ACUTE TOXICITY STUDY OF PEPTIDE HYDROLYSATES FROM WINGED BEAN (Psophocarpus tetragonolobus) SEEDS IN SPRAGUE DAWLEY RATS

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Abstract

Synthesis of peptide hydrolysates derived from edible high-protein foods especially legumes are increasing tremendously. The safety of peptide hydrolysates of winged bean (Psophocarpus tetragonolobus) seeds (PHWB) has not yet been evaluated as the subject of toxicological testing. Therefore, the present study was carried out to investigate the acute oral toxicity effects of aqueous solution of HWB in Sprague dawley rats based on Organization for Economic Co-operation and Development (OECD) Guidelines, Section 423. A total of eighteen Sprague dawley rats aged 8-12 weeks old were randomly segregated into three groups: Group 1, Group 2 and Group 3, which were treated with 0.9% saline, 2000 mg/kg body weight and 5000 mg/kg body weight of PHWB, respectively. The animals were orally administered with aqueous solution of PHWB and were observed for 14 days. The median lethal dose (LD₅₀) of PHWB was found to be higher than 5000 mg/kg of body weight. No mortality, abnormal behaviours, toxic signs, altered feeding pattern and haematological changes were observed among the treated groups which were comparable with the control group. There were also no macroscopic changes of the selected organs and the organs weight. In conclusion, high dosage of PHWB showed no acute toxicity effects. Therefore, the findings had verified that short duration of high dosage consumption of aqueous solution of PHWB is safe as a dietary source. Additional analysis on biochemicals, histopathological parameters of sub-chronic toxicity of PHWB in vivo are yet to be conducted in order to reveal its toxicity effects due to prolonged intake.

Keywords: Winged bean seeds, peptide hydrolysate, acute toxicity, haematological parameters, organ weight

1.0 Introduction

Malaysia is a well-known country which is rich in its biodiversity. Winged bean (Psophocarpus tetragonolobus) is one of the plant species found in Malaysia locally known as ‘kacang botor’. It had been recognised as the potential source of protein since 1975 [1, 18]. The good compositions of its mature seeds give a nutritional advantage to this plant. It has high protein (300-420 g/kg) and oil (150-280 g/kg) contents as compared with soybean (Glycine max) seeds. Its amino acid composition indicates a satisfactory level of lysine and relative deficiencies of cystine and methionine [2, 15].

Progress in hydrolysis technique using proteolytic enzymes has led to the production of hydrolysates from many food proteins [13]. In recent years, peptides hydrolysate from legumes has been recognized as a potential source in the development of functional foods and pharmaceutical industries. Enzymatic protein hydrolysate is widely used in specific formulations with clinical applications because it appears to be more effective than intact protein or free amino acids [6, 22]. However, due to its lower molecular weight, peptide hydrolysates of winged bean seeds have a possibility to become more reactive than the native protein [3]. In line with national policy on food production, PHWB may provide the alternative source of proteins in food industries. Hence, toxicological study of the peptide hydrolysates from these species is extremely necessary. Thus, this study aims to determine the LD₅₀ and acute oral toxicity effects of peptide hydrolysates from winged bean (Psophocarpus tetragonolobus) seeds in Sprague dawley rats to ensure that this peptide hydrolysate is safe to be exploited for human nutrition and health.

2.0 Materials and methods
2.1 Plant materials and preparation of sample

The seeds of winged bean (WB) were harvested on day 75 from the planting date at Agro Technology Farm, Selangor, Malaysia. The seeds were ground to form powder using hammer mill and undergone pre-treatment process. Fifty grams of WB seeds were dissolved in 250 mL of distilled water and shaken in water bath shaker for 15 minutes at 100-150 rpm. The mixture was then transferred into a centrifuge tube and centrifuged at 10 000 rpm for 10 minutes at temperature of 10°C. The supernatant formed was discarded and the residue was collected. The mixture of residues was preheated to 70°C prior to the enzymatic hydrolysis process. The enzymatic hydrolysis process was performed according to the method patented by Nazamid, (2013, Patent no:PI 2013700406) using papain for 6 hours. The pH was maintained at 6.5 by adding 1M of sodium hydroxide (NaOH). The solution was heated at 100°C for 10 minutes to stop the enzymatic reaction. The peptide hydrolysates produced from the enzymatic hydrolysis process were freeze dried and stored at -80°C for further study.

2.2 Experimental animal

Healthy and adult female Sprague dawley rats aged 8-12 weeks old, weighing 180-220 g were purchased from CheNur Sdn Bhd (Selangor, Malaysia) and were housed in the Animal Holding Room, Faculty of Applied Sciences, Universiti Teknologi MARA. All procedures involving using and handling of animals had been approved by the Animal Ethics Committee, Universiti Teknologi MARA, UiTM (Shah Alam, Malaysia) (Ref no: 26/2012). The animals were acclimatised for 5 days prior to the initiation of dosing. They were kept under standard laboratory with 12:12 hours of light and dark cycle. The animals were provided with standard rodent pellet diets and ad libitum supply of water.

