Nutritional composition, antioxidant properties, and toxicology evaluation of the sclerotium of Tiger Milk Mushroom Lignosus tigris cultivar E

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The Tiger Milk Mushroom (Lignosus spp.) is an important medicinal mushroom in Southeast Asia and has been consumed frequently by the natives as a cure for a variety of illnesses. In this study, we hypothesized that Lignosus tigris (cultivar E) sclerotium may contain high nutritional value and antioxidant properties, is nontoxic and a potential candidate as a dietary supplement. The chemical and amino acid compositions of the sclerotium were evaluated and antioxidant activities of the sclerotial extracts were assessed using ferric reducing antioxidant power, 1,1-diphenyl-2-picrylhydrazyl, and superoxide anion radical scavenging assays. Acute toxicity of the L. tigris E sclerotium was assessed using a rat model study. The sclerotium was found to be rich in carbohydrate, protein, and dietary fibers with small amounts of fat, calories, and sugar. The amino acid composition of the protein contains all essential amino acids, with a protein score of 47. The sclerotial extracts contain phenolics, terpenoids, and glucan. The ferric reducing antioxidant power values of the various sclerotial extracts (hot water, cold water, and methanol) ranged from 0.008 to 0.015 mmol min\(^{-1}\) g\(^{-1}\) extract, while the 1,1-diphenyl-2-picrylhydrazyl and superoxide anion radical scavenging activities ranged from 0.11 to 0.13, and \(-2.81\) to \(9.613\) mmol Trolox equivalents g\(^{-1}\) extract, respectively. Acute toxicity assessment indicated that L. tigris E sclerotial powder was not toxic at the dose of 2000 mg kg\(^{-1}\). In conclusion, L. tigris E sclerotium has the potential to be developed into a functional food and nutraceutical.

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Keywords:
Tiger Milk Mushroom
Lignosus tigris
Nutrient
Phenolic
Antioxidant
Acute toxicity
Rat
1. **Introduction**

Mushrooms have been receiving considerable attention because of their nutritional value, culinary characteristics, and medicinal properties. Mushrooms such as *Ganoderma lucidum* (Reishi), *Lentinus edodes* (Shiitake), *Inonotus obliquus* (Chaga), *Agaricus bisporus* (white button), and *Pleurotus eryngii* (King Oyster) are among the mushrooms that are widely consumed for their medicinal properties.

The “Tiger Milk mushroom” is an important indigenous medicinal mushroom in Southeast Asia and China. The mushroom, which consists of at least 3 species (*L. rhinocerotis*, *L. tigris*, and *L. cameronensis*), belongs to the Polyporaceae family. The sclerotium is the part of the mushroom with medicinal value. Previous studies have indicated that the sclerotial extracts of *L. rhinocerotis* contain considerable antioxidant activity while also exhibiting anti-inflammatory, anti-proliferative, and immunomodulatory properties [1-4]. Investigations on the nutritional properties of the sclerotium indicate that it has the potential to be developed into a functional food or nutraceutical [5].

Recently, *L. tigris* has been domesticated (cultivar tigris K) and Yap et al [5] reported on the antioxidant and anti-proliferative activities of the cultivar. They also found that the sclerotium of *L. tigris* showed good prospects for development into functional food and to be included as a dietary component because of its nutritive value and potent superoxide anion scavenging activity. Unfortunately, at room temperature the aqueous extract of *L. tigris* cultivar K quickly turned a dark brown color, perhaps as a result of oxidation. This undesirable property compromises its potential as a functional food or nutraceutical.

A new cultivar of *L. tigris* (cultivar E or *L. tigris* E) has recently been successfully cultivated by Ligno Biotech (Malaysia) using an improved cultivation method. The extract of *L. tigris* E was found to be stable and does not yield the dark brown color upon standing.

Therefore, we aim to investigate the nutritional composition (including amino acid composition of the proteins) and antioxidant properties, as well as assess the acute toxicity of the sclerotium of *L. tigris* using a rat model, as a preliminary step in examining the safety of the mushroom for consumption. We hypothesized that this new cultivar of *L. tigris* E sclerotium is nutritive with good antioxidant properties, nontoxic, and a potential human dietary supplement.

