Acta Biologica Hungarica 60 (3), pp. 281–291 (2009)
DOI: 10.1556/ABiol.60.2009.3.5

STUDY ON GENES WITH ALTERED EXPRESSION IN α-AMANITIN POISONED MICE AND EVALUATION ON ANTAGONISTIC EFFECTS OF TRADITIONAL CHINESE MEDICINES AGAINST TOXICITY OF α-AMANITIN

Q. CHEN,[1a,c] M. CAO,[1b] W.-L. XIANG,[a] Q. SUN,[a] J. ZHANG,[a] R.-T. HOU,[a] Z.-Y. YAN,[c] Z.-R. YANG,[a] J. LIU[a] and J. ZHAO[a]*

[a] Key Laboratory of Biological Resource and Ecological Environment of the Ministry of Education, College of Life Sciences, Sichuan University, Chengdu 610064, PR China
[b] Key Laboratory of Sichuan Academy of Medical Sciences, Sichuan Provincial People's Hospital, Chengdu 610072, PR China
[c] Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu 610041, PR China

(Received: July 9, 2008; accepted: August 18, 2008)

The forward and reverse cDNA subtractive libraries before and after the toxic effect of α-amanitin were constructed by suppression subtractive hybridization and randomly selected clones from each subtractive library were screened by PCR and dot blot hybridization. A total of 85 genes with altered expression were finally identified, with 41 genes from the forward library and 44 from the reverse library. Subsequently, the antagonistic effects of candidate traditional Chinese medicines were evaluated based on the genetic transcription levels of the genes with significant altered expression, including Catnβ, Flt3-L, IL-7r and Rpo2-4. The results indicated that *Silybum marianum* (L.) Gaert and *Ganoderma lucidum* had significant down-regulated effects on the transcription level of Catnβ that was up-regulated by α-amanitin, and the two herbs also up-regulated the transcription levels of Flt3-L and Rpo2-4. *Silybum marianum* (L.) had significant up-regulated effects on the IL-7r that was down-regulated by α-amanitin. These preliminary studies suggested that *Silybum marianum* (L.) and *Ganoderma lucidum* were effective antagonists against the toxicity of α-amanitin.

Keywords: α-Amanitin – antagonist – genes with altered expression – suppression subtractive hybridization – traditional Chinese medicines

INTRODUCTION

The compound α-amanitin, a bicycle-octopeptide molecule, is mainly found in the fruiting body of *Amanita* fungi. It is a major fatal component in poisonous mushrooms leading to death if ingested by people and animals. Pathologic study indicates that α-amanitin is absorbed by hepatic cells through active transport *in vitro*. Nucleolus disintegration, chromatin condensation and decrease of RNA synthesis is

* Corresponding author; e-mail: bio_zj2003@tom.com
[1] Qian Chen and Mei Cao contributed equally to this research.

0236-5383/$ 20.00 © 2009 Akadémiai Kiadó, Budapest

observed in cells within 30 minutes after α-amanitin is absorbed *in vivo* [25]. Due to hepato-enteric circulation, repeated absorption of α-amanitin by hepatic cells would finally induce the failure of liver function [13]. Moreover, the kidney is also damaged when α-amanitin is excreted [33, 39]. Recent researches show α-amanitin does not only noncompetitively affect RNA polymerase to inhibit the mRNA synthesis, but also changes the expression of some important genes [19, 38]. Studies on these genes with altered expression will facilitate exploring effective treatment against α-amanitin poisoning. In the current study, suppression subtractive hybridization (SSH), dot blot hybridization and semi-quantitative PCR are applied to isolate genes with altered expression and they have proven useful for isolation these genes [22, 25].

The candidate traditional Chinese medicines (TCMs), including *Silybum marianum* (L.) Gaert (*S. marianum* (L.) Gaertn, silybin) [3, 29, 34], *Ganoderma sinense* (*G. sinense*) [6], *Ganoderma lucidum* (*G. lucidum*) [6, 18], *Perilla frutescens* (L.) britt var. *frutescens* [23], *Perilla frutescens* var. *criasta* (*P. frutescens* (L.) britt var. *frutescens*) [23] and *Glycyrrhiza uralensis* Fisch [1, 14], are advantageous to maintain functions of liver and kidney, and boost performance of the immune system according to TCM theory. These herbs are used in many hospitals in China to treat the mushroom poisoned patients, which shows some therapeutic effects [15, 35, 37, 40]. Though it is very important to find effective medicines to treat these patients, less is known as to how to evaluate the antagonistic effect of the herbs against α-amanitin's toxicity. In this study, the antagonistic effects of candidate TCMs against the toxicity of α-amanitin was evaluated based on their recovery effects on the genetic transcription levels of the genes with altered expression.

