Full Length Research Paper

**In vivo toxicity study of *Ganoderma boninense***

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**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 data available regarding safety of this mushroom. The present study describes the toxicity of methanol extract of *G. boninense* in *Artemia salina*. The methanol extract of *G. boninense* was tested for in vivo brine shrimp lethality assay. The brine shrimp toxicity test exhibited no significant toxicity result for short-term exposure of this extract (LC₅₀ value of 2.214 mg/ml (6 h) and a significant toxicity results for long-term exposure (LC₅₀ of value 0.640 mg/ml (24 h). The results obtained may suggest that this mushroom is either outright toxic or may have potential anticancer activity. Methanolic extract of *G. boninense* was found to be relatively safe on short-term exposure.

**Key words:** *Ganoderma boninense*, *Artemia salina*, toxicity, mushroom.

**INTRODUCTION**

Mushrooms are a popular and valuable food, low in calories and high in nutritional values and some of them produce substances that have potential medical effects. *Ganoderma* is a traditional Chinese medicine and has been prescribed for treatment of chronic hepatopathy, hypertension, bronchitis, arthritis, neurasthenia and neoplasin in China and other countries of the Orient (Arisawa et al., 1986). However, little information is available about the toxicity of local *Ganoderma boninense* (Pat.) from Ganodermataceae family. In accordance with Chinese tradition, it would be useful against a variety of diseases like hepatopathy, hypertension, bronchitis, arthritis, neurasthenia, neoplasin and so on.

Some studies show that the aqueous extract of *G. boninense* contains chemicals that may be used for treating diabetes mellitus. 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 from the main component of a mycelium fraction of a product obtained by culturing the mycelium of *G. boninense* are able to achieve anticancer effect by inducing the production of interleukin 12 (IL-12). Recently, Mitchell et al. (2009) reported the antioxidant activity of methanolic extract of *G. boninense*. Although this species showed various biological activities, there is no recorded data for detail toxicity against brine shrimp. Toxicity studies play an important role in identification and isolation of new compounds from crude extracts of medicinal mushroom. Careful toxicity studies comparing the activity of whole mushroom extracts are still necessary to determine whether *G. boninense* provide real clinical benefits. Due to the large consumption of *G. boninense* in traditional medicine for prevention and treatment of various types of ailments, probably more data are needed on safety. Hence, the current investigation reports the toxicity of *G. boninense* methanol extract against *Artemia salina*, which has never before been reported in literature.

**MATERIALs AND METHODS**

**Mushroom sample**

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

**Extraction procedure**

The oven dried mushroom was made into powder. The dried samples (5 g) were extracted by stirring with 100 ml of 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 described earlier. Subsequently, the methanolic extracts was evaporated at 50°C to dryness and stored until further use.

**Toxicity testing against the brine shrimp**

**Hatching shrimp**

Brine shrimp eggs, *A. salina*, were hatched in artificial seawater prepared by dissolving 38 g of sea salt in 1 L of distilled water. After a 24-h incubation period at room temperature (22 to 29°C), the larvae were attracted to one side of the vessel with a light source and collected with a pipette. The larvae were separated from the eggs by aliquoting them three times in small beakers containing seawater. Brine shrimp prefer a temperature between 28 to 30°C and salinity of 3035 ppm, a pH of 8 to 9, and strong aeration under a continuous light regime (Al-Amin and Islam, 2005).

**Brine shrimp assay**

The bioactivity of the extract was monitored by the brine shrimp lethality test (Meyer et al., 1982). Dimethyl sulphoxide (DMSO) was used as a solvent and it has been observed that the mortality is almost zero (Al-Amin and Islam, 2005). Samples were dissolved in DMSO and diluted with artificial seawater. Two milliliters of seawater is placed in all the bijoux bottles. A two-fold dilution is carried out to obtain the concentration from 100 to 0.195 mg/ml. Potassium dichromate served as positive control and was prepared by dissolving in artificial seawater to obtain the concentration from 0.1 to 0.9 mg/ml (Sasidharan et al., 2008a). The last bottle was filled with sea salt water and DMSO only, serving as a drug-free control. A suspension of larvae (100 µl) containing about 10 to 15 larvae was added into each bottle and incubated for 24 h. The bottles were then examined and the number of dead larvae in each bottle was counted after 6 and 24 h. The total number of shrimp in each bottle was counted and recorded. The logarithm of concentrations was plotted against the mean percentage mortality, and the concentration that could kill 50% of the larvae (LC₅₀) was determined from the graph (Yoga et al., 2007a).

