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A 90-day subchronic toxicological assessment of *Antrodia cinnamomea* in Sprague–Dawley rats

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ABSTRACT

Antrodia cinnamomea (Ac) is a medicinal mushroom widely used for the treatment of abdominal pain, hypertension and hepatocellular carcinoma, but subchronic toxicity of this material has not yet been investigated. This present study was conducted to assess the 90-day oral toxicity of *A. cinnamomea* from submerged culture in male and female Sprague–Dawley (SD) rats. Eighty rats were divided into four groups, each consisting of ten male and ten female rats. Test articles were administered by oral gavage to rats at 3000, 2200 and 1500 mg/kg BW/day for 90 consecutive days and reverse osmosis water was used as control. All animals survived to the end of the study. During the experiment period, no abnormal changes were observed in clinical signs, body weight and ophthalmological examinations. No significant differences were found in urinalysis, hematology and serum biochemistry parameters between the treatment and control groups. Necropsy and histopathological examination indicated no treatment-related changes. According to the above results, the no-observed-adverse-effect level (NOAEL) of *Antrodia cinnamomea* is identified to be greater than 3000 mg/kg BW/day in Sprague–Dawley rats.

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1. Introduction

Antrodia cinnamomea (Ac) in the Polyporaceae, Basidiomycotine family, is a native mushroom parasitic on the endemic perennial tree, *Cinnamomum kanehirai* Hay in Taiwan (Chang and Chou, 1995). Ac is considered highly expensive due to its rareness and cultivation difficulties of fruit bodies, but has been commonly used as traditional herb material for treatment against abdominal pain, hypertension and hepatocellular carcinoma (Tsai and Liaw, 1985). Many studies have been shown its exclusive biological activity as both supplementary medicines and functional foods (Hseu et al., 2006; Liu et al., 2007; Lu et al., 2007; Yang et al., 2006). In Taiwan, matured fermentation techniques have promoted mass production of Ac and its products have been marketed in the form of mycelium from submerged culture as functional foods for over 10 years. Currently the dried mycelia powder of Ac is considered officially as a dietary supplementation in Taiwan. The mycelia of Ac powder contain 8% polysaccharides with molecular weight 10^6 Da. Its neutral monosaccharides are mannose, glucose and xylose linked by a β-D-glucan chain (Huang and Mau, 2000), which naturally possess a variety of pharmaceutical functions (Chen et al., 2001). Ac has been found with antioxidant properties (Song and Yen, 2002) which may act as a possible chemopreventing agent, while it could also provide protection against CCl_4-induced hepatic toxicity in rats (Song and Yen, 2003). Partially-purified polysaccharides from Ac showed antitumor effects against human leukemic U937 cells by promoting a Th-1 state and killer activities (Liu et al., 2004). Other biological active ingredients include an anti-HBV pyrrolidione (Shen et al., 2005), and the most recent finding of ergostatrien-3β-ol, which possesses anti-inflammatory activity and analgesic effects in formalin-induced mice (Huang et al., 2010). Although several in vitro and in vivo studies regarding the biological functions of Ac has been published, very few toxicological studies were reported. Toxicological evaluation of Ac performed so far included Ames mutagenicity tests, in vitro and in vivo chromosomal aberration tests, the latter in pneumonocytes of CHL mice and a 28 day gavage toxicity test in SD rats have been evaluated (Chen et al., 2001). The results of the chromosomal aberration test showed no dose-dependent cytotoxicity and no significant correlation in numerical aberrations compared to the control group. Results from the 28 day toxicity study suggested that daily treatment with Ac at 2 g/kg BW/day for 28 days did not induce observable toxicopathologic lesions in male and female rats. However, a 90-day subchronic toxicity study has not yet been conducted for a comprehensive safety profile of this potential mushroom. The objective of the present study was to investigate its toxicity in a 90 day subchronic toxicity study to further support its safety upon its possible use as a functional food ingredient.

