Toxicological Evaluation of *Thermoascus aurantiacus* SL16W in Albino Rats: Subchronic Toxicity Study

1Watee Kongbuntad, 2Kanokporn Saenphet, 1Parote Wongputtisin, 3Chatchai Khanongnuch and 3Saisamon Lumyong  
1Programe of Biotechnology, Faculty of Science, Maejo University, 50290 Chiang Mai, Thailand  
2Department of Biotechnology, Faculty of Agro-Industry  
3Department of Biology, Faculty of Science, Chiang Mai University, 50200 Chiang Mai, Thailand

**Abstract:** The purpose of this study was to evaluate the toxicology of crude xylanase enzymes prepared from *T. aurantiacus* SL16W in male Albino rats. Sixty rats aged 6 weeks old were randomly distributed into 4 groups of 15 rats. The 1st group was the untreated control while the 2nd (400 U), 3rd (2000 U) and 4th (4000 U) groups received the crude xylanase enzyme at dose levels of 400, 2000 and 4000 unit/kg body weight/day, respectively. After treatment body weight, organ weight, hematology and plasma chemistry were examined. The results indicated that oral force-feeding of the crude xylanase enzyme did not affect the body and organ weight of the rats. Hemoglobin values in the 2000 U group and hematocrit values in the 2000 U group were slightly decreased compared to the control group. The levels of Blood Urea Nitrogen (BUN), creatinine, aspartate Aminotransferase (AST) and alanine Aminotransferase (ALP) were not significantly different between the groups except that the ALT of the 200 U was significantly lower than the control group.

**Key words:** *Thermoascus aurantiacus*, xylanase, subchronic toxicity, toxicity test, enzyme, Thailand

**INTRODUCTION**

*Thermorascus aurantiacus* is a highly thermophilic fungus which produces enzymes such as xylanases and endoglucanases (Heldt-Hansen, 1997) cellulases and hemicellulases (Ryu and Mandels, 1980; Mandels, 1985; Beguin and Aubert, 1994; Bhat and Bhat, 1997) and endo-xylosanase (Bar and Lindley, 1994). These enzymes are commonly used in many industrial applications (Bhat, 2000). *T. aurantiacus* is also known to produce cellulolytic and xylanolytic enzymes (Kalogeris et al., 2003). Various agriculture substrates-byproducts have been used successfully in Solid State Culture (SSC) for cellulase production from this fungus (Kalogeris et al., 1998; Fujian et al., 2002). In this study, researchers used *T. aurantiacus* SL16W isolated from soil samples from the Chiang Mai province, Thailand by Assoc. Saisamon Lumyong, Department of Biology, Faculty of Science, Chiang Mai University, Thailand. Optimum activity was achieved at high temperatures (45-50°C) and low pH (pH5) in SSC. *T. aurantiacus* SL16W is also believed to be essential in improving the nutritive quality of animals feed. However, the toxicological effects in animals of crude enzyme prepared from *T. aurantiacus* SL16W has not been hitherto studied. Therefore, researchers studied the toxicological effects of the crude enzyme on the body weight, organ weight, hematology and blood chemistry of the albino rat. The objective of this study was to evaluate the toxicity of the crude enzyme which may be used to improve the nutrient efficiency in animals food in the future.

**MATERIALS AND METHODS**

**Animals, housing and diets:** Male Wistar strain rats, 6 weeks old and weighting 230±10 g were obtained from the breeding colony of the National Laboratory Animal Center. These rats were kept in a Striet Hygienic Conventional system. During the 1st week of the acclimation period, each rat was housed individually in a barrier room in stainless-steel wire-mesh cages 37 cm long, 23 cm wide and 21 cm high. Animals were maintained in standard conditions including adequate ventilation an ambient temperature at 25±2°C and a relative humidity of...

**Corresponding Author:** Watee Kongbuntad, Programe of Biotechnology, Faculty of Science, Maejo University, 50290 Chiang Mai, Thailand
60±15% with a 12 h light-dark cycle controlled via an
automatic timer. The animals received commercial
laboratory animal food pellets (Chalempokkapun Co., Ltd.,
No. 082) and acidified filtered water ad lib. The animals
used in this study were kept in accordance with all
applicable animal welfare regulations.

