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REVIEW ARTICLE

AGARITINE: A NATURAL TOXIC AMINO ACID OF CULTIVATED MUSHROOM *AGARICUS* SPP. AND ITS POTENTIAL HEALTH RISK

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Summary

Agaritine, a naturally occurring amino acid and phenylhydrazine derivative found in mushrooms of the genus *Agaricus*, has gained attention due to its potential impact on human health. The presence of the hydrazine moiety in the structure of agaritine plays a crucial role in its toxicological properties. It has raised concerns due to its high reactivity as chemical radicals. Therefore, research is commonly focused on the potential health risks of agaritine and its possible role as a pro-carcinogenic agent. However, some studies did not provide evidence of agaritine's toxicological effects. Therefore, further research is needed to understand agaritine's mechanisms of action and its safe consumption levels in humans. This review aims to provide an overview of current knowledge surrounding agaritine and its potential health risks.

Key words: Agaritine; Hydrazine; Agaricus bisporus; Human health

Introduction

Agaricus spp. is one of the largest genera of macrofungi (1), with many edible and a few poisonous mushrooms, with over 500 members worldwide (2–4). Mushrooms of *Agaricus* species (spp.) belong to the *Agaricaceae*

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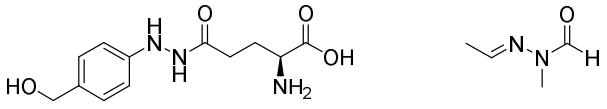
family, and they are generally characterized by small to large basidiomata (fruiting bodies) (5), a fleshy cap from the underside of which grow a series of ray-like gills, annulus, and stem or central stipe (6). Genus *Agaricus* includes the most consumed and cultivated mushroom worldwide (Figure 1), *Agaricus bisporus* (J. E. Lange) Imbach (*A. bisporus*) (1,7), which accounts for 30 % of total mushroom production worldwide (8). However, young fruiting bodies of *A. bisporus* can easily be confused with several deadly species of toadstool, especially *Amanita phalloides* E. M. Fries which is commonly known as the death cap, as well as some other highly poisonous mushrooms (1,9), due to misidentification based on morphological characteristics (10).

Besides various mushrooms of *Agaricus* spp., *A. bisporus*, commonly known as the button mushroom or white mushroom (11), is one of the primary sources of agaritine (12), a naturally occurring α-amino acid and phenylhydrazine derivative (13). The presence of the hydrazine moiety in agaritine is worth considering due to toxicity of hydrazine derivatives. These structures exhibit high chemical reactivity via acting as chemical radicals (14–16). Several studies have described agaritine as a potential carcinogen (17–19). Despite this fact, feeding studies utilizing mushrooms and mushroom extracts have provided no evidence of the toxicological effects of agaritine (13). To the best of our knowledge, there is no direct evidence of a significant health risk in humans associated with consumption of *Agaricus* spp. mushrooms and agaritine. Although effects of agaritine on human health after consumption of agaritine-containing mushrooms are commonly considered insignificant, its potential negative effects are still discussed (20). Moreover, the potential risk of agaritine to human health is increased by the fact that several food supplements on the market contain dried mushrooms or their extracts. These products are commonly recommended as natural remedies for various health conditions and diseases, including cancer (21,22).

Figure 1. Button mushroom or champignon (French for mushroom).

Structure of agaritine

Agaritine, according to International Union of Pure and Applied Chemistry (IUPAC) nomenclature known as (2*S*)-2-amino-5-[2-[4-(hydroxymethyl)phenyl]hydrazinyl]-5-oxopentanoic acid (Figure 2), is a water-soluble derivative of phenylhydrazine. In addition, the compound belongs to the group of α-amino acids, therefore it includes one (*S*)-configured stereocenter. The acylhydrazine structural motif of agaritine is structurally related to formylhydrazine motif of gyromitrin (Figure 2) (13,23), the main poisonous hydrazine derivative present in raw edible wild mushroom *Gyromitra esculenta* (Pers.) Fr. (24–26).



agaritine **gyromitrin**

Figure 2. Structural resemblance of agaritine and gyromitrin.

