Evaluation of the sub-acute toxicity of the sclerotium of *Lignosus rhinocerus* (Cooke), the Tiger Milk mushroom

Sook Shien Lee\(^a\), Nget Hong Tan\(^a\),*, Shin Yee Fung\(^b\), Jayalakshmi Pailoor\(^b\), Si Mui Sim\(^c\)

\(^a\) CENAR and Department of Molecular Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
\(^b\) Department of Pathology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
\(^c\) Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

**A R T I C L E   I N F O**

Article history:
Received 2 June 2011
Received in revised form 15 July 2011
Accepted 4 September 2011
Available online 10 September 2011

**Keywords:**
Lignosus rhinocerus
Sub-acute toxicity
Sclerotium

**A B S T R A C T**

**Ethnopharmacological relevance:** *Lignosus rhinocerus* (known locally as ‘Tiger Milk mushroom’) is the most important medicinal mushroom used by the indigenous communities of Malaysia to treat fever, cough, asthma, cancer, food poisoning and as a general tonic. The sclerotium of the mushroom is the part with medicinal value. *Lignosus rhinocerus* was hitherto unexploited commercially because of limited supply. Recently, the mushroom was successfully cultivated.

**Materials and methods:** Sprague Dawley rats (5 rats/group/sex) were fed orally with 250, 500 and 1000 mg/kg TM02, 1000 mg/kg TM03 as well as 1000 mg/kg wild type *Lignosus rhinocerus* sclerotial powder. Sclerotial powder was orally administered once daily and consecutively for 28 days. Body weight of each animal was measured and any gross behavioral change was observed daily. Hematological and clinical biochemical parameters as well as histopathological analysis were carried out on 29th day.

**Results:** The results showed that oral administration of the sclerotial powder at daily dose of up to 1000 mg/kg had no adverse effect on the growth rate, hematological and clinical biochemical parameters (including renal and liver function parameters). Histological studies showed that the treatments did not induce any pathological changes in the liver, kidney, heart, spleen and lung of the animals.

**Conclusion:** In conclusion, our results show that there was no treatment-related sub-acute toxicity in rats following 28-days oral administration of 250, 500 and 1000 mg/kg TM02, 1000 mg/kg TM03 as well as 1000 mg/kg wild type *Lignosus rhinocerus* sclerotial powder. As the highest tested dose of 1000 mg/kg was not associated with any toxicity concern, the NOAEL dose is higher than 1000 mg/kg.

© 2011 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

*Lignosus rhinocerus* (Tiger Milk mushroom), belongs to the Polyporaceae family, is an important medicinal mushroom in Southeast Asia and China. In Malaysia, it is also known as ‘cendawan susu rimau’ and is the most popular medicinal mushroom used by the indigenous communities of Peninsular Malaysia (Lee et al., 2009). It was used to treat a variety of diseases, including fever, cough, asthma, cancer, food poisoning and as a general tonic (Lee et al., 2009). Phylogenetic analysis indicated that the mushroom is closely related to *Canodermia lucidum* and *Trametes versicolor*, the two most popular medicinal mushrooms used in Asia (Tan et al., 2010).

The sclerotium of *Lignosus rhinocerus* is the part with medicinal value. The existence of this mushroom in the jungle is always solitary and this makes collection of the mushroom’s sclerotia a difficult task. As a result, the sclerotium is costly and its supply is limited. Recently, Tan (2009) reported a successful cultivation of the mushroom in agar, solid and spawn medium with good yield, thus overcoming the supply problem.

The antiproliferative effects of the sclerotium of *Lignosus rhinocerus* have been investigated. Lai et al. (2008) demonstrated that sclerotial polysaccharides from *Polyporus rhinocerus* (synonym of *Lignosus rhinocerus*) exhibited antiproliferative effects on several kinds of leukemic cell lines. Wong et al. (2009) reported that the hot water extract of *Polyporus rhinocerus* exhibited immunomodulatory effect by stimulating human innate immune cells. Our preliminary studies also demonstrated that the cold water extract of *Lignosus rhinocerus* sclerotium exhibited direct cytotoxicity on human breast carcinoma (MCF-7) and human lung carcinoma (A549) cell lines (Lee et al., 2010). Furthermore, Gao et al. (2009) showed that the nondigestible carbohydrates might function as novel prebiotics.