2. **Methods and materials**

2.1. **Sample and chemicals**

Cultivated *L. tigris* E was provided by Ligno Biotech Sdn. Bhd. (Selangor, Malaysia). The fungus was identified by a DNA barcode marker targeting the internal transcribed spacer (ITS) region [6]. Sclerotial powder of the *L. tigris* E was freeze-dried and milled into powder using a 0.2 mm sieve. All the chemicals and reagents used in the experiments were of analytical grade and were purchased from Merck and Co., (NJ, USA), Friendemend Schmidt Chemical (Parkwood, WA, USA) and Sigma Aldrich (St. Louis, MO, USA).

2.2. **Analysis of the *L. tigris* E sclerotial powder**

2.2.1. **Nutritional value and amino acid composition analysis**

Carbohydrate and energy of the sclerotial powder of *L. tigris* E were determined according to Sullivan [7]. Dietary fiber, soluble, and insoluble dietary fibers, mineral, and amino acid composition of protein were determined according to the Association of Official Analytical Chemists (AOAC) [8] methods 985.29, 991.43, 984.27, and 994.12, respectively. Total fat, total sugar, and crude protein content was estimated using an in-house method of ALS Technichem (M) Sdn Bhd, Selangor, Malaysia based on that of Sullivan [7] and Kirk and Sawyer [9]. Moisture content was determined using a moisture analyzer.

2.2.2. **Extraction of the *L. tigris* E sclerotial powder**

Cold water extract (CWE), hot water extract (HWE), and methanol extract (ME) were prepared as described by Yap et al [5]. Total protein content of the extracts was measured using the Bradford method [10] with bovine serum albumin as a standard. Total carbohydrate content of the extracts was measured using the phenol sulphuric acid method as described by Dubois et al [11]. D-glucose was used to construct the standard curve. Each extract was measured in triplicate.

2.2.3. **Estimation of glucan content**

The glucan content of *L. tigris* E sclerotial extracts was determined according to the manual of the Megazyme Mushroom and Yeast Beta-Glucan Assay Kit (Megazyme International Ireland Ltd, Wicklow, UK). Briefly, for determination of the total glucan content, *L. tigris* E extracts were hydrolyzed with concentrated hydrochloric acid (HCl) (37% vol/vol). The pH of the hydrolysate was neutralized with 2 M potassium hydroxide and digested with exo-1,3-β-glucanase (2 U) plus β-glucosidase (0.4 U) in 200 mM of sodium acetate buffer (pH 5.0). To measure the glucan content, GOPOD reagent (glucose oxidase/peroxidase and 4-aminoantipyrine in p-hydroxybenzoic acid and sodium azide) was added and absorbance reading was measured at 510 nm against the GOPOD reagent blank. For determination of the α-glucan content, *L. tigris* E sclerotial extracts were hydrolyzed in 2 M KOH and neutralized with 1.2 M sodium acetate buffer (pH 3.8). Amyloglucosidase (326 U) and invertase (100 U) were added into the hydrolysate and incubated at 40 °C for 30 min. The aliquot was incubated with a mixture of GOPOD at 40°C for 20 min. Absorbance reading was measured at 510 nm against the GOPOD reagent blank. The total glucan and α-glucan contents were calculated by comparing to the D-glucose standard. The β-glucan content was determined by subtracting the α-glucan from total glucan content. Yeast β-glucan supplied in the assay kit was used as positive control. Each extract was measured in triplicate.

2.2.4. **Total phenolic content analysis**

Total phenolic content of *L. tigris* E sclerotial extracts was measured using the Folin–Ciocalteu method with minor modifications [12]. Briefly, 500 μL of 1:10 Folin–Ciocalteau’s phenol reagent was added to 10 μL of sample. The mixture was left at room temperature for 5 min before the addition of 350 μL of 0.115 mg ml⁻¹ sodium carbonate. The mixture was
further incubated for 2 h in the dark and then absorbance at 765 nm was measured. Gallic acid (20-200 μg mL⁻¹) was used as standard. Results were expressed in mg of gallic acid equivalents (GAE). Each extract was measured in triplicate.

