Toxicity of raw and processed roots of *Polygonum multiflorum*

Xiaoqing Wu, Xiaozhen Chen, Qingchun Huang, Dongmei Fang, Guoyou Li, Guolin Zhang,*

*Chengdu Institute of Biology of the Chinese Academy of Sciences, Chengdu, PR China
bGraduate University of the Chinese Academy of Sciences, Beijing, PR China

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

The roots of *Polygonum multiflorum* (Chinese name: He-Shou-Wu, HSW) are used in traditional Chinese medicine for many diseases in processed form or raw state. There are reports dealing with the toxicity of HSW. However, the toxicity is caused by over dosage or by the herb itself remains unclear. We evaluated the toxicity of raw and processed HSW on Kunming (KM) mice. For raw HSW, the toxicity of water decoct is much higher than that of acetone extract. Meanwhile, the toxicity of acetone extract of raw HSW is considerably higher than that of acetone extract of processed HSW. HPLC analyses revealed that the contents of characteristic compounds in raw HSW were changed after processing: the content of 2,3,4′,5-tetrahydroxystilbene 2-O-β-D-glucoside was decreased by 55.8%, whereas the content of emodin was increased by 34.0%. Thus, processing could reduce the toxicity of HSW. Thus, the toxicity of HSW does not depend on the content of anthranoid derivatives, it may be correlated with the content of tetrahydroxystilbene glucosides.

© 2011 Elsevier B.V. All rights reserved.

**Keywords:**
*Polygonum multiflorum*
He-Shou-Wu
Processing
Stilbene
Emodin
Hepatotoxicity

1. Introduction

The roots of *Polygonum multiflorum* (Chinese name: He-Shou-Wu, HSW) are traditional Chinese medicinal herbs used in clinic for many diseases in processed form or raw state [1]. The raw HSW is used for antioxidation and purgation, whereas the processed HSW is used as a tonic and an antiaging agent [1]. There are many HSW-containing products such as Shen-Min, Shou-Wu-Plan, and Shou-Wu-Wan [2]. Reports on adverse effects and hepatotoxicity of HSW products have been increasing since 1990s although the two forms of HSW had always been perceived to be safe for a long time [3–6].

In traditional Chinese medicine, processing is a very important procedure and it is believed that processing could decrease the toxicity and change therapeutic efficacy of Chinese herbal medicine [2]. For HSW, whether the processing can decrease hepatotoxic effects or not remains unclear. In contrast to the unfavorable reports on humans, HSW extracts have been reported to possess no toxic effects at least in liver, and might have elicit useful but limited beneficial effects on liver in vivo [7]. Recent studies demonstrated that the crude extracts of HSW exhibited prominent effects, particularly in cardiovascular diseases [8–10]. Obviously, it is necessary to perform detailed investigations on the hepatotoxicity of HSW for the safe use. Thus, in vivo experiments are warranted to evaluate toxicity difference between the processed and unprocessed forms of HSW. In this study, we performed the comparative study on the toxicity of raw and processed HSW. The toxicity of HSW decreased significantly after being processed. The toxicity of water extract (water decoctum) of raw HSW is much stronger than that of acetone extract of raw HSW. The toxicity of acetone extract of raw HSW (AEUP) exhibited low toxicity, whereas acetone extract of processed HSW (AEPP) displayed no toxicity. HPLC analyses indicated that the contents of characteristic compounds in HSW were changed by processing: the content of 2,3,4′,5-tetrahydroxystilbene-2-O-β-D-glucoside was decreased by 55.8%, whereas the content of emodin was increased by 34.0%. It could be concluded that the toxicity of HSW does not depend on the content of anthranoid derivatives.
2. Materials and methods

Fresh roots of Polygonum multiflorum (HSW) were collected in Emei Mountain of Sichuan Province, P. R. China in November 2005. It was identified by Professor Liangke Song at School of Life Science and Engineering, Southwest Jiaotong University. The voucher specimen (Heshouwu-05-10-25) was deposited at the Herbarium Center of Southwest Jiaotong University. Fresh HSW was dried to give raw HSW. Processed HSW was prepared according to the Pharmacopoeia of the People’s Republic of China (2010 version) [2]: HSW was steamed until the surface turned to dark black (usually it takes about 32 h, Steaming-processing, SP) or steamed with the black bean juice until the surface turned to dark black (usually it takes about 32 h, Bean-processing, BP).