**Solid State Culture (SSC) and crude enzyme preparation:**
*T. aurantiacus* SL16W gram were kept on PDA at 4°C.
Dried corn cobs were prepared from corn cobs boiled for
30 min for three runs to obtain the sugar from a hot air oven
process at 80°C until a constant dry weight was achieved.
The corn was then milled in a hammer mill and passed
through a 0.7 mm mesh-sized sieve before being used as
a carbon source. The soybean meal was prepared with the
same methods as an organic nitrogen source. Ammonium
phosphate was used as the inorganic nitrogen source.
The food component concentrations were calculated to
provide 0.06 g Nitrogen per gram of corn cobs as was
previously reported by Kasin-Ubon. A SSC was carried
out using a 250 mL Erlenmeyer flask containing a carbon
source mixture of 1.7 g corn cobs and 1.3 g soybean meal.
Ammonium phosphate was dissolved with 5.5 mL of
distilled water which was then added to the flask and
mixed. The SSC was autoclaved for 40 min at 121°C and
cooled at room temperature. Each flask was inoculated with
three pieces (0.5 cm²) of 7 days old mycelia disk and
incubated at 50°C for 7 days. The crude enzyme was
extracted using 50 mL of cooled distilled water, mixed and
kept at a temperature of 4°C for 60 min. The solid materials
and fungal biomass were separated by filtration through a
cotton sheet and centrifuged at 1500 rpm for 20 min at
4°C. The supernatant was used as a crude enzyme
solution. This method was carried out according to the
Kasin-Ubon's procedure.

**Experimental design and procedures:** A Complete
Randomized Design (CRD) was used for the experimental
design. Animals were randomly distributed into 4 groups
of 15 rats each. Animals were caged individually in
stainless-steel wire-mesh cages. The 1st group was
untreated while the 2nd, 3rd and 4th groups received the
crude enzyme produced by *T. aurantiacus* SL16W at dose
levels of 400, 2000 and 4000 U/kg body weight/day,
respectively (using the activity of xylanase). The crude
enzyme samples were fed by oral force-feeding with a
feeding needle 3 times a day at 8.00, 12.00 and 16.00
for 60 days. Food pellets and water were given ad lib. The
animals were weighed and three rats/group were randomly
killed by cervical dislocation. An autopsy was done to
determine the toxicity effect at the beginning of the experiment (on day 0) and on day 15, 30, 45 and 60,
respectively. Blood samples were collected from a cardiac
puncture with EDTA-2K anticoagulant tubes for the
haematological examination and in the blood tubes for
plasma chemistry. Haematological parameters included
hemoglobin, haematocrit, white blood cell count and
differential leukocyte count of the animals were
determined by an Automatic Hematology Analyzer.
Plasma chemistry parameters included Blood Urea
Nitrogen (BUN), creatinine, aspartate Aminotransferase
(AST), alanine aminotransferase (ALT) and Alkaline
Phosphatase (ALP) were determined by automatic
analyzer. A necropsy was carried out after the collection
of blood samples. Dead animals were necropsied immediately after their discovery, all organs and tissues
were subjected to gross examination to determine the
toxicity effect of the crude enzyme.

The following organs, heart, liver, lung, spleen, kidneys and adrenal glands were rapidly removed and
weighted. The relative organ weights (g/% body weight)
were also calculated. Throughout the experiment, all
animals were measured daily for food consumption and
body weight.

**Statistical analysis:** The results were analyzed using an
ANOVA procedure of the Statistical Analysis System
Institute (SAS user's guide version 6.12, NC, USA). The
level of significance was taken as p<0.05.

**RESULTS AND DISCUSSION**

**Body weights:** Body weights were compared between the
control and treated groups. There were different levels of
crude enzyme (400, 2000 and 4000 unit/kg body
weight/day) throughout the study periods as shown in
Fig. 1. The body weights in all groups similarly increased
during the 5-45 days of the study periods with no

![Fig. 1: Body weight of rats receiving crude enzyme for 60 days](image-url)
significantly differences between the groups. However, the body weight in the treated groups was slightly greater than in the control group during the last periods, although the different was not found to be statistically significant. Furthermore, the feed intake and feed efficiency ratio were comparable between the control and treated groups which no significant differences. As the results demonstrate, there was no toxicity effect that changed the body weight.

**Organ weight:** After 60 days of oral administration, the organs were rapidly removed for a weight determination. The studied organs were the heart, liver, lung, spleen, kidneys and adrenal glands. The relative organ weights (g/ % body weight) were calculated and the results are shown in Table 1. The liver, heart, kidneys, spleen and kidney weights were not significantly different between treated and the control group. The lung weight of treated groups compared with the control group was found to be significantly greater in the 400 and 2000 U groups than in the control group. However as all of the other results demonstrate, there were no abnormal signs in the organs and their weight changes. These results suggest that the oral force-feeding of crude enzyme was not toxic to the body weights and organs weight of male albino rats.

