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Evaluation of Genotoxicity and Subclinical Toxicity of *Agaricus blazei* Murrill in the Ames Test and in Histopathological and Biochemical Analysis

JIN-BIOU CHANG[1,2*], HSU-FENG LU[3,4*], NIEN-CHIEH LIAO[4], CHING-SUNG LEE[3], MING-YANG YEH[5], CHI-MING LIU[6], MING-TENG CHUNG[7], AU MAN-KUAN[8], JEN-JYH LIN[9,10], MING-FANG WU[11] and JING-GUNG CHUNG[12,13]

[1]Department of Pathology, National Defense Medical Center, Division of Clinical Pathology, Tri-Service General Hospital, Taipei, Taiwan, R.O.C.; [2]Department of Medical Laboratory Science and Biotechnology, Yuanpei University, Hsinchu, Taiwan, R.O.C.; [3]Department of Restaurant, Hotel and Institutional Management, Fu-Jen Catholic University, New Taipei, Taiwan, R.O.C.; [5]Office of Director and Departments of [4]Clinical Pathology, [6]Research and Education, [7]Anatomical Pathology, [8]Orthopedics, Cheng Hsin General Hospital, Taipei, Taiwan, R.O.C.; [9]Graduate Institute of Chinese Medicine, [12]Department of Microbiology and [13]School of Biological Science and Technology, China Medical University, Taichung, Taiwan, R.O.C.; [10]Division of Cardiology, Department of Medicine, China Medical University Hospital, Taichung, Taiwan, R.O.C.; [11]Animal Medicine Center, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Abstract. *This study was conducted in order to assess the safety and tolerability of Agaricus blazei Murrill (ABM) in general toxicological studies by Ames tests in vitro and in 28-day feeding toxicity experiments. There were no dose-dependent increases or decreases in the number of revertant colonies both with and without metabolic activation in Ames tests. Doses of 10, 5 and 0.1 mg/per mouse of ABM daily were administered by oral gavage to mice (n=10) for 28 days. The effects on clinical observations, clinical pathology, and histopathology were evaluated. There were no significant changes in the brain, heart, kidney, liver, spleen, adrenal gland, testes or ovaries visually. With increasing doses, male and female treated mice did not show any gradual elevation of serum concentration in any of the nine items we examined, except for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in females. The AST levels of the treatment by medium or high dose and the ALT levels of the treatment by high dose in females were abnormal in comparison to those of the baseline control group, with significant differences. On studying the histological changes in mice, tissue sections of negative control and experimental groups exhibited no apparent pathological alterations. In summary, the Ames test, pathology determinations, biochemical analysis and routine blood parameters were all normal, except for AST and ALT in females. Results showed that the statistical differences observed in one sex were not observed in the other and were not dose dependent.*

*These Authors contributed equally to this work.

Correspondence to: Dr. Jing-Gung Chung, Department of Biological Science and Technology, China Medical University, No. 91, Hsueh-Shih Rd., Taichung 40402, Taiwan, R.O.C. Tel: +886 422053366 ext. 2161, Fax: +886 422053764, e-mail: jgchung@mail.cmu.edu.tw and Dr. Ming-Fang Wu, Animal Medicine Center, College of Medicine, National Taiwan University, 10F., No.2, Syu-Jhou Road, Taipei 10055, Taiwan, R.O.C. Tel: +886 223123456 ext. 88160, Fax: +886 223911306, e-mail: mfwu5555@ntu.edu.tw

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In recent years, the use of complementary and alternative medicine (CAM) has become increasingly popular among cancer patients in Western countries, with a prevalence as high as 80% (1, 2). Traditional Chinese medicine (TCM), and herbal medicines in particular, have been used in the treatment of cancer for thousands of years in China, Japan, and other Asian countries (3-5). These medicines are widely accepted as current forms of CAM for cancer treatment in the United States and Europe (6, 7). As recent pre-clinical and clinical studies have shown, TCM combined with conventional Western medicine (chemotherapy and radiotherapy) can provide effective supportive care for cancer

patients. TCM has great advantages in terms of increasing the sensitivity to chemo- and radiotherapeutics, to reducing their side-effects, and complications associated with chemotherapy and radiotherapy, and improving patient quality of life and survival time (8). Therefore, an understanding of Chinese herbal medicines is needed by physicians and other health care providers.

Since ancient times, mushrooms have been used as an important nutritional food and therapeutic item throughout the world on account of their composition (9). *Agaricus blazei* Murrill (ABM) popularly known as 'Cogumelo do Sol'in Brazil, and 'Himematsutake' in Japan, is a mushroom native to Brazil, and widely cultivated in Japan for its medicinal uses, hence it is now considered as one of the most important edible, culinary, medicinal and biotechnological species. It was traditionally used to treat many common diseases such as atherosclerosis, hepatitis, hyperlipidemia, diabetes, dermatitis and cancer (10). *In vitro* and *in vivo* ABM has shown immunomodulatory and anti-mutagenic properties, although the biological pathways and chemical substances involved in its pharmacological activities are still not clear (11, 12).