Water extract of HSW: HSW was grinded and decocted twice with water (each 30 min). The resulting filtrates were combined and concentrated to dryness under reduced pressure. The dried extract was re-dissolved and dispersed in warm water and then cooled for intragastric administration to mice.

Acetone extract of HSW: HSW powder was extracted twice with acetone in an ultrasonic water bath (each 30 min). The resultant filtrates were combined, concentrated under reduced pressure to dryness. The dried extract was re-dissolved and dispersed in warm water and then cooled for intragastric administration to mice.

The standard substances of emodin, physcion, and 2,3,4,5-tetrahydroxy-stilbene-2-O-β-D-glucoside (THSG) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products of China (Beijing, China). The purity of all these standards was >98%. Acetonitrile (HPLC grade, Fisher USA), Methanol (HPLC grade, Fisher USA) and Formic acid (GR, Merck, USA) were used for HPLC analysis. The water from Milli-Q water purification system was used for all the solutions and dilutions.

2.1. Animals

Male and female Kunming (KM) mice (SPF grade, weighting 18–20 g) were obtained from Sichuan Provincial Academy of Medical Sciences (Sichuan, China). All animals were maintained in plastic cages under standard environmental conditions at 21 ± 2 °C with relative humidity of 51 ± 10% and light cycle of 12 h. The Mice were fed on a standard mice diet and water ad libitum with the institutional animal care guidelines approved by the Ministry of Science and Technology of China.

2.2. Acute toxicity

Both male and female healthy mice were fasted overnight and just allowed to access to water ad libitum. They were random divided into seven groups (20 animals/group). The mice of the first group (control group) were fed with water only. The mice of groups 2–7 were treated with the water and acetone extracts of raw and processed HSW at the doses of 5, 10, and 20 g/kg of the body weight per day od (counted on the quantity of crude material). The dosages were equivalent to 10, 20 and 40 times of the upper dosage for human recommended in Chinese Pharmacopoeia (0.5 g/kg). The water and acetone extracts of HSW were administrated intragastrically. The dosage was set at a high level to uncover any potential toxicity in order to investigate the hepatic risk. The general behavior, body weight changes, hazardous symptoms, and mortality of mice were monitored for a period of 14 days after treatment. The LD₅₀ values were estimated according to the method described by Lichfield and Wilcoxon [11].

2.3. Subacute toxicity

Male mice were randomly divided into ten groups (20 animals/group). The mice of the first group (Control group) were fed with water only. The mice of groups 2 to 10 were treated with the extracts of HSW as mentioned in Section 2.2. The body weight of the mice was recorded weekly and the water intake by the mice was monitored daily. The signs of abnormalities of the mice were recorded during the treatment period. At the end of the treatment, the mice were fasted overnight and just allowed to access to water ad libitum. Then the mice were anesthetized with ether and the blood samples were obtained by retro-orbital puncture, using capillary tubes for biochemical studies without anticoagulant.

2.4. Histopathologic examination and biochemical analysis

The mice were dissected to collect the livers for histopathologic examination. The livers were fixed in 10% buffered formalin solution for Haematoxylin-Eosin (H&E) staining. The pathological changes of fatty liver and degeneration of hepatocytes were graded: 0, normal; +1, mild degree; +2 moderate degree; and +3, severe degree.

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol (TC), triglycerides (TG), total and direct bilirubin (TBIL), and alkaline phosphatase (ALP) were quantitatively analyzed on the Hitachi clinical analyzer 7020 (Hitachi High-Technologies Corporation, Japan).

2.5. HPLC analysis of raw and processed HSW

2.5.1. Sample preparation

The powder of raw or processed HSW (200 mg) was extracted 3 times with 8 mL of acetone or other solvents in an ultrasonic water bath. The resulting filtrates were combined and transferred to a 25 mL volumetric flask. Appropriate volume of acetone was added to the mark of the volumetric flask. The resulting solution (5 mL) was concentrated under reduced pressure to dryness. The residue obtained was dissolved in 2 mL of methanol, and then filtered through a 0.45 μm filter membrane prior to HPLC analysis.