**Haematological and plasma chemistry:** Blood samples of the rats were collected at 0, 15, 30, 45 and 60 days. Hematological parameters such as hemoglobin concentration, hematocrit, total and differential leucocytes count were examined for comparison between control and treated groups. No significant differences were found at 0, 15, 30 and 45 days. The hematological results at 60 days were significantly different as shown in Table 2. Hemoglobin values in the 4000 U group and hematocrit values in the 2000 U group were slightly decreased compared with the control group but it was not significantly different in 4000 U group while in the 2000 U group was significantly different. The results of total leucocytes values show a significantly increased in the treated groups compared with the control group. Furthermore, the leucocytes count such as eosinophil, neutrophil and basophil were not significantly different in all groups while the monocyte count in the 4000 U group was significantly different compared with the control group. However, lymphocytes were found in the 400 and 2000 U groups only. Plasma chemistry parameters such as BUN, creatinine, AST, ALT and ALP were examined and compared between control group and treated groups as shown in Table 3. The level of BUN, creatinine, AST and ALP were not significantly different while the level of ALT in the 400 U group showed a significant decrease when compared with the control group.

### Table 1: Organ weight of rats which received crude enzymes for 60 days

<table>
<thead>
<tr>
<th>Organ weight</th>
<th>Parameter of organ weights (g) mean±SD</th>
<th>Dose of crude enzyme (Unit/kg body weight/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>3.43±0.04</td>
<td>3.00±0.04</td>
</tr>
<tr>
<td>Heart</td>
<td>0.29±0.02</td>
<td>0.27±0.04</td>
</tr>
<tr>
<td>Lung</td>
<td>0.40±0.02</td>
<td>0.40±0.04</td>
</tr>
<tr>
<td>Kidneys</td>
<td>0.51±0.03</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.19±0.01</td>
<td>0.18±0.01</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>0.01±0.00</td>
<td>0.02±0.00</td>
</tr>
<tr>
<td>L.W.</td>
<td>0.02±0.00</td>
<td>0.02±0.00</td>
</tr>
</tbody>
</table>

### Table 2: Hematology of rats receiving crude enzymes for 60 days

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Parameters of hematology (mean±SD)</th>
<th>Dose of crude enzyme (Unit/kg body weight/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>15.4±0.62</td>
<td>18.3±0.68</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>46.3±1.56</td>
<td>46.3±2.30</td>
</tr>
<tr>
<td>Leucocytes</td>
<td>2.0±0.04</td>
<td>1.56±0.12</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>0.13±0.03</td>
<td>0.13±0.15</td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.66±0.57</td>
<td>0.66±0.57</td>
</tr>
<tr>
<td>Basophil</td>
<td>85.4±10.26</td>
<td>87.6±6.80</td>
</tr>
<tr>
<td>Monocyte</td>
<td>2.66±2.08</td>
<td>5.55±2.51</td>
</tr>
</tbody>
</table>

### Table 3: Plasma chemistry of rats receiving crude enzymes for 60 days

<table>
<thead>
<tr>
<th>Plasma chemistry</th>
<th>Parameters of plasma chemistry (mean±SD)</th>
<th>Dose of crude enzyme (Unit kg⁻¹ body weight day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN</td>
<td>31.0±4.58</td>
<td>27.3±3.78</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.63±0.01</td>
<td>0.76±0.05</td>
</tr>
<tr>
<td>AST</td>
<td>17.06±24.8</td>
<td>14.9±26.0</td>
</tr>
<tr>
<td>ALT</td>
<td>49.66±15.31</td>
<td>52.66±2.08</td>
</tr>
<tr>
<td>ALP</td>
<td>207.2±75.4</td>
<td>287.3±99.0</td>
</tr>
</tbody>
</table>

### CONCLUSION

The results demonstrate that the crude xylanase enzyme prepared from *T. aurantiacus* SL16W shows no toxicity on the body weight, organ weight, haematology and plasma chemistry in male albino rats when they are administered orally for 60 days.

### ACKNOWLEDGEMENTS

Funds for this study were provided by the Graduated School of Chiang Mai University, Chiang Mai, Thailand and Mahasarakham University, Mahasarakham, Thailand.

### REFERENCES