Haematological, biochemical and histopathological aspects of *Hericium erinaceus* ingestion in a rodent model: A sub-chronic toxicological assessment

Hariprasath Lakshmana,b, Jegadeesh Ramana, Pamela Davida,c, Kah-Hui Wonga,c, Murali Naidua,c, Vikineswary Sabaratnama,d,⁎

a Mushroom Research Centre, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia
b Department of Biochemistry, Karpagam University, Coimbatore 641021, India
c Department of Anatomy, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
d Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

**ARTICLE INFO**

Keywords: Toxicity Sub-chronic Hericium Aqueous extract Safety

**ABSTRACT**

Ethnopharmacological relevance: *Hericium erinaceus* is a culinary-medicinal mushroom and has a long history of usage in traditional Chinese medicine as a tonic for stomach disorders, ulcers and gastrointestinal ailments.

Aim of the study: The present investigation was aimed to evaluate the potential toxic effects of the aqueous extract from the fruiting bodies of *H. erinaceus* in rats by a sub-chronic oral toxicity study.

Materials and methods: In this sub-chronic toxicity study, rats were orally administered with the aqueous extract of *H. erinaceus* (HEAE) at doses of 250, 500 and 1000 mg/kg body weight (b.w.) for 90 days. Body weights were recorded on a weekly basis and general behavioural changes were observed. The blood samples were subjected to haematological, biochemical, serum electrolyte, and antioxidant enzyme estimations. The rats were sacrificed and organs were processed and examined for histopathological changes.

Results: No mortality or morbidity was observed in all the treated and control rats. The results showed that the oral administration of HEAE daily at three different doses for 90 days had no adverse effect on the general behaviour, body weight, haematology, clinical biochemistry, and relative organ weights. Histopathological examination at the end of the study showed normal architecture except for few non-treatment related histopathological changes observed in liver, heart and spleen.

Conclusion: The results of this sub-chronic toxicity study provides evidence that oral administration of HEAE is safe up to 1000 mg/kg and *H. erinaceus* consumption is relatively non-toxic.

**1. Introduction**

The World Health Organization (WHO) has asserted that traditional medicines are relied upon by 65%–80% of the world’s population for their primary health care needs (Gao and Watanabe, 2011). Natural products and herbal medicine are considered to be safe in view of long history of use in traditional medicine. Mushrooms are considered as nutritional functional foods and source of physiologically beneficial medicines. Of the 14,000 to 15,000 species of mushrooms in the world, around 700 have medicinal properties. However it has been estimated that there are about 1800 species of mushrooms that have medicinal attributes (Chang et al., 1999).

*Hericium erinaceus* (Bull.: Fr.) Pers., is a culinary-medicinal mushroom that is also known as monkey's head mushroom or lion’s mane mushroom (Hou Tou Gu in Chinese or Yamabushitake in Japanese), is commonly used as medicine or food and has attracted recent attention due to its multi-health benefits. The mushroom belongs to the class Agaricomycetes under the phylum basidiomycota. The mushroom is commonly found in the East Asian countries and has a long history of usage in traditional Chinese medicine for stomach disorders, ulcers and gastrointestinal ailments (Hiwatashi et al., 2010). Recent research on *H. erinaceus* has unearthed several medicinal values and reported widely to possess anti-cancer, anti-microbial, anti-diabetic, anti-hypertensive, antioxidant, gastro-protective, neuro-protective, immuno-modulating, and wound-healing properties (Abdulla et al., 2011; Kim et al., 2012, 2013; Shang et al., 2013; Wong et al.,...
Effects of HEAE in female Sprague Dawley rats.