---

\* Corresponding author. Tel.: +60 379674912; fax: +60 379674957. 
E-mail address: tangethong@yahoo.com.sg (N.H. Tan).

0378-8741/ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved. 
In view of the widespread ethno-botanical usages as well as proven antiproliferative activities of the *Lignosus rhinocerus*, it is believed that the cultivated *Lignosus rhinocerus* will rapidly become a popular health supplement. It is therefore necessary to examine the toxicity of the sclerotium of the mushroom. We report here our investigation on the sub-acute toxicity of the sclerotia of the wild-type and two cultivars of *Lignosus rhinocerus* (termed TM02 and TM03). In our experiments, six groups of male and female rats (5 animals each) were fed orally with various mushroom sclerotial powders. After 28 days, the animals were sacrificed and the blood and organs were collected and analysed. The sub-acute toxicity study was carried out generally in compliance with the guidelines from the Organization of Economic Cooperation and Development (OECD, 1995). The highest dose used was 1000 mg/kg, this was chosen based on a preliminary 7-days acute toxicity studies where male and female rats (n = 5 each) fed with 2000 mg/kg of the sclerotial powder did not reveal any toxicity (unpublished results).

### Materials and methods

#### 2.1. Preparation of *Lignosus rhinocerus* sclerotal powder

Three types of sclerotal of *Lignosus rhinocerus* (wild type, cultivar TM02 and cultivar TM03) were provided by Ligno Biotech Sdn.

#### Table 3

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Control (n = 5)</th>
<th>1000 mg/kg wild type (n = 5)</th>
<th>1000 mg/kg TM02 (n = 5)</th>
<th>500 mg/kg TM02 (n = 5)</th>
<th>250 mg/kg TM02 (n = 5)</th>
<th>100 mg/kg TM02 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (&lt;x&gt;10&lt;sup&gt;6&lt;/sup&gt;/L)</td>
<td>7.60 ± 0.39</td>
<td>6.72 ± 0.30</td>
<td>7.24 ± 0.44</td>
<td>7.22 ± 0.56</td>
<td>6.98 ± 0.08</td>
<td>7.46 ± 0.42</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>13.86 ± 0.69</td>
<td>12.86 ± 0.77</td>
<td>13.66 ± 0.23</td>
<td>13.10 ± 1.10</td>
<td>14.34 ± 0.49</td>
<td>14.08 ± 1.10</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>43.20 ± 2.95</td>
<td>38.00 ± 2.24</td>
<td>40.80 ± 3.11</td>
<td>39.80 ± 3.56</td>
<td>40.00 ± 1.22</td>
<td>40.40 ± 1.95</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>57.00 ± 2.00</td>
<td>56.40 ± 1.14</td>
<td>56.40 ± 2.07</td>
<td>55.20 ± 0.84</td>
<td>58.00 ± 1.22</td>
<td>54.40 ± 2.07</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.20 ± 1.30</td>
<td>19.20 ± 0.45</td>
<td>19.00 ± 1.22</td>
<td>18.20 ± 1.64</td>
<td>20.60 ± 0.55</td>
<td>18.80 ± 1.48</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>32.20 ± 3.11</td>
<td>33.80 ± 0.45</td>
<td>33.60 ± 2.61</td>
<td>32.80 ± 3.03</td>
<td>35.40 ± 0.89</td>
<td>34.60 ± 2.41</td>
</tr>
<tr>
<td>Platelet count (&lt;x&gt;10&lt;sup&gt;9&lt;/sup&gt;/L)</td>
<td>80.00 ± 5.53</td>
<td>753.00 ± 274.14</td>
<td>772.00 ± 119.70</td>
<td>828.00 ± 464.65</td>
<td>925.00 ± 158.59</td>
<td>983.20 ± 191.58</td>
</tr>
<tr>
<td>WBC (&lt;x&gt;10&lt;sup&gt;3&lt;/sup&gt;/L)</td>
<td>6.70 ± 2.38</td>
<td>5.72 ± 1.75</td>
<td>7.66 ± 1.52</td>
<td>7.14 ± 3.79</td>
<td>6.82 ± 3.27</td>
<td>6.82 ± 2.20</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>58.40 ± 6.77</td>
<td>66.00 ± 7.31</td>
<td>59.80 ± 6.94</td>
<td>69.40 ± 12.42</td>
<td>68.40 ± 8.41</td>
<td>68.80 ± 7.92</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>4.00 ± 2.80</td>
<td>2.20 ± 1.92</td>
<td>4.00 ± 1.73</td>
<td>2.80 ± 2.05</td>
<td>3.20 ± 2.17</td>
<td>3.50 ± 2.07</td>
</tr>
<tr>
<td>Basophilin (%)</td>
<td>1.20 ± 0.84</td>
<td>0.60 ± 0.45</td>
<td>2.60 ± 0.42</td>
<td>2.40 ± 0.95</td>
<td>1.50 ± 0.95</td>
<td>1.40 ± 0.95</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>Atypical lymphocyte (%)</td>
<td>0.00 ± 0.00</td>
<td>0.60 ± 0.55</td>
<td>0.60 ± 0.89</td>
<td>0.40 ± 0.55</td>
<td>0.80 ± 0.84</td>
<td>0.40 ± 0.89</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5/group).  
* Significant difference with control (ANOVA, Dunnett’s t (two-sided) test, p < 0.05).
Bhd. (Selangor, Malaysia). The wild type Lignosus rhinocerus sclerotia were collected locally. The fungi were identified by their internal transcribed spacer (ITS) regions of the ribosomal RNA (Tan et al., 2010). Sclerotia were freezing dried and milled into powder using 0.2 mm sieve. The powder is light brown, dry fluffy powder with milk like taste. More than 1 kg of each sample was prepared.

