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Original Article

ACUTE AND SUB CHRONIC TOXICITY STUDY OF ETHANOL EXTRACT OF ANREDERA CORDIFOLIA (TEN.) V. STEENIS LEAVES

C.D. SALASANTI[1], E. Y. SUKANDAR, I. FIDRIANNY

School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia.
Email: citrasalasanti@gmail.com[1]

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ABSTRACT

Objectives: To evaluate acute and sub chronic oral toxicity effects of ethanol extract of *Anredera cordifolia* (Ten.) v. Steenis leaves.

Methods: Extract was prepared by reflux method in ethanol 96%. Acute oral toxicity test was conducted in ddY mice using the conventional test. *A. cordifolia* extract was administered orally at dose ranging between 0.05 – 15 g/kg body weight to experimental mice and observed for any toxic symptoms up to 14 days. Sub chronic toxicity study was evaluated in Wistar albino rats by administration orally *A. cordifolia* extract at doses of 0.1 g/kg body weight, 0.4 g/kg body weight, and 1 g/kg body weight daily for 90 days. Behavior, mortality, and body weight of rats were observed during study period. Relative organ weight, hematology, blood biochemistry, and histopathology were observed at the end of study period.

Results: The *A. cordifolia* extract was well tolerated at the single administration in acute toxicity study. No mortality was observed even at the highest dose of 15 g/kg body weight. In sub chronic toxicity study, *A. cordifolia* extract up to dose of 1 g/kg body weight did not cause mortality and behavioral changes. There were no significantly different in body weight development, relative organ weight, hematology, blood biochemistry in rats treated by *A. cordifolia* extract compared with control group ($p > 0.05$). Histology observations showed that heart, lung, liver, kidney and spleen had no different with the control group.

Conclusion: Beside on acute and sub chronic study, ethanol extract of *A. cordifolia* leaves showed no toxic signs or abnormalities that can be considered to be safe for medical uses.

Keywords: Acute, Sub chronic, Oral toxicity, *Anredera cordifolia.*

INTRODUCTION

Plant derived products have been used for medicinal purposes since the creation of man. Approximately about 80% of the world population today, relies on botanical preparations as medicines to meet their health needs [1]. The problem with the botanical preparations is that most of the plants are been used indiscriminately without adequate information on associated safety/ toxicity risks. Thus for proper knowledge and guidance of these natural products, there is need for scientific documentation on the safety/ toxicity profile on these acclaimed medicinal plants [2]. *Anredera cordifolia* is a medicinal plant that originated from China which is known as the original name *Dhen San Chi* or *Madeira v*ine in South America. In Indonesia, this plant is known as binahong. Binahong is used traditionally to treat various diseases, including skin disease, hypertension, inflammation and gout. Traditional medicine in Colombia and Taiwan use water extract of *A. cordifolia* leaves as antidiabetic drug and analgesics. *A. cordifolia* leaves are reported to contain saponin, flavonoid, quinon, steroid, monoterpenoid and sesquiterpenoid, while the rhizomes are known to contain flavonoid, poliphenol, tannin and steroid. A study has managed to isolate the triterpenoid saponin from *A. cordifolia* leaves which is known as bousingosida A1 [3]. Based on the pharmacology research that the *A. cordifolia* leaves have activity as diuretics [4], nefroprotektor [5], and antioxidants [6].

This study is significant as it predict the safety associated with the use of isolated bioactive agents from medicinal plant [7] and justify the execution of toxicological screening of new pharmaceutical product before approval for human use [8]. This is the first report of toxicological profile of *A. cordifolia* leaves extract.

MATERIALS AND METHODS

Plant materials

Fresh leaves of *A. cordifolia* were collected from Manoko Botanical Garden in Lembang, West Java, Indonesia during January - February, 2013. Plant identification has been done at Bandungense Herbarium, School of Biological Sciences and Technology, Bandung Institute of Technology. This was then chopped into tiny bits of about 2 cm and subsequently dried in a hot air oven and pulverized using a grinding machine. The pulverized sample was extracted with 96% ethanol with reflux method for 3 times (@ 2 hours). Liquid ethanol extract was concentrated by rotary evaporator.