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2. Materials and methods

2.1. Material preparation

A. cinnamomea (BCRC 35398) was purchased from Bioresources Collection and Research Center in Food Industry Research and Development Institute (Hsinchu, Taiwan). A small square of mycelium was cut and transferred onto a potato dextrose agar plate and incubated at 28 °C for 15 days. The strain was cut and inoculated into a 2 l Hinton flask with 1 l medium consisting of glucose 1.0%; soya bean powder 0.5%; peptone 0.5%; $MgSO_4$ 0.01%, and pH was adjusted to 4.0 with 1 mol^{-1} HCl. The whole medium was cultivated at 28 °C on a 100 rev min^{-1} rotary shaker for 7 days and then scaled up to 500 l fermenter with agitation for another 7 days as seed culture. The seed trough was then inoculated into a 50-ton fermenter and cultured for 14 days under the same conditions. The red-brown broth was then freeze-dried under vacuum, ground to powder and stored at room temperature. The proximate composition analysis, including crude protein, total crude lipids, ash, fiber, and moisture contents of the freeze-dried mycelia were determined according to the AOAC official procedures [methods 984.13, 43.275, 968.08, 991.43, and 950.46 B, respectively] (AOAC, 1995). Pyrroledione and ergostatrien-3β-ol were assayed with HPLC following the conditions described by Shen et al. (2005) and Huang et al., 2010.

2.2. Animals

Eighty seven-week-old Sprague–Dawley (SD) rats (BioLASCO, Taiwan) were quarantined for 2 weeks and acclimated in polyethylene cages for at least 5 days prior to being randomly assigned to the control and treatment groups of 10 rats per sex in each group by stratified randomization. The rats were identified by ear punch and housed in pairs. The animals had free access to standard rodent diet (Laboratory Autoclavable Rodent Diet® #5010, PMI Nutrition International, USA) and reverse osmosis water ad libitum, and were maintained at controlled temperature (20–23 °C), relative humidity (40–70%) and light cycle (12 h light/12 h dark). Frequency of ventilation was 10–15 times/h. Body weights of all rats were measured prior to the administration of the test article.

2.3. Study design

This study was performed based on the Organization for Economic Co-operation and Development (OECD) Guideline 408 and was conducted in accordance with Good Laboratory Practices (GLP). The protocol was approved by the Institutional Animal Care and Use Committee (IACUC No. 98-14a) before the beginning of the study. Body weight of all rats was measured before the experiment and then weekly until scheduled necropsy 90 days later. Average measurement of feed and water consumption was conducted weekly during the study period for both male and female rats. *Antrodia cinnamomea* were given daily by gavage to rats at different dosage: 3000 mg/kg (high), 2200 mg/kg (medium), 1500 mg/kg (low), and 0 mg/kg (control). To prevent the adverse effect of gavage, the volume of test sample administered was calculated as 20 mL/kg. Ac was prepared freshly everyday and administered to each rat via a stainless steel ball-tipped gavage needle. The test animals were not first fasted before gavage. Clinical observations were made daily during the experiment period for mortality, morbidity and possible signs of toxicity. At the end of the experiment, all surviving animals were anesthetized with carbon dioxide and euthanized after blood collection.

2.4. Urinalysis

One day before the gross necropsy examination, each rat was placed in metabolic cages for 16 h to collect urine. Immediately after each urine sample is obtained, specific gravity (SG), color, protein, urobilinogen, pH, ketone, bilirubin, glucose, nitrite, and occult blood were analyzed by using a semiquantitative urinalysis system (Urisys 2400, Roche, Basel, Switzerland). Sediments of each urine sample were observed for white blood cell (WBC), red blood cell (RBC), epithelial cell (EP), crystals, and microbes by using a microscope.

2.5. Ophthalmology

An ophthalmological examination was conducted for all rats on the first day of the experiment prior to the administration of Ac and on the 90th day after the experiment. The peripheral and internal structure of both eyes in each rat were examined then evaluated with naked eyes and indirect ophthalmoscopy, respectively.

