Toxicity of Ganoderma boninense methanol extract in mice

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(Received 20 December 2009)

\textit{Ganoderma boninense} Pat. is widely used in China and other countries of the Orient in the traditional treatment of many ailments. However, there is little toxicological information available regarding safety following repeated exposure. The present study describes the toxicity of a methanol extract of \textit{G. boninense} in mice. In an oral acute toxicity study, a methanol extract of \textit{G. boninense} was orally administered to Wister albino mice (female and male) at a dose of 2000 mg/kg body weight (bw) for a period of 14 days. The animals were killed, followed by examination of their organs and blood serum. Administration of the methanol extract \textit{G. boninense} at 2000 mg/kg bw did not produce mortality or significant changes in general behavior, bodyweight, or organ gross appearance. There were no significant differences in general condition, growth, organ weights, hematological parameters, clinical chemistry values, or gross and microscopic appearance of the organs from the treatment groups compared to the control group. \textit{G. boninense} was found to be relatively safe on short-term oral administration.

\textbf{Keywords:} basidiocarps; oral acute toxicity; medicinal mushroom

\textbf{Introduction}

Mushrooms are not only utilized as food but may also be a source for the development of new drugs and nutraceuticals (Yang et al. 2002; Barros et al. 2007). They are considered a good source of protein and phenolic antioxidants, such as variegic acid and diboviquinone (Cheung et al. 2003). \textit{Ganoderma} is a traditional Chinese medicine prescribed for the treatment of chronic hepatitis, hypertension, bronchitis, arthritis, neurasthenia and neoplasin in China and other countries of the Orient (Arisawa et al. 1986; Dudhgaonkar et al. 2009; Majagi and Patil 2009; Chen et al. 2009). However, little information is available on the toxicity of local \textit{Ganoderma boninense} Pat. (Ganodermataceae).

The increasing consumption of natural products and natural chemicals from plants and fungi has motivated scientists to evaluate such chemicals for possible genotoxic, mutagenic and carcinogenic effects (Bellini et al. 2008). Toxicology is the study of the adverse effects of chemical substances on living organisms (Schrager 2008). It is the study of symptoms, mechanisms, treatments and detection of poisoning, especially the poisoning in humans. \textit{G. boninense} may constitute a potentially useful resource for new and safe drugs for the treatment of various ailments. Thus, toxicity studies will play an important role in identification and isolation of new compounds from crude extracts. Toxicological data helps in the selection of natural remedies with potential medicinal properties for future study. Not much is known about \textit{G. boninense}; therefore, research is required on the status, extent, and utilization of \textit{G. boninense}. The present work aims to determine the toxicity of a methanolic extract from \textit{G. boninense} in mice, which to the best of our knowledge has not been reported previously.

\textbf{Materials and methods}

\textbf{Mushroom sample}

The wild fruiting bodies of \textit{G. boninense} were collected from an oil palm plantation in Leong Watt Hin Estate (Malacca, Malaysia) in April 2008. In the field, the fresh basidiocarps were rinsed with tap water to remove debris and epiphytes before being transported to the laboratory. In the laboratory, the basidiocarps were further washed with freshwater and brushed with a soft brush before being dried in an oven at 60°C for 7 days.

\textbf{Extraction procedure}

The oven-dried basidiocarps were powdered. Dried samples (5 g) were extracted by stirring with 100 ml methanol at 25°C at 150 rpm for 48 h and filtered through Whatman No. 4 paper. The residue was then extracted with two additional 100 ml portions of methanol, as

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described above. Subsequently, the methanolic extract were evaporated at 50 °C to dryness and stored until use.

**Oral acute toxicity testing**

**Animals**

The experimental animals used in this study were Swiss albino mice of both sexes, weighing 20–30 g and aged 8–10 weeks. The animals were obtained from the animal shop in Penang, Malaysia. They were housed in cages with free access to water and food except for the short fasting period before oral administration of the extract. All mice were maintained on a 12-h light/dark cycle and constant temperature (22 °C) and humidity. They were allowed to acclimatize to laboratory conditions for 7 days.

