

THE TOXICITY OF A CRUDE ENZYME EXTRACT FROM *GLIOMASTIX MURORUM* IN SWISS ALBINO MICE

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Abstract. A crude enzyme extract from a fungus, *Gliomastix murorum*, could be used in the synthesis of oligosaccharides that are essential to the food and drug industries. This extract may contaminate such products and lead to serious health problems. An investigation on the possible toxicity and mutagenic effect of the extract from this fungal isolate was carried out in Swiss Albino mice. One hundred and 50% of the crude enzyme extract were injected intraperitoneally into the mice every 2 days for 30 days. Normal saline (0.9%), cultivation medium, and cyclophosphamide (80 mg/kg body weight) were given to the control groups. The results indicated that the white blood cell count, serum creatinine, and uric acid of the treated mice were significantly higher than those of the controls ($p < 0.05$), whereas the serum urea-N was lower. For the micronucleus test, mice treated with the extract, especially the group received 100% crude enzyme extract, showed a higher number of micronuclei in polychromatic erythrocytes, as compared to controls. Nevertheless, the micronucleus values were not as high as those found in mice treated with cyclophosphamide, the mutagenic agent. It can be concluded from the results that crude enzyme extract had minor toxic effects on various organ systems tested; more extensive investigation on the safe use of this extract is therefore necessary.

INTRODUCTION

Glycosidase is a group of enzymes produced by some species of plants, animals, and microorganisms. These enzymes are used for synthesizing oligosaccharides. Due to the low cost of processing, the synthesis of oligosaccharides by glycosidase is widely used in many sectors, from pharmaceutical to food industries.

Alpha mannosidase, isolated from a soil fungus (*Gliomastix murorum*), is one of the enzymes in the glycosidase group. Anuchapreeda (1992) reported that the crude enzyme extracted from this fungus showed high enzyme activity (1.464 MU/ml) and specific activity (7.611 MU/mg protein). Moreover, the cost of synthesizing oligosaccharides by using alpha mannosidase was found to be the lowest as compared to other methods. Despite the fact that alpha mannosidase is capable of contaminating products, there are no reports of its toxicological effects. Because of its widespread use in the food and drug industries, research on various aspects related to the toxicity of alpha mannosidase should be considered. In view of this, the present study aimed to test the effect of *G. murorum* crude enzyme extract on Swiss albino mice by means of body weight, white blood cell count, serum urea N, creatinine and uric acid concentration (Watcharasit,

1990). Mutagenic testing by the micronucleus method was also conducted.

MATERIAL AND METHODS

Crude enzyme extraction

The method of extraction was based on Anuchapreeda (1992). *G. murorum* was cultured in potato dextrose agar at 45°C for 5 days. The separated colonies were then incubated in Conyagumannan agar for 4 days and cultured in a cultivation medium in an incubator shaker (200 rpm) at 45°C for 4 days. After shaking, the enzyme was extracted by centrifugation at 12,000 rpm, at 5°C, for 15 minutes.

Animals

Male Swiss albino mice (7-8 week-old) weighing 35-40 g were purchased from the National Laboratory Animal Center, Salaya, Nakhon Pathom and used in the present investigation. They were allowed to acclimatize in the departmental animal laboratory for at least one week prior to the day of experiment. The animal house was maintained at approximate 25±2°C, with 12 hours of illumination.

Experimental design

The animals were divided into 5 experimental groups (10 each) and injected intraperitoneally with crude enzyme extracts every two days, according to the following regimes: 1) normal saline solution at 0.5 ml/day (control), 2) cultivation medium at 0.5 ml/day, 3) 50% crude enzyme extract in cultivation medium at 0.5 ml/day, 4) 100% crude enzyme extract at 0.5

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ml/day, and 5) cyclophosphamide at 80 mg/kg body weight (positive control) for the micronucleus test. The last group was injected 30 hours before being sacrificed. At the end of 30 days, the body weight was measured and the animals were sacrificed and bled by the cardiac puncture technique. The tibia bone was removed for the micronucleus test.

