A Safety Assessment of N-(β-D-Xylopyranosyl) Taurine Sodium Salt in Male and Female ICR Mice

Jeong Soon You, Yun Ju Lee, So Young Kim, Kyung Ja Chang, Hye Jeong Cho, and Sung Hoon Kim

Abbreviations

ANOVA One-way analysis of variance
BW Body weight
H&E Hematoxylin and eosin
HFF Health functional food
LD₅₀ Median lethal dose
SEM Standard error of the mean
SPSS Statistical Product and Service Solution
T-Xyl N-(β-D-Xylopyranosyl)taurine sodium salt

1 Introduction

Taurine has many biological benefits, including metabolic syndrome prevention (Kim et al. 2010), antioxidative (Keys and Zimmerman 1999), anti-obesity (Tsukuiyama-Kasaoka et al. 2006; Kim et al. 2010), and anti-diabetic effects (Cheong and Chang 2013). However, because of various disadvantages of taurine, taurine derivatives were developed and their functions have been verified (Kontny et al. 2007; Budhram et al. 2013). Recently, taurine-carbohydrate derivatives were also developed, and T-Xyl, one such taurine-carbohydrate derivative, exhibited good anti-adipogenic effect in vitro (Cho et al. 2014) and anti-obesity effect in vivo.
Therefore, it seems that T-Xyl has the potential to be developed as a specific ingredient of health functional food (HFF).

Due to its fast renal extraction rate, taurine is considered a non-toxic ingredient. However, it has been reported that rats supplemented with 5% taurine have diarrhea. For the clinical use of T-Xyl in the future, it is very important that we first assess its safety.

Therefore, the objective of this study was to perform a safety assessment of T-Xyl. To evaluate the safety of T-Xyl, we conducted the acute oral toxicity test and determined the LD_{50} of T-Xyl in male and female ICR mice.

2 Methods

2.1 Animals and T-Xyl

ICR mice of both sexes were supplied from Koatech (Pyeongtaek, Korea). All mice were housed at the laboratory animal housing at Inha University and the procedures for mice care were conducted in accordance with the guidelines outlined by the Experimental Animal Ethics Committee of Inha University with a constant 12-h light and dark cycle (09:00 AM to 09:00 PM), controlled temperature (23 ± 1°C), and controlled humidity (55 ± 10%). All mice were fed a commercial pellet diet supplied by DBL (Anseong, Korea) and were provided water ad libitum.

\[ N-(\beta-D-Xylopyranosyl)taurine \] sodium salt (Fig. 1) was synthesized recently with the goal of enhancing the absorption rate and improving the liposolubility and physiological activities in comparison with taurine (Cho et al. 2014).

2.2 The Toxicity Test of Acute Oral Dose

To assess the safety of T-Xyl, we conducted the acute oral toxicity test and determined the LD_{50} of T-Xyl in male and female ICR mice. The acute oral toxicity test of T-Xyl followed modified OECD-420 guidelines (OECD 1987).

Following 1 week of acclimatization, 12 ICR mice of each sex were randomly assigned to three groups of 4 mice each and fed for 14 days. Mice in each group were administered T-Xyl via oral gavage at a single dose of 0 (control), 2,000

Fig. 1 Structure of T-Xyl. The chemical structure of T-Xyl was identified by nuclear magnetic resonance spectroscopy.
(in accordance with OECD guidelines), or 5,000 (in accordance with the harmless material classification standard of the US Environmental Protection Agency) mg T-Xyl/kg BW. The administration volume was 2 ml/100 g BW and distilled water was administered to the control groups.

In addition, we determined the LD₅₀ of T-Xyl. LD₅₀ is the amount of a toxin, given all at once, which causes the death of 50 % of the group of test animals.

### 2.3 Body Weight Measurement and Clinical Signs Observation

Individual body weights were measured and recorded prior to administration of T-Xyl and at 7 and 14 days following T-Xyl or control administration.

