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REVIEW ARTICLE

BOLESATINE, A TOXIC PROTEIN FROM THE MUSHROOM *RUBROBOLETUS* SATANAS

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Summary

Rubroboletus satanas (Lenz) Kuan Zhao & Zhu L. Yang, known as *Boletus satanas* (Lenz) until 2014, and commonly known as the Devil's bolete or Satan's bolete, is a basidiomycete mushroom of the bolete family. Grows in mixed woodlands in the southern, warmer regions of Europe and North America. Satan's bolete generally regarded as a poisonous mushroom, with predominantly gastrointestinal symptoms of nausea and violent vomiting occurring if eaten raw or isufficiently heat-treated. The toxicity of the mushroom corresponds to a toxic protein called bolesatine. Bolesatine is a toxic glycoprotein which has been shown to inhibit protein synthesis in cell-free systems and cell culture and is toxic to rodents. Biology, chemistry, pharmacology and toxicology of bolesatine is discussed in this article.

Key words: Rubroboletus satanas; Boletus satanas; poisoning mushroom; toxic peptide; bolesatine

INTRODUCTION

The estimated number of mushroom species growing in Europe ranges from 2 thousands to 1.5 million (Barney, 1977). Of these species, less than 100 are known to be poisonous (Chang, 1996). Such a mushroom that is ranked among the poisonous is also *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang (2014) commonly known as the Satan's bolete or Devil's boletus. It is a basidiomycete fungus of the bolete family, which was known scientifically as *Boletus satanas* (Lenz) until 2014 (Janda a Kříž, 2016). In the southern regions of Europe and also in Great Britain, it is generally regarded as a poisonous mushroom, with predominantly gastrointestinal symptoms of nausea and violent vomiting occurring if eaten raw or fried. In Britain, this fungus is one of the 10 most toxic mushrooms (Pegler and Watling, 1982). On the other hand, information appears in the unscientific press that, after thorough cooking, the fungus is harmless. The main toxic principle of the mushroom Satan's bolete is considered to be bolesatine, a potent cytotoxic glycoprotein, which has previously been shown to be an inhibitor of protein synthesis in several *in vitro* and *in vivo* systems.

RUBROBOLETUS SATANAS TAXONOMY

Satan's bolete is a mushroom filled with many people's superstitions and bachorks. One of them is his proverbial toxicity, which probably owes the common name. Most people think know this mushroom, but the opposite is true.

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Satan's bolete is a rare mushroom. Information about Satan's toxicity is contradictory and contains many ambiguities. At present, it is predominant among mushrooming peoples that it is not poisonous if the food prepared from it is cooked well. However, due to its rarity and possible confusion with other similar mushrooms, this information can be very misleading. There is much more to those who have heard of than to those who have seen it (Kubička et al., 1980).

In the Czech Republic Satan's bolete is a rare mushroom and it is a great coincidence to meet her. It grows in July to September under deciduous trees on warm limestone substrates (Kubička et al., 1980). It is one of our largest bolete mushroom. (Fig. 1). It can be up to 30 cm in diameter and can weigh up to 1 kg. The compact cap can be up to 30 cm. When young, the surface of the cap is greyish white, when older it tends more to a greenish ochre or leather colour. The stipe is 5–15 cm long and is often very bulbous. Usually it is wider than it is long and when young it is even almost spherical. The free tubes are up to 3 mm long. At first they are pale yellow or greenish yellow before soon reddening and are already entirely purplish red or carmine before full maturity. It has a yellow background covered with a hexagonal close-meshed net that starts bright red and turns dark blood-red and which sometimes reaches to the yellowish base layer (Zeitlmayr, 1976).

Figure 1. *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang, syn. *Boletus satanas* Lenz, commonly known as the Devil's bolete or Satan's bolete. Photo: Václav Burle.