Experimental animals

Male and female ddY mice from the animal house of the PT. Bio Farma, Indonesia were used for the acute toxicity studies. Male and female Wistar albino rats obtained from the animal house of D'Wistar, West Java Indonesia were used for the sub chronic studies. They were housed in wire mesh cages in a well ventilated room, 12 h natural light and 12 h darkness, with free access to water and food (standard rat feed). They were allowed to acclimatize for 1 week before experimentation. This study was conducted according to *Guide for the Care and Use of Laboratory Animals*, Institute for Laboratory Animal Research, National Research Council [9].

Acute toxicity study

Sixty mice each consisting 30 male and female mice, (25-30 g) distributed in to 6 groups of five were accommodated in wire mesh cages. Group 1 served as the control and received carrier solution, CMC Na 1%, only while group 2-6 were administered single doses by oral gavages of plant extract (0.05, 0.3, 2, 5 and 15 g/kg body weight) dissolved in carrier solution. Behaviors were observed at 0 and 30 min and 1, 2, 4 and 24 hours. The animals were monitored for 14 days for any change in activity such as excitation, fatigue, diarrhea, itching, curved tail, shivering, falling of hair and mortality. On the 14th day all animals were sacrificed. Macroscopic presentation of organ was observed for changes in size and color. All the animals had regular supply of food and water until the end of the experiment.

Sub chronic toxicity study

Six groups of 10 rats each were used for this study. Group 1 served as the control animals and was administered carrier solution (CMC

Na 1%) by oral gavages. Groups 2, 3 and 4 served as the test groups and were administered three graded doses (0.1, 0.4 and 1 g/kg body weight) of the plant extracts by oral gavages. Group 5 served as the control satellite and was administered carrier solution (CMC Na 1%) by oral gavages. Group 6 served as the test satellite group and was administered doses 1 g/kg body weight of the plant extracts by oral gavages. Carrier solution and plant extracts were administered daily for 90 days to all animals. All animals in each group was weighed on day 0 and then weekly until termination of the investigation on day 90. Animal in test and control group were sacrificed on the 91th day while satellite group on the 121th day after an overnight fast.

Five micro liters blood was taken from animal tail to determine hematology parameter. There were leukocyte, erythrocyte, hemoglobin (Hb), hematocrit (Ht), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean platelet volume (MPV), red blood cell distribution width (RDW), and platelet (PLT). Blood collected for analysis of biochemistry parameters through the jugular vein. Blood collected from animals at the end of the experiment were transformed to serum and used for determination of serum concentration of glucose, cholesterol, triglyceride, creatinine, urea and aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. All serum biochemistry was performed using the respective analytical kits obtained from PT. Rajawali Nusindo diagnostics, Indonesia. The brain, heart, lungs, liver, kidney, adrenal glands, spleen, stomach, testis, seminal vesicle, uterus, and ovarian were harvested cleaned of blood using distilled water and weighed. The heart, lung, liver, kidney, and spleen were fixed in 10% formalin for histopathology examination. The fixed tissues were then dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5 µm thick sections and then stained using hematoxylin – eosin and observed under microscope.

Statistical analysis

Data obtained were expressed as mean ± standard deviation. The statistical analysis was performed using one-way analysis of variance and LSD post test was conducted to determine significantly different between groups. The significantly different between the mean of the control and treated groups was considered at $p < 0.05$.

RESULTS AND DISCUSSION

In acute toxicity study, the ethanol extract of *A. cordifolia* leaves was well tolerated by the experimental animals even at higher dosages. No mortality was observed even at the highest dose of 15 g/kg body weight. In acute toxicity testing doses higher than 5 g/kg body weight are generally not considered as dose related toxicity [10]. Also in accordance with the Organization for Economic Corporation and Development (OECD) guidelines for oral acute toxicity, an LD_{50} of 2 g/kg and above is categorized as unclassified and hence declared relatively safe [11]. Thus the ethanol extract of *A. cordifolia* was well tolerated and is not toxic at acute administration.