### Table 1
Effects of HEAE on haematological parameters in female Sprague Dawley rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>250 mg/kg of HEAE</th>
<th>500 mg/kg of HEAE</th>
<th>1000 mg/kg of HEAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^12/L)</td>
<td>8.45 ± 0.55</td>
<td>8.60 ± 0.61</td>
<td>8.43 ± 0.57</td>
<td>8.64 ± 0.28</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>14.75 ± 0.78</td>
<td>16.00 ± 1.38</td>
<td>15.60 ± 0.70</td>
<td>16.07 ± 0.75</td>
</tr>
<tr>
<td>HCT (L/L)</td>
<td>0.45 ± 0.02</td>
<td>0.49 ± 0.04</td>
<td>0.48 ± 0.02</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>MCV (FL)</td>
<td>56.00 ± 2.83</td>
<td>57.20 ± 2.28</td>
<td>56.67 ± 0.58</td>
<td>56.83 ± 1.6</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.60 ± 0.99</td>
<td>18.60 ± 0.71</td>
<td>18.43 ± 0.12</td>
<td>18.6 ± 0.43</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.00 ± 0.14</td>
<td>32.54 ± 0.59</td>
<td>32.50 ± 0.20</td>
<td>32.63 ± 0.54</td>
</tr>
<tr>
<td>Platelets</td>
<td>9.78 ± 2.52</td>
<td>9.66 ± 3.17</td>
<td>8.87 ± 2.50</td>
<td>9.48 ± 1.79</td>
</tr>
<tr>
<td>Neutrophils %</td>
<td>95.2 ± 1.95</td>
<td>88.8 ± 1.93</td>
<td>83.0 ± 1.81</td>
<td>87.7 ± 1.18</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>86.41 ± 7.49</td>
<td>87.47 ± 4.43</td>
<td>87.16 ± 9.78</td>
<td>88.15 ± 8.03</td>
</tr>
<tr>
<td>Monocytes %</td>
<td>2.18 ± 0.62</td>
<td>1.70 ± 0.58</td>
<td>2.20 ± 0.48</td>
<td>2.04 ± 0.59</td>
</tr>
<tr>
<td>Eosinophils %</td>
<td>1.95 ± 0.35</td>
<td>2.32 ± 0.59</td>
<td>2.28 ± 0.59</td>
<td>1.88 ± 0.76</td>
</tr>
<tr>
<td>Basophils %</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD (n=6).

### Table 2
Effects of HEAE on serum biochemical parameters in female Sprague Dawley rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>250 mg/kg of HEAE</th>
<th>500 mg/kg of HEAE</th>
<th>1000 mg/kg of HEAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>8.29 ± 0.91</td>
<td>6.77 ± 0.81</td>
<td>8.64 ± 0.43</td>
<td>8.33 ± 0.89</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>2.08 ± 0.22</td>
<td>2.03 ± 0.33</td>
<td>2.20 ± 0.13</td>
<td>1.82 ± 0.18</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.62 ± 0.08</td>
<td>0.66 ± 0.08</td>
<td>0.73 ± 0.09</td>
<td>0.77 ± 0.06</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.35 ± 0.21</td>
<td>1.09 ± 0.23</td>
<td>1.00 ± 0.09</td>
<td>0.97 ± 0.11</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>43.00 ± 3.22</td>
<td>36.83 ± 2.79</td>
<td>40.17 ± 1.47</td>
<td>41.50 ± 1.64</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>5.85 ± 1.50</td>
<td>5.62 ± 1.06</td>
<td>6.00 ± 0.62</td>
<td>6.50 ± 1.25</td>
</tr>
<tr>
<td>Uric acid (mmol/L)</td>
<td>124.83 ± 61.83</td>
<td>137.83 ± 49.75</td>
<td>176.00 ± 35.38</td>
<td>164.00 ± 44.15</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>30.50 ± 2.74</td>
<td>30.5 ± 3.02</td>
<td>30.83 ± 1.17</td>
<td>33.67 ± 2.07</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>0.98 ± 0.34</td>
<td>0.83 ± 0.22</td>
<td>0.73 ± 0.20</td>
<td>0.68 ± 0.18</td>
</tr>
<tr>
<td>Total bilirubin (umol/L)</td>
<td>2.00 ± 0.00</td>
<td>2.00 ± 0.82</td>
<td>2.00 ± 0.82</td>
<td>2.00 ± 0.00</td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>67.67 ± 4.18</td>
<td>63.33 ± 5.54</td>
<td>64.00 ± 4.00</td>
<td>66.17 ± 5.25</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>37.00 ± 9.32</td>
<td>39.67 ± 5.68</td>
<td>45.33 ± 8.87</td>
<td>46.33 ± 5.24</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>71.50 ± 23.59</td>
<td>63.00 ± 19.84</td>
<td>61.00 ± 13.77</td>
<td>58.50 ± 12.03</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>187.83 ± 57.37</td>
<td>143.17 ± 68.60</td>
<td>180.33 ± 68.77</td>
<td>153.33 ± 22.07</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>312.00 ± 85.26</td>
<td>337.00 ± 63.72</td>
<td>353.33 ± 47.08</td>
<td>390.50 ± 83.37</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
<td>&lt; 3</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD (n=6).