2.6. Hematology and serum biochemistry

After overnight fasting, all rats were anesthetized with inhalation of carbon dioxide. Each blood sample was collected by heart puncture and collected in an EDTA blood collecting tube and mixed well. An automatic blood analyzer (Gen. S™, Beckman, California, USA) was applied to the following detection: hematocrit, hemoglobin, RBC, WBC, platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte, neutrophil, monocyte, eosinophil and basophil. Anticoagulated blood samples were analyzed by Blood Coagulation Analyzer (CA-1500, Sysmex, Kobe, Japan) for prothrombin time and activated partial thromboplastin time. The following clinical chemistry parameters were evaluated: alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (γ-GT), albumin, total protein, total bilirubin, creatinine, blood urea nitrogen (BUN), glucose, cholesterol, triglyceride, phosphorus, calcium, chloride, potassium and sodium (by using an automated analyzer (Beckman LX®-20, California, USA)).

2.7. Pathology

All surviving animals at the end of the study were subjected to a complete necropsy. The weights of major organs such as the brain, heart, kidney, liver, spleen, adrenal gland, testes or ovaries were recorded after the removal of peripheral fat tissue. In addition, relative organ weights were calculated according to the formula: Relative organ weight (%) = organ weight (g)/body weight (g) × 100. The peripheral oral cavity, cranial cavity and all tissues and organs in the thoracic and abdominal cavity were examined visually for any abnormality and recorded. Histopathological examinations were performed for the brain, heart, kidney, liver, spleen, adrenals, testes, ovary, aorta, bone marrow, duodenum, jejunum, ileum, caecum, colon, rectum, eyes, esophagus, mammary gland, Harderian gland, trachea, lung, lymph node, pancreas, sciatic nerve, pituitary, prostate gland, salivary gland, skin, spinal cord, stomach, thigh muscle, thymus, thyroid/parathyroid gland, urinary bladder, and the uterus. All the collected tissues mentioned above were fixed in 10% neutral buffered formalin. Preserved organs and tissues were dehydrated, clarified, infiltrated with paraffin and embedded after trimming, forming paraffin tissue blocks and sliced into 5 μm thick sections using a microtome (Leica RM 2145, Nussloch, Germany), then stained with Hematoxylin & Eosin (H&E). Histopathology was conducted using an optical microscope (Opticphot-2, Nikon, Tokyo, Japan). If treatment-related changes were observed in a particular organ or tissue in the high dose group, extended examination was conducted on the corresponding organs of other dose-treated groups.

2.8. Statistical analysis

All data were expressed as mean and standard deviation (SD). Body weight, feed consumption, organ weight, hematology and serum biochemistry analysis were tested by conducting One-Way ANOVA using the SPSS statistical software. Duncan's test was used to determine statistical significance ($P < 0.05$) between the control and treatment groups. Male and female rats were evaluated separately.

3. Results

3.1. Proximate composition

The composition analysis of *Antrodia cinnamomea* submerged culture contains approximately 4% moisture and 96% dry matter, both based on air dry weight; 53% carbohydrate, 24% crude protein, 6% crude fat, 7% crude fiber and 10% ash (Chen et al., 2007). The total carbohydrate content in freeze-dried powder of Ac fermented broth was consistent with the fundamental composition usually found in most of the cultivated mushrooms (Mattila et al., 2002). The active ingredients pyrroledione and ergostatrien-3β-ol were determined at 1168 ppm (w/w) and 2750 ppm (w/w), respectively.

3.2. Body weight and feed intake

No mortalities occurred during the study. Physical and behavioral examinations did not reveal any treatment-related adverse effects after dosing. Lower average body weights were noted for the low dose and high dose group of male rats and for all the treated groups of female rats. However, these differences were not significant compared to their respective control groups ($P > 0.05$) (Fig. 1). The overall feed consumption of animals receiving Ac was similar to that of the control groups and was not statistically significant (data not shown).