2.3 Acute oral toxicity test

The procedure for acute toxicity study followed closely the Guidelines for Testing of Chemicals, Health Effects Test Guidelines on Acute Oral Toxicity- Acute Toxic Class Method, Section 423 (Organization for Economic Cooperation and Development (OECD)) [7]. A total of eighteen rats were randomly segregated into three groups with 6 rats per group. Group 1 was treated with 0.9% saline. Meanwhile, Group 2 and Group 3 were given a single dose of 2000 mg/kg and 5000 mg/kg body weight of PHWB, respectively. Prior to the treatments, the rats were individually marked on the tail and their weights were recorded. All animals were supplied with standard animal pellets purchased from CheNur Supplier Sdn. Bhd. (Selangor, Malaysia) and water was supplied ad libitum during the period of treatment. The animals were observed throughout the experiment with special attention given during the first 4 and 24 hours after dosing. Daily observations were conducted for the following 13 days of the test period. Toxic symptoms, morbidity, mortality, physiological and behavioural changes and numbers of animals that survived were recorded. Also, daily food and water intakes for each of the animals were individually measured. Body weights were measured once a week.

2.4 Haematological parameters

On day 14, the blood from the animals was drawn through cardiac puncture under diethyl ether anaesthesia. Blood samples were collected in heparinized centrifuge tubes and analysed for haematological parameters which included red blood cell (RBC) count using haemocytometer; white blood cell (WBC) count as described previously [4]; packed cell volume (PCV) [5], hemoglobin (Hb) content using an assay kit purchased from Next Gene Sdn Bhd (Selangor, Malaysia) and also mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) based on the mathematical equation for red cell indices.

2.5 Relative organ weight

The organs which included kidney, liver, heart, lung and reproductive organs were removed and washed in 0.9% saline solution, blotted free of blood and immediately weighed on an electronic balance. Organ weights were
expressed in relation to the body weight of the animal (g/100 g of bw). Then, the collected organs were fixed in 10% buffered formalin for subsequent analysis.

2.6 Statistical Analysis

Comparisons between the means of the control and the treated groups were analyzed using One-way Analysis of Variance (ANOVA) adopted from the Social Science Statistical Package (SPSS), Version 18. The experimental data between treated and untreated group were expressed as means ± standard error of the means (SEM), and p<0.05 value was considered as significant.

3.0 Results and discussion

It was observed that the peptide hydrolysates of winged bean (Psophocarpus tetragonolobus) seeds give no mortality, physiological and behavioural changes even at the highest dosage (5000 mg/kg) administered to the animals. This showed that the median lethal dose (LD_{50}) of PHWB exceeded 5000 mg/kg body weight. Based on the Acute Oral Toxicity-Fixed Dose Procedure [7], the PHWB could be classified under the category 5 which is non-toxic. The results showed an increasing trend in body weight gain, food and water intake from control to the highest dosage of PHWB seeds when administered to the experimental animal as demonstrated in Table 1. Mean daily food intake and mean weekly body weight gain in animal administered with highest dosage (11.72% and 18.49%, respectively) were higher compared to the control groups (17.06 g/day and 11.25 g/week). However, no significant differences at p<0.05 were observed in all parameters tested.

Table 2 summarized the changes observed in the haematological parameters. The animals were not significantly affected with the treatment. Profiles of the blood parameters which included the red blood cells count (RBC), white blood cells count (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were not significantly changed as compared with the control animals. However, there is an increase in trend showed in the RBC, Hb and PCV contents from control to dosage 2000 mg/kg and it decrease back from dosage 2000 mg/kg to 5000 mg/kg. Tanuja et al. [19] reported that reduction in haematological values is an indication of anemia which may be due to erythropoiesis, haemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in haematopoietic organs. Besides that, excessive damage of erythrocytes or inhibition of erythrocyte formation may result in a decrease of RBC count [10]. Analysis from this study also showed a reduction in MCV and MCH from control to the highest dosage (5000 mg/kg) administered with PHWB. MCV, MCH and MCHC are known as red cell indicators which depend on the RBC count, Hb concentration and PCV values as reported by Jayakumar et al. [9]. The results revealed an increase in WBC indicated that the animal bodies were fighting against infection. The main function of white blood cells (WBC) is to prevent and combat infection [8]. In contrast, when the body is less able to fight against infections due to problems with bone marrow, it caused the WBC to be very low. This condition known as cytopenia or leukopenia [14].

