TOXICOLOGICAL EVALUATION OF *CLADOPHORA GLOMERATA* KÜTZING AND *MICROSPORA FLOCCOSA* THURET IN ALBINO RATS

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Abstract. This study aimed to evaluate the toxicity of *Cladophora glomerata* and *Microspora floccosa* ethanolic extracts in rats. Acute toxicity was tested with a single oral administration of the extract at a dose of 25 g/kg bd wt. Mortality, behavior, amount of food intake, body weight, and any abnormalities of the visceral organs, were observed. The results showed that the extract caused neither mortality, nor abnormalities. Subchronic toxicity was tested by administering the extract at doses of 0.5 g/kg and 1.0 g/kg for 60 days. Differences in body weight, hematology and blood biochemistry (alanine aminotransferase, ALT; aspartate aminotransferase, AST; blood urea nitrogen, BUN and creatinine, Cre) were not detected among the control and treatment groups. Although the packed cell volume of the male rats treated with 1.0 g/kg extract was significantly lower than the controls (p 0.05), the level was in the standard range for rat hematocrit.

INTRODUCTION

Cladophora glomerata and *Microspora floccosa* are freshwater macroalgae belonging to the Division Chlorophyta. These two species are naturally grown together in the Nan and Kong rivers. They are edible macroalgae so called "Gai" by local people in Nan Province, Thailand. Both of these algae have been used as a food source for centuries in local cultures, but only in the last few years they have become increasingly popular as food products marketed to tourists. *C. glomerata* and *M. floccosa* contain abundant proteins and fibers, and can be valuable sources of vitamins (Yavichai, 2003). Besides being one of the most popular food sources, these two species of algae are believed to offer many important health benefits; for instance, rejuvenating, promoting appetite, and remedying many common maladies.

A small number of algae are highly toxic when consumed (Vasconcelos, 1999; Ouellette and Wilhelm, 2003; Oberholster *et al*, 2004). Although *C. glomerata* and *M. floccosa* have long been eaten and are highly nutritious, an evaluation of the safety of these algae, based on scientific studies, has not been published. The following experiments were designed to evaluate safety through acute toxicity testing by single oral administration of the extract at a dose of 25.0 g/kg bd wt. Subchronic toxicity was tested by administering the extract at doses of 0.5 and 1.0 g/kg for 60 days. The parameters evaluated included toxicological signs, body weight, autopsy, hematology, and blood biochemistry.

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MATERIALS AND METHODS

Extraction of *C. glomerata* and *M. floccosa*

C. glomerata and *M. floccosa* were collected from Nan Province, Thailand. The algae were exposed to sunlight until completely dry, then extracted in 95% ethyl alcohol at room temperature for 1 week. The ethanolic filtrate was then evaporated by rotary evaporation and dehydrated by maltodextrin, the non-toxic dehydrating agent widely used for pharmaceutical purposes (Freers, 2002). The crude extract was kept in a dry place and used after preparation at the required dose in distilled water.

Animals

Male and female Wistar rats (*Rattus norvegicus*), approximately 6 weeks of age and weighing between 140-240g were used. The animals were purchased from the National Laboratory Animal Center, Thailand. They were allowed to acclimatize in the departmental animal facility for 1 week before the start of the experiment. They were fed with water and a standard diet (C.P. 082). The study room was maintained at approximately 25 ± 2°C in a 12-hours light/dark cycle.

Acute toxicity test

Rats were divided into 2 groups (10 each, 5 males and 5 females). They were administered orally a single dose of 25 g/kg. Controls were treated with distilled water. Signs of toxicity and deaths in rats were observed for 24 hours post-treatment. All animals were sacrificed at day7 post-administration. The appearance of the organs was observed and the indexes of each organ was calculated. The organs included the liver, kidney, heart, spleen, ovary, and testes. The behavior, diet and feces of the rats were observed and recorded. The body weights of the rats were measured every 2 days.

Subchronic toxicity test

Rats were randomized into 3 groups (9 each, 4 males and 5 females) and treated orally for 60 days with agal extract at doses of 0.5 and 1.0 g/kg, respectively. Controls were treated with distilled water. At the end of the treatment period, blood samples were collected by cardiac puncture technique. The following analyzed profiles were then evaluated:

(1) Blood chemistry; aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN) and creatinine were measured by automated system (Synchron C5X, Beckman) with the cooperation of the Clinical Chemistry Department, Faculty of Associated Medical Sciences, Chiang Mai University.

(2) Hematological test; hematocrit (PVC), total white blood cell count, and differential cell count were conducted by routine complete blood cell count method (Dacie and Lewis, 1984).

Statistical analysis

Data were expressed as mean ± standard deviation. Student's *t*-test and Kruskal-Wallis one-way analysis of variance test were employed for statistical comparison at p=0.05.

