Cytotoxicity and Acute Oral Toxicity of Ascomycetous Mycoparasitic *Scytalidium parasiticum*

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ABSTRACT: *Scytalidium parasiticum* (anamorph of *Xylogone* species) was described and reported as potential necrotrophic mycoparasite of *Ganoderma boninense* in two previous studies. This mycoparasitic *S. parasiticum* showed capability in reducing *Ganoderma* disease incidences and severity in the nursery. However, the scientific information on toxicity of *S. parasiticum* is limited. In the current study, *S. parasiticum* has been selected for cytotoxicity (MTT assay – yellow tetrazolium salt) and acute oral toxicity in rodent tests to determine the toxicity level of *S. parasiticum*. There were 6 different concentrations of lyophilized *S. parasiticum* tested – 0.063, 0.125, 0.25, 0.5, 1, and 2 mg/mL, in MTT assay. Results from MTT assay illustrated that *S. parasiticum* was considered to be weakly cytotoxicity (Inhibition concentration of 50% at the dose of 2 mg/mL) in V79-4 cells (Chinese hamster lung cells). There was no adverse toxic reaction observed in acute oral toxicity test as well (using Female Sprague-Dawley rats) at the rate of 2000 mg/kg body weight. The findings of the current study demonstrated that *Scytalidium parasiticum* was observed to be relatively safe with low to very low cell cytotoxicity effects and appeared to be none toxic to rats.

KEYWORDS: Basal stem rot; *Ganoderma boninense*; inhibition concentration; necrotrophic mycoparasite

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TRANSACTIONS ON SCIENCE AND TECHNOLOGY

INTRODUCTION

The search for environmental-friendly approaches, including the use of beneficial microbial or potential biocontrol candidates, natural plant-based phenolic compounds, other natural products, and breeding for tolerant materials to manage basal stem rot (BSR) in oil palm caused by *Ganoderma boninense* has been intensified partly due to the lack of single or combined most effective treatments (Chung, 2011; Hushiarian *et al*., 2013; Idris *et al*., 2004; Jee & Chong, 2014). *Ganoderma boninense*, is the most pathogenic *Ganoderma* species among the *Ganoderma* species associated with the disease (Idris, 1999) and its various isolates may also vary in the level of pathogenicity (Kok *et al*., 2013). *Ganoderma boninense* itself was reported to harbour a few fungal isolates (Goh *et al*., 2015a; Marzuki *et al*., 2015). *Scytalidium parasiticum* (teleomorph *Xylogone* species) isolated from *G. boninense* culture (Goh *et al*., 2015) was later found to be a potential necrotrophic mycoparasite to *G. boninense* (Goh *et al*., 2016). *Scytalidium parasiticum* suppressed the growth of *G. boninense* under *in-vitro* studies and reduced both BSR disease incidence and severity in nursery experiments (Goh *et al*., 2016). The information related to toxicity of this newly described fungal isolate is limited. Therefore, the main objective of the current study was to determine the toxicity of *S. parasiticum* fungal culture through cytotoxicity and acute oral toxicity tests. Furthermore, this toxicity information is crucial for determining the safeness of *S. parasiticum* to be applied as biofungicide.

METHODOLOGY

Fungal culture and growth conditions

Scytalidium parasiticum AAX0113 was cultured and maintained on malt extract agar (MEA) at 24°C in the dark for 7 days. Mycelial masses were harvested and transferred into 250-mL Erlenmeyer flask with malt extract broth (MEB). The inoculated MEB flasks were incubated for 2 weeks on an orbital shaker at 150 rpm at 24°C. The mycelia were separated from the liquid medium through filtering the mixture with Corning Falcon Cell strainers. The filtered mycelia were then lyophilized for 48 hours using Labconco freezer dryer (Fisher Scientific, Malaysia) until constant dry weight was obtained. Lyophilized *S. parasiticum* samples were sent to Makmal Bioserasi, Centre for Research & Instrumentation Management (CRIM), Universiti Kebangsaan Malaysia (UKM), Bangi, Selangor, Malaysia for cytotoxicity – MTT assay and acute oral toxicity study. Both MEA and MEB used in this study were purchased from Difco (Becton Dickinson Diagnostics, Sparks, MD, USA).

Cytotoxicity: MTT assay

The experiment was performed on Chinese hamster lung cells (*Cricetulus griseus*, V79-4, CCL-93) from American Type Culture Collection according to the procedures outlined in ISO 10993-1:2009(E), ISO 10093-5:2009(E), ISO 10993-12:2009(E), and Mosmann, (1983). V79-4 Chinese hamster lung cells were exposed to *S. parasiticum* at the concentrations of 0.063, 0.125, 0.25, 0.5, 1, and 2 mg/mL for 24 hours. Optical density (OD) values at 570 nm were recorded. Percent of cell viability (V79-4 lung cells) were calculated with the following formula: [(OD values in the treatment with *S. parasiticum* / mean OD of medium control) x 100]. All the treatments were in triplicates.