Values on relative organ weight of heart, liver, kidney, lung and ovary of treated and untreated groups are shown in Table 3. No significant difference (p>0.05) of the means of relative organ weight were recorded between control and treated groups. However, there was a significant reduction (p<0.05) between 2000 mg/kg (0.65 g) and 5000 mg/kg (0.78 g) in treated groups for relative organ weight of lung. A reduction in internal organ weights after exposure to the substance is reported to be as sensitive indices of toxicity [11, 17, 21, 20]. Michael et al. [12] reported on the contribution and value of organ weight in toxicity studies and mentioned that liver, kidney and heart are selected as the toxicity indicator due to their sensitivity to predict toxicity, frequent target in toxicity studies and correlates well with histopathological changes.

Table 1: Acute toxicity effects of peptide hydrolysate of Psophocarpus tetragonolobus seeds on feeding pattern of rats.
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<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2000 mg/kg</th>
<th>5000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Food (g/day)</td>
<td>17.06 ± 0.37a</td>
<td>18.67 ± 0.54a</td>
<td>19.06 ± 0.92a</td>
</tr>
<tr>
<td>Water (mL/day)</td>
<td>27.08 ± 1.85a</td>
<td>27.45 ± 1.06a</td>
<td>28.60 ± 0.70a</td>
</tr>
<tr>
<td>Weight gain (g/week)</td>
<td>11.25 ± 2.21a</td>
<td>12.92 ± 1.74a</td>
<td>13.33 ± 1.36a</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n=6). Values with different superscript letters in the same row are significantly different (p<0.05).

Table 2: Haematological values of rats administered with peptide hydrolysate of *Psophocarpus tetragonolobus* seeds in acute toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2000 mg/kg</th>
<th>5000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x 10^6)</td>
<td>7.51 ± 0.59a</td>
<td>8.11 ± 0.66a</td>
<td>8.14 ± 0.44a</td>
</tr>
<tr>
<td>WBC (x 10^3)</td>
<td>2.99 ± 0.75a</td>
<td>3.58 ± 0.81a</td>
<td>4.93 ± 1.06a</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>13.20 ± 0.00a</td>
<td>13.75 ± 0.35a</td>
<td>13.48 ± 0.28a</td>
</tr>
<tr>
<td>PCV (%)</td>
<td>33.67 ± 0.67a</td>
<td>35.83 ± 0.31a</td>
<td>34.50 ± 1.61a</td>
</tr>
<tr>
<td>MCV (µm^3)</td>
<td>46.12 ± 3.40a</td>
<td>45.70 ± 3.76a</td>
<td>42.62 ± 1.54a</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>18.12 ± 1.40a</td>
<td>17.42 ± 1.18a</td>
<td>16.77 ± 0.84a</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>39.30 ± 0.78a</td>
<td>38.42 ± 1.12a</td>
<td>39.47 ± 1.94a</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n=6). Values with different superscript letters in the same row are significantly different (p<0.05).

Table 3: Relative organ weight (100g body weight) of rats administered with peptide hydrolysate of *Psophocarpus tetragonolobus* seeds in acute toxicity study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>2000 mg/kg</th>
<th>5000 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart (g)</td>
<td>0.39 ± 0.02a</td>
<td>0.33 ± 0.01a</td>
<td>0.46 ± 0.09a</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>3.12 ± 0.06a</td>
<td>2.89 ± 0.21a</td>
<td>2.98 ± 0.13a</td>
</tr>
<tr>
<td>Kidney (g)</td>
<td>0.69 ± 0.02a</td>
<td>0.65 ± 0.03a</td>
<td>0.68 ± 0.02a</td>
</tr>
<tr>
<td>Lung (g)</td>
<td>0.68 ± 0.04ab</td>
<td>0.65 ± 0.02b</td>
<td>0.78 ± 0.05a</td>
</tr>
<tr>
<td>Ovary (g)</td>
<td>0.07 ± 0.00a</td>
<td>0.06 ± 0.00a</td>
<td>0.07 ± 0.00a</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM (n=6). Values with different superscript letters in the same row are significantly different at p<0.05.

4.0 Conclusion
In conclusion, the peptide hydrolysates of winged bean (*P. tetragonolobus*) seeds are safe and practically are non-toxic when administered at single-high-dosage. The findings show no mortality and no significant alterations in the haematological parameters neither in control nor treated groups might be attributed to minimal toxin found in the aqueous solution of *P. tetragonolobus* peptide hydrolysate. The observation via macroscopic examination and weight measurement on the selected organs also were not significantly affected. It also could be concluded that there were no toxic effects related to the body weight gain, food and water consumption of rats. This evidence is sufficed to assume that the peptide hydrolysates of *P. tetragonolobus* is safe for consumption at or above 5000 mg/kg body weight.

5.0 Acknowledgements

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6.0 References