Hematology and serum biochemistry

The total white blood cell count was conducted according to the method of Watcharasi (1990). The serum analysis followed Temcharoen (1989) and Imchai *et al* (1998). The parameters evaluated in the serum were urea N, creatinine, and uric acid. The preparation of tibia bone marrow smears for micronucleus testing was performed according to Adler (1984), Blakey *et al* (1988), Harper and Legator (1988), and Schmid (1975).

Statistical analysis

Analysis of variance ($p < 0.05$) was applied to all the data, and differences between the means were determined by Student's *t*-test and the F-test for between-treatment comparison of means.

RESULTS

Mice in all groups presented a normal appearance throughout the experimental period. However, the results of mean body weight recorded every two days revealed that mice that received 50% of the crude enzyme extract from *G. murorum* had significantly higher ($p < 0.05$) body weight as compared to other groups (Fig 1).

Hematology and serum biochemistry

The total white blood cell count of mice treated with the cultivation medium and controls were similar. Differences were observed in both groups treated with the crude enzyme extract, which were found to be significantly higher ($p < 0.05$) than controls and cultivation media treated mice (Table 1).

The blood biochemical data are presented in Table 1 and Fig 2. The activity of serum creatinine and uric acid in all treated groups had raised levels as compared to the control groups. The highest value of uric acid was recorded for the mice treated with the higher concentration of crude enzyme extract (9.82 mg/dl). The levels of serum urea N of all treated groups,

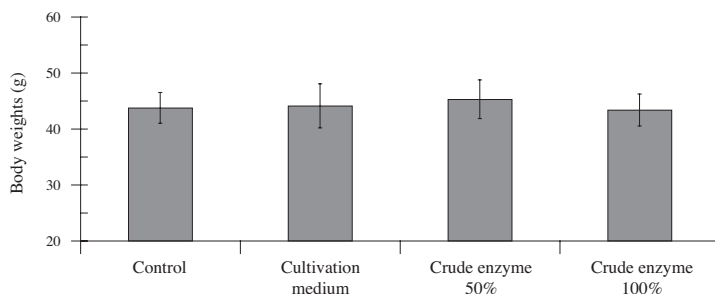


Fig 1- Body weights of mice treated with crude enzyme extract from *Gliomastix murorum* as compared to controls (mean \pm SD). * $p < 0.05$.

Table 1

Serum biochemistry and total white blood cell counts of mice treated with crude enzyme extract from *Gliomastix murorum* as compared to controls (mean \pm SD).

Group	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Total white blood cell (cell/ml)
Control	24.41 \pm 1.37 ^a	3.54 \pm 1.10 ^b	4.33 \pm 0.29 ^c	1225.00 \pm 451.83 ^b
Cultivation medium	19.23 \pm 3.65 ^c	6.45 \pm 2.54 ^a	7.09 \pm 2.32 ^b	1457.5 \pm 596.44 ^b
Crude enzyme 50%	23.93 \pm 6.67 ^b	7.27 \pm 2.01 ^a	7.43 \pm 2.12 ^b	3831.00 \pm 523.93 ^a
Crude enzyme 100%	22.52 \pm 1.37 ^c	6.75 \pm 2.45 ^a	9.82 \pm 2.63 ^a	4300.00 \pm 524.4 ^a

a, b, c = statistical differences at $p < 0.05$

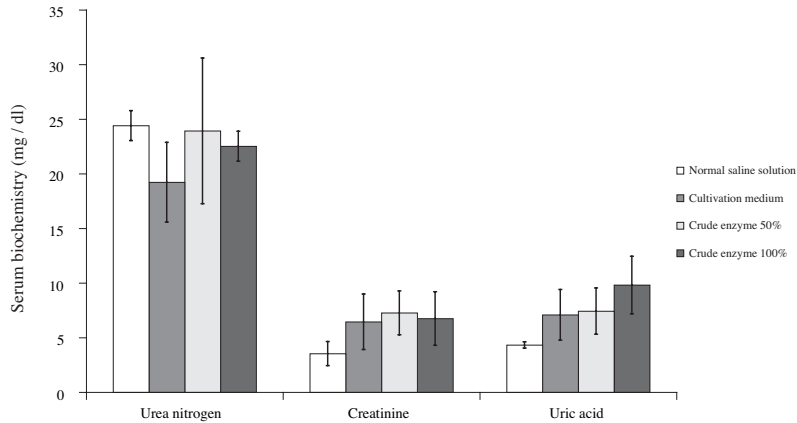


Fig 2- Serum biochemistry of mice treated with crude enzyme extract from *Gliomastix murorum* as compared to controls (mean ± SD).