In sub chronic toxicity study, there was a difference of observation time in the satellite group. The aims of observations on the satellite group were to see any delayed effects or recovery effect after administration of the test substance. There were two groups of satellites are control satellite and highest dose satellite. Toxic effects appear comparable to the dose, if the highest dose did not appear toxic effect it will not appear toxic effects at doses below it.

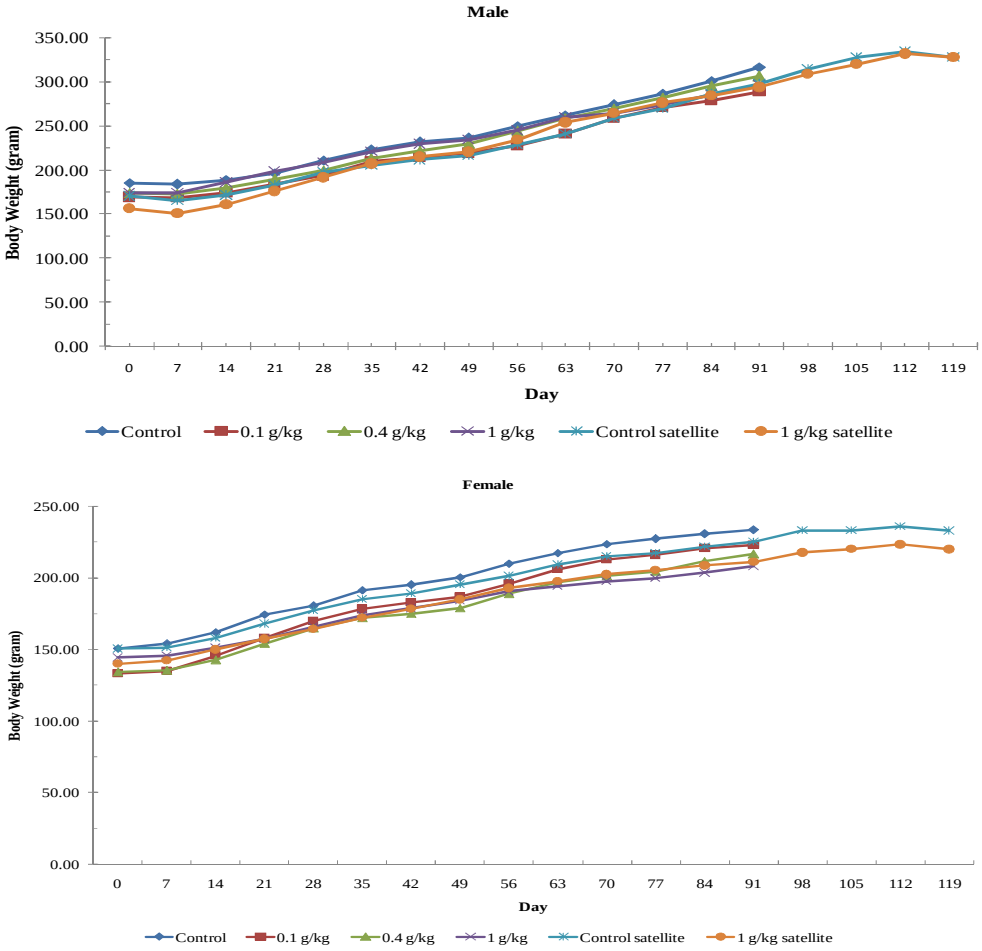

Fig. 1: Profile Body Weight Development in Male and Female Rats for 90 Days after Administration Ethanol Extract of *Anredera cordifolia* Leaves

Changes in body weight can be used as the basis for the assessment of individual response to the effects of drugs [12] and may indicate the side effects of a drug [13]. Profile of body weights of control and *A. cordifolia* extract treated rats at various dose levels are presented in Fig. 1. There was an increasing in body weight in each group of test animals and not significantly different with the control group ($p > 0.05$) so that the ethanol extract of *A. cordifolia* leaves did not affect to animals appetite.