MATERIALS AND METHODS

Chemicals and materials

α-Amanitin (Fluka, SG, Switzerland), Superscript II Reverse Transcriptase (Invitrogen, CA, USA), PCR-Select™ cDNA Subtraction Kit (Clontech, CA, USA), NucleoTrap PCR Suspension Kit (Clontech, CA, USA), Clontech PCR cDNA Synthesis Kit (Clontech, CA, USA), Detector™ HRP Southern Blotting Kit (KPL, MD, USA) Detector™ PCR DNA Biotinylation Kit (KPL, MD, USA), One Step RNA PCR Kit (TaKaRa, Shiga, Japan), Rneasy Mini Kit (Qiagen, Hilden, Germany). The candidate TCMs: *Silybum marianum* (L.) Gaert (*S. marianum* (L.) Gaertn, silybin), *Ganoderma sinense* (*G. sinense*), *Ganoderma lucidum* (*G. lucidum*), *Perilla frutescens* (L.) britt var. *frutescens, Perilla frutescens* var. *criasta* (*P. frutescens* (L.) britt var. *frutescens*) and *Glycyrrhiza uralensis* Fisch (*G. Fisch*) were purchased from Tong-ren Tang Drugstore in China.

Animals

The BALB/c mice (20 ± 2.0 g) were purchased from the Laboratory Animal Research Center of Sichuan University, China.

Synthesis of primers

The primers of SSH were synthesized according to the previous references [20, 22, 24, 36], the others are shown in Table 1.

Table 1
List of primers others than SSH

Catnβ	F:5-GCAACCCTGAGGAAGAAG-3	R:5-CAATGGGAGAATAAAGCAAC-3
Flt3-L	F:5-GGGTTCTTAGAAGAGGAGATG-3	R:5-TCCCAACTCTAGCACCAA-3
IL-7r	F:5-GTGAAAGCAACTGGACGC-3	R:5-TGAGGAAGTGGAGATGGG-3
Rpo2-4	F:5-AACGCCTGCTTGTTCAC-3	R:5-CCCTGAGGAGTATGTGCC-3
β-actin	F:5-ACCGTGAAAAGATGACCC-3	R:5-AGAAGGAAGGCTGGAAAA-3

RNA isolation and cDNA synthesis

The α-amanitin was dissolved in sterile physiological saline. Before injecting α-amanitin, blood samples of mice in α-amanitin administrated group were collected into the test tube with heparin for RNA isolation from the left eyes of each mouse. Then, the mice were i.v. injected with LD_{50} of α-amanitin (0.327 mg/kg body weight) [27]. After 24 h, the analytic blood was collected from the right eye and divided into two parts. The first part was used for the assay on biochemical indicator (BUN > 15 mmol/L, Crea > 40 μmol/L, ALT > 600 U/L and AST > 300 U/L), and the second part was collected into the test tube with heparin for the purification of total RNA. The total RNA was extracted with Trizol reagent and further purified with Rneasy Mini Kit. The purified RNA was 50-fold diluted and its integrity was verified (OD260/280). The relative brightness ratio of RNA was investigated after incubation at 70 °C for 1 h. The synthesis of double-stranded cDNA was performed in accordance with the manual of Clontech PCR cDNA Synthesis Kit [4, 11, 30, 31]. The concentration and OD_{260}/OD_{280} value of cDNA were also investigated.

Suppression subtractive hybridization

The cDNA was digested with *Rsa*I and purified with NucleoTrap PCR Suspension Kit. The forward subtractive library was constructed with the tester cDNA from

α-amanitin treated mice to identify the up-regulated cDNA in α-amanitin treated mice, and reverse subtractive library was constructed by reversing the tester and driver to identify the down-regulated cDNA in α-amanitin treated mice. SSH was performed using PCR-Select™ cDNA Subtraction Kit according to the manufacturer's protocol.

