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Original Article

Acute and Subacute Toxicities of the Ethanol Extract from the Rhizomes of *Cyperus rotundus* Linn.

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Abstract The study was carried out to evaluate the acute and subacute toxicities of the ethanol extract from *Cyperus rotundus* Linn. A single oral administration of the ethanol extract at a dose of 5,000 mg/kg did not produce signs of toxicity, behavioral changes, mortality and differences on gross appearance of internal organs. In subacute toxicity, all rats were received a repeated oral dose of 1,000 mg/kg of the ethanol extract over 14 days. The satellite group was given the ethanol extract in the same period but kept for further 14 days without dosing in order to detect the delayed effects or reversibility of toxic effects. The results showed that the extract did not cause changes in terms of general behaviors, mortality, weight gain, hematological and clinical blood chemistry parameters. The results of gross and pathological examinations showed normal appearance of the internal organs as compared to those of the control group.

Keywords: acute toxicity, *Cyperus rotundus*, subacute toxicity

INTRODUCTION

Cyperus rotundus Linn., family Cyperaceae, with Thai name "Haeo Mu", is a common tropical plant that widely grows in all continents. In Asian countries, the rhizomes of this plant, which are used as traditional folk medicine for the treatment of stomach and bowel disorders, and inflammatory diseases. The plant has been proved to exhibit a number of pharmacological actions such as antibacterial,[1-5] antifungal,[6-8] antiviral,[9,10] and antidiarrhoeal activities.[11] Moreover, the plant is reported to possess analgesic, anti-inflammatory, antipyretic,[12] antimalarial,[13-15] and prostaglandin biosynthesis inhibitory activities.[16] Phytochemical constituents of this plant mainly include terpenoids, saponins, alkaloids and sesquiterpenoids.[17-20] In Korean folk medicine, the rhizomes of this plant have been used as an analgesic, a sedative drug[21] and used for the treatment of inflammatory diseases.[22] Furthermore, the 95% ethanol extract from the rhizome has been recently shown to have an inhibitory effect on human immuno-deficiency virus (HIV) and possess antifungal, antibacterial and immuno-modulating properties.[23]

Toxic effects of the 95% ethanol extract of this plant were previously examined in mice. Akperbekova *et al*.,[24] performed toxicity study in mice by intraperitoneal route using the 95% ethanol extract from the rhizomes, showing the LD_{50} of 90 g/kg. Moshin *et al*.,[25] reported the 95% ethanol extract of the whole plant has no oral toxicity effect at the dose of 3,000 mg/kg. Because pharmacological

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properties of *C. rotundus* are highly attractive to assess for a new drug discovery, it is essential to provide its toxicity and safety data for further development of the plant for therapeutic uses. Thus, this study is aimed to investigate the oral acute and subacute toxicity effects of the ethanol extract from the rhizomes of the *C. rotundus* in rats.

MATERIALS AND METHODS

Plant Material

Cyperus rotundus was collected from Bangkok, Thailand in April 2002. The plant materials were identified in the Pharmaceutical Botany Mahidol (PBM) Herbarium, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The voucher specimen (Fansai 0007) was deposited in the PBM Herbarium and kept at the Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok.

Preparation of the Extract

The rhizomes were cut into small pieces and dried in a hot air oven at 55ºC. The dried materials were ground and macerated in 95% ethanol for 3 days and filtered. The filtrate was evaporated under reduced pressure until dryness. The residue from the filtration was macerated in 95% ethanol again for 3 days and filtered. The filtrate was evaporated with the same procedure and combined with the extract from the first extraction. Thin layer chromatography (TLC) fingerprints of the extract were recorded.

Experimental Animals

Male and female Sprague-Dawley rats (120-160 g) were obtained from the National Laboratory Animal Center, Nakhon Pathom, Thailand. All animals were kept in the room maintained under environmentally controlled conditions of 24 ±1°C and 12-hour dark-light cycle. Before each experiment, the animals were fasted overnight with free access to water.

Acute Toxicity Study

The acute oral toxicity was evaluated in rats as described by the World Health Organization (WHO) guideline[26] and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals.[27] Rats were divided into two groups of ten animals (five males, five females). The ethanol extract (2,500 mg/ml in 10% dimethylsulfoxide, DMSO) was orally administered to rats at a single dose of 5,000 mg/kg body weight, while the control group received only vehicle. The animals were monitored for the appearance of toxicity signs over 14 days. The animals that died within this period were necropsied. All rats were weighed and sacrificed on the 14th day following administration. Finally, the vital organs including heart, lungs, livers, kidneys, spleen, adrenals, sex organs and brain were grossly examined.

