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Determination of Toxic Effects of Crude Xylanase Derived from *Thermoascus aurantiacus* SL16W by Hematology and Blood Biochemistry in Albino Rats

[1]Watee Kongbuntad, [2]Kanokporn Saenphet, [3]Chartchai Khanongnuch and [2]Saisamorn Lumyong
[1]Department of Biotechnology, Faculty of Graduate School,
[2]Department of Biology, Faculty of Science,
[3]Department of Biotechnology, Faculty of Agro-industry,
Chiang Mai University, Chiang Mai, Thailand

Abstract: Xylanase derived from *Thermoascus aurantiacus* SL16W has characteristics of high activity, heat tolerance and exhibits the potential development for industrial feed enzyme. The objective of this study was to determine the toxic effect of crude xylanase on hematological and blood biochemistry in albino rats. Crude xylanase at doses of 750, 1,500 and 4,000 U kg^{-1} body weight (bw) were orally administered to rats for 120 days. Hematological and blood biochemistry levels were investigated in two periods (exposure and recovery). It was found that crude xylanase had no effect on rat's body weight. SGOT levels of treated rats significantly decreased as compared to those of control group ($p \leq 0.05$), while SGPT levels were similar in all groups. BUN levels in treated groups were higher than those of controls in exposure period, but slightly lower in recovery period. However, Creatinine levels in treated groups were not different from control group. It was suggested from the results that crude xylanase is safe for using in animals feed industry.

Key words: Xylanase, *Thermoascus aurantiacus*, fungi, toxins, albino rats

INTRODUCTION

Xylanases have been employed in a variety of applications. Special attention has been given to their use in food industry in order to enhance the digestibility of animal feeding. Most of animal feed ingredients contain non-digested part (Hemicellulose) and some anti-nutritive factors which inhibit feed utilization and growth performance (Bhat, 2000). The main problem of indigestibility was found in monogastric animals, which lacked enzyme to hydrolyze the non-starch polysaccharides (NSPs) (Choct *et al*., 1995; Bedford, 2000). Although thermostable xylanases could be produced by many microbial strains, for instances, fungi, bacteria and actinomycetes, xylanases from thermophilic fungi have gained considerable interests due to their property of thermal stability to withstand against the high temperature of feed manufacturing process and remain active in the gastrointestinal tract of animal. *T. aurantiacus* SL16W, the thermophilic fungi isolated from soil samples in Chiang Mai province, Thailand has been reported to produce high quantity of thermostable xylanase when grown in solid state fermentation (Kasinubon, 2004). This enzyme has characteristics of heat tolerant and exhibited the potential development for industrial feed enzyme. However, the toxicological effects of crude enzyme in laboratory animals have not been studied.

In this report, the chronic toxicity of crude xylanase prepared from *T. aurantiacus* SL16W was determined in albino rats. The investigated parameters included the hematological changes, blood biochemistry and the change of body weight.

MATERIALS AND METHODS

Animal preparation: Wistar rats (*Rattus norvegicus*), approximately 4 weeks old with the average weight of 230±10 g, were obtained from breeding colony of the National Laboratory Animal Center, Salaya, Nokhorn Pathom, Thailand. Those were allowed to acclimatize in the animal facility at Biology Department for at least one week prior to the experiment with the excess feeding of water and standard diet (Chalernpokkapun Co., Ltd. No. 082), *ad libitum*. The experimental unit was maintained in standard condition including, adequate ventilation, ambient temperature at 25±2°C and a relative humidity of 60±15%, with 12 h light-dark cycle controlled via an automatic timer. Used animals in this study were in accordance with all applicable animal welfare regulations.

Corresponding Author: Watee Kongbuntad, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand Tel: 66-53-943346 Fax: 66-53-892259

Microbial strain: The fungal strain used in this study was *Thermoascus aurantiacus* SL16W, the thermophillic fungi isolated from soil sample in Chiang Mai province, Thailand. The fungus was grown on potato dextrose agar (PDA) at 45-50°C for 4 days and stocked at 4°C.