RESULTS

Acute toxicity test

Neither abnormal signs nor deaths among the rats were observed during the week-long experiment period. The oral LD_{50} values of the extracts of *C. glomerata* and *M. floccosa* were > 25.0 g/kg. Body weight, food weight, and autopsy of organs were normal (Fig 1, Table 1).

Subchronic toxicity test

During the experiment, the rats in each group showed no abnormal signs in appearance, behavior, or diet. Although the packed cell volume of male rats treated with 1.0 g/kg extract was significantly lower than the controls (p 0.05), the level was in the standard range for rat hematocrit (Sharp and La Regina, 1998). The other results, including hematological test and blood biochemical assays were normal (Fig 2, Tables 2 and 3).

DISCUSSION

Generally, 20-30 g of *C. glomerata* or *M. floccosa* are consumed by local people as a daily meal. Hence, the human dose for these algae should be 0.5 g/kg, if calculated from a person with bodyweight 60 kg. The

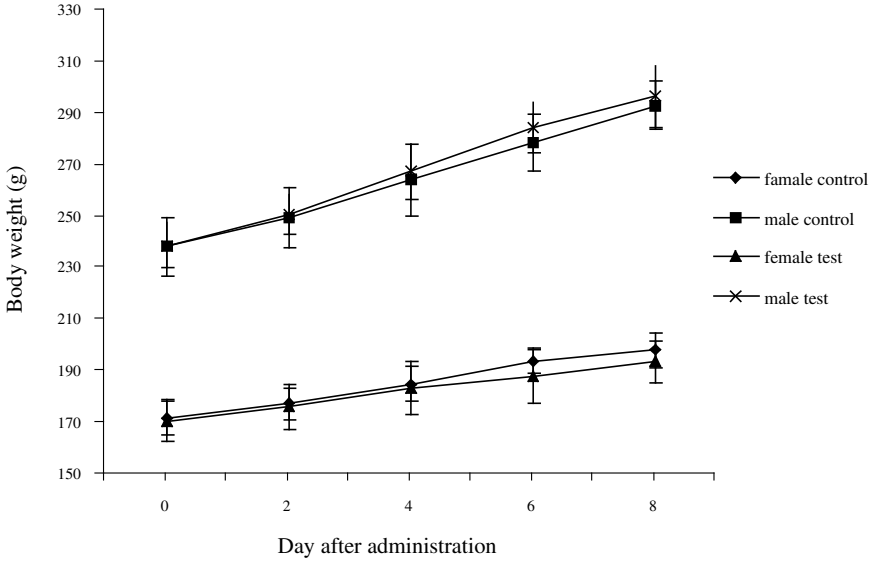

Fig 1- Body weight (g) of female and male rats treated with ethanolic extract of *C. glomerata* and *M. floccosa* at a dose of 25 g/kg and controls.

Table 1
Organ weight (g%) of female and male rats treated with ethanolic extract of *C. glomerata* and *M. floccosa* at a dose of 25 g/kg, and controls.

Sex	Organ	Weight (g%)	
		Control	Test
Female	Liver	4.11 ± 0.477	4.45 ± 0.491[ns]
	Kidney	0.75 ± 0.262	0.78 ± 0.080[ns]
	Heart	0.42 ± 0.060	0.43 ± 0.045[ns]
	Spleen	0.28 ± 0.024	0.26 ± 0.025[ns]
	Ovary	0.07 ± 0.013	0.07 ± 0.008[ns]
Male	Liver	4.37 ± 0.435	4.42 ± 0.231[ns]
	Kidney	0.69 ± 0.061	0.71 ± 0.037[ns]
	Heart	0.38 ± 0.030	0.36 ± 0.013[ns]
	Spleen	0.25 ± 0.019	0.24 ± 0.029[ns]
	Testes	1.17 ± 0.124	1.26 ± 0.319[ns]

ns = not significantly different between groups.