Observations for clinical signs were made every hour for the first 6 h and then once daily for 14 days. Clinical signs included changes in skin, fur, and eyes; respiratory effects; autonomic effects, including salivation, diarrhea, and urination; and central nervous system effects, including tremors, convulsions, relaxation, and coma (Demma et al. 2007).

### 2.4 Sampling and Organ Weight Measurement

At the end of the observation period, the mice were fasted for 12 h before sacrifice. All mice were subject to macroscopic examination. The absolute organ weights of the liver, kidneys, and spleen were determined and the relative organ weights (g/100 g BW) were calculated. Sections of liver tissue were removed for histological evaluation.

### 2.5 Histological Examination of Liver Tissue

Histological examination was performed on sections of liver tissue under a light microscope (Axioskop 2, Zeiss, Jena, Germany) using the paraffin method. The liver tissues were fixed immediately with 10 % buffered formalin after removal, and paraffin-embedded sections were stained with hematoxylin and eosin (H&E).

### 2.6 Statistical Analysis

Data are expressed as means± standard error of the mean (SEM). Statistical evaluations were performed using one-way analysis of variance (ANOVA) followed by Duncan’s multiple range test at p<0.05 for analysis of significant differences. All analyses were performed using the Statistical Product and Service Solution (SPSS) 20.0 software program.
3 Results and Discussion

3.1 Mortality and LD<sub>50</sub>

Among all groups, there was no animal death prior to scheduled sacrifice. Therefore, final mortality was 0% in all experimental groups.

According to the conventional LD<sub>50</sub> tests (Lipnick et al. 1995), generally females are slightly more sensitive than males in their acute toxicity response. Therefore, females are normally used, but we used both sexes. In this study, the LD<sub>50</sub> value of T-Xyl was >5,000 mg/kg BW in both male and female ICR mice.

3.2 Body Weight Measurement and Clinical Signs Observation

The body weight measurements are shown in Table 1. From these results, single-dose administration of T-Xyl had no effect on BW of groups administered T-Xyl compared to control groups. There was no significant difference in the BW from day 0 to day 14 in male or female mice among all groups.

Following single-dose administration, there were no abnormal clinical signs such as changes in skin, fur, or eyes; respiratory effects; autonomic effects, including salivation, diarrhea, and urination; or central nervous system effects, including tremors, convulsions, relaxation, and coma in all groups administered T-Xyl.

3.3 Organ Weight Measurement

At the end of observation period, all animals were sacrificed and major organs were subject to macroscopic examination. No abnormal findings or lesions were found in the liver, spleen, lung, heart, stomach, and kidney of all animals.

Table 1 Body weight of male and female ICR mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>T-Xyl (mg/kg BW)</th>
<th>Number of mice</th>
<th>Days after treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Day 0 (g)</td>
<td>Day 7 (g)</td>
<td>Day 14 (g)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>4</td>
<td>32.8±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>35.8±0.6</td>
<td>36.5±0.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>4</td>
<td>32.8±0.5</td>
<td>34.6±0.9</td>
<td>36.6±0.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>32.9±0.5</td>
<td>35.7±0.7</td>
<td>37.1±0.7</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>4</td>
<td>26.9±0.5</td>
<td>27.5±0.8</td>
<td>28.0±0.5</td>
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</tr>
<tr>
<td></td>
<td>2,000</td>
<td>4</td>
<td>26.7±0.4</td>
<td>27.4±0.7</td>
<td>28.9±0.5</td>
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</tr>
<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>26.9±0.4</td>
<td>27.6±0.9</td>
<td>28.1±0.7</td>
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</tr>
</tbody>
</table>