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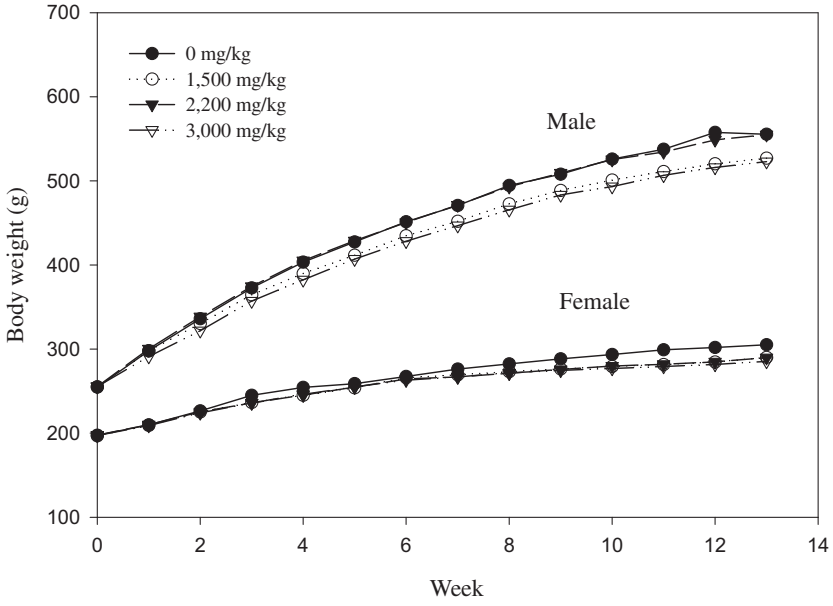

Fig. 1. Body weight changes of male and female SD rats during the 90-day safety assessment.

3.3. Urinalysis

No significant differences in urine sediments or urinalysis response variables were observed between the treatment and control groups of both sexes (data not shown).

3.4. Opthalmology

Opthalmoscopic examinations revealed no abnormality in the treated or control groups during the test period (data not shown).

3.5. Hematology

Only one parameter in each sex was noted to be statistically significant during hematological analysis. Mean corpuscular hemoglobin (MCH) in the high dose of Ac-treated males (18.5 ± 0.3) was significantly higher than in the control (17.9 ± 0.6). In female rats, platelet counts significantly increased in the low (897.5 ± 109.1) and medium dose group (907.9 ± 107.7) compared to the control group (788.9 ± 84.5), Except for the above two significant differences, all other parameters were comparable to the control (data not shown). Results showed the statistical differences observed in one sex were not observed in the other and were found to be non dose-dependent. No significant differences were noticed in other parameters for both sexes.

3.6. Serum biochemistry

In male rats, BUN of the high dose group was significantly lower (11.2 ± 1.4) than the control group (12.3 ± 1.1) (Table 1), and the creatinine level showed a significant increase in both the low dose (0.48 ± 0.04) and medium dose group (0.48 ± 0.03) compared to the control group (0.44 ± 0.04). The cholesterol level showed a significant decrease in the low dose group (52.6 ± 7.0) compared to

Table 1
Serum biochemistry findings in male Sprague–Dawley rats after 90 days of Ac administration.

Items	Dose (mg/kg BW/day)			
	0	1500	2200	3000
AST (U/L)	116.5 ± 18.4[a]	116.1 ± 17.6	131.6 ± 57.5	122.9 ± 12.7
ALT (U/L)	50.4 ± 8.8	52.2 ± 14.2	48.7 ± 8.6	48.4 ± 5.7
ALP (U/L)	90.4 ± 13.3	102.5 ± 22.1	93.2 ± 15.2	102.8 ± 25.1
T. bilirubin (mg/dL)	0.10 ± 0.00	0.14 ± 0.13	0.10 ± 0.00	0.10 ± 0.00
γ-GT (U/L)	3.0 ± 0.0	3.1 ± 0.3	3.0 ± 0.0	3.0 ± 0.0
T. protein (g/dL)	7.2 ± 0.2	7.1 ± 0.4	7.2 ± 0.3	7.0 ± 0.5
Albumin (g/dL)	4.3 ± 0.1	4.3 ± 0.2	4.3 ± 0.1	4.2 ± 0.2
Globulin (g/dL)	2.9 ± 0.2	2.8 ± 0.3	2.9 ± 0.2	2.8 ± 0.3
BUN (mg/dL)	12.3 ± 1.1	12.4 ± 1.4	13.4 ± 1.2	11.2 ± 1.4*
Creatinine (mg/dL)	0.44 ± 0.04	0.48 ± 0.04*	0.48 ± 0.03*	0.43 ± 0.04
Glucose (mg/dL)	156.1 ± 23.5	179.7 ± 44.3	150.7 ± 30.7	150.3 ± 32.8
Triglyceride (mg/dL)	71.3 ± 29.4	58.3 ± 20.7	56.2 ± 28.4	53.1 ± 17.3
Cholesterol (mg/dL)	66.3 ± 12.2	52.6 ± 7.0*	64.5 ± 16.4	55.6 ± 9.9
Sodium (meq/L)	151.1 ± 1.5	150.8 ± 1.9	151.3 ± 1.8	150.1 ± 1.2
Potassium (meq/L)	6.0 ± 0.6	6.2 ± 0.7	6.0 ± 0.3	6.2 ± 1.0
Calcium (meq/L)	11.6 ± 0.3	11.2 ± 0.5	11.3 ± 0.3	11.2 ± 0.5
Chloride (meq/L)	100.0 ± 1.9	100.5 ± 1.3	100.4 ± 2.2	100.2 ± 2.3
Phosphorus (mg/dL)	9.4 ± 0.7	9.0 ± 0.8	9.7 ± 2.1	9.4 ± 1.1