RUBROBOLETUS SATANAS AND ITS TOXICOLOGICAL SECRETS

Satan's bolete toxicity has been witnessed by Professor Harald Othmar Lenz, who professionally described the first mushroom in 1831 (Nilson and Persson, 1977). Circumstances of poisoning and its course are colorfully depicted in his book "Mushrooms edible and similar to them poisonous" Czech mycologist Jan Bezděk (1901).

He writes that on September 12, 1830, Professor Lenz and his friend - a student of medicine, Karel Salzmann - went to mushrooms. Among other things, they found the fetters of Satan's bolete who were interested in it, because in the works of previous scholars this type was not recorded. After poisoning the fungus and finding out how unpleasant the poison was, he gave him the scientific name *Boletus satanas* (German Satanpilz).

The next day at 10 a.m. Professor Lenz tasted a piece of fresh mushroom. The taste was quite pleasant, but he spat out the sponge after the bite. An hour and a half later, a strange feeling resembling a faint stroke struck him for a few minutes. He did not associate him with the effect of a mushroom, and in the afternoon he tasted another piece. In the evening there was general malaise and repeated vomiting. The urge to vomit came slowly but regularly. That night he'd twitched about twenty times, and it was very disturbing. There was an extreme weakness that he could only get up and walk with great difficulty. He healed drinking olive and linseed oil. Only the third day he felt good again.

The fact that Lenz's troubles were a consequence of consuming poisonous mushrooms was not convincing the professor until his friend Karel Salzmann had poisoned himself. He had prepared a dish with bacon, onion, and flour from one Satan's bolete fungus, which he ate for dinner. Health problems occurred about an hour after eating. Painful stomach cramps and severe bloody diarrhea have occurred. The temperature was barely noticeable, the body temperature dropped, and the whole body was hurt. An hour after midnight, a summoned physician Richter of Valtershausen came in, and in the morning Dr. Kerst of Rothy, a member of the medical council, but his condition did not improve and he did not know the advice or the healer. He was thirteen times more likely than the repeat dose of opium to suppress vomiting. Only the third day after poisoning he could get out of bed and walk for a while. Outside the house he dared to go for two more days. Still, he watched the overall weakness that faded after 2-3 weeks.

A similar unpleasant experience with Satan's bolete was also experienced by an important Czech mycologist of the first half of the 19th century, the university Professor MUDr. Julius Vincenc Krombholz. With this mushroom he met in Prague at the marketplace, where the salesman offered him together with a royal bolete (*Butyriboletus regius* formerly *Boletus regius*). Krombholz tasted a small piece of hat and, at the advice of the vendor, bought all of the puppies that he handed over to the painter Šíra to paint them. When he began to observe mild dizziness and nausea after an hour, he did not associate these symptoms with the use of a mushroom. But when he visited the designer again and caught him with his severe abdominal pain and vomited blood because he tasted the larger piece of the mushroom, he did not have the slightest doubt about her toxicity. The Krombholz's student and Krombholz's scribbler were poisoned by the mushroom as they both tasted a bit of a fungus and then a prosecutor who took one mushroom in Krombholz's absence (estimated at about 50 grams) and prepared it for lunch for butter. All nausea, weakness, dizziness, anxiety, blurred vision, hearing loss, tinnitus and violent vomiting have been observed.

As reported in his book Bezdek (1901), the prosecutor, who was found to have freezing in his back, increased sensation of the abdomen, heartbeat, mouth dryness, unexplained thirst and violent vomiting of all fluids received. At midnight, fits of rage and blood appeared in the vomiting and the stool. The patient was treated with abdominal bruising with a mixture of oil and bayonet extract, and the mustard flour cladding was served with barley broth. Reconvalescence was very slow. For a long time he felt nausea, severe abdominal pain, resistance to beverages, and also cold air. The idea of a mushroom dish caused him to get sick more than a month after the event. The Czech mycologist František Smotlacha (Smotlacha, 1947), who in 1908 tasted a bit of Satan's bolete, also describes the poison of Satan in his own preparation.

