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SHORT COMMUNICATION

Comment on "Study of biological activity of *Tricholoma equestre* fruiting bodies and their safety for human"

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Dear Editor,

The paper by Muszyńska et al. [1] entitled "Study of biological activity of *Tricholoma equestre* fruiting bodies and their safety for human" published in the journal of *European Food Research and Technology* (https://doi.org/10.1007/s00217-018-3134-0) presents the results of a study on the composition of *T. equestre* (syn. *T. flavovirens*, and syn. *T. auratum*) and in vitro anti-microbial and antioxidant activities of this mushroom. As reported, *T. equestre* can be a source of some essential minerals and has a favorable ratio of unsaturated-to-saturated fatty acids, while its extracts exhibit antioxidant effects (as shown in DPPH assay) at levels comparable to other mushroom species, but weak anti-microbial activities. Based on in vitro study using human lung carcinoma epithelial cells (A549 line), the authors suggest that ethanolic extract of *T. equestre* may exhibit pro-inflammatory activities. The authors conclude that consumption of this mushroom should be avoided. We raise some points contrary to this view, highlighting that existing toxicological evidence is insufficient to claim *T. equestre* as inedible or poisonous.

The edibility of *T. equestre* has raised number of controversies. A small number of cases of rhabdomyolysis apparently developed following consumption of very large amounts of this mushroom. It was first reported in 2001 [2–4], and claimed to be supported by the results of in vivo experiments in rodents [2, 5–7]. This forced a number of countries (e.g., France, Spain, and Italy) to officially declare *T. equestre* as poisonous, releasing warnings to avoid its consumption. Prior to this, it had a long -history of consumption in many countries with no anecdotal or scientific evidence of toxic effects. It is still considered edible and widely consumed in regions such as Poland [8]. As shown in a recent study, more than half of mushroom foragers in this country have consumed *T. equestre* at least once in their lifetime [9]. This questionnaire survey and additional analysis of Polish registry of mushroom toxicity demonstrate that no rhabdomyolysis was reported in the last decade and that only mild gastrointestinal effects were noted, at a lower frequency than for other well-established edible wild mushrooms, such as *Macrolepiota procera* and *Imleria badia* [10, 11].

Importantly, the evidence from case reports of rhabdomyolysis lack critical information to indicate unambiguously that *T. equestre* was a causative factor of reported clinical effects. This has been recently extensively reviewed [8]. One possible explanation suggests that rhabdomyolysis could be triggered by consumption of morphologically related but genetically distinctive mushroom species to *T. equestre*. A paper by Muszyńska et al. [1] states that two varieties of *T. equestre* can be distinguished: one associated with *Populus* sp. (known as *T. equestre* var. *populinum*) and *Betula* sp. (known as *T. equestre* var. *pallidifolia*). However, the molecular evidence supports the view that the former belongs to *T. frondosae* clade and the latter is also a representative not belonging to the *T. equestre* species complex [11]. *Tricholoma equestre* is in turn a mycorrhizal group associated particularly with coniferous habitat (mainly *Pinus*) [12]. Rhabdomyolysis has also been reported after the consumption of other edible species including *Agaricus bisporus* or members of *Boletus* and *Leccinum* genera [13].

The in vivo models indicated increases in CK levels (a sensitive marker of rhabdomyolysis) at doses of *T. equestre* which are virtually impossible in human. For instance, in

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studies of Nieminen et al. [6, 7], the effects were observed at doses which would be equivalent to daily consumption of 4 kg of fresh *T. equestre* eaten for five consecutive days by 60 kg subject. Lower but still high dosing (equivalent to 3 kg of mushrooms eaten every day) did not cause significant effects in studied animals [6, 7]. Similar effects in mice were observed after administration of similarly high doses of various other mushroom species with well-established edibility such as *Lentinula edodes, Cantharellus cibarius, Albatrellus ovinus, Leccinium versipelle, Flammulina velutipes*, and *Imleria badia* [5, 6, 14, 15]. This implies that the observed reaction may be unrelated to specific species and cannot be used as an isolated evidence to support a notion that *T. equestre* is a cause or rhabdomyolysis. Toxic compounds have not been identified so date in *T. equestre*, in contrary to other related species—*T. terreum* [16].

Finally, the hypothesis that *T. equestre* causes rhabdomyolysis is contradicted by observations in studies employing volunteers consuming this mushroom [6, 9]. The foragers that consumed 300 g of molecularly identified, fried specimens of *T. equestre* showed no changes in hematological and biochemical markers, including aspartate and alanine aminotransferase, bilirubin, and creatine kinase (CK) [9].

The study by Muszyńska et al. [1] employed an in vitro model of human lung carcinoma epithelial A549 cells to investigate whether ethanol extracts of *T. equestre* may alter levels of cyclo-oxygenase-2 (COX-2), cytosolic prostaglandin E synthetase (cPGES), and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Cells were also activated with pro-inflammatory endotoxin lipopolysaccharide (LPS) as well as exposed to *T. equestre* extract after the LPS activation. As stated, *T. equestre* revealed pro-inflammatory and additive effects in the studied model. However, the results—as presented on Fig. 3—clearly demonstrated that extract of *T. equestre* had only a slight effect on increase of COX-2 but did decrease both cPGES and Nrf2 when compared to vehicle control. No additive effect could also be seen as addition of *T. equestre* extract to LPS-activated cells did not elevate the studied parameters compared to the effects induced solely by LPS. It could even be seen that Nrf2 level was slightly decreased which could potentially indicate that *T. equestre* extract may partially prevent LPS-caused enhancement of intracellular reactive oxygen species.