### Table 3
Effects of HEAE on serum electrolytes concentration in female Sprague Dawley rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>250 mg/kg of HEAE</th>
<th>500 mg/kg of HEAE</th>
<th>1000 mg/kg of HEAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloride (mmol/L)</td>
<td>105.00 ± 1.41</td>
<td>104.17 ± 1.83</td>
<td>102.33 ± 1.75</td>
<td>102.00 ± 1.79</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>5.27 ± 0.68</td>
<td>5.08 ± 0.73</td>
<td>4.68 ± 0.80</td>
<td>4.93 ± 0.49</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>141.83 ± 1.33</td>
<td>142.17 ± 0.98</td>
<td>141.17 ± 0.98</td>
<td>141.50 ± 0.55</td>
</tr>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.75 ± 0.33</td>
<td>2.03 ± 0.32</td>
<td>2.32 ± 0.12</td>
<td>2.00 ± 0.17</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.57 ± 0.08</td>
<td>2.63 ± 0.15</td>
<td>2.52 ± 0.08</td>
<td>2.55 ± 0.10</td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD (n=6).

* Statistically significant (P < 0.05) compared to the control group (one-way ANOVA with Tukey's post-hoc test).

Tukey's post hoc test.
natant was freeze-dried and the hot aqueous extract powder of *H. erinaceus* was stored at 4 °C ± 2 °C until further use (Wuilloud et al., 2004).

### 2.2. Experimental animals

Healthy female Sprague Dawley rats aged seven weeks and weighing 150–180 g used in this study were obtained from the Animal Experimental Unit (AEU), Faculty of Medicine, University of Malaya (accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International)). The animals used were nulliparous and non-pregnant. Food and water were provided ad libitum during acclimation and throughout the study. The animals were maintained at 22.0 ± 2 °C and relative humidity around 50–60%. They were housed in polypropylene cages over husk beddings and 12 h light and 12 h dark cycle was maintained throughout the experimental period. All the animal experiments were performed after getting necessary approval from the Faculty of Medicine Institutional Animal Care and Use Committee (FOM IACUC No. 2014-02-14/ANAT/R/RPSD, approval date 25-Feb-2014) of University of Malaya. All efforts were made to minimize both the number of animals used and unwanted stress or discomfort to the animals throughout the experimental procedures.

### 2.3. Experimental design

The study was conducted in accordance with OECD 408 guidelines; 90-d Oral Toxicity Study in Rodents (OECD, 2008). The rats were allowed to acclimate to the housing environment (22.0 ± 2 °C, 50–60% humidity, 12 h light/dark cycle) for 7 days before the start of experimental procedures. A total of twenty-four animals were randomly divided into four groups of six rats per group as follows:

- **Group 1**: Control animals treated with vehicle (normal saline) for 90 days.
- **Group 2**: Treated with 250 mg/kg b.w. of HEAE for 90 days.
- **Group 3**: Treated with 500 mg/kg b.w. of HEAE for 90 days.
- **Group 4**: Treated with 1000 mg/kg b.w. of HEAE for 90 days.

Behavioural changes and mortality were monitored on a daily basis. Body weights were recorded on a weekly basis for a period of 90 days. Blood samples were collected by the retro-orbital puncture under mild ether anaesthesia, with or without anticoagulant (ethylenediamine tetraacetate-EDTA) vials after treatment period. Blood samples containing the anticoagulant were used immediately for the determination of haematological parameters, whereas those without the anticoagulant were centrifuged at 4000 rpm for 10 min at 4 °C, and the serum obtained were stored at −20 °C until analysed for biochemical parameters.

### 2.4. Haematological analysis

Blood samples were analysed for haematological parameters including red blood cell (RBC) count, differential white blood cell (WBC)
count, haemoglobin, haematocrit (Hct), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin (MCHC) using haematology auto-analyser system (XN-3000 haematology analyser, Sysmex).

2.5. Serum biochemical and electrolytes analysis

The level of glucose, total cholesterol, HDL cholesterol, LDL cholesterol, albumin, urea, uric acid, creatinine, bilirubin and triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (γ-GT) and total protein in serum samples were determined using automated biochemistry analyser (ADVIA 2400, Siemens Healthcare). The level of electrolytes (chloride, potassium, sodium, phosphate, calcium) in serum samples were also determined using the above automated analyser.