**Acute toxicity assay**

Two groups of 20 mice (control and test group), containing an equal number of males and females, were formed. A 2000 mg/l/kg body weight (OECD 2000) dose of *G. boninense* extract dissolved in 10% DMSO was administered intragastrically to the test group. The animals in the control group received 10% DMSO. In each case, the volume administered was 10 ml/kg body weight. After administration, the animals were closely observed during the first 6 h, and occasionally thereafter, for 14 days for toxic signs and symptoms or death. The symptoms of toxicity, such as asthenia, hypoactivity (motor activity), anorexia, diarrhea and syncope, were recorded. The animals were weighed daily and anesthetized under CO₂ inhalation at the end of the 14-day experimental period. Blood samples were collected via cardiac puncture into non-heparinized and EDTA-containing tubes for biochemical and hematological analyses, respectively (Petterino and Argentino-Storino 2006). After cardiac puncture, the mice were killed by cervical dislocation. Vital organs were excised, weighed, macroscopically examined and then fixed in 10% formalin for histopathological study.

**Blood analyses**

Hematological and biochemical analyses were performed at the pathology laboratory. Full blood cell counts were determined on a fully automated Abbott Cell-Dyn 3500 hematology analyzer (Abbott Laboratories, Abbott Park, IL, USA), and serum biochemistry tests were performed using a COBAS Integra 800 (Roche, Cologne, Germany).

**Statistical analysis**

Statistical analysis involved use of the Statistical Package for Social Sciences (SPSS). Data are given as the mean±SEM; statistics were performed using t-tests and *p* values less than 5% were considered statistically significant (*p* < 0.05).

**Result**

**General signs and organ body weight index**

No toxic symptoms or mortality was observed in any animals, which lived up to 14 days. Macroscopic examination of the organs of the animals treated with extract showed no changes in color compared to control. Autopsy at the end of the experiment period revealed no apparent changes in any organs. There were no changes either in body weight or in the weight of the principal organs, and all animals exhibited a gain in body weight. Table 1 shows the effect of extract on principal organ weights relative to body weight. Regarding daily consumption of food and water, water intake oscillated around 15 and 20 ml per day in females and males, respectively, for both groups. Food intake exhibited the same pattern in each sex and group. Therefore, the minimum acute fatal dose of the extract of *G. boninense* for mice is higher than 2000 mg/kg body weight.

**Effect of sub-chronic oral administration of *G. boninense* methanol extract on the hematological and biochemical parameters in mice**

The analyzed hematological parameters, which included red blood cell count (RBC), hemoglobin concentration (Hb), haematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular

| Organs  | Control (Males) | 2000 mg/kg extract (Males) | Control (Females) | 2000 mg/kg extract (Females) |
|---------|-----------------|----------------------------|-------------------|-------------------------------|
| Liver   | 6.059 ± 0.642   | 6.991 ± 0.771             | 5.624 ± 0.625     | 7.038 ± 0.556                 |
| Kidney  | 1.882 ± 0.240   | 1.656 ± 0.111             | 1.447 ± 0.108     | 1.339 ± 0.137                 |
| Lung    | 0.748 ± 0.093   | 0.828 ± 0.127             | 1.159 ± 0.108     | 0.891 ± 0.166                 |
| Spleen  | 0.467 ± 0.227   | 0.474 ± 0.127             | 0.409 ± 0.060     | 0.540 ± 0.202                 |
| Heart   | 0.545 ± 0.108   | 0.541 ± 0.144             | 0.492 ± 0.095     | 0.517 ± 0.062                 |

Organ body index was calculated as (organ weight/body weight) × 100%.

*Crude extract of *Ganoderma boninense* was administered to mice at a dose of 2000 mg/kg body weight. Values are mean ± SD (n = 10).

*p > 0.05 (t-test).*
hemoglobin concentration (MCHC), platelets, white blood cell count (WBC), and white blood cell differential count, for mice treated with G. boninense methanol extract (2000 mg/kg) were not significantly different to controls (Table 2). Values remained within normal limits throughout the experimental period. Biochemical analysis indicated that no significant differences were detected for any of the parameters in either the control or G. boninense methanol extract-treated group of male or female mice (Table 3).

**Histopathological study**

No lesions or pathological changes in the organs attributable to treatment with G. boninense methanol extract were determined from pathological examinations (Figures 1–5).