According to enforcement rules of the Health Food Control Act established by the Taiwan Department of Health, health food products should be evaluated for their pharmacological effects and safety by the Ames test and the 28-day food safety assessment for toxicology. In this study, the Ames Salmonella/microsome mutagenicity assay (Salmonella test; Ames test) was used, which is a short-term bacterial reverse mutation assay specifically designed to detect a wide range of chemical substances that can produce genetic damage which leads to gene mutations (13). A positive test indicates that the chemical might act as a carcinogen (although a number of false-positives and false-negatives are known). The procedure is described in a series of papers from the early 1970s by Bruce Ames and his group at the University of California, Berkeley (14-17). Safety is also evaluated by the 28-day subclinical toxicological assessment in order to examine the blood routine, the biochemical activities and the pathological assessment of liver, spleen and kidney.

Materials and Methods

Preparation of ABM and administration dose levels for Ames test in vitro. ABM powder (500 mg), obtained from Chang Gung Biotechnology Corporation, Ltd. (Taipei, Taiwan, ROC), and 10 ml distilled water were mixed thoroughly and filtered (0.22 μm pore size) to provide a solution with a concentration of 50 mg/ml. A series of concentrations was prepared from this stock solution by dilution, namely 3 mg/ml, 6 mg/ml, 12 mg/ml, 25 mg/ml and 50 mg/ml (10, 18).

Bacterial strains. Bacterial strains were provided by the Food Science Institute, Hsinchu, Taiwan. The strains used were *Salmonella typhimurium* TA97 (ΔuvrB/rfa/pKM101), TA98 (ΔuvrB/rfa/pKM101), TA100 (ΔuvrB/rfa/pKM101), TA102 (rfa/pKM101), and TA1535 (ΔuvrB/rfa). Strains were prepared by preculturing for 8 h at 37˚C in a nutrient broth. Strain properties, including their susceptibility to mutagens, were confirmed prior to use in the assays by the National Taiwan University College of Medicine Animal Medicine Center, Taipei, Taiwan, ROC.

Preparation of liver S9 fractions. Rats treated with enzyme-inducing agent β-naphthoflavone were sacrificed by spinal dislocation after 28 days. Briefly, after treatment rat livers were removed, placed in beakers on ice, rinsed with ice-cold homogenization KCl (1.15%) buffer, minced with scissors and then placed in 4 volumes of ice-cold KCl buffer. They were then homogenized with a tissue grinder. The homogenate was transferred to a close-fitting (0.045 mm clearance) Perspex [poly(methyl methacrylate)]/glass homogenizer and homogenized. After diluting the homogenate to 10% with the homogenization buffer and centrifugation at 9000 ×*g*, the microsomal pellets were suspended in KH_2PO_4 buffer pH 7.4 and stored at –80˚C until use.

Bacterial reverse mutation (Ames) assay. The Ames test was used to examine the mutagenicity of ABM. For the plate incorporation method, without metabolic activation, 0.1 ml of the test solutions of various concentrations of ABM, 0.1 ml of fresh bacterial broth and 0.5 ml of sterile buffer were mixed with 2.0 ml of overlay agar. For the assay with metabolic activation, 0.5 ml of metabolic activation mixtures containing an adequate amount of post-mitochondrial fraction was mixed with the overlay agar (2.0 ml), together with the bacteria and test solution. The contents of each tube were mixed and poured over the surface of a plate with minimal glucose agar. The overlay agar was allowed to solidify before incubation. The plate was incubated for 48 h at 37˚C and the number of reverting colonies was then counted. For a proper estimate of variation, triplicate plating was used at each dose level. All plates in a given assay were incubated at 37˚C for 48 h. After the incubation period, the number of reverting colonies per plate was counted.

Agar solvent was used a negative control. The positive control without S9 fraction consisted of 0.5 μg/plate of 4-nitro-o-phenylenediamine for TA97, TA98 and TA100 strains; 0.5 μg/plate of mitomycin C for TA102 strain; and 1 μg/plate of sodium azide for TA1535 strain; for any with S9 fraction, 1 μg/plate of benzo[*a*]pyrene was used for TA97, TA98, TA100 and TA102 strains, and 2-aminoanthracene for TA1535 strain.