Most of the information on the toxicity of Satan's bolete and the clinical signs of poisoning is known from the mycologists themselves who did not hesitate to test the effects of poison on their bodies. However, scientific literature is silent. We have very little information about such poisoning (Kohn and Moťovská, 1997).

BOLESATINE

First attempts to isolate poison and its characteristics have led to the finding that it is a protein (Kretz et al., 1989) and it is therefore not surprising that the heat denatures and loses its biological activity. The poisonous protein was called bolesatine and was found to in vitro inhibit the synthesis of proteins in the cell. Using 14C isotope-labeled bolesatine, its distribution was monitored in the mouse. Shortly after administration, the bolesatine occurs in the stomach and the intestines, kidneys and the liver, and only a little later in the spleen and lungs (Kretz et al., 1991).

Monitoring of bolesatine in subcellular fractions of rat liver and kidney has been shown to be present in all fractions: cytoplasm, mitochondria, ribosomes, microsomes even in the nucleus. It eliminates both the urine and the faeces, and 80% of poison is eliminated in the first 24 hours after intoxication. No decomposition occurs and none of the common enzymes of the protease type (trypsin, chymotrypsin, pronase, proteinase K) hydrolyze neither native nor heat-denatured bolesatine (Kretz et al., 1991a).

Chemistry

In other studies, bolesatine was characterized as a glycoprotein per mole. weighing 63 ± 3 kDa and isoelectric point pI of 8.3 ± 0.1 and one disulphide intrachain bridge (Kretz et al., 1991b). Bolesatine acts as an inhibitor of proteosynthesis, but studies of its toxicity at the molecular level have been found not to function as other inhibitors of this type known as ribosome-inactivating proteins (Kretz et al., 1992a). Bolesatine was characterized as a single-stranded protein and a portion of its amino acid sequence at the N-terminus (NH_2-Thr-Trp-Arg-Ile-Tyr-Leu-Asn-Asn-Gln-Thr -Val-Lys- Leu-Leu-Leu-Pro-Asn-Gly ....) (Kretz et al., 1992b). Bolesatine has lectin properties (Licastro et al., 1993). Lectins form a large group of proteins of non-immune origin that can recognize and bind sugars, both free and glycoprotein-bound or glycolipids, with a high degree of specificity. They do not catalyze any chemical reaction, they do not show enzymatic activity. Lectins are involved in many processes that require specific recognition (immunological reactions, tissue contact, host-pathogen interactions, etc.). The first discovered lectins were for their ability to agglutinate (clumping, precipitating) red blood cells called agglutinins. Many of these are highly toxic. Such a lectin is, for example, a well-known *Ricinus communis* ricin, which was seriously considered as a chemical warfare during the Cold War (Patočka, 2004).

Toxicology

However, the toxicity of bolesatine does not reach the toxicity of ricin. While the mean death rate (LD_{50}) of mouse ricin in parenteral routes is in the range of tens of micrograms /kg (Patocka, 2001), the LD_{50} for mice or rats at intraperitoneal administration is 1 mg/kg and, intravenously, 0.14 mg/kg. The LD_{50} for mice for oral administration of bolesatine is 3 mg/kg (Kretz et al., 1991a). Bolesatine is approximately 100 times less toxic than ricin, but it does not mean it is not a dangerous poison. All substances with an LD_{50} size of less than 5 mg/kg are classified as supertoxic substances (Patočka, 2003).

Clinical Signs

Bolesatine causes dangerous gastroenteritis, a disease characterized by inflammation of the digestive tract, affecting both the stomach and the small intestine, and ultimately resulting in a combination of diarrhea, vomiting, convulsions and abdominal pain. Gastroenteritis leads to losses of fluids and minerals from the body, which can be very serious in risky groups of patients (young children, the elderly, people with other illness) (Kretz et al., 1991).

