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EVALUATION OF ACUTE AND SUBACUTE ORAL TOXICITY OF THE ETHANOL EXTRACT FROM *ANTIDESMA ACIDUM* RETZ.

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Abstract

Toxicity tests of 95% ethanol extract of the root of *Antidesma acidum* were studied in male and female rats. The oral acute toxicity test at 5,000 mg/kg revealed that the ethanol extract did not produce toxic effects on signs, general behavious, mortality and gross appearance of internal organs of rats. Furthermore, the oral sub-acute toxicity test at the dose of 1,000 mg/kg/day displayed no significant changes in body and internal organs' weights, normal hematological and clinical blood chemistry values. Histological examination also showed normal architecture of all internal organs. In conclusion, the ethanol extract of *Antidesma acidum* did not produce any toxicity in oral acute and suba-cute toxicity studies.

Keywords: *Antidesma acidum*; Acute toxicity; Subacute toxicity

Introduction

Antidesma acidum Retz. is a shrub tree in Euphorbiaceae family. This plant is commonly found in many countries of South-East Asia including Thailand known as "Ma-Mao". The decoction of fruit and leaf has long been used for treatment of anemia and stimulation of blood circulation. Moreover, topical application of the leaf and fruit relieves headache and abdominal edema. The bark is used as astringent and analeptic whereas the stem and root is diuretic. The pharmacological activities of *A. acidum* have been reported on anti-HIV and immunomodulating actions (Lousirirojanakul et al., 2003). Since toxicity of *A. acidum* has not been intensively evaluated, this study is undertaken to contribute scientific data on safety of the 95% ethanol extract from *A. acidum* in rats. Thus, both oral acute and subacute toxicities were tested using standard protocols as described by the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals (2001) and World Health Organization (WHO) guideline (2000).

Materials and Methods

Plant material

Antidesma acidum Retz. was collected from Bangkok, Thailand. The voucher specimen (Fansai 0006) was kept at Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok and at Pharmaceutical Botany Mahidol (PBM) Herbarium, Faculty of Pharmacy, Mahidol University, Bangkok. The plant materials were identified by Associate Professor Wongsatit Chuakul, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand.

Preparation of the extract

The whole plant was cut into small pieces and dried in a hot air oven at 55 ºC. The dried materials were ground and macerated in 95% ethanol for three days and filtered. The residue from the filtration was macerated in 95% ethanol again for three days and filtered. The filtrate was evaporated under reduced pressure until dryness.

Animals

Male and female Sprague-Dawley rats, weighing 120-160 g were obtained from the National Laboratory Animal Center, Nakorn Pathom, Thailand. They were all clinically healthy and maintained in environmentally controlled conditions

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at 25 ± 1°C under a 12-hour-dark-light cycle, and given a standard diet and water *ad libitum*, throughout the experimental period. All experimental protocols were approved by the Animal Ethics Committee of Faculty of Medicine, Thammasat University (No. 0001/2003).

Acute toxicity study

The procedure was performed by following the OECD guideline (2001) and WHO guideline (2000). Two groups of ten Sprague-Dawley rat (five males, five females) were orally given a single dose of the ethanol extract at 5,000 mg/kg body weight, while the control group received only vehicle 10% dimethyl sulfoxide (DMSO). The animals were observed for appearance of signs of toxicity at 1, 2, 4 and 6 hour after the administration of test substance, and once daily for 14 days. The visual observations included changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system as well as somatomotor activity and behavioral pattern. The number of survivors was noted after 24 hours, and they were then maintained for a further 14 days. On the 15th day after administration, all surviving rats were weighed and sacrificed. The animals that died during the experiment were necropsied. Next, the internal organs including brain, heart, lungs, livers, kidneys, spleen, adrenals, and sex organs were grossly examined.

