus* might indicate that it may have significantly better antioxidant potential. In the present study, a significantly higher TOS value of *P. arhizus* was observed as compared to *F. pinicola* and *L. sulphureus*. The higher TOS value might be attributed to the presence of higher amounts of oxidative agents in the mushroom due to various environmental and metabolic factors of the region. The OSI value, on the other hand, is the ratio of how much of the oxidant compounds produced by environmental and physiological processes in the structure of the living organism could be tolerated by antioxidant molecules that the organisms produced. The *P. arhizus* OSI level was found to be significantly lower than that of *L. tigrinus*, *C. cylindracea*, *I. geotropa L. nuda*, and *H. leucopus*, and *C. varius*. These findings might demonstrate that *P. arhizus* could be significantly rich in antioxidant compounds that may neutralize oxidant molecules and could produce more antioxidant molecules.

Heavy Metal Content

Fe, Cu, Zn, Pb, Ni, Mn, Cd, and Cr contents of *P. arhizus* were determined with heavy metal analyses and given in mg/kg. The heavy metal contents are given in Table 2 as average±S.D.

Mushrooms accumulate elements in their bodies[31]. The results obtained might indicate significantly (P <0.05) lowest and highest element levels as 9.63–42.7 for Cr, 60.33–95 for Cu, 18.1–103 for Mn, 14.6–835 for Fe, 0.67–5.14 for Ni, 2.71–7.5 for Cd, 2.86–16.54 for Pb, and 29.8–158 mg/kg for Zn[10,31]. Compared to these values, the contents of Ni, Mn, Fe, Pb, and Zn were found to be within the range as indicated in the literature. Cr and Cd content of *P. arhizus* were significantly (P <0.05) higher than literature. In addition, Cu was significantly (P <0.001) lower than the literature range[10,31]. In this context, it is thought that *P. arhizus* may be an indicator for Cr and Cd. Cd is absorbed through the respiratory and digestive tracts. Cd is transported in blood bounded mainly to metallothionein. Metallothioneins are heavy metal-binding proteins that can protect against heavy metal toxicity and oxidative stress. The vascular wall is shown to be a target organ for cadmium deposition[32]. Chronic Cd exposure is associated with hypertension and diabetes[33]. In prospective studies, a potential relationship was shown between blood Cd and blood pressure. Cd exposure speeds up the occurrence of diabetic renal complications[34]. *In vitro* studies showed that low dose Cd levels may contribute to the initiation of pathophysiological changes in the vessel wall. The cardiovascular effects of Cd have been observed in *in-vitro* studies and experimental animal models[35]. Cd has adverse effects on the cardiovascular system. It occurs by promoting atherosclerosis and by inducing cardiac functional failure and metabolic changes[36]. Many pathogeneses have been described for cardiovascular diseases and cadmium relationship. Cd has effects such as partial agonism, vasoconstrictor effect, and inhibition of vasodilator agents such as NO for calcium channels[37]. Despite these described mechanisms, the full effect is unknown. Cd toxicity has an effect on cells through oxidative stress[38]. Upregulation of antioxidant defence in endothelial cells in response to cadmium may define the presence of reactive oxygen species in endothelial cells. Cd infiltrates the vascular wall with

Table 2 — Heavy metal concentration

Elements	Element contents (mg/kg)	Literature ranges (mg/kg)
Cr	73.01±2.43	9.63–42.7
Cu	24.66±1.57	60.33–95
Mn	50.57±1.81	18.1–103
Fe	579.22±10.70	14.6–835
Ni	0.91±0.13	0.67–5.14
Cd	8.96±0.32	2.71–7.5
Pb	3.47±0.27	2.86–16.54
Zn	37.51±1.07	29.8–158

Values are presented as mean±S.D., n=3 (Experiments were made as 3 parallel) direct endocytosis and monocytes[39]. Considering the role of monocytes and macrophages in atherosclerosis, cadmium is thought to accelerate foam cell formation of monocytes and macrophages. In addition, cadmium is thought to impair endothelial integrity with endothelial cell deaths also contributing to vascular inflammation[17].

Conclusion

In the present study, total antioxidant status, total oxidant status, oxidative stress index of *P. arhizus* was determined. *P. arhizus* was found to have high antioxidant potential. In this context, it is thought that the compounds causing the antioxidant effect of *P. arhizus* can be identified and used as an antioxidant source. In addition, *P. arhizus* is thought that it can be taken as an indicator in terms of Cr and Cd element due to the accumulation of Cr and Cd at high levels in the mushroom. In this context, studies have shown harmful effects of heavy metals exposure on the development of CVD. Heavy metals resulting in pathophysiological changes can cause atherogenic events like increased oxidative stress, inflammatory response, and coagulation activity.

Conflict of interest

The authors declare no conflict of interest.

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