Agaritine in macromycetes

The content of agaritine varies depending on the species of mushroom (27) and its processing methods (28). Generally, fresh mushrooms contain higher levels of agaritine (*e.g., A. bisporus* mushrooms with 94–629 mg/kg of agaritine) than canned mushrooms (*e.g., A. bisporus* mushrooms with 1–55 mg/kg of agaritine) (13,29). It was found that agaritine breaks down rapidly when cooked and during the process of freezing (18,30,31). Dried commercial mushrooms contain the highest agaritine values, reaching thousands of milligrams (32). However, agaritine oxidizes rapidly during storage and completely decomposes after 48 hours in aqueous solution (27).

Bioactivity of *Agaricus* spp. and agaritine

Ahn *et al.* (2004) investigated the beneficial effects of *Agaricus blazei* Murill Kyowa (ABMK) consumption on immunological status and quality of life in cancer patients undergoing chemotherapy (33). It was observed that ABMK possesses antimutagenic and antitumor activity. Similarly, Akiyama *et al.* (2011) investigated the effects of agaritine on human leukemic monocytic lymphoma (U937) cells. DNA fragmentation, annexin V expression, and cytochrome c release were all induced by agaritine. Caspase-3, 8, and 9 activities gradually increased after treatment with agaritine. These findings suggest that agaritine induces apoptosis in U937 cells moderately (34). Kim *et al*. (2009) used *A. blazei* as an adjuvant in cancer chemotherapy, and it has yielded a variety of antileukemic bioactive compounds. The *in vitro* antileukemic effects were assessed using MTT and tritiated thymidine incorporation assays. The most effective extract was tested further in nude mice containing human promyelocytic leukemia (NB-4) cells. JAB80E70 extract had the most potent tumor-selective growth inhibitory activity against NB-4 and K-562 human leukemia cells. The fraction induced apoptosis in NB-4 cells, according to DNA fragmentation assays and cell death detection by ELISA (35).

Moreover, Adams *et al*. (2008) investigated impact of *A. bisporus* extract and its major component, conjugated linoleic acid (CLA), on prostate cancer cell lines *in vitro* and the effects of mushroom extracts *in vivo*. *In vitro*, the mushroom extract inhibited cell proliferation across various tested cell lines in a dose-dependent manner and triggered apoptosis within 72 hours of treatment. Similarly, CLA hindered expansion in prostate cancer cell lines. *In vivo*, experimentation involving nude mice revealed that the treatment with mushroom extract led to decreased DU145 and PC3 prostate tumor size and reduced tumor cell proliferation while enhancing tumor cell apoptosis compared to pair-fed controls. Microarray analysis of tumors exhibited significant alterations in gene expression in mice treated with mushroom extract in comparison to control. Gene network analysis pinpointed network changes related to cell death, growth, proliferation, lipid metabolism, the TCA cycle, and immune response (36).

Based on the animal models, agaritine undergoes cleavage in kidneys to toxic metabolites 4-(hydroxymethyl)phenylhydrazine and 4-(hydroxymethyl)benzenediazonium ions. Agaritine is sensitive to oxygen (37) and stable at pH 6.8 but not at pH 1.2. Therefore, it may be unstable in the human stomach, where the pH ranges between 2–3 (26). Kondo *et al*. (2008) provided important information on agaritine metabolism in mice. Agaritine concentrations in mouse plasma and urine were measured by LC/MS/MS techniques. After ingestion, agaritine and metabolites such as diazonium ions and free radicals were observed. Agaritine concentrations in mice peaked 20 minutes after oral administration (4.0 and 40 mg/kg). In 100 minutes, the concentration gradually decreased and returned to baseline. After administration of agaritine at the dose of 40 mg/kg body weight, the maximum concentration, time to maximum concentration, and half-life were 0.37 g/mL plasma, 0.33 h, and 0.71 h, respectively (37,38).