Subacute toxicity study

Three groups of 12 rats (six males, six females) orally received the extract at the dose of 1,000 mg/kg once daily for 14 days, but the control group received vehicle. The satellite group (six males, six females) was given the extract at the dose of 1,000 mg/kg/day for 14 days and kept further for another 14 days in order to detect the reversible or delayed occurrence of toxic effects. All rats were weighed and observed for the appearance of signs of toxicity or behavioral alterations once daily during the experimental period. Daily visual observations were made and recorded systematically similar to those performed as in the acute toxicity study. At the end of each experiment, the rats were fasted 16 hours, and then anesthetized with pentobarbital sodium. Their blood was collected from a common carotid artery for hematological study, including red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet, white blood cell (WBC), neutrophil (PMN), lymphocyte (LYMP), monocyte (MONO), eosinophill (EOS), and basophill (BASO). The serum was separated from the blood and 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 histopathological studies. The following tissues and organs were weighted and examined: 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. All tissues were fixed in 10% buffered formaldehyde solution, and then preserved in 10% neutral buffered formalin solution for histopathological examination.

Statistical Analysis

Results were expressed as mean ± standard error of mean (S.E.M.). 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 were analyzed using Student's *t*-test. *P* values less than 0.05 were considered significant.

Results and discussion

In the acute oral toxicity test, all rats did not exhibit signs of toxicity and mortality after a single oral administration of 95% ethanol extract at the high dose of 5,000 mg/kg. The body weight gain and internal organs' weights were next observed since a decrease in both parameters would indicate the presence of toxicity. The body weight of male rats received the extract on the seventh day was slightly lower than that of the control group, but the body weight gain on the 14th day recovered back to normal. At the end of this study, the average weight and pathological examination of the internal organs showed no signs of abnormalities relative to the control group (data not shown). According to the OECD guideline (2001), the results of acute oral toxicity test gave the median lethal dose (LD_{50}) higher than 5,000 mg/kg body weight suggesting that the 95% ethanol extract from the roots of *A. acidum* is not toxic.

As reported in the OECD guideline, a full study using three dose levels is considered not to be necessary if an acute toxicity test at one dose level of at least 5,000 mg/kg body weight produce no observable toxic effects. Next, a dose of 1,000 mg/kg body weight should be given once daily for 14 days to evaluate the subacute oral toxicity. In our study, the results of subacute oral toxicity showed that the ethanol extract of *A. acidum* at a dose of 1,000 mg/kg for 14 days did not cause mortality, and body weight changes. Moreover, both female and male rats were healthy due to the normal appearance of general behavior, respiratory pattern, cardiovascular signs, motor activities, reflexes, and normal change in skin and fur (data not shown). Some internal organ weights of the satellite group showed significant differences compared to those of the control group (Table 1) because they were further observed for 14 days, but they did not exhibit any gross morphological lesions. Moreover, the difference may result from variation of the size and/or weight of animals' organs (Lillie et al., 1996; Carol, 1995). To confirm these results, all the sampling tissue or internal organs were histological examined and showed no significant changes compared to those of the control. Taken together, all data indicated that the 95% ethanol extract from the roots of *A. acidum* has no toxic effect.

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In the hematological examination, the cells of the blood or blood-forming tissues (especially bone marrow) were observed for changes in their structure and/or numbers of various types of blood cells, including immature cells. As shown in Tables 2 and 3, some of the hematological values such as RBC, HGB, MCV, and WBC of treated rats and satellite rats were slightly different from those of the control group. However, such values are within the normal ranges, thus indicating the result of normal variation among animal groups (Inala et al., 2002; Feldman et al., 2000; Lillie et al., 1996; Barry, 1995).

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 the present study, the administration of the ethanol extract of *A. acidum* markedly caused an increase in the concentration of BUN, creatinine and SGPT in both treated and satellite groups. However, these values were not higher than one fold when compared with those of the control group (Tables 4 and 5). In general, if the clinical blood chemistry values are higher or lower than one fold from the normal values, abnormality of kidney, liver and pancreas's function should be noted (Angkhasirisap et al., 2002; Sacher and McPherson, 2000; Lillie et al., 1996; Barry, 1995; Caisey and King, 1980; Vondruska and Greco, 1973). Thus, in our study, the observed difference suggested normal function of the organs. In addition, the pathological examination of the internal organs was normal in both the control and the treated groups.

In conclusion, the ethanol extract from the roots of *A. acidum* did not cause toxicity in oral acute and subacute toxicity studies in rats. An additional study in chronic toxicity evaluation is needed to further determine the long-term safety of this plant extract.