Solid State Fermentation (SSF): Dried corncob was used as carbon source while soybean meal and ammonium phosphate were used as organic and inorganic nitrogen sources, respectively. Dried corn cob was milled in a Hammer mill and passed through a 0.7 mm mesh-sized sieve before use. The final concentrations of nitrogen was 0.06 g g^{-1} of corncob as described by Kasinubon (2004). SSF was carried out using a 250 mL Erlenmeyer flask containing 1.7 g corncobs and 1.3 g soybean meal. Ammonium phosphate dissolved with 5.5 mL distilled water was mixed in the flask and autoclaved for 20 minutes at 121°C. Three pieces (0.5 cm^2) of 7 days old mycelia disk was incubated in prepared solid state medium at 50°C for 7 days.

Crude xylanase extraction: *T. aurantiacus* mycelia obtained from SSF was extracted by 50 mL of cooled 20 mM phosphate buffer for 60 min. The solid materials and fungal biomass were separated by filtration through a cotton sheet. The filtrate of fungal biomass was centrifuged at 1,500 rpm for 20 min. All described procedures were done at 4°C and crude enzyme (CX) was also kept at the same temperature.

Contamination of aflatoxin and heavy metals was checked before use. Aflatoxin was measured by Immunoaffnity column and HPLC method (Sharma and Marquez, 2001) with the cooperation of Laboratory Center for Food and Agricultural Products Co., Ltd.: LCFA, Laboratory Service Department Chiang Mai office, Thailand. The analyses showed no aflatoxin contamination (Table 1). The determination for heavy metals such as lead (Pb), arsenic (As), cadmium (Cd), copper (Cu) and ferrum (Fe) was conducted by Atomic absorption assay.

Table 1: Chemical composition analysis of extracted CX an inoculated with *T. aurantiacus* SL16W

	Extracted CX from SSF	
Parameters	Non-inoculated	Inoculated
Xylanase activity (U mL^{-1})	NT	179-190
Heavy metals (mg kg^{-1})		
Pb	NT	<0.03
Cu	NT	0.53
Cd	NT	0.02
Fe	NT	0.84
As	NT	0.01
Mycotoxin (as aflatoxin)		
Aflatoxin type B1	ND	ND
Aflatoxin type B1	ND	ND
Aflatoxin type G2	ND	ND
Aflatoxin type G1	ND	ND

LOD (Limit of Detection) for aflatoxin type B1 = 0.05 μg kg^{-1}, B2 = 0.09 μg kg^{-1}, G1 = 0.24 μg kg^{-1} and G2 = 0.07 μg kg^{-1}, ND = Not Detected, NT = Not Test, Lead (Pb), Arsenic (As), Cadmium (Cd), copper (Cu) and Ferrum (Fe)

Experimental design and procedures: Male and female rats were caged individually in stainless-steel wire-mesh cages and randomly distributed into 4 experimental groups (18 each with equal number of male and female). Three groups of rats were treated orally with 750 (CX 750 U), 1, 500 (CX 1, 500 U) and 4, 000 (CX 4,000 U) U mL^{-1} kg^{-1} bw of CX for 120 days. Additional control group received only Distilled Water (DW). Behaviors of the rats were observed and recorded daily. Body weights were measured every 14 days throughout the experimental period. The experiment was conducted in two periods, 120 days of exposure period and 45 days recovery period. At the end of exposure period, 12 rats of each group (6 males, 6 females) were sacrificed for hematological values and blood biochemistry, the rest were kept without administration of CX for 45 days and sacrificed for the same propose.

Assessment of hematology and blood biochemistry: At the end of treatment period, blood samples were collected by cardiac puncture technique and mixed well with anticoagulant (EDTA). The following analyzed profiles were then evaluated:

Hematological test; hemoglobin, hematocrit, total white blood cell count and differential cell count were detected by a routine complete blood cell count method. Blood chemistry; Serum Glutamic Pyruvic Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), alkaline phosphatase (ALP), Blood Urea Nitrogen (BUN) and creatinine (Crea) were measured by automate (Synchron C5X, Beckman) with the cooperation of Clinical Chemistry Department, Faculty of Associated Medical Science, Chiang Mai University.

Statistical analysis: Statistical tests were performed with SPSS software version 11.5. The data were analyzed by analysis of variance (ANOVA) to test the effect of the factor or treatments. Duncan's Multiple Rang Test was used to compare means. The level of significance was taken as $p \leq 0.05$.