Mechanism of action

Bolesatine in addition to inhibiting proteosynthesis, has a mitogenic activity in human lymphocytes that is at least 200-fold higher than that of other lectins (Ennamany et al., 1994). Mitogenic activity of bolesatine is mediated either by activation of the phospholipid/calcium dependent protein kinase or indirectly by activation of InSP3 inositol receptors (Ennamany et al., 1995a). In addition, it induces bolesatine in lipid peroxidation and activates the production of free radicals, thereby contributing to the induction of programmed cell death (Ennamany et al., 1995b). Bolesatine also causes agglutination of red blood cells and platelets (Gachet et al., 1996), which leads to the formation of blood thrombi (clots). The generation of thrombi can be prevented by the administration of aspirin, ticlopidine or heparin, and French authors (Ennamany et al., 1998) believe that these antithrombotic drugs could be used in Satan's bolete poisoning. These drugs, however, can not prevent the death of animals receiving a high dose of bolesatine (Patočka and Burle, 2012).

SATAN'S BOLETE POISONING

The protein nature of the poison explains why the heat treatment of the Satan's bolete leads to the loss of its toxicity. Heat degrades bolesatine like any other protein and loses its toxicity. Therefore, if Satan's bolete is heated

for a long time, the food should be prepared to be safe. As we have already explained, in a raw state, the muhroom is very poisonous. A case has been described where poisoning accompanied by two-hour vomiting and all-day abdominal pain caused only a taste of a piece of raw mushroom without pulsing the pulp (Kašpar, 1921). Preparing any dish from Satan's bolete is also risky. We are never sure that all bolestatin will be denatured in the kitchen. The experience of the writers who first botanically described Satan's honey and tasted food from it does not prove that its consumption was without risk (Bezděk, 1901). Similar results have been reported by later work (Edwards and Henry, 1989; Köppel, 1993; Pore, 1993; Schenk-Jaeger et al., 2012).

BOLESATINE AND PROTEOSYNTHESIS

The influence of bolesatine ion proteosynthesis is difrent form other cytostatics. Bolesatine cannot be included in the group of protein synthesis inhibitors of plant origin known as ribosome-inactivating proteins (RIPs). Bolesatine also does not have a direct effect on elongation factors but hydrolyses nucleoside triphosphates, GTP and ATP with consequent inhibition of protein synthesis. Thus, bolesatine should be classified as a nucleoside triphosphate phosphatase, rather than as a direct inhibitor of protein synthesis. The mechanism whereby bolesatine affects protein synthesis probably involves GTP hydrolysis rather than EF-2 inhibition (Ennamay et al., 1995c).

BOLESATINE PERSPECTIVES

Mushrooms are a valuable source of novel lectins with unique specificities and potentials for biotechnological and biomedical applications. Lectins recognizing sugar moieties in cell walls or cell membranes alter the membrane physiology and trigger biochemical changes in the cell. Thus, various applications of lectins have been described, for example as tools to identify aberrant glycans expressed by neoplastic cells and as antitumor agents by inducing apoptosis by various mechanisms (Sarup Singh et al., 2016). It may help to exploit these biomolecules, as for as bolesatine, as potential novel antitumor drugs in near future.

BOLEVENINE

A toxic protein, called bolevenine, was isolated from the toxic mushroom *Boletus venenatus* based on its lethal effects on mice. On SDS-PAGE, in either the presence or absence of 2-mercaptoethanol, this protein showed a single band of approximately 12 kDa. In experiments based on gel filtration and MALDI-TOFMS, its relative molecular mass was estimated to be approximately 30 kDa, indicating that the protein consists of three identical subunits. This toxin exhibited its lethal activity following injection at 10 mg/kg into mice. The N-terminal amino acid sequence was determined up to 18 (NH_2-Thr-Trp-Ser-Ala-Phe-Leu-Asn-Asn-Gln-Ser-Val-Lys-Leu-Ala-Met-Leu-Leu-Pro ....) and found to be similar to the previously reported bolesatine (Matsuura et al., 2007).