2.2. Animals and feeding of Lignosus rhinocerus sclerotial powder

Five male (7 week old) and five female (7–8 week old) Sprague Dawley (SD) rats were used for each treatment. The animals were supplied by Chenur Supplier (Selangor, Malaysia). The Lignosus rhinocerus sclerotial powder was suspended in distilled water as vehicle. The animals were kept under standard conditions (temperature at 22 ± 2 °C, 12 h light, 12 h dark), and given water ad libitum. Animals were used only after 14 days of acclimisation. The animals were divided into twelve groups of 5 each as follows: group 1 is the control group that received vehicle only throughout the entire test period. Animals in group 2, 3, 4, 5 and 6 (5 rats/group/sex) daily

![Fig. 1. Body weight of male rats treated with 1000 mg/kg TM02 (Lignosus rhinocerus cultivar TM02) and the control group. Body weight is shown as mean ± SD (n = 5). Black color is control group, white color is the 1000 mg/kg TM02 treated group.](image)

![Fig. 2. Heart of female rats subjected to various treatments showing normal cardiac muscle fibres (H and E stain x 100). (A) Control; (B) wild type Lignosus rhinocerus, 1000 mg/kg; (C) TM02, 1000 mg/kg; (D) TM02, 500 mg/kg; (E) TM02, 250 mg/kg; (F) TM03, 1000 mg/kg.](image)
received 1000 mg/kg wild type *Lignosus rhinocerus*, 1000 mg/kg TM02, 500 mg/kg TM02, 250 mg/kg TM02 and 1000 mg/kg TM03, respectively. The animals were fed orally with the sclerotial powder once daily, and consecutively for 28 days. Body weight of each animal was measured and any gross behavioral change was observed daily. The doses were selected according to OECD guidelines. For TM02, 500 mg/kg and 250 mg/kg were selected to demonstrate if there is any dose related response.

2.3. Blood analysis

At the end of day 28, rats were fasted for 18 h. At day 29, rats were anaesthetised with ketamine (45 mg/kg) and xylazine (4.5 mg/kg). Blood samples were withdrawn using cardiac puncture. Hematological examinations and clinical biochemistry were performed using Advia 2120 Hematology System (Siem, Germany) and Advia 2400 Chemistry System (Siem, Germany), respectively. The parameters for hematological examination include red blood cell count, hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count and atypical lymphocyte count. Biochemical tests included glucose, urea, creatinine, calcium, sodium, potassium, total cholesterol, total protein, albumin, serum glutamic oxaloacetic transaminase (SGOT or AST) and serum glutamic pyruvic transaminase (SGPT or ALT).