It is suggested, that *in vivo* the activation process of agaritine is initiated by removing γ-glutamyl group, catalyzed by a γ-glutamyl transpeptidase located in the liver and kidney. This enzymatic reaction results in the release

of 4-(hydroxymethyl)phenylhydrazine (HMPH), a free hydrazine compound, and leads to the formation of 4-(hydroxymethyl)benzenediazonium (HMBD) ion through enzymatic oxidation (Figure 3). Research findings indicate that agaritine is rapidly metabolized and eliminated from the bloodstream (37,39).

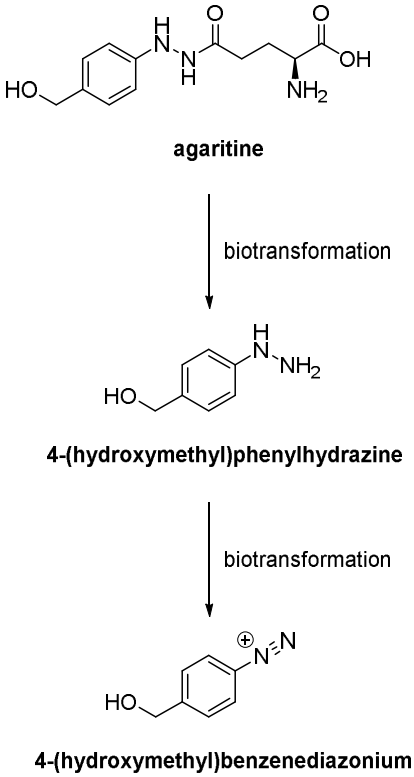


Figure 3. Metabolism of agaritine to 4-(hydroxymethyl)phenylhydrazine (HMPH) and 4-(hydroxymethyl)benzenediazonium (HMBD).

The enzyme system γ-glutamyltransferase in button mushrooms facilitates the breakdown of agaritine into glutamate and HMPH. It's worth noting that HMPH is an unstable compound that is not easily detectable, while HMBD is detected up to a level of 0.6 ppm and is considered mutagenic. 4-(Hydroxymethyl)phenyl radical, a carbon-centered radical, is generated from the 4-(hydroxymethyl)benzenediazonium salt. This radical causes DNA strand breaks, contributing to its carcinogenic properties. Further carcinogenic compounds related to agaritine have also been identified. Specifically, β-*N*-[γ-L-(+)-glutamyl]-4-(carboxy)phenylhydrazine (GCPH), a compound structurally similar to agaritine which bears carboxylic group at *para*-position of benzene ring, also contains a nitrogen-nitrogen bond. This gives rise to another carcinogenic compound, *p*-hydrazinobenzoic acid, through phenyl radical formation. The presence of unidentified metabolites adds complexity to the data, as they might contribute to toxicity. The specific breakdown products of agaritine remain unexplored. An investigation is needed to understand agaritine derivatives or metabolites in mushrooms. While current data suggest potential weak carcinogenicity, particularly in mice, there's a lack of data for humans or other primates (37,39,40).

Carcinogenicity and mutagenicity studies

Numerous feeding studies have consistently generated further evidence concerning the mutagenicity and carcinogenicity of an agaritine-containing diet (37,39). Notably, the chronic consumption of lyophilized button mushrooms also demonstrated carcinogenic effects. A study using the *lacI* transgenic mouse mutation assay (Big Blue mice) shows that only the crude agaritine extracts showed significant genotoxic effects (41). However, weak genotoxicity was later confirmed using orally administered ^{14}C-ring-labelled agaritine, the conclusion was that

agaritine is a mild carcinogen. A comprehensive risk assessment study in mice concluded that agaritine and its derivatives are carcinogenic (42).

Roupas *et al*. (2010) concluded that agaritine from cultivated *A. bisporus* mushrooms possesses no known toxicological risk to healthy humans. No studies show a direct link between agaritine consumption and any carcinogenic effects observed in humans, and agaritine holds the classification of International Agency for Research on Cancer (IARC) group 3, denoting it is "not classifiable as to its carcinogenicity to humans." However, it was stated that a carcinogenic risk to humans could not be ruled out (13). As a result, the cumulative lifetime cancer risk associated with agaritine consumption in mushrooms has been approximated to be around 10^{-5} (37,43).