<sup>a</sup>Values are expressed as means ± SEM; ns not significant
Table 2  Organ weights of male and female ICR mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>T-Xyl (mg/kg BW)</th>
<th>Number of mice</th>
<th>Liver (g)</th>
<th>Spleen (g)</th>
<th>Lung (g)</th>
<th>Kidney (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0</td>
<td>4</td>
<td>1.5±0.06*</td>
<td>0.1±0.02</td>
<td>0.2±0.01</td>
<td>0.5±0.03*</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>4</td>
<td>1.5±0.07</td>
<td>0.1±0.01</td>
<td>0.2±0.01</td>
<td>0.5±0.03</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>1.5±0.07</td>
<td>0.1±0.01</td>
<td>0.2±0.01</td>
<td>0.5±0.02</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>4</td>
<td>1.1±0.10</td>
<td>0.1±0.02</td>
<td>0.2±0.01</td>
<td>0.3±0.02*</td>
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<tr>
<td></td>
<td>2,000</td>
<td>4</td>
<td>1.2±0.07</td>
<td>0.1±0.00</td>
<td>0.2±0.01</td>
<td>0.3±0.01</td>
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<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>1.1±0.07</td>
<td>0.1±0.01</td>
<td>0.2±0.00</td>
<td>0.3±0.03</td>
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</tbody>
</table>

*Values are expressed as means±SEM; ns not significant

Table 3  Relative organ weights of male and female ICR mice

<table>
<thead>
<tr>
<th>Sex</th>
<th>T-Xyl (mg/kg BW)</th>
<th>Number of mice</th>
<th>Liver (g)</th>
<th>Spleen (g)</th>
<th>Lung (g)</th>
<th>Kidney (g) g/100 g BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>0</td>
<td>4</td>
<td>4.2±0.1*</td>
<td>0.3±0.04</td>
<td>0.6±0.02</td>
<td>1.4±0.06*</td>
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<tr>
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<td>2,000</td>
<td>4</td>
<td>4.3±0.2</td>
<td>0.3±0.03</td>
<td>0.6±0.02</td>
<td>1.4±0.03</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>4.2±0.2</td>
<td>0.3±0.03</td>
<td>0.6±0.04</td>
<td>1.3±0.10</td>
</tr>
<tr>
<td>Female</td>
<td>0</td>
<td>4</td>
<td>4.2±0.4</td>
<td>0.4±0.03</td>
<td>0.6±0.03</td>
<td>1.2±0.06*</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>4</td>
<td>4.4±0.2</td>
<td>0.4±0.02</td>
<td>0.6±0.04</td>
<td>1.1±0.01</td>
</tr>
<tr>
<td></td>
<td>5,000</td>
<td>4</td>
<td>4.1±0.2</td>
<td>0.4±0.04</td>
<td>0.6±0.02</td>
<td>1.1±0.07</td>
</tr>
</tbody>
</table>

*Values are expressed as means±SEM; ns not significant

In addition to macroscopic evaluation, the major organs of the mice were also weighed. Organ weight is a basic measurement to diagnose whether the organ was exposed to the toxic substance or not. The liver, spleen, lung, and kidney are the major organs affected by metabolism caused by toxic substances (Dybing et al. 2002). Compared to the control group, the organ weights of liver, spleen, lung, and kidney from the mice administered T-Xyl indicated no significant changes (Tables 2 and 3).

3.4 Histopathological Examination

Because the liver is very important in the metabolism of toxic substances, we conducted microscopic examination of liver sections. No abnormal findings or lesions upon macroscopic examination were observed.

In this study, no significant differences in histopathology of liver tissues between the experimental groups and the control group were observed (Figs. 2 and 3)
Fig. 2 Light microscopy of hepatocytes in male ICR mice. (a) ×100 magnification (b) ×200 magnification. Images are representative H&E-stained liver sections. No significant differences were found in liver histology between the different treatment groups.

Fig. 3 Light microscopy of hepatocytes in female ICR mice. (a) ×100 magnification (b) ×200 magnification. Images are representative H&E-stained liver sections. No significant differences were found in liver histology between the different treatment groups.

4 Conclusion

In this study, we assessed the safety of T-Xyl in male and female ICR mice. Our results showed that the LD_{50} value of T-Xyl was >5,000 mg/kg BW and that an acute oral administration of T-Xyl was not toxic in male or female ICR mice. Therefore, oral administration of T-Xyl has the potential to be safe for clinical use, but further toxicity studies of repeated oral dosing are needed.
Acknowledgements  We thank Dong-A Pharmaceutical Co. for the donation of taurine.

References