2.6. Serum antioxidant enzymes and lipid peroxidation

Measurement of serum antioxidant activities malondialdehyde (MDA) levels were carried out using standard assay methods. Catalase (CAT) and superoxide dismutase (SOD) activity was determined using EnzyChrom™ standard colorimetric determination kits according to the methods of Zhu et al. (2012) and Janknegt et al. (2007), respectively. The reduced glutathione level was measured according to the method of Soon and Tan (2002). The MDA level was quantified using the Biovision colorimetric assay kit.

2.7. Harvesting of organs and histopathological examination

Animals were sacrificed after blood collection and major organs including heart, liver, kidney, spleen, lung and brain were quickly excised and washed with saline. The organs absolute and relative weights were calculated. For histopathological examination, the organs were washed with normal saline to remove blood clot and blotted to dryness and pieces of the tissues approximately 1 cm³ were fixed in 10% neutral formalin buffer. The fixed tissues were subjected to routine histological processing and stained with haematoxylin-eosin dye and permanent mounts of the tissue samples were prepared as detailed by Bancroft and Cook (1984). The sections were microscopically examined using an inverted microscope (Nikon Eclipse TS100, Nikon Instruments Inc., Melville, NY, USA).

2.8. Statistical analysis

All data were subjected to One-way Analysis of Variance (ANOVA), and Tukey’s multiple comparison test was performed to evaluate the significance of difference in mean values of various treatment groups, using SPSS version 22 (SPSS Inc., Chicago, IL, USA). The values are presented as mean ± S.D. and the P value < 0.05 was considered significant.

3. Results

3.1. Effect of HEAE on the general behaviour and body weight of rats

No significant changes were observed in general behaviour and no other major abnormal clinical observations were seen at any time point of this study. There was no mortality recorded in all the treatment groups during the 90 days of administration of HEAE. Similarly, no significant differences (P > 0.05) in the body weight were recorded between the control and HEAE-treated groups (Fig. 1).
3.2. Effect of HEAE on haematological parameters

The results of different haematological parameters of rats in the experimental and control groups at the end of the 90-d HEAE treatment is presented in Table 1. The results showed that there was no significant difference (P > 0.05) in the haematological indices (RBC, haemoglobin, HCT, MCV, MCH, MCHC, platelets, total WBC and differential count) values of the HEAE treated group rats compared to the control group of rats.

3.3. Effect of HEAE on serum biochemical parameters

The results of different serum biochemical parameters of rats in treated and control groups at the end of the 90-d HEAE treatment is presented in Table 2. The glucose level was significantly decreased (P < 0.05) in rats treated with 500 mg/kg of HEAE compared to the control group. A significant decrease (P < 0.05) in the level of albumin was seen in rats treated with 250 mg/kg of HEAE relative to the control group rats. A significant increase (P < 0.05) in the HDL cholesterol level was observed in rats treated with higher doses (500 and 1000 mg) of HEAE compared to the control rats, however the total cholesterol and LDL cholesterol were significantly different (P < 0.05) in rats treated with 1000 mg/kg of HEAE only when compared to the control rats. Other parameters were within the normal limits.

3.4. Effect of HEAE on serum electrolytes

There was no significant effect observed with HEAE treatment compared with control on the serum electrolytes (Table 3).

3.5. Effect of HEAE on antioxidant enzymes

The effect of HEAE on different antioxidant enzymes is presented in Fig. 2. The antioxidant indices CAT, SOD and GSH showed significant (P < 0.05) and dose-dependent increase in the serum level of treated rats compared to the control rats. The level of MDA decreased as the dose of HEAE increased and exhibited a significant decrease (P < 0.05) compared to control rats.

3.6. Effect of HEAE on relative organ weights

Organs such as heart, liver, kidneys, lung, spleen and brain were weighed in all groups of HEAE treated rats and control rats, and is presented in Table 4. There was no significant change in the relative organ weights observed between the HEAE treated and control rats at the end of the study (P > 0.05).

3.7. Effect of HEAE on histopathology of vital organs

Histological analysis did not reveal any treatment related toxicological changes in the liver, kidney, brain, heart, lung and spleen (Figs. 3–8). However, some non-treatment changes such as mononuclear cell infiltration and diffuse glycogen infiltration in the liver, focal mononuclear cell infiltration in the myocardium and congestion of cells in the spleen were observed.