2.4. Histopathological analysis

After blood collection, vital organs including liver, spleen, heart, lung and kidney were removed and preserved in 10% buffered formalin. The tissues were dehydrated by serial ethanol solution, cleared with xylene, paraffin embedded, sectioned and stained with haematoxylin and eosin. Light microscopic examinations of multiple tissue sections from each organ were performed.
2.5. Statistical evaluation

All data were expressed as mean ± standard deviation and analysed using One-way Analysis of Variance (ANOVA). Statistical differences between the means of control and treatment groups were determined using Dunnett’s t (two-sided) test. In case of variance heterogeneity, Dunnett’s T3 test was used. The homogeneity of variances was calculated using Levene statistics. Results were considered significant at p < 0.05.

3. Results

3.1. General observations

Oral administration of the three Lignosus rhinocerus samples (Wild type, TM02 and TM03) at all the doses used did not produce any abnormality in fur, eyes color, piloerection, locomotor activity or diarrhea in all the treated rats, and there were no death observed. Fig. 1 shows the growth rate for male rats treated with 1000 mg/kg of TM02, and that of the control group. Table 1 shows that the net body weight gain of the all the treated groups were not significantly different from the control animals (p > 0.05).

The male and female rats were fed orally with various types and doses of Lignosus rhinocerus sclerotal powder for 28 consecutive days. The values for body weight gain are expressed as mean ± SD (n = 5/group/sex). The weight gain between the various groups was not significantly different (p > 0.05).

3.2. Blood analysis

3.2.1. Hematological examinations

Tables 2 and 3 show the results of the hematological examinations of the blood samples of the treated and control groups for male and female rats, respectively, after the 28 days treatment.

Fig. 4. Liver of female rats subjected to various Lignosus rhinocerus treatments showing normal architecture with normal hepatocytes and portal tracts (H and E stain × 100). (A) Control; (B) wild type Lignosus rhinocerus, 1000 mg/kg; (C) TM02, 1000 mg/kg; (D) TM02, 500 mg/kg; (E) TM02, 250 mg/kg; (F) TM03, 1000 mg/kg. Arrows showed the position of portal tracts.
Fig. 5. Spleen of female rats subjected to various treatments showing normal histology (H and E stain × 40). (A) Control; (B) wild type Lignosus rhinocerus, 1000 mg/kg; (C) TM02, 1000 mg/kg; (D) TM02, 500 mg/kg; (E) TM02, 250 mg/kg; (F) TM03, 1000 mg/kg.

Generally, there were no significant differences in the values of red blood cell count, hemoglobin, PCV, MCV, MCH, MCHC, platelet count, WBC and differential leucocyte counts between rats from all the treated groups and the control group. The only exception was for female rats of the 250 mg/kg TM02 treatment group, the mean corpuscular volume (MCH) of the 250 mg/kg TM02 treatment group (20.60 ± 0.55) was slightly higher than that of the control group (18.20 ± 1.30, p < 0.05) (Table 3).

3.3. Clinical biochemistry

The results of clinical biochemistry are shown in Tables 4 and 5, respectively, for male and female rats. Generally, there were no significant differences (p > 0.05) in the levels of serum glucose, urea, creatinine, calcium, sodium, potassium, total cholesterol, total protein, albumin, SGOT and SPGT between the treated groups and the control group, except for the following:

- Glucose levels in male group treated with 1000 mg/kg TM03 (5.94 ± 0.47 mmol/L) were significantly lower than that of the control group (8.02 ± 0.94 mmol/L, p < 0.05).
- Calcium levels in the male group treated with 1000 mg/kg wild type Lignosus rhinocerus (2.36 ± 0.03 mmol/L) were slightly lower than the control group (2.49 ± 0.03 mmol/L, p < 0.05).
- Total protein level in the male group treated with 1000 mg/kg wild type Lignosus rhinocerus (69.60 ± 1.67 mmol/L) was slightly higher than the level in control group (64.00 ± 1.58 mmol/L, p < 0.05).

3.4. Histopathological analysis

Microscopic examinations of the vital organs including heart, kidney, liver, spleen and lung of rats in all treated groups and the control groups did not reveal any pathological changes as a result of the 28 days oral feedings. Figs. 2–6 show the histological sections...
Fig. 6. Lung of female rats subjected to various treatments showing interstitial inflammatory cell infiltrate of mainly lymphocytes (H and E stain × 40). (A) Control; (B) wild type Lignosus rhinocerus, 1000 mg/kg; (C) TM02, 1000 mg/kg; (D) TM02, 500 mg/kg; (E) TM02, 250 mg/kg; (F) TM03, 1000 mg/kg.