Table 1: Organ weights of rats in subacute oral toxicity of the ethanol extract of *Antidesma acidum* Retz.

	Organ weights (g)		
	Control	*A. acidum* [a]	*A. acidum* [b]
Female			
Lung	1.08 ± 0.04	1.13 ± 0.08	1.26 ± 0.07*
Heart	0.85 ± 0.03	0.76 ± 0.02	0.93 ± 0.06
Liver	5.98 ± 0.22	5.63 ± 0.37	6.52 ± 0.55
Spleen	0.54 ± 0.02	0.54 ± 0.03	0.67 ± 0.06*
Adrenal	0.03 ± 0.00	0.03 ± 0.00	0.05 ± 0.00*
Kidney	0.84 ± 0.03	0.77 ± 0.04	0.94 ± 0.04*
Ovary	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.00
Male			
Lung	1.27 ± 0.05	1.18 ± 0.05	1.35 ± 0.04
Heart	1.13 ± 0.04	1.01 ± 0.04	1.23 ± 0.06
Liver	8.42 ± 0.38	7.24 ± 0.20	9.61 ± 0.41
Spleen	0.76 ± 0.04	0.67 ± 0.06	0.83 ± 0.06
Adrenal	0.03 ± 0.00	0.04 ± 0.00	0.06 ± 0.02*
Kidney	1.09 ± 0.04	0.97 ± 0.02	1.29 ± 0.04*
Testis	1.22 ± 0.03	1.38 ± 0.04	1.78 ± 0.02*

Values are expressed as mean ± S.E.M., n = 10.
* Significantly different from control, $p<0.05$.
a: A group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days.
b: A satellite group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

Table 2: Hematological values of rats in subacute toxicity of the ethanol extract of *Antidesma acidum* Retz.

	Control	*A. acidum* [a]	*A. acidum* [b]
Female			
RBC ($x10^6$/μl)	7.78 ± 0.25	7.77 ± 0.55	7.74 ± 0.23
HGB (g/dl)	14.97 ± 0.47	14.92 ± 1.04	14.60 ± 0.37
HCT (%)	36.50 ± 4.88	33.67 ± 6.99	43.67 ± 1.08
MCV (fl)	57.67 ± 0.33	57.70 ± 0.68	56.47 ± 0.75
MCH (pg)	19.20 ± 0.30	19.22 ± 0.11	18.88 ± 0.24
MCHC (g/dl)	33.30 ± 0.51	33.37 ± 0.35	33.50 ± 0.59
Platelet ($x10^5$/μl)	7.97 ± 0.67	7.74 ± 1.14	8.00 ± 1.08
Male			
RBC ($x10^6$/μl)	7.08 ± 0.45	7.83 ± 0.27*	8.05 ± 0.16*
HGB (g/dl)	13.83 ± 0.80	15.00 ± 0.64	15.40 ± 0.35*
HCT (%)	36.50 ± 6.21	38.33 ± 5.04	46.67 ± 1.15
MCV (fl)	61.48 ± 0.40	60.10 ± 0.38	57.97 ± 0.51*
MCH (pg)	19.52 ± 0.29	19.10 ± 0.33	19.25 ± 0.10
MCHC (g/dl)	31.77 ± 0.30	31.80 ± 0.44	33.23 ± 0.35
Platelet ($x10^5$/μl)	9.31 ± 0.89	10.49 ± 0.82	9.39 ± 0.75

Values are expressed as mean ± S.E.M., n = 10.
* Significantly different from control, $p<0.05$.
a: A group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days.
b: A satellite group was given the extract of *A. acidum* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

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Table 3: Differential white blood cell count values of rats in subacute toxicity of the ethanol extract of *Antidesma acidum* Retz.