2.2.5. Determination of total terpenoid content
Total terpenoid content in the L. tigris E sclerotium extracts was estimated according to the method described by Ghorai et al [13]. Linalool (0-500 mg mL⁻¹) in methanol was used to construct the standard curve. Briefly, 200 μL of the extract or standard solution was added to 1.5 mL of chloroform. The mixture was vortexed thoroughly, incubated at room temperature for 3 min before the addition of 100 μL of concentrated sulfuric acid. The reaction mixture was then incubated in the dark at room temperature for 2 h (for standard solution the incubation time was only 5 min). The supernatant was discarded gently without disturbing the brown-reddish precipitate. The precipitate was then re-dissolved in 1.5 mL of 10% (vol/vol) methanol and absorbance at 538 nm was measured. Each extract was measured in hexaplicate.

2.2.6. Antioxidant assays
The measurement of ferric reducing antioxidant power (FRAP) of the extracts was carried out as described by Benzie and Strain [14]. Iron (II) sulfate (FeSO₄) was used to construct calibration curve and results were expressed as mmol ferric reducing activity min⁻¹ g⁻¹ of extract. 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was carried out as described by Cos et al [15] with some modifications. Briefly, 25 μL of extract (concentrations ranged from 0-16 mg mL⁻¹) was added to 150 μL of 0.04 mg mL⁻¹ DPPH solution in methanol. The mixtures were incubated in the dark for 30 min and absorbance was measured at 515 nm using a plate reader. The DPPH radical scavenging activity was calculated using the following equation:

\[
\text{DPPH inhibition (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance control}} \times 100
\]

The concentration of the extracts required to scavenge 50% (IC₅₀) of the total DPPH radicals was also determined. Superoxide anion (SOA) radical scavenging activity was determined according to the procedure described by Siddhuraju and Becker [16], with minor modifications. Fifty μL of 468 μmol/L⁻¹ nicotinamide adenine dinucleotide, 150 μmol/L⁻¹ nitroblue tetrazolium and 60 μmol/L⁻¹ phenazine methosulfate (all were prepared in 0.1 μmol/L⁻¹ phosphate buffers, pH 7.4) were added to 50 μL of extract (concentrations ranged from 0-1 mg mL⁻¹). The reaction mixture was incubated for 10 min at 25 °C in the dark and absorbance at 570 nm was measured. Trolox (a-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard to construct a calibration curve in DPPH and SOA radical scavenging assays. Rutin and quercetin were used as positive controls for all assays. Each extract was measured in triplicate.

2.3. Acute toxicity study

2.3.1. Animals
Female Sprague-Dawley (SD) rats, 7 to 8 weeks old, were used in the acute toxicity study. The rats were obtained from the Animal Experimental Unit, University of Malaya. The rats were housed in standard environmental conditions (temperature 22°C ± 2°C under 12 h light/dark cycle and relative humidity 60% ± 10%) and fed with rodent standard diets and water ad libitum. All protocols in this study were approved by the Institutional Animal Care and Use Committee, University of Malaya (UM IACUC – Ethics reference no. 2013-09-17/MOL/R/TNH).

2.3.2. Treatment
The acute toxicity study was carried out according to the guidelines of the Organization for Economic Co-operation and Development for testing of chemicals, TG 425 (adopted – 3 October 2008) [17]. The rats were fasted overnight before dosing. A single dose of 2000 mg kg⁻¹ of L. tigris E sclerotum powder dissolved in distilled water was administered orally to the rats (n = 5) at a ratio of 10 mL kg⁻¹ on day 1. Rats in the control group received only distilled water (10 mL kg⁻¹). After the treatment, food was withheld for 4 h. The rats were observed for acute toxicity signs and behavioral changes at 30 min after dosing and periodically during the first 24 h (with special attention during the first 4 h), and once daily further for a period of 14 days. The body weight of the rats was recorded on day 1, day 7, and day 14.