These results do not support a view that *T. equestre* has an additive pro-inflammatory action, but rather imply that it has no promising pharmaceutical activity to attenuate effects induced by pro-inflammatory factors such as LPS. This cannot be used to conclude that *T. equestre* has human toxicity. Nor should it be compared to the effects observed in vivo in mice in which extremely high doses of mushrooms caused elevation of CK. One should also note that CK is a sensitive marker of myocyte injury [17] and cancer lung A549 cell line does not constitute a relevant model to study such effect. Moreover, the anti-inflammatory properties of mushrooms are mostly attributed to polysaccharides, and these require extraction with hot water or mixtures of specific enzymes [18]. Ethanol extraction will in turn lead to extraction of phenolic compounds that possess antioxidant properties, and as expected exposure of A549 cells (including when cells were activated with LPS) led to decreased levels of Nrf2, one of key molecule responding to oxidative insults [19]. Last but not least, ethanolic extracts of raw *T. equestre* is not how this mushroom is consumed, so one should not extrapolate the results to the human situation.

In summary, there is insufficient evidence that correctly identified *T. equestre* is a cause of rhabdomyolysis or any other specific toxic event. As long as the results presented by Muszyńska et al. [1] provide an interesting perspective on nutritional value of this species, the in vitro experiments in A549 cells do not provide evidence that *T. equestre*, in the manner consumed by humans, is significantly pro-inflammatory or toxic. We also recommend that *T. equestre* should be identified with molecular methods in all future investigations.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Compliance with ethics requirements This paper does not contain any studies with human or animal subjects.

References

1. Muszyńska B, Kała K, Radović J, Sułkowska-Ziaja K, Krakowska A, Gdula-Argasińska J, Opoka W, Kundakovic T (2018) Study of biological activity of *Tricholoma equestre* fruiting bodies and their safety for human. Eur Food Res Technol 244:2255–2264
2. Bedry R, Baudrimont I, Deffieux G, Creppy EE, Pomies JP, Ragnaud JM, Dupon M, Neau D, Gabinski C, De Witte S, Chapalain JC, Godeau P, Beylot J (2001) Wild-mushroom intoxication as a cause of rhabdomyolysis. New Eng J Med 345:798–802
3. Chodorowski Z, Waldman W, Sein Anand J (2002) Acute poisoning with *Tricholoma equestre*. Przegląd Lekarski 59:386–387
4. Laubner G, Mikulevičienė G (2016) A series of cases of rhabdomyolysis after ingestion of *Tricholoma equestre*. Acta Med Litu 23:193–197
5. Nieminen P, Kirsi M, Mustonen AM (2006) Suspected myotoxicity of edible wild mushrooms. Exp Biol Med 231:221–228

6. Nieminen P, Mustonen AM, Kirsi M (2005) Increased plasma creatine kinase activities triggered by edible wild mushrooms. Food Chem Toxicol 43:133–138
7. Nieminen P, Kärjä V, Mustonen AM (2008) Indications of hepatic and cardiac toxicity caused by subchronic *Tricholoma* flavovirens consumption. Food Chem Toxicol 46:781–786
8. Rzymski P, Klimaszyk P (2018) Is the yellow knight's mushroom edible or not? A systematic review and critical viewpoints on the toxicity of *Tricholoma equestre*. Compr Rev Food Sci Food Saf 17:1309–1324
9. Klimaszyk P, Rzymski P (2018) The yellow knight fights back: toxicological, epidemiological, and survey studies defend edibility of *Tricholoma equestre*. Toxins 10:468
10. Gawlikowski T, Romek M, Satora L (2015) Edible mushroom-related poisoning, a study on circumstances of mushroom collection, transport, and storage. Hum Exp Toxicol 34:718–724
11. Moukha S, Férandon C, Beroard E, Guinberteau J, Castandet B, Callac P, Creppy E, Barroso G (2013) A molecular contribution to the assessment of the *Tricholoma equestre* species complex. Fungal Biol 117:145–155
12. Christensen M, Heilmann-Clausen J (2013) The genus of *Tricholoma*. Fungi of Northern Europe, 4 edn. Svampetryk, Tilst, p 228
13. Chwaluk P (2013) Rhabdomyolysis as an unspecyfic symptom of mushroom poisoning—a case report (text in Polish). Przegląd Lekarski 70:684–686
14. Nieminen P, Kärjä V, Mustonen AM (2009) Myo- and hepatotoxic effects of cultivated mushrooms in mice. Food Chem Toxicol 47:70–74
15. Mustonen AM, Määttänen M, Kärjä V, Puukka K, Aho J, Saarela S, Nieminen P (2018) Myo- and cardiotoxic effects of the wild winter mushroom (Flammulina velutipes) on mice. Exp Biol Med 243:639–644
16. Yin X, Feng T, Shang JH, Zhao YL, Wang F, Li ZH, Dong ZJ, Luo XD, Liu JK (2014) Chemical and toxicological investigations of a previously unknown poisonous European mushroom *Tricholoma terreum*. Chemistry 20:7001–7009
17. Huerta-Alardin AL, Varon J, Marik PE (2005) Bench-to-bedside review: rhabdomyolysis—an overview for clinicians. Crit Care 9:158–169
18. Friedman M (2016) Mushroom polysaccharides: chemistry and antiobesity, antidiabetes, anticancer, and antibiotic properties in cells, rodents, and humans. Foods 5:80
19. Ma Q (2013) Role of nrf2 in oxidative stress and toxicity. Annu Rev Pharmacol Toxicol 53:401–426