Conclusions and future perspectives

In summary, agaritine, a phenylhydrazine derivative found in *Agaricus* spp. mushrooms, has raised significant concerns due to its potential impact on human health, particularly its reactivity as chemical radicals. Effects of agaritine, similar as other bioactive compounds, are dose-dependent. Thus, it is not surprising that higher dose is associated with several inappropriate health conditions. On the other side, agaritine may also provide beneficial effects on health. On the basis of studies summarized herein, there is no direct connection between consumption of agaritine-containing mushrooms and agaritine side effects in humans. However, further research is crucial to comprehensively understand agaritine's mechanisms of action and its safe consumption levels. Investigations should focus on elucidating agaritine's precise interaction mechanisms, conducting robust human studies to assess health outcomes, refining risk assessment models, exploring dose-response relationships, considering cooking effects, accounting for human variability, updating regulatory guidelines, and enhancing public awareness. This comprehensive approach will enable a more accurate assessment of agaritine's potential risks and benefits, informing better decisions regarding its consumption and role in human health promotion.

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Author Contributions

Conceptualization, J.P. and U.K.K.; writing—original draft preparation, L.M., U.K.K., M.M., P.O. and J.P.; writing—review and editing, M.M., L.M., O.J.M., Z.N., U.K.K., B.P., P.O. and J.P.; visualization, J.P. and U.K.K.; supervision, J.P. All authors have read and agreed to the published version of the manuscript.

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Adherence to ethical standards

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References

1. Zhang K, Pu Y-Y, Sun D-W. Recent advances in quality preservation of postharvest mushrooms (Agaricus bisporus): A review. Trends in Food Science & Technology. 2018;(78):72–82. DOI: 10.1016/j.tifs.2018.05.012
2. Thongklang N, Hoang E, Rodriguez Estrada AE, et al. Evidence for amphithallism and broad geographical hybridization potential among Agaricus subrufescens isolates from Brazil, France, and Thailand. Fungal Biol. 2014;118(12):1013–1023. DOI: 10.1016/j.funbio.2014.10.004