CONCLUSION

Bolesatine is a glycoprotein (single chain, Mr 63,000 ± 3000, pI 8.3 ± 0.1, disulphide intrachain bridge) from *Rubroboletus satanas*, a toxic mushroom which causes serious gastroenteritis. This glycoprotein possesses a mitogenic activity on human lymphocytes at very low concentrations, whereas higher concentrations inhibits protein synthesis in vitro in several systems. Bolesatine and similar fungal toxic proteins, such as bolevenine, have the potential of new drugs against cancer. As shown by Basset et al. (1995) bolesatine inhibits *in vitro* protein synthesis in a concentration-dependent manner in a cell line from a radiation-induced thymic lymphosarcoma (SP2/O). *In vivo*, in Balb/c mice having ascitic tumour induced by the i.p. preinjection of SP2/O cells allows a remission of 50% and 30%, respectively. Treated mice survived 120 days after the treatment, i.e. 90 days after the death of control animal.

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CONFLICT OF INTEREST

Author declare no conflict of interest.

REFERENCES

1. Barney DL. Growing mushroom commercially - Risks and Opportunities. University of Idaho, 1977; 8 pp. Available at: http://www.cals.uidaho.edu/edcomm/pdf/cis/cis1077.pdf
2. Basset L, Ennamany R, Portail J P, Kretz O, Deffieux G, Badoc A, Guillemain B, Creppy E E. Effects of bolesatine on a cell line from the SP2/O thymic lymphosarcoma. Toxicology. 1995;103(2):121-125.
3. Bezděk J. Mushrooms edible and similar to them poisonous. Prague, Own cost. 1901. p. 33-38.
4. Chang S T. 1996. Mushroom research and development - equality and mutual benefit. Mush Biol Mush Prod. 1996;2:1-10.
5. Edwards J N, Henry J A. Medical problems of mushroom ingestion. Mycologist. 1989;3(1):13-15.
6. Ennamany R, Bingen A, Creppy E E, Kretz O, Gut J P, Dubuisson L, Balabaud C, Bioulac Sage P, Kirn A. Aspirin and heparin prevent hepatic blood stasis and thrombosis induced by the toxic glycoprotein Bolesatine in mice. Hum Exp Toxicol. 1998;17(11):620-624.
7. Ennamany R, Kretz O, Badoc A, Deffieux G, Creppy E E. Effect of bolesatine, a glycoprotein from Boletus satanas, on rat thymus in vivo. Toxicology. 1994;89(2):113-118.
8. Ennamany R, Kretz O, Creppy E E. Effect of bolesatine on phospholipid/calcium dependent protein kinase in Vero cells and in rat thymus. Arch Toxicol. 1995a;69(9):624-630.
9. Ennamay R, Lavergne J P, Reboud J P, Dirheimer G, Creppy E E. Mode of Action of Bolesatine, a Glycoprotein from Boletus Satanas Lenz, Mechanistic Approaches. Toxicology Letters. 1995c;78(S1):31-32.
10. Ennamany R, Marzetto S, Saboureau D, Creppy E E. Lipid peroxidation induced by bolesatine, a toxin of Boletus satanas: implication in m5dC variation in Vero cells related to inhibition of cell growth. Cell Biol Toxicol. 1995b;11(6): 47-354.
11. Gachet C, Ennamany R, Kretz O, Ohlmann P, Krause C, Creppy E E, Dirheimer G, Cazenave J P. Bolesatine induces agglutination of rat platelets and human erythrocytes and platelets in vitro. Hum Exp Toxicol. 1996; 15(1): 26-29.
12. JandaV, Kříž M. Rubroboletus satanas f. crataegi, validly published name for santhoid form of Rubroboletus satanas. Czech Mycology 2016;68(1),109-110.
13. Kašpar F. Poisoning with the Satan's bolete. In Smotlacha F. Magazine of Czechoslovak Mushrooms. Prague, B. Kočí Publishing House, 1921. Year II. 1920-1921, p. 287. Article In Czech.
14. Köppel C. Clinical symptomatology and management of mushroom poisoning. Toxicon. 1993;31(12):1513-1540.
15. Kretz O, Barbieri L, Creppy E E, Dirheimer G. Inhibition of protein synthesis in liver and kidney of mice by bolesatine: mechanistic approaches to the mode of action at the molecular level. Toxicology. 1992a;73(3):297-304.
16. Kretz O, Creppy E E, Dirheimer G. Characterization of bolesatine, a toxic protein from the mushroom Boletus satanas Lenz and it's effects on kidney cells. Toxicology. 1991b;66(2):213-224.
17. Kretz O, Creppy E E, Boulanger Y, Dirheimer G. Purification and some properties of bolesatine, a protein inhibiting in vitro protein synthesis, from the mushroom Boletus satanas Lenz (Boletaceae). Arch Toxicol Suppl. 1989;13,422-427.
18. Kretz O, Creppy E E, Dirheimer G. Disposition of the toxic protein, bolesatine, in rats: its resistance to proteolytic enzymes. Xenobiotica. 1991a;21(1):65-73.
19. Kretz O, Reinbolt J, Creppy E E, Dirheimer G. Properties of bolesatine, a translational inhibitor from Boletus satanas Lenz. Amino-terminal semence determination and inhibition of rat mitochondrial protein synthesis. Toxicol Lett. 1992b; 64-65 Spec No: 763-766.
20. Licastro F, Morini M C, Kretz O, Dirheimer G, Creppy E E, Stirpe F. Mitogenic activity and immunological properties of bolesatine, a lectin isolated from the mushroom Boletus satanas Lenz. Int J Biochem. 1993; 25(5): 789-792.
21. Matsuura M, Yamada M, Saikawa Y, Miyairi K, Okuno T, Konno K, Uenishi J, Hashimoto K, Nakata M. Bolevenine, a toxic protein from the Japanese toadstool Boletus venenatus. Phytochemistry. 2007; 68(6): 893-898.
22. Nilson S, Persson O. Fungi of Northern Europe 1: Larger Fungi (Excluding Gill-Fungi). Penguin; 1977. p. 104. ISBN 0-14-063005-8.
23. Kohn R, Mot'ovská Z. [Mushroom poisoning--classification, symptoms and therapy]. Vnitr Lek. 1997;43(4):230-233. Article in Slovak.