Table 4
Clinical biochemistry parameters of male rats treated with various types and doses of Lignosus rhinocerus samples for 28 days.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Control (n = 5)</th>
<th>1000 mg/kg wild type (n = 5)</th>
<th>1000 mg/kg TM02 (n = 5)</th>
<th>500 mg/kg TM02 (n = 5)</th>
<th>250 mg/kg TM02 (n = 5)</th>
<th>1000 mg/kg TM03 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.02 ± 0.94</td>
<td>6.28 ± 0.78</td>
<td>7.58 ± 1.21</td>
<td>6.78 ± 1.69</td>
<td>6.50 ± 1.18</td>
<td>5.94 ± 0.47*</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>7.52 ± 1.40</td>
<td>7.40 ± 0.78</td>
<td>6.16 ± 0.96</td>
<td>7.46 ± 1.95</td>
<td>6.5 ± 0.86</td>
<td>6.36 ± 1.08</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>54.80 ± 6.50</td>
<td>47.80 ± 11.37</td>
<td>49.80 ± 3.49</td>
<td>48.00 ± 3.32</td>
<td>46.40 ± 16.26</td>
<td>48.60 ± 4.04</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.49 ± 0.03</td>
<td>2.36 ± 0.03*</td>
<td>2.43 ± 0.10</td>
<td>2.16 ± 0.28</td>
<td>2.40 ± 0.12</td>
<td>2.39 ± 0.06</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>143.60 ± 1.14</td>
<td>146.00 ± 1.41</td>
<td>144.60 ± 0.55</td>
<td>144.00 ± 0.74</td>
<td>144.00 ± 2.79</td>
<td>144.60 ± 0.55</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.80 ± 0.29</td>
<td>4.34 ± 0.30</td>
<td>4.70 ± 0.34</td>
<td>4.98 ± 0.74</td>
<td>4.52 ± 0.57</td>
<td>4.50 ± 0.84</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.58 ± 0.33</td>
<td>1.44 ± 0.32</td>
<td>1.38 ± 0.34</td>
<td>1.34 ± 0.34</td>
<td>1.40 ± 0.23</td>
<td>1.40 ± 0.22</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>64.00 ± 1.58</td>
<td>60.60 ± 1.67*</td>
<td>66.60 ± 2.61</td>
<td>61.00 ± 4.30</td>
<td>67.60 ± 4.28</td>
<td>66.20 ± 3.11</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>33.00 ± 0.71</td>
<td>35.60 ± 2.07</td>
<td>35.00 ± 1.58</td>
<td>31.80 ± 2.17</td>
<td>34.60 ± 2.41</td>
<td>34.60 ± 1.52</td>
</tr>
<tr>
<td>SGOT (AST) (IU/L)</td>
<td>214.60 ± 19.77</td>
<td>250.60 ± 49.97</td>
<td>221.20 ± 33.00</td>
<td>246.80 ± 33.25</td>
<td>271.00 ± 42.47</td>
<td>267.60 ± 36.56</td>
</tr>
<tr>
<td>SGPT (ALT) (IU/L)</td>
<td>62.40 ± 6.35</td>
<td>57.80 ± 11.37</td>
<td>57.00 ± 12.98</td>
<td>58.60 ± 7.89</td>
<td>64.00 ± 8.69</td>
<td>60.00 ± 4.85</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5/group).
* Significant difference from control (ANOVA, Dunnett’s t (two-sided) test, p < 0.05).
of heart, kidney, liver, spleen and lung of female rats subjected to the different treatments. For all the treated rats, the heart shows normal cardiac muscle fibres, the kidney showed normal glomeruli, tubules and interstitium; and the liver showed normal architecture with normal hepatocytes and portal tracts, while the spleen also showed normal histology. The lungs of all treated groups and control groups showed peribronchial and interstitial inflammatory cell infiltrate of mainly lymphocytes, at similar degree, and there was no dose-related response for the TM02 groups (Fig. 6). The same histological pictures for lung were obtained for male or female rats without any treatment (not shown). Histological pictures of the organs of the male rats were similar to those of the female and are not shown.