RESULTS AND DISCUSSION

General behavior: No mortality was observed. During the 165 days of experiment period, rats in each group did not show abnormal signs of behavior in all treatments. The

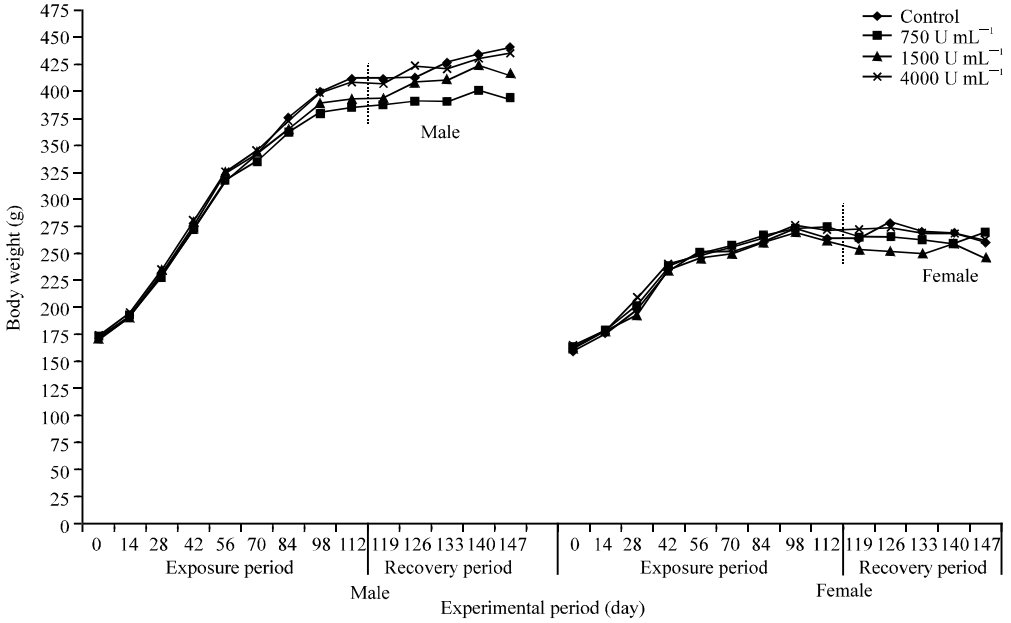

Fig. 1: Body weights of albino rats treated with CX in two experimental periods. Means are given

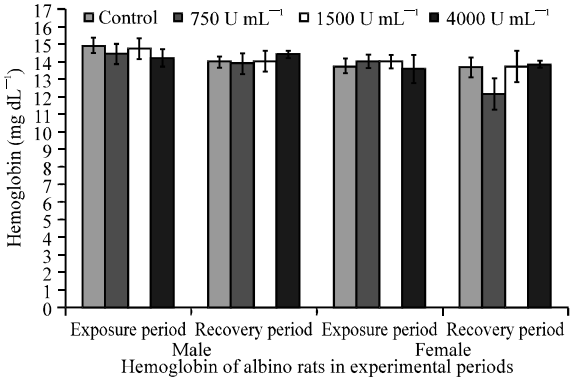

Fig. 2: Hemoglobin of albino rats treated with CX in two experimental periods. Means and Standard Deviations (SD) are given. a-b = significantly difference at p≤0.05

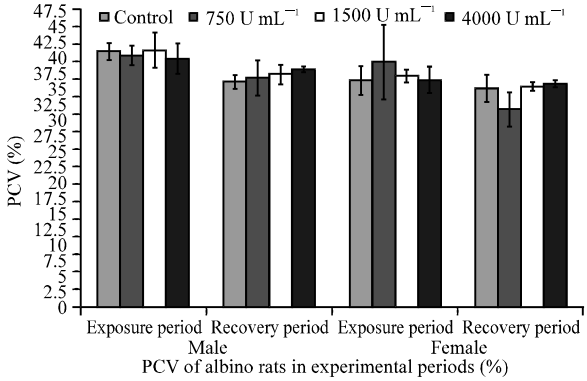

Fig. 3: Packed red cell percent (% hematocrit or PCV) of rats treated with CX in chronic. Means and Standard Deviations (SD) are given. a-b = significantly difference at p≤0.05

animals appeared healthy and did not exhibit any weight loss, as body weights of the treated group were not significantly different from the untreated as control group (Fig. 1).