Subacute Toxicity Study

The method was conducted according to the WHO guideline[26] and the OECD guideline.[28] Due to no sign of acute toxicity at the dose of 5,000 mg/kg, a dose of 1,000 mg/kg given daily for 14 days was chosen in the subacute toxicity test. Briefly, the 1,000 mg/ml extract in 10% DMSO was orally administered at 1,000 mg/kg body weight to rats (ten males, ten females) once daily over 14 days, but the control group received vehicle. The satellite group of each sex (ten males, ten females) was given the extract at the dose of 1,000 mg/kg body weight over 14 days and kept for other 14 days after the treatment in order to detect a delayed occurrence of toxic effect.

During the experimental period, all rats were observed for the appearance of toxicity signs or behavioral alterations (respiration, motor activities, convulsion, reflexes, ocular signs, cardiovascular signs, gastrointestinal signs, etc.). At the end of each experiment, the rats were fasted 12 hours, and then anesthetized with ether. Their blood was collected from a common carotid artery and the serum was separated for hematological study. The following parameters were measured: levels of glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total bilirubin, direct bilirubin, alkaline phosphatase (ALP), serum glutamic-oxaloacetic transaminase (SGOT), and serum glutamic-pyruvic transaminase (SGPT).

After the blood collection, the animals were immediately sacrificed for tissue examinations. The following tissues and organs were weighed, examined, and then fixed in 10% buffered formaldehyde solution: heart, lungs, thymus, livers, kidneys, spleen, adrenals, small intestine, stomach and duodenum, muscle with sciatic nerve, thoracic spines, brain, eyes, sex organs, uterus and epididymis. The fixed organs from all animals were examined by histological method.

Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M.). In subacute toxicity, statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test. The data obtained from acute toxicity studies was analyzed using Student's *t*-test. *P* values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

In the acute toxicity test at the dose of 5,000 mg/kg, all rats did not exhibit signs of toxicity and mortality after a single oral administration of 95% ethanol extract from the rhizomes of *C. rotundus.* The body weight gain and internal organs' weights were next observed since a decrease in both parameters would indicate the presence of toxicity.[29-31] The body weight gain of male rats received the extract was slightly lower than that of the control group but the difference was not significant (Table 1). At the end of this study, the average weight of the internal organs (Table 2) and condition of the color and the texture were normal and not statistically different from the control group. According to the OECD guideline for testing of chemicals, the results of acute toxicity suggested that the 95% ethanol extract from the rhizomes of *C. rotundus* is fairly non-toxic.

Results of the subacute toxicity showed that administration of the ethanol extract from the rhizomes of *C. rotundus* at a dose of 1,000 mg/kg daily over 14 days did not cause mortality or behavioral changes. As shown in Table 3, no statistical difference from the control was also detected on the body weight gains. The weights of some internal organs of both male and female rats in the satellite group were found to be statistically different from those of the treated and the control groups (Table 4), which may be due to variation of the size and/or weight of animals' organs.[32] However, histological examination is then needed to confirm the characteristic of the all tissues. Hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effects such as hemolysis. Some of the hematological values of male rats in the satellite group were significantly different from those of the control group (Table 5). Nonetheless, the significant changes in all of the parameters were in the normal range. The age of animals in the satellite group may account for such variation.[33,34] The differential white blood cell count values showed no difference between the control and

Table 1. Body weights of rats in acute toxicity of the ethanol extract from the rhizomes of *C. rotundus* (5,000 mg/kg)

	Body weight (g)			Weight gain (g) on day 14
	Day 0	Day 7	Day 14	
Female				
Control	104.00 ± 9.80	145.40 ± 8.91	159.60 ± 5.38	55.60 ± 4.92
C. rotundus	102.80 ± 1.50	129.20 ± 5.08	159.20 ± 4.45	56.40 ± 4.45
Male				
Control	129.40 ± 6.43	178.00 ± 7.62	215.40 ± 5.47	86.00 ± 3.33
C. rotundus	123.60 ± 2.64	152.40 ± 3.92	192.80 ± 3.83	69.20 ± 5.24

Values are expressed as mean ± S.E.M., n = 5. There were no significant differences at $p < 0.05$.