	Control	*A. acidum* [a]	*A. acidum* [b]
Female			
WBC (x10^3/μl)	2.67 ± 0.30	4.22 ± 0.53*	2.75 ± 0.54
PMN (%)	14.17 ± 1.35	20.00 ± 2.70	16.83 ± 3.88
LYMP (%)	82.50 ± 1.18	75.83 ± 3.25	73.50 ± 3.62
MONO (%)	1.50 ± 0.62	2.17 ± 0.79	4.00 ± 0.86
EOS (%)	1.83 ± 0.40	2.17 ± 0.91	3.17 ± 0.95
BASO (%)	0.00 ± 0.00	0.00 ± 0.00	2.50 ± 1.96
Male			
WBC (x10^3/μl)	2.56 ± 0.22	3.85 ± 0.53*	3.85 ± 0.32*
PMN (%)	18.00 ± 3.66	26.33 ± 5.07	19.33 ± 4.93
LYMP (%)	80.00 ± 3.47	70.67 ± 5.13	78.17 ± 5.00
MONO (%)	1.50 ± 0.43	1.50 ± 0.56	1.67 ± 0.56
EOS (%)	0.83 ± 0.40	1.50 ± 0.43	1.17 ± 0.48
BASO (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

Values are expressed as mean ± S.E.M., n = 10
* Significantly different from control, $p<0.05$.
a: A group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days.
b: A satellite group was given the extract of *A. acidum* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

Table 4: Clinical blood chemistry values of female rats in subacute toxicity of the ethanol extract of *Antidesma acidum* Retz.

	Control	*A. acidum* [a]	*A. acidum* [b]
Glucose (mg/dl)	92.83 ± 5.29	74.33 ± 5.85	120.50 ± 12.64
BUN (mg/dl)	17.50 ± 1.12	22.67 ± 1.17*	20.83 ± 1.56
Creatinine (mg/dl)	0.35 ± 0.02	0.42 ± 0.03	0.43 ± 0.03
Total protein (g/dl)	5.17 ± 0.07	5.27 ± 0.10	5.10 ± 0.13
Albumin (g/dl)	2.63 ± 0.09	2.77 ± 0.10	2.68 ± 0.07
Total bilirubin (mg/dl)	0.15 ± 0.02	0.36 ± 0.11	0.32 ± 0.06
Direct bilirubin (mg/dl)	0.06 ± 0.03	0.22 ± 0.13	0.19 ± 0.06
SGOT (U/l)	119.67 ± 16.74	169.00 ± 17.22	149.50 ± 15.66
SGPT (U/l)	30.67 ± 2.14	33.83 ± 1.99	32.50 ± 1.75
ALP (U/l)	103.33 ± 7.08	119.00 ± 24.87	85.33 ± 8.90

Values are expressed as mean ± S.E.M., n = 10.
* Significantly different from control, $p<0.05$.
a: A group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days.
b: A satellite group was given the extract of *A. acidum* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

Table 5: Clinical blood chemistry values of male rats in subacute toxicity of the ethanol extract of *Antidesma acidum* Retz.

	Control	*A. acidum* [a]	*A. acidum* [b]
Glucose (mg/dl)	106.67 ± 2.65	118.00 ± 19.81	120.00 ± 10.81
BUN (mg/dl)	13.83 ± 0.60	15.67 ± 0.80	16.83 ± 0.75*
Creatinine (mg/dl)	0.37 ± 0.02	0.35 ± 0.03	0.45 ± 0.04*
Total protein (g/dl)	4.88 ± 0.12	5.20 ± 0.30	4.98 ± 0.05
Albumin (g/dl)	2.33 ± 0.09	2.57 ± 0.17	2.47 ± 0.03
Total bilirubin (mg/dl)	0.20 ± 0.04	0.30 ± 0.10	0.19 ± 0.06
Direct bilirubin (mg/dl)	0.05 ± 0.05	0.12 ± 0.08	0.05 ± 0.05
SGOT (U/l)	124.50 ± 16.38	126.83 ± 18.62	129.67 ±± 17.50
SGPT (U/l)	33.83 ± 1.08	37.00 ± 2.10	47.50 ± 2.68*
ALP (U/l)	165.50 ± 11.27	153.00 ± 9.64	155.00 ± 11.41

Values are expressed as mean ± S.E.M., n = 10.
* Significantly different from control, $p<0.05$.
a: A group was treated with the extract of *A. acidum* at 1,000 mg/kg/day for 14 days.
b: A satellite group was given the extract of *A. acidum* at 1,000 mg/kg/day for 14 days followed by no treatment for 14 days.

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