Screening and sequencing of subtractive cDNA

The secondary PCR products were purified and cloned into pMD18-T vector. The ligation products were transformed into *E. coli* JM109, and the recons were screened on agar plates containing ampicillin, X-gal and IPTG. The PCR products of the cDNAs obtained from forward and reverse subtractive libraries were digested with *Rsa*I, respectively. The digested cDNAs were labeled with biotin using Detector™ PCR DNA Biotinylation Kit and identified by dot blot hybridization with Detector™ HRP Southern Blotting Kit according to manufacturer's protocol. The dot blot hybridization was performed with probes generated from forward and reverse subtractive library, respectively. The positive clones were identified when blots probed with the subtracted tester demonstrated signal intensity tenfold stronger than blots probed with the subtracted driver, and vice versa. The positive clones were randomly picked out for gene sequencing and the sequences were searched for homology in Genebank at NCBI.

Evaluation antagonistic effects of candidate TCMs

Based on fold change values of dot blot hybridization, four genes (Catnβ, Flt3-L, IL-7r and Rpo2-4) were selected to evaluate the antagonistic effects of candidate TCMs against the toxicity of α-amanitin. The decoctions of candidate TCMs were prepared (0.5 mg/ml silybin solution, 0.33 g/ml *G. sinense,* 0.33 g/ml *G. lucidum,* 75 mg/ml *P. frutescens* (L.) britt var. *frutescens* and 25 mg/ml *P. frutescens* var. *criasta* mixed with 30 mg/ml *G. Fisch*). The BALB/c mice were randomly divided into seven groups (Ten mice per group) and i.v. injected with 0.327 mg/kg α-amanitin (Seven groups: α-amanitin group, silybin group, *G. sinense* group, *G. lucidum* group, *P. frutescens* (L.) britt var. *frutescens* group, *P. frutescens* var. *criasta* mixed with *G. Fisch* group, physiological saline group). In 12 h after α-amanitin injection, the decoctions of the candidate herbs were intragastrically administrated to mice of corresponding groups with a dose of 0.02 ml/g body weight for three times per day [28]. The α-amanitin and physiological saline groups were treated with physiological saline. The total RNA was isolated from each group with and without the intragastric administration of candidate herbs. Each RNA sample was reverse-transcribed with One Step RNA PCR Kit following the user's instructions (Primers are shown in Table 1). Expression of β-actin was used to normalize the amount of the four genes presenting in each semi-quantitative PCR experiment. The antagonistic effects of the

candidate TCMs against the toxicity of α-amanitin were evaluated based on their recovery effects on the transcription levels of the four genes with altered expression [16, 26].

RESULTS

Suppression subtractive hybridization

Tester cDNA, which was digested with *Rsa*I, was ligated with Adaptor1 and Adaptor2R. The subtracted cDNAs were greatly specifically enriched after the nested PCR. Total 146 clones were identified with 77 from forward subtractive library and 69 from reverse one.

Screening and sequencing of subtractive cDNA

All the 146 clones were hybridized with probes made from forward and reverse subtractive library. The 41 positive spots were identified from forward hybridization and 44 positive spots from reverse hybridization (Figs 1 and 2). The 9 positive clones marked with arrows were selected for sequencing and homology searching. The up-regulated expression genes in α-amanitin treated mouse were D14716, NM_007563 and NM_019946. The down-regulated ones were NM_013520, AK013055, AK013732, NM_007645, NM_016974 and NM_021718.

Evaluation antagonistic effects of candidate TCMs

After candidate herbs intervened, the mice showed different changes of clinical symptoms, especially in the groups of *S. marianum* (L.) Gaertn and *G. lucidum*.

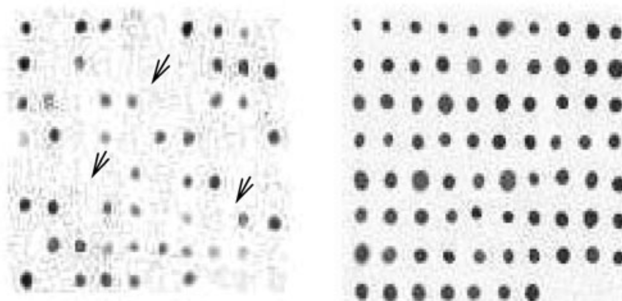

Fig. 1. The 77 clones selected from forward subtractive library were identified by dot blot hybridization. Left: Probe was made from the cDNA of reverse subtractive library. Right: Probe was made from the cDNA of forward subtractive library. Arrows showed the sequenced positive clones