The relative organ weights of control and *A. cordifolia* extract treated rats at various dose levels are presented in Table 1. The relative organ weight of brain, heart, and adrenal gland in male rats were significantly different. The relative organ weight of cardiac and adrenal gland of 1 g/kg satellite group were significantly different with control satellite ($p < 0.05$) and the relative organ weight of brain of 1 g/kg satellite group were significantly different with 1 g/kg group ($p < 0.05$). The different only found in male rats. In female rats there were no significantly different in relative organ weight between groups that treat *A. cordifolia* extract with control group. The relative organ weight value is influenced by the weight of organs and body of animals, the different in relative organ weight may be due to variations in the internal organs and body weight of each animal [14-15].

Table 1: The relative organ weight of rats treated with *Anredera cordifolia* extract

% Relative organ weight	Treatment					
	Control	*Anredera cordifolia* ethanol extract			Control satellite	0.1 g/kg satellite
		0.1 g/kg	0.4 g/kg	1 g/kg		
Male						
Brain	0.589 ± 0.057	0.599 ± 0.025	0.571 ± 0.036	0.620 ± 0.033	0.563 ± 0.033	0.565 ± 0.043*
Heart	0.310 ± 0.020	0.316 ± 0.013	0.293 ± 0.017	0.315 ± 0.021	0.324 ± 0.031	0.298 ± 0.022**
Lungs	0.568 ± 0.068	0.692 ± 0.117	0.634 ± 0.120	0.689 ± 0.181	0.637 ± 0.162	0.632 ± 0.141
Liver	0.567 ± 0.025	0.571 ± 0.033	0.548 ± 0.028	0.574 ± 0.018	0.573 ± 0.028	0.548 0.033
Kidneys	0.572 ± 0.027	0.577 ± 0.036	0.561 ± 0.045	0.577 ± 0.019	0.577 ± 0.029	0.579 ± 0.022
Adrenal glands	0.015 ± 0.002	0.015 ± 0.002	0.015 ± 0.003	0.018 ± 0.003	0.016 ± 0.003	0.020 ± 0.005**
Stomach	1.118 ± 0.435	1.245 ± 0.619	1.226 ± 0.517	1.131 ± 0.344	1.510 ± 0.389	1.225 ± 0.470
Spleen	0.227 ± 0.067	0.191 ± 0.031	0.189 ± 0.031	0.202 ± 0.043	0.195 ± 0.025	0.186 ± 0.040
Testis	0.896 ± 0.084	0.957 ± 0.130	0.895 ± 0.088	0.989 ± 0.091	0.898 ± 0.068	0.915 ± 0.088
Seminal vesicles	0.399 ± 0.078	0.384 ± 0.045	0.407 ± 0.063	0.391 ± 0.116	0.398 ± 0.055	0.395 ± 0.049
Female						
Brain	0.830 ± 0.098	0.786 ± 0.131	0.825 ± 0.053	0.869 ± 0.089	0.792 ± 0.124	0.803 ± 0.060
Heart	0.313 ± 0.035	0.313 ± 0.020	0.318 ± 0.021	0.333 ± 0.033	0.306 ± 0.028	0.312 ± 0.019
Lungs	0.683 ± 0.168	0.922 ± 0.548	0.887 ± 0.314	0.801 ± 0.201	0.719 ± 0.120	0.750 ± 0.137
Liver	2.790 ± 0.390	2.590 ± 0.710	2.924 ± 0.264	2.924 ± 0.234	3.043 ± 0.371	2.943 ± 0.373
Kidneys	0.565 ± 0.074	0.575 ± 0.032	0.574 ± 0.047	0.576 ± 0.057	0.567 ± 0.037	0.564 ± 0.038
Adrenal glands	0.025 ± 0.007	0.027 ± 0.005	0.028 ± 0.004	0.029 ± 0.008	0.025 ± 0.004	0.027 ± 0.006
Stomach	1.278 ± 0.480	1.256 ± 0.293	1.165 ± 0.289	1.203 ± 0.272	1.347 ± 0.497	1.273 ± 0.394
Spleen	0.229 ± 0.134	0.256 ± 0.087	0.229 ± 0.043	0.266 ± 0.049	0.240 ± 0.084	0.222 ± 0.031
Ovarian	0.049 ± 0.017	0.038 ± 0.010	0.049 ± 0.015	0.049 ± 0.010	0.047 ± 0.013	0.054 ± 0.016
Uterus	0.217 ± 0.086	0.210 ± 0.101	0.213 ± 0.086	0.213 ± 0.050	0.261 ± 0.094	0.217 ± 0.069