[a] Data expressed as mean ± S.D., $n = 10$.
* Significant different from control group ($P < 0.05$).

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Table 2
Serum biochemistry findings in female Sprague–Dawley rats after 90 days of Ac administration.

Items	Dose (mg/kg BW/day)			
	0	1500	2200	3000
AST (U/L)	115.7 ± 40.7[a]	107.8 ± 23.6	95.3 ± 13.8	113.0 ± 31.5
ALT (U/L)	38.5 ± 8.5	37.9 ± 8.4	32.8 ± 4.7	45.0 ± 22.6
ALP (U/L)	45.9 ± 12.5	51.5 ± 16.6	44.3 ± 6.2	50.0 ± 15.3
T. bilirubin (mg/dL)	0.09 ± 0.03	0.08 ± 0.04	0.08 ± 0.04	0.09 ± 0.03
γ-GT (U/L)	2.6 ± 0.8	2.6 ± 0.8	2.6 ± 0.8	2.6 ± 0.8
T. protein (g/dL)	7.7 ± 0.3	7.4 ± 0.4	7.8 ± 0.2	7.7 ± 0.4
Albumin (g/dL)	4.7 ± 0.2	4.6 ± 0.3	4.7 ± 0.2	4.8 ± 0.3
Globulin (g/dL)	3.0 ± 0.1	2.8 ± 0.2	3.0 ± 0.1	2.9 ± 0.2
BUN (mg/dL)	14.6 ± 2.5	13.9 ± 2.5	14.2 ± 1.4	13.8 ± 1.6
Creatinine (mg/dL)	0.55 ± 0.09	0.52 ± 0.07	0.54 ± 0.07	0.51 ± 0.06
Glucose (mg/dL)	195.3 ± 33.5	179.4 ± 38.0	198.6 ± 41.5	175.3 ± 42.8
Triglyceride (mg/dL)	47.3 ± 12.3	47.1 ± 8.5	49.5 ± 7.6	46.7 ± 11.2
Cholesterol (mg/dL)	60.4 ± 10.8	69.2 ± 17.1	75.0 ± 14.4*	57.4 ± 9.6
Sodium (meq/L)	147.4 ± 1.4	146.6 ± 1.2	146.3 ± 1.0	147.3 ± 1.3
Potassium (meq/L)	6.4 ± 0.5	6.4 ± 0.8	6.6 ± 0.6	6.5 ± 0.4
Calcium (meq/L)	11.5 ± 0.4	11.2 ± 0.5	11.5 ± 0.6	11.6 ± 0.4
Chloride (meq/L)	101.5 ± 2.2	100.9 ± 1.4	101.6 ± 1.4	101.5 ± 2.6
Phosphorus (mg/dL)	8.0 ± 1.1	7.7 ± 1.2	7.7 ± 0.8	7.8 ± 0.9

[a] Data expressed as mean ± S.D., $n = 10$.
* Significant different from control group ($P < 0.05$).

the control group (66.3 ± 12.2) for males, while a significant increase was observed in the medium dose group (75.0 ± 14.4) compared to the control group (60.4 ± 10.8) for females (Table 2). Results showed the statistical differences observed in one sex were not observed in the other and were found to be non dose-dependent. No significant differences were found in other parameters.