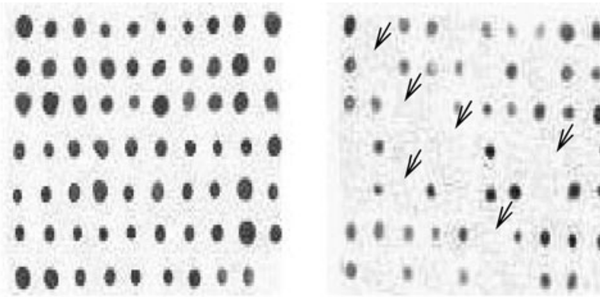

Fig. 2. The 69 clones selected from reverse subtractive library were identified by dot blot hybridization. Left: Probe was made from the cDNA of reverse subtractive library. Right: Probe was made from the cDNA of forward subtractive library. Arrows showed the sequenced positive clones

Comparing with the poisoned mice, the mice of these two groups behaved actively and moved quickly. The comparison of the variation data for the mouse weight of each group showed that mice in the *S. marianum* (L.) Gaertn, *G. lucidum* and *P. frutescens* (L.) britt var. *frutescens* groups significantly recovered their weight after the administration 36 h of these herbs.

Based on their significant fold change values in dot blot hybridization, the four genes were selected to evaluate the antagonistic effects of candidate TCMs against the toxicity of α-amanitin. After the herbs administered, the expression levels of these genes were analyzed according to the results of semi-quantitative PCR (Figs 3–6).

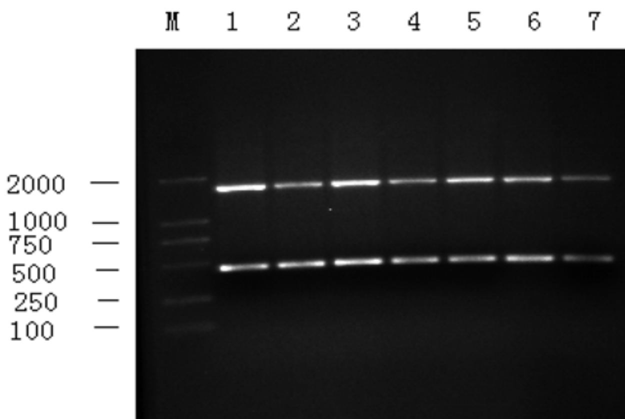

Fig. 3. Semi-quantitative PCR analysis of Catnβ expression after candidate herbs administrated. (M) DNA Marker DL2000; (1) cDNA from α-amanitin group; (2) silybin group; (3) *G. sinense* group; (4) *G. lucidum* group; (5) *P. frutescens* (L.) britt var. *frutescens* group; (6) *P. frutescens* var. *criasta* with *G. Fisch* group; (7) physiological saline group

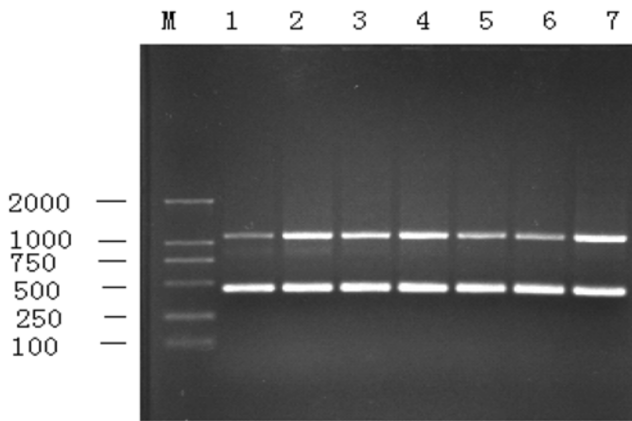

Fig. 4. Semi-quantitative PCR analysis of Flt3-L expression after candidate herbs administrated. (M) DNA Marker DL2000; (1) cDNA from α-amanitin group; (2) silybin group; (3) *G. sinense* group; (4) *G. lucidum* group; (5) *P. frutescens* (L.) britt var. *frutescens* group; (6) *P. frutescens* var. *criasta* with *G. Fisch* group; (7) physiological saline group

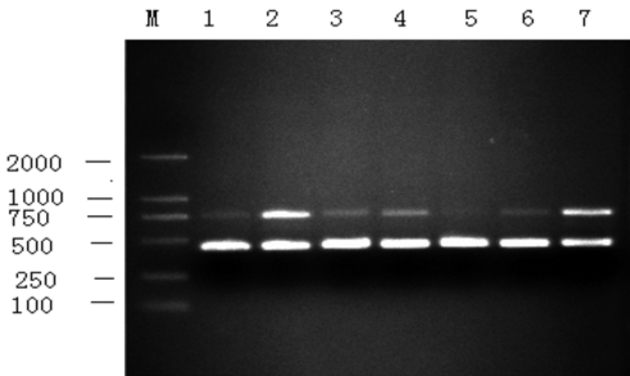

Fig. 5. Semi-quantitative PCR analysis of IL-7r expression after candidate herbs administrated. (M) DNA Marker DL2000; (1) cDNA from α-amanitin group; (2) silybin group; (3) *G. sinense* group; (4) *G. lucidum* group; (5) *P. frutescens* (L.) britt var. *frutescens* group; (6) *P. frutescens* var. *criasta* with *G. Fisch* group; (7) physiological saline group