2.3.3. Blood and histopathological analysis
At the end of the experiment, the rats were fasted overnight and anaesthetized with ketamine (55 mg kg⁻¹) and xylazine (5 mg kg⁻¹). Blood samples were collected by cardiac puncture for hematological and biochemical analysis. After blood collection, the rats were euthanized by intraperitoneal injection of an overdose of 200 mg kg⁻¹ sodium pentobarbital. Selected vital organs (heart, lung, liver, spleen, and kidney) were excised and preserved in 10% buffered formalin. The tissues were dehydrated by serial ethanol solution, cleared with xylene, paraffin embedded, sectioned, and stained with hematoxylin and eosin. Light microscopic examinations of multiple tissue sections from each organ were performed for evidence of toxicity. Parameters which indicated signs of toxicity in the selected organs include degeneration, necrosis, and inflammation of cardiac muscle fibers; inflammation in the lung tissue including inflammatory exudate in the alveolar or interstitial spaces; evidence of degeneration, necrosis of renal tubules or glomerular changes; changes in liver architecture, hepatocytes including fatty change and congestion and presence of inflammatory cell infiltrate in the portal tracts or any evidence of cholangitis; and changes in spleen architecture or evidence of infection.

2.4. Statistical analyses
Statistical analysis was performed using IBM SPSS Statistics 22. All data were expressed as means ± SEM. Data for total phenolic content, terpenoids content and antioxidant tests were analyzed using one-way analysis of variance followed by least significant difference (LSD) post hoc test to evaluate the differences between the mean values in the experiment groups. The homogeneity of variances was calculated using Levene statistics. In case of variance heterogeneity, Tamhane’s T2 was used. Data from the toxicity study were analyzed using the independent-samples t test. According to the Organization for Economic Co-operation...
and Development guidelines [17], the sample size of 5 animals per group is sufficient to obtain a significant result. All differences were considered statistically significant at the P < .05 level.

3. Results

3.1. Nutritional value and amino acid composition of the sclerotium of L. tigris cultivar E

The results of the proximate and mineral composition analysis are shown in Table 1. The dominant components of the mushroom sclerotium are carbohydrate and protein which account for 73.8% (of which 17.7% is dietary fiber, mostly insoluble) and 16.1%, respectively, of the dry weight. Total fat, sugar, ash, and moisture contents are 1.7%, 5.6%, 0.8%, and 6.0%, respectively. Of the minerals analyzed, potassium content was the highest, at about 2180 mg kg⁻¹ of the dried weight (DW) of sclerotial powder. This was followed by magnesium (436 mg kg⁻¹ DW) and sodium (12 mg kg⁻¹ DW) as the most insoluble) and 16.1%, respectively, of the dry weight. Total fat, sugar, ash, and moisture contents are 1.7%, 5.6%, 0.8%, and 6.0%, respectively. Of the minerals analyzed, potassium content was the highest, at about 2180 mg kg⁻¹ of the dried weight (DW) of sclerotial powder. This was followed by magnesium (436 mg kg⁻¹ DW) and sodium (12 mg kg⁻¹ DW) as the most insoluble component. Using the Joint FAO/WHO/UNU [18] suggested pattern of amino acid requirements for a preschool child as reference, the chemical score was 47, with lysine and tryptophan as first and second limiting amino acids.

3.2. Extraction yields, total protein, and carbohydrate content of L. tigris sclerotial extracts

Hot water extraction of the L. tigris sclerotial powder yielded the highest amount of extracted materials (386 g kg⁻¹ dry weight, 50.5% carbohydrate, and 3.2% protein), followed by cold water extraction (240 g kg⁻¹ dry weight, 40.6% carbohydrate, and 3.2% protein). Methanol extraction, on the other hand, yielded the lowest amount of extracted materials, with only 80 g kg⁻¹ dry weight (24.6% carbohydrate, 0.2% protein).

3.3. Glucan content of the of L. tigris sclerotial extracts

Table 3 shows the glucan content of the HWE, CWE, and ME. HWE has the highest total glucan content. For both HWE and CWE, the majority (>90%) of the glucan was β-glucan. For the methanol extract, however, β-glucan was the major glucan component.