Acute oral toxicity test

The study was conducted using female Sprague-Dawley rats (weighing 190 to 260 g) subjected to 2000 mg/kg body weight of *S. parasiticum* test substance (oral suspension was prepared: 2.0 g of lyophilized *S. parasiticum* in 10.0 mL of pre-filtered water). Acute oral toxicity test was performed according to OECD, (2002) and ISO 10993-11:2006(E). Control rats were administrated with 10 ml/kg body weight of pre-filtered water only. Body weights were taken before the experiment started and before the test ended. General clinical features or appearance and behavioural observations were recorded up to day 14. At the end of the study, necropsy was conducted on all the control and treated rats. Organ weights were recorded. All the treatments (control and treated rats were subjected to pre-filtered water only and *S. parasiticum* in pre-filtered water, respectively) were in triplicates. Each individual replicate was with one rat.

Statistical analyses

Differences among means percent of cell viability (V79-4 Chinese hamster lung cells) subjected to 6 different concentrations of *S. parasiticum* were analyzed using Tukey's test at $P = 0.05$ in Minitab 16 (Minitab Inc., State College, PA). Differences in means percent of increment in body weights and the relative organ weight to body weight for female Sprague-Dawley rats obtained from control and treated animals were analyzed using *t*-test at $P = 0.05$.

RESULT AND DISCUSSION

Cytotoxicity

Results obtained through MTT assay (yellow tetrazolium salt) showed that *Scytalidium parasiticum* was considered to be relatively weak cytotoxicity (Inhibition concentration of 50% at the dose of 2 mg/mL) in V79-4 cells (Chinese hamster lung cells) (Figure 1). When the V79-4 cells were treated with *S. parasiticum* at the concentrations that ranged from 0.06 to 1 mg/mL, the percent of cell viability was above 50% (Figure 1). At the concentrations of 0.06, 0.125, and 0.25 mg/mL of *S. parasiticum*, the percent of cell viability was significantly higher compared to the treatment with 2.00 mg/mL of *S. parasiticum* (Figure 1). Based on the cytotoxicity categories outlined by Abbas *et al.* (1984), *S. parasiticum* is considered only weakly toxicity to rats. Abbas *et al.* (1984) outlined three different categories of cytotoxicity levels and they were high cytotoxicity (with lethal concentration (LC) value of 50% between 0.01 and 5 ug/mL test substance), moderate cytotoxicity (with LC value of 50% between 5 and 250 ug/mL), and weak cytotoxicity (with LC value of 50% between 250 and 5000 ug/mL).

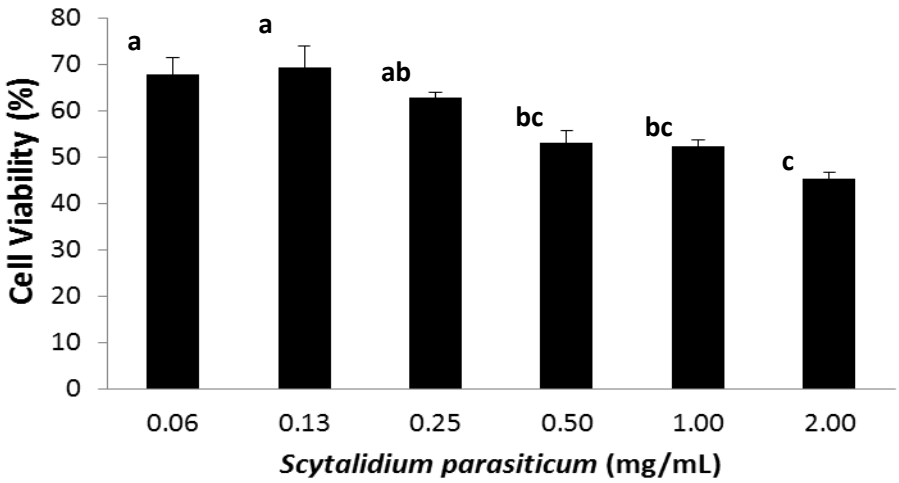

Figure 1. Percent of cell viability (V79-4 Chinese hamster lung cells) at six different *Scytalidium parasiticum* concentrations after 24 hours exposure duration. Data are means and standard errors. Solid bars with the same lowercase letter are not significantly different between treatments at six separate concentrations of *S. parasiticum* inoculum (mg/mL) at $P = 0.05$ with Tukey's test.