3. Thongklang N, Nawaz R, Khalid AN, et al. Morphological and molecular characterization of three Agaricus species from tropical Asia (Pakistan, Thailand) reveals a new group in section Xanthodermatei. Mycologia. 2014;106(6):1220–1232. DOI: 10.3852/14-076
4. He M-Q, Chen J, Zhou J-L, et al. Tropic origins, a dispersal model for saprotrophic mushrooms in Agaricus section Minores with descriptions of sixteen new species. Sci Rep. Nature Publishing Group; 2017;7(1):5122. DOI: 10.1038/s41598-017-05203-5
5. Sitotaw R, Lulekal E, Abate D. Ethnomycological study of edible and medicinal mushrooms in Menge District, Asossa Zone, Benshangul Gumuz Region, Ethiopia. J Ethnobiol Ethnomed. 2020;16(1):11. DOI: 10.1186/s13002-020-00361-9
6. Joshi M, Bhargava P, Bhatt M, et al. Mushrooms of Gujarat. Springer Nature; 2022.
7. Firenzuoli F, Gori L, Lombardo G. The Medicinal Mushroom Agaricus blazei Murrill: Review of Literature and Pharmaco-Toxicological Problems. Evid Based Complement Alternat Med. 2008;5(1):3–15. DOI: 10.1093/ecam/nem007
8. Royse D. A global perspective on the high five: Agaricus, Pleurotus, Lentinula, Auricularia & Flammulina. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8); 2014.
9. Lewinsohn D, Lurie Y, Gaon A, et al. The epidemiology of wild mushroom poisoning in Israel. Mycologia. 2023;115(3):317–325. DOI: 10.1080/00275514.2023.2177471
10. Karlson-Stiber C, Persson H. Cytotoxic fungi--an overview. Toxicon. 2003;42(4):339–349. DOI: 10.1016/s0041-0101(03)00238-1
11. ten Have R, Wijngaard H, Ariës-Kronenburg NAE, et al. Lignin degradation by Agaricus bisporus accounts for a 30% increase in bioavailable holocellulose during cultivation on compost. J Agric Food Chem. 2003;51(8):2242–2245. DOI: 10.1021/jf021131h
12. Gry J, Andersson C, Krüger L, et al. Mushrooms traded as food. Copenhagen: Nordic Council of Ministers; 2012.
13. Roupas P, Keogh J, Noakes M, et al. Mushrooms and agaritine: A mini-review. Journal of Functional Foods. 2010;2(2):91–98. DOI: 10.1016/j.jff.2010.04.003
14. Ross AE, Nagel DL, Toth B. Evidence for the occurrence and formation of diazonium ions in the Agaricus bisporus mushroom and its extracts. J Agric Food Chem. 1982;30(3):521–525. DOI: 10.1021/jf00111a028
15. Spencer PS. Hypothesis: Etiologic and Molecular Mechanistic Leads for Sporadic Neurodegenerative Diseases Based on Experience With Western Pacific ALS/PDC. Front Neurol. 2019;10:754. DOI: 10.3389/fneur.2019.00754
16. Toth B. Synthetic and naturally occurring hydrazines as possible cancer causative agents. Cancer Res. 1975;35(12):3693–3697.
17. Toth B, Sornson H. Lack of carcinogenicity of agaritine by subcutaneous administration in mice. Mycopathologia. 1984;85(1–2):75–79. DOI: 10.1007/BF00436706
18. Hashida C, Hayashi K, Jie L, et al. [Quantities of agaritine in mushrooms (Agaricus bisporus) and the carcinogenicity of mushroom methanol extracts on the mouse bladder epithelium]. Nihon Koshu Eisei Zasshi. 1990;37(6):400–405.
19. Singh K, Kumari A. Mushroom Toxins and Health Hazards Associated with Them. Mycotoxins and Mycotoxicoses. Singapore: Springer Nature Singapore; 2022.
20. Masroor M. Do Mushrooms also Cause Cancer in Human? EC Emergency Medicine and Critical Care. 2021;5:20–27.
21. Hess J. Mushrooms, Snacks, and Dairy Foods: Health Impacts, Consumption Patterns, and Dietary Guidance. 2018
22. Weisburger J, Barnes W, Czerniak R. Mutagens and carcinogens in food. Diet, nutrition and cancer: a critical evaluation. 1st Edition. CRC Press; 2018.
23. Lagrange E, Vernoux J-P. Correction: Lagrange, E.; Vernoux, J.-P. Warning on False or True Morels and Button Mushrooms with Potential Toxicity Linked to Hydrazinic Toxins: An Update. Toxins 2020, 12, 482. Toxins (Basel). 2022;14(5):345. DOI: 10.3390/toxins14050345
24. Coulet M, Guillot J. Poisoning by Gyromitra : a possible mechanism. Med Hypotheses. 1982;8(4):325–334. DOI: 10.1016/0306-9877(82)90024-x
25. Patocka J, Pita R, Kuca K. Gyromitrin, mushroom toxin of Gyromitra spp. Mil Med Sci Lett. 2012;81:61–67. DOI: 10.31482/mmsl.2012.008
26. Hajslová J, Hájková L, Schulzová V, et al. Stability of agaritine - a natural toxicant of Agaricus mushrooms. Food Addit Contam. 2002;19(11):1028–1033. DOI: 10.1080/02652030210157691