4. Discussion

Changes in body weight have been used as an indicator of adverse effects of drugs and chemicals (Hilly et al., 2004; Mukinda and Eagles, 2010). The similar growth pattern as shown by body weight gain (Table 1, Fig. 1) indicate that oral administration of the three *Lignosus rhinoceros* sclerotal powder at a daily dose of up to 1000 mg/kg had no adverse effect on the growth of the SD rats.

It has been established that the highest overall concordance of toxicity in animals with humans is hematological parameters (Olson et al., 2000). Our studies show that there were no significant difference between the hematological parameters of rats fed with wild type, TM02 and TM03 *Lignosus rhinoceros* sclerotal for 28-days and that of the control groups, except for 250 mg/kg TM02 treatment, where the MCH of the female rats was slightly higher than that of the control group. The value, however, was within the maximum normal range established for SD rats at 18–19 week old (Petterino and Argentino-Storino, 2006). Besides, other related hematological parameters such as red blood cell count, hemoglobin, PCV, MCV and MCHC of the rats in the same group were all normal. Therefore, it can be concluded that *Lignosus rhinoceros* sclerotal had no adverse effect on the hematological parameters of the rats.

Clinical biochemistry studies also show that generally the various *Lignosus rhinoceros* treatments did not affect the renal functions (urea and creatinine levels), hepatic functions (albumin, total protein, SGOT and SGPT), serum electrolytes (copper, sodium, potassium) as well as glucose and total cholesterol levels. Though treatment by 1000 mg/kg TM03 lowered the blood glucose levels of the male rats to 5.94 mmol/L (p < 0.05), it does not necessarily mean that the TM03 has a hypoglycemic effect, as the value still fell within the reference range established by Petterino and Argentino-Storino (2006). Besides, the blood glucose levels for sample TM02 (all 3 doses) and 1000 mg/kg treated—wild type *Lignosus rhinoceros* were both within normal range. In the 1000 mg/kg wild type *Lignosus rhinoceros* treatment group, though the calcium and total protein levels in the male group were slightly different from the control group (p < 0.05), the levels also fell within the reference range (Petterino and Argentino-Storino, 2006) and thus these minor differences are unlikely to be of any clinical significance.

Histological examinations supported the conclusions from clinical biochemistry studies that oral feeding of up to 1000 mg/kg of TM02, TM03 and wild type *Lignosus rhinoceros* sclerotal powder did not induce any renal or liver damages. In addition, the various treatments also did not induce any pathological changes in heart and spleen of the rats. The observed peribronchial and interstitial inflammatory cell infiltration observed in all treatment groups were not induced by the *Lignosus rhinoceros* sclerota, as the same infiltration was observed also in both control groups (male and female rats) as well as rats without any treatment. Also results from TM02 treatments where 3 different doses were tested, the inflammatory response in the lung was not dose-related. We believe that the inflammatory response may be caused by the housing environment of the animal supplier who used wood chips bedding for the animals (during the experimental period, however, the animals were housed on paper crumbs). The particle-generating characteristics of the wood chips bedding may be the cause of the lung inflammation, as in vivo mouse model showed that repeated airway exposure to wood dust can elicit lung inflammation (Määttä et al., 2006).

In conclusion, our results showed that there is no treatment-related sub-acute toxicity in rats following 28-days oral administration of 250, 500 and 1000 mg/kg TM02, 1000 mg/kg TM03 as well as 1000 mg/kg wild type *Lignosus rhinoceros* sclerotal powder; as measured by hematological, clinical biochemistry as well as weight and general observations. Histological examinations of heart, kidney, spleen, lung and liver of animals treated with up to 1000 mg/kg of the three samples of *Lignosus rhinoceros* sclerotal powder for 28 days did not reveal any pathology concerns. As the highest tested dose of 1000 mg/kg was not associated with any toxicity concern, the NOAEL dose is higher than 1000 mg/kg.