Mutagenicity was evaluated based on the rule reported previously by Claxton *et al*. (19). The value of the positive control should be significantly higher than that of the negative control. Mutagenicity was judged to be positive when the revertants in the test plates increased more than two-fold compared with those of the negative control. All the tests of this experiment were performed in triplicate.

Preparation of ABM and administration dose levels for 28-day safety assessment in vivo. ABM powder and distilled water were mixed thoroughly at 60˚C for 10 min, then cooled to room temperature and left for 5 h with stirring at 200 rpm to form solutions of low (0.5 mg/ml), medium (25 mg/ml) and high (50 mg/ml) concentrations. The ABM solution was filtered before use (10).

Animals. Forty BALB/c male mice and forty BALB/c female mice (ten/group) were supplied from the National Taiwan University College of Medicine Animal Medicine Center (our own breeding

colony), and were four weeks of age weighing 20-25 g at the beginning of the study. The animals were housed singly in an animal room with a 12-hour light/dark cycle at a temperature and relative humidity range of 20±2˚C and 75±15%, respectively. The animals were acclimated for at least two weeks prior to testing. They were fed orally with Laboratory Rodent Diet 5001 manufactured by PMI Nutrition International (St. Louis, MI, USA) during the acclimation period and throughout the study.

Study design. Animals used in the present study were maintained in accordance with the guidelines approved up by the National Science Council of the Republic of China and the Committee for the Purpose of Control and Supervision of Experiments on Animals. Experiments were performed according to law, regulations and guidelines for animal experiments in Taiwan, which are in agreement with the Helsinki declaration. Male mice were randomized and allocated into control (group 1) and experimental (second to fourth groups) groups of ten animals each. Groups 2, 3 and 4 were orally administered high (10 mg/0.2 ml), medium (5 mg/0.2 ml) and low (0.1 mg/0.2 ml) doses of ABM daily for 28 days. Female mice were also randomized and allocated similarly into groups of ten animals each. Experimental groups were orally administered the three different dose levels daily. Negative controls were fed with distilled water.

Complete blood count (CBC) analysis. At the end of the 28-day period, animals were fasted for at least 15 h and then placed in metabolism cages one day before clinical pathological evaluation. Via orbital bleeding at the end of the experiments, 20 μl whole blood samples were collected by EDTA capillary tube. Hematological parameters included erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelet count (PLT), total white blood cell (WBC) and differential leukocyte count (DC). Mean corpuscular hemoglobin concentration (MCHC) was also calculated. Blood smears were prepared and evaluated. The CBCs were determined on a Medonic CA530 Vet automation instrument produced by Boule Medical AB (Stockholm, Sweden). Wright-Giemsa-stained blood smears from all animals were examined microscopically for confirmation of automated results and evaluation of cellular morphology.

Serum biomarker analysis. At the end of the experiment, animals were fasted for at least 15 hours and then placed in metabolism cages one day before clinical pathological evaluation. Blood was collected *via* cardiac puncture (0.2 ml with 10 U/ml heparin), allowed to clot, and centrifuged (1000 ×*g*, 10 min, room temperature) for biochemical tests. The clinical biochemical values analyzed were: serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), total bilirubin (T-Bil), blood urea nitrogen (BUN), blood creatinine, total cholesterol (T-Cho), fasting glucose, total serum protein (T-Pro) and albumin, all carried out on an Arkray Spotchen SP-4410 clinical chemistry analyzer using reagents manufactured by Arkray, Inc. (Kyoto, Japan).

Histopathological assessment of organs. All surviving animals at the end of the study were subjected to a complete necropsy after sacrifice under anesthesia by CO_2. Major organs such as the brain, heart, kidney, liver, spleen, adrenal gland, testes, and ovaries, were weighted and examined visually for any abnormality after the removal of peripheral fat tissue. Histopathological examinations were performed for the kidney, liver and spleen. All the collected tissues mentioned above were fixed in 10% neutral buffered formalin. Preserved organs and tissues were dehydrated, clarified, and paraffin embedded after trimming, forming paraffin tissue blocks; these were the sliced into 5 μm-thick sections using a microtome (Leica RM 2145; Leica, Nussloch, Germany), then stained with hematoxylin and eosin (H&E). Histopathology was conducted using an optical microscope by a pathology specialist and the changes were evaluated.

Statistical analysis. All data of hematological and serum biochemical analyses are expressed as the mean and standard deviation (SD). Treated and control groups were compared using a one-way analysis of variance (ANOVA). The student's *t*-test was used to compare different dose treatment groups when one way ANOVA was significant. Male and female mice were evaluated separately, and differences among groups were judged to be significant at a probability value of $p<0.001$.