**Discussion**

The toxicity of G. boninense extract in mice was examined, and its potency was qualitatively and quantitatively evaluated by histopathology and oral acute toxicity studies. It should be emphasized that toxic effects of natural products on host cells must be considered, as a substance may exhibit an apparent biological activity by virtue of its toxic effect on the cells (Sasidharan et al. 2008). In addition, experimental screening is important to ascertain the safety and efficacy of natural products and to establish the active component of these natural remedies (Ogbonnia et al. 2008).

In the acute toxicity study of the extract, no mortality was recorded and no changes in animal behavior or in organ weights were observed in the treated group. The starting dose was at a level most likely to produce mortality in some of the treated animals (OECD 2000). Thus, the minimum fatal dose of crude extract for mice was greater than 2000 mg/kg, which is the single high dose recommended in the OECD (2000) guidelines for testing oral acute toxicity. Thus, G. boninense did not cause any apparent acute toxicity.

The hematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status in both animals and humans (Adeneye et al. 2006). After 14 days of treatment

### Table 2. Hematology values of mice treated with *Ganoderma boninense* methanol extract for 14 days.

| Unit                        | Treatment                        | Control             | *Ganoderma boninense* methanol extract (200 mg/ml) |
|-----------------------------|----------------------------------|---------------------|---------------------------------------------------|
| Male                        |                                  |                     |                                                   |
| White blood cell count      | $10^{9}$/l                       | 8.58 ± 2.10         | 8.60 ± 1.65                                       |
| Neutrophils                 | %                                | 1.83 ± 0.46         | 1.75 ± 0.23                                       |
| Lymphocytes                 | %                                | 5.87 ± 1.48         | 5.83 ± 1.39                                       |
| Monocytes                   | %                                | 0.34 ± 0.14         | 0.31 ± 0.04                                       |
| Eosinophils                 | %                                | 0.20 ± 0.09         | 0.21 ± 0.03                                       |
| Basophils                   | %                                | 0.44 ± 0.10         | 0.43 ± 0.20                                       |
| Red blood cell count        | $10^{12}$/l                      | 8.17 ± 0.43         | 8.15 ± 0.73                                       |
| Hemoglobin                  | g/l                              | 15.12 ± 1.33        | 15.21 ± 1.22                                      |
| Hematocrit                  | %                                | 0.72 ± 0.07         | 0.73 ± 0.04                                       |
| Mean red blood cell volume  | fl                               | 87.54 ± 4.89        | 86.79 ± 3.89                                      |
| Mean corpuscular            | pg                               | 18.35 ± 2.11        | 18.24 ± 2.01                                      |
| Mean corpuscular Hb concen. | g/l                              | 20.78 ± 0.64        | 20.62 ± 0.57                                      |
| Platelets                   | $10^{9}$/l                       | 994.22 ± 210.24     | 989.21 ± 201.12                                   |
| Mean platelet cell volume   | fl                               | 8.23 ± 0.64         | 8.19 ± 1.02                                       |
| Female                      |                                  |                     |                                                   |
| White blood cell count      | $10^{9}$/l                       | 7.99 ± 2.01         | 8.10 ± 1.89                                       |
| Neutrophils                 | %                                | 1.35 ± 0.19         | 1.34 ± 0.14                                       |
| Lymphocytes                 | %                                | 5.94 ± 1.17         | 5.93 ± 1.17                                       |
| Monocytes                   | %                                | 0.36 ± 0.20         | 0.33 ± 0.11                                       |
| Eosinophils                 | %                                | 0.24 ± 0.15         | 0.26 ± 0.17                                       |
| Basophils                   | %                                | 0.12 ± 0.15         | 0.13 ± 0.11                                       |
| Red blood cell count        | $10^{12}$/l                      | 7.89 ± 0.34         | 7.91 ± 0.78                                       |
| Hemoglobin                  | g/l                              | 14.98 ± 0.27        | 14.83 ± 0.89                                      |
| Hematocrit                  | %                                | 0.75 ± 0.132        | 0.76 ± 0.17                                       |
| Mean red blood cell volume  | fl                               | 90.63 ± 6.54        | 90.84 ± 7.14                                      |
| Mean corpuscular Hb         | pg                               | 18.53 ± 0.76        | 18.82 ± 0.93                                      |
| Mean corpuscular Hb concen. | g/l                              | 20.71 ± 2.13        | 20.64 ± 2.14                                      |
| Platelets                   | $10^{9}$/l                       | 1057.00 ± 111.21    | 1061.00 ± 124.73                                  |
| Mean platelet cell volume   | fl                               | 7.73 ± 0.88         | 7.81 ± 0.79                                       |