2.5.2. Chromatography and calibration

A Shimazu (Japan) LC-10A series HPLC-DAD system consisting of a vacuum degasser, binary pump, thermo stated column compartment, a C18 column (5 μm, 4.6 mm × 250 mm, hypersil, USA) and DAD was used to obtain chromatograms and UV spectra. The mobile phase consisted of acetonitrile (A) and water (B) was used: 10–40% A, 0–35 min; 40%–100% A, 35–60 min. The flow rate was 1.0 mL/
min and column temperature was maintained at 30 °C. DAD detector was set at 290 nm wavelength. The loading volume was 10 μL.

The calibration curves, precision and recoveries for the component analysis were examined and proven to be validated.

2.6. Statistics

Statistical analysis was performed using an unpaired t-test. P values lower than 0.05 were considered statistically significant. All data were expressed as mean ± S.E.M. for all experiments.

3. Results

3.1. Acute toxicity of HSW

No toxicity or death was observed in mice treated by oral route at doses up to 100 g/kg with HSW’s acetone extracts during 14 days of observation (Table 1). The LD50 could not be estimated in our experiments. The possible LD50 is higher than 100 g/kg. That means the LD50 was at least equivalent to 200 times of the upper dose of human stipulated in Chinese Pharmacopoeia (0.5 g/kg).

3.2. Subacute toxicity of HSW

3.2.1. Survival analysis

No toxicity or death was recorded in the mice treated with AEUP-H and AEPP-2 during the 28 consecutive days (Table 1). However, some mice treated with AEUP died. Statistics analysis revealed that the death ratio is dose-dependent (Table 1). During 7 days of observation, 14, 5, and 2 mice died after treatment with WDUP for 7 days at high, mediate, and low dosages, respectively. Meanwhile no mice died after treatment with AEUP (Tables 1 and 2). The results indicated that the possible toxicity order of HSW extracts is WDUP > AEUP > AEPP-1 ≈ AEPP-2. Thus, the toxicity of HSW decreases significantly after being processed.

3.2.2. Body weight, food, and water consumption analysis

The toxicity signs were found in WDUP-treated (Table 3) and AEPP-treated groups (Table 3). The absolute body weight of all mice treated with the WDUP and AEUP for 7 consecutive days decreased, respectively, by 18.9%–44.6% and 4.0%–17.6%, depending on the dosages. Whereas neither absolute body weight nor body weight gain was affected by AEPP-1 and AEPP-2 administration at all dosages after 28 consecutive days of treatment (Table 3). A same trend was observed in the case of food and water consumption (Tables 1 and 2 in the Supporting Information). The results supported that the toxicity of HSW decreases significantly after being processed.

3.2.3. Biochemical analysis

Table 4 summarizes the results of the biochemical characteristics in livers of mice treated with different extracts of HSW. The AEPP-1 and AEPP-2 administration at all dosages did not induce any changes in AST, ALT, TC, ALP, TG and TBIL levels. However, an increase of 121%, 72% in ALT serum levels were observed within the animals treated with the AEUP-H and AEUP-M, respectively. The serum levels of the AST of the mice treated with AEUP-H, AEUP-M, and AEUP-L increased by 138%, 102%, and 51% respectively, in comparison to control group. The results indicated that hepatotoxicity was dose-dependent.

3.3. HPLC analysis of HSW

The major constituents in raw and processed HSW (Fig. 1) were identified by comparing with the standard compounds and the published data[12]. The compounds of the six characteristic peaks in Fig. 1 were unambiguously identified as 2,3,4′,5-tetrahydroxystilbene-2-O-β-D-glucoside (THSG, 1), emodin-8-O-β-D-glucoside (EG, 7), physcion-8-O-β-D-glucoside (PG, 3), emodin (4), chrysophanol (5), and physcion (6) (Fig. 2).

As shown in Fig. 1A and B, it is obvious to figure out the differences between the water and the acetone extracts of raw HSW. The major compounds of the water extract of

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-H*</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-M*</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-L*</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 2

Number of survival mice treated with water decocta of raw HSW (WDUP).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-H*</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-M*</td>
<td>15</td>
</tr>
<tr>
<td>WDUP-L*</td>
<td>15</td>
</tr>
</tbody>
</table>

* H, M, and L represent the dosage level: H-high, 20 g of the crude material; M-median, 10 g of the crude material; L-low, 5 g of the crude material.
HSW are THSG and EG (Fig. 1A). However, the contents of THSG and EG decreased significantly (Fig. 1B and C). Meanwhile the contents of compounds 5 and 6 increased markedly after being processed.