Hematology: Hb and %PCV of rats in both sexes received all doses of crude xylanase for 120 days were not significantly different from those of control group (Fig. 2 and 3) and those detected levels were in the standard range of rat's hematology (Sharp and La Regina, 1998). These results suggested that crude xylanase produced from *T. aurantiacus* SL16W had no effect on erythropoiesis in albino rats. The non-toxic effect of crude xylanase from *T. aurantiacus* SL16W on Hb and %PCV was coincide with the reports of acute and subchronic toxicity test in male albino rats (Kongbuntad *et al.*, 2004a, b).

Differential white blood cell count of male rats treated with crude xylanase were not different from those of controls in both exposure and recovery periods (Table 2). In female rats, neutrophils in CX1, 500 U group was significantly higher than that of controls while basophils in CX750 U group and lymphocyte in CX1, 500 U group were significantly lower than those of controls at p≤0.05

Table 2: Differential white blood cell counts of male albino rats treated with CX in two experimental periods. Means and Standard Deviations (SD) are given

	Hematological values of male albino rat (mean of % LW±SD), two periods							
	Exposure period (U mL^{-1} kg^{-1} bw)				Recovery period (U mL^{-1} kg^{-1} bw)			
Parameters	DW	CX 750	CX 1,500	CX 4,000	DW	CX 750	CX 1,500	CX 4,000
Total WBC (cu mm^{-1})×10^3	2.63±1.20	2.60±0.91	2.21±0.58	2.46±0.53	1.93±1.10	2.23±0.56	2.33±0.37	1.96±0.68
Eosinophils	5.03±2.49	7.53±3.30	7.12±5.57	6.86±1.45	5.50±0.52	6.73±2.35	5.13±1.24	3.80±0.79
Neutrophils	49.8±12.60	56.13±6.78	59.75±5.68	56.25±4.57	54.56±4.48	59.70±4.53	58.56±5.92	62.83±4.95
Basophils	0.50±0.1	0.52±0.08	0.31±0.09	0.73±0.08	0.20±0.00	0	0	0.26±0.00
Monocyte	1.12±0.85	1.08±0.64	0.78±0.09	0.72±0.08	1.80±0.70	1.10±0.05	0.66±0.28	1.30±0.30
Lymphocyte	43.61±12.64	34.63±4.44	32.03±5.62	35.43±4.87	37.93±4.05	32.50±5.47	35.26±5.08	31.80±5.72

LW = Live Weight, WBC = White Blood Cell, CX = Crude Xylanase, DW = Distilled Water. In each row, a-c = Significantly difference at p≤0.05

Table 3: Differential white blood cell count of female albino rats treated with CX in two experimental periods. Means and Standard Deviations (SD) are given

	Hematological values of female albino rat (mean of % LW±SD), two periods							
	Exposure period (U mL^{-1})				Recovery period (U mL^{-1})			
Parameters	DW	CX 750	CX 1,500	CX 4,000	DW	CX 750	CX 1,500	CX 4,000
Total WBC (cu mm^{-1})×10^3	1.71±0.46	1.90±0.92	2.10±0.58	1.42±0.31	0.93±0.20	1.06±0.51	1.17±0.61	1.33±0.64
Eosinophils	10.11±3.96	8.93±6.22	9.26±3.76	8.91±3.86	10.23±4.42	10.63±6.27	14.63±2.40	7.66±4.29
Neutrophils	47.18±4.61^a	54.45±12.03^{ab}	57.56±3.22^b	49.96±8.39^{ab}	53.43±6.83	48.80±2.53	46.96±5.09	40.16±7.51
Basophils	0.33±0.02^a	0.30±0.01^b	0.36±0.01^a	1.03±0.05^{ab}	0	0	1.00±0.08	0.56±0.01
Monocyte	0.66±0.05	1.33±0.82	0.92±0.03	0.52±0.07	1.00±0.03	0	0	1.10±0.08
Lymphocyte	41.70±5.64^a	33.58±6.39^{ab}	31.88±4.16^b	39.56±6.36^{ac}	35.33±8.95	37.56±2.33	37.40±8.18	49.83±2.92