Results

Table I shows the results of the mutagenicity of ABM treatment using the Ames test. Compared to the negative control, the ABM solutions with S9 and without S9 did not affect bacterial growth. The values with S9 were lower than these without S9 for all the TA100 strains. There were no dose-dependent increases or decreases in the number of revertant colonies neither with nor without metabolic activation. Generally, mutagenicity was negative in all strains with and without the S9 mix, as shown in Table I.

There were no mortalities nor emaciation during the course of study and all animals appeared to be active and healthy during the course of the study. Physical and behavioral examinations did not reveal any treatment-related adverse effects after dosing. Weekly mean body weight and body weight gain for all groups that consumed ABM were comparable to the control values. Sometimes light brown feces were found in low, medium or high doses treatment groups, but these findings were considered to be non-adverse as all animals recovered by the end of the study. The overall feed consumption of animals receiving ABM was not statistically significantly different from that of the control groups.

The potential changes in toxicity associated with the ABM treatment were assessed at the biochemical, hematological and histopathological levels. The serum concentrations of the biochemical markers ALT, AST, BUN, T-Bil and creatinine were obtained in order to evaluate the liver and renal functions. In addition, the histopathological changes in the target organs such as liver, spleen and kidney were evaluated.

For the male groups, six items, namely RBC, Hb, Hct, MCV, MCH and MCHC were not altered by exposure to the three different doses of ABM (Table II). No increase in PLT count was observed after administration of the high or medium dose, but the mean PLT count of animals in the low-dose treatment groups was higher than those of the control group

Table I. *The counts of total colonies counts (CFU) including spontaneous revertant colonies that appeared on plates with different concentrations of ABM were measured by the Ames test. As positive controls without S9 (–S9), 0.5 μg/plate of 4-nitro-o-phenylenediamine for TA97, TA98 and TA100 strains, 0.5 μg/plate of mitomycin C for TA102 strain and 1 μg/plate of sodium azide for TA1535 strains were used. As positive controls but with S9 mixtures (+S9), 1 μg/plate of benzo [a] pyrene was used for TA97, TA98, TA100 and TA102 strains, and 2-aminoanthracene (1 μg/plate) for TA1535 strains were used. Control solvent was used as the negative control.*

Strain	Mix	Positive control	ABM					Negative control
			5 mg/plate	2.5 mg/plate	1.2 mg/plate	0.6 mg/plate	0.3 mg/plate	
TA97	–S9	342±8	139±12	136±11	137±10	134±8	132±10	136±15
	+S9	339±8	147±6	144±9	146±7	147±5	143±9	141±5
TA98	–S9	164±8	28±7	25±11	26±6	24±8	27±10	23±4
	+S9	181±9	25±6	23±6	24±3	26±1	25±2	22±3
TA100	–S9	887±7	164±9	163±7	161±11	162±12	164±9	158±6
	+S9	791±15	157±13	156±7	154±8	158±11	155±12	156±9
TA102	–S9	575±10	164±8	163±7	161±9	162±12	164±9	158±6
	+S9	683±10	287±12	282±16	284±18	286±19	285±14	281±16
TA1535	–S9	221±7	15±2	13±4	11±4	11±2	14±2	13±2
	+S9	282±7	24±5	22±5	21±5	21±2	23±4	22±4

Table II. *Results of mean hematological counts of male mice administered with different doses of ABM for 28-days.*

Dose (mg/kg/day) Parameter	Control	Low (0.5 mg/ml)	Medium (25 mg/ml)	High (50 mg/ml)
Erythrocyte count (10^6/mm^3)	10.8±0.3	11.3±0.5	10.8±0.3	10.8±0.5
Hemoglobin concentration (g/dl)	16.10±0.54	16.73±0.59	16.13±0.37	15.97±0.64
Hematocrit (%)	51.2±1.8	53.3±2.0	50.7±0.9	51.0±2.0
Mean corpuscular volume (μm^3)	47.4±0.9	47.3±0.4	47.1±0.9	47.1±0.4
Mean corpuscular hemoglobin (pg)	14.90±0.26	14.83±0.21	14.96±0.37	14.74±0.16
Mean corpuscular hemoglobin concentration (g/dl)	31.50±0.18	31.39±0.30	31.78±0.29	31.31±0.21
Platelet count (10^3/mm^3)	607±60	688±69$^{\#}$	633±50	641±44
White blood cell count (10^3/mm^3)	7.9±2.6	8.2±2.1	5.1±0.8$^{\#}$	5.8±1.8$^{\#}$
Differential leukocyte count				
Lymphocytes (%)	89±6	86±7	86±5	87±7
Granulocytes (%)	7±2	8±2	7±1	9±3

Significantly different from the control group at $^{\#}p<0.05$.