The contents of the characteristic components THSG and emodin were usually found to be correlated with the diversified therapeutical efficacy and the hepatotoxicity of the unprocessed and processed HSW. Quantitative analyses revealed that the contents of the major compounds in the processed HSWs (SP and BP) were consistent with each other (Table 3 in the Supporting Information). After being processed, the THSG content is decreased by 55.8%, whereas the emodin content was increased by 34.0% (Table 3 in the Supporting Information).

4. Discussion

HSW was first recorded in herbal “Kaibao Bencao” issued by the imperial court of the Song Dynasty in 974. It has been used in processed form and raw state. Several reports

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th>20 g/kg/day^a</th>
<th>10 g/kg/day^a</th>
<th>5 g/kg/day^a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight</td>
<td>Decrease (%)</td>
<td>Body weight</td>
<td>Decrease (%)</td>
</tr>
<tr>
<td>0</td>
<td>23.56 ± 0.87</td>
<td>0</td>
<td>23.68 ± 1.53</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td></td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>24.20 ± 0.75</td>
<td>2.7</td>
<td>19.51 ± 1.29</td>
<td>0.4</td>
</tr>
<tr>
<td>14</td>
<td>25.50 ± 0.77</td>
<td>8.2</td>
<td>17.25 ± 1.79</td>
<td>2.7</td>
</tr>
<tr>
<td>21</td>
<td>27.06 ± 0.48</td>
<td>14.8</td>
<td>15.18 ± 1.32</td>
<td>35.9</td>
</tr>
<tr>
<td>28</td>
<td>28.83 ± 0.51</td>
<td>22.4</td>
<td>13.68 ± 1.82</td>
<td>42.2</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± SEM (n = 15 animals/group).

a. WDUP: Water decocta of unprocessed HSW.
b. AEUP: Acetone extracts of unprocessed HSW.
c. AEPP-1: Acetone extracts of HSW processed by steaming processing (SP).
d. AEPP-2: Acetone extracts of HSW processed by bean steaming process (BP).
A. Dosage was counted with the quantity of crude material.
* P < 0.05, comparison between the control and HSW treated groups.
** P < 0.01, comparison between the control and HSW treated groups.

**Table 4**

Effect of doses of HSW acetone extracts by oral route on serum parameters in male mice for 28 consecutive days.

<table>
<thead>
<tr>
<th>Treatment^a</th>
<th>ALT^b (U/L)</th>
<th>AST^b (U/L)</th>
<th>ALP^b (U/L)</th>
<th>TG^b (mmol/L)</th>
<th>TC^b (mmol/L)</th>
<th>TBIL^b (μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56.75 ± 16.18</td>
<td>124.80 ± 33.41</td>
<td>156.16 ± 66.20</td>
<td>1.46 ± 0.22</td>
<td>3.16 ± 0.37</td>
<td>6.81 ± 0.25</td>
</tr>
<tr>
<td>AEUP-H^a</td>
<td>296.45 ± 84.83**</td>
<td>211.45 ± 76.79</td>
<td>155.65 ± 40.11</td>
<td>0.82 ± 0.25</td>
<td>2.51 ± 0.28</td>
<td>6.45 ± 0.74</td>
</tr>
<tr>
<td>AEUP-M^b</td>
<td>188.52 ± 33.77</td>
<td>167.33 ± 48.39</td>
<td>152.66 ± 33.77</td>
<td>0.81 ± 0.21</td>
<td>2.03 ± 0.39</td>
<td>6.90 ± 0.31</td>
</tr>
<tr>
<td>AEPP-1-H^c</td>
<td>51.88 ± 8.29</td>
<td>105.81 ± 26.33</td>
<td>167.33 ± 48.39</td>
<td>1.23 ± 0.47</td>
<td>2.51 ± 0.28</td>
<td>6.45 ± 0.74</td>
</tr>
<tr>
<td>AEPP-1-M^d</td>
<td>52.25 ± 9.80</td>
<td>124.90 ± 26.33</td>
<td>134.50 ± 45.25</td>
<td>1.05 ± 0.43</td>
<td>2.84 ± 0.39</td>
<td>6.76 ± 0.32</td>
</tr>
<tr>
<td>AEPP-1-L^e</td>
<td>47.25 ± 5.99</td>
<td>125.50 ± 20.45</td>
<td>146.33 ± 48.38</td>
<td>1.09 ± 0.40</td>
<td>3.24 ± 0.58</td>
<td>6.92 ± 0.32</td>
</tr>
<tr>
<td>AEPP-2-H^f</td>
<td>51.63 ± 6.02</td>
<td>118.54 ± 16.87</td>
<td>151.66 ± 33.77</td>
<td>1.21 ± 0.42</td>
<td>2.56 ± 0.45</td>
<td>7.11 ± 0.43</td>
</tr>
<tr>
<td>AEPP-2-M^g</td>
<td>52.15 ± 8.21</td>
<td>112.12 ± 49.59</td>
<td>131.16 ± 27.33</td>
<td>1.06 ± 0.48</td>
<td>2.89 ± 0.35</td>
<td>7.05 ± 0.29</td>
</tr>
<tr>
<td>AEPP-2-L^h</td>
<td>48.75 ± 6.99</td>
<td>105.34 ± 26.15</td>
<td>146.83 ± 26.85</td>
<td>1.04 ± 0.45</td>
<td>3.09 ± 0.38</td>
<td>6.73 ± 0.26</td>
</tr>
</tbody>
</table>

