

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

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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-mannanase (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. Saisamorn 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 stain 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 Strict 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

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 corncobs were prepared from corncobs boiled for 30 min for three run 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 corncob 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 corncobs 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

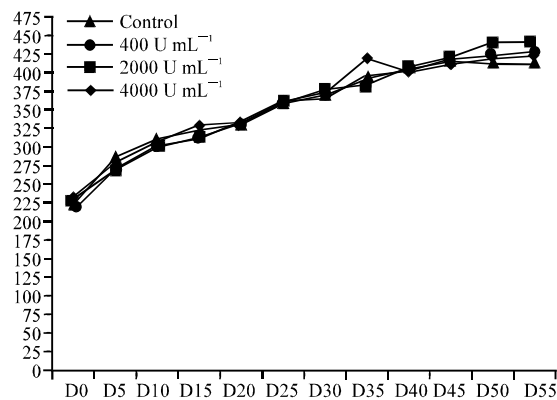


Fig. 1: Body weight of rats receiving crude enzyme for 60 days

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. Haematological parameters such as hemoglobin concentration, hemaotocrit, 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 haematological 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: Organs weight of rats which received crude enzymes for 60 days

Parameter of organ weights (g/% LW)	Organ weights (g) mean±SD			
	Dose of crude enzyme (Unit/kg body weight/day)			
	0	400	2000	4000
Liver	3.43±0.20	3.39±0.23	3.10±0.04	3.27±0.31
Heart	0.29±0.00	0.27±0.02	0.27±0.03	0.30±0.04
Lung	0.40±0.02 ^c	0.49±0.02 ^a	0.42±0.04 ^{ab}	0.46±0.01 ^{ab}
Kidneys	0.51±0.03	0.54±0.04	0.54±0.03	0.55±0.04
Spleen	0.19±0.01	0.19±0.02	0.18±0.01	0.22±0.04
Adrenal glands	0.01±0.00	0.02±0.00	0.02±0.00	0.02±0.00

LW: Life body weight. In each row, different letter differ significantly p<0.05

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

Parameters of hematology	Hematology (mean±SD)			
	Dose of crude enzyme (Unit/kg body weight/day)			
	0	400	2000	4000
Hemoglobin (mg dL ⁻¹)	15.50±0.62 ^{ab}	15.36±0.51 ^{ab}	16.10±0.40 ^a	14.83±0.68 ^b
Hematocrit (%)	49.33±0.57 ^a	47.33±1.15 ^{ab}	46.33±1.15 ^b	47.33±2.30 ^{ab}
Total leucocytes (cu/mm)×10 ²	3.53±1.61 ^a	3.00±1.56 ^a	1.96±1.33 ^{ab}	4.00±1.05 ^b
Eosinophil	11.33±0	6.33±2.30	5.33±4.16	14.33±1.15
Neutrophil	0.66±0.57	2.00±1.73	1.33±2.30	1.66±0.57
Basophil	85.30±10.26	89.00±1.73	87.60±6.80	83.00±1
Monocyte	2.66±2.08 ^{ab}	2.33±2.08 ^{ab}	5.55±2.51 ^a	1.00±0.1 ^b
Lymphocyte	0	0.33	0.33	0

WBC: White Blood Cell. In each row, different letters differ significantly p<0.05

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

Parameters	Plasma chemistry (mean±S.D)			
	Dose of crude enzyme (Unit kg ⁻¹ body weight day ⁻¹)			
	0	400	2000	4000
BUN	31.0±4.58	27.0±5.29	27.33±3.78	30.33±1.52
Creatinine	0.83±0.11	0.66±0.05	0.76±0.05	0.73±0.10
AST	176±46.48	114.66±23.45	149±26.05	132.33±40.01
ALT	49.66±13.61 ^a	34.0±1 ^b	52.66±2.08 ^a	44.33±4.72 ^{ab}
ALP	207±7.54	174.0±45.13	287.33±99.6	186.66±39.57

BUN: Blood Urea Nitrogen, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Pphosphatase. In each row different letter differ significantly p<0.05

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.

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