3.4. Total phenolic and terpenoid content

The total phenolic and terpenoid content of the various extracts of L. tigris are shown in Table 4. The amount of phenolic and terpenoid compounds in ME was the highest (7.43 ± 0.09 mg GAE g⁻¹ extract; 11.14 ± 2.63 g Linalool g⁻¹ extract, respectively) compared to CWE and HWE. When expressed in mg GAE g⁻¹ of dry weight of sclerotial powder, the amount of phenolic compounds was highest in HWE and was about 3 times higher than that of ME. Total terpenoids content in CWE (2.62 ± 0.47) was higher than that in HWE (1.70 ± 0.16) and ME (0.90 ± 0.21) when expressed in terms of g Linalool g⁻¹ of dry weight of sclerotial powder.

3.5. Antioxidant activity

The ability of L. tigris extracts to reduce ferric ion and scavenge free radicals was summarized in Table 5. The HWE and ME had the same value of FRAP (0.015 mmol g⁻¹ extract) and this was higher than that of the CWE. In terms of DPH radical scavenging ability, HWE, CWE, and ME extracts exhibited comparable activity.

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scavenging activities (Fig. 1). The CWE and HWE exhibited strong extracts showed dose-dependent superoxide anion radical activity in comparison to the positive controls (quercetin and exhibited relatively low FRAP value and DPPH radical scavenging mean body weight and weight gain were observed (Fig. 2).

In fur, eye color, piloerection, locomotor activity, or diarrhea of acute toxicity in the rats. No abnormality was observed in the treated rats. Similarly, no treatment-related effects on the sodium level in between the treated and control groups. Only the sodium level in the treated group (145.00 ± 1.58 mmol L⁻¹) was slightly lower (P < .05) than in the control group (145.60 ± 1.82 mmol L⁻¹).

3.6. Acute toxicity of L. tigris sclerotial powder

3.6.1. General observations

The test dose of 2000 mg kg⁻¹ did not cause mortality or any sign of acute toxicity in the rats. No abnormality was observed in fur, eye color, piloerection, locomotor activity, or diarrhea in the treated rats. Similarly, no treatment-related effects on mean body weight and weight gain were observed (Fig. 2).

3.6.2. Hematological and biochemical parameters

The hematological and biochemical parameters of the blood samples of the treated and control rats were summarized in Tables 6 and 7. No significant differences (P > .05) were observed in the values of red blood cell count, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular hemoglobin concentration, mean corpuscular hemoglobin concentration, platelet count, white blood cell and differential leucocyte counts between rats from the treated and control groups. Similarly, there were no significant differences (P > .05) in the levels of serum glucose, urea, creatinine, calcium, potassium, total cholesterol, total protein, albumin, aspartate aminotransferase, and alanine transaminase between the treated and control groups. Only the sodium level in the treated group (143.00 ± 1.58 mmol L⁻¹) was slightly lower (P < .05) than in the control group (145.60 ± 1.82 mmol L⁻¹).

3.6.3. Tissue examination

Light microscopic examination of the vital organs including heart, lung, liver, spleen, and kidney of rats in the treated and control groups did not reveal any pathological changes as a result of the single dose oral feeding. The histologic sections of heart, lung, liver, spleen, and kidney of the control and treated rats are illustrated in Fig. 3. In both the control and treated rats, the heart shows normal cardiac muscle fibers; lungs reveal a normal alveolar structure and no treatment-related inflammatory response was observed; kidneys show normal glomeruli, tubules, and interstitial space; and liver shows normal hepatocytes and lobular architecture with no

Table 3 – Total glucan, α-glucan, and β-glucan contents in the various L. tigris E extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total glucan</th>
<th>α-glucan</th>
<th>β-glucan</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWE</td>
<td>277.58 ± 0.45⁺</td>
<td>253.18 ± 3.43⁺</td>
<td>24.39 ± 3.01⁺</td>
</tr>
<tr>
<td>HWE</td>
<td>483.34 ± 8.68⁶</td>
<td>439.97 ± 9.51³</td>
<td>43.37 ± 4.82α</td>
</tr>
<tr>
<td>ME</td>
<td>146.52 ± 3.88⁸</td>
<td>68.77 ± 1.10⁹</td>
<td>77.74 ± 3.92⁹</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column indicate the mean values are significantly different, according to analysis of variance and post hoc LSD test (P < .05).