(p=0.0247), although this was not significantly increased above the baseline control value, probably on account of the great variability of the male group (Table II). Mice receiving ABM did not show any statistically significant decrease or increase in WBC levels of the low dose but a statistically significant decrease at medium and high dose. There were no exposure-related changes in the differential leukocyte counts, inclusive of lymphocytes and granulocytes (p=0.0012).

Female mice treated with increasing doses of ABM did not show any gradual elevation or decrease of MCV, MCH and MCHC (Table III). Although for the WBC, Hb and Hct items, it was observed that treatment groups gave higher results compared with control groups, these were not statistically significant (p>0.001) (Table III). For the female groups, no increase in PLT was observed after administration of low or medium dose. The mean PLT of the high-dose treatment group was slightly higher than that of the control group but did not reach statistically significant difference compared to the baseline control value, probably on account of the great variability. The leukocyte data are shown in Table III. Mice did not show any statistical decrease or increase in total leukocytes on exposure to ABM. These were no exposure-related changes in the differential leukocyte counts (p=0.2742).

Male mice treated with increasing doses of ABM did not show any elevation of serum AST, ALT, T-Bil, BUN, CRE, T-Cho, glucose, T-Pro and albumin concentrations (Table IV). Although the AST levels recorded in the male control group (251±79 IU/l) and high-dose treatment group (278±41 IU/l) were different, this was not significant statistically.

Treated female mice did not show any difference from the control group in serum T-Cho concentration (Table V). No

Table III. *Results of mean hematological counts of female mice administered with different doses of ABM for 28-days.*

Dose (mg/kg/day) parameter	Control	Low (0.5 mg/ml)	Medium (25 mg/ml)	High (50 mg/ml)
Erythrocyte count ($10^6/mm^3$)	11.5±0.5	11.7±0.3	12.1±0.5	12.1±0.6
Hemoglobin concentration (g/dl)	17.08±0.68	17.52±0.56	17.89±0.59	18.39±1.58
Hematocrit (%)	52.1±2.6	53.7±1.8	55.2±2.0	54.9±2.9
Mean corpuscular volume (μm^3)	45.4±0.5	45.7±0.5	45.7±0.9	45.5±0.8
Mean corpuscular hemoglobin (pg)	14.86±0.22	14.91±0.19	14.82±0.31	14.88±0.25
Mean corpuscular hemoglobin concentration (g/dl)	32.79±0.40	32.60±0.19	32.46±0.27	32.74±0.61
Platelet count ($10^3/mm^3$)	496±55	542±70	523±74	589±91
White blood cell count ($10^3/mm^3$)	6.10±1.43	5.88±1.69	7.17±1.88	6.69±1.28
Differential leukocyte count				
Lymphocytes (%)	85±5	86±5	81±5	83±9
Granulocytes (%)	10±2	9±2	12±4	11±4

Table IV. *Mean serum biochemical values of male mice administered with different doses of ABM for 28 days.*

Dose (mg/kg/day) parameter	Control	Low (0.5 mg/ml)	Medium (25 mg/ml)	High (50 mg/ml)
Aspartate aminotransferase (IU/l)	251±79	253±58	270±44	278±41
Alanine aminotransferase (IU/l)	65±17	69±35	54±10	61±13
Total bilirubin (mg/dl)	1.01±0.45	0.83±0.32	0.95±0.46	0.98±0.35
Blood urea nitrogen (mg/dl)	22±4	20±2	22±4	19±3
Blood creatinine (mg/dl)	0.63±0.25	0.53±0.32	0.55±0.17	0.54±0.17
Total cholesterol (mg/dl)	72±9	76±8	76±6	78±7
Fasting glucose (mg/dl)	94±19	89±13	95±23	86±12
Total serum protein (g/dl)	4.63±0.31	4.66±0.27	4.72±0.36	4.85±0.27
Albumin (g/dl)	2.10±0.12	2.18±0.13	2.13±0.07	2.15±0.11

Table V. *Mean serum biochemical values of female mice administered with different doses of ABM for 28 days.*

Dose(mg/kg/day) parameter	Control	Low (0.5 mg/ml)	Medium (25 mg/ml)	High (50 mg/ml)
Aspartate aminotransferase (IU/l)	258±27	272±32	427±71*	396±69*
Alanine aminotransferase (IU/l)	94±13	88±12	142±23*	112±26
Total bilirubin (mg/dl)	2.41±0.81	2.44±1.48	2.80±0.99	1.87±0.71
Blood urea nitrogen (mg/dl)	31±3	35±3	32±4	37±10
Blood creatinine (mg/dl)	0.62±0.16	1.28±0.50#	0.61±0.09	0.99±0.21
Total cholesterol (mg/dl)	77±11	82±9	83±7	78±17
Fasting glucose (mg/dl)	114±31	104±21	131±24	121±21
Total serum protein (g/dl)	5.68±0.41	6.07±0.85	6.17±0.56	5.56±0.57
Albumin (g/dl)	2.22±0.08	2.29±0.12	2.28±0.13	2.28±0.13