Comparing the expression levels of these genes with β-actin in each group, each herb had different recovery effects on the genetic transcription levels of these genes with altered expression caused by α-amanitin. *S. marianum* (L.) Gaertn, *G. lucidum* and *P. frutescens* var. *criasta* with *G. uralensis* Fisch had significant down-regulated effects to Catnβ which was up-regulated by α-amanitin, and the ratios of Catnβ transcription amount to β-actin were 0.71, 0.80 and 0.85, respectively, which were

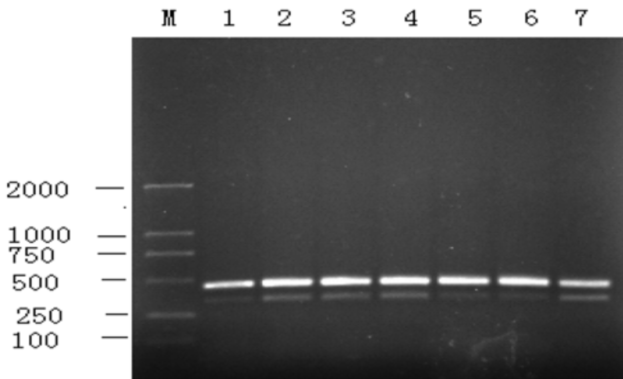

Fig. 6. Semi-quantitative PCR analysis of Rpo2-4 expression after candidate herbs administrated. (M) DNA Marker DL2000; (1) cDNA from α-amanitin group; (2) silybin group; (3) *G. sinense* group; (4) *G. lucidum* group; (5) *P. frutescens* (L.) britt var. *frutescens* group; (6) *P. frutescens* var. *criasta* with *G. Fisch* group; (7) physiological saline group

far lower than 1.28 of α-amanitin group. *S. marianum* (L.) Gaertn and *G. lucidum* had up-regulated effect on the transcription of Flt3-L gene. After administration of these two herbs, the ratios of Flt3-L transcription amount to β-actin were 0.83 and 0.79, respectively, which were close to 0.88 of the physiological saline group. The transcription of IL-7r in the *S. marianum* (L.) Gaertn group was significantly up-regulated, which transcription amount to β-actin increased from 0.36 to 0.78 that is close to 0.83 of the physiological saline group. In addition, administration of *G. lucidum*, *G. sinense* and *S. marianum* (L.) Gaertn had a significant influence on the transcription of Rpo2-4. After administration of these herbs, the ratios of Rpo2-4 transcription amount to β-actin increased to 0.58, 0.56 and 0.50 from 0.23 of α-amanitin group. These results suggested that *S. marianum* (L.) Gaertn and *G. lucidum* had strong antagonism against the toxicity of α-amanitin.

DISCUSSION

The compound α-amanitin could cause severe damage to liver and kidney and change the expression of some important genes. The results of SSH and dot blot hybridization revealed that there were 41 genes with altered expression in forward subtractive library and 44 ones in reverse subtractive library. Meanwhile, for the significant fold change values in dot blot hybridization, four genes with important biological functions were selected to evaluate the antagonistic effectiveness of candidate TCMs against the toxicity of α-amanitin.

The four genes with altered expression: Flt3-L, IL-7r, Catnβ and Rpo2-4, that were involved in the important functions of regulating immune system, cell signaling pathway and gene transcription were identified in α-amanitin poisoned mice by