Table 2. Organ weights (in grams) of rats in acute toxicity of the ethanol extract from the rhizomes of *C. rotundus* (5,000 mg/kg)

	Control	*C. rotundus*
Female		
Lung	0.89 ± 0.02	0.88 ± 0.02
Heart	0.69 ± 0.03	0.66 ± 0.02
Liver	7.97 ± 0.63	6.49 ± 0.21
Spleen	0.54 ± 0.01	0.59 ± 0.02
Adrenal	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.82 ± 0.02	0.77 ± 0.03
Ovary	0.05 ± 0.00	0.04 ± 0.00
Male		
Lung	1.06 ± 0.04	1.09 ± 0.03
Heart	0.88 ± 0.02	0.80 ± 0.02
Liver	10.18 ± 0.41	9.67 ± 0.43
Spleen	0.81 ± 0.02	0.83 ± 0.03
Adrenal	0.02 ± 0.00	0.03 ± 0.00
Kidney	1.15 ± 0.06	1.04 ± 0.03
Testis	1.19 ± 0.04	1.11 ± 0.02

Values are expressed as mean ± S.E.M., n = 5. There were no significant differences at $p < 0.05$.

Table 3. Body weights of rats in subacute toxicity of the ethanol extract from the rhizomes of *C. rotundus*

	Body weight (g)			Weight gain (g) on day 14
	Day 0	Day 14	Day 28	
Female				
Control	136.00 ± 94.38	175.33 ± 3.13	-	39.67 ± 4.55
C. rotundus[a]	130.40 ± 6.67	168.00 ± 9.65	-	37.60 ± 3.54
C. rotundus[b]	133.20 ± 4.50	166.60 ± 4.19	204.00 ± 7.15	33.40 ± 1.99
Male				
Control	146.00 ± 6.55	221.67 ± 7.03	-	75.67 ± 2.75
C. rotundus[a]	139.00 ± 9.40	202.67 ± 9.78	-	63.67 ± 6.60
C. rotundus[b]	150.67 ± 7.62	230.17 ± 5.71	288.67 ± 3.13	79.50 ± 5.85

Values are expressed as mean ± S.E.M., n = 10. There were no significant differences at $p < 0.05$.
[a] A group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days.
[b] A satellite group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days followed by no treatment for 14 days.

Table 4. Organ weights (in grams) of rats in subacute toxicity of the ethanol extract from the rhizomes of *C. rotundus*

	Control	*C. rotundus*[a]	*C. rotundus*[b]
Female			
Lung	1.08 ± 0.04	1.03 ± 0.06	1.14 ± 0.06
Heart	0.85 ± 0.03	0.86 ± 0.04	1.02 ± 0.06*
Liver	5.98 ± 0.22	6.50 ± 0.20	7.07 ± 0.51*
Spleen	0.54 ± 0.02	0.57 ± 0.03	0.68 ± 0.03*
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Kidney	0.84 ± 0.03	0.84 ± 0.01	0.83 ± 0.03
Ovary	0.05 ± 0.00	0.06 ± 0.01	0.07 ± 0.01
Male			
Lung	1.27 ± 0.05	1.23 ± 0.05	1.35 ± 0.07
Heart	1.13 ± 0.04	1.08 ± 0.05	1.18 ± 0.08
Liver	8.42 ± 0.38	7.94 ± 0.37	9.81 ± 0.89
Spleen	0.76 ± 0.04	0.69 ± 0.04	0.68 ± 0.08
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
Kidney	1.09 ± 0.04	1.01 ± 0.05	1.30 ± 0.06*
Testis	1.22 ± 0.03	1.33 ± 0.06	1.58 ± 0.08*

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days.
[b] A satellite group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days followed by no treatment for 14 days. * Significantly different from control, $p < 0.05$.

the treated groups (Table 6). Therefore, the ethanol extract of *C. rotundus* rhizome did not produce subacute toxicity. The clinical blood chemistry values were used to analyze kidney function (BUN and creatinine), liver function (total protein, albumin, total and direct bilirubins, SGOT, SGPT and ALP) and pancreas function (glucose). In both female and male satellite groups, some clinical blood chemistry values such as creatinine, BUN, SGPT and ALP were statistically different from those of the control group. In general, if the clinical blood chemistry values differ more or less than one fold from the normal values, abnormality of kidney, liver and pancreas's function should be noted.[33,35-37] However, in our study, the observed differences were less than one fold (Table 7), suggesting normal function of the organs. In addition, the histological examinations of the kidney and liver (Figure 1), lung, heart, spleen, adrenal grand, thymus, stomach and duodenum, small intestine, ovary, uterus, testis, epididymis, muscle and nerve, thoracic spine, eyes and brain were normal in both the control and the treated groups.