Data arranged as: mean ± SEM ($n = 5$/sex).
with *G. boninense* there were also no treatment-related changes in the hematological parameters between the control and treatment group, indicating that the *G. boninense* methanol extract does not affect hematopoiesis and leucopoiesis in mice. The orally administrated dose of the extract was non-toxic and did not interfere with the production of circulating red blood cells, white blood cells or platelets. Moreover, there were no significant differences in biochemical parameters of the groups treated with *G. boninense* methanol extract (2000 mg/kg p.o.) compared to control. The lack of significant alterations in the levels of ALT, AST, creatinine and BUN are good indicators of liver and kidney functions (Hilaly et al. 2004), which suggests that acute administration of *G. boninense* methanol extract did not alter the hepatocytes and kidneys of mice. Bilirubin concentration has been used to evaluate chemically induced hepatic injury. Besides various normal functions, liver excretes the breakdown product of hemoglobin, namely bilirubin, into bile. It is well known that necrotizing agents can injure the hepatic parenchyma causing a large increase in bilirubin content (Plaa and Hewitt 1982). *G. boninense*
Figure 2. (a) The kidney of a control mouse, stained with hematoxylin and eosin, showing renal corpuscle (RC), collecting tubule (CT), distal tubule (DT) and proximal tubule (PT). (b) The kidney of a treated mouse, stained with hematoxylin and eosin, interlobular artery (IA), aorto artery (AA) and renal corpuscle (RC).

Figure 3. (a) The lung of a control mouse, stained with hematoxylin and eosin, showing bronchiole (B), alveolar sac (AS), alveoli (A) and blood vessel (BV). (b) The lung of a treated mouse, stained with hematoxylin and eosin, showing terminal bronchiole (TB), alveolar sac (AS), alveoli (A) and blood vessel (BV).

Figure 4. (a) The spleen of a control mouse, stained with hematoxylin and eosin, showing white pulp (WP) and red pulp (RP). (b). The spleen of a treated mouse, stained with hematoxylin and eosin, showing white pulp (WP), red pulp (RP), trabeculum (T) and central artery (CA).
extract was non-toxic as evidenced by the normal level of bilirubin in the serum of the treated group. In the present study, histopathological evaluation of acute oral ingestion of *G. boninense* methanol extract did not adversely affect the morphology of organs in mice. This agrees with the results of biochemical analysis, and oral administration of 2000 mg/kg/day for 14 days was well tolerated by the treated mice.

The safety of natural medicines is of paramount importance, as not much is known about many natural products used in traditional medicine (Moshi 2007). Some studies have shown that an aqueous extract of *G. boninense* contains chemicals that may be used for treating diabetes mellitus (Chee 2005). Results from this study showed that rate of glucose clearance in animals treated with *G. boninense* extract was significantly faster than the saline control (Chee 2005). Moreover, according to Akikuni (2002), compositions of the main component obtained by culturing the mycelium of *G. boninense* were capable of achieving an anticancer effect by inducing the production of interleukin 12 (IL-12). This research finding concurs with the above studies, signifying that this fungus was not toxic to humans.

Oral acute toxicity testing in mice could be used to evaluate natural remedies for different pharmacological activities, taking into account the basic premise that pharmacology is simply toxicology at a lower dose (Sasidharan et al. 2008a). A toxic substance might elicit interesting pharmacological effects at a lower non-toxic dose. Toxicity results from animals will be crucial in definitively judging the safety of this *Ganoderma* sp., as and when they are found to have sufficient potential for development into pharmacological products (Moshi 2007). The present results suggest the possibility of other, hitherto unreported, biological activities, which requires further investigation as a potential source of antimicrobial compounds, etc.

**Conclusion**

A methanol extract of *G. boninense* was not toxic to mice (single high dose >2000 mg/kg body weight). This is significant and supports the suggestion that a methanol extract of *G. boninense* could be used to developed natural-based medicines.

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