Significantly different from the control group at $^*p<0.001$, $^\#p<0.05$.

increase in fasting glucose levels was observed after administration of different doses of ABM for the female groups (Table V). As for total serum protein and albumin concentrations, there was no significant difference between the serum concentrations in the control group and those found in mice treated with increasing doses of ABM (Table V). Renal biomarkers included BUN and creatinine. Female mice treated with increasing doses of ABM did not show any gradual elevation of serum concentrations of BUN and creatinine, except for the low-dose treatment (Table V). The

blood creatinine concentrations for the females were significantly increased (1.28±0.52 mg/dl) after administration of the low dose in comparison to those of the control group (0.62±0.16 mg/dl) (p=0.0011) (Table V). For the female groups, no increase in AST levels was observed after administration of the low dose but at medium (427±71 IU/l) and high dose (396±69 IU/l), the mean serum AST concentrations were significantly (p<0.001) higher than those of the control group (258±27 IU/l). ALT concentrations were increased (142±23 IU/l) after administration of the medium dose in comparison to those of the baseline of the control group (94±13 IU/l) with significant differences (p<0.001) (Table V). The value of T-Bil was elevated by the therapy of the medium dose (2.80±0.99 mg/dl) although this did not reach statistical significance compared with the baseline control value, probably due to the large variability (Table V).

The results from the subclinical toxicity study did not show any changing trends of dose dependency on individual body weight or individual organ weight inclusive of liver, spleen and kidney after 28 days of ABM administration compared with control group. We also examined other organs, including lung, brain, pancreas, ovary, uterus, testis by eye and no tumor mass was found.

Liver, spleen and kidney tissue sections were stained for all groups with H&E. The histopathological assessments in liver, spleen and kidney were performed for the control and the experimental groups.

Histopathological examination of liver sections from mice treated with different concentrations of ABM revealed non-significant hepatocellular necrosis in centrilobular regions, without any signs of vascular or inflammatory changes. No apoptotic morphology, such as nuclear chromatin margination and apoptotic bodies were observed, and normal hepatocytes were observed primarily in centrilobular regions. Representative microphotographs did not show any nuclear pyknosis, vascular congestion involving the portal triad and dilation of central vein, nor fatty change in liver parenchyma. The histopathological analysis of the liver revealed no signs of toxicity after administration of ABM (Figure 1).

The spleen did not show any significant loss of volume, and nor was any splenic hyperplasia observed. These was no micronodular lymphoid infiltrate located in white pulp, nor variable red pulp infiltration, marginal zone differentiation, or follicular replacement by neoplastic cells. The histological assessment of the spleen revealed normal morphology and no vascular changes at any dose of ABM. Furthermore, no inflammatory changes were observed at any dose in the control and experimental groups (Figure 1).

Normal histology of the glomerulus and tubules was found in kidney tissue of mice that received control, and ABM treatment. ABM did not induce any vascular or inflammatory changes, or signs of vascular congestion, tubular necrosis or glomerular atrophy, which is a degenerative phenomenon. No areas of red blood cells extravasating into the interstitium and amidst the spaces between the tubules were found (Figure 1).

Discussion

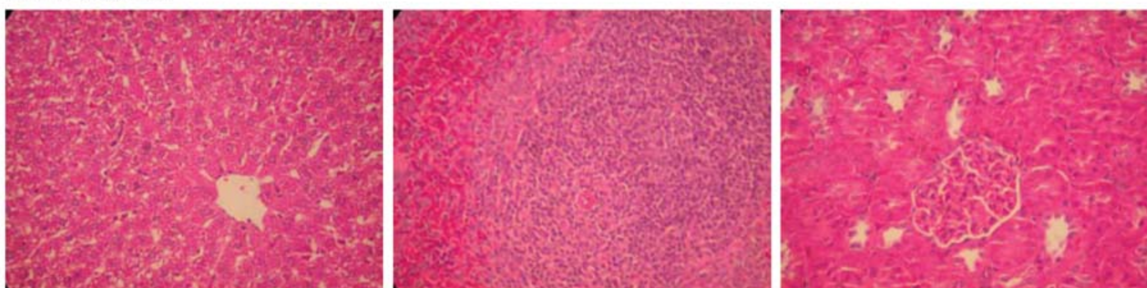
The use of dietary supplements is increasing globally and this includes the use of plant food supplements. A variety of factors may be influencing this increased consumption, including the increasing number of older people in society, mistrust in conventional medicine and the perception that natural is healthy.