LW = Live Weight, WBC = White Blood Cell, CX = Crude Xylanase, DW = Distilled Water. In each row, a-c = Significantly difference at p≤0.05

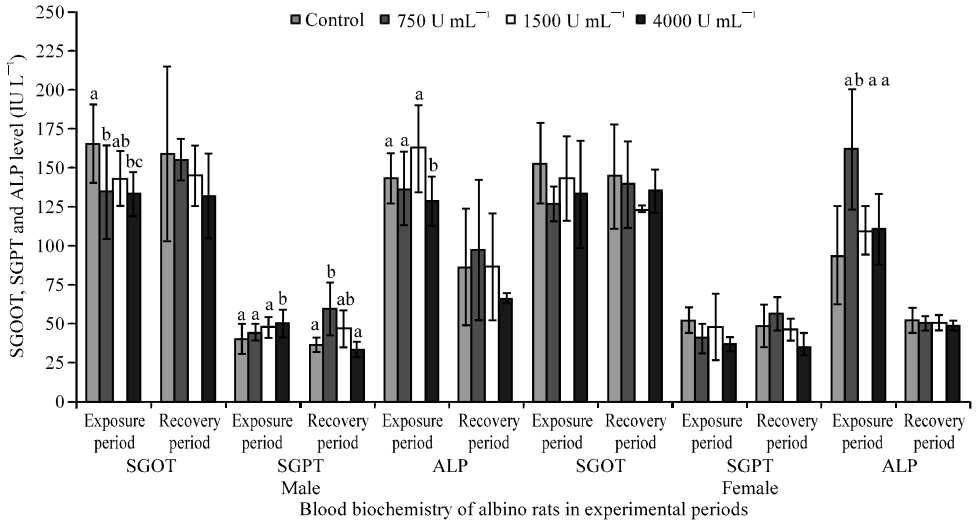

Fig. 4: Serum glutamic oxaloacetic transaminase (SGOT; AST), serum glutamic pyruvic transaminase (SGPT; ALT) and alkaline phosphatase (ALP) of albino rats treated with CX in two experimental periods. Means and Standard Deviations (SD) are given. a-c = significantly difference at p≤0.05

(Table 3) in exposure period. Nevertheless, those white blood cell values were restored in recovery period. Eosinophils and monocytes, however, were similar to those of controls in both periods.

Janaki and Sashidhar (2000) demonstrated that changing of differential white blood cell parameters could be used as an indicator for the toxicity of tested substance on circulating system, hematopoiesis and leucopoiesis while changes in PCV and eosinophils could be a sign of anemia. Brown (1980) suggested that the changes in number of some kinds of white blood cells might be caused by the infection of bacteria at the mean time.

The normal levels of Hb, %PCV and differential white blood cell count obtain from our results indicated that orally administration of the crude xylanase had no toxic effect on hematological system of rats in both sexes.