4. Discussion

The consumption of medicinal plants has increased in recent years. The safety profile on medicinal plants is essential as safety of herbal medicine use has recently been questioned due to reports of illness and
fatalities (Park et al., 2010). To identify the safety of consuming products from medicinal plants, systematic studies on its toxicity (if any) must be conducted. Hot water extraction of mushrooms is the traditionally practiced method in Chinese medicine (Mark Stengler, 2005). However, methanol extraction is the common method for isolation of antioxidant and antimicrobial compounds from mushrooms (Cheung and Cheung, 2005), as these compounds have polyphenolic in nature and can easily dissolve in methanol (Orhan et al., 2007). The present study evaluated the safety of *H. erinaceus* for human consumption and in traditional practice, hence the hot water extraction was prepared.

*Hericium erinaceus* and its components have attracted interest in the medical research during the past two decades because of its vast biological and clinical properties. A previous acute toxicity study showed that the aqueous extract of *H. erinaceus* was well tolerated and safe up to the highest dose (5 g/kg) tested in the study (Wong et al., 2013). Another sub-acute toxicity study reported that the no-observed-adverse-effect level of erinacine A-enriched *H. erinaceus* is greater than 3 g/kg body weight (Li et al., 2014). However, this safety assertion may not be applicable when HEAE is administered for a long period. To our knowledge, no systematic investigation is available on the effects of prolonged consumption of *H. erinaceus*. The present study was conducted to determine the sub-chronic toxic effects of HEAE using a rat model to provide information on the safety of this mushroom as food and medicine. Hence, considering the sub-acute toxicity data, 1000 mg/kg was taken as the highest dose to observe any adverse effect of HEAE in rats administered orally for a period of 90 days.

The present sub-chronic oral toxicity study of HEAE showed no mortality at all doses tested throughout the course of the study. General behaviour and body weights are one of the critical indicators for the evaluation of early signs of toxicity (Sireeratawong et al., 2008). In the present study no adverse behavioural changes were noticed throughout the course of study. The treated rats gained weight with age and there was no significant change (P > 0.05) in the body weight compared to the control rats. The oral administration of HEAE at a dose of up to 1000 mg/kg for 90 days had no adverse effect on the growth of rats. The results of haematological examination further supported the consumption of *H. erinaceus*. Haematological examination is an important indicator for predicting the value for human toxicity when the toxicity data on animal studies is applied in clinical research (Olson et al., 2000). In this study, no significant change (P > 0.05) in the haematological parameters was observed in all the doses of HEAE treated rats compared to control rats.

The results of serum biochemistry analysis showed a significant decrease (P < 0.05) in glucose levels in rats treated with 250 and 500 mg/kg of HEAE. However, this significant change was not observed in rats treated with 1000 mg/kg of HEAE. Similarly, a significant decrease (P < 0.05) in albumin level in rats treated with 250 mg/kg was not observed in rats treated with 500 and 1000 mg/kg. This suggests that the observed change in the glucose and albumin level is not treatment related. The HEAE treatment caused a significant decrease (P < 0.05) in the level of total cholesterol, LDL and a significant increase (P < 0.05) in the level of HDL when compared to control rats. This change may be attributed to the antihyperlipidemic activity of the active ingredients in HEAE, which is in support of a previous work done by Lian et al. (2013). Hyperlipidemia is one of the major risk factors for atherosclerosis, which leads to coronary artery disease (Puster et al., 2005). Hence, HEAE may contribute in the management of coronary heart diseases.

Urea and creatinine are important biomarkers of kidney dysfunction (Mukinda and Eagles, 2010). In the present study, the level of urea and creatinine showed no significant difference (P > 0.05) between the
Fig. 6. Micrographs of the cardiac muscle sections obtained from SD rats treated with various doses of aqueous extract of *Hericium erinaceus* (HEAE) (400x). (A) Untreated control