27. Schulzova V, Hajslova J, Peroutka R, et al. Agaritine content of 53 Agaricus species collected from nature. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. 2009;26(1):82–93. DOI: 10.1080/02652030802039903
28. Andersson HC, Hajslová J, Schulzová V, et al. Agaritine content in processed foods containing the cultivated mushroom (Agaricus bisporus) on the Nordic and the Czech market. Food Addit Contam. 1999;16(10):439–446. DOI: 10.1080/026520399283830
29. Fischer B, Lüthy J, Schlatter C. [Rapid determination of agaritin in commercial mushrooms (Agaricus bisporus) with high performance liquid chromatography]. Z Lebensm Unters Forsch. 1984;179(3):218–223. DOI: 10.1007/BF01041897
30. Liu J-W, Beelman R, Lineback D, et al. Agaritine Content of Fresh and Processed Mushrooms [Agaricus bisporus (Lange) Imbach]. Journal of Food Science. 1982;47(5):1542–1544.
31. Koge T, Komatsu W, Sorimachi K. Heat stability of agaritine in water extracts from Agaricus blazei and other edible fungi, and removal of agaritine by ethanol fractionation. Food Chemistry. Food Chemistry 2011;126:1172–1177. DOI: 10.1016/j.foodchem.2010.11.153
32. Sharman M, Patey AL, Gilbert J. A survey of the occurrence of agaritine in U.K. cultivated mushrooms and processed mushroom products. Food Addit Contam. 1990;7(5):649–656. DOI: 10.1080/02652039009373930
33. Ahn W-S, Kim D-J, Chae G-T, et al. Natural killer cell activity and quality of life were improved by consumption of a mushroom extract, Agaricus blazei Murill Kyowa, in gynecological cancer patients undergoing chemotherapy. Int J Gynecol Cancer. 2004;14(4):589–594. DOI: 10.1111/j.1048-891X.2004.14403.x
34. Akiyama H, Endo M, Matsui T, et al. Agaritine from Agaricus blazei Murrill induces apoptosis in the leukemic cell line U937. Biochim Biophys Acta. 2011;1810(5):519–525. DOI: 10.1016/j.bbagen.2011.02.010
35. Kim C-F, Jiang J-J, Leung K-N, et al. Inhibitory effects of Agaricus blazei extracts on human myeloid leukemia cells. J Ethnopharmacol. 2009;122(2):320–326. DOI: 10.1016/j.jep.2008.12.025
36. Adams LS, Phung S, Wu X, et al. White button mushroom (Agaricus bisporus) exhibits antiproliferative and proapoptotic properties and inhibits prostate tumor growth in athymic mice. Nutr Cancer. 2008;60(6):744–756. DOI: 10.1080/01635580802192866
37. Lagrange E, Vernoux J-P. Warning on False or True Morels and Button Mushrooms with Potential Toxicity Linked to Hydrazinic Toxins: An Update. Toxins (Basel). 2020;12(8):482. DOI: 10.3390/toxins12080482
38. Kondo K, Watanabe A, Akiyama H, et al. The metabolisms of agaritine, a mushroom hydrazine in mice. Food Chem Toxicol. 2008;46(3):854–862. DOI: 10.1016/j.fct.2007.10.022
39. Spencer PS, Okot C, Palmer VS, et al. Nodding syndrome: A key role for sources of nutrition? eNeurologicalSci. 2022;27:100401. DOI: 10.1016/j.ensci.2022.100401
40. Ferrucci V, Boffa I, De Masi G, et al. Natural compounds for pediatric cancer treatment. Naunyn Schmiedebergs Arch Pharmacol. 2016;389(2):131–149. DOI: 10.1007/s00210-015-1191-5
41. Shephard SE, Gunz D, Schlatter C. Genotoxicity of agaritine in the lacI transgenic mouse mutation assay: evaluation of the health risk of mushroom consumption. Food Chem Toxicol. 1995;33(4):257–264. DOI: 10.1016/0278-6915(94)00142-b
42. Shephard SE, Schlatter C. Covalent binding of agaritine to DNA in vivo. Food Chem Toxicol. 1998;36(11):971–974. DOI: 10.1016/s0278-6915(98)00076-3
43. Gry J, Andersson C. Mushrooms traded as food. Vol II sec 2: Nordic risk assessments and background on edible mushrooms, suitable for commercial marketing and background lists for industry, trade and food inspection. Risk assessments of mushrooms on the four guidance lists: Nordic Council of Ministers; 2014.