In conclusion, the ethanol extract from the rhizomes of *C. rotundus* did not cause oral acute and subacute toxicities in rats. An additional study in chronic toxicity evaluation is needed to determine the long-term safety of the extract.

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Table 5. Hematological values of rats in subacute toxicity of the ethanol extract from the rhizomes of *C. rotundus*

	Control	*C. rotundus*[a]	*C. rotundus*[b]
Female			
Red blood cells ($x10^6/\mu l$)	7.78 ± 0.25	7.89 ± 0.15	8.28 ± 0.21
Hemoglobin (g/dl)	14.97 ± 0.47	15.13 ± 0.26	15.20 ± 0.38
Hematocrit (%)	44.00 ± 1.41	43.80 ± 1.80	45.60 ± 1.17
Mean corpuscular volume (fl)	57.67 ± 0.33	57.67 ± 0.77	57.54 ± 0.42
Mean corpuscular hemoglobin (pg)	19.20 ± 0.30	19.18 ± 0.25	18.34 ± 0.16
Mean corpuscular hemoglobin concentration (g/dl)	33.30 ± 0.51	33.33 ± 0.26	33.00 ± 0.33
Platelet ($x10^5/\mu l$)	7.97 ± 0.67	7.99 ± 1.11	8.77 ± 0.94
Male			
Red blood cells ($x10^6/\mu l$)	7.08 ± 0.45	7.45 ± 0.32	8.15 ± 0.10*
Hemoglobin (g/dl)	13.83 ± 0.80	14.50 ± 0.44	15.60 ± 0.14*
Hematocrit (%)	49.00 ± 1.12	43.17 ± 5.09	46.00 ± 0.52
Mean corpuscular volume (fl)	61.48 ± 0.40	60.75 ± 0.62	56.55 ± 0.72*
Mean corpuscular hemoglobin (pg)	19.52 ± 0.29	19.52 ± 0.31	19.12 ± 0.14
Mean corpuscular hemoglobin concentration (g/dl)	31.77 ± 0.30	32.15 ± 0.51	33.98 ± 0.45
Platelet ($x10^5/\mu l$)	9.31 ± 0.89	10.53 ± 0.58	9.45 ± 1.22

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days.
[b] A satellite group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days followed by no treatment for 14 days. * Significantly different from control, $p < 0.05$.

Table 6. Differential white blood cell count values of rats in subacute toxicity of the ethanol extract from the rhizomes of *C. rotundus*

	Control	*C. rotundus*[a]	*C. rotundus*[b]
Female			
White blood cell (x10^3/µl)	2.67 ± 0.30	3.17 ± 0.49	2.59 ± 0.39
Neutrophil (%)	14.17 ± 1.35	15.33 ± 2.76	13.80 ± 1.62
Lymphocyte (%)	82.50 ± 1.18	75.67 ± 5.72	80.20 ± 1.46
Monocyte (%)	1.50 ± 0.62	1.33 ± 4.36	1.80 ± 0.97
Eosinophil (%)	1.83 ± 0.40	1.73 ± 0.71	1.80 ± 0.58
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Male			
White blood cell (x10^3/µl)	2.56 ± 2.22	2.43 ± 3.86	3.05 ± 4.61
Neutrophil (%)	18.00 ± 3.66	20.83 ± 5.50	16.33 ± 2.87
Lymphocyte (%)	80.00 ± 3.47	74.17 ± 6.51	80.50 ± 3.90
Monocyte (%)	1.50 ± 0.43	1.17 ± 0.54	1.00 ± 0.52
Eosinophil (%)	0.83 ± 0.40	0.85 ± 0.12	0.87 ± 0.80
Basophil (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days.
[b] A satellite group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days followed by no treatment for 14 days. There were no significant differences at $p < 0.05$.