Table 4 – L. tigris E sclerotial extract yields, total phenolic, and terpenoid contents

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolic content</th>
<th>Total terpenoid content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg GAE g⁻¹ extract</td>
<td>g Linalool g⁻¹ extract</td>
</tr>
<tr>
<td>CWE</td>
<td>5.64 ± 0.32⁺</td>
<td>10.93 ± 1.97⁺</td>
</tr>
<tr>
<td>HWE</td>
<td>4.55 ± 0.14⁶</td>
<td>4.42 ± 0.43α</td>
</tr>
<tr>
<td>ME</td>
<td>7.43 ± 0.09⁹</td>
<td>11.14 ± 2.63⁹</td>
</tr>
</tbody>
</table>

Different superscript letters in the same column indicate the mean values are significantly different, according to analysis of variance and post hoc LSD test (P < .05).

3.6.4. Antioxidant activities of the L. tigris E sclerotial extracts

Table 5 – Antioxidant activities of the L. tigris E sclerotial extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>FRAP value</th>
<th>Trolox equivalent antioxidant capacity (TEAC, mmol TE g⁻¹ extract)</th>
<th>DPPH</th>
<th>Superoxide anion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(mmol ferric reducing activity min⁻¹ g⁻¹ extract)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CWE</td>
<td>0.008 ± 0.001⁺</td>
<td>0.011 ± 0.001⁺</td>
<td>9.613 ± 0.149⁺</td>
<td>9.613 ± 0.149⁺</td>
</tr>
<tr>
<td>HWE</td>
<td>0.015 ± 0.001⁺</td>
<td>0.013 ± 0.001⁺</td>
<td>8.365 ± 0.267⁺</td>
<td>8.365 ± 0.267⁺</td>
</tr>
<tr>
<td>ME</td>
<td>0.015 ± 0.000⁹</td>
<td>0.011 ± 0.001⁺</td>
<td>8.365 ± 0.267⁺</td>
<td>8.365 ± 0.267⁺</td>
</tr>
<tr>
<td>Rutin</td>
<td>3.042 ± 0.34²</td>
<td>4.237 ± 0.719³</td>
<td>13.847 ± 0.174³</td>
<td>13.847 ± 0.174³</td>
</tr>
<tr>
<td>Quercetin</td>
<td>5.709 ± 0.139⁴</td>
<td>9.179 ± 0.463⁷</td>
<td>14.007 ± 0.596⁷</td>
<td>14.007 ± 0.596⁷</td>
</tr>
</tbody>
</table>

All the values were expressed as means ± SD (n = 3). Different superscript letters in the same column indicate the mean values are significantly different, according to analysis of variance and post hoc LSD test (P < .05). Rutin and quercetin were used as positive controls.
congestion of sinusoids and fatty changes seen. Normal spleen histologic structures were also observed in all the rats.

4. Discussion

Mushrooms have been consumed as a delicacy and are known for their nutritive value. Like other edible mushrooms, the L. tigris mushroom sclerotium contains a high amount of carbohydrate, protein, and dietary fibers but is low in fat, calories, and sugar. It is interesting to note that L. tigris cultivar E sclerotium had double the amount of proteins and 5 times higher dietary fiber content compared to the earlier cultivar, L. tigris K [19]. Mushrooms are a good source of proteins and contain amino acid compositions that are comparable to plant proteins [20]. The amino acid scores of all the essential amino acids except lysine are comparable to or higher than chicken eggs, cow’s milk, and beef in FAO reference patterns [18]. Lysine is the limiting amino acid in the sclerotium protein and this is similar to that reported for L. rhinocerotis [5]. L. tigris E sclerotium is a good source of nonstarch polysaccharides as it is composed mostly of insoluble fiber with very low amount of soluble fiber (less than 1%). A study on the intake of high fiber diets has revealed that it can reduce the risk of diverticulosis and colon cancer [21]. Furthermore, dietary fiber substances such as β-glucans and polysaccharide-proteins have been reported to be able to stimulate immune systems and exert anti-tumor, immunomodulatory, and anti-cancer activities [3,22,23]. The ash content in the L. tigris E sclerotium was slightly higher than the former cultivar L. tigris K and was comparable to that of other common edible mushrooms [20]. Potassium was the main constituent, constituting about 27.3% of the ash. High potassium and low sodium contents, with K/Na ratio of 181.67 implies that L. tigris E sclerotium is a good diet for maintaining the right balance of electrolytes in the body.