SSH and dot blot hybridization. After administration the TCMs to these poisoned mice, different recovery effects on the genetic transcription levels of these genes were observed. Compared with the physiological saline group, Flt3-L and IL-7r were significantly up-regulated after *S. marianum* (L.) Gaertn and *G. lucidum* administrated. Flt3-L is a hematopoietic growth factor, which has a profound effect on the mobilization and expansion of stem cells and progenitors. It plays an important role in both dendritic cell (DC) and natural killer (NK) cell differentiation from hematopoietic progenitor cell in liver, bone marrow and thymus. It promotes the generation of large number of CD11+ cells, DCs, plasmacytoid dendritic cells and CD14+ cells. T cell-mediated immunity could be promoted by Flt3-L [4, 5, 28]. IL-7r is an indispensable cytokine receptor in immune system and it is important for the expansion and differentiation of lymphocytes [2, 12, 21]. *S. marianum* (L.) Gaertn and *G. lucidum* could up-regulate Flt3-L and IL-7r, which were down-regulated by α-amanitin, thus *S. marianum* (L.) Gaertn and *G. lucidum* were benefit for recovering the normal functions of Flt3-L and IL-7r. The gene Catnβ, up-regulated by α-amanitin, is a member of intracellular glucoproteins. It is essential for normal liver growth and development. Moreover, it is identified as a component of the Wnt signaling pathway, which is highly susceptible to alteration in Catnβ stability. Wnt signaling pathway is crucial for sustaining the balance between proliferation and differentiation throughout embryogenesis and postnatal life of organism [7, 10, 17]. Therefore, the Catnβ gene expression and its biological function were recovered by *S. marianum* (L.) Gaertn, *P. frutescens* var. *criasta* with *G. uralensis* and *G. lucidum*. Besides, *S. marianum* (L.) Gaertn, *G. sinense* and *G. lucidum* could up-regulate the expression of gene Rpo2-4, which was down-regulated by α-amanitin. Rpo2-4 is the 14 kDa subunit of RNA polymerase. It plays a key role in gene transcription and in maintaining their normal function. Many works have demonstrated that α-amanitin not only noncompetitively inhibits the transcription activity of RNA polymerase in eukaryotes, but also suppresses the expression of RNA polymerase [8, 9, 32]. *S. marianum* (L.) Gaertn, *G. sinense* and *G. lucidum* helper the recovery of the important biological function of Rpo2-4 in organism.

The candidate TCMs have been used to treat patients poisoned by poisonous mushroom in many hospitals in China, which reveals they have some therapeutic effects. In addition, they have effects of maintaining functions of liver and kidney and boosting performance of the immune system according to the theories of TCM. Therefore, the candidate TCMs were selected and its antagonistic effectiveness was evaluated based on the analysis of semi-quantitative PCR. The research revealed that *Silybum marianum* (L.) Gaert and *G. lucidum* can significantly recover the expression levels of Flt3-L, IL-7r, Catnβ and Rpo2-4 in α-amanitin poisoned mice, which indicated that they were the effective antagonists against the toxicity of α-amanitin.

ACKNOWLEDGEMENTS

This project was supported by Chinese National Programs for High Technology Research and Development (2007AA021506) and National Science and Technology Pillar Program in the Eleventh Five-year Period (2007BAD81B04).