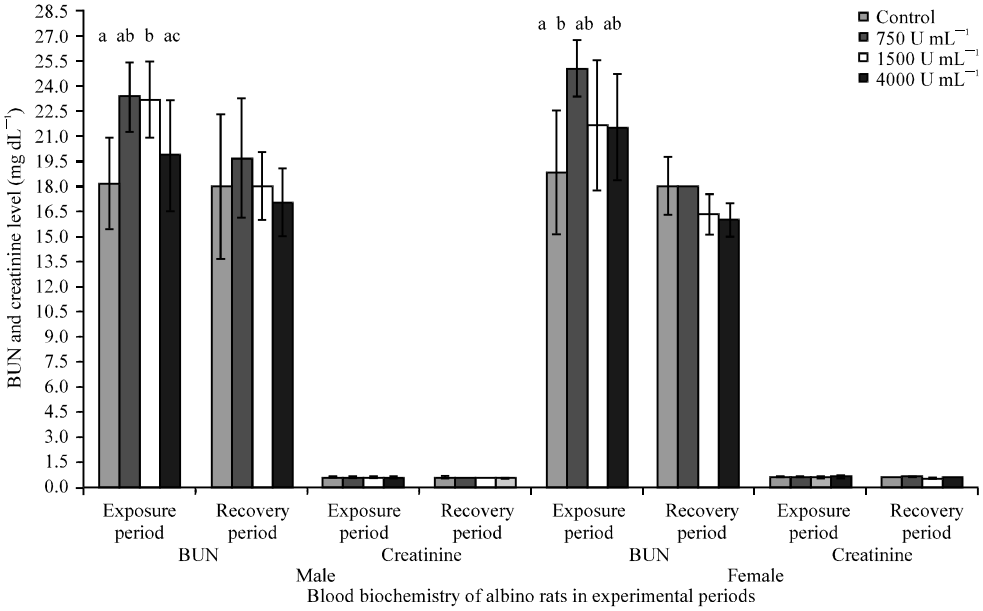

Fig. 5: Blood Urea Nitrogen (BUN) and creatinine of albino rats treated with CX in two experimental periods. Means and Standard Deviations (SD) are given. a-c = significantly difference at $p \leq 0.05$

Blood biochemistry: The SGOT, SGPT and ALP levels were determined (Fig. 4). SGOT level of male rats in CX750 U and CX4,000 U groups in exposure period significantly decreased as compared to control group ($p \leq 0.05$). In CX1, 500 U group the value was lower than that of control group, but no statistical difference was presented. In recovery period, SGOT level of all treated group was similar to that of control group. SGOT level in female rats, was not different from that of controls and remained constant in both periods.

In exposure period, SGPT level of male rats in CX750 U and CX1, 500 U groups slightly higher than that of control, but no statistical differences were presented. In CX4, 000U group, however, the value was significantly higher than that of other groups ($p \leq 0.05$). In recovery period, SGPT level of male rats in CX750U group was significantly higher than that of control at ($p \leq 0.05$), while CX1, 500 U and CX4,000 U groups were not different from control group. SGPT level of female rats of all groups was similar in both periods.

ALP level of male rats in exposure period revealed that only the CX4, 000U group was significantly lower than the control group ($p \leq 0.05$). Although ATP level was lower in CX750 U group and higher in CX1, 500 U group, the statistical difference from control group was not presented. Also, ALP level in all treatment groups was restored in recovery period. In female rats, only CX750 U group had significantly higher ALP level as compared to controls in exposure period ($p \leq 0.05$) and it was restored in recovery as well.

The assessment of pathological changes and toxic effects are also characterized by an increase in liver and kidney weight, high levels of biochemical substances as well as some specific enzymes in serum, such as ALP, SGOT and SGPT (Fauci *et al.*, 1998; Wildmann, 1984). The tendency of lower ALP level in treated rats as compared to controls suggests that pathological change of liver function had not occurred.

BUN levels of male rats in CX750 U and CX1, 500 U groups in exposure period showed the significantly higher value as compared to control group at $p \leq 0.05$ (Fig. 5), while the level of CX4,000 U group slightly increased but not significantly different from that of control group. In recovery period, BUN level in CX750 U group also increased with no statistical difference as compared to control group. The value was similar to controls in 1,500 U group, but in CX4,000 U group it was lower than that of controls with no statistical difference. Creatinine level of male rats in all groups was not significantly different from that of controls in both periods.

BUN level of female rats in all treated group was higher than that of controls in exposure period, but only CX750 U group showed the significant difference ($p \leq 0.05$). The value of BUN in all groups was lower

than that of control in recovery period, but no statistical difference were presented. Creatinine level, however, was similar in all groups. Although the higher BUN levels of treated rats in exposure period may indicate the malfunction of kidney, the lower level of this parameter in recovery period revealed no detrimental effect of crude xylanase on kidney's cells. Moreover, the similar values of Creatinine level in treated and control groups clearly indicated that the crude xylanase did not cause severe damage on kidney of albino rats. Janaki and Sashidhar (2000) suggested an emphasis on liver and kidney that the liver plays an important role in xenobiotic function, while kidneys are sites for reabsorption.

The results of this study indicated that administration of crude xylanase derived from *T. aurantiacus* SL16W which grown in solid state fermentation to albino rats for 120 days gives no toxic effect on hematological values, as well as on liver's and kidney's functions. Such crude xylanase, therefore, should be used safely in animal feed industry. The extensive research and development of this non-harmful xylanase as a new animal feed enzyme in industrial level are needed.

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