![Graph of superoxide anion radical scavenging activity](image)

Fig. 1 – Superoxide anion radical scavenging activity of L. tigris E sclerotial extracts. The radical scavenging activity are expressed as percentage inhibition (means ± SD, n = 3). Rutin and quercetin were used as positive controls.

![Graph of body weight and weight gain](image)

Fig. 2 – Body weight and weight gain of rats treated with 2000 mg/kg L. tigris E and the control group. Body weight is shown as means ± SD (n = 5). Data were analysed using SPSS, independent-samples t test. No significant difference in the body weight and weight gain between control and treatment groups (P > .05).
Among the extraction methods, water extraction produced the highest yield of extracted material compared to methanol extraction. The high yield of water extraction suggests that *L. tigris* E sclerotial powder contains mainly water-soluble substances of high polarity. The composition of the cold water extracts of *L. tigris* (in terms of carbohydrate and protein content) was comparable to that of *L. rhinoceroticus*, but the hot water extracts of *L. tigris* E had much lower protein content (3.2%) compared to *L. rhinoceroticus* (41.3%) [24].

The glucan content of the *L. tigris* E sclerotium as estimated from the hot water extract was higher than the reported glucan content of the medicinal mushrooms *Ganoderma lucidum*, *Ganoderma applanatum*, *Lentinus edodes*, and *Trametes versicolor* [25]. However, the content of α-glucan (about 91%) and β-glucan (about 9%) in the CWE and HWE differs from the glucan composition of the *L. rhinocerotis* extracts as reported by Lau et al [26], who reported that β-glucan was the dominant form (82%-93%). Both α-glucan and β-glucan have been reported to exhibit immunomodulation activity [27,28].

Table 6 – Hematological parameters of female rats treated with single dose of *L. tigris* E sclerotial powder (2000 mg kg⁻¹)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 5)</th>
<th>Treatment (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (×10¹² L⁻¹)</td>
<td>6.98 ± 0.36</td>
<td>6.78 ± 0.36</td>
</tr>
<tr>
<td>Haemoglobin (G DL⁻¹)</td>
<td>14.32 ± 0.93</td>
<td>14.14 ± 0.72</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>42.40 ± 2.07</td>
<td>41.20 ± 1.92</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>60.80 ± 1.92</td>
<td>61.60 ± 1.82</td>
</tr>
<tr>
<td>MCH (PG)</td>
<td>20.40 ± 0.89</td>
<td>21.00 ± 0.71</td>
</tr>
<tr>
<td>MCHC (G DL⁻¹)</td>
<td>33.80 ± 0.45</td>
<td>34.20 ± 0.84</td>
</tr>
<tr>
<td>Platelet count (X 10¹² L⁻¹)</td>
<td>841.80 ± 129.17</td>
<td>784.00 ± 104.74</td>
</tr>
<tr>
<td>WBC (X 10³ L⁻¹)</td>
<td>7.76 ± 1.52</td>
<td>8.62 ± 0.90</td>
</tr>
<tr>
<td>Neutrophil (%)</td>
<td>27.80 ± 5.26</td>
<td>22.40 ± 6.03</td>
</tr>
<tr>
<td>Lymphocyte (%)</td>
<td>66.20 ± 7.09</td>
<td>68.60 ± 7.47</td>
</tr>
<tr>
<td>Monocyte (%)</td>
<td>5.40 ± 1.52</td>
<td>7.00 ± 2.12</td>
</tr>
<tr>
<td>Eosinophil (%)</td>
<td>0.60 ± 1.34</td>
<td>2.00 ± 2.00</td>
</tr>
<tr>
<td>Basophil (%)</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>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 5/group). There was no significant difference between control and treatment groups, according to the SPSS, independent-samples t test (P > 0.05). Abbreviations: LTE, *L. tigris* cultivar; RBC, red blood cell; PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration, WBC, white blood cell.