(*) significantly different with 0.1 g/kg group ($p < 0.05$); **) significantly different with control satellite group ($p < 0.05$)

Table 2: The hematology value of rats treated with *Anredera cordifolia* extract

Hematology Parameter	Treatment					
	Control	*Anredera cordifolia* ethanol extract			Control satellite	0.1 g/kg satellite
		0.1 g/kg	0.4 g/kg	1 g/kg		
Male						
Leukocyte (x 10^3/ mm^3)	4.35 ± 0.77	4.51 ± 1.39	4.44 ± 1.08	4.61 ± 1.09	4.53 ± 1.87	4.34 ± 2.19
Erythrocyte (x 10^6/ mm^3)	1.96 ± 0.61	1.95 ± 0.61	1.97 ± 0.41	1.94 ± 0.39	1.93 ± 0.53	1.59 ± 0.38
Ht (%)	7.34 ± 2.47	7.39 ± 2.39	7.38 ± 1.62	7.38 ± 1.50	7.41 ± 1.31	6.26 ± 1.32
Hb (g/dl)	6.24 ± 130	6.53 ± 1.16	6.28 ± 1.19	6.35 ± 1.12	6.16 ± 1.01	5.81 ± 1.04
RDW (%)	7.63 ± 0.82	7.80 ± 0.92	7.93 ± 0.39	7.71 ± 0.70	7.51 ± 0.67	7.46 ± 0.59
MCV (fl)	37.88 ± 0.43	37.86 ± 0.40	37.95 ± 0.24	38.00 ± 0.22	37.81 ± 0.38	38.05 ± 0.35
MCH (pg/ cell)	33.49 ± 2.96	31.61 ± 3.13	33.33 ± 2.73	32.63 ± 1.97	34.70 ± 1.56	34.99 ± 1.41
MCHC (g/ dl)	86.85 ± 9.58	85.90 ± 11.51	85.23 ± 7.13	85.14 ± 5.50	88.19 ± 7.32	91.73 ± 4.46
Platelet (x 10^2/ mm^3)	17.21 ± 1.39	16.00 ± 3.01	16.07 ± 2.15	14.91 ± 0.98	16.20 ± 1.54	16.15 ± 1.52
MPV (fl)	21.83 ± 0.46	21.79 ± 0.34	21.91 ± 0.15	21.86 ± 0.41	21.85 ± 0.23	21.84 ± 0.18
Female						
Leukocyte (x 10^3/ mm^3)	3.34 ± 0.54	3.96 ± 0.63	4.00 ± 0.48	3.68 ± 0.90	3.29 ± 0.97	2.97 ± 0.60
Erythrocyte (x 10^6/ mm^3)	1.72 ± 0.31	1.86 ± 0.38	1.84 ± 0.37	1.65 ± 0.46	1.41 ± 0.51	1.41 ± 0.48
Ht (%)	6.43 ± 1.36	7.05 ± 1.52	6.98 ± 1.49	5.08 ± 1.86	5.11 ± 0.66	4.53 ± 0.69
Hb (g/dl)	5.63 ± 0.66	6.01 ± 0.72	6.05 ± 0.50	5.56 ± 0.79	5.01 ± 0.62	5.00 ± 0.73
RDW (%)	8.14 ± 3.46	7.91 ± 0.98	8.04 ± 0.84	7.54 ± 0.82	7.37 ± 1.14	7.05 ± 0.87
MCV (fl)	37.86 ± 0.39	37.93 ± 0.46	38.02 ± 0.37	37.71 ± 0.36	37.50 ± 0.71	37.30 ± 0.34
MCH (pg/ cell)	31.00 ± 2.90	32.42 ± 3.62	33.00 ± 3.86	33.96 ± 4.26	34.55 ± 4.31	36.38 ± 5.37
MCHC (g/ dl)	80.31 ± 9.03	83.77 ± 11.55	84.93 ± 11.73	87.47 ± 11.99	90.79 ± 11.96	95.95 ± 14.59
Platelet (x 10^2/ mm^3)	12.15 ± 2.05	14.27 ± 2.25	14.03 ± 1.48	14.04 ± 2.58	10.60 ± 1.59	12.32 ± 2.41
MPV (fl)	22.02 ± 0.44	21.96 ± 0.28	21.98 ± 0.53	21.99 ± 0.38	21.92 ± 0.32	21.79 ± 0.32