3.7. Pathology

No treatment-related changes were observed for tissues of the peripheral cavity, thymus, heart, lung, liver, kidney, gastrointestinal tract, spleen, brain and the reproductive system in the treated and control groups (data not shown). During the postmortem organ examination, no significant difference was observed in male rats for absolute organ weight, but as for the females, the weight of the heart in the medium dose group significantly decreased (0.93 ± 0.04) compared to the control group (1.04 ± 0.10), however, no significant differences were found in either sex for the relative organ weight compared to the control group (data not shown). Histopathological examination was performed on 40 rats (ten rats per sex from the control and high dose group). No significant treatment-related changes were observed for both Ac-treated and control groups for females. In males, mononuclear cell infiltration (focal) was found in the heart, incidence rate of the control and high dose group were 3/10 and 2/10, respectively. Minimal to mild tubular regeneration was found in the kidney of the male control group (incidence rate of 2/10). Only one male rat in the control group presented focal and mild seminiferous tubular atrophy in the testes, but the above results were not considered relevant to the test article treatment (Table 3).

4. Discussion

The results from the 90-day subchronic toxicity study did not show any changing trends of dose dependency on individual body weight or individual organ weight after 90 days of Ac administration, which correlates to the Ac toxicology study results of Chen et al. (2001). Opthalmoscopic results showed no abnormality. No particular lesions were found during the gross necropsy for major organs and tissues. The absolute heart weight in females from the medium dose group was significantly lower compared with the control, but no significant changes in heart-to-body weight ratio was found. This finding was not associated with other clinical data, and no indication of pathological abnormality was noted. Therefore, this change is considered to be of no toxicological significance. Hematology results showed no changes from the Ac administration in all parameters, except for a significant increase of MCH in the high dose for male rats, and a significant increase of platelet counts in the low and medium dose group for female rats compared to

Table 3
Result of histopathological examination.

Organ	Lesion	Group			
		Control		High dose	
		Male	Female	Male	Female
Heart	Infiltration, mononuclear cell, focal, minimal[a]	3/10[b]	–	2/10	–
Kidney	Regeneration, tubule, focal, minimal to slight	2/10	–	–	–
Testes	Atrophy, seminiferous tubule, focal, slight	1/10	N	–	N

(–) No effect. N: No tissue.
[a] Degree of lesions was graded from one to five depending on severity: 1 = minimal (<1%); 2 = slight (1–25%); 3 = moderate (26–50%); 4 = moderate/severe (51–75%); 5 = severe/high (76–100%).
[b] Incidence: affected rats/total examined rats ($n = 8$–10).

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their respective control groups. Clinical chemistry findings showed significant decrease of BUN in the high dose group and a significant increase of creatinine in the low and medium dose group. Total cholesterol level was also found to be significantly lower in the low dose group for male rats, while significantly higher level of cholesterol was found in the medium dose for female rats. The above findings are changes within the range observed in normal SD rats (OECD, 1998), and the changes were not found to be dose-dependent. Therefore, it is concluded that the results are not due to Ac administration. Histopathological examination showed mononuclear cell infiltration and tubular regeneration in the treated group, and one male rat in the control group presented mild seminiferous tubular atrophy. No positive correlations of the degree and incidence rate of the changes between the treated and control groups were noted, and histopathological changes were non-specific.

5. Conclusion

In this present study, the 90-day subchronic toxicity test showed no systemic toxicity attributable to Ac administration, and Ac from submerged culture showed no significant toxicity even at the highest dose of 3000 mg/kg BW/day in SD rats. In conclusion, the toxicity in a 90 day subchronic toxicity study of *Antrodia cinnamomea* does not raise concern with respect to possible use as a functional food ingredient, provided that the margin of safety between the NOAEL now established and the estimated intake resulting from the proposed uses and use levels would be adequate.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

AOAC, Official Methods of Analysis, 16th ed., Association of Official Analytical Chemists, Washington, DC, 1995.