Free radical scavenging is one of the known mechanisms by which antioxidants prevent oxidative damage and inhibit lipid oxidation. The IC₅₀ values of the *L. tigris* E extracts as measured by DPPH scavenging activity (10.35-12.82 mg mL⁻¹) were comparable or slightly lower than most medicinal mushrooms such as *Agrocybe* spp. (IC₅₀ = 9.559 mg mL⁻¹), *Lentinus edodes* (IC₅₀ = 19.093 mg mL⁻¹), *Volvariella volvacea* (IC₅₀ = 17.832 mg mL⁻¹) and *Pleurotus* spp. (IC₅₀ = 15.422-31.5 mg mL⁻¹) [33].

Superoxide radicals produced through metabolic process are known to be harmful to cellular components. Superoxide radicals can interact with other molecules to generate secondary ROS (eg, hydroxyl radicals, hydrogen peroxide, and single oxygen), either directly or prevalently through enzyme or metal catalyzed processes [34]. Overproduction of superoxide radicals could induce oxidative damage in lipids, proteins, and DNA, leading to degenerative diseases in humans [35]. It is therefore interesting to note that the *L. tigris* E extracts exhibited potent SOA radical scavenging activities at concentrations below 1.5 mg mL⁻¹. These data are in contrast to the ferric reducing and DPPH radical scavenging abilities of the extracts, and suggest that the extracts may contain other nonphenolic compounds that are able to effectively scavenge SOA. The SOA radical scavenging activities of the water extracts (8.365-9.613 mmol TE g⁻¹ extract) were comparable to those of *L. rhinocerotis* extracts and higher than those for *L. tigris* K cultivar (4.82-7.98 mmol TE g⁻¹ extract) [5,19]. A negative inhibition of SOA at concentrations above 500 μg mL⁻¹ in the ME was found; the same phenomenon has been observed in *L. rhinoceroticus* and *L. tigris* K extracts [5,19].

In the acute toxicity assessment, administration of *L. tigris* E sclerotial powder at a single dose of 2000 mg kg⁻¹ caused neither treatment-related signs of toxicity nor mortality in the tested rats. All the treated rats survived beyond the 14-day observation period and the growth rate was similar to that of healthy controls.
the control rats. The mushroom sclerotial powder did not show any adverse effect on the hematological parameters as no significant difference was observed between the treated and control rats. In terms of clinical biochemical parameters, the single dose treatment of \( L. \) tigris E had no effect on the blood glucose, total cholesterol, serum electrolytes, and renal and liver functions. Although the sodium level in the treated rats was significantly lower than that in the control rats, the level of clinical biochemical parameters, including blood glucose, total cholesterol, serum electrolytes, and renal and liver functions, did not show any significant difference between the treated and control rats.

Fig. 3 – Hematoxylin and eosin stains (100× magnifications) of (i) heart (cardiac muscle fibers), (ii) lung, (iii) liver, (iv) spleen and (v) kidney of rats orally fed with (A) distilled water and (B) 2000 mg kg\(^{-1}\) of \( L. \) tigris E sclerotial powder. No significant histopathological abnormality was observed in the organs of the control and treated rats. ‘T’ and ‘V’ shows the alveolar space and lung interstitium, respectively; ‘W’ and ‘X’ shows the position of the hepatocytes and portal tracts in liver, respectively; ‘WP’ and ‘RP’ are the white pulp and red pulp in spleen, respectively; ‘Y’ and ‘Z’ show the position of glomerulus and tubule in kidney, respectively.

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was within the normal reference range [36,37]. Histopathological examination showed that there were no lesions or pathological changes in the heart, lung, liver, spleen, and kidney tissues. Therefore, it can be concluded that the L. tigris E sclerotal powder did not exhibit acute toxicity at the dose of 2000 mg kg\(^{-1}\).

In summary, as we hypothesized, L. tigris E sclerotal is high in nutritional value with good antioxidant properties. It is also nontoxic when assessed by acute toxicity evaluation, and thus has the potential to be developed as a functional food and nutraceutical. However, to support the safety assessment of L. tigris E sclerotal as a dietary supplement for humans, evaluation of subacute, and chronic toxicity of the sclerotium is necessary.

**Conflict of interest**

There is no conflict of interest.

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