Acute toxicity

General appearance and behavioural observations recorded over the 14-day interval showed there was no difference between female Sprague-Dawley rats treated with *S. parasiticum* at 2000 mg/kg body weight and control (with 10 ml/kg body weight of pre-filtered water). General clinical observations, namely skin and fur, eyes, respiratory effect, mucous membrane, motor activity, tremor, convulsion, walking behaviours, and diarrhoea were normal for both treated and control animals (Data not shown). At the end of 14 days, there was no mortality or any toxic symptoms. Furthermore, necropsy results also showed no notable abnormalities in all eight separate organs, namely brain, kidneys, lungs, liver, stomach, spleen, heart, and pancreas. There were no significant differences in body weights between rats treated with *S. parasiticum* and control (Data not shown). Percent of increment in body weights in rats for treated and control showed no significant difference (Table 1). Body weight increment was noticed to be slightly higher in the rats

treated with *S. parasiticum* (Table 1). In term of relative weight (%) (Percent of organ weight to body weight), there was no significant difference between treated and control rats for most of the organs, except brain (P = 0.044) and lungs (P = 0.015) (Figure 2). However, the range of both brain (1.70 to 1.80 g) and lungs (1.95 to 2.30 g) was within the range of organ weights for female Sprague Dawley rats reported by Piao *et al*. (2013).

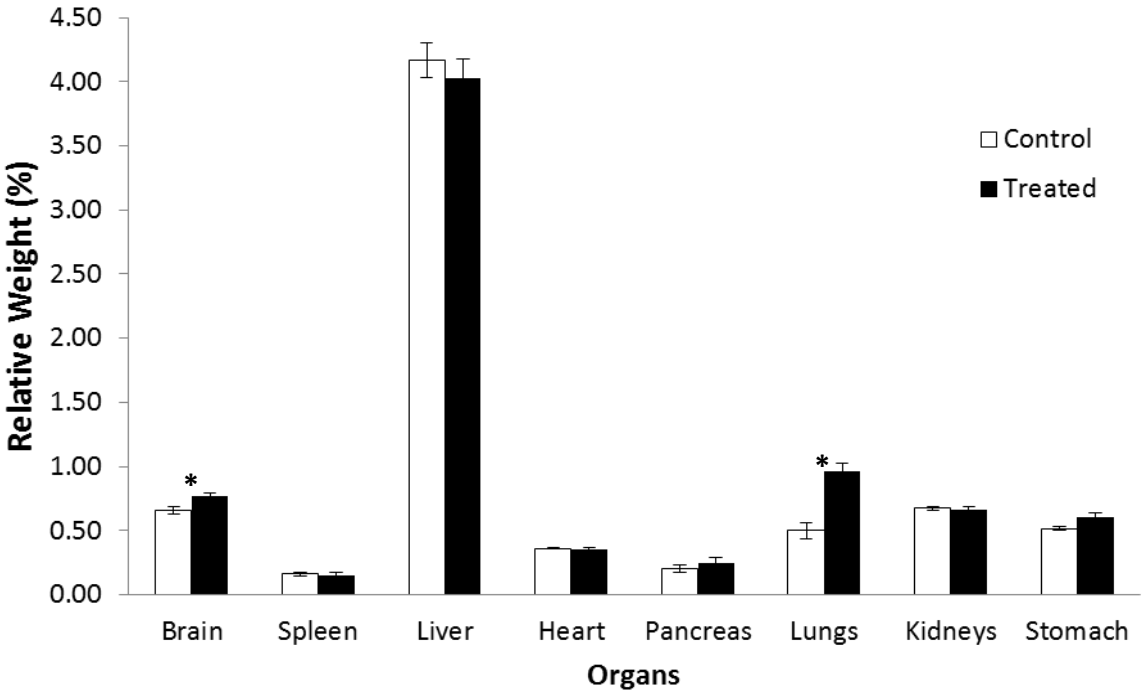

Figure 2. Relative organ weight to body weight (%) for eight separate organs in female Sprague-Dawley rats for control and treatment with 2000 mg/kg body weight of *Scytalidium parasiticum.* Data are means of relative organ weight to body weight and standard errors (n = 3). Each organs was analyzed separately. * refers to significant different between control and treated experiments at P = 0.05 with t-test.

Table 1. Percent of increment in body weights for female Sprague-Dawley rats in control and treated with *Scytalidium parasiticum*

Treatment	Percent of body weight increment*
Control	8.78 ± 5.07 a
With *S. parasiticum*	17.58 ± 10.15 a

*Data are means and standard errors (n = 3). Means for the percent of body weight increment in female Sprague-Dawley rats with the same lowercase letter are not significantly different between treatments with *S. parasiticum* and control at P = 0.05, with t-test.

CONCLUSION

In conclusion, *S. parasiticum* was determined to have only weak cytotoxicity effect on V79-4 cells (Chinese hamster lung cells) with inhibition concentration at 50% using 2 mg/mL of *S. parasiticum. Scytalidium parasiticum* showed no adverse toxicity effect or reaction at 2000 mg/kg body weight of female Sprague Dawley rats tested in the study.

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