Bone marrow is the one of the most sensitive targets for toxic compounds. Bone marrow is the main place where blood cells are produced. The hematology parameter value of control and *A. cordifolia* extract treated rats at various dose levels are presented in

Table 2. There were no significantly different in hematological parameters value between groups that treat *A. cordifolia* extract with control group. Hematological parameter values of groups that treat *A. cordifolia* extract showed similar with control group ($p > 0.05$), so it can be concluded that the administration of the *A. cordifolia* extract did not affect the hematological profile and blood formation process.

Liver and kidney function can be seen from blood biochemistry result. AST and ALT were biochemistry parameters that can be used in describing the condition of the liver function. Increasing AST and ALT levels can be indicated liver damage [16]. Creatinine and urea levels can be used in describing the function of the kidneys. Increasing creatinine and urea level may represent a defect in the kidney [17-18]. The blood biochemistry level of control and *A. cordifolia* extract treated rats at various dose levels are presented in Table 3. There were no significantly different in the AST, ALT, creatinine, and urea levels between groups that treat *A. cordifolia* extract with control group ($p>0.05$), so that the *A. cordifolia* extract did not affect the liver and kidney function.

A number of factors have been identified as contributing to the formation of atherosclerotic plaques and the resulting impairment of coronary arterial blood flow. Among these, blood levels of lipids and lipoproteins play an important role [19]. There were no significantly different in the cholesterol and triglyceride levels between groups that treat *A. cordifolia* extract with control group ($p>0.05$). Sub chronic administration of *A. cordifolia* did not affect the serum level of the cholesterol and triglycerides. This indicates that the extract did not present a risk of hypercholesterolemia and hypertriglyceridemia. However, cholesterol levels in female rats at 0.1 g/kg satellite group were significantly different to the 0.1 g/kg group. Cholesterol levels of 0.1 g/kg satellite group were smaller than 0.1 g/kg group.

Table 3: The biochemistry value of rats treated with *Anredera cordifolia* extract