**Calculations and statistics**

The mean results of logarithms of concentrations against brine shrimp mortality were plotted using the Microsoft Excel computer program, which also presents regression equations. The regression equations were used to calculate the LC₅₀ value. Extracts giving LC₅₀ values greater than 1.0 mg/ml were considered to be nontoxic (Sasidharan et al., 2008a).

**RESULTS**

The result of the toxicity evaluation of the crude extract against *A. salina* is shown in Figures 1 and 2. Maximum mortalities took place at a concentration of 100 mg/ml whereas least mortalities were at 0.195 mg/ml concentration. The extract showed no significant toxicity against brine shrimp as the LC₅₀ value was 2.21 mg/ml at 6 h of incubation (Figure 1). However, the extract showed moderate toxicity to the shrimps with LC₅₀ value below 1 mg/ml after 24 h incubation (Figure 2). Figure 3 shows a light microscope micrograph of the death of *A. salina* after treatment with the methanol extract of *G. boninense*. Potassium dichromate, which acts as positive control, showed significant toxicity to brine shrimps (LC₅₀ < 1 mg/mL). The LC₅₀ of potassium dichromate at 6 and 24 h were 0.464 mg/ml (Figure 4) and 0.216 mg/ml (Figure 5), respectively. The mortality for negative control, which contains only DMSO and artificial seawater, was
Figure 2. Toxic effects of the *G. boninense* methanol extract after 24 h using brine shrimp lethality assay.

Figure 3. Light micrograph of the *G. boninense* extract-treated *A. salina*.

Figure 4. Toxic effects of potassium dichromate after 6 h using brine shrimp lethality assay.
observed to be nearly zero.

**DISCUSSION**

Brine shrimp lethality test has been used as a preliminary tool to evaluate the toxicity of the *G. boninense*. This assay is based on the ability to kill laboratory-cultured *A. nauplii* brine shrimp (Carballo et al., 2002). The procedure determines lethal concentrations of active compounds in brine medium. The activities of a broad range of active compounds are manifested as toxicity to the shrimp. As mentioned by Yoga et al. (2007b), this test is a convenient preliminary toxicity test because the brine shrimp is highly sensitive to a variety of chemical substances. Moreover, it avoids unnecessary use of animals in laboratories, because the LC$_{50}$ and LD$_{50}$ values show a good relationship between in vivo tests, with a rate of $r = 0.85$. In addition, this method requires small quantities of the samples that can be tested on a large scale. The extracts are considered inactive when all nauplii survive at a concentration of 1000 µg/ml (Meyer et al., 1982).

With respect to the effect of the time of exposure in the brine shrimp assay, no significant changes in toxicity were detected at 6 h of exposure compared with the positive control, potassium dichromate, which exhibited significant toxicity (LC$_{50}$ value < 1.0 mg/ml) against the brine shrimp as shown in Figures 4 and 5. However, results at 24 h showed that extract was quite toxic to the shrimps. Since the test is also used to identify potential anticancer substances, the results may mean that this mushroom is either outright toxic or may have potential anticancer activity (Moshi, 2007). Brine shrimp larvae are speculated as rapidly growing and developing creatures, so any extract that kills them probably interferes with some aspect of cell growth and differentiation (Hanson, 2005). Nevertheless, further anticancer studies are needed to determine the effects of this extract on cytotoxicity of cell line.

Our previous study described the toxicity of a methanol extract of *G. boninense* in mice (Sasidharan et al., 2010). Methanol extract of *G. boninense* was orally administered to Wister albino mice (female and male) at a dose of 2000 mg/kg for a period of 14 days. The animals were sacrificed, followed by examination of their organs and blood serum. Administration of the methanol extract *G. boninense* at 2000 mg/kg did not produce mortality or significant changes in the general behaviour, bodyweight, or organ gross appearance of mice. There were no significant differences in the general condition, growth, organ weights, haematological parameters, clinical chemistry values, or gross and microscopic appearance of the organs from the treatment groups as compared to the control group. *G. boninense* was found to be relatively safe on short-term oral administration.

**Conclusions**

*G. boninense* was found to be relatively safe on short-term exposure. This is interesting and lends support to the suggestion that methanol extract of *G. boninense* can be used to developed natural product base medicine with further detail animal model studies. However, further toxicity studies are needed to determine the effects of this extract on chronic oral toxicity, animal foetus, pregnant animals, and their reproductive capacity to complete the safety profile of this extract.

**REFERENCES**


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