CAM is defined as a medical intervention which is not widely taught at medical schools or is not generally available in hospitals (20). The use of CAM has become popular among many cancer patients all over the world. A summary of 26 surveys conducted across 13 countries estimated the prevalence of the use of CAM at 31.4% of all cancer patients, ranging from 7 to 64% (21), and this market may be growing as fast as 30% annually in the USA (22).

The incidence of acute hepatitis is mostly limited to a single or very few clinical case report(s) among cancer patients who have undergone chemotherapy with episodic consumption of ABM, although no such severe acute hepatitis or fatal liver failure was observed in many placebo-controlled clinical studies involving ABM intake (23). It is noteworthy that the most extensively consumed species of mushroom, Agaricus bisporus, contains a number of aromatic hydrazines, among which the most abundant is agaritine, β-N-(γ-L(+)glutamyl)-4-(hydroxymethyl) phenylhydrazine, being present at concentrations as high as 1.7 mg/g raw mushroom (23-30). Following the studies of Toth and Erickson (31) in which this mushroom was shown to induce tumors at a number of sites in mice, hydrazines such as agaritine were considered as being most likely responsible for the carcinogenicity induced by this mushroom, since hydrazines are an established class of chemical carcinogens (32). Agaritine has been shown to induce adenomas and adenocarcinomas in the lungs of mice when administered through drinking water (33). It has also been shown to induce mutation in DNA of the bacterium *Salmonella typhimurium* (34). Upon mammalian ingestion, agaritine is metabolized into its highly reactive diazonium ion (35). The mutagenic activity of the diazonium ion is due to its reaction with oxygen to produce hydrogen peroxide, which then covalently modifies DNA through a radical mechanism (36). Agaritine itself has also been shown to covalently bind to DNA *in vivo* (37). Agaritine is a weak carcinogen, however, and estimates for cumulative lifetime risk from mushroom consumption are approximately 1 in 10,000 (38).

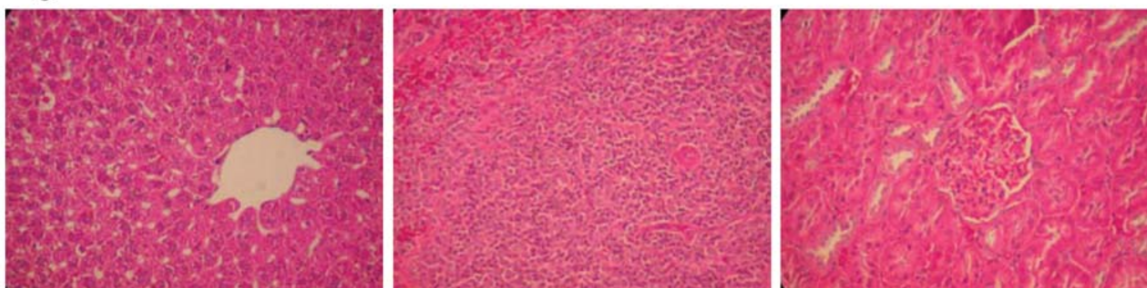
Despite the numerous reports regarding the preclinical safety and efficacy of ABM derivatives (39), further evaluation is