Table 7. Clinical blood chemistry values of rats in subacute toxicity of the ethanol extract from the rhizomes of *C. rotundus*

	Control	*C. rotundus*[a]	*C. rotundus*[b]
Female			
Glucose (mg/dl)	96.40 ± 4.78	89.20 ± 1.89	110.20 ± 8.93
BUN (mg/dl)	17.50 ± 1.12	19.50 ± 1.48	19.60 ± 1.94
Creatinine (mg/dl)	0.35 ± 0.02	0.43 ± 0.02	0.48 ± 0.02*
Total protein (g/dl)	5.17 ± 0.07	5.57 ± 0.33	5.34 ± 0.20
Albumin (g/dl)	2.63 ± 0.09	2.93 ± 0.26	2.80 ± 0.09
Total bilirubin (mg/dl)	0.15 ± 0.02	0.13 ± 0.09	0.14 ± 0.02
Direct bilirubin (mg/dl)	0.06 ± 0.03	0.05 ± 0.02	0.03 ± 0.06
SGOT (U/L)	119.67 ± 16.74	182.50 ± 17.43	161.00 ± 30.38
SGPT (U/L)	30.67 ± 2.14	31.00 ± 1.38	30.20 ± 2.27
ALP (U/L)	103.33 ± 7.08	92.00 ± 8.65	69.40 ± 7.30*
Male			
Glucose (mg/dl)	106.67 ± 2.65	104.17 ± 9.79	114.00 ± 11.99
BUN (mg/dl)	13.83 ± 0.60	15.33 ± 1.33	19.17 ± 1.14*
Creatinine (mg/dl)	0.37 ± 0.02	0.35 ± 0.02	0.43 ± 0.02
Total protein (g/dl)	4.88 ± 0.12	4.83 ± 0.13	4.90 ± 0.11
Albumin (g/dl)	2.33 ± 0.09	2.32 ± 0.08	2.43 ± 0.06
Total bilirubin (mg/dl)	0.20 ± 0.04	0.15 ± 0.03	0.18 ± 0.04
Direct bilirubin (mg/dl)	0.05 ± 0.05	0.07 ± 0.05	0.05 ± 0.04
SGOT (U/L)	124.50 ± 16.38	127.50 ± 18.50	128.67 ± 14.90
SGPT (U/L)	33.83 ± 1.08	33.33 ± 3.37	44.33 ± 3.31*
ALP (U/L)	165.50 ± 11.27	146.00 ± 6.50	124.83 ± 5.90*

Values are expressed as mean ± S.E.M., n = 10.
[a] A group was given the ethanol extract of *C. rotundus* rhizomes at 1,000 mg/kg daily over 14 days.
[b] A satellite group was given the ethanol extract of *C. rotundus* rhizome at 1,000 mg/kg daily over 14 days followed by no treatment for 14 days. * Significantly different from control, $p < 0.05$.

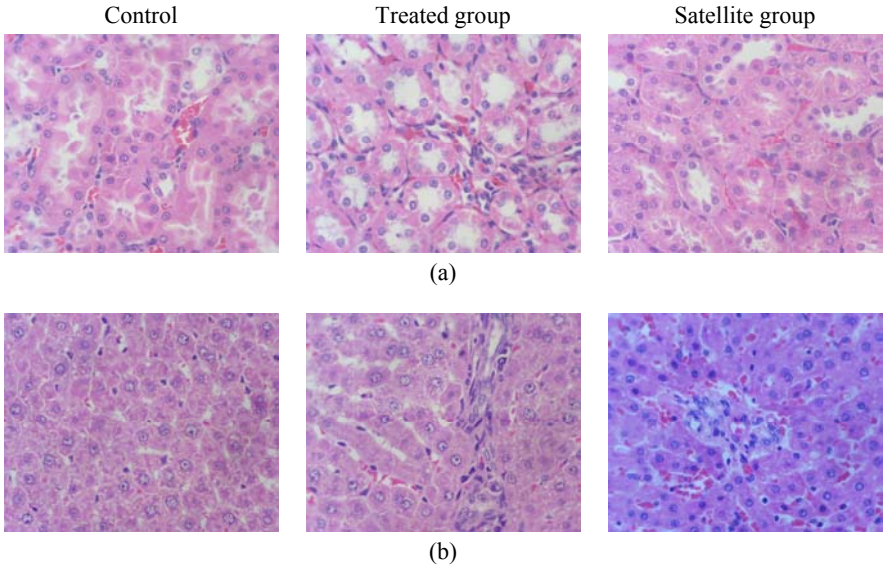

Figure 1. The histology of kidney (a) and liver (b). No significant damage was detected in any treatment groups.

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