As the 28-day sub-acute toxicity testing using repeated doses is generally accepted as a satisfactory test to assess any possible health hazard for chemicals, our results indicate that sclerotal from the wild type, the cultivars TM02 and TM03 of *Lignosus rhinoceros* were devoid of toxicity, even at a daily dose of 1000 mg/kg. It should be noted that at the moment, the recommended daily consumption of the *Lignosus rhinoceros* sclerotal as neutraceutical is only approximately 5–10 mg/kg (assuming the average BW is 50 kg). Nevertheless, further studies on its effect on fertility, its possible teratogenic effect on the offspring, its genotoxicity and mutagenicity are necessary to firmly establish the safety of the consumption of this medicinal mushroom.

Table 5: Clinical biochemistry parameters of female rats treated with various types and doses of *Lignosus rhinoceros* sample.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>Control (n = 5)</th>
<th>1000 mg/kg, wild type (n = 5)</th>
<th>1000 mg/kg TM02 (n = 5)</th>
<th>500 mg/kg TM02 (n = 5)</th>
<th>250 mg/kg TM02 (n = 5)</th>
<th>1000 mg/kg TM03 (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>6.78 ± 0.07</td>
<td>6.20 ± 0.73</td>
<td>7.54 ± 2.68</td>
<td>6.74 ± 0.82</td>
<td>6.54 ± 0.89</td>
<td>7.16 ± 0.89</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>7.26 ± 0.85</td>
<td>6.80 ± 0.69</td>
<td>9.66 ± 2.04</td>
<td>8.24 ± 0.68</td>
<td>8.36 ± 0.72</td>
<td>8.80 ± 1.10</td>
</tr>
<tr>
<td>Creatinine (µmol/L)</td>
<td>47.20 ± 4.15</td>
<td>42.00 ± 2.45</td>
<td>46.80 ± 9.76</td>
<td>47.40 ± 12.22</td>
<td>33.80 ± 6.76</td>
<td>43.00 ± 14.75</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.55 ± 0.08</td>
<td>2.58 ± 0.12</td>
<td>2.42 ± 0.08</td>
<td>2.47 ± 0.07</td>
<td>2.54 ± 0.11</td>
<td>2.48 ± 0.08</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>140.60 ± 1.52</td>
<td>143.40 ± 1.14</td>
<td>140.40 ± 1.34</td>
<td>142.40 ± 1.34</td>
<td>142.40 ± 1.52</td>
<td>141.20 ± 1.48</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.64 ± 0.71</td>
<td>4.08 ± 0.26</td>
<td>4.78 ± 0.54</td>
<td>4.38 ± 0.49</td>
<td>4.20 ± 0.41</td>
<td>4.18 ± 0.25</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>1.88 ± 0.52</td>
<td>1.78 ± 0.13</td>
<td>1.64 ± 0.30</td>
<td>1.52 ± 0.31</td>
<td>2.12 ± 0.33</td>
<td>1.72 ± 0.38</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>73.80 ± 5.59</td>
<td>70.00 ± 3.74</td>
<td>71.20 ± 1.92</td>
<td>68.60 ± 5.03</td>
<td>74.40 ± 3.65</td>
<td>72.4 ± 2.51</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>38.40 ± 4.16</td>
<td>37.80 ± 1.79</td>
<td>37.00 ± 1.58</td>
<td>36.40 ± 4.28</td>
<td>41.80 ± 3.35</td>
<td>37.4 ± 3.97</td>
</tr>
<tr>
<td>SGPT (AST) (IU/L)</td>
<td>126.20 ± 28.45</td>
<td>144.80 ± 25.12</td>
<td>160.00 ± 36.03</td>
<td>179.60 ± 45.81</td>
<td>164.60 ± 34.85</td>
<td>180.2 ± 38.49</td>
</tr>
<tr>
<td>SGPT (ALT) (IU/L)</td>
<td>44.60 ± 7.89</td>
<td>55.60 ± 8.02</td>
<td>46.80 ± 3.56</td>
<td>43.00 ± 5.79</td>
<td>63.60 ± 23.11</td>
<td>47.8 ± 7.79</td>
</tr>
</tbody>
</table>

Values are mean ± SD (n = 5/dose). There was no significant difference between control and treatment groups.
Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This research is supported by Post Graduate Research Grant (PPP) PS243/2010B and High Impact Research Grant (J20014-73807) from University of Malaya. The authors would like to express gratitude to staff from Laboratory Animal Center, Faculty of Medicine, University of Malaya for their assistance.

References