Biochemistry Parameter		Treatment					
		Control	*Anredera cordifolia* ethanol extract			Control satellite	0.1 g/kg b.w satellite
			0.1 g/kg	0.4 g/kg	1 g/kg		
Male							
ALT	U/L	50.67 ± 14.17	50.78 ± 12.59	51.98 ± 15.29	51.13 ± 15.14	55.03 ± 14.98	50.34 ± 7.32
AST	U/L	175.59 ± 23.14	178.10 ± 27.45	175.72 ± 24.02	153.35 ± 39.57	162.37 ± 27.62	154.23 ± 18.24
Creatinine	mg/dL	0.38 ± 0.13	0.43 ± 0.06	0.39 ± 0.10	0.39 ± 0.09	0.36 ± 0.09	0.35 ± 0.09
Urea	mg/dL	53.33 ± 7.52	45.96 ± 9.99	53.87 ± 8.94	45.23 ± 9.68	41.30 ± 2.96	39.40 ± 6.98
Glucose	mg/dL	151.60 ± 9.06	156.23 ± 32.63	151.80 ± 29.04	150.99 ± 20.47	149.34 ± 29.23	168.98 ± 47.73
Total Cholesterol	mg/dL	56.11 ± 23.06	62.23 ± 29.68	56.41 ± 33.41	65.50 ± 28.53	54.10 ± 6.21	58.99 ± 14.91
Triglyseride	mg/dL	110.64 ± 33.77	105.44 ± 21.71	120.83 ± 17.35	113.33 ± 39.89	135.24 ± 26.08	138.53 ± 22.05
Female							
ALT	U/L	47.90 ± 6.82	45.11 ± 9.74	40.35 ± 5.94	47.99 ± 9.93	39.09 ± 6.30	43.23 ± 9.46
AST	U/L	147.76 ± 20.26	139.25 ± 24.25	140.95 ± 16.78	145.18 ± 21.59	130.90 ± 17.53	137.20 ± 11.99
Creatinine	mg/dL	0.46 ± 0.16	0.42 ± 0.14	0.37 ± 0.11	0.38 ± 0.14	0.48 ± 0.08	0.43 ± 0.07
Urea	mg/dL	62.15 ± 16.34	59.46 ± 10.92	63.39 ± 8.93	59.76 ± 7.92	52.41 ± 10.03	49.36 ± 7.21
Glucose	mg/dL	127.61 ± 20.25	136.06 ± 29.08	142.22 ± 20.13	135.11 ± 18.01	137.49 ± 16.29	141.06 ± 22.92
Total Cholesterol	mg/dL	49.12 ± 15.37	42.93 ± 14.40	52.15 ± 12.52	61.97 ± 28.58	40.75 ± 14.30	40.42 ± 12.41*
Triglyseride	mg/dL	99.53 ± 31.81	101.57 ± 33.73	110.52 ± 33.56	100.51 ± 25.20	97.03 ± 33.11	121.77 ± 27.69

*) significantly different with 0.1 g/kg group ($p < 0.05$)

Impaired of insulin action and/or inadequate insulin secretion leads to hyperglycemia [20]. The management of hyperglycemia is an important observation in cases of diabetes and often this is carried out by oral administration of hypoglycemic agents. There were no significantly different in the glucose levels between groups that treat *A. cordifolia* extract with control group ($p>0.05$). Repeated administration of *A. cordifolia* extract did not affect the serum level of the glucose. This indicates that the extract did not affect carbohydrate metabolism or blood glucose regulation system.

Histology observations performed on heart, pulmonary, liver, kidney, and spleen. In histology observation of heart, liver, and kidney were not different with the control group. This is consistent with the levels of AST, ALT, creatinine, and urea of groups that treat *A. cordifolia* extract were not significantly different to the control group . In histological observation of lung and spleen were not different with the control group.

CONCLUSION

LD_{50} of ethanol extract of *A. cordifolia* leaves was above 15 g/kg body weight. Repeated administration of ethanol extract of *A. cordifolia* leaves at doses 0.1, 0.4, and 1 g/kg body weight orally for 90 days showed no mortality, did not change the behavior, did not affect blood profile, biochemistry parameters, and not toxic to the organs of male and female rats.