REFERENCES

1. Abe, M., Akbar, F., Hasebe, A., Horiike, N., Onji, M. (2003) Glycyrrhizin enhances interleukin-10 production by liver dendritic cells in mice with hepatitis. *J. Gastroenterol. 38,* 962–967.
2. Akashi, K., Kondo, M., von Freeden-Jeffry, U., Murray, R., Weissman, I. L. (1997) Bcl-2 Rescues T Lymphopoiesis in interleukin-7 receptor–deficient mice. *Cell 89,* 1033–1041.
3. Anil, K. T., Chapla, A., Rana, P. S., Kenneth, R. S., Michael, L. G., Rajesh, A. (2003) Silibinin downregulates survivin protein and mRNA expression and causes caspases activation and apoptosis in human bladder transitional-cell papilloma RT4 cells. *Biochem. Bioph. Res. Co. 312,* 1178–1184.
4. Bray, B. J., Goodin, M. G., Inder, R. E., Rosengren, R. J. (2001) The effect of retinol on hepatic and renal drug-metabolising enzymes. *Food Chem. Toxicol. 39,* 1–9.
5. Chen, W., Anissa, S. H. C., Amanda, J. D., Lin, X. Q., Bruce, R. B., Jeffrey, S. M. (2005) FLT3 Ligand administration after hematopoietic cell transplantation increases circulating dendritic cell precursors that can be activated by CpG oligodeoxynucleotides to enhance T-cell and natural killer cell function. *Biol. Blood Marrow Tr. 11,* 23–34.
6. Chiu, S. W., Wang, Z. M., Leung, T. M., Moore, D. (2000) Nutritional value of Ganoderma extract and assessment of its genotoxicity and antigenotoxicity using comet assays of mouse lymphocytes. *Food Chem. Toxicol. 38,* 173–178.
7. Clevers, H. (2006) Wnt/β-Catenin signaling in development and disease. *Cell 127,* 469–480.
8. Damgaard, J., Balslev, Y., Møllgaard, K., Wassermann, K. (1996) Ongoing activity of RNA polymerase? Precludes chromatin collapse and DNA fragmentation in chinese hamster ovary cells. *Biochem. Bioph. Res. Co. 227,* 677–683.
9. Englek, K. A., Lewis, E. W., Howard, B. H. (1998) Mechanisms of replication-deficient vaccinia virus/T7 RNA polymerase hybrid expression: Effect of T7 RNA polymerase levels and α-amanitin. *Virology 243,* 331–339.
10. Feng, J., Christopher, J. P., Branko, S. (2006) Gene expression profile of quiescent and activated rat hepatic stellate cells implicates Wnt signaling pathway in activation. *J. Hepatol. 45,* 401–409.
11. Floersheim, G. L. (1976) Tolerance to lethal doses of *Amanita phalloides* induced by small doses of the mushroom. *Toxicol. Appl. Pharm. 37,* 455–459.
12. Fukuyama, S., Hiroi, T., Yokota, Y., Paul, D. R., Yanagita, M., Kinoshita, N., Terawaki, S., Shikina, T., Yamamoto, M., Kurono, Y. (2002) Initiation of NALT organogenesis is independent of the IL-7R LTβR, and NIK signaling pathways but requires the Id2 gene and CD3-CD4+CD45+ cells. *Immunity 17,* 31–40.
13. Gundala, S., Wells, L. D., Milliano, M. T., Talkad, V., Luxon, B. A., Brent, A. N. (2004) The hepatocellular bile acid transporter Ntcp facilitates uptake of the lethal mushroom toxin α-amanitin. *Arch. Toxicol. 78,* 68–73.
14. Hye, G. J., Ho, J. Y., Sung, J. P., Ae, R. M., Young, C. C., Shin, K. K., Hyo, K. C. (2002) Hepatoprotective effects of 18-beta-glycyrrhetinic acid on carbon tetrachloride-induced liver injury: inhibition of cytochrome P450 2E1 expression. *Pharmacol. Res. 46,* 221–227.
15. Huang, M. F., Guo, L., Zheng, J., Jiang, Y. (2007) Study advances in pefilla frutescens. *China Food Addit. 5,* 85–89.
16. Jorgensen, S. M., Kleveland, E. J., Grimholt, U., Gjoen, T. (2006) Validation of reference genes for real-time polymerase chain reaction studies in atlantic salmon. *Mar. Biotechnol. 8,* 398–408.
17. Kundu, J. K., Choi, K. Y., Surh, Y. J. (2006) α-catenin-mediated signaling: A novel molecular target for chemoprevention with anti-inflammatory substances. *BBA-Rev. Cancer 1765*, 14–24.
18. Lin, S. B., Li, C. H., Lee, S. S., Kan, L. S. (2003) Triterpene-enriched extracts from *Ganoderma lucidum* inhibit growth of hepatoma cells via suppressing protein kinase C, activating mitogen-activated protein kinases and G2-phase cell cycle arrest. *Life Sci. 72,* 2381–2390.
19. Loranger, A., Tuchweber, B., Gicquaud, C., St-Pierre, S., Côté, M. G. (1985) Toxicity of peptides of *Amanita virosa* mushrooms in mice. *Fund. Appl. Toxicol. 5,* 1144–1152.
20. Lü, B. J., Xu, J., Zhu, Y. M., Zhang, H., Lai, M. D. (2007) Systemic analysis of the differential gene expression profile in a colonic adenoma-normal SSH library. *Clin. Chim. Acta 378,* 42–47.