24. Kubička J, Erhart J, Erhartová M. [Toxic mushrooms]. Prague, Avicenum. 1980, 248 pp. P. 30, 45-46, 206. Article in Czech.
25. Patočka J. Abrin and ricin - two dangerous poisonous proteins. ASA Newletter. 2001;85:20-22.
26. Patočka J, Burle V. [Satan's Bolete]. Vesmír 2012;91(10):574. Article in Czech.
27. Patočka J. (Ed.) [Military Toxicology]. Prague, Grada 2004. pp. 178. ISBN 8024706083. Article in Czech.
28. Pegler DN, Watling R. British toxic fungi. Bull Brit Mycol Soc. 1982;16,66-75.
29. Pore RS. Mushroom poisoning. In Fungal Infections and Immune Responses. Springer US, 1993; p. 493-519.
30. Sarup Singh R, Preet Kaur H, Rakesh Kanwar J. Mushroom lectins as promising anticancer substances. Current Protein Peptide Sci. 2016;17(8),797-807.
31. Schenk-Jaeger K M, Rauber-Lüthy C, Bodmer M, Kupferschmidt H, Kullak-Ublick G A, Ceschi A. Mushroom poisoning: A study on circumstances of exposure and patterns of toxicity. Europ J Int Med. 2012;23(4):e85-e91.
32. Smotlacha F. [Atlas of edible and inedible mushrooms]. Prague, Melantrich, 1947. 297 pp. Article in Czech.
33. Zeitlmayr L. Wild Mushrooms: An Illustrated Handbook. Garden City Press, Hertfordshire, 1976; p. 102. ISBN 0-584-10324-7.