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REFERENCES

1. Polasa, K., and Nirmala, K. Ginger: Its Role in Xenobiotic Metabolism. ICMR Bull., 2003; 33 Suppl 6: 57-63.
2. Deng, J.F. Clinical Toxicity of Herbal Medicine in Taiwan. Proceedings of the 7th International Conference on Health

Problems Related to the Chinese in North America, July 1-3, 1994, New York, USA.
3. Lemmens, R.H.M.J. and Bunyapraphatsara, N. Plant Resources of South-East Asia No. 12(3): Medicinal and Poisonous Plants 3. Leiden, Netherlands: Backhuys Publication; 2003. p. 72-73.
4. Sukandar E.Y., Sutjiatmo, A.B., and Sari, N.P. Diuretic Effect of Ethanol Extract of Anredera cordifolia (Ten) v. Steenis in Westar Rat. Proceeding 38th Meeting of National Working Group, International conference on Medicinal Plants, July 21-22, 2010, Surabaya, Indonesia.
5. Sukandar, E.Y., Fidrianny, I., and Adiwibowo, L.F. Efficacy of Ethanol Extract of *Anredera cordifolia* (Ten) Steenis Leaves on Improving Kidney Failure in Rats. International Journal of Pharmacology, 2011; 7 Suppl 8: 850-855.
6. Djamil, R., Suwarso, W.P., Sumaryono, W., dan Hanafi, M. Antioxidant Activity of Flavonoid from Andredera Cordifolia (Ten) Steenis Leaves, International Reseach Jounal of Pharmacy 2012: 3 Suppl 9: 241-243.
7. McNamara, B. Concept in Health Evaluation of Commercial and Industrial Chemical. In: New Concepts in Health Evaluation, Mehiman, M.A., R.E. Shapiro and H. Blumental (Eds.). Washington DC.: Hemisphere; 1976.
8. Echobichon, D.J. The Basis of Toxicity Testing. Boca Ranton, Florida: CRC Press; 1992.
9. National Research Council of The National Academies. Guide for the Care and Use of Laboratory Animals. Washington, DC.: National Academy Press: 1996. ISBN-10: 0-309-05377-3.
10. Hayes, A.W. Guidelines of Acute Oral Toxicity Testing. 2nd ed. New York: Raven Press Ltd.; 1987. p. 185.
11. Organization for Economic Cooperation and Development. OECD Guidelines for testing of chemicals. Test guideline 423. Acute Oral Toxicity – Acute Toxic Class Method, adopted 17/12/2001.
12. Winder, C.V., Lembke, L.A., and Stephens, M.D. Comparative bioassay of drugs in adjuvant-induced arthritis in rats: Flufenamic acid, mefenamic acid and phenylbutazone. Arthritis Rheum 1969; 12 Suppl 5: 472-452.
13. Teo, S., Stirling, D., Thomas, S., Hoberman, A., Kiorpes, A., and Khetani, V. A 90 day oral gavage toxicity studies of D-methyl penidate and DL methyl penidate in Sprague Dawley rats. Toxicology 2002; 179 Suppl 3: 183-196.
14. Levine, B.S. Derelanko, M.J., and Hollinger, M.A., editors. Animal Clinical Pathology. In CRC Handbook of Toxicology. USA: CTC Press, Inc.; 1995. p. 318-365.
15. Bailey, S.A., Zidell, R.H., and Perry, R. W. Relationships between Organ Weight and Body/Brain Weight in the Rat: What is The Best Analytical Endpoint? Toxicol Pathol 2004; 32 Suppl 4: 448-466.
16. Marshal, W. J. Clinical Chemistry. 3rd ed. London: Mosby; 1995. p. 231-232.
17. Wolf, P.L. Methods and Technique in Clinical Chemistry. New York: John Willey and Sons Inc.; 1972. p. 135-136, 159, 189-190, 377-382.
18. Kanter, M.W. Clinical Chemistry. Indiana: The Bobbs-Merrill Company Inc; 1975. p. 65, 74-75, 111-114, 184-186.
19. Shaila, H.P., Udopa, S.L., and Udopa, A.L. Hypolipidemic effect of Terminalia arjuna in cholesterol fed rabbits. Fitotherapia 1997; 68: 405-409.
20. Bailey, C.J. Potential new treatment for type 2 diabetes. Trends Pharmacol. Sci. 2000; 21 Suppl 7: 259-265.