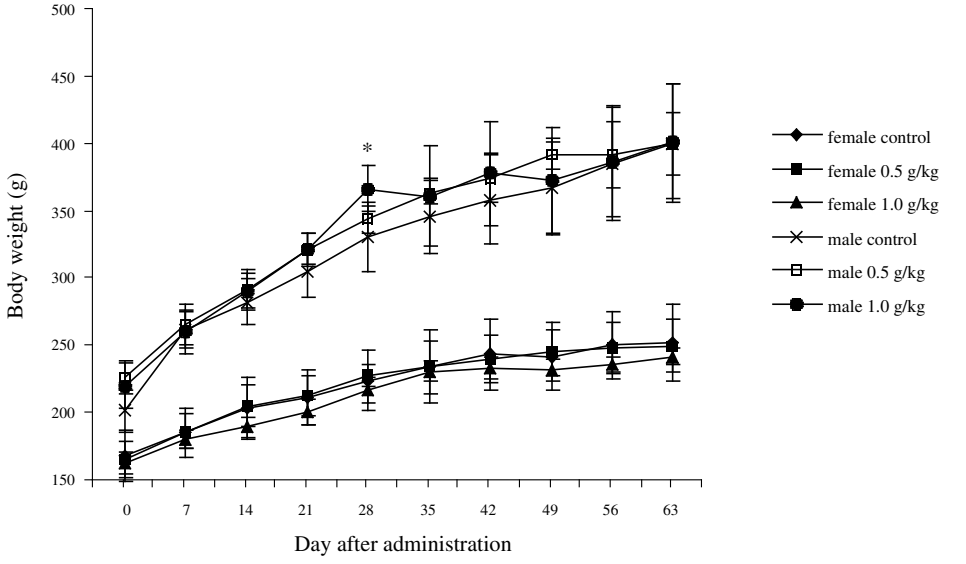

Fig 2- Body weight (g) of female and male rats treated with ethanolic extract of *C. glomerata* and *M. floccosa* at doses of 0.5 g/kg and 1.0 g/kg, compared with controls; *significantly different from the control group (p 0.05).

dose of extract used in our acute toxicity test for rats (25.0 g/kg) was equivalent to 50 times the human dose. This dose did not induce any toxicity sign or death in rats. Likewise, our results from subchronic toxicity testing indicated the non-toxicity of the extract. The normal levels of BUN, creatinine, AST, and ALT indicated that neither *C. glomerata* nor *M. floccosa* altered kidney or liver function. The numbers of white blood cells, which were not significantly different from the controls, revealed normal function of the hematological system. Although the packed cell volume of the male rats treated with 1.0 g/kg of extract was significantly lower than the controls (p 0.05), the level was in the standard range for rat hematocrit (Sharp and La Regina, 1998).

Table 2
Biochemical values of female and male rats treated with ethanolic extract of *C. glomerata* and *M. floccosa* at doses of 0.5 and 1.0 g/kg for 60 days, compared with controls.

Sex	Groups	Blood biochemical assays			
		AST(IU/l)	ALT(IU/l)	BUN(mg/dl)	Creatinine(mg/dl)
Female	Control	169.00 ± 59.189	39.00 ± 9.695	35.20±5.704	0.70 ± 0.071
	0.5 g/kg	165.00 ± 46.138^{ns}	40.00 ± 9.557^{ns}	34.60±3.787^{ns}	0.70 ± 0.100^{ns}
	0.1 g/kg	186.00 ± 46.804^{ns}	39.00 ± 5.620^{ns}	32.80±11.732^{ns}	0.72 ± 0.178^{ns}
Male	Control	137.00 ± 28.355	32.50 ± 4.950	29.00±2.000	0.60 ± 0.081
	0.5 g/kg	121.00 ± 32.078^{ns}	33.50 ± 3.536^{ns}	27.67.±3.055^{ns}	0.58 ± 0.050^{ns}
	1.0 g/kg	116.00 ± 23.643^{ns}	32.50 ± 0.707^{ns}	25.67±3.786^{ns}	0.60 ± 0.082^{ns}

ns = not significantly different between groups

Table 3
Hematological values of rats treated with ethanolic extract of *C. glomerata* and *M. floccosa* at doses of 0.5 and 1.0 g/kg for 60 days, compared with controls.

Sex	Group	Hematocrit (%)	Total white blood cell count (cell/mm^3)	Differential white blood cell (%)				
				Neutrophil	Eosinophil	Basophil	Lymphocyte	Monocyte
Female	Control	43.00 ± 1.73^a	3266.67 ± 153.81^a	8.40±4.28^a	1.80±0.45^a	0.20±0.45^a	87.20±4.55^a	2.40±0.55^a
	0.5 g/kg	40.80 ± 3.96^a	2300.00 ± 916.52^a	15.00±8.37^a	2.20±1.30^a	0.20±0.45^a	80.80±8.47^a	2.20±1.64^a
	1.0 g/kg	42.00 ± 3.08^a	3333.33 ± 1184.62^a	11.80±9.12^a	1.60±0.89^a	0.40±0.55^a	80.80±8.47^a	2.40±1.95^a
Male	Control	46.00 ± 2.16^a	5033.33 ± 1300.50^a	11.75±3.10^a	0.75±0.50^a	0.00±0.00^a	85.50±3.11^a	2.00±0.45^a
	0.5 g/kg	43.50 ± 1.29^{a,b}	5300.00 ± 500.00^a	11.25±5.85^a	1.25±0.50^a	1.25±2.50^a	84.25±5.56^a	2.00±3.37^a
	1.0 g/kg	40.75 ± 3.86^b	2800.00 ± 916.51^a	13.25±2.36^a	0.75±0.50^a	1.25±2.50^a	81.75±5.85^a	3.00±2.71^a

a, b = significant difference at p 0.05

The results of this study could scientifically support the supposition that the general use of *C. glomerata* or *M. floccosa* in daily meals is safe for humans. Since consumption of health products based directly on natural materials has recently shown a rapid sales growth in Thailand, extensive research and development of these non-harmful, edible algae as new natural products with particular nutritional importance are needed.

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