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Lack of subchronic toxicity of an aqueous extract of *Agaricus blazei* Murrill in F344 rats

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Abstract

Agaricus blazei Murrill, an edible mushroom, is widely used as a functional food due to its possible medicinal effects. Aqueous extracts are also used as food additive to provide an agreeable bitter taste. As a part of its safety assessment, the present 90-day subchronic toxicity study was performed in F344 rats. To establish a no-observed-adverse-effect level (NOAEL), rats were fed powder diet containing *A. blazei* Murrill aqueous extract at dose levels of 0 (basal diet), 0.63, 1.25, 2.5 and 5% (maximum) for 90 days. During the experiment, there were no remarkable changes in general appearance and no deaths occurred in any experimental group. Although serum blood urea nitrogen was slightly but significantly increased in males of the 2.5 and 5% groups, no related histopathological changes were observed in the kidney, and serum creatinine levels were rather reduced, suggesting the increase of blood urea nitrogen to be of little toxicological significance. Hematology, organ weight measurement and histopathological observation revealed no test compound-related toxicological changes. In conclusion, *A. blazei* Murrill extract even at 5% in the diet (2654 mg/kg b.w./day for male rats and 2965 mg/kg b.w./day for female rats) did not cause remarkable adverse effects in F344 rats. Thus, the NOAEL was concluded to be 5% in the diet.

Keywords: *Agaricus blazei*; Murrill extract; Subchronic toxicity; F344 rat; Food additive

1. Introduction

Agaricus blazei Murrill, an edible mushroom belonging to the *Agaricaceae* family, is native to southern Brazil. It is popularly known as "Himematsutake" in Japan. At present, *A. blazei* Murrill is widely used as a medicinal and functional food rather than for nutrition proposes because of its potent medicinal properties. Although it was traditionally consumed as tea, various kinds of healthy foods containing *A. blazei* Murrill and/or its extracts are also produced in some countries such as Brazil, China and Japan and traded in the world. Aqueous extracts from the mycelium, fruit bodies or culture solution of *A. blazei* Murrill have been approved in Japan as food additive giving a bitter taste (MHLW, 1996a; JETRO, 2004).

A. blazei Murrill has tested negative for mutagenicity in the Ames, micronucleus, chromosome aberration, sister chromatid exchange, and comet assays (Osaki et al., 1994; Menoli et al., 2001; Luiz et al., 2003;

Abbreviations: AlB, albumin; AlP, alkaline phosphatase; AlT, alanine aminotransferase; AsT, aspartate aminotransferase; A/G, albumin/globulin ratio; BUN, blood urea nitrogen; Ca, calcium; Cl, chloride; CRN, creatinine; γ-GT, γ-glutamyl aminotransferase; Hb, hemoglobin; Ht, hematocrit; K, potassium; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; Na, sodium; NOAEL, no-observed-adverse-effect level; P, inorganic phosphate; PLT, platelets; RBC, red blood cell count; T. Bil, total bilirubin; T. Cho, total cholesterol; TP, total protein; WBC, white blood cell count.

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Guterrez et al., 2004), but a comprehensive toxicological evaluation of extracts has hitherto not been conducted. Therefore, a 90-day subchronic toxicity study in F344 rats was here performed as part of a safety assessment for food additive use.

2. Materials and methods

2.1. Experimental animals and housing conditions

The protocol for this study was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences. Specific pathogen-free F344/DuCrj male and female rats (5 weeks old) were purchased from Charles River Japan (Kanagawa, Japan) and acclimated for 1 week prior to commencement of the test. The animals were randomly allocated to five groups, each consisting of 10 males and 10 females, housed in a room with a barrier system, and maintained under the following conditions: temperature of 24 ± 1 °C, relative humidity 55 ± 5%, ventilation frequency of 18 times/h and a 12 h light/dark cycle. The animals were housed in plastic cages (5 rats/cage) on soft chip bedding (Sankyo Labo-service, Tokyo, Japan). Throughout the experiment, chips were renewed every 3 or 4 days and rats were given tap water ad libitum.

2.2. Test compound and administration dose levels

A. blazei Murrill extract (ABM-EG3), a brown dry powder, was kindly provided by Iwade Mushroom Institute (Mie, Japan). The mycelium and fruit bodies of *A. blazei* Murrill (Iwade strain 101) harvested in Paraguay and Brazil were extracted with hot water and solvent, and concentrated in vacuum. The test material ABM-EG3 comprised 30% of the extract, and 70% of soluble dextrin and starch. By an enzymatic analysis, ABM-EG3 proved to include 4.7% of β-glucan as an indicator of mushroom constituents. On the basis of a preliminary 2-week feeding study (data not shown), the maximum dose level was set as 5% in compliance with guidelines for food additives (MHLW, 1996b). As the high, middle and low dose levels, 2.5, 1.25 and 0.63% were set with a common ratio of 2. The requisite amount of test compound for each dose was weighed and mixed with powdered basal diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo) and the test compound/diet admixture was supplied ad libitum for 90 days. The animals in the control group received the basal diet only. Dry powder of *A. blazei* Murrill was found to hardly denature over 2 years under refrigeration. Therefore, diets were prepared at a forage supplier Oriental Yeast Co., Ltd., and preserved in the dark at 4 °C prior to use.

2.3. Observation and examination methods

Clinical signs and general appearance were noted once daily, and body weights and food consumptions were measured once a week. Before the day of necropsy, the animals were deprived of food overnight. They were necropsied after blood samples were collected from the abdominal aorta under ether anesthesia.

Hematological parameters, measured with an automated hematology analyzer (Sysmex M-2000, TOA Medical Electronics Co., Ltd., Hyogo, Japan) were: white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelets (PLT). The distributions of white blood cells were assessed with a blood cell automatic analyzer (MICROX HEG-120A, Omron Co., Tokyo).

Serum biochemical parameters, measured at SRL Inc. (Tokyo, Japan) using sera frozen after centrifugation of blood, were: total protein (TP), albumin/globulin ratio (A/G), albumin (AlB), total bilirubin (T. Bil), total cholesterol (T. Cho), blood urea nitrogen (BUN), creatinine (CRN), calcium (Ca), inorganic phosphate (P), sodium (Na), potassium (K), chloride (Cl), aspartate aminotransferase (AsT), alanine aminotransferase (AlT), alkaline phosphatase (AlP) and γ -glutamyl aminotransferase (γ-GT).

At necropsy, all the organs were examined macroscopically, and the brain, heart, lungs, liver, spleen, adrenals, kidneys and testes were weighed. In addition to the organs mentioned above, the cranium with nasal cavity, pituitary, eyeballs, Harderian glands, spinal cord, salivary glands, thymus, stomach, small and large intestine, caecum, pancreas, urinary bladder, skin, mammary gland, lymph nodes, sternum, trachea, esophagus, thyroid gland, tongue, femoral muscle and bone, trigeminal and ischiatic nerves, epididymis, seminal vesicles, prostate and coagulating glands, uterus, ovary and vagina were fixed in 10% buffered formalin solution. Histopathological examination was carried out only on the 5% and basal diet (control) groups for both sexes. The organs of those groups were routinely processed for embedding in paraffin, sectioned and stained with hematoxylin and eosin.

2.4. Statistical analysis

The data for relative organ weights, hematology and serum biochemistry were analyzed statistically as follows. The Bartlett's test was applied to test homogeneity of variance between groups. If no significant heterogeneity was detected, one-way analysis of variance was applied. If significant heterogeneity of variance was detected, the Kruskal–Wallis's test was conducted. If parameters were found to significantly differ between