Male control



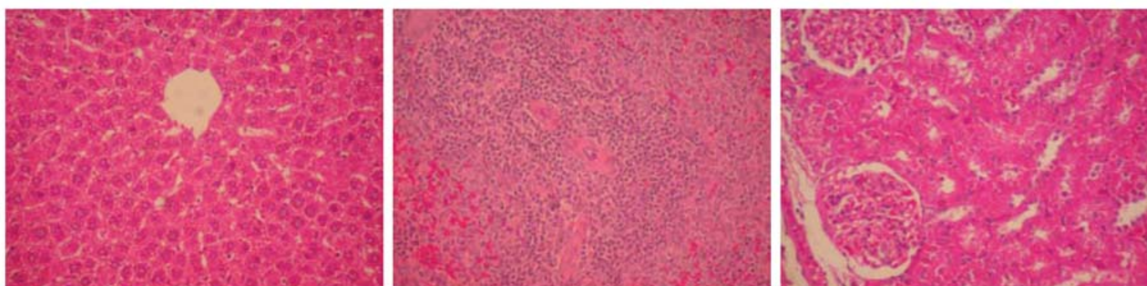
Liver Spleen Kidney

High dose treatment for male



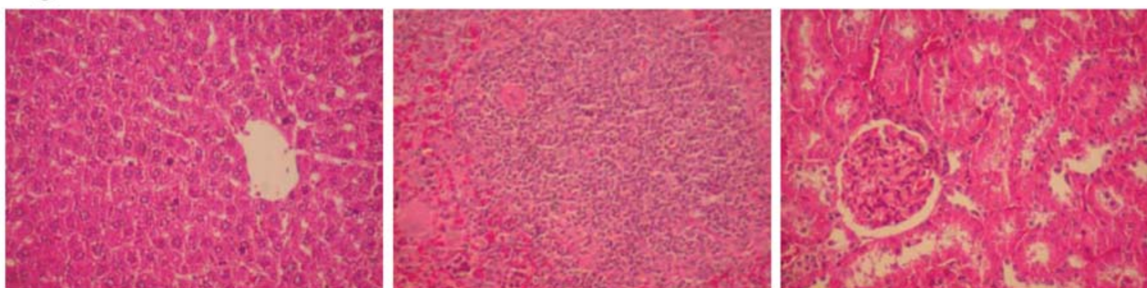
Liver Spleen Kidney

Female control



Liver Spleen Kidney

High dose treatment for female



Liver Spleen Kidney

Figure 1. *Representative photomicrographs of liver, spleen and kidney from mice treated with high dose ABM, compared with those of the control groups. After ABM treatment, liver sections revealed no hepatocellular necrosis in centrilobular regions and were without any signs of vascular or inflammatory changes. Signs of toxicity were not found. The spleen did not show any hyperplasia, nor was there any micronodular lymphoid infiltrate located in white pulp, nor variable red pulp infiltration, marginal zone differentiation or follicular replacement by neoplastic cells. The histological assessment of the spleen displayed normal morphology and did not reveal any vascular changes. Photomicrographs also showed there were no lesions involved in the cortical and the medullary zones of kidney.*

needed on account of many subspecies and various manufactures presenting different contents. Here, we have completed an oral safety study using ABM. The goal of this study was to determine the suitability of ABM for future use in food safety studies. The Ames test is used worldwide as an initial screen to determine the mutagenic potential of new chemicals and drugs because there is a high predictive value for rodent carcinogenicity when a mutagenic response is obtained.

Although mutagenicity was not shown in any of the strains with and without the S9 mix, in this experiment, we cannot definitively state whether this particular extract is capable of causing cancer or not. The Ames test is useful as a screening tool for setting priorities because it is an inexpensive and quick way to help single out chemicals that should be subjects of further testing. More extensive testing, such as chromosome aberration test, micronucleus test, 28-day subacute feeding toxicity test or 90-day subchronic feeding toxicity test, is needed to determine whether a chemical is likely to produce cancer in humans. Because the micronucleus test and 90-day subchronic feeding toxicity test are so labor-intensive and time-consuming, we chose to carry out a 28-day subacute feeding toxicity test to support food safety of ABM.

The results from the 28-day subchronic toxicity study did not show any dose-dependent trends on individual body weight or individual organ weight after 28 days of administration. For the male and female groups on blood routine tests, all the eight items exclusive of WBC counts of male were not altered by treatment to three different doses of ABM (Table II and III). Although exposure of male mice to ABM did not cause any statistically significant decrease or increase in total leukocytes counts, we take notice that p=0.0012 is near to the cut-off p-value of 0.001, this decrease of WBC by medium- or high-dose treatment cannot be neglected.

With increasing doses of ABM, male mice did not show any gradual elevation of serum biochemical concentration in any of the nine items examined (Table IV). Treated female mice did not exhibit any differences from the control group in T-Bil, BUN, T-Cho, fasting glucose, or total serum protein and albumin concentrations (Table V). In creatinine levels observed after administration of different doses in the female groups, attention should be paid that p=0.0011 is near to the cut-off p-value of 0.001. This suggests that the renal function is possibly impaired by the low-dose treatment. The AST levels of the mice treated with medium or high dose and the ALT levels of those treated with high dose are abnormal in comparison to those of the control group, with significant differences. Results showed the statistical differences observed in one sex were not observed in the other and were not dose-dependent. No significant differences were noticed in other parameters for either sex.

ABM (Himematsutake) is widely used among cancer patients in Japan, however, only few cases causing liver damage were reported. On account of different side-effects from modern medical therapy, ABM should be safe and its concurrent use with conventional therapies should be evaluated, especially for female patients. So far as we know, there are no serious side-effects reported for normal persons.

In summary, based on our findings, no infiltration, aggregation, necrosis and atrophy were found in the control and the experimental sections of liver, spleen and kidney. Tissue sections of negative control and experimental groups exhibited no apparent pathological alterations (Figure 1). Upon microscopic examination, there was no hepatic histopathological change correlated with the elevated transaminase findings for female mice treated with medium- or high-doses, and liver weights and macroscopic appearances were normal. In female mice, the creatinine level of the low-dose group was borderline significantly higher than that of the control group but normal histology of the glomerulus and tubules was found in kidney tissues.

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