21. Moniuszko, M., Smith, Y. E., Venzon, D., Stevceva, L., Nacsa, J., Tryniszewska, E., Tsai, W. P., Franchini, G. (2006) Decreased number of CD4+ and CD8+ T cells that express the interleukin-7 receptor in blood and tissues of SIV-infected macaques. *Virology 356,* 188–197.
22. Nowak, T. S., Woodards, A. C., Jung, Y., Adema, C. M., Loker, E. S. (2004) Identification of transcripts generated during the response of resistant *Biomphalaria glabrata* to *Schistosoma mansoni* infection using suppression subtractive hybridization. *J. Parasitol. 90,* 1034–1040.
23. Osakabe, N., Yasuda, A., Natsume, M., Sanbongi, C., Kato, Y., Osawa, T., Yoshikawa, T. (2002) Rosmarinic acid, a major polyphenolic component of *Perilla frutescens,* reduces lipopolysaccharide (LPS)-induced liver injury in D-galactosamine (D-GalN)-sensitized mice. *Free Radical Bio. Med. 33,* 798–806.
24. Pan, D., He, N. H., Yang, Z. Y., Liu, H. P., Xu, X. (2005) Differential gene expression profile in hepatopancreas of WSSV-resistant shrimp *(Penaeus japonicus)* by suppression subtractive hybridization. *Dev. Comp. Immunol. 29,* 103–112.
25. Petrov, P., Sekeris, C. E. (1971) Early action of α-amanitin on extranucleolar ribonucleoproteins, as revealed by electron microscopic observation. *Exp. Cell Res. 69,* 393–400.
26. Saed, G. M., Kruger, M., Diamond, M. P. (2006) Enhanced matrix metalloproteinase expression by Tisseel in mesothelial cells, normal peritoneal fibroblasts, and adhesion fibroblasts. *Eur. J. Plast. Surg. 28,* 472–479.
27. Shi, Y., Mei, S. C. (2002) Methods of administration and calculating the dose. In: Jiang, D. P. (ed.) *Medicine Animal Experiment Utility Manual*. China Agriculture Press, Beijing, pp. 24–31.
28. Sian, E. N., Winter, S., Mottram, R. Miyan, J. A., Whetton A. D. (1999) Flt3 ligand can promote survival and macrophage development without proliferation in myeloid progenitor cells. *Exp. Hematol. 27,* 663–672.
29. Skottová, N., Vagera, Z. S., Vecera, R., Urbánek, K., Jegorov, A., Simánek, V. (2001) Pharmacokinetic study of iodine-labeled silibinins in rat. *Pharmacol. Res. 44,* 247–253.
30. Sobel, E. S., Wang, F., Butfiloski, E., Croker, B., Roberts, S. M. (2006) Comparison of chlordecone effects on autoimmunity in (NZBxNZW) F1 and BALB/c mice. *Toxicology 218,* 81–89.
31. Turton, J. A., Andrews, C. M., Havard, A. C., Robinson, S., York, M., Williams, T. C., Gibson, F. M. (2002) Haemotoxicity of thiamphenicol in the BALB/c mouse and Wistar Hanover rat. *Food Chem. Toxicol. 40,* 1849–1861.
32. Vanácová, S., Tachezy, J., Ullu, E., Tschudi, C. (2001) Unusual diversity in α-amanitin sensitivity of RNA polymerases in trichomonads. *Mol. Biochem. Parasit. 115,* 239–247.
33. Vogel, G., Braatz, R., Mengs, U. (1979) On the nephrotoxicity of α-amanitin and the antagonistic effects of silymarin in rats. *Inflamm. Res. 19,* 221–226.
34. Vogel, G., Tuchweber, B., Trost, W., Mengs, U. (1984) Protection by silibinin against Amanita phalloides intoxication in beagles. *Toxicol. Appl. Pharm. 73,* 355–362.
35. Xiao, G. L., Li, X. M., Li, Z. J., Zhang, J., Li, J. (2006) 103 Patients with Amanita mushroom poisoning treated by Lingzhi Decoctio. *J. TCM Univ. Hunan 5,* 44–45.
36. Xu, D. Q., Xiong, Y. Z., Ling, X. F., Lan, J., Liu, M., Deng, C. Y., Jiang, S. W., Lei, M. G. (2005) Identification of a differential gene HUMMLC2B between F1 hybrids Landrace × Yorkshire and their female parents Yorkshire. *Gene 352,* 118–126.
37. Yao, X. L., Zhu, H. L., Lv, X. L. (2005) Inhibition effect on the Perilla extracts to hemolytic reaction induced by H_2O_2 and LPO formation in liver homogenate of mice. *J. Tianjin Univ. TCM 3,* 126–128.
38. Zhao, J., Cao, M., Zhang, J., Sun, Q., Chen, Q., Yang, Z. R. (2006) Pathological effects of the mushroom toxin α-amanitin on BALB/c mice. *Peptides 27,* 3047–3052.
39. Zhao, J., Zhang, J., Hou, R. T., Li, D. X., Yang, Z. R. (2003) Advances in the studies on toxins of Amanita. *Chem. Ind. Forest Prod. 23,* 90–96.
40. Zhang, X. W., Zhao, Z. Y. (2006) Therapeutic observation on compound glycyrrhizin for medicamentous liver lesion induced by chemotherapy. *China Pharm. 18,* 1408–1409.