Chang, T.T., Chou, W.N., 1995. *Antrodia cinnamomea* sp. nov. on *Cinnamomum kanehirai* in Taiwan. Mycology Research 99, 756–758.

Chen, C.C. 2007. Functional activities and safety of *Antrodia camphorate*. Ph.D. thesis, National Tsing Hua University, Hsinchu, Taiwan.

Chen, C.C., Lin, W.H., Chen, C.N., Hsu, S.J., Huang, S.C., Chen, Y.L., 2001. Development of *Antrodia camphorata* mycelium with submerge culture. Fungi Science 16, 7–22.

Hseu, Y.C., Chen, S.C., Tsai, P.C., Chen, C.S., Lu, F.J., Chang, N.W., Yang, H.L., 2006. Inhibition of cyclooxygenase-2 and induction of apoptosis in estrogen-nonresponsive breast cancer cells by *Antrodia camphorata*. Food and Chemical Toxicology 45, 1107–1115.

Huang, G.J., Huang, S.S., Lin, S.S., Shao, Y.Y., Chen, C.C., Hou, W.C., Kuo, Y.H., 2010. Analgesic effects and the mechanisms of anti-inflammation of ergostatrien-3β-ol from *Antrodia camphorate* submerged whole broth in mice. Journal of Agricultural and Food Chemistry 58, 7445–7452.

Huang, L.C., Mau, J.L., 2000. Antioxidant properties and polysaccharide composition analysis of *Antrodia camphorate* and *Agaricus blazei*. M.Sc. thesis, National Chung Hsing University, Taichung, Taiwan.

Liu, D.Z., Liang, Y.C., Lin, S.Y., Lin, Y.S., Wu, W.C., Hou, W.C., Su, C.H., 2007. Antihypertensive activities of a solid-state culture of *Taiwanofungus camphorates* (Chang-Chih) in spontaneously hypertensive rats. Bioscience Biotechnology Biochemistry 71, 23–30.

Liu, J.J., Huang, T.S., Hsu, M.L., Chen, C.C., Lin, W.S., Lu, F.J., Chang, W.H., 2004. Antitumor effects of the partially purified polysaccharides from *Antrodia camphorata* and the mechanism of its action. Toxicology and Applied Pharmacology 201, 186–193.

Lu, Y.C., Huang, C.C., Huang, C.J., Chu, S.T., Chi, C.C., Su, H.H., Hsu, S.S., Wang, J.L., Chen, I.S., Liu, S.I., Huang, J.K., Ho, C.M., Kuo, S.J., Jan, C.R., 2007. Effects of *Antrodia camphorate* on viability, apoptosis, $[Ca2^{+}]I$, and MAPKs phosphorylation in MG63 human osteosarcoma cells. Drug Development Research 68, 71–78.

Mattila, P., Salo-Vaananen, P., Konko, K., 2002. Basic composition and amino acid content of mushrooms cultivated in Finland. Journal of Agricultural and Food Chemistry 50, 6419–6422.

OECD, 1998. 90-Day Oral Toxicity Studies in Rodents. OECD 408.

Shen, C.C., Yang, H.C., Huang, R.L., Chen, C.C., Chen, C.C., 2005. Anti-HBV principle from the culture broth of *Antrodia camphorate* (Strain CCRC-35396). Journal of Chinese Medicine 16, 57–61.

Song, T.Y., Yen, G.C., 2002. Antioxidant properties of *Antrodia camphorate* in submerged culture. Journal of Agricultural and Food Chemistry 50, 3322–3327.

Song, T.Y., Yen, G.C., 2003. Protective effects of fermented filtrate from *Antrodia camphorate* in submerged culture against CCl_4-induced hepatic toxicity in rats. Journal of Agriculture and Food Chemistry 51, 1571–1577.

Tsai, Z.T., Liaw, S.L., 1985. The Use and the Effect of Ganoderma. San Yun Press, Taichung, Taiwan. p. 116.

Yang, H.L., Chen, C., Chang, W.H., Lu, F.J., Lai, Y.C., Chen, C.C., Hseu, T.H., Kuo, C.T., Hseu, Y.C., 2006. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by *Antrodia camphorate*. Cancer Letters 231, 215–227.

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