Fig. 7. Micrographs of the lung sections obtained from SD rats treated with various doses of aqueous extract of *Hericium erinaceus* (HEAE) (400x). (A) Untreated control rats. (B) Rats treated with 250 mg/kg. (C) Rats treated with 500 mg/kg. (D) Rats treated with 1000 mg/kg.
HEAE treated rats and control rats. Similarly, no significant difference among groups was observed in the level of bilirubin, triglyceride and total protein. Thus, the HEAE administration on rats did not show any adverse toxic effects. This was further supported by the level of biomarker enzymes determined. Levels of serum biomarker enzymes in the liver (AST, ALT, and ALP) are widely used as sensitive markers to evaluate any toxic effects in the liver (Mukinda and Syce, 2007). Increased ALT levels in the serum reflect hypertrophy and other conditions of the liver cells (Hall et al., 2011). The biomarker AST, apart from being an indicator of liver toxicity, is also associated with other disorders of organs such as heart and muscle (Ozer et al., 2008). ALP is abundant in the cells lining the biliary duct of the liver and used in the diagnosis of biliary obstruction (Burtis and Ashwood, 2001). In the present study, the level of AST, ALT and ALP were within the limits and no significant difference (P > 0.05) was observed among the groups. Another evidence on the safety of HEAE was witnessed in the level of serum electrolytes (chloride, potassium, sodium, phosphate and calcium). No significant difference (P > 0.05) was observed among the HEAE treated and control rats.

Decrease in the levels of CAT, SOD, GSH activities and increase in the level of MDA signifies impaired antioxidant activity and increased oxidative stress, which reduces the capacity of the body to fight against free radicals (Parejo et al., 2002). In the present study, a significant increase (P < 0.05) in the levels of CAT, SOD, GSH and decrease in the level of MDA was observed between the HEAE treated rats and control rats. This suggests that HEAE has beneficial effect in increasing the antioxidant defence of the body and may contribute in the management and prevention of degenerative diseases. In support to our study, Han et al. (2013) reported that *H. erinaceus* can significantly decrease lipid peroxidation level and increase antioxidant enzymes activities in experimental animals. Another study reported that the chloroform subfraction of the methanol extract showed high flavonoid content and the electrospray ionization (ESI) LC-MS/MS analysis showed four phenolic compounds (4-hydroxybenzoic acid, syringic acid, 4-coumaric acid, and ferulic acid) responsible for the antioxidant properties of the *H. erinaceus* (Li et al., 2012). *Hericium erinaceus* polysaccharide (HEP) significantly decreased lipid peroxidation level and increased antioxidant enzymes in experimental animals (Han et al., 2013).

The safety of HEAE oral administration on Sprague Dawley (SD) rats for 90 days was confirmed by the relative organ weights and histopathological results. A change in organ weights is another indicator for toxic effects of drugs (Michael et al., 2007). The results revealed no statistically significant differences in the relative organ weights of liver, kidney, heart, brain, spleen and lungs. The results also revealed no treatment related adverse histopathological changes. Few histopathological changes were observed in the sections of the liver, heart and spleen. However these changes are not treatment related as they are also observed in control rats.

The no observed adverse effect level (NOAEL) was determined as > 1000 mg/kg of HEAE in this study. Thus, the human equivalent dose (HED) calculated using body surface area (Reagan-Shaw et al., 2007) is 162.16 mg/kg/day for adults and 240 mg/kg/day for children. Hence, for a 70 kg adult the HED is 11.3 g/day of HEAE.

5. Conclusions

In summary, the oral administration of HEAE up to a dose of 1000 mg/kg neither caused any mortality, nor were any adverse effects noticed in the haematological and biochemical parameters in rats studied for a period of 90 days. The body and organ weights of HEAE treated rats showed no significant changes compared to the control rats. Finally, the histopathological studies did not reveal any micro-

![Fig. 8. Micrographs of the spleen sections obtained from SD rats treated with various doses of aqueous extract of Hericium erinaceus (HEAE) (200x). (A) Untreated control rats. (B) Rats treated with 250 mg/kg. (C) Rats treated with 500 mg/kg. (D) Rats treated with 1000 mg/kg. Arrows → indicate splenic congestion.](image-url)
scopic alterations related to treatment given. The study reveals that this mushroom is relatively safe for human consumption and supports its traditional practice. However, since there is a paucity of information on its numerous potential characteristics that may contribute to its nutraceutical and health benefits, clinical trial protocols are much needed to further confirm its safety and effectiveness.

Acknowledgments

This research was supported by University of Malaya grant, UMRG RG-269-13AFR and University of Malaya High Impact Research Grant UM.C/625/1/HIR/MoE/SC/02. The lead authors thank the University of Malaya for the postdoctoral fellowships.

References


Gao, P.F., Watanabe, K., 2011. Introduction of the world health organization project of traditional practice. However, since there is a paucity of information on the international classification of traditional medicine. Zhong Xi Yi Jie He Xue Bao 9 (3), 1161–1164.