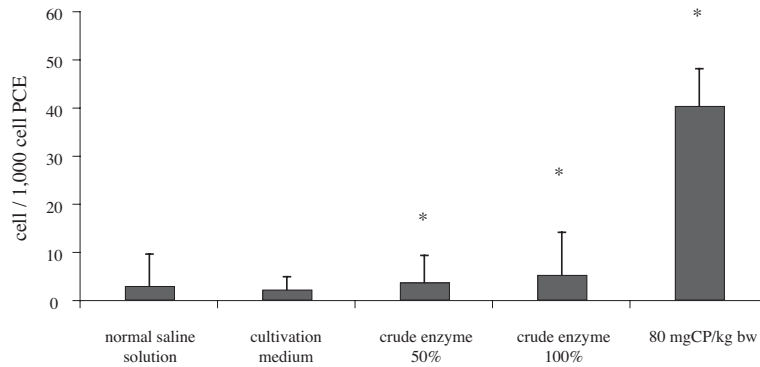


Fig 3- Frequencies of polychromatic erythrocytes (PCEs) with micronucleus (MPCEs) detected in bone marrow of mice treated with crude enzyme extract from *Gliomastix murorum* as compared to controls (mean ± SD). $p < 0.05$

however, were significantly lower than those of controls (Fig 2).

Micronucleus test

According to the micronucleus test, the polychromatic erythrocytes (PCEs) from bone marrow were examined for micronuclei. The micronucleated polychromatic erythrocytes per 1,000 cells were counted. The results shown in Fig 3 demonstrated that the mice that received crude enzyme extract in both concentrations had significantly higher ($p < 0.05$) numbers of micronucleated PCE (MPCEs) compared to the controls, and the increase of MPCEs was in a concentration-dependent manner. Nevertheless, the number of MPCEs in all crude enzyme treated groups was significantly lower than those of the positive controls, the cyclophosphamide treated group (Fig 3).

DISCUSSION

The data of mice body weight indicated that intraperitoneal injection of crude enzyme extract from *G. murorum* did not alter feeding behavior or the nutrient metabolism of the mice. Moreover, the 50% crude enzyme extract may have activated the food consumption of mice, as seen in the highest body weight as compared to the other groups (Fig 1).

A markedly high number of white blood cells were observed in mice treated with the crude enzyme that reflects the impurity of the extract. Some substances in the extract may have acted as foreign bodies and activated the immune response of the mice by increasing the number of white blood cells.

We found that there was a decreased level of serum

urea N in all treated groups as compared to controls. This result revealed that the crude enzyme extract may lead to the dysfunction of hepatocytes in transforming ammonia to urea, as suggested by Lohleka (1990) concerning acute liver destruction caused by some toxic substances. This evidence should be clarified by histological investigation that will be conducted in our further research. The levels of creatinine in all treated groups, even in the mice that received the cultivation medium, were higher than those of controls. However, levels of creatinine in those treated mice were similar. Such an increase could be caused by the cultivation media or crude enzyme. Elevation of creatinine levels in treated mice has been associated with the high levels of uric acid. These data indicated impairment of kidney function in excreting creatinine and uric acid that could be attributed to cell necrosis, changes in cell membrane permeability, or urinary obstruction (Hepler, 1988).

The numbers of micronuclei found in both groups of mice that received crude enzyme extract (0.36% and 0.51%, respectively) were relatively high compared to the normal frequency of micronuclei that spontaneously occurred in laboratory mice (0.12-0.41%) as reported by Wild (1988). The frequency of micronucleus induced by cyclophosphamide, a premutagen that we used as a positive control, was markedly high (4.02%). The low ability of the crude enzyme to induce micronucleus formation, as compared to cyclophosphamide, may confirm that the crude enzyme is not the mutagenic agent. It could be concluded from our results that crude enzyme extract from *G. murorum* had minor toxic effects on various organ systems tested. More intensive work on its toxicity should be further investigated.

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