^a H, M, and L represent the dosage level: H-high, high dosage (20 g/kg); M-median, median dosage (10 g/kg); L-low, low dosage (5 g/kg).

^b ALT — alanine aminotransferase, AST — aspartate aminotransferase, ALP — alkaline phosphatase, TG — triglycerides, TC — total cholesterol, TBIL — total bilirubin.

^c All the values are expressed as mean ± S.E.M. (n = 15 animals/group).

* P < 0.05, comparison between the control and PM treated groups.
** P < 0.01, comparison between the control and PM treated groups.
described severe hepatic dysfunctions caused by the intake of HSW products in human [3,13,14]. Many factors including the prescribed dose, the prescribed medicine form, the condition being treated, the age and gender of the patient and other treatments have impact on the toxicity. The results described here are consistent with those hepatotoxicity...
reports in human. The possible hepatotoxicity order of the HSW extracts is WDUP > AEUP > AEPP-1 ≈ AEPP-2.

The raw HSW have effect on the hepatic function in mice (subacute pretreatment (28-day), a daily dose: 10 and 20 g/kg), which was supported by number of survived animals, body weight change, plasma makers of hepatic function, and other hematological parameters. A daily dose of 5 g/kg in a mouse equals 300 g HSW intake per day for a 60-kg human individual. While the normal recommended dosage of HSW for humans is 10–30 g. Therefore, the above results provided essential information for the consumers that the use of raw HSW within the recommended dose range could be safe. At the highest dose (20 g/kg) of raw HSW, considerable numbers of mice died within 7 days. This result indicated that the toxic effect increased in proportion to the dosages. On the other hand, no hepatotoxicity was found after repetitive administration of processed HSW to mice for 4 weeks at the dosages of 5, 10, and 20 g/kg of body weight per day. Taken together, the above in vivo experiments suggested that the processing significantly decreased the toxicity of raw HSW.

In contrast to previous data [15], no new peak in processed HSW was observed in our HPLC analyses. However, the contents of characteristic compounds varied in raw and processed HSW. Anthraquinones were believed to cause the hepatotoxicity for a long time in previous reports [3,13]. But the previous studies about toxic effects of HSW contradicted hepatotoxic findings reported in humans [5]. Unfortunately the origin of HSW used in this study was not authenticated and the experimental period was relatively short (10-day). And, it was still uncertain that the HSW used in this study was raw or processed. We found that the toxicity of raw HSW is significantly higher than that of processed HSW. The nontoxic processed HSW indicated that processing could significantly decrease the toxicity of raw HSW.

5. Conclusion

In summary, the processing significantly decreased the toxicity of raw HSW. Subacute daily intake of processed HSW extract did not induce any hepatotoxicity. The processing altered the contents of chemical composition. After being processed, the THSG content was decreased by 55.8%, but the emodin content was increased by 34.0%. The toxicity of HSW does not depend on the content of anthranoid derivatives.

Acknowledgment

This work was financially supported by the National Basic Research Program of China (973 Program, No. 2009CB522804).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.fitote.2011.12.012.

References

